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Technical Note

Determination of Reserpine by Circular Dichroism

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INTRODUCTION

Reserpine is indicated in the treatment of mild hypertension and certain mental disorders (1). *Rauwolfia serpentina* root is a natural source of reserpine. Two other antihypertensives, rescinnamine and deserpidine, are also present in the root (2). In addition to these active components, *R. serpentina* contains as many as 22 other alkaloids including ajmaline, ajmalicine, raubasinine, reserpiline, serpentine, and yohimbine (3).

Analytical determinations of reserpine have been achieved by colorimetric (4), fluorometric (5,6), and electrochemical (7,8) detection methods. Gas chromatography (GC) (9) and gas–liquid chromatography (GLC) (10) have also been employed as assay procedures. High-performance liquid chromatography (HPLC) has some utility but is limited to determination of the relative concentrations of the constituents present in multicomponent pharmaceutical formulations (11) and in *R. serpentina* root extracts (12). Most of the methods reported depend upon derivatization steps to eliminate interferences. Results are reported for only the reserpine group alkaloids (reserpine and rescinnamine), which are usually calculated as total reserpine.

Selective analytical determinations based on circular dichroism (CD) are possible because an analyte must possess both absorbance and optical activity. The degree of selectivity has been sufficient in many cases that neither chromatographic separation nor a lengthy workup is necessary. Various pharmaceutical preparations have been assayed for their active constituents using CD. Examples include Demerol (13), Seconal (14), penicillin-V tablets, and penicillin-V broth (15). More complex samples, such as plant extracts, have also been directly assayed for as many as four CD active analytes (16–18).

Reserpine and three other indole alkaloids of *Rauwolfia serpentina* exhibit CD activity. In this work the CD spectra of these four compounds have been characterized and the data used for direct determination of reserpine, first in prepared laboratory mixtures and subsequently in pharmaceutical preparations.

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MATERIALS AND METHODS

Chemicals and Instrumentation. The indole alkaloids yohimbine hydrochloride (Aldrich Chemical Co.), reserpine, rescinnamine, and ajmaline (Sigma Chemical Co.) and the diuretic substances furosemide and hydrochlorothiazide (Sigma Chemical Co.) were used without further purification. Standard *Rauwolfia serpentina* was obtained from the United States Pharmacopeial Convention and was used without further purification. Forty-milligram furosemide tablets (Lasix, Hoechst-Roussel), 0.25-mg reserpine tablets (Serpasil, Ciba) and 50-mg *Rauwolfia serpentina* tablets (Raudixin, Squibb) were all obtained from a local pharmacy. All solvents and other reagents used in the extractions were of analytical-grade quality.

CD measurements were made on a JASCO-500A/DP500N automatic spectropolarimeter/data processor combination. The instrument was calibrated daily with a standard solution of androsterone in dioxan as recommended. Instrument parameters were selected to give optimum signal-to-noise ratios.

Laboratory Mixtures. Chloroform stock solutions of reserpine, rescinnamine, and ajmaline were used for the preparation of in-house reserpine–ajmaline and reserpine–rescinnamine binary mixtures.

Reserpine Tablets. Individual tablets were reduced to a fine powder by shaking in a Wig-L-Bug. This powder was extracted with chloroform, and the resulting solution agitated for 45 min. Centrifugation was required to reduce the amount of suspended particulate matter.

Rauwolfia serpentina. Fifty milligrams of USP root powder or an entire *Rauwolfia serpentina* tablet was extracted according to the procedure described by Cieri (12), but with the following modifications. The volume of H_2SO_4 was reduced to 25 ml and the $CHCl_3$ portions were reduced from 30 to 10 ml. Pooled $CHCl_3$ extracts from the Raudixin tablets were washed with one 10-ml portion of 0.1 *N* NaOH to remove interferences due to the dyes used in the coating (5). The $CHCl_3$ fraction was evaporated under air to a volume of approximately 5 ml. Anhydrous $CaCl_2$ was added to remove any remaining moisture. The $CHCl_3$ was decanted and combined with $CHCl_3$ washings of the $CaCl_2$. The sample was then diluted to a final volume of 10 ml. The extraction procedure was carried out under reduced lighting to avoid photooxidation (19).

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RESULTS AND DISCUSSION

CD is a modified form of absorption spectrophotometry in which the selectivity in detection is determined by the fact that there is chirality in the molecular structure. The chirality is of qualitative significance, but the absorption is the basis for quantitative analysis. The experimental difference inherent to CD is that the incident beam is divided into two circularly polarized components, both of which traverse the medium containing the analyte, and both are absorbed, but to different extents. The experimentally measured parameter of ellipticity is directly proportional to this absorbance difference. CD calibration curves are plots of measured ellipticities (degrees) versus analyte concentrations (moles per liter) and obey the Beer–Lambert Law at all wavelengths. The slopes of these curves are referred to as molar ellipticities (θ_M). For mixtures, the observed total experimental ellipticity is equal to the sum of the products $\theta_M C_M$ for all CD active components, where C_M is the analyte concentration.

The molecular structures of the four CD-active indole alkaloids are given in Fig. 1. All four absorb light in the 300- to 210-nm range. Since CD activity occurs only in the vicinity of an absorption band, CD spectra were obtained for the 370- to 240-nm region. A distinctive CD spectrum was observed for each alkaloid (Fig. 2). Reserpine's spectrum exhibits a single negative Cotton band which maximizes at 266 ± 2 nm ($\theta_M = -262° \cdot M$ cm), while a single positive band maximizing at 269 ± 2 nm ($\theta_M = +62° \cdot M$ cm) is observed for yohimbine hydrochloride. Chloroform solutions of ajmaline exhibited bands centered at 298 ± 2 nm ($\theta_M = -70° \cdot M$ cm) and at 255 ± 2 nm ($\theta_M = +394° \cdot M$ cm). Rescinnamine produced three Cotton bands centered at 322 ± 2 nm ($\theta_M = +57° \cdot M$ cm), 297 ± 2 nm ($\theta_M = -131° \cdot M$ cm), and 274 ± 2 nm ($\theta_M = -153° \cdot M$ cm).

The determination of reserpine in Serpasil tablets was straightforward. Reserpine content was calculated using the relationship $C_M = \psi/\theta_M b$, where C_M is the analyte concentration, ψ is the measured ellipticity at the wavelength maximum, θ_M is the molar ellipticity, and b is the cell path length. Averaging the results from six separate tablets yielded a value of 0.249 ± 0.034 mg, compared with a labeled content of 0.250 mg/tablet. When the reserpine content was expressed as a percentage of the total tablet weight, the variation among tablets was less than 0.02%. Diuretics are often prescribed in conjunction with antihypertensives, and often both medications are combined in a single formulation. The diuretics, hydrochlorothiazide and furosemide, absorb in the UV but are achiral. The presence of either diuretic did not interfere with the determination of reserpine.

A more complex extraction procedure was needed for the determination of reserpine present in the root material due to the large number of other alkaloids present. It has been demonstrated (12) that ajmaline, yohimbine, and other alkaloids remain in the H_2SO_4 fraction, while reserpine and rescinnamine transfer to the chloroform fraction. Reserpine content was calculated as before. Determinations made on the USP samples yielded a reserpine content of 0.165 ± 0.002%. Raudixin tablets were determined by CD assay to

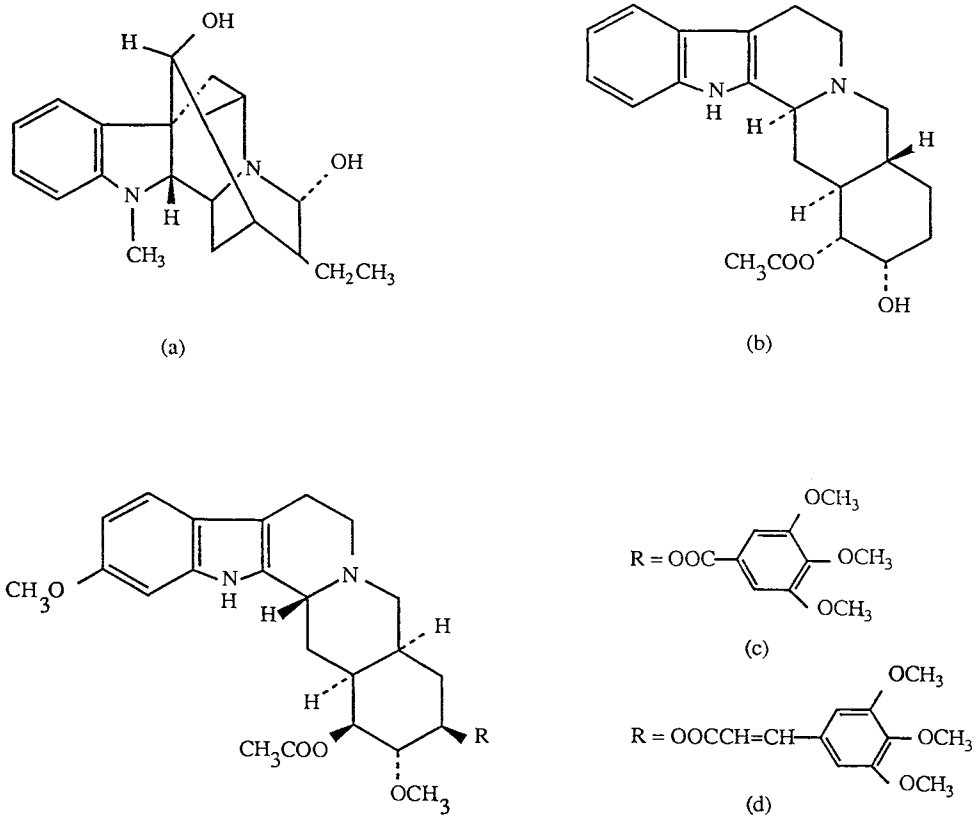

Fig. 1. Molecular structures of the indole alkaloids (a) ajmaline, (b) yohimbine, (c) reserpine, and (d) rescinnamine.

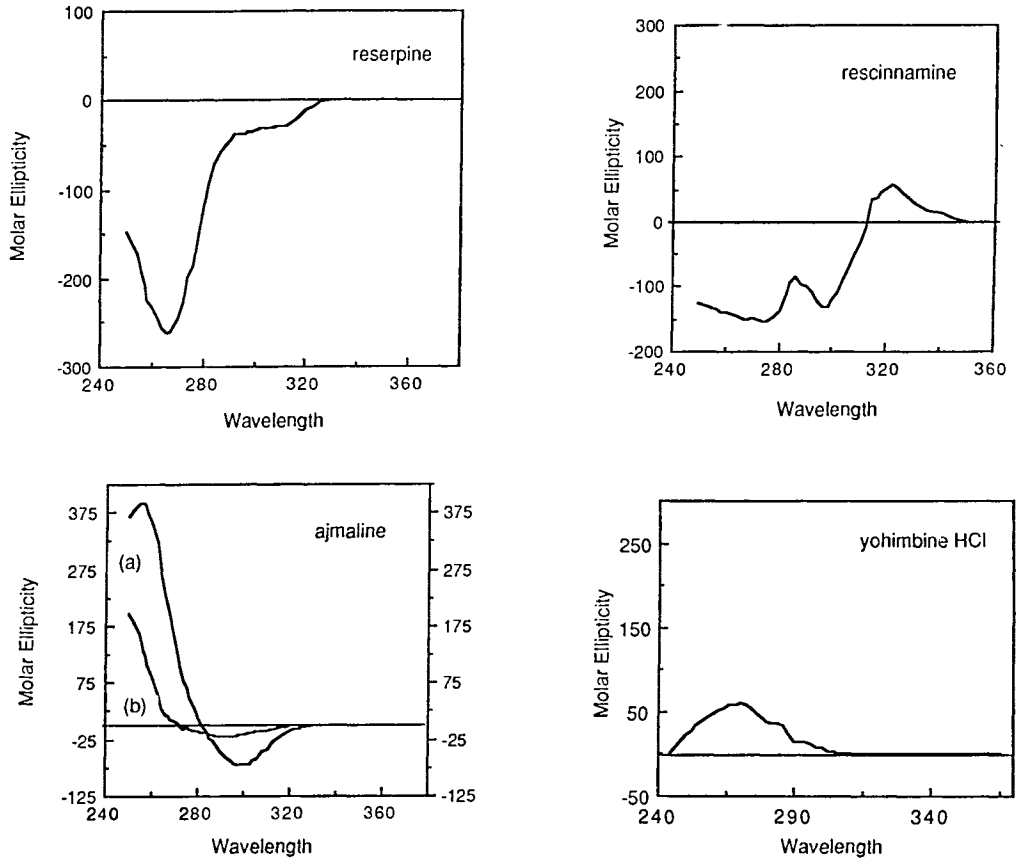

Fig. 2. CD spectra of the indole alkaloids reserpine, rescinnamine, yohimbine HCl, and ajmaline (a) in $CHCl_3$ and (b) in methanol–sulfuric acid.

contain 0.164 ± 0.017% reserpine. Both of these values fall within the accepted concentration range of 0.15–0.20% reserpine. For these calculations a root content of 50 mg/tablet was assumed.

The positive Cotton band at 322 nm typical for rescinnamine was not present in the spectra for the root extracts. Any contribution to the total signal from rescinnamine at 297 or 274 nm was concealed by the very large reserpine signal. Reserpine is present in such a large excess and has such a high θ_M value that the rescinnamine contribution to the total signal height is negligible. This made direct determination of rescinnamine in the USP samples or the Raudixin tablets impossible.

The limits on the detectability of the minor component in a binary mixture can be estimated from the study of laboratory mixtures. This was done for mixtures of reserpine–rescinnamine and reserpine–ajmaline. A simple curve-fitting program (18) was used to analyze the experimental data. The program simulates the experimental curve by adding weighted contributions from the standard curves. Agreement between theoretical percentage and calculated percentage was good for both sets of mixtures. Optimum results were obtained with mixtures of 75% reserpine and 25% ajmaline or rescinnamine. Calculated compositions were in error by no more than 0.8%. Poor results were obtained from those mixtures containing 10% reserpine. Calculation yielded a reserpine content of only 2.5% (a 75% error). The corresponding calculated ajmaline or reserpine content was 97.5% (a relative error of 9% compared with the theoretical value of 90%). The 90/10, 50/50, and 25/75 percentage reserpine/percentage ajmaline or rescinnamine mixtures exhibited errors as great as 20% in estimation of the minor component, while the error in the estimation of the major component did not exceed 6%.

This work is a further endorsement of the utility of CD in the analysis of pharmaceutically important substances based upon the selectivity inherent to the method. CD detection allowed the extraction procedure to be simplified, resulting in a reduced analysis time. Additional time savings can be achieved with automation. Determinations did not require derivatization of the analytes or addition of internal standards. Based on these advantages, consideration of the application of CD to quality control in the pharmaceutical industry is strongly recommended.

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