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Determination of Cocaine by Circular Dichroism

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A procedure is described for the direct quantitative analysis of L-cocaine using circular dichroism spectropolarimetry. Samples investigated were either prepared in-house or had been confiscated. Preparation of the samples for analysis is minimal and the time for analysis is competitive with that for other approved methods.

In standard laboratory procedure, drug identification is done in two steps. A broad screening procedure is used at the first level. In this, wet chemistry spot tests (*1*) and microcrystalline tests (*1*) are used to classify an unknown substance into a particular generic group. The tests are empirical and relatively nondiscriminatory and by themselves are insufficient evidence for positive identification. In the second level of testing, instrumental methods are commonly used (*2–11*). By use of these methods sufficient corroborative evidence can be used for positive, and if necessary, quantitative identification. Quantitative identifications are seldom done for one basic reason. Total separation of the components of the mixture is almost always a prerequisite. This introduces an unfavorable time element into the analysis which can reach critical proportions when case loads are heavy. However in most states qualitative identification is considered to be sufficient evidence for a successful conviction in cases of possession of federally controlled substances.

Cocaine is somewhat unique in that many state statutes specify that only extracts from the naturally occurring coca plant (*Erythoxylum coca*) are controlled substances. When written in this vague way, the statute is interpreted to mean that only L-cocaine and its derivatives are included in the schedule of dangerous drugs. Possession of either the D-isomer alone or of the DL-racemic mixture is not illegal because neither form occurs naturally in the coca plant. The D-isomer is not known to be physiologically active and to date has not been resolved from the enantiomeric mixture. Although the racemate is physiologically active because of the presence of the L-isomer, conviction in a court of law demands proof that the confiscate contains the L-isomer. This can easily be done qualitatively with a polarimeter, if all other optically active substances are first removed.

Our principal objective in the analysis of samples of dangerous drugs is to perform the determinations quickly, directly, and quantitatively. Separation procedures should be kept to an absolute minimum. One method that achieves this goal is circular dichroism (CD) spectropolarimetry (*12–15*) which provides specificity due to measured optical rotation and a quantitative result by visible–UV absorption spectrophotometry. Only those substances which are chiral and which absorb light can be quantitated by CD. L-Cocaine is one of those drug molecules that satisfy these prerequisites.

CD spectra are obtained from substances in solution in an achiral solvent. Dissolution is the only sample preparation envisaged (*14, 15*). Separation of insoluble material by either filtration or centrifugation is the extent of the separation procedure required.

Table I. Quantitative Determinations of L-Cocaine in Mixtures [a]

sample	% composition by weight [b]	by CD [c]	by OSBI [d]
I	31.9	31.3	
II	43.3	44.4	
III	1.9	1.5	
IV	0.5	0.47	
1		71.9	66.0
2		42.1	42.5
3		2.5	
4		19.8	
5		40.9	40.0
6		40.5	
7		14.6	
8		19.7	
9		20.2	
10		22.5	
11		28.0	
12		23.5	

[a] Compositions are reported as percentages of cocaine as free base. [b] Roman numerals refer to in-house prepared cocaine–lactose mixtures. [c] Results are averaged for at least four determinations at 278 and 245 nm. [d] OSBI refers to independent determinations made at the Criminalistics Laboratory at the Oklahoma State Bureau of Investigation.

The CD spectrum of L-cocaine is described and results of the quantitative determinations of the drug in prepared mixtures and in unknown confiscated samples are reported.

EXPERIMENTAL SECTION

L-Cocaine hydrochloride was obtained from the National Institute for Drug Abuse via the Research Triangle Institute and was used without purification. Solutions were prepared in absolute methanol, in distilled water, and in 0.1 M aqueous HCl. The principal absorption band in the UV spectrum of L-cocaine, due to the presence of the aromatic ring, occurs at 275 nm with a shoulder at 285 nm. Reagent grade lactose (Mallinckrodt Inc.) was used as a diluent in the preparation of mixtures of predetermined composition. Confiscates 1–5 (Table I) were generously provided by the Criminalistics Laboratory of the Oklahoma State Bureau of Investigation as trial samples. Confiscates 6–12 were delivered in the custody of an officer from the Oklahoma City Police Department and the results were subsequently introduced as evidence.

CD measurements were made on a Cary 61 spectropolarimeter. Sample sizes ranged from 1 to 10 mg and were dissolved in 25 mL of distilled water or absolute methanol stock solutions. Insoluble materials were separated by centrifugation and were not identified. Solutions appropriately diluted from the stock were placed in a 1-cm cell and the spectra were measured against a solvent blank.

RESULTS AND DISCUSSION

The CD spectra of L-cocaine in absolute methanol and in distilled water are shown in Figure 1. The principal Cotton

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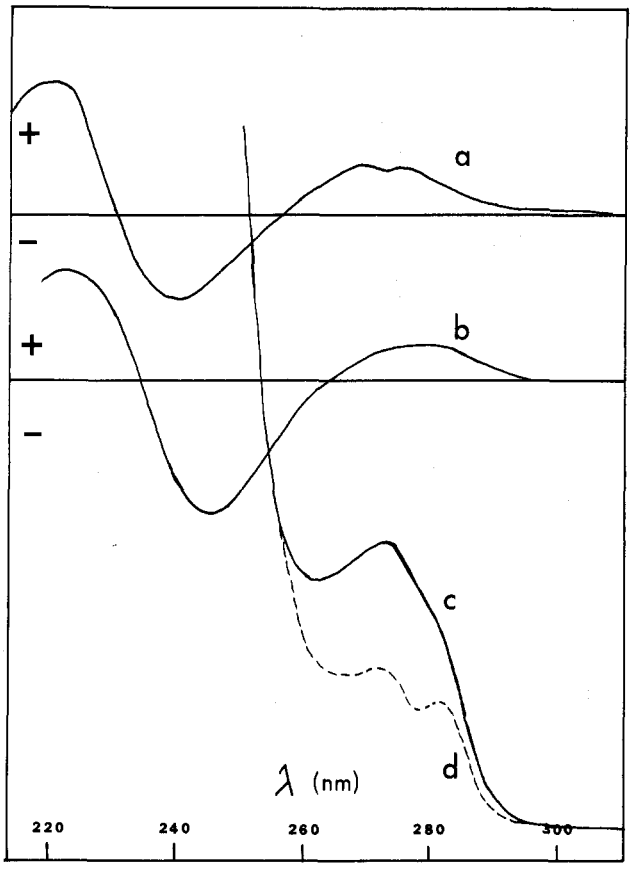

Figure 1. UV spectra of L-cocaine in arbitrary units: (a) CD spectrum in methanol $[\theta]_{278} = 9$, $[\theta]_{245} = -38$; (b) CD spectrum in water $[\theta]_{278} = 11.5$, $[\theta]_{245} = -55$; (c) absorption spectrum in water, $\epsilon = 1040$; (d) absorption spectrum in methanol.

bands observed are at 278 nm (positive), at 245 nm (negative), and at 222 nm (positive). A separation of the 278-nm positive band into two maxima is observed in methanol. In dilute acid the spectra are analogous to that in water differing only in the magnitude of the bands. No further work was done on acidic solutions.

Loss of base line stability at short wavelengths and excessive absorption by the chromophore discouraged the use of the 222-nm band for quantitation. Instead the 278-nm and 245-nm bands were preferred. The ordinate of a CD spectrum is called the ellipticity ψ_{exp} which is related to the difference in the molar absorption coefficients of the left ϵ_L and the right ϵ_R circularly polarized components of the plane polarized incident light beam by the equation

$$[\theta] = \frac{\psi_{exp} L}{\text{mol}} = 3300(\epsilon_L - \epsilon_R) \qquad (1)$$

where $[\theta]$ is the molar ellipticity coefficient. A CD spectrum is simply a modified absorption spectrum which shows positive and negative maxima depending upon the relative magnitudes of the two extinction coefficients (eq 1). Thus the data obey the Beer–Lambert–Bougert law (BLB), Figure 2. The molar ellipticity coefficients $[\theta]$ for L-cocaine, calculated from the BLB slope are +11.5 and −55.0 at 278 nm and 245 nm, respectively, in aqueous solution. For this particular instrument the limit of detection is on the order of 1×10^{-5} M, calculated from the 245-nm band.

The definition we have used for the molar ellipticity in terms of solution concentrations (eq 1) is not that used in the older literature (*16*). Instead we prefer to use an equation totally analogous to the BLB equation. Results obtained for the prepared mixtures and for the 12 confiscates are given in Table I. Agreement with the prepared samples is excellent.

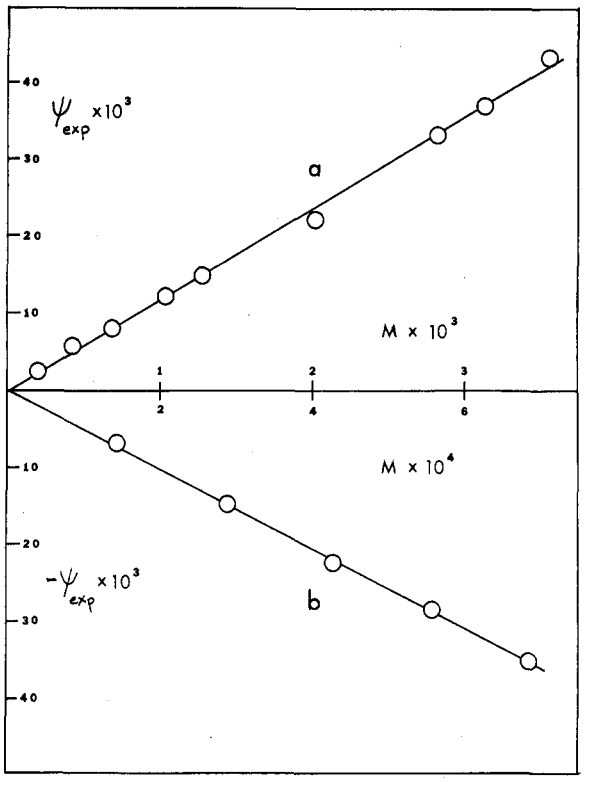

Figure 2. Plot of experimental ellipticity ψ_{exp} vs. cocaine concentration at (a) 278 nm and (b) 245 nm.

Column 4 of Table I contains the results of the only quantitative determinations performed at the Oklahoma Bureau of Investigation and which we have for comparison with our results.

Preparations of drug samples for illicit use follow a general pattern of being progressively diluted as distribution becomes more extensive. The ballast materials are usually starches and simpler sugars such as lactose. Additives are also present, some to provide a secondary physiological sensation, others to make identifications more difficult, because of similarities in their physical properties when compared to the drug. Methods for quantitative determinations presently in use are protracted because, after separation, derivatization is often necessary, as it is in gas chromatography and mass spectrometry, and the instrument must be repeatedly calibrated for each drug.

We have demonstrated that quantitative determination of L-cocaine can effectively be done with a minimum of sample preparation. The linear dynamic range is wide and reproducibility is excellent. Insoluble materials are probably starch although no positive identification was made. Among the usual additives present in cocaine confiscates are lidocaine, procaine, and benzocaine. Each of these molecules contains the benzene ring chromophore, which absorbs in the same UV range as the phenyl ring in cocaine. None of the compounds is chiral and therefore all are CD inactive. Lactose is optically active but does not absorb in the same UV range and it too is not CD active. There is no experimental interference from any of these compounds which simplifies the determinations.

Absorption by an achiral substance in the wavelength region of the Cotton bands of a chiral compound does reduce the intensity of the transmitted light, which is observed experimentally as a decrease in signal to noise ratio. Confiscate 5 contained a large excess of an intensely colored dyestuff and in appearance the sample was a viscous tar and difficult to handle. The presence of L-cocaine in this sample had escaped detection for a long period of time by the approved methods (*17*). The sample was readily soluble in water and L-cocaine

was quickly identified and quantitated by CD spectropolarimetry.

In terms of time for analysis, CD compared favorably with methods presently in use. The time required is equivalent to the time needed to obtain an absorption spectrum. As a library of CD spectra is gradually accumulated, the need for screening procedure will be reduced. Determinations are not dependent on instrument parameters. The only information needed is the molar ellipticity coefficient [θ]; cf. the molar absorption coefficient ϵ in absorption spectrophotometry. Total separation is not a prerequisite although it might be anticipated in the future as newer interfering additives are found. Derivatization prior to analysis is unnecessary. The principal difficulty will arise when more than one CD active substance is present. Then the combined spectrum must be deconvoluted or total separation must be performed.

It cannot be stated with absolute certainty that only the L-enantiomer was present in the confiscated drug samples. Any DL-racemate would remain undetected. What is calculated to be L-cocaine might not represent the total cocaine present in the sample. The CD evidence was admitted in Oklahoma County Court and accepted by the defense attorney as proof of the presence of L-cocaine in the confiscates 6–12.

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Identification of Cocaine and Phencyclidine by Solute-Induced Circular Dichroism

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A complexation equilibrium between cycloheptaamylose (β-cyclodextrin) and either L-cocaine or phencyclidine (PCP) causes a structural disymmetry to be induced into the drug molecules. The accompanying solute-induced circular dichroism spectra can be used for analytical identification. The equilibrium constant for the L-cocaine–sugar complex has been calculated as a function of temperature.

Circular dichroism (CD) is the effect caused by the simultaneous absorption and rotation of an incident beam of plane polarized light measured as a function of wavelength (*1*). The phenomenon is usually associated with birefringent substances which, materially speaking, are structurally disymmetric either at the molecular level in solution (chiral) or in the macroscopic solid state. In the present context this will be referred to as the intrinsic CD.

The same phenomenon can be induced into achiral molecules. Referred to as the extrinsic CD, it can be induced in one of three ways: by applying a static uniform magnetic field (*2*), abbreviated to MCD; by dissolving the achiral solute in a chiral solvent, such as a cholesteric liquid crystal (*3–5*); and by dissolving the achiral solute in a chiral solution (*6*, *7*), i.e., an achiral solvent in which is dissolved a chiral cosolute. The second and third methods involve specific complexation interactions between the achiral and chiral moieties. For the first and third methods the magnitude of the extrinsic CD is generally small and careful data assimilation is necessary. For the second approach the extrinsic CD is generally large but the experimental procedure is very inconvenient (*5*) because of the physical properties of the anisotropic and metastable solvent.

Previous work from this laboratory has demonstrated that CD can be successfully applied to the identification and quantitative determination of dangerous drugs in aqueous media (*8–10*). In the expanding search for even greater specificity in identification we had used a liquid crystalline mixture of cholesteryl chloride and cholesteryl nonanoate (*5*) to induce an extrinsic CD. Although the specificity was increased, we are sceptical that quantitation can ever be successfully accomplished by this method.

In this work we report preliminary results for solute-induced extrinsic CD spectropolarimetry applied to drug analysis. The chiral system is an aqueous solution of cycloheptaamylose, or β-cyclodextrin, which acts as the chiral host to the drug. The drugs for which results are reported are chiral, L-cocaine, and achiral, PCP. Complexation between guest (drug) and host is specific. The data are treated in terms of a 1:1 complexation equilibrium reaction and thermodynamic parameters are reported for the cocaine–sugar complexation.

EXPERIMENTAL SECTION

L-Cocaine and PCP as hydrochloride salts were supplied by the National Institute for Drug Abuse via the Research Triangle

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