as it was not possible to photograph it before it disintegrated.

Further confirmation was obtained by hydrolyzing the diphosphate area in 1 N HCl for 16 hr. The material obtained from experiments using $C^{14}O_2$, CN^- and CO_2, $C^{14}N^-$ was shown to be identical with synthetic hamamelonic acid (made from HmDP) by cochromatography in one dimension in butanol–propionic acid–water.

Conclusions

It has been shown that the new material which appears in photosynthesis experiments with algae by the use of high concentrations of cyanide is hamamelonic acid diphosphate together with some epimer. This compound has not been detected in normal photosynthesis experiments without cyanide addition. A substance which is chromatographically rather similar but not identical has been detected by Moses[34] in normal photosynthesis experiments. This material on dephosphorylation and *reduction* by KBH_4 yields hamamelonic acid and its isomers.

The source of hamamelonic acid diphosphate is clearly a cyanohydrin reaction on RuDP. In order to form appreciable amounts of HmDP, it is essential to have a light period between the cyanide addition and the injection of boiling alcohol to kill the algae.[15] Furthermore, the net fixation of radiocarbon as PGA diminishes when the algae are illuminated after the addition of cyanide. The amount of radiocarbon fixed as hamamelonic acid diphosphate is consistent with the amount disappearing from the pentose phosphates and the PGA pools. This can be interpreted if it is assumed that the addition of cyanide has little effect on the ability of the algae to reduce PGA at least for an initial short period of time. Then in the light period after the cyanide addition, PGA is reduced to triose phosphate which cycles into RuDP and is trapped by the cyanide. The principal effect of the cyanide would then be to block the uptake of CO_2, probably by inhibition of the carboxydismutase system,[14] and trap the RuDP as it accumulated from other intermediates of the photosynthetic cycle. In addition, it can be deduced that the reaction involving the phosphorylation of RuMP is also occurring during the light period after the addition of cyanide. This is consistent with the finding of Kandler,[35] who has taken his results on cyanide inhibition to indicate that a light phosphorylation is not inhibited by high concentrations of cyanide in the first few minutes. However, the validity of this method of determining phosphorylation rates must be re-examined in the light of the broad reactivity of CN^- here demonstrated.

It also has been demonstrated that a facile reaction occurs between RuDP and KCN to produce HmDP. It is feasible that this reaction occurs readily because the intermediate cyanohydrin can cyclize to give an imino-lactone which should undergo rapid hydrolysis.

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[34] V. Moses, unpublished work from this Laboratory.

[35] O. Kandler, *Naturwiss.*, **42**, 390 (1955).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Condensation of 2-Nitroethanol with the D-Aldopentoses

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D-*gluco*-Heptulose, D-*manno*-heptulose, D-*galacto*-heptulose, D-*talo*-heptulose, 2,7-anhydro-β-D-*ido*-heptulopyranose and 2,7-anhydro-β-D-*altro*-heptulopyranose (sedoheptulosan) have been obtained by application of the 2-nitroethanol synthesis to the appropriate D-aldopentoses.

A preliminary communication[1] from this Laboratory reported the condensation of sodium *aci*-2-nitroethanol with D-arabinose to give, after hydrolysis of the intermediate sodium *aci*-2-deoxy-2-nitroheptitols, D-*gluco*-heptulose and D-*manno*-heptulose. The synthesis now has been extended to D-lyxose, D-xylose and D-ribose to give, respectively, D-*galacto*-heptulose plus D-*talo*-heptulose, 2,7-anhydro-β-D-*ido*-heptulopyranose and 2,7-anhydro-β-D-*altro*-heptulopyranose (sedoheptulosan). Although the yields (5–22%) by this method are not high, the relative simplicity of the synthesis rates it favorably with other available methods for obtaining the higher-carbon ketoses.

2,7-Anhydro-β-D-*ido*-heptulopyranose has been synthesized previously[2] from D-xylose through consecutive condensations with nitromethane and formaldehyde, followed by hydrolysis of the resulting *aci*-2-deoxy-2-nitroheptitols. However, the present synthesis using 2-nitroethanol provides the anhydroheptulose with greater ease and in considerably higher yield.

The laboratory preparation of 2-nitroethanol is accomplished readily by the condensation of nitromethane with formaldehyde,[3] and the product can be isolated safely by co-distillation with diphenyl ether.[4] Extraction of the separated, crude 2-nitroethanol thus obtained with an equal volume of hexane or low-boiling petroleum ether, to remove diphenyl ether, then gives a product (50% yield based on formaldehyde) of sufficient purity for condensation with the sugars.

Experimental

D-*gluco*-Heptulose and D-*manno*-Heptulose.—To a suspension of 10 g. of D-arabinose in 24 ml. of 2-nitroethanol

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[1] J. C. Sowden, THIS JOURNAL, **72**, 3325 (1950).

[2] J. K. N. Jones, *J. Chem. Soc.*, 3643 (1954).

[3] I. M. Gorsky and S. P. Makarow, *Ber.*, **67**, 996 (1934); J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., THIS JOURNAL, **71**, 2463 (1949).

[4] H. T. Roy, Jr. (to the General Tire and Rubber Co.), U. S. Patent 2,710,830, June 14, 1955; W. E. Noland, H. I. Freeman and M. S. Baker, THIS JOURNAL, **78**, 188 (1956).

and 50 ml. of anhydrous methanol was added a solution of 3 g. of sodium in 50 ml. of anhydrous methanol. Upon shaking, the pentose dissolved at once and within a few minutes the precipitation of the sodium *aci*-2-deoxy-2-nitroheptitols began. After 5 hours of shaking, the mixture was cooled to −20° and filtered. Following washing with cold methanol, ether and low-boiling petroleum ether, the sodium salts were dissolved in 90 ml. of cold water and the solution was added rapidly, dropwise, to a stirred solution containing 8 ml. of sulfuric acid in 10 ml. of water at 0°. During the addition of the first 60 ml. of the sodium salt solution, an additional 10 ml. of sulfuric acid also was added dropwise to the reaction mixture. Fifteen minutes after all reagents had been added, the solution was deionized,[5] decolorized and concentrated to a sirup. Seeding of this sirup with D-*gluco*-heptulose[6] yielded 4.6 g. of crystals (m.p. 140–145°, $[\alpha]^{20}\text{D}$ −32° in water, *c* 2) which contained an appreciable amount of D-arabinose. Accordingly, the total product was redissolved in 300 ml. of water and treated with 4 ml. of bromine in the presence of 20 g. of barium carbonate. After 18 hours at room temperature, excess bromine was removed by aeration and the mixture was filtered and deionized. The effluent was titrated to a stable phenolphthalein end-point with 0.2 *N* sodium hydroxide and again deionized. The titration and deionization were repeated on the effluent and the latter was then concentrated to a sirup. Seeding with D-*gluco*-heptulose yielded 1.6 g. (11.4%) of this heptulose[7] in nearly pure condition, m.p. 168–169°, $[\alpha]^{20}\text{D}$ +64.4° in water, *c* 4. Seeding of the residual sirup with D-*manno*-heptulose then yielded 0.66 g. (4.7%) of the latter heptulose,[8] m.p. 151–152°, $[\alpha]^{20}\text{D}$ +29.5° in water, *c* 4. Further seeding of the residue gave 0.75 g. (5.3%) of the mixed heptuloses, m.p. 145–148°, $[\alpha]^{20}\text{D}$ +44°.

2,7-Anhydro-β-D-*ido*-heptulopyranose.—Five grams of D-xylose was condensed with 2-nitroethanol, and the resulting sodium *aci*-nitroalcohols were hydrolyzed, under the same conditions as those described above for D-arabinose. No attempt was made to remove pentose, but rather the deionized, sirupy product was heated in 50 ml. of 0.2 *N* hydrochloric acid at 100° for 2 hours. Barium hydroxide octahydrate (8 g.) then was added and the heating was continued for 45 minutes to destroy reducing sugars. The sirup obtained following deionization and concentration was seeded with 2,7-anhydro-β-D-*ido*-heptulopyranose to yield 1.21 g. (18.9%) of the anhydroheptulose,[9] m.p. 168–169°, $[\alpha]^{20}\text{D}$ −40.3° in water, *c* 2.

2,7-Anhydro-β-D-*altro*-heptulopyranose (Sedoheptulosan).—Five grams of D-ribose was condensed with 2-nitroethanol as described above, except that the reaction mixture was allowed to stand at −20° for 27 hours to increase the precipitation of the sodium salts. The latter were processed further as described in the experiment with D-xylose. Crystallization of the final sirup from absolute methanol yielded 0.31 g. (4.8%) of sedoheptulosan,[10] m.p. 154–156°, $[\alpha]^{20}\text{D}$ −140° in water, *c* 3. Benzoylation of the residual sirup provided 0.18 g. (0.9%) of the corresponding tetrabenzoate,[11] m.p. 161–162°.

D-*galacto*-Heptulose and D-*talo*-Heptulose.—Five grams of D-lyxose was condensed with 2-nitroethanol, the resulting sodium *aci*-nitroalcohols were hydrolyzed, and unchanged pentose was removed by bromine oxidation as described above for D-arabinose. The resulting sirup (4.2 g.) did not crystallize when seeded with D-*galacto*-heptulose. Accordingly, it was chromatographed on a column of powdered cellulose (Whatman standard grade, 3.5 × 32 cm.) using the top layer of 1-butanol (50 v.)–ethanol (15 v.)–water (40 v.) as the developing solvent. Twenty fractions of 50 ml. each were collected, and all gave a positive test for ketose with orcinol–trichloroacetic acid reagent. Concentration of the individual fractions to sirups and seeding with D-*galacto*-heptulose produced crystals in fractions 4–6 and 9–15. A total of 0.54 g. (7.7%) of this heptulose[12] (m.p. 105–107°, $[\alpha]^{20}\text{D}$ +81° equil. in water, *c* 2) was obtained. The residual sirups were combined and seeded with D-*talo*-heptulose to yield 0.12 g. (1.7%) of the latter heptulose,[13] m.p. 138–139°, $[\alpha]^{20}\text{D}$ +15.8° equil. in water, *c* 1.

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[5] The ion exchange resins used in this work were Amberlite IR-100, a product of Rohm and Haas Co., Philadelphia, Pa., and Duolite A-4, a product of Chemical Process Co., Redwood City, Calif.

[6] The authors are indebted to Dr. Nelson K. Richtmyer, N. I. A. M. D., National Institutes of Health, for seeding crystals of the heptuloses and anhydroheptuloses described in this paper.

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[13] J. W. Pratt and N. K. Richtmyer, *ibid.*, **77**, 6326 (1955).

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[CONTRIBUTION FROM THE SCHOOL OF PHARMACY OF THE UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, VARIAN ASSOCIATES, AND THE UNIVERSITY OF PENNSYLVANIA]

Steric Effects on the Nuclear Magnetic Resonance Spectra of Some Cyclohexanone, Indanone and Camphor Compounds

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Cyclohexanone has two peaks whose areas are in the ratio of 4:6 suggesting that the hydrogens adjacent to the carbony are different from the others. There is no evidence of a difference between equatorial and axial hydrogens which would result in a 5:5 ratio. 2-Chlorocyclohexanone has multiplets in the ratio 1:2:6 as would be expected. 1-Chloro-2-indanone has three sharp peaks in the ratio 1:2:4, while the spectrum of 1-bromo-2-indanone is very similar except the peak arising from the two hydrogens on the number 3 atom is lower and broader possibly due to an indirect steric effect of the larger bromine or to the magnetic anistropy of the bromine. α-Chlorocamphor has a doublet with 5 c.p.s. spacing while α′-chlorocamphor has a singlet shifted about 20 c.p.s. Thus both the magnitude of the chemical shift and whether the spin–spin coupling takes place in this case appears to depend on the configuration. Bromine in these ring compounds causes a greater chemical shift to lower field strengths than does chlorine, contrary to the behavior in aliphatic compounds. In α,α′-dibromocamphor, peaks due to three different methyl hydrogens are present while only two are present in the α-bromocamphor indicating that the close approach of the α′-bromine is affecting one of the *gem*-dimethyl groups making it different from the other *gem*-dimethyl group.

High resolution NMR spectra of cyclohexanone, 2-chlorocyclohexanone, 1-chloro-2-indanone, 1-bromo-2-indanone, D-α-chlorocamphor, D-α′-chlorocamphor, D-α-bromocamphor and D-α,α′-dibromocamphor were obtained at 40 mc. in a magnetic field of approximately 9400 gauss with the aim of correlating shifts in the spectra with expected steric effects. The compounds were all studied in dilute solution in carbon tetrachloride, the zero of reference being taken as pure benzene in an external annular