# 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).

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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.