lute methanol; dec. 148–155°, $[\alpha]^{25}$D +54⁻ (initial, extrapolated) → −36° (c 2.3, water), X-ray powder diffraction data[21]: 6.55vw, 5.91m, 5.21w, 4.65m, 4.28vw, 4.05vs(1), 3.80vw, 3.60m, 3.42s(3), 3.29vw, 3.16m, 3.06w, 2.85m, 2.67w, 2.61s(2), 2.31w, 2.27vw, 1.95m, 1.77w, 1.74vw, 1.61w.

The substance reduced Benedict solution and exhibited a positive ninhydrin reaction.

Anal. Calcd. for $C_5H_{12}ClNO_4$: C, 32.36; H, 6.52; N, 7.55. Found: C, 32.34; H, 6.44; N, 7.97.

COLUMBUS 10, OHIO

2-Deoxy-D-ribose. II.[1] The Synthesis of 2-Deoxy-D-ribose 5-Phosphate

BY DONALD L. MACDONALD AND HEWITT G. FLETCHER, JR.

The conversion of 2-deoxy-D-ribose to the crystalline di-(cyclohexylammonium) salt of 2-deoxy-D-ribose dimethyl acetal 5-phosphate is described. Mild acid hydrolysis of this acetal affords 2-deoxy-D-ribose 5-phosphate.

While 2-deoxy-D-ribose (2-deoxy-D-*erythro*-pentose) was recognized as a component of deoxyribonucleic acid some twenty-nine years ago,[2,3] only in the last few years has the role of this sugar in various biological systems attracted widespread attention. In particular, the phosphoric acid esters of 2-deoxy-D-ribose have been shown to be important intermediates in a variety of biochemical transformations. Friedkin and Kalckar,[4] for instance, demonstrated that the enzymatic phosphorolysis of guanine deoxyriboside afforded a 2-deoxy-D-ribose 1-phosphate which Friedkin[5] isolated as the barium and cyclohexylamine salts. Numerous other workers[6–11] have extended our knowledge of this highly labile substance. Manson and Lampen[6] demonstrated the existence of a mutase capable of converting 2-deoxy-D-ribose 1-phosphate to the 5-phosphate; the reverse reaction was found by Racker[12] who isolated the 5-phosphate as its barium salt. Racker,[12] as well as others,[8,13] has also obtained the substance through the acid hydrolysis of 2'-deoxyadenosine 5'-phosphate while Agranoff and Brady[14] described the phosphorylation of 2-deoxy-D-ribose with a ribokinase from calf liver, suggesting that 2-deoxy-D-ribose 5-phosphate was formed although this product was not actually isolated.

A deoxyribosephosphate aldolase was purified and described by Racker.[12] More recently, Domagk and Horecker[15] have shown that *Lactobacillus plantarum*, grown on D-glucose and adapted to 2-

deoxy-D-ribose, has an active deoxyribosephosphate aldolase and crude extracts from this bacteria have been used by these authors to prepare 2-deoxy-D-ribose 5-phosphate as its barium or calcium salt in about 50% purity. Subsequently, an essentially pure barium salt has been obtained by this process.[16]

Klenow and Emberland[17] showed that 2-deoxy-D-ribose 1-phosphate was consumed in a system which was capable of converting D-ribose 1-phosphate to D-ribose 1,5-diphosphate, while Tarr[18] found that a crude fish muscle purine riboside phosphorylase possessing phosphoribomutase activity, acting on a mixture of 2-deoxy-D-ribose 1-phosphate and D-ribose 1,5-diphosphate, afforded 2-deoxy-D-ribose 1,5-diphosphate as well as 2-deoxy-D-ribose 5-phosphate, D-ribose 1-phosphate and D-ribose 5-phosphate.

None of the phosphates of 2-deoxy-D-ribose appears to have been made by strictly chemical means.[18a] Allerton, Overend and Stacey announced[19] the synthesis of the 3- and 5-phosphates of this sugar some years ago, but experimental details of their work have not, apparently, been published. With 2-deoxy-D-ribose readily accessible through a simplified preparation[1] we have turned our attention to the chemical synthesis of its 5-phosphate which will now be described.

The mercaptals of the pentoses offer the most direct route to 5-substituted derivatives of these sugars and, as a number of mercaptals of 2-deoxy-D-ribose have been described by Zinner,[20] the present synthesis was patterned after those previously described for D-glyceraldehyde 3-phosphate[21] and D-erythrose 4-phosphate.[22] The free sugar or, more conveniently, its anilide[1] was converted to the sir-

(1) 2-Deoxy-D-ribose. I: H. W. Diehl and H. G. Fletcher, Jr., *Arch. Biochem. Biophys.*, **78**, 386 (1958).

(2) P. A. Levene, L. A. Mikeska and T. Mori, *J. Biol. Chem.*, **85**, 785 (1930).

(3) For reviews of the chemistry of 2-deoxy-D-ribose see (a) W. G. Overend and M. Stacey in "The Nucleic Acids" (E. Chargaff and J. N. Davidson, eds.), Academic Press, Inc., New York, N. Y., 1955, vol. I, p. 9; and (b) W. G. Overend and M. Stacey, *Advances in Carbohydrate Chem.*, **8**, 45 (1953).

(4) M. Friedkin and H. M. Kalckar, *J. Biol. Chem.*, **184**, 437 (1950).

(5) M. Friedkin, *ibid.*, **184**, 449 (1950).

(6) L. A. Manson and J. O. Lampen, *ibid.*, **191**, 95 (1951).

(7) L. A. Manson and J. O. Lampen, *ibid.*, **193**, 539 (1951).

(8) C. E. Hoffmann and J. O. Lampen, *ibid.*, **198**, 885 (1952).

(9) H. M. Kalckar, *Biochim. et Biophys. Acta*, **12**, 250 (1953).

(10) M. Friedkin and D. Roberts, *J. Biol. Chem.*, **207**, 245, 257 (1954).

(11) H. L. A. Tarr, *Can. J. Biochem. and Physiol.*, **36**, 517 (1958).

(12) E. Racker, *J. Biol. Chem.*, **196**, 347 (1952).

(13) A. Kornberg, I. Lieberman and E. S. Simms, *ibid.*, **215**, 389 (1955).

(14) B. W. Agranoff and R. O. Brady, *ibid.*, **219**, 221 (1956).

(15) G. F. Domagk and B. L. Horecker, *ibid.*, **233**, 283 (1958).

(16) B. L. Horecker and W. E. Pricer, Jr., private communication.

(17) H. Klenow and R. Emberland, *Arch. Biochem. Biophys.*, **58**, 276 (1955).

(18) H. L. A. Tarr, *Chemistry & Industry*, 562 (1957).

(18a) Added in proof May 4, 1959. S. Lewak, R. Derache and L. Szabó [*Compt. rend.*, **248**, 1837 (1959)] have very recently announced a chemical synthesis of 2-deoxy-D-ribose 5-phosphate from 3-O-methyl-D-glucose 6-phosphate.
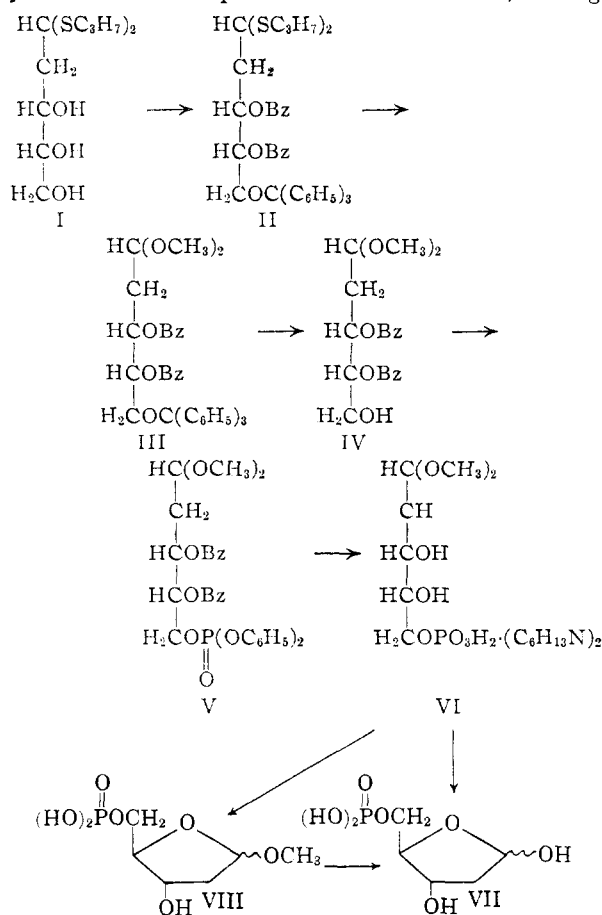
(19) R. Allerton, W. G. Overend and M. Stacey, *ibid.*, 952 (1952).

(20) H. Zinner, H. Nimz and H. Venner, *Chem. Ber.*, **90**, 2696 (1957).

(21) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **77**, 3329 (1955).

(22) C. E. Ballou, H. O. L. Fischer and D. L. MacDonald, *ibid.*, **77**, 5967 (1955).

upy diisopropyl mercaptal[23] (I) which was then successively tritylated and benzoylated to yield crystalline 3,4-di-O-benzoyl-2-deoxy-5-O-triphenylmethyl-D-ribose diisopropyl mercaptal (II) in 57% yield.[24] Demercaptalation in methanol, using

HC(SC₃H₇)₂   HC(SC₃H₇)₂

$$
\begin{array}{ccc}
\text{HC(SC}_3\text{H}_7)_2 & & \text{HC(SC}_3\text{H}_7)_2 \\
| & & | \\
\text{CH}_2 & & \text{CH}_2 \\
| & & | \\
\text{HCOH} & \longrightarrow & \text{HCOBz} & \longrightarrow \\
| & & | \\
\text{HCOH} & & \text{HCOBz} \\
| & & | \\
\text{H}_2\text{COH} & & \text{H}_2\text{COC(C}_6\text{H}_5)_3 \\
\text{I} & & \text{II}
\end{array}
$$

$$
\begin{array}{ccc}
\text{HC(OCH}_3)_2 & & \text{HC(OCH}_3)_2 \\
| & & | \\
\text{CH}_2 & & \text{CH}_2 \\
| & & | \\
\text{HCOBz} & \longrightarrow & \text{HCOBz} & \longrightarrow \\
| & & | \\
\text{HCOBz} & & \text{HCOBz} \\
| & & | \\
\text{H}_2\text{COC(C}_6\text{H}_5)_3 & & \text{H}_2\text{COH} \\
\text{III} & & \text{IV}
\end{array}
$$

$$
\begin{array}{ccc}
\text{HC(OCH}_3)_2 & & \text{HC(OCH}_3)_2 \\
| & & | \\
\text{CH}_2 & & \text{CH} \\
| & & | \\
\text{HCOBz} & \longrightarrow & \text{HCOH} \\
| & & | \\
\text{HCOBz} & & \text{HCOH} \\
| & & | \\
\text{H}_2\text{COP(OC}_6\text{H}_5)_2 & & \text{H}_2\text{COPO}_3\text{H}_2\cdot(\text{C}_6\text{H}_{13}\text{N})_2 \\
\quad\quad \| & & \\
\quad\quad \text{O} & & \\
\text{V} & & \text{VI}
\end{array}
$$

mercuric chloride and mercuric oxide,[25] converted the mercaptal to the dimethyl acetal III, a substance obtained in 85% yield in two crystalline, interconvertible forms. In sharp contrast to the ease of conversion of the diisopropyl mercaptal II to the dimethyl acetal III, the ethylene mercaptal analogous to II was exceedingly inert and conditions for its conversion to an acetal were not found. The stability of ethylene mercaptals and the lability of diisopropyl mercaptals have been noted by Zinner, Brandner and Rembarz.[26]

Hydrogenolysis of the trityl group in 3,4-di-O-benzoyl-2-deoxy-5-O-triphenylmethyl-D-ribose dimethyl acetal (III) with palladium-on-charcoal in dioxane–methanol gave 3,4-di-O-benzoyl-2-deoxy-D-ribose dimethyl acetal(IV) which was not isolated but phosphorylated directly in pyridine solu-

tion with diphenyl phosphorochloridate. No attempt was made to isolate the product V, the phenyl groups being removed with hydrogen in the presence of Adams catalyst and the acyl groups cleaved with alkali to give 2-deoxy-D-ribose 5-phosphate dimethyl acetal, isolated as its crystalline di-(cyclohexylammonium) salt (VI). The yield in the sequence of reactions from III to VI was variable, at best about 25%.[27]

Conversion of the di-(cyclohexylammonium) salt of the dimethyl acetal of 2-deoxy-D-ribose 5-phosphate (VI) to the free phosphate was carried out in aqueous solution through the use of an excess of Amberlite IR-120H⁺. Paper chromatography demonstrated that the dimethyl acetal disappeared completely at room temperature in about 45 minutes. While some 2-deoxy-D-ribose 5-phosphate (VII), identified by comparative chromatography with authentic material, is formed at once, the major product is a non-reducing, periodate-stable sugar phosphate which is slowly converted to the 5-phosphate although some is still present after the reaction has progressed for four days at room temperature, suggesting that an equilibrium may be involved. Addition of methanol to the reaction mixture increases the concentration of this intermediate and lends support to the view that the substance is methyl 2-deoxy-D-ribofuranoside 5-phosphate (VIII).

The course of the hydrolysis in water was followed using the hypoiodite oxidation of Auerbach and Bodländer.[28] This assay, however, gave abnormally high results and it was subsequently found that pure 2-deoxy-D-ribose itself afforded values some 10–18% higher than expected. Friedkin[5] has noted a similar difficulty with this sugar. It should be pointed out that 2-deoxy-D-glucose reacts normally and quantitatively with hypoiodite. A sample of the dimethyl acetal VI which had been hydrolyzed for 48 hours at room temperature was assayed by Mr. W. E. Pricer of this Institute using deoxyribosephosphate aldolase,[15] alcohol dehydrogenase and glycerol phosphate dehydrogenase, the results showing that about 90% of the theoretical quantity of VII was present.

Experimental[29]

3,4-Di-O-benzoyl-2-deoxy-5-O-triphenylmethyl-D-ribose Diisopropyl Mercaptal (II).—Ten grams of 2-deoxy-D-ribose

(23) H. Zinner, H. Nimz and H. Venner (ref. 20) obtained this substance in crystalline form and we are indebted to Dr. Zinner for seed crystals. However, in our hands, crystallization was slow, entailing considerable loss, so that use of the sirupy material proved more practicable.

(24) The corresponding 3,4-di-O-acetyl derivative has recently been reported by H. Zinner, H. Nimz and H. Venner, Chem. Ber., **91**, 638 (1958).

(25) H. A. Campbell and K. P. Link, J. Biol. Chem., **122**, 635 (1938).

(26) H. Zinner, H. Brandner and G. Rembarz, Chem. Ber., **89**, 800 (1956); H. Zinner and H. Brandner, ibid., **89**, 1507 (1956).

(27) Isolation of crystalline triphenylmethane in 75% yield demonstrated that the trityl group had been removed satisfactorily. After the ditritylation but prior to phosphorylation a sample of the material was debenzoylated and then subjected to paper electrophoresis at pH 8.6 in borate buffer. Two components were readily detectable by periodate–benzidine spray. Furthermore, the mother liquors from the crystallization of the cyclohexylammonium salt (VI) always contained a considerable quantity of non-reducing unphosphorylated sugar. These results suggest that methyl 3,4-di-O-benzoyl-2-deoxy-D-riboside is formed when III is detritylated, possibly owing to acidity in the catalyst. Attempts to avoid such a side reaction through the addition of various alkaline substances (pyridine, sodium bicarbonate, sodium acetate, barium carbonate) unfortunately inhibited the hydrogenation. Similarly, when dioxane alone was used as a solvent either with Adams catalyst or palladium-on-charcoal, the reduction failed, in these and the above-mentioned experiments the starting material (III) was recovered in 50–95% yield. The inhibitory action of alkalies on platinum- and palladium-catalyzed hydrogenations has been studied by V. M. Clark, G. W. Kirby and A. R. Todd, J. Chem. Soc., 3039 (1958).

(28) F. Auerbach and E. Bodländer, Angew. Chem., **36**, 602 (1923).

(29) All melting points are corrected.

anilide,[1] 13.5 ml. of isopropyl mercaptan and 12 ml. of concentrated hydrochloric acid were mixed at 0° and then stirred magnetically at room temperature for 1 hr. The mixture was worked up as described by Zinner, *et al.*,[20] and the resulting sirupy 2-deoxy-D-ribose diisopropyl mercaptal (11.06 g., 86%) dried by azeotroping benzene from it several times *in vacuo*. The mercaptal was then dissolved in anhydrous pyridine (110 ml.), the solution cooled in ice and 12.1 g. (1.05 molar equivs.) of trityl chloride added. After standing at room temperature for 24 hr., the mixture was cooled to 0°, benzoyl chloride (15 ml.) added dropwise, and the whole left at 0° overnight. The excess of benzoyl chloride was then destroyed with a little ice and, after dilution with methylene chloride, the solution was washed successively with water, 1 *N* sulfuric acid, saturated aqueous sodium bicarbonate and water. Moisture was removed with sodium sulfate and the solution concentrated *in vacuo* to a viscous sirup which was dissolved in ten parts of warm methanol, seeded[30] and cooled slowly to give colorless crystals (19.8 g., 57% based on the anilide) melting at 105–107° and rotating $[\alpha]^{20}$D $-39.7°$ (*c* 2.8, CHCl$_3$). In some runs recrystallization, including treatment with decolorizing carbon, was necessary.

Anal. Calcd. for C$_{44}$H$_{46}$O$_5$S$_2$ (718.93): C, 73.50; H, 6.45; S, 8.92. Found: C, 73.33; H, 6.56; S, 8.76.

3,4-Di-*O*-benzoyl-2-deoxy-5-*O*-triphenylmethyl-D-ribose ethylene Mercaptal.—A sample (1.89 g.) of 2-deoxy-D-ribose ethylene mercaptal,[20] dissolved in dry pyridine (10 ml.) was successively treated with trityl chloride (2.63 g.) and benzoyl chloride (4 ml.), as described for the isopropyl analog above. The product crystallized readily; after two recrystallizations from propyl alcohol it weighed 4.88 g. (82%), melted at 147–148° and rotated $[\alpha]^{20}$D $-25.1°$ (CHCl$_3$, *c* 4.1).

Anal. Calcd. for C$_{40}$H$_{36}$O$_5$S$_2$ (660.81): C, 72.70; H, 5.49; S, 9.70. Found: C, 72.64; H, 5.52; S, 9.64.

3,4-Di-*O*-benzoyl-2-deoxy-5-*O*-triphenylmethyl-D-ribose Dimethyl Acetal (III).—Yellow mercuric oxide (16.1 g., 74 mmoles) was added to a solution of 13.36 g. (18.6 mmoles) of 3,4-di-*O*-benzoyl-2-deoxy-5-*O*-triphenylmethyl-D-ribose diisopropyl mercaptal (II) in 500 ml. of boiling, dry methanol. The hot solution was stirred vigorously (magnetic stirrer) while a solution of 15.1 g. (56 mmoles) of mercuric chloride in 60 ml. of methanol was added over a period of 1 min.; stirring was continued while the suspension was refluxed for 15 min. While still hot, the solution was filtered, the solid matter being washed with hot, dry methanol. A little yellow mercuric oxide was added to the combined filtrate and washings and these were then concentrated *in vacuo* to a sirup which was dissolved in methylene chloride (250 ml.) and filtered. The filtrate was washed successively with 100-ml. portions of water, 10% aqueous potassium iodide (2 ×) and water (3 x) and dried with sodium sulfate. Solvent was removed *in vacuo* and the residue crystallized from *ca.* 20 parts of methanol. One recrystallization from the same solvent afforded 10.37 g. (88%) of the dimethyl acetal as minute, irregular prisms, m.p. 63–67° (hot-stage), $[\alpha]^{20}$D $-18.2°$ (CHCl$_3$, *c* 2.0).

Anal. Calcd. for C$_{40}$H$_{38}$O$_7$ (630.70): C, 76.17; H, 6.07. Found: C, 75.94; H, 6.37.

In another experiment a second crystalline form of the substance, stout, flat prisms, m.p. 105–106°, $[\alpha]^{20}$D $-18.2°$ (CHCl$_3$, *c* 2.0) was obtained. Subsequently it was found that either form could be obtained from methanol solution by using the appropriate seeds.

Anal. Calcd. for C$_{40}$H$_{38}$O$_7$ (630.70): C, 76.17; H, 6.07. Found: C, 75.96; H, 6.43.

Di-(cyclohexylammonium) 2-Deoxy-D-ribose Dimethyl Acetal 5-Phosphate (VI).—Two grams of 3,4-di-*O*-benzoyl-2-deoxy-5-*O*-triphenylmethyl-D-ribose dimethyl acetal (III) was dissolved in 15 ml. of purified dioxane[31] and the solution diluted with 35 ml. of dry methanol. One gram of 10% palladium-on-charcoal,[32] which had been saturated with hydrogen and washed thoroughly with methanol, was then added, and the mixture shaken with hydrogen at atmos-

pheric pressure and room temperature for 20 hr. The catalyst was removed by centrifugation, washed with methanol, and the solution concentrated *in vacuo* at 40°. The sirupy residue, usually containing crystals of triphenylmethane, was dissolved in dry pyridine (10 ml.) and the solution cooled in ice. Diphenyl phosphorochloridate (1.5 ml.) was added and, after 10 min. at 0°, the solution was left at room temperature for 18 hr. Excess phosphorylating agent was then decomposed at 0° through the addition of a little water and the mixture diluted with 100 ml. of methylene chloride. It was washed successively with 1% aqueous sodium sulfate (2 × 200 ml.), 1 *M* sulfuric acid, saturated aqueous sodium bicarbonate and, finally, again with sodium sulfate solution. Moisture was removed with sodium sulfate and the solution concentrated *in vacuo* at 40°, methanol being added to and then evaporated (*in vacuo*) from the resulting sirup. The viscous residue (2.0 g.) was dissolved in 50 ml. of absolute ethanol and reduced with hydrogen at room temperature and pressure using 0.5 g. of Adams catalyst. Frequently, the hydrogen uptake was very slow, in which case the catalyst was removed by centrifugation and replaced with a fresh 0.5-g. portion. When the reduction was complete (16–18 hr.) the solution was filtered from the catalyst, made alkaline through the addition of 10 ml. of 1 *N* sodium hydroxide and left at room temperature overnight. The solvent was then removed *in vacuo*, water (30 ml.) added and the mixture extracted with methylene chloride and ether. The aqueous solution was then passed slowly through a column (1.8 × 14 cm.) of Amberlite IR-120 in the cyclohexylammonium form, 150 ml. of water being used for washing the resin. The percolate was concentrated *in vacuo* at 40°, methanol being distilled twice from the residue which then was a crystalline mass: 1.95 g. This was dissolved in 8 ml. of methanol, the solution diluted with 125 ml. of ether and left at 5° to afford the cyclohexylammonium salt as a mass of exceedingly fine, flexible needles; washed with ether and dried, these weighed 0.31 g. (21%). Recrystallized (with 80% recovery) from methanol–ether the product had m.p. 145–150° dec. and showed $[\alpha]^{20}$D $-10.3°$ in water (*c* 2.0). Chromatography on Whatman No. 1 paper using propyl alcohol–ammonia–water (6:3:1, v./v.)[33] revealed but one component (either before or after recrystallization) of R_f 0.55 using either molybdate spray for phosphate[33,34] or periodate–benzidine spray for sugars.[35] In a phosphate buffer of *p*H 7 the substance consumed 1.04 molar equivalents of periodate. For analysis the material was dried *in vacuo* over magnesium perchlorate at room temperature for 20 hr.

Anal. Calcd. for C$_{19}$H$_{43}$O$_8$N$_2$P (458.53): C, 49.77; H, 9.45; N, 6.11; P, 6.76. Found: C, 49.99; H, 9.46; N, 6.06; P, 6.51.

The crystalline salt was also obtained in comparable yields using an alternative procedure described recently.[21,22] The aqueous solution remaining after extraction with methylene chloride and ether was cooled to 0°, acidified by stirring with an excess of Amberlite IR-120H and the resin removed by filtration and washed with ice-water. The solution was extracted with ether and then made basic by the addition of cyclohexylamine, the salt then being crystallized as already described. Owing to the extreme lability of the acetal (see below), these operations must be conducted rapidly and the solution kept cold.

2-Deoxy-D-ribose 5-Phosphate (VII).—A solution of 25.5 mg. of the di(cyclohexylammonium) salt in 5 ml. of water was treated with 250 mg. of Amberlite IR-120H resin which had previously been dried *in vacuo* over KOH. The suspension was swirled for *ca.* 1 min and then left at room temperature. Aliquots (0.2 ml.) were removed at intervals, added to 0.05 ml. of 10% aqueous pyridine to stop the reaction, and chromatographed on Whatman No. 1 paper using isopropyl alcohol–ammonia–water (7:1:2),[36] the components being detected by molybdate spray and periodate–benzidine spray. Examination of the chromatograms thus obtained revealed that the acetal (R_f 0.34) disappeared quite rapidly, none being detectable after about 0.75 hr. Concomitantly, two phosphates were produced: one of R_f 0.27 and the other of R_f 0.13. No inorganic

(30) Seed crystals were first obtained by keeping a sample in propyl alcohol at +5° for about 2 weeks.

(31) L. F. Fieser, "Experiments in Organic Chemistry," 3rd. ed., D. C. Heath & Co., Boston, Mass., 1955, p. 285, method a.

(32) R. Mozingo in "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 685.

(33) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(34) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(35) M. Viscontini, D. Hoch and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

(36) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 2040 (1953).

phosphate (R_f 0.07) was liberated. Comparative chromatography with an authentic, biochemically derived sample of 2-deoxy-D-ribose 5-phosphate demonstrated that the component of R_f 0.13 was identical with this substance. The component of R_f 0.27 was not detectable either with periodate–benzidine spray or tetrazolium blue spray.[37] As the reaction progressed this component very slowly diminished while the proportion of 2-deoxy-D-ribose 5-phosphate increased. However, even after 2–3 days some of the former was still present. After 4 days a sample of the solution showed $[\alpha]^{20}$D +19° (c 0.47) based on the theoretical yield of 2-deoxy-D-ribose 5-phosphate.

In one experiment, after the hydrolysis had progressed for 2 days, an aliquot of the solution was diluted with an equal volume of methanol and left for 4 hr. at room temperature.

Chromatography then revealed a large increase in the quantity of the component of R_f 0.27.

A sample of the dimethyl acetal (11.1 μmoles/ml.) which had been hydrolyzed for 48 hr. as described above was assayed using deoxyribosephosphate aldolase.[15] D-Glyceraldehyde 3-phosphate (9.66 μmoles/ml.) and acetaldehyde (10.0 μmoles/ml.) were formed. A Dische diphenylamine test[15] indicated 10.6 μmoles/ml. of 2-deoxypentose.

(37) W. J. Mader and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952).

The Synthesis by Plants of a New Disaccharide Containing 2-Deoxy-D-glucose[1]

BY G. A. BARBER

A new series of oligosaccharides containing 2-deoxy-D-glucose is synthesized when this monosaccharide is administered to plants. The predominant compound was isolated and crystallized. Evidence is presented that its structure is 6-O-(β-D-fructofuranosyl)-2-deoxy-D-glucose.

In an experiment designed to help to elucidate the mechanisms of flavonol glycosylation in plants, 2-deoxy-D-glucose was administered through the roots to buckwheat seedlings. Paper chromatography of an aqueous ethanol extract of the seedlings revealed the presence of several compounds with the mobilities of simple oligosaccharides which gave the Kiliani[2] reaction for deoxy sugars. The fastest moving of these seemed to be present in considerably higher concentration than the others as evidenced by the intensity of its reaction. The predominant compound was eluted from the chromatograms, hydrolyzed and again chromatographed. After hydrolysis, 2-deoxyglucose and fructose were the only compounds detected.

These results suggested that several oligosaccharides containing 2-deoxyglucose had been formed. Similar results were obtained with extracts of other plants to which the sugar had been administered. These included seedlings of corn, wheat, sorghum and cucumber, and the flower buds and leaves of corn, sorghum, tobacco and *Impatiens sultani*.

Procedures were developed for the isolation of milligram quantities of the predominant compound in crystalline form. It was found to be a disaccharide of 2-deoxyglucose and fructose, the most probable structure being 6-O-(β-D-fructofuranosyl)-2-deoxy-D-glucose.

Experimental

Materials.—3,5-Diaminobenzoic acid and 2-deoxy-D-glucose were purchased from the Aldrich Chemical Co. The sugar was recrystallized from a mixture of methanol and acetone before use as an analytical standard. Methyl-2-deoxy-α-D-glucopyranoside was synthesized by the method of Hughes, Overend and Stacey.[3] Whatman No. 1 chromatography paper was used unless otherwise indicated. Invertase was the "melibiase-free" product of the Nutritional Biochemical Corp. It did not hydrolyze maltose under the conditions of these experiments.

Biosynthesis and Extraction.—The top segments of a number of mature tobacco plants (*Nicotiana tabacum*, var. Havana Seed) consisting of stem, two or three small leaves and several flower buds were cut from the plants. The base of each segment was immersed in a small vial containing 5 ml. of 0.05–0.1 M 2-deoxyglucose and allowed to absorb the solution in the diffuse light of the laboratory for about 48 hr. Water was added to maintain the volume as the solution was absorbed. The segments were ground in a Waring Blendor in a mixture of hot ethanol and 0.1 M potassium phosphate buffer, pH 7.0 (4:1 by volume), about 100 ml. being used per tobacco plant segment. The mixture was heated on a steam-bath for 5 minutes and filtered with the aid of Celite. The residue was heated again in the same volume of ethanol buffer, filtered and the two filtrates were combined. The extract was stirred with approximately 1 g. of activated carbon (Darco G-60), filtered and evaporated to a small volume *in vacuo* at 45°.

Isolation.—The concentrated extract from 3 plants was washed on to a 2.5 × 7 cm. carbon and Celite column prepared as described by Whistler and Durso.[4] The column was further washed with 500 ml. of water, and the compound was eluted with 500 ml. of 5% ethanol. To eliminate contaminating sucrose and traces of 2-deoxyglucose, the eluate was evaporated to a minimal volume and applied in a streak to washed Whatman 3 MM paper. The chromatograms were developed in a descending direction for three days with the organic phase of butanol, ethanol and water[2] (9:1:10 by volume). Under these conditions, 2-deoxyglucose usually ran off the chromatograms, while sucrose appeared 5 cm. or more below the new disaccharide. Marker strips cut from the chromatograms were sprayed with the Kiliani reagent to locate the compound. The appropriate section of paper was cut out and the substrate was eluted with water.

Crystallization.—The eluates were combined and evaporated to a small volume *in vacuo* at 35°. The concentrated solution was transferred to a conical centrifuge tube and evaporated to a sirup at room temperature under a stream of air. The sirup was dissolved in several ml. of absolute

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(2) S. Aronoff, "Techniques of Radiobiochemistry," The Iowa State College Press, Ames, Iowa, 1956, pp. 94–118.

(3) I. W. Hughes, W. G. Overend and M. Stacey, *J. Chem. Soc.*, 2846 (1949).

(4) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).