Immunopolysaccharides. Part III. The Dimethyl Ethers of* L-*Rhamnopyranose.*

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[Reprint Order No. 6046.]

The nature of a di-*O*-methyl-L-rhamnopyranose, obtained from a methyl ether of the *Pneumococcus* Type II specific polysaccharide by hydrolysis, has been investigated. It was distinguished from the known 2 : 3- and 3 : 4-di-*O*-methyl-L-rhamnopyranose by chemical and other studies and was identified as the one remaining structural isomer, 2 : 4-di-*O*-methyl-L-rhamnose. Its synthesis from methyl 4-*O*-methyl-α-L-rhamnopyranoside has been achieved by a method involving the formation and controlled methanolysis of trifluoroacetyl derivatives.

L-Rhamnose was first recognised as a constituent sugar of the *Pneumococcus* Type II specific polysaccharide by Stacey (*Quart. Rev.*, 1947, **1**, 217) in 1947, and since that time its presence has been confirmed by Beiser, Kabat, and Schor (*J. Immunol.*, 1952, **69**, 297) and by Butler and Stacey (following paper) who have carried out a preliminary structural investigation of the polysaccharide. From the products of hydrolysis of the methylated polysaccharide there was isolated a di-*O*-methyl-L-rhamnose (A), further methylation of which followed by acidic hydrolysis gave the known 2 : 3 : 4-tri-*O*-methyl-L-rhamnose which established that the diether was unsubstituted at $C_{(5)}$. In order that the diether might be assigned a definite structure comparative studies were made with the known 2 : 3- and 3 : 4-di-*O*-methyl-L-rhamnose and are described below.

3 : 4-Di-*O*-methyl-L-rhamnose was obtained by the method of Haworth, Hirst, and Miller (*J.*, 1929, 2469) while for the synthesis of the 2 : 3-derivative reference was made to the work of Brown, Hough, and Jones (*J.*, 1950, 1125). Methyl 4-*O*-benzoyl-2 : 3-di-*O*-methyl-α-L-rhamnopyranoside, prepared by complete methylation of methyl 4-*O*-benzoyl-α-L-rhamnopyranoside with silver oxide and methyl iodide, was hydrolysed with aqueous-alcoholic alkali. The resulting glycoside gave 2 : 3-di-*O*-methyl-L-rhamnose in good yield on acidic hydrolysis, and it was shown to contain a minute amount of the 3 : 4-diether owing presumably to migration of the benzoyl group during methylation. This will be the subject of a subsequent communication. The 2 : 3-diether was purified by conversion into the crystalline aniline derivative (Percival and Percival, *J.*, 1950, 690) and regeneration on treatment of the latter with benzaldehyde.

* Part II, *J.*, 1954, 2395.

The dimethylrhamnose (A) and 3 : 4-di-*O*-methyl-L-rhamnose had the same melting point and the melting points of mixtures of the two were variable. However, appreciable differences between the specific rotations of the three diethers were found. On the paper chromatogram 2 : 3-di-*O*-methyl-L-rhamnose was readily distinguished by its slow rate of movement and red colour reaction with aniline trichloroacetate reagent (Brown *et al.*, *loc. cit.*). Mixtures of the 2 : 3-diether with each of the other two were separated in a reasonably satisfactory way for, although slight overlapping of the spots sometimes occurred, the component sugars could be recognised by their distinctive colours (A, brown; 3 : 4-, green-brown). On the other hand but little separation of (A) and the 3 : 4-diether could be achieved. The use of paper ionophoresis in borate buffer (Foster, *Chem. and Ind.*, 1952, 828) led to rapid and complete separation of 3 : 4-di-*O*-methyl-L-rhamnose from the other two ethers.

Confirmation that ether (A) presented no contiguous hydroxyl groups was given by its failure to reduce sodium metaperiodate. Under similar conditions the 3 : 4-diether consumed 1 mol. of the oxidising agent, a result in agreement with the value given by Hirst, Hough, and Jones (*J.*, 1949, 3145) but at variance with that of Brown *et al.* (*loc. cit.*) (0·7 mol.). The latter group state that 2 : 3-di-*O*-methyl-L-rhamnose is oxidised to the extent of 0·1 mole/mole.

Properties of the di-O-methyl-L-rhamnopyranoses and their derivatives.

	Free sugar							*N*-Phenylglycosylamine		2 : 4-Dinitrophenylhydrazone	
L-Rhamnose ether		M. p.	$[\alpha]_D^{20}$ in H_2O	R_G	Colour *	M_G	P †	M. p	$[\alpha]_D^{20}$ in EtOH	M. p.	$[\alpha]_D^{20}$ in dioxan
2 : 3-Me_2	Syrup	—	44·3°	0·83	Red	0·02	0·0	136—137°	147·8 → 42·8	168°	45·4°
2 : 4-Me_2	Hygroscopic solid	91—93°	10·6	0·87	Brown	0·02	0·0	141—142·5°	136 → 4	164—165	39·0
3 : 4-Me_2	Needles	91—92	18·0 (equm)	0·88	Green-brown	0·4	1·0	—	—	170	75·6

All rotations are positive in degrees.

* On development with aniline trichloroacetate. † Periodate consumed (mole/mole).

The three diethers formed crystalline 2 : 4-dinitrophenylhydrazones having very similar melting points : these were differentiated by their mixed melting points and their specific rotations. The ether (A) furnished a *N*-phenylglycosylamine which was distinguished from the corresponding derivative of 2 : 3-di-*O*-methyl-L-rhamnose by its optical rotational behaviour in methanol and by the mixed melting point. No crystalline *N*-phenylglycosylamine could be prepared from the 3 : 4-diether nor a crystalline phenylosazone from ether (A).

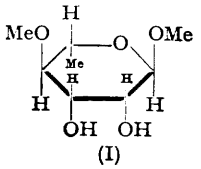
From these results, which are summarised in the Table, it was evident that the ether (A) was the other possible isomeric di-*O*-methylrhamnopyranose, *viz.*, the 2 : 4-derivative. This conclusion was supported by a synthesis of the sugar achieved as described below and already briefly described (Butler, Lloyd, and Stacey, *Chem. and Ind.*, 1954, 107).

Methyl 2 : 3-*O*-*iso*propylidene-α-L-rhamnopyranoside was prepared by Levene and Muskat's method (*J. Biol. Chem.*, 1934, **105**, 431) employing sulphuric acid and anhydrous copper sulphate as condensing agents. Use of zinc chloride gave lower yields but in one experiment the product was obtained crystalline, with m. p. 36°, $[\alpha]_D^{20}$ −15·9° (*c*, 1·6 in acetone), which has not been previously reported. Methylation with methyl iodide and silver oxide, followed by removal of the ketal grouping with methanolic hydrogen chloride, yielded hygroscopic prisms of methyl 4-*O*-methyl-α-L-rhamnopyranoside (I), from which with trifluoroacetic anhydride in the presence of sodium trifluoroacetate (Bourne, Tatlow, and Tatlow, *J.*, 1950, 1367) methyl 4-*O*-methyl-2 : 3-bis-*O*-trifluoroacetyl-α-L-rhamnopyranoside (II) was obtained in good yield.

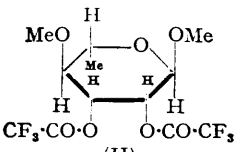
In its reaction with methanol the trifluoroacetate (II) resembled other trifluoroacetates previously reported (Bourne, Tatlow, and Tatlow, *loc. cit.*), dissolution in the alcohol

leading to complete deacetylation with quantitative regeneration of the ether (I). The de-esterification was remarkably rapid and, as indicated polarimetrically, was complete after about 25 min. at room temperature. The method of partial removal of trifluoroacetyl employed by Bourne, Stacey, Tatlow, and Tatlow (*J.*, 1951, 826) was inapplicable to synthesis of the 3 : 4-dimethyl ether owing to the high solubility in alcohol of the methyl rhamnosides. Attempts to precipitate a monotrifluoroacetate from various alcoholysis reagents with light petroleum or carbon tetrachloride were unsuccessful. Trial experiments indicated that very little methanolysis occurred in the presence of a large excess (6 volumes) of carbon tetrachloride from which the alcohol could be rapidly removed by distillation under reduced pressure.

When de-esterification was conducted in methanol–carbon tetrachloride (65 : 35 v/v), the final product, recovered by evaporation, was again methyl 4-*O*-methylrhamnoside (I). When the methanolysis was effectively terminated at the point of maximum optical rotation, by pouring into a large volume of carbon tetrachloride, there was obtained, on evaporation of the solvent, a syrup which had the methoxyl content of a monotrifluoroacetate (III). During the early stages of methanolysis, starting material could be isolated from the reaction mixture. As deacylation proceeded this became more difficult and after a time only syrups could be obtained. This point was always reached well before the mixture had attained maximum optical rotation and, since the diester (II) was normally readily crystallisable, even in the presence of an equal weight of the diol (I), much more rapid de-esterification of the diester than of the monotrifluoroacetate(s) was again indicated. From the form of the methanolysis curve it seemed likely that the monoester obtained was essentially a single molecular species rather than a mixture of the 2- and the 3-monotrifluoroacetate. The methanolysis curve was reproducible and curves of similar form were recorded on replacement of the carbon tetrachloride with other diluents such as chloroform or ether, the rate of methanolysis being related to the concentration of methanol employed. It was clear that when pure methanol was used reaction occurred so rapidly that the initial increase in optical rotation could not be accurately recorded.



(I)



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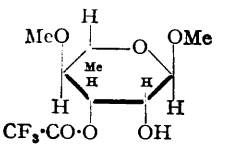
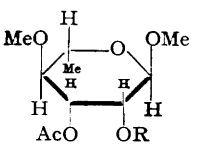


(III)



(IV); R = H
(V); R = Me

The monotrifluoroacetate (III) was acetylated with pyridine and acetic anhydride, and methanolysis of the product afforded syrupy methyl 3-*O*-acetyl-4-*O*-methyl-α-L-rhamnoside (IV). This was converted into methyl 3-*O*-acetyl-2 : 4-di-*O*-methyl-α-L-rhamnoside (V) by silver oxide and methyl iodide and then, after Zemplen deacetylation, into the reducing sugar by acidic hydrolysis. The main product, as determined chromatographically, was a dimethyl-L-rhamnose and this was separated from traces of 4-*O*-methyl- and tri-*O*-methyl-L-rhamnose by chromatography on paper strips. It was predominantly 2 : 4-di-*O*-methyl-L-rhamnose but ionophoresis (Foster, *loc. cit.*) revealed that a small amount of 3 : 4-di-*O*-methyl-L-rhamnose was also present.

On treatment with hot ethanolic aniline the product formed crystalline 2 : 4-di-*O*-methyl-*N*-phenyl-L-rhamnosylamine identical with that derived from the methylated *Pneumococcus* Type II polysaccharide. On acid hydrolysis the rhamnosylamine yielded a product showing M_G 0·02 and no trace of 3 : 4-di-*O*-methyl-L-rhamnose (M_G 0·4). The method of synthesis precluded the formation of any 2 : 3-di-*O*-methyl-L-rhamnose; the structure assigned to the dimethyl rhamnose from the Type II *Pneumococcus* was thus fully confirmed.

The above synthesis was not quite so unequivocal as that of Bourne, Stacey, Tatlow, and Tatlow (*loc. cit.*) in the glucose series. An analogous reaction sequence was involved and we consider the mechanism of the displacement of the residual trifluoroacetyl groups

from $C_{(3)}$ to $C_{(2)}$ is the same. Of the possible mechanisms by which removal of trifluoroacetyl (methanolysis) could proceed, the rate-controlling step would be a nucleophilic attack by MeO^- or $MeO^{\delta-}$, $H^{\delta+}$. This would be aided by an electrophilic substituent at the α-position in the alcohol. Thus methanolysis would be expected to proceed more rapidly at $C_{(2)}$, to which is attached the strongly electronegative, potentially reducing group, than at $C_{(3)}$. This interpretation would also explain the relatively high stability of monotrifluoroacetates compared with that of polyesters with adjacent trifluoroacetyl groups (Bourne, Tatlow, and Tatlow, *loc. cit.*).

The monotrifluoroacetate obtained by selective methanolysis of the diester (II) is regarded as having the structure, methyl 4-*O*-methyl-3-*O*-trifluoroacetyl-α-L-rhamnoside (III). Thus in order to obtain the monoacetate (V), and finally the 2 : 4-diether (A), migration of an acyl group must occur at some stage. This may have taken place during methylation (IV ⟶ V) but it is considered more likely that transposition of the trifluoroacetyl group occurred during acetylation of (III), as has been observed in the glucose series.

It is now recognised that the spatial orientation of substituents in pyranoside sugars may be an important factor in determining rates of interaction (cf. Foster, Martlew, and Stacey, *Chem. and Ind.*, 1953, 825). Even in unimolecular reactions steric effects may be very considerable (Newth and Phillips, *J.*, 1953, 2904) and it is likely that the rates of methanolysis of carbohydrate trifluoroacetates are in some measure governed by stereochemical factors. However, until a wider, quantitative study has been made, not only of reaction rates but also of reaction mechanism, analysis in such terms of the cases under discussion would be premature.

Experimental

Unless otherwise stated, all operations involving trifluoroacetates were conducted with dry reagents under anhydrous conditions.

2 : 3-*Di*-O-*methyl*-N-*phenyl*-L-*rhamnosylamine*.—Slightly impure 2 : 3-di-*O*-methyl-L-rhamnose prepared by essentially the method of Brown, Hough, and Jones (*loc. cit.*) was converted according to Percival and Percival's directions (*loc. cit.*) into the *N*-phenylrhamnosylamine, m. p. 136—137°, $[\alpha]_D^{20}$ +147·8° ⟶ +42·8° in 70 hr. (*c*, 0·4 in EtOH) (Found : C, 63·2; H, 7·8; N, 5·4. Calc. for $C_{14}H_{21}O_4N$: C, 62·9; H, 7·9; N, 5·2%). Percival and Percival gave m. p. 138—139°.

2 : 3-*Di*-O-*methyl*-L-*rhamnose*.—The above derivative (0·036 g.) was treated with water (6 ml.) and freshly distilled benzaldehyde (0·066 g.) with stirring by carbon dioxide; the mixture was heated at 90° for 1½ hr., cooled, and extracted with ether. Evaporation of the aqueous solution gave syrupy 2 : 3-di-*O*-methyl-L-rhamnose, which was chromatographically and ionophoretically homogeneous and had $[\alpha]_D^{21}$ +44·3° (*c*, 0·8 in H_2O). Schmidt, Plankenhorn, and Kubler (*Ber.*, 1942, **75**, 579) gave $[\alpha]_D$ +47·7°, Percival and Percival $[\alpha]_D$ +40°, and Brown, Hough, and Jones $[\alpha]_D$ +42·5°.

Properties of 2 : 3-, 2 : 4-, *and* 3 : 4-*Di*-O-*methyl*-L-*rhamnose*.—The three compounds were examined together on paper chromatograms, the mobile phase being the organic layer of *n*-butanol–ethanol–water (4 : 1 : 5), and the developer aniline trichloroacetate. For R_G values and colours see the Table, which includes M_G values obtained by ionophoresis in borate buffer at pH 10 (Foster, *loc. cit.*).

Weighed samples of 2 : 4- and 3 : 4-di-*O*-methyl-L-rhamnose were treated with standard (0·25M or 0·15M) sodium metaperiodate at room temperature. The extent of oxidation was determined by treatment with excess of sodium hydrogen carbonate followed by potassium iodide solution and titration of the liberated iodine with sodium arsenite (Muller and Friedberger, *Ber.*, 1902, **35**, 2652). 3 : 4-Di-*O*-methyl-L-rhamnose utilised 1·00, 1·03 mols. after 3 hr. and 1·00 mol. after 3·5 hr., and 2 : 4-di-*O*-methyl-L-rhamnose 0·01 mol. after 3 or 5 hr. Control determinations were in close agreement.

2 : 4-*Di*-O-*methyl*-L-*rhamnose* 2 : 4-*Dinitrophenylhydrazone*.—2 : 4-Di-*O*-methyl-L-rhamnose (0·120 g.) was heated with 2 : 4-dinitrophenylhydrazine (0·065 g.) in 50% (v/v) acetic acid (0·84 ml.) at 90° for 12 min. On cooling lemon-yellow needles were deposited. Water (2·1 ml.) was added, and after 3 hr. at room temperature the precipitate was filtered off and washed with water. Recrystallisation from ethanol afforded the 2 : 4-*dinitrophenylhydrazone* (0·070 g.), m. p. 164—165° (decomp.), $[\alpha]_D^{23}$ +39·0° (*c*, 1·0 in dioxan) (Found : C, 45·4; H, 5·6; N, 15·3. $C_{14}H_{20}O_8N_4$ requires C, 45·2; H, 5·4; N, 15·1%).

2 : 3-*Di*-O-*methyl*-L-*rhamnose* 2 : 4-*Dinitrophenylhydrazone*.—2 : 3-Di-*O*-methyl-L-rhamnose formed a hydrazone less readily than the 2 : 4-isomer, requiring 45 min. at 90—95° under the above conditions. The product separated as an orange oil which slowly crystallised on trituration and addition of water and recrystallised as lemon-yellow needles, m. p. 168° (decomp.), $[\alpha]_D^{23}$ +45·4° (*c*, 0·6 in dioxan), from a small volume of ethanol (Found : C, 44·9; H, 5·6; N, 15·4%).

3 : 4-*Di*-O-*methyl*-L-*rhamnose* 2 : 4-*Dinitrophenylhydrazone*.—A solution of 3 : 4-*O*-methyl-L-rhamnose (0·110 g.) in 50% acetic acid (0·94 ml.) was heated with 2 : 4-dinitrophenylhydrazine (0·075 g.) on the water-bath at 50° for 65 min. The mixture was cooled and the material in suspension (hydrazone and some unchanged hydrazine) was removed, washed with water, and recrystallised from ethanol, yielding 3 : 4-*di*-O-*methyl*-L-*rhamnose* 2 : 4-*dinitrophenylhydrazone* (0·044 g.), lemon-yellow needles, m. p. 170° (decomp.), $[\alpha]_D^{23}$ +75·6° (*c*, 1·0 in dioxan) (Found : C, 45·1; H, 5·6; N, 15·5%).

Admixture of any two of the above described 2 : 4-dinitrophenylhydrazones depressed the m. p. by about 20°.

2 : 4-*Di*-O-*methyl*-N-*phenyl*-L-*rhamnosylamine*.—Treatment of 2 : 4-di-*O*-methyl-L-rhamnose (0·170 g.) with aniline (0·120 g.) in boiling ethanol (2·0 ml.) for 4 hr. followed by removal of the solvent in a vacuum-desiccator gave a L-*rhamnosylamine* (0·076 g.), m. p. 141—142·5° after recrystallisation from light petroleum (b. p. 40—60°), $[\alpha]_D^{18}$ +136° ⟶ +4° in 24 hr. (*c*, 0·5 in EtOH) (Found : C, 62·9; H, 7·7; N, 5·3; OMe, 23·1. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·2; OMe, 23·2%).

Methyl 4-O-*Methyl*-α-L-*rhamnopyranoside*.—Methyl 4-*O*-methyl-2 : 3-*O*-*iso*propylidene-α-L-rhamnopyranoside (Levene and Muskat, *loc. cit.*) (6·4 g.) was heated in dry methanol (235 ml.), containing 1% of hydrogen chloride, under reflux for 1 hr. The solution was neutralised with silver carbonate, filtered, boiled with charcoal, filtered again, and evaporated in a vacuum to a colourless syrup. This crystallised on trituration with ether and exhaustive recrystallisation from ether–light petroleum (b. p. 60—80°) and finally from ether furnished *methyl* 4-O-*methyl*-α-L-*rhamnopyranoside* (2·5 g.) as hygroscopic white needles, m. p. 60—61°, $[\alpha]_D^{16}$ −87·3° (*c*, 1·1 in MeOH) (Found : C, 50·3; H, 8·6; OMe, 32·0. $C_8H_{16}O_5$ requires C, 50·0; H, 8·3; OMe, 32·3%).

Methyl 4-O-*Methyl*-2 : 3-*bis*-O-*trifluoroacetyl*-α-L-*rhamnopyranoside*.—Methyl 4-*O*-methyl-α-L-rhamnopyranoside (1·01 g.), when added to the gelatinous mixture of trifluoroacetic anhydride (3·3 ml.) and sodium trifluoroacetate (0·35 g.), underwent immediate vigorous reaction and required use of a reflux condenser. After 1 hr. at room temperature, the mixture was heated under reflux for 50 min., then distilled under slight vacuum with several portions of carbon tetrachloride until trifluoroacetic acid was no longer detectable. The residue was extracted with carbon tetrachloride and evaporation of the filtered extracts gave pale crystals. Prisms of *methyl* 4-O-*methyl*-2 : 3-*bis*-O-*trifluoroacetyl*-α-L-*rhamnopyranoside* (1·564 g.), obtained from light petroleum (b. p. 100—120°), had m. p. 98·5—99°, $[\alpha]_D^{16}$ −51·6° (*c*, 2·0 in $CHCl_3$) (Found : C, 37·7; H, 3·8; F, 28·3; OMe, 16·2. $C_{17}H_{14}O_7F_6$ requires C, 37·5; H, 3·7; F, 29·7; OMe, 16·2%). It could also be purified by sublimation, was soluble in the common organic solvents (but decomposed in alcohols) and insoluble in water, was stable for long periods when carefully purified and stored *in vacuo* over P_2O_5, but slowly decomposed in air.

Methanolysis.—(*a*) *In methanol*. Reaction of the bistrifluoroacetate (0·050 g.) in methanol (4·00 ml.) was followed by means of the change in optical rotation. The solvent was then removed under reduced pressure. The residual colourless methyl 4-*O*-methyl-α-L-rhamnopyranoside crystallised under ether and had m. p. and mixed m. p. 58—59° (Found : OMe, 32·8%).

(*b*) *In methanol–carbon tetrachloride* (65 : 35, *v*/*v*). Methanolysis of the diester in methanol–carbon tetrachloride (65 : 35, v/v) was followed polarimetrically. Periodically aliquot parts were mixed with 6 vols. of carbon tetrachloride and the solvents removed in a vacuum. Before maximum optical rotation had been reached, syrups or partly crystalline mixtures were obtained from which some starting material could be isolated during the early stages of methanolysis. Working up at the point of maximum rotation gave syrupy *methyl* 4-O-*methyl*-3-O-*trifluoroacetyl*-α-L-*rhamnopyranoside*, $[\alpha]_D^{21}$ −75·8° (*c*, 1·2 in $CHCl_3$) (Found : OMe, 21·7. $C_{10}H_{15}O_6F_3$ requires OMe, 21·5%). After maximum rotation had been reached the product was methyl 4-*O*-methyl-α-L-rhamnopyranoside, the yield being quantitative when the rotation had become constant.

Methyl 3-O-*Acetyl*-4-O-*methyl*-α-L-*rhamnopyranoside*.—Acetic anhydride (0·36 ml.) was added to a solution of methyl 4-*O*-methyl-3-*O*-trifluoromethyl-α-L-rhamnopyranoside (0·70 g.) in pyridine (1·8 ml.). After 2 days at room temperature the volatile components were removed by distillation and co-distillation with carbon tetrachloride under diminished pressure and the

residual syrup was dissolved in methanol–carbon tetrachloride (3 : 1, v/v) (10 ml.) and kept at room temperature until the rotation became constant, the changes being : $[\alpha]_D^{20}$ −39·4° (initial); −38·7° (0·5 hr.); −37·2° (1 hr.); −33·9° (4 hr.); −31·7° (20 hr.); −31·7° (24 hr.). After evaporation the syrup (0·54 g.) obtained contained only a trace of fluorine [sodium fusion and treatment with cerous nitrate (Simons and Ramler, *J. Amer. Chem. Soc.*, **1943**, **65**, 389)]. The *ester* had $[\alpha]_D^{21}$ −55·4° (*c*, 1·0 in $CHCl_3$) (Found : C, 49·7; H, 7·3; Ac, 19·0; OMe, 22·6. $C_{10}H_{18}O_6$ requires C, 51·3; H, 7·7; Ac, 18·4; OMe, 26·5%).

Methyl 3-O-*Acetyl*-2 : 4-*di*-O-*methyl*-α-L-*rhamnopyranoside*.—The product from the previous experiment was methylated by two successive treatments with methyl iodide and silver oxide at 45° for 16 hr. The *product* had $[\alpha]_D^{22}$ −51·1° (*c*, 0·8 in $CHCl_3$) (Found : OMe, 37·1. $C_{11}H_{20}O_6$ requires OMe, 37·5%).

Methyl 2 : 4-*Di*-O-*methyl*-α-L-*rhamnopyranoside*.—The preceding syrupy methylation product (0·25 g.) was dissolved in dry methanol, and a small piece of sodium added. After 18 hr. the specific rotation had become constant and the solution was treated with solid carbon dioxide and evaporated to dryness, yielding a viscous amber *rhamnoside* (0·205 g.), $[\alpha]_D^{22}$ −68° (*c*, 4·1 in MeOH) (Found : OMe, 46·0. $C_9H_{18}O_5$ requires OMe, 45·1%).

2 : 4-*Di*-O-*methyl*-L-*rhamnose*.—A solution of the methyl di-*O*-methyl-L-rhamnopyranoside (0·190 g.) from the above experiment in 0·5N-hydrochloric acid (14·5 ml.) was heated on a boiling-water bath for 140 min.; the optical rotation had then become constant. The solution was neutralised with silver carbonate, filtered, and evaporated at 40—45° *in vacuo*, yielding an amber syrup (0·163 g.). On the paper chromatogram three spots were detected, the main component (R_G 0·86) corresponding to di-*O*-methyl-L-rhamnose. The other two spots, of much lower intensity, were due to mono- and tri-*O*-methyl-L-rhamnose (R_G 0·68, 0·99).

The di-*O*-methyl-L-rhamnose fraction was separated chromatographically on a larger scale employing 5 sheets (30 × 57 cm.) of Whatman No. 1 filter paper. A solution of the product in methanol was applied as a line of spots along the base line of each chromatogram. After irrigation for 17 hr. with *n*-butanol–ethanol–water (4 : 1 : 5; v/v) marginal and central strips were removed and developed. Horizontal strips containing the dimethyl sugar were cut from the remainders of the papers and extracted (Soxhlet) with ether. Evaporation of the extract furnished a viscous syrup (0·078 g.) which on drying *in vacuo* (P_2O_5) crystallised as hygroscopic fibrous needles, m. p. 80° (unsharply), $[\alpha]_D$ +3° (*c*, 1·0 in EtOH). Ionophoresis revealed, in addition to 2 : 4-di-*O*-methyl-L-rhamnose, a minute amount of 3 : 4-di-*O*-methyl-L-rhamnose.

Treatment of the product (0·065 g.) with ethanolic aniline as described above led to the formation of 2 : 4-di-*O*-methyl-*N*-phenyl-L-rhamnosylamine (0·010 g. after three recrystallisations), m. p. 141—142·5° alone or mixed with the specimen derived from the methylated Type II *Pneumococcus* polysaccharide. The samples showed similar optical rotational behaviour and had identical *X*-ray powder photographs (the latter investigation was kindly made by Dr. P. Cucka) (Found : C, 62·9; H, 7·9%).

Hydrolysis. The *N*-glycosylamine (0·5 mg.) was hydrolysed by N-hydrochloric acid at room temperature for 24 hr. A spot of the solution was transferred to the base line of an ionophoresis paper and there dried in a current of cold air. Ionophoresis showed M_G 0·02. No 3 : 4-di-*O*-methyl-L-rhamnose (M_G 0·4) was detected. A preliminary experiment using 2 : 3-di-*O*-methyl-*N*-phenyl-L-rhamnosylamine had indicated that under the conditions described above hydrolysis of the anilide to the parent sugar occurred.

The authors thank Professor M. Heidelberger for helpful discussions. The expenses of the investigation were covered by grants from Imperial Chemical Industries Limited and from the special Research Fund of this University.

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[*Received, January 20th*, 1955.]