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SYNTHESIS OF SOME 5-*O*-SUBSTITUTED 3-DEOXYOCT-2-ULOSONIC ACIDS, AND THEIR RESPONSE TO THIOBARBITURATE DURING ACIDIC HYDROLYSIS

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ABSTRACT

5-*O*-(α-L-Rhamnopyranosyl)-, -(β-D-glucopyranosyl)-, -(2-acetamido-2-deoxy-β-D-glucopyranosyl)-, and -(β-D-glucopyranosyluronic acid)-3-deoxyoctulosonic acids have been synthesised in low yields by condensation of the appropriate 2-*O*-glycosyl-D-arabinose with oxalacetic acid. Their responses in the thiobarbiturate reaction during hydrolysis with acid are different and may be used for structural studies.

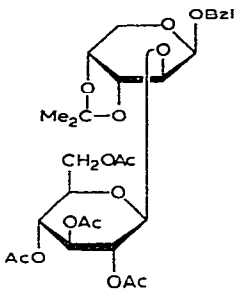
INTRODUCTION

Endotoxins of Gram-negative bacteria are considered to be lipopolysaccharides[1] in which the polysaccharide and lipid A moieties are usually linked by the ketosidic bond of 3-deoxyoctulosonic acid (KDO), which is easily cleaved by very mild (pH 3.4, 100°) hydrolysis with acid. Analysis of the polysaccharide moiety is then usually accomplished by reactions requiring treatment with more concentrated acid. 3-Deoxyaldulosonic acids are readily degraded by acid[2] through a series of reactions in which the hydroxyl functions of the aldulosonate participate. 5-*O*-Substituted 3-deoxyaldulosonic acids have been identified in several bacterial endotoxins[3,4]. It was therefore of interest to investigate the behaviour of such 3-deoxyaldulosonic acids in acidic media. The reactions of 3-deoxy-5-*O*-methyloctulosonic acid[5] allow no predictions to be made on the behaviour of 3-deoxyaldulosonic acids bearing acid-labile substituents. Hence, β-D-glucopyranosyl- (**9**), α-L-rhamnopyranosyl- (**10**), 2-acetamido-2-deoxy-β-D-glucopyranosyl- (**11**), and -β-D-glucopyranosyluronic acid) (1→5)-(3-deoxyoctulosonic acids) (**12**) were synthesised as a series having glycosidic bonds of progressively increasing stability to acidic hydrolysis. The stabilities of these disaccharides during acidic hydrolysis were examined and their behaviour in the thiobarbiturate reaction[1,7] was established.

RESULTS AND DISCUSSION

As the naturally occurring[6] 3-deoxy-D-*manno*-octulosonic acid is not easily accessible in amounts that would make it an attractive starting material for synthesis, the glycosylated 3-deoxyoctulosonic acids were obtained by condensation[7] of the appropriate 2-*O*-glycosyl-D-arabinose with oxalacetic acid in alkaline medium. We have shown by the reaction sequence used previously[8] that the condensation of the D-glucosyl derivative **8** gave a mixture of the D-*gluco* and D-*manno* isomers in the ratio of ~1:3, which it was not considered necessary to separate for the present study.

The glycosylated arabinose derivatives **1** and **2** were obtained by condensing benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside[20] with the acetylglycosyl halides of α-D-glucose and α-L-rhamnose, respectively. Removal of the protecting groups from **1** and **2** by conventional methods gave 2-*O*-β-D-glucopyranosyl-β-D-arabinose (*via* **6**) and 2-*O*-α-L-rhamnopyranosyl-D-arabinose, respectively.



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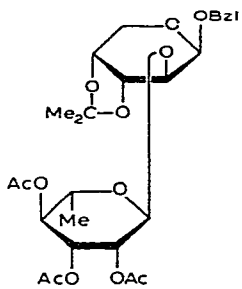

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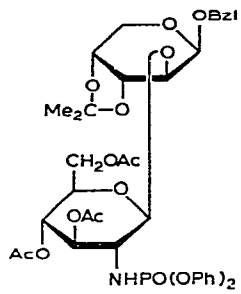

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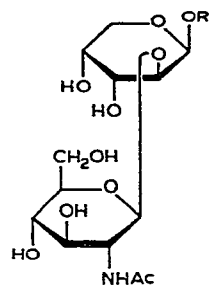

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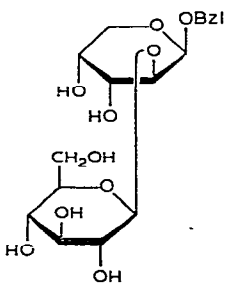
5 R = H



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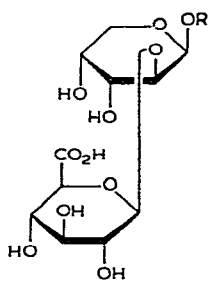
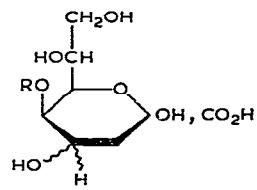


7 R = Bzl
8 R = H



9 R = β-D-glucopyranosyl
10 R = α-L-rhamnopyranosyl
11 R = 2-acetamido-2-deoxy-β-D-glucopyranosyl
12 R = β-D-glucopyranosyluronic acid

Attempted condensation of the arabinoside with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucosyl bromide failed. However, the protected disaccharide **3** was readily obtained when the arabinoside was treated with 3,4,6-tri-*O*-acetyl-2-deoxy-2-

(diphenylphosphoramido)-α-D-glucopyranosyl bromide[9]. Catalytic hydrogenation[10] failed to remove the diphenylphosphoryl group, as did the attempted transesterification to give the dibenzylphosphoramidate[11]. However, treatment of **3** with alkali[12] followed by Amberlite IR-120(H^+) resin, with *N*-acetylation of the product *in situ*, gave benzyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-arabinopyranoside (**4**). Hydrogenolysis of **4** gave the crystalline disaccharide **5**, which showed no mutarotation.

2-*O*-(β-D-Glucopyranosyluronic acid)-D-arabinose (**8**) was obtained by oxidation[13] of **6** to give **7**, followed by hydrogenolysis of the benzyl group; the aldobiouronic acid (**8**) was isolated as the amorphous ammonium salt.

The α-glycosidic linkage expected for the rhamnoside was confirmed by ^{13}C-n.m.r. spectroscopy[14]. The linkage of the glucosylarabinoside **6** was β as expected, since D-glucose was quantitatively released upon treatment with β-D-glucosidase. It follows that the (glucosyluronic acid)arabinose is also β-linked. The assignment of a β-glycosidic linkage to **4** was also based on ^{13}C-n.m.r. spectroscopy[15].

To obtain the 5-*O*-substituted 3-deoxyoctulosonic acids **9**–**12**, each of the foregoing disaccharides was condensed[16] with oxalacetate at pH 11. It is usual to monitor the formation of octulosonates by the thiobarbiturate test[17] and to stop the reaction at the time of maximal yield, but this procedure could not be followed here, because the 2-*O*-glycosylated arabinoses gave a strong red colour when treated with periodate and thiobarbiturate. The reaction was therefore arbitrarily stopped after

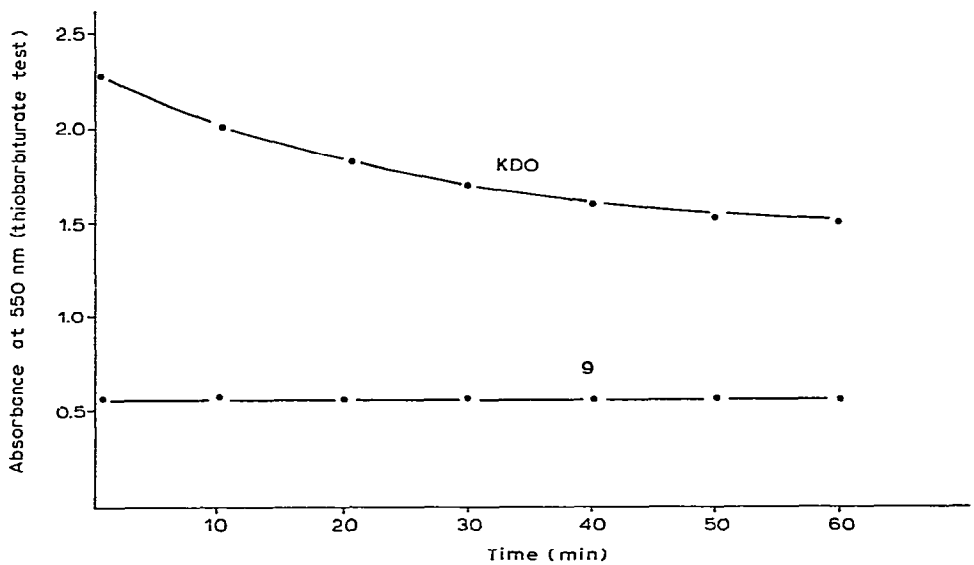


Fig. 1. Development of the response of 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO) and of 3-deoxy-5-*O*-(β-D-glucopyranosyl)oct-2-ulosonic acid (**9**) in the thiobarbiturate test during treatment with acetic acid of pH 3.4 at 100°.

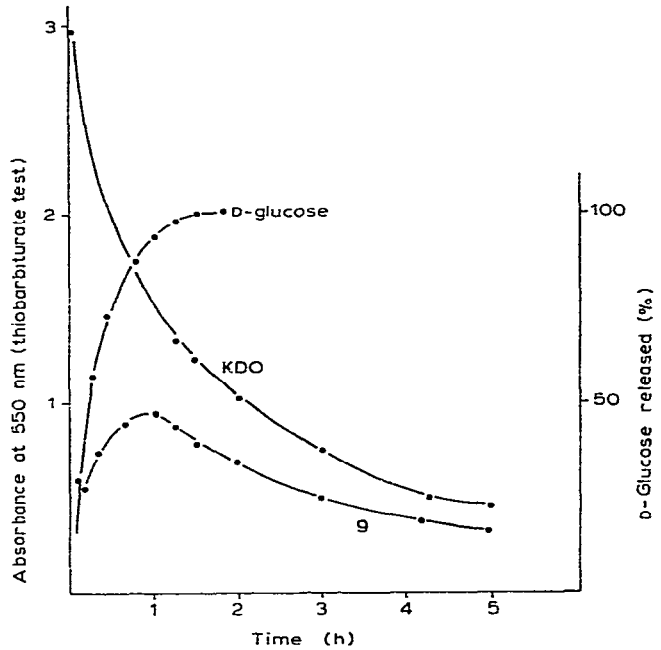


Fig. 2. Development of the response of 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO) and of 3-deoxy-5-*O*-(β-D-glucopyranosyl)oct-2-ulosonic acid (**9**) in the thiobarbiturate test during treatment with M HCl at 100°. Kinetics of the simultaneous release of D-glucose from the disaccharide.

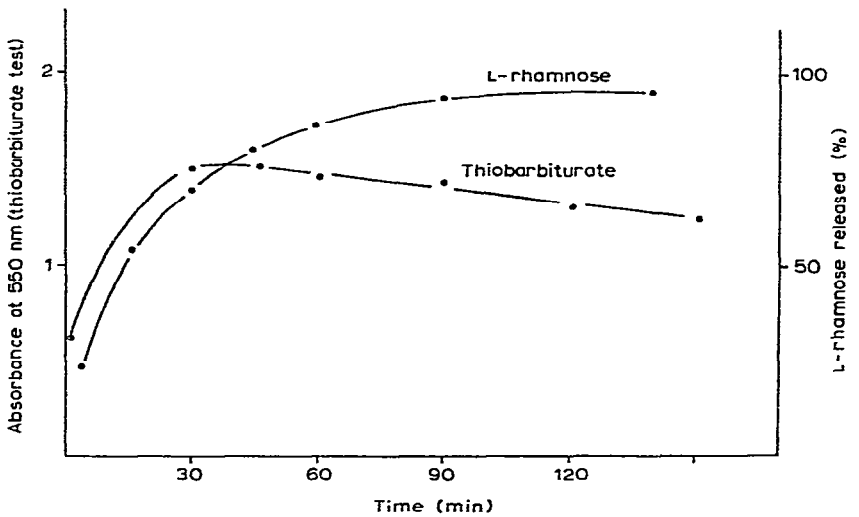


Fig. 3. Development of the response of 3-deoxy-5-*O*-(α-L-rhamnopyranosyl) oct-2-ulosonic acid (**10**) in the thiobarbiturate test during treatment with 0.1M HCl at 100°. Kinetics of the simultaneous release of L-rhamnose from the disaccharide.

2 h, as this reaction time gave the best results for the analogous synthesis of 3-deoxy-5-*O*-methyloctulosonic acid[5]. The glycosylated 3-deoxyoctulosonic acids **9–12**

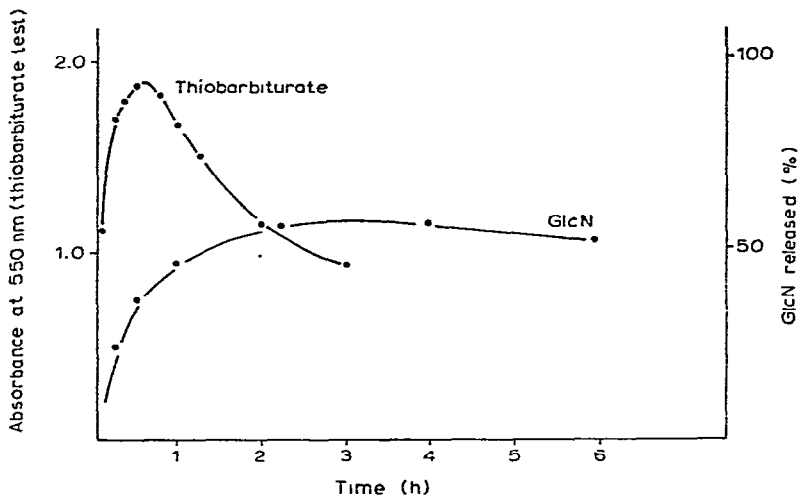


Fig. 4. Development of the response of 5-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-3-deoxyoct-2-ulosonic acid (**11**) in the thiobarbiturate test during treatment with M HCl at 100°. Kinetics of the simultaneous release of 2-amino-2-deoxy-D-glucose.

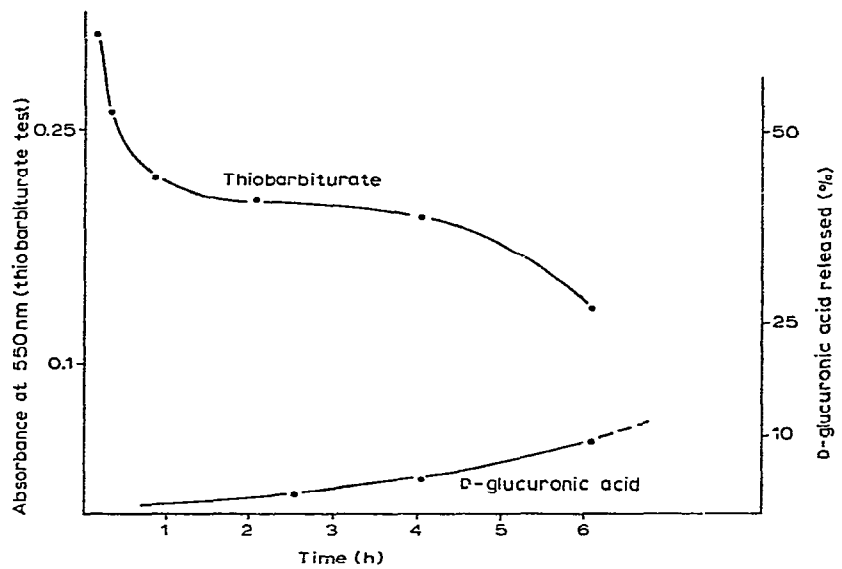


Fig. 5. Development of the response of 3-deoxy-5-*O*-(β-D-glucopyranosyluronic acid)-oct-2-ulosonic acid (**12**) in the thiobarbiturate test during treatment with M HCl at 100°. Kinetics of the simultaneous release of D-glucuronic acid.

(mixed D-*gluco* and D-*manno* isomers) were isolated by ion-exchange chromatography in yields of 7–10% as their amorphous ammonium salts.

The effect of acid on **9–12** is shown in Figs. 1–5. When 3-deoxy-D-*manno*-octulosonic acid was treated with acetic acid (pH 3.4) at 100° (the conditions generally used for the cleavage of the polysaccharide and the lipid moieties of endotoxins), its response in the thiobarbiturate reaction decreased by ~40%, whereas that of the 5-*O*-D-glucosyl (Fig. 1) and those of the other 5-*O*-glycosylated 3-deoxyoctulosonic acids remained constant. However, the molar absorption coefficient of the 5-*O*-glycosylated 3-deoxyoctulosonic acids (and of 3-deoxy-5-*O*-methyloctulosonic acid[5]) is only about one-seventh of that of the free, unsubstituted acids.

In the more stringent conditions generally used for the hydrolysis of polysaccharides, the four compounds behaved very differently, as monitored by the thiobarbiturate reaction. Thus, when treated with M HCl at 100°, the response of free KDO decreased by ~45% within 60 min. Under similar conditions, 95% of the D-glucose was released from 5-*O*-D-glucosyl-KDO and the response in the thiobarbiturate test passed through a maximum (~2.5 times the initial value), but this response was less than one-third of that given by an amount of free KDO equivalent to that present in the disaccharide and exposed to the same acidic treatment (Fig. 2).

The cleavage of the linkage in L-rhamnosyl-KDO was too fast to follow in M acid. The kinetics of the thiobarbiturate reaction and of the release of L-rhamnose on hydrolysis with 0.1M HCl at 100° are shown in Fig. 3. The maximal value of the molar absorbancy is about the same as that observed for D-glucosyl-KDO treated with M HCl; it was 2.5 times the initial value and was reached in 30 min, when only ~70% of the linkages had been cleaved. It is noteworthy that the intensity of the thiobarbiturate reaction decreased considerably more slowly in this case than with D-glucosyl- and 2-acetamido-2-deoxy-D-glucosyl-KDO when M acid was used for the hydrolysis.

Treatment of 2-acetamido-2-deoxy-D-glucosyl-KDO with M HCl at 100° cleaved ~55% of the linkages (Fig. 4); the response in the thiobarbiturate reaction had a maximum (~1.7 times the initial value) at about 30 min and returned to the initial value when the release of 2-amino-2-deoxyglucose, estimated by the Elson–Morgan reaction, had reached its final stages. The Morgan–Elson reaction indicated the presence of only negligible amounts of 2-acetamido-2-deoxyglucose.

A very different type of curve was found for the (D-glucosyluronic acid)-KDO (Fig. 5). Whereas only very small amounts of D-glucuronic acid, estimated as reducing sugar[18], were released by treatment with M HCl at 100°, the intensity of the response in the thiobarbiturate reaction decreased to about two-thirds of the initial value in 1 h, remained nearly constant for the next 3 h, and declined thereafter.

The terminal KDO molecule of a specific polysaccharide isolated from bacterial endotoxins is often substituted in position 5. The foregoing data on the monitoring of the thiobarbiturate reaction should be helpful in obtaining preliminary indications as to the nature of the penultimate sugar substituting the terminal KDO molecule. It can be expected that pentoses, hexoses, and heptoses, if present as pyranosides, will give curves similar to Fig. 1, and that 6-deoxyhexoses will resemble rhamnosyl-KDO.

On the other hand, it is likely that, in the thiobarbiturate test, the hydrolysis of α-linked 2-acetamido-2-deoxyglucosyl-KDO will have kinetics different from those of a β-linked disaccharide, on account of the much faster hydrolysis of the latter[19]. Comparisons of the thiobarbiturate reaction during the treatment with M HCl of 2-acetamido-2-deoxy-β-D-glucopyranosyl-, β-D-glucopyranosyl-, and (β-D-glucopyranosyluronic acid)-KDO are shown in Fig. 6.

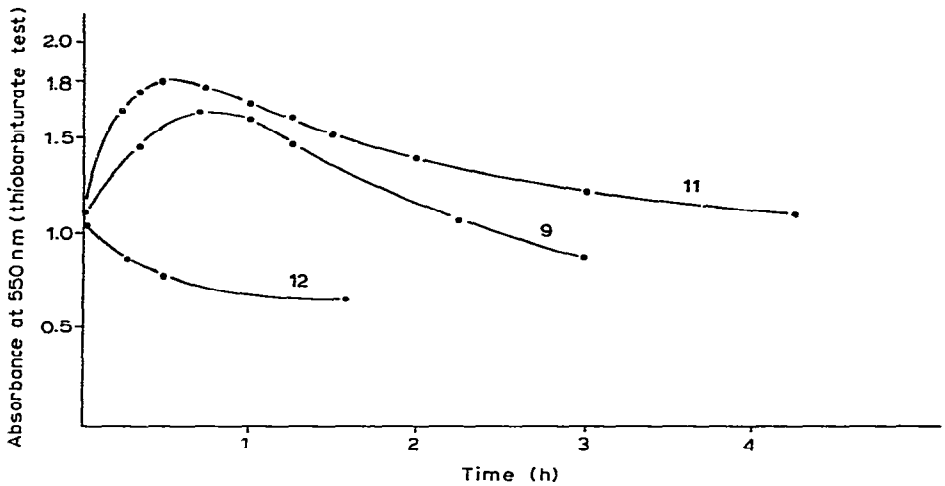


Fig. 6. Effect of M HCl at 100° on the disaccharides **9**, **11**, and **12**. At intervals, samples (0.25 ml) were removed from the equimolar solutions, cooled (0°), and successively treated with 0.5M H_2SO_4 (0.5 ml) and M NaOH (0.25 ml). 70mM $NaIO_4$ (0.5 ml) was added and, after 30 min, 2% sodium arsenite in 0.5M HCl (0.5 ml). Thiobarbiturate reagent[17] (4 ml) was added, and the mixture was kept at 100° for 10 min and then cooled. The dye formed was extracted with redistilled cyclohexanone (3.6 ml), and the absorbance was read at 550 nm against distilled cyclohexanone.

EXPERIMENTAL

Benzyl 3,4-O-isopropylidene-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-diphenylphosphoramido-β-D-glucopyranosyl)-β-D-arabinopyranoside (**3**). — To a solution of benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside[20] (2.8 g) in anhydrous benzene (30 ml) were added powdered mercuric cyanide (2.55 g), anhydrous calcium sulphate (1 g), and molecular sieve (4 Å; 1.7–2.4 mm), and the mixture was stirred for 1 h. After the addition of 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenylphosphoramido-α-D-glucosyl bromide[9] (6 g, prepared immediately before use), the mixture was stirred at room temperature for 24 h and monitored by t.l.c. (silica gel; carbon tetrachloride–ethyl acetate, 1:1). If necessary, further amounts of the bromide were added and the stirring was continued for another 24 h. Solids were collected, and washed with warm benzene (50 ml). The solution was washed with ice-cold, saturated, aqueous sodium hydrogen carbonate and water, dried, and concentrated. The residue was crystallised from ether

and then from ethanol to yield **3** (3.1 g, 38%), m.p. 143°, $[\alpha]_D^{20}$ −18° (*c* 1, chloroform) (Found: C, 58.3; H, 5.75; N, 1.7; P, 3.8. $C_{39}H_{46}NO_{15}P$ calc.: C, 58.65; H, 5.75; N, 1.75; P, 3.9%).

Benzyl 2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-arabinopyranoside (**4**). — M NaOH (125 ml) was added to a solution of **3** (2.5 g) in acetone (125 ml), and the mixture was kept first at room temperature (4 h) and then at 4° overnight. Acetone was removed, the residual aqueous solution was diluted to 600 ml with water, and its pH brought to 8 with acetic acid. A suspension of 5% acetic anhydride in water (12 ml) was added, the pH adjusted to 7.5 with saturated, aqueous sodium hydrogen carbonate, and the mixture kept at 100° for 10 min. The solution was cooled, and then deionised with Amberlite IR-120 (H^+) and Dowex 1x8 (CO_3^{2-}) resins. The columns were eluted with water until no more neutral sugars could be detected in the effluent with phenol–sulphuric acid[21]. The effluent was concentrated and the residue was crystallised from a small amount of ethanol to yield **4** (975 mg, 64%), m.p. 265°, $[\alpha]_D^{20}$ −130° (*c* 0.5, water) (Found: C, 52.2; H, 6.5; N, 3.0. $C_{20}H_{29}NO_{10}.H_2O$ calc.: C, 52.1; H, 6.7; N, 3.0%).

2-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-arabinose (**5**). — A solution of **4** (0.5 g) in water (25 ml) was adjusted to pH 3.5 with acetic acid. 10% Palladised charcoal (40 mg) was added and the mixture was stirred in an atmosphere of hydrogen overnight. After removal of the solids, the solvent was evaporated, and the residue (280 mg, yield 80%) was crystallised from aqueous ethanol to give **5**, m.p. 102°, $[\alpha]_D^{20}$ −36° (*c* 0.7, water) (Found: C, 44.0; H, 6.4; N, 3.9. $C_{13}H_{23}NO_{10}$ calc.: C, 44.2; H, 6.5; N, 4.0%).

5-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-3-deoxyoctulosonic acid (*mixed* D-gluco *and* D-manno *isomers*) (**11**). — To ice-cold water (5 ml) were added, alternately, 10M NaOH and oxalacetic acid (600 mg) so as to maintain the pH between 8 and 10 and, thereafter, finely powdered **5** (2 g). When the disaccharide had dissolved, the pH of the solution was adjusted to 11, and the mixture was stored for 2 h during which time the temperature gradually reached ~20°. The yellow solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and passed through a column (1 × 2 cm) of Dowex 1x8 (AcO^-) resin. The column was washed with water until the effluent was free of neutral sugars[21], and then eluted with 0.25M pyridinium acetate buffer (pH 3.5). Fractions (5 ml) giving a positive reaction in the Warren test were combined, adjusted to pH 6 with pyridine, and lyophilised. The slightly yellow residue was dissolved in 3 ml of water and the pH of the solution was brought to 7.5 with M ammonia. The ammonium salt of **5** (260 mg, 12%), precipitated by the addition of acetone, had $[\alpha]_D^{20}$ +17° (*c* 0.42, water) (Found: C, 38.5; H, 6.4; N, 6.1. $C_{16}H_{30}N_2O_{13}.2H_2O$ calc.: C, 38.8; H, 6.8; N, 5.8%).

Benzyl 3,4-O-isopropylidene-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-arabinoside (**2**). — To a solution of benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (2.8 g) in anhydrous benzene (30 ml) were added powdered mercuric cyanide (2.55 g), anhydrous calcium sulphate (1 g), and molecular sieve (4 Å), and the mixture was stirred. 2,3,4-Tri-*O*-acetyl-α-L-rhamnosyl bromide[22] (3.53 g) was added

and the stirred mixture was kept at room temperature for 24 h. It was then filtered, benzene (75 ml) was added, and the solution was washed successively with ice-cold water, saturated, aqueous sodium hydrogen carbonate, and water, dried, and concentrated. A solution of the residue in ethyl acetate–hexane (1:1) was passed through a column (400 × 45 mm) of silicic acid (Mallinckrodt, 100 mesh) and eluted with the same solvent. Fractions (9 ml) were collected, and those containing the disaccharide were combined and concentrated to yield **2** (4.5 g, 90%); the analytical sample (amorphous) was purified by t.l.c. on silica gel (Found: C, 58.8; H, 6.8. $C_{27}H_{36}O_{12}$ calc.: C, 58.7; H, 6.5%).

Benzyl 2-O-α-L-rhamnopyranosyl-β-D-arabinopyranoside. — A solution of **2** (4.1 g) in 0.1M methanolic sodium methoxide was kept at 0° for 45 min, and then decationised with Amberlite IR-120 (H^+) resin. The solid was collected and washed with methanol, and the solution was concentrated. The residue was treated with 5mM oxalic acid (100 ml) at 100° for 30 min. The solution was cooled, neutralised with Amberlite IR-45 (HO^-) resin, and filtered, and the filtrate and washings were concentrated. The residue was crystallised from a small amount of ethanol to give the title compound (2.6 g, 92%), m.p. 203°, $[\alpha]_D^{20}$ −188° (*c* 1.4, water) (Found: C, 56.2; H, 6.6; O, 37.3. $C_{18}H_{36}O_9$ calc.: C, 56.0; H, 6.7; O, 37.3%).

2-O-α-L-Rhamnopyranosyl-D-arabinose. — A solution of the foregoing glycoside (1 g) in acidified (pH 4) water was hydrogenated as for **4**. The title disaccharide was very hygroscopic and did not crystallise. It was homogeneous in t.l.c. using butanone–acetic acid–methanol (6:2:2), and in p.c. using butan-1-ol–acetic acid–water (4:1:5, organic phase) and butan-1-ol–pyridine–water (6:4:2) (Found: C, 43.1; H, 7.3. $C_{11}H_{19}O_9.H_2O$ calc.: C, 43.4; H, 7.2%).

3-Deoxy-5-O-(α-L-rhamnopyranosyl)octulosonic acid (**10**). — The foregoing rhamnosyl-arabinose (1.5 g) was condensed with oxalacetic acid (400 mg) as described for **11**. The amorphous ammonium salt (180 mg, 10%) had $[\alpha]_D^{20}$ −9° (*c* 0.52, water) (Found: C, 38.5; H, 6.6; N, 2.8. $C_{14}H_{27}NO_{12}.2H_2O$ calc.: C, 38.5; H, 6.9; N, 3.2%).

Benzyl 3,4-O-isopropylidene-2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-arabinopyranoside (**1**). — Benzyl 3,4-*O*-isopropylidene-β-D-arabinoside (5.6 g) was dissolved in benzene (300 ml) and nitromethane (300 ml), and two-thirds of the solvents were distilled off at atmospheric pressure. To the cooled solution was added mercuric cyanide (5.1 g) followed by acetobromoglucose (8.22 g), and the mixture was stirred for 72 h. More acetobromoglucose (2.05 g) was added and stirring was continued for 24 h. The reaction was monitored by t.l.c. (silica gel; ethyl acetate–benzene–methanol, 35:15:10). Benzene was added (500 ml), the solids were removed, and the filtrate and washings were sequentially extracted with ice-cold, saturated, aqueous sodium hydrogen carbonate and water, and then dried and concentrated. The residue was crystallised from ether and then from ethanol to yield **1** (7.6 g, 63%), m.p. 154°, $[\alpha]_D^{20}$ −105° (*c* 1.5, chloroform) (Found: C, 56.9; H, 6.2; O, 36.8. $C_{29}H_{38}O_{14}$ calc.: C, 57.0; H, 6.2; O, 36.8%).

2-O-β-D-Glucopyranosyl-β-D-arabinose. — A solution of **4** (3.05 g) in methanolic sodium hydroxide (0.1M, 300 ml) was stirred at 4° for 18 h and then passed through a

column of Amberlite IR-120 (H^+) resin, and the effluent and washings were concentrated. The residue was dissolved in oxalic acid (5mM, 150 ml) and hydrogenated over 10% palladium-on-carbon. The mixture was filtered, and the filtrate was kept at 100° for 1 h, cooled, neutralised with Amberlite IR-45 (HO^-) resin, filtered, and concentrated to dryness. The residue was crystallised from aqueous methanol to give the title compound (1.4 g, 90%), m.p. 189–190°, $[\alpha]_D^{20}$ −96° (150 sec), −67° (36 min, equilibrium) (*c* 1, water) (Found: C, 42.1; H, 6.4. $C_{11}H_{22}O_{12}$ calc.: C, 42.3; H, 6.4%).

3-Deoxy-5-O-(β-D-glucopyranosyl)oct-2-ulosonic acid (**9**). — Oxalacetic acid (264 mg) was dissolved in water (2 ml) at 0° by dropwise addition of 10M NaOH with stirring, and the final pH was brought to 10. A solution of glucosyl-arabinose (1 g) in water (22 ml) was added and the pH of the ice-cold solution was brought to 11 with 10M NaOH. The stirred mixture was allowed to reach room temperature (2 h) before neutralisation with Amberlite IR-120 (H^+) resin. Solids were removed, the filtrate was passed through a column (1 × 10 cm) of Dowex 1x8 (CO_3^{2-}) resin, and the column was washed with water until no neutral sugars (unreacted glucosyl-arabinose) were detected. The column was then eluted with ammonium hydrogen carbonate (0.04M); fractions (3 ml) giving a positive thiobarbiturate reaction were combined, neutralised with Amberlite IR-120 (H^+) resin, filtered, and lyophilised. The slightly yellow, solid residue was dissolved in a small volume of water, and the ammonium salt of the title compound precipitated with acetone. This material was adsorbed on a column (45 × 530 mm) of cellulose (equal amounts of Whatman CF1 and CF11). Acetone–water (15:85, 400 ml) was passed through the column, followed by acetone–water mixtures: 25:75 (300 ml) and then 35:75 (250 ml). Fractions (4 ml) containing the disaccharide were combined and concentrated to ~2 ml, and the ammonium salt (235 mg) of **9** was precipitated by the addition of acetone. It had $[\alpha]_D^{20}$ +29° (*c* 0.4, water) (Found: C, 38.25; H, 6.5; N, 3.5. $C_{14}H_{27}NO_{13}.H_2O$ calc.: C, 38.6; H, 6.7; N, 3.2%).

Benzyl 2-O-(β-D-glucopyranosyluronic acid)-β-D-arabinoside (**7**). — Benzyl 3,4-*O*-isopropylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-arabinopyranoside (5 g) was deacetylated with methanolic sodium methoxide (0.1M, 250 ml) at 4° for 18 h. The solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. To a solution of the residue in water (50 ml) was added Adams' platinum catalyst (3 g), and oxygen was bubbled through the stirred solution at 68°; the pH was maintained at 8 by the addition of 0.5M sodium hydrogen carbonate. When the oxidation (monitored by paper electrophoresis at pH 5) was complete, the cooled solution was filtered, decationised with Amberlite IR-120 (H^+) resin, and stored at 100° for 15 min before being neutralised with ammonia and adsorbed onto a column (220 × 25 mm) of Dowex 1x8 (AcO^-) resin. The column was washed free of neutral sugars and then eluted with pyridinium acetate buffer of pH 3.5 (0.25M, 500 ml; 0.5M, 500 ml; M as required). Fractions (10 ml) giving a positive test for neutral sugars were combined and lyophilised. Water (60 ml) was added to the dry residue, the pH was adjusted to 7.2 with ammonia solution, and the volume of the solution was diminished to ~7 ml. The ammonium salt (1.75 g, 49%)

of **7**, precipitated by trituration with acetone, had $[\alpha]_D^{20}$ −143° (*c* 0.43, water) (Found: C, 46.8; 6.35; N, 2.8. $C_{18}H_{27}NO_{11}.1.5H_2O$ calc.: C, 46.95; H, 6.5; N, 3.0%).

2-O-(β-D-Glucopyranosyluronic acid)-D-arabinose (**8**). — A solution of **7** (1 g) in water (100 ml) was decationised with Amberlite IR-120 (H^+) resin, filtered, and hydrogenated over 10% palladium-on-carbon. When hydrogen uptake had ceased, the catalyst was removed, the pH of the solution adjusted to 7.2 with ammonia solution, and its volume diminished to ~3 ml. Compound **8** (750 mg, 94%) was precipitated upon addition of acetone and had $[\alpha]_D^{20}$ −78° (*c* 0.7, water) (Found: C, 36.0; H, 6.0; N, 3.85. $C_{11}H_{21}NO_{11}.H_2O$ calc.: C, 36.1; H, 6.4; N, 3.8%).

3-Deoxy-5-O-(β-D-glucopyranosyluronic acid)oct-2-ulosonic acid (**12**). — To ice-cold water (5 ml) were added, alternately, 10M NaOH and oxalacetic acid (400 mg) so as to maintain a pH of 8–10. To the stirred, ice-cold solution was added powdered 2-*O*-(β-D-glucopyranosyluronic acid)-β-D-arabinose (950 mg), the pH was adjusted to 11, and the reaction was allowed to proceed for 2 h, during which time the mixture reached room temperature. After the addition of sufficient Amberlite IR-120 (H^+) resin to bring the pH to 7, solids were removed and the solution was percolated through a column (1 × 12 cm) of Dowex 1x8 (AcO^-) resin (100–200 mesh). The resin was washed with water (100 ml) and then eluted with 0.25M pyridinium acetate buffer (pH 3.5) to remove (glucopyranosyluronic acid)arabinose, followed by 2M pyridinium acetate of the same pH to recover **12**. Fractions containing the disaccharide (Warren test) were combined and concentrated to dryness. The residue was then dissolved in water (10 ml) and lyophilised. The solid obtained was redissolved in water (50 ml), the pH was brought to 7.2 with ammonia solution, and the solution was concentrated to ~2 ml and triturated with acetone. The precipitated ammonium salt of **12** (110 mg, 8.4%), collected by centrifugation, had $[\alpha]_D^{20}$ +2° (*c* 0.9, water) (Found: C, 36.05; H, 6.4; N, 6.0. $C_{14}H_{28}N_2O_{14}.H_2O$ calc.: C, 36.0; H, 6.2; N, 6.2%).

REFERENCES

1 O. LÜDERITZ, O. WESTPHAL, A.-M. STAUB, AND H. NIKAIDO, in E. WEINBAAUM, S. KADIS, AND S. J. AJL (Eds.), *Microbial Toxins*, Vol. IV, Academic Press, New York, 1971, pp. 145–224.
2 B. A. DMITRIEV, L. V. BAKINOVSKII, AND N. K. KOCHETKOV, *Dokl. Akad. Nauk SSSR*, 193 (1970) 1304–1307 [*Chem. Abstr.*, 74 (1971) 23,084x]; D. CHARON AND L. SZABÓ, *J. Chem. Soc. Perkin Trans. 1*, (1973) 1175–1179.
3 G. HÄMMERLING, O. LÜDERITZ, O. WESTPHAL, AND P. H. MÄKELÄ, *Eur. J. Biochem.*, 22 (1971) 331–344; W. DRÖGE, V. LEHMANN, O. LÜDERITZ, AND O. WESTPHAL, *ibid.*, 14 (1970) 175–184; N. A. FULLER, M.-C. WU, R. G. WILKINSON, AND E. C. HEATH, *J. Biol. Chem.*, 248 (1973) 7938–7950.
4 K. SUGIMOTO AND R. OKAZAKI, *J. Biochem. (Tokyo)*, 62 (1967) 373–383.
5 D. CHARON AND L. SZABÓ, *Eur. J. Biochem.*, 29 (1972) 184–187.
6 C. HERSHBERGER AND S. B. BINKLEY, *J. Biol. Chem.*, 243 (1968) 1578–1584.
7 J. W. CORNFORTH, M. E. FIRTH, AND A. GOTTSCHALK, *Biochem. J.*, 68 (1958) 57–61.
8 D. CHARON AND L. SZABÓ, *J. Chem. Soc. Perkin Trans. 1*, (1976) 1628–1632.
9 L. ZERVAS AND S. KONSTAS, *Chem. Ber.*, 93 (1960) 435–446.
10 D. BUNDLE AND N. SHAW, *Carbohydr. Res.*, 21 (1972) 211–217.
11 K. HEYNS, K. PROPP, R. HARRISON, AND H. PAULSEN, *Chem. Ber.*, 100 (1967) 271–279.
12 R. IWAMOTO AND Y. IMANAGA, *Carbohydr. Res.*, 24 (1972) 133–139.
13 C. L. MEHLTRETTER, *Adv. Carbohydr. Chem.*, 8 (1953) 231–249.

14 D. E. DORMAN AND J. D. ROBERTS, *J. Am. Chem. Soc.*, 92 (1970) 1355–1361; W. VOELTER, V. BILIK, AND E. BREITMAIER, *Collect. Czech. Chem. Commun.*, 38 (1973) 2054–2071.
15 P. COLSON AND R. R. KING, *Carbohydr. Res.*, 47 (1976) 1–13.
16 J. W. CORNFORTH, M. E. DAINES, AND A. GOTTSCHALK, *Proc. Chem. Soc. London*, (1957) 25–26.
17 A. WEISSBACH AND J. HURWITZ, *J. Biol. Chem.*, 234 (1959) 705–709.
18 J. E. HODGE AND B. T. HOFREITER, *Methods Carbohydr. Chem.*, 1 (1962) 386–388.
19 J. E. SHIVELY AND H. E. CONRAD, *Biochemistry*, 9 (1970) 33–43.
20 C. E. BALLOU, *J. Am. Chem. Soc.*, 79 (1957) 165–166.
21 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
22 E. FISCHER, M. BERGMANN, AND A. RABE, *Ber.*, 53 (1920) 2362–2388.