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THE STRUCTURE OF SITSIRIKINE - A NEW ALKALOID FROM VINCA ROSEA LINN

James P. Kutney and R. T. Brown

Department of Chemistry, University of British Columbia,

Vancouver 8, Canada.

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In connection with our interest in Vinca alkaloids we have elucidated the structure of a new alkaloid, sitsirikine. This communication describes our results which allow us to assign structure I to this alkaloid.

Sitsirikine was first isolated as one of the minor alkaloids from Vinca rosea Linn by the Lilly group (1) who were able to isolate this alkaloid as a crystalline sulfate ($C_{21}H_{26}O_3N_2 \cdot \frac{1}{2}H_2SO_4$). The free base was only obtained as an amorphous powder and on the basis of infrared comparison with β-yohimbine, these workers suggested that sitsirikine may represent a new yohimbine isomer. However work in our laboratory reveals that the original alkaloid reported by the Lilly group is a mixture of at least two substances - sitsirikine and the corresponding dihydro derivative, and that this alkaloid is a close relative of the tetracyclic corynantheine group. Our evidence for the assignment of structure I to this alkaloid is presented below.

After numerous unsuccessful attempts, the alkaloid was obtained crystalline in acetone although it solvated strongly with this solvent. The purest material melting at 181° analyzed for $C_{21}H_{26}O_3N_2 \cdot CH_3COCH_3$. The unsolvated alkaloid was subsequently obtained crystalline from aqueous methanol m.p. 206-208°, $[\alpha]_D^{26}$ -58° (CH_3OH) and analyzed well for $C_{21}H_{26}O_3N_2$. It must be emphasized that the purest sample which we could obtain from a variety of purification attempts, although behaving as a pure substance,

always indicated a trace impurity on thin layer chromatoplates and this impurity was subsequently shown to be due to dihydrositisirikine (II). The ultraviolet spectrum (in CH_3OH) of sitsirikine indicated an unsubstituted indole chromophore (λ_{max} 226 mμ (36,000), 282 mμ (8000) and 290 mμ (6500)) and the infrared spectrum (Nujol) with bands at 2.98μ (NH and/or OH) and 5.86μ (>C=O) provided evidence for the nature of the oxygen atoms. The NMR spectrum (in CD_3COCD_3) showed signals at 4.7 τ (olefinic H), 6.1 τ (H attached to carbon bearing an oxygen) and a very sharp spike at 6.38 τ (OCH_3). The molecular formula assigned to sitsirikine was further supported by elemental analyses on a crystalline picrate, m.p. 226–228° (dec) and finally substantiated by a mass spectral molecular weight determination (354).

The presence of a hydroxyl group in sitsirikine suggested from the infrared and NMR data, was readily confirmed by the preparation of a crystalline monoacetate, $C_{23}H_{28}O_4N_2$, m.p. 198°, $[\alpha]_D^{26}$ -26° (CH_3OH). The NMR spectrum of sitsirikine acetate was particularly instructive and apart from the expected appearance of a new sharp signal at 8.02 τ (CH_3CO-), the broad signal at 6.1 τ present in the NMR spectrum of the alkaloid, shifted downfield and now appeared at 5.6 τ. This shift provided strong evidence that the formation of sitsirikine acetate involved the acetylation of a primary alcohol (2).

The presence of a double bond in the original alkaloid was ascertained by catalytic reduction (Pd in CH_3OH) which provided dihydrositsirikine (II). This substance crystallized from acetone with one mole of solvent, m.p. 180° and analyzed for $C_{21}H_{28}O_3N_2 \cdot CH_3COCH_3$. Recrystallization from aqueous methanol afforded unsolvated dihydrositsirikine, $C_{21}H_{28}O_3N_2$, m.p. 215°, $[\alpha]_D^{26}$ -55° (CH_3OH), λ_{max} 226 mμ (41,000), 282 mμ (8900) and 290 mμ (7500). The NMR spectrum of dihydrositsirikine showed complete disappearance of the olefinic signals and the infrared spectrum, with a strong band at 5.85μ, excluded any conjugation between the carbonyl function and the double bond present in

sitsirikine. The molecular formula assigned to dihydrositsirikine was confirmed by elemental analyses on a crystalline picrate, m.p. 228-230° (dec), acetate, $C_{23}H_{30}O_4N_2$, m.p. 187°, $[\alpha]_D^{26}$ -31° (CH_3OH) and p-bromobenzoate, $C_{28}H_{31}O_4N_2Br$, m.p. 174°, as well as a molecular weight determination (356) by the mass spectrometric method.

The nature of the olefinic linkage in the original alkaloid was suggested by the NMR spectrum of the reduction product which indicated a new C-methyl signal at 9.07 τ thereby providing evidence for a terminal double bond. This fact was confirmed by ozonolysis of sitsirikine which provided formaldehyde, identified by paper chromatography of its 2,4-dinitrophenylhydrazine derivative (3). A conventional Kuhn-Roth determination on dihydrositsirikine indicated 0.93 moles C-methyl while a modified Kuhn-Roth (4), which yielded propionic acid, established that the new C-methyl group was, in fact, present in a C-ethyl function. These series of experiments established that a vinyl group was present in sitsirikine.

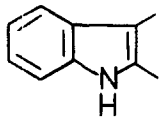
Since dihydrositsirikine could be obtained very pure, it was used as the starting material in all subsequent studies.

The nature of the carbonyl group was ascertained when dihydrositsirikine was subjected to hydride reduction ($LiAlH_4$ in tetrahydrofuran) and the product was a crystalline diol (III), $C_{20}H_{28}O_2N_2$, m.p. 203°, $[\alpha]_D^{26}$ -3° (CH_3OH). The infrared spectrum of the diol showed no carbonyl absorption and the NMR spectrum (CD_3COCD_3) indicated a complete absence of the methoxyl signal which had occurred as a very sharp spike at 6.38 τ in all previous spectra. This evidence established that a carbomethoxy group was present in the alkaloid structure.

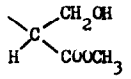
When dihydrositsirikine diol was treated with acetone containing a trace of p-toluenesulfonic acid, a crystalline acetonide (m.p. 105-109°) was obtained. The NMR spectrum ($CDCl_3$) of this derivative was instructive since the signal due to the methylene protons on the two oxygen-bearing carbon atoms (6.25 τ)

was split into a doublet thereby indicating that one proton must be present on the adjacent carbon atom.

This series of experiments established, beyond any doubt, that sitsirikine possessed a tetracyclic skeleton and the following features:



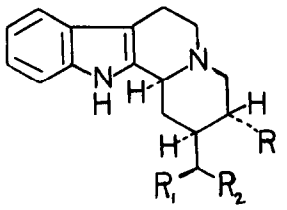
$-CH=CH_2$



During the course of the chemical investigations, a detailed mass spectrometric analysis of sitsirikine and various derivatives was undertaken. The mass spectrum of dihydrositsirikine run by the direct inlet procedure (5) was most helpful and is discussed in some detail. The significant peaks occur at m/e 356 (M^+), 355 (M-1), 338 ($M-H_2O$), 325 ($M-CH_2OH$), 253 (M- ), 184, 170, 169, and 156. It was immediately apparent from the last four peaks mentioned that rings A, B and C of the sitsirikine skeleton were of the type encountered in the yohimbine and related alkaloid classes (6).

Pertinent information regarding the ring skeleton was obtained from semi-micro dehydrogenation experiments. Dehydrogenation of dihydrositsirikine with lead tetraacetate afforded a gummy product which exhibited an ultraviolet spectrum in excellent agreement with that of various tetradehydroyohimbine derivatives (7) (λ_{max} 253, 308, and 365 mμ in neutral or acid solution; λ_{max} 284 and 328 mμ in alkaline solution).

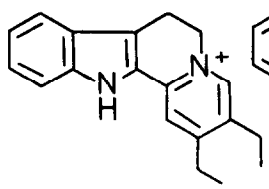
Palladium dehydrogenation (10% Pd/C, 280°) of dihydrositsirikine hydrobromide provided a mixture of products from which, by careful thin layer chromatography, a small amount of a gummy product which showed an ultraviolet absorption in complete agreement with that of 5,6-dihydroflavocoryline hydrochloride (IV) (8) (λ_{max} 221, 312, 386 mμ ; λ_{min} 215, 277, 338 mμ) could be isolated. This experiment provided the first piece of evidence for the entire skeleton present in sitsirikine. Final confirmation for the ring



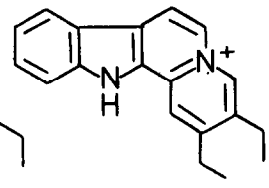
I, R = $-CH=CH_2$, R_1 = $COOCH_3$, R_2 = CH_2OH

II, R = $-CH_2CH_3$, R_1 = $COOCH_3$, R_2 = CH_2OH

III, R = $-CH_2CH_3$, R_1 = R_2 = CH_2OH



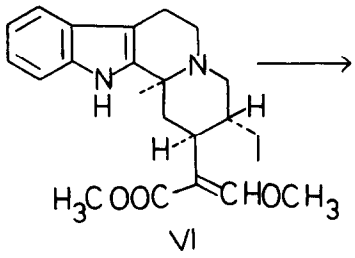
IV



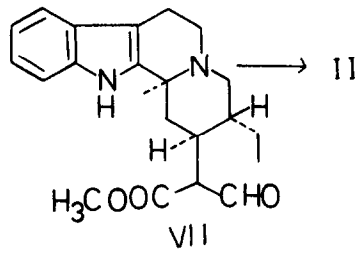
V

skeleton was obtained when the palladium dehydrogenation product was reacted with 2,3-dichloro-5,6-dicyano-p-benzoquinone and the resulting gummy product, possessing a completely aromatic ring system, was isolated. This substance exhibited a highly informative ultraviolet spectrum (λ_{max} 237, 291, 345, 385 mμ ; λ_{min} 274, 304, 373 mμ) which was in agreement with that reported for flavocorylline hydrochloride (V)(8). In spite of the minute amounts of dehydrogenation products which were available and which prevented complete characterization, the ultraviolet data established the ring skeleton and furthermore, strongly suggested that sitsirikine was probably a close relative of the corynantheine class of indole alkaloids.

This last suggestion was confirmed by the following sequence of reactions. Dihydrocorynantheine (VI) was converted to desmethyldihydrocorynantheine (VII) according to the published procedure (9) and when this latter substance was subjected to reduction by means of sodium borohydride, the



VI



VII

II

product was identical in every respect with dihydrositsirikine. Having

established the structure of dihydrositsirikine, structure I could be immediately assigned to sitsirikine.

The isolation of sitsirikine is of considerable biogenetic interest because of its close relationship to the corynantheine alkaloids on the one hand and to such alkaloids as polyneuridine (6) on the other. It is also of interest to note that dihydrocorynantheol, which is descarbomethoxydihydrositsirikine has been recently reported in nature (10).

Acknowledgements

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REFERENCES

(1) G. H. Svoboda, M. Gorman, N. Neuss and A. J. Barnes, J. Pharmaceutical Sciences, 50, 409(1961).

(2) L. M. Jackman, Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, Pergamon Press, 1959, p. 55.

(3) D. F. Meigh, Nature, 170, 579(1952).

(4) H. Bickel, H. Schmid and P. Karrer, Helv. Chim. Acta, 38, 649(1955).

(5) J. F. Lynch, J. M. Wilson, H. Budzikiewicz and C. Djerassi, Experientia, 19, 211(1963).

(6) L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, J. Am. Chem. Soc. 84, 2161(1962).

(7) F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, J. Am. Chem. Soc. 77, 3547(1955).

(8) K. B. Prasad and G. A. Swan, J. Chem. Soc. 2024(1958).

(9) P. Karrer, R. Schwyzer and A. Flam, Helv. Chim. Acta, 35, 851(1952).

(10) B. Gilbert, L. D. Antonaccio and C. Djerassi, J. Org. Chem. 27, 4702 (1962).