of other animals. Oxycellulose may prove useful as a fractionation medium for other purposes, where, for reasons of molecular structure or size, compounds are not amenable to separation with ion-exchange resins.

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RECEIVED MAY 7, 1951

SEDOHEPTULOSE IN PHOTOSYNTHESIS BY PLANTS[1]

Sir:

Although its function has not been ascertained, the general occurrence of sedoheptulose,[2] D-altroheptulose, in the succulent plants is well established. This sugar has not been identified in the majority of the members of the plant kingdom, but it now appears possible that its phosphate esters may perform a vital function during photosynthesis.

We have isolated labeled sedoheptulose monophosphate in $C^{14}O_2$ photosynthesis products of all the plants thus far studied in this laboratory (*Chlorella, Scenedesmus, Rhodospirillum rubrum,* and the leaves of barley seedlings, soybean, alfalfa, sugar beet, spinach and geranium). It is invariably found as monophosphate esters. At least two such esters have been observed in radiograms of C^{14}-labeled *Scenedesmus*. The major one is associated with fructose monophosphate while the minor one is inseparable, as yet, from glucose monophosphate. Sedoheptulose may be liberated enzymatically from its phosphates during the killing of the plant, but it has not been observed to accumulate in amounts exceeding the steady state concentrations of these phosphates. This suggests its participation only as a phosphate in most plants. These sedoheptulose phosphates are formed prior to hexose phosphates in the cases examined kinetically in this laboratory. In a typical experiment, one-second photosynthesis in $C^{14}O_2$ by barley seedling leaves, the distribution of radioactivity among the neutral compounds obtained upon phosphatase hydrolysis of the mixed phosphates was as follows: 43% in fructose, 47% sedoheptulose and 7% in glucose.

Sedoheptulose, isolated chromatographically[3] from phosphatase ("Polidase") hydrolysates of similarly separated phosphate esters,[3] was identified by the following tests. (1) Two-dimensional paper co-chromatography with authentic sedoheptulose[4] showed identical positions of the sugar and the radioactivity. The position of the authentic specimens was determined by resorcinol spray test. (2) The radioactive sugar in tracer concentrations is readily converted to sedoheptulosan by five-minute heating in 1 *N* hydrochloric acid. It was identified by co-chromatography with sedoheptulosan prepared similarly from an authentic specimen. (3) The equilibrium constant of the dehydration of the radioactive compound was found to be 4.0 as reported by LaForge and Hudson[2] for sedoheptulose. (4) Catalytic hydrogenation gave D-β-mannoheptitol, which was identified by co-chromatography with an authentic specimen prepared from sedoheptulose. (5) Periodate oxidation of both the hexose and the heptitol gave the expected amounts of products. The sedoheptulose obtained from five minutes $C^{14}O_2$ photosynthesis by soy bean leaves gave 14.4% of formaldehyde activity, 28% glycolic acid activity and 55% of formate activity. The heptitol obtained from this compound had a formate/formaldehyde ratio of 3.1 compared to an expected 2.5 for uniform labeling.

The examination of the kinetics of formation of the phosphate esters involved in $C^{14}O_2$ fixation[5] and a detailed description of the identification will be published.

This early synthesis of sedoheptulose in CO_2 fixation and its stereochemical deviation from that of glucose strongly suggests its participation in a C_2 regenerative system for the primary CO_2-acceptor rather than as a hexose precursor. The predominant role of malic acid in "succulent metabolism" may well be related to the accumulation of sedoheptulose in these plants.

(1) This work was sponsored by the United States Atomic Energy Commission.

(2) F. B. LaForge and C. S. Hudson, *J. Biol. Chem.*, **30**, 61 (1917).

(3) A. A. Benson, J. A. Bassham, M. Calvin, T. Goodale, V. Haas and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

(4) A sample was kindly supplied by Mr. F. W. Putman of the Division of Plant Nutrition of this University.

(5) A. A. Benson, S. Kawaguchi and M. Calvin, to be published.

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THE STRUCTURE OF JERVINE. II.[1] DEGRADATION TO PERHYDROBENZFLUORENE DERIVATIVES

Sir:

In a recent publication,[1] in which it was shown that the double bond conjugated with the inert keto group in jervine cannot occupy the 8,9-position as postulated by Jacobs and Sato,[2] we implied that this alkaloid does not have a normal steroid nucleus. We now present some of the evidence on which this assertion is based.

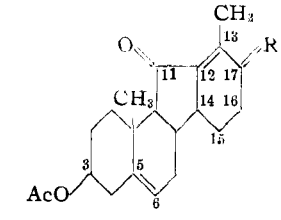
Jervine on treatment with acetic anhydride and zinc chloride at 140° yielded a dienone $C_{28}H_{30}O_3$, I (m.p. 186–188°,[3] $[\alpha]^{25}D$ −101°,[3] $\lambda_{max.}^{alc.}$ 300 mμ (4.4)[3]; calcd.: C, 78.02; H, 8.48; acetyl, 12.2; found: C, 78.14; H, 8.58; acetyl, 11.5), while isojervine was merely acetylated under these conditions. I was cleaved by chromic acid into acetaldehyde and the yellow 1,4-diketone $C_{21}H_{26}O_4$, II (m.p. 181–183°; $[\alpha]^{22}D$ −234°; $\lambda_{max.}^{alc.}$ 267 mμ (4.16); 415 mμ (1.77); calcd.: C, 73.66; H, 7.62; acetyl, 12.6; found: C, 73.53; H, 7.87; acetyl, 13.5). Monoxime: (m.p. 243–245°). Alkali readily converted II into the phenol III (diacetate, m.p. 207–209°; $[\alpha]^{24}D$ −139°; calcd. for $C_{28}H_{26}O_5$: C, 72.23; H, 6.85; acetyl, 22.5; found: C, 72.58; H, 6.94; acetyl, 20.6), the ultraviolet charac-

(1) Paper I of this series: O. Wintersteiner, M. Moore, J. Fried and B. M. Iselin, *Proc. Nat. Acad. Science*, in press.

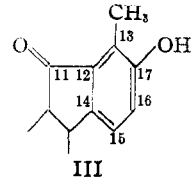
(2) W. A. Jacobs and Y. Sato (a) *J. Biol. Chem.*, **175**, 57 (1948); (b) **181**, 55 (1949).

(3) All melting points corrected; all rotations in chloroform; ultraviolet data; figures in parentheses denote log ϵ.

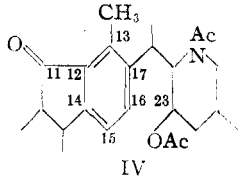
teristics of which ($\lambda_{max.}^{alc.}$ 255 mμ (3.98), 330 mμ (3.51)) were identical with those of 6-keto-β-estradiol,[4] inclusive of the shifts produced by alkali and by acetylation of the phenolic group. Other compounds derived from II are the 12,13-dihydro derivative (m.p. 171–173°, $[\alpha]^{24}D$ −208°), the free diketo alcohol (m.p. 170–171°, $[\alpha]^{22}D$ −220°), and the Δ^4-3-ketone (m.p. 196–199°; $[\alpha]^{22}D$ +8°), all giving correct analyses.



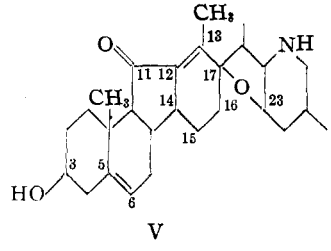
I, R = $CHCH_3$
II, R = O



III



IV



V

O,N-Diacetyljervine with acetic anhydride, acetic acid and sulfuric acid at 24° yielded besides a sulfonic acid the indanone IV (m.p. 239–240°; $[\alpha]^{24}D$ −29°; calcd. for $C_{33}H_{43}O_6N$: C, 72.10; H, 7.89; found: C, 71.82; H, 7.98), identified as such by the ultraviolet spectrum ($\lambda_{max.}^{alc.}$ 251 mμ (4.08), 300 mμ (3.30)), and the fact that the amorphous product obtained on hydrogenation (PtO_2, acetic acid, uptake 2 moles) exhibited a spectrum similar to that of neoergosterol ($\lambda_{max.}^{alc.}$ 268 mμ (2.63)) which reverted to that of IV on chromic acid oxidation. The acetoxy group formed by acetolysis of the oxidic ring is placed at C_{23} on the strength of the evidence adduced by Jacobs and Sato[2b] and additional facts ascertained by us.

The position of the methyl group (C_{13}) in I–IV is arbitrarily assigned. In all the compounds described the 11-keto group is inert toward keto reagents, as it is in jervine. Such hindrance would not obtain in the analogous perhydrochrysene structures carrying the keto group at C_{12}. The presence in jervine itself of the perhydrobenzfluorene nucleus receives support from the nature of the hydrocarbons $C_{20}H_{16}$ and $C_{22}H_{20}$, evidently homologs of 1,2-benzfluorene, found among the selenium dehydrogenation products.[5] Barring rearrangements in the acetolysis reactions jervine would then appear to be V, or perhaps the $\Delta^{12,14}$-double bond isomer of V. We prefer C_{17} to C_{16} as the other point of attachment of the oxide bridge on account of the ease which which double bond formation takes place at that site not only in the reaction leading to IV but also, as will be reported later, in the sulfuric acid-catalyzed acetolysis of tetrahydrojervine derivatives.

(4) B. Longwell and O. Wintersteiner, *J. Biol. Chem.*, **133**, 219 (1939).

(5) W. A. Jacobs, L. C. Craig and G. L. Lavine, *ibid.*, **141**, 51 (1941).

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IDENTIFICATION OF RIBULOSE IN $C^{14}O_2$ PHOTOSYNTHESIS PRODUCTS[1]

Sir:

The intermediates involved in carbon dioxide fixation by plants are largely phosphorylated hydroxy acids and sugars. A compound observed during the first few seconds of $C^{14}O_2$ photosynthesis in all the plants investigated in this laboratory has now been identified as ribulose (adonose) diphosphate.

The diphosphate ester occupies a paper chromatographic position near that of fructose and glucose 1,6-diphosphates[2] and 2,3-diphosphoglyceric acid. A monophosphate[3] ester which gives the same labeled sugar upon phosphatase ("Polidase") hydrolysis occupies a chromatographic position intermediate between triose phosphates[2] and the hexose monophosphates. In young cultures of *Scenedesmus* the concentration of the diphosphate approaches that of phosphoglycerate.

Independent evidence of the phosphorus content of ribulose diphosphate was obtained from measurements of C^{14}/P^{32} ratios in chromatographically separated compounds derived from *Scenedesmus* saturated with P^{32} (12 hours equilibration in radiophosphate) and C^{14} (35 minutes photosynthesis in $C^{14}O_2$). The measured ratios (samples were counted when the ratios were near unity for optimum accuracy) were all multiplied by an appropriate factor to give 3.0 for phosphoglycerate, 5.1 for glucose monophosphate, 5.8 for fructose plus sedoheptulose monophosphate, and 2.0 for ribulose diphosphate. The calculated value for ribulose diphosphate is 2.5.

The chromatographic position of the radioactive sugar R_f(phenol) = 0.60; R_f(butanol–propionic acid–water[2]) = 0.27, corresponds exactly to that of ribulose prepared by epimerization of ribose or arabinose in pyridine. No common hexoses or tetroses have such a position.

The radioactive sugar resists bromine but is cleaved by oxygen, particularly under basic conditions such as in diethylamine solutions or on anion exchange resins. Radioactive glycolic, glyceric and a polyhydroxy acid (presumably erythronic[4]) are obtained upon air oxidation. These products are those expected from ribulose oxidation. The labeled diphosphate was found to be oxidized by air in diethylamine solutions to give phosphoglyceric and phosphoglycolic acids as major products. These were identified by chromatography of the hydroly-

(1) This work was sponsored by the United States Atomic Energy Commission.

(2) A. A. Benson, J. A. Bassham, M. Calvin, T. Goodale, V. Haas and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

(3) B. L. Horecker and P. Z. Smyrniotis, *Arch. Biochem.*, **29**, 232 (1950).

(4) J. U. Nef, O. F. Hedenberg and J. W. E. Glattfeld, THIS JOURNAL, **39**, 1638 (1917).