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NMR Spectroscopic Determination of Preferred Conformations of Quinidine and Hydroquinidine

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Abstract □ NMR spectra of quinidine (I), hydroquinidine (II), and their respective acetyl derivatives (III and IV) were compared. The chemical shifts of some protons in I differed from those of their counterparts in II. These values were concentration dependent in I and II; they were similar in III and IV but not concentration dependent. The implications of these findings and the correlation of the NMR data with the preferred conformations are discussed.

Keyphrases □ Quinidine—conformation, NMR spectroscopic analysis, concentration dependence □ Hydroquinidine—conformation, NMR spectroscopic analysis, concentration dependence □ NMR spectroscopy—quinidine and hydroquinidine, conformations, concentration dependence

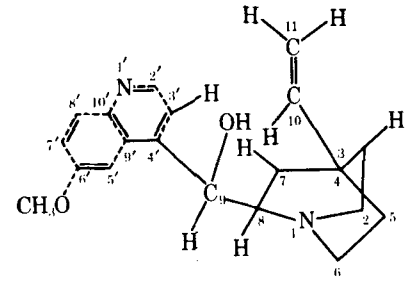
Of all of the cinchona alkaloids, quinidine (I) remains the most important because of its extensive use as an antiarrhythmic agent. Hydroquinidine (II) is an impurity found in commercial I and also has antiarrhythmic activity. The absolute configuration of all asymmetric carbon atoms in these molecules has been assigned by different methods including NMR spectroscopy (1–4). The evidence of these studies is considered conclusive.

No comprehensive studies have been carried out concerning the conformation of the different fragments of I and II. Knowledge concerning the preferred stable conformation of these compounds facilitates structural elucidation of the metabolic products. The objectives of this work were to analyze the NMR spectra of I and II and their 9-acetyl derivatives III and IV, stressing the importance of acetylation, and to discover new information related to their structure and conformation.

EXPERIMENTAL

Spectra were obtained at 100 MHz using an analytical NMR spectrometer[1] equipped with an automatic recorder. Tetramethylsilane in deuterochloroform was used as the internal standard; the chemical shift of its protons does not appear in the spectra. Pure I and II were obtained from commercial quinidine sulfate[2]. TLC and preparative TLC were carried out on aluminum oxide[3] plates. The developing solvent was ethyl acetate.

Purification of Commercial Quinidine—Commercial quinidine sulfate (500 mg) was dissolved in water (20 ml). A slight excess of sodium bicarbonate was added, and the free base was extracted in a separator by three 30-ml portions of chloroform. The chloroform solution was filtered through anhydrous sodium sulfate and evaporated.



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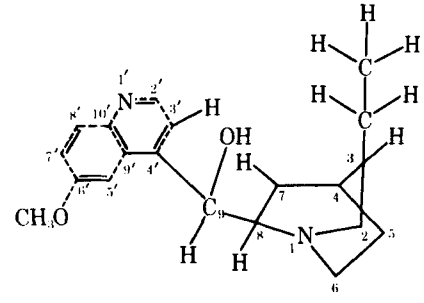

II

Portions of the free base (30 mg) were chromatographed on aluminum oxide plates. The fluorescing material was viewed under UV light and divided into three fractions. The upper strip was scraped and dispersed in 20 ml of 0.1 *N* H_2SO_4. Sodium bicarbonate (240 mg) was added, and pure quinidine was extracted with chloroform. The mixture was transferred to a separator, and the chloroform solution was filtered through anhydrous sodium sulfate and evaporated. A readily crystallized pure quinidine was obtained after solvent removal, mp 170–171°, R_f 0.135 (chloroform–ethyl acetate, 2:1). The NMR spectrum is shown in Fig. 1.

The lower strip, containing II contaminated by small quantities of I, was scraped and extracted as described. Pure II was obtained by a repetition of the chromatographic procedure. Recrystallization from methanol yielded crystals, mp 169° [lit. (5) mp 169 and 174°]; R_f 0.1 (chloroform–ethyl acetate, 2:1). The NMR spectral data are given in Table I.

Acetylation—Pure quinidine (40 mg) was dissolved in pyridine (1 ml), and acetic anhydride (1 ml) was added. The reaction mixture was allowed to stand at room temperature for 24 hr. Solvents were removed with an air stream, and the solid residue was dissolved in 30 ml of chloroform. The

[1] Varian A-100 D.
[2] Quinidine sulfate USP, Sigma Chemical Co., St. Louis, Mo.
[3] Aluminum oxide GF 254, Stahl, Merck.

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Table I—Chemical Shift Values [a] (in Parts per Million) of the Protons of Quinidine (I) and Hydroquinidine (II) at Various Concentrations (Milligrams per 0.5 ml)

Proton	I				II			
	15 mg	30 mg	60 mg	100 mg	13 mg	30 mg	60 mg	100 mg
H-2′	8.59 (d) (J = 4 Hz)	8.52 (d) (J = 4 Hz)	8.45 (d) (J = 4 Hz)	8.38 (d) (J = 4 Hz)	8.63 (d) (J = 4 Hz)	8.59 (d) (J = 4 Hz)	8.47 (d) (J = 4 Hz)	8.44 (d) (J = 4 Hz)
H-8′	7.94 (d) (J = 9 Hz)	7.90 (d) (J = 9 Hz)	7.86 (d) (J = 9 Hz)	7.83 (d) (J = 10 Hz)	7.95 (d) (J = 10 Hz)	7.93 (d) (J = 10 Hz)	7.88 (d) (J = 9 Hz)	7.87 (d) (J = 9 Hz)
H-3′	7.49 (d) (J = 4 Hz)	7.47 (d) (J = 4 Hz)	7.46 (d) (J = 4 Hz)	7.46 (d) (J = 4 Hz)	7.52 (d) (J = 4 Hz)	7.51 (d) (J = 4 Hz)	7.47 (d) (J = 4 Hz)	7.46 (d) (J = 4 Hz)
H-10	6.00 (m)	6.03 (m)	6.03 (m)	6.06 (m)	—	—	—	—
H–C-9–OH	5.57 (d) (J = 4 Hz)	5.57 (d) (J = 4 Hz)	5.57 (a)	5.58 (a)	5.62 (d) (J = 4 Hz)	5.59 (a)	5.55 (m)	5.54 (m)
H-11	5.09	5.09	5.09 (d) (J = 3 Hz)	5.08 (d) (J = 3 Hz)	—	—	—	—
H-11	4.95 (d) (J = 3 Hz)	4.96	4.94	4.93	—	—	—	—
CH_3O–	3.84	3.81	3.79	3.79	3.81	3.80	3.80	3.79

[a] d = doublet; m = multiplet; and a = ill-defined doublet.

chloroform solution was transferred to a separator, washed with 20 ml of water, and filtered without delay through anhydrous sodium sulfate since it was prone to hydrolysis; R_f 0.315 (chloroform–ethyl acetate, 2:1). The NMR spectrum is given in Fig. 1.

Acetylation of II was carried out under the same conditions described for acetylation of I; R_f 0.315 (chloroform–ethyl acetate, 2:1).

RESULTS AND DISCUSSION

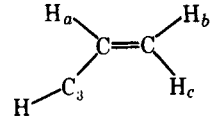
The protons of I–IV may be classified on the basis of their chemical shifts by comparison with literature data (6, 7).

NMR Spectral Analysis of I and III—The spectra of I and III (Fig. 1) were determined at a concentration of 30 mg/0.5 ml.

Aromatic Protons—In I, H-2′ appeared as a doublet at δ 8.52 (J = 4 Hz) and was split by H-3′; therefore, the doublet with the same splitting constant at δ 7.47 was assigned to H-3′. After acetylation, H-2′ appeared at δ 8.70. The H-3′ shifted to a higher field at δ 7.27, indicating the H-2′ and H-3′ were in the vicinity of the acetoxy group. The H-3′ was within the shielding zone and H-2′ was within the deshielding zone of the carbonyl cone (Fig. 2).

The H-8′ proton resonated at δ 7.90 (J = 9 Hz) in I and at δ 7.99 in III and was split by H-7′. The splitting of H-8′ by H-5′ was negligible.

Vinylic Protons—These protons in I appeared as two separate groups: the methine proton at C-10 and the two methylene protons at C-11:



These findings and those of others (6) with regard to the chemical shifts and J values are not in accord with the theoretical data (8). Thus, the H_a proton resonated at δ 6.03 as a multiplet instead of at δ 5.30, a field 0.73 ppm lower than the theoretical value. The H_b and H_c protons absorbed at δ 5.09 and 4.96 (0.44 and 0.33 ppm downfield). Furthermore, the splitting constants between H_a and H_c (*trans* coupling) and between H_a and H_b (*cis* coupling) were barely discernible. The theoretical values for the *trans* coupling are 11–20 Hz, and those for the *cis* coupling are 6–14 Hz. No doubt these differences in the chemical shifts may be associated with the spatial orientation of the vinylic group under the influence of the aromatic ring in the quinoline moiety.

Replacement of the hydrogen in the OH group at C-9 by $COCH_3$ affected the chemical shifts of the methylene vinylic protons. The *cis* proton H_b resonated at δ 5.16, and the *trans* proton H_c appeared as a double doublet at δ 5.02 [$J(H_c - H_a)$ = 6 Hz, and J (H_c – $H_{c\text{-}3}$) = 2 Hz].

A proton alpha to the OH group at C-9, appearing as a doublet, resonated at δ 5.57 (J = 4 Hz) in the vicinity of the vinylic methine proton. After acetylation, the chemical shift and the coupling constant were altered, and the proton at C-9 absorbed at δ 6.55 (J = 7 Hz). The vicinal coupling between the protons of C-8 and C-9 corresponded to a three single-bond system, H–C–C–H, in which the J value depended on both the dihedral angle and the electronegativity of the substituents (9). In I, this system was disubstituted by both a hydroxyl and nitrogen. Therefore, it is not surprising that both the chemical shift and the J value were altered after acetylation. The bulk of the $OCOCH_3$ and its electronegativity differed from that of the OH group. The actual dihedral angle is determined from the steric interactions experienced by the quinoline molecule in this vicinity and from the mutual interactions of the π-systems.

Assignment of Preferred Stable Conformation of Quinidine Molecule: Rotamer Populations—NMR data of I and II showed that the preferred stable conformation of I around the C-4′–C-9 and the C-8–C-9 bonds occurred when the quinoline ring was arranged spatially so that its nitrogen atom was on the same side as the vinylic group and opposite the nitrogen atom in the quinuclidine ring. The proton at C-3′ was in the neighborhood of the O–R group at C-9 (R = H, $COCH_3$). In III, this proton was within the shielding zone of the anisotropic acetoxy group and the proton at C-2′ was within the deshielding zone of this group.

The vinylic group plane was almost perpendicular to the quinoline ring and extended upwards to the deshielding zone of the aromatic ring. Since the methine proton H_a was more exposed to the aromatic magnetic field, it was deshielded to a greater extent than the vinylic protons at H_b and H_c.

This preferred stable conformation establishes the dihedral angle between the C-8 and C-9 protons and places the OH group at C-9 between the vinylic proton at C-10 and the C-2 proton alpha to the nitrogen in the quinuclidine ring. In addition, the hydrogen at C-3′ is positioned between the two vinylic protons at C-10 and C-11, minimizing nonbonded interactions. These observations appear to indicate the predominance of one rotamer.

NMR Spectra of I and III in Mixtures—Two mixtures were prepared, one rich in I and the other rich in III. The most striking change in the NMR analysis of these mixtures was the appearance of two separate

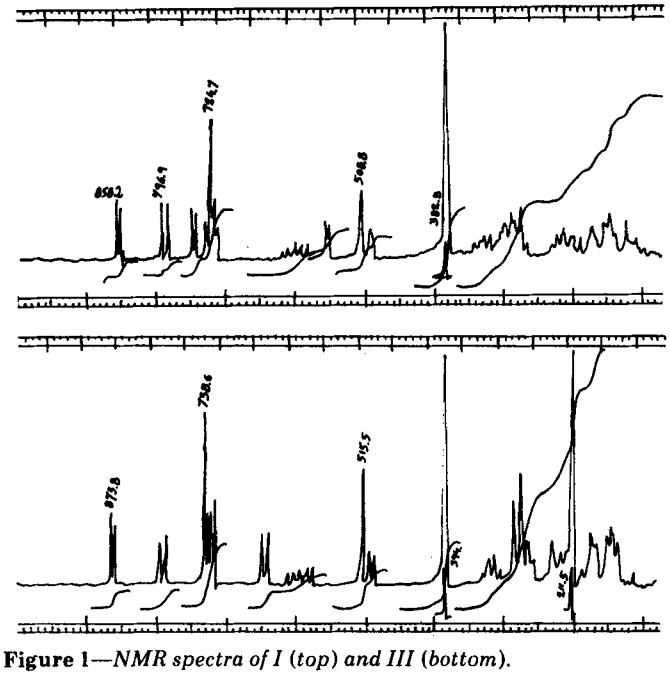


Figure 1—*NMR spectra of I (top) and III (bottom).*

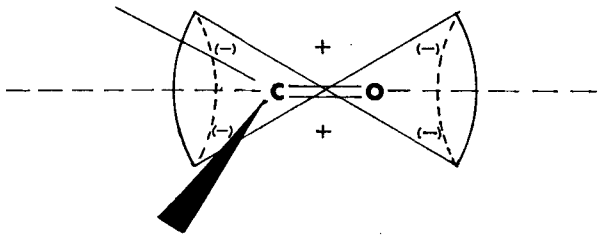


Figure 2—*Carbonyl cone.*

peaks for the methoxy protons (Figs. 3 and 4). Integration makes it possible to estimate with great accuracy the relative amounts of each component. Another pronounced deviation was found in the chemical shifts of the aromatic and vinylic protons of both I and III, which were different from those found in the NMR spectra of the pure substances, apparently as a result of slight intermolecular interactions.

Comparison of NMR Spectra of I and II and Concentration Dependence of Their Chemical Shifts—A comparison of the NMR spectra of I and II shows that the chemical shift values of certain protons were concentration dependent. When these NMR spectra were recorded at various concentrations (Table I), the data indicated that the chemical shifts of some protons of I and II differed at the same concentration and that they were concentration dependent in both alkaloids. Therefore, the comparison of the NMR spectra of I and II was made with dilute solutions to minimize the concentration effect on the chemical shift values and to produce a clear contrast.

The aromatic protons H-2′, H-3′, and H-8′ in II appeared at lower fields in comparison to the corresponding protons in I, demonstrating the shielding effect of the vinylic group which was absent in II. This finding is in keeping with the preferred stable conformation assigned to I. The proton alpha to the OH group at C-9 in II appeared at a lower field when compared to its counterpart in I, suggesting that the shielding effect of the vinylic group also extends to this proton in I.

At higher concentrations, the influence of intermolecular forces due to molecular association becomes more pronounced and may blur the mentioned mutual intermolecular effects of the various groups. This association is apparently initiated through hydrogen bonding between the C-9 OH groups in the same molecules of both alkaloids. Blocking these OH groups by acetylation prevents molecular association, as reflected by the appearance of identical chemical shifts in all concentrations of III and IV.

Additional proof that molecular association is brought about by hydrogen bonding between C-9 OH groups is shown by the change in the peaks of the proton alpha to the OH group at C-9. In dilute solution, this peak in both I and II appeared as a clear doublet, but it became ill defined at higher concentrations. This result implies a change in the dihedral angle between the protons of C-8 and C-9 during association. In contrast, the proton alpha to the acetoxy group in both III and IV appeared as a clear doublet in all concentrations, suggesting that no association takes place in these molecules where the OH groups are blocked by the bulky acetyl groups. The rotations about the C-8–C-9 bonds are now more difficult; thus, the chemical shifts of III and IV are similar.

The diamagnetic shift of H-2′, H-3′, and H-8′ observed in I was related to the vinylic group. The enhancement of this effect as the concentration was increased suggests that, as the association initiated by the hydrogen bonding proceeds, the molecules approach each other so that the aromatic rings and the quinuclidine rings of the same molecules tend to overlap. In other words, the association may be seen as being head to head and tail to tail. Additional evidence supporting this mode of association can be found in the change of the multiplicity and the chemical shift values

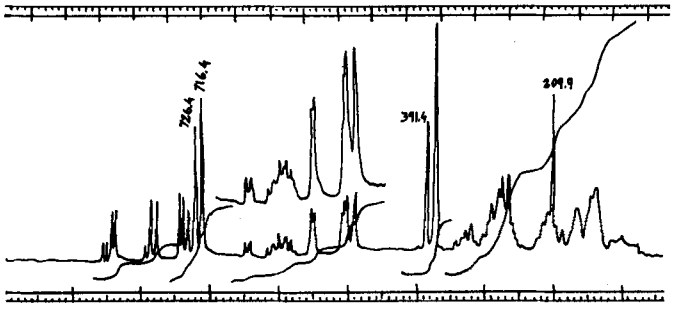


Figure 3—*NMR spectrum of mixture rich in I.*

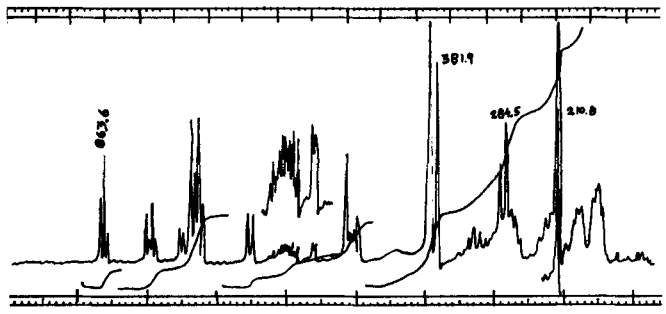


Figure 4—*NMR spectrum of mixture rich in III.*

of the vinylic protons (Table I). These pronounced alterations may be explained on the basis of the overlapping of the two systems of the vinylic groups as well. It is not surprising, therefore, that published NMR spectra show the methylene proton of the vinylic group at a lower field both as a singlet (6) and a doublet (10). This alteration in the methylene proton signal shapes encountered in I was absent in III, which provides further evidence that in I the association extends up to the vinylic group. No sign of association was observed in III and IV.

In II, the aromatic protons resonated at lower fields when compared to their counterparts in I, and this finding was related to the absence of the shielding effect of the vinylic group. However, a diamagnetic effect on some protons in II was observed when the concentration was increased. This effect indicates that one π-system is sufficient to affect the chemical shift values of the pertinent protons. Thus, the intermolecular association in II probably takes place in a similar fashion to that of the molecules of I, that is, head to head and tail to tail.

Commercial I containing II exhibits a similar NMR spectrum to that of pure I. On the basis of the data presented in Table I, II present in commercial I as a contaminant in small quantities should have appeared as a separate entity in the NMR spectrum of the mixture. The fact that this was not the case gives further evidence that the two molecules have similar steric structures and that pronounced association takes place between I and II, compared to the lesser association occurring between I and III. This phenomenon emphasizes the importance of hydrogen bonding in the association process.

The similarity in the NMR spectra of III and IV suggests that the conformations of both molecules are similar. The variations in the chemical shifts for I and II when passing from dilute to more concentrated solutions were more striking than the variations found in the two molecules at the same concentration. This finding suggests that the preferred stable conformations in I and II are similar. The small changes that were observed may be attributed to minor rotations about the C-8–C-9 bond causing movement of the quinoline ring toward the quinuclidine nitrogen and away from the ethyl group at C-3. This minimizes the steric interaction between the quinoline moiety and the ethyl group protons.

Acetylation Advantages—Since the chemical shift of the OH proton is highly dependent on the solvent, temperature, and concentration, its absorption may occur almost anywhere in the NMR spectrum. Therefore, it is not surprising that different investigators have reported varying data concerning the chemical shift of this OH proton (5). Thus, the diagnostic value of this proton is very limited. Acetylation of the OH groups at C-9 proved to be of great advantage for the following reasons:

1. The protons of the $OCOCH_3$ group have definite chemical shift values (δ 2.12 in III).
2. The proton alpha to the $OCOCH_3$ group at C-9 departs from the vinylic proton zone and appears separately downfield; it is more discernible and any changes in the vicinity of this proton can be more easily analyzed by NMR spectroscopy.
3. The NMR spectral data of III and IV are not concentration dependent, so these derivatives are more reliable in determining molecular structures and in quantitative analysis.

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Cytotoxicity of Modified Indole Alkaloids

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Abstract □ Indole alkaloids of the iboga series were structurally modified by incorporation of a 3,4-dimethoxybenzyl or -benzoyl unit so that they contained the N–O–O triangle required for antileukemic activity according to the triangulation hypothesis. The cytotoxicities of the modified alkaloids in the *in vitro* P-388 system were not significantly increased over the unmodified alkaloids, suggesting that the triangulation hypothesis does not apply in this series at least.

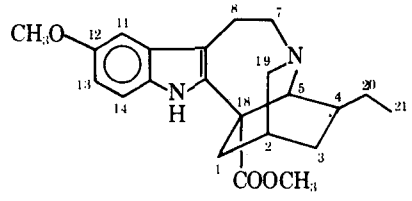
Keyphrases □ Indole alkaloids—semisynthetic, cytotoxicity, structure–activity relationships □ Antineoplastic agents, potential—modified indole alkaloids, cytotoxicity, structure–activity relationships □ Structure–activity relationships—modified indole alkaloids, antineoplastic activity □ Voacangine—derivatives, cytotoxicity, structure–activity relationships □ Catharanthine—derivatives, cytotoxicity, structure–activity relationships

In an earlier paper (1), the cytotoxicity of various bisindole alkaloids of the voacamine type was shown to be strikingly dependent on the detailed structure of the alkaloid and, in particular, on the position of the vobasane unit attachment to the iboga unit of the bisalkaloid. The antileukemic activity of various compounds, including the bisindole alkaloids vincristine and vinblastine, can be correlated with the structural feature of an N–O–O triangle of defined dimensions in these molecules (2). It was possible that indole alkaloids of modest antileukemic activity could be modified to contain the N–O–O triangle and might then show an increased antileukemic activity. This paper presents the results of some studies directed toward this question.

RESULTS AND DISCUSSION

The iboga alkaloids voacangine (I) and catharanthine were selected as the parent alkaloids for structural modification. Voacangine, which was available from previous isolation studies on *Tabernaemontana arborea* (3), shows a weak cytotoxicity in the P-388 cell culture system. This system, using a leukemia-derived cell line, was selected for the initial bioassay, with *in vivo* testing available at a later stage for promising candidates.

Reaction of I with 3,4-dimethoxybenzyl alcohol yielded the dimethoxybenzyl derivatives II and III, together with unreacted I and more highly substituted products. Assignment of Structures II and III to the two major substitution products was done primarily on the basis of PMR and mass spectra. Compound II showed singlets in its PMR spectrum at δ 6.89 and 6.84 ppm, assignable to H-14 and H-11, respectively. In III, the relevant signals appeared as doublets at δ 7.14 and 6.90 ppm, corresponding to H-14 and H-13, respectively. The mass spectra of both



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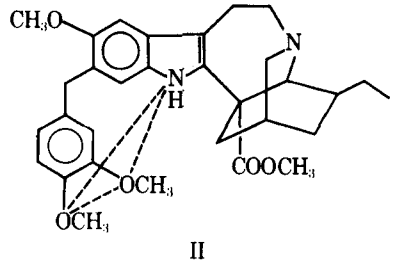

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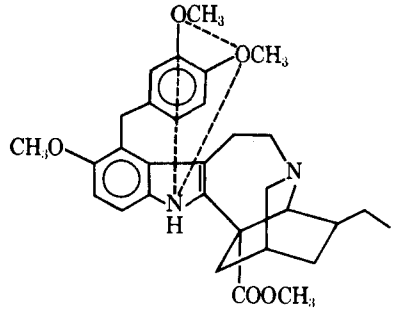

III

compounds showed molecular ion peaks at *m/e* 518 and typical peaks for iboga alkaloids at *m/e* 136 and 124 (4).

Reduction of I with lithium aluminum hydride yielded the known compound voacanginol (5). Esterification of voacanginol with 3,4-dimethoxybenzoyl chloride yielded the ester IV, which had NMR and mass spectra consistent with its assigned structure. Coupling of IV with vobasinol yielded the bisalkaloid V; its PMR spectrum was essentially a superimposition of the vobasane spectrum on that of IV. Coupling of the vobasine molecule to the 13′-position of IV (rather than the 11′-position) was indicated by the singlet resonances assignable to the 11′- and 14′-protons and by the broadening of the resonance of the I methoxyl group at δ 3.93 ppm due to hydrogen bonding (6).

Conversion of catharanthine to its epoxy lactam (VIII) was effected by published procedures (7, 8). Coupling of the intermediate iodolactone (VII) with 3,4-dimethoxybenzyl alcohol yielded the 3,4-dimethoxybenzyl derivative (IX). The assignment of Structure IX to the coupled product