pounds were identified by comparison in parallel chromatographic experiments with authentic samples.

No attempt was made to refine this procedure as a preparative method. It was carried out primarily to confirm the structure of the product as synthesized using method 1.

Preparation of 5′-Adenylyl Methylphosphonate (AMP-PCH_3). Method 1.—A mixture of methylphosphonic acid (7.3 g., 76 mmoles), AMP·$2H_2O$ (2.7 g., 7.1 mmoles), 60 ml. of pyridine, and 7.5 ml. of water was stirred at room temperature until a clear solution was obtained. DCC (60 g., 290 mmoles) in 60 ml. of pyridine was added and the mixture was stirred vigorously for 24 hr. After 1, 3, and 20 hr. pyridine solutions of 30, 15, and 7.5 g., respectively, of DCC (1 ml. of pyridine/g. of DCC) were added. After 24 hr. the dicyclohexylurea was filtered off with suction and was washed with several portions of water.

The filtrate and washings were adjusted to pH 8 with 2 *N* NaOH and passed at a rate of about 2 ml./min. onto a Dowex-1 anion-exchange column (formate form; 200–400 mesh, 2% cross-linked) containing 250 ml. of resin in a glass column 3 cm. in diameter. The resin was washed with 3 l. of water to remove the residual pyridine, until the optical density of the eluate was less than 0.05 at 260 mμ.

The technique of stepwise elution was utilized (flow rate 2 ml./min.) using successively 2.7 *N* formic acid and 0.5 *M* ammonium formate in 4 *N* formic acid as the eluting media. The 2.7 *N* formic acid eluate (total volume 1.4 l.) contained AMP together with a small amount of DAPP and made up 46% of the total optical density eluted from the column. The 0.5 *M* ammonium formate–4 *N* formic acid eluate (total volume 800 ml.) contained the product, 5′-adenylyl methylphosphonate, in an amount corresponding to 54% of the total ultraviolet-absorbing material eluted from the column. The absolute yield as calculated from optical density measurement, was 3.22 mmoles (45% based on starting AMP).

The fraction containing the product was treated directly with 30 g. of acid-washed Norit A charcoal. The charcoal was filtered on a Büchner funnel and exhaustively washed with water (to pH 6) to remove nonultraviolet-absorbing impurities. The product was eluted with 200 ml. portions of an aqueous ethanol–ammonia solution containing ethanol–ammonia–water (50:1:49) until 90%, 2.9 mmoles as calculated from absorbancy at 260 mμ, of the material on the charcoal had been removed. The ethanol was removed from the combined eluates on a rotary evaporator at 35° at 20 mm. and the remaining aqueous solution was lyophilized to a colorless glass. This material was dissolved in 45 ml. of water, barium bromide (5.0 ml. of a 1 *M* solution) was added, and the barium salt was precipitated by the slow addition of 6 volumes of 95% ethanol. The gummy white precipitate was collected by centrifugation, washed twice with ethanol and twice with ether, and dried *in vacuo*. This material was reprecipitated from ethanol–water and dried at room temperature at 0.01 mm., yield 1.45 g. (33% based on starting AMP).

Anal. Calcd. for $C_{11}H_{15}BaN_5O_9P_2 \cdot 2H_2O$: C, 22.14; H, 3.21; N, 11.75; total P, 10.39; phosphate P, 5.19; mol. wt., 596; adenine–strong acid–weak acid, 1.0:2.0:0.0. Found: C, 22.43; H, 3.42; N, 11.67; total P, 10.13; phosphate P, 5.01; spectral equiv. wt., 600 (λ_{max} 259 mμ, λ_{min} 229 mμ at pH 7.0); adenine–strong acid–weak acid, 1.0:2.0:0.0 (pK_a', 4.07).

Paper chromatography using solutions of barium salt gave single clean spots: solvent system A, R_f 0.70, R_{AMP} 0.97; solvent system B, R_f 0.56, R_{AMP} 0.80. The product gave a positive reaction when the chromatograms were sprayed with periodate–benzidine spray.

Hydrolysis of the product with 2.5 *N* NaOH for 30 min. at 100° caused degradation to AMP which was detected and identified by paper chromatography (R_f in solvent B, 0.45).

The Synthesis of 5′-Adenylyl Methylphosphonate. Method 2.—A mixture of methylphosphonic acid (80 mg., 0.87 mmole) and AMP-NH_2 (380 mg., 0.67 mmole) was dissolved in 2 ml. of dry pyridine and 3 ml. of formamide and the solution was allowed to stand for 5 days at room temperature. The reaction mixture was treated with an equal volume of water and extracted with several portions of ether. The resulting aqueous solution was used for analysis by paper chromatography in solvent system B as described below for the DCC time–course studies. At the end of the reaction three ultraviolet-absorbing materials, AMP, AMP-NH_2, and AMP-PCH_3, were present to the extent of 55, 8, and 37%, respectively. The compounds were identified by comparison in parallel chromatogram experiments with authentic samples. As in the case of AMP-PCH_2Cl no attempt was made to refine this procedure as a preparative method. It was carried out primarily to confirm the structure of the product prepared by method 1.

Time–Course Studies of the Dicyclohexylcarbodiimide-Mediated Reactions of AMP with Methylphosphonic and Chloromethylphosphonic Acids.—The reactions of AMP with methylphosphonic acid and chloromethylphosphonic acid in the presence of DCC in aqueous pyridine were studied under conditions identical with those described above for the preparation of 5′-adenylyl chloromethylphosphonate.

Aliquots (*ca.* 0.2 ml.) of the reaction mixtures were treated with an equal volume of water and the solid dicyclohexylurea was removed by centrifugation. The supernatant solution was extracted with several portions of ether and was analyzed by means of paper chromatography using solvent system B. After development of the chromatograms the ultraviolet-absorbing areas were cut out and eluted with 10 ml. of 1.0 *N* HCl. The absorbancy of each solution at 260 mμ was determined using as blanks eluates from adjacent areas of the chromatograms as described by Khorana.[13] The yield of material in each area was calculated in terms of per cent of total eluted ultraviolet-absorbing material. DAPP was not detected by this procedure and was ignored in the calculations. Typical results are given in Table I.

Structure of a Di-*O*-benzylidene-3-*O*-methyl-D-glucitol[1]

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The di-*O*-benzylidene compound obtained by condensing 3-*O*-methyl-D-glucitol with benzaldehyde at room temperature in the presence of sulfuric acid as a catalyst is demonstrated to be 2,4:5,6-di-*O*-benzylidene-3-*O*-methyl-D-glucitol. The formulation is in agreement with the theoretical prediction that 3-*O*-methyl-D-glucitol would form a relatively strong β C-ring and a weaker α C-ring on condensation with benzaldehyde.

In a previous communication[2] it was reported that 3-*O*-methyl-D-glucitol, a sirup, could be obtained as a crystalline di-*O*-benzylidene derivative by condensing it with benzaldehyde in the presence of sulfuric acid as a catalyst. The positions of the benzylidene residues however, were not determined.

Apart from the general interest in determining the structure of the compound that results from the reaction between 3-*O*-methyl-D-glucitol and benzaldehyde, the structure is of interest in that it reflects a preferential reaction of benzaldehyde with four of the five available specifically oriented hydroxyl groups in the 3-*O*-methyl-D-glucitol molecule.

The structure of the di-*O*-benzylidene-3-*O*-methyl-D-glucitol was determined as follows. The free hydroxyl

(1) This investigation was supported in part by Public Health Service Research Career Program Award (No. 5-K3-GM-19, 470-01).

(2) F. A. H. Rice and A. R. Johnson, *J. Am. Chem. Soc.*, **81**, 4419 (1959).

group was methylated by reaction of the sodium salt with methyl iodide. The resulting nicely crystalline di-*O*-benzylidenedi-*O*-methyl-D-glucitol, on treatment with aqueous acetic acid, yielded a mono-*O*-benzylidenedi-*O*-methyl-D-glucitol which consumed 1 molar equiv. of periodate. The products of the periodate cleavage were formaldehyde and a crystalline compound which analyzed correctly for a monobenzylidenedimethyl-*aldehydo*-pentose monohydrate. The di-*O*-methylpentose was obtained by removing the benzylidene group with aqueous acetic acid. The di-*O*-methylpentose was a sirup and had an optical rotation equal and opposite to that reported for 3,5-di-*O*-methyl-D-xylose.[3] The melting point of the *p*-bromophenylosazone agreed with that reported for 3,5-di-*O*-methyl-D-xylose *p*-bromophenylosazone.[3,4] The optical rotation was, however, opposite in sign. The di-*O*-methylpentose must therefore be 3,5-di-*O*-methyl-L-xylose.

The identification of the di-*O*-methylpentose as 3,5-di-*O*-methyl-L-xylose is strengthened by the fact that in order to obtain a benzylidenedi-*O*-methylpentose from the periodate oxidation of a benzylidenedi-*O*-methyl-D-glucitol in which one of the methoxy groups is on C-3, oxidation must take place either between C-1 and C-2 or between C-5 and C-6. In the first case a benzylidenedi-*O*-methyl-D-arabinose with one of the methoxy groups on C-2 would result. Oxidation between C-5 and C-6 would lead to the formation of a benzylidenedi-*O*-methyl-L-xylose with one of its methoxy groups on C-3. The di-*O*-methylpentose has to be therefore a di-*O*-methyl-L-xylose with one of its methoxy groups on C-3 or a di-*O*-methyl-D-arabinose with one of its methoxy groups on C-2. Of the possibilities only 3,5-di-*O*-methyl-L-xylose and its derivatives have reported melting points and optical rotations in agreement with those found.

Insofar as only the 2- and 4-hydroxyl groups on the di-*O*-methylpentose are available for condensation with benzaldehyde, the structure of the *aldehydo*-di-*O*-methylpentose is 2,4-benzylidene-3,5-di-*O*-methyl-*aldehydo*-L-xylose. It follows therefore, that the benzylidenedi-*O*-methyl-D-glucitol must be 2,4-benzylidene-1,3-di-*O*-methyl-D-glucitol, and the di-*O*-benzylidenedi-*O*-methyl-D-glucitol must be 2,4:5,6-di-*O*-benzylidene-1,3-di-*O*-methyl-D-glucitol. The di-*O*-benzylidene derivative formed by the reaction of 3-*O*-methyl-D-glucitol with benzaldehyde is therefore 2,4:5,6-di-*O*-benzylidene-3-*O*-methyl-D-glucitol.

It is of interest that the structure of the di-*O*-benzylidene-3-*O*-methyl-D-glucitol is in agreement with that predicted by the rules of Hann and Hudson.[5] From a consideration of these rules it might be expected that a β C-ring[6] would be favored and hence a relatively stable 2,4-benzylidene acetal would result. With the formation of the β C-ring the presence of the methoxyl group on C-3 would prevent the formation of the 1,3-acetal or β C-ring and only the α C-ring or 5,6-benzylidene acetal remains possible. The latter or α C-ring configuration should be much less stable than the β C-ring and, indeed, it is the α C-ring that is the most readily broken by aqueous acetic acid to leave the acetal with the β C-ring configuration.

(3) R. A. Laidlaw, *J. Chem. Soc.*, 2941 (1952).
(4) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 331 (1933).
(5) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 1909 (1944).
(6) The nomenclature used is that proposed by S. A. Barker and E. J. Bourne [*Advan. Carbohydrate Chem.*, **7**, 137 (1952)].

Experimental

Methylation of Di-*O*-benzylidene-3-*O*-methyl-D-glucitol.—Two grams of di-*O*-benzylidene-3-*O*-methyl-D-glucitol, m.p. 130–131°, $[\alpha]^{20}D$ +24° (*c* 3, EtOH), was dissolved in 50 ml. of anhydrous diethyl ether and treated with an excess of sodium metal (0.4 g.). The mixture was allowed to react at room temperature for 2 hr. and was then heated under reflux for 3 hr. At the end of this time the evolution of gas had ceased. The mixture was then concentrated to dryness under reduced pressure at 40–50° and the resulting solid mass was treated with an excess (40 ml.) of methyl iodide, heated under reflux for 4 hr., and allowed to stand at room temperature overnight. The mixture was then concentrated to dryness under reduced pressure at 40–50° and the solid mass was extracted three times with 25-ml. portions of anhydrous diethyl ether. The ether extracts were combined and shaken in a separatory funnel five times with 5-ml. portions of distilled water. The ether phase was then dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under reduced pressure at 40–50°. The resulting product was crystallized from 95% ethanol. After two recrystallizations from the same solvent the product (yield about 2 g.) had m.p. 99–100°,[7] $[\alpha]^{20}D$ +29.2° (*c* 1, EtOH), and analyzed correctly for a di-*O*-benzylidenedi-*O*-methyl-D-glucitol.

Anal. Calcd. for $C_{22}H_{26}O_6$: C, 68.37; H, 6.79; OMe, 16.06. Found: C, 68.09; H, 6.99; OMe, 16.64.

Preparation of the Mono-*O*-benzylidenedi-*O*-methyl-D-glucitol.—An amount of 1.8 g. of the di-*O*-benzylidenedi-*O*-methyl-D-glucitol was suspended in 10 ml. of a 1:1 (v./v.) mixture of glacial acetic acid and water and dissolved by heating the mixture in a water bath at 90° for 1 hr. The solution was allowed to cool to room temperature and was then extracted twice in a separatory funnel with 20-ml. portions of diethyl ether. The ether solution was washed three times with 5-ml. portions of water, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under reduced pressure at 40–50°. The resulting sirup was crystallized from 95% ethanol: yield about 250 mg., m.p. 160–161°, $[\alpha]^{20}D$ +20.9° (*c* 1, EtOH).

Anal. Calcd. for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43; OMe, 20.81. Found: C, 60.41; H, 7.33; OMe, 21.04.

The aqueous acetic acid phase was concentrated to dryness under reduced pressure at 40–50° and yielded a glassy material that could not be obtained crystalline. The sirup showed approximately the correct analysis for a di-*O*-methyl-D-glucitol. It was probably contaminated with some mono-*O*-benzylidenedi-*O*-methyl-D-glucitol.

Anal. Calcd. for $C_8H_{16}O_6$: C, 45.70; H, 8.63; OMe, 29.93. Found: C, 48.74; H, 8.92; OMe, 33.98.

The solidified sirup was optically inactive at a concentration of 20 mg. in 2 ml. of ethanol in a 1-dm. tube. It was not investigated further.

Reaction of Sodium Metaperiodate with the Mono-*O*-benzylidenedi-*O*-methyl-D-glucitol.—The mono-*O*-benzylidenedi-*O*-methyl-D-glucitol was treated with sodium metaperiodate in the usual manner[8] and was found to consume after 5 hr. 1 mole of periodate per mole of compound and to liberate formaldehyde. The other product of the reaction was isolated in the following manner. The mono-*O*-benzylidenedi-*O*-methyl-D-glucitol (100 mg.) was dissolved in 10 ml. of water containing 200 mg. of sodium metaperiodate and allowed to stand at room temperature for 5 hr. The aqueous solution was then extracted with ten 10-ml. portions of chloroform in a separatory funnel. The chloroform was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under reduced pressure at 40–50°. The resulting sirup crystallized from ether–petroleum ether (b.p. 40–60°) weighed approximately 75 mg., m.p. 114–115°. A solution of 14 mg. in 2 ml. of chloroform showed no optical activity. The compound gave the correct analysis for the monohydrate of a mono-*O*-benzylidenedi-*O*-methyl-*aldehydo*-pentose.

Anal. Calcd. for $C_{14}H_{18}O_5 \cdot H_2O$: C, 59.14; H, 7.09; OMe, 21.83. Found: C, 59.42; H, 7.08; OMe, 22.53.

The compound, when treated with hydroxylamine hydrochloride and sodium acetate in the usual manner, formed an oxime,

(7) All melting points were taken on the Kofler melting point block.
(8) E. L. Jackson, *Org. Reactions*, **2**, 314 (1944).

m.p. 135–136°, after recrystallization from 50% (v./v.) aqueous ethanol.

Hydrolysis of the Monohydrate of the Mono-*O*-benzylidenedi-*O*-methyl-*aldehydo*-pentose to Form 3,5-Di-*O*-methyl-L-xylose.—The mono-*O*-benzylidenedi-*O*-methyl-*aldehydo*-pentose monohydrate (80 mg.) was suspended in 2 ml. of aqueous acetic acid (1:1 by volume) contained in a flask fitted with a reflux condenser and the mixture was heated in a boiling water bath for 4 hr. At the end of this time no solid particles could be seen; the solution, however, had a cloudy appearance and had an odor of benzaldehyde. The solution was cooled to room temperature, diluted with 5 ml. of water, and extracted four times with 10-ml. portions of ether to remove the liberated benzaldehyde. The aqueous solution was then evaporated to dryness under reduced pressure at 40–50°. The resulting sirup, when dried under high vacuum over P_2O_5 overnight, weighed 50 mg. It analyzed for a di-*O*-methylpentose and had an optical rotation of $[\alpha]^{20}D$ −11° (*c* 1, $CHCl_3$). The optical rotation is of the same magnitude but opposite in sign to that reported for 3,5-di-*O*-methyl-D-xylose.[3,4]

Anal. Calcd. for $C_7H_{14}O_5$: C, 47.18; H, 7.92; OMe, 34.84. Found: C, 47.21; H, 7.85; OMe, 34.02.

Preparation of the *p*-Bromophenylosazone of the 3,5-Di-*O*-methyl-L-xylose.—The 3,5-di-*O*-methyl-L-xylose (36 mg.) was treated after the manner of Levene and Raymond[4] with 120 mg. of *p*-bromophenylhydrazine in 2 ml. of aqueous acetic acid (1:1 v./v.). The solution was heated in the boiling water bath for 0.5 hr., then cooled. The thick oil that had separated was recovered by decanting off the aqueous phase. The oil was then crystallized from aqueous methanol, m.p. 107°, $[\alpha]^{20}D$ +30° (*c* 1, C_5H_5N–EtOH, 2:3 v./v.) after 12 hr.

Anal. Calcd. for $C_{19}H_{22}Br_2N_4O_3$: N, 10.89; OMe, 12.07. Found: N, 10.70; OMe, 11.81.

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An Improved, Stereoselective Synthesis of 2-Amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (Muramic Acid)[1]

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Reaction of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside with an excess of DL-chloropropionic acid in the presence of sodium hydride gave 76% of crystalline benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (muramic acid derivative) and 1–3% of the 3-*O*-(L-1-carboxyethyl) derivative (isomuramic acid derivative). Removal of the benzylidene group by weak acid hydrolysis gave benzyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside, which forms easily a lactone at C-4. Hydrogenolysis of the benzyl group of the free acid gave 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (*N*-acetylmuramic acid) in 92% yield, from which 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (muramic acid) could be obtained in 70% yield.

2-Amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose or muramic acid (IX) has been found to be a constituent of numerous cell walls of microorganisms.[3] It was first isolated in 1956 by Strange and Dark[4] and it was first synthesized by Strange and Kent.[5] This synthesis and the following ones are based on the formation of an ether link between D-lactic acid and the hydroxyl group at C-3 of 2-amino-2-deoxy-D-glucose (D-glucosamine). Most syntheses start from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside; after formation of the sodium salt at C-3, this compound is condensed with various α-halogenopropionic acid derivatives. The use of racemic α-halogenopropionic acid derivatives leads to the formation of the D- and L-3-ethers (muramic and isomuramic acid derivatives) and necessitates a chromatographic separation which is carried out either at the final stage on the mixture of muramic and isomuramic acid,[5] or on the mixture of the methyl 2-acetamido-2-deoxyglycosides.[6] Both methods give low yields since many reactions are carried out before final purification. By the use of an L-α-chloropropionic acid derivative, formation of the isomuramic acid derivative is avoided, and improved yields are given. The preparation of the required reagent is, however, cumbersome.[7,8] The chromatographic separation of the isomers can also be sidestepped by use of the alloxazine derivative of 2-benzamido-2-deoxy-5,6-*O*-isopropylidene-D-glucofuranose as starting material and separating the D- and L-isomers by fractional crystallization.[9,10]

Since both the starting material and the condensing agent possess an asymmetric structure, conditions in which formation of one of the isomers would be preferred were investigated. Therefore a careful separation of the two resulting isomers was made by chromatography at the stage of the benzylidene methyl ester derivatives. Variations of the nature of the aglycone showed the influence of the group attached at C-1 on the stereoselectivity of the reaction at C-3.[2] Thus, when the benzyl α-D-glycoside was used the reaction was practically stereospecific giving in high yield the known benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(methyl D-1-ethylcarboxylate)-α-D-glucopyranoside (IV).[11] The fact that practically only one of the two isomers of DL-chloropropionic acid takes part in the reaction presents a stereoselectivity of a degree which

(1) Amino Sugars XLII. This is publication no. 385 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston, Mass. This investigation was supported by a research grant from the National Science Foundation (Grant 9-2312). A preliminary communication has been published.[2]

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