THE USE OF SOLVENT AFFECTED CIRCULAR DICHROISM IN THE ANALYSIS OF DANGEROUS DRUGS

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PREFACE

The objective of this study was to develop methods to more easily characterize dangerous drugs using circular dichroism.

I wish first to express my gratitude to Dr. Neil Purdie for his patience, understanding and invaluable guidance throughout this study, and for his assistance in the preparation of this manuscript. I also wish to thank all the members of my faculty committee, Drs. Rutledge, Mottola and Rockley, for their help and encouragement throughout my tenure at this university. Special thanks are due to Mr. Heinz Hall for the manufacture of the SACD cells, Mr. Ewald Friedle for the installation of the instrument, my fellow graduate students and to the faculty and staff of the Chemistry Department for their encouragement.

I would also like to express gratitude to my parents and Grandmother for their patience and unfailing encouragement throughout the long years.

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CHAPTER I

INTRODUCTION

Linked to the recent expansion of the pharamaceutical industry, and in turn, the now common availability of drugs, is the great increase in the use and abuse of drugs, both pharmaceutical and illicit. The case loads of health care facilities and forensic laboratories have correspondingly increased. In 1974 it was estimated that in the United States between 15 and 20 million urine samples were analyzed for drugs of abuse (1). The problem is not just an American one (2). In 1977 clinical laboratories in West Berlin recorded that of 3000 requests for analysis, fully 2400 were for drugs, and that a high percentage of these were for the narcotic analgesic, heroin (3) which is a synthetic derivative of morphine, a naturally occurring opiate.

This problem of drug useage and abuse which is manifested in the large clinical and forensic case loads mentioned above has led to an increased interest in the methods for the analytical determination of drugs. The methods now most commonly used for drug analysis include color tests (4,5), microcrystalline tests (1), and immunoassay techniques (6). Instrumental methods include such diverse methods as Nuclear Magnetic Resonance (NMR)

(4), mass spectrometry (7, 8, 9), its combinations with gas and liquid chromatography (9, 10), and spectrophotometric methods such as IR, fluorescence, and UV absorption (11, 12, 13, 14).

The reduction in the number of steps in multistep analyses, is of high priority in the development of alternative methods of drug analysis. Thus the development of a method of analysis which can both identify and quantitate an unknown druga in a less protracted way is of great interest.

Ultraviolet spectrophotometry has long been regarded as a very straight-forward experimental technique, and the quantitation of compounds using this method is well established. However, the absorption spectra of drugs obtained from UV, with few exceptions, consist of broad featureless bands. Thus, qualitative identification of a mixture of UV absorbers is hopeless without prior separation. However, UV spectrophotometry uses one property of most drugs that is to its advantage in drug analysis. This is that while most drugs absorb light in the UV region of the spectrum, many diluents commonly added to illlicit drugs do not absorb. A modification that is often used with UV spectrophotometry is the use of different solvents and different aqueous pH in order to impart a change in the spectrum of the solute, and hopefully to better distinguish between similar solutes (14). The change is often manifested by shifts in the wavelength of maximum absorption, as well as by the change in spectral shape.

A property exhibited by most illicitly used drugs that has been mostly ignored in the development of new or alternate analytical methods for drug analysis is that of optical activity. Measurement of the angle of rotation of plane polarized light at the sodium-D emission line has been a standard test in clinical and forensic laboratories. This test has proven valuable in the confirmation of compounds with only one physiologically active isomer, as in the case of cocaine, but is not considered sufficient to uniquely identify an anonymous compound.

If the rotation of plane polarized light is measured over a range of wavelengths, even more discriminantion among drugs can be made. This technique is called Optical Rotatory Dispersion (ORD). It was first reported by Biot in 1817 (15) and has since been extensively developed and used by Djerassi (16) since the 1950's. ORD spectra display anomalous dispersion (the Cotton Effect) over the wavelength region of molecular absorption of light. This phenomenon allows for greater distinction than UV spectrophotometry due to the fact that the spectra display negative, positive, and zero dispersions which provide more information than a like UV spectrum. However, these characteristic anomalous dispersions are always superimposed on the rising or falling "plane" dispersion, making it difficult to distinguish small changes of rotation in this region.

If the difference in the optical density of the left and right moving circularly polarized components of the

light is measured against wavelength after having passed through an optically active chromophore at the region of its absorption, the phenomenon of circular dichroism (CD) is observed. The circularly polarized light after absorption and rotation is no longer circular in polarization, but is elliptically polarized. The other method of measuring CD is to measure the ellipticity, which is defined as the tangent of the ratio of the long axis to the short axis of the transmitted light.

Like ORD, the measurement of CD requires that a molecule be chiral but unlike ORD, the molecule must also have a chromophore that absorbs light. The CD spectrum (Cotton Effect) is observed only over the wavelength range of chromophoric absorption. Most of the commonly abused drugs fulfill both criteria. CD also has a measurement advantage over ORD. For a 1° observation of optical rotation in ORD at 360 nm in a 1 dm cell, the instrument must measure the difference in indices of refraction of around 10^{-8}, while for CD, under similar circumstances, a difference of molar extinction coefficients of between 10^{-2} and 10^{-3} must be measured for a 1° change in ellipticity (17). As the CD spectra appear only over the absorption region, and the spectra are based on a flat baseline, unlike that of ORD, the detection of low probability or hiden transitions is facililtated. Circular dichroism, like ORD was first observed in the middle of the last century. Haedinger (18) detected the effect in solids in 1847, while Cotton (19, 20)

in 1895 reported its observation in solutions. Cotton measured the CD both by finding the differences in the absorption coefficients of the circularly polarized components, and by the measurement of ellipticity.

Although circular dichroism can offer distinction among different families of drugs, distinction among drugs that are structurally similar is often difficult. However, just as UV spectra can be altered by changing the solvent from polar to non-polar or from acidic to basic, in order to extract more informatmion from the sample, so CD spectra can also be affected by similar means.

Several methods of altering CD spectra have been explored. These methods generally use an asymmetric solvent or specific molecular interactions to either induce chirality into non-chiral molecules, or to affect already chiral molecules in order to alter their CD spectra. Spectra thus altered might provide even greater distinction for drug analysis than do the natural CD spectra. Tokura et al. (21, 22, 23), used a method they called induced circular dichroism (ICD). Their method was to use a chiral molecule to induce chirality into a second, non-chiral molecule by the close molecular association of these two co-solutes. Anisotropic solvents of liquid crystals have been used by several groups of investigators to induce or enhance chirality into solute molecules (24-34). Crone and Purdie (35) were able to alter the CD spectra of opiates by changing the solution pH. Two groups (36, 37) have used

cyclodextrin sugars to induce optical activity into barbiturates and naphthaleane derivatives respectively. These methods which have the property of changing or creating CD spectra, and causing them to become more complex and distinctive might be valuable analytical methods for the identification of dangerous drugs.

Statement of the Problem

This study was undertaken in order to determine if circular dichroism can be used as an analytical method, and then to develop and evaluate different methods of solvent affected circular dichroism (SACD), and their use as an analytical techniques for the purpose of drug identification and quantitation. The three methods of SACD are: 1) the use of liquid crystal circular dichroism (LCCD), 2) the use of solid state circular dichroism (SSCD), and 3) the use of a cyclodextrin sugar as a co-solute in water. The results from the above methods have been compared with the more normal CD spectra of these drugs which is due to their molecular chirality, in isotropic solvents. These techniques and their relative analytical capabilities are discussed in the following chapters.

CHAPTER II

BACKGROUND AND THEORY

The phenomenon of circular dichroism (CD) is a direct result of the differential absorption by a substance of left and right circularly polarized light. In order for the effect to be observed two criteria must be met: 1) the light that is used must be circularly polarized; 2) the compound of interest must (a) absorb light, and (b) exhibit optical activity. These criteria will be discussed in greater detail.

Circularly Polarized Light

Circularly polarized light is produced by the separation of plane polarized light into two components. Plane polarized light is first produced from an ordinary beam of unpolarized monochromatic light. Plane polarization is accomplished in most modern instruments with a birefringence polarizer such as the Rochon polarizer (38) which is made from crystals which exhibit a uniaxial optical axis. The polarizers are produced by cutting the crystals and crossing the uniaxial optical axes. The polarizer operates, as do others of its type (38), by resolving a non-polarized light beam into two plane polarized beams. One is called the

ordinary ray which is polarized orthogonally to the other, which is called the extraordinary ray (Fig. 1). The latter ray is typically masked out in normal applications. The polarizers used in most modern instruments are made of uniaxial crystals of potassium dihydrogen phosphate (KH_2PO_4) which have an optical transmission range to 185 nm (39) much farther into the ultraviolet region than other uniaxial crystals.

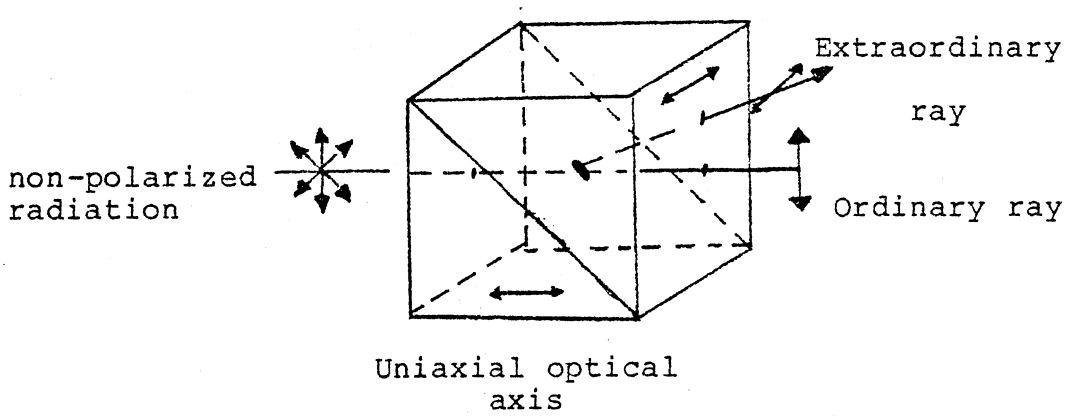


Figure 1. Rochon Type Birefringence Plane Polarizer Showing the Two Resolved Plane Polarized Beams of Light.

Plane polarized light, which consists of an electric wave orthogonal to a magnetic wave, is most commonly depicted as a sine wave (with the magnetic wave commonly

omitted for clarity) (Fig. 2 diagram 1).

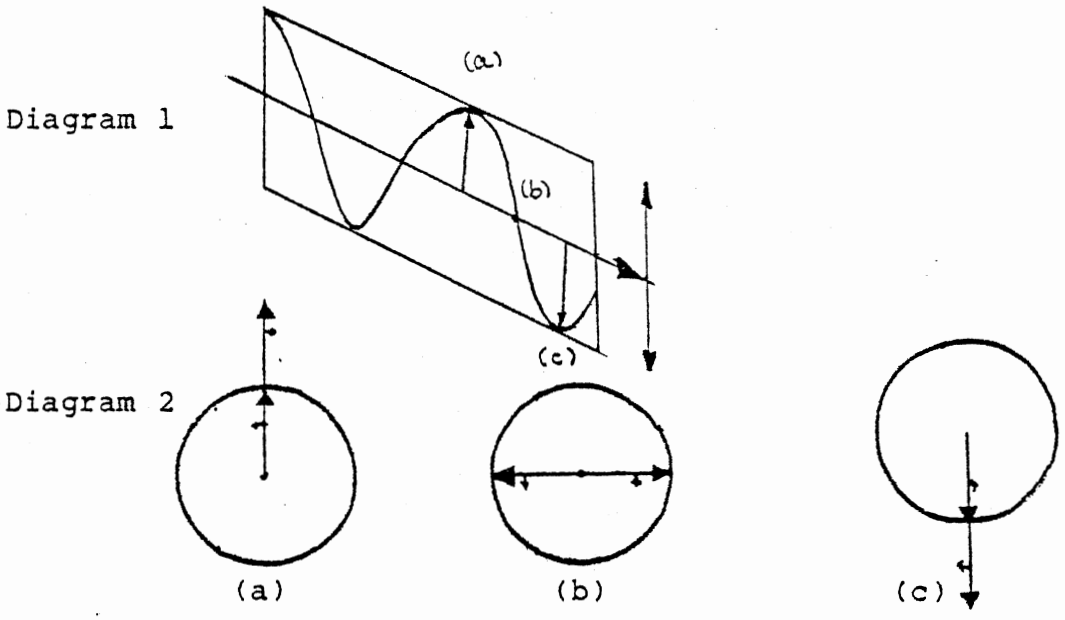


Figure 2. Plane Polarized Light Depicting the Summation of Circular Components Which Result in Plane Polarization. The Circular Vectors are Shown in Diagram 2 and the Results of Their Summation are Represented by the Sine Wave in Diagram 1.

If viewed along the axis of propagation the electric vector would be observed to rise to the maximum positive amplitude at (a), fall to zero at (b) with time, then decrease to the maximum negative amplitude at (c), a phenomenon which is repeated with time.

Plane polarized light can be thought of in terms of two, in phase, circularly polarized components of opposite

sense (Fig. 2 diagram 2) i.e. rotating clockwise and counter-clockwise respectively. The vector summations of the two in-phase components result in plane polarized light, where the amplitude of the wave is a function of the angle of the rotating vector components from the arbitrary vertical plane or axis of propagation.

If the material traversed by the plane polarized light is circularly birefringent, meaning that the indices of refraction for the circular components are unequal, the two circular components of light will propagate through the material at unequal speeds. This will cause the polarization plane of the transmitted light to be rotated by an angle from its original plane of polarization (Fig. 3). The angle is defined by the following relationship (40):

$$\alpha = \frac{\pi}{\lambda} (n_L - n_R) \qquad (2.1)$$

where is the wavelength of light in cm, and n_L and n_R are the indices of refraction for the left and right circularly polarized components respectively.

The variation of with wavelength is called optical rotatory dispersion (ORD). ORD curves are called "plane" if light absorption is absent and "anomalous" if measured over an absorpation band, in which case they are called Cotton effect curves, after their discoverer (16).

If the two circularly polarized components also undergo unequal absorption, that is, when the substance traversed by the light has unequal molar extinction coefficients for the

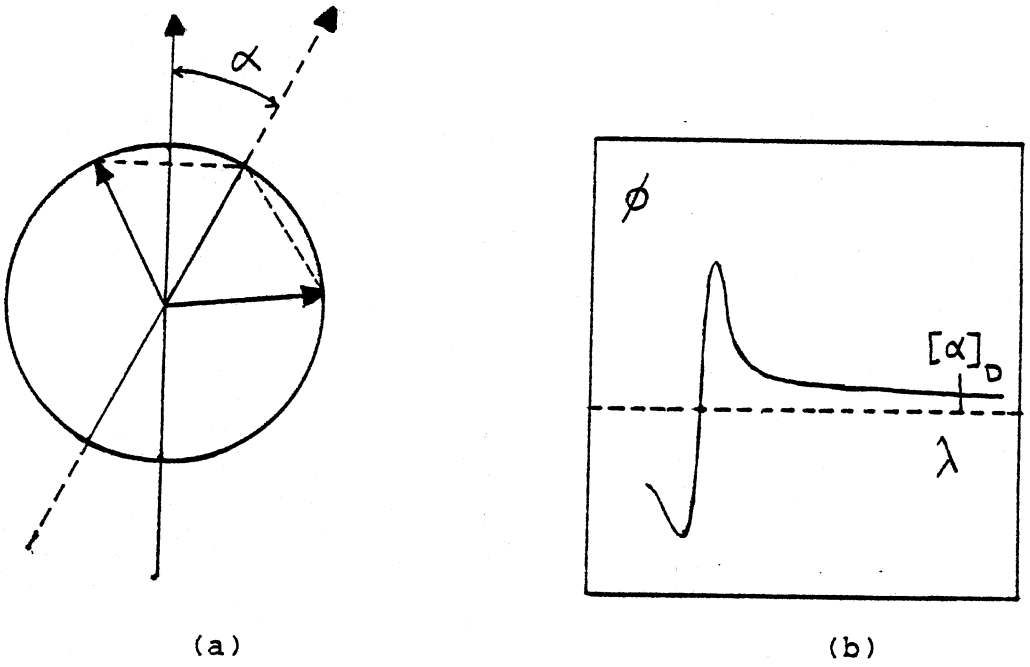


Figure 3. Diagram (a) Represents the Angle of Rotation Caused by the Unequal Speed of Propagation of the Circular Components of Light Through a Circularly Birefringent Sample. In this Case the Left Component has Traversed the Sample More Slowly than the Right Component. Diagram (b) Shows the Plane and Anomalous Regions of the ORD Curve.

left and right circularly polarized components ($\epsilon_L = \epsilon_R$), then the rotating vector components are absorbed or attenuated to different degrees. In this case both components continue to propagate in a circularly polarized manner along the optical axis, but the summations of the two components now trace out the circumference of an ellipse rather than a plane as a function of time (Fig. 4). The ellipse may also be rotated out of the original plane of polarization due to circular birefringence in the medium the light traverses. The experimental ellipticity of the elliptically polarized light is defined by the following relation (40):

$$\psi_{ob} = \tan^{-1} \frac{OB}{OA} \qquad (2.2)$$

where $_{ob}$ is the angle of ellipticity, and OB and OA are the long and short axes of the ellipse respectively. The variation of ellipticity with wavelength is called circular dichroism (CD). The molecular ellipticity, analogous to the molar extinction coefficient of absorption spectrophotometry, is defined by the following relation (40):

$$[\theta] = \frac{\psi_{ob}\ M.W.}{100\ b\ c} \simeq 3300(\epsilon_L - \epsilon_R) \qquad (2.3)$$

where b is the pathlength in dm, c the concentration of the absorbing medium in g/dL (41), M.W. the molecular weight of the absorbing species, and $_L$ and $_R$ the molar extinction coefficients of the solute for the left and right

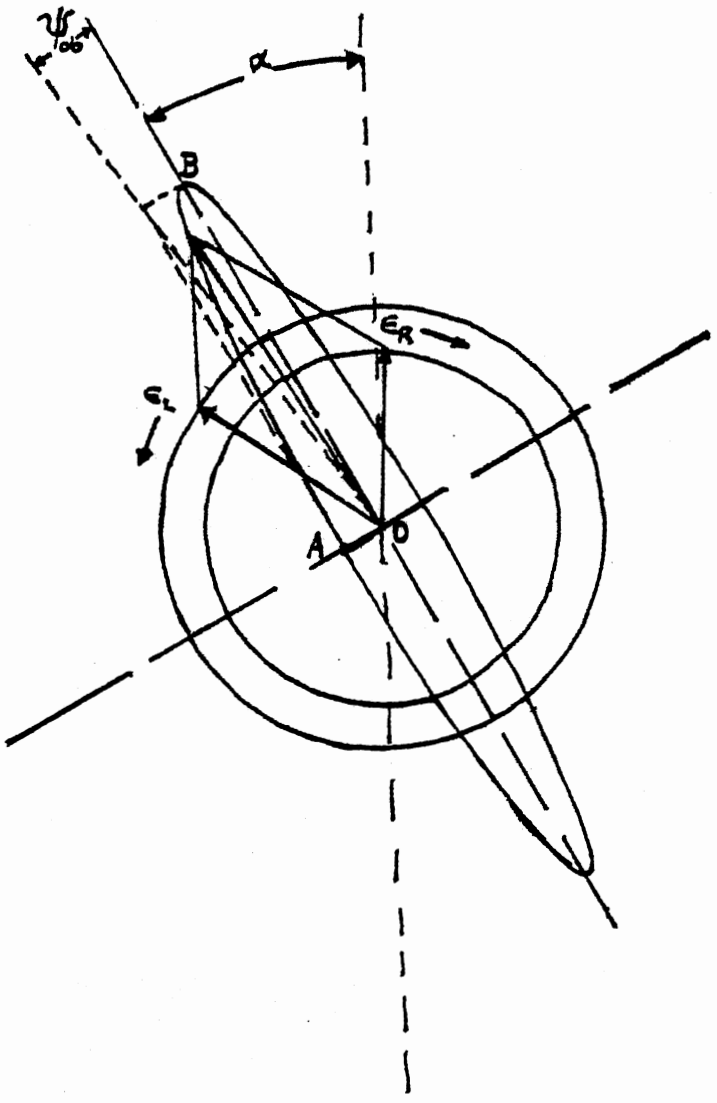


Figure 4. Elliptically Polarized Light, Where ψ_{ob} is the Observed Ellipticity, the Angle of Rotation and ϵ_L and ϵ_R are the Molar Extinction Coefficients of the Circularly Polarized Light Components

circularly polarized components respectively, of the incident light.

We have discarded this older, more cumbersome definition which is based on polarimetry, and have adopted a definition for [O] that is more in accordance with the Beer-Lambert law for absorption spectroscopy, vis.

$$[\theta] = \frac{\psi_{ob}}{b \cdot c} \simeq 3300\ (\epsilon_L - \epsilon_R) \qquad (2.4)$$

where the concentration M is in molarity and the pathlength b is in cm.

For CD, unlike ORD, there is no signal on the instrumental baseline outside the absorption regions of the chromophores, that is, outside the Cotton effect regions, because when $\epsilon_L = \epsilon_R$, $\psi = 0$ (eq. 2.4). CD bands therefore have a discreteness that is particularly useful in observing small overlapping or "hidden" polarized transitions, and thus become useful in the identification of molecules and in the assignment of structural conformations.

CD spectra are characterized by their ellipticity maxima, which may be either positive or negative (λ^+_{max}, λ^-_{max}), their minima (λ^+_{min}, λ^-_{min}) and the crossover points of zero ellipticity (λ_o).

Molecular Requirements for CD

The great majority of chemical compounds are not chiral, and so do not give rise to a CD spectrum. For those

molecules which do exhibit a CD spectrum in an isotropic (non-chiral) medium, the molecules must both absorb light and be optically active. Optical activity in molecules can be divided into three general groups (42):

1. Chromophores which are inherently disymmetric, such as twisted conjugated systems and aromatic molecules which have become non-planar due to steric strain.
2. Coupled oscillators formed by non-conjugated dienes.
3. Perturbed symmetrical chromophores such as those having asymmetric carbons close to the chromophores.

Compounds which are described by these groups which of themselves produce CD spectra are referred to as exhibiting intrinsic CD. Other types of asymmetry deal with macromolecular structure, such as helicity and have been thoroughly reviewed by Chan et al. (43).

Methods Used to Induce Chirality

Just as in absorption spectrophotometry, the CD spectra of any chiral compound is affected by the solvent in which it is dissolved. Solvation effects, pH, and solvent polarity all affect the CD spectra by the same well known mechanisms that affect absorption spectra (43). For those compounds which do exhibit CD spectra in isotropic media, but which are structurally very similar the CD spectra are

found to be quite similar. Just as differences in the CD spectrum of achiral compounds are often observed by changing solvents the usefulness of inducing even more asymmetry into these molecules can be of analytical importance in distinguishing among structurally similar molecules. CD induction can be theoretically accomplished by exposing any molecule which has a chromophore to an asymmetric environment (25). This is referred to as the extrinsic CD. The most popular method of inducing an asymmetric environment thus far has been the use of magnetic circular dichroism (MCD) (44), where a magnetic field, which is helically symmetric and thus anisotropic, is applied along the direction of propagation of the circularly polarized light.

The other major method of producing the asymmetric environment is by the close molecular association of the molecule under study with either solvent or co-colute molecules which are of themselves asymmetric or which aggregate in an asymmetric manner. Modification of this latter method have been used in this study.

Magnetic Circular Dichroism

In the case of a naturally non-optically active molecule, absorptions of the left and right circularly polarized components are equal and no net CD is observed. If the substance is placed in the magnetic field described above, the medium will exhibit a selective response to each of the circularly polarized components of the incident light (45).

The effect, often called the Faraday effect (45) is the summation of three terms, the A, B, and C terms, related to three different electronic situations in a given electronic transition. These arise when either the ground and/or the excited electronic states, originally degenerate, are split by the magnetic field. If these different electronic transitions are stiumlated by the opposite circularly polarized components and occur at different frequencies, the effect is known as the Zeeman effect, which gives rise to an S shaped curve and is described by the A term. The C term arises when the electronic populations of the sublevels in the ground state of an ion or paramagnetic molecule are perturbed by the magnetic field. The rare B term is due to non-degenerate ground state terms which introduce different attenuations of the left and right circularly polarized transitions (45). "A" terms in MCD have no isotropic CD counterpart and thus offer completely new information. This is also the case with non-chiral molecules from which MCD spectra may be produced.

Solvent-Solute and CO-Solute Interactions which Induce Chiral Behavior

Liquid Crystals. More than five percent of all organic compounds exhibit liquid crystalline behavior. This behavior is characterized by one or more thermodynamically metastable intermediate phases (or mesophases) which appear to possess a combination of the physical properties of the

solid and liquid phases. Compounds with these properties have the ability to flow like a liquid but also have a substantial long-range order, like that of a solid (46). The long range structure of the liquid crystals is sensitive to temperature and magnetic and electric fields. Some liquid crystals are known to exhibit unprecedented optical rotatory power. All of these properties make these compounds valuable for many applications.

When liquid crystals are used as solvents, one finds that dissolved organic molecules assume the same long-range order and are thus affected by the same forces that affect liquid crystals, which in turn will affect the CD spectra of the solutes. Therefore, liquid crystals, especially those exhibiting optical rotation, are valuable solvents for the study of the optical dichroic properties of the solute.

Liquid crystals can be divided into two major categories (46), both of which have been used in CD studies. These are the lyotropic and thermotropic liquid crystal systems.

Lyotropic liquid crystals are composed of molecules which have both polar and non-polar portions, and are wetted by either a polar or a non-polar solvent. Examples of this type of liquid crystal are the micellar detergent molecules in water. Lyotropic liquid crystals have been used in conjunction with CD (47), as a chirality inducing solvent. However, the macromolecular structure of the liquid crystal is known to change dramatically with the ratio of the liquid

crystal to the wetting solvent (46). If the aggregation of the liquid crystal is changed, the very specific liquid crystal-solute interactions are altered, and the CD spectra of the solutes are also changed. As yet, this method has not been extensively used.

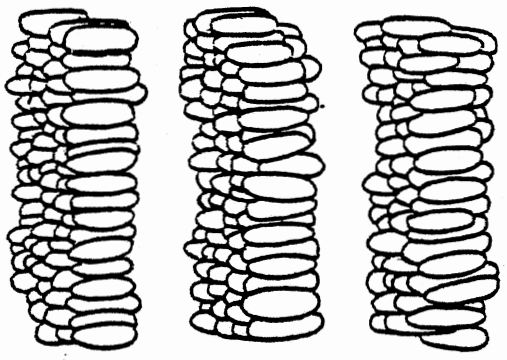
Thermotropic liquid crystals are compounds which exhibit liquid crystalline properties at the solid to liquid phase transition. The liquid crystal characteristics change in response to temperature changes (46). The one or more mesophases (48) found between the solid and isotropic liquid phases are the source of the directionally dependent or anisotropic properties of the liquid crystals.

There are three general categories of thermotropic mesophases, all of which, if exhibited by a compound, are thermodynamically reversible, and all of which can exhibit supercooling effects. The most highly ordered is the smectic mesophase which exhibits order in two dimensions (Fig. 5a). The nematic mesophase (Fig. 5b) exhibits order in only one direction, and is generally more fluid than the smectic mesophase. A more complex mesophase, sometimes considered as a special case of the nematic class, is the cholesteric or twisted nematic mesophase, and is associated with colorful iridescence. This mesophase, which occurs only for optically active compounds (48), can be considered as a nematic mesophase with a helical macrostructure. This macrostructure can be thought of as a series of nematic mesophases stacked one on another, with a small regular

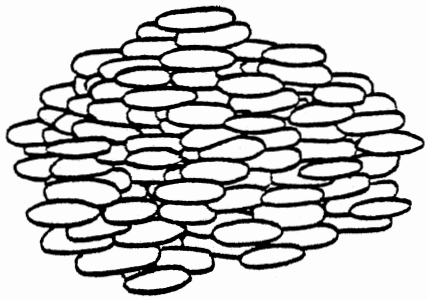
angle of rotation around the axis of the stack (Fig. 5c). The stack rotation is characterized by the sense, which can be either to the left or right, and by the pitch (P) which is defined as twice the distance between layers in which the molecular long axis point in the same direction. The pitch (P) is a temperature dependant experimental parameter, and can be determined by absorption spectroscopy from the wavelength of maximum reflectivity (λ_{max}) and by the relation (30):

$$\lambda_{max} = nP \qquad (2.5)$$

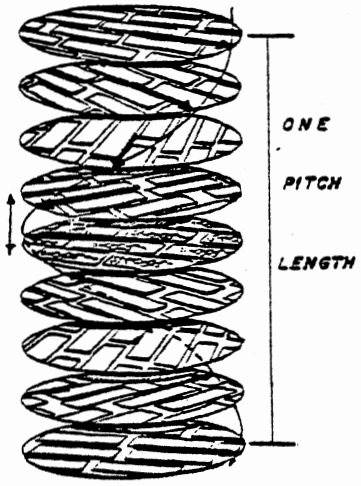
where n is the index of refraction of the medium. The cholesteric mesophase is highly anisotropic and can both induce optical activity into non-chiral molecules, and either enhance or affect the CD signal from chiral molecules which have been dispersed in it. The sign and magnitude of the induced or enhanced CD is dependent on the sense of the cholesteric helix on its pitch (32), on the temperature (31), and on the concentration of chiral or non-planar molecules dispersed within it. The induced CD is a direct result of the fact that the solute molecules are forced into the helicoidal macrostructure of the cholesteric mesophase (25, 32), and/or that the solute molecules are exposed to this anisotropic arrangement of solvent molecules (27), both of which contribute to the induced or enhanced CD signal. The solute-solvent aggregation has been found to be predominantly composed of London dispersion forces, with smaller



(a) Smectic



(b) Nematic



(c) Cholesteric

Figure 5. Thermotropic Liquid Crystal Mesophases. Arrows Represent Order Directors; P is the Pitch Length of the Cholesteric Stack.

contributions from repulsive interactions (32).

Although several types of cholesteric liquid crystals exist, the most useful as a solvent for observations in the UV spectral range are the cholesteryl derivatives, as they possess only a single olefinic chromophore. Cholesteryl derivatives of different sense are available. The sense of cholesteryl cholride is left-handed, while the cholesteryl esters of fatty acids, such as nonanoic and lauric acids are raight-handed (25). Control of the sense and pitch of the liquid crystal solvent can be accomplished by varying the cholride to ester composition, as liquid crystals of opposite sense are mixed together. Control of the pitch and helicoidal sense and the extension of the useful mesophasic temperature range result from mixing liquid crystals of opposite sense. The helicoidal structure can be totally cancelled at a specific chloride to ester composition to leave a nematic or "totally compensated" cholesteric mixture (32). The mixture used in this study was a partially compensated right-handed solvent of cholesteryl chloride and cholesteryl nonanoate.

The observed induced or enhanced ellipticity (also called chirality amplification) increases up to the point of total compensation or nematicity. At this point, the induced or enhanced CD disappears. This extrinsic CD is also dependent on the texture of the liquid crystal with the Grandjean texture being responsible for the effect (27). Over moderate concentration ranges, the extrinsic

ellipticity is linear with concentration (27). Liquid crystal circular dichroism (LCCD) is so named to include both the enhancement and inducement effects. It was done in this study by allowing a chiral or non-chiral molecule to be perturbed by the cholesteric macrostructure, as described above. Conversely, much the same effect can be achieved by perturbing a nematic liquid crystal with a chiral solute molecule (34).

The Use of a KBr Matrix for Solid State Circular Dichroism (SSCD)

The use of solids and solid matrices containing microcrystalline solutes in IR spectrophotometry is an accepted and well used procedure. The method has been used in the UV range in conjunction with circular dichroism for single crystals, where a crystal of the sample of interest is grown, and cut along a crystal plane (49, 50, 51, 52). CD has been used with mulls (53), heat streached films (54), and with solutes suspended in pressed KBr pellets (55, 56, 57). MCD (49, 50) has also been used to investigate solid samples.

Several authors (56, 57) have observed that the SSCD of various compounds was different from the corresponding solution CD and one author (51) found that the spectra of compounds in KBr differed from those in the single crystal

CD measurements. Bosnich and Harrowfield (56) believed that the KBr matrix method was the closest approximation to non-molecular interaction model which they described as a "dilute hypothetical gas". They stated that the observed optical activity in the microcrystalline or KBr supported samples was due only to the molecular configuration of the solute. Kuroda and Saito (51) compared the single crystal CD with that of the KBr matrix CD and of their compounds in an isotropic liquid solvent. They concluded that the solvent effects seen in the solution CD were not present in the SSCD spectra. Imanishi and Insemura (57) and later Ito and Takagi (55) found some anisotropic effects in their KBr spectra. This they believed was due to shear and creep of the KBr sample during the compression stage, and was a result of their sample preparation procedures. It is apparent that if the sample is well mixed and powdered, that the action of compression or sintering of the KBr matrix is isotropic, and the CD spectra that are observed are due only to the chirality of the molecule (53).

Bosmich and Harrowfield (56) found that a linear relationship exists between the observed ellipticity and the concentration of the solute. This relationship however, exists only below concentrations of 5%. At 15% the signal becomes roughly constant with concentration, and spectra are only identifiable up to twice that concentration.

The Use of Cyclodextrin Sugar as a Method for Affecting Circular Dichroism Spectra

A third method of producing an asymmetric environment for both chiral and non-chiral molecules is accomplished via the use of achiral co-solutes which form a molecular complex with the compound under study. Cyclodextrin sugars behave as chiral co-solutes by forming inclusion complexes with many compounds, including pharmaceuticals (59, 60, 61, 62, 63).

Cyclodextrins are formed from the hydrolytic breakdown of starch by the action of the enzyme amylase of B. macerans (59). The cyclodextrins thus produced are homogeneous cyclic molecules composed of α-(1, 4) linked D-glucopyranose units which are arranged in the shape of a torus, one side of which is narrower at the opening than the other (64) (Fig. 6).

The cyclodextrins, also known as Schardinger dextrins (58) after their discoverer, are designated by Greek letters that denote the number of glucopyranose units in the torus. The smallest of these is alpha-cyclodextrin or cyclohexaamylose, which has six units. Each succeeding letter denotes one more glucopyranose unit. Only the first three, alpha-, beta-, and gamma-, are common, and all are optically active. All are UV transparent, and thus display no CD spectra making them ideal extrinsic CD co-solutes.

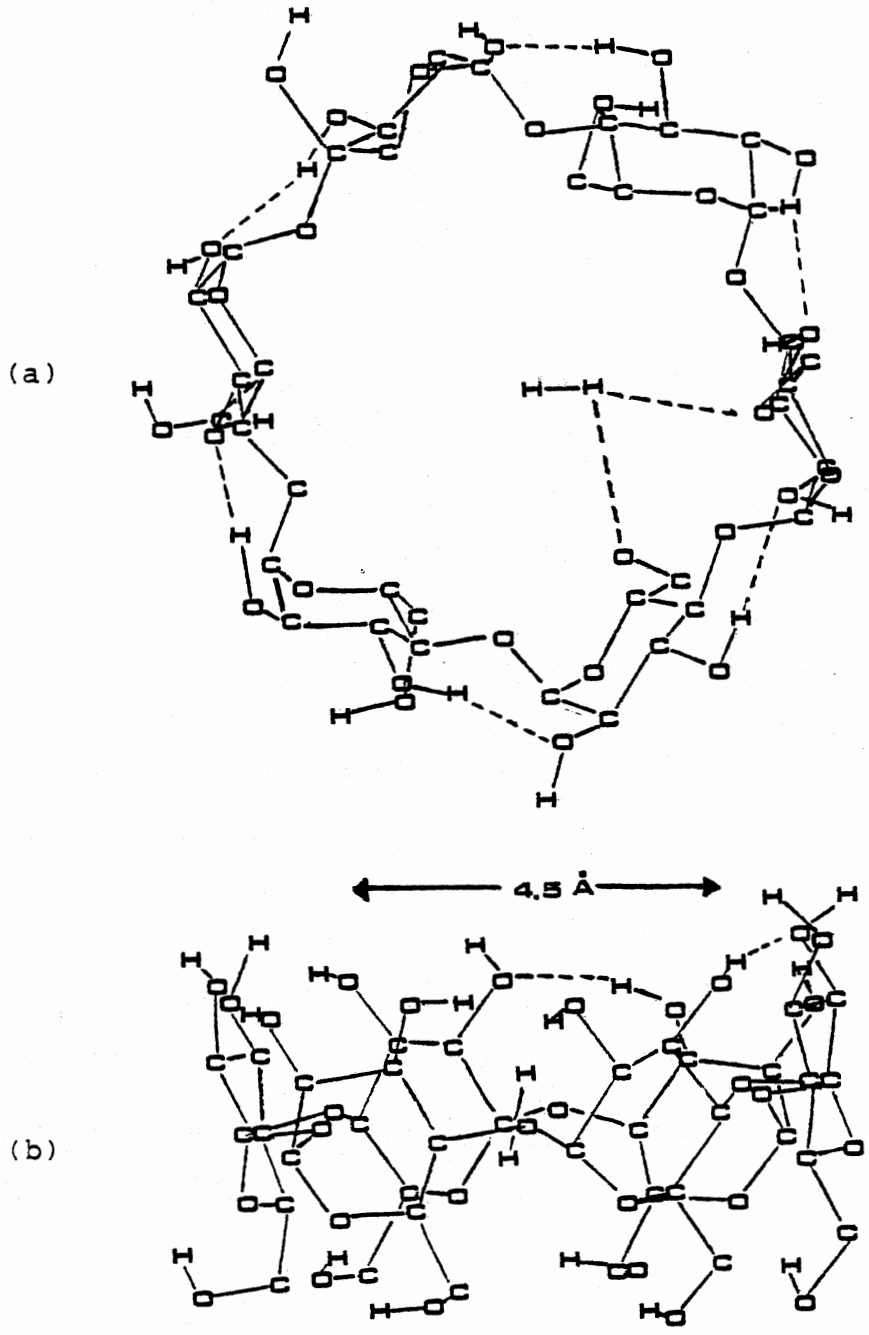


Figure 6. Alpha-cyclodextrin with Included Hydrogen Gas as Shown From One End (a) and From the Side (b). (65)

The cyclodextrins have different cavity sizes that enable complexation with different compounds. Alpha-cyclodextrin, with a cavity diameter of about 4.5 Å is known to form inclusion complexes with halogen gasses and small mono- and di-substituted benzenes. Beta-cyclodextrin, with a cavity diameter of 7.0 Å is large enough to accommodate almost any organic molecule, including those with benzene chromophores (59), and even barbiturate molecules (65). Gamma-cyclodextrin, which has not been extensively studied, can form inclusion complexes with large organic molecules (59), of a size similar to that of 1-anilinonapthalene sulfonate (64). All of the cyclodextrins are about 6.7-7 Å thick (64).

The cyclodextrin molecule is arranged such that all of the primary hydroxyl groups are found on the narrow opening side of the torus, while the secondary hydroxyl groups are found on the wider side.

The interior of the torus is comprised of aliphatic C-H groups and is relatively non-polar, which explains the ability to form inclusion complexes with aromatic molecules. The entire molecule is maintained in a rigid configuration by intramolecular hydrogen bonding. In water the torus is somewhat flattened, with one of the glucopyranose units turned out of the plane, apparently to facilitate hydrogen bonding with included water molecules (65). This is especially true in the case of alpha-cyclodextrin.

Through the cyclodextrins are optically active, they

lack a chromophore and so exhibit no CD in the visible or UV regions. But the cyclodextrins have been found to induce CD spectra into flat achiral aromatic molecules such as the naphthalene derivatives (37). This effect may be due to either or both the asymmetric environment, or to the distortion of the included molecules due to the very close association with the cyclic sugar, with the latter effect more likely.

The forces that cause the inclusion activity are not completely understood, however it is believed that hydrogen bonding, Van der Waals and London dispersion forces are important (67) especially because of the small size of the cavity. There is also the effect of favorable enthalpy which is associated wither with the release of "high-energy" water or by the release of strain energy by the torus as it moves from the flattened disposition to a more rounded shape upon inclusion (68).

The inclusion complex, which usually has a stoichiometry of 1:1 is not static. The substrate molecules exchange rapidly. The included molecules are also believed to have different orientations relative to the torus axis (69) but the orientations of each included substrate are specific enough to produce constant and individual spectral.

Inclusion rate constants have been measured by various methods (70, 36) and equilibrium constants for various included drugs were found by Thakker et al. (36).

These last three methods have been used in this study

in order to find a usable and facile method for the analysis of drugs using the enhanced spectra induced by solvent affected circular dichroism.

CHAPTER III

EXPERIMENTAL

Instrumental

All of the circular dichroism measurements were made on a CARY model 61 spectropolarimeter. This instrument was modified for difference measurements with the addition of the CARY model 6101 Difference CD Accessory which allows the instrument to be used as a single pass instrument for studies with non-chiral solvents, or as a double beam instrument for use with solvents which exhibit CD. The instrument has a wavelength range of from 185 to 800 nm, and sensitivity from 0.01 to 2.00 degrees full range over a 25.4 cm (10 in) chart. This theoretically allows a deflection of as small as 10^{-4} ° to 2° ellipticity to be observed.

The instrument is purged with nitrogen, which is boiled from the liquid state from a Dewar in order to prevent the production of ozone from oxygen by the instruments 450 W high pressure xenon arc lamp.

Wavelength calibration is performed by measuring the emission lines of a standard fluorescent lamp. The ellipticity scale is calibrated using a 0.1% by weight solution of D-10 camphorsulfonic acid in water. Absorption by this solution is associated with an electronic transition which

produces an ellipticity of +0.31° at 290 nm using a 1 cm path length cell.

Ultraviolet spectral measurements were preferentially performed on a CARY model 14 double beam recording spectrophotometer. This instrument has a UV range from 185 to 400 nm, the most common region for drug absorbances.

All the sample cells used, excepting the thermostatted LCCD cells, could be transferred directly from the CARY-61 to the CARY-14 for analysis of the same samples.

Temperature control for the CD measurements was provided by a Haake constant temperature controller Model F which utilizes circulating water from an internal resevoir for the heat exchange fluid. Temperatures from -1° to 105° can be maintained to $\pm$ 0.02°C. A five minute thermal equilibrium time was observed after each temperature change in all temperature controlled experiments.

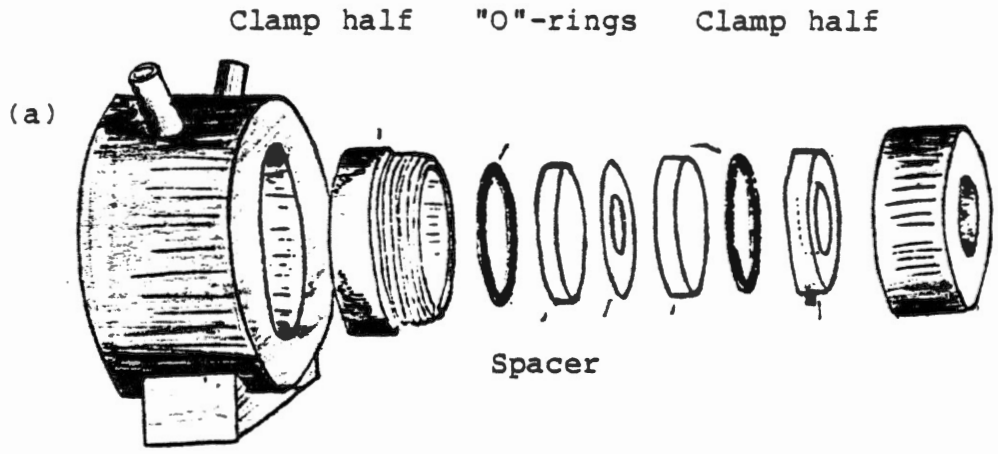
Two balances were used for mass measurements. A Sartorius balance model 2403 was used for routine mass measurements. For smaller masses, a CHAN model 2000 RG electrobalance was used. This instrument was calibrated within a wider range to measure between 0 and 10 $\pm$ 0.05 mg. Each balance was calibrated before each use.

The cells for both the LCCD and SSCD experiments were of local design and manufacture. The LCCD cells consisted of two 1/8in thick optical quartz flats (Oriel) held apart at a constant sample thickness of 12.7 μm by a self-adhesive spacer material (Connecticut Hard Rubber Co.). The

two quartz flats were then pressed together by two threaded circular clamp halves (Fig. 7a). A torque spacer was included to prevent the flats from rotating, and rubber "O"-rings were used to distribute the pressure evenly over the flats. Thermostatted water jackets were added to provide the necessary temperature control. The entire apparatus was mounted on a carriage compatable with the CARY-6101 CD Difference Accessory.

The SSCD cells were actually portions of a KBr press of local manufacture which had been designed for ultra-thin (ca. 0.2 mm) KBr windows. It consisted of two cylindrical anvils which fitted into either side of a hollow cylinder or compression collar which also served as the sample holder (Fig. 7b). The KBr was pressed in the device and, after the removal of the two anvils, the collar containing the window was simply placed in the CARY-6101 sample carriage for analysis.

Standard cylindrical 1 cm quartz cuvettes were used for the isotropic CD experiments and all the cyclodextrin-complexation experiments. All of the equilibrium experiments were conducted in standard 1 cm rectangular cuvettes which were thermostatted with water circulating from the temperature controller through a CARY accessory water jacketed cell holder.



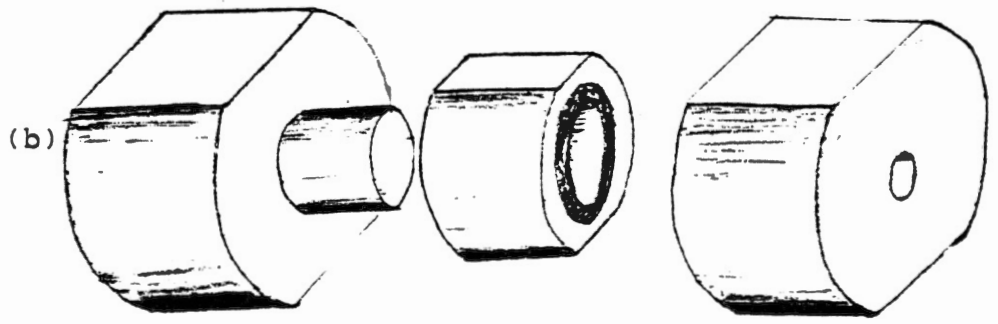


Figure 7. (a) Liquid crystal Circular Dichroism Cell.
(b) Solid State Circular Dichroism Cell.

Chemicals

The isotropic solvents were distilled water and spectral grade organic reagents which were used without further purification.

The LCCD solvent consisted of a partially compensated, twisted nemataic (cholesteric), thermotropic liquid crystalline mixture of cholesteryl chloride and cholesteryl nonanoate in 1:1.63 molar ratio (30, 31, 32) respectively (Aldrich Chemical Co.). The cholesteryl nonanoate was used without further purification. The cholesteryl chloride (98%) was repeatedly recrystallized from dry isohexane or from a solution of chloroform in methanol.

The SSCD solvent used was unrefined IR grade KBr (Sigma). The KBr and the solute material were dried at 140°C for at lest 48 hr prior to their use in the SSCD experiment.

The cyclodextrin experiments were conducted using an aqueous solution composed of two co-solutes, the drug to be studied, and the cyclodextrin sugar. The cyclodextrin sugar that was used was beta-cyclodextrin (Aldrich) which was used without further purification. This cyclic sugar has a low solubility in water (1.85 g/100 mL) (64), which limited the maximum useful concentration of the other co-solute.

Most of the solutes used in these experiments were pure drug standards, and were used without purification. Drugs on the free base form were used exclusively in the LCCD

experiments due to solubility problems with the liquid crystals. Both the free base and acid salt forms of the drugs were used in the other experiments. The drug samples were provided by Mallinckrodt Inc., Research Triangle Institute (RTI), the National Institute of Drug Abuse (NIDA) and U.S. Pharmacopeia. All illicit samples were used either as they were obtained or were extracted by a simple procedure. These samples were donated by the Oklahoma State Bureau of Investigation (O.S.B.I.), and by the Oklahoma City Police Department.

Experimental Procedures

Isotropic CD Measurements

For the qualitative experiments, the solute drugs were weighed into volumetric flasks which were filled to the mark with an isotropic solvent. The samples were then shaken and analyzed without further manipulation. If sample dilution was necessary, this was done by transfer with a 2 mL Gilmont "Micrometer" syringe model 7844 into a second volumetric flask, which was then filled to the mark.

Samples were run against a solvent CD baseline which was repeated several times during the experiment to insure against baseline drift. In many of the isotropic experiments, the samples were prepared such that a less sensitive full range, or sensitivity could be used. This procedure minimized baseline drift problems.

For quantitative isotropic experiments, ellipticity vs. concentration curves were prepared inaccordance with the Beer-Lambert law. The ellipticity vs. concentration curve standards were prepared by weighing a sample of the compound to be studied into a volumetric flask which was then filled to the mark with the appropriate solvent. This sample was diluted by transfer into other volumetric flasks with the Gilmont volumetric syringe to produce the concentration series. These samples were then run on the same day in order to minimize any day to day ellipticity changes in the instrument.

For the quantitative isotropic experiments, a constant wavelength ellipticity determination was developed. Measurements were made at the wavelength of maximum ellipticity for each particular compound. Then samples of the compound under investigation were alternataely run against the solvent baseline. Each measurement was continued for at least three minutes. This method acts as a signal averaging procedure which effectively decreases uncertainty in these measurements. So, very dilute samples can be run at high sensitivity, high period, and very low speed, effectively increasing the sensitivity of the instrument over that of a standard CD spectrum measurement. The ellipticity is measured as the distance between the centers of the sample and baseline traces.

LCCD Experiments

The liquid crystal components were weighed into separate volumetric flasks to prepare concentrated solutions in chloroform. Predetermined aliquots were then transferred by Gilmont syringe into a third volumetric flask so that the mole ratio of 1:1.63 cholesteryl chloride to cholesteryl nonanoate could be obtained. A concentrated solution of the drug was prepared in a like manner. A predetermined aliquot of the drug solution was transferred by a second Gilmont syringe into the volumetric flask containing the liquid crystal aliquots to make a mole ratio of solute to solvent equal to 1:150 (ca. 1-4 mgm/0.24 g solvent). The resultant cholesteric mesophase of this solvent mixture was stable over several hours, and had an overall right-handed helical sense. The optimum solvent to solute ratio was arrived at experimentally and represented a ration at which all the solute drugs were soluble in the liquid crystal solvent. After the removal of the choloroform by slow evaporation over low heat and with vigorous stirring, 20 μL of the now isotropic liquid crystal mixture ($T \simeq 70°C$) was transferred with a preheated 100 μL graduated Lambda pipet to the center of the preheated quartz flat. The second heated quartz flat was quickly and firmly pressed onto the spacer-equipped flat to obtain a uniform thickness of liquid crystal over the cell pathlength. The torque spacer was inserted and the cell clamps were tightened to a pre-set pressure. The entire cell was inserted into the cell

holder-water jacket assembly which was already thermostatted to 42.5 $\pm$ 0.02°C. The second, or reference cell was assembled simultaneously, and in the same manner, excluding the addition of the drug. Each cell was visually inspected for a homogeneous red reflection from the liquid crystal solvent. If any flaws were observed the cell was remade. Between assemblies the quartz flats were washed with chloroform and stored dry.

SSCD Spectra

The KBr solvent and all samples were dried at 140°C for at least 48 hours prior to the analysis. The samples were weighed to an approximate ratio of 100:1 KBr to drug, with a total sample weight of 80 mg. The sample was intimately ground together in a fine agate mortar. Drugs in the salt and free base form could be used with equal ease. These samples were placed in a desiccator until ready to be pressed. The KBr was pressed for about 2 min at 1000 lb/in^2, and then at 2000 lb/in^2 (7×10^5 and $1.\times 10^6$ kg/m^2 respectively). This produced, in most cases, very thin (ca. 0.2 mm) and transparent sample windows. The sample windows were inspected for clarity. Those which were opaque were rejected and a new window was prepared. The samples in their protective collars were again stored in the desiccator until the spectra could be run. After a second inspection for clarity, the samples were transferred to the CARY 6101 CD sample carraige and the CD spectra recorded.

These experiments were run in the normal or single beam mode, using a KBr spectrum as the baseline.

Spectra Characterization

For the most part, CD spectra were unavailable for the drugs of interest. So before, proceeding to the technically more difficult LCCD and SSCD methods the CD spectra for the drugs dissolved in isotropic solvents, eg. water and alcohols were obtained. The characteristic features were collected and compared and molar ellipticity data were obtained from calibration curves. A wavelength range of 220-350 nm was normally used. In a similar manner, the characteristic LCCD spectra for all the available opiates, and SSCD spectra for seven opiates were taken and their characteristic features were recorded.

In conjunction with the LCCD characterization experiments, several experiments into the effect of the liquid crystalline solvent upon the CD spectra were undertaken. The effect of changing the temperature of the liquid crystalline solvent on the LCCD ellipticity was studied using codeine at the standard 150:1 solvent molecular ratio. After the cell was prepared in the standard way and the LCCD spectrum obtained, the temperature of the LCCD cell was progressively raised in about five degree intervals up to the isotropic temperature. An LCCD spectrum was run at each temperature after an appropriate time was allowed for thermal equilibration.

In order to determine the effect of temperature on this mixture, an analogous experiment was carried out in which the solvent was reversed to be 1.63:1 (ca. 0.6365 g cholesteryl chloride, 0.3647 g cholesteryl nonanoate) in favor of cholesteryl chloride over cholesteryl nonanoate. Codeine (ca. 0.0038 g) was again used as the solute, but at a solvent to solute molecular ratio of 89:1.

Another anticipated difficulty in quantitating LCCD spectra is the effect on the CD spectrum of the solvent pitch, and the change in pitch with solution concentration. Accordingly several experiments were performed which measured the change in the cholesteric pitch at varying concentrations of codeine. The samples of varying codeine concentration were made up separately and the pitch was determined by observing the wavelength of maximum relfection, λ_{max}, on the CARY-14 spectrophotometer. λ_{max} which is found for this solvent mixture in the near IR, is related to the helical pitch by equation (2.5).

Quantitative Experiments

The use of circular dichroism as a quantitative tool has apparently not been extensively exploited, especially in the case of drugs. Therefore, several experiments into the quantitative nature of both isotropic and solvent affected circular dichroism were performed.

Since CD is a modification of absorption spectrophotometry, the test for quantitative behavior is to establish if

the dependence of ellipticity with concentration is linear in a manner analogous to the Beer-Lambert law. Non-linear behavior could be indicative of specific complexation reactions.

The quantitative isotropic experiment involved the identification and quantitation of cocaine in spectral grade methanol. For this experiment, an ellipticity curve was prepared using an L-cocaine hydrochloride (Mallinckrodt) standard, which was used without further purification. The unknown samples that were used were unseparated and unpurified illicit cocaine samples. Five of these samples were donated by the O.S.B.I., and six others were from a case under investigation by the Oklahoma City Police Department and in the care of an officer of that origanization. The illicit samples, which ranged in weight from 1-10 mg, were dissolved in 25 mL of methanol or water, and the ellipticities were measured at 245 nm in the manner described above. The confiscated samples also contained some solid diluents, probably starches or sugars, which were both UV and CD transparent and could not be identified.

The same type of experiment was performed for codeine and morphine using LCCD. The samples were prepared using the standard method described above, and run on the CARY-6101 as usual. The object of the experiment was to determine if the ellipticity vs. concentration curve was linear. A non-linear but reproducible calibration curve could still be used for quantitative determination. The results were

different for codeine and morphine.

An investigation into the nature of a complexation equilibrium was possible in the reaction between beta-cyclodextrin and two drugs, L-cocaine which is chiral, and phencyclidine hydrochloride (PCP) which is non-chiral and therefore CD transparent. The ellipticity of the negative CD band maximizing at 245 nm of L-cocaine changes sign to positive in the presence of beta-cyclodextrin, while complexed PCP exhibits an induced or extrinsic CD spectrum in the presence of the cyclic sugar.

CHAPTER IV

EXPERIMENTAL RESULTS

Spectra Characterization

Isotropic CD Spectra

The aromatic ring is the principal absorption chromophore for all the drugs studied and electronic transitions in the UV region are the mechanisms for most of the observed CD bands present in all the following spectra. The prominent transitions for the aromatic ring in the 220-350 nm wavelength range are the ^1L_a (or $^1B_{1u}$) transition occurring at 220-260 nm and the ^1L_b (or $^1B_{1u}$) transition at 270-290 nm. These appear in the UV absorption spectra of the opiates (and in the spectra of most drugs) as a shoulder and a peak respectively. The UV spectra of the opiates are indistinguishable one from another. Not surprisingly, the isotropic CD spectra of most of the studied drugs are superficially similar in appearance, with the Cotton bands for the ^1L_a transition generally negative, and the Cotton bands for the ^1L_b transition either positive or negative (75).

The CD spectra for the available opiates were measured in ethanol to provide a basis for comparison with those from

solvent affected circular dichroism. CD maxima, crossover points, and molar ellipticity data for these compounds are presented in Table I, and the spectra in Figure 8.

The CD bands which are most prominent in the opiates may be divided into three basic types: 1) 220-260 nm, usually positive band associated with the ^1L_a aromatic transition, 2) 270-290 nm, generally negative band in the region of the ^1L_b aromatic transition, 3) 300-305 nm negative CD band which can be associated with the π^*n transition, which is observed only with those opiates with a ketonic functional group.

From their intrinsic CD spectra in isotropic media, the opiates may be separated into two groups: 1) morphine, nalorphine, the acetyl morphines, codeine and dihydrocodeine (the morphine-like compounds) exhibit almost identical CD spectra, although heroin and dihydrocodeine have smaller positive band amplitudes. 2) compounds with an extra chromophore such as thebaine (Fig. 9), which has a conjugated C-ring, and hydrocodone and naloxone which have ketonic functional groups in addition to the aromatic chromophore. This extra chromophoric group is easily distinguishable in the CD spectra. However, the CD spectra of naloxone and hydrocodone are almost identical to each other, with only minor differences at 240 and 280 nm.

Two non-opiate drugs were run in both methanol and distilled water. From the comparison of these spectra (Fig. 10), the analytical advantages of the CD spectrum over the

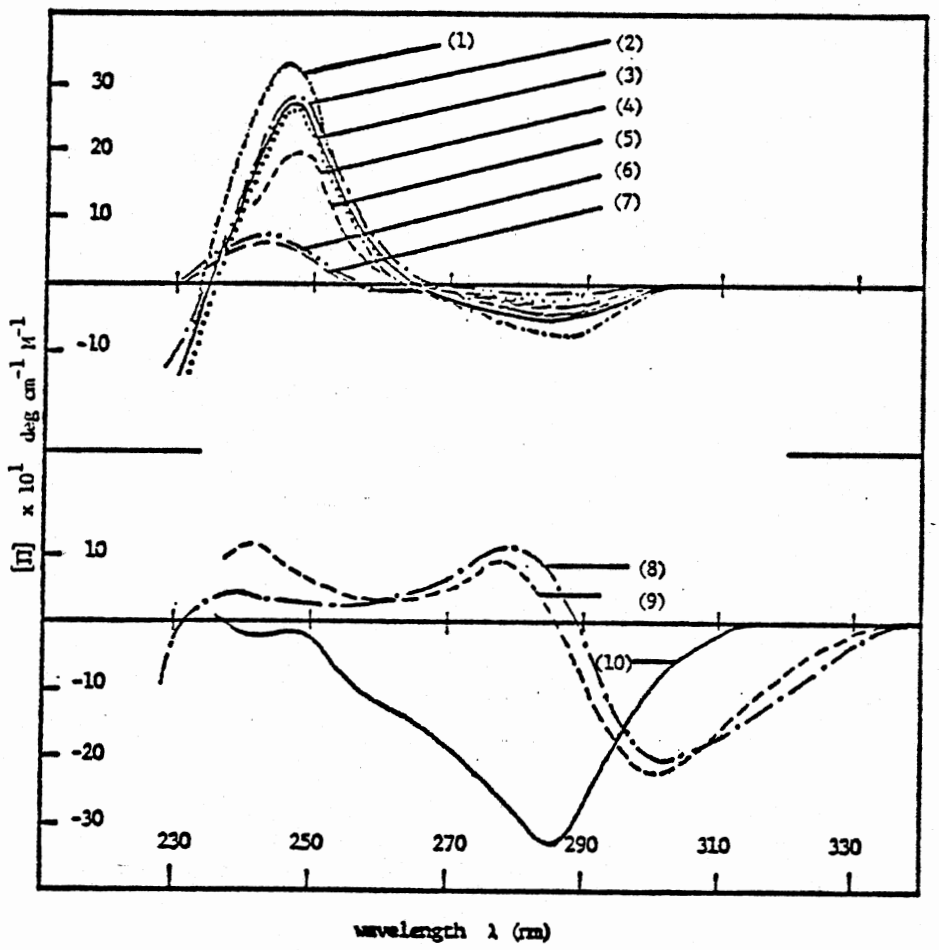


Figure 8. Ethanol CD Spectra of (1) Nalorphine, (2) Codeine, (3) Morphine, (4) 6-MAM, (5) 3-MAM, (6) Dihydrocodeine, (7) Heroin, (8) Naloxone, (9) Hydrocodone, and (10) Thebaine

TABLE I

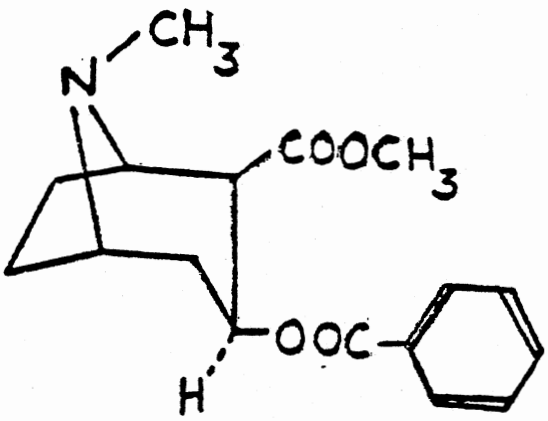
ETHANOL CD DATA

Compound	λmax (nm)	[θ]†	[π]††	λ_o (nm)	λmax (nm)	[θ]	[π]	λmax (nm)	[θ]	[π]
Morphine	288	- 85.5	- 67.5	265	246	411.3	348.3	-	-	-
Nalorphine	288	- 94.8	- 74.8	263	246	406.1	320.6	-	-	-
6-MAM	288	- 56.3	- 44.4	265	247	322.3	254.4	-	-	-
3-MAM	287	- 46.0	- 36.3	266	246	225.1	117.7	-	-	-
Heroin	286	- 18.4	- 14.6	250	232	78.3	61.8	-	-	-
Codeine	286	- 98.3	- 77.6	265	247	493.3	389.2	-	-	-
Dihydrocodeine	287	- 63.4	- 50.0	257	243	121.0	95.5	-	-	-
Thebaine	286	- 541	- 427	-	245*	-150.0	-118.4	-	-	-
Naloxone	303	-260.7	-205.8	289	280	145.5	114.8	240	54.6	43.1
Hydrocodone	301	-358.5	- 283	286	278	144	113	241	181	142

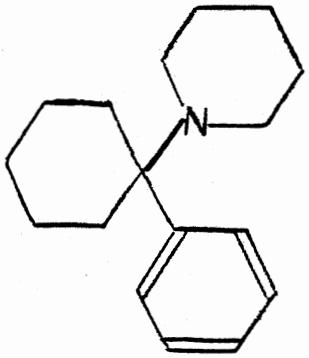
†Units are: deg $cm^{-1}M^{-1}$

††Units are: deg $cm^{-1}m^{-1}$

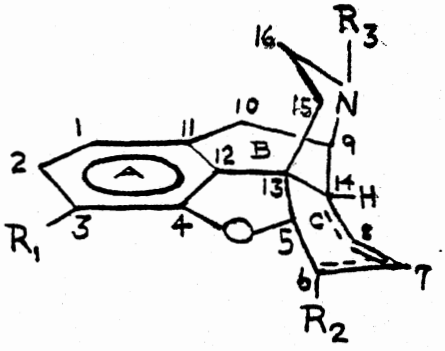
*shoulder



cocaine



phencyclidine (PCP)



morphine alkaloids:
Derivatives used have the following R subsitiuents: morphine, R_1=R_2=OH, 7-8 double bond; codeine, R_1=OCH_3, R_2= OH, 7-8 double bond; dihydroodeine, R_1=OCH_3, R_2=OH, 7-8 saturated; 3-MAM, R_1=$OCOCH_3$, R_2= OH, 7-8 double bond; 6-MAM, R_1=OH, R_2=$OCOCH_3$, 7-8 double bond; heroin, R_1=R_2=$OCOCH_3$, 7-8 double bond; hydrocodone, R_1=OCH_3 R_2=O, saturated C ring; thebaine, R_1= R_2=OCH_3, 6-7 and 8-17 double bonds; R_3 for all above, =CH_3; naloxone, R_1=OCH_3, R_2=O, saturated C ring; nalorphine, R_1=R_3=OH, 7-8 double bond; R_3 for these last = $CH_2CH=CH_2$

Figure 9. Structures of Cocaine, PCP, and the Morphine-Like Opiates

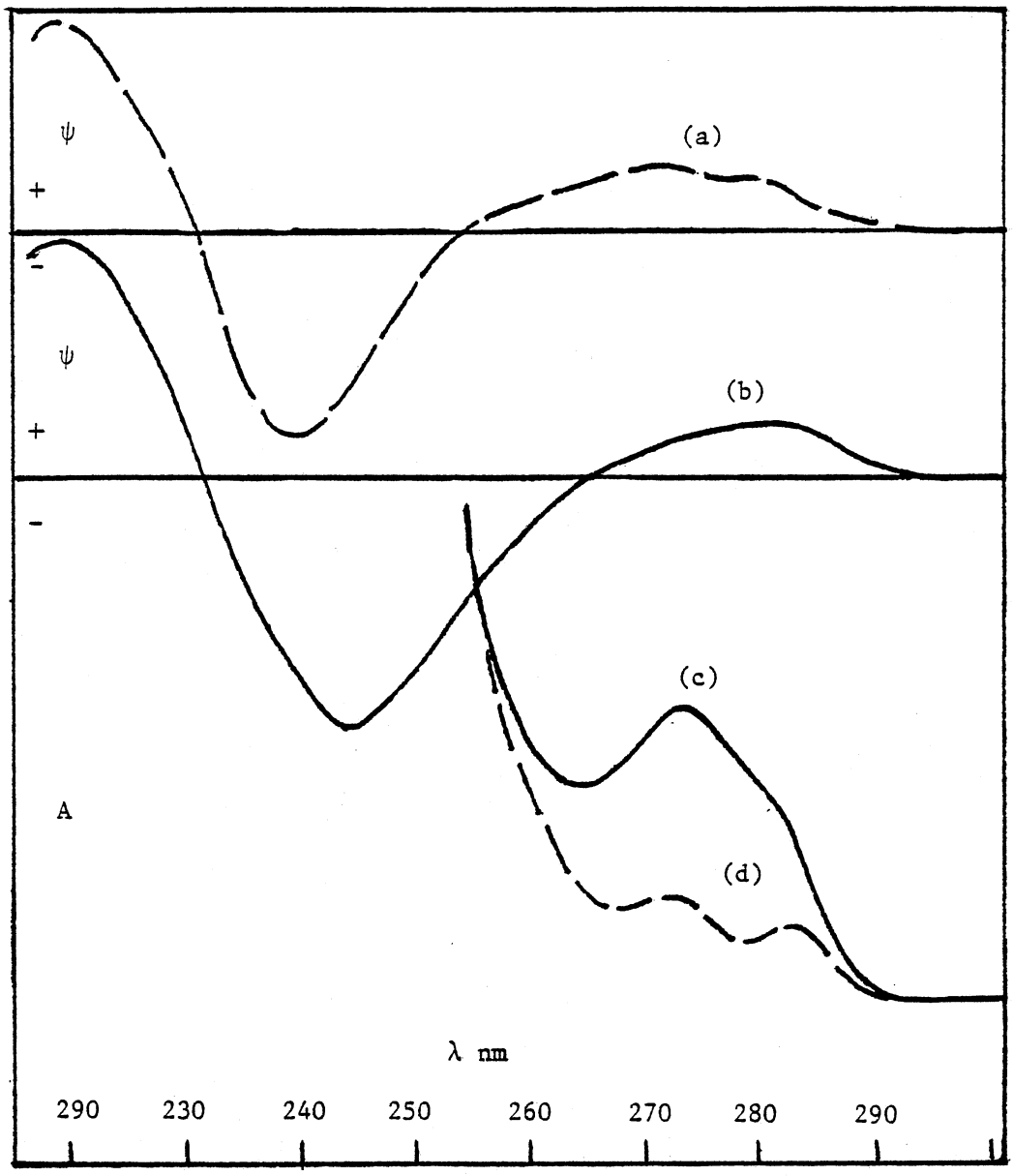


Figure 10. Spectra of L-Cocaine in Methanol ——, and Distilled Water ---. (a) CD Spectrum $[\theta]_{278} = 9.0$; $[\theta]_{245} = -38$; (b) CD Spectrum in Water $[\theta]_{278} = 11.5$; $[\theta]_{245} = -55$; (c) Absorption Spectrum in Water, $\epsilon = 1.040$; (d) Absorption Spectrum in Methanol

UV spectrum can be seen in the new information obtained. The principal Cotton effect bands for cocaine are a positive band maximizing at 220 nm, a negative band with a 245 nm maximum, and a positive 278 nm maximum of a band with measured molar ellipticities of +55, -55, and +11 /(M cm) respectively. Separation of the 278 nm band into two maxima is observed in both the UV and CD spectra of the compound in methanol.

All of the drug spectra measured show a very intense and sometimes unpolarized $^{1}E_{1u}$ transition at wavelengths below 230 nm. Since this band occurs near the usable limit of the instrument and solvent absorption wave- length range, it is frequently impossible to observe the maximum and sometimes the sign of the band, regardless of dilution.

LCCD Experiments

TheLCCD spectra of all the opiates were run several times using the standard right-handed helical liquid crystalline solvent and at the standard molecular solvent to solute ratio, except for the narcotic antagonists nalorphine and naloxone which were run at a molecular ratio of 300:1 (ca. 0.8 mg drug/0.25g solvent) (Fig. 11, 12, 13). Those opiates earlier classified in the first group are now easily distinguishable among themselves by their crossover points and negative CD bands. Of interest are the spectra of morphine, 3-monoacetyl morphine (3-MAM), 6-monoacetylmorphine (6-MAM), and heroin, which show a progressive loss of positive

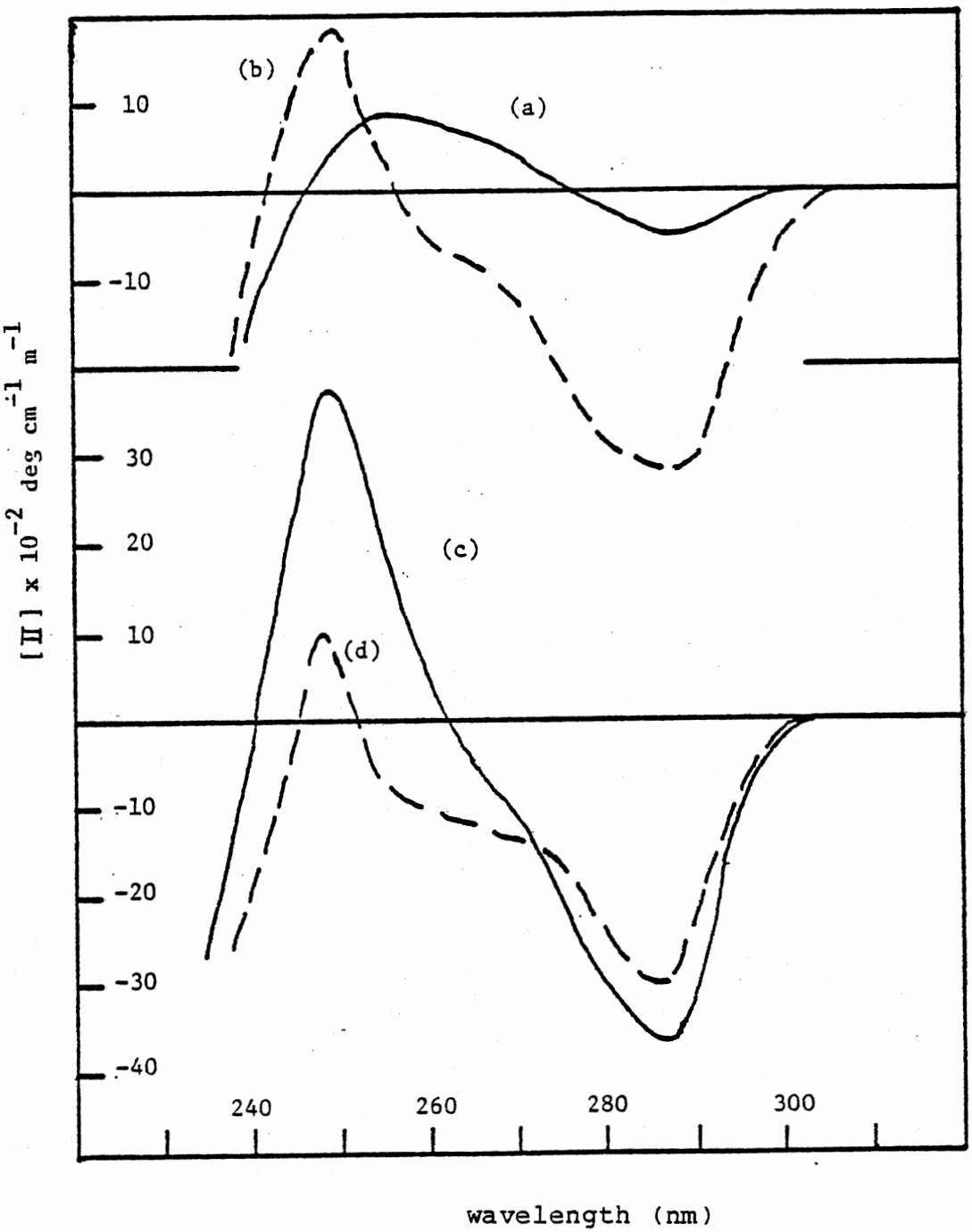


Figure 11. LCCD Spectra of (a) Morphine, (b) Nalorphine, (c) Codeine, (d) Dihydrocodeine

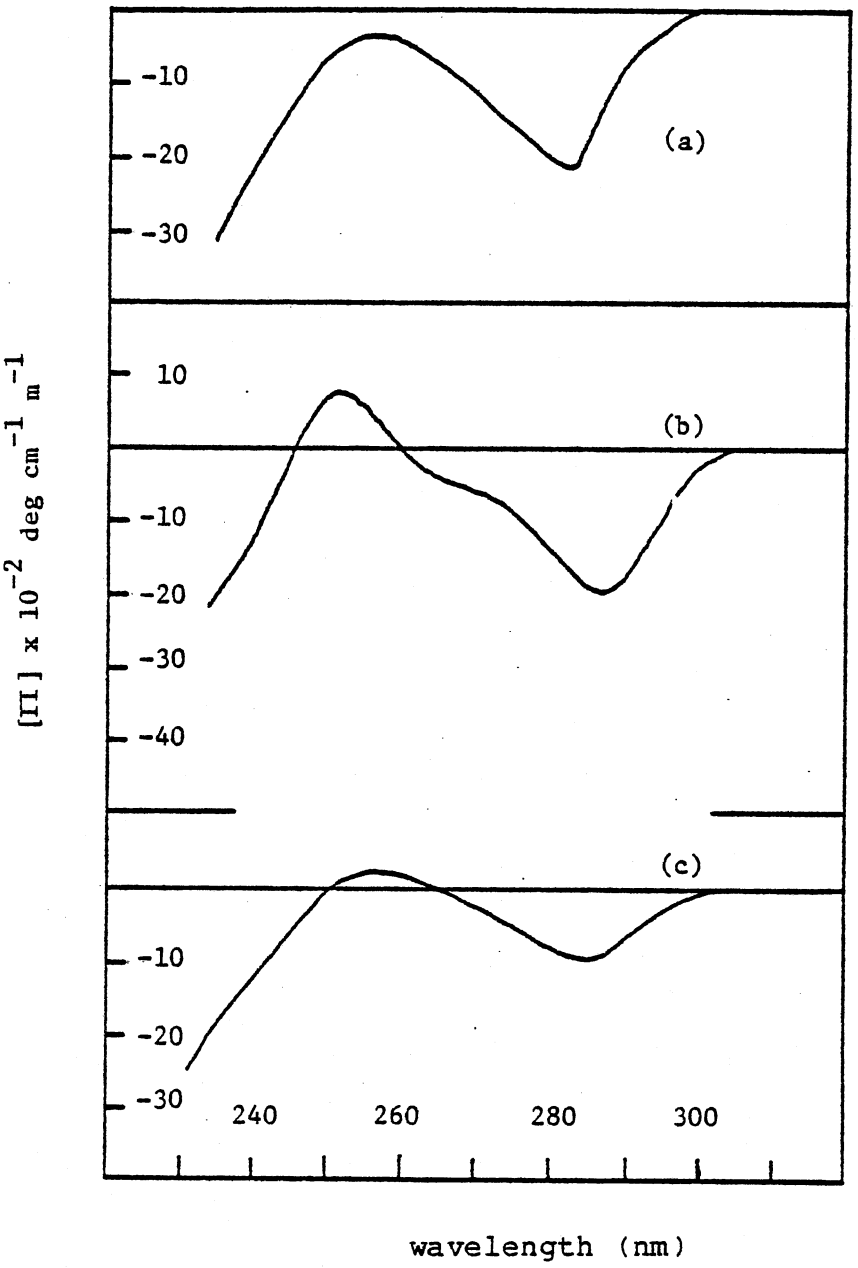


Figure 12. LCCD Spectra of (a) Heroin, (b) 6-MAM, (c) 3-MAM

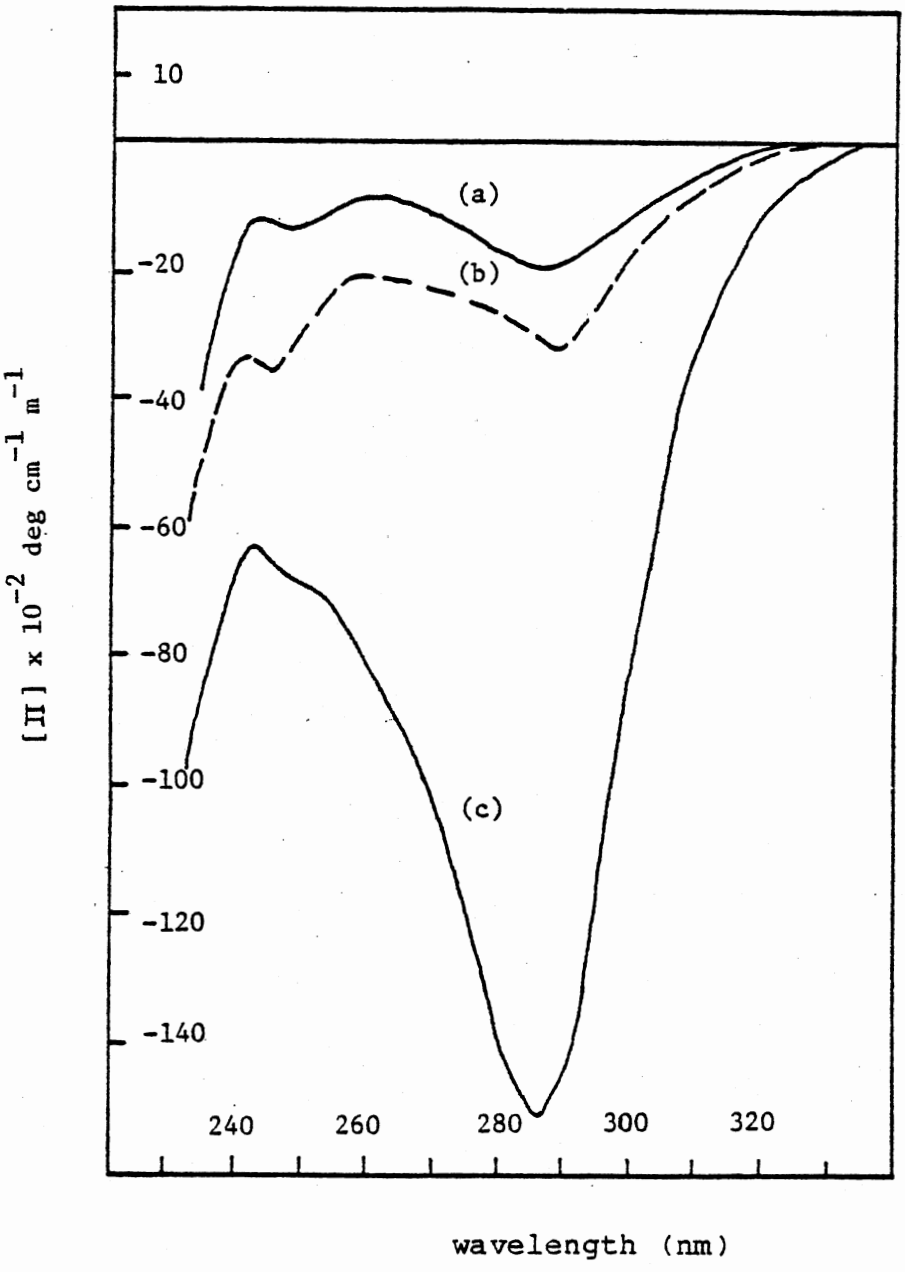


Figure 13. LCCD Spectra of (a) Hydrocodone, (b) Naloxone, (c) Thebaine

character at the 230-260 nm CD band.

Although thebaine is still distinguishable, the extrinsic and intrinsic CD spectra are nearly identical. Naloxone and hydrocodone are still indistinguishable. The LCCD spectra of these are identical except for amplitude. However, naloxone is a narcotic antagonist and is unlikely to be found in any illicit sample. It is of interest that the prominent intrinsic ketonic CD band characteristic of these compounds is lost in the extrinsic spectra under what appears to be preferential enhancement of the previously unseen CD band caused by the ^1L_b aromatic transition.

In order to simplify the LCCD sample preparation and data presentation the normalized ellipticity was re-defined as a molal quantity (i.e. molality instead of molarity being used as the concentration term) according to the equation:

$$[\Pi] = \frac{\Psi_{ob}}{m \cdot b} \qquad (4.1)$$

where m is the concentraion in moles solute/kg solvent, and b is the path length in cm. Molal ellipticity data are presented in Table II. It was learned that the extrinsic CD caused by the liquid crystal solvent not only changed but also magnified or enhanced the intrinsic CD signal of the drugs. Table III presents $[\Pi]$ values for both LCCD and the isotropic spectra were obtained by multiplying molar ellipticity values by the solvent density, and so are not entirely accurate. However, this error is found to be on

TABLE II

LCCD DATA

Concentrations at Molecular Ratio 150:1 Solvent to Drug					
Compound	max (nm)	$[\pi]$	λ_o (nm)	max (nm)	$[\pi]$
Morphine	289	- 510	272	255	812
Nalorphine*	287	- 1730	256	246	9190
6-MAM	288	- 2133	260	250	689
3-MAM	283	- 1040	263	255	246
Heroin	283	- 2164	-	-	min(nm)=256
Codeine	287	- 3629	262	249	3725
Dihydrocodeine	285	- 2988	250	246	1062
Thebaine	287	-15074	-	248†	-6847
Naloxone*	289	- 3130	-	245†	-2390
Hydrocodone	287	- 1928	-	247†	-1338

*These had a molecular concentration ratio 300:1
†shoulder

TABLE III

ELLIPTICITY ENHANCEMENT OF LCCD,
BY ELECTRONIC TRANSITIONS
AND PEAK SHIFTS

Compound	λmax (nm)	$[\pi]^{ECD}$	λmax (nm)	$[\pi]^{LCCD}$	$[\pi]^{LCCD}/[\pi]^{ECD}$	
Morphine	278	- 67.5	289	- 510	7.6	
	246	348.3	255	812	2.3	
Nalorphine	287	- 74.8	287	- 3228	43.2	
	246	320.6	249	1839	5.7	
6-MAM	288	- 44.4	288	- 2133	48.0	
	247	254.4	250	689	2.7	
3-MAM	287	- 36.3	289	- 510	14.0	
	266	117.7	255	246	2.1	
Heroin	275	- 14.6	283	- 2164	148.2	
Codeine	286	- 77.6	287	- 3629	46.8	
	247	389.2	249	3725	9.6	
Dihydrocodeine	287	- 50.0	285	- 2988	59.8	
	243	95.5	246	1062	11.1	
Naloxone	303	-205.8	289	- 3130	15.2	
	240	- 43.1	245†	- 239	56.5	total
Hydrocodone	301	-283.0	287	- 1428	6.8	
	241	142.0	247†	- 1339	10.4	total
Thebaine	286	-427	287	-15074	35.3	
	245†	-150	248†	- 6847	45.7	

†shoulder

the order of 10^{-3} to $1-^{-5}$% in ellipticity for the concentrations used (ca. $1-^{3}$ to 10^{5} M).

Two investigations of the temperature dependence of LCCD spectra were carried out for codeine. In the first (Fig. 14), the standard liquid crystal solvent was used. Spectra were taken at 39°, 56°, 68°, and 74°C. This experiment showed that an increase in temperature is accompanied by a decrease in LCCD signal intensity. The LCCD spectrum shape or ratio of band maxima however did not change until after the liquid crystal to liquid phase transition temperature was reached, at which point the characteristically exaggerated LCCD spectra of codeine dissappeared, leaving the quite different and very much less intense intrinsic CD spectrum of codeine, very similar to its CD spectrum in ethanol. The second experiment (Fig. 15) was performed on the solvent mixture of the opposite sense. The LCCD spectrum of codeine is inverted, which demonstrates the importance and the power of the solvent helicoidal sense in determining the LCCD spectra. The 220-240 nm LCCD band ($^{1}L_{a}$ transition) appears to be made up of two transitions of different polarization which are now divided by the solvent-solute interaction. In contrast, the same inverse relationship of LCCD signal intensity vs. temperature that was observed in the previous experiment still holds true.

An experiment to investigate the effect of pitch and solute concentration on the LCCD spectra was carried out on

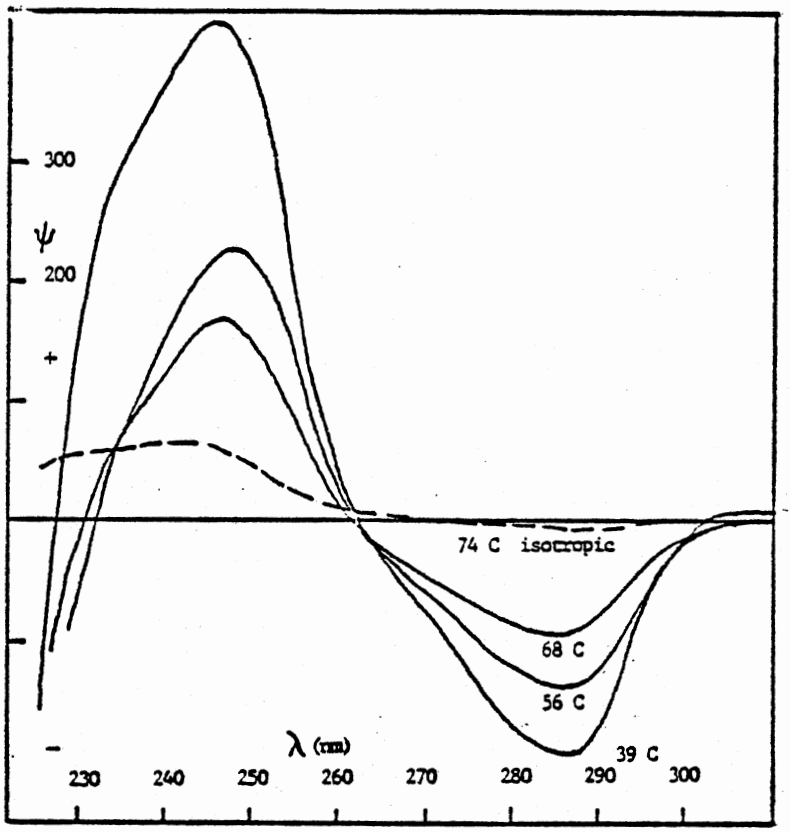


Figure 14. LCCD Spectra of Codeine in the 1:1.65 Molar Ratio Cholesteryl Chloride-Cholestryl Nonanoate Liquid Crystal Solvent. Extrinsic Enhancement is Twenty-three Times the Intrinsic CD (Isotropic CD). Temperature was Raised to obtain Each Spectrum

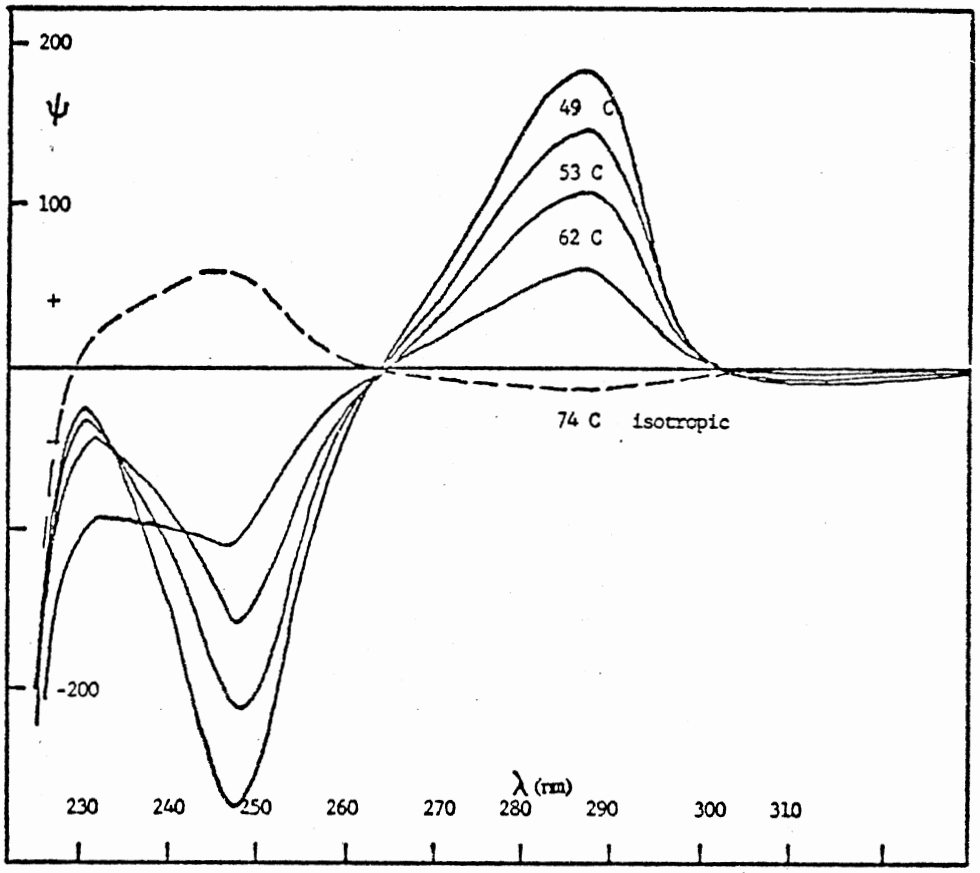


Figure 15. LCCD Spectra of Codeine in the 1.65:1 Molar Ratio Cholesteryl Chloride-Cholesteryl Nonanoate Liquid Crystal Solvent Showing Inversion Due to the Solvent Sense. Temperature was Raised to Obtain Each Spectrum. The Intrinsic CD is Seen at Temperatures Above that of the Phase Transition

the standard liquid crystal solvent. Four samples of liquid crystal solvent with increasing codeine concentrations were run and the pitch band reflections were measured (Table IV). These measurements show that the pitch does change at low solute concentration, but on further increases in the solute concentration, no change is observed. The overall pitch expansion in going from zero to the highest solute concentration amounts to only 20% of the solvent value.

SSCD Experiments

The SSCD technique was investigated in order to produce the identification characteristics of the more difficult LCCD technique but without the difficulties of sample preparation and necessity for temperature control. However, due to the non-reproducibility of the pressing operation which led to random sample window thickness, not even molal ellipticities could be satisfactorally calculated. Even so, qualititative distinction is possible (Table V).

The SSCD spectra for mprphine sulfate, codeine and 3-MAM are qualitatively similar (Fig. 16, 17), and would prove difficult to distinguish one from another. The SSCD spectra of the other compounds are uniquely different and are similar to their isotropic CD spectra, excepting heroin which displays an LCCD like spectrum with a reproducibly sharp negative band with a maximum at 283 nm. The spectrum of thebaine displayed some linear dichroism as the sample was rotated in the instrument. It is interesting to note that

TABLE IV

EFFECT OF CONCENTRATION OF SOLUTE ON PITCH

Codeine Concentration in drug: solvent molecular ratio	Pitch*
0	400
1:150	420
1:500	480
1:80	480

*From pitch band reflection measurements

TABLE V

SSCD ELLIPTICITY MAXIMA AND CROSSOVER POINTS

Compound	$-\lambda$ max (nm)	λ_o (nm)	$+\lambda$ max (nm)
Morphine			252
Morphine Sulfate	+290	275	250
6-MAM	286	267	257
3-MAM	287	262	255
Heroin	283,242 min.		
Thebaine	287	270, 260	265
Hydrocodone	328,222	293, 266	283
Codeine	287	267	252

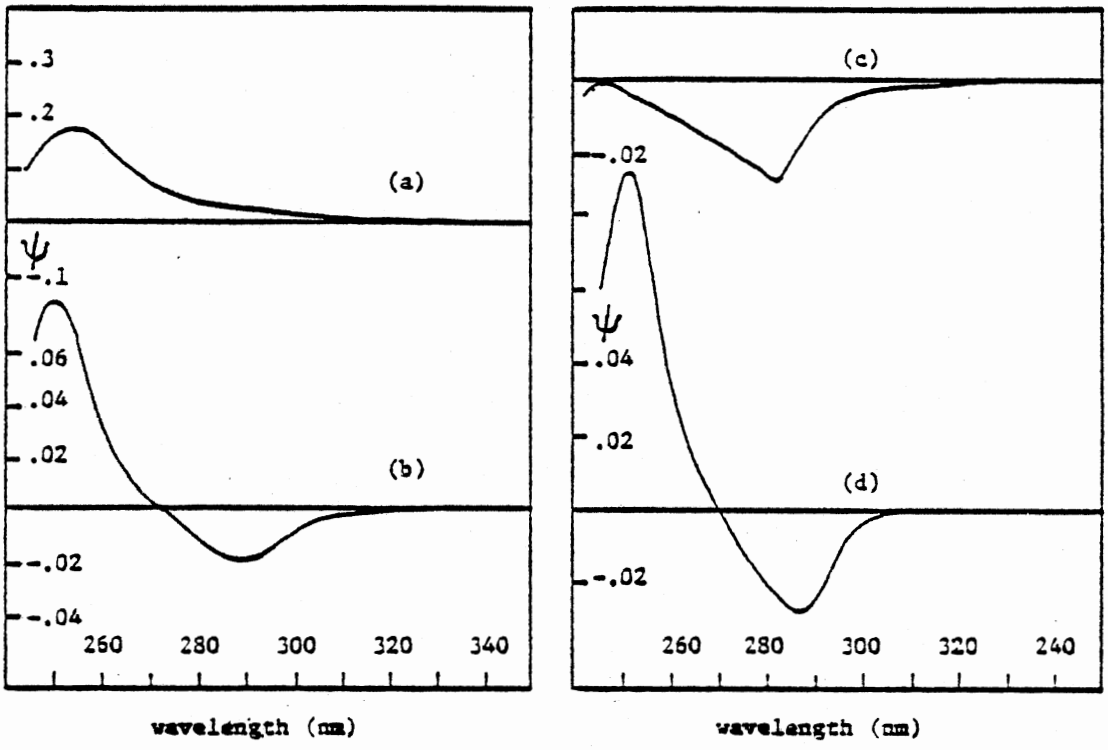


Figure 16. SSCD Spectra of (a) Morphine Base, (b) Morphine Sulfate, (c) Heroin, (d) Codeine, at a Concentration of 0.8 mg/KBr

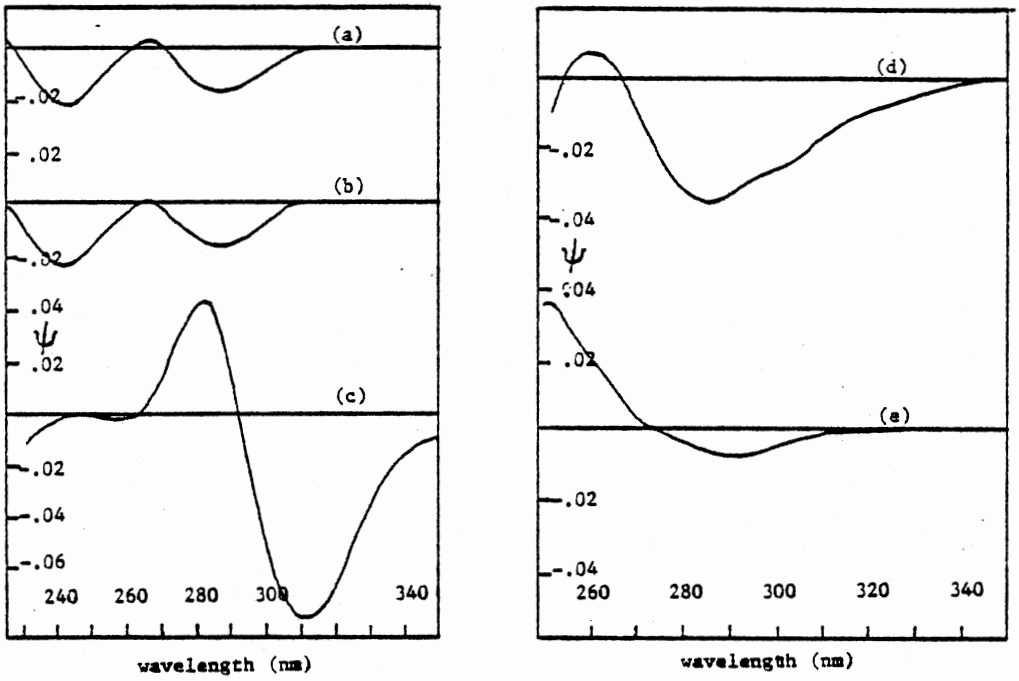


Figure 17. SSCD Spectra of (a) Thebaine, (b) Thebaine (Sample Rotated 90°), (c) Hydrocodone, (d) 6-MAM, and (e) 3-MAM, all at Concentrations of 0.8 mg/ 80mg KBr

SSCD can distinguish morphine from its own sulfate salt.

Quantitative Experiments

Isotropic Experiment

As was noted before, a CD spectrum is simply a modified absorption spectrum which displays positive and negative bands which are dependent upon the magnitudes of the left and right molar absorption coeffieients (equation 2.4). Thus quantitation using the Beer-Lambert law should be applicable.

A series of experiments were performed using L-cocaine HCL in methanol. Ellipticity vs. concentration calibration curves were obtained for data from the wavelengths of the two CD maxima at 245 and 278 nm. The band with the maximum at 220 nm was not used due to excessive noise at that wavelength. Both calibration curves were found to be linear (Fig. 18), with slopes, representing molar ellipticities, of -55° /M at 245 nm and $+11^{\circ}$ /M at 278 nm. Of these two bands, the 245 nm band was chosen for quantitation due to its greater molar ellipticity value and less likehood of interference from the absorption by other additives in anomymous mixtures. The limit of detection for this band was calculated to be 3.98×10^{-5}M using Kaiser's method (71). This method defines a signal confidence limit by the following equation:

$$\psi_D = \overline{\psi}_{bl} + 3s^*_{bl} \qquad (4.2)$$

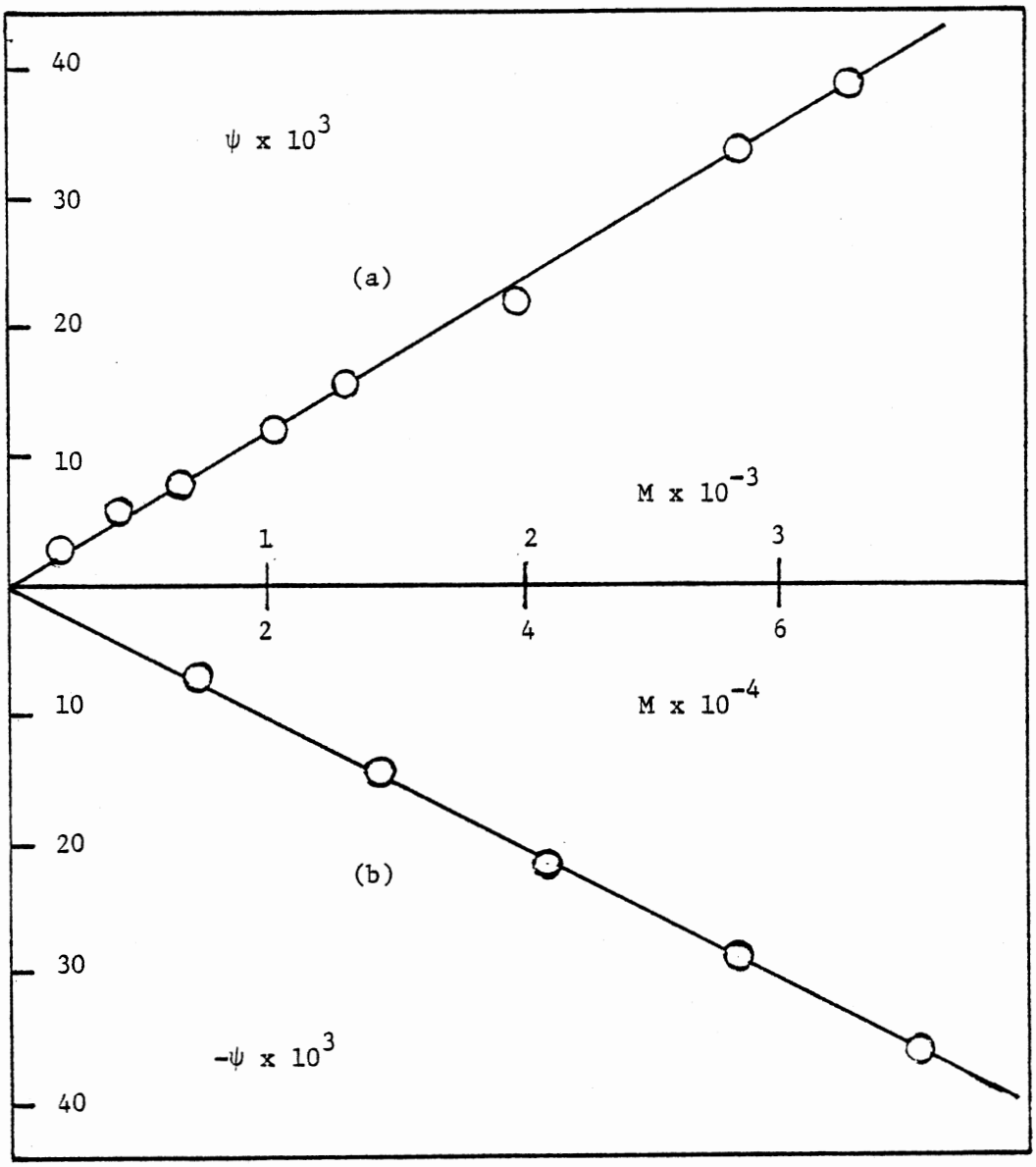


Figure 18. Plot of Experimental Ellipticity ψ_{exp} vs. Concentration of L-Cocaine at (a) 278 nm and (b) 245 nm

where ψ_D is the lower bound of signal confidence, and $\bar{\psi}_{bl}$ and s^*_{bl} the average and standard deviation of the observed ellipticity of at least 20 blank or solvent runs at the wavelength of measurement. If the $\log_{10}$ concentration is plotted against the experimental ellipticity, ψ_D marks the point below which no ellipticity signals can be considered accurate. The antilog of the point where the extrapolated calibration curve of this graph enters this 'forbidden' region is a measure of the limit of detection (71).

Twelve unknowns of illicit origin were analyzed for cocaine content calculated as percent by weight of cocaine free base (Table VI) using data at 245 nm. The Roman numerals refer to in-house mixtures of L-cocaine and lactose which were used to determine the accuracy of the method. Of the illicit samples numbers 1-5 were provided by the O.S.B.I. and 6-12 were provided by the Oklahoma City Police Department, and were evidence from a case under their investigation.

Confiscate 5 presented an interesting problem involving what may be the use of a diluent designed specifically to confuse the analysis. Indeed, the presence of L-cocaine in this sample had escaped detection by the approved methods for a long period of time (72). The sample was a viscous tar, 'black' in color, due to the presence of a large excess of intensely colored dyestuff. A UV spectrum of the sample revealed that the absorption bands of the dye effectively

TABLE VI

QUANTITATIVE DETERMINATIONS OF
L-COCAINE IN MIXTURES

	Concentrations as Percentage L-Cocaine Free Base		
Sample	%Composition[a] by weight	%Composition[b] by CD	%Composition[c] by O.S.B.I.
I	31.9	31.3	--.-
II	43.3	44.4	--.-
III	1.9	1.5	--.-
IV	0.5	0.47	--.-
1	--.-	71.9	66.0
2	--.-	42.1	42.5
3	--.-	2.5	--.-
4	--.-	19.8	--.-
5	--.-	40.9	40.0
6	--.-	40.5	--.-
7	--.-	14.6	--.-
8	--.-	19.7	--.-
9	--.-	20.2	--.-
10	--.-	22.5	--.-
11	--.-	28.0	--.-
12	--.-	23.5	--.-

[a] Roman Numberals Refer to In-house Prepared Cocaine-Lactose Mixtures.
[b] Results are Averaged for at Least Four Determinations at 278 and 245 nm.
[c] O.S.B.I. Results were Performed by the O.S.B.I. Using Other Anatylical Methods.

masked the characteristic absorption bands of L-cocaine. However, the dyestuff was CD transpareant and did not interfere with the facile identification of the L-cocaine in the sample.

The other samples were easily identified and quantitated by the usual procedure and compared favorably with results from judicially acceptable analytical methods employed by the O.S.B.I. The results for confiscates 6-12 were accepted as evidence for the presence of L-cocaine in the samples by the defense attorney in the case of the State of Oklahoma vs. Gary Eugene Fisher, Oklahoma County Court, November, 1980. Our laboratory was involved because State law requires proof that the L-isomer of cocaine was present in the confiscate. Optical dichroism is the easiest experimental way to confirm this.

It should be noted that this study represents the direct quantitative analysis of an illicit drug mixture without the benefit of any prior qualitative tests, extraction, or separation procedures. For such a direct analysis, CD is superior to UV spectrophotometry.

Solvent Affected Experiments

The quantitative aspects of the LCCD technique were evaluated using codeine and morphine as solutes. A calibration curve was prepared for codeine by varying the solvent to solute ratio from 25:1 to 175:1 (Fig. 19, Table VII). The curve is linear with a slope corresponding to a molal

TABLE VII

EXPERIMENTAL ELLIPTICITIES (ψ) VS. MOLAL CONCENTRATION (m) FOR MORPHINE AND CODEINE IN LIQUID CRYSTAL SOLVENT

Compound	mg solute	10^2m	$-\psi$ at 286 nm	K	$\log_{10}$ [m]
Codeine	0.80	0.53	0.030		-2.3
	1.60	1.07	0.064		-2.0
	1.80	1.20	0.120		-1.9
	3.20	2.14	0.181		-1.7
	4.30	2.84	0.272		-1.5
	6.40	4.24	0.310		-1.4
Morphine	0.26	0.29	0.010	19.9	
	0.26	0.29	0.009_2	26.3	
	0.39	0.42	0.014	16.2	
	0.45	0.49	0.014	20.6	
	0.45	0.49	0.014	18.2	
	0.58	0.63	0.017	18.2	
	0.62	0.68	0.018	18.6	
	0.62	0.68	0.017	19.2	
	0.77	0.84	0.020_6	16.8	
				$K = \overline{19.6} \pm 2.0$	

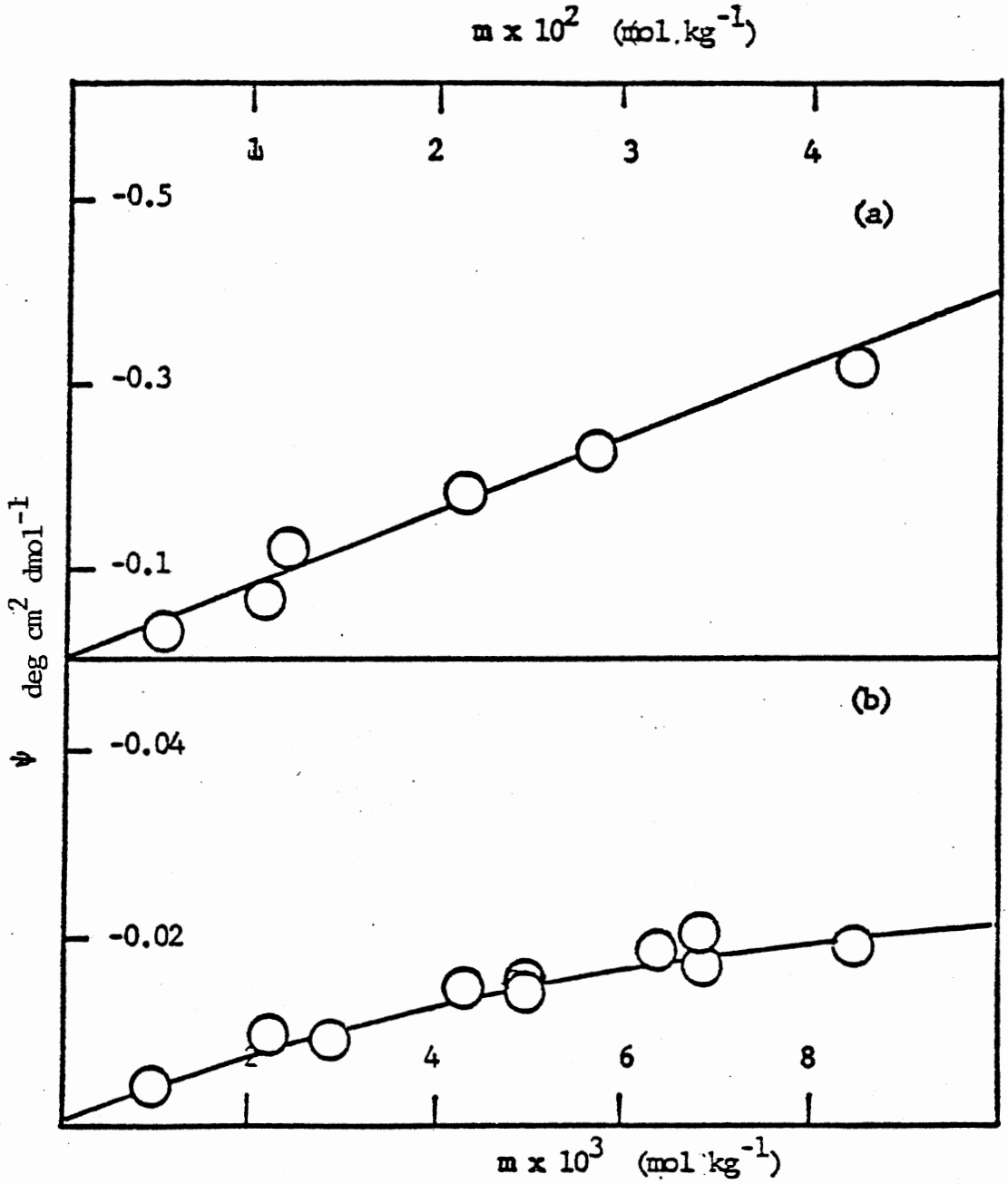


Figure 19. Experimental Ellipticity vs. Molal Concentration for (a) Codeine and (b) Morphine in the Cholesteric Liquid Crystal Solvent at 42.5°C

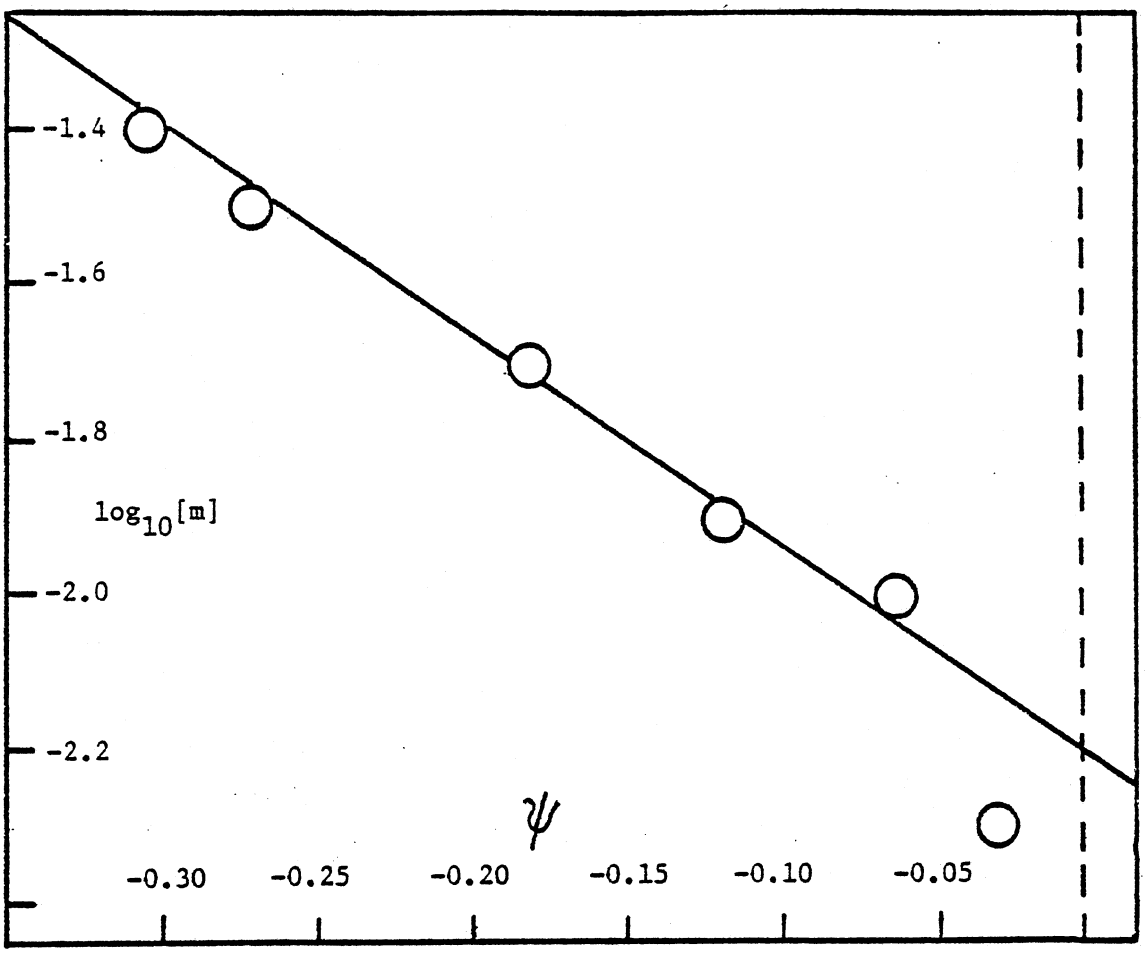


Figure 20. Graph Used to Determine the Limit of Detection for Codeine in the Liquid Crystal Solvent Using Kasier's Method. The Dashed Line is $\psi_D = .0031° \psi$, the Limit of Detection is 1.7×10^{-3}m

ellipticity of -3622°ψ/m b. The limit of detection determined again by Kaiser's method is 1.7 x 1^{-0-3}m (ca. 0.13 mg codeine/0.25 g solvent) (Fig. 20). The detection limits for all compounds is inversely proportional to their molal ellipticity values, which theoretically make LCCD more sensitive than isotropic CD. That this is not so can be attributed to the large absorptivity of the liquid crystal solvent in the UV region.

The calibration curve for morphine, on the other hand, is not linear. Departure from linearity may be the result of a self-association reaction. The data were analyzed in terms of a dimerization equilibrium, $2M \rightleftharpoons M_2$, where M is the morphine monomer, and M_2 represents the dimer. This is not unexpected, as morphine has both 3- and 6-hydroxyl functional groups spacially adjacent to the dihyrodfuran oxygen and is the most likely opiate to participate in a dimerization reaction via intermolecular hydrogen bonding.

The observed or experimental ellipticity for the morphine solutions in liquid crystal is presumed to be the sum of two terms:

$$\psi_{ob} = [\pi]_M(m-2x) + [\pi]_{M_2}x \qquad (4.2)$$

such that both the monomer and the dimer contribute to the overall signal. $[\pi]_M$ and $[\pi]_{M_2}$ are the ellipticities of the monomer and dimer, m is the total analytical molal concentration and x is the molal concentration of dimer. $[\pi]_M$ was measured as the tangent of the calibration curve

at zero solute concentration, and $[\pi]_{M_2}$ was taken as the normalized elliptitcity value from the plateau of the calibration curve, at high concentration. These values are -4.6 and +14.5 respectively (Fig. 16). The dimerization constant K_d, given by:

$$K_d = \frac{x}{(m-2x)^2} \qquad (4.3)$$

K_d was calculated for all points on the calibration curve. A resultant averaged value of 19.6 $\pm$ 2.0 was obtained.

Isotropic Complexation Reaction Experiments

The effects of the interaction between drugs and cyclodextrin sugars and the resultant effect on the CD spectra of these drugs was also investigated in a quantaitative manner. The interactions of beta-cyclodextrin with the drugs L-cocaine and PCP were studied.

The intrinsic CD spectrum of L-cocaine is observed to change upon the addition of beta-cyclodextrin. As the sugar concentration is progressively increased at a constant L-cocaine concentration, the 245 nm negative CD band in the region of the ^1L_a aromatic transition is observed to decrease, change sign, and finally increase to a positive value (Fig. 21). The sugar concentration is in excess of the L-cocaine concentration throughout, changing from 2 to 100 times the concentration of L-cocaine. the resultant CD

spectrum , at high sugar concentration, is reminiscent of the UV absorption specrtrum of cocaine. As an interesting contrast, no changes are observed in the UV absorption spectrum of L-cocaine as beata-cyclodextrin is added over the same concentration range (Fig. 22).

These changes in the region of the ^1L_a aromatic transition band for L-cocaine have been interpreted in terms of a 1:1 drug to cyclodextrin sugar complexation equilibrium reaction which is favored only when the sugar is in large excess. This equilibrium process may be represented by the reaction:

$$C + S \rightleftharpoons CS \qquad (4.4)$$

where C is L-cocaine, S reparesents the cyclodextrin sugar, and CS the 1:1 complex. In the reaction model it is assumed that $[\theta]_C$ and $[\theta]_{CS}$, the molar ellipticities of L-cocaine and the complex respectively are invariant with concentration and temperature.

The observed ellipticity is again assumed to arise from contributions from both L-cocaine and the complex:

$$\psi_{ob} = [\theta]_C[C] + [\theta]_{CS}[CS] \qquad (4.5)$$

where a value of $[\theta]_C$ at 245 nm equal to $-55° \psi/M$ b was used. The complex formation constant K is given by:

$$K = \frac{[CS]}{(C_c-[CS])(C_s - [CS])} \qquad (4.6)$$

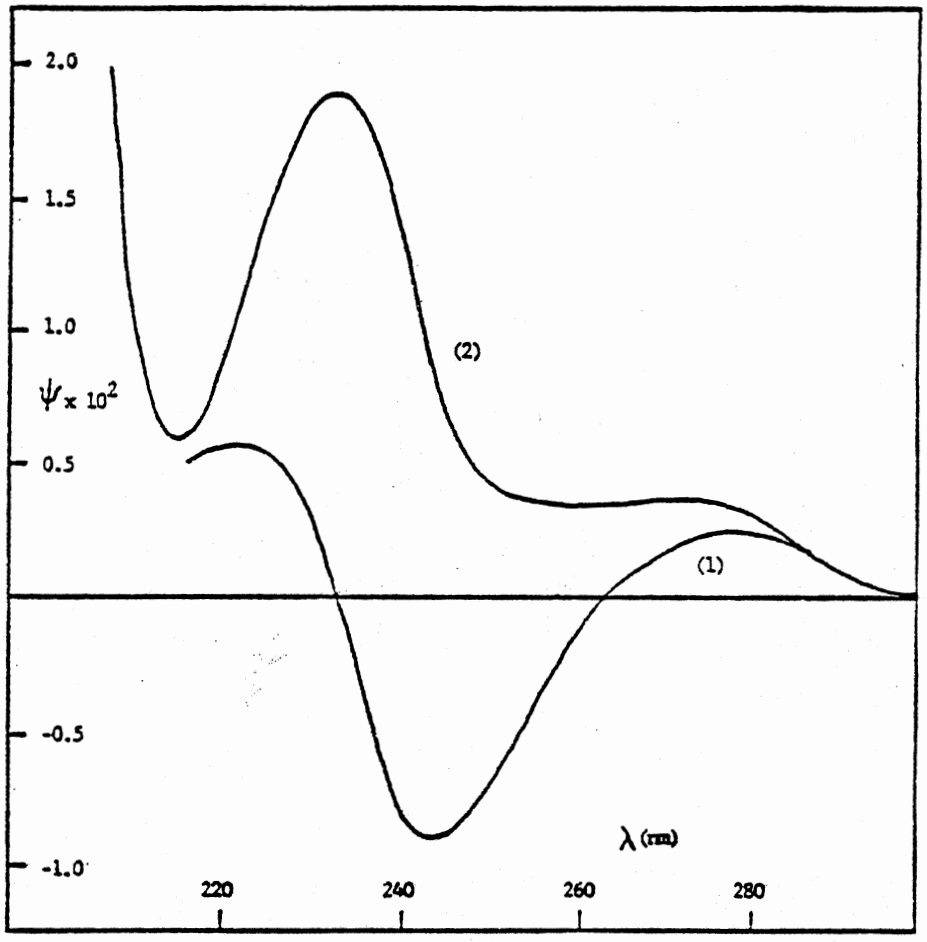


Figure 21. CD Spectra of (1) 2.25×10^{-4} L-Cocaine in Water, (2) 2.25×10^{-4}M L-Cocaine in 10^{-2}M Beta-Cyclodextrin

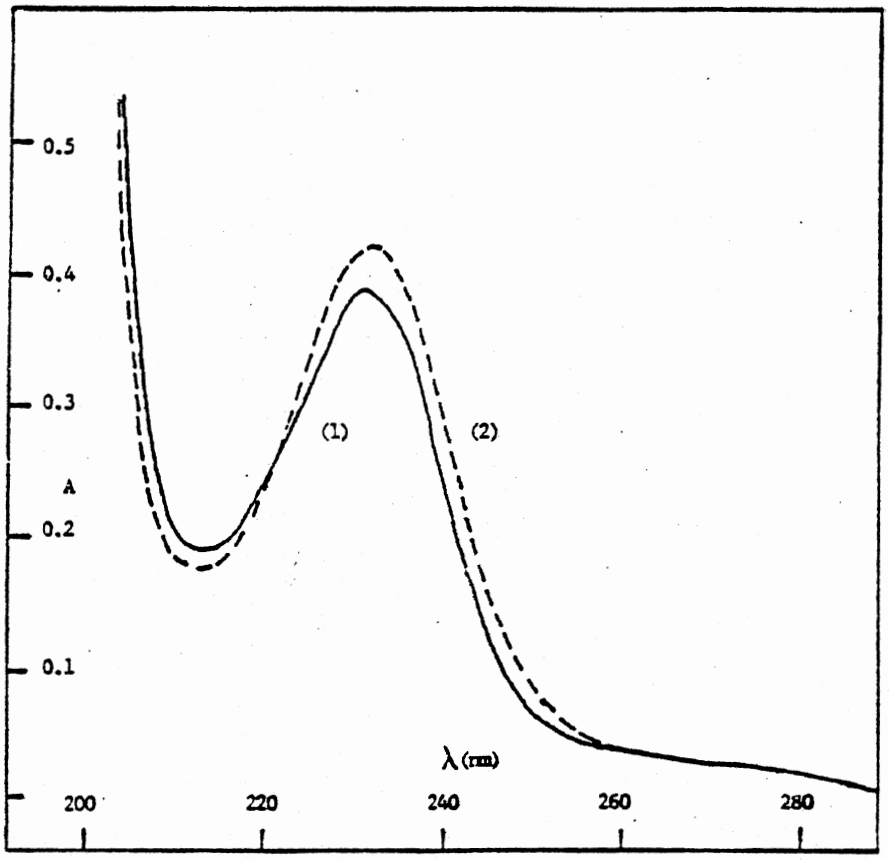


Figure 22. UV Spectra of (1) L-Cocaine at 2.25×10^{-4} M in Water, (2) L-Cocaine at 2.25×10^{-4} M in 10^{-2} M Beta-Cyclodextrin in Water

where C_c and C_s represent the total analytical concentrations of L-cocaine and beta-cyclodextrin respectively. If [CS] from equation (4.5) is substituted into equation (4.6) the only unknown values are $[\theta]_{CS}$ and K. In a simple programmed routine, K was calculated by choosing several values of $[\theta]_{CS}$ between +10 and +1000. The data consisted of a series of ψ_{ob} measured as C_s was increased at constant C_c. The calculated K values were plotted vs. C_s and the curve with zero slope, the minimum deviation in K was used to determine a value for the complexation constant (Fig. 23). The experiment was repeated for different values of C_c. The calculated best value for $[\theta]_{CS}$ = +85 which yeilded the corresponding best value for K of 423 at 25°C.

To better understand the thermodynamics of the complexation equilibrium reaction, the experiments were conducted in replicate at 15°, 25°, 35°, and 40°C (Table VIII), with data taken at 245 nm, and with the best value of +85, assumed to be invarient with temperature. The resultant K values are 447 $\pm$ 30, 423 $\pm$ 15, 353 $\pm$ 30, 319 $\pm$ 30 respectively. The errors were found from the variance of the calculated K values from the mean. A value of -3.56 kcal was calculated for $\Delta H°$ form the slope of the ln K vs (T^{-1}) plot. A value of $\Delta G° = -3.58$ kcal was calculated from the relationship $\Delta G° = -RT\ln K$, at 25°C. The entropy calculated from the Gibbs equation, $\Delta S° = (\Delta H° - \Delta G°)/T$ is equal to 0.07 cal mol $^{-1}$ K^{-1}.

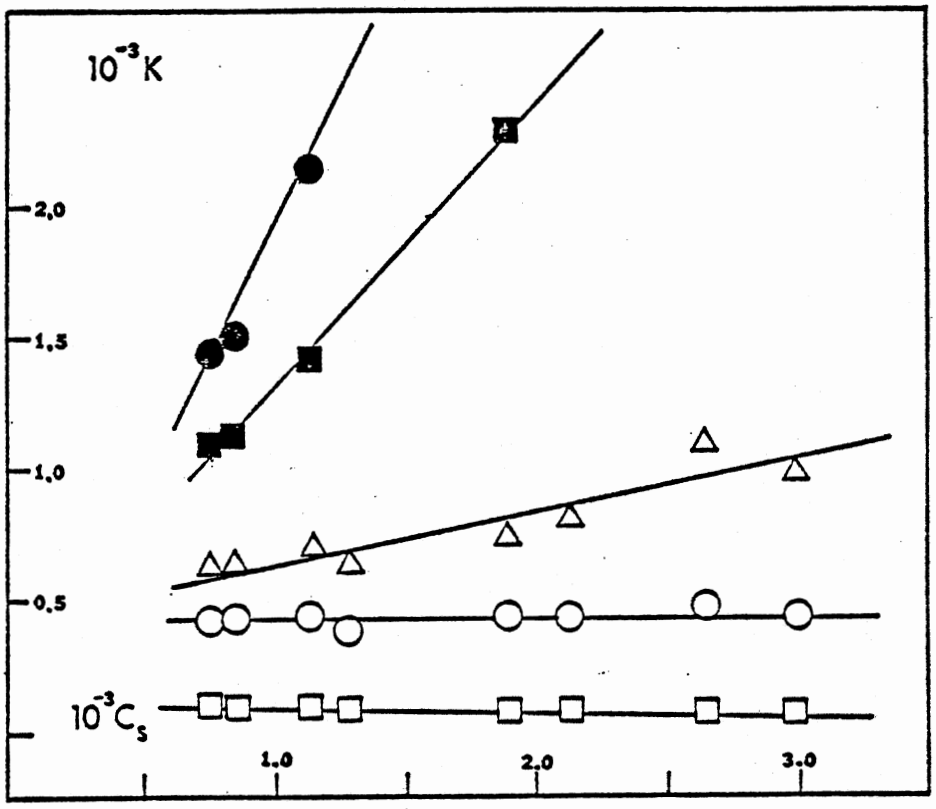


Figure 23. Variation of the Calculated Complex Formation Constant K with Concentration (M) of Beta-Cyclodextrin in Water. Lines represent $[\theta]_{CS}$ Values of +10, +20, +50, +85, +500 From Top to Bottom

TABLE VIII

EXPERIMENTAL ELLIPTICITIES OF THE COCAINE-BETA-CYCLODEXTRIN COMPLEX AT DIFFERENT CYCLODEXTRIN CONCENTRATION AND AT DIFFERENT TEMPERATURES

[Cocaine]	[β-cyclodextrin]	$\psi_{15°C} \times 10^3$	$\psi_{25°C} \times 10^3$	$\psi_{35°C} \times 10^3$	$\psi_{40°C} \times 10^3$
7.60×10^{-5}	7.61×10^{-4}	-1.61	-1.70	-1.86	-2.05
	1.14×10^{-3}	-0.390	-0.745	-1.28	-1.39
	1.90×10^{-3}	+0.570	+0.410	0	-0.270
	2.66×10^{-3}	+1.75	+1.71	+0.700	+0.700
8.55×10^{-5}	8.55×10^{-4}	-1.43	-1.69	-2.24	-1.90
	1.28×10^{-3}	-0.210	-1.01	-1.02	-1.80
	2.14×10^{-3}	+1.31	+0.887	+0.585	-0.145
	2.99×10^{-3}	+2.35	+1.93	+1.20	+0.800

Similar experiments were carried out using PCP as the drug co-solute. Although PCP has no intrinsic CD it does exhibit an extrinsic CD spectrum in the presence of an excess of beta-cyclodextrin. Exactly as before a 1:1 PS complex was assumed, where P and S represent PCP and the sugar respectively. The molar ellilpticities were again assumed to be invariant with temperature and concentration.

For this experiment, the mathematical model is simpler because $[O]_P = 0$, thus any CD spectrum that is seen is only the extrinsic CD of the PS complex. For the complex the observed ellipticity is given by:

$$\psi_{ob} = [\theta]_{PS}[PS] \tag{4.8}$$

and the complex formation constant K, in its expanded form is given by:

$$K = \frac{\psi_{ob}/[\theta]_{PS}}{(C_P - \psi_{ob}/[\theta]_{PS})(C_s - \psi_{ob}/[\theta]_{PS})} \tag{4.9}$$

where C_P is the total analytical concentration of PCP. The same experimental procedure used in the cocaine-cyclodextrin experiment in spite of the simpler mathematical model. This arises because, as before, the complexation is favored when one reactant is in excess, in this experiment, when $C_s > C_P$. But the extrinsic CD signal of the PCP-cyclodextrin complex is very small compared to that of the L-cocaine-cyclodextrin complex. Further, the concentration of PCP in the reactant solution that would be required to

increase the small extrinsic signal is limited by the low solubility of the beta-cyclodextrin. As a result, a value of $K \simeq 10$ is the best estimate of the complex formation constant that can be made. The errors transmitted to $\Delta H°$ and $\Delta S°$ are so great than any interpretation of the values is meaningless.

CHAPTER V

DISCUSSION

This study was undertaken in order to develop and evaluate three methods of solvent affected circular dichroism (SACD). The impetus behind the development of SACD techniques is to improve the ability of the already powerful technique of circular dichroism to distinguish among very similar molecules. This is accomplished by the use of selected solvents which can, by diverse specific intramolecular interactions, affect the CD spectrum of a solute compound, often improving its distinction among compounds very similar to it.

In addition to the enhanced resolving capabilities, this technique should also offer the speed of analysis and ease of sample preparation usually associated with absorption spectrophotometry, of which CD is a modified form. Such a technique would prove valuable to the heavily taxed clinical and forensic laboratories.

Isotropic CD

It is easily seen that the isotropic CD spectra of the opiates show the similarities within the first, or morphine-like group and within the ketonic group more than their

minor differences, even though characterization among families of drugs is easily accomplished. However, quantitation of compounds able to produce an intrinsic CD spectrum is more easily done even in unseparated mixtures of non-chiral drug diluents, making this a rapid and valuable analytical technique.

LCCD

Almost complete qualitative distinction can be done using LCCD due to the very specific intramolecular interactions between the liquid crystal solvent and the dispersed solute. This is the cause of the spectral changes observed for the very structurally similar opiates, and allows for their facile identification.

The most striking aspect of LCCD is the large solute signal enhancement over that of the isotropic CD spectra. The intrinsic CD due to the chirality of the solute molecule itself is amplified to 5 to 150 times by the extrinsic CD due to the effect of the solvent. This chirality amplification (74) is explained simply as a consequence of the constructive alignment of the polarized translations of the solute molecules to conform with the helicoidal structure of the solvent. Those transitions which are so aligned are enhanced, and those which are not aligned are not enhanced, as is evident in the example of the disappearance of the intrinsic 301 nm CD band of naloxone and hydrocodone in the LCCD spectra. The enhancement effect is not due to having

more molecules in the samples. Using a simple calculation to determine the number of moles in a beam of light with a cross section of 1.27 cm, it is found that 5×10^{-7} moles of heroin in the LCCD cell produce about 150 times the signal as do 6×10^{-5} moles of heroin in an isotropic solvent. Cell path lengths are 12.5 μm and 1 cm respectively.

It was first thought that the pitch of the liquid crystal might have a profound effect on the amount of enhancement by the LCCD signal. It is known that chiral, and non-planar molecules do tend to disrupt the helicoidal order of the liquid crystal (36, 37, 38). Such a disruptive effect, it was thought, might be expected with the opiates which are both chiral and have a rigid "T" shape. However, through several experiments with pitch band reflection vs. concentration of codeine, it was found that the pitch of the liquid crystal was not changed by more than 20% indicating that pitch does not play a significant role in the enhancement of ellipticity.

If a LCCD sample is heated, the signal intensity is observed to decrease in a monotonic fashion until the mesophase to liquid phase transition takes place (Fig. 17). This phenomenon is common to both helical senses of liquid crystals (Fig. 18).

There are two possible reasons for this observation. The effect could be due to an increase or decrease in the helical pitch of the solvent with temperature, or it could

be due to the thermal randomization of the helical macrostructure of the solvent, and hence the randomization of the solute orientations. We found that the pitch of the left-handed solvent, that with a predominance of cholesteryl chloride, decreases with a rise in temperature, while the right-handed solvent used in our studies exhibited an increase in pitch with increasing temperature. Since the decrease in signal is common to both senses, the latter effect of thermal randomization is undoubtedly dominant over any effect due to the pitch change.

In the left-handed solvent, the LCCD spectra of codeine is almost completely inverted in sign (Fig. 18), the exception being that the 249 nm band seems to consist of two transitions of different polarization. The effect of the inverted spectrum is indicative of the importance of the helicoidal sense to an LCCD spectrum.

Although no unknowns were run, quantitation using LCCD was found to be possible using the Beer-Lambert law, although only for pure drug compounds in the free base form. Even with the lower limits of detection made possible due to the ellilpticity enhancement of LCCD, the above experimental limitations severely limit the quantitative usefulness of this method. Information about the behavior of the drug molecule in the liquid crystalline solvent from the shape of the calibration curves was obtainable. Morphine, unlike the more soluble codeine did not exhibit a linear calibration curve. This is probably because morphine and the other

opiates not substituted at the 3-position are more likely to undergo some kind of self-association or interaction with some other fairly polar molecule. Morphine is also the least soluble, and one of the most polar of the opiates studied in the predominantly non-polar liquid crystal solvent. That this molecule, like benzoic acid, should form a dimer in the presence of a more non-polar solvent is not competely surprising. Since codeine is substituted at position-3 it would not be expected to form such a complex and so would have a greater chance to exhibit linear behavior in an ellipticity vs. concentration curve.

Errors in the calculations of the molal ellipticities were found from the variance from the average of the LCCD measurements for those opiates not expected to dimerize. For those compounds analyzed after the technique was established, the error was on the order of 9% of the total signal, with hydrocodone the most precise, at 2% of the total signal. For the earlier data, errors as high as 30% of the total signal were not uncommon, as can be seen in the low concentration values in the codeine calibration curve. The cause of this error is due in large part to the intractability of the liquid crystal solvent system which is sensitive to temperature, aging, texture of the liquid crystal, purity, and to the cleanness of the quartz cell flats. The solvent is also chiral, necessitating difference measurements, and it also absorbs strongly in the UV region of the spectrum.

SSCD

The original purpose behind the SSCD technique was to shorten and simplify the nearly 120 min sample preparation time of the LCCD technique and to alleviate the need for long repetitive scans that are often necessary with LCCD. The actual SSCD sample preparation time is only about 20 min, with somewhat shorter scan times. Both are a consequence of the fact that if kept dry the SSCD sample will not change with time as did the liquid crystal solvent, a phenomenon which affected the LCCD spectra. However, a drying time of at least one and preferably two days is required for the solute and solvent. Although volatile drugs are very difficult to analyze using SSCD, both the salt and free base forms of most drugs could be used without prejudice.

The SSCD spectra only unequivocally identify four of seven opiates, however it does reveal the striking difference between morphine base and morphine sulfate. From X-ray analysis, both the drug and its salt crystallize into a helix of the same handedness. Thus there is no apparent structural reason for this phenomenon.

Apparently, as discussed in Chapter II, if the sample is prepared carefully, the system is truely isotropic, and the spectra seen would be similar to those in a dilute gas phase. The small but real problem of linear dichroism, which causes deviations in the spectra on rotation of the sample, is associated with the pressing procedure. This

problem is localized to small areas within the KBr disc and can be largely alleviated by careful sample preparation procedures.

Unfortunately, the sample preparation methods cannot be expected to be perfectly reproducible, as there is no way of guarenteeing the thickness of the sample, although it can be approached by using exactly the same amount of sample each time.

The worst problem with SSCD was the sensitivity of the sample to moisture. The samples are hygroscopic, and upon becoming wet, or if a wet solute is used the KBr disc becomes cloudy, indicating the growth of larger light scattering centers. Thus, the transmission of the UV circularly polarized light is attenuated by absorption or complete scattering. This problem can be alleviated by different and imporved drying methods and the use of Nujol oil to protect the surfaces of the sample windows from moisture during its analysis in the instrument.

This method still requires very careful sample preparation and exacting sample handling techniques. The ability to distinguish among only four of seven structurally similar opiates indicates that the method is not as specific as that of LCCD. Although some simplification of the sample preparation technique is realized, the results are not convencing enough to recommend SSCD as an effective analytical procedure. Nevertheless it is undoubltedly very useful for structure determination of solutes in solid phase.

Cyclodextrin-Complex CD

The third method of SACD that was studied involved the use of cyclodextrin sugars as a co-solute with drugs in water. This is the simplest method of the three, requiring only that the weights of the two co-solutes used in the sample be known, and taking only 10 to 15 minutes for the entire sample preparation procedure.

The major drawback to the system is the small concentration of the co-solute complex due to the low solubility of the sugar, and the requirement that the sugar concentraiton be nearly fifty times that of the drug in order to favor the formation of the complex.

As was observed in Chapter II, the two cyclic sugars alpha- and beta-cyclodextrin, due to their cavity sizes will not form inclusion complexes with any of the opiates. Further, while beta-cyclodextrin is known to form cocaine, PCP and other amphetamine-like compounds, alpha-cyclodextrin will not.

However, the unique change that is imparted to chiral-molecules such as L-cocaine may prove to be a simple method for their characterization by CD. This is certainly the case for non-chiral drugs such as PCP. The technique has also been used to induce CD into racemic mixtures such as DL-methadone (75), and into molecules as simple as benzoic acid. It has not as yet been established if greater distinction among drugs is possible. Perhaps gamma-cyclodextrin will clathrate the opiates. The greatest innovation of the

method however, is that it extends the easily quantitatable technique of CD in isotropic media to include achiral molecules. Any criticism that CD is applicable only to chiral compounds, and that its versitility is therefore limited, is no longer valid.

The extrinsic CD encountered in this method appears to be caused by inducing disymmetry into the electronic transitions of the included aromatic moiety. The magnitude of the extrinsic CD seems to be an effect of 1) the chirality, and 2) the rigidity of the remainder of the guest molecule.

Calculation of the formation constants at different temperatures for the L-cocaine-beta-cyclodextrin comples, from which we were able to estimate the thermodynamic properties from CD measurements appears to be the first example, although many measurements of this kind have been performed using other methods. These data are not unexpected and compare favorably with observations by Bender (64) who found the enthalpy and entropy changes for the 1-adamantane-carboxylate complexes with alpha- and beta-cyclodextrin. He associated entropy driven complexation mainly with the transfer of the included substrate from an aqueous to a non-polar environment. However, stabilization of the inclusion complex by more classical apolar binding was associated with large and favorable enthalpy changes which far outweighed the importance of the contribution by a much smaller entropy change, which is the case with the L-cocaine-beta-cyclodextrin complex. The most likely contributions to the

apolar binding were attributed to Van der Waals and London dispersion forces, and to the release of "high energy water" from the interior of the cyclodextrin molecule to accommodate the guest molecule. The relative importance of each of these contributions is unknown (69), however, the favorable enthalpy change and small entropy change found are consistent with hydrophobic interactions within the cavity upon complextion (69).

The aromatic ring in both PCP and L-cocaine is monosubstituted, and from a niave proposition that the inclusion by beta-cyclodextrin would be analogous for both, it might have been expected that the formation constants for PCP and L-cocaine would have been more consistent. The 40-fold difference between them emphasizes the complexity of the interaction and demonstrates the specificity of the complex with different molecules. The specificity is of prime concern for the identification of drugs using this type of SACD. This method promises wide scope in that it can induce CD into such complex molecules as cocaine, and also into such simple molecules as benzoic acid. One needs only to add the drug to an aliquot of the saturated cyclodextrin solution.

Conclusions and Suggestions for Further Work

Of the three methods of solvent affected Circular dichroism, the one that offers the most specificity, easiest identification and largest extrinsic CD signal is LCCD.

However, the time of analysis, and the complexity of the sample preparation system do not lend themselves to any convenient use for the bulk analysis of drugs. Due to the problem of reproducibility and the intractability of the solvent system we feel that the use of LCCD for quantitative, or even bulk qualitative analysis may never be successfully accomplished.

Solid state CD appears useful for limited qualitative-analysis but is unikely ever to be used quantitatively. This system, although it can be used on drugs in either the free base or salt form, can only be used on drugs that can be effectively dried. This makes the analysis of the more volatile drugs very difficult. Further, like LCCD the sample preparation is often not trivial, although not as difficult as that for LCCD.

By far the easiest and most promising form of SACD studied is the use of cyclodextrin sugar complexation.

Although the method is limited by the solubility of the cyclic sugar, which is a problem where the signal is small as it is with PCP, drugs in both the free base and salt form can be analyzed with equal facility using standard, widely used volumetric procedures. The instrumentation needs no specially designed equipment, or even difference capability. Preliminary results with racemic mixtures show promise for their identification as well.

The problem of complexation of the larger more rigid molecules such as the opiates, we feel, can be alleviated by

the use of gamma-cyclodextrin, which with a larger cavity can be expected to accommodate the larger drug molecule. Further study using gamma-cyclodextrin is being planned.

Studies into the use of cyclodextrin induced CD with unseparated illicit samples holds promise of success. although the cyclic sugar can complex with other moieties, the aromatic chromophore seems to be perferred, and is common to all illicit drugs. Although non-drug molecules may be complexed and made optically active, unless they possess a chromophore they will not exhibit a conflicting CD signal.

It may be that SACD using pre-prepared solutions of beta- and gamma-cyclodextrins can be used to both identify and then quantitate illicit drug samples all in the same step, that of adding the solution and running the CD spectrum. This method would take the place of the more usual analytical route of preliminary detection by the use of spot or color tests, separation of the drug from the matrix, concentration and finally identification and quantitation by some other method such as GLC.

This lab has recently demonstrated the use of isotropic CD in the direct analysis of biofluids such as urine and blood. To this has been added a quick separation method using miniaturized reversed phase liquid chromatography which is capable of both separation and concentration. Given only partial separation and concentration of any drug sample, and the use of CD as a detector, and with

confirmation by spectrum alteration of the inducement of extrinsic CD by cyclodextrin-complexation SACD, any drug containing sample could be both qualitatively and quantitatively analyzed rapidly. This technically simple and yet powerful combination of easy techniques holds great potential for the analysis of dangerous drugs.

A SELECTED BIBLIOGRAPHY

1. Mule, S. J., J. Chromatog. Sci., 12, 245 (1974).

2. O'Keefe, C., Proc. Int. Symp. Inst. Appl. in Forensic Drug Chem., U.S.D.E.A., p. 6 (1978).

3. Paulig, G., Proc. Int. Symp., Inst. Appl. in Forensic Drug Chem. U.S.D.E.A., p. 41 (1978).

4. Taylor, J. F. in "Narcotic Drugs", D. H. Clouet (ed.) Plenlum Press, New York, (1971).

5. Clark, C. C., Anal. Chem. 36, 1967 (1974).

6. Mule, S. J., J. Chromatog. Sci. 12, 245 (1974).

7. Herman, G. J., and M. N. Kan, Biomed. Mass Spectrom. 1, 350 (1974).

8. Milne, G. W. A., H. M. Fales, and T. Axenrod, Anal. Chem., 43, 185 (1971).

9. Nakamura, G., T. Noguchi, D. Jackson, and D. Banks, Anal. Chem., 44, 408 (1972).

10. Arpino, P. J., and G. Guiochon, Anal. Chem., 51, 682A (1979).

11. Siek, T. J., J. Forensic Sci., 19, 193 (1974).

12. Siek, T. J., J. Forensic Sci., 20, 18 (1975).

13. Siek, T. J., J. Forensic Sci., 21, 525 (1976).

14. Sunshine, I. (ed.), "Handbook of Analytical Toxicology", Chemical Rubber Company, Cleveland, p. 213 (1969).

15. Biot, J. B., Mem. Acad. Sci. Toulouse, 2, 41 (1817).

16. Djerassi, C., "Optical Rotatory Dispersion, Applications to Organic Chemistry", McGraw-Hill, New York, (1960).

17. Lambert, J. B., H. F. Shurell, L. Verbit, R. G. Cooks, and G. H. Stout, "Organic Structural Analysis", MacMillian, New York, p. 329 (1976).

18. Haedinger, W., Ann. Phys., 70, 531 (1847).

19. Cotton, A., Compt. Rend., 120, 989 (1895).

20. Cotton, A., Ann. Chim. Phys., 8, 347 (1896).

21. Takenaka, S., M. Sugigama, and N. Tokura, J. C.S. Perkin II, 555 (1976).

22. Takenaka, S. , K. Kondo, and N. Tokura, J. C. S. Perkin II, 1520 (1975).

23. Takenaka, S., K. Kondo, and N. Tokura, J. C. S. Perkin II, 1749 (1974).

24. Saeva, F. D., P. E. Sharpe, and G. R. Olin, J. Am. Chem. Soc., 95, 7656 (1973).

25. Saeva, F. D., and J. J. Wysocki, J. Am. Chem. Soc., 93, 5928 (1971).

26. Saeva, F. D., G. R. Olin, R. F. Ziolo, and P. Day, J. Am. Chem. Soc., 101, 5419 (1979).

27. Saeva, F. D., D. E. Sharpe, and G. R. Olin, J. Am Chem Soc., 7660 (1973).

28. Kuball, H.-G., M. Acimis, and J. Altschuh, J. Am. Chem Soc., 101, 20 (1979).

29. Sakamoto, K., R. Yoshida, M. Hatano, and T, Tachibana, J. Am Chem Soc., 100, 6896 (1978).

30. Sackmann, E., and J. Voss, Chem. Phys. Lett., 14, 528 (1972).

31. Sackmann, E., and H. Mohwald, J. Chem. Phys., 58, 5407 (1973).

32. Sackmann, E., P. Krebs, H. U. Rega, J. Voss, and H. Mohwald, Mol. Cryst. and Liq. Cryst., 24, 283 (1973).

33. Samori, B., J. Phys. Chem., 83, 375 (1979).

34. Gottarelli, G., B. Samori, U. Folli, and G. Torre, J. Phys. Collog. (Orsay, Fr.), C3-25 (1979).

35. Crone, T. A, and N. Purdie, Anal. Chem., 53, 17 (1981).

36. Thakkar, A. L., P. B. Kuehn, J. H. Perrin, and W. L. Wilham, J. Pharm. Sci., 61, 1891 (9172).

37. Harata, K., and H. Uedaira, Bull. Chem. Soc. Jpn., 48, 375 (1975).

38. Abu Shumays, A., and J. J. Duffield, Anal. Chem., 38, 29A (1966).

39. CARY-61 Manual, Varian Co. p. 11-7.

40. Lambert, J. B., Shurrell, L. Verbit, R. G. Cooks, and G. H. Stout, "Organic Structural Analysis", MacMillian, New York, (1976).

41. Salvadori, P., and F. Ciardelli in "Fundamental Aspects and Recent Developments in Optical Rotatory Dispersion and Circular Dichroism", D. Salvadori, and F. Ciardelli (eds.) Heydon, New York, p. 21 (1973).

42. Crabbe, P. "ORD in Chemistry and Biochemistry", Academic Press, New York, p. 21 (1972).

43. Chan, R. S., C. Ingold, and V. Prelog, Angew. Chem. Internat. Edit., 5, 385 (1966).

44. Crabbe, P. "ORD in Chemistry and Biochemistry", Academic Press, New York, p. 155 (1972).

45. Briat, B. in "Fundamental Aspects and Recent Developments in Optical Rotatory Dispersion and Circular Dichroism", D. Salvadori, and F. Ciardelli (eds.) Heydon, New York, p. 375-401 (1973).

46. Brown, G. Am. Scientist, 60, 64 (1972).

47. Sakamoto, K., R. Yoshita, M. Hatano, and T. Tachibana, J. Am. Chem. Soc. 100, 6898 (1978).

48. Van Metwe, J. P., Eastman Org. Chem. Bull., 45, 1 (1973).

49. Wong, K. Y., D. Songupta, and D. T. Makey, J. Phys. Chem. Solids, 35, 313 (1979).

50. Wong, K. Y., and N. P. Manson, J. Phys. Chem. Solids, 38, 1017 (1977).

51. Kuroda, R., and Y. Saito, Bull. Chem. Soc. Jpn., 49, 433 (1976).

52. Yang, M. C-L., and R. A. Palmer, J. Am. Chem. Soc., 97, 5930 (1975).

53. Kahn, P. C., and S. Beychock, J. Am. Chem. Soc., 90, 4168 (1968).

54. Sarko, A., and C. Fischer, Biopolymers, 12 2189 (1973).

55. Ito, N., and T. Takagi, Biochem. Biophys. Acta., 221, 430 (1970).

56. Bosnich, b., and J. Harrowfield, J. Am. Chem. Soc., 94, 3425 (1972).

57. Imanishi, A., and T. Isemura, J. Biochem., 65, 309 (1969).

58. Private communication with Dr. G. Mains, Dept. of Chemistry, Oklahoma State University.

59. Cohen, J., and J. L. Lach, J. Pharm. Sci., 52, 132 (1963).

60. Lach, J. L., and J. Cohen, J. Pharm. Sci., 52, 137 (1963).

61. Lach, J. L., and W. A. Pauli, J. Pharm. Sci., 55, 32 (1966).

62. Lach, J. L., and T-F. Chin, J. Pharm. Sci., 53, 69 (1964).

63. Pauli, W. A., and J. L. Lach, J. Pharm. Sci., 54, 1745 (1965).

64. Bender, M. L., and M. Domiyama, "Cyclodextrin Chemistry", Springer-Verlag, New York, (1978).

65. Saenger, W., M. Noltmeyer, P. C. Manor, B. Hingerty, and B. Klar, Bioorg. Chem., 5, 187 (1978).

66. Bender, M. L., and M. Domiyama, "Cylodextrin Chemistry", Springer-Verlag, New York, p. 24 (1978).

67. Bender, M. L., and M. Domiyama, "Cylodextrin Chemistry", Springer-Verlag, New York, p. 23 (1978).

68. Bender, M. L., and M. Domiyama, "Cyclodextrin Chemistry", Springer-Verlag, New York, p. 17 (1978).

69. Cramer, F., W. Saenger, and H-Ch. Spatz, J. Am. Chem. Soc., 89, 14 (1967).

70. Rohrback, R. P., L. J. Rodriquez, E. M. Eyring, and J. F. Wojeck, J. Phys. Chem., 81, 944 (1977).

71. Kaiser, H., Pure and Appl. Chem., 34, 35 (1973).

72. Flynt, D., O.S.B.I., personal communication.

73. Bowen, J. M., H. McMorrow, and N. Purdie, to be published.

74. Pirkle, W. H., and P. L. Rinaldi, J. Org. Chem., 45, 1379 (1980).

75. McMorrow, H., and N. Purdie, unpublished results.

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