COMMUNICATIONS TO THE EDITOR

FORMATION OF XYLULOSE PHOSPHATE FROM RIBOSE PHOSPHATE IN SPLEEN EXTRACTS

Sir:

We wish to report the identification of D-xylulose phosphate arising from the action of a spleen extract upon D-ribose 5-phosphate. Approximately 20% of the starting ribose phosphate was ultimately isolated in the form of the free ketopentoses, ribulose (14%) and xylulose (6%). The latter material was originally suspected in the reaction mixture on the basis of its relatively slow rate of color development in the cysteine-carbazole reaction[1] and this property was extremely helpful in following the isolation procedure.

The extract used in these experiments, a 50–95% saturated ammonium sulfate fraction of mouse spleen homogenate, was incubated with ribose phosphate at 37° for 40 minutes. The reaction was stopped with perchloric acid and the precipitated protein removed by centrifugation. The reaction products were dephosphorylated with a partially purified potato phosphatase[2] and the mixture de-ionized. In order to facilitate the separation of the two ketopentoses, the unreacted aldopentose was destroyed by bromine oxidation[3] and the solution again de-ionized. This material was then chromatographed on a Dowex-1-Borate column[4] which separated the remaining pentoses into two clearly defined peaks. The free sugars were recovered from the borate complex by a low temperature distillation with methyl alcohol to remove the volatile methyl borate.[5]

Examination of the orcinol spectrum of the first peak showed it to be identical with that of authentic xylulose, exhibiting a 540/670 ratio of 0.34.[4] The unknown gave a strongly positive cysteine–carbazole reaction in which the time for maximal color development at 540 mμ was somewhat in excess of two hours and closely paralleled the rate with known xylulose. A reducing sugar determination on this fraction indicated the presence of 90 micromoles of pentose which was in excellent agreement with the value obtained by the cysteine–carbazole assay.

The optical rotation, based upon the above concentration, gave a value of $\alpha^{20}D$ −27° (*c* 1.0, H_2O). Authentic D-xylulose has a rotation of $\alpha^{20}D$ −33.2°.[6] Paper chromatography, in a saturated phenol–water solvent, produced a single spot with an R_f = 0.54. Authentic xylulose gave the same R_f; both spots developed a characteristic gray-purple color when sprayed with the orcinol-trichloroacetic reagent.[7] This color is readily distinguished from ribulose which turns pink and shows a strong orange fluorescence in the ultraviolet. The crystalline phenylosazone of the unknown was prepared and melted at 161–162°. Pure D-xylosazone melted at 161–162°. Equal portions of the two derivatives were mixed and recrystallized. The melting point of this product was 161.5–162.5°. On the basis of this evidence, it would appear that the unknown sugar was D-xylulose.[8]

The identity of the second peak was established as ribulose by its behavior in the orcinol and cysteine-carbazole reactions and by paper chromatography in a saturated phenol-water solvent. Approximately 230 micromoles were recovered in this peak.

A mechanism for the formation of xylulose phosphate from ribulose phosphate, in the presence of transketolase, depends upon the apparent lack of *cis–trans* specificity of this enzyme with regard to the condensation of active glycolaldehyde and phosphoglyceraldehyde.[9] It is of interest, however, to note that the transketolase activity of the extract used in these experiments was too slight to be detected in the transketolase assay[9] under conditions where the original homogenate reacted strongly.

(1) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

(2) A. Kornberg, unpublished procedure.

(3) B. L. Horecker, P. Z. Smyrniotis and J. E. Seegmiller, *J. Biol. Chem.*, **193**, 383 (1951).

(4) J. O. Lampen, *ibid.*, **204**, 999 (1953).

(5) L. P. Zill, J. X. Khym and G. M. Cheniae, THIS JOURNAL, **75**, 1339 (1953).

(6) O. T. Schmidt and R. Treiber, *Ber.*, **66**, 1765 (1933).

(7) R. Klevstrand and A. Nordal, *Acta Chem. Scand.*, **4**, 1320 (1950).

(8) In a personal communication, Dr. Dische of Columbia University, has informed us that upon incubation of ribose phosphate in hemolysates of human erythrocytes, he has obtained a substance which behaves like xylulose on a paper chromatogram.

(9) B. L. Horecker, P. Z. Smyrniotis and H. Klenow, *J. Biol. Chem.*, **205**, 661 (1953).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
UNITED STATES PUBLIC HEALTH SERVICE
BETHESDA, MARYLAND
GILBERT ASHWELL
JEAN HICKMAN

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ALKALOID STUDIES. VI.[1] CUAUCHICHICINE, A NEW DITERPENOID ALKALOID

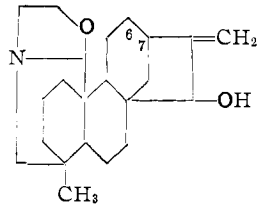
Sir:

The outstanding researches of Wiesner and collaborators[2] on the alkaloids of *Garrya veatchii* have culminated in the structure elucidation of veatchine (I) and garryine (II). Recognizing the close similarity of these alkaloids with atisine (and iso-atisine)[3], the Canadian workers proposed[2c] structures analogous to those of veatchine and garryine but with the hydroxyl-bearing ring terminating at C-6 rather than C-7. Pelletier and

(1) Paper V, C. Djerassi, J. J. Beereboom, S. P. Marfey and S. K. Figdor, THIS JOURNAL, **77**, January (1955).

(2)(a) K. Wiesner, S. K. Figdor, M. F. Bartlett and D. R. Henderson, *Can. J. Chem.*, **30**, 608 (1952); (b) K. Wiesner, W. I. Taylor, S. K. Figdor, M. F. Bartlett, J. R. Armstrong and J. A. Edwards, *Ber.*, **86**, 800 (1953); (c) K. Wiesner, R. Armstrong, M. F. Bartlett and J. A. Edwards, *Chemistry & Industry*, **132**, (1954); and THIS JOURNAL, **76**, Dec. (1954).

(3) For a review see E. S. Stern in R. H. F. Manske and H. L. Holmes, "The Alkaloids," Academic Press, Inc., New York, N. Y., 1954, Vol. IV, p. 275.



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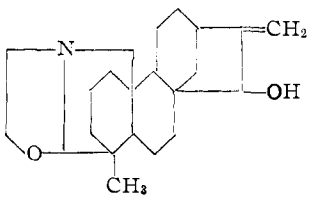
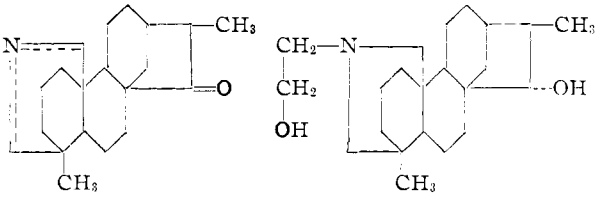


II

Jacobs[4] have presented supporting evidence in favor of the atisine structure.

In connection with a study of the alkaloids of the bark of the Mexican tree *Garrya laurifolia* ("cuauchichic"), we have isolated in crystalline form a new alkaloid isomeric with garryine, veatchine and atisine which we have named cuauchichicine. We should like to record the pertinent experiments which demonstrate that this alkaloid is an additional member of this biogenetically important class of diterpenoid alkaloids and that it possesses a modified phyllocladene skeleton typical of veatchine (I) and garryine (II).

Cuauchichicine was obtained by counter-current distribution of the crude alkaloid fraction at *p*H 7.4 followed by crystallization from methanol: m.p. 143–145°, $[\alpha]_D$ −76° ($CHCl_3$), $\lambda_{max}^{CHCl_3}$ 5.78 μ but no free OH or NH bands, no high selective absorption in the ultraviolet; (*Anal.* Calcd. for $C_{22}H_{33}NO_2$: C, 76.92; H, 9.68; N, 4.08. Found: C, 76.75; H, 9.79; N, 4.26); hydrochloride, m.p. 259–262° (*Anal.* Calcd. for $C_{22}H_{34}ClNO_2$: C, 69.53; H, 9.02; Cl, 9.33; N, 3.69. Found: C, 69.44; H, 9.32; Cl, 9.47; N, 3.89). A Kuhn-Roth determination carried out parallel with veatchine (I)[5] (found, 2.87; calcd. for 1 C-CH_3, 4.37) and cuauchichicine (found, 5.04) shows that the latter contains two C-methyl groups. In contrast to veatchine, garryine and atisine, cuauchichicine does not possess an exocyclic methylene group as demonstrated by ozonization experiments. The infrared carbonyl band at 5.78 μ can be attributed to a five-membered ring ketone, which could be characterized as the *oxime*, m.p. 192–194° (*Anal.* Calcd. for $C_{22}H_{34}N_2O_2$: C, 73.70; H, 9.56; N, 7.81. Found: C, 73.27; H, 9.89; N, 7.60). Pyrolysis of the alkaloid with selenium at 290° afforded *pyrolysis base A (III)* (m.p. 133–136°, identical infrared spectrum with authentic material[5]), already isolated[2b] by Wiesner, *et al.*, from similar treatment of veatchine (I), thus accounting for five of the six rings of cuauchichicine. Sodium borohydride or lithium aluminum hydride reduction of the alkaloid (V) yielded a tetrahydro (dihydroxy) derivative, m.p. 175–177°, $[\alpha]_D$ −86.7° ($CHCl_3$) (*Anal.* Calcd. for $C_{22}H_{37}NO_2$: C, 76.03; H, 10.73; N, 4.03. Found: C, 76.11; H, 10.71; N, 4.43; 2 active H; *pK* 6.84 (cellosolve–20% water)), which proved to be identical (mixture melting point, rotation and infrared comparison) with *tetrahydroepiveatchine* (IV) (observed values in our laboratory for authentic material[5]: m.p. 175–178°, $[\alpha]_D$ −85.2°), obtained earlier[2b] by partial synthesis from pyrolysis base A (III). The *pK* value (10.98)[6] of cuauchichicine is comparable to that of veatchine (I) (*pK* 11.5)[2a] rather than that of garryine (II) (*pK* 8.7) thus indicating that the oxazolidine ring is fused to C-17 rather than C-16. The above described data are only compatible with expression V[7] for cuauchichicine.



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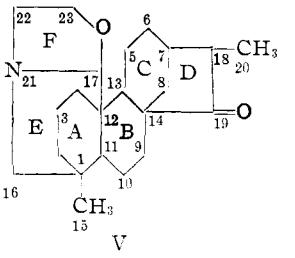

V

DEPARTMENT OF CHEMISTRY
WAYNE UNIVERSITY
DETROIT, MICHIGAN
CARL DJERASSI
C. R. SMITH[8a]
S. K. FIGDOR[8b]

INSTITUTO DE QUIMICA
UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO
MEXICO, D. F.
J. HERRAN
J. ROMO

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(4) S. W. Pelletier and W. A. Jacobs, THIS JOURNAL, **76**, 4496 (1954).

(5) We are indebted to Prof. K. Wiesner, University of New Brunswick, for this sample.

(6) This value is obtained (cellosolve–20% water) if the alkaloid is titrated immediately with 0.1 *N* HCl. If the solution is allowed to stand under nitrogen for 12 hours prior to titration, two breaks in the titration curve are observed corresponding to *pK* 11.15 and 8.80.

(7) In view of the structural similarity with the diterpenes, we are employing a numbering system based on that of abietane (*cf.* W. Klyne, *J. Chem. Soc.*, 3072 (1953)).

(8) (a) Eli Lilly Predoctorate Research Fellow 1953–1954; (b) Postdoctorate Research Fellow at Wayne University, 1953–1954.

SYNTHESIS OF PICROPODOPHYLLIN[1]

Sir:

We wish to report the successful conversion of synthetic DL-*β*-apopicropodophyllin[2] to picropodophyllin,[3] and thereby the completion of a total synthesis of picropodophyllin. Saponification of DL-*β*-apopicropodophyllin furnished DL-*α*-apopodophyllic acid[4] melting at 170–171°. *Anal.* Calcd. for $C_{22}H_{22}O_8$: C, 63.7; H, 5.3. Found: C, 63.5; H, 5.3. The infrared and ultraviolet absorption curves of the DL-acid were the same, respectively, as those determined for authentic *α*-apopodophyllic acid. Treatment of the racemic acid with

(1) This work has been supported by grants-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) W. J. Gensler, C. M. Samour and Shi Yi Wang, THIS JOURNAL, **76**, 315 (1954).

(3) *Cf.*, W. Borsche and J. Niemann, *Ann.*, **494**, 59 (1932); *Ber.*, **65**, 1633, 1790 (1932); E. Späth, F. Wessely and E. Nadler, *ibid.*, **65**, 1773 (1932); **66**, 125 (1933).

(4) Saponification of optically active *β*-apopicropodophyllin has been shown by Robertson and Waters[5] and again by Schrecker and Hartwell[6] to give optically active *α*-apopodophyllic acid.

(5) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 83 (1933).

(6) A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **74**, 5676 (1952).