

THE STRUCTURES OF OVATINE, LINDHEIMERINE, AND GARRYFOLINE,
C₂₀-DITERPENOID ALKALOIDS OF GARRYA OVATA VAR. LINDHEIMERI TORR.

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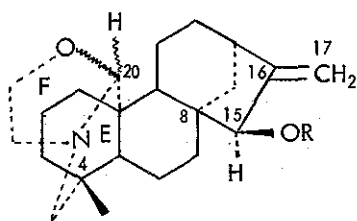
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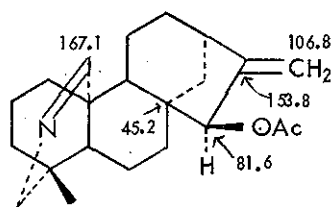
Chemical investigation of alkaloids from Garrya ovata var. lindheimeri Torr. has led to the isolation and characterization of two new C₂₀-diterpenoid alkaloids, ovatine (1) and lindheimerine (2), as well as the alkaloid garryfoline (3). Ovatine and garryfoline each exists as a mixture of C(20)-epimers; these alkaloids were chemically interconverted in high yield.

In a continuing search for tumor inhibitory natural products from plants, we have examined the crude extracts from the bark and leaves of Garrya ovata var. lindheimeri Torr,¹ which have shown confirmed antitumor activity *in vivo*. We report the isolation and structure determination of two new C₂₀-diterpenoid alkaloids, ovatine (1) and lindheimerine (2). Along with these new alkaloids we have isolated the known alkaloid, garryfoline² (3) which, like ovatine, was found to exist as a mixture of C(20)-epimers as we predicted.³

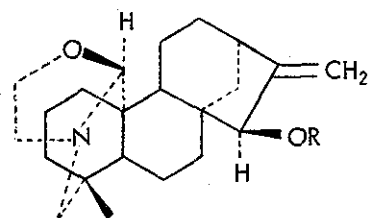
The major alkaloid, ovatine, was isolated as a crystalline compound, mp 113-114° (corrected), by a combination of pH gradient and column chromatographic techniques. Ovatine, C₂₄H₃₅NO₃ (mass spectrum and elemental analysis), $[\alpha]_D^{22} - 79.4^\circ$ (c 1.0 CHCl₃), shows ir absorption at 1735 and 1235 (acetate), 1660 (double bond) and 1100 (ether) cm⁻¹. The ¹H nmr spectrum shows two sharp singlets of unequal intensity⁴ at δ 0.72 and δ 0.80 in 1:3 ratio, respectively, for the C(4)-CH₃ group, one singlet for an acetoxy group at δ 2.15



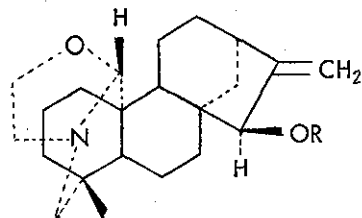
- 1 R = Ac Ovatine
 3 R = H Garryfoline



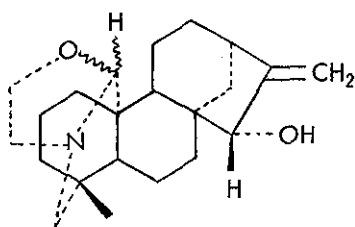
- 2 Lindheimerine



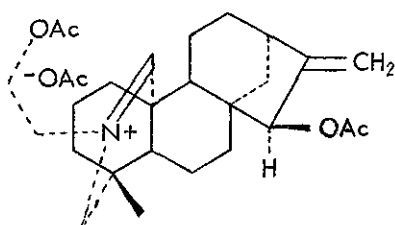
- 1A R = Ac
 3A R = H



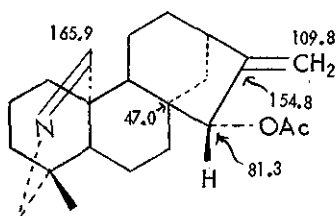
- 1B R = Ac
 3B R = H



- 4 Veatchine



- 5



- 6

and a broad singlet at δ 2.60 for the N-CH₂-C group. The spectrum also exhibits two broad doublets centered at δ 4.88 and δ 5.14 for the exocyclic double bond and two broad singlets at δ 3.95 and δ 4.25 in a 1:3 ratio, respectively, for the C(20)-proton. The ¹³C nmr data of ovatine (**1A** and **1B**) showed the presence of one methyl, one acetoxy, nine methylene, four methine groups, and three tetrasubstituted carbon atoms together with two olefinic and one carbonyl carbon atoms. The ¹³C nmr spectra of ovatine and garryfoline in CDCl₃ at room temperature showed the presence of two different sets of signals for the oxazolidine ring F, the piperidine ring E, the C(4)-methyl group and other carbon atoms, a result which indicated the existence of a mixture of epimers at the C(20)-position in these alkaloids. It should be noted that early work⁵ on the configuration of garryfoline assumed, without evidence, a β -configuration for the hydrogen at C(20). Similar results were also observed earlier in the case of normal-type oxazolidine ring-containing alkaloids: veatchine (**4**) in solution³ as well as in the solid state,⁶ and atisine.³ Comparison of the ¹³C and ¹H nmr spectra of ovatine with those of garryfoline indicated that the only difference between these two alkaloids was an additional acetoxy group at C(15). Mild basic hydrolysis of ovatine⁷ afforded the known alkaloid garryfoline (**3**), indicating the position of the acetoxy group is at C(15) in ovatine.

Treatment of garryfoline with acetic anhydride and pyridine at r.t. afforded the unusual product **5** in quantitative yield instead of the expected acetylation product, ovatine. The latter compound also gave compound **5** upon treatment with acetic anhydride and pyridine. To confirm the structure of ovatine, garryfoline was converted into ovatine via lindheimerine in two steps in an overall yield of 80%.⁸ Thus refluxing compound **5** in chloroform gave lindheimerine (**2**) in 90% yield. The latter afforded ovatine upon treatment with ethylene oxide in acetic acid in 89% yield. The structure of ovatine can be represented as an epimeric mixture at C(20) of **1A** and **1B**, with the **1A** epimer predominating just as in the case of the closely related alkaloid, veatchine.^{3,6}

The minor alkaloid, lindheimerine, C₂₂H₃₁NO₂, [α]_D²⁴ - 113.8° (c 1.0 CHCl₃), was isolated as an amorphous compound. The ir spectrum exhibits absorption at 1735 and 1230 (acetate), 1645 (imine) and 1660 (double bond) cm⁻¹. The ¹H nmr spectrum indicates the presence of the Me group (3H, s) at δ 0.82, an acetoxy group (3H, s) at δ 2.18 and the N-CH₂-C group as a singlet at δ 3.42. The spectrum also shows two broad singlets for the exocyclic double bond and a broad singlet at δ 8.0 for the C(20) imine proton. The ¹³C

nmr spectrum of lindheimerine in CDCl_3 at room temperature revealed the presence of one acetoxy, one methyl, one imine, seven methylene, three methine groups and three quaternary carbons together with two olefinic and one carbonyl carbon atoms. The pattern of ^{13}C chemical shifts in lindheimerine was similar to that of the known compound 6⁹ except for a few changes. Comparison of the ^{13}C chemical shifts of C(8), C(15), C(16) and C(17) in lindheimerine with those of compound 6 afforded evidence for the presence of a β -acetoxy group at the C(15) position in lindheimerine. Therefore, we assign structure 2 to lindheimerine. Finally, the structure was confirmed by comparison with the internal Hofmann degradation product of compound 5, which was found to be identical with lindheimerine (2).

It is interesting to note that lindheimerine occurs in extremely small quantity in comparison with ovatine. Since ovatine and lindheimerine can be interconverted, we suggest that lindheimerine may be a biogenetic precursor of ovatine. These alkaloids are being tested for their antitumor activity.

REFERENCES

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7. Mild basic hydrolysis of ovatine gave the major product garryfoline as well as isogarryfoline as a minor product (10%) as expected. During basic hydrolysis garryfoline can be easily isomerized to isogarryfoline.
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9. N. V. Mody and S. W. Pelletier, Tetrahedron, 1978, in press.

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