405. *The Constitution of the Disaccharide of Glycyrrhinic Acid.*

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Glycyrrhinic acid has been studied by periodate oxidation and by degradation of *trimethyl glycyrrhinate pentamethyl ether* and shown to have the structure (XIII) in which two glucopyruronic acid residues are joined by a β-1′ : 2-link. A method for the identification of methylated uronic acid derivatives has been elaborated in which they are converted into methylated aldoses by making use of lithium aluminium hydride to effect the reduction $CO_2Me \longrightarrow CH_2{\cdot}OH$.

Glycyrrhinic acid, the acidic saponin of liquorice root, contains the triterpene acid glycyrrhetic acid attached glycosidically to a disaccharide composed of two hexuronic acid units. The disaccharide was isolated in the form of its methylglycoside dimethyl ester by Voss and Pfirschke (*Ber.*, 1937, **70**, 132). They did not investigate its structure and were not able to decide with certainty the nature of the uronic acid present; indeed, the rapid and extensive decarboxylation which they found to occur when the saponin was hydrolysed with dilute acids made it seem doubtful if any of the known uronic acids could be present. Earlier work had led Tschirsch and Gauchmann (*Arch. Pharm.*, 1908, **246**, 554) to think that the uronic acid was D-glucuronic acid, and Bergmann (*Biochem. Z.*, 1933, **267**, 304) to suggest that it was D-mannuronic acid.

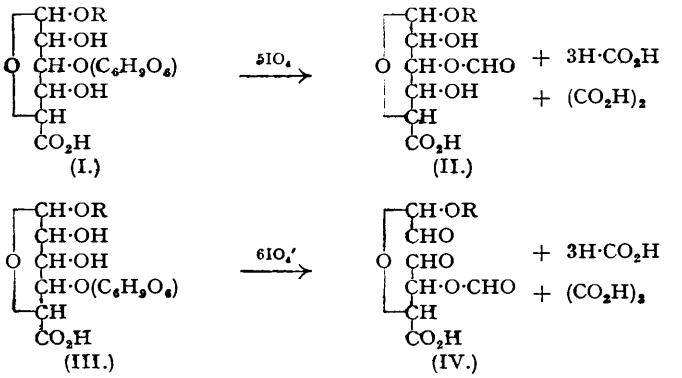
At first we were interested in glycyrrhinic acid as a possible model for centelloside, a saponin-like substance which we isolated from *Centella asiatica*; centelloside contains a hydroxylated triterpene carboxylic acid aglycone attached to a polysaccharide system. It soon became clear, however (Lythgoe and Trippett, *Nature*, 1949, **163**, 258), that the link between the aglycone and the carbohydrate in centelloside involves the carboxyl group of the former, not, as is usual in triterpene saponins, the alcoholic hydroxyl at $C_{(3)}$, so that an ordinary saponin could not be expected to provide an exact model for centelloside. Since little seems to be known about the oligosaccharides of saponins in general, and glycyrrhinic acid is relatively accessible and presents some interesting features, we decided to determine the nature of its sugar component. Our results are described in the present paper.

The oxidation of glycyrrhinic acid by sodium metaperiodate provided useful structural information. The aglycone, glycyrrhetic acid, was unaffected, but the intact glycoside con-

sumed 8 moles of the reagent. No formaldehyde was produced in the reaction, but 6 equivalents of strong acid were liberated, one of which was due to 1 mole of oxalic acid. These results cannot be reconciled with structures containing 2- or 5-ketohexuronic acid units, and support the views of earlier workers that the saponin contains an aldohexuronic acid.

Huebner and his collaborators (*J. Biol. Chem.*, 1945, **159**, 502; *J. Amer. Chem. Soc.*, 1947, **68**, 1621) found that bornyl-D-glucuronoside consumed 5 moles of periodate and liberated 4 equivalents of strong acid, one of which was due to 1 mole of oxalic acid; at the end of the reaction the aglycone was present as bornyl formate. The interpretation of these results which the authors provided shows the important part which the hydroxylation of suitably activated methylene groups may play in oxidations of this kind. There is an obvious resemblance between their results with bornylglucuronoside and our own with glycyrrhinic acid; both consume large amounts of the oxidant, and from both oxalic acid is liberated. At least one of the hexuronic acid units of glycyrrhinic acid must be pyranose to account for the formation of oxalic acid; in fact, both must be so, for no structure containing a furanose unit can be written which would consume more than 6 moles of oxidant. The formation of only 1 mole of oxalic acid indicated that only one of the hexuronic acid residues was degraded completely; this might leave its glycosidic carbon atom as a formyl group attached to the other unit, which would be thereby protected from complete oxidation. This point will be clear if reference is made to the structures (I) and (III), the behaviour of which on oxidation seems easily predictable.

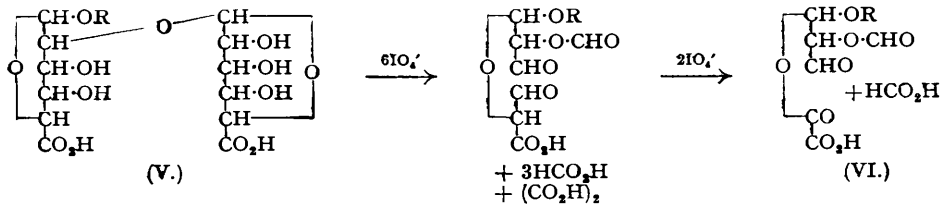
In the dihexuronoside (I) the two uronic acid residues are joined by a 1′ : 3-link. The residue written as $C_6H_9O_6$ should be completely oxidised in the way described by Huebner and his collaborators, giving (II), the formyl group of which should protect it against further attack. The dihexuronoside (III), in which the uronic acid residues are joined by a 1′ : 4-link, should suffer complete oxidation of the residue remote from the aglycone, and the residue proximate to the aglycone should consume an additional mole of oxidant to give (IV).



It was desirable that these ideas should be confirmed by experiment, since they would, if correct, exclude the structures (I) and (III) for glycyrrhinic acid. In order to test whether our experimental conditions caused degradation of a glucuronoside in a way similar to that observed by Huebner and collaborators, *triacetyl* cyclo*hexyl*-β-D-*glucuronoside* was synthesised by methods similar to those used for other uronic acid derivatives by Stacey and his co-workers (*J.*, 1939, 1529; *J.*, 1944, 131; compare Luckett and Smith, *J.*, 1940, 1114). The compound obtained by the removal of the acetyl groups from the synthetic triacetate consumed 5 moles of periodate. A representative dihexuronoside was synthesised from *hepta-acetyl* cyclo*hexyl*-β-D-*maltoside* by converting it into 2 : 3 : 2′ : 3′ : 4′-*penta-acetyl* cyclo*hexyl*-β-D-*maltoside*, which was oxidised by potassium permanganate to *penta-acetyl* 4-(α-D-*glucuronosyl*) cyclo*hexyl*-β-D-*glucuronoside*. The compound (III; R = C_6H_{11}) obtained on removal of the acetyl groups consumed, as expected, 6 moles of periodate.

There remained the possibility that the uronic acid residues of glycyrrhinic acid might be united by a 1′ : 2-link, as in (V), the oxidation of which might be expected to proceed as shown. When 8 moles of periodate have been reduced, the compound (VI) should result; it seemed probable that this would resist further attack, although, since it is an oxalic acid half ester, the possibility that it could undergo hydrolysis in the acidic reaction medium and so be oxidised more extensively, cannot altogether be discounted. However, since during the formation of

(VI) 5 equivalents of strong acid and a further 1 mole of oxalic acid would be formed, as actually found for glycyrrhinic acid, the structure (V) was adopted provisionally and gave valuable guidance in later experiments which proved it correct.

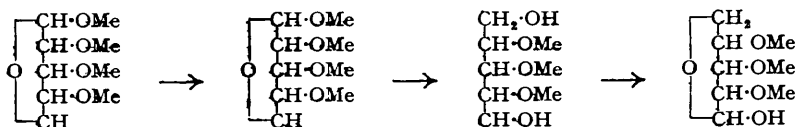


(V.) (VI.)

Thinking that some of the difficulties which Voss and Pfirschke encountered in characterising the uronic acid of glycyrrhinic acid might so be avoided, we preferred to work with methylated compounds. Methylation of the saponin as described in the Experimental section gave a crystalline *trimethyl glycyrrhinate pentamethyl ether*. This, like the saponin itself, could not be methanolised completely with 8% methanolic hydrogen chloride; after prolonged boiling the principal products were methyl glycyrrhetate and a *methyldihexuronoside dimethyl ester pentamethyl ether* possessing a resistant inter-uronic link. Spoehr (*Arch. Biochem.*, 1947, **14**, 153) found that alginic acid, which mineral acids hydrolyse only slowly and with extensive decarboxylation, can be hydrolysed with relatively little decomposition if 90% formic acid is used as the reagent. Both trimethyl glycyrrhinate pentamethyl ether and the methyldihexuronoside dimethyl ester pentamethyl ether were hydrolysed successfully by this reagent and, after the hydrolysis product from either compound had been treated first with methanolic hydrogen chloride and then with methanolic ammonia, two methylated carbohydrate fragments were isolated as crystalline amides. That more soluble in benzene (amide *A*) was the amide of a methylhexuronoside trimethyl ether and had m. p. 157—163°, $[\alpha]_D$ +106° (water). The less soluble component (amide *B*) was the amide of a methylhexuronoside dimethyl ether and had m. p. 193°. The latter was deaminated by nitrous fumes, and the resulting acid methylated with methyl iodide and silver oxide. Treatment of the product with methanolic ammonia gave a product identical with amide *A*, hence the two uronic acid units of glycyrrhinic acid must be identical.

Up to the present the amides of only three methylhexopyruronoside trimethyl ethers have been described. That derived from D-glucuronic acid has m. p. 183°, $[\alpha]_D$ +138° (water) (Smith, *J.*, 1939, 1724); that derived from D-mannuronic acid has not been obtained crystalline (compare Ault, Haworth, and Hirst, *J.*, 1935, 517); that derived from D-galacturonic acid has m. p. 154°, $[\alpha]_D$ +139° (water) (Luckett and Smith, *loc. cit.*). It appeared that amide *A* from glycyrrhinic acid could be identical with none of these, if the constants found for it were reliable. The rather wide temperature range over which it melted gave us some anxiety on this score, and we attempted to purify it by repeated recrystallisation and sublimation, but its constants remained unaltered, so we considered it to be pure. Experiments described below, by which its identity was established, show that we were mistaken in this.

In the past, methylhexuronoside trimethyl ethers have been identified by converting them into the corresponding trimethoxyglutardiamides. The sequence of reactions shown below affords an alternative method of identification which is quite general and has marked advantages when rather small amounts of material are available for investigation. The reactions used give very high yields; the end-product from any methyl hexopyruronoside trimethyl ether is a trimethyl pentopyranose; at least one of the two enantiamorphous trimethyl ethers corresponding to each of the four aldopentoses is known and crystalline, and these compounds are moreover, readily identified by paper chromatography.

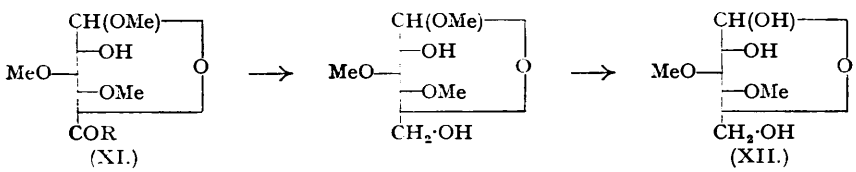


(VII.) (VIII.) (IX.) (X.)

In order to apply this method, amide *A* was deaminated with nitrous fumes and the free acid converted by diazomethane into its methyl ester, which was purified by distillation.

Treatment of the methyl ester (VII) with lithium aluminium hydride gave the primary alcohol (VIII) as a gum which failed to give the naphtharesorcinol test, showing that the reduction was complete. Hydrolysis with dilute acid removed the glycosidic methyl group from (VIII), and the crude reaction product was reduced with hydrogen and Raney nickel to the partly methylated hexitol (IX). The reduction product, used without preliminary purification, consumed 60% of the amount of sodium metaperiodate which would have been required had each of the preceding steps given a theoretical yield; the reaction product (X) crystallised, and was identified as 2 : 3 : 4-trimethyl L-xylose by direct comparison with authentic material and by paper chromatography.

Amide *A* must thus have been derived from either D-glucuronic or L-iduronic acid, and its high dextrorotation argued strongly against the second alternative. Mixtures of it with the authentic amide of methyl-α-D-glucuronoside trimethyl ether melted between 163° and 183°, and in spite of the wide divergence in the constants of the two samples there can be no doubt that they were essentially the same substance; clearly the sample from the saponin was contaminated by a small quantity of the β-isomer. We had been led by the literature (*e.g.*, Smith, *loc. cit.*) to believe that when 2 : 3 : 4-trimethyl D-glucuronic acid is boiled with methanolic hydrogen chloride the α-form of the methylglycoside methyl ester predominates very greatly in the product. Our experience shows that this predominance is by no means always so great as we had expected, and it is noteworthy that Hirst and Jones (*J.*, 1938, 1174) isolated from a methylated aldobiuronic acid a sample of the amide of methyl-D-glucuronoside trimethyl ether having m. p. 158°, $[\alpha]_D$ +60° (water), which was a mixture of α- and β-forms which could not be separated by crystallisation. Since amide *B* from glycyrrhinic acid gave, when converted into amide *A*, material with a low optical rotation, it seems likely that, in spite of its sharp melting point, it too may be an α-form contaminated with traces of the β-isomer.



(XI.) (XII.)

If the interpretation of the periodate oxidation experiments given above is correct, amide *B* must be the amide of 3 : 4-dimethyl methyl-D-glucuronoside (XI; R = NH_2). It was converted into the methyl ester (XI; R = OMe) which was reduced with lithium aluminium hydride to the corresponding alcohol, acidic hydrolysis of which gave crystalline 3 : 4-dimethyl D-glucose. This was identified by comparison with a specimen kindly supplied by Drs. Bell and Greville of the Biochemistry Department. They have shown (forthcoming publication) that their material is identical with the original product of Dewar and Fort (*J.*, 1944, 496), but that the latter authors gave incorrect values for its optical rotation. Our product had the same optical constants as those found by Bell and Greville.

Glycyrrhinic acid thus contains two 1′ : 2-linked glucuronic acid units. With regard to the stereochemistry of the glycosidic links Voss and Pfirschke suggested that the difficultly methanolysed interuronic link was of the β-, the other of the α-type; we find that the former assignment is supported by optical-rotation data.

Isorotation rules have often been applied to disaccharides; the method used here, which applies to disaccharides of the trehalose type and to methyl-glycosides of reducing disaccharides, but more particularly to the fully methylated derivatives of both classes, is merely an extension of that of Charlton, Haworth, and Hickinbottom (*J.*, 1927, 1527; compare Haworth and Hirst, *J.*, 1930, 2615). We make the assumption, which is supported by considerable evidence, that methyl and glycosyl groups make approximately equal contributions to the rotation of a monosaccharide in which they are present as substituents, not only when they are substituted at the glycosidic centre of the latter, but also at other positions. If this assumption is justified, the molecular rotation [*M*] of a disaccharide of the type under discussion can be expressed in terms of the " *B* " values of its two components and the " *A* " value of a methyl group; it is then possible to define a parameter the value of which is given by the expression $S = [M] - B_1 - B_2$, such that *S* will depend only on the stereochemistry of the two glycosidic centres. Where both of these have an α-configuration *S* should have a value approximately the same as $2A_{Me}$; where both have a β-configuration $S \approx -2A_{Me}$; where one has an α-, the other a β-configuration $S \approx$ zero.

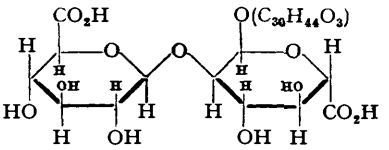
The extent to which the original assumption is justified can be judged from the table, in which some *S* values, relating to aqueous solutions and the sodium D line, are assembled. All those methylated derivatives for which the necessary data are available have been included. The following values, calculated from rotations recorded in Beilstein's Handbuch, IVth Edition, vol. XXXI, have been used in the calculations: "*A*" values: Me, +18,700°. "*B*" values: glucose, +12,100°; galactose, +19,000°; methylated glucose, +16,300°; methylated galactose, +21,000°.

Compound.	$[\alpha]_D$ (in water).	$[M]$.	*S*.
αα-Trehalose	+197°	+67,400°	+43,200°
Methyl-α-D-gentiobioside	+65·5	+23,300	− 900
Methyl-β-D-maltoside	+78·8	+28,100	+ 3,900
Heptamethyl methyl-β-D-maltoside	+88·1	+40,000	+ 7,400
Heptamethyl methyl-β-D-melibioside	+97·8	+44,000	+ 7,100
Heptamethyl methyl-β-D-lactoside	−16·9	− 7,700	−45,000
Heptamethyl methyl-β-D-cellobioside	−19·1	− 8,700	−41,300
Heptamethyl methyl-β-D-gentiobioside	−36	−16,400	−49,000

No fully methylated representative with two α-links is available, so the *S* value (+43,200°) for αα-trehalose has been included, and may be compared with the theoretical value, +37,400°. Values of *S* for compounds containing one α- and one β-link range from −900° to +7,400° (theoretical, zero); those for compounds with two β-links range from −41,300° to −49,000° (theoretical, −37,400°). Individual variations from the theoretical values in each class are, as might be expected, considerable; but this does not invalidate the point we wish to establish, which is that *S* values do allow disaccharide derivatives to be assigned unambiguously to one of three classes, depending only on the types of the glycosidic links present.

In order to apply this method to the problem in hand, the "*B*" value for a fully methylated D-glucuronoside methyl ester is required. Smith (*loc. cit.*) records for methyl-α-D-glucuronoside methyl ester trimethyl ether, $[\alpha]_D$ +87°, corresponding to $[M]$ +23,000°; this value is undoubtedly somewhat low, since it relates to a syrupy product prepared by a method which must lead to the presence of small amounts of the β-isomer. By subtracting A_{Me} (+18,700°) we obtain for the desired *B* value +4,300°, which again is probably too low, but is unlikely to be sufficiently so to invalidate configurational deductions from it. It is of interest as a test to apply this *B* value to the 6-(β-D-glucuronosyl) methyl-β-D-galactoside methyl ester hexamethyl ether obtained from gum arabic by Challinor, Haworth, and Hirst (*J.*, 1931, 258). This compound has $[\alpha]_D$ −21° ($[M]$ −9,800°), giving $S = -9,800 - 4,300 - 21,000 = -35,100°$. Although this value is, as expected, somewhat low numerically, it is sufficient to show unambiguously the presence of two β-links in the aldobiuronoside.

Applying the method now to the methyldiglucurosonide methyl ester pentamethyl ether from glycyrrhinic acid, which has $[\alpha]_D$ +38·5° ($[M]$ +18,600°) we obtain $S = +18,600 - 2(4,300) = +10,000°$; this value will be rather higher than the true one for reasons already given, but it is sufficiently close to the values obtained for compounds containing one α- and



(XIII.)

one β-link to show that the diglucuronoside belongs to the same class. The α-link is clearly that which attaches the glycosidic methyl group, which was introduced by a method known to give rise predominantly to glycosides of the α-type. The inter-uronic acid link must therefore, as suggested by Voss and Pfirschke, be of the β-type, and glycyrrhinic acid must have the structure (XIII).

Experimental.

Isolation of Glycyrrhinic Acid.—The method of Tschirsch and Cederburg (*Arch. Pharm.*, 1907, **245**, 97) was used, with modifications only in detail, for the isolation of glycyrrhinic acid from commercial "glycyrrhizinum ammoniacale," and proved more satisfactory than that of Voss, Klein, and Sauer (*Ber.*, 1937, **70**, 122). From the commercial drug (1 kg.) crude ammonium glycyrrhinate was obtained which separated from glacial acetic acid as light brown crystals (80 g.), $[\alpha]_D^{17}$ +40° (*c*, 3·0 in 40% alcohol). This material was converted into the lead salt and thence into the free acid, which was obtained pure and colourless by recrystallisation from glacial acetic acid and had $[\alpha]_D^{17}$ +46·2° (*c*, 1·5 in alcohol). The pure acid, treated with alcoholic ammonia, gave ammonium glycyrrhinate as colourless needles (from

alcohol), $[\alpha]_D^{17}$ +43·2° (*c*, 2·9 in 40% alcohol). Voss, Klein, and Sauer record for this ammonium salt, $[\alpha]_D^{17}$ +43·3° (in 40% alcohol).

Periodate Oxidation of Glycyrrhinic Acid.—To a solution of glycyrrhinic acid (74·6 mg.) in a little water containing sodium hydroxide (1 equiv.) 0·23M-sodium metaperiodate (10 c.c.) was added, and the solution was diluted to 50 c.c. and kept at room temperature, aliquots being withdrawn for titration at intervals. The oxidation was complete after 24 hours, 8·27 mols. of oxidant per mol. of saponin having been consumed, and 6·2 equivs. of strong acid (methyl-red indicator) liberated. No formaldehyde could be found in the solution by the method of Reeves (*J. Amer. Chem. Soc.*, 1941, **63**, 1476).

In another experiment, ammonium glycyrrhinate (177·5 mg.) dissolved in water (5 c.c.) was treated with 0·23M-sodium metaperiodate (25 c.c.) and the solution kept at room temperature for 24 hours to complete the oxidation. The filtered solution was then treated with N-hydrochloric acid (6 c.c.) and iodate and periodate ions removed by titration with 1·2N-sodium arsenite. The solution was buffered to pH 7 by adding sodium acetate, treated with 5% aqueous calcium chloride (1 c.c.), and kept overnight. The precipitated calcium oxalate, collected, washed, and dissolved in warm dilute sulphuric acid, required 18·56 c.c. of 0·02N-potassium permanganate for its oxidation; this corresponds with 0·88 mols. of oxalic acid per mol. of saponin. Under similar conditions methyl-β-D-galacturonoside gave 0·80 mol. of oxalic acid per mol. of glycoside.

2 : 3 : 4-*Triacetyl* 6-*Trityl* cyclo*Hexyl-β-D-glucoside.*—*cyclo*Hexyl-β-D-glucoside (4·7 g.; Pacsu, *J. Amer. Chem. Soc.*, 1930, **52**, 2568) and triphenylmethyl chloride (5 g.) dissolved in dry pyridine (30 c.c.) were heated on a boiling water-bath for 2 hours, the solution was cooled to 0°, and acetic anhydride (12 c.c.) added. After being kept at room temperature overnight the solution was poured slowly into stirred ice-water (2 l.). The suspension was stirred for a further hour, and the solid collected and crystallised from ethanol. The 2 : 3 : 4-*triacetyl* 6-*trityl* cyclo*hexyl-β-D-glucoside* (9 g., 72%) had m. p. 146—147°, $[\alpha]_D^{17}$ −26·8° (*c*, 4·5 in chloroform) (Found: C, 70·8; H, 6·9. $C_{37}H_{42}O_9$ requires C, 70·7; H, 6·8%).

2 : 3 : 4-*Triacetyl* cyclo*Hexyl-β-D-glucoside.*—To a solution of the above trityl derivative (8·8 g.) in glacial acetic acid (35 c.c.) at 10° a solution of hydrogen bromide in acetic acid (3·1 c.c.; saturated at 0°) was added, and the solution was shaken for 1 minute, and filtered directly into ice-water (250 c.c.). The resulting suspension was extracted with chloroform, and the extract washed with sodium hydrogen carbonate solution and then with water and evaporated under reduced pressure. Crystallisation of the residue from ether gave the triacetate (5 g., 90%), m. p. 112—113°.

2 : 3 : 4-*Triacetyl* cyclo*Hexyl-β-D-glucuronoside.*—A solution of the above triacetate (2·3 g.) in glacial acetic acid (20 c.c.) was added to a stirred solution of potassium permanganate (0·5 g.) in dry acetone (20 c.c.) and stirring continued for 40 hours whilst more permanganate (1·5 g. in 60 c.c. of acetone) was added gradually. The suspension obtained after the addition of water (40 c.c.) was centrifuged and the supernatant solution evaporated under reduced pressure. The residual syrup was dissolved in chloroform, and the solution washed with dilute sulphuric acid and with water, dried, and evaporated. The syrup obtained in this way crystallised, and recrystallisation from ether–light petroleum (b. p. 60—80°) gave the *glucuronoside* (0·7 g., 30%) as fine needles, m. p. 176—177° (Found: C, 54·4; H, 7·0. $C_{18}H_{26}O_{10}$ requires C, 53·8; H, 6·5%). Treatment with diazomethane gave the *methyl* ester, long needles (from alcohol), m. p. 135—137°, $[\alpha]_D^{17}$ −36° (*c*, 2 in chloroform) (Found: C, 55·0; H, 6·9. $C_{19}H_{28}O_{10}$ requires C, 54·8; H, 6·7%). For periodate oxidation triacetyl *cyclo*hexyl-β-D-glucuronoside (45 mg.) was dissolved in dry methanol (10 c.c.) containing sodium methoxide (from 50 mg. of sodium). The solution was kept at 0° for 6 hours and then neutralised with N-hydrochloric acid, and the alcohol evaporated under reduced pressure. After the addition of 0·23M-sodium metaperiodate (5 c.c.) the solution was made up to 25 c.c. and kept at room temperature, the progress of the oxidation being followed by the titration of aliquots. Reaction ceased after 60 hours, when 5·1 mols. of oxidant per mol. of glucuronoside had been consumed.

Penta-acetyl 4-(α-D-*Glucuronosyl*) cyclo*Hexyl-β-D-glucuronoside.*—Interaction of acetobromomaltose and *cyclo*hexanol in the usual manner gave *hepta-acetyl* cyclo*hexyl-β-D-maltose*, m. p. 137—139°, $[\alpha]_D^{17}$ +41·5° (*c*, 2 in chloroform) (Found: C, 52·3; H, 6·6. $C_{32}H_{46}O_{18},H_2O$ requires C, 52·4; H, 6·6%). *cyclo*Hexyl-β-D-maltoside was obtained only as a syrup by the action of methanolic sodium methoxide on the hepta-acetate; it was converted by methods exactly similar to those described above into 2 : 3 : 2′ : 3′ : 4′-*penta-acetyl* cyclo*hexyl-β-D-maltoside*, m. p. 159—160° (Found: C, 53·4; H, 6·9. $C_{28}H_{42}O_{16}$ requires C, 53·0; H, 6·7%). By oxidation with potassium permanganate the *penta-acetyl* cyclo*hexyldihexuronoside* was obtained. It separated from alcohol as crystals which sintered at 175°, m. p. 185—187°, $[\alpha]_D^{18}$ +47·2° (*c*, 2·2 in chloroform) (Found: C, 49·7; H, 6·5. $C_{28}H_{38}O_{18},H_2O$ requires C, 49·4; H, 5·9%). Treatment with diazomethane gave the *dimethyl* ester, m. p. 169—171° (from alcohol) (Found: C, 52·5; H, 6·4. $C_{30}H_{42}O_{18}$ requires C, 52·2; H, 6·1%).

For the periodate oxidation the penta-acetyl *cyclo*hexyldihexuronoside (75 mg.) was deacetylated with methanolic sodium methoxide, the neutralised solution freed from methanol and after the addition of 0·23M-sodium metaperiodate (5 c.c.) diluted to 25 c.c. and kept at room temperature. The oxidation was complete after 36 hours, 6·0 mols. of oxidant per mol. of the diglucuronoside having been consumed.

Trimethyl Glycyrrhinate Pentamethyl Ether.—Ammonium glycyrrhinate (4 g.) dissolved in 40% alcohol (50 c.c.) was treated with 0·8N-thallous hydroxide (8 mols. per mol. of saponin) and the pale yellow precipitate collected, washed, and dried to constant weight at room temperature over phosphoric oxide. The finely powdered thallous derivative was heated under reflux with methyl iodide, with exclusion of light and moisture for 8 hours, and the suspension filtered. The filtrate was evaporated, the residue extracted with chloroform, and the chloroform solution evaporated to a syrup. Two further methylations of the syrupy product with methyl iodide and silver oxide gave a *product* which crystallised from alcohol in flat leaves. The pure material (2·8 g., 60%) had m. p. 275—276°, $[\alpha]_D^{18}$ +34·3° (*c*, 1·1 in

chloroform) (Found: C, 62·8; H, 8·0; OMe, 25·2. $C_{56}H_{78}O_{16},H_2O$ requires C, 63·0; H, 8·4; OMe, 26·1%).

Methanolysis of Trimethyl Glycyrrhinate Pentamethyl Ether.—A suspension of the above compound (4·6 g.) in 8% methanolic hydrogen chloride (150 c.c.) was heated under reflux for 12 hours, and all dissolved. The hydrogen chloride was neutralised with freshly prepared silver carbonate, the suspension filtered, and the filtrate evaporated under reduced pressure to a partly crystalline residue. After as much as possible of the methyl glycyrrhetate had been removed by crystallisation from the minimum amount of alcohol the mother-liquors were evaporated under reduced pressure to a syrup, which by distillation at 10^{-3} mm. furnished two fractions. Fraction 1 (0·4 g.), b. p. 120—140° (bath-temp.), had OMe, 52·9%; Fraction 2 (0·9 g.), b. p. 170—190°, had OMe, 49·3%. When Fraction 2 was dissolved in warm light petroleum (b. p. 60—80°) the cooled solution deposited the *methyldihexuronoside dimethyl ester pentamethyl ether* as long rods, m. p. 94—95°, $[\alpha]_D^{17}$ +36° (*c*, 2 in chloroform); +38·5° (*c*, 1 in water) (Found: C, 50·3; H, 7·2; OMe, 50·0. $C_{20}H_{34}O_{13}$ requires C, 49·8; H, 7·1; OMe, 51·4%).

Hydrolysis of the Methyldihexuronoside Dimethyl Ester Pentamethyl Ether with 90% Formic Acid.—The above dimethyl ester (1 g.) and 90% formic acid (30 c.c.) were heated together under reflux for 16 hours and after evaporation of the solution under reduced pressure the last traces of formic acid were removed by repeated evaporation of the residue with alcohol. The syrupy product was heated under reflux for 12 hours with 2% methanolic hydrogen chloride, the acid was removed by treatment with silver carbonate, and solvents were removed from the filtered solution under reduced pressure. When the residual syrup was distilled at 10^{-4} mm. only one fraction (0·7 g.) was obtained, b. p. 130—140° (bath-temp.); this material was kept at 0° for 4 days with methanolic ammonia (10 c.c.; saturated at 0°). When the solvent was removed a solid remained which was extracted with hot benzene (100 c.c.). The residue was recrystallised from ethyl acetate, giving the *methylhexuronoside dimethyl ether amide* (0·13 g.), m. p. 191—193°, $[\alpha]_D^{17}$ +100° (*c*, 2·5 in water) (Found: C, 46·2; H, 7·1; N, 6·0; OMe, 38·3. $C_9H_{17}O_6N$ requires C, 46·0; H, 7·2; N, 6·0; OMe, 39·5%). Evaporation of the benzene extract and crystallisation of the residue from light petroleum (b. p. 100—120°) gave the amide of a methylhexuronoside trimethyl ether (0·4 g.) as long needles, m. p. 157—163°, $[\alpha]_D^{18}$ +106° (*c*, 1 in water), +85° (*c*, 0·3 in chloroform) (Found: C, 48·6; H, 7·3; N, 5·5. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·6; N, 5·6%). These constants were altered neither by sublimation in a vacuum nor by fractional crystallisation from light petroleum (b. p. 100—120°).

Conversion of the Amide of the Methylhexuronoside Dimethyl Ether into that of the Methylhexuronoside Trimethyl Ether.—A stream of dry nitrous fumes was passed through a solution of the amide of the methylhexuronoside dimethyl ether (0·1 g.) in glacial acetic acid (5 c.c.) till it assumed a permanent green colour. The solution was kept at 0° for 2 hours and then evaporated under reduced pressure, and the residue evaporated repeatedly with alcohol. Methylation of the syrupy product with methyl iodide and silver oxide in the usual way gave a syrup which was kept at 0° for 4 days with methanolic ammonia (3 c.c.; saturated at 0°). Solvents were removed and the residue was crystallised from light petroleum (b. p. 100—120°), the amide of the methylhexuronoside trimethyl ether being obtained as long needles, m. p. 163°, $[\alpha]_D^{17}$ +110° (*c*, 1·2 in water).

Identification of the Methylhexuronoside Trimethyl Ether by the Conversion of its Amide into a Trimethyl Pentopyranose.—The amide of the methylhexuronoside trimethyl ether was deaminated with nitrous fumes as described above and the syrupy acid dissolved in ether and treated with an excess of diazomethane dissolved in ether. When the esterification was complete the solvent was removed and the product distilled at 10^{-3} mm. The main fraction (0·5 g.), b. p. 120° (bath-temp.), was dissolved in dry ether and the solution, after the addition of lithium aluminium hydride (0·5 g.), heated under reflux for 2 hours. The strongly cooled solution was treated with an excess of dilute sulphuric acid, and the acid solution extracted continuously with ether for 24 hours. Evaporation of the extract gave a colourless syrup which was heated at 95° for 3 hours with N-hydrochloric acid. The acid was removed by treatment with silver carbonate, and silver ions by treatment with hydrogen sulphide; when the filtered solution was evaporated under reduced pressure a colourless syrup remained which had strong reducing properties. It was dissolved in water (50 c.c.) and hydrogenated at 120°/100 atm. for 12 hours in the presence of Raney nickel catalyst (1 g.). After the catalyst had been removed and the solvent evaporated a non-reducing syrup (0·5 g.) remained which was dissolved in water and treated with a 10% excess of 0·23M-sodium metaperiodate. After 12 hours N-hydrochloric acid (5 c.c.) was added and the iodate and excess of periodate were removed by titration with 1·2N-sodium arsenite. The product, isolated by continuous extraction with ether, was a syrup (0·3 g.) which had strong reducing properties. Paper chromatography of this material against the four trimethyl pentopyranoses in both *n*-butanol and *s*-collidine showed that it contained one reducing substance only, which in these solvents travelled at the same speed as trimethyl D-xylopyranose. The syrup crystallised from ethyl acetate, giving trimethyl L-xylopyranose, m. p. 85—86°, undepressed on admixture with authentic material, m. p. 91°, but depressed on admixture with the D-isomer.

Conversion of the Amide of the Methylhexuronoside Dimethyl Ether into a Dimethyl Hexose.—The amide of the methylhexuronoside dimethyl ether (0·3 g.) was deaminated with nitrous fumes as already described and the free acid heated under reflux with 2% methanolic hydrogen chloride (30 c.c.) for 12 hours so as to convert it into the methyl ester. This was isolated by removing the acid with silver carbonate and evaporating the filtered solution under reduced pressure to a syrup. A solution of the latter in dry ether (20 c.c.) was added slowly to a solution of lithium aluminium hydride (0·5 g.) in ether (20 c.c.) which was maintained under reflux. Heating was continued for 2 hours, the cooled solution was treated with an excess of dilute sulphuric acid, and the syrupy product (0·3 g.) isolated by continuous extraction with ether. It was heated at 100° for 3 hours with N-hydrochloric acid, the acid was removed with silver carbonate, and silver ions were removed with hydrogen sulphide. Evaporation of the finally filtered solution under reduced pressure gave a syrup which crystallised from *iso*propyl

acetate in long needles, m. p. 119—120°, $[\alpha]_D^{18}$ +114·3° (5 minutes) ⟶ +77·5° (final value, 9 hours) (*c*, 1 in water) (Found : C, 46·1; H, 7·9. Calc. for $C_8H_{16}O_8$: C, 46·1; H, 7·7%). An authentic sample of 3 : 4-dimethyl D-glucose kindly supplied by Drs. Bell and Greville had m. p. 113°, $[\alpha]_D^{18}$ +98° (8 minutes) ⟶ +77·8° (final value, 9 hours) (water). Paper chromatography of the two samples in *n*-butanol and *s*-collidine gave additional confirmation of their identity.

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