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Circular Dichroism Spectra of Opium Alkaloids in Aqueous Media

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Ten derivatives from the morphine and codeine opium alkaloids series have been investigated by circular dichroism (CD) spectropolarimetry in aqueous HCl and NaOH and in a buffer solution of pH 8.6. Qualitative and quantitative distinction is possible and is most effectively done in the buffered medium. Molar ellipticity coefficients are reported for all derivatives. The greatest differences in the spectra on changing pH are observed for the morphine derivatives and are associated with the ionization of the phenolic group in the 3-position of the molecules.

One usually associates the technique of circular dichroism (CD) spectropolarimetry with the qualitative determination of the absolute configuration of a chiral molecule whose identity is known (*1*). As a general rule, corroboration of the absolute configuration by X-ray diffraction is still preferred.

Little has been done with CD in the identification of anonymous molecules, either qualitatively or quantitatively. This is somewhat surprising in the cases where the suspected molecule is known to be chiral, for example, in the identification of drugs of abuse. A case in point is the fact that a legally permissible test for the natural or L isomer of cocaine is a microcrystalline test wherein recognition is made by microscopic examination (*2*). Since CD is a measure of the difference in *absorption* of left and right circularly polarized light, it is conceivable that the analytical potential of CD has not been developed because it was suspected that the same limitations inherent to absorption spectroscopy would exist; i.e., bands are broad and structureless.

In previously reported work we described the qualitative identification by CD of derivatives of the morphine group of the opium alkaloids placed in KBr pellets (*3*) and in a cholesteric liquid crystal solvent (*4*). Distinction, it was thought, would be difficult because the UV absorption spectra of the narcotics are very similar (*5*), as are their CD spectra in isotropic solvents such as ethanol or chloroform (*6*, *7*). The two anisotropic media, KBr and liquid crystal, were introduced to modify the spectra and to make identification possible as a result. Both methods ultimately will have the potential of becoming quantitative when properly developed.

Another way to modify spectra is to change the pH of aqueous solutions (*8*). This procedure is commonly used to assist in the assignment of bands to particular electronic transitions in the absorbing molecules. In a series of papers Siek et al. (*5*, *9*, *10*) have accumulated an extensive inventory of UV absorption spectra for drugs and toxic substances in aqueous acid and base media and have made tentative assignments. The point is also very firmly made that analytical distinction among subgroups is possible, but distinction among members within a subgroup is not.

In this work, CD spectra have been obtained for some 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 (3MAM), 6-monoacetylmorphine (6MAM), 3,6-diacetylmorphine (heroin), codeine, dihydrocodeine, thebaine, hydrocodone, and naloxone. Concentration studies have been done for all of the derivatives. It is apparent that qualitative and quantitative distinction is possible among these members of the opium alkaloid derivatives, which makes the method more analytically advantageous than conventional absorption spectroscopy.

EXPERIMENTAL SECTION

Morphine, codeine, 3MAM, 6MAM, nalorphine, and thebaine were obtained as pure forms of the free bases. Dihydrocodeine, heroin, nalorphine, naloxone, and hydrocodone were obtained as pure forms of the hydrochloride salts. Morphine was also available as a pure sulfate salt. Heroin was supplied from two separate batch samples. Decimolar HCl and NaOH were used in the form of commercially prepared secondary standard solutions supplied by Ricca Chemical Co. and by Harleco Inc., respectively. The buffer solutions were prepared by use of pHydrion capsules.

Solution concentrations were on the order of (0.5 to 5) $\times 10^{-4}$ M which is the optimum operable range consistent with maximum instrument sensitivity and solute absorptivity. Samples were weighed on a Cahn Electrobalance Model No. 2000 RG. In concentration studies, solutions were prepared both by direct weighings and by progressive dilutions of standardized stock solutions. Hydrolysis reactions were observed to occur for the acetyl derivatives in basic media. Rates of reaction are perceptibly slow in the 8.6 buffer medium and are not a serious problem to a quantitative study. Hydrolyses are rapid in 0.1 M NaOH solutions.

CD spectra were recorded on a Cary 61 spectropolarimeter. The instrument was purged with gas from a liquid nitrogen reservoir deoxygenated by passage over a column of molecular sieve. Where samples of free base and salt were available, both were used to prepare solutions. No difference was observed between the spectra for base and salt in each of the three media, provided that at pH

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Table I. Structural Properties of Compounds Used in Study

	R_1	R_2	R_3	R_4	unsaturation in C-ring
morphine	OH	OH	CH_3	H	C(7)-C(8)
nalorphine	OH	OH	$CH_2CH{=}CH_2$	H	C(7)-C(8)
3MAM	$OCOCH_3$	OH	CH_3	H	C(7)-C(8)
6MAM	OH	$OCOCH_3$	CH_3	H	C(7)-C(8)
heroin	$OCOCH_3$	$OCOCH_3$	CH_3	H	C(7)-C(8)
codeine	CH_3O	OH	CH_3	H	C(7)-C(8)
dihydrocodeine	CH_3O	OH	CH_3	H	saturated
thebaine	CH_3O	CH_3O	CH_3	H	C(6)-C(7), C(8)-C(14)
hydrocodone	CH_3O	O	CH_3	H	saturated
naloxone	OH	O	$CH_2CH{=}CH_2$	OH	saturated

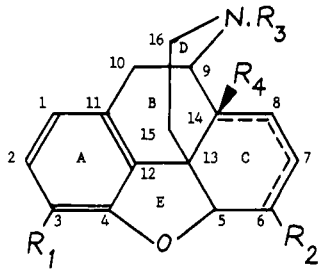

Figure 1. General formula for the morphine and codeine alkaloids.

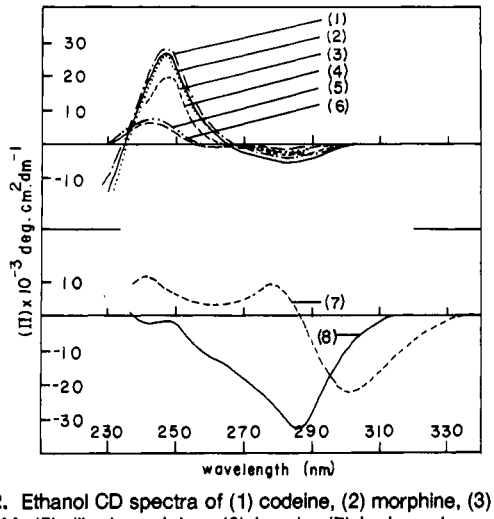

Figure 2. Ethanol CD spectra of (1) codeine, (2) morphine, (3) 6MAM, (4) 3MAM, (5) dihydrocodeine, (6) heroin, (7) hydrocodone, and (8) thebaine.

8.6 there was enough buffer capacity to prevent excessive increase in acidity on dissolving the salts.

RESULTS

The general molecular formula for the morphine and codeine derivatives of the opium alkaloids can be represented by Figure 1. The molecule approximates to a T-shape (*11*, *12*) such that rings A and B are almost perpendicular to rings C and D. This configuration tends to bring substituents on position 6 into the sphere of influence of the aromatic ring A. Any through space interaction with the A-ring chromophore might be expected to be weak. From X-ray structural analyses (*11*, *12*), ring A is known to be distorted apparently to meet the steric restrictions imposed by the dihydrofuran ring E. The compounds used in this study are listed in Table I.

The CD spectra in Figure 2 are of the alkaloids dissolved either in absolute or in 95% ethanol. Ellipticities have been normalized to 1 M concentration. The level of distinction among the compounds is not conclusive being comparable to what is possible from comparing UV absorption spectra of the compounds in the same solvent.

In Figures 3–9, the normalized CD spectra for each of the compounds in acid, base, and buffered solutions are shown.

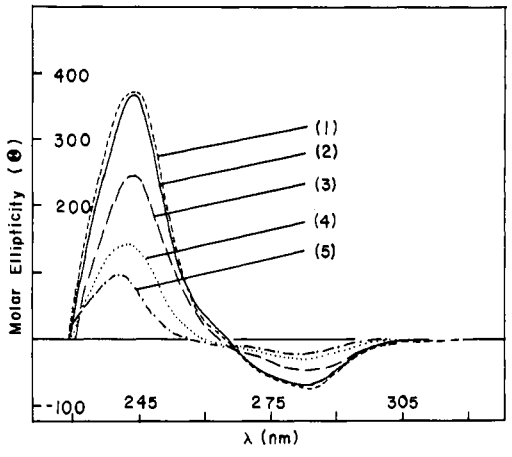

Figure 3. CD spectra of morphine group in aqueous HCl: (1) morphine, (2) nalorphine, (3) 3MAM, (4) 6MAM, (5) heroin.

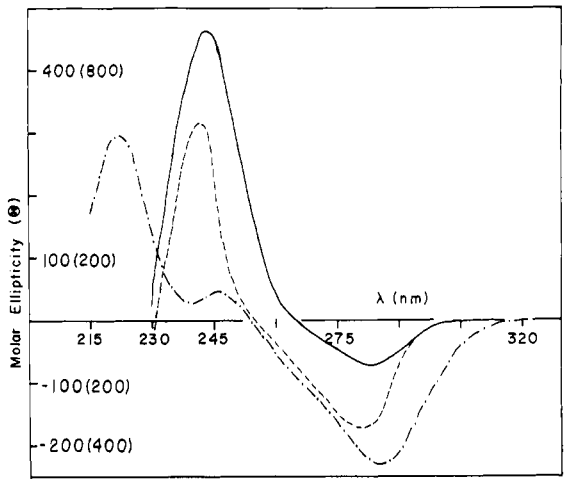

Figure 4. CD spectra of codeine group in aqueous HCl: (—) codeine, (- - -) dihydrocodeine, (– • –) thebaine. Parenthetical [Θ] apply only to thebaine.

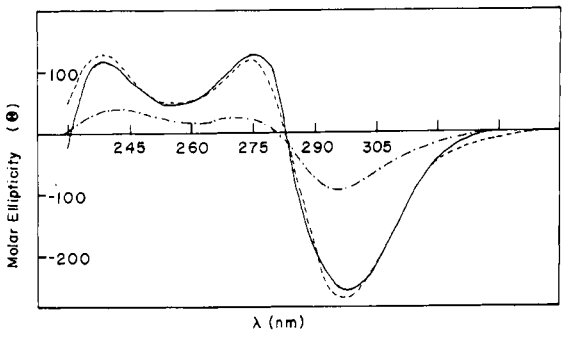

Figure 5. CD spectra of hydrocodone: (—) in HCl, (- - -) in 8.6 buffer, (– • –) in NaOH.

The printed spectra are averages of four to five independent experiments. In general terms the spectra show a relatively

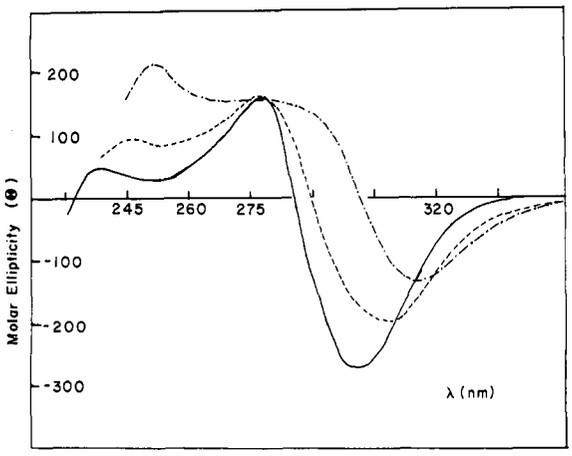

Figure 6. CD spectra of naloxone: (—) in HCl, (- - -) in 8.6 buffer, (-·-) in NaOH.

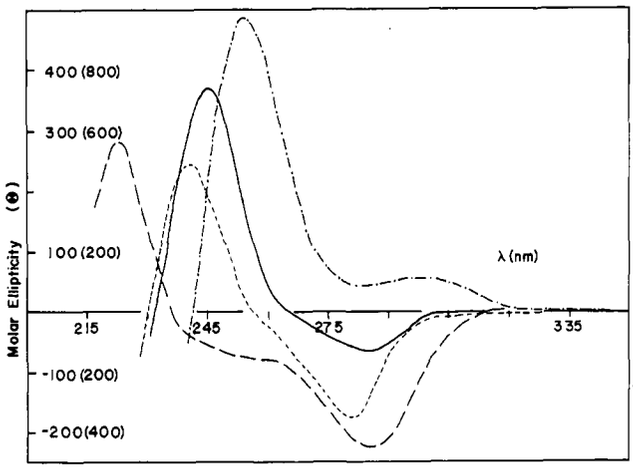

Figure 7. CD spectra of morphine and codeine groups in aqueous NaOH: (-·-) morphine, nalorphine, and acetyl derivatives, (—) codeine, (- - -) dihydrocodeine, (– – –) thebaine. Parenthetical [θ] apply only to thebaine.

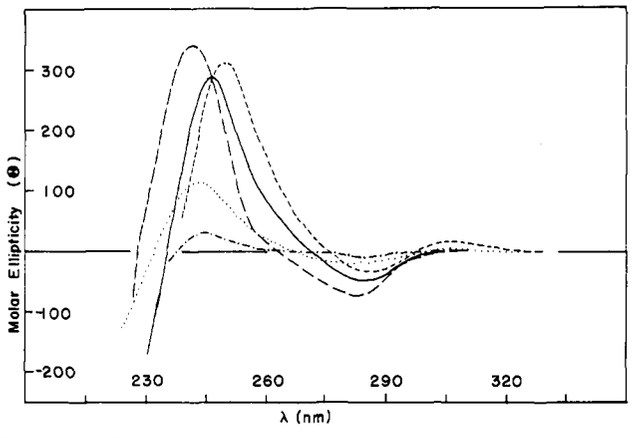

Figure 8. CD spectra of morphine group in 8.6 buffer: (- - -) morphine, (—) nalorphine, (– – –) 3MAM, (···) 6MAM, (-·-) heroin.

small negative Cotton effect in the wavelength range 280–290 nm attributed to a $\pi^* \leftarrow \pi$ transition of type ^1L_b, and a larger positive Cotton effect around 240–255 nm attributed to another $\pi^* \leftarrow \pi$ transition designated by ^1L_a. The ketone chromophores in hydrocodone and naloxone probably contribute to the signals observed in excess of 300 nm as an $\pi^* \leftarrow$ n transition. The *N*-vinyl group in naloxone and nalorphine does not appear to change the spectra significantly from the corresponding *N*-methyl derivatives.

In Table II are collected the wavelength parameters for maximum ellipticities, both positive λ_{max}^+ and negative λ_{max}^-, and for zero ellipticities λ^0 in the spectra of all the compounds in all media. Bands which have no corresponding maxima in the UV absorption spectra are designated by a superscript (*). Molar ellipticities calculated from the slope of the line which describes the linear dependence of ellipticities on solution concentration are listed in Table III. Representative of the linear plots are those shown in Figure 10 for morphine, codeine, and hydrocodone in the buffered solution. Included also in Table III are the ratios of molar ellipticities expressed as the positive maximum divided by the negative maximum.

Compounds which have either OH or $OCOCH_3$ in the 3-position show a red shift for λ_{max}^+ on going from acidic to basic media. Where OCH_3 is present in position 3, both λ_{max} values are invariant with pH. The results are consistent with trends observed in UV absorption maxima and are most likely at-

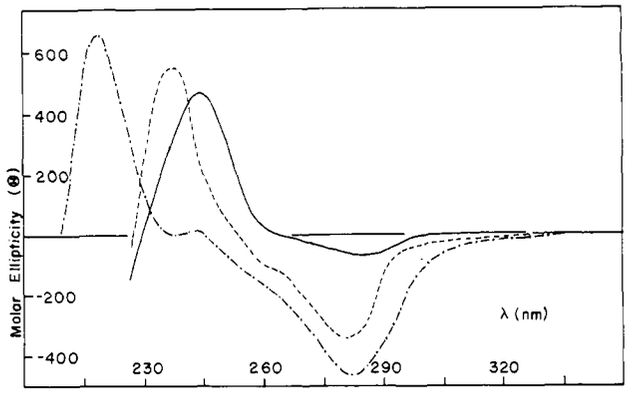

Figure 9. CD spectra of codeine group in 8.6 buffer: (—) codeine, (- - -) dihydrocodeine, (-·-) thebaine.

Table II. CD Spectral Parameters[a] for the Opium Alkaloids in Aqueous Media

	solution	λ_{max}^+	λ_{max}^-	λ^0
morphine	HCl	244	285	230, 263
	8.6	247, 302*	285	236, 272, 296
	NaOH	253, 285, 300*		253
nalorphine	HCl	244	286	229, 263
	8.6	246, 300*	284	235, 272, 296
	NaOH	254, 284, 295*		254
3MAM	HCl	245*	285*	229, 264
	8.6	242*	283*	228, 263
	NaOH	hydrolysis to morphine		
6MAM	HCl	242	284	229, 262
	8.6	244*, 300*	281*	232, 268
	NaOH	hydrolysis to morphine		
heroin	HCl	238	280*	227, 258
	8.6	243*	283*	238, 269
	NaOH	hydrolysis to morphine		
codeine	HCl	244	285	230, 264
	8.6	244	284	230, 264
	NaOH	244	284	230, 264
dihydrocodeine	HCl	240*	281	230, 256
	8.6	240*	281	230, 256
	NaOH	240*	281	230, 256
thebaine	HCl	221*, 246*	286	254
	8.6	219*, 242*	283	245
	NaOH	221*	285	236
hydrocodone	HCl	237*, 274*	295*	230, 283
	8.6	239*, 274*	295*	229, 281
	NaOH	238*, 274*	295*	230, 280
naloxone	HCl	239*, 278	300*	232, 286
	8.6	247*, 278	307*	289
	NaOH	252*, 278	315*	302

[a] Wavelengths are in nm.

Table III. Molar Ellipticities[a] for the Opium Alkaloids in Aqueous Media

	solution	$[\Theta]_{max}^{+}$	$[\Theta]_{max}^{-}$	ratio[b]
morphine	HCl	372	−66	5.6
	8.6	313, 14	−40	7.8
	NaOH	458, 52		
nalorphine	HCl	366	−70	5.2
	8.6	288, 5	−49	5.9
	NaOH	487, 55		
3MAM	HCl	245	−47	5.2
	8.6	346	−68	5.1
	NaOH	hydrolysis to morphine		
6MAM	HCl	135	−27	5.0
	8.6	138	−19	6.9
	NaOH	hydrolysis to morphine		
heroin	HCl	64	−18	3.6
	8.6	63	−21	5.0
	NaOH	hydrolysis to morphine		
codeine	HCl	463	−75	6.2
	8.6	405	−68	6.0
	NaOH	349	−74	4.7
dihydrocodeine	HCl	314	−170	1.8
	8.6	553	−340	1.6
	NaOH	244	−175	1.4
thebaine	HCl	596, 92	−461	1.3
	8.6	655, 20	−431	1.5
	NaOH	564	−450	1.3
hydrocodone	HCl	115, 125	−265	0.43, 0.47
	8.6	128, 106	−275	0.47, 0.39
	NaOH	37, 21	−93	0.40, 0.23
naloxone	HCl	50, 158	−275	0.18, 0.57
	8.6	98, 161	−200	0.49, 0.81
	NaOH	107, 77	−67	1.6, 1.1

[a] Defined as the experimental value divided by the molar concentration. [b] Absolute value of $[\Theta]_{max}^{+}$ divided by $[\Theta]_{max}^{-}$.

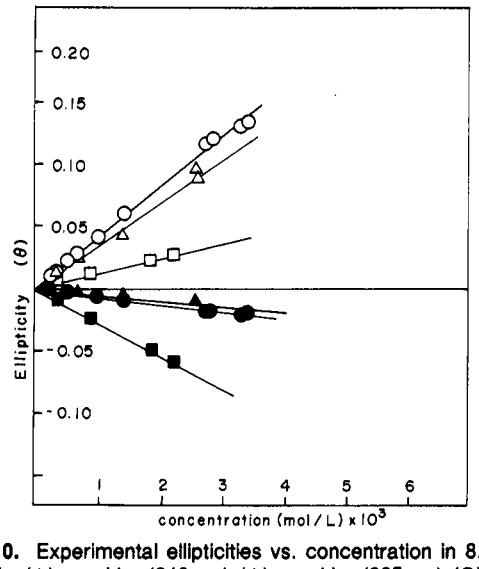

Figure 10. Experimental ellipticities vs. concentration in 8.6 buffer solution for (△) morphine (248 nm), (▲) morphine (285 nm), (○) codeine (244 nm), (●) codeine (285 nm), (□) hydrocodone (274 nm), and (■) hydrocodone (295 nm).

tributable to dissociation of the phenol proton. All of the acetyl derivatives are fairly stable at pH 8.6, but in more basic solutions they hydrolyze to morphine which dissociates at high pH.

Another feature associated with the ionization of the phenolic group is the gradual emergence of a positive Cotton effect at ca. 300 nm for morphine, nalorphine, and 6MAM. 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. It is significant too that naloxone shows an exaggerated red shift of λ_{max}^{-} while hydrocodone does not. Naloxone has the 3-OH substituent; hydrocodone has the 3-methoxy substituent.

DISCUSSION

For the purposes of the discussion, the compounds are divided into three subgroups, viz., the morphine group, the codeine group, and the ketone group. They are arranged this way in Table II.

The first most obvious development in the CD compared to the UV absorption spectra in all solvents is the separation of the shoulder at ca. 240 nm, the ^1L_a band, from the broad and very intense short wavelength absorption band, the ^1B band, whose maximum is usually around 210–220 nm. From Figure 2, we see that the transition associated with the ^1L_a band produces a positive Cotton effect, and the ^1B band an, as yet, unexplored negative Cotton effect. The λ^0 separating these bands occurs around 230 nm. A second λ^0 occurs around 250–265 nm and separates the ^1L_a from the negative band of the ^1L_b transition. There are, as a result, more parameters which might be used for analytical identification. Nevertheless, in ethanol as solvent, Figure 2, distinction is not easy, and the little that has been gained over absorption spectral data is that thebaine and hydrocodone are readily recognizable by their dominating intense negative ellipticity bands.

In water as solvent, the greatest similarity in the spectra is observed for the compounds in strong acid, Figures 3–6. There the phenolic group is undissociated, the acetyl substituents are not hydrolyzed, and the ring nitrogen is not protonated. As it was in ethanol, some qualitative distinction is observed where the C-ring is modified, for example, for thebaine (conjugated diene), dihydrocodeine (saturated), and the ketones (saturated). The remainder could only be distinguished by knowing the molar ellipticities of the pure compounds, which severely limits the method in the analysis of mixtures. Thebaine is uniquely defined by its 221 nm positive band, dihydrocodeine by its unique λ^0, which is 15 nm blue shifted from the others, and the ketones by their persistently intense negative ellipticities, which are probably due to overlapping $\pi^* \leftarrow \pi$ and $\pi^* \leftarrow$ n transitions (*6*, *7*) in the region of 280–320 nm.

The conditions are least favorable for qualitative distinction in strongly basic media. There morphine, nalorphine, and the acetyl derivatives, all of which are rapidly hydrolyzed to morphine, have identical spectra, Figure 7. Codeine and dihydrocodeine are readily distinguishable from any member of the morphine group, because both λ_{max} values are unchanged from the values in acid media, and the negative ^1L_b band persists. The two compounds are again distinguishable from each other by the 15 nm difference in λ^0 and by the difference in the ratios of the maxima. Between acid and basic media there is only one significant change in the thebaine spectrum which is the change in sign of the low-intensity band at 245 nm. Without a fully developed quadrant-rule model, the structural reasons for this reversal in sign are unclear.

Major differences occur in the naloxone spectrum on changing pH, Figure 6. Band maxima are red-shifted 15 nm in base, and the molar ellipticities are more positive or less negative. The changes in λ^0 and λ_{max} are especially obvious and are possibly a consequence of the emerging positive band due to phenol ionization; cf. morphine. In contrast hydrocodone, with no ionizable phenol group, shows no changes in λ_{max} values, and the ellipticity changes toward less negative and less positive values, Figure 5. The two compounds are easily recognizable.

The most promising single condition for qualitative identification is the pH 8.6 buffer solution medium, Figures 8 and 9. Codeine and dihydrocodeine, thebaine, and hydrocodone and naloxone are again individually recognizable by the pa-

rameters already described. Members of the morphine group are now distinguishable also by the many changes in the ^1L_a band, by the separation of the λ^0 values, and by evolution of the positive band at 300 nm for morphine, nalorphine, and 6MAM. There is also a fairly general decrease in molar ellipticity values on raising pH which is greatest for heroin and difficult at this time to interpret in terms of structural changes. 3MAM is a singular exception to the trend.

The evolution of the positive band at 300 nm is interesting from a structural point of view. It is certainly associated with the formation of the phenolate ion as the pH is raised. There is good reason to believe that the entire spectrum is red shifted with increasing pH, and the maximum of the ^1L_b band occurs at 300 nm. The spectrum at pH 8.6 is then a superposition of the spectra for the –OH form and the $-O^-$ form. The change in sign of the ^1L_b band may be accounted for by the following explanation. From X-ray diffraction it is known that the aromatic ring is forced out-of-plane by the steric restrictions imposed on the molecules by the atoms at the annular bridge head (*11*, *12*). On the other hand, delocalization of the oxygen negative charge might assume steric priority causing the aromatic ring to become flat and therefore to dominate in the structure determination. Changes in sign would properly be interpreted as movement of substituents from one sector into another in a viable quadrant-rule model. The critical pivot points are at C(5) and C(13).

These points are probably critical too in transmitting the effect of modifying the C-ring over to the transitions in the aromatic ring. The effect is not observed in UV absorption spectra where one can see that the spectrum of thebaine in ethanol is very similar to those of the morphine group compounds (*5*). Distinction by CD is as much a consequence of the inherent rigidity of the molecule so that small structural changes produce enlarged conformational reorientations.

In summary CD spectropolarimetry has been successfully applied to the distinction of 10 opiates. Compared to the alternative methods described from this laboratory, where the compounds are placed either in KBr pellets (*3*) or in a cholesteric liquid crystalline solvent (*4*), the present method is straightforward and easily has the advantage of becoming quantitative. It is necessarily restricted to the study of chiral compounds, and in this regard the anisotropic cholesteric solvent has the advantage, being applicable to the study of both achiral molecules and racemic mixtures. CD using KBr pellets is of particular value where the compound is insoluble and where it is important to preserve the integrity of the sample.

Although distinction is greatest in pH 8.6 buffer solutions, greater versatility is obtained by looking at results from both acidic and basic media. This may become necessary as the data base is extended to include other opiates and related compounds. Using different media might well be essential also in the analyses of mixtures, where work has already begun.

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Excitation Frequency Dependence of Resonance-Enhanced Inverse Raman Band Shapes

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The theoretical equation governing the shape of a resonance-enhanced inverse Raman band is derived. The band shape is shown to depend on the difference between the pump laser frequency and the frequency of the resonant electronic transition. The theory is shown to describe acridine orange and β-carotene spectra obtained with the argon ion laser lines between 488 nm and 514.5 nm as pump lasers.

During the last 3 years inverse Raman spectroscopy and Raman gain spectroscopy have emerged as useful methods for obtaining the Raman spectra of highly luminescent materials. The utilization of various modulation/demodulation schemes (*1*–*3*) provides much better detection limits than direct measurements of absorption or gain. We have recently shown that one modulation/demodulation method, ac-coupled inverse Raman spectrometry, (*1*) gives detection limits comparable to those obtained by spontaneous Raman spectroscopy (*4*).

Recently, we reported the first resonance-enhanced Raman spectra obtained by a modulation scheme (*5*). In that paper we demonstrated that resonance-enhanced inverse Raman spectra of a highly luminescent molecule, acridine orange, could easily be obtained by ac-coupled inverse Raman spectrometry. However, the spectra were inverted. That is, they appeared in apparent gain rather than in absorption. Only tentative explanations were offered for the unexpected results.

In this paper we report the dependence of inverse Raman band shapes on pump laser frequency and derive the governing equation which describes the band shapes. We show that, in general, inverse Raman bands change from positive Lorent-

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