823. *Sophorose and Its Derivatives.*

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An improved preparation of sophorose from the pods of *Sophora japonica* L. is described. New derivatives (sophoritol and its nona-*O*-acetate, and sodium sophoronate) have been prepared. Additional evidence that sophorose is 2-*O*-β-D-glucopyranosyl-D-glucose is produced.

Sophorose can be obtained by the hydrolysis of kæmpferol sophoroside, a pigment occurring in the pod of *Sophora japonica* L.[1] and of stevioside, the sweet principle of *Stevia rebaudiana* Bertoni.[2] It has been isolated from the acid reversion products of D-glucose,[3] from the "hydrol" of sweet potato,[4] and from the fragmentation products of a pyrodextrin.[5] Chemical[6] and enzymic[7] syntheses of sophorose have also been described.

The best source of sophorose is the pod of *S. japonica*. *Sophora* is a large genus of Leguminoseæ with about 75 species of world-wide distribution,[8] and although *S. japonica* appears to be the only species which has been used as a source of sophorose it is possible that others may also produce this disaccharide.

We have improved the preparation of sophorose from *S. japonica*; instead of isolating

[1] (*a*) Rabaté and Charaux, *Bull. Soc. Chim. biol.*, 1938, **20**, 454; Rabaté and Dussy, *ibid.*, 1938, **20**, 459, 467; (*b*) Freudenberg, Knauber, and Cramer, *Chem. Ber.*, 1951, **84**, 144.

[2] Vis and Fletcher, jun., *J. Amer. Chem. Soc.*, 1956, **78**, 4709.

[3] (*a*) Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309; (*b*) Peat, Whelan, Edwards, and Owen, *J.*, 1958, 586.

[4] Aso and Shibasaki, *Tôhoku J. Agric. Res.*, 1955, **6**, 159.

[5] Wolfrom, Thompson, and Ward, *J. Amer. Chem. Soc.*, 1959, **81**, 4623.

[6] (*a*) Freudenberg, Toepffer, and Anderson, *Ber.*, 1928, **61**, 1750; Freudenberg and Soff, *Ber.*, 1936, **69**, 1245; (*b*) Gakhokidze, *J. Gen. Chem. U.S.S.R.*, 1941, **11**, 117; *Chem. Abs.*, 1941, **35**, 5467; (*c*) Haq, Ph.D. Thesis, Wales, 1957.

[7] (*a*) Peat, Whelan, and Hinson, *Nature*, 1952, **170**, 1056; (*b*) Walker, Pellegrino, and (in part) Khan, *Arch. Biochem. Biophys.*, 1959, **83**, 161.

[8] Dandy, personal communication.

kæmpferol sophoroside, we applied column chromatography to the mixture of sugars obtainable by a mild acid hydrolysis of the juice or of an aqueous extract of the pods.

The aqueous extract of *S. japonica* pods contained polysaccharides (there is no previous record of them and studies of them are now in progress), and after their removal sophorose was isolated as described in an overall yield of 0·4%; previous yields reported were 0·3% [1a] and 0·08%.[1b] The disaccharide was identified by comparison of its β-octa-*O*-acetate with an authentic specimen.

Sophoritol was prepared by the reduction of sophorose with sodium borohydride,[9] a reagent to which glycosides are stable,[10] and was isolated as its crystalline nona-*O*-acetate. Deacetylation of the acetate gave sophoritol as a syrup. Sophoritol isolated directly after reduction of sophorose was also a syrup.

Gakhokidze [6b] prepared a glucobionic acid from the 2-*O*-β-D-glucopyranosyl-D-glucose, which he had synthesised, but did not describe its properties. Our preparation of sodium sophoronate is, therefore, the first to be characterised. Sophorose was oxidised by the method of Hudson and Isbell; [11] after chromatography on charcoal, sodium sophoronate crystallised with 1·5 mol. of water.

Both sophoritol and sodium sophoronate have high M_G values (0·77 and 1·05 respectively). This increase of M_G when sophorose is converted into its open-chain derivatives, sophoritol and sodium sophoronate, where in the latter case the carboxyl group increases the effect, parallels that observed by Côté for fucobioses.[12] We have observed the same effect with glucobi-itols and glucobionates prepared from other glucobioses. The degree of enhancement of M_G is related to the type of glycosidic linkage.[12]

Other derivatives are sophorose phenylhydrazone,[6b] octa-*O*-acetyl-α-sophorose,[14] α-acetobromosophorose,[1b,2,6a] methyl α-sophoroside,[6a] and methyl hepta-*O*-acetyl-α-sophoroside.[6a]

With regard to the structure of sophorose, Rabaté's methylation studies [14] of sophorose and of kæmpferol sophoroside, although incomplete, indicated the presence of a pyranose ring in the non-reducing moiety of the disaccharide. Because sophorose did not form a disaccharide osazone and reacted feebly with Fehling's solution, Rabaté concluded that the D-glucose units were joined by a 1,2-glycosidic linkage, to which he assigned the β-configuration since the compound was hydrolysed by almond emulsin,[1a] an enzyme preparation containing a β-glucosidase.[15] By a direct comparison of the α-acetobromo-derivatives, Freudenberg and his associates [1b] identified the natural compound with a synthetic β-1,2-glucobiose, the glycosidic linkage of which was deduced mainly from the fact that the disaccharide, when treated with phenylhydrazine, gave glucosazone.[6a] Of the two definitive syntheses of 2-*O*-β-D-glucopyranosyl-D-glucose, one [6b] yielded a product whose properties do not agree with the accepted values for sophorose (see Table), the other,[6c] a product which was insufficiently characterised; neither of these compounds was compared directly with the natural product. Therefore, although the structure of sophorose has been elaborated to a certain degree, no rigid proof of structure is described in the literature. As additional evidence that sophorose is 2-*O*-β-D-glucopyranosyl-D-glucose, we describe below the results of periodate oxidations of sophorose, sophoritol, and sodium sophoronate.

These oxidations were carried out in 40mM- and in 0·4mM-sodium periodate, concentrations which afford a ready distinction between the reducing and non-reducing end of a disaccharide.[16] In the oxidation of sophorose or its derivatives, or of sophoritol or

[9] Abdel-Akher, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691.
[10] Whelan and Morgan, *Chem. and Ind.*, 1955, 1449; Lee, *ibid.*, 1959, 1455.
[11] Hudson and Isbell, *J. Amer. Chem. Soc.*, 1929, **51**, 2225.
[12] Côté, *J.*, 1959, 2248.
[13] Clancy, unpublished results.
[14] Rabaté, *Bull. Soc. chim. France*, 1940, **7**, 565.
[15] Tauber, *J. Biol. Chem.*, 1932, **99**, 257; Pigman (Ed.), "The Carbohydrates," Academic Press Inc. New York, 1957, p. 575.
[16] Clancy and Whelan, *Chem. and Ind.*, 1959, 673.

Properties of sophorose and octa-O-acetyl-β-sophorose obtained from various sources.

Source	Sophorose monohydrate M. p.	Sophorose monohydrate $[\alpha]_D$ in H_2O *	Octa-*O*-acetyl-β-sophorose M. p.	Octa-*O*-acetyl-β-sophorose $[\alpha]_D$ in $CHCl_3$	Ref.
Present work	196—198°	+19·1°	193—194°	−3·2°	
Sophora japonica	195—196	22·6	—	—	1*a*
Sophora japonica	—	18·6	192	−2·8	11
Sophora japonica	195—196	20 †	—	−8	1*b*
Stevia rebaudiana	—	—	192	—	2
Acid + glucose	—	—	191—192 ‡	−3·8	3*a*
" Hydrol "	—	—	188	—	4
Pyrodextrin	—	—	186	—	5
Chemical synthesis (Freudenberg)	180	19·9	192	−3·8	6*a*, 1*b*
,, (Gakhokidze)	176—178	27·5	189—190	−40·5	6*b*
,, (Haq)	—	21 †	—	—	6*c*
Almond emulsin + glucose	—	18·4	189·5	−3	7*a*
Acetobacter rancens enzyme + glucose	—	26	189·5	−8	7*b*

* Equilibrium value. † Anhydrous. ‡ Corr.

sodium sophoronate, especially by 0·4mM-periodate, it is to be expected that the large reducing end-group will have a steric effect [17] on the oxidation of the non-reducing end; the amount of periodate reduced by the non-reducing end should be considerably less than that reduced by methyl β-D-glucopyranoside (0·25 mol. after 7 hr. in 0·4mM-periodate [16]). The results obtained with sophorose in 0·4mM-periodate, namely, 1·5 mol. of oxidant consumed and 0·3 mol. of formaldehyde liberated after 7 hr., are, therefore, almost wholly due to oxidation of the reducing end and are consistent with a 2-substituted D-glucopyranose. Sophoritol yielded 1 mol. of formaldehyde with 0·4mM- and 40mM-periodate, but consumed 2·8 mol. of oxidant in the dilute periodate and 5 mol. in the more concentrated periodate (7 hr.); these results are in agreement with expectation (1, 3, and 5 mol., respectively) for a 2- or 5-*O*-D-glucopyranosyl-D-glucitol. A distinction between these alternatives is afforded by the oxidation of sodium sophoronate in 0·4mM-periodate: in this experiment, 1 mol. of formaldehyde was liberated and 3 mol. of oxidant were consumed in 1 hr., which excludes a 1,5-glycosidic linkage. Finally, the β-configuration of the glycosidic linkage is confirmed by the low rotation ($[\alpha]_D$ −18·6°) of sophoritol (2-*O*-α-D-glucopyranosyl-D-glucitol has $[\alpha]_D$ +81° [18]), and by hydrolysis of sophorose to glucose by almond emulsin. It is, therefore, rigidly proved that sophorose is 2-*O*-β-D-glucopyranosyl-D-glucopyranose.

Experimental

General Methods.—Adsorption chromatography was carried out on columns of equal parts by weight of B.D.H. activated charcoal powder and Celite 535,[19] which were thoroughly washed with water to remove acidic impurities before use.

Paper partition chromatography by the descending technique [20] was carried out on Whatman no. 3MM paper, which has a large capacity and so facilitates detection of traces of impurities. The following solvent systems were used: (*a*) butan-1-ol–acetic acid–water (4 : 1 : 5 v/v),[21] and (*b*) ethyl acetate–pyridine–water (10 : 4 : 3 v/v).[22] The spraying reagents were aniline diphenylamine,[23] silver nitrate–sodium hydroxide,[24] alkaline triphenyltetrazolium chloride.[25] R_{Gl} is the rate of travel relative to glucose.

Ionophoresis [26] was carried out in borate buffer at pH 10; reducing sugars were detected

[17] Garner, Goldstein, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1958, **80**, 1206.
[18] Barker, Gómez-Sánchez, and Stacey, *J.*, 1959, 3264.
[19] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.
[20] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.
[21] Partridge, *Biochem. J.*, 1948, **42**, 238.
[22] Whistler and Hickson, *Analyt. Chem.*, 1955, **27**, 1514.
[23] Buchan and Savage, *Analyst*, 1952, **77**, 401.
[24] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[25] Wallenfels, *Naturwiss.*, 1950, **37**, 491.
[26] Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 81.

with the aniline–diphenylamine spray,[23] and non-reducing materials with the periodate–benzidine spray.[27]

All solutions were evaporated under reduced pressure below 40°.

Oxidations were carried out in amber bottles at room temperature in 40mM- and in 0·4mM-sodium metaperiodate.[16] Periodate consumption was measured by oxidation of acidified potassium iodide,[28] and formaldehyde by chromotropic acid.[29]

Isolation of Sophorose.—Dried pods (730 g.) of *S. japonica* and water (2 l.) were heated on a boiling-water bath for 20 min. The softened fruit was then pulverised in a Waring blender to give a slurry from which the seeds were easily separated; these were discarded. Water (2 l.) was added to the slurry and the mixture again heated on a boiling-water bath for 20 min. The cooled mixture was centrifuged and the residue was washed with sufficient water to make a thin slurry and was then re-centrifuged. The combined supernatant liquids (about 6 l.) were concentrated to about 1 l. The concentrated solution was centrifuged at 7500 r.p.m. to remove fine particles and the clear supernatant liquid further concentrated to about 400 ml. The concentrate, a very dark brown solution, was added dropwise with stirring to ten times its volume of absolute ethanol. The precipitate, which included polysaccharide material, was removed on the centrifuge. Addition of diethyl ether (500 ml.) to the supernatant liquid precipitated more polysaccharide, which was removed in the same way. The clear supernatant liquid was concentrated to a yellow syrup (140 g.), which was heated in 22·5mN-sulphuric acid (8 l.) on a boiling-water bath for 1 hr. The hydrolysate was neutralised while still hot with 0·4N-barium hydroxide, filtered, and concentrated to about 750 ml. This solution was adsorbed on a charcoal–Celite column (78 × 7·5 cm.), which was eluted first with water (10 l.) and then by gradient elution with aqueous ethanol (V = 20 l., and X = 0·25 in the formula of Alm, Williams, and Tiselius [30]). Fractions of 500 ml. were collected and the elution pattern of the column was established by analysing 1 ml. of each fraction for sugar by the phenol–sulphuric acid method.[31] Fractions within a given peak were combined and concentrated. Portions of the solid residues from each peak were analysed on chromatograms with solvent *b*. Sophorose was the disccharide component which reacted strongly with the aniline–diphenylamine spray, slowly with the silver nitrate–sodium hydroxide spray, but not with the alkaline triphenyltetrazolium chloride spray.[32]

The first peak (fractions nos. 5—20), with water, was due to a mixture (67 g.), mainly of approximately equal amounts of glucose and fructose. The second peak (fractions nos. 32—40), at about 5% ethanol, was due to a mixture (834 mg.) of an unidentified disaccharide and sophorose. Crystalline sophorose (3 g.) was obtained from the eluate of the third peak (fractions nos. 41—52), at about 7% ethanol. Subsequent peaks (fractions nos. 53—61, 214 mg., and 62—90, 904 mg.) contained some sophorose and various unidentified oligosaccharides.

Sophorose, recrystallised from 80% (v/v) aqueous methanol in long needles (2·8 g.), was chromatographically pure and had $[\alpha]_D^{18}$ +19·1° (in H_2O; *c* 1·2) (stable), m. p. 196—198°, M_G 0·26 (lit.,[26] M_G 0·24), R_{Gl} 0·51 and 0·61 in solvents *a* and *b* respectively.

Enzymic Hydrolysis of Sophorose.—To the sugar (10 mg.), dissolved in water (1 ml.), was added 2% (w/v) aqueous almond emulsin [15] (0·25 ml.), and the mixture was incubated under toluene for 48 hr. at 30°. The digest was then examined by paper chromatography in solvents *a* and *b*. Sophorose, laminaribiose, and cellobiose were each hydrolysed to glucose, while maltose and methyl α-D-glucopyranoside were unaffected.

Octa-O-*acetyl*-β-*sophorose.*—Sophorose (250 mg.) was acetylated with sodium acetate–acetic anhydride. The product, worked up in the usual way, gave a chloroform-soluble syrup, which after two crystallisations from ethanol gave crystals (184 mg.) having $[\alpha]_D^{18}$ −3·2° (in $CHCl_3$; *c* 2·5), m. p. 193—194° undepressed on admixture with an authentic specimen kindly provided by Dr. W. J. Whelan.

Nona-O-*acetylsophoritol.*—Sophorose (200 mg.), dissolved in water (20 ml.), was added to 1% (w/v) aqueous sodium borohydride [9] (20 ml.) and kept at room temperature for 48 hr. The pH of the mixture was then adjusted to 4·8 with 0·1N-sulphuric acid and the whole

[27] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.
[28] Hughes and Nevell, *Trans. Faraday Soc.*, 1948, **44**, 941.
[29] Frisell, Meech, and Mackenzie, *J. Biol. Chem.*, 1954, **207**, 709; Clancy and Whelan, unpublished work.
[30] Alm, Williams, and Tiselius, *Acta Chem. Scand.*, 1952, **6**, 826.
[31] Dubois, Gilles, Hamilton, Rebers, and Smith, *Analyt. Chem.*, 1956, **28**, 350.
[32] Bell and Dobonder, *J.*, 1954, 2866; Feingold, Avigad, and Hestrin, *Biochem. J.*, 1956, **64**, 351.

evaporated to dryness. The residue was further dried *in vacuo* at 56° overnight and then acetylated with sodium acetate–acetic anhydride. The chloroform-soluble syrup (230 mg.), isolated in the usual way, deposited crystals from ethanol, which after one more crystallisation yielded pure *nona-O-acetylsophoritol* (142 mg.), $[\alpha]_D^{20}$ −21° (in $CHCl_3$; *c* 2·5), m. p. 151—152° (Found: C, 49·0; H, 5·9; Ac, 53·3. $C_{30}H_{42}O_{20}$ requires C, 49·9; H, 5·9; 9Ac, 53·6%).

Sophoritol.—(*a*) Nona-*O*-acetylsophoritol (119 mg.) was de-*O*-acetylated with dry methanol saturated with ammonia [33] (15 ml.). The product (63 mg.) was the uncrystallisable sophoritol and was chromatographically pure. It had $[\alpha]_D^{26}$ −18·6° (in H_2O; *c* 0·75), the concentration being determined by the method of acid hydrolysis to glucose.[34]

(*b*) Sophorose (51·2 mg.) was reduced as described in (*a*). The mixture was then percolated through a column (7 × 1·2 cm.) of Amberlite 1R-120 (H^+) ion-exchange resin, which was washed with water until the eluate contained no more carbohydrate [tested with a 0·2% (w/v) solution of anthrone in concentrated sulphuric acid [35]]. The combined eluates from the column were freed from boric acid by repeated addition and distillation of methanol. The final ethanol-soluble syrup (49·8 mg.), which did not crystallise, was identical with that of (*a*) when examined by paper chromatography and by ionophoresis. The alcohol had $[\alpha]_D^{20}$ −18·2° (in H_2O; *c* 0·2) (concentration determined by acid hydrolysis to glucose [34]), M_G 0·77, R_{Gl} 0·51, and 0·57 in solvents *a* and *b* respectively.

Sodium Sophoronate.—Bromine (0·13 ml.) was added to a solution of sophorose (240 mg.) and barium benzoate (400 mg.) in water (10 ml.), and the oxidation was conducted in the dark at room temperature for 48 hr.[11] The excess of bromine was removed by cautiously adding 98—100% formic acid dropwise to the reaction mixture. The colourless mixture was then treated with 10% (w/v) aqueous sodium sulphate (5 ml.), and the precipitated barium sulphate and benzoic acid were filtered off. The clear filtrate was adjusted to pH 6 with 3N-sodium hydroxide and evaporated. The solid residue was dissolved in water (5 ml.) and adsorbed on a charcoal–Celite column (44 × 2 cm.), which was then eluted with water. Inorganic salts were removed in the first litre of the eluate (tested with silver nitrate solution), and sodium sophoronate in the next 1·5 l., which was concentrated to a syrup. This syrup was extracted with 80% (v/v) aqueous methanol, filtered, and concentrated to a syrup (190 mg.), which crystallised from aqueous ethanol. The large crystals of hydrated *sodium sophoronate* (154 mg., 73%) had $[\alpha]_D^{20}$ −4·2° (20 min.) ⟶ −5·1° (in H_2O; *c* 1), m. p. 199—200° decomp.), M_G 1·05 R_{Gl} 0·40 and 0·13—0·17 (a double spot) in solvents *a* and *b* respectively [Found: C, 35·1; H, 5·8; loss at 85° *in vacuo* over P_2O_5 in 18 hr., 6·6 (constant). $3C_{12}H_{21}O_{12}Na,2H_2O$ requires C, 35·4; H, 5·9; H_2O, 6·6%)].

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[33] Fischer and Bergmann, *Ber.*, 1917, **50**, 1047.
[34] Pirt and Whelan, *J. Soc. Food Agric.*, 1951, **2**, 224.
[35] Drewood, *Analyt. Chem.*, 1946, **18**, 499.