CIRCULAR DICHROISM: AN ALTERNATIVE METHOD OF DRUG ANALYSIS

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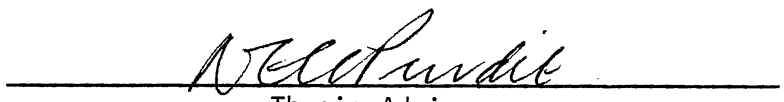

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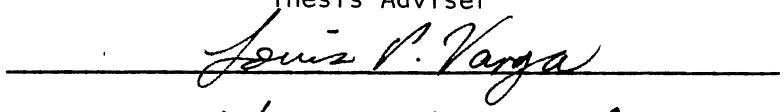

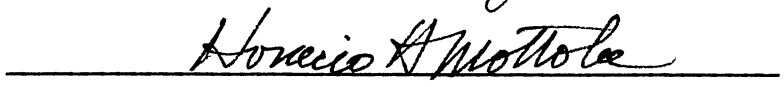

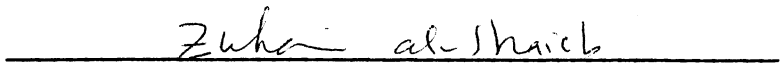

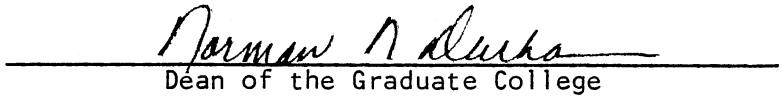

Dean of the Graduate College

PREFACE

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CHAPTER I

INTRODUCTION

Statement of the Problem

Drug abuse continues to be one of the major areas of concern to law enforcement officials. In 1980, there were approximately 7000 cases involving drugs in Oklahoma alone. Samples related to drug abuse account for approximately half of the man hours allocated in the state crime laboratories.[1] Police administrators and crime laboratory managers are constantly seeking new methods that will hasten the analysis of samples without a significant loss in accuracy and without error in their identification.

An investigation was undertaken to determine if circular dichroism (CD) spectropolarimetry could be utilized to prove positive identification of members of the opium alkaloid group and to determine if the spectra gave results which were quantitative and reproducible. Circular dichroism has often been used as a method for the qualitative determination of the absolute configuration of a chiral molecule whose identity is known, or to establish the position of a particular substituent on a molecule.[2,3] However, little has been done with CD in identification of anonymous molecules, either qualitatively or quantitatively. In retrospect, this is somewhat surprising, especially in cases where the suspected molecule is known to be chiral, as is the case for many drugs of abuse. No references were found in the literature which provided molar

ellipticity values for any of the drugs of abuse in aqueous media. Therefore, both qualitative and quantitative examinations were conducted in the present investigation.

Previously, the qualitative identification by CD of derivatives of the morphine group of the opium alkaloids in mixtures with KBr in pellets[4] and in a cholesteric liquid crystal solvent[5] have been investigated. The former method has not been shown to be quantitative while the latter is quite tedious and is quantitative only with excellent technique.

It has generally been thought that ultraviolet-visible spectrophotometry has limited application as an analytical tool because absorption bands, with a few exceptions, are broad and unstructured. On the other hand, the technique is both simple and quantitative. In the past, changes of solvent and of the pH of the solution have been used to modify the spectra.[6] This variation makes it possible to identify the group to which the confiscated drug sample belongs, but does not allow for specific identification of the drug. Siek et al.[7,8,9] have accumulated an extensive collection of UV absorption spectra for drugs and toxic substances in aqueous acidic and basic media and in ethanol.

In the experiments for this work, CD spectra have been obtained for several of the opium alkaloids dissolved in dilute acid, dilute base, and in a solution buffered to pH 8.6. Results are reported for morphine, nalorphine, 3-monoacetylmorphine (3 MAM), 6-monoacetylmorphine (6 MAM), 3,6-diacetylmorphine (heroin), codeine, dihydrocodeine, ethylmorphine, thebaine, hydrocodone, naloxone, oxycodone, oxymorphone, and hydromorphone. Concentration studies have been performed for most of these derivatives. The results from the experiments show that the spectra

obtained using CD are much more definitive than those obtained by conventional UV absorption spectroscopy. CD, therefore, should have more analytical potential than UV absorption.

Alternative Methods for Analysis

Prior to World War II, the primary methods of drug identification were titrimetry, microcrystal tests, color tests, and gravimetric methods. The first three types of tests are empirical and somewhat non-specific while the latter method is only used to assay pharmaceutical samples of known origin.

During the early sixties there was an emphasis on developing quicker and better methods of analysis, as the number of drug samples increased dramatically. This led to the development of instrumental methods for drug analysis such as IR and UV spectroscopy, gas and liquid chromatography, and ultimately gas chromatography/mass spectrometry.

Although some non-instrumental methods are still being used in most crime laboratories, they are gradually being replaced by instrumental methods. This is due in large measure to the demand for tests which give unique results for a drug. It is of paramount importance in the American jurisprudence system that a particular drug be distinguishable from others. Although many of the older tests give reactions which are obvious, their chemistry is not well understood. Even today the chemistry and physics of the instrumental methods are better known than those of microcrystal and color tests.

Physical Properties

Some advantage can be made of the physical properties of the drug

groups. Properties, such as solubility in dilute acids and bases and organic solvents, aid in a preliminary classification of drugs into three categories: acid soluble, base soluble, and neutral drugs.

The opium alkaloids possess the following structural and physical properties:[10]

1. A tertiary aliphatic amino group
2. Several contain a phenolic hydroxyl group
3. All are weak bases (pKa = 6.6 - 9.5)
4. All are somewhat soluble in organic solvents.

The phenolic substituted narcotics are soluble in strong aqueous alkali solutions, a consequence of the ionization to form the phenolate ion. This property can be useful in separating the members of the codeine group from those of the morphine group by extraction from a basic medium into chloroform. The members of the codeine group are extractable into chloroform while those of the morphine group are not.

Several authors[11,12,13] have cited various extraction procedures which are used to concentrate the drug when it is known to be present in urine, blood or plasma, and also to reduce interferences.

A necessary preparation step which precedes identification by instrumental means is to separate the drug from diluents and binders. This is true regardless of whether the sample is a pharmaceutical preparation or is of clandestine origin. Typically, this is accomplished by an organic solvent extraction followed by a back extraction into an acidic aqueous medium. More recently other methods, both instrumental and noninstrumental, are being developed in order to replace aqueous extraction procedures as the means of separation.

For example, the introduction of XAD-2 resin has added a new dimension to drug extraction and concentration procedures.[12,14-16] Large samples of urine can be extracted rapidly and therapeutic levels of narcotics, barbiturates, or tranquillizers detected. In this procedure water soluble organic species are adsorbed onto the non-ionic copolymer structure and are subsequently eluted with an appropriate organic solvent. The resin can be used either in the form of a column or as a slurry. The method is often used in conjunction with thin layer chromatography (TLC), which is useful in separating the drugs of interest. Alumina or Fluorosil can later be used to further "clean up" samples. However, loss of a portion of the drugs of interest might result.[17]

Cation exchange has also been used to isolate morphine from opium, illicit narcotic mixtures, pharmaceutical preparations, and toxicological extracts.[23] Anion exchangers have also been used for the analysis of narcotics. In the process, the salts are converted into free bases, which are eluted with an organic solvent.

Paper chromatography has also been used to a lesser extent. It does not possess the flexibility of some of the other methods. Nakamura[24] was able to successfully separate morphine from 3- and 6-monoacetylmorphine, 3, 6-diacetylmorphine, and codeine.

Thin Layer Chromatography

Thin layer chromatography (TLC) is one of the most, if not the most, widely used non-instrumental analytical tools for the separation and identification of drugs and their metabolites[11,12,16-20] Separation schemes are important for analysis of most of the drugs since each drug may have several metabolites or the drugs are present in combination.

Masoud[21] devised a separation scheme for most of the common illicit drugs which combined R_f values obtained from the study of various solvent systems with chromogenic reactions with spray reagents for the purpose of identification of the drugs present. However, since R_f values can change from day to day, it was necessary to run standards simultaneously with the unknowns.[22] Sometimes the material was spotted and developed in duplicate[20] for both visualization by spray reagents and quantitation by UV spectroscopy. Drug levels of 0.1 mg/ml were detectable by UV spectroscopic analysis of the solution.

One approach for the separation of compounds with close R_f values is to expand TLC to two-dimensional chromatography, using two different solvent systems. The preparation of dansyl derivatives resulted in the ability to detect picogram quantities of drugs.[12]

Several of the drugs fluoresce under UV light, which aids in the location of the spots. Quinine, a common adulterant with morphine, can be successfully separated and quantitated by detecting its fluorescence at 254 nm.

An alternate procedure is to separate the drugs on TLC plates which have been impregnated with a fluorescent binder. The drugs appear as dark spots on a bright background.

Color Tests

Color tests serve a vital role in the identification of drugs. They can be either in the form of spray reagents for preliminary identification of drugs separated by TLC or in the form of spot tests for small quantities of the suspected drug. The number of spray reagents used for chromogenic detection are probably more numerous than the number of

solvent systems which have been tried for separations by TLC. For example, Taylor[23] has listed 25 reagents commonly used in conjunction with TLC and their expected color reactions. It has been shown[25,26] that some spray reagents react only with specific functional groups, thus aiding in a classification of the unknown drug with a specific drug group. A few examples of drug groups and spray reagents used to identify those groups are: Zwikker's test for barbiturates; the cobalt thiocyanate reagent for cocaine; and the Duquenois-Levine reagent for THC and other cannabinoids. Spot tests are usually performed on 1-2 mg of the solid, although the drug can be extracted from urine, evaporated to dryness, and tested.

The three most commonly used spot tests for members of the opium alkaloid groups are the Marquis, the Mecke, and the Frohde reagents. The Marquis test yields shades of purple for all opiates, whereas the other two yield various shades ranging from green to brown.[23]

Although color tests and spot tests are quick and fairly easy to perform, Masoud[26] listed three problems inherent to them. These included the occurrence of false positives or negatives, their lack of specificity, and the difficulty of interpreting some results. One author[21] stated that multiple TLC analyses with chromogenic detection of the drug was equal to IR spectroscopy in relevancy, analytical power, and utility. However, most authors listed spot tests only as screening tests.

Overspraying the TLC plate with successive spray reagents is possible, although care must be observed as to the order in which they are used. Bastos et al.[25] devised a separation scheme for drugs using TLC

in conjunction with successive spray reagents, whereby over 100 drugs were classified and tentatively identified.

Microcrystal Tests

In its simplest form a microcrystal test is performed by adding one drop of reagent to one drop of test solution on a microscope slide, stirring and observing the crystal formation through a microscope. The volatility of the test solution is sometimes a problem. This has been overcome, in part, by the hanging microdrop technique.[23]

It is generally agreed that microcrystal tests, although more sensitive and specific than color tests, are unsuitable as a primary means of identification of unknown compounds because they cannot be incorporated into a basis for an identification scheme. That is, a given class of compounds does not give a unique set of crystal formations. Also, sometimes crystal formations are not stable with time. Temperature, concentration, and pH changes, as well as impurities, may cause variability in crystal formation.[18,23] Several hundred reagents have been used for the precipitation of alkaloids in microcrystal tests. However, a small fraction of that number is all that is necessary to carry out meaningful drug analyses. Most microcrystal reagents are halides of heavy metals and organic acids or their salts.[23] These reagents have become much more expensive in the past few years.

Most microcrystal tests are performed by comparing the results for a standard of the drug with the results obtained from the sample under approximately the same growing conditions. Microcrystal tests are often technique sensitive and thus require extensive training before an analyst can become proficient in their use.

Gas Chromatography

The most widely used instrumental method is probably gas chromatography (GC). GC had been found by some to be impractical for screening procedures for biological fluids, but has often been used for a confirmatory test.[12] Usually, extraction and concentration steps are necessary before placing the sample on to a GC column. Otherwise, the front of the column would soon be plugged with involatile salts and proteins.

However, Dezan and Fasanello[28] have shown that, by using an internal standard, they could quantitate illicit samples of heroin by hydrochloride without prior extraction. Narcotics are usually separated on a nonpolar column, such as 1-2% SE 30. Care must be taken in choosing the proper column packing material. In many instances compounds separated with difficulty on SE-30 were readily separated on the more polar stationary phases.

One commonly encountered example of this is morphine, which is difficult to elute in a reasonable amount of time at a nondestructive temperature.[29,30] Opiates in biological fluids, especially after they have been metabolized to the glucuronide, are difficult to elute from most GC columns, due to the higher polarity of the molecule in this state.[31] Acid or enzyme hydrolysis can be used to hydrolyze the sample, followed by a derivatization of the sample.

Other problems associated with gas chromatography are the lack of identification capabilities and the necessity to recalibrate daily. The first problem can be alleviated somewhat by analyzing a duplicate sample at a different temperature or on a column with a slightly different polarity.[31] Since there are often only small differences in retention times between similar drugs, it is also necessary to analyze standards

along with the samples. In order to compare results from one laboratory to the next, relative retention times are often used with codeine having an arbitrary RT of unity.[12,13,30]

The discriminatory power of the GC is increased by preparing anhydride derivatives. Relative retention times are increased for any narcotics which can be derivatized.[12,13,32] Two fluorinated derivatives, heptafluorobutyric anhydride (HFBA) and pentafluoropropionic anhydride (PFPA) have been found to be useful in the improvement of sensitivity. These have been used in conjunction with an electron-capture detector to improve sensitivity to 40-200 pg.[12]

Mass Spectrometry

Mass spectrometry (MS), especially coupled to gas chromatography, has increased in popularity in applications to the field of drug analysis. The most common form of ionization is still electron impact (EI), although chemical ionization (CI) and chemical exchange ionization (CE) have proven to have greater potential for drug analysis. Complex spectra caused by severe fragmentation is the greatest drawback to mass spectrometry using EI as the ionization source. Clandestine samples are often composed of a mixture of drugs. Unfortunately, the spectra of these mixtures cannot readily be separated to determine the various components in the mixture. Chemical ionization and chemical exchange techniques, which impart a much lower ionization energy to the sample, cause less fragmentation of the sample when compared with EI. In fact, the parent peak is usually the major peak, with perhaps a few other less intense peaks. Usually the identities of the components of the mixture can be deduced from these spectra.[33] However, when soft ionization

mass spectrometry techniques are used, there is a loss of sensitivity. CE is only about 5% as sensitive and CI only 25% as sensitive as EI.[34] The sensitivity is often still enough to allow identification of most drugs found in illicit mixtures.

Gas Chromatography/Mass Spectrometry

Usually mass spectrometers are interfaced to gas chromatographs in crime laboratories. This accentuates the strong points of both methods, i.e., the separation powers of gas chromatography with the identification capabilities of mass spectrometry. First, any drugs of sufficient volatility are separated by gas chromatography. Then, the substance giving rise to each peak is identified by EI or CI mass spectrometry. Smith[35] separated the components of opium samples by GC/MS. He was able to produce mass spectra which identified morphine, codeine, papaverine, and thebaine. However, thebaine eluted poorly and was thought to have undergone some type of thermal rearrangement. In contrast solid probe analysis of material separated by TLC gave a mass spectrum which was very similar to that from a thebaine standard. Other investigators[36] were able to identify and quantitate 2 ug samples of street heroin.

Finkle and Taylor[37] compiled a reference data system in 1972 for phenethylamine derivatives, barbiturates, opiate and synthetic narcotics, and urine metabolites. All spectra were coded as to peak maxima and the information stored in a computer index system, alphabetically, by drug name, and by the base peak location. This index has been expanded and updated to include 450 drugs and metabolites.[38] Experimentation has begun on the coupling of liquid chromatography to mass spectrometry.[39] The only drug reported, strychnine, is identifiable from the observed

mass spectrum. Many problems remain to be worked out before the technique can become an efficient method for drug analysis.

High Pressure Liquid Chromatography

One of the latest techniques applied to drug identification is high-pressure liquid chromatography (HPLC). Liquid chromatography avoids the two problem areas encountered in the use of gas chromatography for drug analysis, that is, the low volatility and tendency of the drugs to undergo thermal decomposition. Also with HPLC, the polarity of the mobile phase is easily changed, which changes the retention properties of the column. Wu and Siggia[40] demonstrated that better resolution and shorter retention times could be obtained for some opium alkaloids by the dynamic coating technique. Ziegler et al.[41] indicated that no precolumn was necessary in cases where alkaloids were first trapped on an amberlite XAD-2 resin and then eluted with methanol. They used both calibration curves and internal standards in an effort to obtain quantitative results. Usually isocratic conditions were chosen rather than a gradient elution technique.[42]

Often, it is advantageous to use a fluorimetric detector rather than a conventional UV detector. Fluorimetric detection of LSD and morphine can improve the sensitivity up to 100-fold.[42] Peterson et al.[43] have established that morphine, as well as other drugs, can be separated by HPLC and quantitated amperometrically if there is a 3-hydroxyl group present on the drug. Compounds such as codeine, which do not have the 3-hydroxyl group present, give no signal. Thus, this technique is not only useful for quantitative information, but also aids in the identification of the various substituents in the mixture.

Ultraviolet Spectroscopy

UV spectroscopy is recognized as one of the most important methods for the preliminary identification of drugs of abuse. The spectra obtained by this method have a fairly simple structure, which usually allows identification of the drug group to which the confiscated sample belongs. As previously mentioned, Siek and coworkers[7,8,9] have compiled a significant number of drugs into a reference index. These UV spectra, catalogued by the drug groups, show the similarities of spectra among drugs which belong to the same group. Clarke[44] and Sunshine[6] have also made significant contributions in the compilation of UV spectra.

A quantitative assay of the drug can usually be obtained once the drug has been identified and separated from other substituents. The one prerequisite for UV spectroscopy is that the drug possess a chromophore which will absorb light in the UV region. Fortunately, most drugs, including all of the opium alkaloids, contain such a chromophore. However, there is a great deal of similarity among their spectra, making positive identification of a particular drug almost impossible. Some degrees of deconvolution of spectral bands can be achieved by derivative spectroscopy.[45,46] Second and even higher derivatives enhance the band resolution and reveal hidden bands. Although it is useful to attempt to correlate band shape to molecular structure, the primary intent is to obtain new parameters for solute identification. Also, changes of solvent and pH have been used to modify the electronic structure, often resulting in a change in the position of the wavelength at maximum absorption. These shifts have been catalogued for transitions associated with particular chromophores in the molecule.[47] This principle has been

especially useful in helping to identify the opium alkaloids by UV spectroscopy. Deconvolution of the absorption bands present in the UV region by CD, if possible, should make identification of individual opium alkaloids easier.

Infrared Spectroscopy

Infrared spectroscopy has been called the "fingerprint" technique for drug identification. Few methods have the specificity that this method affords. IR is often used as the final method for the identification of an unknown compound.[48]

The method has one inherent problem. In order to obtain reproducible spectra, the sample must be purified and be in the same state (free base, salt, amorphous) as the drug sample used to obtain the reference spectrum. Functional group characteristics can be assigned for the IR spectrum of the unknown, but major bands present must be compared to those in a reference spectrum of the drug before positive identification can be obtained. A fractional percentage of impurities can smear the bands and make identification difficult. Impurities at the five percent level can make identification of the sample impossible by IR spectroscopy.

Clarke[44] and Sunshine,[6] as well as others, have compiled infrared spectra with indices for drug-related compounds. The drugs are indexed by the major band positions and coded for comparison with other spectra obtained at a later date.

Immunoassay Techniques

Some of the newest methods for drug identification involve immuno-

assay techniques. These include radio immunoassay (RIA), enzyme multiplied immunoassay (EMIT), free radical assay (FRAT), and hemagglutination-inhibition (HI) techniques. Each of these methods incorporate the concept of a competition between labeled haptens (drugs) and free haptens for complexation sites on the antibody. However, the method for detecting the bound drug varies from one method to the next.

Some of the advantages of these techniques are as follows:[12]

1. Detection of drugs directly in biofluids.
2. High degree of sensitivity.
3. Tests are easily and quickly performed.
4. Procedures lend themselves toward automation.

However, there are two particular (or principle) disadvantages:

1. There is a lack of specificity for several of the tests (Cross reactions occur).
2. The cost of the test per drug is rather expensive, since there must be individual test kits prepared for each drug or each class of drugs.

Spector and Seider,[49] using a RIA technique, found that codeine was just as sensitive to the antibody as morphine. Other researchers[50] employing the EMIT and FRAT techniques have also arrived at the same conclusion. It was also discovered that heroin and morphine glucuronide, as well as higher concentrations of nalorphine and meperidine, give reactions similar to morphine. It is a fortunate result that the test is sensitive to morphine glucuronide, since this is the major metabolite for heroin. However, further tests must be performed to confirm the presence of a particular opiate. For all of these techniques it is

necessary to prepare standard calibration curves using known amounts of the pure drug in the same concentration range as that of the unknowns.

It appears that these techniques are being used more and more by medical technologists and researchers while being shunned somewhat by crime laboratories. The preference reverts back to the lack of specificity, which necessitates the performance of a confirmatory test for the crime laboratory before positive identification of a particular drug is complete.

CHAPTER II

THEORY OF OPTICAL ACTIVITY

The Discovery of Optical Activity

Biot (1812) discovered that quartz crystals rotated a plane of linearly polarized light (51) and subsequently came to the conclusion that several other materials also were found to possess the same properties. Fresnel (52) attributed this optical rotation, α, to the difference in refractive indices for left, n_ℓ and right, n_r circularly polarized light.

Pasteur, in 1848, separated a racemic mixture of double sodium ammonium paratartrate under an optical microscope and showed that the right hemihedral crystals rotated plane polarized light to the right, whereas the left hemi-hedral crystals rotated plane polarized light to the left to the same degree. He recognized that a chiral crystal structure arose from the chirality of the molecules themselves and that the resultant optical activity could have contributions both from the inherent molecular activity and the chirality of the crystals (53).

Using crystals of amethyst quartz, Haidinger (54) showed that if $n_\ell > n_r$ for circularly polarized light at wave lengths where the optically active medium is transparent, the absorption index, k, should be larger for left than for right circularly polarized light at absorption frequencies. André Cotton, in 1895 made similar observations regarding

solutions of copper(II) and chromium(III) (+)- tartrate (55). It was Cotton who demonstrated that the special shape of the optical rotation (ORD) curves of optically active solutions at absorption bands was due to the differential absorption of left and right circularly polarized light, that is, circular dichroism (CD). Both anomalous dispersion and circular dichroism have come to be known as "Cotton effects" (56).

Theoretical Advances

Fresnel, in 1825, developed the first phenomenological theory of optical activity. Soon afterward, Maxwell developed the fundamental equations for the electromagnetic theory of light on which all classical arguments of optical phenomena rests.

Other important theoretical advances were Kuhn's (57) Coupled Oscillator Theory and Rosenfeld's (58) development of the quantum origin of optical activity. Others, including Condon (59), Kirkwood (60), Moffitt and Moscowitz (61) and Schellman (62) made further adaptations of Rosenfeld's theories to better correlate molecular interaction with light based upon spectroscopic evidence. Tinaco (63) then extended the quantum theory of optical activity to cover both dynamic and static coupling and to give the quantum theory more general applicability.

The semiclassical approximation usually gives fairly accurate results, as shown by some experimental results from a fairly recent paper by Moscowitz (64). For a more in-depth discussion of the history of optical spectroscopy, the reader is referred to the recent monograph by Charney (65) or the excellent discussion on optical rotatory power by Mason (66).

Correlation of Optical Activity

Any beam of light has electric and magnetic fields at right angles to each other and perpendicular to the direction of propagation of the beam. The interaction of these fields with electromagnetic radiation produces various phenomena termed "spectroscopy".

From a theoretical standpoint, there is a definite correlation between optical absorption, circular dichroism and optical rotatory dispersion. The first two are related by g, the "anisotropy factor" (67), while the strength of ORD is related to the CD absorption spectrum by the Kronig-Kramers transforms, both of which will be discussed in greater detail in a later section.

Rotatory Dispersion

Unpolarized light may be described as an array of plane waves that are randomly oriented with respect to a plane perpendicular to the direction of propagation. The electric field of each plane wave in this array is represented by a vector whose amplitude is given by

$$E = E_o \cos 2\omega \left(t - \frac{nz}{c}\right) \qquad (1)$$

where E_0 is the maximum amplitude of the wave, ω is the angular velocity of the wave at time t, n is the refractive index in a transparent region, z is the pathlength of the medium in centimeters and c is the velocity of light in a vacuum.

This plane wave is usually depicted as oscillating in a sinusoidal manner with a maximum amplitude of E_0 and a periodicity of 2π. Such a wave is shown in Figure 1.

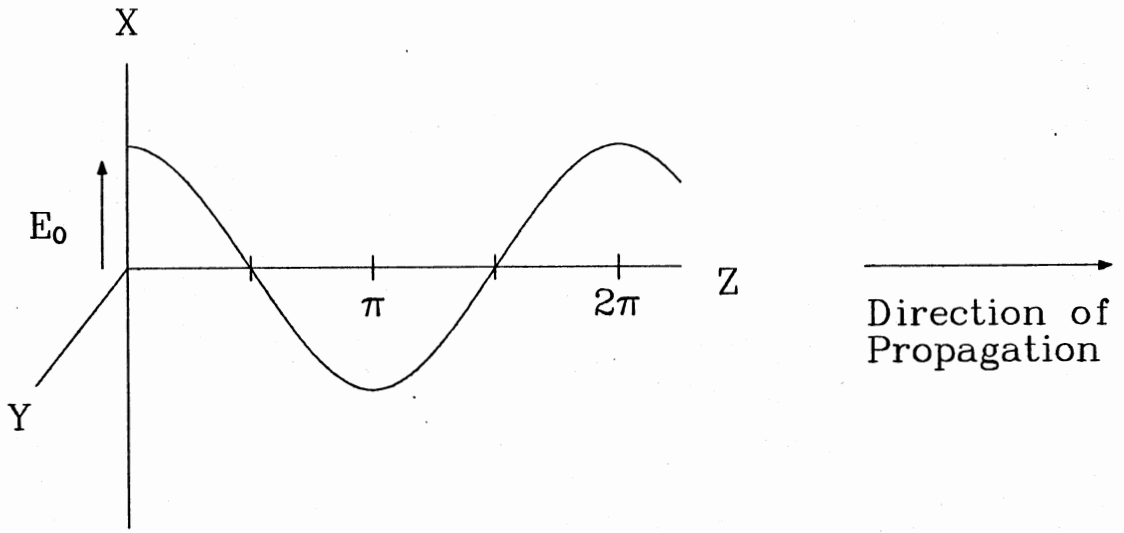


Figure 1. Electromagnetic Field Component E
of an Electromagnetic Wave

The wavelength is related to the frequency of the light by $\omega = c/\lambda$, if λ is measured in vacuum.

Plane polarized light may be resolved into two circular components with equal amplitudes. At any given time, the envelope of the amplitudes has the form of a right-hand helix about the direction of propagation for right circularly polarized light (Figure 2) and of a left-handed helix for left circularly polarized light.

A simple description of the two waves is given by

$$E_r = E_o (i \cos\alpha_r - j \sin\alpha_r) \tag{2}$$

$$E_l = E_o (i \cos\alpha_l + j \sin\alpha_l) \tag{3}$$

and their vector sum can be shown to be

$$E = E_o \, i \cos\alpha \tag{4}$$

where

$$\alpha = 2\pi\omega\left[t - \frac{nz}{c}\right] \tag{5}$$

and i is the unit vector in the direction of the x axis.

At any given point in time, the vector amplitudes appear, to an observer looking toward the direction of propagation, to be rotating clockwise for right and counter-clockwise for left circularly polarized light (Figure 3). Initially, the two circularly polarized light waves have the same angular velocity in opposite directions. After entering an isotropic medium (one having a single refractive index) both components are decelerated to the same extent. Upon emerging from the medium,

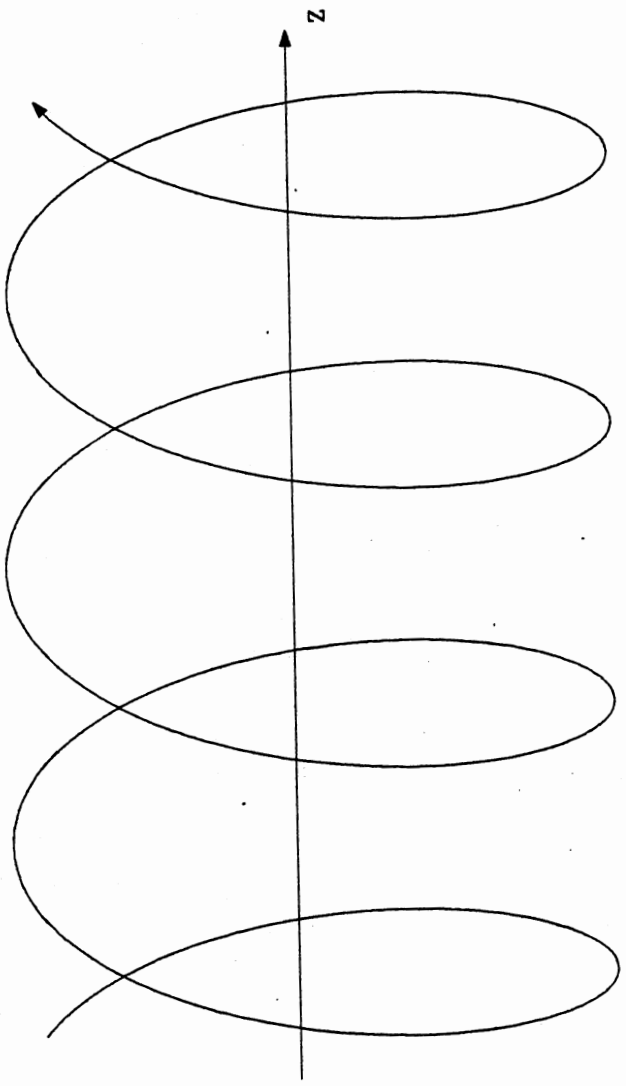


Figure 2. Right Circularly Polarized Light

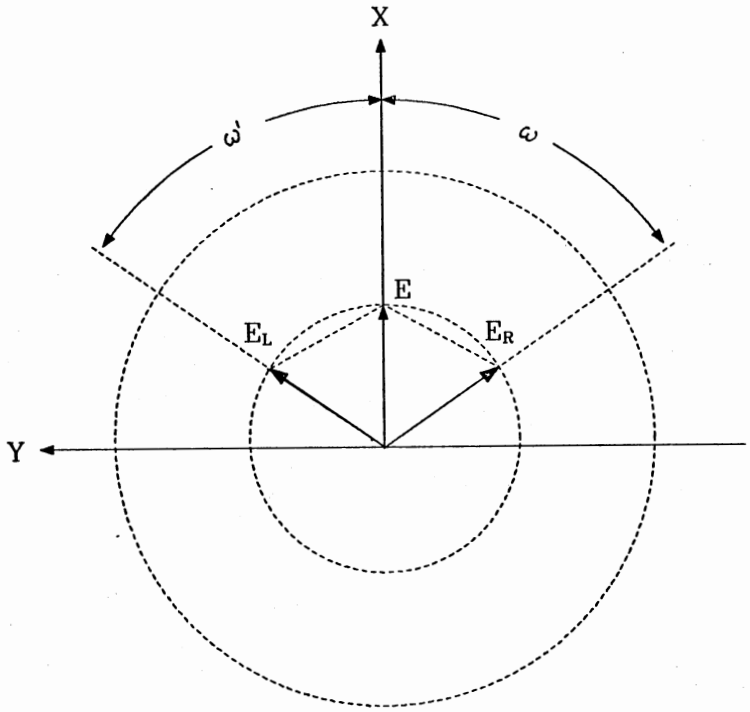


Figure 3. Projection of Right and Left Circularly Polarized Light and the Resulting Field Vector E

they are still in phase and the vector summation of the two components results in a plane polarized light wave which has undergone no rotation. However, if the indices of refraction are different for the two beams, one of the components will lag behind the other, the lag being proportional to the difference in the two indices of refraction and the length of the path through the material and inversely proportional to the wavelength of the light, as shown by Fresnel (52). Thus, it has been shown (66) that:

$$\alpha = \frac{\pi z}{\lambda} (n_{\ell} - n_r) \tag{6}$$

where α is the angle (in radians) by which the plane polarized wave has been rotated in transversing the optical medium of thickness z (measured with the same units as λ) and λ is the wavelength of the incident light.

To obtain the specific rotation $[\alpha]$, where $[\alpha]$ is in the more common experimental units of degrees per decimeter, π radians is replaced by 180 degrees and z cm by 1 dm, resulting in

$$[\alpha] = \frac{\alpha}{c} \cdot \frac{1800}{\pi} \tag{7}$$

or,

$$[\alpha] = \frac{1800}{\lambda C} (n_{\ell} - n_r) \tag{8}$$

where C is the concentration in grams/cm^3. The molecular rotation, Φ, is defined as

$$\Phi = \frac{[\alpha] \cdot M}{100} \tag{9}$$

where M is the molecular weight of the optically active substance. The molecular rotation is used for comparison of rotations of different substances on a mole-to-mole basis (79).

Circular Dichroism

After passing through an optically active medium, both the left and right polarized vectors not only exhibit circular birefringence due to the difference in indices of refraction, but are differentially absorbed as well. Thus, in the spectral region in which optically active absorption bands are present, the length of vector E_R is no longer equal to E_L, and the resultant vector, E, traces out an ellipse rather than a circle, as seen in Figure 4.

However, the major axis, a, is the sum of the amplitudes of both circular components upon emerging from the optically active medium while the minor axis, b, is the difference of the two components. Thus,

$$\tan\psi = \frac{E_R - E_L}{E_R + E_L} \tag{10}$$

The right side of Equation 10 can be shown (65) to be equal to the hyperbolic tangent, $\tanh \pi (K_L - K_R)Z/\lambda$. The hyperbolic tangent of ψ is very nearly equal to ψ for values of ψ less than one. The measured ellipticity is very rarely greater than one because the difference in the absorptions indices, $K_L - K_R$, is rarely greater than 10^{-6}. Consequently,

$$\psi = \frac{\pi Z}{\lambda} (K_L - K_R) \tag{11}$$

measured in radians.

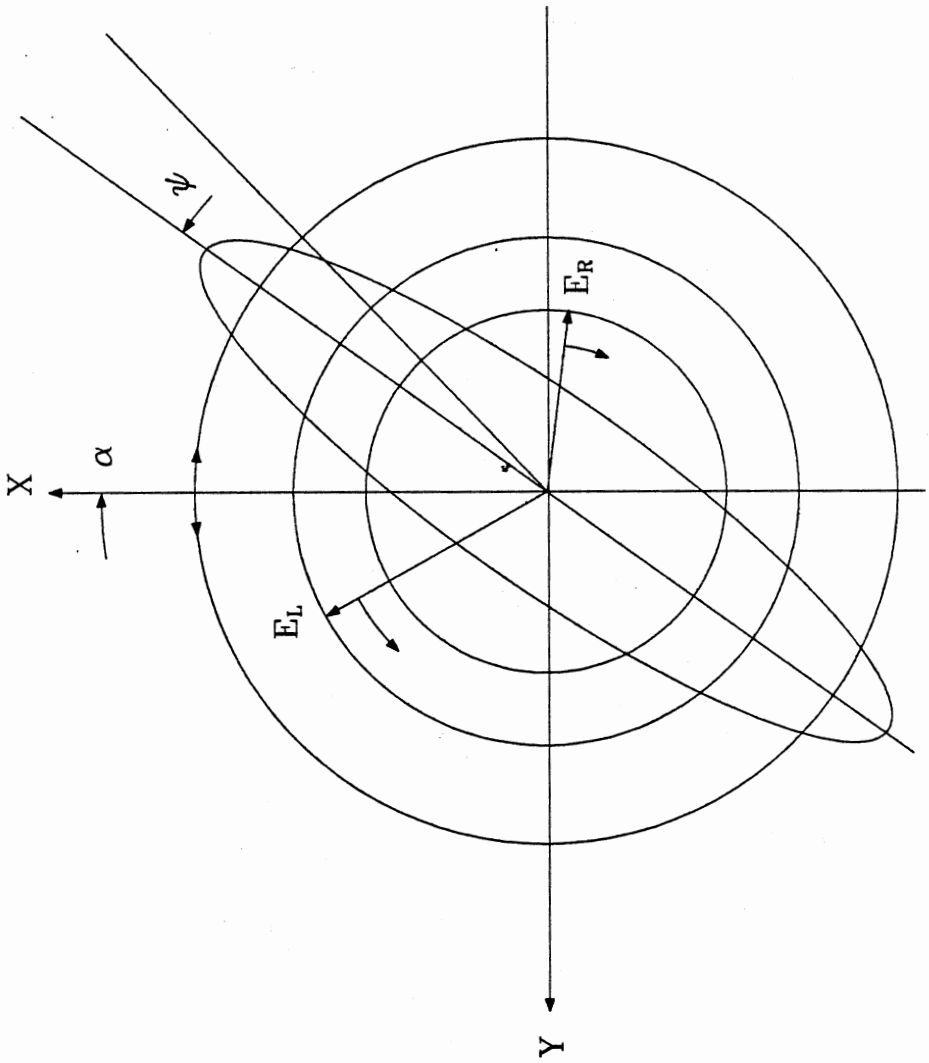


Figure 4. Elliptically Polarized Light

The decadic molar extinction coefficient is related to the absorption index (65) by Equation 12.

$$k = \frac{2.303\lambda\ CE}{4\ \pi} \tag{12}$$

where C is the concentration in moles per liter. From Equations 11 and 12.

$$k = \frac{2.303\ Cz}{4\ \pi}\ (E_L - E_R) \tag{13}$$

By definition,

$$\Delta\varepsilon = E_L - E_R \tag{14}$$

thus,

$$\psi = \frac{2.303\ Cz}{4\ \pi}\ \Delta\varepsilon \tag{15}$$

The generally preferred unit of measurement is degrees of ellipticity, θ, which is obtained by multiplying by the number of degrees per radian:

$$\theta = \psi\ \frac{360}{2\ \pi} \tag{16}$$

By substituting Equation 15 into Equation 16,

$$\theta = 32.95 \cdot Cz\ \Delta\varepsilon \tag{17}$$

(in degrees). The molar ellipticity, $[\theta]$, is defined as the degrees of ellipticity divided by the molarity of the solution and the path length in centimeters.

$$[\theta] = \frac{32.95\ Cz}{Cz}\ \Delta\varepsilon = 32.95\ \Delta\varepsilon \qquad (18)$$

Correlation Between Molar Ellipticity and Molecular Ellipticity

Following the same convention as optical rotatory dispersion, the specific ellipticity (68) is defined by Equation 19.

$$[\psi] = \frac{\psi}{l \cdot c} \qquad (19)$$

where ψ is measured in degrees, l is the pathlength in decimeters, and c is the concentration in grams per cubic centimeter of solution.

Similarly, the molecular ellipticity (20) is defined as

$$\theta_M = \frac{[\psi] \cdot M}{100} \qquad (20)$$

where the resulting molecular ellipticity is in units of degrees-centimeters squared per decimole. It is unfortunate that the notation $[\theta]$ was chosen for the molecular ellipticity (64) since the brackets usually denote a measurement having a molar concentration. However, henceforth in this thesis, the notation $[\theta]$ will be used to denote the molar ellipticity, as previously defined.

The transformation from degrees-liters per centimeter-mole to degrees-centimeter square per decimole can be easily made, resulting in Equation 21.

$$\theta_M = \frac{100}{Cz}\ \theta = 100[\theta] \qquad (21)$$

Thus the value obtained for the molecular ellipticity, θ_M, is always one

hundred times the value of the molar ellipticity. This fact is also easily verifiable from the usual equation given for the molecular ellipticity (64).

$$\theta_M = 3300\ \Delta\varepsilon, \tag{22}$$

an answer one hundred times greater than that in Equation 18. Charney (70) reached the same conclusion, although there was no mathematical proof given. Since the equations to determine the molar ellipticity were derived from the basic equations of optical activity, they give an answer which has the proper order of magnitude.

Molecular Origin of Optical Activity

The perturbations involving the interaction of molecules with a light wave (absorption, dispersion, optical rotatory dispersion and circular dichroism) have a common phenomenological origin (71, 72). Some of the more important equations regarding these interactions will be given, although the derivation of these equations is beyond the scope of this thesis.

Stereochemical Requirements

There are three basic stereochemical symmetry conditions that lead to optical activity (68). To have optical rotatory power the molecule should not possess a center of inversion, a plane of symmetry or an alternating rotating-reflexion axis of symmetry. For ORD, measurements of optical activity can be made and interpreted outside of absorption regions by means of Drude's equation (73). However, circular dichroism measurements can only be made if there is a chromophore present in the

spectral region under investigation. Thus, ORD measurements can render information about absorption bands outside the range of the instrument. On the other hand, CD measurements are usually more easily interpreted, since there is less overlap of absorption bands and often additional absorption bands are apparent that are "hidden" in the absorption and ORD curves.

There are three different types of optically active chromophores, the designation of which sometimes overlap (74).

1. The inherently disymmetric chromophores, which include non-planar aromatic substances and twisted conjugated systems, such as twisted biphenyls.

2. The coupled oscillators formed by two non-conjugated chromophores such as homo-conjugated dienes and β, γ-ketones.

3. The perturbed symmetrical chromophores, such as a double bond, a saturated carbonyl or aromatic ring.

The Interaction of Polarized Light With Matter

Both absorption and circular dichroism phenomena arise due to charge displacements induced by a perturbing light wave. Such movements of charge cause induced and electrical dipoles. The rotational strength, R_K, is related to these induced electric and magnetic dipoles by Equation 23.

$$R_K = \mu_e^k \cdot \mu_m^k = \mu_e^k \, \mu_m^k \cos\gamma \qquad (23)$$

where μ_e^k and μ_m^k are the electric and magnetic dipoles, respectively, in c.g.s. units, and γ is the angle between the vectors of these two components. If a molecule possesses either a center of inversion or a

reflection plane of symmetry, the rotational strength will be equal to zero, and there will be no optical activity. Hence, the rotational strength is a direct reflection of the asymmetric bond strengths surrounding a molecule. From an empirical viewpoint, the rotational strength may be obtained from the area under the corresponding CD absorption band (75):

$$R_K = \frac{3\hbar c}{8\pi^3 N_i} \int_o^\infty \frac{\theta_k(\lambda)}{\lambda} d\lambda \qquad (24)$$

where R_K is the rotational strength in c.g.s. units, $\hbar$ is Planck's constant, c is the speed of light in a vacuum, N_i is the number of absorbing molecules per cubic centimeter and $\theta_k(\lambda)$ is the ellipticity at a given wavelength.

Similarly, the dipole strength, D_k, is proportional to the area under the absorption band (76):

$$D_K = \frac{3\hbar c}{8\pi^3 N_i} \int_o^\infty \frac{K_K(\lambda)}{\lambda} d\lambda \qquad (25)$$

where the dipole strength is in c.g.s. units and K_K is the absorption coefficient for the Kth transition at a given wavelength.

The classical oscillator strength, f, giving the number of electrons promoted in the transition responsible for the absorption band (therefore, no units) is related to the dipole strength by the expression:

$$f_K = 0.476 \times 10^{30} \nu D_K \qquad (26)$$

where ν is the frequency of the Kth transition.

The ratio of the strength of the CD band to the absorption band is

called the anisotropy factor, g, which also includes shape factors for both bands. If both transitions are approximated by Gaussian curves, then:

$$g = \frac{4nR_K}{\beta D_K} = \frac{\Delta\varepsilon}{\varepsilon} \text{ (by definition)} \tag{27}$$

Where,

$$\beta = \frac{n^2+2}{3} \tag{28}$$

which yields

$$g = 4n \frac{R_K}{D_K} \tag{29}$$

if the refractive index, n, is approximately unity. The greater the transition is magnetically allowed, relative to the dipole strength, the greater will be the anisotropy factor and the greater the difference in the wavelength of the CD maximum and the absorption maximum.

Finally, the Kronig-Kramers transform is used to go from the absorptive property, circular dichroism, to the dispersive property, optical rotation. The form of the transform is dependent upon the CD band shape (22). For Gaussian bands the Kronig-Kramers transform is (77):

$$M_{\theta,m} = \frac{48\ B\ N_i\ \lambda_m R_K}{\hbar c\ \Delta m} \left[\exp[-X^2] \int_o^X \exp X^2\, dX + \frac{\Delta m}{2(\lambda+\lambda_m)}\right] \tag{30}$$

where λ_m is the wavelength of the CD maximum, Δm is the exponential half-width, $X = (\lambda-\lambda_m)/\Delta m$ and the other terms have the same meaning as

when they were previously defined.

As a corollary to the Kronig-Kramers theorem, an equation has been proposed to relate the molecular amplitude, a, of a rotatory dispersion curve to the dichroic absorption, $\Delta\varepsilon$, of a circular dichroism curve:

$$a = 40.28\ \Delta\varepsilon \tag{31}$$

where a has been defined as the difference in the molecular rotation at the extremum of longer wavelength $[\Phi_1]$, and the molecular rotation at the extremum of shorter wavelength, $[\Phi_2]$, divided by 100, as shown in Equation 32.

$$a = \frac{[\Phi_1] - [\Phi_2]}{100} \tag{32}$$

In terms of the molar ellipticity, $[\theta]$

$$a = 1.22\ [\theta] \tag{33}$$

However, it should be remembered that these relationships in Equations 31 and 32 should only be regarded as semi-quantitative, since they were obtained for the $\pi^* \leftarrow n$ transition of saturated carbonyl chromophores and should only be used with caution for other chromophores (74).

CHAPTER III

EXPERIMENTAL

Instrumental

All of the spectral data were measured on a Varian Cary 61 Spectropolarimeter. This instrument was modified for differential measurements by the addition of the Cary Model 6101 Difference Accessory, which allowed for the direct subtraction of the CD spectra of either solvent or solution background. The instrument has a wavelength range from 185 to 800 nm and a sensitivity from 0.01 to 2.00 degrees full scale over a 25.4 cm (10 in.) chart, which should allow for a deflection as small as 2×10^{-5} degree ellipticity to be observed. However, this degree of sensitivity was not obtained in actual experimental work due to instrumental deterioration with age and to a drift in the baseline with time. Baseline potentiometers were reset daily in an effort to achieve a flat baseline. A calibration spectrum of ellipticity versus wavelength was made for the baseline and used to correct experimental data, if necessary. For most compounds, a full scale value of 0.1 or 0.2 degree ellipticity was sufficient for accurate measurements.

The instrument was purged with nitrogen gas, boiled, from a liquid nitrogen Dewar. A nitrogen purge is required to prevent the accumulation of ozone in the instrument caused by the absorption of UV-radiation by atmospheric oxygen external to the 450 W high-pressure xenon arc lamp, which is the source of the incident radiation. Wavelength calibra-

tion was performed using a standard fluorescent lamp. The ellipticity scale was calibrated at two wavelengths using the CD maxima recommended for a 0.10% W/W solution of recrystallized D-10-camphorsulfonic acid in distilled water and a 0.05% by W/W solution of reagent grade D-pantoylactone in distilled water. The former solution has an electronic transition with a recommended ellipticity for calibration equal to +0.31 degree at 290 nm for a 1 cm path length cell, while the value for pantoylactone is -0.66 degree at 200 nm.

A block diagram outlining the basic components of the instrument is given in Figure 5. A current-regulated power supply drives the 450 W xenon arc lamp. The propagating light beam passes through a slit assembly which is automatically programmed as a function of wavelength. A double monochromator on a single rotatable mounting disperses the radiation from the lamphouse into discrete wavelengths. The bandwidth of the radiation exiting the monochromator is determined by the exit slit-width which may either be set manually or is automatically controlled by the slit control program.

After exiting the monochromator the light radiation is collected by an achromatic triplet lens. Following this lens, the monochromatic radiation passes through a Rochon polarizer fabricated from two ammonium dihydrogen phosphate prisms with a 60-degree angle of incidence on the refracting surface. The polarizer separates the incident beam into two linearly polarized components. The ordinary component, which is polarized in a horizontal direction, is transmitted with no deviation along the optical axis. The extraordinary component, vertically polarized, is deviated from the optical axis and blocked from the optical path by a mask, after passing through the electro-optic modulator. The

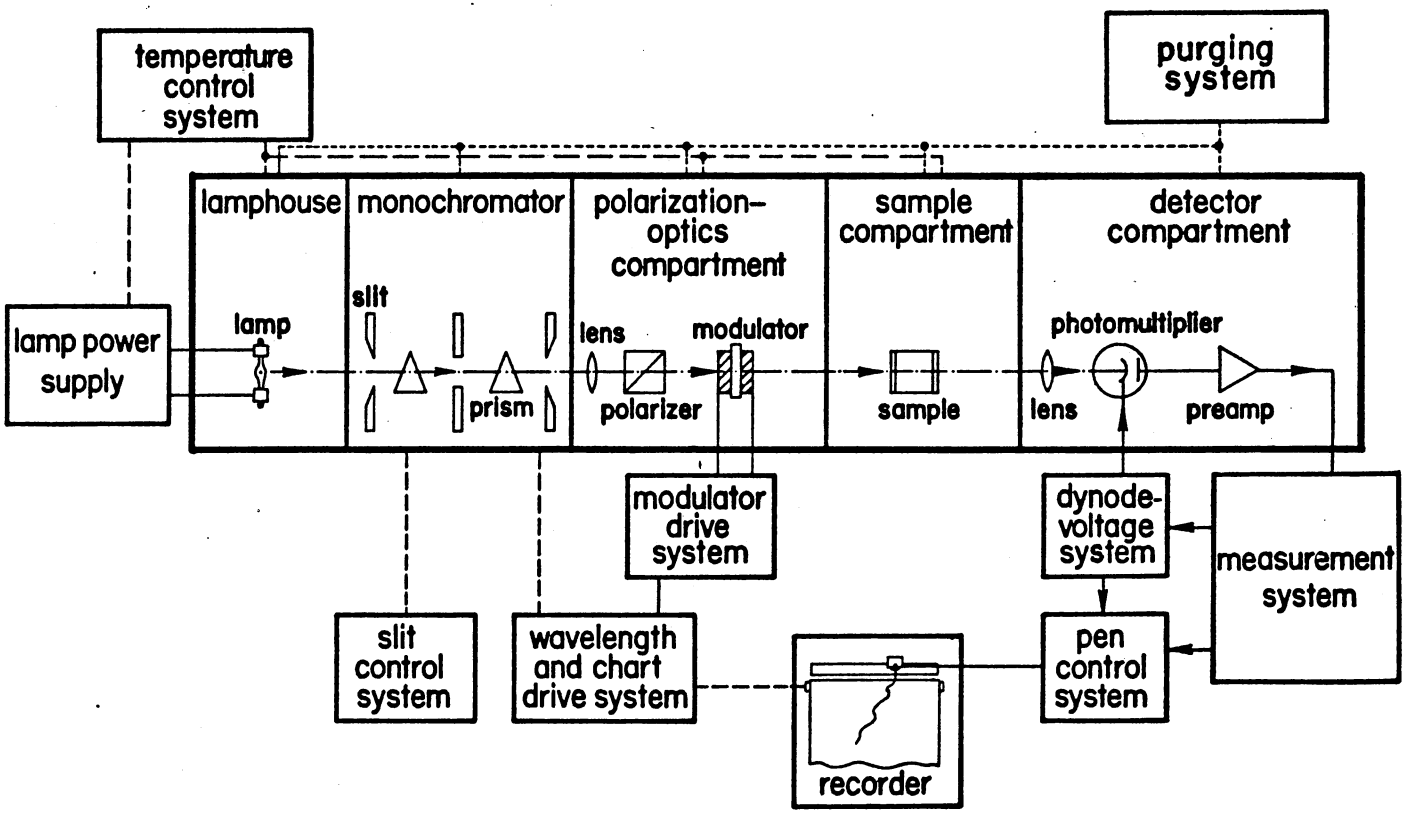


Figure 5. Simplified Diagram of a Cary Model 61 Spectropolarimeter
(Reprinted by Permission of Varian Instruments)

ordinary beam of radiation continues through the electro-optic modulator which is a specially designed Pockel cell made from potassium dideuterium phosphate (KD_2*PO_4). The modulator is excited by a square-wave voltage supply of alternating current, which alternates the state of polarization of the beam between left circularly and right circularly polarized.

The circularly polarized light enters the sample compartment where it passes through the sample cell. Standard one centimeter path length quartz cells, two centimeters in diameter, were used throughout the experiments.

In the "normal" mode, the light radiation enters the detector compartment which contains a lens, photomultiplier, and preamplifier. The light impinging on the photomultiplier is converted to a dc voltage signal. The magnitude of the output current is proportional to the intensity of the incident light source on the cathode and the gain of the photomultiplier stages. The gain is determined by the "dynode voltage" which is regulated to maintain the output anode current nearly constant. The signal from the photomultiplier is then amplified by the preamplifier and sent to the measurement system.

In the "differential" mode, the radiation emerging from the sample compartment is reflected by the mirror, passes through the reference cell, and is detected by a second photomultiplier tube and preamplifier system. After being reflected from the mirror, the circularly polarized radiation has the opposite ellipticity compared to the non-reflected light radiation. Therefore, if both the sample and reference cells contain the same solvent, their CD signals cancel and only the signal due to the solute is recorded.

Sample Preparation

A. Standard Materials

Morphine, codeine, 3-monoacetylmorphine (3-MAM), 6-monoacetylmorphine (6-MAM), nalorphine, and thebaine were obtained as pure forms of the free bases, with the exception of 3-monoacetylmorphine. Within six months after the research was initiated, it was determined that the 3-monoacetylmorphine had a significant impurity present, probably morphine. This casts doubt as to the purity of the sample at the time spectra were obtained.

Heroin (3,6-diacetylmorphine), nalorphine, naloxone, oxycodone and oxymorphone were obtained as pure forms of the hydrochloride salts (heroin was supplied as two batch samples). Dihydrocodeine and hydrocodone were made available as the bitartrate salts. Morphine was also available as a pure sulfate salt.

Where standards of both free base and salt were available, both were used to prepare solutions. No differences were observed between the shape of the spectra for base and salt in each of the three media, providing that at pH 8.6 there was enough buffer capacity to prevent an excessive increase in acidity upon dissolving the salt. A correction for molecular weight resulted in the two forms having the same molar ellipticity values.

Samples were weighed on a Cahn Electrobalance Model No. 2000 RG, which is capable of accurately weighing samples to a hundredth of a milligram. The usual sample size was 2 to 10 milligrams diluted to 50 ml for stock solutions.

Decimolar HCl and NaOH were prepared by dilution of commercially

available secondary standard solutions supplied by Ricca Chemical Company and Harleco, Inc., respectively. The buffer solutions were prepared using pHydrion capsules, according to the recommended procedure of the manufacturer.

Dilute solutions on the order of 5×10^{-5} to 5×10^{-4} M of each drug were prepared in decimolar HCl and the spectra recorded over an optimum concentration range consistent with maximum instrument sensitivity and solute absorptivity. Subsequently, sufficient base, usually NaOH, was added to make the solution approximately decimolar in base and the spectrum recorded. Other spectra were recorded for the drug at the same concentration in a pH 8.6 aqueous buffer solution.

This buffer solution was chosen since it is the optimum pH for the extraction of morphine and other similar alkaloids into an organic solvent. The acidic and basic spectra were recorded on five different samples, often on different days, for each drug. The results were digitized, recorded and analyzed statistically to determine if analytical distinction is possible among drug samples.

Dilutions were made of the stock solutions using calibrated pipets and volumetric flasks. A spectrum was obtained for each dilution for each compound.

Rather than prepare all solutions by dilution of a stock, some solutions were prepared by weighing samples directly and dissolving them in decimolar acid or base or pH 8.6 buffer solution. The two methods gave comparable results.

Graphs of concentration versus maximum ellipticity were prepared from the results of these experiments. The data showed a linear dependence over the concentration ranges previously mentioned. Solutions

whose maximum ellipticity values were less than 3×10^{-3} degree usually resulted in a poor signal-to-noise ratio. On the other hand, if the maximum CD signal exceeded 0.5 degree, the proportion of the radiation that was absorbed was too high relative to the CD signal, which resulted in significant amplifier gain noise. These experimental limits determine the dynamic range of the instrument which varies from compound to compound.

B. Prepared Mixtures

Dilute solutions of mixtures of CD active drugs were investigated to determine if their CD spectra are additive. These samples contained one to four milligrams each of two or three compounds. They were dissolved in 25-50 ml of aqueous 0.1 M HCl or aqueous 0.1 M NaOH. The drug combinations and their amounts are listed in Table I.

The dilute solutions of each drug were added together in equal amounts and mixed thoroughly. The concentrations shown are those existing before the solutions were mixed together. The initial stock solutions were split so that the concentrations of the dilute basic and acidic solutions were the same. CD spectra were then obtained in the conventional manner. The spectra obtained experimentally were compared to those obtained by adding the CD spectral response every five nm for each component in the mixture of both dilute acidic and basic solutions.

C. Unknowns

One important aspect of this investigation was to determine the accuracy and precision attainable for analyses of actual confiscated samples. Three types of unknowns were studied. These included in-house

TABLE I

SYNTHETIC MIXTURES OF MORPHINE, CODEINE, QUININE AND THEBAINE

Mixture	Drug	Initial Concentration, M	Solvent	Figure No.
#1	Morphine	3.42×10^{-4}	Acid	23
	Codeine	3.35×10^{-4}	Base	24
#2	Morphine	1.37×10^{-4}	Acid	
	Codeine	1.34×10^{-4}		
	Quinine	3.25×10^{-5}	Base	
#3	Morphine	1.72×10^{-4}	Acid	25
	Codeine	3.58×10^{-5}		
	Thebaine	3.12×10^{-5}	Base	26

preparations, heroin confiscates, and pharmaceutical preparations.

In-House Preparations

Samples of heroin, morphine, codeine and thebaine diluted with lactose, which is CD inactive, were prepared in-house by other laboratory personnel. Their identities and concentrations were unknown to the analyst. However, that of the heroin sample was of the same order of magnitude as would typically be found in street samples. The other three drugs were chosen for two reasons. First, they are the major constituents present in opium. It was hoped that opium samples could eventually be determined directly by CD spectroscopy. There was also a considerable data base for morphine and codeine from which to obtain exact molar ellipticity values.

In all, four sets of unknowns were prepared, each of a different concentration or method of preparation, to determine their effects on the analytical results. The first two sets contained samples with either single components or two component mixtures. The concentration of the drug components in the first set was 1.5 - 2.5% (W/W), while that of the second was 9 - 14%. Both of these samples were mixed stirring by hand.

The analytical procedure was similar to that for obtaining the molar ellipticity values of the standards. Three to four milligram aliquots of the samples were dissolved in 25 ml of aqueous 0.1 M HCl and used in determinations. Another CD spectrum of each sample was taken after the addition of NaOH to each solution. Those with single drug components were easily identifiable in the mixture from their CD spectra.

The last two sets contained only a single drug component. For the third set of unknowns, the entire samples were dissolved in 0.1 M HCl and the CD spectra recorded. The samples were then diluted in 0.1 M HCl or 0.2 M NaOH and additional CD spectra were obtained. For the last set of unknowns there was a greater effort to achieve homogeneity of samples. The solid samples were mechanically mixed then transferred to plastic vials, and shaken in a Wig-L-Bug device. The effects of drug component, sample size, solvent and sampling method upon the accuracy and precision attainable for samples of this type were estimated from these sets of data.

The two samples containing different mixtures of two optically active drugs diluted with lactose, were prepared at the same time as those containing only one optically active drug. Solutions were prepared and spectra obtained in the same manner as mentioned before. The identification and quantitation of the components in these samples were naturally more difficult. The identities of the possible components were initially deduced from the CD spectra. Using the instrument in the differential mode, spectra were recorded in dilute aqueous acidic and basic solutions for various concentrations of one of the suspected components in the reference cell. This amounts to a spectral subtraction procedure and the process was repeated until the identities of both components were confirmed. Quantitation was done by an iterative procedure in which molar ellipticities for maxima for both components in both acidic and basic solutions, and wavelengths of the crossing points were successively compared, until the difference between the experimental and calculated spectra was a minimum.

Heroin Unknowns

Clandestine samples of heroin were examined to establish if they could be successfully analyzed by CD spectroscopy. Four samples of heroin were provided by the Oklahoma State Bureau of Investigation from their dead case file. Three of the samples were typical of what is known as "brown" heroin; the fourth was a white specimen which proved to have a very high heroin content. The last specimen was the only one of the four which had been quantitated by the OSBI.

The sample size used for identification and quantitation ranged from 3 to 20 mg, depending upon the composition by weight of drug in the sample. The samples were dissolved in either 25 ml of aqueous 0.1 M HCl or aqueous buffer solution with a pH equal to 8.6. The aqueous solutions were centrifuged prior to analysis. if there was no extraction step involved in the analysis. The drug was positively identified from the CD spectra of the samples in acid. The CD spectra of the samples in pH 8.6 buffer solution were found to have more variability, both from a qualitative and quantitative standpoint. Five different experimental strategies were tried to determine the best method for the determination of heroin in the samples. Quantitative determinations were first made from the solutions of heroin in 0.1 M HCl or pH 8.6 buffer solution and then as morphine upon the addition of a pellet of sodium hydroxide. An alternative method was to preferentially extract heroin from 20 ml of pH 8.6 buffer solution into 25 ml of chloroform and then to back-extract the drug into 25 ml of 0.1 M HCl prior to analysis. Only a very small amount of morphine is extracted under these conditions, whereas the extraction efficiency for heroin is in excess of 95%. The fifth method to quantify the sample was to add a pellet of

sodium hydroxide to the acid extract and then to measure the heroin composition after complete hydrolysis to morphine.

Gas Chromatographic Analysis

Gas chromatography was chosen as a comparative method for heroin determinations. The method has been used extensively in the past for the analysis of confiscated drug samples. Experiments were done on a Hewlett-Packard 7620-A with a six foot silane-treated glass column. The column material was SP-2100 on 80/100 Supercoport; the oven temperature was 260°C, the FID temperature 325°C and the injector port temperature 280°C. Helium was used as the carrier gas at a flow rate of 30 ml per minute. Five to twenty milligrams of the confiscated drug samples were extracted with 20 ml of chloroform and filtered through calcium chloride prior to the gas chromatographic analysis.

For consistency, the instrument was calibrated using a set of dilutions, ranging in concentration from 100 to 1000 µg/ml, of a stock heroin solution which had been subjected to the same extraction procedure as the samples. Aliquots of 1.2 ± 0.05 microliters of the chloroform solutions were injected onto the column using a 10 microliter syringe. Peak heights were measured as the difference between the baseline to peak maximum. Analyses were performed in duplicate.

Pharmaceutical Preparations

Three types of samples containing codeine or dihydrocodeine were analyzed to determine the efficiency of CD spectroscopy for the analysis of pharmaceutical preparations. These included in-house preparations of codeine and lactose, solid dispensary items (tablets

and capsules) and two cough syrups.

Two samples of codeine were diluted with lactose and given to the investigator for quantitation. Precise molar ellipticity values were established from two samples of codeine of known concentration. These two samples gave reproducible values of +438 and -73 degrees $\cdot l \cdot mol^{-1}$ for the ^1L_a and ^1L_b transitions respectively. As Table VI indicates, values of +460 and -74 degrees $\cdot l \cdot mol^{-1}$ were obtained as an average of several samples.

Four commercial pharmaceutical preparations were quantitated by CD spectroscopy for their codeine content. All four of these preparations contained codeine phosphate in conjunction with one or more other drugs (Table II). Acetaminophen, aspirin and caffeine all absorb strongly in the UV region. This made the direct analysis of codeine in these samples virtually impossible, due to the loss of signal intensity and an increase in noise in the spectral region of interest. One of the tablets from each sample was weighed, crushed and mixed by hand. A 50-80 milligram aliquot was weighed and easily dissolved in an aqueous 0.1 M HCl solution.

Attempts to quantitatively elute the codeine from an acidic liquid chromatography column packed with alumina with chloroform failed. A similar procedure had worked in the past for pharmaceutical preparations containing codeine. A second sample, in aqueous 0.1 M HCl, was washed twice with 25 ml of ether to remove the acetaminophen. The solution was then made basic with sodium hydroxide, extracted into 25 ml of chloroform and back-extracted into 25 ml of 0.1 M HCl. The solution was quantitated and yielded low results. However, by performing the extraction first, and then washing once with ether, there was 95% recovery of the

TABLE II

NOMINAL COMPOSITION OF PHARMACEUTICAL PREPARATIONS

Preparation	Composition	
Phenaphen[a], Tylenol #3[a]	Codeine Phosphate	30 mg
	Acetaminophen	300 mg
Tylenol[a] #4	Codeine Phosphate	60 mg
	Acetaminophen	300 mg
Fiorinal[a] #3	Codeine Phosphate	30 mg
	Aspirin	200 mg
	Phenacetin	130 mg
	Caffeine	40 mg
	Butalbital	50 mg
Synalgos DC[a]	Dihydrocodeine Bitartrate	16 mg
	Aspirin	194 mg
	Phenacetin	162 mg
	Caffeine	30 mg
	Promethazine HCl	6.3 mg
Phenergan VC[a]	Codeine Phosphate	2.0 mg[b]
	Promethazine HCl	6 mg[b]
	Phenylephrine	5 mg[b]
	Potassium Guaiacol Sulphonate	44 mg[b]
	Citric Acid	60 mg[b]
	Sodium Citrate	197 mg[b]
FC's Cough Syrup	Codeine Phosphate	476 mg[c]
	Terpene Hydrate	
	Phenylpropanolamine	333 mg[c]
	Chlorpheniramine maleate	36 mg[c]

[a]Registered trademark.

[b]Per 5 ml.

[c]Per 8.0 fluid oz.

codeine, which was quantitated from a good CD spectrum. This procedure was then used for the analysis of all four pharmaceutical preparations. Later, a capsule containing Synalgos DC® was also analyzed using the same procedure and good results were obtained. After the ether wash it is necessary to drive off any residual ether in the aqueous 0.1 M HCl solution by gentle heating and reconstituting the solution to its original volume. The residual ether had a strong UV absorption which contributed to the loss of signal quality.

Two preparations of cough syrup were analyzed for their codeine content. The conventional analysis of the codeine in these samples is hindered by several UV active drugs and dyes, some of which are listed in Table II. The high viscosity hinders any extraction procedure. These two samples were analyzed two ways, either by dilution of one ml of cough syrup in 25 ml aqueous 0.1 M HCl or after extraction from 25 ml basic solution into 25 ml chloroform and back extraction into 25 ml aqueous 0.1 M HCl.

CHAPTER IV

EXPERIMENTAL RESULTS

The general formula for the morphine and codeine derivatives of the opium alkaloids is shown in Figure 6. There are five rings in the structure which, by convention, are designated A through E. The molecule is approximately T-shaped,[79,80] such that the plane containing rings A and B is almost perpendicular to the plane of the rings C and D.

The derivatives studied in these experiments are listed in Table III. The functional groups involved fall into five categories:

1. -OH, $-OCH_3$, $-OCH_2CH_3$, $-OOCCH_3$; substituents at C(3).
2. -OH, $-OCH_3$, $-OOCCH_3$; substituents at C(6).
3. $-CH_3$, $CH_2CH{=}CH_2$; substituents on nitrogen.
4. -H, -OH; substituents on C(14).
5. Saturation or unsaturation at C(7)-C(8) in ring C, except for thebaine, which has a conjugated diene structure.

Spectral Characterization

A comparison of the UV absorption spectra of the alkaloids in dilute acid and base indicates that there is some modification of the molecular structure induced by the change in pH of the solvent medium. However, there is still a lack of analytical distinction among most of the drugs as shown in Table IV.[9]

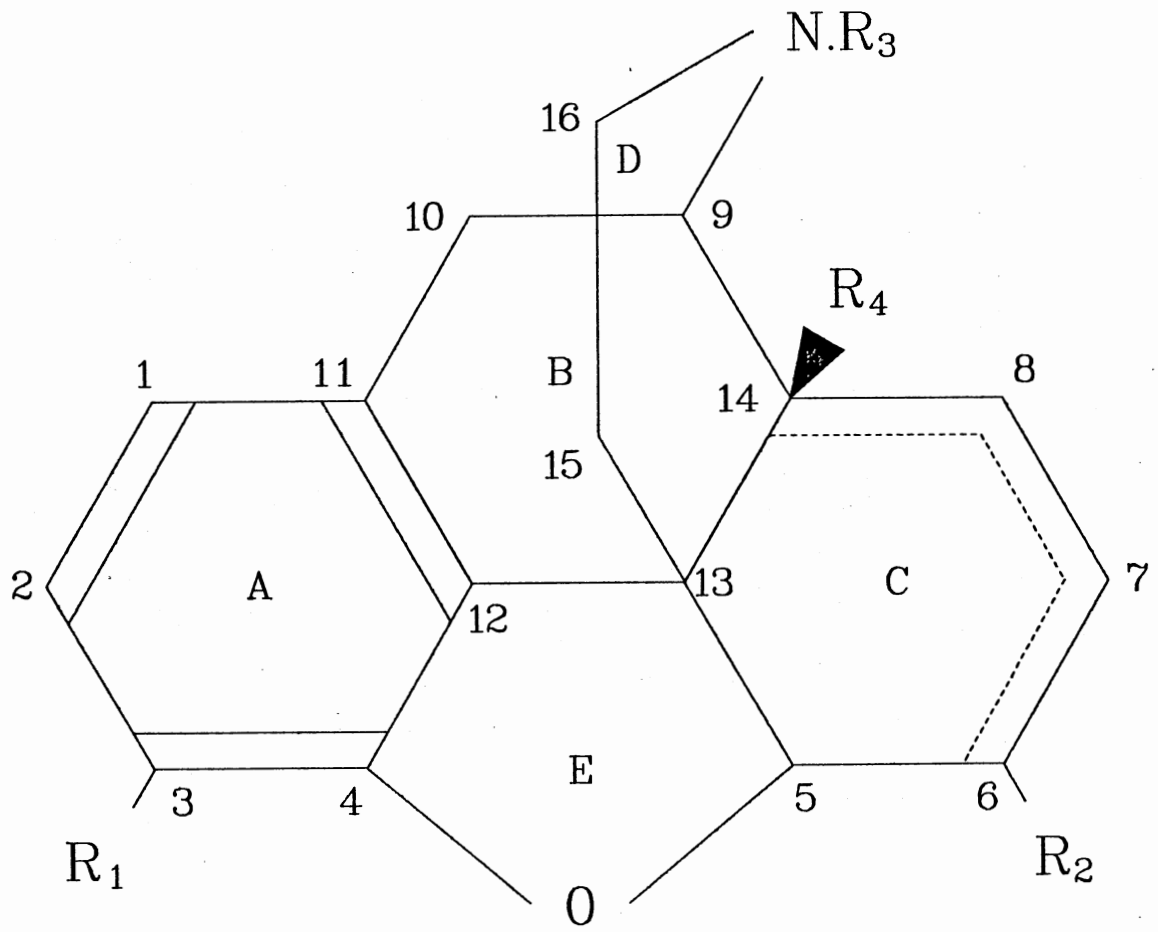


Figure 6. General Structural Formula for the Morphine and Codeine Alkaloids

TABLE III

STRUCTURE OF ALKALOIDS STUDIED

Alkaloid	R_1	R_2	R_3	R_4	C-Ring
Morphine	OH	OH	CH_3	H	C7-C8
Nalorphine	OH	OH	$CH_2CH{=}CH_2$	H	C7-C8
3-MAM	CH_3COO	OH	CH_3	H	C7-C8
6-MAM	OH	CH_3COO	CH_3	H	C7-C8
Heroin	CH_3COO	CH_3COO	CH_3	H	C7-C8
Hydromorphone	OH	O	CH_3	H	Saturated
Oxymorphone	OH	O	CH_3	OH	Saturated
Naloxone	OH	O	$CH_2CH{=}CH_2$	OH	Saturated
Codeine	CH_3O	OH	CH_3	H	C7-C8
Dihydrocodeine	CH_3O	OH	CH_3	H	Saturated
Hydrocodone	CH_3O	O	CH_3	H	Saturated
Oxycodone	CH_3O	O	CH_3	OH	Saturated
Ethylmorphine	CH_3CH_2O	OH	CH_3	H	C7-C8
Thebaine	CH_3O	CH_3O	CH_3	H	C6-C7; C8-C14

TABLE IV

UV ABSORPTION DATA FOR SEVERAL OPIUM ALKALOIDS[9]

Alkaloid	Wavelength Max	
	Aqueous Acid	Aqueous Base
Morphine	210, 240s, 280s, 284	251, 298
Nalorphine	210, 240s, 280s, 284	251, 298
3-Acetylmorphine	205, 274s, 278	251, 298
6-Acetylmorphine	210, 240s, 285	251, 297
3,6-Diacetylmorphine	204, 235s, 278.5, 274s	251s, 298
Hydromorphone	207s, 235s, 282	235s, 290
Oxymorphone	281	240s, 292
Naloxone	230s, 280	239s, 292
Codeine	211, 240s, 278s, 284	238s, 278s, 284
Dihydrocodeine	230s, 278s, 284	277s, 283
Hydrocodone	204, 228s, 280	279
Oxycodone	205, 227s, 280	280
Ethylmorphine	211, 241s, 279s, 284	279s, 284
Thebaine	228s, 284	228s, 284

The characterization of these same alkaloids in ethanol by CD spectroscopy adds little in the way of identification,[81] as shown in Figure 7. Two distinct bands are observed for codeine, morphine, 6-monoacetylmorphine, dihydrocodeine, and heroin. Although there are three different substituents on the aromatic chromophore, the spectra for all six compounds bear a strong resemblance to one another. Heroin and dihydrocodeine are distinguishable from the other compounds but not from each other. Changes are noted in the spectra for hydrocodone and thebaine, which are related to additional chromophores present in the C ring.[9,85,87] Other morphine ketones (not shown) would probably have a spectrum similar to hydrocodone.

In Figures 8 through 17 the normalized CD spectra are shown for each of the compounds in dilute acid, dilute base, and in solutions buffered to pH equal to 8.6. The printed spectra are representative of the average of three to five independent experiments with two exceptions, ethylmorphine and 3-monoacetylmorphine. Only a very limited amount of the former drug was available and the latter deteriorated so rapidly that replication was not possible.

The important parameters used to characterize the spectra are the wavelengths where there are positive (λ^{+}_{max}) and negative (λ^{-}_{max}) ellipticity maxima (or minima) and also the wavelengths where the ellipticity values are zero (λ°) referred to as "cross-over points."

The data for these parameters for all the alkaloids studied in all three media are collected together in Table V. Bands which have no corresponding maxima in the UV absorption spectra are designated by an asterisk (*). The data have been analyzed by standard statistical procedures. The results of this analysis are discussed in Chapter VI.

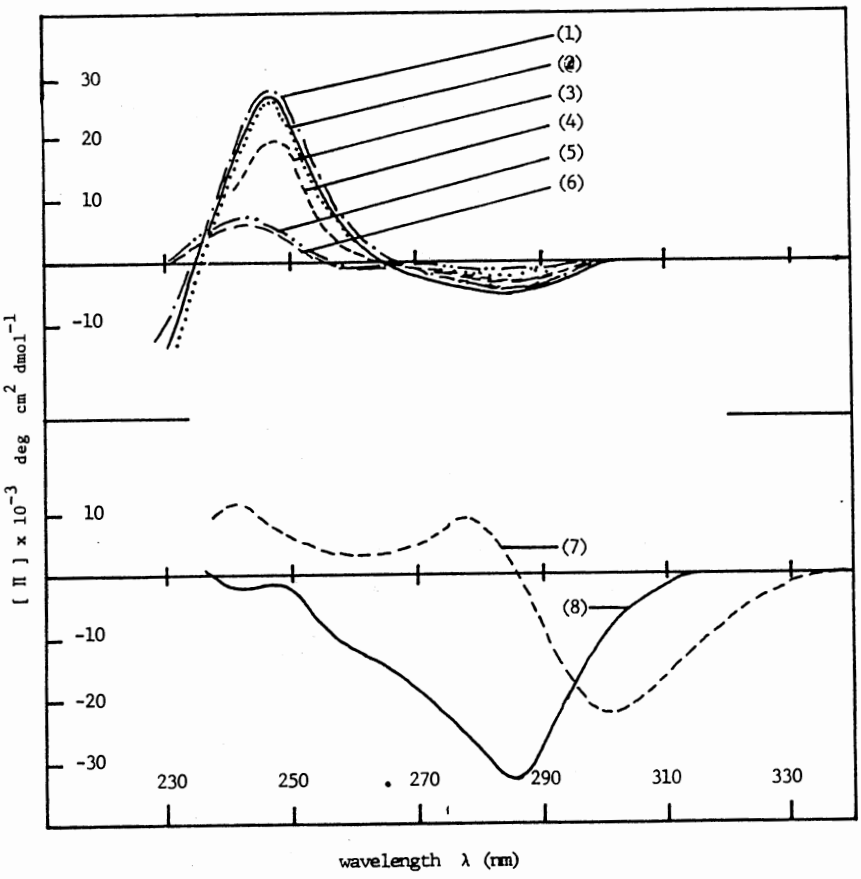


Figure 7. Ethanol CD Spectra of (1) Codeine, (2) Morphine, (3) 6-MAM, (4) 3-MAM, (5) Dihydrocodeine, (6) Heroin, (7) Hydrocodone, and (8) Thebaine

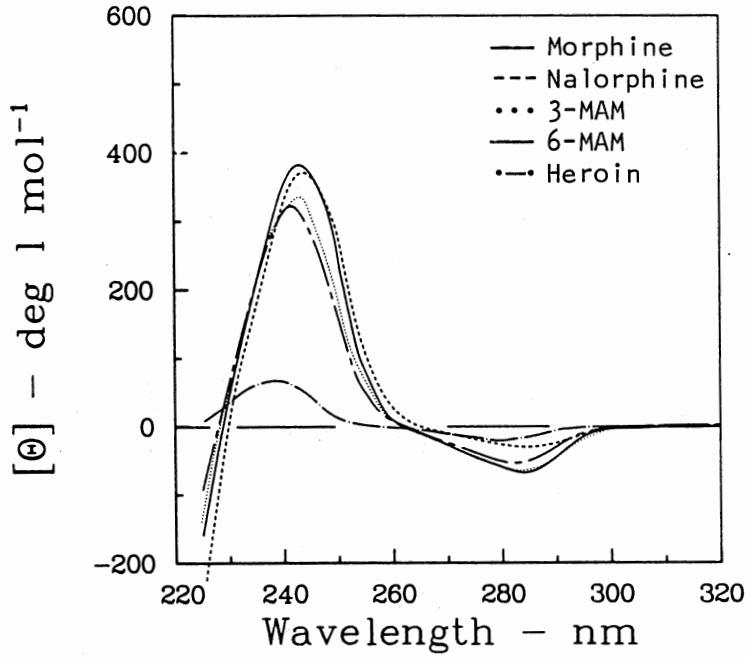


Figure 8. Morphine Group, 0.1 M HCl

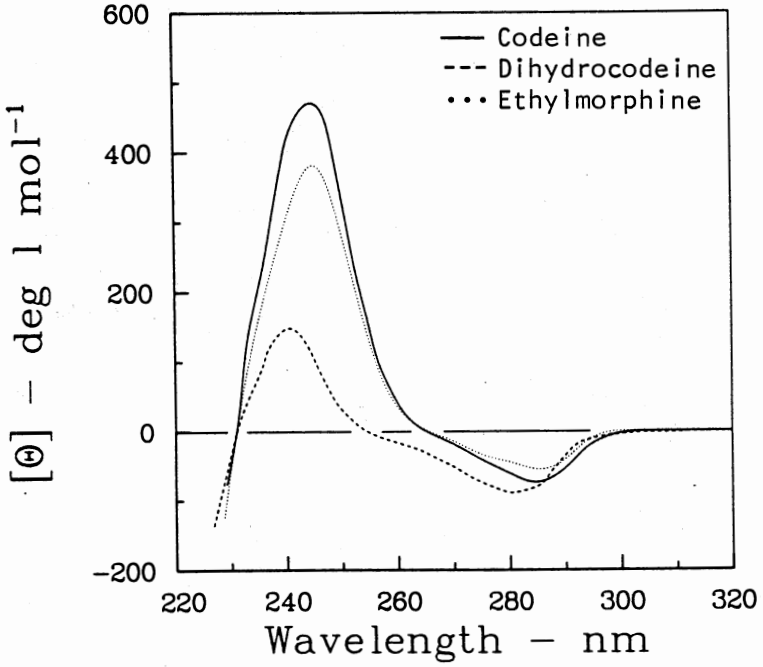


Figure 9. Codeine Group, 0.1 M HCl

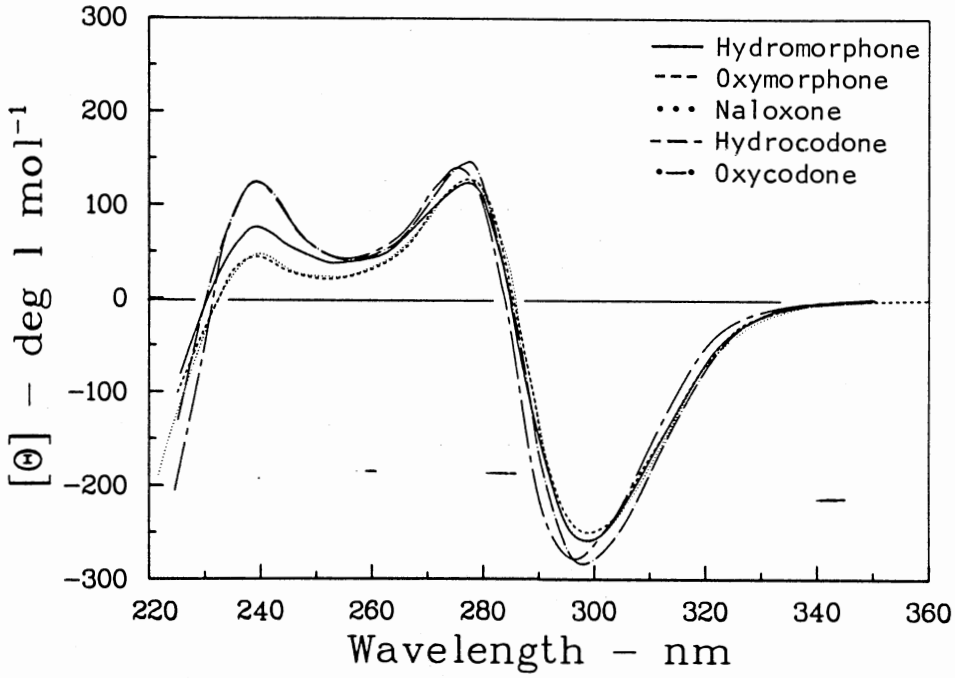


Figure 10. Ketone Group, 0.1 M HCl

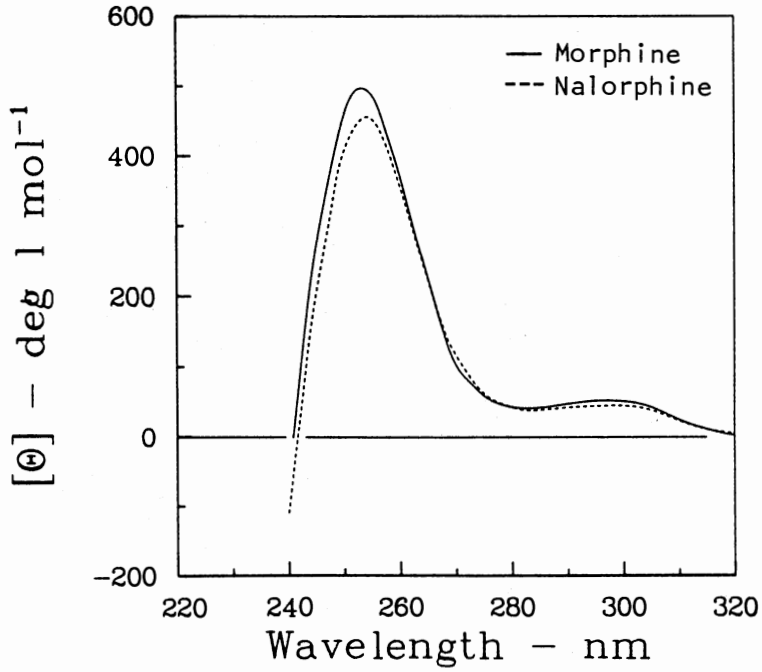


Figure 11. Morphine Group, 0.1 M NaOH

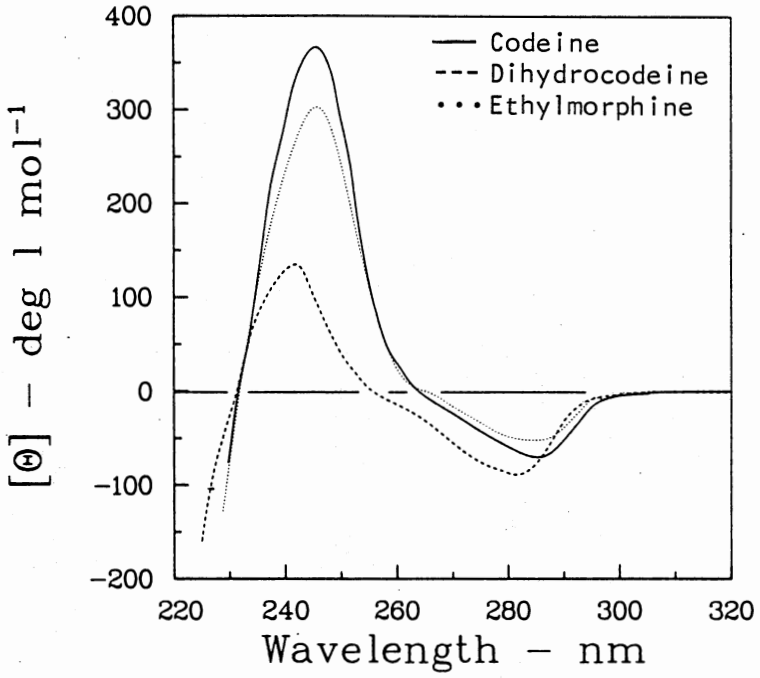


Figure 12. Codeine Group, 0.1 M NaOH

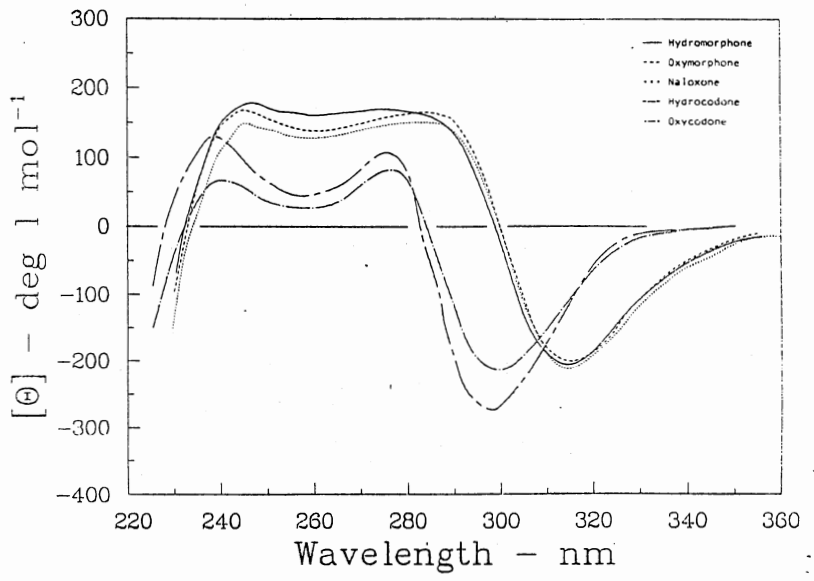


Figure 13. Ketone Group, 0.1 M NaOH

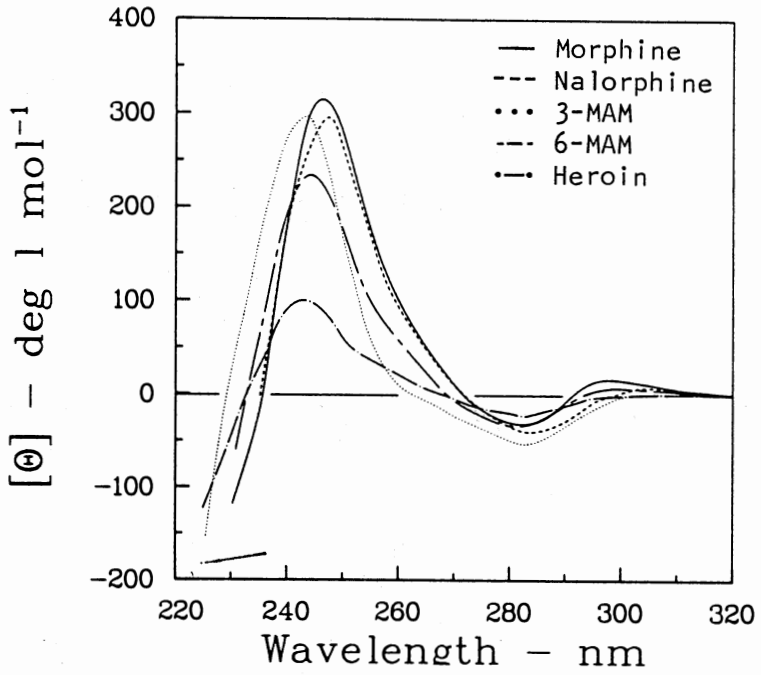


Figure 14. Morphine Group, pH 8.6 Buffer

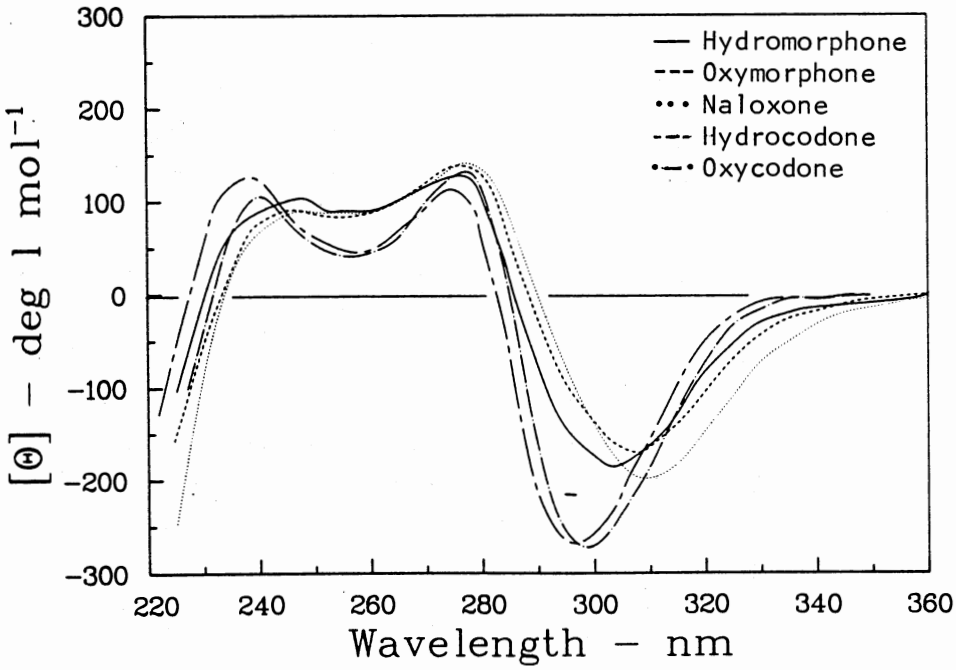


Figure 15. Codeine Group, pH 8.6 Buffer

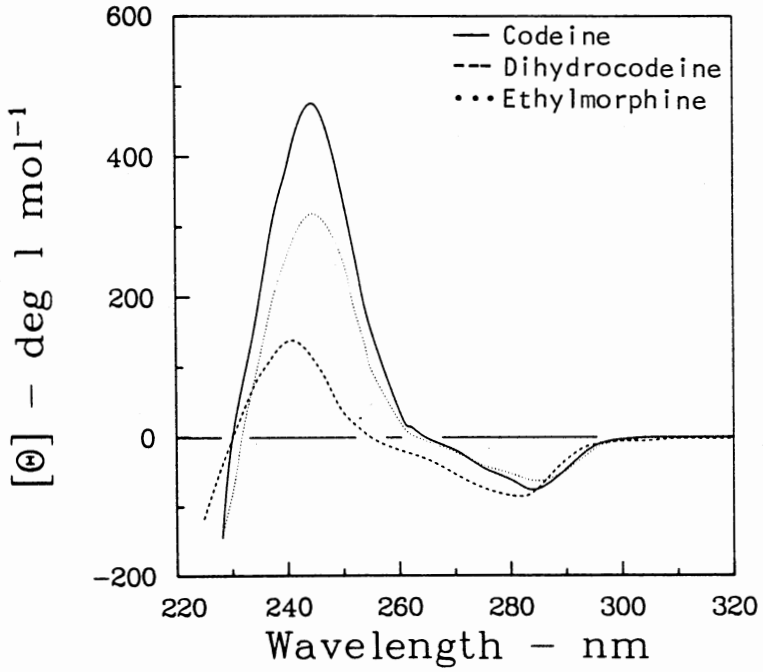


Figure 16. Codeine Group, pH 8.6 Buffer

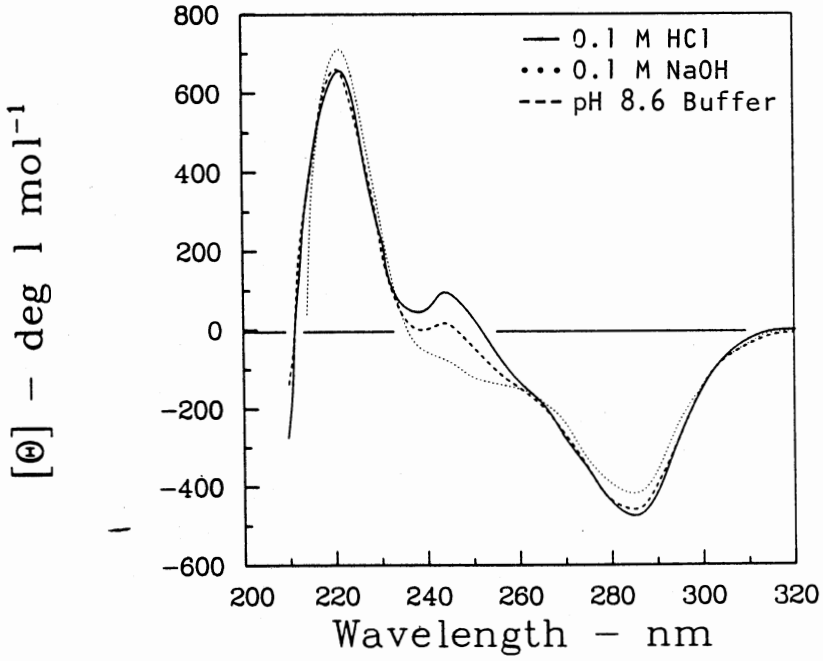


Figure 17. Thebaine

TABLE V

CD SPECTRAL PARAMETERS FOR THE OPIUM ALKALOIDS IN AQUEOUS MEDIA

Drug	Solution	λ^{+}_{Max}	λ^{-}_{Max}	λ°
Morphine	HCl	243	284	229, 263
	8.6	247, 301	285	237, 273, 295
	NaOH	253, 298*	283**	241
Nalorphine	HCl	245	285	230, 265
	8.6	247, 305*	285	236, 272, 299
	NaOH	254, 298	282	242
3-Acetylmorphine	HCl	244*	285	229, 263
	8.6	244, 303	284	232, 267, 294
	NaOH	Hydrolysis to Morphine		
6-Acetylmorphine	HCl	243	284	229, 263
	8.6	244,* 302*	282	232, 268, 294
	NaOH	Hydrolysis to Morphine		
Heroin	HCl	238	280	221, 260
	8.6	243	283	234, 270
	NaOH	Hydrolysis to Morphine		
Hydromorphone	HCl	239, 276*	299*	230, 284
	8.6	246, 276*	304*	231, 286
	NaOH	246, 275*	314*	232, 299
Oxymorphone	HCl	239,* 277	299*	232, 285
	8.6	247, 276	309	234, 290
	NaOH	246, 285*	315*	233, 300
Naloxone	HCl	239, 277	299*	233, 285
	8.6	249, 279	308*	234, 289
	NaOH	247, 285	315*	235, 300
Codeine	HCl	244	285	231, 264
	8.6	244	284	231, 262
	NaOH	244	284	230, 263
Dihydrocodeine	HCl	240	281	231, 253
	8.6	241	281	230, 256
	NaOH	240	281	231, 255
Ethylmorphine	HCl	245	285	231, 265
	8.6	245	285	231, 263
	NaOH	245	285	231, 264
Hydrocodone	HCl	240, 276*	297*	232, 284
	8.6	237, 276*	297*	227, 284
	NaOH	239,* 276	297*	229, 283
Oxycodone	HCl	240, 277	298*	230, 285
	8.6	240, 276	298*	231, 285
	NaOH	240,* 277	300*	232, 284
Thebaine	HCl	221, 245*	285	211, 252
	8.6	221, 243*	285	211, 247
	NaOH	221*	285	236

** Positive Minimum.

Molar Ellipticity Values

Molar ellipticity values ($[\theta]_\lambda$) calculated from the slopes of the linear correlations of ellipticity maxima at wavelengths λ with solution concentrations are listed in Table VI. Good linear correlations were obtained for all alkaloids over the concentration range of interest, with slightly better results being obtained from dilute acidic and dilute basic media than from pH 8.6 buffer. The more intense ^1L_b transition resulted in slightly better data reproducibility.

Graphs of ellipticity maxima versus molar concentrations are included for morphine, codeine, and thebaine as representative examples of the linear correlations that are obtainable in the three media (Figures 18 through 22). More details of the correlation coefficients for all of the alkaloids are available in Chapter VI.

Also included in Table VI are the ratios of molar ellipticities expressed as the positive maximum divided by the negative maximum for each alkaloid. Where two positive bands occur, the ratio has been shown for each.

The ^1E electronic transition, which is an "allowed" transition, has much greater intensity than the forbidden ^1L_a and ^1L_b electronic transitions. The ^1E transition of morphine and codeine were investigated to determine if the added spectral parameters would facilitate their identification. Since the ^1E transition is much stronger than the ^1L_a transition, the limit of detection should be greatly improved. Representative spectra for both drugs in dilute acidic and basic media are shown in Figures 23 and 24. Although the limit of detection was improved by approximately a factor of two, this feature was offset by other liabilities.

TABLE VI

MOLAR ELLIPTICITIES[a] FOR THE OPIUM ALKALOIDS IN AQUEOUS MEDIA

Drug	Solution	$[\theta]^{+}_{Max}$	$[\theta]^{-}_{Max}$	Ratio[b]
Morphine	HCl	383	-67	5.8
	8.6	294, 16.5	-33	8.9, 0.50
	NaOH	493, 52		9.5
Nalorphine	HCl	364	-60	6.1
	8.6	297, 7.4	-42	7.1, 0.18
	NaOH	447, 47		9.5
3-Acetylmorphine	HCl	334	-63	5.3
	8.6	265	-44	6.0
	NaOH	Hydrolysis to Morphine		
6-Acetylmorphine	HCl	316	-55	5.7
	8.6	247, 8.7	-38	6.5, 0.24
	NaOH	Hydrolysis to Morphine		
Heroin	HCl	70	-20	3.5
	8.6	102	-20	5.1
	NaOH	Hydrolysis to Morphine		
Hydromorphone	HCl	76, 120	-251	0.30, 0.48
	8.6	99, 129	-188	0.53, 0.69
	NaOH	181, 170	-203	0.89, 0.84
Oxymorphone	HCl	44, 129	-251	0.18, 0.51
	8.6	104, 140	-167	0.62, 0.84
	NaOH	168, 167	-203	0.83, 0.83
Naloxone	HCl	43, 128	-257	0.17, 0.50
	8.6	81, 134	-188	0.43, 0.71
	NaOH	150, 152	-210	0.71, 0.72
Codeine	HCl	462	-74	6.2
	8.6	395	-73	5.4
	NaOH	359	-70	5.2
Dihydrocodeine	HCl	143	-87	1.6
	8.6	141	-82	1.7
	NaOH	134	-83	1.6
Ethylmorphine	HCl	374	-59	6.3
	8.6	315	-61	5.2
	NaOH	299	-57	5.2
Hydrocodone	HCl	127, 137	-273	0.47, 0.50
	8.6	138, 117	-265	0.52, 0.44
	NaOH	128, 107	-272	0.47, 0.39
Oxycodone	HCl	119, 139	-268	0.44, 0.52
	8.6	98, 123	-248	0.40, 0.50
	NaOH	69, 85	-216	0.32, 0.39
Thebaine	HCl	645, 88	-469	1.4, 0.18
	8.6	659, 20	-455	1.4, 0.04
	NaOH	700	-89, -423	7.87, 1.7

[a] Defined as the experimental value divided by the molar concentration.

[b] Absolute value of $[\theta]^{+}$, max divided by $[\theta]^{-}$, max.

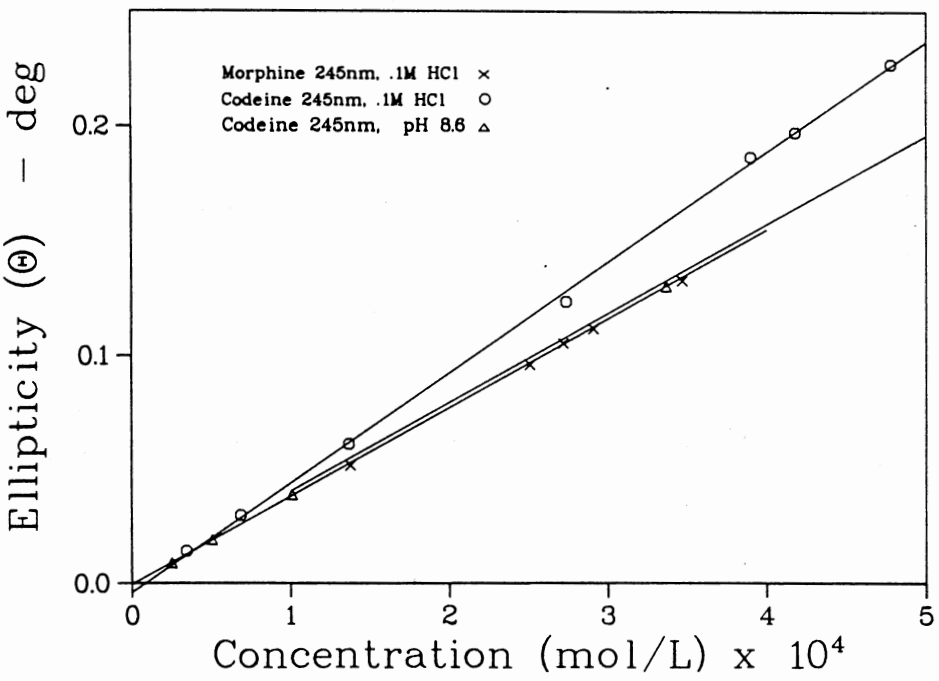


Figure 18. Ellipticity Maxima Versus Concentration--
Morphine and Codeine, λ = 245 nm

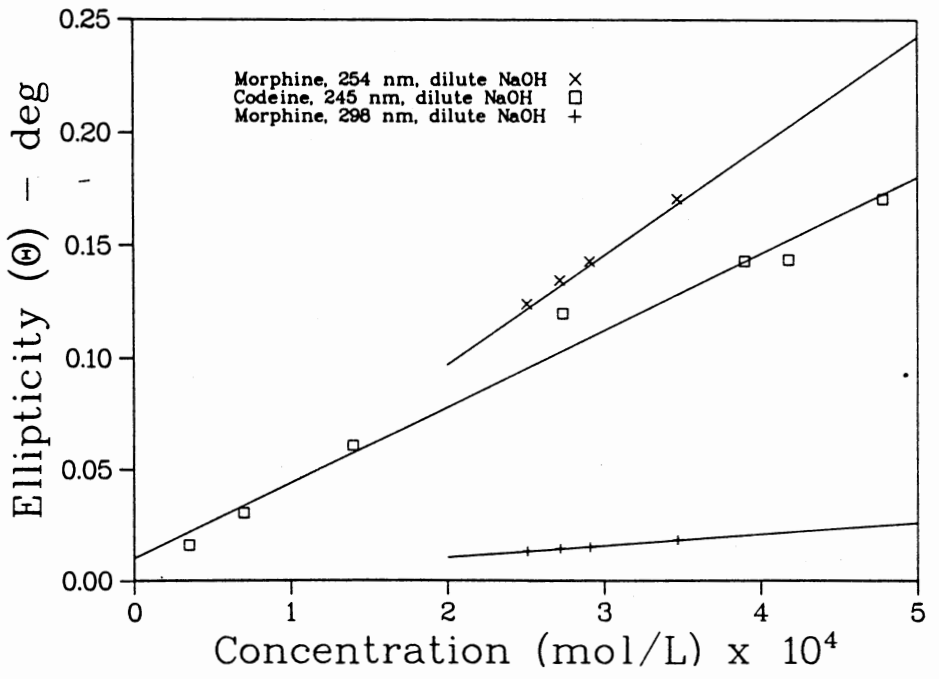


Figure 19. Ellipticity Maxima Versus Concentration--
Morphine and Codeine, λ = 245, 254 nm

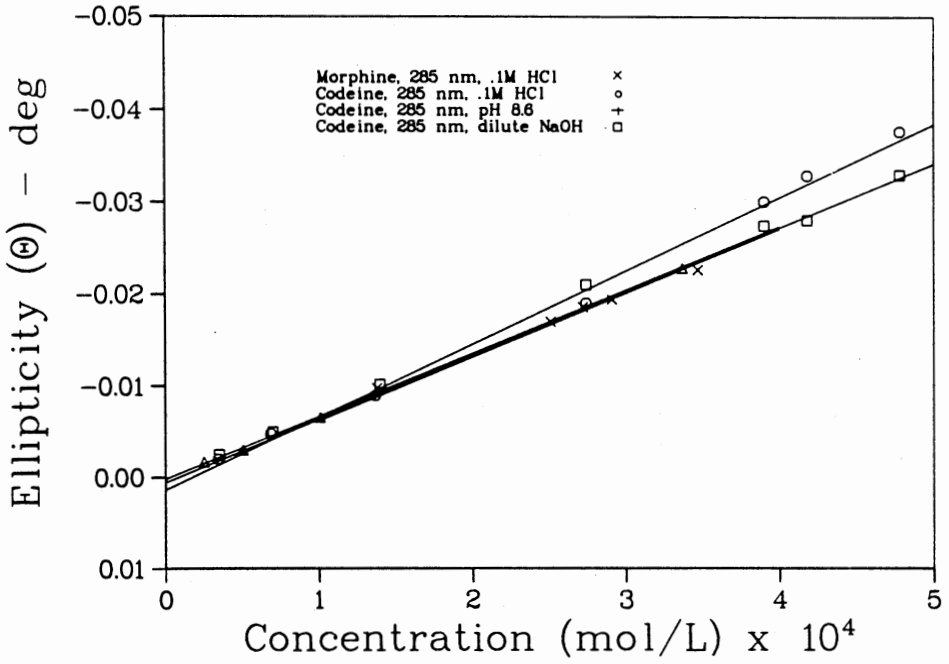


Figure 20. Ellipticity Maxima Versus Concentration--Morphine and Codeine, λ = 285 nm

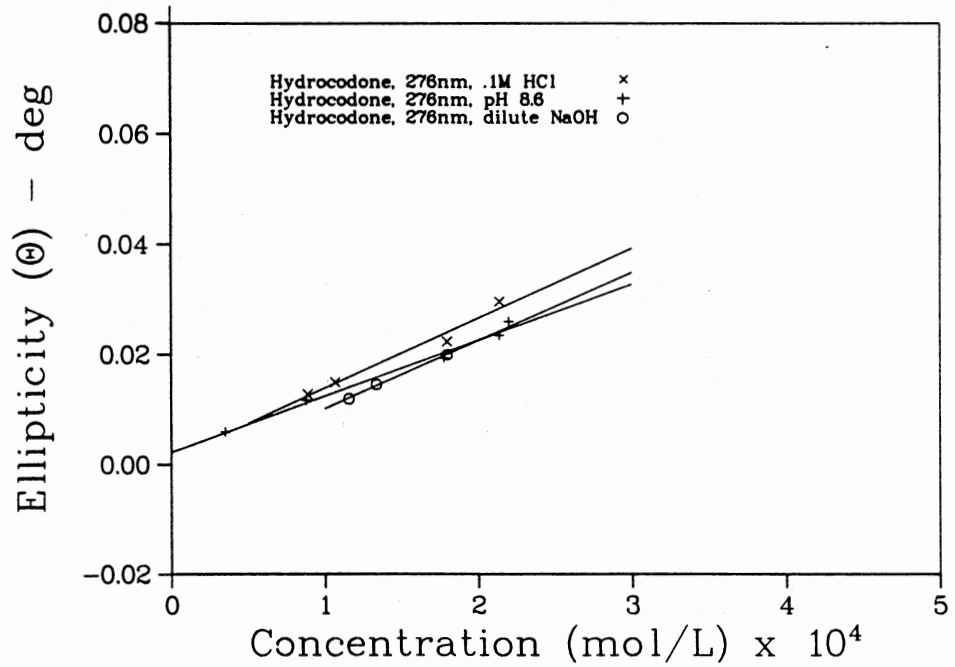


Figure 21. Ellipticity Maxima Versus Concentration --Hydrocodone, λ = 275 nm

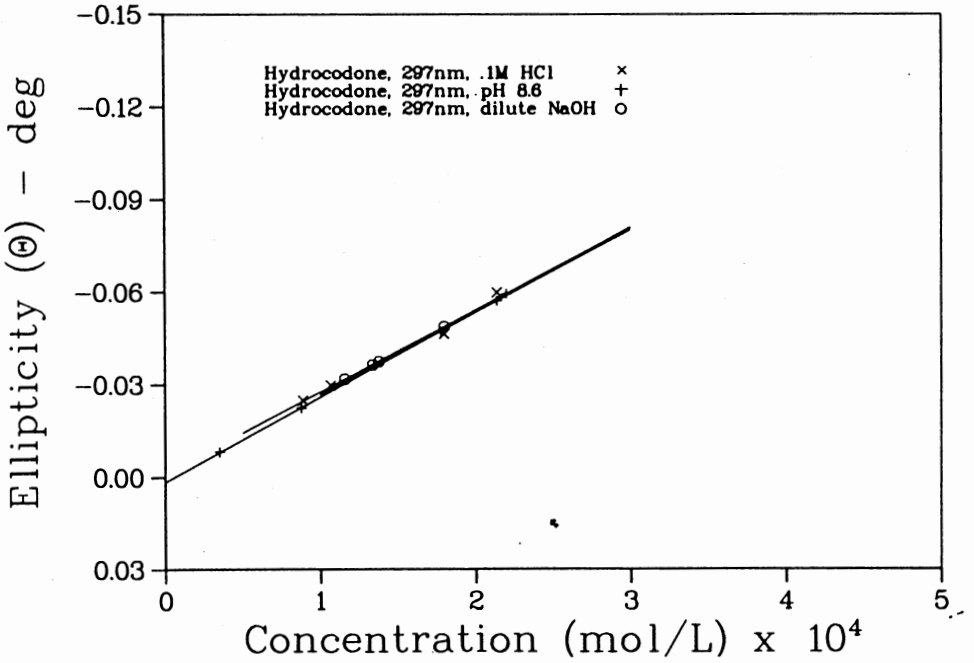


Figure 22. Ellipticity Maxima Versus Concentration--
Hydrocodone, λ = 297 nm

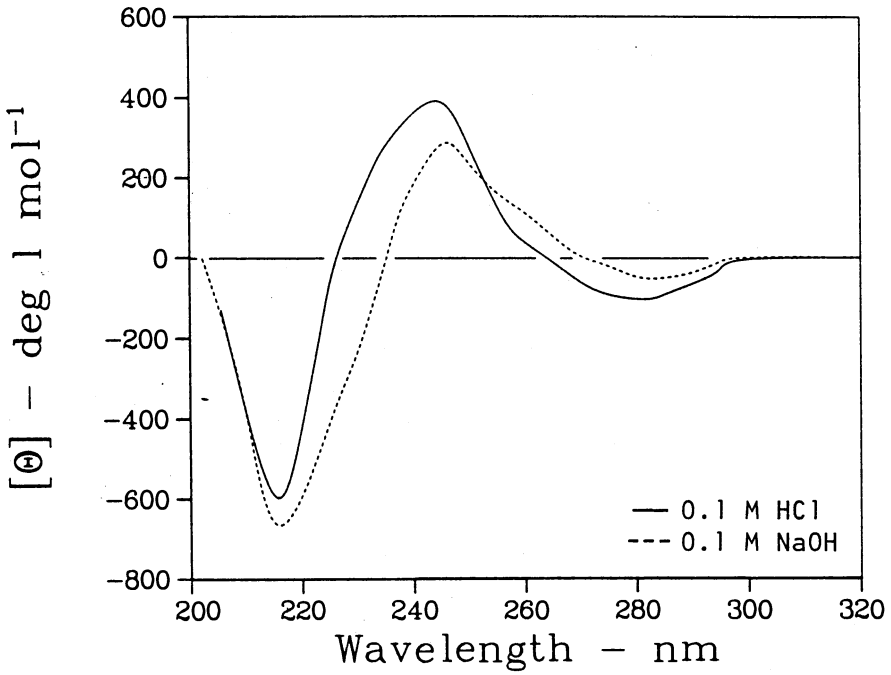


Figure 23. Morphine CD Spectra Including the ^1E Transition

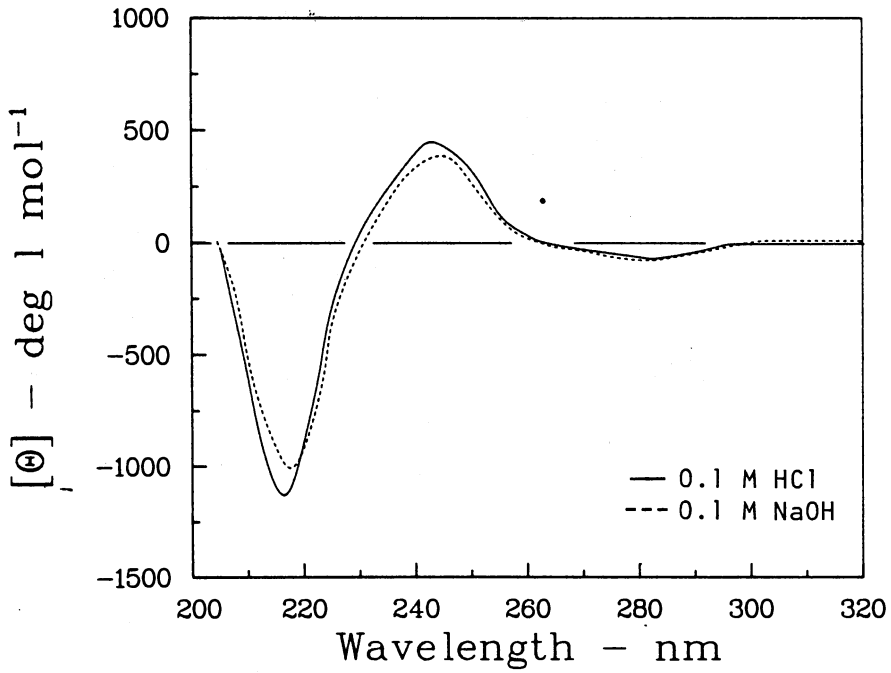


Figure 24. Codeine CD Spectra Including the ^1E Transition

The data used to calculate the molar ellipticities from ^1E transition were found to have more variability than those for the ^1L_a transition. The problem may be that a partial loss of illumination at these wavelengths, due to the presence of oxygen, causes fluctuation in the CD signal. It was believed that the information obtained was not worth the additional effort. No attempt was made to study this transition for the other opiates.

Synthetic Mixtures

Three different mixtures of morphine, codeine, thebaine, and quinine were studied to determine if their spectra are additive. The first three drugs are the principle components of opium. Quinine is a typical adulterant found in clandestine heroin samples. Since CD is simply a modified form of absorption spectrophotometry, additivity of spectra should apply within the constraints normally associated with Beer's Law and deviations from it. Theoretically, the principle components of a sample of opium could then be identified and quantitated.

The results obtained by the addition of the drug components together for both acidic and basic media are shown, in Figures 25 through 28, for two of the mixtures. The agreement between theoretical and experimental results was good in all three cases. However, there sometimes appeared to be a wavelength discrepancy in the experimental maximum at 245 nm in relation to the theoretical model.

It is probable that instrumental pen lag would account for part of this problem. At a period setting of 10, there was as much as 2 nm difference in the measurement of the wavelength of maximum positive ellipticity. This also resulted in a rounding of the peak and decrease in

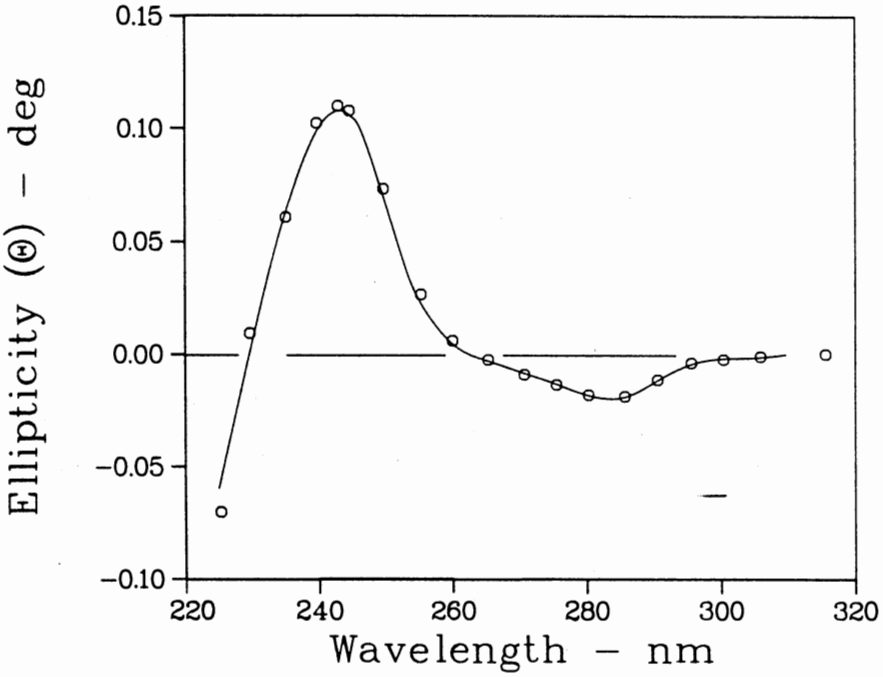


Figure 25. Synthetic Mixture of Morphine and Codeine, Dilute Acidic Medium, 1.4×10^{-4} M Each

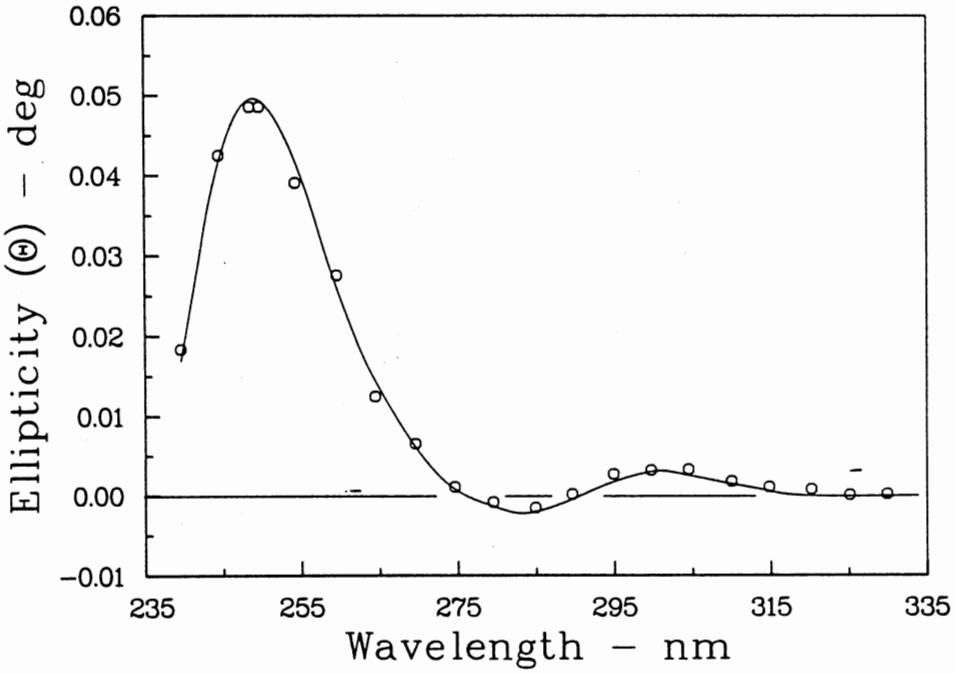


Figure 26. Synthetic Mixture of Morphine and Codeine, Dilute Basic Medium, 1.4×10^{-4} M Each

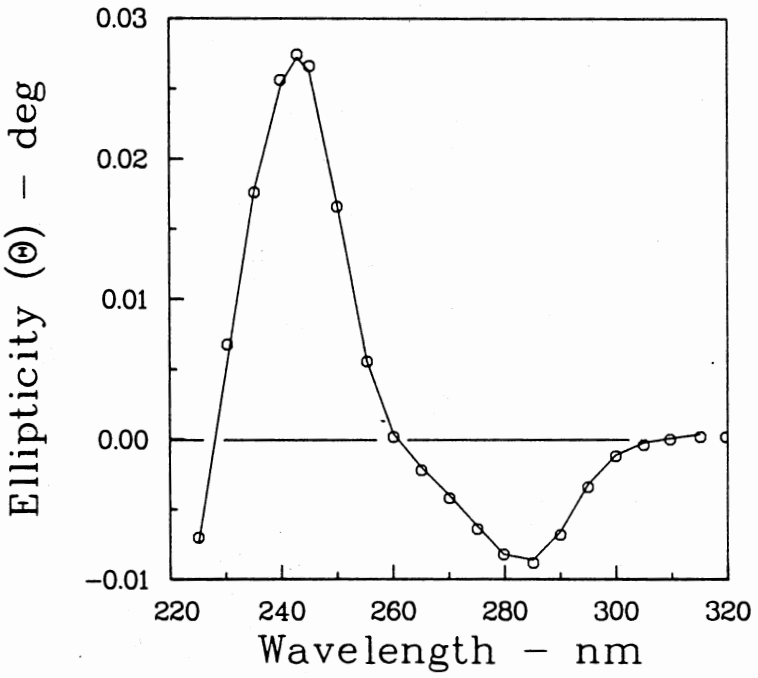


Figure 27. Synthetic Mixture of Morphine, Codeine, and Thebaine, Dilute Acidic Medium; Morphine, 1.7×10^{-4} M; Codeine, 3.7×10^{-5} M; Thebaine, 3.1×10^{-5} M

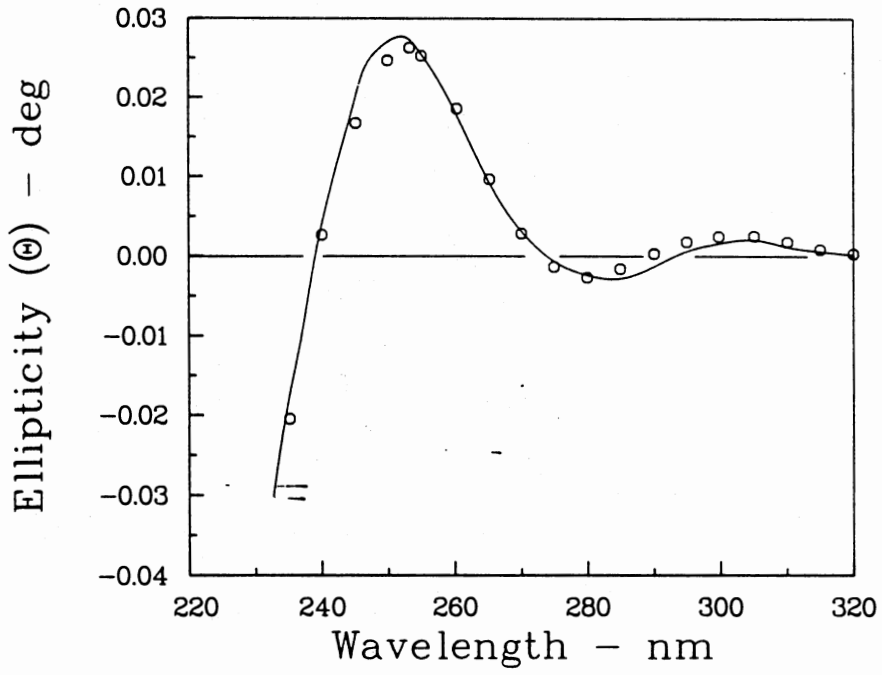


Figure 28. Synthetic Mixture of Morphine, Codeine, and Thebaine, Dilute Basic Medium; Morphine, 1.7×10^{-4} M; Codeine, 3.7×10^{-5} M; Thebaine, 3.1×10^{-5} M

ellipticity by as much as five percent. However, this problem was not detected until a later date.

These experiments with synthetic mixtures confirmed that CD absorption bands are additive in nature. They did not show, however, to what extent CD spectra could be subtracted from one another and the level of accuracy to be expected in determinations.

Unknowns

In-House Preparations

The in-house heroin sample of unknown concentration was analyzed to determine the best calculation method for accurate quantitation (Table VII). Since the molar ellipticities are so much greater for morphine than for heroin, the hydrolyzed sample gave results which had greater accuracy. Regarding heroin in 0.1 M HCl, the accuracy obtained using data for the 245 nm maximum was better than that from data taken at the 285 nm maximum. This may be due in part to baseline drift. Efforts to compensate for any changes in the baseline from zero ellipticity could not always be completely accomplished. Any undetected change in baseline would cause a measurement error, which is magnified as the signal size decreases.

The results from the analysis of the samples containing codeine, morphine, and thebaine, either individually or as mixtures, are shown in Tables VIII through XI.

There are some general remarks which can be made in reference to these experiments. The most significant reasons for differences between sample results were the effects of sample size and sampling technique. The best results, by far, were for the most concentrated samples (Set 4,

TABLE VII

QUANTITATIVE DETERMINATION OF HEROIN SAMPLE[a]

Wavelength, nm	Amount, mg	Percentage	Rel. Error, %
243	4.61	31.3	8.9
283	4.43	30.1	12.3
254	4.77[b]	32.4	5.6
298	4.77[b]	32.4	5.6

[a] 34.3% heroin.

[b] Hydrolyzed to morphine.

TABLE VIII

QUANTITATIVE DETERMINATION OF DILUTE SAMPLES (SET 1)

Sample	Drug	Trial 1 %	Trial 2 %	Trial 3 %	Average, %	Actual, %	Diff. %	Rel. Error, %
#1	Morphine	1.5	1.6	1.7	1.6	2.2	-0.6	-27
#2	Thebaine	2.0	1.8	1.8	1.9	1.5	+0.4	+29
	Morphine	1.4	1.6	1.5	1.5	2.6	-1.1	-42
#3	Codeine	2.0	2.2		2.1	1.5	+0.6	+40
#4	Thebaine	2.3	2.5		2.4	2.7	-0.3	-11
#5	Codeine	1.3				1.7	-0.4	-15
	Morphine	1.4				1.7	-0.3	-11

TABLE IX

QUANTITATIVE DETERMINATION OF SAMPLES MIXED BY HAND (SET 2)

Sample No.	Drug	Trial 1, %	Trial 2, %	Trial 3, %	Average %	Actual, %	Diff. %	Rel. Error, %
1, Acid	Codeine	10.0	20.7	17.9	16.2 ± 5.5	13.9	2.3	+15
1, Base	Codeine	10.8	22.0	18.8	17.2 ± 5.8		3.3	+16
2, Acid	Morphine	8.0	7.9	8.3	8.1 ± 0.2	10.6	-2.5	-24
2, Base	Morphine	8.4	8.7	8.4	8.5 ± 0.2		-2.1	-20
3, Acid	Codeine	10.2	11.8	9.9	10.6 ± 1.0	9.9	+0.7	+10
	Morphine	6.7	7.8	6.5	7.0 ± 0.7	8.7	-1.7	-24
3, Base	Codeine	10.1	11.4	9.9	10.5 ± 0.8	9.9	+0.6	+10
	Morphine	6.7	7.5	6.5	6.9 ± 0.5	8.7	-1.8	-21

TABLE X

QUANTITATIVE DETERMINATION OF SAMPLES MIXED BY SHAKER (SET 3)

Sample No.	Drug	Wave-length	Trial 1	Trial 2	Trial 3	Average %	Actual %	Diff. %	Rel. Error, %
1	Codeine	245	13.6	13.6	13.6	13.6 ± 0.0	14.7	-1.1	-7.5
		285	15.8	19.1	14.3	16.4 ± 2.5	14.7	+1.7	+10.2
2	Morphine	245	8.2	8.3	9.0	8.5 ± 0.4	10.3	-1.8	-17.5
		285	10.3	8.3	8.9	9.2 ± 1.0	10.3	-1.1	-10.7
3	Thebaine	221	11.0	12.2	11.8	11.7 ± 0.6	14.0	-2.3	-16.4
		285	10.9	12.5	11.4	11.6 ± 0.8	14.0	-2.4	-17.1

TABLE XI

QUANTITATIVE DETERMINATION OF MORE CONCENTRATED SAMPLES (SET 4)

Sample No.	Drug	Direct, Acid, mg	Direct, Base, mg	Diluted, Acid, mg	Average mg	Actual mg	Diff. mg	Rel. Error, mg
1	Codeine	4.55	4.85	4.67	4.69	4.83	-0.14	-2.9
2	Morphine	2.20	2.35	2.23	2.26	2.29[a]	-0.08	-2.7
3	Thebaine	2.21	2.35	2.21	2.26	2.26	0.00	-0.1

[a]As morphine sulfate; equal to 2.34 mg morphine base.

approximately 3×10^{-4} M). These samples were two to four times more concentrated than those in Sets 2 or 3. Since the entire sample was dissolved in 0.1 M HCl, there was also no doubt over whether the sample chosen was representative of the whole. There is a definite sampling problem for the samples mixed by hand. The codeine samples, in particular, showed a large variance among the three trials. For the other samples mixed by hand there is as great, or greater, variance between trials than there is for results taken in different solvents. The variance factor between trials is improved for the samples mixed by shaking in the Wig-L-Bug.

There was no obvious trend in these sets of experiments which indicated that any one drug could be more accurately quantitated than another; that the ^1L_a transition data gave significantly better results than the ^1L_b transition data; or that one solvent medium was better than another. There may be statistical significance in the data for these two parameters, but they are not easily discernible. It appears that the factors interact occasionally to increase or decrease the variance. One such example is the 285 nm versus 245 nm transition for codeine in 0.1 M HCl. From Table X it appears that the 245 nm transition affords better accuracy and precision. Tendencies such as these will be discussed in greater depth, for a larger population, in Chapter VI.

There was also no decrease in accuracy or precision for mixtures having two optically active species, as compared to those having a single optically active compound. Apparently, the error in the molar ellipticities cause less variability in the answers obtained than do the other parameters discussed.

From the analysis of the very dilute solutions (1-3 X 10^{-5} M), it is apparent that there is some loss of accuracy. However, all weight percentages, except one, were to within 0.6 percent of the actual value. Thus, mixtures of optically active compounds may be quantitated, although perhaps not to the desired degree. The accuracy may be sufficient for some applications of this method, at these concentration levels.

Heroin Confiscates

Four samples of heroin seized by the OSBI were quantitated by CD spectroscopy. Gas chromatographic analyses were performed on all four samples as an alternate procedure for comparison among methods. First, four replicates of heroin sample No. 4 were examined by CD to determine their spectral reproducibility and quantitative accuracy. The drug present was identified as heroin from data for the samples dissolved in 0.1 M HCl. The CD data resulted in higher quantitative values as compared to GC data for all four replicates, as shown in Table XII. The replicates dissolved in the pH 8.6 buffer medium resulted in the poorest accuracy and precision. At times, the values were twice those obtained via the hydrolysis product. As would be predicted from previous heroin samples, the most accurate (as compared to GC data) and most reproducible results were obtained for the samples hydrolyzed in dilute NaOH.

The data for the other three heroin confiscates dissolved in 0.1 M HCl also resulted in higher values as compared to GC, as shown in Table XIII. This is due to an enhanced experimental ellipticity presumably caused by the presence of either 6-monoacetylmorphine or morphine. Heroin is prepared by the acetylation of morphine. Samples of heroin will usually contain small amounts of unreacted morphine and 6-MAM, as

TABLE XII

QUANTITATIVE DETERMINATION OF HEROIN CONFISCATE NO. 4[a]

As Heroin	As Heroin	As Morphine
0.1 M HCl	8.6 Buffer	Dilute NaOH
104 ± 1.7%[b] (238 nm)	117% (243 nm)	81.3 ± 2.2%[b] (254 nm)
110 ± 13.7%[b] (280 nm)	123% (283 nm)	84.4 ± 0.4%[b] (298 nm)

[a]Results presented as percent by weight of heroin free base.

[b]Standard deviation for three samples.

TABLE XIII

QUANTITATIVE DETERMINATION OF HEROIN CONFISCATES[a] BY CD

	As Heroin[b]		As Morphine[c]		As Heroin	
OSBI No.	Direct	Extracted	Direct	Extracted	GC	OSBI[d]
1			0.7, 2.6[e]	0.6	0.8	
2	7.7	7.3	5.6	5.1	4.9	
3	4.0	3.2	1.5	1.4	1.1	
4	99.3[f]	79.2	73.4[g]	59.2	73.4	75

[a]Results presented as percent by weight of heroin free base.

[b]0.1 MHCl.

[c]Excess NaOH.

[d]OSBI is the Oklahoma State Bureau of Investigation.

[e]Quantitated after $CHCl_3$ wash.

[f]Standard deviation = 3.6 (N = 5).

[g]Standard deviation = 0.5 (N = 5).

gas chromatography seemed to confirm. Since the molar ellipticity for the 245 nm band is approximately four to five times greater for 6-MAM and morphine than for heroin, even a small impurity will result in a high result for heroin determination.

Better results were obtained from the data for the samples hydrolyzed in dilute sodium hydroxide, as shown in column four. All of the acetylated morphine derivatives are hydrolyzed to morphine. Any acetylated derivative present as an impurity would be included in the quantitation as morphine, but on a one-to-one basis. Therefore, the answers would still be high as compared to the GC analysis, as the data indicate. Sample No. 4 was determined by GC to have heroin as the main constituent, with a small amount (approximately 3%) of a CD active impurity, probably 6-MAM. The direct analysis from the hydrolyzed sample should be in close agreement with the GC analysis.

The results in column three indicate that the separation of heroin by chloroform extraction was incomplete, resulting in values which are still high as compared to GC determination.

Ideally, the best results should be obtained by the hydrolysis of the extracted heroin samples. Any initially unreacted morphine would not be extracted and 6-MAM, only partially. The results from Sample Nos. 1 through 3 would indicate that some, but not all, of the acetylation by-products have been removed. The results from the extract of Sample No. 4 might be low due to incomplete extraction of the large sample size.

The direct analysis of Sample No. 1 was nullified, and that of No. 3 hampered by a significant quantity of a UV absorbing but CD transparent compound. Therefore, an aliquot of Sample No. 1 was dissolved in 0.1 N sodium hydroxide solution and washed with chloroform. This improved the

signal-to-noise ratio significantly, which allowed the CD spectrum to be obtained. Still, the results from the hydrolyzed sample were high when compared to the GC analysis.

GC analyses revealed that each sample had its own unique formulation containing both UV and CD absorbing components. The retention times and peak heights were both very reproducible. Table XIV gives the average retention time and peak height for each component in the chromatogram. All four samples contained peaks with the same retention times (R.T.) as heroin and 6-MAM. Heroin has the greatest peak height at a retention time of 7.9 minutes. Since 6-MAM is a by-product of the preparation of heroin, it is highly probable that it is represented by the peak at 6.2 minutes. A small peak appeared at 11.5 minutes in all four samples. Considering the relatively long retention time, this peak might represent a small amount of unreacted morphine. The presence of both would cause the results for heroin, from CD, to be higher. Sample No. 3 contained a significant impurity with a R.T. of 4.3 minutes. If the compound represented by this peak is CD active, then it is understandable why the direct analysis of this sample resulted in a much larger value than that of the hydrolyzed product. The compound with a R.T. of 2.4 minutes must be a major CD transparent, but UV absorbing compound. Otherwise it would probably dominate the CD spectrum.

Chromatograms for the 1 μg/μℓ heroin standard, confiscates No. 2 and No. 3, are shown in Figure 29. The heroin GC calibration curve is shown in Figure 30. The lower three calibration standards, plotted versus peak height, were linear.

A positive deviation from linearity was observed for the highest calibration standard. All of the unknowns were similar in concentration

TABLE XIV

QUANTITATIVE DETERMINATION OF HEROIN[a]
CONFISCATES BY GC

OSBI No.	2.4	4.3	Retention Time, Minutes 6.2[b]	7.9[c]	11.5[d]
1	Off scale	---	3.0	23	2.5
2	---	---	9.0	163	1.0
3	Off scale	19	3.5	31	<1.0
4	---	<1	8.0	168	<1.0

[a]Results presented as average peak heights, from the baseline, in millimeters; attenuation 20 for Nos. 1-3; attenuation 40 for No. 4.

[b]Probably 6-MAM.

[c]Heroin.

[d]Possibly morphine.

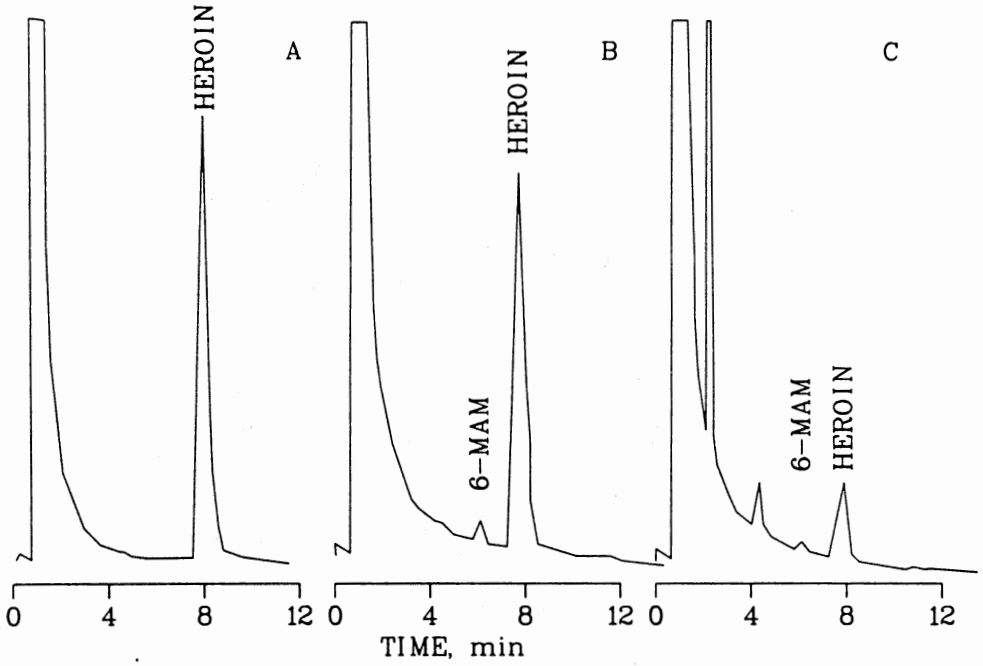


Figure 29. Chromatograms of one ug/ml Heroin Standard and Two Heroin Confiscates; A--1 ug/ul Standard; B--Heroin Confiscate No. 2; C--Heroin Confiscate No. 3

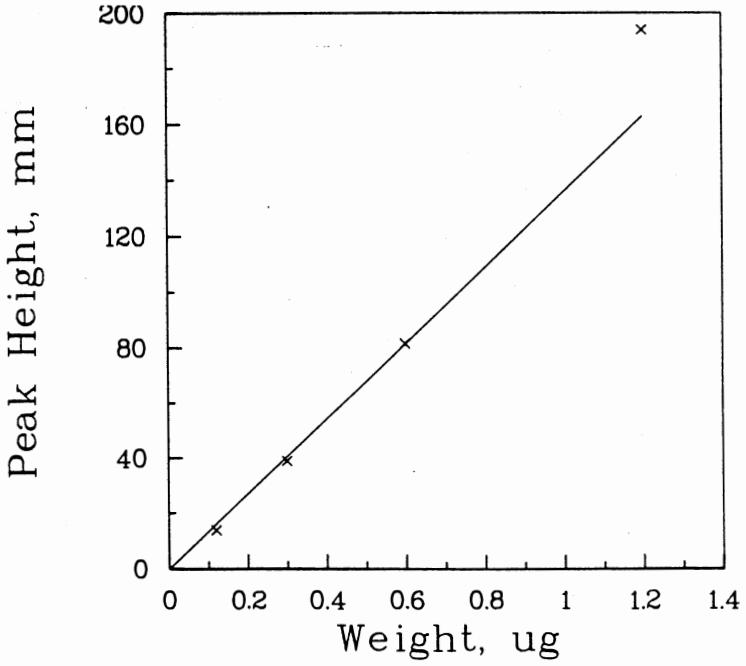


Figure 30. GC Calibration Curve

to one of the calibration standards, which would improve the accuracy of the analysis.

From these results one can easily conclude that heroin cannot be accurately quantitated directly in 0.1 M HCl solutions. Also, acetylated impurities are not easily detected by CD if their concentrations are not in excess of 10 percent by weight to that of the heroin present. After the samples are hydrolyzed to morphine it is impossible to state from which acetylated derivative they originated. These are the most serious drawbacks to the use of CD spectropolarimetry for the analysis of mixtures containing heroin.

No attempt was made to identify the other components present in the solid confiscates. Unless they are CD active, they will not interfere in the determination of heroin. An exception to this is a strongly absorbing material in the UV region. Such a case in point is confiscate No. 1. Part of the UV-absorbing material had to be removed by extraction before a satisfactory CD spectrum could be obtained. The problem is entirely one of there not being enough radiative throughput to obtain a sufficiently noise-free signal.

Compounds which are optically active but which do not absorb in the near-UV, such as the mono- and polysaccharides, present no problem in the qualitative identification of the CD active components.

Sugars, starches, and dyes are common diluents encountered in heroin confiscates. Dyes, in particular, present problems in the analysis of these samples by conventional methods. In the samples under investigation, three of the four confiscates had a coloration in the chloroform extract which would probably be UV absorbing, but which presented no problem in the analyses by CD spectroscopy.

Pharmaceutical Preparations

The results obtained for the quantitative analyses of the codeine samples of unknown concentration, of the pharmaceutical preparations, and of the cough syrups were very encouraging. The results from duplicate or triplicate analyses were more reproducible and more accurate than those for the heroin samples of unknown concentration. In the case of the cough syrups, the samples could be quantitated on simple dilution in aqueous 0.1 M HCl.

Using average values of +460 and -74 degrees $\cdot l \cdot mol^{-1}$ for the two CD transitions resulted in precise quantitation of the two samples of codeine mixed with lactose. These values were five percent lower than those using the molar ellipticities of +438 and -73 degrees $\cdot l \cdot mol^{-1}$, obtained from the two codeine standards. The average values were used in all calculations for the codeine samples. The results are listed in Table XV.

Fairly good precision was observed within the duplicate or triplicate analyses of each sample. There was evidence for greater variation among different formulations. The analyses were made on four separate occasions, over a three-week period, in a fairly random manner. The results should therefore not be biased by instrumental performance. No data were found for the tolerance regarding the amount of codeine that should be present in each tablet. It is certainly possible that the Phenaphen® tablet analyzed contained an excess of codeine phosphate while that of the Fiorinal® No. 3 was deficient.

The dihydrocodeine present in Synalgos DC® was also accurately quantitated using the same standard extraction procedure on a 53 mg aliquot of the capsule. Using data from both the 239 and 281 nm

TABLE XV

QUANTITATIVE DETERMINATION OF CODEINE PREPARATIONS[a]

Sample	Stated Amount, mg	Experimental, mg	Percent Difference
Unknown No. 1	2.08[b]	2.09[b]	0.0
Unknown No. 2	0.98[b]	0.98[b]	0.0
Phenaphen	30.00	32.50[c]	+8.3
Tylenol No. 3	30.00	29.10 ± 4.30[d]	-3.0
Tylenol No. 4	60.00	56.30[c]	-6.2
Fiorinal No. 3	30.00	26.30 ± 4.30[d]	-12.4

[a]As codeine phosphate, except as noted.

[b]Codeine base.

[c]Duplicate analyses.

[d]Triplicate analyses.

transitions, 15.5 mg were detected as compared to a nominal composition of 16.0 mg.

Even with an extraction procedure involved in the analysis, the separation process was much simpler than that for the quantitation by UV spectroscopy. The extraction process does not have to be 100 percent efficient if the other components are not CD active. The molar extinction coefficients are high for aspirin and caffeine, which necessitates their complete removal before quantitation can be accomplished by UV absorption spectroscopy.

One of the reasons for the analyses of these samples was that consistently low results were obtained by other investigators using HPLC. It is not known if this problem was resolved, but it is encouraging that an alternate convenient method is available.

Two cough syrups containing codeine were analyzed, both directly in 0.1 M HCl and after extraction. The results are shown in Table XVI. In the direct analysis of the Phenergan VC, the signals were very noisy. Data from 285 nm transition yielded a value of 2.07 mg/ml while that from the 245 nm transition data were 1.62 mg/ml. The discrepancy might be attributed to instrument limitations in signal amplification at two different wavelengths. An accurate quantitative determination was effected in the direct analysis of the second sample using data from the 245 nm transition of codeine, although the signal was somewhat noisy. Data from the 285 nm transition resulted in low values. This was probably caused by another CD active drug which partially canceled the signal due to codeine at this wavelength.

TABLE XVI

QUANTITATIVE DETERMINATION OF CODEINE IN COUGH SYRUPS[a]

Preparation	Extracted, mg/ml	Nonextracted, mg/ml	Expected, mg/ml
Phenergan VC	2.00[b] ± .02	1.84[c]	2.00
FC's	1.97[c]	1.97[c]	2.00

[a]As codeine phosphate.

[b]Triplicate analyses.

[c]Duplicate analyses.

After extraction very good CD spectra were obtained, and the codeine was accurately quantitated from either transition for both preparations, as shown in Table XVII.

Thus, cough syrups containing codeine phosphate can be accurately quantitated after a simple extraction procedure. This should be a much simpler method than that for HPLC and more qualitatively acceptable than GC. It should be reiterated that the method is no more difficult than UV absorption spectroscopy. Once the instrument has been calibrated and the molar ellipticity coefficients have been determined, there is no need for daily calibration.

Limits of Detection

There were no experiments designed to test the limits of detection of these compounds. Molar ellipticities were calculated from data for solutions with sufficient concentrations to reduce experimental error. These linear calibration curves were extrapolated to the limit of detection, using the same definition as that by Kaiser.[78]

In the analyses of these compounds the limit of detection is affected both by baseline drift and noise. Drift in the baseline was measured by maintaining the instrument at a single wavelength and then measuring the change in ellipticity with time. From these measurements, an estimate was made of the drift in the baseline at the average wavelengths of the ellipticity maxima. The average noise in the baseline was measured from several spectra to determine the analytical noise present at the average wavelengths of the ellipticity maxima. These values were then used in the following formula for the limit of detection:

TABLE XVII

LIMITS OF DETECTION[a] -X10^{6}M

Drug	Solvent	245 nm	285 nm	300 nm
Morphine	HCl	5.2	19.0	
	NaOH	4.1		19.0
Nalorphine	HCl	5.5	22.0	
	NaOH	4.5		21.0
3-Acetylmorphine	HCl	6.0	21.0	
	8.6	7.6	30.0	
6-Acetylmorphine	HCl	6.3	24.0	
	8.6	8.1	30.0	
Heroin	HCl	29.0	65.0	
	8.6	20.0	65.0	
Hydromorphine	HCl	27.0	11.0	4.0
	NaOH	20.0	7.6	5.0
Oxymorphone	HCl	45.0	10.0	4.0
	NaOH	19.0	7.8	5.0
Naloxone	HCl	20.0	10.0	3.9
	NaOH	25.0	8.6	4.8
Codeine	HCl	4.3	18.0	
	NaOH	5.6	19.0	
Dihydrocodeine	HCl	14.0	15.0	
	NaOH	15.0	16.0	
Ethylmorphine	HCl	5.3	22.0	
	NaOH	6.7	23.0	
Hydrocodone	HCl	16.0	9.5	3.7
	NaOH	16.0	12.0	3.7
Oxycodone	HCl	17.0	4.9	3.7
	NaOH	29.0	6.0	4.6
Thebaine	HCl	3.1	15.0	2.1
	NaOH	2.9	15.0	2.4

[a]Using criterion of Kaiser[79]: $X_D = \bar{X}_{bl} + 3s_{bl}$.

$$X_{D,\lambda} = \bar{X}_{bl} + 3\ S_{bl}$$

where

$X_{D,\lambda}$ = measurement at the limit of detection (degree) at a given wavelength;

$\bar{X}_{bl}$ = average value of the drift at a given wavelength; and

S_{bl} = standard deviation of the noise at a given wavelength.

CHAPTER V

DISCUSSION

Characterization of Spectra

General Parameters

The CD spectra in aqueous solutions upon a change in pH show changes in wavelength maxima similar to those observed for UV absorption spectroscopy. There is the added benefit that broad bands are often resolved into two or more component bands with a significant improvement in resolution. This is due, at least in part, to there being both positive and negative CD bands.

Since energy absorption is a prerequisite to CD activity, the bands observed in the CD spectra for the opiate drugs are assigned to the standard electronic transitions for the aromatic ring chromophore. The large positive Cotton effect in the wavelength range 238-255 nm is attributed to a $\pi^* \leftarrow \pi$ electronic transition of symmetry ^1L_a and the smaller negative Cotton effect around 275-290 nm is attributed to a second $\pi^* \leftarrow \pi$ electronic transition designated by ^1L_b. The third aromatic transition, the very intense ^1B transition around 210-220, shows an even larger negative Cotton effect, with the exception of thebaine, where a moderately large positive value is observed.

The ketone functional group chromophores in hydromorphone, oxymorphone, naloxone, hydrocodone and oxycodone are associated with the

negative Cotton bands which occur at 300-315 nm as an $\pi^* \leftarrow n$ transition. The N-vinyl group present in naloxone and nalorphine does not seem to affect the spectra significantly when compared to the corresponding N-methyl derivatives, except for a slight loss in intensity of the CD band.

Both λ_{max} values are invariant with pH for alkaloids which have OCH_3 present at C(3). Alkaloids which have either OH or $COOCH_3$ in the 3-position show a red shift for λ_{max} on going from acidic to buffered to basic media, and to differing degrees for the different transitions. The results are consistent with trends observed in UV absorption spectroscopy and are most likely attributable to the dissociation of the phenol proton, and acetate ester hydrolysis.

Further modifications of the spectra were evident in alkaloids with a ketone functional group at C(6) and thebaine, which has a conjugated C-ring. These are summarized in Table V first for the morphine group, including those members with a ketone group at C(6) or an N-vinyl group, and second, the codeine group, which includes those members with a ketone functional group, and finally, thebaine.

All of the acetyl derivatives are fairly stable at pH 8.6, but at higher pH rapid hydrolysis to morphine occurs in the form of the phenolate ion. Accordingly, the spectra for the acetylated alkaloids in solutions buffered to pH 8.6 must be obtained within a few hours to insure accurate quantitative results.

Analytical Distinction by CD Spectral Parameters

The improvement in CD spectral resolution, as compared to that of UV absorption spectra, affords a significant improvement in qualitative

distinction among the opiates. This improvement is enhanced by changes in the pH of the solvent medium.

The greatest similarity in CD spectral parameters is observed in a strong acid solution, Figures 8-10 and Figure 17a. At acid concentrations in excess of 0.1 M HCl, there appear to be no significant spectral differences as the acidity is increased.

Under these strongly acidic conditions, the phenolic group is undissociated, the acetyl substituents are not hydrolyzed, and the ring nitrogen is protonated. As it was for the alkaloids dissolved in ethanol, some qualitative distinction is observed when the C-ring is modified; for example, thebaine (conjugated diene) and dihydrocodeine (saturated). Thebaine is uniquely identified in this study by its positive band at 221 nm, heroin by its ^1L_a band at 238 nm, and dihydrocodeine by its unique λ^o at 253 nm. All of the ketone derivatives have similar spectra, as do the members of the morphine and codeine groups with OH at C(6). These members could possibly be distinguished by the ratio of their molar ellipticity values, which, however, severely limits the ability for qualitative identification in the event that a mixture is under investigation.

Qualitative analysis of the acetylated derivatives is not possible in base, since all acetylated derivatives are rapidly hydrolyzed to morphine. The members of the morphine group having a ketone group at C(6) can be readily distinguished from the corresponding members of the codeine group by the significant red shift in the $\bar{\lambda}_{max}$ of only the morphine ketones in base, but there is no distinction possible within each group. Again, thebaine and dihydrocodeine have unique spectra that separate them from the other drugs studied. The CD spectra in basic

media are shown in Figures 11-13 and Figure 17b.

The most promising single condition for qualitative identification is the pH 8.6 buffer solution medium (Figure 14-16 and 17c). All members of the codeine group are now distinguishable by their λ_{max} and λ^{o} values, with the exception of codeine and ethylmorphine. The only difference in structure between the two is an ether methoxy versus ethoxy functional group at C(3). Distinction between the two alkaloids may be accomplished by close inspection of the intensities of the respective λ_{max} values at different pH values. Note that hydrocodone can be distinguished from oxycodone by small but significant differences in peak locations. The wavelength maxima and zero ellipticity values are slightly red-shifted for oxycodone in relation to hydrocodone.

Members of the morphine group are now distinguishable by changes in the $^{1}L_{a}$ band, by the separation of the λ^{o} values, and by the emergence of the positive band at 300 nm for morphine, nalorphine and 6-acetylmorphine. The exceptions to this may be morphine versus nalorphine, and oxymorphone versus naloxone. In both cases an N-vinyl group is substituted for an N-methyl group.

There is an overall decrease in molar ellipticity values within the morphine group in both dilute acid and pH 8.6 buffer media in the following progression: morphine > nalorphine > 3-monoacetylmorphine > 6-monoacetylmorphine > 3,6-diacetylmorphine (heroin). This may be related to an increase in molecular weight. Relative intensities in CD bands are interpreted by empirical structural models such as the octant rule (87). A decrease in signal with increasing molecular weight might be interpreted as an increasing component in a negative octant.

A feature associated with the ionization of the phenolic group is

the gradual emergence of a positive Cotton effect at approximately 300 nm for morphine, nalorphine and 6-acetylmorphine. This band grows at the expense of the negative band around 285 nm as the pH is increased. At high pH the usually negative band for the ^1L_b transition no longer exists. Also, since the band at 300 nm for hydromorphone and oxymorphone is probably due to overlapping $\pi^* \leftarrow \pi$ and $\pi^* \leftarrow n$ transitions, the 15 nm red-shift may be solely due to the formation of the phenolate ion. Further evidence for this is the lack of a red-shift for the ketone-containing members of the codeine group.

Ratio of Molar Ellipticity Values

Ratios of the maximum ellipticities can often be a useful adjunct to the procedures for identifying an anonymous alkaloid. These ratios and how they change with pH, in conjunction with the absolute molar ellipticities measured, have the potential of providing a unique identification.

Distinction is possible among the members of the morphine group by comparison of the band ratios, with the possible exception of morphine and nalorphine. The ellipticity ratio in acid is 6.1 for nalorphine and 5.8 for morphine. However, in the absence of other CD active species, the distinction should be made statistically meaningful by performing multiple scans. The small positive Cotton effect at 300 nm may have insufficient reproducibility for quantitative purposes. Distinction among the three keto-morphine derivatives can be made by virtue of the ratios of the ^1L_a and ^1L_b transitions in both dilute acid and pH 8.6 buffer solutions.

A decrease in molar ellipticity with increasing molecular weight

is again observed between codeine and ethylmorphine. The intermediate, dihydrocodeine, does not conform to the trend. This is perhaps due to a more dominant effect caused by a change in the basic codeine structure on saturating the C-ring.

Dihydrocodeine and codeine are easily distinguishable from each other, while ethylmorphine is most easily recognizable by the ratio of the ^1L_a to ^1L_b transitions in pH 8.6 buffer. Hydrocodone and oxycodone can be distinguished from each other in the same manner. For hydrocodone the ^1L_a band has the greater ellipticity while for oxycodone the ^1L_b transition has the greater ellipticity.

In the final analysis, it is possible to distinguish between all of the alkaloids in this study using both wavelength and intensity parameters. Only experience will tell if this continues to hold true as more alkaloids are included in the data base. Any identification problems encountered must be between compounds that are fairly close in structure. It has been shown (81) that different drug groups have significantly different CD spectra. Therefore, it would seem very unlikely that some drug foreign to the group in question would give identical results.

Molecular Origin of Spectral Changes

By changing the substituent present on the ethanophenanthrene alkaloid structure, some insight can be gained into their effect on molecular structure. Similar studies have been performed for several steroids (68) and the phthalideisoquinoline alkaloids (82). The latter study showed that CD spectra can be deconvoluted into gaussian bands corresponding to discrete transitions.

Since the alkaloids are basically T-shaped, the substituents on C(6) and the π-electrons of the unsaturated bands are actually much closer to the aromatic nucleus than is readily apparent from a conventional drawing of the molecules. Therefore, throughspace interactions may be invoked which are just as important as conjugation in interpreting changes in spectra.

Effect of the Oxygen Bridge

The morphine alkaloids have a fairly rigid structure with most of the flexibility in the C-ring. This rigidity increases the optical activity. For example, the aromatic amines and isoquinoline alkaloids have very small CD signals as compared to the morphine alkaloids. The same effect was noticed in a series of rigid versus flexible indane derivatives (86). However, the authors noted that morphinan derivatives still had enough rigidity to produce optical activity on the order of one-third of that of the corresponding morphine alkaloids (86). In morphine (79) and codeine (83), steric restriction due to the oxygen bridge forces C(12) out of the aromatic plane of symmetry as shown by x-ray crystallography. In contrast, for 3-methoxy-N-methylmorphinan (84) and tetrahydrodesoxycodeine (85), where the oxygen bridge is missing, the aromatic ring is almost flat. There is also a slight distortion of the bond angles for the C-ring in morphine and codeine as compared to the morphinan compounds. In summary, the oxygen bridge introduces enough steric rigidity to produce unanticipated distortions in the adjacent B- and C-rings.

Group Present at C(3)

In acidic media the phenolic group at C(3) is protonated and the A-ring should have basically the same conformation as was described in the x-ray studies. In morphine (79) and codeine (83), the C-ring assumes a boat conformation while for (+)- 3-methoxy-N-methylmorphinan (84) the C-ring exists as the chair conformer. In the process of formation of the phenolate ion, the delocalization of the oxygen negative charge might assume steric priority, causing the ring to flatten. This change will be transmitted by way of a conformational change in the dihydrofuran ring to the remainder of the molecule, where a change in conformation of the C-ring is expected to occur with C(5) and C(13) as the pivot points.

DeAngelis and Wildman (87) have developed a sector rule to make empirical comparisons for aromatic molecules. Using their basic rules for illustration, the projections of tetrahydrodesoxycodeine, codeine, and morphine (in the phenolate form) are shown in Figure 31. Note that there is only a slight shift in quadrant locations of the atoms for the three compounds from this perspective. Weiss and Rüll (85) have pointed out that compounds without the bridge oxygen have weaker negative ^1L_a and ^1L_b transitions whereas compounds with the bridge oxygen, such as morphine and codeine, have a moderately large positive ^1L_a transition and a negative ^1L_b transition. Although it is possible, it is difficult to conceive that such minimal conformational changes can produce such radical spectral changes.

From this projection, the change in the conformation of the C-ring is not easily seen. If the projection is turned 90 degrees and viewed from above the aromatic ring (Figure 32), the orientation of the C(7)-

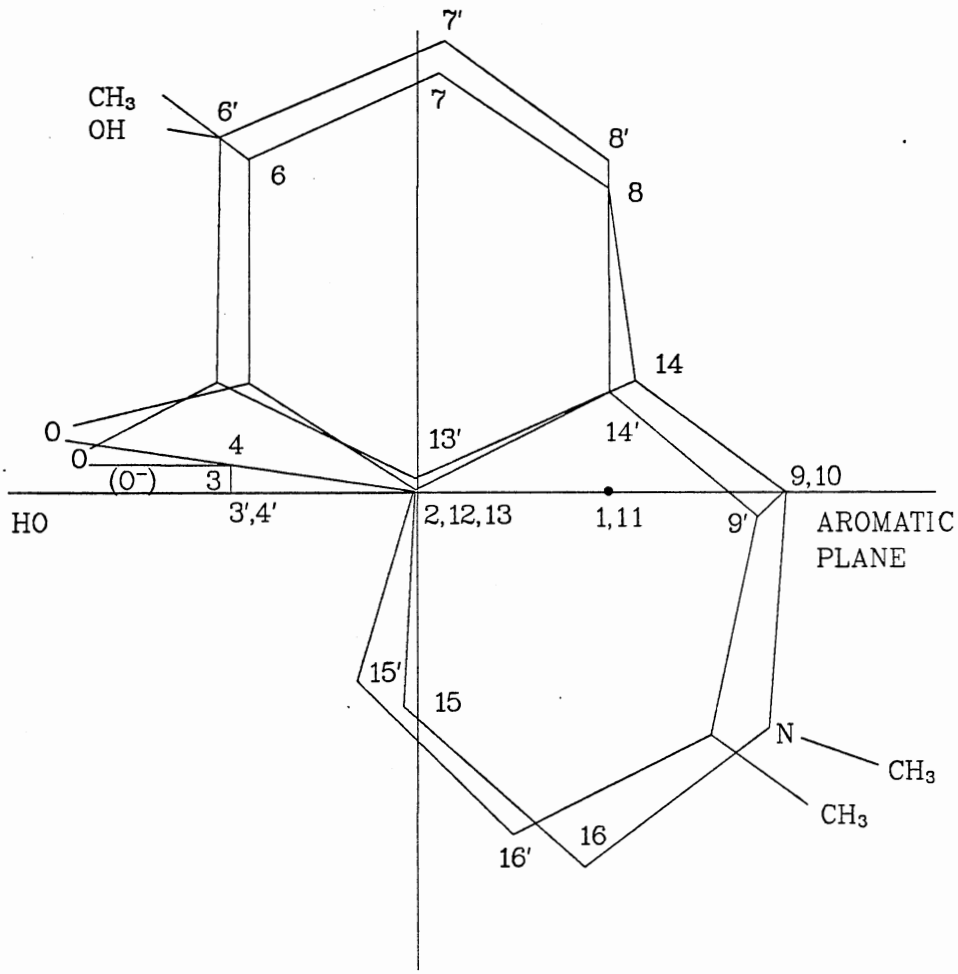
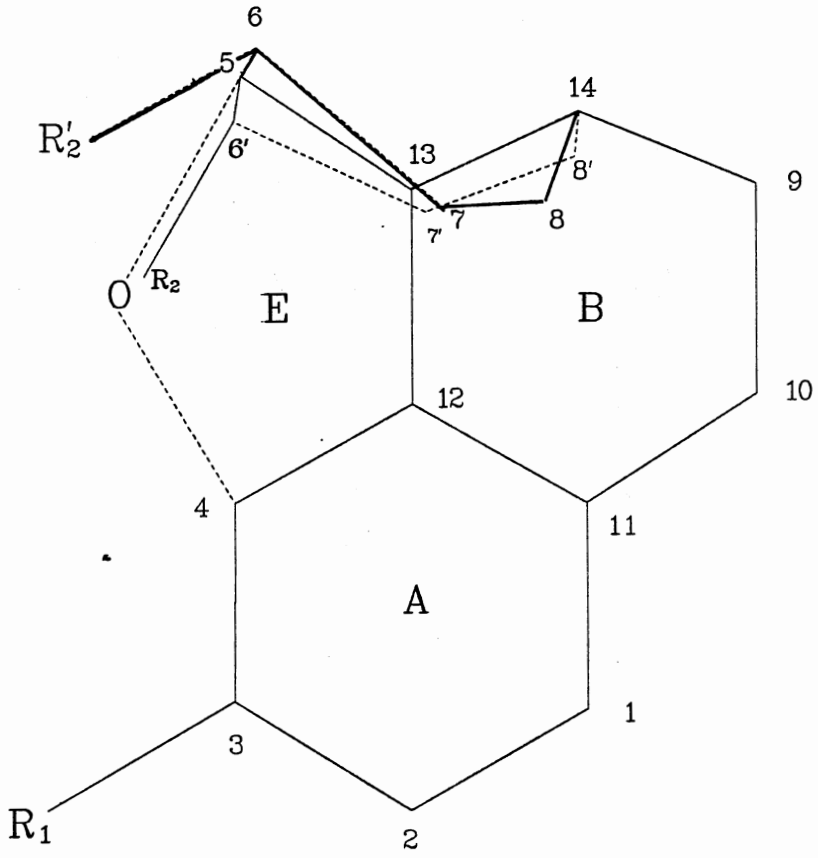


Figure 31. Projection of Tetrahydrodesoxycodeine (Primed), Codeine, and Morphine as Viewed by DeAngelis and Wildman Method



D RING OMITTED

---------- MORPHINE STRUCTURE

—— MORPHINAN STRUCTURE

Figure 32. Projection of Morphine as Viewed From Above the Aromatic Ring

C(8) bond is observed to have changed considerably on inversion of the C-ring conformation. If the morphine molecule as the phenolate ion assumes the morphinan conformation, it is easily seen that orbital overlap between the diene π-electrons with the π-electrons of the aromatic nucleus would change significantly from acidic to basic conditions. For the morphinans, one must also take into account that the C(7)-C(8) bond is saturated. Crabbe has pointed out (86, Table X) that there is a significant difference in the CD spectral properties of tetrahydrodesoxycodeine and dihydrodesoxycodeine D, its unsaturated analog. This adds additional evidence to the concept that there is orbital overlap between the π-electrons of the unsaturated linkage and the aromatic nucleus, and that changing the conformation changes the overlap of the π-orbitals.

Hydroxyl Versus Ketone on C(6)

The substitution of a ketone group at C(6) in place of the hydroxyl group causes an increase in the rigidity of the molecule. Looking at the molecule from the perspective of a quadrant projection, the ketone group is shifted to a more positive position in the quadrant, which should result in an increase in the intensity of the CD signal. However, this is not the result observed; there is a change in sign of the ^1L_b band, which cannot be effectively explained by a quadrant rule.

The Diene Structure

Thebaine was the only alkaloid studied with a C-ring conjugated diene structure, which imparts the most rigidity of all the compounds studied. Again, a sector rule does not adequately describe the

observed Cotton effects. The ^1L_a band should become more positive and the ^1L_b more negative. The compound does exhibit an intense negative ^1L_b transition, but the ^1L_a transition is fairly weak. The Cotton effect for it may be partially canceled by the much more intense ^1E and ^1L_b Cotton effects.

The conjugated diene structure is β to the aromatic nucleus, which should allow for effective orbital overlap, especially in a rigid structure such as thebaine. Interactions of this type have been observed for several aromatic compounds (61, 86). It is also possible to have through space orbital interaction due to the geometry of the molecule. Thebaine was the only compound studied which has a positive ^1E transition. The change to a diene structure may change the chirality of the conjugated system. Such changes have been observed in skewed styrenes (14).

Vinyl Versus Methyl Group on N

In all of the morphine and codeine alkaloids the N-methyl group is equatorial to the molecule.

Other structural changes have little steric affect upon the nitrogen ring. There is no reason to believe that a conformational change occurs on N-substitution by a vinyl group. No substituents on the nitrogen ring appear to have a significant effect upon the aromatic chromophore.

CHAPTER VI

STATISTICAL ANALYSIS

Statistical procedures were used to evaluate the data generated from the CD spectra. Two basic questions were examined: "What are the variances in the CD spectral parameters and molar ellipticities?" Secondly, "Are these parameters sufficiently different to allow unique identification of the compound in question?"

Statistical Parameters

Representative data for most of the compounds were analyzed by a standard statistical procedure for analysis of variance, both among samples and between compounds. The average wavelength values for positive and negative ellipticity maxima are tabulated in Table V. The corresponding standard deviations, calculated from the replicate mean square, are listed in Table XVIII. The standard deviations of the molar ellipticities are listed in Table XIX. No statistical data are included for the acetylated compounds in base due to their hydrolysis to morphine. There were insufficient data for the statistical analyses of ethylmorphine and morphine sulfate in base and of ethylmorphine, morphine sulfate and thebaine in pH 8.6 buffer. For those samples where only duplicate analyses were performed, the average wavelength values are listed in Table V and the average molar ellipticities in Table VI, but no statistical data are listed in Tables XVIII and XIX.

TABLE XVIII

STANDARD DEVIATIONS OF CD SPECTRAL PARAMETERS

Drug	Solvent	$\lambda_{Max, 245}$ Y2	$\lambda_{Max, 285}$ Y3	$\lambda_{Max, 300}$ Y4	λ^{o}_{230} Y5	λ^{o}_{250} Y6	λ^{o}_{280} Y7
Morphine	Acid	.4	.6		.3	.3	
	Base	.4	.3	.7	.4		
	8.6 Buffer	.2	.5	.0	.5	1.2	.6
Nalorphine	Acid	.3	.2		.0	.0	
	Base	.4	.2	.1	.1		
	8.6 Buffer	.1	.0	1.0	.3	.4	1.0
3-MAM	Acid	.7	.1		.8	1.7	
	8.6 Buffer	---	---	---	---	---	---
6-MAM	Acid	.3	.6		.2	.7	
	8.6 Buffer	1.2	1.0	1.4	1.0	1.4	.1
Heroin	Acid	.3	.0			1.0	
	8.6 Buffer	.7	.5		1.3	.5	
Hydro-Morphone	Acid	.2	.1	.2	.1		.1
	Base	.3	.0	.2	.4		.2
	8.6 Buffer	.7	.7	.7	.4		.4
Oxymorphone	Acid	.2	.1	.1	.2		.2
	Base	.3	.1	.2	.4		.5
	8.6 Buffer	1.5	.2	1.0	.2		.9
Morphine Sulfate	Acid	.6	.5		.7	.3	
	8.6 Buffer	---	---	---	---	---	---

TABLE XVIII (Continued)

Drug	Solvent	$\lambda_{Max, 245}$ Y2	$\lambda_{Max, 285}$ Y3	$\lambda_{Max, 300}$ Y4	λ^{o}_{230} Y5	λ^{o}_{250} Y6	λ^{o}_{280} Y7
Naloxone	Acid	.3	.2	.2	.2		.2
	Base	.7	.7	.4	.4		.3
	8.6 Buffer	.8	.5	.6	.3		.4
Codeine	Acid	.7	.7		.6	.6	
	Base	.7	1.0		.9	.4	
	8.6 Buffer	.5	.4		.4	1.9	
Dihydro-Codeine	Acid	.6	1.0		.4	1.3	
	Base	.4	.3		.7	1.5	
	8.6 Buffer	.3	.3		.7	1.5	
Ethyl-Morphine	Acid	.3	.3		.4	.9	
	Base	---	---		---	---	
	8.6 Buffer	---	---		---	---	
Hydrocodone	Acid	.3	.4	.7	.2		.1
	Base	.9	.6	.3	.6		.4
	8.6 Buffer	.9	.5	.4	1.1		.2
Oxycodone	Acid	.3	.1	.1	.2		.0
	Base	.5	.3	.4	.2		.2
	8.6 Buffer	.7	.7	.6	.3		.2
Thebaine	Acid	.5	.5	.4	.5	.5	
	Base	.5		.4	.5	.5	

TABLE XIX

STANDARD DEVIATIONS OF MOLAR ELLIPTICITIES

Drug	Solvent	$[\theta]_{245}$	$[\theta]_{285}$	$[\theta]_{300}$
Morphine	Acid	2.0 (0.5)	0.9 (1.3)	0.4 (1.3)
	Base	3.4 (0.7)		0.4 (0.7)
	8.6 Buffer	20.0 (6.8)	3.6 (10.9)	---
Morphine Sulfate	Acid	2.6 (0.7)	1.6 (2.4)	
	8.6 Buffer	12.4 (3.9)	4.6 (6.9)	
Nalorphine	Acid	5.9 (1.6)	2.3 (3.9)	
	Base	16.8 (3.8)		2.3 (4.9)
	8.6 Buffer	5.0 (1.7)	1.4 (3.4)	1.2 (16.0)
3-MAM	Acid	----	----	
	8.6 Buffer	----	----	
6-MAM	Acid	2.6 (0.8)	1.5 (2.7)	
	8.6 Buffer	13.2 (5.3)	0.7 (1.9)	4.7 (54.0)
Heroin	Acid	2.3 (3.3)	0.7 (3.5)	
	8.6 Buffer	7.0 (6.9)	0.6 (3.0)	
Hydromorphone	Acid	3.0 (3.9)	3.4 (2.7)	5.7 (2.3)
	Base	2.8 (1.5)	3.7 (2.2)	6.3 (3.1)
	8.6 Buffer	3.1 (3.3)	8.5 (6.6)	4.5 (2.4)
Oxymorphone	Acid	1.9 (4.2)	0.9 (0.7)	2.3 (0.9)
	Base	1.8 (1.1)	1.0 (0.6)	2.1 (1.0)
	8.6 Buffer	1.4 (1.4)	2.4 (1.7)	2.7 (1.7)
Naloxone	Acid	2.2 (5.1)	2.2 (1.7)	1.6 (0.6)
	Base	8.0 (5.3)	8.8 (5.8)	4.0 (1.9)
	8.6 Buffer	5.2 (6.5)	4.0 (3.0)	6.7 (3.6)
Codeine	Acid	15.0 (3.2)	2.2 (3.0)	
	Base	14.3 (3.8)	1.0 (1.4)	
	8.6 Buffer	32.0 (8.1)	5.0 (7.0)	
Dihydrocodeine	Acid	5.8	1.0 (1.1)	
	Base	9.0 (6.7)	4.1 (4.8)	
	8.6 Buffer	3.2 (2.3)	2.8 (3.5)	
Ethylmorphine	Acid	----	----	
	Base	7.2 (2.4)	0.3 (0.6)	
	8.6 Buffer	----	----	

TABLE XIX (Continued)

Drug	Solvent	$[\theta]_{245}$	$[\theta]_{285}$	$[\theta]_{300}$
Hydrocodone	Acid	4.9 (3.9)	8.6 (6.3)	9.9 (3.6)
	Base	5.7 (4.5)	3.8 (3.6)	3.0 (1.0)
	8.6 Buffer	11.6 (8.4)	5.9 (5.0)	2.7 (1.0)
Oxycodone	Acid	1.9 (1.6)	1.5 (1.1)	1.2 (0.4)
	Base	3.2 (4.7)	2.2 (2.5)	2.2 (1.0)
	8.6 Buffer	1.9 (1.9)	3.3 (2.6)	2.1 (0.8)
Thebaine*	Acid	12.8 (2.0)	5.5 (6.3)	11.4 (2.4)
	Base	20.2 (2.9)	11.1 (12.7)	13.5 (3.2)

*Maxima are at 221, 245 and 285 nm.

For comparison between drugs, the variance is divided (partitioned) into variances for drugs, replicates and, if available, duplicates. The replicate mean square is probably the best indicator of variations in wavelength maxima and molar ellipticities on a day-to-day basis. For samples where duplicates have been analyzed, the mean square values for drugs will not be the same as the mean square values for replicates. For most samples analyzed in duplicate, especially those in pH 8.6 buffer, a large part of the variability in the data was partitioned into the variance of the duplicates. This resulted in the variance of the replicates being lowered for these samples.

The standard deviations of CD parameters for naloxone in pH 8.6 buffer are extreme examples of this trend. The variance for duplicate samples (in a statistical sense) was thirty times as great as that for replicates. Thus, the analysis of variance (ANOVA) for replicates indicated that all analyses were of a common drug, whereas for duplicates it indicated that they were not of a common drug.

For most of the compounds, either five or ten replicates were analyzed on the same date. This gives an estimate of the experimental error in the analytical procedure, since each replicate is an independent analysis. For the compounds replicated ten times (Table XX), only codeine showed any significant differences in molar ellipticities or wavelength maxima as compared to the overall averages listed in Table VI. However, one explanation for the lack of difference for thebaine was the small number of examinations performed, except for the replicate study.

For the compounds replicated five times (Table XXI), the members of the ketone group showed a significant improvement in variance. Small

TABLE XX

MOLAR ELLIPTICITIES FOR TEN REPLICATES

Drug	Solutions	$[\theta]^{+}_{Max}$	$[\theta]^{-}_{Max}$
Morphine	HCl	383 ± 3.1 (0.8%)[a]	-66.8 ± 1.0 (1.5%)
		493 ± 4.2 (0.9%)	
		52.3 ± 0.7 (1.3%)	
Codeine	HCl	478 ± 3.0 (0.6%)	-78.0 ± 0.9 (1.1%)
	NaOH	359 ± 12 (3.9%)	-69.0 ± 1.0 (1.4%)
Thebaine	HCl	650 ± 11 (1.7%)	-472 ± 4.4 (0.9%)
	NaOH	87.6 ± 4.8 (5.5%)	-93.3 ± 5.7 (6.1%)
		700 ± 27 (3.8%)	-423 ± 13 (3.1%)

[a]Relative standard deviation for all values.

TABLE XXI

MOLAR ELLIPTICITIES FOR FIVE REPLICATES

Drug	Solvent	Max	Molar Ellipticity
Morphine	0.1 M HCl	245	382 ± 4.0 (1.1%)
		285	-67.0 ± 1.0 (1.4%)
	Base	253	492 ± 5.2 (1.1%)
		300	52.3 ± 0.7 (1.2%)
Nalorphine	0.1 M HCl	244	364 ± 5.2 (14.%)
		285	-59.5 ± 2.1 (3.5%)
	Base	254	447 ± 15 (3.4%)
		298	47.4 ± 2.0 (4.3%)
	pH 8.6	247	297 ± 4.7 (1.6%)
		285	-41.6 ± 1.4 (3.4%)
		303	7.4 ± 1.1 (14%)
Hydromorphone	Acid	238	75.8 ± 4.0 (5.3%)
		276	120 ± 7.0 (6.1%)
		298	-251 ± 8.2 (3.3%)
	Base	238	181 ± 2.5 (1.4%)
		275	170 ± 3.3 (1.9%)
		313	-203 ± 6.3 (3.1%)
Oxymorphone	.014 M HCl	238	44.4 ± 0.2 (0.3%)
		277	129 ± 0.8 (0.6%)
		297	-251 ± 2.3 (0.9%)
	Base	246	168 ± 1.6 (1.0%)
		287	167 ± 0.8 (0.5%)
		315	-203 ± 1.9 (0.9%)
	pH 8.6	247	112 ± 0.7 (0.6%)
		277	155 ± 1.0 (0.6%)
		309	-184 ± 1.5 (0.8%)
Naloxone	.1 M HCl	240	44.5 ± 1.6 (3.5%)
		277	126 ± 1.0 (0.8%)
		300	-253 ± 3.8 (1.5%)
	Base	247	144 ± 1.7 (1.2%)
		287	145 ± 3.4 (2.4%)
		315	-210 ± 4.5 (2.2%)

TABLE XXI (Continued)

Drug	Solvent	Max	Molar Ellipticity
	pH 8.6	248	77.7 ± 4.6 (5.9%)
		277	132 ± 1.0 (0.7%)
		308	-195 ± 4.7 (2.4%)
Dihydrocodeine	0.1 M HCl	241	297 ± 5.0 (1.7%)
		281	-179 ± 2.0 (1.1%)
	pH 8.6	241	139 ± 1.2 (0.9%)
		282	-85.6 ± 1.4 (1.6%)
	Base	241	125 ± 2.1 (1.7%)
		281	-81.5 ± 0.9 (1.1%)
Hydrocodone	0.1 M HCl	240	124 ± 2.1 (1.7%)
		276	142 ± 2.7 (1.9%)
		298	-278 ± 2.7 (1.0%)
	Base	238	129 ± 3.7 (2.9%)
		276	107 ± 2.4 (1.9%)
		297	-273 ± 0.9 (0.3%)
	pH 8.6	239	126 ± 2.1 (1.7%)
		276	109 ± 0.7 (0.7%)
		298	-264 ± 1.6 (0.6%)
Oxycodone	.02 M HCl	241	120 ± 0.8 (0.7%)
		277	139 ± 1.4 (1.0%)
		298	-268 ± 1.2 (0.4%)
	Base	240	69.2 ±.2.6 (3.8%)
		277	86.1 ± 3.1 (3.6%)
		300	-218 ± 3.2 (1.5%)
	pH 8.6	240	105 ± 1.5 (1.4%)
		277	131 ± 1.2 (0.9%)
		298	-271.8± 2.3 (0.9%)

differences in pH from one solution to the next may affect spectral parameters. By performing these examinations in a consistent and expeditious manner, the variance was reduced in the replicated experiments. The lack of variability in the other data is an indication that, for most of the compounds, the variance between day-to-day experiments is less than the experimental error.

The instrumental error was very low for duplicate samples analyzed on the same day. Samples of dihydrocodeine and oxycodone were analyzed five times in succession. The average deviation ranged from (0.4 - 1.9) x 10^{-4} degree of ellipticity.

Statistical data for morphine and nalorphine in 0.1 M HCl, both individually and compared to each other, are given in the Appendix as examples.

CD Spectral Parameters

The variance of the CD spectral parameters are important in the qualitative analyses of samples, since these parameters are the primary means of sample identification. Changes in molar ellipticities upon a change in pH are of secondary importance in this respect.

In most cases the variance between drugs was much larger than that for replicates or duplicates. This is an indicator that, indeed, the different drugs have unique spectra.

Overall, the acidic solutions resulted in spectral parameters with the lowest standard deviations. The analysis of the drugs in basic solutions produced results which were better than those analyzed in pH 8.6 buffer. For the majority of samples, the standard deviations of the CD spectral parameters was less than one nanometer. There were two

notable exceptions to this generality. There were some compounds in pH 8.6 buffer whose spectral parameters exceeded one nanometer, especially the two positive transitions in the ketone-bearing compounds.

The second exception involves those drugs with CD spectra which have slowly decreasing values at the cross-over points, such as the 250 nm and 255 nm cross-over points for heroin and dihydrocodeine, respectively. Any uncertainty in the baseline is translated into a large deviation for the location of λ^{o}. For samples whose CD spectrum approach the baseline at a steep angle, such as morphine in dilute acid, this uncertainty is not a serious problem.

Significant differences were found among the CD spectral parameters for all the drugs in at least one of the media, with one exception. The parameters for codeine and ethylmorphine were found to be almost identical. The available ethylmorphine spectral data were insufficient to generate an adequate statistical analysis. Even naloxone and oxymorphone could be distinguished from each other by careful inspection of CD spectral parameters in base and pH 8.6 buffer.

Molar Ellipticities

There was much more variability in the molar ellipticities between drugs than in any other CD spectral parameters. This usually led to even greater statistical differences between drugs. The relative average deviations in the molar ellipticities ranged in value from 0.5 to 54.0%, with an average value of 3.5%. This average deviation is comparable to that found for UV spectroscopy.

These parameters are of little value in the identification of a compound. However, as previously mentioned, the ratios of ellipticities

for different transitions and for changes in solvent can be of importance as a means of identification. Thus, the ratios listed in Table III can reliably be used as an additional means of identification in most instances.

From the standard deviations listed in Table XVIII, it is apparent that some transitions have much better CD spectral reproducibility than others. In the absence of mixtures, these transitions should be used for quantitative analysis of samples. Also, dilute aqueous HCl is the preferred solvent for most quantitative analyses. Overall, the relative average deviation for compounds dissolved in 0.1 M HCl averaged 2.5%. Measurement error is further reduced by the judicious choice of the transition from which the sample is quantitated. This degree of accuracy is certainly adequate for most drug analyses.

Correlation Coefficients

Correlation coefficients were calculated for all compounds where there were sufficient data for each compound, over a concentration range from $(0.30 - 3.5) \times 10^{-4}$ M (Table XXII). These coefficients indicate the degree of linearity of ellipticity versus concentration. The closer the value is to ±1.0, the better the correlation of the data to a linear regression model. For most peak maxima having molar ellipticities greater than ±30, the correlation coefficients exceeded 0.9900; in fact, they often approached ±1.0. The transitions with molar ellipticities whose absolute value was greater than 200 resulted in a slightly better linear fit of data than those whose ellipticities were less.

The transitions of the ketone-bearing compounds with positive molar

TABLE XXII

CORRELATION COEFFICIENTS FOR MOLAR ELLIPTICITIES

Drug	Solvent	245	285	300
Morphine	HCl	0.9997	-0.9991	
	NaOH	0.9998		0.9970
Nalorphine	HCl	0.9987	-0.9991	
	8.6	0.9997	-0.9964	---
	NaOH	0.9999		0.9915
6-Acetyl Morphine	HCl	0.9999	-0.9999	
	8.6	0.9970	-0.9893	0.9043
Heroin	8.6	0.9996	-0.9993	
Hydromorphone	8.6	0.9718	0.9849	-0.9986
Oxymorphone	8.6	0.9759	0.9946	-0.9979
Naloxone	8.6	0.9899	0.9974	-0.9877
Codeine	HCl	0.9996	-0.9982	
	8.6	0.9999	-0.9998	
	NaOH	0.9910	-0.9971	
Dihydrocodeine	HCl	0.9960	-0.9997	
	8.6	0.9997	-0.9989	
	NaOH	0.9930	-0.9998	
Hydrocodone	HCl	0.9935	0.9874	-0.9934
	8.6	0.9910	0.9956	-0.9998
	NaOH	0.8984	0.9987	-0.9999
Oxycodone	8.6	0.9899	0.9974	-0.9877

ellipticities had less correlation than similar transitions for other compounds. This is probably a result of greater standard deviations in the molar ellipticities of these compounds.

CHAPTER VII

CONCLUSIONS

Analytical Applications of CD

In this study the analytical value of CD spectropolarimetry to the identification of compounds in aqueous solutions of varying pH has been confirmed. All compounds studied were uniquely identified by CD spectral parameters or molar ellipticity values. Although analytical distinction is the best in pH 8.6 buffer, accurate quantitation is best achieved in 0.1 M HCl. In this medium, quantitative analysis can result in less than 2.5% error. Separation or derivatization are usually not required unless there is a strongly UV absorbing compound present. The instrument does not need to be calibrated daily. All checks of the instrument calibration showed very little change in ellipticity over a period of a year.

Compared to alternative methods for CD analysis such as in a KBr pellet matrix or in a cholesteric liquid crystalline solvent, the present method is straightforward and quantitative. The analytical technique is the same as that used in analyses by UV absorption spectroscopy. However, the technique is usually limited to chiral compounds, which absorb radiation unless a solvent is used which can induce chirality, such as a cyclic long-chain sugar. An anisotropic cholesteric liquid crystalline solvent has the advantage of being applicable to both the study of achiral molecules and racemic mixtures.

The lack of CD signal for achiral molecules can be an advantage or a disadvantage, depending upon the circumstances. In the event that an analysis is being made for heroin in the presence of methapyrilene, the analysis is facilitated by CD. If, however, the analyst desires to know the identity of all components in the mixture, additional analytical methods must be employed.

In the event that the compound under investigation is not CD-active, another method of identification must be devised. Drugs which are achiral because of inherent molecular symmetry, e.g., PCP, or because they occur as a racemic mixture, e.g., dl-methadone, cannot be directly determined by CD in an isotropic solvent. They can be determined by association with a chiral co-solute if the equilibrium constant for the molecular association is known.

The analyses of heroin samples showed that the determination becomes much more complicated if there is an indication of drug mixtures being present, where both are CD-active. If one component has considerably less CD signal intensity in one of the aqueous media, at a given wavelength, the problem is easily resolved. The identification and quantitation of codeine and morphine in combination by deconvolution of the combined spectra in a basic medium is one example of the use of this technique.

If the spectra are fairly complex or indistinct, an additional analytical method, such as gas or liquid chromatography, should be used for quantitative analysis.

Pharmaceutical preparations containing codeine and dihydrocodeine were readily quantitated, either directly by dilution or after a basic

extraction procedure. Even with an extraction step, the analysis time is a half hour or less.

To date, only a limited number of drug groups have been examined. There are probably several drug groups containing compounds which have little or no CD activity. Again, this is an advantage if the compound is not one being sought, but negates the method somewhat if this component is to be quantitated.

The following flowchart (Figure 33) is given as a review and as a guideline for the analysis of an unknown sample by CD spectroscopy. It does not include many alternative methods which might be useful for the analysis of drug components.

Further Areas of Research

It is certain that other drug groups should be investigated to determine if CD spectropolarimetry is a viable method for their analyses. All drug groups examined to date have little similarity in their spectral characteristics. If this trend continues, perhaps unique identification of other drug compounds is also possible.

It would probably be beneficial to vary the pH and record changes in molar ellipticities as well as CD spectral parameters. Perhaps then structural features could be better correlated with spectral response.

With the use of modern CD instruments, it should be possible to detect CD active drugs at much lower concentrations. Perhaps trace concentrations of drugs in blood and urine can be detected by CD within the next few years. Preconcentration efforts may be of value in this effort. Research in this vital area of clinical analysis has already begun.

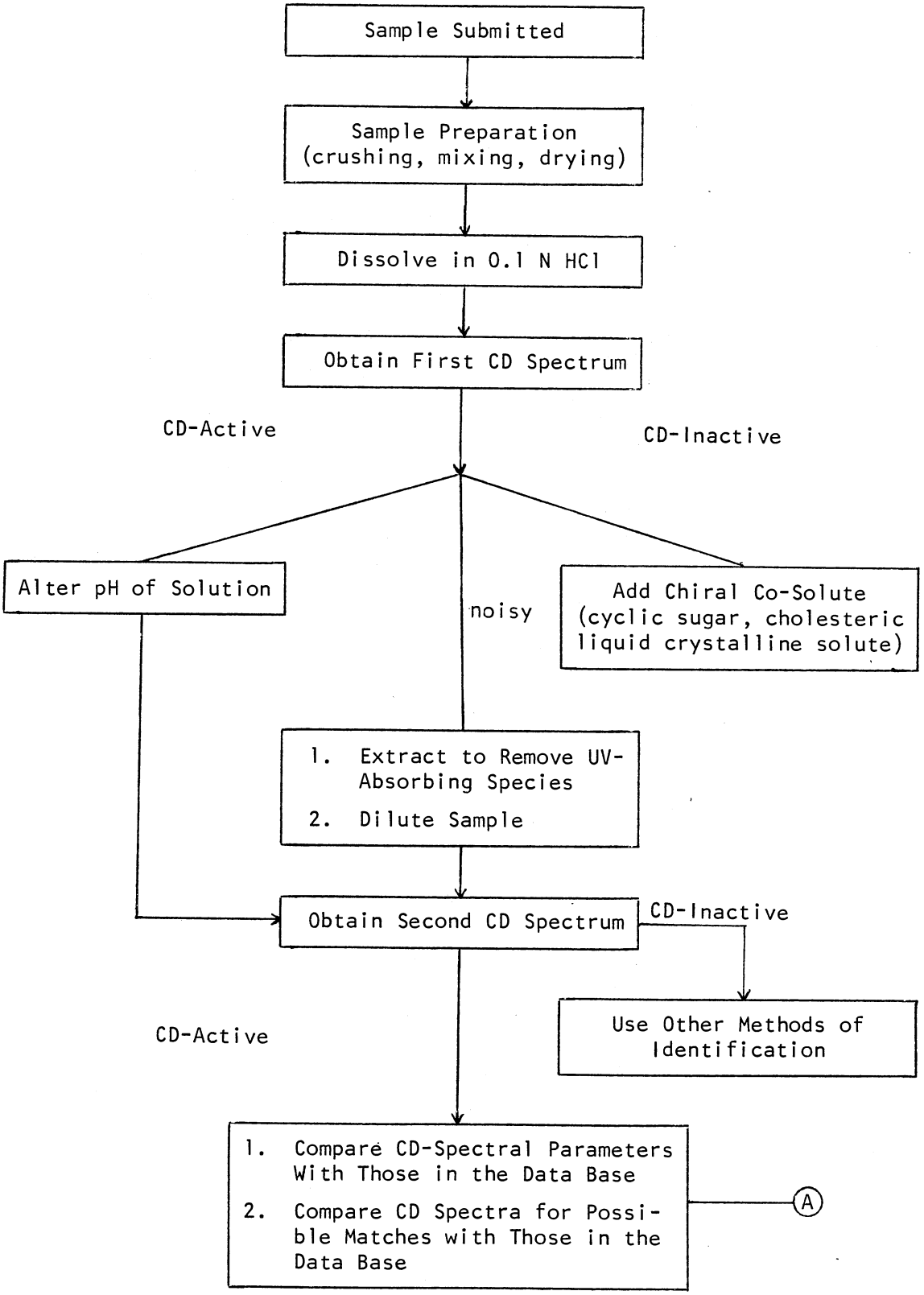


Figure 33. Identification Scheme for the Analysis of Drugs by CD Spectroscopy

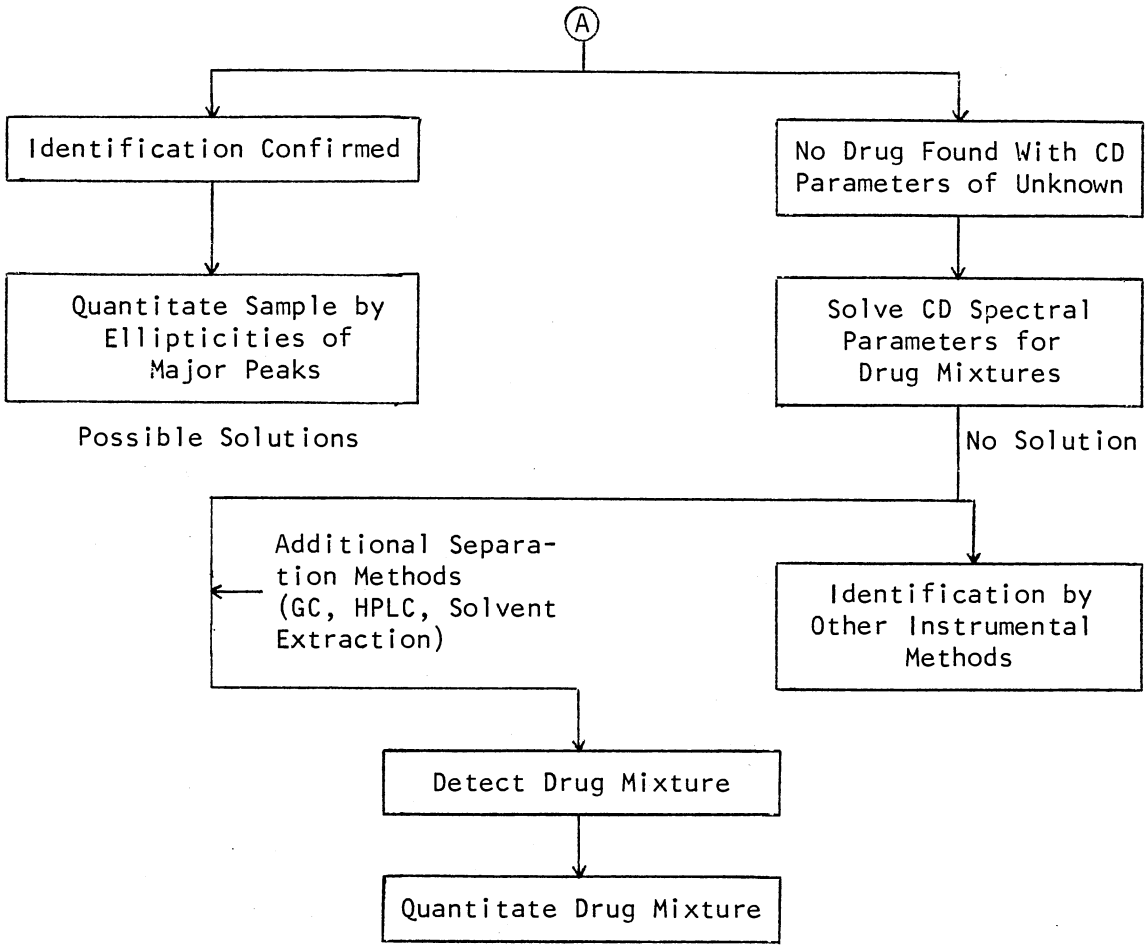


Figure 33. (Continued)

Concluding Remarks

In reality, research has only begun to determine the applications of CD spectropolarimetry to the analysis of drug components, whether in dispensary form or in biological fluids. Many more comprehensive research efforts need to be performed before an accurate assessment of the analytical capabilities of this technique can be made.

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APPENDIX

STATISTICAL ANALYSIS SYSTEM

DRUG=MOR SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: Y2

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	9	1.44400000	0.16044444	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		STD DEV		Y2 Mean
Corrected Total	9	1.44400000			0.00000000		243.16000000

Source	DF	Anova SS	F Value	PR > F
Rep	9	1.44400000		
Dup(Rep)	0	0.00000000		

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Rep	9	1.44400000		

DRUG=MOR SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: THETA245

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	9	96.90000000	10.76666667	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		STD Dev		Theta245 Mean
Corrected Total	9	96.90000000			0.00000000		383.10000000

Source	DF	Anova SS	F Value	PR > F
Rep	9	96.90000000		
Dup(Rep)	0	0.00000000		

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Rep	9	96.90000000		

DRUG=MOR SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: Y3

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	9	2.80500000	0.31166667	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Y3 Mean
Corrected Total	9	2.80500000			0.00000000		284.00000000

Source	DF	Anova SS	F Value	PR > F
Rep	9	2.80500000	.	.
Dup(Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Rep	9	2.80500000	.	.

DRUG=MOR SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: THETA275

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	9	10.68400000	1.18711111	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Theta275 Mean
Corrected Total	9	10.68400000			0.00000000		-66.76000000

Source	Df	Anova SS	F Value	PR > F
Rep	9	10.68400000	.	.
Dup(Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	Df	Anova SS	F Value	PR > F
Rep	9	10.68400000		

DRUG=NAL SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: Y2

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	4	0.32800000	0.08200000	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Y2 Mean
Corrected Total	4	0.32800000			0.00000000		244.52000000

Source	DF	Anova SS	F Value	PR > F
Rep	4	0.32800000	.	.
Dup(Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Rep	4	0.32800000	.	.

DRUG=NAL SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: THETA245

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	4	137.00000000	34.30000000	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Theta245 Mean
Corrected Total	4	137.20000000			0.00000000		364.00000000

Source	DF	Anova SS	F Value	PR > F
Rep	4	137.20000000	.	.
Dup(Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Rep	4	137.20000000	.	.

DRUG=NAL SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: Y3

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	4	0.20000000	0.05000000	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Y3 Mean
Corrected Total	4	0.20000000			0.00000000		284.90000000

Source	DF	Anova SS	F Value	PR > F
Rep	4	0.20000000	.	.
Dup(Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Rep	4	0.20000000	.	.

DRUG=NAL SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: THETA275

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	4	21.58000000	5.39500000	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Theta275 Mean
Corrected Total	4	21.58000000			0.00000000		-59.50000000

Source	DF	Anova SS	F Value	PR > F
Rep	4	21.58000000	.	.
Dup(Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Dup(Rep) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Rep	4	21.58000000	.	.

SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: Y2

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	14	7.93733333	0.56695238	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Y2 Mean
Corrected Total	14	7.93733333			0.00000000		243.61333333

Source	DF	Anova SS	F Value	PR > F
Drug	1	6.16533333	.	.
Rep(Drug)	13	1.77200000	.	.
Dup(Drug*Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Rep(Drug) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Drug	1	6.16533333	45.23	0.0001

SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE Y2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT

ALPHA LEVEL = .05 DF=0 MS=0

GROUPING	MEAN	N	DRUG
A	244.520000	5	NAL
B	243.160000	10	MOR

SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: THETA245

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	14	1399.73333333	99.98095238	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Theta245 Mean
Corrected Total	14	1399.73333333			0.00000000		376.86666667

Source	DF	Anova SS	F Value	PR > F
Drug	1	1165.63333333	.	.
Rep(Drug)	13	234.10000000	.	.
Dup(Drug*Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Rep(Drug) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Drug	1	1165.63333333	64.73	0.0001

SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: Y3

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	14	4.41333333	0.31523810	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		Y3 Mean
Corrected Total	14	4.41333333			0.00000000		284.46666667

Source	DF	Anova SS	F Value	PR > F
Drug	1	1.40833333	.	.
Rep(Drug)	13	3.00500000	.	.
Dup(Drug*Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Rep(Drug) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Drug	1	1.40833333	6.09	0.0282

SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE Y3

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT

ALPHA LEVEL=.05 DF=0 MS=0

GROUPING	MEAN	N	DRUG
A	284.000000	5	NAL
B	284.250000	10	MOR

SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: THETA275

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	14	207.95600000	14.85400000	99999.99	0.0000	1.000000	0.0000
Error	0	0.00000000	0.00000000		Std Dev		THETA275 Mean
Corrected Total	14	207.95600000			0.00000000		-64.34000000

Source	DF	Anova SS	F value	PR > F
Drug	1	175.69200000	.	.
Rep(Drug)	13	32.26400000	.	.
Dup(Drug*Rep)	0	0.00000000	.	.

Tests of Hypotheses Using the Anova MS for Rep(Drug) as an Error Term

Source	DF	Anova SS	F Value	PR > F
Drug	1	175.69200000	70.79	0.0001

SOLV=ACID

ANALYSIS OF VARIANCE PROCEDURE

MEANS

Rep	Drug	N	Y2	Y3	Y5	Y6	THETA245	THETA275
1	MOR	1	243.800000	283.800000	228.700000	262.500000	382.000000	-67.7000000
2	MOR	1	243.500000	283.500000	229.000000	262.300000	376.000000	-65.8000000
3	MOR	1	243.000000	284.100000	228.800000	262.500000	387.000000	-68.5000000
4	MOR	1	243.700000	285.000000	228.800000	262.700000	386.000000	-66.3000000
5	MOR	1	243.100000	284.300000	229.200000	263.000000	380.000000	-66.9000000
6	MOR	1	243.300000	285.000000	229.200000	263.200000	384.000000	-66.6000000
7	MOR	1	243.000000	283.800000	228.500000	263.000000	384.000000	-68.3000000
8	MOR	1	242.700000	284.000000	228.500000	263.000000	382.000000	-65.2000000
9	MOR	1	242.700000	284.000000	228.300000	263.000000	386.000000	-66.0000000
10	MOR	1	242.800000	285.000000	228.400000	262.700000	384.000000	-66.3000000
1	NAL	1	244.300000	285.000000	230.000000	265.000000	357.000000	-56.2000000
2	NAL	1	244.300000	285.000000	230.000000	265.000000	364.000000	-59.0000000
3	NAL	1	244.500000	285.000000	230.000000	265.000000	361.000000	-59.9000000
4	NAL	1	244.500000	284.500000	230.000000	265.000000	372.000000	-59.7000000
5	NAL	1	245.000000	285.000000	230.000000	265.000000	368.000000	-62.7000000

VITA 2

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