Circular Dichroism Spectra of Opium Alkaloids in a Cholesteric Liquid Crystalline Solvent System

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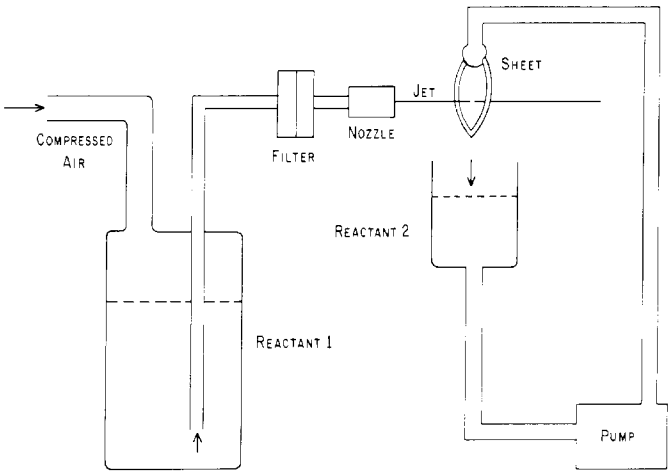

Figure 1. Jet flow kinetics apparatus.

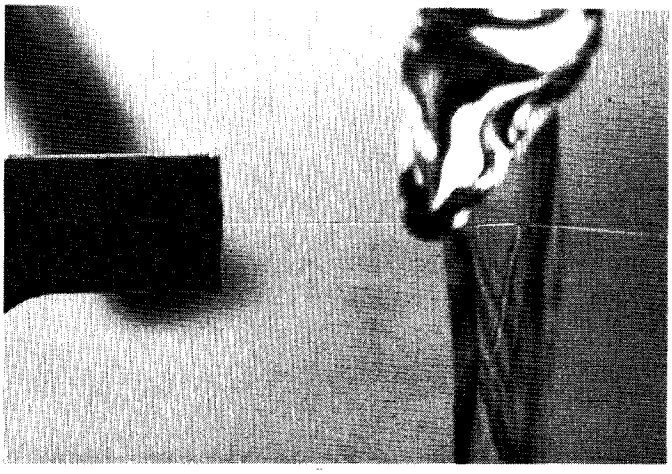

Figure 2. Photograph of the jet passing through the sheet. The diameter of the jet is 80 μm. It passes through the sheet from left to right. The fan-shaped glass jet which forms the sheet is shown in part at the top of the photograph.

about 2 cm downstream from the sheet. Averaging several measurements, we obtained a rate constant $k = 150$ mol^{-1} L s^{-1}, which considering the crudeness of our method is in surprisingly good agreement with the previously determined value of 127 mol^{-1} L s^{-1}.

The purpose of our present apparatus was to demonstrate the possibility of this technique. The performance of the apparatus can be greatly improved with several design changes. Using smaller nozzles it is relatively easy to produce 10 μm diameter jets with speeds of about 40 m/s. With such jets the mixing time is expected to be less than 1 μs. A spectrometer using fiber optics is now being assembled to measure optical absorption and fluorescence along the jet. A spatial resolution of 10 μm will be obtained with the device. At a jet velocity of 40 m/s this corresponds to a time resolution of 2.5×10^{-7} s.

With a 10-μm jet and a speed of 40 m/s, the reactant flow rate from the jet is about 4 μL s^{-1}. We estimate that a typical measurement can be completed in 10 s. Therefore, the amount of reactant used is 40 μL, which is less than but comparable to the amount required for a stopped-flow reaction measurement. The amount of reactant required for the formation of the sheet is greater by about a factor of 10.

Some of the specialized techniques developed for ink jets which have been used previously in flame spectrometric experiments (*10*) may also be valuable for the jet kinetic studies. It is well-known that jets break up into droplets downstream from the nozzle. The size of the droplets is comparable to the diameter of the jet. The formation and spacing of the droplets can be controlled by pressure modulating the fluid at the nozzle entrance (*11*). Individual droplets can then be observed downstream with a synchronized stroboscopic light. Selected droplets can be electrostatically charged, removed from the stream, and collected. These techniques may be useful for example in biological studies of protein or cell kinetics.

ACKNOWLEDGMENT

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Circular Dichroism Spectra of Opium Alkaloids in a Cholesteric Liquid Crystalline Solvent System

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It is generally understood that visible-ultraviolet spectrometry has limited application as an analytical tool because absorption bands, with a few exceptions, are both broad and unstructured (*1-3*). On the other hand, in uncomplicated systems, the technique is both simple and quantitative (*1-3*). This in itself is good reason for the continued interest in developing and modifying the method to include more complex systems.

Changes of solvent and solution pH are commonly used to modify electronic spectra. The usual outcome is a shift in

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position of the wavelength of maximum absorption. These shifts have been cataloged for transitions associated with particular chromophores in the molecules (*1–3*). In another direction spectral deconvolution can be accomplished to some degree by derivative spectroscopy (*4, 5*). Second and higher derivatives of the absorption spectra enhance resolution and reveal the existence of hidden bands as new information. More is expected from this approach with the newest generation of visible–UV spectrophotometers with derivative attachments added. The goal is the same in both modifications, which is to provide more spectral parameters to aid in the process of solute recognition.

Circular dichroism (CD) spectropolarimetry goes a step further. Here the difference in absorption between left and right circularly polarized light is measured (*3*) and the resultant ellipticity is plotted as a function of wavelength. CD spectra are inherently more informative than conventional absorption spectra because ellipticities can be positive, negative, or zero, and preliminary deconvolution of the broad bands often results. Further modification is then possible with either or both of the aforementioned procedures.

In the first work (*6*) we published results for the identification of some eight opium alkaloids from the CD spectra taken of the drugs in pressed KBr pellets. The point was made that no distinction was possible if the drugs were dissolved in the usual isotropic solvents (*7, 8*). In this work spectra of the same opiates are compared for the compounds dissolved in the chiral, anisotropic, partially compensated, liquid crystalline mixture of cholesteryl chloride and cholesteryl nonanoate.

Cholesteric liquid crystals have been used fairly extensively as solvents (*9–12*) in CD studies. The solvents introduce chiroptical behavior into achiral molecules and enhance the ellipticities observed for chiral molecules over those observed in isotropic solvent media, a phenomenon sometimes referred to as chirality amplification (*13*). Conversely an achiral nematic liquid crystal system can assume chiroptical properties on the addition of a chiral compound (*14*). Solvation interactions are very specific and have the potential for distinguishing absolute configurations (*13, 14*).

In this work the specificity of the drug–solvent interaction is tested as a potentially useful analytical technique for distinguishing among structurally similar molecules all of which possess the same aromatic ring chromophore. The compounds to be described are morphine, codeine, 3-monoacetylmorphine (3-MAM), 6-monoacetylmorphine (6-MAM), 3,6-diacetylmorphine (heroin), dihydrocodeine, hydrocodone, and thebaine. All eight derivatives are individually distinguishable by their CD spectra. A concentration study for morphine and codeine shows possible dimer formation for the former compound.

EXPERIMENTAL SECTION

Chemicals. The solvent system used is a partially compensated mixture (*9–12*) of cholesteryl chloride (98%, Aldrich Chemical Co.) and cholesteryl nonanoate (97%, Aldrich Chemical Co.). The chloride was purified by repeated recrystallization from isohexane; the nonanoate was used without further purification.

Morphine, codeine, 3-MAM, 6-MAM, and thebaine were obtained as pure forms of the free base. Dihydrocodeine, heroin, and hydrocodone were extracted from pure forms of the hydrochloride salts by standard procedures, i.e., from aqueous buffered solution into ether. Solutions were prepared by using only the free bases since solubility of the salts is limited. Reagent grade ethanol was used to prepare drug solutions to obtain conventional CD spectra for comparison purposes.

Solutions. The composition of the partially compensated mixture used is 1.63:1 in favor of cholesteryl nonanoate. Appropriate weights are used to prepare chloroform stock solutions which are stored tightly sealed and refrigerated. The composition of a stock solution is checked systematically over the period of time it is in use. An appropriate aliquot of stock solution is added to a known weight of opiate, and the chloroform is removed by slow evaporation with vigorous stirring. A standardized procedure produces a uniform and readily reproducible sample for analysis. The liquid crystal solvent for the reference cell is prepared in precisely the same way from an equivalent aliquot of stock, no drug being added. Each aliquot uses less than 0.25 g of solvent and ca. 1 mg of alkaloid. In preparation of the samples, a 20-μL aliquot of the cholesteric mixture, in an isotropic state, T = 72 °C, is pressed between quartz plates. A sample thickness of 12.5 μm is established by a self-adhesive spacer. Careful temperature control is important to the experimental consistency. Experiments are conducted at 42.5 ± 0.2 °C, at which temperature the solvent mesophase is maintained for a number of hours, and the drug solubility is improved.

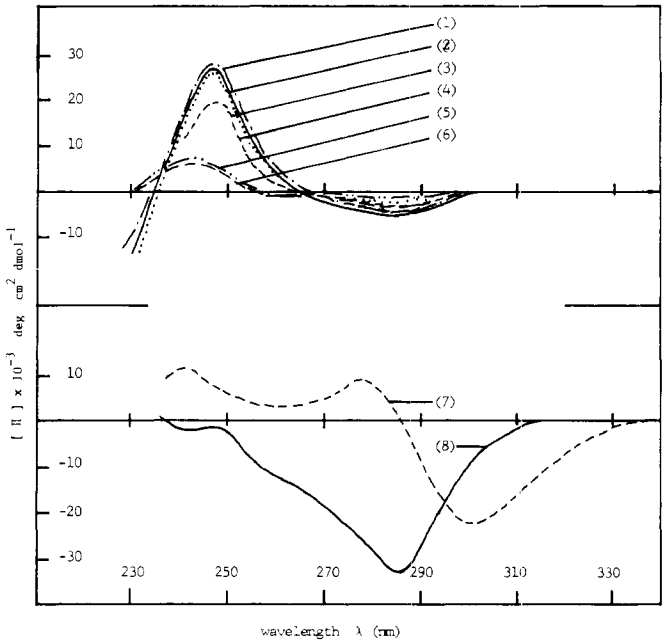

Figure 1. Ethanol CD spectra of (1) codeine, (2) morphine, (3) 6-MAM, (4) 3-MAM, (5) dihydrocodeine, (6) heroin, (7) hydrocodone, and (8) thebaine.

CD spectra were recorded on a Cary 61 spectropolarimeter. The instrument was purged by using gas from a liquid nitrogen reservoir deoxygenated by passage through a pyrogallol scrubber. Sample cells were of local manufacture and details can be provided. After the instrument was preconditioned, a spectrum can be obtained in less than 1 h from the time of preparation of the chloroform sample for evaporation. This time includes setting the base line for differential measurement of the spectra. The spectral limit is around 230 nm, because at this wavelength absorption by the solution is excessive, even though the instrument is operated in the difference mode.

RESULTS AND DISCUSSION

The CD spectra of the drugs in ethanol are well established (*7, 8*). Our data are reproduced here (Figure 1) to point out the spectral similarities. Spectra have been normalized to one molal concentration. Two distinct bands are observed for morphine, codeine, dihydrocodeine, 3-MAM, 6-MAM, and heroin. These have been assigned to the $^1B_{2u}$ (or ^1L_b) transition (negative ellipticity) and to the $^1B_{1u}$ (or ^1L_a) transition (positive ellipticity) involving the aromatic ring chromophore, ring A (Figure 2).

Although there are three different substituents on the aromatic chromophore, the spectra bear a strong resemblance to one another. In fact for morphine, codeine, and 6-MAM, the spectra are quantitatively identical. The spectrum for 3-MAM differs only in the magnitude of the ellipticity of the band at short wavelength. Heroin and dihydrocodeine are distinguishable from these four but not from each other. For

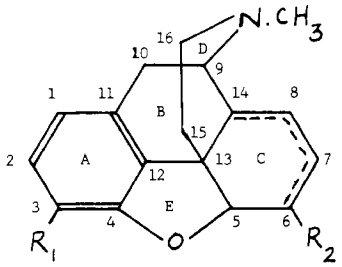

Figure 2. Basic structure of the morphine alkaloids. Derivatives used have the following R substituents: morphine, $R_1 = R_2 = OH$, 7–8 double bond; codeine, $R_1 = OCH_3$, $R_2 = OH$, 7–8 double bond; dihydrocodeine, $R_1 = OCH_3$, $R_2 = OH$, 7–8 saturated; 3-MAM, $R_1 = OCOCH_3$, $R_2 = OH$, 7–8 double bond; 6-MAM, $R_1 = OH$, $R_2 = OCOCH_3$, 7–8 double bond; heroin, $R_1 = R_2 = OCOCH_3$, 7–8 double bond; hydrocodone, $R_1 = OCH_3$, $R_2 = O$, saturated C-ring; thebaine, $R_1 = R_2 = OCH_3$, 6–7 and 8–14 double bonds.

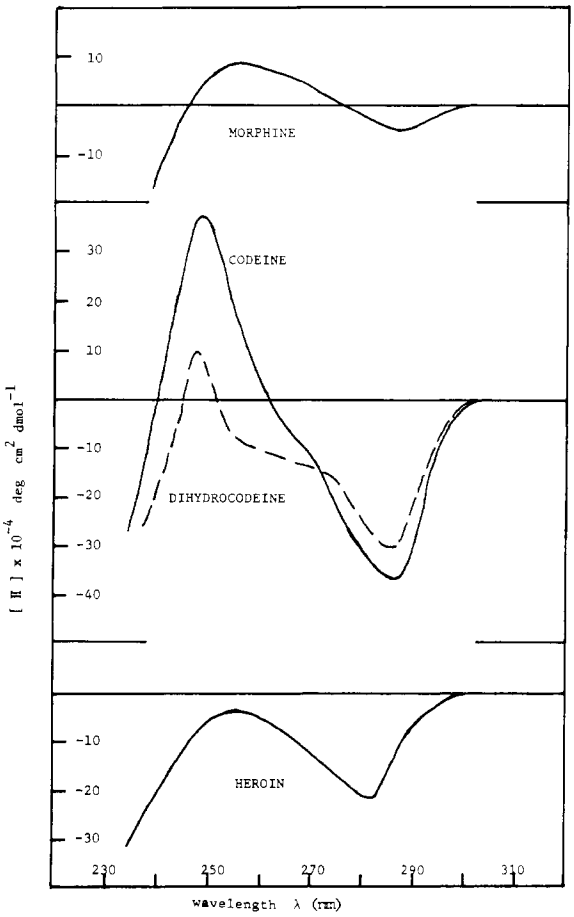

Figure 3. LCCD spectra of morphine, codeine, dihydrocodeine, and heroin at a concentration of 1:150 parts solvent.

hydrocodone and thebaine, broadening of the bands of the spectra toward the visible is observed and is associated with the additional chromophores in the C ring (*7*, *8*, *15*).

The liquid crystal circular dichroism (LCCD) spectra are shown in Figures 3–5. Data are plotted as molal ellipticities [II] defined by

$$[\Pi] = \frac{\psi \cdot \mathrm{MW} \cdot \mathrm{g\ solvent}}{100 \cdot d(\mathrm{dm}) \cdot \mathrm{g\ solute}} \qquad (1)$$

where ψ is the experimental ellipticity and d(dm) is the path length. To avoid possible error due to incomplete solution for any one of the derivatives, we obtained all spectra shown from solutions which are in a 150:1 solvent to solute ratio which is well below saturation for morphine, the least soluble derivative.

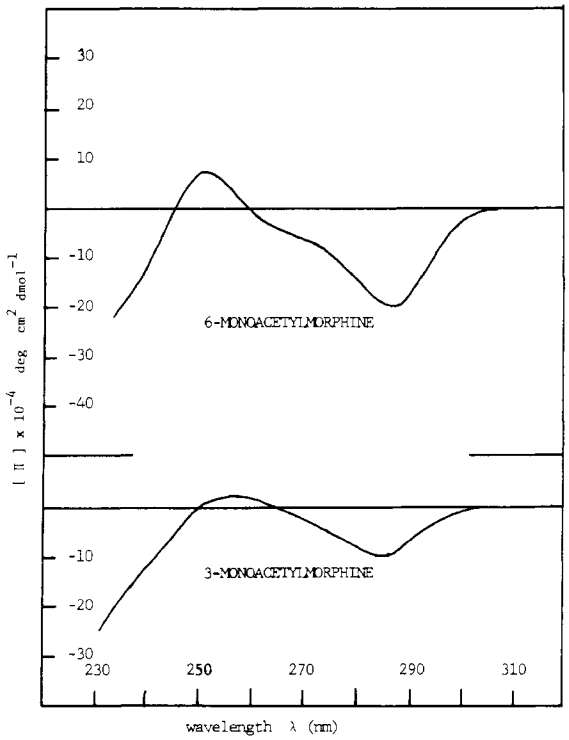

Figure 4. LCCD spectra of 6-MAM and 3-MAM at a concentration of 1:150 parts solvent.

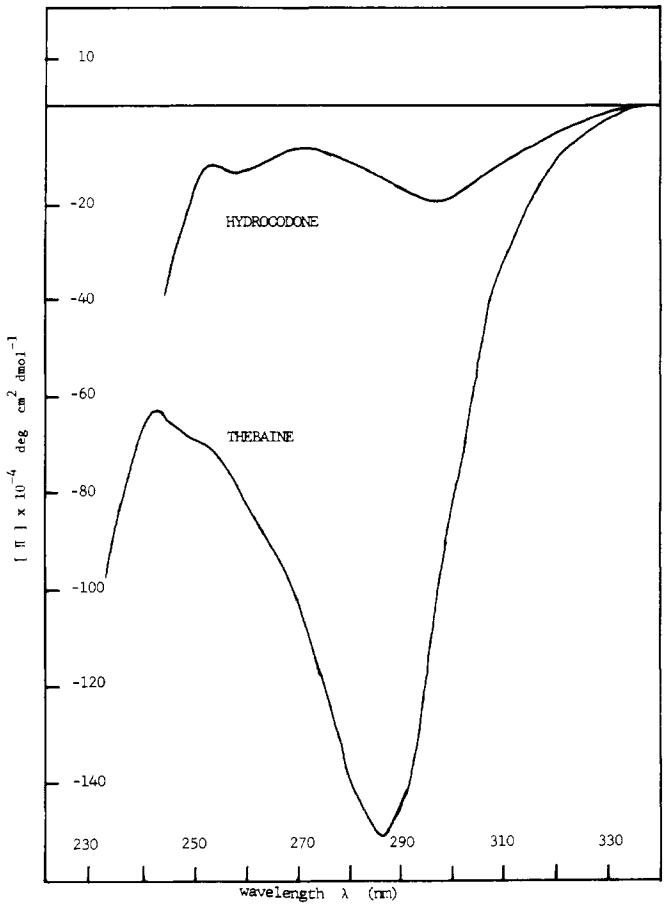

Figure 5. LCCD spectra of hydrocodone and thebaine at a concentration of 1:150 parts solvent.

It is seen from Figure 3 that the molal ellipticity of codeine is much greater than that for morphine. However, even at very low codeine concentrations the spectrum is still distinct from that of morphine because the wavelengths of zero el-

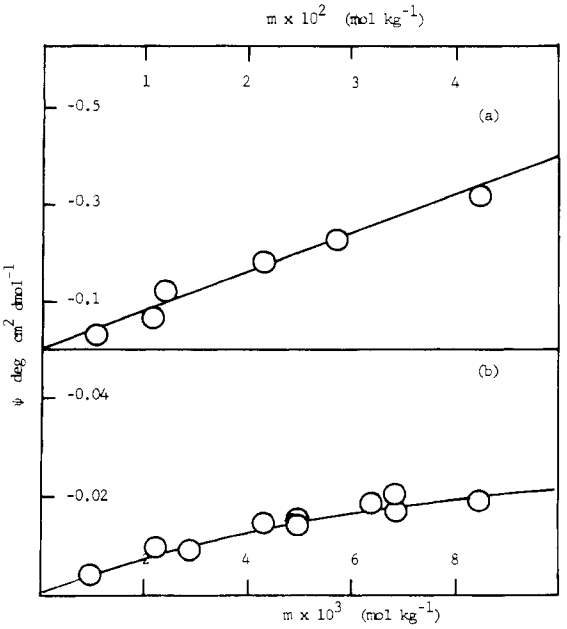

Figure 6. Observed ellipticity ψ vs. molal concentration for (a) codeine and (b) morphine in solution with 1:1.63 cholesteryl chloride:cholesteryl nonanoate at 42.5 °C.

lipticity are concentration independent and separated by 15 nm.

A quantitative study of the concentration dependence of ellipticity was made for morphine and codeine, Figure 6. Good linearity is observed for codeine up to saturation which is approximately 0.2 *m* or 12 "solvent molecules" for every codeine molecule. No significant interaction between solute molecules appears to occur. Departure from linearity is observed for morphine and the limit of solubility is much lower. The data were analyzed in terms of a dimerization equilibrium, $2M \rightleftharpoons M_2$, by using the equation for the apparent ellipticity

$$\psi = [\Pi_M](C - 2x) + [\Pi_{M_2}]x \qquad (2)$$

where $[\Pi_M]$ and $[\Pi_{M_2}]$ are the respective molal ellipticities and C is the total morphine concentration. From a short reiterative calculation, values obtained for $[\Pi_M]$, $[\Pi_{M_2}]$ and K for dimerization are −4.6, +14.5, and 19.6 ± 2, respectively, at 42.5 °C. Data are given in Table I.

The basic structure of the morphine derivatives of the opium alkaloids is not planar but approximates to a T-shaped disposition of the A and B rings to the C and D rings. Nevertheless, good solubility in the liquid crystal is achieved in spite of the tightly layered structure of the solvent. Disruption of this layered structure with increasing concentration is insignificantly small and apparently monotonic. The relative insolubility of morphine may well be due to the polarity imparted by the phenolic OH in the 3-position.

The first most obvious difference in the spectra on changing solvents is the signal enhancement produced. Compare Figure 1 with Figures 3–5. In addition a second chirality is introduced by the solvent which affects the already existing bands, intrinsic to the chiral solutes, in different ways. Signal enhancement is simply a consequence of the cooperative alignment and preferred orientation imposed on the solutes by the helically arranged solvent molecules (*9–12*). The effect is general. Spectral changes, on the other hand, must reflect subtle differences in specific molecular associations between solute and receptor, i.e., specific solvation.

One can see that because of specific solvation, the normalized LCCD spectra for morphine, codeine, dihydrocodeine, 3-MAM, 6-MAM, and heroin differ enough that the drugs are easily distinguishable. Reviewing the structures relative to the parent morphine, Figure 2, one can see that for some only

Table I. Experimental Ellipticities (ψ) vs. Molal Concentration (m) for Morphine and Codeine in Liquid Crystal Solvent[c]

mg solute	$10^2 m$, mol kg^{-1}	$-\psi$ (at 286 nm)	K
		Codeine[a]	
0.80	0.53	0.030	
1.60	1.07	0.064	
1.80	1.20	0.120	
3.20	2.14	0.181	
4.30	2.84	0.272	
6.40	4.24	0.310	
		Morphine[b]	
0.26	0.29	0.010	19.9
0.26	0.29	0.009_2	26.3
0.39	0.42	0.014	16.2
0.45	0.49	0.014	20.6
0.45	0.49	0.014	20.6
0.58	0.63	0.017	18.2
0.62	0.68	0.018	18.6
0.62	0.68	0.017	19.2
0.77	0.84	0.020_6	16.8
			$\bar{K} = 19.6 \pm 2.0$

[a] Weight of solvent equals 0.5 g. [b] Weight of solvent equals 0.25 g. [c] $[\Pi] = -6.3 \times 10^5$ for codeine.

the C-ring has been modified, yet distinction is possible. If one presumes that the same solvent receptor sites are offered to all solutes, then the observed distinction is even more remarkable.

On reviewing the spectral changes brought about by specific solvation, we see that in overall appearance the CD spectra of morphine are least affected by the solvent change, consistent with the idea that solvation is unfavorable in the liquid crystal. Besides the ellipticity enhancement, inflections in the codeine and dihydrocodeine spectra at 270 nm signify partial separation of a hidden band, and, as a result, the ^1L_a band is considerably narrower. These compounds differ only in the degree of unsaturation of the C-ring, yet can be distinguished from each other by the difference in the ratios of the maxima in the spectra.

The same ^1L_a band changes sign in the heroin spectrum so that there is no evidence for any positive Cotton effect over the range of wavelengths studied. The analytical identification of heroin by LCCD is totally unique. There is a structural progression in the series morphine, 3-MAM, 6-MAM, and heroin which is transmitted into a gradual diminution of the positive character of the ^1L_a band. In a preliminary experiment it is observed that an equimolar mixture of the 3- and 6-MAMs does not reproduce the heroin spectrum emphasizing the uniqueness of each solvation interaction. It is premature to interpret these changes, but it appears that substituents in position 3 have a greater influence on the ^1L_a band under these particular orientation conditions.

For hydrocodone, Figure 5, one sees a shift in the long wavelength band to 286 nm in LCCD and the loss of the positive Cotton effect at shorter wavelengths. Since none of the other compounds produce a similar wavelength shift, the change in maximum wavelength is probably associated with the $\pi^* \leftarrow$ n transition of the ketone chromophore (*7, 8*), to which is attributed the absorption band around 303 nm in the UV absorption spectrum (*15*). The shift may be apparent and explained in terms of a preferential orientation of the transition dipole for the overlapping ^1L_b band by the solvent which concomitantly deemphasizes the Cotton effect associated with the $\pi^* \leftarrow$ n transition of the ketone. For thebaine, Figure 5, the spectrum is all negative in both solvents. Again there is a tendency toward a more negative Cotton effect for the ^1L_a transition. The reason is not yet clear and requires new

information on related compounds.

Dimerization by morphine and not by codeine implicates the 3-OH group in the association mechanism. The supply of 3-MAM was too limited to make a comparison study. Intermolecular hydrogen bonding involving the OH group and the dihydrofuran ring oxygen is a likely structural model for the association. The interesting result is that the molal ellipticity of the dimer is greater in magnitude and opposite in sign to that for the monomer. This result might be rationalized in terms of a sector rule theorem (*8*), although such a model has not been prepared for solutes in structured solvents. Structural determinations by X-ray (*16, 17*) diffraction have detected distortion of the plane of the aromatic ring in the monomer. How hydrogen bonding might affect planarity is beyond speculation at this time.

In summary it has been successfully demonstrated that cholesteric liquid crystal solvents interact very specifically with dissolved solutes to the extent that compounds of similar molecular structure can be distinguished by their LCCD spectra. The technique of differential LCCD is fairly routine with reproducible spectra readily obtainable for chiral as well as for achiral molecules. Cholesteric solvents offer a new concept in the study of solute–solvent interactions in that each layer in the helical stack approximates to a surface, and their unique orientation allows one the use of electronic absorption spectroscopy as a probe for the interactions.

ACKNOWLEDGMENT

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Water-Cooled Torch for Inductively Coupled Plasma Emission Spectrometry

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One of the disadvantages in the use of the inductively coupled plasma (ICP) is the large consumption of argon gas. Typically, 10–20 L/min of argon flows are required for the total of plasma, auxiliary, and carrier gases.

Among several proposals to decrease the argon consumption (*1–3*), a water-cooled torch described by Kornblum et al. (*3*) permitted the most drastic reduction, down to 2 L/min. However, their torch had serious drawbacks resulting from the complexity of the design, the difficulty involved in introducing sufficient amounts of samples and hence the high detection limits, and the relatively large phosphate interference on the calcium emission. Water-cooled torches have also been described by Zil'bershtein (*4*) and Britske et al. (*5*) for a high-power ICP (40 MHz, 4 kW). The latter authors operated it at low total gas consumption (3.5–4 L/min) and obtained good analytical results in the determination of rare earth and some other refractory elements.

The torch described by Kornblum et al. (*3*) had the inlet and outlet of the cooling water at the bottom of the water jacket, and the structure became complicated in trying to prevent air or steam bubbles from lingering. These may destroy the torch by local overheating. Though they excluded designs in which the water supply was mounted on top of the load coil, such designs are possible if the torch is positioned into the coil from the top end of it before attaching the spray chamber to the torch.

The most promising torch we constructed requires 4 L/min of the plasma gas and 0.8 L/min of the carrier gas. Though it requires more argon than those described by Kornblum et al., it has definite advantages in that it can be used with a conventional nebulizer and that the detection limits are superior and interference effects are comparable to those of conventional ICP torches.

EXPERIMENTAL SECTION

Instruments. Detection limits and interference effects were measured by using an 0.5-m grating monochromator (Nippon Jarrell Ash, JE-50E, grating 1200 grooves/mm, slit width 10 μm) with a photomultiplier (HTV, R-106) and an electrometer (Shimadzu Seisakusho, SE-41). A conventional plasma power supply (Nippon Koshuha, 27 MHz, crystal controlled) with a coupling unit (Shimadzu Seisakusho) and a concentric glass neubulizer with a dual-barrel spray chamber were used without modification. Axial emission profiles from the plasma were measured by using a photodiode array spectrometric system described previously (*6*).

Torch Design. Three torches we constructed are shown in Figure 1. They are made of silica glass except for a Teflon sleeve and rubber O-rings to position a central sample tube which carries sample aerosols. A water flow of 2.5 L/min was sufficient to run the plasma at the radio frequency (rf) power from 1 to 1.8 kW with any designs shown in Figure 1. The temperature increase of the cooling water was 2–5 °C depending on the power.

With the design in Figure 1a, the plasma was easily initiated at 4 L/min of the plasma gas when the carrier gas was not introduced. When the latter flow was slowly increased, the plasma gas flow had to be decreased at the same time to prevent the plasma from extinguishing. A hole is generated in the plasma at about 0.3 L/min of the carrier gas and 1 L/min of the plasma gas. However, when the carrier gas was further increased and the sample uptake became appreciable, the plasma suddenly shrank and became like a glowing "O-ring", and only a tail flame of the plasma could be seen at the top end of the torch from a horizontal direction.

This change of the plasma shape was caused by the excessive introduction of molecular substances such as water and air, because the latter introduction into the discharge zone from the top end of the torch increased as the plasma gas was decreased. To prevent the air from entering the discharge zone, we attached a silica collar tube on top of the water jacket as shown in Figure

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