The Structure of Lindenianine from *Lupinus lindenianus*

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Lindenianine, a new alkaloid from *Lupinus lindenianus*, was shown to be (−)-13β-hydroxy-10-oxosparteine by chemical as well as spectroscopic methods. The preferred conformations of this alkaloid and its 13-oxo derivative were discussed by measuring their CD spectra.

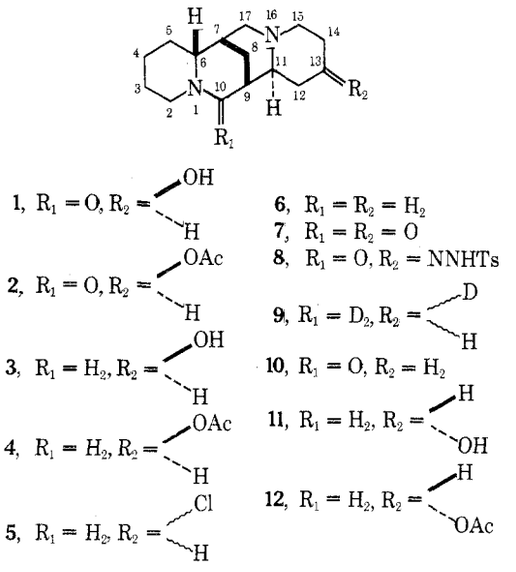
The lupin alkaloids occur in a wide variety of plants of the Leguminosae family and find some use in the treatment of cardiac arrhythmias. Lindenianine, $C_{15}H_{24}N_2O_2$ (*m/e* 264), was isolated as the major alkaloid from the aerial portion of *Lupinus lindenianus*. It behaves as a monoacidic base, giving a monopicrate. Its ir spectrum showed bands at 3330 (OH) and 1620 cm^{-1} (lactam CO), accounting for the two oxygen atoms. It could be readily acetylated[2] with acetic anhydride–pyridine at room temperature to afford an *O*-acetyl derivative **2**, and hence its hydroxyl group is not tertiary. It does not possess a carbinolamine moiety in its molecule since it did not react with sodium borohydride in methanol and did not form anhydronium salts with acids.

The mass spectrum of lindenianine gave fragment peaks at *m/e* 247 ($M^+ - 17$), 236 ($M^+ - 28$), 207 ($M^+ - 57$), 152, 138, 137, 136, 134, 97, 96, and 84, which suggested that it possesses the sparteine or isosparteine skeleton.[3]

Lindenianine was very resistant to the reduction by lithium aluminum hydride and failed to undergo clean reduction, leading to a mixture of unidentifiable products after refluxing with the excess reagent in dioxane for 24 hr. It did not absorb any hydrogen in the presence of platinum oxide in acetic acid, but in acid solution (2 *N* aqueous HCl) it took up 2 mol of hydrogen to afford a deoxo derivative **3**, the ir spectrum of which showed the absence of a lactam carbonyl function. Attempts to eliminate the hydroxyl group of lindenianine by dehydration with phosphorus pentoxide under varying conditions led to a complex mixture of unidentifiable products. The chlorination of **3** with thionyl chloride and subsequent reduction of the resulting chloro derivative **5** with sodium in boiling ethanol yielded a deoxodeoxy derivative **6**, $[\alpha]D$ −16° (*c* 0.9, ethanol). It formed a crystalline dipicrate, mp 182–183°, the identity of which was made by direct comparison (mixture mp, rotation, ir, and mass spectra) with a dipicrate of natural (−)-sparteine.

Lindenianine was readily oxidized by the Oppenauer method to a keto lactam **7**: ir 1715 (six-membered CO) and 1624 cm^{-1} (lactam CO). Since it did not exhibit any characteristic pH dependence of β-dicarbonyl compounds in its uv spectrum, the keto and the lactam carbonyl functions should not be in the 1,3 arrangement. Reduction of **7** with sodium borohydride in methanol gave back lindenianine. Therefore, the hydroxyl group in lindenianine must possess the thermodynamically stable equatorial orientation. In the nmr spectrum of lindenianine, the proton attached to the carbon atom bearing the hydroxyl group occurs at δ 3.70 ppm as a broad multiplet with a width of *ca.* 40 Hz. The great magnitude[4] of the width of this signal suggested that this proton is not only axially oriented (hence the hydroxyl group is equatorially oriented) but also it is in coupling with at least four neighboring protons. Therefore, we tentatively assumed that this equatorial hydroxyl group must be located at either C-3 or C-4, or either C-13 or C-14

Chart I



in the sparteine skeleton. In order to remove reductively the carbonyl group of **7**, we prepared the tosylhydrazone **8**[5] and treated it with lithium aluminum hydride. The product[6] isolated was found to be (−)-sparteine **6**, which was also obtained by reduction of **5** with sodium in ethanol. In order to label with deuterium the sites of attachment of both the keto and the lactam carbonyl functions in **7**, **8** was also reduced with lithium aluminum deuteride,[7] whereby trideuterated sparteine **9** (22% d_0; 10% d_1; 18% d_2; 48% d_3) was obtained. It is known[3] that upon electron bombardment the sparteine molecule is broken down between the 7–17 and 9–11 (or the 6–7 and 10–9 linkages), and between the 6–7 and 1–10 (or the 17–16 and 9–11 linkages) to produce the characteristic fragment ions,[8] as shown in Figure 1 (see Experimental Section). In the mass spectrum of sparteine-d_3 (Figure 2), however, the *m/e* 137 peak is shifted by 2 mu to *m/e* 139. This indicated that two deuterium atoms have been incorporated in the fragment a to give rise to an ion d. The *m/e* 98 peak corresponding to a fragment b′ now suffered a 2-mu shift to *m/e* 100 (fragment e or f). The ion at *m/e* 99 may be formed from a fragment g or h (incorporation of two deuterium atoms in b) or i (incorporation of one deuterium in b′). The absence of appreciable amounts of the ions at *m/e* 140 and 101 confirmed that three deuterium atoms have not been incorporated in the fragment ions a and b′. Hence the trideuterated product from the above deuterium labeling must be sparteine-*3*- or *-4-d*$_1$*,15,15-d*$_2$ or *-3-* or *-4-d*$_1$*,17,17-d*$_2$, or sparteine-*13*- or *-14-d*$_1$*,2,2-d*$_2$ or *-13-* or *-14-d*$_1$*, 10,10-d*$_2$.

With lithium aluminum hydride **7**[9] was reduced to **3**. On Wolff–Kischner reduction[10] **7** afforded in good yield a product, $[\alpha]D$ −12° (*c* 2.2, MeOH), which showed the pres-

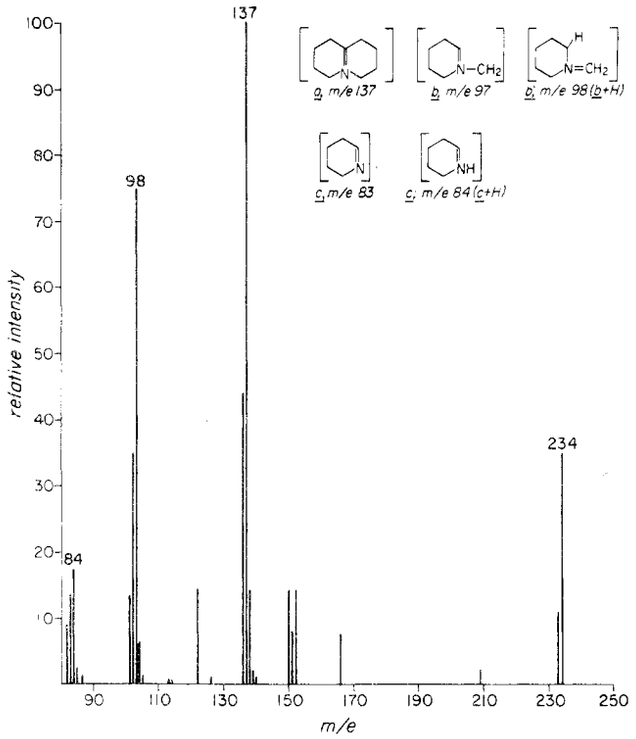


Figure 1. The mass spectrum of (−)-sparteine **6**.

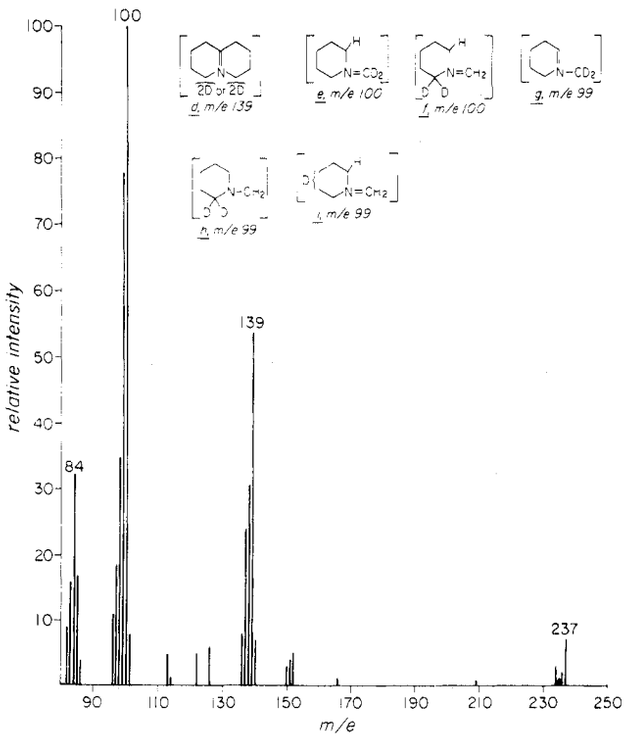
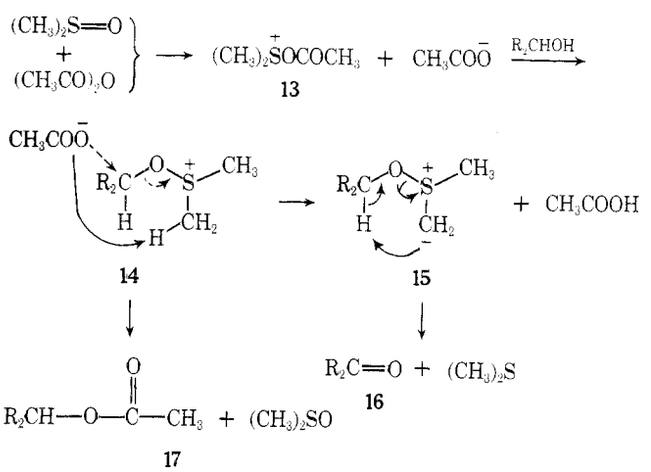


Figure 2. The mass spectrum of trideuterated sparteine **9**.

ence of a lactam carbonyl group but did not exhibit a keto carbonyl group in its ir spectrum. This product proved to be identical with (−)-aphylline, **10**.[11] Therefore, if lindenianine possessed an equatorial hydroxyl group at C-13, then deoxolindenianine should be identical with (−)-13β-hydroxysparteine, **3**[12] derivable from its 13α-hydroxy epimer **11**.[13] We attempted to epimerize the 13α-hydroxyl group of **11** according to the procedure of Bohlmann.[14] However, we failed to obtain the same result[15] as Bohlmann. Since oxidation of deoxolindenianine and (−)-13α-hydroxysparteine, **11**, might afford identical 13-oxo derivative, we also tried to oxidize the former by the Oppenauer method, but a mixture of two difficultly separable products[16] was formed. We therefore employed the acetic anhydride–dimethyl sulfoxide mixture for the oxidation of **11** since it has proved to be a very useful reagent[17] for oxidation of hydroxyl functions in sensitive molecules such as indole alkaloids. However, we obtained a product which possessed the molecular formula $C_{17}H_{28}N_2O_2$ (*m/e* 292) and did not show a carbonyl but an acetate band at 1733 cm^{-1} in its ir spectrum. It proved to be different from the *O*-acetyl derivative **12** prepared from **11** by the usual acetic anhydride–pyridine method but identical with *O*-acetyldeoxolindenianine, **4**. Therefore, the dimethyl sulfoxide–acetic anhydride mixture apparently caused the inversion of the 13-hydroxyl group of (−)-sparteine from the axial to the equatorial configuration to yield **4**. The above result established not only the position of the hydroxyl group of deoxolindenianine as C-13 but also its equatorial configuration, and thus lindenianine is (−)-13β-hydroxy-10-oxosparteine, **1**.

It is interesting to envisage the mode of formation of **4** from **11** in the above oxidation. We assume that the acyloxysulfonium salt **13**, which is formed from the dimethyl sulfoxide–acetic anhydride mixture, reacts with an alcohol to give an alkoxysulfonium salt **14**. The intermediate **14** would then yield ylide **15**, which, through a cyclic transition state, would decompose to a carbonyl derivative **16** and dimethyl sulfide. If, in this case, the acetate anion, which coexists, attacks the somewhat polarized carbon atom bearing the axial alkoxyl group of **14** from the rear side, then the product formed must be an equatorial *O*-acetyl derivative **17** whose configuration of its acetoxyl group has been inverted.[18] The sequence of these reactions is depicted in Scheme I.

Scheme I



We have also studied the conformations[19] of lindenianine, **1**, and its 13-oxo derivative **7** by measuring their CD spectra. **1** and **7** can adopt either of the following three conformations **18**, **19**, and **20**, and **21**, **22**, and **23**, respectively. As shown in Figure 3, the CD spectrum in isooctane or acetonitrile of **7** exhibits two absorption maxima at 296 nm ([θ] −4830) and 215 nm ([θ] +26,750), or 290 nm ([θ] −5430) and 217 nm ([θ] +27,800), respectively. The absorption maximum in the low-wavelength region may be due to the amide n → π^* transition (in ring B) and the one in the long-wavelength side to the carbonyl n → π^* transition[20] (in ring D). In addition, there is seen another CD band with a low intensity around 246 nm in both solvents, which, however, upon addition of one drop of hydrochloric acid

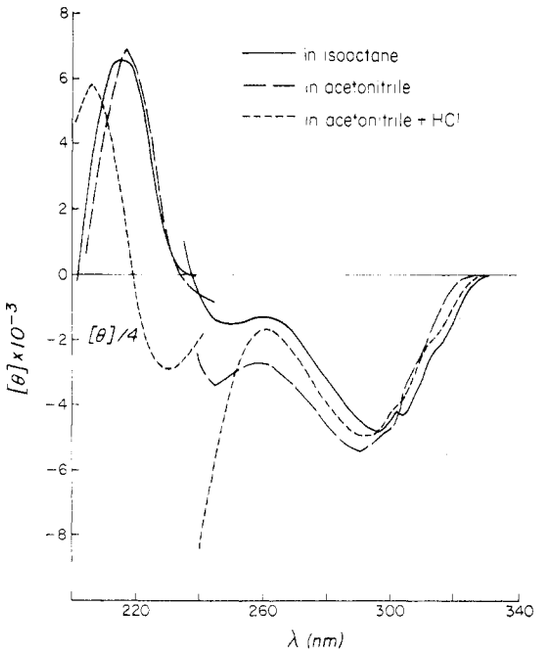


Figure 3. The CD curve of 13-oxolindenianine **7.**

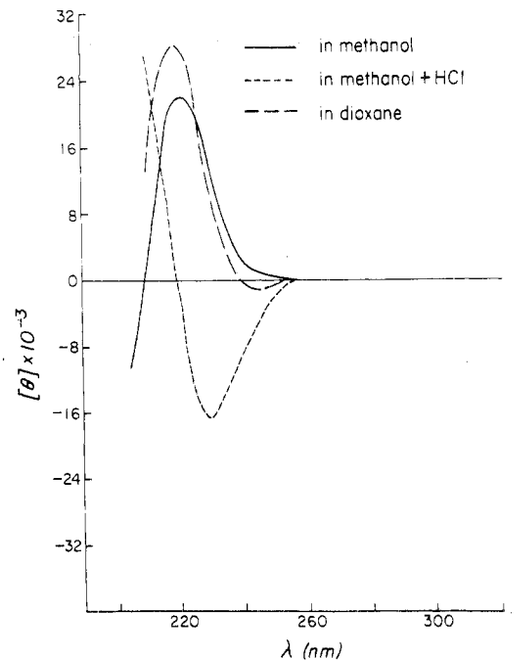


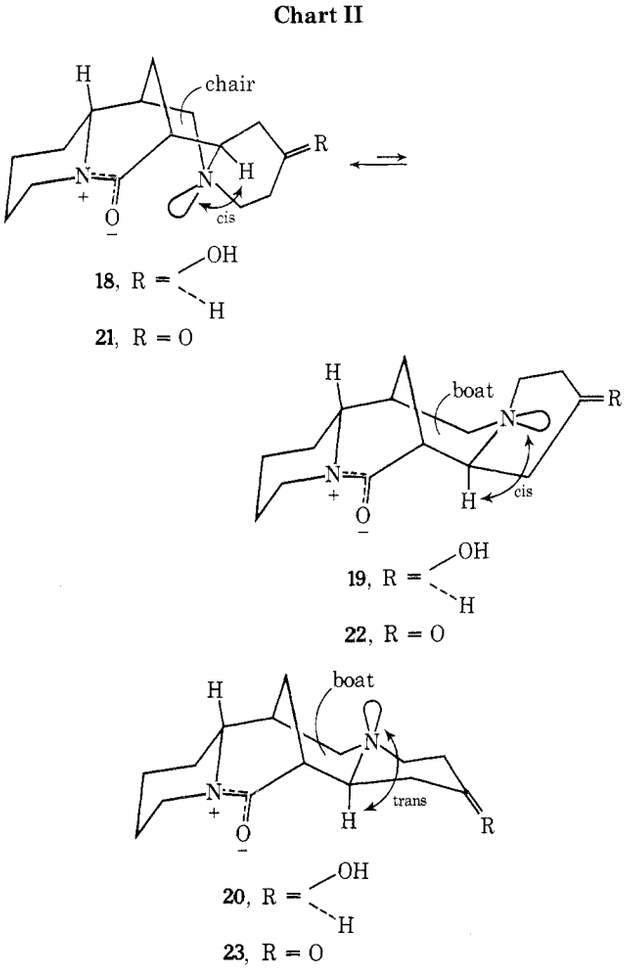
Figure 4. The CD curve of lindenianine **1.**

disappears. A similar band is also seen in the CD spectrum of **1** in dioxane but not in a protonic solvent such as methanol (see Figure 4). This band apparently arises from the transannular interaction[21] between the lone pair orbital of the nitrogen atom at the C/D ring junction and the amide n $\rightarrow \pi^*$ orbital. The finding of such a CD band clearly excludes the possibility of the ring C as a boat conformation.[22] The sign of the Cotton effect due to the amide n $\rightarrow \pi^*$ transition is in agreement with that[23] predicted, and we conclude that lindenianine **1** and its 13-oxo derivative **7** adopt the conformation **18** and **21**,[24] respectively.

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Ir spectra were taken on a Perkin-Elmer 337 spectrometer in KBr disks and nmr spectra obtained on a Varian A-60 instrument in deuteriochloroform and chemical shifts are reported in parts per million downfield from internal TMS (δ scale). Rotations were measured at 23° with a Zeiss polarimeter (0.01°). Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6H at 70 eV using a direct inlet system. The mass spectra of some of the compounds were determined as the picrates. In the mass spectrometer the picrates were thermally decomposed to the picric acid and the base component, the mass spectra of which, by virtue of the difference in vaporization, could be recorded without overlapping. For column chromatography Merck standardized alumina, activity II–III was used. For tlc Merck silica gel G was used and development was effected with chloroform–ethylamine (100:1–5, v/v). The spots were identified by spraying Dragendorff's reagent. All organic extracts were dried over anhydrous magnesium sulfate and evaporated under reduced pressure below 40°. Microanalyses were carried out by A. Bernhardt Microanalytical Laboratory, 5521 Elbach über Engelskirchen, West Germany.

Chart II



Isolation of Lindenianine. The finely powdered aerial portion (13 kg) save flowers, collected in the Andean mountains, was extracted exhaustively with boiling methanol and the methanol extracts were concentrated *in vacuo* to a small volume. Water was added, the solution was basified with concentrated aqueous sodium hydroxide, and the alkaloid was extracted with chloroform. The chloroform extracts were evaporated and ether was added, whereupon the alkaloid crystallized out. After recrystallization from chloroform–ether it showed mp 212–213° (yield, 20 g); $[\alpha]_D$ −11° (*c* 1.3, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{24}N_2O_2$: C, 68.15; H, 9.15; N, 10.60. Found: C, 68.36; H, 9.34; N, 10.30. It formed a picrate, mp 160–162° (from acetone–ether). *Anal.* Calcd for $C_{15}H_{24}N_2O_2 \cdot C_6H_3N_3O_7$: C, 51.11; H, 5.52; N, 14.19. Found: C, 50.95; H, 5.22; N, 14.15.

Acetylation of Lindenianine with Acetic Anhydride–Pyridine. Lindenianine (0.1 g) was acetylated with acetic anhydride (5 ml) and dry pyridine (10 ml) at room temperature overnight. **2** was obtained as a viscous oil: *m/e* 306 (M^+) and 240 ($M^+ - CH_3COOH$); ir 1730 (acetate CO) and 1238 cm^{-1} (acetate C–O); nmr δ 1.98 (3 H, s, CH_3CO). It did not form a crystalline picrate.

Catalytic Hydrogenation of Lindenianine. Lindenianine (0.3

g) in hydrochloric acid (2 *N*; 20 ml) was hydrogenated using platinum oxide. Two molar equivalents of hydrogen were taken up in 24 hr. The catalyst was filtered off, and the filtrate was basified with aqueous sodium hydroxide and extracted with chloroform. On evaporation **3** (0.2 g) was obtained, which was crystallized from ether to show mp 172–174°, $[\alpha]D$ −13° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{26}N_2O$: C, 71.95; H, 10.47; N, 11.19. Found: C, 71.63; H, 10.21; N, 10.87. It formed a dipicrate, mp 118–121° (from acetone–ether). *Anal.* Calcd for $C_{15}H_{26}N_2O{\cdot}2C_6H_3N_3O_7$: C, 45.76; H, 4.55; N, 15.80. Found: C, 45.55; H, 4.33; N, 15.68.

Acetylation of 3 with Acetic Anhydride–Pyridine. **3** (70 mg) was treated with acetic anhydride (3 ml) and dry pyridine (6 ml) at room temperature overnight. **4** was obtained as an oil which formed a dipicrate, mp 137–140° (from acetone–ether). *Anal.* Calcd for $C_{17}H_{28}N_2O_2{\cdot}2C_6H_3N_3O_7$: C, 46.40; H, 4.56; N, 14.93. Found: C, 46.25; H, 4.31; N, 14.65.

Chlorination of 3. **3** (100 mg) was dissolved in acetone and treated with a few drops of concentrated hydrochloric acid until the solution became just acid. The solution was then evaporated *in vacuo* to dryness and the residue was refluxed with thionyl chloride (0.13 ml) for 50 min. Ice–water was added and the solution was basified with aqueous sodium hydroxide and extracted with chloroform. **5** was obtained as an oil (80 mg): *m/e* 270 and 268 (M^+).

Reduction of 5 with Sodium in Ethanol. A solution of **5** (59 mg) in ethanol (6 ml) was gently boiled and sodium (0.7 g) was added in small portions during 5 min. The product **6** was extracted with chloroform and obtained as an oil (30 mg), $[\alpha]D$ −16° (*c* 0.9, ethanol); dipicrate, mp 182–183° (from acetone–ether), identical with (−)-sparteine dipicrate (rotation, mixture mp, ir, and mass spectra).

Oxidation of Lindenianine by the Oppenauer Method. Lindenianine (100 mg) in dry benzene (10 ml) was added under nitrogen to a stirred, ice-cooled mixture of potassium *tert*-butoxide (300 mg) and 9-fluorenone (200 mg) in dry benzene (8 ml) during 10 min. The solution was stirred at room temperature under nitrogen for an additional 3 hr. The product was taken up in 5% hydrochloric acid and the acid solution was basified with aqueous sodium hydroxide and extracted with chloroform. **7** was obtained as yellow crystals (60 mg): mp 173–176° (from ether), $[\alpha]D$ −116° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.44; H, 8.31; N, 10.36.

Reduction of 7 with Sodium Borohydride. A solution of **7** (100 mg) and sodium borohydride (446 mg) in methanol (30 ml) was kept standing at room temperature overnight. The product (90 mg) was crystallized from ether to show mp 194–196°, identical with lindenianine (rotation, mixture mp, ir, and mass spectra).

Reduction of 7 with Lithium Aluminum Hydride. **7** (120 mg) and lithium aluminum hydride (450 mg) were heated under reflux in dry dioxane for 24 hr. The excess reagent was decomposed with water and the complex treated with concentrated aqueous potassium hydroxide. The solution was dried over anhyd magnesium sulfate, filtered, and evaporated to yield an oil (100 mg), which was crystallized from acetone, mp 161–165°; dipicrate, mp 96–95°, identical with **3** dipicrate (mixture mp, ir, and mass spectra).

Preparation of 8. **7** (350 mg) and tosylhydrazine (0.51 g) in methanol (12 ml) were heated under reflux for 4 hr. After removal of the solvent *in vacuo*, the product was chromatographed over alumina. Elution with chloroform yielded **8** as an amorphous solid (300 mg) which showed a single spot on tlc.

Reduction of 8 with Lithium Aluminum Hydride. **8** (240 mg) in dry dioxane was heated under reflux with lithium aluminum hydride (1.2 g) for 24 hr. The product (140 mg) was obtained, which formed a dipicrate, mp 178–180°, identical with (−)-sparteine dipicrate (mixture mp, ir, and mass spectra).

Reduction of 8 with Lithium Aluminum Deuteride. **8** (280 mg) was reduced with lithium aluminum deuteride (1.4 g) as above. The product was chromatographed over alumina (16 g) and elution with benzene–chloroform (9:1) yielded an oil (100 mg) which formed a dipicrate, mp 113–115°.

Wolff–Kischner Reduction of 7. **7** (100 mg) and 99% hydrazine (0.49 ml) in diethylene glycol (20 ml) were heated with stirring at 110° for 30 min under nitrogen. Sodium hydroxide (0.2 g) was then added cautiously and the solution was heated at 116° for 1 hr and then at 216° for 3.5 hr. The product **10** was isolated in the usual way and obtained as an oil (80 mg). After distillation at 0.2 mm and 130° (bath temperature), it had $[\alpha]D$ −12° (*c* 2.2, MeOH). The ir spectrum of this compound in $CHCl_3$ was identical with that of *dl*-aphylline. It formed a picrate, mp 110–113° (from acetone–ether), and a perchlorate, mp 190–195° (from acetone–ether).

Oxidation of 11 with DMSO and Acetic Anhydride. To a solution of **11** (120 mg) in dimethyl sulfoxide (1.5 ml) [dried over Molecular Sieves (Type 4A, Fisher)] was added acetic anhydride (1 ml) and the mixture was stirred at room temperature for 24 hr. The mixture was then treated with ethanol (1 ml), stirred for 1 hr, and diluted with water (0.5 ml). After basification with concentrated aqueous ammonia and extraction with chloroform, the product was obtained as an oil which formed a dipicrate, mp 146–148° (yield, 20 mg), identical with *O*-acetyldeoxolindenianine **4** dipicrate, but not identical with (−)-13α-acetoxysparteine **12** dipicrate, mp 233–235°, prepared from **11** by the usual acetic anhydride–pyridine method (mixture mp, ir, and mass spectra).

Acknowledgment. We thank Mrs. M. Cerhalmi de Hazos for the determination of the ir, uv, nmr, and mass spectra. Part of this work was supported by a research grant from the "Fundación José María Vargas," Caracas.

Registry No.—**1,** 52539-65-8; **1** picrate, 52539-66-9; **2,** 52539-67-0; **3,** 15402-87-6; **3** dipicrate, 52539-71-6; **4,** 15358-42-6; **4** dipicrate, 52539-72-7; **5,** 52539-68-1; **6,** 90-39-1; **6** dipicrate, 6160-11-8; **7,** 52539-69-2; **8,** 52539-70-5; **10,** 577-37-7; **10** picrate, 52610-72-7; **10** perchlorate, 52610-71-6; **11,** 24181-95-1.

References and Notes

(1) (a) Instituto Venezolano de Investigaciones Cientificas; (b) Universidad de Los Andes.
(2) It could not be tosylated with tosyl chloride–pyridine at room temperature. Refluxing with tosyl chloride–pyridine led to the formation of an inseparable mixture.
(3) D. Schumann, N. Neuner-Jehle, and G. Spiteller, *Monatsh. Chem.*, **99,** 390 (1968).
(4) For similar references, see Y. Kawazoe, Y. Sato, T. Okamoto, and K. Tsuda, *Chem. Pharm. Bull. (Tokyo)*, **11,** 328 (1963); J. I. Musher, *J. Amer. Chem. Soc.*, **83,** 1146 (1961).
(5) L. Caglioti and M. Magi, *Tetrahedron*, **19,** 1127 (1963); L. Caglioti and P. Grasselli, *Chem. Ind. (London)*, 153 (1964).
(6) In contrast to the fact that lindenianine itself was very resistant to this reduction, not only the keto but also the lactam carbonyl groups were reduced in this case.
(7) M. Fischer, Z. Pelah, D. H. Williams, and C. Djerassi, *Chem. Ber.*, **98,** 3236 (1965).
(8) In the case of nondeuterated (−)-sparteine **6,** the ions corresponding to the fragments c′, b′, and a occur at *m/e* 84 (rel intensity 17%), 98 (75), and 137 (100), and in the case of sparteine-d_3 (**9**), a series of these fragment peaks appear at *m/e* 84 (rel intensity 32%), 98 (35), 99 (77), and 100 (100).
(9) See ref 6.
(10) *A priori*, the Wolff–Kischner reduction did not appear promising since **7** bears, besides a keto carbonyl group, a lactam function in its molecule, which might very probably be hydrolyzed in the alkaline reaction medium.
(11) We are indebted to Professor Bohlmann who kindly compared the ir spectrum of our sample with that of his synthetic *dl*-aphylline in chloroform solution.
(12) Note that the 13β-hydroxyl group is equatorial if the ring D is in the chair form. (−)-13β-Hydroxysparteine has not been isolated from nature.
(13) Note that the 13α-hydroxyl group is axial if the ring D is in the chair form. Dr. Goosen, University of Port Elizabeth, South Africa, kindly sent us a specimen of **11.**
(14) F. Bohlmann, E. Winterfeldt, and H. Brackel, *Chem. Ber.*, **91,** 2194 (1958).
(15) Under varying conditions, no appreciable amount of the tosylate was formed. In all cases tried, we either recovered starting material or obtained an inseparable mixture.
(16) The Oppenauer oxidation may have caused epimerization of the C/D ring juncture to produce also 13-oxo-α-isosparteine [for a similar case, see F. Bohlmann, E. Winterfeldt, O. Schmidt, and W. Reusche, *Chem. Ber.*, **94,** 1767 (1961)].
(17) J. D. Albright and L. Goldman, *J. Amer. Chem. Soc.*, **89,** 2416 (1967).
(18) We also treated (−)-13β-hydroxysparteine, **3,** with dimethyl sulfoxide–acetic anhydride under the same condition, but the ir spectrum of the crude product did not show any carbonyl or acetate band. After chromatography, we failed to separate any identifiable product.
(19) The conformations of the C_{15} lupin alkaloids have been discussed in some detail by means of nmr [F. Bohlmann and D. Schumann, *Tetrahedron Lett.*, 2435 (1965)] as well as ir spectra [M. Wiewiorowski, O. E. Edwards, and M. D. Bratek-Wiewiorowski, *Can. J. Chem.*, **45,** 1447 (1967)].
(20) According to the Octant rule, while conformation **22** gives a positive Cotton effect, both conformations **21** and **23** exhibit a negative Cotton effect, assuming that in either case the ring D is in a chair form.
(21) Similar examples of this kind of transannular interaction have been reported previously [T. Nakano, T. H. Yang, and S. Terao, *J. Org. Chem.*, **29,** 3441 (1964); Z. Horii, M. Ikeda, Y. Tamura, S. Saito, M. Suzuki, and K. Kodera, *Chem. Pharm. Bull. (Tokyo)*, **12,** 1118 (1964)]. Also see R. C. Cookson and N. S. Wariyar, *J. Chem. Soc.*, 2302 (1956); H. Labhart and G. Wagnière, *Helv. Chim. Acta*, **42,** 2219 (1959); W. A. Ayer, B. Altenkirk, R. H. Burnell, and M. Moinas, *Can. J. Chem.*, **47,** 449 (1969);

and R. C. Cookson, J. Henstock, and J. Hudec, *J. Amer. Chem. Soc.*, **88**, 1060 (1966).

(22) In conformations **19, 20,** and **22, 23,** the methylene bridge at the B/C ring junction interferes with the interaction between the lone pair of the nitrogen at the C/D ring junction and the amide chromophore.

(23) We applied the lactone sector rule [J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 7211 (1965)] to the amide n $\rightarrow \pi^*$ Cotton effect [see H. Wolf, *Tetrahedron Lett.*, 1075 (1965), and A. F. Beecham, *Tetrahedron Lett.*, 4897 (1969)]. It is interesting to note that, upon addition of hydrochloric acid, the amide n $\rightarrow \pi^*$ absorption band at 220 nm in the CD spectra of **7** and **1** changes in sign from positive to negative (see Figures 3 and 4). This phenomenon has not yet been reported on the amide chromophore, and we are studying it in more detail by measuring the CD spectra of related amide compounds. We are indebted to Dr. K. Kuriyama, Shionogi Research Laboratory, Shionogi & Co., Ltd., Osaka, Japan, for the determination of the CD spectra and valuable comments.

(24) This conformation is in agreement with that suggested for aphylline from its ir studies by Bratek-Wiewiorowski, *et al.* (see reference 19). In the ir spectra of **1** and **7,** a Bohlmann trans band with a low intensity appears at 2820 cm^{-1}. Note that in conformations **18** and **19** there is only one hydrogen on carbons attached to N-16 and in a trans-diaxial relation to its lone pair of electrons, and in conformation **20** there are three such hydrogens. According to the criterion made originally by Bohlmann [F. Bohlmann, *Chem. Ber.*, **91**, 2157 (1958)], the occurrence of trans bands requires the presence of at least two such hydrogens.

Phlebicine, a New Biphenylbisbenzylisoquinoline Alkaloid from *Cremastosperma polyphlebum*

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Phlebicine, a new bisbenzylisoquinoline alkaloid from *Cremastosperma polyphlebum*, has been assigned structure 1 on the basis of spectroscopic evidence, oxidative degradation, and conversion to the known alkaloid rodiasine (3).

Although alkaloids have been isolated from many genera of the family Annonaceae,[1] the genus *Cremastosperma* has not previously been investigated phytochemically. We now report the isolation of the new alkaloid phlebicine from the bark of the Amazonian species *Cremastosperma polyphlebum* (Diels) Fries, and evidence in support of the assignment of structure **1** to phlebicine.

Countercurrent fractionation of the phenolic bases from *C. polyphlebum,* followed by crystallization from chloroform–methanol, gave white needles of phlebicine, mp 195°; the composition $C_{37}H_{40}N_2O_6$ was determined by mass spectrometry.

The infrared spectrum (KBr) of phlebicine showed the absence of a carbonyl band, but a band at 3400 cm^{-1}, attributable to a nonchelated hydroxyl, was observed.

The nmr spectrum of phlebicine showed the presence of three aromatic methoxyls at δ 3.80, 3.70, and 3.38, as well as two methylimino groups at δ 2.57 and 2.27. In the aromatic region, three one-proton singlets were observed at δ 6.69, 6.28, and 6.20, in addition to a total of six protons of higher multiplicity.

Treatment of phlebicine with excess diazomethane afforded *O,O*-dimethylphlebicine (**2**), mp 163–165°. The nmr spectrum of the latter now showed the presence of five methoxyls in the δ 3.3–3.9 region. Phlebicine is therefore a diphenolic base.

The optical rotatory dispersion curve of phlebicine (Figure 1) shows peaks at 297 and 252 nm, and troughs at 272 and 231 nm. It strongly resembles the reported ORD curve of rodiasine (**3**),[2] and therefore suggests that phlebicine is also a bisbenzylisoquinoline alkaloid which contains the structural feature of a biphenyl linkage.

In accord with the presence of a biphenyl system in the molecule, attempted sodium–ammonia cleavage of *O,O*-dimethylphlebicine gave no characterizable products. However, *O,O*-dimethylphlebicine was successfully cleaved by photochemical air oxidation,[3] followed by borohydride reduction. Two crystalline products were isolated. The first product, derived from the two tetrahydroisoquinoline units of the alkaloid ether **2,** was the known isocarbostyril **4.**[3] The second product was the biphenyl diol **5,** which was identical with the borohydride reduction product of the known dialdehyde **6.**[4] Consequently, *O,O*-dimethylphlebicine must be assigned structure **2,** which is identical with that of *O*-methylrodiasine,[2,5] ignoring the stereochemistry at the two asymmetric carbons. Finally, incomplete methylation of phlebicine was found to afford a mono-*O*-methylphlebicine, which was identical in all respects, including optical properties, with authentic rodiasine (**3**).[2,5,6] Phlebicine is, therefore, one of the four possible isomeric *O*-demethylrodiasines.

The position of the second phenolic hydroxyl group in phlebicine was determined by a comparative nmr and mass spectrometric study of phlebicine and its products of deuteration, deuteriomethylation, O-acetylation, and O-ethylation.

The mass spectrum of rodiasine (**3**) shows an intense peak at *m/e* 198, attributable to the doubly charged ion **7.**[2,6] In phlebicine, the corresponding ion (**8**) appears at *m/e* 191, whereas in *O,O*-diethylphlebicine (**9**) and in *O,O*-bis(dideuteriomethyl)phlebicine (**10**) similar ions (**11** and **12**) appear at *m/e* 205 and 199, respectively. The second hydroxyl of phlebicine must, therefore, be present in one of the tetrahydroisoquinoline units of the molecule.

When phlebicine was subjected to base-catalyzed deuteration,[7,8] it was converted into a dideuteriophlebicine (**13**). Since the oxygenation pattern of phlebicine is identical with that of rodiasine, the two deuteriums of dideuteriophlebicine must have been introduced ortho to each of the two phenolic hydroxyls of phlebicine. The deuteration of phlebicine brings about a change in its nmr spectrum, in which both a one-proton singlet at δ 6.20 and a one-proton doublet at δ 6.78 (J = 8 Hz) vanish. The latter doublet must arise from H-13′ of the biphenyl unit of phlebicine. The replaceable singlet must represent a proton at either C-5 or C-5′ of the bisisoquinoline unit of the molecule.

The mass spectrum of dideuteriophlebicine (**13**) shows peaks due to the undegraded bisisoquinoline unit at *m/e*