270. *The Chemical Synthesis of Polysaccharides. Part II.* The Chemical Synthesis of Nigerose.*

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Nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) has been synthesised by reaction between 3 : 4 : 6-tri-*O*-acetyl-1-chloro-β-D-glucopyranose and 1 : 2-5 : 6-di-*O*-*iso*propylidene-3-*O*-sodio-α-D-glucofuranose. Minor products are kojibiose, a trehalose, and an unidentified diglucose.

The chemical synthesis of *O*-α-D-glucopyranosyl-(1 ⟶ 3)-D-glucose was achieved in 1946 by Gakhokidze,[1] by treatment of a mixture of 4 : 6-*O*-benzylidene-1 : 2-*O*-*iso*propylidene-α-D-glucopyranose and 2 : 3 : 4 : 6-tetra-*O*-acetyl-D-glucose with zinc chloride and phosphoric oxide. The properties of the sugar and a crystalline acetyl derivative were recorded. The isolation of the disaccharide was not reported again until 1953, when Barker, Bourne, and Stacey [2] obtained it from a partial hydrolysate of the fungal polysaccharide nigeran, and gave it the name nigerose. The structure was established [2] by conversion of the sugar into the same osazone as is formed by turanose (3-*O*-α-D-glucopyranosyl-D-fructose), and the crystalline β-octa-*O*-acetyl derivative [3] has been used to characterise the sugar. Recently Barker, Bourne, O'Mant, and Stacey [4] provided further

* Part I, *J.*, 1956, 4543.

[1] Gakhokidze, quoted by Evans, Reynolds, and Talley, *Adv. Carbohydrate Chem.*, 1951, **6**, 27; see also *J. Gen. Chem. U.S.S.R.*, 1949, **19**, 2100; *Chem. Abs.*, 1950, **44**, 3913.

[2] Barker, Bourne, and Stacey, *J.*, 1953, 3084.

[3] Peat, Whelan, and Hinson, *Chem. and Ind.*, 1955, 385.

[4] Barker, Bourne, O'Mant, and Stacey, *J.*, 1957, 2448.

structural evidence by means of methylation analysis. After the isolation from nigeran, nigerose was obtained from a variety of sources (see Table). With two exceptions all these specimens and their acetyl derivatives have the same specific optical rotations as the material from nigeran and these values are very different from those reported by Gakhokidze.[1] The exceptions are specimens of the free sugar synthesised by Pazur and his co-workers [5,6] who effected the acid- and enzyme-catalysed transfer of a glucose unit from maltose to glucose. Their products had the same $[\alpha]_D$ as Gakhokidze's specimen and gave turanosazone on treatment with phenylhydrazine, but the acetyl derivatives were not prepared. Despite the many reports of the isolation of nigerose the only definitive chemical synthesis has been that by Gakhokidze. We have resolved the conflict by a chemical synthesis of 3-*O*-α-D-glucopyranosyl-D-glucose.

The desired linkage has the α-configuration. The synthesis of α-glucosidic disaccharides is difficult and it is only in the last few years that sucrose,[7] maltose,[8] αα-trehalose,[9] and kojibiose [10] (2-*O*-α-D-glucopyranosyl-D-glucose) have been chemically synthesised, though always in yields of only a few per cent. In each case the compound used to form the α-glucosyl group in the disaccharide was 3 : 4 : 6-tri-*O*-acetyl-1 : 2-anhydro-α-D-glucopyranose (Brigl's anhydride [11]). β-Linked disaccharides are usually synthesised by the Koenigs–Knorr reaction but for the synthesis of an α-diglucose by this method a tetra-*O*-acetyl-β-halogenoglucose would be required and the known examples of this type are unstable. However, 3 : 4 : 6-tri-*O*-acetyl-1-chloro-β-D-glucopyranose [12] is reasonably stable and was chosen in preference to Brigl's anhydride since it allowed the use of the 3-*O*-sodio-derivative of the second reactant, 1 : 2-5 : 6-di-*O*-*iso*propylidene-α-D-glucofuranose, in the attempt to increase the reactivity of the 3-hydroxyl group. This group is rather unreactive, as can be seen by comparing the yields of gentiobiose [12] (74%; β-1 : 6-linked diglucose) and laminaribiose (9·5%,[13] *ca.* 2%,[14] β-1 : 3-linked diglucose) obtained by the Koenigs–Knorr reaction. The ultimate yield of nigerose (see below) was not, however, greater than the yields of disaccharides synthesised from Brigl's anhydride. Competition was expected from self-condensation of the halogenoglucose through its free 2-hydroxyl group, forming kojibiose,[10,15] and this, in fact, happened (see below).

The two reactants were refluxed in toluene, conditions used by Gilbert, Smith, and Stacey [16] in a similar type of condensation with di*iso*propylidenesodioglucose. The products were deacetylated and fractionated on charcoal–Celite into free sugars and *iso*propylidene derivatives. It had been hoped to obtain a di-*O*-*iso*propylidene derivative of nigerose but the isolation of 1 : 2-*O*-*iso*propylidene-D-glucose showed that some loss of acetone had taken place. (When synthesising laminaribiose from acetobromoglucose and di*iso*propylideneglucose, Freudenberg and Oertzen [14] encountered the same behaviour, some 40% of the di*iso*propylideneglucose being decomposed into the mono*iso*propylidene derivative.) The acetone residues were therefore removed with acid from the mixed derivatives of glucose and nigerose, and 231 mg. (1·8%) of chromatographically pure disaccharide were finally obtained. Much more nigerose was undoubtedly present, but it proved impossible preferentially to hydrolyse the *iso*propylidene residues without severing the inter-sugar linkage, either under the conditions chosen or those described by

[5] Pazur and Budovich, *J. Amer. Chem. Soc.*, 1956, **78**, 1885.
[6] Pazur, Budovich, and Tipton, *ibid.*, 1957, **79**, 625.
[7] Lemieux, *Canad. J. Chem.*, 1953, **31**, 949.
[8] Lemieux and Huber, *J. Amer. Chem. Soc.*, 1956, **78**, 4117.
[9] Lemieux and Bauer, *Canad. J. Chem.*, 1954, **32**, 340.
[10] Haq and Whelan, *Nature*, 1956, **178**, 1221.
[11] Brigl, *Z. physiol. Chem.*, 1921, **116**, 1.
[12] Reynolds and Evans, *J. Amer. Chem. Soc.*, 1938, **60**, 2559.
[13] Bächli and Percival, *J.*, 1952, 1243.
[14] Freudenberg and Oertzen, *Annalen*, 1951, **574**, 37.
[15] Barker, Bourne, Grant, and Stacey, *Nature*, 1956, **178**, 1221.
[16] Gilbert, Smith, and Stacey, *J.*, 1946, 622.

Freudenberg and Oertzen [14] and Bächli and Percival [13] for the hydrolysis of the corresponding derivatives of laminaribiose.

Properties of nigerose, nigerosazone and β-nigerose octa-O-acetate obtained from various sources.

Source	Nigerose, $[\alpha]_D$ in H_2O	Nigerosazone, m. p.	β-Nigerose octa-*O*-acetate m. p.	$[\alpha]_D$ in $CHCl_3$	Ref.
Present work	135°	—	151—152°	84°	—
Nigeran	136	204—206°	149—150	72	2, *a*
Amylopectin	—	—	151—153	80	*b*
*iso*Lichenin	138·8	—	149	84·9	*c*
Floridean starch	136·9	—	147—148	86·6	*d*
Acid + glucose	—	—	155—157 *	78	*e*
Aspergillus niger enzyme + glucose	134	—	151—152	75	3
Schizosaccharomyces pombe enzyme + glucose	—	—	150—150·5	—	
Koji extract + glucose	135	205	150	80	*g*
Chemical synthesis (Gakhokidze)	84·8	—	149	41·3	1
Acid + glucose + maltose	87	205—206	—	—	5
Aspergillus oryzae enzyme + glucose + maltose	89	204—205	—	—	6

* Corr.

(*a*) Barker, Bourne, and O'Mant, *Chem. and Ind.*, 1955, 425; (*b*) Wolfrom and Thompson, *J. Amer. Chem. Soc.*, 1956, **78**, 4116; (*c*) Morgan, Ph.D. Thesis, Wales, 1956; (*d*) Peat, Turvey, and Evans, *Nature*, 1957, **179**, 261; (*e*) Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309; (*f*) Shibasaki, *Tohôku J. Agric. Res.*, 1955, **6**, 171; (*g*) Matsudo and Aso, *ibid.*, 1954, **5**, 123.

Properties of Synthetic Nigerose.—The synthetic disaccharide had $[\alpha]_D$ 145° (in H_2O) and formed a crystalline β-acetyl derivative having the same properties as all other such preparations, except that obtained by Gakhokidze [1] (see Table). The sugar obtained by deacetylation of the acetate had $[\alpha]_D$ 135°. Again this is in line with all reported values except those of Gakhokidze and Pazur *et al.* The somewhat higher value of $[\alpha]_D$ of the whole synthetic preparation suggested that it was not completely pure, although paper chromatography and ionophoresis revealed only one component, having the same behaviour as a sample of nigerose from *iso*lichenin.[17]

It is therefore confirmed that the sugar isolated from nigeran by Barker *et al.*[2] and having the same properties as samples isolated by all later workers except Pazur *et al.* (see Table) is 3-*O*-α-D-glucopyranosyl-D-glucose. The fact that Pazur *et al.* obtained turanosazone from their preparations suggests that nigerose was present but it must have been impure. This might explain Gakhokidze's findings but the properties of his acetyl derivative are unlike those of any known octa-*O*-acetyl derivative of a glucose disaccharide and the magnitude of its $[\alpha]_D$ excludes the possibility that this was the unknown α-nigerose octa-*O*-acetate.

Minor Products of the Reaction.—There were three other disaccharide products of the condensation reaction. One, kojibiose (50 mg.), was found uncombined with acetone, indicating that it arose solely from the reaction between two molecules of 3 : 4 : 6-tri-*O*-acetyl-1-chloro-β-D-glucose, as already mentioned. The second was a trehalose (73 mg.), probably the αα-isomer; this was also formed along with kojibiose when Brigl's anhydride was polymerised.[10] The third product (*X*) (46 mg.) had the same R_F value as isomaltose and gentiobiose and its $[\alpha]_D$ value (83°) suggested that it might be a mixture of the two sugars ($[\alpha]_D$ 122° and 9·6°, respectively) but the properties of its crystalline β-octa-*O*-acetate (m. p. 192°; $[\alpha]_D$ 30°) distinguish it from any known diglucose acetate. It may perhaps be 5-*O*-α-glucosylglucose, which has not yet been described. This could be formed by reaction between the chloroglucose and the 5-hydroxyl group of 1 : 2-*O*-*iso*propylidene-glucose, shown to be a by-product of the reaction.

[17] Morgan, Ph.D. Thesis, Wales, 1956.

Experimental

3 : 4 : 6-*Tri*-O-*acetyl*-1-*chloro*-β-D-*glucopyranose*.—This was prepared from β-glucose penta-*O*-acetate by Brigl's method,[11] as modified by Lemieux and Huber.[18] It had m. p. 157—158° (lit.,[11] m. p. 158°).

1 : 2-5 : 6-*Di*-O-iso*propylidene*-3-O-*sodio*-D-*glucofuranose*.—1 : 2-5 : 6-Di-*O*-*iso*propylidene-D-glucofuranose (10 g.) was treated with sodium (0·88 g., 1 mol.), as by Gilbert *et al.*,[16] in the apparatus designed by Philip, Scherer, and Field.[19]

Synthesis of Nigerose.—The 3-sodio-compound from 10 g. of sugar (as above) was dissolved in sodium-dried toluene (50 ml.), and the chloroglucose (14·5 g.) was added, some of which did not at first dissolve. After 48 hours' shaking at room temperature a sample gave a positive test for chloride and, tested on treatment with alkali (deacetylation) and then with hot dilute sulphuric acid (removal of acetone), followed by paper chromatography, gave a faint disaccharide spot but there was much unchanged glucose. Accordingly the solution was heated under reflux for 11 days, during which it became dark brown. The cooled mixture was filtered and evaporated. The residue was dissolved in a 0·5N-solution of sodium hydroxide in 50% acetone (200 ml.) and kept at 0° for 48 hr. After neutralisation with 3N-sulphuric acid the solution was applied to a charcoal–Celite column (150 × 5 cm.; equal parts by weight of B.D.H. " activated charcoal " and Celite No. 535) which was eluted by the gradient method.[20] A reservoir of water (10 l.) was connected to the top of the column, about 6 ft. below, and the level in this reservoir was automatically maintained by feeding in 60% aqueous ethanol with stirring. The fractions (150—200 ml. each) were collected automatically and after passage through a Seitz filter their optical rotations (4 dm. tube) were measured. Paper chromatography was also used as a guide to the identity of the substances. Each fraction was examined before and after treatment with 0·01N-sulphuric acid at 100° for 5 min. *iso*Propylidene derivatives were rendered visible by spraying the chromatogram with 10% trichloroacetic acid and heating it for 15 min. at 100° to remove the *iso*propylidene groups before spraying with benzidine–trichloroacetic acid.[21] Fractions 1—22 (1·2 g.) contained glucose; fractions 23—36 (2·13 g.) contained kojibiose (see below); fractions 37—98 (3·5 g.) contained mono- and di-*iso*propylideneglucose and a substance migrating between these compounds. Acid hydrolysis of this mixture gave glucose and nigerose. When fraction 98 had been collected the column was eluted with ethanol (2 l.), which removed 1·3 g. of carbohydrate. This was combined with fractions 37—98 and attempts were made preferentially to remove *iso*propylideneglucoses by extraction with solvents. The ethanolic extract deposited 1 : 2-*O*-*iso*propylideneglucose, which after several recrystallisations from methanol had m. p. 160° and $[\alpha]_D$ −11·2° (in EtOH; *c* 0·22) (lit.,[22] m. p. 161—162·5°, $[\alpha]_D$ −11·8°). The remaining material (4·7 g.) was heated in 0·01N-sulphuric acid (30 ml.) at 100° for 15 min. The solution was neutralised by aqueous sodium hydroxide and adsorbed on charcoal–Celite (100 × 3·5 cm.), and glucose was eluted with water. 7·5% Ethanol (1·8 l.) was then applied and the optically active material obtained was combined and evaporated. The residue (549 mg.) was found by chromatography to contain nigerose, a reducing sugar moving slightly faster than glucose, and mono*iso*propylideneglucose. These were separated by chromatography on six sheets of thick filter paper in butan-1-ol–acetic acid–water (4 : 1 : 5) into nigerose (67 mg.), reducing sugar (33 mg.), and mono*iso*propylideneglucose (289 mg.). The charcoal column was then eluted with 80% ethanol (3 l.), and 2·78 g. of carbohydrate were obtained. It was evident that hydrolysis of the *iso*propylidene derivatives was far from complete. Accordingly the 80% ethanol extract was heated in 0·015N-sulphuric acid at 100° for 35 min. After neutralisation and fractionation on charcoal–Celite (66 × 2·5 cm.) as before, the 7·5% ethanol extract (0·383 g.) contained a sugar (*X*) moving with isomaltose (and gentiobiose), along with nigerose and a trace of glucose. Fractionation on thick filter paper (5 sheets) gave *X* (46 mg.) and nigerose (164 mg.). The total yield of nigerose was therefore 231 mg. (1·8% based on the di*iso*propylideneglucose).

Characterisation of Nigerose.—On paper chromatography and paper ionophoresis in borate

[18] Lemieux and Huber, *Canad. J. Chem.*, 1953, **31**, 1040.
[19] Philip, Scherer, and Field, *Rayon Textile Monthly*, Oct., 1941, p. 51.
[20] Alm, *Acta Chem. Scand.*, 1952, **6**, 1186; Bacon and Bell, *J.*, 1953, 2528.
[21] Bacon and Edelman, *Biochem. J.*, 1951, **48**, 114.
[22] Fischer and Rund, *Ber.*, 1916, **49**, 93.

buffer (pH 8·7), with spray reagents to detect reducing and non-reducing sugars,[23] nigerose behaved as a single component, having the same R_F and M_G values as a sample from *iso*lichenin.[17]

Nigerose (90 mg.) was acetylated with sodium acetate–acetic anhydride, and the product was worked up in the usual way, the chloroform-soluble syrup (125 mg.) depositing crystals (from ethanol), m. p. 147—148°, which after two further crystallisations melted at 151—152° and had $[\alpha]_D$ +84° (in $CHCl_3$; *c* 0·42) (Found: C, 49·8; H, 5·9. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6%). The mixed m. p. with β-nigerose octa-*O*-acetate obtained from *iso*lichenin [17] (see Table) was 150°. The acetate (8·39 mg.) was dissolved in dry methanol saturated with ammonia (2 ml.) and left for 2 hr. at room temperature. The ammonia and methanol were then removed by storage over concentrated sulphuric acid and solid potassium hydroxide. The residue was dissolved in water (5 ml.) and α_D (1 dm. tube) was measured (0·114°). The free sugar therefore had $[\alpha]_D$ +135° (*c* 0·08). The specific optical rotation of the original sugar, based on determination of concentration by acid hydrolysis to glucose,[24] was +145° (*c* 0·05; 4 dm. tube).

Minor Disaccharide Products Formed during the Synthesis of Nigerose.—(a) *Kojibiose and trehalose.* Fractions 23—36 (2·13 g.; see above), obtained during the first fractionation of the reaction mixture, contained a disaccharide together with traces of trisaccharide and mono*iso*propylideneglucose. The fraction was adsorbed on charcoal–Celite (100 × 2·6 cm.) which was eluted with water and then 7·5% ethanol to remove the disaccharide (732 mg.). To ensure freedom from nigerose and *X* (see below) the material was fractionated by ionophoresis [23] on three sheets of thick filter paper (18¼ × 22½ in.) in 0·02M-borate buffer (pH 8·7) for 16 hr. with an applied potential of 9 v/cm. The main disaccharide component was eluted with water; the pH was adjusted to 5 with hydrochloric acid and the sodium borate removed on charcoal.[23] The disaccharide fraction (275 mg.) had the same R_F and M_G values as authentic kojibiose [10, 15] and likewise failed to react on paper with triphenyltetrazolium chloride spray reagent. A test was made for the presence of αα-trehalose, which had been formed with kojibiose in the synthesis of this sugar from Brigl's anhydride.[10] The two sugars have the same R_F value. The kojibiose was destroyed by heating the sugar with 0·4N-barium hydroxide for 3 hr. at 100°. After neutralisation with sulphuric acid, paper chromatography showed a non-reducing sugar to be present in the position previously occupied by kojibiose. The sugars were separated by converting the kojibiose into the methyl furanoside, following the general procedure described by Barker, Bourne, and O'Mant.[25] The fraction (240 mg.) was dissolved in 4% (w/w) methanolic hydrogen chloride and stored at room temperature. The optical rotation (1 dm. tube) became constant after 4 hr. (1·13° ⟶ 0·99°). Freshly prepared silver carbonate (7 g.) was added and, when neutral, the mixture was centrifuged, the solid was washed with methanol, and the combined solutions were evaporated over freshly washed and dried barium carbonate. 80% Aqueous methanol extracted from this residue a product (584 mg.) still containing some silver salts. This was fractionated on four sheets of thick filter paper and the zones corresponding to the two non-reducing disaccharide components, trehalose (73 mg.) and methyl kojibioside (R_F equal to that of glucose; 157 mg.) were eluted and evaporated. The furanoside was dissolved in 0·01N-hydrochloric acid (156 ml.), and stored at 42—48°. Samples were removed at intervals, neutralised with silver carbonate, and examined by chromatography. At 64 hr. the methyl furanoside had disappeared and was replaced by the benzidine-reducing disaccharide. After 72 hr. the whole solution was neutralised and worked up as before. The product contained a trace of glucose which was removed by chromatographic fractionation on thick filter paper. The final product weighed 50 mg. The chromatographic and ionophoretic properties and the infrared spectrum were identical with those of kojibiose.[15]

(b) *Disaccharide* X. This was obtained when the synthetic *iso*propylidenedisaccharides were hydrolysed with 0·015N-sulphuric acid (see above). It had the same R_F and M_G values as isomaltose but had $[\alpha]_D$ +83° (in H_2O; *c* 0·03) and with sodium acetate–acetic anhydride gave an acetate, m. p. 192°, $[\alpha]_D$ +30° (in $CHCl_3$; *c* 0·07).

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[23] Peat, Whelan, and Roberts, *J.*, 1957, 3916.
[24] Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224.
[25] Barker, Bourne, and O'Mant, *Chem. and Ind.*, 1955, 425.