Solvothermal Crystallization of Organic Compounds and Natural Products

by

WONG Wan Yee

A Thesis Submitted to
The Hong Kong University of Science and Technology
in Partial Fulfillment of the Requirements for
the Degree of Master of Philosophy
in Chemistry

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WONG Wan Yee
Solvothermal Crystallization of Organic Compounds and Natural Products

by

WONG Wan Yee

This is to certify that I have examined the above MPhil thesis and have found that it is complete and satisfactory in all respects, and that any and all revisions required by the thesis examination committee have been made.

Prof. Ian D. WILLIAMS
Thesis Supervisor

Prof. Xiao-Yuan LI
Head of Department of Chemistry

Department of Chemistry
29 August 2006.
Dedication

to

my family and friends
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<table>
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<th>Term</th>
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<tr>
<td>Benzoic Acid</td>
<td>BA-H</td>
</tr>
<tr>
<td>2-bromobenzoic acid/ 2-bromobenzoate</td>
<td>2BrBA-H/ 2BrBA</td>
</tr>
<tr>
<td>4-chlorobenzoic acid/ 4-chlorobenzoate</td>
<td>4ClBA-H/ 4ClBA</td>
</tr>
<tr>
<td>4-bromobenzoic acid</td>
<td>4BrBA-H</td>
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<tr>
<td>3-hydroxybenzoic acid</td>
<td>3OHBA-H</td>
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<tr>
<td>p-Toluic acid</td>
<td>4MeBA-H</td>
</tr>
<tr>
<td>4-aminobenzoic acid</td>
<td>4NH₂BA-H</td>
</tr>
<tr>
<td>(dl)-tetrahydropalmatine</td>
<td>(dl)THP</td>
</tr>
<tr>
<td>(l)-tetrahydropalmatine</td>
<td>(l)THP</td>
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<tr>
<td>Tetrahydropalmatine</td>
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<tr>
<td>Palmatine</td>
<td>P</td>
</tr>
<tr>
<td>Dihydropalmatine</td>
<td>DHP</td>
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<tr>
<td>isophthalic acid/ isophthalate</td>
<td>13BA-H₂/ 13BA-H</td>
</tr>
<tr>
<td>meso-tartaric acid</td>
<td>MesoTA-H₂</td>
</tr>
<tr>
<td>D-tartaric acid</td>
<td>D-TAR</td>
</tr>
<tr>
<td>4-nitrophenylacetic acid</td>
<td>4npaa</td>
</tr>
<tr>
<td>2-nitrophenylacetic acid</td>
<td>2npaa</td>
</tr>
<tr>
<td>Cambridge Structural Database</td>
<td>CSD</td>
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<tr>
<td>X-ray diffraction</td>
<td>XRD</td>
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<tr>
<td>One dimensional</td>
<td>1-D</td>
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<tr>
<td>Two dimensional</td>
<td>2-D</td>
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<tr>
<td>Variation Temperature Powder XRD</td>
<td>VT-PXRD</td>
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<tr>
<td>Thermal gravimetric analysis</td>
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Solvothermal Crystallization of Organic Compounds and Natural Products

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The Hong Kong University of Science and Technology

Abstract

This thesis continues the exploration of our group’s research into the application of solvothermal crystallization methods to organic compounds, with specific reference to natural products. The background to these studies is presented in Chapter 1.

In this work we have taken Tetrahydropalmatine as an example of a natural product, available in two free base forms as both a racemic mixture ((dl)THP) and enantiopure isomer ((l)THP) and investigated their hydrothermal stability, hydrolytic breakdown products and the use of solvothermal conditions as a facile approach to producing stable salt and neutral co-crystal derivatives of these parent forms.

Chapter 2 describes the hydrothermal crystallization and subsequent single crystal X-ray structural determinations of eight salts of tetrahydropalmatine, including compounds with organic or inorganic counter-anions, hydrated or anhydrous formulations and either as racemic crystals or involving only enantiopure l-(-)-tetrahydropalmatiniunm cations. The hydrochloride salt of this was used to establish the absolute structural configuration of this species and another chiral salt obtained from reaction with D-tartaric acid and l-THP indicates a plausible way to resolve the racemic mixture of dl-THP.

Chapter 3 describes further crystallization of racemic dl-THP with other organic acids to examine the effect of the pKa of the organic acid. In this case a
series of benzoic acids were used with varying substituents which affect the
electronic properties and the acidity of the carboxylic acid functionality. It was
found in a study of eight benzoic acid adducts that three species with pKa below 4.0
formed 1:1 salts with proton transfer to the THP moiety and +NH---O⁻ hydrogen
bonds involving the THP nitrogen atom. Conversely benzoic acids with pKa of
4.00 and higher formed 1:1 neutral molecular adducts with no proton transfer.
These crystals usually possessed N:---HO hydrogen bonds involving the THP
nitrogen. The cross-over in product type is marked and the 4-chloro and 4-bromo
compounds. One conclusion from these studies is that many salts prepared for
pharmaceutical formulations might in fact be neutral co-crystals and that care should
be taken in considering the pKa of ionizable groups.

Chapter 4 discusses the use of ethanol solvent in place of water in many of
these reactions between THP and organic acids. The resulting compounds were
typically found to involve the hydrolysis product palmatine which is an oxidized
form of THP and involves loss of hydrogen from rings B and C to form conjugated
aromatic rings. Five organic salts of palmatine (which is cationic) were isolated and
structurally characterized. The process of decomposition may involve an
intermediate dihydropalmatine which has partial dehydrogenation of THP, with loss
of hydrogen atom from the chiral center C13. This compound can be formed in
good yield and purity after hydrothermal reaction of THP at 120°C for 6 days, or at
140°C for shorter time periods.

Finally in Chapter 5 two new polymorphs of organic compounds produced by
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Understanding of these various issues such as salt and neutral co-crystal formation, hydrolytic breakdown products and high temperature polymorph formation and energetics, as well as the possibility of facile formation of derivatives, e.g. esters, are of importance to application of solvothermal crystallization methodology to pharmaceutical engineering. Perhaps the most important message however is that with the sparing aqueous solubility of many organic compounds such as drugs and natural products, a hydrothermal crystallization approach can offer a novel but facile method of producing single crystals of sufficient size (>100microns) to routinely allow X-ray structure determination and thus aiding product identification.
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1.1 Crystal Engineering of Organic Compounds

In chemistry, a crystal is defined as a solid in which the constituent atom, molecules or ions are packed in a regular ordered, repeating pattern can be considered in terms of a unit cell extending in all three spatial dimensions. Intermolecular interactions found in a crystal structure lead to a minimization of the free energy of the entire atomic arrangement through the crystal.\(^1\)

Crystal engineering is the design and synthesis of solid-state structures with desired properties, based on an understanding and exploitation of intermolecular interactions. The two main strategies currently in use for crystal engineering are based on hydrogen bonding and coordination bonds. These may be understood with key concepts such as the supramolecular synthon and the secondary building unit.

Crystal engineering of organic compounds is a rapidly expanding discipline as revealed by the recent appearance of several international journals in which the topic plays a major role. These include CrystEngComm from the Royal Society of Chemistry and Crystal Growth and Design from the American Chemical Society.

The term ‘crystal engineering’ was first used for organic materials in 1971 by Schmidt in connection with photodimerisation reactions in crystalline cinnamic acids.\(^2\) Since this initial use, the meaning of the term has broadened considerably to include many aspects of solid-state supramolecular chemistry. A useful modern definition is that provided by Desiraju, who in 1989 defined crystal engineering as “the understanding of intermolecular interactions in the context of crystal packing and the utilization of such understanding in the design of new solids with desired physical and chemical properties”.\(^3\)\(^4\)
Since many of the bulk properties of molecular materials are dictated by the manner in which the molecules are ordered in the solid state, it is clear that an ability to control this ordering would afford control over these properties.

Crystal engineering of organic compounds relies on noncovalent forces to achieve the organization of molecules and ions in the solid state. Much of the initial work on purely organic systems focused on the use of hydrogen bonds,\textsuperscript{4} though with the more recent extension to inorganic systems,\textsuperscript{5,6} the coordination bond has also emerged as a powerful tool. Other intermolecular forces such as $\pi \ldots \pi$ and halogen...halogen interactions have all been exploited in crystal engineering studies, and ionic interactions can also be important. However, the two most commonly used strategies in crystal engineering exploit hydrogen bonds and coordination bonds.

The term “synthon” was introduced by Corey in 1967 in an article entitled “General Methods for the Construction of Complex Molecules”.\textsuperscript{7} The term was traditionally used to represent key structural features in a target molecules in organic synthesis.\textsuperscript{8} Since the definition of a synthon is so general and flexible, in the later years, Corey himself in 1988,\textsuperscript{9} and Seebach in 1990,\textsuperscript{10} degenerated into a descriptor of synthetic intermediates. Now, by applying the connotation emblem in supramolecular sense, supramolecular synthon is defined as structural units within supermolecules which can be formed and/or assembled by known or conceivable synthetic operations involving intermolecular interactions.\textsuperscript{8}

By analogy with the retrosynthetic approach to organic synthesis, Desiraju coined the term “Supramolecular synthon” to describe building blocks that are common to many structures and hence can be used to order specific groups in the solid state.\textsuperscript{8} The carboxylic acid dimer represents a simple supramolecular synthon, though in practice this is only observed in approximately 30% of crystal structures in which it
is theoretically possible. The supramolecular synthon approach has been successfully applied in the synthesis of one-dimensional tapes, two-dimensional sheets and three-dimensional structures. The CSD today contains atomic positional parameters for nearly 300 000 crystal structures, and this forms the basis for heuristic or synthon based or "experimental" crystal engineering.

1.2 Hydrothermal Crystallization of Organic Compounds

The term "hydrothermal" implies the crystallization or reaction is carried out in water at temperature higher than 100°C. By extension of meaning, the term "solvothermal" can be used when the organic solvents or mixed organic-aqueous solvents are used instead of water. Since the temperature is usually above the boiling point of the solvent, the reaction will be normally carried out in a Teflon-lined safety bomb or acid-digestion bomb (Figure 1-1). It is designed to withstand the autogenous pressure, which will be generated by the solvent vapor of water, or solvent when it is heated up inside the oven when the % fill is less than 50%. The typical temperature is constrained below 240°C as beyond this temperature the Teflon lining of the cups becomes soft leading to permanent deformation of the cups and the possibility of the loss of the pressure seal of the reaction.

Historically the technique has been applied to synthesis and crystallization of materials which have low solubility in water at ambient temperature, but which is enhanced at the high temperatures (and possibly higher pressures) available above the solvent's normal boiling point. For example purely inorganic framework materials such as silicates, alumino-silicates or alumino-phosphates have been prepared by this method, with the intriguing feature that the presence of organic
moieties, which are also only sparingly soluble in water at room temperature can be added to the synthesis and help to modify the inorganic phase produced either through their direct incorporation or indirect influence on crystallization conditions. Hydrothermal methodologies have thus been commonly used in the synthesis of zeolites\textsuperscript{11} and other porous inorganic solids.\textsuperscript{12} More recently they have been applied to mixed organic-inorganic hybrid materials,\textsuperscript{13-14} which have been advanced by my group for example in the area of organically modified manganese vanadates,\textsuperscript{15} or organic salts of polyoxometallate/ borate clusters.\textsuperscript{16} Extension of the hydrothermal synthetic method was applied to the formation of metal coordination polymers\textsuperscript{17} such as "HKUST-1" [Cu\textsubscript{3}(TMA)\textsubscript{2}(H\textsubscript{2}O)\textsubscript{3}]n a prototypical microporous MCP,\textsuperscript{18} which has received considerable interest with respect to its cooperative magnetism,\textsuperscript{19} active metal sites\textsuperscript{20} and for its exceptional hydrogen storage capability.\textsuperscript{21} The use of organic ligands with metal coordinating centers allows some degree of architectural control and design, work in our group has indicated the important role played by hydrothermal parameters such as temperature, time and pH on the product phase type.\textsuperscript{22} Our results from various systems are compatible with higher connectivity frameworks resulting from high temperature, since ancillary coordination of solvent is reduced.\textsuperscript{23-26} Cheetham has also explored the effect of such parameters in depth on the cobalt succinate polymer system.\textsuperscript{27}

Interestingly in our own studies combining organic carboxylic acids and organic pyridine bases as co-ligands we occasionally also found the formation of organic products without the incorporation of metallic ions.\textsuperscript{28} At first this seemed a distraction and unfortunate side-product, however most interesting was the fact that in the system 4,4-bipyridine and pyromellitic acid (benzene-1,2,4,5-tetracarboxylic acid) three different organic side-products were found, with 2:1, 1:1 and 1:2
molecular ratio. Impressively these could be later formed phase pure in high yield simply by mixing the reagents in appropriate stoichiometry and concentration. The hydrothermal crystallization also allowed their formation as extremely large single crystals which enabled neutron diffraction studies to be carried out.\textsuperscript{29-30}

Further studies of ‘Hydrothermal Crystallization of Organic Compounds’ both as co-crystals and salts as described above or for pure single phase compounds was then explored by Dr. F. L-Y. Shek in her PhD thesis work at HKUST, 2004.\textsuperscript{31} She found that numerous co-crystals could be formed between organic acids and bases, that enhanced crystal growth could frequently be observed for sparingly to moderately soluble compounds and that for her examination of neutron diffraction studies of short N-H-O hydrogen bonds deuteration of exchangeable protons was readily achieved using D\textsubscript{2}O as solvent in place of water. Furthermore she was also able to initiate study of hydrothermal and solvothermal crystallization methodology to study the artemisinin family of natural product derived anti-malarial drugs. A key finding was not only that ‘hot’ water was a surprisingly good solvent system to effect crystallization of many of these compounds (eg artemisinin itself, arte-ether) but that hydrolytic decomposition products (dihydro-artemisinin) or transition to high temperature polymorphic forms (artemether) could also be identified by the approach. This involved heating concentrated solutions, cooling and re-examining the powder X-ray diffraction patterns of the resulting filtered solid products.
Hydrothermal method provides an alternative way for crystal growth of the organic co-crystallization rather than the conventional solvent technique. By hydrothermal reaction method, the high temperature and pressure generated inside would increase the solubility of the substances and enhance the reaction between the acids and bases. The cooling of the reaction would lower the solubility of the resulting compounds and hence provide a medium for crystallization to occur. The products would be isolated by filtration through plastic funnel. The products can then be readily compared by analysis of their Powder XRD patterns, which serves as 'fingerprints' for particular crystalline phase; therefore, the progress of the reaction can be readily monitored as a function of time and temperature, or other features such as concentration, pH etc.
The features of hydrothermal crystallization of organic compounds are summarized below:

(1) avoid the incompatible solubilities of precursor molecules.

(2) provide high temperature and pressure for the reaction condition.

(3) Enhance crystal growth (the crystal size of resultant compounds which are large enough to carry out neutron analysis for a number of samples).

(4) Allow high reagent loading of the precursors.

(5) Obtain high yield of the resulting co-crystals from starting reagents with low room temperature aqueous solubility.

(6) Provide rapid equilibrium between the solids and solution

(7) Allow multiple phases possible with stoichiometric control for mixed crystal.

1.3 Natural Products and Pharmaceuticals – Application of Crystallization

1.3.1 Solid-state Formulation of Pharmaceuticals

In the modern pharmaceutical industry the discovery of promising lead compounds for therapy is the beginning of a lengthy and costly process in drug development leading to a commercialized product. One important step after the identification of a pharmacophore or active compound is the evaluation of its solid state properties involving not just toxicology, but also solubility, absorption, stability and shelf-life, examination of its transport and metabolism and so on. Since the primary approach in western medicine is to identify single efficacious compounds make them pure and cost effective manner and then to prepare them in a form ideally for oral use the solid
state properties of the compound in question is of key importance. As has been eluded to in the above section on organic crystal engineering molecules in the solid state actually have relatively weak forces holding them together in the solid state, with the hierarchy of energies of a) coulombic charge interaction b) hydrogen bonds c) permanent dipole-dipole interactions and d) van der Waals (fluctuating dipole) interactions. Since the individual nature of these interactions b-d) are all weak and must be summed over possibly numerous individual contacts for an organic molecule of even modest complexity the result, perhaps unsurprisingly, is that almost all molecules can pack in a number of different ways with similar potential energy. The existence of different solid state packing arrangements for the same molecule or molecules) is called polymorphism and will be discussed at greater length in Section 1.5. The high probability that a change of crystallization conditions can often allow access to different polymorphs, or sometimes different pseudo-polymorphs\textsuperscript{71-74} involving different quantities of water or co-solvent is of grave importance to drug companies.\textsuperscript{75} Ignorance of this may lead them to run the risk of either a) inconsistent batch processing, in which meta stable polymorphs may be replaced by more stable ones, b) variable/non-optimal solubility or absorption properties for the form of the drug c) non-optimal shelf life for the compound\textsuperscript{79} d) the possible loss of exclusive patent rights\textsuperscript{80} if new solid state forms of active drugs with substantially different or superior pharmaco-properties than those for the registered form are discovered by competitors or generic drug manufacturers.

Atomoxetine is a highly selective norepinephrine reuptake inhibitor. Atomoxetine HCl hydrochloride has been approved for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in children and adults. Meta-stable crystal form is isolated by rapid crystallization.\textsuperscript{76} One of the example of b) is Norvir\textsuperscript{77,78} which is
Abbott's novel protease inhibitor for human immunodeficiency virus (HIV). Two years after the launch of Norvir to the market, some lots of Norvir capsules failed a dissolution specification due to existence of another crystal form. In a classic patent case the pharmaceutical company GlaxoSmithKline defended its patent for the polymorph type II of the active ingredient in Zantac against competitors while that of the polymorph type I had already expired\textsuperscript{81,82}. Company Bristol-Myers Squibb sued a generic company which marketed the hemihydrate antibiotic cefadroxil, claiming the hemihydrate was converted to monohydrated in body before dissolves\textsuperscript{80}.

Given the high cost of development for a modern drug this latter problem means that big pharma must get these issues of crystalline forms of a compound comprehensively studied. Nowadays this will involve high throughput screening of crystallization conditions for the parent compound from numerous solvents and solvent mixtures, as well as attempts to form salt derivatives by use of ionizable groups, wherever possible. In the case of bases these will often be protonated from the free base and then formulated as inorganic salts such as hydrochloride, bicarbonate or hydrogenphosphate, or more commonly as organic salts of non-toxic organic acids, such as citrate, maleate, tartrate, succinate or benzoate.\textsuperscript{83} It is important to realize that each of these products will have their own different solubilities, stabilities and polymorphic behaviors so that effectively each salt derivative may need to be viewed as an independent pharmaco-material. Recently there has been growing awareness that salt formation is not the only possibility of forming more complex solids incorporating molecules of interest. The increased knowledge of crystal engineering of neutral molecular co-crystals has led to investigations of forming drug co-crystals\textsuperscript{84,85} and at least one big pharmaceutical company has now begun screening protocols for drug-co-crystals.
The investigations in this thesis will relate primarily to the application of hydrothermal crystallization to a natural product compound tetrahydropalmatine (THP). This is available in both racemic (+/-) and enantiopure chiral (-) forms. Our studies will show the general applicability of the hydrothermal and solvothermal methods to growing crystals to seek polymorphs formed at higher temperatures, as well as new salts or neutral co-crystals. These experiments have clear application to real world drug formulation issues.

1.3.2 Compound Identification of Natural Products

What actually is a “natural product?” The Dietary Supplement Health and Education Act (DSHEA), passed in 1994, defined natural products as “dietary supplements” and defined the term “dietary supplement” as a product containing one or more of the following ingredients: Vitamin, mineral, herb or other botanical, amino acid, dietary substance for use by humans to supplement the diet by increasing the total daily intake, a concentrate, metabolite, constituent, extract, or combination of the listed ingredients. The product must be intended for ingestion in pill, capsule, tablet, or liquid form and cannot be marketed for use as a conventional food or as the sole component of a meal or diet. According to the DSHEA definition, natural products include herbals or plant products, such as feverfew and ginkgo biloba, and nonplant products, such as glucosamine, coenzyme Q10, creatine, and others.

For most of human history, resources for treating disease were limited. Plant remedies were used long before written history. Plant products still in use today have been found in Neanderthal burial sites from 60,000 years ago. Early discoveries of natural products like morphine and quinine in the 1800s helped found the field of phyto-, or plant-, chemistry. Today approximately 25% of modern drugs contain one
or more active ingredients originally derived from plants, although the plant source may since have been replaced by a synthetic chemical process. The exploration of new sources of drugs is also highly active from natural product isolation, since many plant species have yet to be evaluated for their novel or unique organic constituents. Critical to this process is the identification of these often highly complex carbon skeletons arranged with a bewildering variety of functional groups and with often numerous chiral sites in which the 3D stereochemistry must be determined. Natural product identification involves a mix of nmr and high resolution mass spectra, along with the prior knowledge of related compounds and their chemical structures. In this work we would like to re-emphasize that single crystal X-ray structure determination should actually where possible always be the identification method of choice, which is frequently not case is partly due to historical reasons and partly due to the lack of efforts afforded to growing good quality single crystals for the structure determination. New methodologies for crystal growth, such as hydrothermal discussed in section 1.2 may be of help in this regard.

The example of the THP system, which will be studied herein, as well as the previous work in our group on the artemisinin family of anti-malarial compounds will serve to indicate that hydrothermal crystallization can be applied successfully to natural product compounds to obtain specimens capable of single crystal X-ray crystal structure determination. Modern X-ray diffractometers are equipped with CCD X-ray detectors that enable specimen size of about 0.1mm on edge (100microns) to be studied. The technique involves the diffraction of X-rays by an individual crystal with a single orientation of its ordered molecular array. Diffracted X-ray beams are due to reinforced scattering from electrons which have repeat spacings related to the crystal lattice. Thus the geometry of the diffracted positions relates to
the unit cell of the crystal which is effectively the smallest repeat unit of the crystal involving translational symmetry vectors. Measurement of the diffraction peak positions thus gives rise to a unique unit cell for the material and subsequent measurement of the various diffracted peak intensities can eventually be used to model the electron density distribution within this unit cell, and thus obtain molecular structure since electrons are predominantly associated with the atomic positions and scale with the atomic number \(Z\) of the elements concerned.

The result of a 12 hour data collection for a standard organic molecule would be a detailed knowledge of the 3D arrangement of the molecule (with precise and accurate bond lengths angles and torsions) as well as information on the packing of this molecule with its neighbors in the solid state. Such information and the degree of certainty to which it can be held goes well beyond the information obtained from numerous multi-nuclear nmr spectra or even high resolution mass spectra and thus single crystal X-ray structure determination is the most powerful method for structure elucidation for all organic solid state compounds including pharmaceuticals and natural products. In the case of products of organic synthesis crystal structures have only rarely been determined since historically the degree of difficulty and length of time for structure determination was long (1 week to 1 month in the 1970s) and the use of definite reaction conditions and knowledge of the product types was usually known and later confirmed by \(^1\text{H}\) and \(^{13}\text{C}\) nmr spectra and with the additional support of mass spectra. Unfortunately the renaissance of natural product isolation and identification came at a time when high field nmr instruments were being developed and high resolution mass spectrometers could identify the empirical formula of molecular species. Thus scientists in the field became expert in the use of nmr and in the main left X-ray crystallography as an occasional technique when large
crystals fortuitously formed or to be attempted only when difficult problems intractable by the nmr/ms approach were encountered.

Given the advances of the single crystal method over the past 30 years in X-ray detection as well as computational advance in crystal structure solution and refinement, this is unfortunate since much better detailed information could be obtained from a single X-ray structure analysis than a host of nmr experiments. A further enormous advantage for crystallography is the determination of stereochemistry of chiral centers, which is often of critical importance for natural product identification, but problematic or ambiguous using nmr techniques alone. The X-ray diffraction experiment gives 3D molecular structure, thus the relative stereochemistry of different chiral centers in a crystal can be directly observed. For enantiopure materials the absolute stereochemistry can be determined by anomalous scattering effects, which for use of standard Molybdenum-Kα radiation (λ = 0.7107 Å) requires the presence of ‘heavy’ atoms. These could be halogens such as Br or I, but the third period elements such as P, S or Cl can suffice for relatively small organic molecules. The anomalous scattering effect works by effectively either adding or subtracting small increments from the expected scattering intensity for the diffraction peak depending on the absolute spatial arrangement. If longer wavelength Copper Kα radiation is used (λ = 1.5401 Å) then the presence of oxygen atoms can be sufficient to give an absolute structure. Another approach which is frequently used is that a derivative, salt or co-crystal can be formed with a species of known absolute configuration eg (-)-camphor which can be used to establish the correct stereochemistry for the other chiral centers.

Crystal structure determinations of natural products are of course thus recommended, however whilst the experiment has become more facile it still
requires single crystal specimens in order to be carried out. One of our key goals in this work is to establish the feasibility of using hydrothermal and related methods to crystallize natural product derivatives and salts readily and using only small quantities of material. This latter is critical in the early investigation of secondary metabolites extracted and purified from plants since the pure compounds isolated may be only 10mg from several kg of raw material.

One final point relating to chirality is that some natural products can be found in racemic form from certain sources, yet enantiopure from others. Tetrahydropalmatine (THP) is such a case. Clearly if a source yielding the racemate is plentiful and cheap and that giving the enantiomer relatively expensive it is worth developing methodology to resolve the racemic mixture through separate crystallization of the enantiomers. One approach which could be used for THP, which is a base is to co-crystallize as a salt of a chiral acid such as L-tartaric acid. The principle is then that (+)-THP and (-)-THP species must form diastereomeric (non-equivalent) salts with L-tartrate ions. These will have different 3D packing arrangements and different physical properties such as melting point and solubility. This latter fact can be used to effect a separation of the (+) and (-) forms of the THP.

1.3.3 Tetrahydropalmatine – A Natural Product from TCM

Tetrahydropalmatine (THP) was first described as a constituent of a Chinese herb in a German report published in 1923. For purposes of historical reference, this was the same year that a German study about another alkaloid from a Chinese plant -ephedrine - established a new drug of choice for treatment of asthma. THP belongs to this rich tradition of pharmacological research, though it has not, until recently, been well publicized. The original source of THP in pharmacological research was
the tuber of *Corydalis turtschaninovii* Bess., commonly called corydalis (Chinese: yanhusu or yuanhu). Isolation and characterization of THP from various sources continued for about a decade after the initial work, primarily in China and Japan, as part of a concerted effort to use Western methods of analysis to reveal the nature of traditional remedies. By 1960, the isolated compound had already gained use in clinical trials for treatment of pain, and alternative sources of THP, such as various species of *Stephania*, notably *Stephania rotunda*, had been identified.\(^{39}\) *Corydalis* species contain dl-tetrahydropalmatine, while *Stephania* species contain only l-tetrahydropalmatine.\(^{40,41}\)

THP(5,8,13,13a-tetrahydro-2,3,9,10-tetramethoxy-6H dibenzo[a,g]quinolizine) is an isoquinoline alkaloid, one of many such compounds that are known for analgesic and tranquilizing properties. Within that large group, this compound belongs to the protoberberine series. Alkaloids in this series are used extensively in the Orient,\(^{39}\) mainly for the treatment of infections (e.g., berberine), cancer (e.g., berbamine), and pain (e.g. stephanolide and THP). They are characteristically yellow, have four linked benzene rings with a nitrogen atom joining two ring pairs, and are modified variously via two oxygen atoms at each end. THP may owe some of the strength of its physiologic effects, compared to closely related structures, to its symmetry and to its solubility in fat (a property conferred by the four terminal methyl groups shielding
each oxygen atom).

The physiological effects of THP are essentially the same as those of the herb corydalis, which is analgesic, sedative, tranquilizing, and slightly hypnotic.\textsuperscript{42} dl-Tetrahydropalmatine potentiates or antagonizes the action of various drugs that influence the central nervous system.\textsuperscript{39} It significantly potentiates the hypnotic action of cyclobarbital and it increases sensitivity to the convulsant effect of strychnine.

The mechanism of action of THP has been investigated.\textsuperscript{39,42-44} It depresses cortical and subcortical electrical activity, especially in the motor areas of the brain. The action on neurotransmitters was shown to be different from that of either reserpine or morphine. Other studies have shown that dl-tetrahydropalmatine and l-tetrahydropalmatine deplete the levels of three neurotransmitters: dopamine, norepinephrine, and serotonin, with a corresponding increase in most of their metabolites. This finding suggests that the compound acts as a short-lasting monoamine depleter. The other berberine compounds, some of which could bind to M-cholinergic receptors, do not have an effect on mono-amines in the brain. In other words, the action of THP is unique.

1.4 Hydrogen Bonding in Organic Crystals

The hydrogen bond was discovered almost 100 years ago.\textsuperscript{45} The discovery of the hydrogen bond cannot be attributed to a single author and no genuine “first paper” can be quoted. Historical surveys can be found in the books cited below.\textsuperscript{45-52} The reason for this long-lasting interest lies in the eminent importance of hydrogen bonds for the structure, function, and dynamics of a vast number of chemical systems,
which range from inorganic to biological chemistry.

A far-sighted early definition is that of Pimentel and McClellan, who essentially wrote that “...a hydrogen bond exists if 1) there is evidence of a bond, and 2) there is evidence that this bond sterically involves a hydrogen atom already bonded to another atom”\textsuperscript{45} From a modern viewpoint, Thomas Steiner modified point 2, such as by requiring that X-H acts as a proton (not electron) donor. Therefore, the following definition is proposed: An X-H...A interaction is called a “hydrogen bond”, if 1) it constitutes a local bond, and 2) X-H acts as proton donor to A.\textsuperscript{53}

1.4.1 Categories of Strong, Moderate and Weak Hydrogen Bonds

Hydrogen bonds can be classified into three different categories of “Strong”, “Moderate” and “Weak” which was described by Prof. Jeffrey\textsuperscript{49} The nature of hydrogen bonds appear to be a wider range of interatomic interactions than what is observed for covalent or ionic bonds or van der Waals forces. Unlike moderate and weak hydrogen bonds, strong hydrogen bonds are quasi-covalent in nature. The general properties of these of hydrogen bond categories are listed in Table 1-1. The numerical data are guiding values only. Prototypes of each category have distinctly different properties. It is impossible to set borderlines between those categories or use it too stringently.

<table>
<thead>
<tr>
<th>D—H—A interaction</th>
<th>Strong</th>
<th>Moderate</th>
<th>Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond lengths</td>
<td>D—H \approx H—A</td>
<td>D—H &lt; H—A</td>
<td>D—H \ll H—A</td>
</tr>
<tr>
<td>H—A (Å)</td>
<td>~1.2-1.5</td>
<td>~1.5-2.2</td>
<td>2.2-3.2</td>
</tr>
<tr>
<td>D—A (Å)</td>
<td>2.2-2.5</td>
<td>2.5-3.2</td>
<td>3.2-4.0</td>
</tr>
<tr>
<td>Bond angles (°)</td>
<td>175-180</td>
<td>130-180</td>
<td>90-150</td>
</tr>
<tr>
<td>Bond energy (kal mol\textsuperscript{-1})</td>
<td>14-40</td>
<td>4-15</td>
<td>&lt; 4</td>
</tr>
</tbody>
</table>
Table 1-1: Properties of strong, moderate, and weak hydrogen bonds following the classification of Jeffrey.49,54

1.4.2 Potential Energy of Hydrogen Bonds

\[
\text{X} \quad \cdots \quad \text{H} \quad \cdots \quad \text{Y}
\]

\[E \quad \cdots \quad r_{XY} \]

Figure 1-2: The schematic diagram of the potential energy wells for the three different types of hydrogen bonds: A) the double-well potential, B) the low-barrier potential, and C) the single well potential.55

Potential energy of hydrogen bonds can be investigated from infrared spectroscopy. In Figure 1-2, the potential energy wells of three different categories of hydrogen bonding (strong, moderate and weak) are illustrated. Curve A represents weak or double-well H-bonds where there are two minima in which the hydrogen atom is closer to one of the donor. Curve B indicates moderate or low barrier
H-bonds where the potential energy surface is quite flat and the equilibrium position of the hydrogen atoms becomes environmental sensitive. Curve C shows very strong or single-well H-bonds where the two minima become more equal and the hydrogen atom is symmetrically fixed between the two donor atoms for example pentachlorophenol/4-methylpyridine,\textsuperscript{56} maleate or phthalate monoanions.\textsuperscript{57} Research on strong hydrogen bonds was pioneered by spectroscopists carrying out vibrational studies in solution.\textsuperscript{58-60} A key finding of spectroscopy is that very strong hydrogen bonds are formed only if the pKa values of the partners are suitably matching. If the pKa values are very different, either a moderate X-H...Y or an ionic X'...H-Y\textsuperscript{+} hydrogen bond is formed, both of which are not very covalent. The quasi-covalent situation occurs in a certain "critical" range of ΔpKa, the numerical characteristics of which depend on the particular system.\textsuperscript{60}

1.4.3 Descriptors of the Hydrogen Bond

\[
\begin{array}{c}
\text{D} \\
\text{H} \\
\text{---A---X}
\end{array}
\]

The three scalar quantities used as descriptors to define the geometry of hydrogen bonding are the D-H covalent bond length, the H...A hydrogen bond length and the D...A hydrogen bond distance. These quantities define D-H...A--X hydrogen bond angle θ. All these features have been discussed extensively.\textsuperscript{461} The general information can refer to Table 1-1. In a multifurcated hydrogen bond, a donor forms hydrogen bonds with more than one acceptors simultaneously.\textsuperscript{53} The terms "bifurcated" and "trifurcated" are commonly used to describe the arrangements in Scheme b and c respectively.\textsuperscript{53}
Figure 1-3: Schematic diagram of the geometry of different hydrogen bonds: a) Normal hydrogen bond with one acceptor. b) Bifurcated hydrogen bond. c) Trifurcated hydrogen bond.

We have eluded to the importance of non-covalent interactions in determining packing in molecular crystals. Hydrogen bonds are among the most energetically important and thus can dominate inter-molecular packing arrangements. The pKa's of ionizable groups in both pharmaceutical and natural product species also have a critical role to play in determining whether crystal engineering attempts result in ionic salt formation, which basically guarantees the co-crystallization of cationic and anionic molecular species through the need for overall charge neutrality of the solid, or neutral molecular 'co-crystals'. In this case molecules may also decide to pack together in the solid state but whilst in the case of salts proton transfer may occur from an organic acid to an organic base and form say a (+)N-H—O(-) hydrogen bond, in the neutral co-crystal proton transfer may not occur and a N—H—O hydrogen bond may be found. Study of hydrogen bonds may thus play a critical role in the compound type and its physical properties – for example salts may be anticipated to have higher melting or decomposition temperatures. The possibility of different hydrogen bond networks of roughly equivalent energetic value is also of importance in many of the polymorphic forms of organic crystals that can be found, a topic which will be discussed at further length below.

1.5 Polymorphism of Organic Compounds
Polymorphism is defined as the phenomenon where the same chemical substance that can be existed in different crystalline forms. In recent article, polymorphism corresponds to the existence of different crystalline patterns for the same compound.

Crystal polymorphism is encountered in all areas of research involving solid substances. Free energy differences between polymorphic forms of a given substance are generally around a few kJ mol\(^{-1}\) and the process of crystallization is affected by many physical parameters (e.g. nature of the solvent, cooling and stirring rates, temperatures, pressure and presence of impurities), minor variations in preparation conditions can tip the balance in favour of crystallization of a polymorph which is not necessarily the thermodynamically stable one.\(^1\)

Like most chemical processes, crystallization in polymorphic system is governed by a combination of thermodynamic and kinetic factors. According to thermodynamic theory, crystallization must result in overall decrease in the free energy of the system. This means that, in general, the crystal structures that appeared will be those with the greater (negative) lattice (free) energies. The relative stability of two polymorphs depends on their free energies, the more stable polymorph probably having a lower free energy. The Gibbs free energy (\(G\)) of a substance is expressed by the following equation:

\[
G = H - TS
\]

where \(H\) is the enthalpy of the system while \(S\) is the entropy of the system.

Thermodynamic considerations also include Ostwald’s law\(^6\) of stages which tells us that at high supersaturation, the most soluble (least stable) metastable form will always appear first. This form will subsequently dissolve and transforms into a more stable one. The cycle will continue until the most thermodynamically stable (least
soluble) form formed.

The polymorphic transformations can be distinguished into two principle types: monotropic and enantiotropic \(^{63}\). The relationship is shown by the energy versus temperature (E/T) diagram introduced into crystallography by Buerger\(^{64}\) in 1951.

![Energy versus temperature (E/T) diagram of enantiotropic situation.](image)

*Figure 1-4: Energy versus temperature (E/T) diagram of enantiotropic situation.*\(^{43}\)

The diagram of enantiotropic situation is displayed in Figure 1-4. G is the Gibbs free energy and H is the enthalpy. The Roman numberals indicate the two polymorphs; m.p. is the melting point and t.p.\(III/II\) is the transition point between the two polymorph.

The theoretical derivation and practical application of this diagram have been described and discussed by Burger and Ramberg\(^{65,66}\) and by Grunenber et al.\(^{67}\)

At absolute zero, TS vanishes so that the enthalpy is equal to the Gibbs free energy. That means at absolute zero, the most stable polymorphic form would be the one which has the lowest Gibbs free energy.

Above absolute zero, the entropy will play a role which may differ for two polymorphs so that the free energy as a function of temperature follows a different trajectory for the two polymorphs represented by \(G_1\) and \(G_II\) curves.
In enantiotropic system: The two curves cross at the thermodynamic transition point t.p.\(\text{II/III}\), but the energy \(\Delta H_{\text{II/III}}\) is required to be input for the phase transition, which must be endothermic for the transition above t.p. and exothermic below t.p. for the situation that is depicted in Figure 1-4. The endothermic solid-to-liquid transitions at the melting points may be understood in the same way, with \((\Delta H_{\text{II}} \text{ and } \Delta H_{\text{III}})\) indicating the respective enthalpies of fusion. Since the t.p.\(\text{II/III}\) is lying at a temperature below the melting points for the two polymorphs, the situation is enantiotropic.\(^{68}\)

The monotropic situation is represented in Figure 1-5. In this case, there is no transition point below the melting points of the two polymorphs. The phenomenon is different from the enantiotropic system in that there is a reversible transition point from one phase to another without going through the gas or liquid phase.

![Figure 1-5: Energy versus temperature (E/T) diagram of monotropic situation.\(^{63}\)](image)

In monotropic system: If the thermodynamic relationship is one of monotropism that is the two modifications are not interconvertible.\(^{69}\) Only at thermodynamic transition points can two forms have the same stability and hence coexist as a mixture at equilibrium. At any other temperature there will be a thermodynamic tendency to transform to the more stable structure. This means that the polymorphic
mixtures will have a limitation of lifetimes, but only at the thermodynamic transition point, the kinetic transformation plays a role in those lifetimes.

With the knowledge of polymorphic phase relationship in enantiotropic and monotropic systems, the crystallization processes can be better controlled in order to obtain the desired polymorph while the undesired form can be prevented.

1.6 Thesis Objectives and Methods

The scope and limitations of applying hydrothermal and solvothermal crystallization techniques to a natural product system will be explored. The system chosen for focus is tetrahydropalmatine (THP) a constituent of certain Traditional Chinese Medicinal remedies, as mentioned in section 1.3. Samples of this were kindly provided by Prof. Hong Xue of the Department of Biochemistry, HKUST. Two solid forms were provided, racemic (+/-)-THP and the pharmaco-active enantiopure (-)-THP. Our goals were to explore the feasibility of growing crystals of both racemic and chiral THP salts and co-crystals in a facile procedure with high yield of recovery, phase purity and of sufficient size (>100 micron) to allow single crystal X-ray structure determinations to be carried out in a routine manner.

Several specific goals were set:

1. To find salts or co-crystals of THP which were stable and anhydrous or free of other solvents of crystallization.
2. To determine the absolute stereochemistry of (-)-THP from a co-crystal or salt involving Cl or P atoms.
3. To explore the feasibility of a resolution of racemic THP through the preferential formation of a chiral salt or co-crystal.
4. To look at the hydrolytic stability of THP and see whether any of the hydrolytic breakdown products could be isolated.
5. Since THP is a base to explore the possibility of forming both salts and co-crystals as a series in which the pKa of organic acids with which it is reacted is varied through the pK range of the THP.

6. To further explore the scope of hydrothermal crystallization in the formation of novel polymorphic forms.

Hydrogen bonds are of key biological significance, affecting the stability, structure and reactivity of almost all bio-molecules. Hydrogen bonds having double minimum proton potential show polarizabilities as a result of shifts of proton within these bonds.\textsuperscript{53} In a certain ‘critical’ range of $\Delta pK_a$, the systems can be found in crystals as molecular adducts linked by hydrogen bonds O–H...N, as ionic adducts linked by $N^+\text{H...O}^-$ interactions, or even as an equilibrium between the two.\textsuperscript{70}

Towards this end we have studied and measured such hydrogen bonds in a number of dl-tetrahydropalmatine((dl)THP) co-crystals with various benzoic acids. From the X-ray diffraction data, the proton position will be interpreted and tried to co-relate the pKa value of the (dl)THP with different benzoic acid involved. At the same time, we will also study of those N-H...O hydrogen bonds which is of fundamental importance and may have wider implications for biological systems, such as enzyme.

Salt formation is a simple means to differ significantly with respect to physicochemical and thus, biopharmaceutical properties of drugs containing one or more ionisable groups. Several common counter ions are selected for salt making with tetrahydropalmatine. We will investigate at forming different THP salts through hydrothermal and solvothermal crystallization, for enhancing the stability and solubility of THP.

Hydrothermal reaction technique will be used to explore the feasibility for synthesis of palmatinium salt and dihydropalmatine from tetrahydropalmatine. And
we will also use hydrothermal crystallization which can provide a thermodynamic approach for searching the thermodynamically stable polymorphic forms of organic compound. It may provide a new way for the crystallization of natural products and drugs, which still have no acceptable crystals for X-ray diffraction structural determination.

1.6.1 Characterization methods

In this thesis, the structural description of the organic co-crystal compounds is very important. In order to study the structure of the mixed co-crystals, single crystal X-ray diffraction technique is used. This technique can allow us to have the detail information about the crystal structure like the bonding distances, angles among each atoms, the intermolecular interactions formed and also the packing motifs. The basic principle of this technique is by using the diffraction behavior of the crystal. Crystals with different compounds will usually give different diffraction patterns-different peak positions and different intensities. By using this diffraction pattern, the structure model of the compound will be predicted preliminarily. Then by refining the model structure, which can be considered as comparing the calculated diffraction pattern of the model structure to the experimental diffraction pattern, the model structure will be adjusted. Finally, the best-fit model will be the most accurate structure of the compound.

In this thesis, unless further specify, the crystal data and reduction was carried by Bruker-Nonius D8 diffractometer equipped with Bruker-Nonius Apex CCD detector with computer program SMART for unit cell indexing and data collection and SAINT for data reduction. The structure solution, refinement, figures and the summary tables of the structures were carried out and prepared by a computer programs package SHELXTL version 6.10 or Mercury 1.4.1 which free download
from CCSD website http://www.ccdc.cam.ac.uk.

Although single crystal X-ray diffraction is a very powerful tool to investigate the structure of unknown compounds, it has the limitation to study the properties of the compounds and also the purity of the samples in whole batch. Elemental analysis (EA) is a very useful method that used to check the composition consistency and the purity of the compound in the whole batch of the crystal. The result provides the weight percentage of carbon, hydrogen, and nitrogen in the compound. Some other elemental analysis can also be done; but in this thesis, only carbon, hydrogen and nitrogen analysis have been done.

Single crystal XRD could analyze one single crystal only, Powder X-Ray Diffraction (Powder XRD) is necessary to check the rest of the sample to see whether they are the same to the crystal structure or not. Powder XRD can also provide us a fast way to check the phase purity and also help us to monitor the rate of phase transition, rough estimation of different phase distribution. To check the powder XRD pattern, a simulated powder XRD pattern of the crystal structure is necessary to be generated. In the thesis, computer programs Mercury 1.4.1 are used to generate the simulated pattern. The experimental powder XRD patterns of the samples in this thesis were done by Philips PW 1830 with 3kW Cu anode. Variable Temperature PXRD is also used to analysis the phase transition in various temperatures. This analysis is carried out by PANalytical X'pert Pro with 40kW 40mA Cu radiation.

Other techniques such as Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) are used to investigate the thermal stability and the melting points of some of the selected samples respectively. Thermal gravimetric analysis was carried out by Perkin-Elmer TGA 7 Thermogravimetric Analyzer. Differential scanning calorimetry was carried out by Perkin-Elmer DSC 1
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Chapter 2  Salt Formation for Tetrahydropalmatine:

Seeking Stable Formulations and Chiral Resolution

2.1  Introduction

2.2  Preparation and crystal structures of tetrahydropalmatine salts

   Compound 2.1 [(dl)THP-H][Maleate-H]

   Compound 2.2 [(dl)THP-H][Citrate-H₂][H₂O]

   Compound 2.3 [(dl)THP-H][H₃PO₄][H₃PO₄][H₂O]

   Compound 2.4 [(dl)THP-H][NO₃][H₂O]

   Compound 2.5 [(l)THP-H][NO₃] 2[H₂O]

   Compound 2.6 [(l)THP-H][Cl][H₂O]

   Compound 2.7 [(l)THP-H]₂[D-TAR] 8[H₂O]

2.3  Discussion

2.4  Experimental

2.5  References

2.6  Crystal Data Summary
2.1 Introduction

Derived from various Traditional Chinese Medicinal herbs – Tetrahydropalmatine THP C_{21}H_{23}NO_{4} is a dopamine receptor antagonist used as anti-anxiolytic agent (H. Xue, HKUST 2004).\textsuperscript{1} It is a chiral molecule and can occur as both racemic and active (-)-forms. Salt formation is a simple means to differ significantly with respect to physicochemical and thus, biopharmaceutical properties of drugs containing one or more ionizable groups. In recent years increasing efforts have been devoted to modify/optimize drug performance through salt formation as opposed to more complex molecular modifications\textsuperscript{2}, and salt selection has become an important part of early drug development\textsuperscript{3,4}. Several common anions used to prepare pharmaceutical salts are selected\textsuperscript{4} for salt making with tetrahydropalmatine. We have looked at forming salts of this through hydrothermal and solvothermal crystallization, for enhanced stability and solubility. A wide range of salts have been prepared using various organic and inorganic acids. Finally chiral resolution of (+/-)-THP appears to be possible by using D-tartaric acid, as discussed in the preparation of compound 2.7.
2.2 Preparation and crystal structures of Tetrahydropalmatine salts

The facile crystallization of eight salts of THP prepared by hydro- or solvothermal approach are described in this section. Compounds 2.1, 2.2 are for organic salts of racemic THP. These are the hydrogen-maleate 2.1 and dihydrogen-citrate 2.2 salts of the N-protonated tetrahydropalmatinium cation.

Whilst the citrate salt is aquated the maleate is anhydrous, an advantage for solid state formulation of a pharmaceutical. Compounds 2.3-2.6 are formed from racemic THP and the inorganic phosphoric and nitric acids respectively. From the phosphate system an intriguing exotic formulation with dihydrogen phosphate anion, a neutral phosphoric acid molecule and a water of crystallization is found. The nitrate form exists just as a monohydrate. The enantiopure chiral (-)-THP was found to be typically more difficult to crystallize under our standard conditions, which reflects the higher aqueous solubility of the salts of this species compared to its racemic form. Once again a nitrate salt could be formed 2.5, however this is now found as a dehydrate and the hydrochloride salt 2.6 has also been prepared, again as a monohydrate. This compound does indeed confirm the absolute configuration for the chiral handedness of the (-)-THP skeleton, due to anomalous scattering arising from the presence of the chloride ion.

Finally a chiral organic salt 2.7 was prepared from D-Tartaric acid and (-)-THP indicating a potential method of chiral resolution for racemic (+/-)-THP.
Compound 2.1 [(dl)THP-H][Maleate-H]

Stoichiometric 2:1 ratio of dl-tetrahydropalmatine and maleic acid were mixed in 1ml of water and heated hydrothermally at 110°C for three hours. Yellow bar crystal compound 2.1 was obtained. The single crystal X-ray analysis showed that the compound was in triclinic crystal system of space group P-1. One tetrahydropalmatinium and one maleate are found in the asymmetric unit which give the formula [(dl)THP-H][Maleate-H] (Figure 2-1).

![Diagram of Compound 2.1]

Figure 2-1: The thermal ellipsoid diagram of 2.1 with 40% displacement probability.

In the maleic acid, there are two carboxylic acid groups. One of the carboxylic acid is deprotonated but the other is not. We find that the C-O bond difference in O31-C31-O32 is 0.022Å while the difference in O33-C34-O34 is 0.084Å. The smaller bond length difference reveals that the carboxylate group O31-C31-O32 was deprotonated. The maleate is connected with the tetrahydropalmatinium forming N1-H1...O32 heteronuclear hydrogen bond with a distance of 2.702Å. Except this
intermolecular hydrogen bond, an intramolecular hydrogen bond is observed between O33-H33A… O31 with O…O distance 2.431Å which is linear and strong. The packing of compound 2.1 is shown in Figure 2-2.

Figure 2-2: The packing diagram of compound 2.1 along b-axis.

**Compound 2.2 [(dl)THP-H][Citrate-H]4.5[H2O]**

Equal molar of dl-tetrahydropalmatine and citric acid were put in 1ml of water and heated hydrothermally at 140°C for one and a half hour. Yellow bar crystal compound 2.2 was obtained and characterized by single crystal X-ray analysis. The compound 2.2 is in triclinic crystal system of space group P-1. Two tetrahydropalmatinium and one citrate are found in the asymmetric unit which give the formula [(dl)THP-H][Citrate -H][H2O]4.5 (Figure 2-3).
Figure 2-3: The thermal ellipsoid diagram of 2.2 with 50% displacement probability, without showing the water molecules. For sake of clarity, disorder group is omitted.

When comparing the C-O bond length difference of the three carboxyl groups in citric acid, we found that the difference between O31-C31 and O32-C31 is 0.014Å while the difference between O34-C36 and O35-C36 is 0.05Å which suggested that they have a delocalized structure. However, the bond length difference between O36-C35 and C35-O37 is 0.094Å which denoted that it is protonated. The citrate has a disorder carboxyl group at atoms C32A, C31A, O31A and O32A with 30% site occupancy. While atoms C31, C32, O31 and O32 shown in Figure 2-3 have 70% site occupancy. Two tetrahydrodropalmatiniums join with a citrate molecule by heteronuclear N-H...O hydrogen bond at N1-H1B...O34, N2-H2A...O35 with distance 2.715Å and 2.708Å respectively. Another intramolecular hydrogen bond is found in O32...H33A-O33 with O...O bond distance 2.808Å. The disorder atom O32 with of the citrate is further connecting with the nearby citrate by homonuclear hydrogen bond at O36 with distance 2.589Å. The hydrogen bond only find in O32 with higher portion instead of lower portion disorder atom. Figure 2-4 shows the packing diagram of compound 2.2 without showing the water molecules for clarity. Those water molecules are sought between the THP and citrate by hydrogen bonds.
Figure 2-4: Packing diagram of compound 2.2 with omitting water.

Compound 2.3 [(dl)THP-H][H$_2$PO$_4$] [H$_3$PO$_4$]H$_2$O

Compound 2.3 was prepared by mixing (dl)THP and phosphoric acid(H$_3$PO$_4$) in 0.5ml water. The mixture was heated hydrothermally at 110° for 4 hours. Colourless bar crystals were formed and the structure was confirmed by single crystal XRD analysis. It possesses triclinic crystal system of space group P-1. In Figure 2-5, the asymmetric unit and the environment around the acid is shown. We find one tetrahydropalmatinium, one phosphate, one phosphoric acid and one water molecular contained in the asymmetric unit and give the formula [(dl)THP-H][H$_2$PO$_4$] [H$_3$PO$_4$]H$_2$O
Figure 2-5: The 50% thermal ellipsoid diagram of 2.3 with the environment. (Proton omitted on the THP)

Figure 2-6: P-O bond lengths in the phosphoric acid in compound 2.3.

The phosphoryl bond lengths vary from 1.495 to 1.515Å and the phosphohydroxyl bond lengths from 1.530 to 1.577Å. Protons are located refer to the P-O bond length shown in Figure 2-6. P2-O23 bond length is 1.527Å which not falls in the range of mentioned above but it is more close to the range of phosphohydroxyl bond. The tetrahydropalmatinium is linked with the phosphate by the heteronuclear N1-H1B...O14 hydrogen bond with distance 2.662Å. The
phosphate further forms a dimer with another phosphoric acid by the linkage O11-H11...O24 and O23-H23...O12 with hydrogen bond distance 2.572Å and 2.464Å respectively. These two phosphoric acids have totally eight oxygen atoms which can easily form hydrogen bonds with the surrounding molecules. In phosphate P1, O13 acts as hydrogen bond donor interact with O14 in phosphate P1 in the next unit. Atom O22 in phosphoric acid P2 also hydrogen bonded to O3 of the methoxyl group in the nearby (dl)THP. A hydrate molecule can be hydrogen bond acceptor for atom O21 of the acid and hydrogen bond donor for atom O24 of the acid. By using those homonuclear hydrogen bond among the acid and the hydrate, one dimensional chain is built (Figure 2-7).

![Figure 2-7: 1D Chain formed by the phosphoric acid and hydrates along c-axis in compound 2.4.](image)

Except these moderate hydrogen bonds, relatively weak bifurcated hydrogen bonds are observed in the water molecules which donate the hydrogen to both atom O1 and O2 in the different methoxyl groups in the (dl)THP. In Figure 2-8, we can see that the (dl)THP seeks between the 1-D chains by using this weak bifurcated hydrogen bonds and the N1-H1B...O14 hydrogen bond forming a 2-D sheet. The hydrogen bond distances and angles formed by compound 2.3 are illustrated in Table 2-1.
Figure 2-8: The packing diagram of compound 2.3.

<table>
<thead>
<tr>
<th></th>
<th>D(D...A)/Å</th>
<th>(DHA)/°</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-H...A</td>
<td>2.662</td>
<td>163.87</td>
</tr>
<tr>
<td>N1-H1B...O14</td>
<td>2.572</td>
<td>171.90</td>
</tr>
<tr>
<td>O11-H11...O24</td>
<td>2.678</td>
<td>166.97</td>
</tr>
<tr>
<td>O13-H13...O14#1</td>
<td>2.541</td>
<td>164.97</td>
</tr>
<tr>
<td>O21-H21...O1W#2</td>
<td>2.632</td>
<td>169.87</td>
</tr>
<tr>
<td>O22-H22...O3#3</td>
<td>2.464</td>
<td>166.27</td>
</tr>
<tr>
<td>O23-H23...O12</td>
<td>2.719</td>
<td>175.15</td>
</tr>
<tr>
<td>O1W-H1WA...O24#4</td>
<td>2.908</td>
<td>155.81</td>
</tr>
<tr>
<td>O1W-H1WB...O1#5</td>
<td>3.026</td>
<td>134.37</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms:

#1 -x+1, -y+1, -z   #2 x, y-1, z   #3 x+1, y-1, z
#4 -x+2, -y+1, -z   #5 -x+1, -y+1, -z+1

Table 2-1: Hydrogen bond table for compound 2.3

Inorganic Salts of Racemic THP:

**Compound 2.4 [(dl)THP-H][NO₃][H₂O]**

(dl)THP and nitric acid(HNO₃) were mixed into 1ml of ethanol and then heated 110° for 4 hours by solvothermal method. Yellow bar crystal was isolated from the reaction mixture. Compound 2.4 has a triclinic crystal system with space group P-1. In the asymmetric unit, one tetrahydropalmatinium, one nitrate and one water molecule are found (Figure 2-9). Thus, the formula is [(dl)THP-H][NO₃]H₂O.
Figure 2-9: The 50% thermal ellipsoid diagram of compound 2.4.

As shown in Figure 2-10, this compound differs with the compound 2.4 shown above. The tetrahydropalmatinium joined with the hydrate instead of the acid with distance 2.834Å. This hydrate further interacts with O21 of the nitrate O1W-H1WA...O21 with O...O distance 2.772Å. Another hydrogen atom of O1W joined with nearby atom O22 from the nitrate of the next unit. The nitrates and hydrates are capped by the (dl)THP. The capped unit is linked up by weak interaction C4-H4A...O3 between the two (dl)THPs with bond distance 3.186Å which forming a 1D chain.
Inorganic Salts of Chiral THP

Compound 2.5 [(l)THP-H][NO₃]2[H₂O]

A different nitrate salt was formed with (l)THP which was prepared by heating (l)THP and nitric acid in 1ml of water at 110° for 3 hours hydrothermally. Colourless rod crystal compound 2.5 was collected and characterized by single crystal XRD analysis. It belongs to the monoclinic crystal system with chiral P2₁ space group. The content of the asymmetric unit is shown in Figure 2-11 as one tetrahydropalmatinium, one nitrate and two water molecules are observed which gives the formula [(l)THP-H][NO₃][H₂O]₂.
Heteronuclear N1-H1B...O21 hydrogen bond is found between the nitrate and the tetrahydropalmatinium with N...O distance 2.745Å. Atom O1, O2 of the methoxyl groups in the THP linked to H2WA-O2W by weak bifurcated hydrogen bonds with distances of 2.955Å and 3.199Å respectively. Besides the above hydrogen bond interaction, O2W also linked with H1WA-O1W with another hydrogen bond. Considering the two hydrates, the O...O length of O1W-H1WA...O2W is 2.787Å, and that of O2W-H2WB...O1W is 2.818Å. A 1D water chain is constructed with these two hydrogen bond linkages (Figure 2-12). This 1D infinite helically water chain screw along the b-axis.

![Figure 2-12](image)

_Figure 2-12: 1D water chain is formed in compound 2.5 viewed along c-axis._

This water chain links up the nitrate by O1W and O22 with distance 2.784Å and tetrahydropalmatinium by the bifurcated hydrogen bond mentioned above. Together with the heteronuclear hydrogen bond, a 2-D hydrogen-bonded network is formed (Figure 2-13).

![Figure 2-13](image)

_Figure 2-13: The packing diagram of compound 2.5 along a-axis with all the protons omitted._
Compound 2.6 [(I)THP][Cl][H₂O]

The hydrochloride salt 2.6 was prepared by heating (I)THP and hydrochloric acid in ethanol hydrothermally at 110° for 6 hours. Yellow bar crystals of (2.6) were obtained. From the single crystal XRD analysis, compound 2.6 belongs to the monoclinic crystal system in space group P2₁. One chloride, one tetrahydropalmatinium cation and one water molecule are observed in the asymmetric unit (Figure 2-14).

![Figure 2-14: The thermal ellipsoid diagram of 2.6 with 50% displacement probability.](image)

Although the compound does not appear in the Cambridge Structural Database, a Science Citation Index search reveals that the compound has in fact been previously structurally characterized and reported.⁶ The crystal packing is shown in Figure 2-15. The published crystals were grown from methanol by slow evaporation, which is different from our preparation.
Towards a resolution for Racemic THP:

**Compound 2.7: [(l)THP-H]_2[D-TAR]_8[H_2O]**

The THP molecule has a chiral centre at atom C13. (+/-)-THP is a racemic mixture which contain 50% of (+) and 50% of (-) enantiomer. Therefore, it can occur as racemic, active(+) or active(-) form. Many studies have indicated that drug enantiomers may have different pharmacodynamic and pharmacokinetic properties due to stereoselective interaction with optically active biological macromolecules.\textsuperscript{7-11}

It has reported that the analgesic activity of (l)THP is much higher than that of (d)THP.\textsuperscript{12,13} Chiral resolution of racemic THP with cheap chiral reagent (D)-tartaric acid(D-TAR) and (L)-tartaric acid has been tried compared with expensive chiral separation by using chiral high-performance liquid chromatograph with our result of formation of hydrated 2:1 salt [(l)THP-H]_2[D-TAR]._8[H_2O] (2.7) in 85% yield (Figure 2-16). The packing diagram of 2.7 is shown in Figure 2-17. For sake of clarity, water molecules are omitted. Separation of rac-THP with D- or L-tartaric acid appears practicable and further study will be carried out in the future.
Figure 2-16: The thermal ellipsoid diagram of 2.7 without showing the water molecules.

Figure 2-17: Packing diagram of 2.7 along a-axis without showing water molecules.
2.3 Discussion

Figure 2-18: The structure of tetrahydropalmatine.

The THP salts consists mainly of the tetracyclic ring system from A to D (Figure 2-20). Both of the rings A and D are planar with average deviations from the least-squares planes listed in Table 2-2. In compound 2.2, there are two THP in the asymmetric unit. 2.2:N1 and 2.2:N2 in Table 2-2 represent the THP contains atom N1 and another THP contains atom N2 respectively in compound 2.2. The dihedral angle between the least-squares planes of phenyl rings A and D range from 18° to 34° (Table 2-2). It is compared with the value found in (dl)THP (25.8°)\(^{14}\) and (l)THP monohydrate (32.6°).\(^{15}\)

<table>
<thead>
<tr>
<th>Compound (Dihedral angle A/D)(^\circ)</th>
<th>Avg. Deviation from the least-squares plane σ/Å</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ring A</td>
</tr>
<tr>
<td>2.1</td>
<td>34.47 ( 0.06 )</td>
</tr>
<tr>
<td>2.2:N1</td>
<td>18.40 ( 0.09 )</td>
</tr>
<tr>
<td>2.2:N2</td>
<td>24.25 ( 0.09 )</td>
</tr>
<tr>
<td>2.3</td>
<td>31.15 ( 0.12 )</td>
</tr>
<tr>
<td>2.4</td>
<td>19.17 ( 0.14 )</td>
</tr>
<tr>
<td>2.5</td>
<td>25.25 ( 0.19 )</td>
</tr>
<tr>
<td>2.6</td>
<td>20.82 ( 0.07 )</td>
</tr>
<tr>
<td>2.7</td>
<td>18.92 ( 0.14 )</td>
</tr>
</tbody>
</table>

Table 2-2: Dihedral angles and average deviations from the least-squares planes of
ring A and ring D.

The two phenyl rings A and D have methoxyl substituents at C16, C17, C7 and C8 but those methoxyl groups are not in the same geometry on the rings. At C8, C16, C17, methoxyl groups are nearly co-planar with their respective phenyl ring, A and D, while the methoxyl group at C7 is rotated out of the plane of ring D by around 100° which avoids the steric hindrance with adjacent methylenic C5 atom.

The ring conformation of the two non-planar rings B and C can be described as follow. Pure half-chair conformation are commonly observed in Ring C with adjacent ring atoms N1 and C13 above and below the plane of the remaining four atoms, respectively. For ring B, a half-chair conformation also dominates which N1 and C4 are out-of-plane atoms, however, there is a tendency towards an envelope form, with C4 as the out-of-plane atom. All have this kind of conformation similar to the published THP structure\textsuperscript{14,15} and most of the THP salts reported in this chapter except in compound 2.4 that ring B tends to be an envelope conformation and the ring C have slightly distortion.

Although compounds 2.4 and 2.5 are both nitrate salts, compound 2.4 is different from compound 2.5. Because the starting reagents are different of THP with (dl)THP in 2.4 while (l)THP in 2.5, and finally the amount of water molecules included in the two compounds are not the same. Two water molecules are found in compound 2.5 while only one water molecules presents in compound 2.4. The two water molecules interact though hydrogen bonds build a 1-D water chain in 2.5 but this chain cannot be found in compound 2.4 and as result to a totally different packing is observed. The more efficient packing with less included water is reasonable for the racemic form compared to its chiral analog. If the chiral form could find an efficient packing itself the implication would be that the racemate would spontaneously
resolve into an 'agglomerate' containing 50% left and 50% right handed crystals. Actually this has not been found to be the case in any of the THP systems we have studied to-date. In compound 2.5, the proton on nitrogen, N-H is directly bonded to the oxygen of nitrate while in compound 2.4, there is a water molecule sitting between the tetrahydropalmatinium and the nitrate ion. In this case, the proton in the tetrahydropalmatinium N-H...O hydrogen bonded to the water molecules, then the proton in the water molecule further forms O-H...O hydrogen bond to the oxygen of nitrate ion. The tetrahydropalmatinium forms hydrogen bond with the water molecule first instead directly links to the nitrate ion. It is different from most of the other THP salts that the other THP hydrogen bond to the counter ion directly even with the present of water molecules.

In the examples presented, the majority exist as hydrated forms. Water molecules can act as proton donor and acceptor which link up with others molecules in the compounds. In a multifurcated hydrogen bond, a donor forms hydrogen bonds with more than one acceptor simultaneously. Bifurcated hydrogen bond is observed in compounds 2.3 and 2.5 because a proton from water molecule is hydrogen bonded to with O1 and O2 on the two methoxyl groups. In these two bifurcated hydrogen bonds, all the acceptors are oxygen in the methoxyl groups. Normally, the hydrogen bonds formed in bifurcated system is weak in long donor and acceptor bond distance.\(^{16}\) The hydrogen bond distance and the angle \(\theta <(\text{DHA})\) in the bifurcated hydrogen of compound 2.3 and 2.5 is summarized in (Table 2-3). These bifurcated hydrogen bonds are more likely to be identified as symmetric bifurcated hydrogen bonds. Other than 1-D water chain, water is also forms hydrogen bonded with nitrate ion to form ring pattern in compound 2.4 but only 1-D chain found in the hydrogen interaction between water and phosphate ion in compound 2.3.
<table>
<thead>
<tr>
<th>D-H...A</th>
<th>D(D...A)/Å</th>
<th>&lt;(DHA)°</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound 2.3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O1W-H1WB...O1</td>
<td>2.908</td>
<td>155.81</td>
</tr>
<tr>
<td>O1W-H1WB...O2</td>
<td>3.026</td>
<td>134.37</td>
</tr>
<tr>
<td><strong>Compound 2.5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>O2W-H1WA...O2</td>
<td>3.199</td>
<td>139.12</td>
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*Table 2-3: Bifurcated hydrogen bond table for compound 2.4 and 2.6.*

Intramolecular hydrogen bonds can be formed between donor and acceptor groups in the same molecule when the molecular configuration and conformation bring them within hydrogen bond geometry. Compound 2.1 and 2.2 both contain an intramolecular hydrogen bond in hydrogenmaleate and citrate respectively. In compound 2.1, the O-H...O intramolecular hydrogen bonding are found between the two carboxylic acid and the carboxylate of hydrogenmaleate in with distance 2.431Å but O-H...O intramolecular hydrogen bond is observed between the carboxylate and hydroxyl groups with bond distance 2.808Å in compound 2.2. Formation of intramolecular hydrogen bond helps to release the negative charge in the hydrogenmaleate and citrate. It is obvious to see that compound 2.2 has a relatively longer bond distance when comparing with compound 2.1. It is probably due to the atom O32A of the citrate in compound 2.2 is further hydrogen bonded with the nearby citrate. Similar short intramolecular hydrogen bonds between carboxylic acid and carboxylate groups have been studied in detail by neutron diffraction and ab initio calculations in maleate ions. It is not surprising that strong hydrogen bond is observed inside the hydrogenmaleate ions of compound 2.1. The studies claim that the hydrogenmaleate ion contains a short strong hydrogen bond where the position of the hydrogen atom is sensitive to the crystallographic environment, and has been widely studied with varying cations. The hydrogen atom was observed as centered
within the hydrogen bond in the case of the imidazolium salt\textsuperscript{18} but occupying an asymmetric position when the cation is calcium\textsuperscript{19}. It is difficult to determine whether the protons is symmetric or not in compound 2.1 as X-ray diffraction can not accurate locate the position of hydrogen atom. More analysis such as neutron diffraction is required to determine the behavior of the proton in this intramolecular bond.

Compound 2.1 and 2.2, together with compound 3.2[(dl)THP-H][salicylate] and 3.5[(dl)THP][BA-H] discussed in the next chapter, totally four organic reagents. Maleic acid, citric acid, salicylic acid and benzoic acid are used for the formation of THP salts in 2.1, 2.2, 3.2 and adduct in 3.5. Maleic acid and citric acid both possess more than one ionizable acid group but they are not fully utilized. The same situation also pertains in compound 3.2 and 3.5. The organic acids form direct hydrogen bonds to the THP. However, neither of those compounds can form a crystal packing with higher dimensionality by using the organic acids because the intramolecular hydrogen bond limits the chance to have interaction with other molecules. Apart from organic reagents, small size of the inorganic acid are used in compound 2.3, 2.4 and 2.5 which have less directional hydrogen bond between the THP. More dimensionality packing can be achieved by hydrates cooperate with the acid and THP. There is an exception case in 2.6, mono-atomic chloride ion is used that is difficult to contribute templating effect to the packing. From the TGA data, no trend and improvement in stability can be observed. Although no special findings are observed in the TGA study, the investigation can be continued by analysis the physical properties such as melting point. THP in all the above structures are hydrogen bonded with either acid or water, giving rise to ionization. Often the solubility of the ionized solute is much greater than the intrinsic solubility of the neutral species.
When compare THP salts with original THP, THP only possess weaker dipole moment interaction that contributes to solubility. The potential improvement in solubility can help to solve the problem in low and variable bioavailability of natural products or drugs caused by insufficient water solubility. Moreover, hydrothermal and solvothermal methods provide a possible easy way for salt making.

**Chiral resolution of (l)THP with D(-)-tartaric acid or L(+) -tartric acid**

The initial results suggest that application of D-tartaric acid to a racemic mixture of (+/-)-THP should be able to produce solid of formula 2.7. In this the preferred material (-)-THP would be isolated as its hydrated D-tartrate salt. The exact conditions needed to effect this with high yield and efficiency are yet to be determined and it may be that an ambient temperature resolution will work effectively. The critical feature to be determined from our experimentation is that the D-TAR anions pack more efficiently with (-)-THP-H cations than the L-TAR analogs which under similar crystallization conditions give a clear solution indicating a higher solubility for the putative L-TAR salt. In order to carry out the resolution a larger quantity of rac-THP will be required however our findings imply that this should be a feasible process once concentration and temperature parameters have been optimized for segregated crystallization.
2.4 Experimental

Preparation and Characterization

Materials: (dl)THP and (l)THP are obtained from Prof H. Xue of Biochemistry Department HKUST without further purification before use. All other organic and inorganic acid are purchased from Acros Organics and Aldrich Chemical Company and used without further purification.

Compound 2.1

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.008g (0.07mmol) of maleic acid were mixed well in 1ml of de-ionized water. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were collected under suction filtration. Yellow bar crystals were filtered and dried in atmosphere with 80% yield. Elemental analysis: (calculated) %C=63.684, %H=6.199, %N=2.970 (Found) %C=66.3, %H=6.53, %N=2.970.

Compound 2.2

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.03g (0.14mmol) of citric acid monohydrate were mixed well in 1ml of de-ionized water. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 140°C for one and a half hours in the oven. The autoclave was cooled to ambient temperature. The products were collected under suction filtration. Yellow bar crystals were filtered and dried in atmosphere with 44% yield. Elemental analysis: (calculated) %C=58.590, %H=6.860, %N=2.850 (Found) %C=56.9 %H=6.41, %N=2.75.
Compound 2.3

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.01ml of phosphoric acid (0.14mmol) were mixed well in 0.5ml of de-ionized water. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for four hours in the oven. The autoclave was cooled to ambient temperature. The products were collected under suction filtration. Colourless bar crystals were filtered and dried in atmosphere with 68% yield. Elemental analysis: (calculated) %C=47.020, %H=6.012, %N=2.611 (Found) %C=41.2, %H=5.58, %N=2.611.

Compound 2.4

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.01ml of nitric acid (0.14mmol) were mixed well in 1ml of ethanol. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for four hours in the oven. The autoclave was cooled to ambient temperature. The products were collected under suction filtration. Yellow bar crystals were filtered and dried in atmosphere with 78% yield. Elemental analysis: (calculated) %C=57.923, %H=6.249, %N=6.433 (Found) %C=58.6, %H=6.33, %N=6.22.

Compound 2.5

0.05g (0.14mmol) of l-tetrahydropalmatine and 0.01ml of nitric acid (0.14mmol) were mixed well in 1ml of de-ionized water. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were collected under suction filtration. Colourless rod crystals were filtered and dried in
atmosphere with 40% yield. Elemental analysis: (calculated) %C=55.622, %H=6.446, %N=6.177 (Found) %C=64.1, %H=6.66, %N=4.53.

**Compound 2.6**

0.05g (0.14mmol) of l-tetrahydropalmatine and 0.015ml (0.14mmol) of hydrochloric acid were mixed well in 0.5ml of ethanol. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for six hours in the oven. The autoclave was cooled to ambient temperature. The products were collected under suction filtration. Yellow bar crystals were filtered and dried in atmosphere with 68% yield. Elemental analysis: (calculated) %C=61.685, %H=6.655, %N=3.425 (Found) %C=60.5, %H=6.41, %N=3.22.

**Compound 2.7**

0.050g (0.134mmol) (l)THP and 0.014g (0.067mmol) D(-)tartaric acid were transferred into a 23ml Teflon cup. They were mixed with 10 drops of water solution. The Teflon cup was rotated in order to make all the reaction components to get wet. The Teflon cup was sealed in a steel autoclave and heated at 110°C for 6 hours. The autoclave was cooled was to ambient temperature. Colourless giant plate crystals were obtained. A similar procedure using L(+)Tartaric acid gave solution under the same conditions.

**2.5 References:**


### 2.7 Crystal Data Summaries for 2.1 to 2.3

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<td>Colourless bar</td>
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<td>100(2) K</td>
<td>100(2) K</td>
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<td>18.6001(10)</td>
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## Crystal Data Summaries for 2.4-2.7

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Chapter 3  Products of dl-Tetrahydropalmatine with Benzoic Acids: Salt Formation versus Neutral Co-crystals

3.1  Introduction

3.2  Preparation and Crystal structures of (dl)-THP - benzoic acid products

Compound 3.1 [(dl)THP-H][2BrBA][H₂O]

Compound 3.2 [(dl)THP-H][salicylate]

Compound 3.3 [(dl)THP-H][4ClBA]

Compound 3.4 [(dl)THP][4BrBA-H][0.5[H₂O]]

Compound 3.5 [(dl)THP][BA-H]

Compound 3.6 [(dl)THP][3OHBA-H]

Compound 3.7 [(dl)THP][4MeBA-H]

Compound 3.8 [(dl)THP][4NH₂BA-H][0.2[H₂O]]

3.3  Discussion

3.4  Experimental

3.5  References

3.6  Crystal Data Summary
3.1 Introduction

In Chapter 2 the formation of a number of crystalline salts of both racemic and chiral THP was described. The yield and purity of these was usually high under optimal conditions and both organic and inorganic mineral acids could be used to effect proton transfer to the N-acceptor of the THP free base, forming the tetrahydropalmitininium cation which typically crystallized with a variety of partly deprotonated forms of poly-acids such as hydrogenmaleate, dihydrogen citrate or hydrogensalicylate. Only in the case of D-tartrate which formed a 2:1 [(l)-THP-H]:[D-TAR] salt was the 1:1 cation anion stoichiometry broken.

In Chapter 1 we alluded to the fact that both salt and neutral co-crystal formation were now being explored by drug companies in optimizing pharmaceutical formulation for particular organic compounds. In this Chapter we present the intriguing results that reaction of racemic THP with a series of benzoic acids with varying pKa value affords either ionic salts or neutral co-crystals dependent on the acidity of the benzoic acid functionality. This feature is readily changed by altering the donor or acceptor substituents on the phenyl ring of the benzoic acid. Most impressive is that the product crystals fall into the two categories extremely cleanly with a cross over of structure type found between the 4-Chloro-benzoic acid product (salt) and 4-Bromobenzoic acid product (neutral co-crystal). The differences in pKa of the two halobenzoic acids are extremely close (3.98 and 4.00 respectively) but are in the correct order for the product types found. The results imply that crystal engineering of adduct crystals may be taken to a high level and furthermore the subtle change of proton transfer in the two compounds almost certainly implies that many compounds formulated previously as ‘salts’ in pharmaceutical product screening may in fact on closer inspection turn out to be neutral co-crystals.
The role of the hydrogen bond in this phenomenon is also of central importance. Hydrogen bonds are of key biological significance, affecting the stability, structure and reactivity of almost all bio-molecules. Whilst OH…O hydrogen bonds, as occur in water, have been studied in depth, less attention has been paid to the intriguing chemically asymmetric N…H…O system. These hydrogen bonds are of crucial importance in many proteins and a deeper understanding of them is needed.

Hydrogen bonds having double minimum proton potential show polarizabilities as a result of shifts of proton within these bonds.\textsuperscript{1} An interesting example are adducts of amines and phenols with suitably small values of $\Delta pK_a$ [$\Delta pK_a = pK_a(\text{NH}^+) - pK_a(\text{OH})$].\textsuperscript{2} In a certain ‘critical’ range of $\Delta pK_a$, the systems can be found in crystals as molecular adducts linked by hydrogen bonds O–H…N, as ionic adducts linked by N’–H…O’ interactions, or even as an equilibrium between the two.\textsuperscript{3} Towards this end we have studied and measured such hydrogen bonds in a number of dl-tetrahydropalmatine ((dl)THP) co-crystals with various benzoic acids as will be discussed. From the X-ray diffraction data, the proton position can unambiguously be differentiated and its position is directly related to the pKa of the benzoic acid involved. One caveat to this finding is that clearly the ‘solid state’ pKa of the benzoic acid should closely follow that of the literature value which is obtained from solution equilibria. For this to be true the plane of the carboxylate group should be more or less co-planar with the phenyl ring in the solid state. Severe out-of-plane ‘twists’ whilst destabilizing are in fact possible in the solid. These would reduce the degree of delocalization of charge for the carboxylate anion. Thus the conjugate base would be destabilized to a greater degree in this case rendering the material ‘less acidic’ than otherwise anticipated.
3.2 Preparation and crystal structures of (dl)THP with various benzoic acids

**Compound 3.1 [(dl)THP-H][2BrBA][H₂O]**

Stoichiometric 1:1 ratio of dl-tetrahydropalmatine and 2-bromobenzoic acid(2BrBA-H) were mixed in 1ml of water and heated hydrothermally at 110°C for 3 hours. Colourless bar crystal compound 3.1 was obtained. The single crystal x-ray diffraction analysis shows that the compound is in triclinic crystal system with space group P-1. One protonated dl-tetrahydropalmatinium, one deprotonated 2-bromobenzoate and one water are found in the asymmetric unit which give the formula [(dl)THP-H][2BrBA][H₂O] (Figure 3-1).

![Chemical structure of Compound 3.1](image)

*Figure 3-1: The thermal ellipsoid diagram of 3.1 with 50% displacement probability.*

As X-ray diffraction cannot locate the hydrogen position accurately, to have better prediction of hydrogen position, subsidiary evidence is needed. In this case, we can compare the C-O bond length difference in the carboxylate group. As when the carboxylate group is deprotonated, both C-O bond lengths should be nearly the same.
In this crystal structure, C-O bond difference is about 0.023Å which consisting a delocalized environment results from the deprotonation of the acid group. Normal N1-H1...O31 hydrogen bond is formed with N...O distance 2.617Å and bond angle 165°. The O1W water molecule is hydrogen bonded to the O32 forming O1W-H1WA...O32 with distance of 2.795Å. The carboxylate group has a torsion angle of about 60° from the aromatic ring. In the packing diagram along b-axis (Figure 3-2), it shows that the two acid capped between the (dl)THP pair by weak hydrogen bond and van der Waals force.

![Figure 3-2: The packing diagram of compound 3.1 along b-axis.](image)

**Compound 3.2 [(dl)THP-H][Salicylate-H]**

Equal molar amount of dl-tetrahydropramatine and salicylic acid were mixed in 2ml of water and heated at 140°C for 3 hours by hydrothermal method. Yellow bar crystal compound 3.2 was formed and further analyzed by single crystal X-ray diffraction. The compound has the triclinic crystal system of space group P-1. One protonated dl-tetrahydropramatinium and one salicylate are found in the asymmetric unit which give the formula [(dl)THP-H][salicylate] (Figure 3-3).
The C-O bond lengths on the carboxylate group are 1.268 Å and 1.230 Å with difference of 0.038 Å which denote that the carboxylate group is deprotonated. Two type of hydrogen bond are found in the crystal structure. Normal N-H...O intermolecular hydrogen bond is formed in O31...H1B-N1 with O...N distance 2.710 Å and bond angle 173°. And the other type is intra-molecular O-H...O hydrogen bond in O33-H33B...O31 with bond distance 2.536 Å. The carboxylate group is coplanar to the aromatic ring of salicylate and form so called “Resonance-Assisted hydrogen bonding”

This hydrogen-bonding is appeared between molecules with conjugated multiple π-bonds. The packing diagram of compound 3.2 is shown in Figure 3-4.
Figure 3-4: The packing diagram of compound 3.2.

Compound 3.3 [(dl)THP-H][4ClBA]

By putting stoichiometric 1:1 ratio of dl-tetrahydropalmatine and 4-chlorobenzoic acid (4ClBA-H) into 1ml of water and was heated hydrothermally at 110°C for 3 hours, yellow bar crystal compound 3.3 was collected. Compound 3.3 is in monoclinic crystal system of space group P2₁/c by single crystal X-ray analysis. One protonated dl-tetrahydropalmatinium and one deprotonated 4-chlorobenzoate are found in the asymmetric unit which give the formula [(dl)THP-H][4ClBA] (Figure 3-5).
Figure 3-5: The thermal ellipsoid diagram of 3.3 with 40% displacement probability.

The C-O bonds on carboxylate are 1.275Å and 1.224Å with difference 0.051Å. A little short O31...H1B-N1 is formed with O...N distance 2.585Å. The carboxylate group has torsion angle of 5° which was nearly coplanar to the aromatic ring of the benzoic acid. A weaker halogen Cl...Cl interaction is observed between two 4-chlorobenzoic acid with distance 3.442Å. By using this interaction, the acid formed a dimer shown in Figure 3-6. From Figure 3-7, we can see that the packing diagram of compound 3.3.
Figure 3-6: The packing diagram of 4-chlorobenzoates showing the Cl...Cl short contacts in compound 3.3.

Figure 3-7: The packing diagram of compound 3.3 along a-axis with protons omitted.
Compound 3.4 [(dl)THP][4BrBA-H]0.5[H₂O]

Stoichiometric ratio 1:2 of dl-tetrahydropalmatine and 4-bromobenzoic acid (4BrBA-H) were added in 1ml of water and heated hydrothermally at temperature 140°C for 1.5 hour. Yellow plate crystal compound 3.4 was obtained. 4BrBA-H is an analogue compound of 4ClBA-H, and we might expect it will give iso-structure co-crystal. However, the single crystal X-ray diffraction analysis shows that the compound crystallizes in the different orthorhombic crystal system with the space group Pbca. One dl-tetrahydropalmatine, one 4-bromobenzoic acid and half water are found in the asymmetric unit, which give the formula [(dl)THP][4BrBA-H][H₂O]₀.₅ (Figure 3-8).

![Diagram](image)

*Figure 3-8: The thermal ellipsoid diagram of 3.4 with 50% displacement probability.*

The C-O distance lies on carboxylic acid are 1.297Å and 1.213 Å, they have
difference 0.084Å implies that there is no proton transferred. Similar to 3.3 compound, a relative short O31...H1B-N1 hydrogen bond is formed with O...N distance 2.585Å which is a little bigger than that in 3.3. The carboxylate group has torsion angle of 169° to the aromatic ring. The bromine atom is disordered into two positions. Atom Br1A has 70% site occupancy and atom Br1B has 30% site occupancy. The O1W water molecule is hydrogen bonded to the O31 of the carboxylic acid forming O1W-H1WA...O31 with bond distance of 2.746Å. Figure 3-9 shows the packing of co-crystal 3.4, we can see that the two acids are capped between two tetrahydropalmatine by weak hydrogen bond and van der Waals force.

Figure 3-9: The packing diagram of compound 3.3 viewing along a-axis with omitting of disorder bromine atom.

Compound 3.5 [(dl)THP][BA-H]

Equal stoichiometric ratio of dl-tetrahydropalmatine and benzoic acid (BA-H) were mixed in 1ml of water and heated hydrothermally at temperature 140°C for 1.5
hours. Colourless rod crystals of compound 3.5 were obtained. The single crystal X-ray diffraction analysis shows that the compound belongs to the triclinic crystal system with space group P-1. This compound contains one dl-tetrahydropalmatine and one benzoic acid in the asymmetric unit and has the formula [(dl)THP][BA-H] (Figure 3-10).

Figure 3-10: The thermal ellipsoid diagram of 3.4 with 50% displacement probability.

The C-O bond lengths on the carboxylate group are 1.323Å and 1.211Å with difference of 0.111 Å which denotes that there is no proton transferred from the carboxylate group. As both of the molecules are neutral, it is an adduct. Normal intermolecular hydrogen bond is formed in O31-H31A...N1 with O...N distance 2.624Å. The carboxylic acid has the torsion angle 9° to the aromatic ring. Figure 3-11 shows the packing of compound 3.5.
Figure 3-11: The packing diagram of compound 3.5 along c-axis.

Compound 3.6 [(dl)THP][3OHBA-H]

By mixing dl-tetrahydropalmatine and 3-hydroxybenzoic acid (3OHBA-H) in stoichiometric 1:1 ratio in 1ml of water and was heated hydrothermally at 110°C for 3 hours, colourless rod crystal compound 3.6 is obtained. The single crystal X-ray diffraction analysis shows that the compound is in triclinic crystal system of space group P-1. From the Figure 3-12, one dl-tetrahydropalmatine and one 3-hydroxybenzoic acid are found in the asymmetric unit which give the formula of the neutral molecular co-crystal [(dl)THP][3OHBA-H].
Figure 3-12: The thermal ellipsoid diagram of 3.6 with 40% displacement probability.

Different from 3.2 [(dl)THP-H][Salicylate-H] crystal, the C-O distance lies on carboxylic acid are 1.314Å and 1.213Å. They have more than 0.101Å difference which implies that no proton is still on the acid group. This adduct has normal in O31-H31B...N1 hydrogen bond with O...N distance 2.608Å. The carboxylic acid has torsion angle of 13° which is slightly twisted from the aromatic ring. Instead of forming intramolecular O-H...O hydrogen bond as in 3.2 salicylate co-crystal, intermolecular O-H...O hydrogen bond is formed between O33 and O3 of THP with bond distance 2.785 Å. One-dimensional hydrogen bond linked chains are formed between the (dl)THP and 3-hydrobenzoic acid (Figure 3-13). These chains are overlayed with each other forming a packing in Figure 3-14.
Figure 3-13: The chain of co-crystal 3.6 viewing along c-axis.

Figure 3-14: The packing diagram of co-crystal 3.6.

**Compound 3.7 [(dl)THP][4MeBA-H]**

Equal stoichiometric ratio of dl-tetrahydropalmatine and p-Toluic acid (4MeBA-H) were mixed in 1ml of water and heated hydrothermally at 110°C for 3 hours. Yellow plate crystal compound 3.7 was obtained and solved by single crystal X-ray diffraction analysis. The compound 3.7 belongs to the monoclinic crystal system with space group P2_1/n. One dl-tetrahydropalmatine and one p-Toluic acid are found in the asymmetric unit which give the formula [(dl)THP][4MeBA-H] (Figure 3-15).
Figure 3-15: The thermal ellipsoid diagram of 3.7 with 50% displacement probability.

The C-O bond lengths on the carboxylic acid group are 1.302 Å and 1.219 Å with difference of 0.083 Å which denote that there is no proton transferred from the carboxylic acid to the (dl)THP. As both of the molecules are neutral, it is an adduct. Normal intermolecular hydrogen bond is formed in O31-H31...N1 with O...N distance 2.586 Å. The carboxylic acid has the torsion angle 3° that almost coplanar to the aromatic ring. The packing of this compound is illustrated in Figure 3-16.
Figure 3-16: The packing diagram of compound 3.7 along b-axis.

**Compound 3.8 [(dl)THP][4NH₂BA-H]0.2[H₂O]**

By adding dl-tetrahydropalmatine and 4-aminobenzoic acid (4NH₂BA-H) in equal ratio in 1ml of water and heating hydrothermally at 110⁰C for 4.5 hours, yellow bar crystal compound 3.8 was obtained. The crystal structure was solved by single crystal X-ray diffraction and showing that it is in monoclinic crystal system with space group P2₁/n. In the asymmetric unit, one dl-tetrahydropalmatine, one 4-aminobenzoic acid and 0.2 water are found which give the formula [(dl)THP][4NH₂BA-H][H₂O]₀.₂ (Figure 3-17).
Figure 3-17: The thermal ellipsoid diagram of 3.8 without water with 50% displacement probability.

There is no proton transferred from the acid to the N of (dl)THP as C-O distances on carboxylic acid are 1.309Å and 1.214Å which have the difference 0.095Å. This adduct have normal N…H-O hydrogen bond which is formed in O31-H31…N1 with O…N distance 2.638Å and bond angle 169°. The carboxylic acid has torsion angle of is coplanar to the aromatic ring. Homonuclear O-H…O hydrogen bond is formed between water and O31 of the carboxylic acid with bond distance 2.834Å. N-H…O hydrogen bond is formed between the amino of the 4-aminobenzoic acid to one of the methoxyl of the (dl)THP in N2-H2A…O1 with bond distance 3.010Å. Combined with normal and weak hydrogen bond, one-dimensional chains are formed (Figure 3-18).
3.3 Discussion

Overall from eight different benzoic acids eight new crystalline adducts were formed with racemic THP, compounds 3.1-3.8. The benzoic acid substituent groups were at the 2,3, or 4-position and either methyl, halide, phenol OH or amino NH₂. Whilst close analogs such as 4-Cl and 4-Br might have reasonably been expected to give the same overall crystalline structure these in fact were completely different, whilst surprisingly certain pairs that would appear unlikely to be related e.g. benzoic acid itself and the 3-OH analog and the 4-Me and 4-NH₂ formed isostructural pairs. Before discussing the key issue of protonation state and salt formation versus neutral adduct formation some attention will be paid to the isostructurality issue.
Isostructurality

Isostructurality is having the same structure but with different chemical formula in which individual atoms or groups can be substituted at the same position in the structure. When looking into the crystal data of crystal structure 3.5 and 3.6 formed by benzoic acid and 3-hydroxybenzoic acid respectively with (dl)THP, it shows that they have similar crystal cell parameters (Table 3-1) and form similar crystal packing structure (Figure 3-19). The blue line shows the close contact between the molecules of the compound 3.5 and 3.6 in the range from 2.7Å to 3.4Å.

<table>
<thead>
<tr>
<th>Compound</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>α</th>
<th>B</th>
<th>γ</th>
<th>Volume</th>
<th>Space group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>10.7575</td>
<td>10.8776</td>
<td>11.9569</td>
<td>92.502</td>
<td>106.995</td>
<td>114.522</td>
<td>1195.30</td>
<td>P-1</td>
</tr>
<tr>
<td>3.6</td>
<td>10.7398</td>
<td>10.8177</td>
<td>12.1156</td>
<td>93.794</td>
<td>107.207</td>
<td>114.391</td>
<td>1195.54</td>
<td>P-1</td>
</tr>
</tbody>
</table>

*Table 3-1: Crystal cell parameters of compound 3.4 and 3.5.*
Figure 3-19: *Comparison of packing in compounds 3.4 and 3.5 along b-axis.*

In general isostructurality is found for compounds with different substituent groups (e.g. Br or I). It is rather unexpected to find this in the case of substitution of OH for H, since there is a clear difference in size and also the fact that OH substituents would be expected to participate in hydrogen bonds that cannot be formed for the H substituted parent compound. However they both had the triclinic crystal system with space group P-1 and are isostructural to each other, as further supported by their almost identical theoretical powder XRD patterns.(Figure 3-20).
Structurally, the 3-hydroxybenzoic acid in compound 3.6, the 3 position of the aromatic ring is a hydroxyl group which acts as the hydrogen bond donor making a relatively strong homonuclear hydrogen bond with atom O3. While the benzoic acid found in compound 3.5, the 3 position is only proton, it does not form any strong or normal hydrogen bond interactions. However, weak C-H...O interactions are found between the methoxyl group of THP and the benzoic acid (Figure 3-21). These weak C-H...O interactions replace the role of hydroxyl group in compound 3.6, hold the THP in place in compound 3.5. One way of explaining the isostucturality of the pair is that the parent benzoic acid compound is rather inefficiently packed in that particular region of space and since the 3-OH can make a tighter H-bond contact to the methoxy group of the THP the two structures end up with almost identical packing arrangements.
Figure 3-21: Different acids used in compound 3.5 (left) and 3.6 (right).

Another pair of compounds 3.7 and 3.8 also appear to be isostructural since they have the same monoclinic space group P2₁/n and with just small deviation in unit cell parameters as shown in Table 3-2. Once again the similarity of the two crystal structures can be demonstrated by comparison of their packing diagrams (Figure 3-22) and calculated powder XRD pattern (Figure 3-23). This also denotes that they are isostructural to each other.

<table>
<thead>
<tr>
<th>Compound</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>α (°)</th>
<th>β (°)</th>
<th>γ (°)</th>
<th>Volume</th>
<th>Space group</th>
</tr>
</thead>
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<tr>
<td>3.7</td>
<td>14.6410</td>
<td>11.4136</td>
<td>16.2593</td>
<td>90</td>
<td>112.799</td>
<td>90</td>
<td>2504.75</td>
<td>P2(1)/n</td>
</tr>
<tr>
<td>3.8</td>
<td>14.3975</td>
<td>11.2795</td>
<td>16.2993</td>
<td>90</td>
<td>112.909</td>
<td>90</td>
<td>2438.17</td>
<td>P2(1)/n</td>
</tr>
</tbody>
</table>

Table 3-2: Crystal cell parameters of compound 3.7 and 3.8.
Figure 3-22: Similar packing of compound 3.7 (top) and 3.8 (bottom) are shown.
Figure 3-23: Theoretical powder XRD patterns of compound 3.7 and 3.8.

In Figure 3-24, it shown that the two acids are quite similar that both contains para-substituent in the benzoic acid. There is a moderate N-H…O hydrogen bond between amine group of acid and methoxyl of THP in compound 3.8 which cannot be found in compound 3.7. However, this interaction is replaced by weak C-H…O interaction in compound 3.7 which leads to similar overall packing of the two compounds.

Figure 3-24: Different acids used in compound 3.7(left) and 3.8(right).
Salt and Neutral Adduct Tautomers

Most importantly the question of whether there is proton transfer between acid and the THP base to form a salt with $^+$N-H---:O$' Hydrogen bonds or not leaving neutral co-crystal adducts with N:---H-O H-bonds to be formed will now be addressed. These two cases can be seen as essentially molecular pair 'tautomers'.

Hydrogen bonds are usually thought to arise from electrostatic attraction between an O-H or N-H or H-F dipole and the electron density on a nearby O or N or F. Hydrogen bonds may have extra stabilization, often viewed as arising from a covalent character, if the two contributing resonance forms, O-H...N and N...H-O, are of equal or nearly equal energy.\textsuperscript{6} This is more likely if the two donor atoms have the same basicity (matched pKa values) and if the hydrogen is centered between them. Indeed, symmetric hydrogen bonds seem to be stronger than asymmetric ones.\textsuperscript{7}

Examination of the co-crystals form from (dl)THP with various benzoic acids shows an interesting solid state pKa phenomenon. For more acidic benzoic acids used in crystals compounds 3.1, 3.2 and 3.3, transferring of proton to the nitrogen occurs and salts are formed with C-O bond length difference on each carboxylate moiety with small deviation. However, the comparatively less acidic acid used in compounds 3.4, 3.5, 3.6, 3.7 and 3.8, the distinct difference of the C-O bond length existed in each carboxylic acid indicated the presence of one C=O and one C-O bond. Neutral molecular adducts were formed with N...HO hydrogen bond in these compounds. Table 3-3 shows the difference in C-O bond distance increases as the pKa of the acid increased. A significant change in the $\Delta$(C-O) appears between compounds 3.3 and 3.4 and indicates this structural switch exists between pKa 3.98 and 4.0 from the 4-Cl and 4-Br derivatives.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Benzoic Acid</th>
<th>pKa Value</th>
<th>Proton Position</th>
<th>Δ(C-O) (Å)</th>
<th>Stoichiometry</th>
<th>Bond distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>2-Br</td>
<td>2.88</td>
<td>N-H...O</td>
<td>0.023</td>
<td>1:1 H₂O</td>
<td>2.617</td>
</tr>
<tr>
<td>3.2</td>
<td>2-OH</td>
<td>2.98</td>
<td>N-H...O</td>
<td>0.038</td>
<td>1:1</td>
<td>2.710</td>
</tr>
<tr>
<td>3.3</td>
<td>4-Cl</td>
<td>3.98</td>
<td>N-H...O</td>
<td>0.051</td>
<td>1:1</td>
<td>2.585</td>
</tr>
<tr>
<td>3.4</td>
<td>4-Br</td>
<td>4.00</td>
<td>N...H-O</td>
<td>0.084</td>
<td>1:1 0.5H₂O</td>
<td>2.585</td>
</tr>
<tr>
<td>3.5</td>
<td>H</td>
<td>4.19</td>
<td>N...H-O</td>
<td>0.111</td>
<td>1:1</td>
<td>2.624</td>
</tr>
<tr>
<td>3.6</td>
<td>3-OH</td>
<td>4.30</td>
<td>N-H...O</td>
<td>0.101</td>
<td>1:1</td>
<td>2.608</td>
</tr>
<tr>
<td>3.7</td>
<td>4-Me</td>
<td>4.37</td>
<td>N-H...O</td>
<td>0.083</td>
<td>1:1</td>
<td>2.586</td>
</tr>
<tr>
<td>3.8</td>
<td>4-NH₂</td>
<td>4.92</td>
<td>N-H...O</td>
<td>0.095</td>
<td>1:1 0.25H₂O</td>
<td>2.638</td>
</tr>
</tbody>
</table>

*Table 3-3: Summary of Benzoic acid Adducts with racemic THP.*

The pKa of 4-chlorobenzoic acid and 4-bromobenzoic acid is slightly different as they possess different substituent at the para position. The Chloro group has stronger electron-withdrawing ability than bromo group. The stronger of such inductive effect in chloro group promoting the removal of the protons. Moreover, pKa is defined as the ability of an ionizable group of an organic compound to donate a proton (H⁺) in an aqueous media. The lower pKa acids would prefer donation of protons. This phenomenon also can be observed in our results. In compounds 3.1, 3.2 and 3.3, with more acidic benzoic acids bearing electron-withdrawing substituents, protons are transferred to (dl)THP to form salts. However, electron donating groups such as Me and NH₂ groups present in compounds 3.7 and 3.8 respectively result in higher value of pKa resulting in a lower tendency for proton transfer.

Compounds 3.2 and 3.6 contain substituted hydroxyl group at ortho and meta position respectively. The pKa of benzoic acid with ortho-OH, i.e. salicylic acid, is much lower than that in meta-OH isomer. In the structure of compound 3.2, an intramolecular interaction is formed between the carboxyl and the substituted group.
From the literature, this intramolecular hydrogen bond in the ortho anion so called "resonance-assisted hydrogen bonding" confers a high degree of electronic stability on this ion with increased electrostatic interaction between the protonic hydrogen and an oxygen atom with a higher negative charge. Furthermore, inductive effect in ortho position and meta position is also different. The closeness between the groups makes the electrostatic effect larger. Hence, protons are transferred to (dl)THP-H instead of the salicylate in compound 3.2. Similarly a THP salt is formed in compound 3.1 (2-Br) but not for compound 3.4 (4-Br).

It is expected that the donor–acceptor distance should increase if the bond has a double-minimum potential well, and decrease for a single minimum potential well. In this system, the shortest bond distances between N and O are found in compounds 3.3 and 3.4 which are both 2.585 Å but are either side of the proton switch. Finally, the C-O bond length difference within the carboxylate groups can be used to determine whether the group is protonated or not, though the $\Delta_{C-O}$ is only marginally different for the 4-Cl and 4-Br cases. An intriguing question remains whether a perfect pKa match between the THP and a donor acid group could be found with a very short ‘single well’ type N-H-O hydrogen bond and studies to examine this will be conducted.

**Supramolecular Effects**

The ortho substitution in benzoic acid has relatively strong steric effect to the carboxylate or carboxylic acid. However, this moderate intramolecular H-bond helps to reduce the torsion between the carboxylate and aromatic ring probably resulted in smaller O…O repulsion. When comparing the torsion angle in compound 3.1 which has a bromine ortho substituent, due to the lack of such moderate intramolecular hydrogen bond and strong steric effect from the heavy bromine atom, the distortion
is expected to be much larger that is 60°. This distortion is absent in compound 3.2 and the carboxyl is coplanar to the aromatic ring (Figure 3-25).

![Compound 3.1](image)

![Compound 3.2](image)

*Figure 3-25: Different torsion angle between the carboxylate and the aromatic ring in Compound 3.1 and 3.2.*

![Structure](image)

*Figure 3-26: Structure of tetrahydropalmatine.*

The hydrogen bond patterns observed in the crystal packing are either found in 1-D chain or in 2-D network. The crystal packing of the compounds are not totally identical but apparently some of them have similar features although the details are different. In those co-crystals, the four methoxyl groups linked to the phenyl ring of the (dl)THP have the dihedral angles similar to the published structure.\(^{12,13}\) The dihedral angles of C15-C16-O1-C21, C1-C17-O2-C18, C9-C8-O4-C20 are almost
coplanar to the phenyl ring while the C6-C7-O3-C19 is rotated out of plane with the
dihedral angel about 105° to avoid steric hindrance with the adjacent methylenic C5
atom. The dihedral angle of C6-C7-O3-C19 is 98.07° 13 in published structure of
(dl)THP. As the oxygen in these methoxyl groups can form weak C-H...O interaction
that slightly alters the dihedral angle. In compounds 3.1, 3.4, 3.7, 3.8, a weak
interaction exists between O4 and C15 in two (dl)THP forming a dimer like structure
with dihedral angle in C9-C8-O4-C20 of 9.21°, 17.41°, -16.44°, -15.92° respectively
that somewhat twisted away from the phenyl ring. Within this pair of (dl)THP,
C-H...π interaction weak interaction is also found between the chiral centre C13 and
the phenyl ring.

From the result, all of the acids are interacted with (dl)THP with N...H...O
hydrogen bond. Different substituted benzoic acids are able to alter the packing of
the crystals. In compounds 3.6 and 3.8, the substituent of OH and NH2 groups in the
benzoic acids able to act as hydrogen bond donor that help to link up the molecules
to form a 1-D chain (Figure 3-27).

\[ \text{Figure 3-27: 1-D chains formed in compound 3.6 and compound 3.8.} \]
The OH group is also present in compound 3.2 and involved in an intramolecular hydrogen bond. Intramolecular hydrogen bonding reduces the ability of the hydroxyl to interact with other molecules. Other substituents in the benzoic acids cannot form any other interactions to higher the dimensionality in the crystal packing. Not only the substituents, but also the carboxylate can further hydrogen bonded to water. The oxygen atom of the water molecule in 3.1, 3.4, 3.8 also can acts as hydrogen bond acceptor due to the two lone pair while it also can donate the pair of hydrogen. Although different substituents are contained in the benzoic acids, the arrangement of the water is roughly the same that it seeks between a pair of acids (Figure 3-28). That pair of acid with inversion of each other are interacting with this water. However, those hydrates just form one hydrogen bond with the carboxylate that cannot further modify the packing by hydrogen bond interaction.

Figure 3-28: Waters seeks between a pair of acid with inversion of each other in compound 3.8.
3.4 Experimental

Preparation and Characterization

Materials: (dl)THP is obtained from Prof H. Xue of Biochemistry Department HKUST without further purification before use. All other benzoic acid are purchased from Acros Organics and Aldrich Chemical Company and used without further purification.

**Compound 3.1**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.028g (0.14mmol) of 2-bromobenzoic acid were mixed well in 1ml of de-ionized water. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Colourless bar crystals were filtered and dried in atmosphere with 82% yield. Elemental analysis: (calculated) %C=58.542, %H=5.614, %N=2.438 (Found) %C=58.9, %H=5.59, %N=2.47.

**Compound 3.2**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.02g (0.15mmol) of salicylic acid are mixed well in 2ml of de-ionized water. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 140°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals were filtered and dried in atmosphere with 78% yield. Elemental analysis: (calculated) %C=68.139, %H=6.331, %N=2.837 (Found) %C=68.5, %H=6.46, %N=2.94.
Compound 3.3

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.022g (0.14mmol) of 4-chlorobenzoic acid are mixed well in 1ml of de-ionized water. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 140°C for one and a half hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals were filtered and dried in atmosphere with 75% yield. Elemental analysis: (calculated) %C=65.684, %H=6.047, %N=2.801 (Found) %C=66.1, %H=5.91, %N=2.74.

Compound 3.4

0.025g (0.07mmol) of dl-tetrahydropalmatine and 0.028g (0.14mmol) of 4-bromobenzoic acid are mixed well in 1ml of de-ionized water. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 140°C for one and a half hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow plate crystals were filtered and dried in atmosphere with 47.5% yield. Elemental analysis: (calculated) %C=58.542, %H=5.614, %N=2.438 (Found) %C=59.0, %H=5.23, %N=2.40.

Compound 3.5

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.017g (0.14mmol) of benzoic acid are mixed well in 1ml of de-ionized water. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 140°C for one and a half hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Colourless rod crystals were filtered and dried in atmosphere with 83% yield. Elemental analysis: (calculated) %C=70.423,
%H=6.543, %N=2.933 (Found) %C=70.4, %H=6.62, %N=2.81.

**Compound 3.6**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.019g (0.14mmol) of 3-hydroxybenzoic acid are mixed well in 1ml of de-ionized water. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Colourless rod crystals were filtered and dried in atmosphere with 64% yield. Elemental analysis: (calculated) %C=68.139, %H=6.331, %N=2.837 (Found) %C=68.1, %H=6.39, %N=2.85.

**Compound 3.7**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.02g (0.15mmol) of p-Toluic acid are mixed well in 1ml of de-ionized water. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow plate crystals were filtered and dried in atmosphere with 48% yield. Elemental analysis: (calculated) %C=70.856, %H=6.766, %N=2.849 (Found) %C=70.7, %H=6.86, %N=3.10.

**Compound 3.8**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.019g (0.14mmol) of 4-aminobenzoic acid are mixed well in 2ml of de-ionized water. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for four and a half hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals were
filtered and dried in atmosphere with 68% yield. Elemental analysis: (calculated)

%C = 67.657, %H = 6.590, %N = 5.635 (Found) %C = 67.6, %H = 6.59, %N = 5.31.
3.5 References


### 3.6 Crystal Data Summary

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100
Chapter 4  Products from Hydrolytic Breakdown of THP:

Synthesis of Palmatine Salts and Dihydropalmatine

4.1 Introduction

4.2 Preparation and structures of palmatine salts and dihydropalmatine

Compound 4.1 [P][citrate-H$_2$]

Compound 4.2 [P][salicylate-H][H$_2$O]

Compound 4.3 [P][4BrBA]3[H$_2$O]

Compound 4.4 [P][13BA-H]2[H$_2$O]

Compound 4.5 [P][succinate]0.25[H$_2$O]

Compound 4.6 [P][MesoTAR-H]H$_2$O

Compound 4.7 Dihydropalmatine

4.3 Discussions

4.4 Experimental

4.5 References

4.6 Crystal Data Summary
4.3 Introduction

In the introductory Chapter it was explained that our group's earlier studies of the hydrothermal crystallization of artemisinin type compounds revealed that hydrolysis reactions could occur. A good example is that of the anti-malarial drug dihydroartemisinin which could be cleanly transformed into deoxyartemisinin in aqueous pyridine over a period of ca. 1 week. In this process the peroxy linkage in DHA is lost (a reduction) and the hemi-acetal of the DHA is converted to a lactone (an oxidation). Overall the process effectively removes H₂O from the compound. The natural product tetrahydropalmatine (THP) has several functional sites which could be subject to hydrolysis at elevated temperatures. It has aromatic rings A and D which possess methoxy substituents which might hydrolyse to phenol and cause resulting oxidation. The central rings B and C are reduced and oxidation of these might be possible leading to an extension in the conjugated π-system of the molecule. One possibility in this respect would be oxidation to the parent compound palmatine.

Palmatine (Figure 4-1) is a kind of alkaloid¹ has been found in plants of various families, and mainly presents in the rhizomes of Fibrarurea Tinctoria Lour. These medicinal plants have been used as folk medicine in treatment of jaundice, dysentery, hypertension, inflammation and liver-related diseases.²³. The previous studies have shown that palmatine exerted protective effect on hepatocytes⁴ and inhibited the process of gene expression and gene transcription leading to its usefulness as a therapeutic agent⁵. It is extremely similar to the alkaloid tetrahydropalmatine, which come from the same origin as palmatine, in overall chemical structure but has a more extended aromatic system.

While using palmatine as the query searching in the Cambridge Structural Database (CSD), there are only two hits of structures being reported with different
counter ions which are palmatine chloride phenol solvate monohydrate\textsuperscript{6} and palmatine iodide\textsuperscript{7}. According to the literatures, the crystals of both salts are obtained by slow evaporation of the solvent at room temperature by using traditional method for growing crystals.

![Chemical structures of Tetrahydropalmatine, Palmatine, and Dihydropalmatine](image)

Figure 4-1: Chemical structure of tetrahydropalmatine, palmatine and dihydropalmatine.

The present Chapter discusses the formation of several new palmatine salts
4.1-4.6 that have arisen by solvothermal reaction of tetrahydropalmatine with various acids. Other than palmatinium salts, partial dehydrogenation of THP has also been found to occur to yield a new compound 4.7, dihydropalmatine in good yield under appropriate hydrothermal conditions (Figure 4-1). To our knowledge this has not been isolated before in plant extracts. The molecular structures of all compounds reported were determined by single crystal X-ray diffraction.

4.2 Preparation and structures of palmatinium salts and dihydropalmatine

Compound 4.1 \([\text{P}]^+\text{[Citrate-H}_2]\)

Stoichiometric 2:1 ratio of dl-tetrahydropalmatine and citric acid were mixed in 0.5ml of ethanol and heated solvothermally at 110°C for 6 hours. Yellow bar crystal compound 4.1 was obtained. The single crystal X-ray analysis showed that the compound was in triclinic crystal system of space group P-1. One palmatinium (P) and one citrate are found in the asymmetric unit which give the formula \([\text{P}]^+\text{[citrate-H}_2]\) (Figure 4-2).

![Figure 4-2: The thermal ellipsoid diagram of 4.1 with 50% displacement probability.](image)

Citric acid possesses three carboxylic acid groups that can be possible to be a tri
anion. When comparing the C-O bond distances on those carboxylic acid groups, it has a difference of 0.008Å between C36-O34 and C36-O35 which consisting a delocalized environment resulted from the deprotonation of the acid group. While in the other two carboxylic acids, the C-O bond difference is about 0.1Å that implies no transfer of proton. There are two types of hydrogen bond exist in the acid, intramolecular and intermolecular hydrogen bonds. Two moderate homonuclear intramolecular hydrogen bonds are observed in O33-H33...O34 and O36-H36A...O35. Atom O36 behaves as hydrogen donor and is hydrogen bonded to O35 with bond distance 2.497Å. Atom O33 in the hydroxyl group acts as the hydrogen bond donor and it is hydrogen bonded to atom O34 with bond distance 2.579Å. Atom O34 is further joined with O31 in another citrate in next unit forming moderate intermolecular hydrogen bond O31-H31A...O34 with bond distance of 2.644Å. This interaction links up the acids forming a negative charge 1-D chain. In figure 4-3, it shows that the acid chain extends between channels of palmatinium ions.

Figure 4-3: The packing diagram of compound 4.1.
Compound 4.2 \([P]^+[\text{Salicylate}]2[H_2O]\)

Equal molar quantities of (dl)THP and salicylic acid were mixed in 1ml ethanol and heated at 110° for 3 hours by solvothermal method. Yellow bar crystal compound 4.2 was collected and was characterized by single crystal XRD analysis. It possesses monoclinic crystal system with P2_1/C space group. One palmatinium, one salicylate and two water molecules are found in the asymmetric unit (Figure 4-4). It has the formula \([P]^+[\text{salicylate}]2[H_2O]\).

![Figure 4-4: The thermal ellipsoid diagram of 4.2 with 50% displacement probability.](image)

Salicylate has the ortho hydroxyl group that can link with atom O31 to form homonucleus intramolecular hydrogen bond O33-H33A...O31. The C-O bond difference in carboxylate is 0.064Å which denotes that it has a delocalized environment. The carboxylate is nearly coplanar to the aromatic rings and the
hydroxyl group also lies in the same plane as the carboxylate. Atom O32 in carboxylate acts as the hydrogen bond acceptor for two water molecules forming O1W-H1WB...O32 and O2W-H2WA...O32 moderate hydrogen bond with bond distance 2.780Å and 2.790Å respectively. O1W further interacts with the atom O2W in the next asymmetric unit by hydrogen bond O1W-H1WA...O2W with distance 2.790Å. Moderate hydrogen bond O2W-H2WB...O31 is observed between the water and the salicylate with bond length 2.715Å. The hydrogen bond data is summarized in Table 4-1.

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Symmetry transformations used to generate equivalent atoms:
#1 -x+1, y-1/2, -z+3/2

Table 4-1: Hydrogen bond table for compound 4.2

The salicylate is hydrogen bonded to water forming a thick negatively charged 1-D chain and 12-membered hydrogen bonded ring is observed. (Figure 4-5). In the packing diagram of compound 4.2 (Figure 4-6), the positively charge palmatinium is in between the negative acid chains.
Figure 4-5: 1-D chain composed of salicylate and water in compound 4.2 along c-axis.

Figure 4-6: Packing diagram of compound 4.2 along a-axis with the hydrogen omitted.
Compound 4.3 \([\text{P}^+\text{[4BrBA]}\text{3[H}_2\text{O}]}\)

A mixture of dl-tetrahydropalmatine and 4-bromobenzoic acid (4BrBA-H) in stoichiometric 2:1 ratio in 1ml of ethanol was heated solvothermally at 110°C for 3 hours. Yellow rod crystal compound 4.3 was formed. Compound 4.3 has the monoclinic crystal system in space group P2_1/n. In the asymmetric unit, one palmatinium, one 4-bromobenzoate and three water molecules are observed (Figure 4-7). It has the formula \([\text{P}^+\text{[4BrBA]}\text{3[H}_2\text{O}]}\). 

![Figure 4-7: The thermal ellipsoid diagram of 4.3 with 50% displacement probability.]

The deprotonation of the carboxylic acid can be confirmed by the C-O distances difference on carboxylate which is 0.019Å. The dihedral angle between the carboxylate and the aromatic ring is 11.82°. Atom O32 is hydrogen bonded to O1W with O1W-H1WB...O32 bond distance 2.8030Å. O1W further interacts with O2W with O1W-H1WA...O2W bond distance 2.750Å. O2W also behaves as hydrogen bond donor forming hydrogen bond to O31 and atom O3W which formed moderate hydrogen bond O2W-H2WA...O31 and O2W-H2WB...O3W in distance 2.778Å and 2.787Å respectively. Atom O3W used up a pair of protons to form hydrogen
bondings. Two moderate hydrogen bonds O3W-H3WA...O1W, O3W-H3WB...O31 with bond distance 2.778Å and 2.804Å respectively are linked up among the two waters and acid.

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Symmetry transformations used to generate equivalent atoms:
#1 -x+3/2, y+1/2, -z+1/2   #2 -x+3/2, y-1/2, -z+1/2

Table 4-2: Hydrogen bond table for compound 4.3.

In the packing diagram of compound 4.3, 12-membered hydrogen bonded ring is observed which is constructed by five water molecules together with atom O31 from the carboxylate (Figure 4-8). This ring links up the benzoates forming a thick negatively charged 1-D tape that let the palmatinium sit in between the chains.

Figure 4-8: Packing diagram of compound 4.3 along a-axis.
**Compound 4.4 [P]=[13BA-H]2[H2O]**

Equimolar quantities of (dl)THP and isophthalic acid (13BA-H2) were added to 1ml of ethanol and were heated solvothermally at 110° for one and a half hours. Yellow rod crystal compound 4.4 was obtained and further analyzed by single crystal XRD. Compound 4.4 have the crystal system triclinic with space group P-1. One palmatinium, one isophthalate and two water molecules are observed in the asymmetric unit (Figure 4-9) which give the formula [P]=[13BA-H][H2O]2.

*Figure 4-9: The thermal ellipsoid diagram of 4.4 with 50% displacement probability.*

When comparing the C-O bond distances, the bond differences of O31-C37-O32 and O33-C38-O34 are 0.056Å and 0.045Å respectively. These carboxylate consist of delocalized environments which are nearly co-planar to the benzene ring. As palmatinium possesses one positive charge, and the isophthalate must bear a negative charge for balancing. H31 and H33 hence have only 50% site occupancy. The postions of the acidic hydrogen are quite interesting. After looking into the environment of
atom O31 and O33 carefully, there are two possibilities of the hydrogens. One possibility is the H31 and H33 are sitting in the special position which are the mid-way between the oxygen atoms to generate (O31-H31-O31) and (O33-H33-O33) strong intermolecular O-H-O hydrogen bond with O…O distance 2.454Å and 2.490Å respectively. The other possibility is both H31 and H33 hydrogen are flipping between two carboxylate groups with the average position in the special position. As X-ray diffraction is not a good technique to determine the position of hydrogen atom, the answer may be available using neutron diffraction. O1W acts as hydrogen bond donor to form hydrogen bond with O32 and O34 in bond distance of 2.766Å and 2.815Å respectively. O1W also can be a hydrogen acceptor of the nearby water forming O2W-H2WA…O1W moderate hydrogen bond with bond distance of 2.767Å.

<table>
<thead>
<tr>
<th>D-H…A</th>
<th>D(D…A)/Å</th>
<th>〈DHA〉°</th>
</tr>
</thead>
<tbody>
<tr>
<td>O31-H31…O31#1</td>
<td>2.454</td>
<td>173.09</td>
</tr>
<tr>
<td>O33-H33…O33#2</td>
<td>2.490</td>
<td>165.19</td>
</tr>
<tr>
<td>O1W-H1WA…O32#3</td>
<td>2.766</td>
<td>166.31</td>
</tr>
<tr>
<td>O1W-H1WB…O34#2</td>
<td>2.815</td>
<td>176.48</td>
</tr>
<tr>
<td>O2W-H2WA…O1W</td>
<td>2.767</td>
<td>147.39</td>
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</table>

Symmetry transformations used to generate equivalent atoms:

#1 -x+1, -y+2, -z+2  #2 -x+2, -y+2, -z+1  #3 -x+1, -y+2, -z+1

Table 4-3: Hydrogen bond table for compound 4.4

Through hydrogen bond interactions shown in Table 4-3, a 2-D negative charge sheet is formed (Figure 4-10). From the packing diagram of compound 4.4 (Figure 4-11), we find that the positive charged palmatinium is sought between the isophthalate sheet.
Figure 4-10: Isophthalate and water formed a 2-D negatively charged sheet along b-axis.

Figure 4-11: Packing diagram of compound 4.4 along a-axis.

**Compound 4.5 [P][succinate]0.25[H₂O]**

Compound 4.5 was prepared by adding equimolar quantities of (l)THP and succinic acid into 1ml of ethanol. The mixture was heated at 110° for 3 hours solvothermally and then yielded yellow bar crystal compound 4.5. The crystal was further analyzed by single crystal XRD and found that it belongs to the monoclinic crystal system in P2₁/c space group. One palmatinium, one succinate and a quarter of
water molecule are found in the asymmetric unit which shows in Figure 4-12. From the asymmetric unit, we can deduce the formula $[\text{P}^\text{I}][\text{succinic acid}][\text{H}_2\text{O}]_{0.25}$ for compound 4.5.

![Diagram of molecular structure](image)

*Figure 4-12: The thermal ellipsoid diagram of 4.5 with 50% displacement probability.*

C-O bond difference in the carboxylate O31-C31-O32 and O33-C34-O34 are 0.063Å and 0.059Å. We can interpret these two carboxylate are deprotonated. After examining the environment of atoms O31 and O33, it is found that atom O31 interacts with O33 in the nearby acid by sharing a proton H31C. This O31-H31-O33 interaction is almost linear and has strong and short O...HO hydrogen bond distance 2.477Å. As the protons H31C are shared by two carboxylic acid groups, they just have 50% site occupancy during the refinement. It gives two carboxylic acid groups totally one proton that lead to the negatively charged succinate.
By using these two hydrogen bonds, a succinate chain is formed. Another intermolecular hydrogen bond is observed between the carboxylate and the hydrate. Atom O34 is joined with the water molecule O1W with relatively weaker hydrogen bond O1W-H1WD...O34 with bond distance 2.854Å. The succinate chain interact with the water formed a 1-D negatively charged chain. (Figure 4-13) In between these chains, positively charged stack of palmatinium is found.

![Packing diagram of compound 4.5 along c-axis.](image)

**Compound 4.6 \([P]^+\text{[MesoTAR-H]}\text{H}_2\text{O}\)**

Stoichiometric 2:1 of (l)THP and meso-tartaric acid(MesoTAR-H\text{2}) were added in 10 drops of water and heated hydrothermally at 110\(^\circ\)C for 6 hours. Yellow bar crystal compound 4.6 was obtained. The single crystal X-ray diffraction analysis shows that it has triclinic crystal system of space group P\(-1\). One palmatinium, one meso-tartrate and one hydrate are found in the asymmetric unit which gives the formula \([P]^+\text{[MesoTA-H]}\text{H}_2\text{O}\)
Figure 4.14: The thermal ellipsoid diagram of 4.6 with 50% displacement probability.

By comparing C-O bond distance, they have difference of 0.037Å and 0.048Å respectively in O31-C32-O33 and O35-C34-O36 indicating the delocalization environment. However, after examining the environment around atom O31, we find that protons H31 is shared in O31-H31-O31. Atom O31 is hydrogen bonded to O31 in the next asymmetric unit through symmetry operation by protons H31 to form strong hydrogen bond. Moreover, the same situation is found in O35. The same situation is also observed in proton H35 forming hydrogen bond in O35-H35-O35. As the protons H31 and H35 are shared between two carboxylic acid groups, they just have 50% site occupancy and this gives two carboxylic acid groups totally one proton to have the negatively charged meso-tartrate. Here are the details of the hydrogen bond in compound 4.6 (Table 4-4).
D-H...A    D(D...A)/Å    <(DHA)°
O31-H31...O31#1  2.481    164.34
O33-H33...O1W#2  2.611    174.96
O34-H34...O3    2.858    146.83
O34-H34...O36   2.643    116.78
O35-H35...O35#3  2.476    175.99
O1W-H1WA...O36  2.695    145.18
O1W-H1WB...O32  2.659    175.89

Symmetry transformations used to generate equivalent atoms:
#1 -x+1, -y, -z+1   #2 x+1, y, z   #3 -x+1, -y, -z

Table 4-4: Hydrogen bond table for compound 4.6

In the packing of compound 4.6, the meso-tartaric acid connects itself through O...H...O strong hydrogen bonding that constructs negative charge chain. These chains further link up with each other by forming normal hydrogen bond with the hydrates (Figure 4-15). Water acts as both hydrogen bond donors and acceptor that form a hydrogen-bonded layer with tartrate.

Figure 4-15: 2-D network is formed by the tartrate and water in compound 4.6 along b-axis.
In Figure 4-16, the positive charged palmatiniums are found between negative charged mono-deprotonated meso-tartarate chain. A bifurcated hydrogen bond motif is observed in H34 forming hydrogen bond in O34-H34…O3 and O34-H34…O36. Indirect intramolecular hydrogen bonding can occur involving a water molecule. The water molecule links between two carboxylate in the tartrate by hydrogen bond in O1W-H1WA…O36 and O1W-H1WB…O32.

Figure 4-16: Packing diagram of compound 4.6 along b-axis.

Compound 4.7 Dihydropalmatine

Besides palmatine salts mentioned above, a new compound, dihydropalmatine(DHP) was also synthesized. It was prepared by mixing 0.05g (l)THP in water heated hydrothermally 110° for six days. Yellow bar crystal compound 4.7 was obtained with acceptable yield and phase purity. After single crystal XRD analysis, compound 4.7 has the crystal system monoclinic in space
group P2\textsubscript{1}/c. Figure 4-17 shows the content in the asymmetric unit.

![Molecular structure diagram](image)

**Figure 4-17:** The thermal ellipsoid diagram of 4.7 with 50% displacement probability.

Compared with THP, two protons at C12 and C13 have been removed. C13-C12 bond length is 1.361\textdegree. This is achiral since dehydrogenation of the chiral center C13 has occurred. In Figure 4-18, it shows that the experimental powder XRD pattern of DHP matches with the theoretical one. And DHP is the sole products. However, long duration of heating leads to part of the reactants decomposition and causes contamination on the product. For the Fast Atom Bombardment Mass Spectroscopy analysis, expected molecular ion peak is found at m/z=352.1567(Figure 4-19). In the TGA analysis, there is an obvious drops in weight % at about 240\textdegree C which is slightly higher than (dl)THP and (l)THP which are around 220\textdegree C.
Figure 4-18: Comparison between the experimental and theoretical powder XRD patterns of DHP

Figure 4-19 Mass spectrum of DHP

In the crystal packing of DHP no hydrogen bonds can be observed since there are no strong H-bond donors. Molecules are held together by weak CH---O hydrogen bond between the methoxyl group and van der Waals interactions.
4.3 Discussion

THP

Palmatinium
Figure 4-20: Thermal ellipsoid plots of THP, palmatine and DHP.

The palmatinium salts and DHP consist mainly of the tetracyclic ring system from A to D. (Figure 4-20) The rings A, C and D are planar in palmatine while only rings A and D are planar in DHP. The average deviations from the least-squares planes of palmatinium salts and DHP are listed in Table 4-5. Large deviation in ring C found in 4.7 is caused by the partially delocalization in N1, C13 and C12 indicated by the bond lengths in Figure 4-21. This leads ring C having an envelop conformation dominated by C5 as an out-of-plane atom but the remaining atoms almost co-planar. For ring B, an envelop conformation is adapted with only C4 being out of the plane.

Figure 4-21: The thermal ellipsoid diagram of 4.7 with 50% displacement probability
showing bond lengths.

For palmatine, ring B dominates with half-chair conformation with C3 and C4 as the out of plane atoms, however, there is a tendency towards an envelope form, with C4 as the out-of-plane and that is in the analogous to the published THP crystal structures. The dihedral angle between the least-squares plane of phenyl rings A and D is range from 15° to 19° (Table 4-5). The angles are smaller than the value found in the case of (dl)THP (25.8°)⁸ and (l)THP monohydrate (32.6°).⁹

<table>
<thead>
<tr>
<th>Compound</th>
<th>(Dihedral angle A/D)/°</th>
<th>Avg. Deviation from the least-squares plane σ/Å</th>
<th>Ring A</th>
<th>Ring C</th>
<th>Ring D</th>
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<tbody>
<tr>
<td>4.1</td>
<td>14.99 (0.09)</td>
<td>0.0068</td>
<td>0.0078</td>
<td>0.0029</td>
<td></td>
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<tr>
<td>4.2</td>
<td>14.82 (0.10)</td>
<td>0.0131</td>
<td>0.0131</td>
<td>0.0067</td>
<td></td>
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<tr>
<td>4.3</td>
<td>14.83 (0.03)</td>
<td>0.0045</td>
<td>0.0082</td>
<td>0.0024</td>
<td></td>
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<tr>
<td>4.4</td>
<td>17.48 (0.07)</td>
<td>0.0057</td>
<td>0.0150</td>
<td>0.0044</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>18.63 (0.08)</td>
<td>0.0007</td>
<td>0.0135</td>
<td>0.0024</td>
<td></td>
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<tr>
<td>4.6</td>
<td>17.95 (0.09)</td>
<td>0.0043</td>
<td>0.0098</td>
<td>0.0091</td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td>18.82 (0.08)</td>
<td>0.0025</td>
<td>0.1260</td>
<td>0.0041</td>
<td></td>
</tr>
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Table 4-5: Dihedral angles and average deviations from the least-squares planes of ring A, C and D.

As shown in Figures 4-19, methoxyl substituents were found at C16, C17, C7 and C8 with different geometry. At C8, C16, C17, methoxyl groups are nearly co-planar with their respective phenyl ring, A and D, while C7 rotates out of the plane of ring D by around 96°-123° which avoids the steric hindrance with the adjacent methylenic C5 atom. This rotation of the methoxyl group is analogous to the structure of THP.

The crystal packing of the palmatine salts are similar to each other. Heteronuclear N...HO or NH⁺...O hydrogen bond interaction discussed in chapter 2 and 3 does not form between the palmatine and the corresponding acids. Because the nitrogen in the positively charge palmatine can no longer be the proton acceptor. The positively charged palmatine are placed between the negatively charged acid or solvent acid
network. The connection between them is not only the van der Waals contact, but also the static electric interaction. Another general phenomenon found in the palmatine is that palmatine tends to pack in stack (Figure 4-22).

![Diagram of palmatine molecules](image)

**Figure 4-22: Stack of palmatinium in compound 4.5.**

Except compounds 4.1 and 4.7, the rest of the palmatine salts are hydrated. Water formed hydrogen bond with the acid and this modifies the pattern. In compounds 4.2, 4.3 and 4.5, 1-D chains are observed. In fact, water in molecule 4.5 do not involve in the main chain but they are sandwiched in between 4.2 and 4.3. The water molecules act as adjacency to connect the salicylate and 4-bromobenzoate in compounds 4.2 and 4.3 respectively forming thick 1-D chain through hydrogen bondings. Water molecules involved in the three hydrogen bonds provide a pair of proton as donor and a lone pair of electron as acceptor. Graph set analysis of hydrogen bonded ring $R^4_5(12)$ is found in compound 4.2 which is shown in Figure 4-5. The water molecules link up the acid and water in the next asymmetric unit forming hydrogen-bonded ring and the ring contains three hydrates and carboxylates from two acids. Hydrates are capped by acids forming an infinite 1-D chain. In compound
4.3, two water molecules and the carboxylate of the 4-bromobenzoate form graph set analysis of $R^3_3(8)$ hydrogen-bonding arrangement (Figure 4-23i). It also forms hydrogen-bonding rings which have an $R^5_6(12)$ hydrogen-bonding arrangement (Figure 4-23ii). Although the asymmetric unit of compound 4.3 only has three water molecules, by symmetry operation, water molecules can interact with a carboxylate forming a six-member ring. Hydrates lie between the 4-bromobenzate forming a sandwich packing.

![Diagram](image)

*Figure 4-23: Hydrogen arrangement i and ii forms by hydrogen bonds.*

As shown in Figure 4-24, the negative charge 1-D chains in compounds 4.2 and 4.3 are sandwiched between the stacks of palmatiniums in the same way. For the sake of clarity, hydrogen atoms are omitted. 4.2 and 4.3 have similar packing and both are in the monoclinic crystal system. However, they have an difference in the unit cell parameter especially in $a$ and $c$ and shown in Table 4-6. From their theoretical powder XRD patterns in Figure 4-25, the patterns are also different. Some dissimilar peaks are observed between 5° and 15°. Although the packing of 4.2 and
4.3 are similar, powder XRD patterns and the graph set analysis shows that they are not isostructure to each other.

![Diagram of packing structures](image)

*Figure 4-24: (left) Packing diagram of compound 4.2 along a-axis. (right) Packing diagram of compound 4.3 along c-axis.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Space group</th>
<th>a(Å)</th>
<th>b(Å)</th>
<th>c(Å)</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>Vol.(Å³)</th>
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<tr>
<td>4.2</td>
<td>P2(1)/c</td>
<td>15.292</td>
<td>7.3795</td>
<td>22.263</td>
<td>90°.</td>
<td>94.673°</td>
<td>90°.</td>
<td>2504.0</td>
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<tr>
<td>4.3</td>
<td>P2(1)/n</td>
<td>10.9530</td>
<td>7.3607</td>
<td>33.607</td>
<td>90°.</td>
<td>91.4850°</td>
<td>90°.</td>
<td>2708.6</td>
</tr>
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</table>

*Table 4-6: Unit cell parameter of compound 4.2 and 4.3.*
Figure 4-25: Theoretical powder XRD patterns of compound 4.2 and 4.3.

Besides the 1-D chains found in the palmatinium salts, 2-D layers of hydrated acid can also be observed in compounds 4.4 and 4.6. In compound 4.4, two water molecules are located between the isophthalate chains (Figure 4-10). The packing of the isophthalate chains is different from the crystal structure of isophthalic acid with double hydrogen bonded cyclic dimers zig-zag chain(Figure 4-26).\textsuperscript{10} Moreover, the 2-D sheet in compound 4.4 is negatively charge instead of neutral in the crystal structure of isophthalic acid.

Figure 4-26: The isophthalic acid forms infinite zig-zag chain through strong intermolecular hydrogen bonding from the carboxyl groups.

A common feature is that strong intermolecular hydrogen bonds are observed
along the chain. When there is a crystallographic center or mirror plane of symmetry between the oxygen atoms, the hydrogen atoms half-occupy the sites across the symmetry element with very short O...O distances.\textsuperscript{11} Shared protons are located between two O31 and two O33 in compound 4.4 which are the crystallographic center of \( i \) (Figure 4-9). Besides compound 4.4, shared proton is also observed in compound 4.5. The proton sits between two carboxylate but it is unlike that in 4.4 that was located in the symmetry center. The same kind of homonucleus strong hydrogen bonds is observed in 2-D layer of compound 4.6. Information of strong hydrogen bond in compounds 4.4, 4.5 and 4.6 are showed in Table 4-7. Mid-way protons may possibly exist in these strong hydrogen bonds but X-ray data cannot provide enough information to confirm the location of the proton. To analyses the protons position accurately, more powerful technique such as neutron diffraction would be required.

<table>
<thead>
<tr>
<th></th>
<th>D-H...A</th>
<th>D(D...A)/Å</th>
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<td>Compound 4.4</td>
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<td>O31-H31...O31#1</td>
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<td>2.476</td>
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<td>Compound 4.5</td>
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</tr>
<tr>
<td>O35-H35...O35#5</td>
<td>2.476</td>
<td>175.99</td>
<td></td>
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</table>

Symmetry transformations used to generate equivalent atoms:
\#1 -x+1, -y+2, -z+2 \hspace{1cm} \#2 -x+2, -y+2, -z+1 \hspace{1cm} \#3 x-1, y, z
\#4 -x+1, -y, -z+1 \hspace{1cm} \#5 -x+1, -y, -z

Table 4-7: Hydrogen bond data of the shared proton for compound 4.4 and 4.5

In most of the cases shown in this chapter, it is rather difficult to obtain pure phase of the salt which leads to difficulty in further study of the properties of the palmatinium salt. For example, in the reaction for compound 4.4, 4.4 coexist with (dl)THP-13BA-H co-crystals. Same situation happens in the case of compound 4.2. Other case like compound 4.1, it coexists with amorphous yellow powder that leads
to difficulty in further analysis. Seeking for optimum condition to obtain a pure phase is still in need for production of palmatine salt efficiently. In fact, from the single crystal X-ray diffraction result, there is no denying that hydrothermal and solvothermal method is a possible way for dehydrogenation of tetrahydropalmatine to palmatine or dihydropalmatine. It provides a simple and direct way of dehydrogenation of tetrahydropalmatine.

The chemistry behind the formation of palmatine salt is still not completely clear. Solvothermal crystallization with ethanol is used in most of the formation of palmatine salts. It may possibly due to the increased solubility of different phases in ethanol compared to water. In general the use of hydrothermal method under similar conditions of temperature and time produced just THP salts and co-crystals as in chapter 2 and 3, and no oxidized P⁺ products can be found. Whilst the higher solubility in ethanol probably promotes the rate of dehydrogenation reactions, this can occur in water. The product 4.6 was rather surprisingly produced from highly concentrated conditions and meso-tartaric acid.

The partial dehydrogenation that occurs in the synthesis of DHP also takes place in aqueous medium. DHP is formed over a long period 6days at 110°C. It can also be synthesized from (dl)THP by heating hydrothermally with 1ml of water at the higher temperature of 140°C for 1 day.

The conversion of THP molecules to DHP and P⁺ clearly is promoted by the high temperature and high pressure by hydrothermal synthesis. It is also probably accelerated by acid or base. The formation of DHP may be an intermediate step on the way to P⁺. This may be reasonable if its high temperature solubility in ethanol is high so that it is never isolated from these preparations. In acid conditions initial protonation of the THP nitrogen might allow for deprotonation at the adjacent ring
junction carbon C(13) with later reduction and proton elimination from the nitrogen. The mechanisms of such processes remain to be explored more fully but are clearly driven by the formation of thermodynamically more stable molecules, as well as elimination processes which decreases the free energy of the system through a higher entropy gain.

Whilst the preparation of the palmatine salts is of no particular value by this route since palmatine is directly available the decomposition of THP to DHP is of potential interest since the medicinal properties of this compound are yet to be evaluated.

4.4 Experimental

Preparation and Characterization

Materials: (dl)THP and (l)THP are obtained from Prof H. Xue of Biochemistry Department HKUST without further purification before use. All other acid are purchased from Acros Organics and Aldrich Chemical Company and used without further purification.

**Compound 4.1**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.015g (0.078mmol) of citric acid were mixed well in 1ml of ethanol. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals and yellow powder were filtered and dried in atmosphere.

**Compound 4.2**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.02g (0.15mmol) of salicylic acid were mixed well in 1ml of ethanol. Then, the mixture was transferred to a 23ml
Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals were filtered and dried in atmosphere.

**Compound 4.3**

0.025g (0.07mmol) of dl-tetrahydropalmatine and 0.028g (0.14mmol) of 4-bromobenzoic acid were mixed well in 1ml of ethanol. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow rod crystals were filtered and dried in atmosphere.

**Compound 4.4**

0.05g (0.14mmol) of dl-tetrahydropalmatine and 0.023g (0.14mmol) of isophthalic acid were mixed well in 1ml of ethanol. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for one and a half hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow rod crystals were filtered and dried in atmosphere.

**Compound 4.5**

0.05g (0.14mmol) of l-tetrahydropalmatine and 0.016g (0.14mmol) of succinic acid were mixed well in 1ml of ethanol. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for three hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals were filtered and dried in atmosphere.

**Compound 4.6**

0.05g (0.14mmol) of l-tetrahydropalmatine and 0.01g (0.07mmol) of meso-tartaric
acid were mixed well in 10 drops of de-ionized water. Then, the mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for six hours in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals were filtered and dried in atmosphere.

**Compound 4.7**

0.05g (0.14mmol) of l-tetrahydropalmatine were put in 1ml of de-ionized water. Then, the solution was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 110°C for six days in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Yellow bar crystals were filtered and dried in atmosphere. Elemental analysis: (calculated) %C=71.369, %H=6.560, %N=3.963 (Found) %C=69.8, %H=5.95, %N=3.96.

**4.5 References**

1. Li, M. *Zhongcaoyao* 1982, 13(9), 403.


### 4.6 Crystal data summary

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<th>Compound</th>
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<th>4.3</th>
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<td>thp36</td>
<td>thp41</td>
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<td>C_{27}H_{29}N_{11}</td>
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<tr>
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<td>Monoclinic</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P-1</td>
<td>P2(1)/c</td>
<td>P2(1)/n</td>
<td>P-1</td>
</tr>
<tr>
<td>a (Å)</td>
<td>7.7073(18)</td>
<td>15.292(4)</td>
<td>10.9350(7)</td>
<td>7.9402(10)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>13.013(3)</td>
<td>7.3795(19)</td>
<td>7.3607(5)</td>
<td>11.5504(14)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>13.181(3)</td>
<td>22.263(6)</td>
<td>33.607(2)</td>
<td>15.0106(18)</td>
</tr>
<tr>
<td>α</td>
<td>94.481(4)°</td>
<td>90°</td>
<td>90°</td>
<td>80.484(2)°</td>
</tr>
<tr>
<td>β</td>
<td>104.172(4)°</td>
<td>94.673(6)°</td>
<td>91.4850(10)°</td>
<td>87.368(2)°</td>
</tr>
<tr>
<td>γ</td>
<td>100.350(4)°</td>
<td>90°</td>
<td>90°</td>
<td>75.115(2)°</td>
</tr>
<tr>
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<td>1250.6(5), 2</td>
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<td>2708.6(3), 4</td>
<td>1312.1(3), 2</td>
</tr>
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<td>D_{ca}(g/cm³)</td>
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<td>1.394</td>
<td>1.487</td>
<td>1.401</td>
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<tr>
<td>F(000)</td>
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<td>1112</td>
<td>1256</td>
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<td>0.3 x 0.3 x 0.2</td>
<td>0.4 x 0.3 x 0.2</td>
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<td>12698</td>
<td>17007</td>
<td>10531</td>
</tr>
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<td>Independent reflections, R(int)</td>
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<td>4399,0.2657</td>
<td>6305,0.0233</td>
<td>4554,0.0216</td>
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<td>4399 / 0 / 344</td>
<td>6305 / 0 / 352</td>
<td>4554 / 0 / 361</td>
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<td>Goodness-of-fit on F2</td>
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<td>0.681</td>
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<td>1.041</td>
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<td>R1 [1&gt;2σ(I)], wR2</td>
<td>0.0467, 0.1090</td>
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<td>0.0333, 0.0806</td>
<td>0.0449, 0.1343</td>
</tr>
<tr>
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<td>0.2888, 0.1284</td>
<td>0.0447, 0.0861</td>
<td>0.0585, 0.1426</td>
</tr>
<tr>
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<td>0.380 and -0.282</td>
<td>0.277 and -0.288</td>
<td>0.769 and -0.269</td>
<td>0.327 and -0.255</td>
</tr>
<tr>
<td>Compound</td>
<td>4.5</td>
<td>4.6</td>
<td>4.7</td>
<td></td>
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<td>Identification Code</td>
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<td>thp6</td>
<td>thp44a</td>
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<td>C_{25} H_{39} N O_{11}</td>
<td>C_{21} H_{32} N O_{4}</td>
<td></td>
</tr>
<tr>
<td>Description</td>
<td>Yellow bar</td>
<td>Yellow bar</td>
<td>Yellow bar</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>100(2) K</td>
<td>100(2) K</td>
<td>100(2) K</td>
<td></td>
</tr>
<tr>
<td>Crystal system</td>
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<td>Triclinic</td>
<td>Monoclinic</td>
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</tr>
<tr>
<td>Space group</td>
<td>P2(1)/c</td>
<td>P-1</td>
<td>P2(1)/a</td>
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</tr>
<tr>
<td>a (Å)</td>
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<td>7.3590(10)</td>
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<tr>
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<td>11.3996(15)</td>
<td>7.1255(8)</td>
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</tr>
<tr>
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<td>22.819(3)</td>
<td>13.6241(18)</td>
<td>25.411(3)</td>
<td></td>
</tr>
<tr>
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<td>90°.</td>
<td>95.173(2)°.</td>
<td>90°.</td>
<td></td>
</tr>
<tr>
<td>β</td>
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<td>91.620(2)°.</td>
<td>97.405(2)°.</td>
<td></td>
</tr>
<tr>
<td>γ</td>
<td>90°.</td>
<td>98.545(3)°.</td>
<td>90°.</td>
<td></td>
</tr>
<tr>
<td>Volume(Å³), Z</td>
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<td>1124.6(3), 2</td>
<td>1762.0(3), 4</td>
<td></td>
</tr>
<tr>
<td>Dcal (g/cm³)</td>
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<td>1.534</td>
<td>1.332</td>
<td></td>
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<tr>
<td>F(000)</td>
<td>1000</td>
<td>548</td>
<td>752</td>
<td></td>
</tr>
<tr>
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<td>0.2 x 0.1 x 0.1</td>
<td>0.30 x 0.28 x 0.25</td>
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</tr>
<tr>
<td>Reflections collected</td>
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<td>8150</td>
<td>8031</td>
<td></td>
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<tr>
<td>Independent reflections, R(int)</td>
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<td>3887, 0.0325</td>
<td>2931, 0.0391</td>
<td></td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
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<td>3887 / 0 / 334</td>
<td>2931 / 0 / 235</td>
<td></td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>0.977</td>
<td>1.151</td>
<td>0.978</td>
<td></td>
</tr>
<tr>
<td>R1 [I&gt;2sigma(I)], wR2</td>
<td>0.0636, 0.1487</td>
<td>0.0797, 0.1684</td>
<td>0.0413, 0.0914</td>
<td></td>
</tr>
<tr>
<td>R1 (all data), wR2</td>
<td>0.1216, 0.1675</td>
<td>0.0990, 0.1761</td>
<td>0.0703, 0.1000</td>
<td></td>
</tr>
<tr>
<td>Largest diff. peak and hole (e Å⁻³)</td>
<td>0.598 and -0.260</td>
<td>0.394 and -0.405</td>
<td>0.201 and -0.212</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 5  Hydrothermal Crystallization of Organic Compounds: Polymorphism in Nitrophenylacetic acids

5.1 Introduction

5.2 Preparation and discussion of NPAA polymorphs

5.2.1 Preparation and results of polymorphs of 4-nitrophenylacetic acid

5.2.2 Discussion on 4-nitrophenylacetic acid polymorphs

5.2.3 Preparation and results of polymorphs of 2-nitrophenylacetic acid

5.2.4 Discussion on 2-nitrophenylacetic acid polymorphs

5.3 Solvothermal synthesis of 4-nitrophenylacetate Esters

5.4 Experimental

5.5 References

5.6 Crystal Data Summary
5.1 Introduction

Crystallization is the main method of purification in the pharmaceutical industry. However, this method may crystallize the solid in more than one packing arrangement. Polymorphism is defined as the phenomenon where the same chemical substance that can exist in different crystalline form. Polymorphs can differ in solubility, dissolution rate, stability, and mechanical properties and may exhibit different bioavailability. Crystallization, however, is a complex process, and the ability to control the crystallization of polymorphic systems may be limited. Many factors can influence the crystallization of polymorphs. Such factors are related to the thermodynamic stability or to the nucleation and growth kinetics of the polymorphic forms.

In this chapter, we are using hydrothermal method as a technique to explore the feasibility of searching the polymorphs of organic compounds. In screening the hydrothermal crystallization of approximately 200 organic compounds we found two compounds 4-Nitrophenylacetic acid and 2-Nitrophenyl acetic acid which gave new powder X-ray diffractograms after hydrothermal heating, yet which had not chemically decomposed. The new powder patterns implied that a change of polymorph had occurred in the two cases and we discuss the details of these findings in this chapter.
5.2 Preparation and discussion of NPAA polymorphs

5.2.1 Preparation of Polymorphs of 4-nitrophenylacetic acid

0.18g of 4-nitrophenylacetic acid (4npaa) and 2ml of water were heated at 80°C, 120°C and 160°C for 1 day. The product from at 80°C were rod-like crystals which were shown by powder XRD to have the same structure as the starting material. This is a published structure with refcode SEMTAF in the Cambridge Structural Database (CSD) and henceforth will be referred to by us as Form I. The product from 120°C showed a quite different powder diffractogram and microscopuc inspection indicated this was a pure new phase with plate-like crystals. This will be referred to as Form II. Both phases Form I and II can clearly be differentiated by the powder XRD patterns.(Figure 5-1). The crystal quality of Form II as prepared in this manner was not good enough for a single crystal X-ray analysis. However, after fine tuning of the crystallization conditions, acceptable single crystals of Form II were obtained from more concentrated solution with 0.18g of 4npaa in 1ml water at 120°C. The crystal structure was subsequently determined and found to be a new polymorph of 4npaa. Finally the powder diffractogram of the products from heating at 160°C for 1 day were found to contain peaks from both Form I & Form II of the 4npaa. This is initially surprising but can be rationalized in that a) the 80°C product probably resulted from incomplete dissolution of the starting material and that re-cooling leads to iso-structural precipitation and that Form I is preferred from water at temperatures below 80°C; b) the 120°C product indicates the Form II
material is the preferred form to crystallize out of hot water. This is likely to occur in the range 120–>80°C since otherwise Form I should have been produced; c) the mixed 160°C product could be formed using the assumption that at 160°C most of the 4npaa has dissolved. Over time any remaining Form I will dissolve and be replaced by Form II. Cooling this solution will then result in the formation of more Form II initially, however this is only crystallizing in a short time period upon cooling and that at some lower temperature in water the Form I then begins to crystallize. The isolated solids are then observed as a mixture of Form I and II.

![XRD graph](image_url)

*Figure 5-1: The powder XRD patterns of Form I and Form II 4npaa polymorphs were compared.*

By changing the temperature, new peaks at two-theta around 7° and 19° at 100°C indicated the appearance of the Form II (Figure 5-2). When the reaction was further heated at 180°C, majority of Form I appeared with minor phase of Form II. This is consistent with probable complete dissolution at high temperature, formation of Form II upon cooling to ca. 80°C and then the majority of the material crystallizing as Form I upon further cooling to ambient temperature.
At temperature higher than 180°C, only an oily solution can be obtained indicating the ultimate decomposition of 4npaa.

![XRD patterns of 4npaa crystallized under different temperatures](image)

*Figure 5-2: The powder XRD patterns of the 4npaa crystallized under different temperature.*

A further reaction has been performed under refluxing condition as a comparison with the product from hydrothermal reaction. After refluxing 0.18g 4npaa with 2ml water at 100°C for 1 day, the reaction mixture was filtered and dried. Powder XRD was used for characterization. Only Form I product was observed (Figure 5-3). This implies that the onset of the phase transformation of Form I to Form II as the solid in equilibrium with solution is in the range between 100°C and 120°C.
Figure 5-3: The powder XRD pattern shows that only Form I observed reflux at 100°C for 1 day.

In order to determine the stability of the two crystalline forms and determine whether they could interconvert in the solid state Variable Temperature Powder XRD (VT-PXRD) were measured from 35°C to 125°C at 5°C intervals for both Form I and Form II under atmospheric pressure. No phase change can be observed for Form I (Figure 5-4) and this implied that Form II cannot be obtained by heating the starting material alone for a short duration or without using water as solvent. However by contrast Form II converts to Form I beginning at 60°C and the change is effectively complete by 70°C. (Figure 5-5).
Figure 5-4: VT-PXRD pattern of Form I, showing no phase change.
Figure 5-5: VT-PXRD patterns of Form II showing phase change to Form I at 60°C.
Further experiments indicate that the phase change from Form I to Form II observed in the hydrothermal reaction after 1 day at 120°C can be finished in as little as 3 hours. This is traced by taking PXRD patterns of the product samples after heating successively for longer time periods of one hour. (Figure 5-6).

Figure 5-6: Powder XRD pattern shows that pure Form II 4nppaa can be obtained at 120°C for 3 hours.
5.2.2 Discussion on 4-nitrophenylacetic acid polymorphs

The single crystal X-ray analysis shows that Form I (published one) & the new Form II polymorph of 4npaa have different unit cell parameters. The unit cells of the two polymorphs are compared in Table 5-1:

<table>
<thead>
<tr>
<th>4npaa(Form I):</th>
<th>4npaa(Form II):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal system: orthorhombic</td>
<td>Crystal system: monoclinic</td>
</tr>
<tr>
<td>Space group: Pbca</td>
<td>Space group: P2₁/c</td>
</tr>
<tr>
<td>Unit Cell:</td>
<td>Unit Cell:</td>
</tr>
<tr>
<td>a = 15.096(1)Å  α = 90°</td>
<td>a = 6.1322(12)  α = 90°</td>
</tr>
<tr>
<td>b = 7.1500(5)Å  β = 90°</td>
<td>b = 5.0909(10)  β = 95.787(4)°</td>
</tr>
<tr>
<td>c = 15.923(2)Å  γ = 90°</td>
<td>c = 25.303(5)  γ = 90°</td>
</tr>
<tr>
<td>V = 1718.7(1)Å³</td>
<td>V = 785.9(3)Å³</td>
</tr>
</tbody>
</table>

Table 5-1: The unit cell parameters of the Form I and Form II polymorphs of 4npaa.

From the unit cell data, it is very clear that the two polymorphic compounds exist with quite different molecular volumes. In Form I, it is in orthorhombic crystal system with space group Pbca with Z = 8. The molecular volume is ca. 214.7 Å³. However, in Form II, it is in monoclinic crystal system with space group P2₁/c and Z = 4 with a smaller molecular volume of 196.5 Å³. In both cases there is only one molecule found in the asymmetric unit in those two polymorphs which shown in Figure 5-7.
Figure 5-7: Thermal ellipsoid diagram with 50% displacement possibility of 4npaa.

Interesting even though the unit cell dimensions and symmetries are dissimilar, the structural arrangement of the molecule is interacted by the same dimer synthon (Figure 5-8). The dimer is formed by moderate O3-H7...O4 hydrogen bond with bond distance 2.613 Å\(^4\) in Form I and O3-H3B...O4 bond distance of 2.675 Å in Form II.

Figure 5-8: The same dimer synthon is found in Form I and Form II polymorph.

The angle between the plane of the NO\(_2\) and the best plane of the benzene ring group is 7.25°\(^4\) for Form I; while in Form II, it has a smaller torsion angle with 4.62°. The dihedral angle between the plane of the carboxylic group and the plane of the benzene ring is 69.90°. Dihedral angle between the plane of the carboxylic group is 89.34° in Form II. The whole backbone of 4npaa is not a very rigid molecule that consists of carbons near the carboxylic acid that is the flexible part. The rotation of this flexible part would result in the formation of different dihedral angle towards the benzene ring. Different conformation of the molecules would occur and this causes polymorphism due to the different arrangement of the molecule in the packing.
structure. The difference in the torsional angles of Form I & form II polymorph is shown in Table 5-2.

<table>
<thead>
<tr>
<th>Torsional angle:</th>
<th>Form I:</th>
<th>Form II:</th>
</tr>
</thead>
<tbody>
<tr>
<td>O3-C8-C7-C3</td>
<td>171.5°</td>
<td>169.98°</td>
</tr>
<tr>
<td>O4-C8-C7-C3</td>
<td>10.2°</td>
<td>10.75°</td>
</tr>
<tr>
<td>C2-C3-C7-C8</td>
<td>105.9°</td>
<td>85.79°</td>
</tr>
<tr>
<td>C4-C3-C7-C8</td>
<td>74.6°</td>
<td>93.67°</td>
</tr>
</tbody>
</table>

*Table 5-2: Some of the torsional angles chosen from Form I and Form II in 4npaa.*

Based on the difference shown above, we would expect the Form I and Form II polymorphs are packed differently. The packing of the two polymorphs are shown in Figure 5-9.

![Figure 5-9: The packing of the two polymorphs Form I (left) and Form II (right).](image)

In the packing of Form I, a sheet of π-stacked hydrogen bonded dimers is observed. However, there are no π-π stacking interactions in the packing of Form II.

The melting point and the enthalpy change of the two polymorphs were further studied using Differential Scanning Calorimetry. (Figure 5-10). The Form I polymorph has melting point of 154°C and enthalpy value of 132.4 J/g. This is consistent with the literature showing that 4npaa has melting point of 154-155°C. However, in the DSC analysis of the Form II polymorph, two peaks are observed
instead of just one peak in Form I. At the transition temperature 67.9°C (peak 1) Form II undergoes an endothermic solid-solid transition to Form I. The heat absorbed is delta H\textsubscript{II\rightarrow I} for that transition. The thermodynamic transition point falls at a temperature below the melting point. This is the thermodynamic definition of an enantiotropic polymorphism system.\textsuperscript{5} By the Heat-of-transition rule, if an endothermic phase change is observed at a particular temperature, the transition point lies below that temperature, and the two polymorphs are enantiotropically related.\textsuperscript{6,7} This transition temperature is also corresponding to the result of VT-PXRD of Form II (Figure 5-5) which phase change to Form I is found around 60°C. Peak 2 indicates Form I melts at 155.1°C.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5-10.png}
\caption{The melting and enthalpy value of the polymorphs of 4nppa.}
\end{figure}
5.2.3 Preparation of Polymorphs of 2-nitrophenylacetic acid

\[
\begin{array}{c}
\text{NO}_2 \\
\text{COOH}
\end{array}
\]

Strangely of the more than 200 organic compounds studied by our group for high temperature polymorphs produced by hydrothermal crystallization, the second system to demonstrate this after 4npaa was its isomer 2npaa. This was studied in a similar fashion:

0.18g of 2-nitrophenylacetic acid (2npaa) and 3ml of water were heated at 100, 110, 120, 140 and 160°C for 1 day. These resulting product solids were filtered off after cooling back to ambient temperature and their powder XRD diffractograms measured. Crystals of the rod-like Form I starting material were found in the 100°C product, however a second phase type Form II began to appear in the PXRD of the 110°C sample, New peaks at two-theta around 15°, 17° and 25° at 80°C indicated the appearance of the Form II (Figure 5-12). This could be isolated in essentially phase-pure form in the material obtained from 120°C. The new phase crystal was determined by single crystal X-ray diffraction and indeed found to be a new polymorphic form of 2npaa. Pure Form II is difficult to obtain by using higher temperature at 120°C at this concentration (Figure 5-11), since Form I reappeared at 140°C and 160°C. For higher temperatures, only oily black liquid can be obtained indicating the compound had decomposed. The re-emergence of Form I at higher temperatures presumably can be explained in a manner to that of 4npaa. The Form II is completely dissolved and cooling from these higher temperatures passes through the ‘precipitation range’ of Form II maybe by super-saturation until Form I crystallizes at temperatures below 110°C.
Figure 5-11: The powder XRD pattern shows the difference of the two polymorphic form of 2-nitrophenylacetic acid.

Figure 5-12: The powder XRD patterns of the 2npaa crystallized under different temperature.

As a comparison with hydrothermal reaction, the reaction was performed under reflux. 0.18g of 2npaa with 3ml water was refluxed at 100°C for 1 day. The product was characterized by Powder XRD and was a mixture of Form I and II (Figure 5-13).
Figure 5-13: The powder XRD pattern shows the mixture of Form I and II in reflux.

VT-PXRD were carried out for both Form I and Form II in a similar way to those described above for the 4npaa system. Once again no phase change can be observed from Form I (Figure 5-14) and it implied that Form II cannot be obtained by heating the starting material alone within a short time or transformed in solid state. Once more Form II transforms in the solid state to Form I commencing at 60°C (Figure 5-15). In this case the phase change occurred at wide range of temperature Phase I and phase II were found between 60°C and 90°C and the transformation was completed at 95°C. It should be remembered that the protocols for measuring VT-PXRD was to incrementally heat every 5 minutes, so that the range of temperatures seen with Form I/II mixtures may simply indicate the relatively slow transformation kinetics in this case compared to 4npaa.
Figure 5-14: VT-PXRD pattern of Form I under atmospheric pressure at different temperatures.
Figure 5-15: VT-PXRD patterns of Form II with phase change to Form I from 60 °C.
Effect of Concentration:

By heating at 120°C for 1 day but varying the concentration of the reaction the effect on the isolated products was examined with the following interesting results. For solvent volumes of 2mL and below, (i.e. higher concentrations) Form I was isolated as the majority solid product with only a trace of Form II, whilst for solvent volumes of 3mL or more (lower concentrations) Form II was almost exclusively isolated as seen in the Powder XRD patterns (Figure 5-16). Visual improvement in the degree of crystallinity of Form II can be found with lower concentration reaction.

In general the use of higher concentrations has been found to assist crystallization of kinetic products whilst more dilute conditions should favor less soluble thermodynamic products. As applied to the two polymorphs of 2npaa this provides rather a dilemma in the explanation since the solid state results indicate that Form II is meta stable and transforms to Form I upon heating. However we also find that it is Form II which is produced in water at elevated temperatures and so under these conditions it appears this is indeed the thermodynamically preferred solid phase.

Figure 5-16: Powder XRD patterns of 2npaa from different conc. at 120°C for 1 day.
Effect of Time:

By varying the duration of the reaction, the reaction time for observing the occurrence of Form II can be shortened to 2 hours at 120°C (Figure 5-17). However, for longer reaction time to 2 or 3 days, only Form I product is obtained (Figure 5-18).

Figure 5-17: Powder XRD patterns of 2-nitrophenylacetic acid with reaction time of 1, 2 and 3 hours at 120°C.

Figure 5-18: Powder XRD patterns of 2npaa from 120°C for 1, 2 and 3 days.
5.2.4. Discussions on 2-nitrophenylacetic acid Polymorphs

The crystal structures of Form I and Form II of 2npaa have been confirmed by a single crystal X-ray analysis. Structure of Form I is found to be identical to that from a recent published result by C. Glidewell et al in April 2006. The unit cell parameters from I and II polymorphs of 2npaa are shown in Table 5-3.

<table>
<thead>
<tr>
<th>2npaa(Form I)</th>
<th>2npaa(Form II)</th>
</tr>
</thead>
<tbody>
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<td>Crystal system: monoclinic</td>
<td>Crystal system: monoclinic</td>
</tr>
<tr>
<td>Space group: P2_1/c</td>
<td>Space group: P2_1/n</td>
</tr>
<tr>
<td>Unit Cell:</td>
<td>Unit Cell:</td>
</tr>
<tr>
<td>a= 9.390(4)Å  α=90°</td>
<td>a= 9.7723(12)  α=90°</td>
</tr>
<tr>
<td>b= 9.513(4)Å  β=114.665(7)°</td>
<td>b= 7.0949(9)  β= 96.735(2)°</td>
</tr>
<tr>
<td>c= 10.025(4)Å  γ=90°</td>
<td>c= 11.7416(15)  γ=90°</td>
</tr>
<tr>
<td>V= 813.8(6)Å³</td>
<td>V= 808.47(18) Å³</td>
</tr>
</tbody>
</table>

*Table 5-3: The unit cell parameters of the Form I and Form II polymorphs of 2npaa.*

From the data, it is very clear that the two polymorphic compounds exist in two crystalline patterns for the same compound of 2npaa. Both 2npaa polymorphs are in the same monoclinic crystal system with the same space group of P2_1/c for Form I and Form II polymorphs. Z is 4 in both polymorphs. There is only one molecule found in the asymmetric unit for two polymorphs which shown in Figure 5-19.
Figure 5-19: Thermal ellipsoid diagram with 50% displacement possibility of 2npaa.

Although the unit cell dimensions are different, the molecules are linked up by moderate linear O3-H3B...O4 hydrogen bond with bond distance 2.687Å and 2.623Å corresponding to Form I and Form II. The hydrogen of the carboxylic group is hydrogen bonded to the carboxylic group in another asymmetric unit forming a dimer synthon which is also found in the 4npaa system (Figure 5-8).

The torsional angle between the benzene ring and the plane of the NO$_2$ group is 29.31° for Form I while 13.34° for Form II which is smaller than that in Form I. The angle between the plane of the carboxylic group and the plane of the benzene ring is 87.19° in Form I but 79.59° in Form II. Similar to 4npaa, the 2npaa is also a flexible molecule due to the rotation of the acetic acid group. It leads to the difference in the torsional angles in the polymorphs shown in Table 5-4.

<table>
<thead>
<tr>
<th>Torsional angle:</th>
<th>Form I:</th>
<th>Form II:</th>
</tr>
</thead>
<tbody>
<tr>
<td>O3-C8-C7-C5</td>
<td>159.06°</td>
<td>165.56°</td>
</tr>
<tr>
<td>O4-C8-C7-C5</td>
<td>22.31°</td>
<td>15.04°</td>
</tr>
<tr>
<td>C4-C3-C7-C8</td>
<td>99.01°</td>
<td>107.44°</td>
</tr>
<tr>
<td>C6-C3-C7-C8</td>
<td>83.04°</td>
<td>73.41°</td>
</tr>
</tbody>
</table>

*Table 5-4: Some of the torsional angles chose from Form I and Form II in 2npaa.*
Based on the differences shown above, we would expect that Form I and Form II polymorphs are packed differently. The packing of the two polymorphs are shown in Figure 5-20. In Form II, stacks of aromatic ring are line up side by side that pack differently from the packing of Form I that the dimer are linked up by C-H...O hydrogen bond. 

![Figure 5-20: The packing difference of the two polymorphs Form I(left) and Form II(right) along a-axis.](image)

The melting point and the enthalpy change of the two polymorphs are further studied (Figure 5-21). The DSC analysis of Form I and Form II polymorphs are very similar. The Form I polymorph has melting point of 139.2°C and enthalpy value of 133.3 J/g. In Form II, the melting point is 139.2°C which is the same as Form I and the enthalpy value is 116.8 J/g. The melting point of 2npaa report in the literature is range from 137°C to 140°C. Different from 4npaa, there is no solid-solid transition temperature can be observed in Form II of 2npaa in DSC. However, based on the VT-PXRD of 2npaa, phase transferred occurred from temperature 60°C to 90°C gradually in large range from Form II to Form I. And this may explain why there is no sharp temperature transition being observed in DSC of Form II.
Figure 5-21: The melting and enthalpy value of the polymorphs of 2npaa.

5.3 Solvothermal Synthesis of 4-Nitrophenylacetate Esters

In order to compare with our results of polymorph formation from hydrothermal crystallization, the use of solvothermal conditions was also applied to the system 4npaa in order to investigate whether Forms I, II or a new polymorph would be formed. Surprisingly new diffraction patterns were found using both methanol or ethanol as solvent, but the resulting single crystal X-ray structure determination revealed these to be the esters 5.1 (methyl 4-nitrophenylacetate) and 5.2 (ethyl 4-nitrophenylacetate) which can be formed in good yield and purity by solvothermal reaction of methanol and ethanol respectively with 4npaa at 120°C.

Compound 5.1 belongs to the orthorhombic crystal system and non-centrosymmetric space group Pna2₁. One molecule of methyl 4-nitrophenylacetate is found in the asymmetric unit (Figure 5-22).
Compound 5.2 is also orthorhombic but has a different non-centric space group Pca2\(_1\). The packing is more complex with two ethyl 4-nitrophenylacetate molecules found in the asymmetric unit. The structure of one of these molecules of ethyl 4-nitrophenylacetate is shown below.

In the packing of compound 5.1 (Figure 5-24) no hydrogen bond interaction can be observed since there are no H-bond donor groups. Nevertheless, \(\pi-\pi\) interaction is observed between the aromatic rings forming a 1-D stack.
Figure 5-24: Packing diagram of compound 5.1 along c-axis.

Within the crystal lattice of compound 5.2, there is no strong interaction can be found except the weak van der Waals interaction (Figure 5-25).

Figure 5-25: Packing diagram of compound 5.2 along a-axis.

Similar solvothermal reactions were undertaken for 2npaa, new peaks also can be found by powder XRD analyses. But no single crystal can be obtained for structural determination. Nmr of the resulting solids indicated these were also esterified products. Esterification is one of the most important reactions in organic synthesis.⁹
Simple condensation between a carboxylic acid and an alcohol is the most straightforward way to this end. Thus, numerous methods have been reported, yet they are not necessarily satisfactory from a practical point of view, since the reaction is an equilibrium. Problems such as low yield, not atom economy also appeared in our result. The use of solvothermal method can in certain cases give an effective preparative route, which is enhanced if the solubility of the ester product is low in the solvent (alcohol/water mixture) used in the reaction. Indeed the equilibrium can then be driven forward by precipitation of the ester solid. This depletes the solution concentration of ester leading to more formation by Le Chatelier’s Principle.

In esterification, base or acid catalysts which are inevitably employed in this reaction. Under such conditions, the tolerance of a wide spectrum of functional groups that is often required in modern synthetic chemistry is not always achievable. Solvothermal reaction may provides another possible way to solve this problem albeit that the acid and alcohol groups must withstand the high reaction temperatures.

5.4 Experimental:

Preparation and Characterization

Materials: 4-nitrophenylacetic acid and 2-nitrophenylacetic acid were purchased from Aldrich Chemical Company and used as received without further purification.

4npaa (FormII):

0.18g (1mmol) of 4npaa was put in 1ml of de-ionized water. The mixture was transferred to a 23ml Teflon cup and sealed in a steel autoclave and heated at 120°C for one day in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. Colourless plate crystals were filtered and
dried in atmosphere with 86.7% yield. Elemental analysis: (calculated) %C=53.044, %H=3.895, %N=7.732 (Found) %C=52.17, %H=3.895, %N=7.732.

2npaa (FormII):

0.18g (1mmol) of 2npaa was put in 1.5ml of de-ionized water. The mixture was transferred to a 23ml Telfon cup and sealed in a steel autoclave and heated at 120°C for one day in the oven. The autoclave was cooled to ambient temperature. The products were under suction filtration. colourless needle crystals were filtered and dried in atmosphere with 84.5% yield.

Compound 5.1

0.18g (1mmol) of 4npaa was put in 1ml of methanol. The mixture was transferred to a 23ml Telfon cup and sealed in a steel autoclave and heated at 120°C for one day in the oven. The autoclave was cooled to ambient temperature. The products solution was under recrystallization. Colourless rod crystals were obtained and dried in atmosphere.

Compound 5.2

0.18g (1mmol) of 4npaa was put in 1ml of ethanol. The mixture was transferred to a 23ml Telfon cup and sealed in a steel autoclave and heated at 120°C for one day in the oven. The autoclave was cooled to ambient temperature. The products solution was under recrystallization. Colourless bar crystals were obtained and dried in atmosphere.

5.5 References

1993.


### 5.6 Crystal Data Summary

<table>
<thead>
<tr>
<th>Compound</th>
<th>4npaa-Form II</th>
<th>2npaa-FormII</th>
<th>5.1</th>
<th>5.2</th>
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<tr>
<td>Identification code</td>
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<td>yee44</td>
<td>yee39</td>
<td>yee35</td>
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<td>Empirical formula</td>
<td>C₈H₁₀NO₄</td>
<td>C₈H₁₀NO₄</td>
<td>C₈H₇NO₄</td>
<td>C₁₀H₁₄NO₄</td>
</tr>
<tr>
<td>Description</td>
<td>Colourless plate</td>
<td>colourless needle</td>
<td>Colourless rod</td>
<td>Colourless bar</td>
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<tr>
<td>Temperature</td>
<td>100(2) K</td>
<td>100(2) K</td>
<td>100(2) K</td>
<td>100(2) K</td>
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<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
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<td>Orthorhombic</td>
<td>Orthorhombic</td>
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<tr>
<td>Space group</td>
<td>P2(1)/n</td>
<td>P2(1)/n</td>
<td>Pna2(1)</td>
<td>Pca2(1)</td>
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<tr>
<td>a (Å)</td>
<td>6.1322(12)</td>
<td>9.7723(12)</td>
<td>7.3955(6)</td>
<td>15.8802(12)</td>
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<tr>
<td>b (Å)</td>
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<td>7.0949(9)</td>
<td>15.1722(13)</td>
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<td>c (Å)</td>
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<td>11.7416(15)</td>
<td>7.9035(6)</td>
<td>24.834(2)</td>
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<td>α</td>
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<td>90'.</td>
<td>90'.</td>
<td>90'.</td>
</tr>
<tr>
<td>β</td>
<td>90'.</td>
<td>90'.</td>
<td>90'.</td>
<td>90'.</td>
</tr>
<tr>
<td>γ</td>
<td>90'.</td>
<td>90'.</td>
<td>90'.</td>
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<tr>
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<td>808.47(18), 4</td>
<td>886.82(12), 4</td>
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<td>Dcal(g/cm³)</td>
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<td>1.488</td>
<td>1.462</td>
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<td>F(000)</td>
<td>376</td>
<td>376</td>
<td>408</td>
<td>880</td>
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<td>Crystal size mm³</td>
<td>0.35 x 0.25 x 0.03</td>
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<td>1392, 0.0203</td>
<td>1994, 0.0191</td>
<td>2729, 0.0342</td>
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<td>1392 / 0 / 118</td>
<td>1994 / 1 / 127</td>
<td>2729 / 1 / 271</td>
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<tr>
<td>Goodness-of-fit on F²</td>
<td>0.989</td>
<td>1.016</td>
<td>1.024</td>
<td>1.039</td>
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<td>R1 [I&gt;2sigma(I)], wR2</td>
<td>0.0376, 0.0672</td>
<td>0.0302, 0.0742</td>
<td>0.0294, 0.0786</td>
<td>0.0691, 0.1384</td>
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<tr>
<td>R1 (all data), wR2</td>
<td>0.0819, 0.0781</td>
<td>0.0384, 0.0765</td>
<td>0.0317, 0.0804</td>
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<td>Largest diff. peak and hole (eÅ⁻³)</td>
<td>0.253 and -0.187</td>
<td>0.181 and -0.144</td>
<td>0.219 and -0.154</td>
<td>0.198 and -0.173</td>
</tr>
</tbody>
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