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TERATOGENIC COMPOUNDS OF *VERATRUM CALIFORNICUM* (DURAND)—VI.

THE STRUCTURE OF CYCLOPAMINE

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Abstract—The teratogenic steroidal alkaloid, cyclopamine, from *Veratrum californicum* responsible for cyclopian and related central nervous system malformations in sheep was investigated. Data from i.r., NMR, mass spectrometry and other means identified cyclopamine as 11-deoxojervine.

CYCLOPIAN and related central nervous system defects in sheep are caused by ingestion of the plant *Veratrum californicum* (Liliaceae) on the 14th day of gestation.[1] The defect is due to an alkaloid, readily isolated from the plant,[2] to which we have given the trivial name cyclopamine.[3,4]

We describe here structural investigations which have established the identification of cyclopamine as 11-deoxojervine (II, Fig. 1) contrary to our earlier suppositions.[3] The close structural similarity of cyclopamine with jervine (I, Fig. 1) is not unexpected since the latter is also teratogenic in the same way.[5]

The acquired data were consistent with the assignment of the cyclopamine structure as 11-deoxojervine as follows. The i.r. spectrum of pure cyclopamine[2] suggested a Δ^5,3βol steroidal system (1050–1057 cm^{-1}) about the same 3500 hydroxyl absorption as jervine—and only about half that of veratramine suggesting a single rather than two OH groups, peaks at 927, 984 and 1118 suggesting a ring oxygen system (the ether bridge) as with jervine. It had no aromatic ring absorption in the u.v. region like jervine and unlike veratramine (IV, Fig. 1). Thus, unlike veratramine, ring D was not aromatic. The probable empirical formula from elemental analysis and molecular weight was $C_{27}H_{41}O_2N$. This matched well with the elemental analyses from an average of twelve determinations reported herein.

NMR integration suggested 41–42 protons. The following NMR assignments could be made: two overlapping doublets (J=6·0 and 6·6) and a singlet at 0·88–0·96δ (9 protons—3 methyl groups—C_{19}, C_{21}, and C_{26}); a singlet at 1·63δ (3 protons—1 methyl group at C_{18}); a multiplet (triplet?) at 5·35δ (1 proton—C_6 olefinic proton); a multiplet at 3·4δ (1 proton—C_3 proton). The foregoing assignments are consistent with Shoolery's rules[6] and with

[1] W. BINNS, J. L. SHUPE, R. F. KEELER and L. F. JAMES, *J. Am. Vet. Med. Assoc.* **147**, 839 (1965).
[2] R. F. KEELER, *Phytochem.* **7**, 303 (1968).
[3] R. F. KEELER and W. BINNS, *Can. J. Biochem.* **44**, 819 (1966).
[4] R. F. KEELER and W. BINNS, *Can. J. Biochem.* **44**, 829 (1966).
[5] R. F. KEELER and W. BINNS, *Teratology* **1**, 5 (1968).
[6] J. N. SHOOLERY and M. T. ROGERS, *J. Am. Chem. Soc.* **80**, 5121 (1958).

assignments made for verarine,[7] veralkamine,[8] jervine[9] and jervane derivatives.[10] The data were consistent with the assignment of the cyclopamine structure as 11-deoxojervine.

The physical constants reported by us previously[2] were similar to those reported by Masamune for 11-deoxojervine.[11] Melting points and optical rotation were within 4 degrees. I.r., u.v. and elemental analysis were essentially similar although not identical. Calculation for "double bonds and rings"[12] gave 8 for cyclopamine.

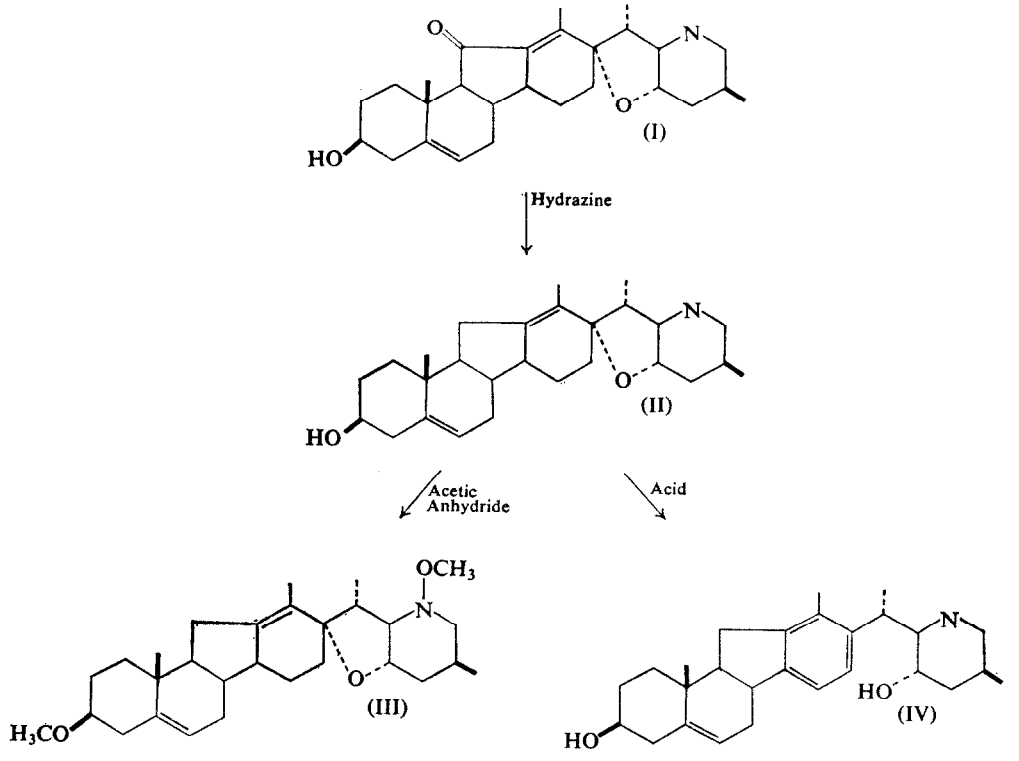
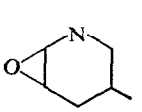


FIG. 1. STRUCTURES OF JERVINE I, CYCLOPAMINE (11-DEOXOJERVINE) II, *O-N*-DIACETYLCYCLOPAMINE III, AND VERATRAMINE IV.

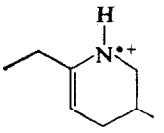
Mass spectrometry fragmentation assignments are as follows. The *m/e* 411 is the parent peak with 412 and 413 as P+1 and P+2, respectively. The *m/e* 396 peak is 411 minus a CH_3 group. The single 340 peak is 411 minus an NH—CH_2—$CH(CH_3)$—CH_2 group. The *m/e* 326 is the 411 minus a CH_2—NH—CH_2—$CH(CH_3)$—CH_2 group. The 310 is the 411 minus a CHO—CH_2—$CH(CH_3)$—CH_2—NH group. The 298 is the 411 minus the group (V). The 125 peak results from the opening of the tetrahydrofuran ring and leads to the radical

[7] J. TOMKO and S. BAUER, *Coll. Czech. Chem. Commun.* **29,** 2570 (1964).
[8] J. TOMKO, A. VASSOVA, G. ADAM, K. SCHREIBER and E. HOHNE, *Tetrahedron Letters* **40,** 3907 (1967).
[9] T. MASAMUNE, M. TAKASUGI, M. GOHDA, H. SUZUKI, S. KAWAHARA and T. IRIE, *J. Org. Chem.* **29,** 2282 (1964).
[10] T. MASAMUNE, N. SATO, K. KOBAYASHI, I. YAMAZAKI and Y. MORI, *Tetrahedron* **23,** 1591 (1967).
[11] T. MASAMUNE, Y. MORI, M. TAKASUGI, A. MURAI, S. OHUCHI, N. SATO and N. KATSUI, *Bull. Chem. Soc. Japan,* **38,** 1374 (1965).
[12] M. D. SOFFER, *Science* **127,** 880 (1958).

ion (VI) which is further stabilized by loss of a hydrogen to give the 124 peak, or of a methyl group to give the 110 peak. These fragmentation assignments are consistent with that reported for jervine,[13] and expected for 11-deoxojervine.



(V)



(VI)

Treatment of cyclopamine with acid produced veratramine as with 11-deoxojervine.[11] This confirmed the C nor D homo steroid ring system with a terminal piperidine ring and with $C_{18, 19, 21 \text{and } 26}$ methyl groups.

Acetylation of cyclopamine produced a derivative (III, Fig. 1) with physical constants essentially identical to those reportedly produced on similar treatment of 11-deoxojervine.[11]

Finally, the i.r. spectrum and other physical constants of 11-deoxojervine prepared by the Wolf-Kishner reduction of jervine[11] and those of isolated cyclopamine were identical.

Thus, cyclopamine, the compound responsible for prenatal cyclopian malformations in lambs resulting from maternal ingestion of *V. californicum*,[1] is 11-deoxojervine (II, Fig. 1).

EXPERIMENTAL

Preparation of Cyclopamine

Cyclopamine was prepared[2] and additionally recrystallized from methanol–water and acetone–water and then dried at 150°. It had m.p. 237–238, $[\alpha]_D^{25} -48$ (C = 1 per cent in methanol: $CHCl_3$ (2:1); u.v. had 250 nm shoulder with strong end absorption. It had a single spot on TLC. The i.r. in KBR pellet had γ_{max} 3400, 3250, 2900, 2860, 1450, 1118, 1067, 1060, 1042, 984, 927 and 809.

(Found: C, 78·96; H, 10·29; N, 2·96. Calc. for $C_{27}H_{41}O_2N$: C, 78·78; H, 10·04; N, 3·40%.)

NMR Analysis

The NMR spectrum was taken in deuteriochloroform at 60 Mc with trimethylsilane as the internal standard.

Mass Spectrum Analysis

The mass spectrum was taken on the commercial RMU-6D instrument using the direct inlet system at a temperature of 130° and electron energy at 70 eV.

Treatment of Cyclopamine with Acid

A 20 mg sample of cyclopamine was swirled in 10 ml of 0·6% HCl, filtered to remove undissolved sample, and incubated at 38°. Time interval aliquots were examined by TLC for evidence of conversion of cyclopamine to veratramine. Within 24 hr virtually all the cyclopamine was converted to a product with R_f identical to veratramine. The i.r. spectrum of the 24 hr product was identical to that of veratramine.[2]

O-N-Diacetylcyclopamine[11] had m.p. 194–196, $[\alpha]_D^{25} = -5$ (in ethanol), i.r. in KBR pellet had γ_{max} 3550, 3460, 2940, 1722, 1655, 1640, 1440, 1254, 1240, 1030, 817.

Wolf-Kishner Reduction of Jervine

Jervine was reduced[11] to 11-deoxojervine. The product had m.p. 234–236, $[\alpha]_D^{25} -44$, (C = 1 per cent in methanol: $CHCl_3$ (2:1)); u.v. had 250 nm shoulder with strong end absorption. It had a single spot on TLC. The i.r. in KBR had γ_{max} 3400, 3250, 2900, 2860, 1450, 1118, 1067, 1060, 1042, 984, 927 and 809.

General Methodology

Melting points, optical rotations, u.v. and i.r. spectra, TLC, carbon, hydrogen, and nitrogen determinations were made as previously described.[2]

[13] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structural Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, p. 21, Holden Day, San Francisco (1964).