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# STRUCTURE–TASTE RELATIONSHIPS IN (1→2)-LINKED DISACCHARIDES*

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ABSTRACT

Nine (1→2)-linked glycosyl-D-glucoses were synthesized by condensing the appropriate *O*-acetylated glycopyranosyl bromides with 1,3,4,6-tetra-*O*-acetyl-α-D-glucose. The D-xylo-, D-gluco-, and D-galactosyl bromides formed approximately equal amounts of the α- and β-linked disaccharides, whereas the L-arabino-, L-rhamno-, and D-mannosyl analogs formed one major product having O-1′ and O-2′, respectively, *cis*, *trans*, and *trans*. Five of the nine crystalline disaccharide peracetates are new. All were deacetylated and compared, in 3% aqueous solutions, with 0.5, 1.0, and 1.5% aqueous solutions of sucrose for sweetness and with 0.05% aqueous caffeine for bitterness. Although none scored a sweetness value as high as that of 1% sucrose, each of the four sweetest members of the series had a C*1* (D) or (L) conformation and each had an axial O-1′ atom *cis* to O-2′. The less sweet members of the series had O-1′ and O-2′ in a *trans* relationship, either diaxial or diequatorial, and were substantially more bitter than the sweeter group of disaccharides.

## INTRODUCTION

As part of the Northern Regional Laboratory's research to develop new sweetening agents of low-caloric value to replace those of doubtful healthfulness, we have examined the occurrence of (1→2)-linked glycosyl-D-glucoses in such intensely sweet plant glycosides as stevioside, osladin, glycyrrhizin, and the dihydrochalcone derivatives of naringin and neohesperidin.

Although the carbohydrate component contributes to the sweetness of these glycosides, the saccharide itself need not be sweet. Of the two more common disaccharide components, neohesperidose and sophorose, the former is either tasteless or faintly sweet[1,2] and the latter is only mildly sweet[3]. Hodge and Inglett[4] have discussed the function of the disaccharide components of such glycosides.

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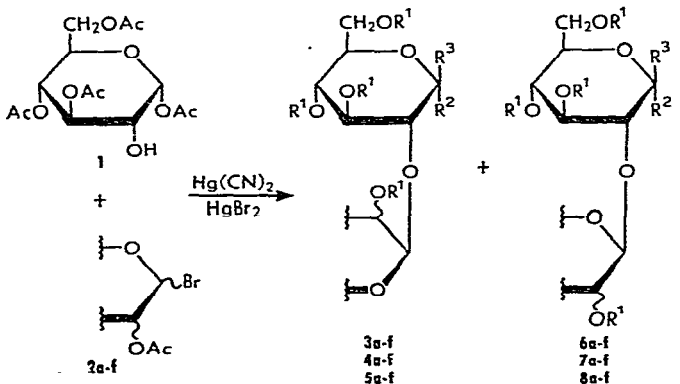
As a class, the (1→2)-linked glycosyl-D-glucoses occur in complex glycosides of plant origin, in natural polymers, and as byproducts of microbial action. Despite this wide distribution, they have not been studied intensively as a group, and several members of the present series were unreported or incompletely characterized previously.

## RESULTS AND DISCUSSION

Although we considered it unlikely that any of the reported or untasted members of the group would be intensely sweet, a series of nine structurally related (1→2)-linked glycopyranosyl-D-glucopyranoses were selected and prepared for a trained taste-panel to determine their relative sweetness values. The glycopyranosyl groups were: 2-*O*-α-D-xylosyl- (**5a**), 2-*O*-α-D-glucosyl- (kojibiose[5,6] **5b**), 2-*O*-β-L-arabinosyl-[7] (**5c**), 2-*O*-α-D-galactosyl-[7,8] (**5d**), 2-*O*-α-D-mannosyl- (**5f**), 2-*O*-β-D-xylosyl- (sambubiose[9], **8a**), 2-*O*-β-D-glucosyl- (sophorose[3,6,10] **8b**), 2-*O*-β-D-galactosyl[11]- (**8d**), and 2-*O*-α-L-rhamnosyl- (neohesperidose[1,12], **8e**). Of these, **5a** and **5f** are novel, although **5f** has been detected in a phosphomannan hydrolyzate (without characterization)[13].

As shown in Scheme 1, the method of Helferich and Zirner[8] was followed to prepare the disaccharides. Under the reaction conditions, which were modified slightly from those reported previously[1,6], **1** was converted into disaccharide products in yields varying from 69 to 96%. The actual product mixture, however, depended upon the acetylated glycosyl bromide added. For D-xylo-, D-gluco-, and D-galactosyl bromides (**2a, b, d**), the ratio of 2-*O*-α- to 2-*O*-β-linked products was approximately 1:1, and overall yields did not exceed 77%. The remaining glycosyl bromides, however,




a = D-Xylopyranosyl-
b = D-Glucopyranosyl-
c = L-Arabinopyranosyl-
d = D-Galactopyranosyl-
e = L-Rhamnopyranosyl-
f = D-Mannopyranosyl-

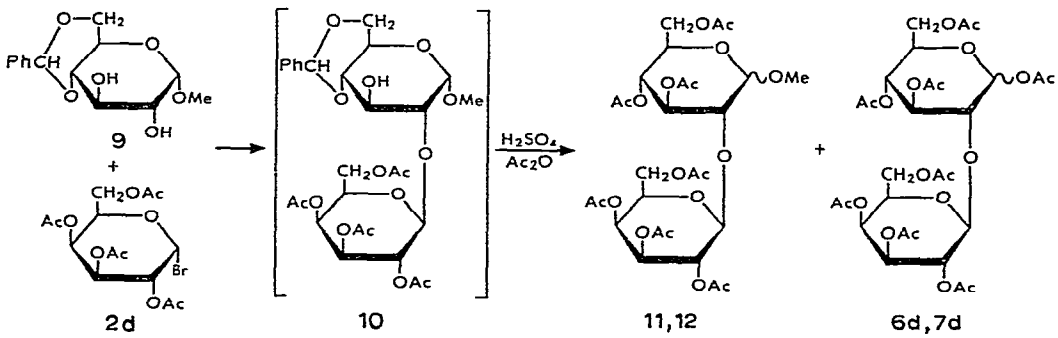
3,6 $R^1$ = Ac, $R^2$ = OAc, $R^3$ = H
4,7 $R^1$ = Ac, $R^2$ = H, $R^3$ = OAc
5,8 $R^1$ = H, $R^2$, $R^3$ = H, OH

Scheme 1.

formed only one major product each, **5c**, **5f**, and **8e**, but the overall yields were higher.

Substantial proportions of the products present could not be isolated in pure form by fractional crystallization. Nevertheless, adequate quantities of each product, except for **8d**, were obtained readily from the mixtures.

Efforts to isolate **8d**, either as **6d** or **7d**, were unsuccessful. However, adaptation of the Coxon and Fletcher[10] procedure for the preparation of sophorose proved satisfactory. Although difficulties encountered with the acetolysis step (Scheme 2) were unexpected, they did not hinder the isolation of **7d**. Similar difficulties were experienced when β-sophorose octaacetate (**7b**) was prepared later by this same procedure.




Scheme 2.

Wherever possible, physical properties of each acetylated disaccharide were compared with values from the literature, and each was examined by n.m.r. spectroscopy to verify the structural assignments. Previously unreported peracetates **4a**, **4c**, **4f**, **7a**, and **7d** were characterized.

Purified samples of each peracetate were deacetylated. After the unsubstituted disaccharides had been examined by g.l.c. and by n.m.r. spectroscopy in methyl sulfoxide-$d_6$, the direction and extent of mutarotation were determined.

Aqueous, equilibrated solutions of each disaccharide were tasted as 3% w/v solutions in water and compared with 0.5%, 1.0%, and 1.5% w/v aqueous solutions of sucrose. The results, expressed as average sweetness, are shown in Table I. The approximate relative sweetness of a given entry can be calculated by dividing the equivalent sucrose concentration by the concentration of the test solution. For relative bitterness, similar calculations can be applied to the entries in Table II.

A 0.05% aqueous solution of caffeine was used to estimate bitterness. Great differences were noted in the ability of panel members to detect bitterness, as can be judged by the relatively large values for the average deviation from the average entries of Table II. As an example, four panel members judged **5b** to be free of bitterness whereas three members assigned a value of 1.0 for this sample. Despite these great

TABLE I

SWEETNESS OF EQUILIBRATED AQUEOUS SOLUTIONS OF D-GLUCOSE AND 2-*O*-GLYCOSYL-D-GLUCOSES[a]

| *Glycosyl group in compound* | *Structure no. in scheme 1* | *Average[b,c] sweetness* | *O-1', O-2' configuration* |
|---|---|---|---|
| D-Glucose[d] | | 0.9 | |
| α-D-Xylo- | 5a | 0.8 | *cis* |
| α-D-Gluco- | 5b | 0.7 | *cis* |
| β-L-Arabino- | 5c | 0.7 | *cis* |
| α-D-Galacto- | 5d | 0.5 | *cis* |
| β-D-Gluco- | 8b | 0.25 | *trans* |
| β-D-Galacto- | 8d | 0.15 | *trans* |
| β-D-Xylo- | 8a | 0 | *trans* |
| α-D-Manno- | 5f | 0 | *trans* |
| α-L-Rhamno- | 8e | 0 | *trans* |

[a]As 3% w/v solutions kept for 18 h before tasting. [b]1% sucrose = 1.0. [c]Average deviation from the average is ±0.2 throughout. [d]As 2% w/v solution.

TABLE II

BITTERNESS OF EQUILIBRATED AQUEOUS SOLUTIONS OF D-GLUCOSE AND 2-*O*-GLYCOSYL-D-GLUCOSES[a]

| *Glycosyl group in compound* | *Structure no. in scheme 1* | *Average bitterness[b]* | *O-1', O-2' configuration* |
|---|---|---|---|
| β-D-Galacto- | 8d | 1.6 ±0.9 | *trans* |
| α-D-Manno- | 5f | 1.2 ±0.6 | *trans* |
| β-D-Xylo- | 8a | 1.0 ±0.6 | *trans* |
| α-L-Rhamno- | 8e | 0.8 ±0.6 | *trans* |
| β-D-Gluco- | 8b | 0.7 ±0.8 | *trans* |
| α-D-Xylo- | 5a | 0.6 ±0.5 | *cis* |
| α-D-Gluco- | 5b | 0.4 ±0.5 | *cis* |
| α-D-Galacto- | 5d | 0 | *cis* |
| β-L-Arabino- | 5c | 0 | *cis* |
| D-Glucose[c] | | 0 | |

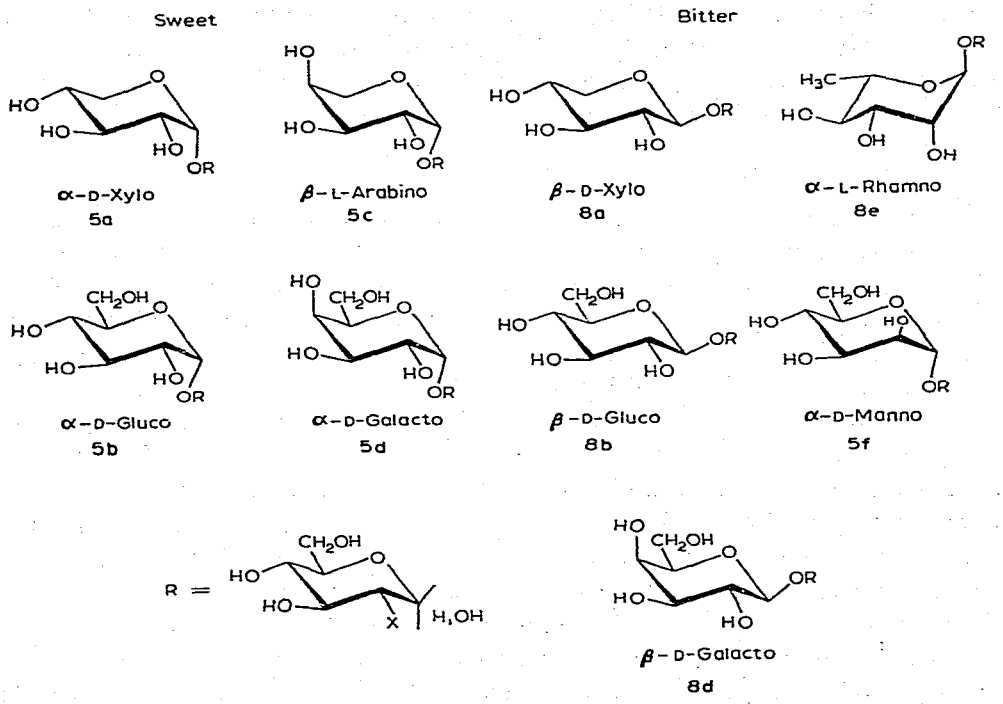
[a]As 3% w/v solutions kept for 18 h before tasting. [b]0.05% caffeine solution = 2.0. Wide variations in bitterness threshold noted in panel members is reflected in the average deviation from average calculations. [c]As 2% w/v solution.

differences in threshold sensitivity to bitterness, the listed order of bitterness is considered to be accurate.

Although it is well established that the subjective intensity of sweetness and bitterness is related to the concentration of the sample being tested, and that either taste in a sample can depress the ability of a panel member to sense the level of the other taste, no attempt was made to adjust the panel findings mathematically[14]. All disaccharides were tasted at a fixed concentration and the values shown in Tables I and II reflect the interaction of sweet and bitter tastes.

Previous investigations with monosaccharides, either as free sugars or as glycosides, have indicated that sweetness and bitterness may be related to certain structural factors[15–17]. Among these factors are: presence of bulky hydrophobic substituents, configuration at certain carbon atoms, introduction of a deoxy center, and the ability to form intramolecular hydrogen bonds with nearby groups. When the data of Tables I and II were evaluated in terms of structure and conformation of the disaccharide's nonreducing moiety, parallel relationships noted were qualitative.

In Scheme 3, the structure and anticipated conformation of each nonreducing moiety are shown. Each conformation is consistent either with well established principles or with n.m.r. data (when two conformers were possible).




Scheme 3.

Despite structural differences at C-4′ or C-5′, the four sweetest disaccharides* of Table I (**5a–d**) have a common structural characteristic: O-1′ is axial and *cis* to O-2′. When the configuration of either oxygen atom is inverted to a *trans* relationship, a substantial decrease in sweetness and a marked increase in bitterness are registered. Each of the other five members of the series shows the *trans* relationship.

Although we were not able to isolate disaccharides containing D-mannose or L-rhamnose having O-1′ and O-2′ in a *cis* relationship (that is, the respective β-D-

*Primed numbers refer to the nonreducing sugar residue.

and β-L-forms), it is probable they would also be sweeter than the *trans* forms tested. The prediction is based on the report by Tsuzuki and Mori[18] that β-L-rhamnose (*cis* O-1′–O-2′) is much sweeter than its α-L isomer.

Changes of sweetness and bitterness caused by inverting the configuration at C-4′ are much smaller than those caused by inversions at C-1′ or C-2′. Slight decreases in sweetness were noted, however, when O-4′ was changed from an equatorial to an axial disposition in relatively sweet derivatives. The presence or absence of a C-5′ hydroxymethyl group was similarly judged to be of minor importance to sweetness when pentose/hexose analogs were compared (**5a/5b** and **5c/5d**).

The results are also in agreement with tests conducted recently by Hodge and coworkers[19] on a series of methyl aldopentopyranosides and aldohexopyranosides, and serve to reinforce the growing body of evidence that certain structural features in carbohydrates are far more important in eliciting sweet or bitter taste-responses than other similar features located at different sites in the sugar molecule.

## EXPERIMENTAL

*General methods.* — N.m.r. spectra were measured at 100 MHz on a Varian HA-100 spectrometer with tetramethylsilane ($\tau = 10.0$) as the internal standard. Chemical shifts and coupling constants are first-order, measured directly from spectral spacings. A Hewlett–Packard research chromatograph, Model 5750 equipped with an electronic integrator, was employed for g.l.c. Columns were 1/8-in. o.d. × 6 ft stainless-steel tubing packed as follows: (A) 3% JXR (dimethyl silicone) on Gas Chrom Q (100–120 mesh), and (B) 3% OV-17 (phenyl methyl silicone) on Gas Chrom Q (100–120 mesh). Column programming was isothermal at 160° for monosaccharides and at 220° for disaccharides, with helium as the carrier gas and with flame-ionization detection. All acetylated derivatives were deacetylated and converted into trimethylsilyl ethers approximately 18 h before injection. Melting points were determined in capillary tubes. Optical rotations were measured at 546.1 nm in a 0.2-dm cell with a Bendix recording polarimeter, Model 1169. Multiplication of the reported specific rotations by 0.85 would allow comparison with data reported at the D-line of a sodium lamp. Pyridine was removed from organic phases by repeated washing with 5% aqueous cupric sulfate, and acetic acid was removed with aqueous sodium hydrogen carbonate. Solutions were evaporated under diminished pressure. Precoated plates of Silica Gel F-254 (E. Merck, Darmstadt, Germany) were used for t.l.c. Layer thicknesses were 0.25 and 2.0 mm for analytical and preparative separations, respectively. For column chromatography, Baker-Analyzed Silica Gel (J. T. Baker Chemical Co., Phillipsburg, N.J.) was used without pretreatment. All chromatographic solvents were proportioned on a v/v basis. Calcium hydride was used to dry acetonitrile.

*Starting materials.* — 1,3,4,6-Tetra-*O*-acetyl-α-D-glucose (**1**), 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (**2a**), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2b**), 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide (**2c**), 2,3,4,6-tetra-*O*-acetyl-

α-D-galactopyranosyl bromide (**2d**), 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**2e**), 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**2f**), and methyl 4,6-*O*-benzylidene-α-D-glucoside were prepared by well established procedures[20–25].

*Preparation and isolation of disaccharides.* — By a modification of the procedure of Helferich and Zirner[8], the disaccharides were prepared on a 5- to 200-fold scale. A 1-mmole quantity of **1** was dissolved in a magnetically stirred solution containing 0.5-mmole portions of anhydrous mercuric bromide and mercuric cyanide in 3–4 ml of acetonitrile, and the resultant solution was treated with 1.3–1.4 mmoles of **2a–f**. The reaction mixture was kept for 18 h at 25°, examined by t.l.c. (either 9:1 chloroform–acetone or ether, 1 ascent), and then evaporated and the residue dissolved in a solution of benzene (5–6 ml) and pyridine (1 ml). Mercuric salts were precipitated with hydrogen sulfide and removed by filtration through Celite 535. The filtrate was evaporated and then kept for 48 h at 25° in a mixture of acetic anhydride (2 ml) and pyridine (1 ml). Excess anhydride was destroyed with cold methanol. The sample was evaporated, the residue dissolved in ethyl acetate, and the solution freed of water-soluble materials. Yields of disaccharides were determined by quantitative g.l.c. analysis on columns A or B, and calculated on the basis of moles of **1** converted into disaccharides. The product mixture was then added as a 50% w/v solution in benzene to a column of silica gel packed with benzene and the column was sequentially eluted with benzene and mixtures of benzene–ethyl acetate (85:15, 7:3, and then 1:1). Up to 100 g of products could be fractionated on 500 g of gel in this manner. The monosaccharide fraction was almost completely eluted by benzene on heavily loaded columns before ethyl acetate was added to the eluant, and the products **3a–f** tended to be eluted faster than the **6a–f** derivatives in the 85:15 eluant, as judged by t.l.c. (7:3 hexane–acetone, 2 ascents; or 100:3 benzene–methanol, 2 ascents). When necessary, forms **3** or **6** were converted into forms **4** or **7** in two steps: First of all, each gram of product was treated for 75 min at 25° with 1.5 ml of 30% hydrogen bromide in acetic acid and then chloroform was added, water-soluble products were removed, and the solutions were dried, and evaporated. Secondly, each gram of glycosyl bromide intermediate was treated for 24 h at 25° with a solution of mercuric acetate (1 g) in acetic acid (10 ml). After the acetic acid had been evaporated, mercuric salts were removed as already described. Purified disaccharide peracetates were deacetylated during 48 h at −5° with barium methoxide (0.1M) in methanol. The solutions were neutralized with Amberlite IRC-50 ($H^+$) resin and the resin filtered off. The filtrate was evaporated, decolorized with Darco G-60 in water, and re-evaporated.

*Hepta-O-acetyl-2-O-α-D-xylopyranosyl-β-D-glucopyranose* (**4a**). — Approximately equal amounts of **3a** and **6a** were formed in an overall yield of 77% when **1** (69 g) was condensed with **2a** (91 g). Neither disaccharide could be crystallized after acetylation and fractionation techniques had been applied. Each was then converted into the β-D-gluco form (**4a, 7a**) by previously discussed techniques and freed of hydrolysis products. Fractional crystallization at 25° from methanol gave **4a** (35 g); m.p. 174–175°, $[\alpha]_{456}^{25}$ +118° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 4.29 triplet, $J_{3',4'}$ 10 Hz, H-3'), 4.34 (doublet, $J_{1,2}$ 8.5 Hz, H-1), 4.55 (doublet, $J_{1',2'}$

3.8 Hz, H-1'), 5.14 (doublet of doublets, $J_{2',3'}$ 10 Hz, H-2'), 6.23 (triplet, $J_{2,3}$ 8.5 Hz, H-2), and 6.70 (multiplet, H-5).

*Anal.* Calc. for $C_{25}H_{34}O_{17}$: C, 49.51; H, 5.65. Found: C, 49.23; H, 5.85.

Deacetylation gave crystalline **5a** from aqueous ethanol; m.p. 190–195°, $[\alpha]_{456}^{25}$ +194° → +165° (24 h, *c* 1.1, water); n.m.r. data (methyl sulfoxide-$d_6$): $\tau$ 4.91 (doublet, $J_{1,2}$ 3.5 Hz, H-1'), and 5.23 (doublet, $J_{1',2'}$ 3.5 Hz, H-1).

*Hepta*-O-*acetyl*-2-O-β-D-*xylopyranosyl*-β-D-*glucose* (**7a**). — Crude **7a** (27 g) separated at −5° from the liquors previously processed for **4a**. One recrystallization from ethanol gave pure **7a**; m.p. 142–143°, $[\alpha]_{456}^{25}$ −9.8° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 4.28 (doublet, $J_{1,2}$ 8.5 Hz, H-1), 4.66 (triplet, $J_{3,4}$ 9 Hz, H-3), 5.36 (doublet, $J_{1',2'}$ 6 Hz, H-1'), 6.30 (triplet, $J_{2,3}$ 8.5 Hz, H-2), 6.60 (multiplet, H-5), and 6.79 (doublet of doublets, $J_{4',5'ax}$ 7.5 Hz, $J_{5'eq,5'ax}$ 12.2 Hz, H-5'ax).

*Anal.* Calc. for $C_{25}H_{34}O_{17}$: C, 49.51; H, 5.65. Found: C, 49.60, H, 5.86.

Deacetylation of **7a** (10 g) gave crystalline **8a** from ethanol; m.p. 179–181° (lit.[9] 202–203°), $[\alpha]_{456}^{25}$ +43.5° → 33.9° (24 h, *c* 1, water); n.m.r. data (methyl sulfoxide-$d_6$): $\tau$ 4.99 (doublet, $J_{1,2}$ 3.3 Hz, H-1) and 5.76 (doublet, $J_{1',2'}$ 7.5 Hz, H-1').

*Octa*-O-*acetyl*-α-*kojibiose* (**3b**). — Condensation of **1** (43 g) with **2b** (67.5 g) gave approximately equal amounts of **3b** and **6b**. The overall yield of disaccharides was 71%, as estimated by g.l.c. Pure **3b** (10 g) was crystallized from methanol; m.p. 164–165° (lit.[6] 168–169°), $[\alpha]_{456}^{25}$ +175° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 3.74 (doublet, $J_{1,2}$ 4 Hz, H-1), 4.33 (triplet, $J_{3,4}$ 9.8 Hz, H-3), 4.97 (doublet of doublets, $J_{2',3'}$ 10 Hz, H-2'), 5.19 (doublet, $J_{1',2'}$ 3.5 Hz, H-1'), 6.49 (doublet of doublets, $J_{2,3}$ 9.8 Hz, H-2). Deacetylation afforded α-kojibiose (**5b**) from aqueous ethanol; m.p. 181–183° (lit.[26] 187–188°), $[\alpha]_{456}^{24}$ +168° → +145° (*c* 1, water); n.m.r. data (methyl sulfoxide-$d_6$): $\tau$ 4.90 (doublet after addition of $D_2O$, $J_{1,2}$ 4 Hz, H-1), and 5.19 (doublet, $J_{1',2'}$ 3.7 Hz, H-1').

*Octa*-O-*acetyl*-β-*sophorose* (**7b**). — The crystallization liquors from **3b** were evaporated, treated with 30% hydrogen bromide in glacial acetic acid as already described, and the product crystallized from ether. The crude hepta-*O*-acetyl-α-sophorosyl bromide (34 g) was converted into **7b** without further examination. Crystallization from ethanol gave pure **7b** (25 g); m.p. 189–190° (lit.[10] 191–192°), $[\alpha]_{456}$ −2.9° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 4.37 (doublet, $J_{1,2}$ 8.1 Hz, H-1), 5.55 (doublet, $J_{1',2'}$ 7.7 Hz, H-1'), 6.37 (triplet, $J_{2,3}$ 8.5 Hz, H-2), and 6.82 (multiplet, H-5). Crystalline α-sophorose·$H_2O$ (**8b**) was obtained from aqueous ethanol; m.p. 186–188° (lit.[10] 185–188°), $[\alpha]_{456}^{42}$ +35.7° → +23.6° (*c* 1, water); n.m.r. data (methyl sulfoxide-$d_6$): $\tau$ 4.87 (doublet after $D_2O$ had been added, $J_{1,2}$ 3 Hz, H-1) and 5.72 (doublet after $D_2O$ had been added, $J_{1',2'}$ 7 Hz, H-1').

*Hepta*-O-*acetyl*-2-O-β-L-*arabinopyranosyl*-α-D-*glucose* (**3c**). — Condensation of **1** (57 g) and **2c** (69 g) gave a 96% yield of **3c** and **6c**, of which **3c** formed 87% of the total. The mixture was acetylated, fractionated, and then crystallized from ether to yield **3c**: m.p. 100–102°, $[\alpha]_{456}^{25}$ +203.6° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 3.73 (doublet, $J_{1,2}$ 4 Hz, H-1), 4.34 (triplet, $J_{3,4}$ 9.8 Hz, H-3), 5.00 (doublet,

$J_{1',2'}$ 3.5 Hz, H-1'), $\tau$ 6.49 (doublet of doublets, $J_{2,3}$ 9.8 Hz, H-2), and 6.54 (doublet of doublets, $J_{4',5'ax}$ 2 Hz, $J_{5'eq,5'ax}$ 13 Hz, H-5'ax).

*Anal.* Calc. for $C_{25}H_{34}O_{17}$: C, 49.51; H, 5.65. Found: C, 49.27; H, 5.86.

Deacetylation gave **5c** from aqueous ethanol; m.p. 235–236° (lit.[7] 210–220°), $[\alpha]_{456}^{25}$ +189° → +224° (24 h, *c* 1.1, water); n.m.r. data (methyl sulfoxide-$d_6$): $\tau$ 4.87 (doublet, $J_{1',2'}$ 3 Hz, H-1') and 5.55 (doublet, $J_{1,2}$ 7.5 Hz, H-1).

*Octa*-O-*acetyl*-2-O-α-D-*galactopyranosyl*-α-D-*glucose* (**3d**). — A 1:1 product mixture of **3d** and **6d** was formed in 69% yield from **1** (65.2 g) and **2d** (100 g). The disaccharide fraction, after silica gel fractionation, was partially crystallized from ethanol to yield **3d** (13.4 g); m.p. 175–177° after one recrystallization from methanol (lit.[8] 176.5–177.5°), $[\alpha]_{456}^{25}$ +176° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 3.69 (doublet, $J_{1,2}$ 3.8 Hz, H-1), 4.32 (triplet, $J_{3,4}$ 9.8 Hz, H-3), 4.98 (multiplet, H-1'), and 6.41 (doublet of doublets, $J_{2,3}$ 9.8 Hz, H-2). A 10-g portion was deacetylated, isolated as a glass, and used without crystallization, $[\alpha]_{456}^{24}$ +170° (*c* 1, water).

*Octa*-O-*acetyl*-2-O-β-D-*galactopyranosyl*-β-D-*glucose* (**7d**). — Efforts to form and isolate **7d** from **3d** in a manner analogous to that used for **7b** did not yield a pure product. In an alternative synthesis, **9** (80.5 g) was condensed with **2d** (145 g) by the method of Coxon and Fletcher[10]. The product mixture did not yield crystalline **10** from 2-ethoxyethanol, and was therefore dissolved in 350 ml of a solution containing sulfuric acid (4% v/v) in acetic anhydride. After 6 h of stirring at 25°, the acetolysis product-mixture was isolated as described for sophorose and separated from monosaccharide byproducts on a silica gel column. G.l.c. (column A, 220°) indicated that approximately one-half of the original **10** had been converted into a mixture of **6d**, **7d**, and a mixture of nonreducing compounds believed to be methyl hepta-*O*-acetyl-2-*O*-β-D-galactopyranosyl-α,β-D-glucosides (**11**, **12**). The nonreducing disaccharides were not characterized, but contained seven acetyl groups and one methoxyl group (as judged by n.m.r. spectroscopy).

A 55-g portion of the acetolysis mixture was dissolved in a solution containing piperidine (50 ml) in anhydrous tetrahydrofuran (210 ml) and the solution was stirred at 25°. Reaction progress was monitored by t.l.c. (9:1 chloroform–acetone) for 3 h, acetic acid (50 ml) was then added, and the final solution was evaporated. A solution of the residue in ethyl acetate (800 ml) was freed of water-soluble materials, dried, and evaporated. The residual syrup (48 g) was fractionated on a silica gel column with two mixtures of benzene–ethyl acetate; 17:3 for the nonreducing components (25 g) and 1:1 for the O-1 deacetylated product (20 g). The latter product was reacetylated, converted into the glycosyl bromide intermediate, and treated with mercuric acetate to form **7d**. Crystallization from ethanol gave pure **7d**, m.p. 150–151°, $[\alpha]_{456}^{25}$ +17.6° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 4.32 (doublet, $J_{1,2}$ 8 Hz, H-1), 4.71 (triplet, $J_{3,4}$ 9 Hz, H-3), 5.45 (doublet, $J_{1',2'}$ 8 Hz, H-1'), 6.28 (triplet, $J_{2,3}$ 8.5 Hz, H-2), and $\tau$ 6.69 (multiplet, H-5).

*Anal.* Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.66. Found: C, 49.26; H, 5.84.

A 10-g sample was deacetylated, and isolated as a glass; $[\alpha]_{456}^{24}$ +47° (*c* 1, water).

*Hepta*-O-*acetyl*-β-*neohesperidose* (**7e**). — Condensation of **1** (65 g) with **2e**

(100 g) gave a mixture of **3e** and **6e** in a yield of 91%, of which **6e** formed 75–80% of the total. The entire mixture was acetylated, reacted to invert the configuration at C-1 of each D-glucose residue as already described, and then fractionated on a silica gel column. Crystalline **7e** was obtained from aqueous ethanol; m.p. 147–149° (lit.[1,12] 152–153°, 148°), $[\alpha]_{456}^{25}$ +3.0° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 4.30 (doublet, $J_{1,2}$ 8.3 Hz, H-1), 5.05 (doublet, $J_{1',2'}$ 3.8 Hz, H-1'), 6.42 (triplet, $J_{2,3}$ 8.5 Hz, H-2), 6.59 (multiplet, H-5), and 8.76 (doublet, C-Me). Deacetylation gave **8e**, isolated as a glass, $[\alpha]_{456}^{24}$ −3.3° (24 h, *c* 1, water).

*Octa*-O-*acetyl*-2-O-α-D-*mannopyranosyl*-β-D-*glucose* (**4f**). — Condensation of **1** (42 g) and **2f** (64 g) gave **3f** almost exclusively in an overall yield of 89% (as disaccharides). Acetylation and fractionation of the mixture gave **3f** as a syrup. A 48-g portion was converted into **4f**, via the glycosyl bromide intermediate, and refractionated. A portion (18 g) was crystalline from ether. Recrystallization from ethanol gave pure **4f**; m.p. 148–149°, $[\alpha]_{455}^{25}$ +48.3° (*c* 1, chloroform); n.m.r. data (benzene-$d_6$): $\tau$ 4.40 (doublet, $J_{1,2}$ 8.5 Hz, H-1), 4.95 (doublet, $J_{1',2'}$ 2 Hz, H-1'), 6.41 (triplet, $J_{2,3}$ 9 Hz, H-2), 6.67 (multiplet, H-5).

*Anal.* Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.66. Found: C, 49.80, H, 5.93.

A 10-g portion of **4f** was deacetylated and isolated without further examination, $[\alpha]_{456}^{25}$ +75.5° (*c* 1, water).

*Taste tests.* — Seven persons were selected for their ability to detect the sweet taste of 0.5% w/v solutions of sucrose in water and to evaluate consistently both the sweet and bitter tastes of methyl β-D-glucopyranoside at 3% concentration in water. Six of the panel members were already experts at evaluating bitter taste in soybean products.

Panel sittings were conducted in private booths under controlled lighting, relative humidity (50%), and temperature (25°). Each (1→2)-linked disaccharide was evaluated as a 3% w/v solution in charcoal-filtered tap water. Each solution was equilibrated for 18 h at +5°, warmed to 25°, and presented in 7- to 10-ml portions in glass beakers to panel members. When necessary, the disaccharide solutions were filtered before evaluation to remove small amounts of a fine, white precipitate. No precipitate formed in distilled water.

Tasters were instructed to taste first with the tip of the tongue for sweetness, and then to roll the solution to the back of the tongue to evaluate bitterness. Ratings in tenths between the concentrations of the reference sucrose solutions (0.5, 1.0, and 1.5% w/v) in the same water were encouraged. No more than four compounds were evaluated at one sitting of the panel.

Bitterness was evaluated on a scale of 0, 1, 2, 3 wherein 2 was judged to be equivalent in bitterness to a reference 0.05% caffeine solution. A rating of 3 designated all degrees of stronger bitterness than the reference solution.

The average deviation from the average was calculated for both sweetness and bitterness ratings for each compound. Occasional aberrant ratings that varied more than three times the average deviation from the average were arbitrarily omitted from the final calculations. Relative sweetness to sucrose (= 1.0) can be calculated for a

sample by dividing the equivalent sucrose rating by the concentration of the test solution.

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