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The Synthesis of Agarobiose[*1]

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Agarobiose, 3, 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose, was synthesized by a series of reactions involving the condensation of 1, 2-*O*-isopropylidene-3, 6-anhydro-L-galactopyranose with tetra-*O*-acetyl-α-D-galactosyl bromide, deacetylation with sodium methoxide, and the hydrolytic removal of the *O*-isopropylidene group. The resulting disaccharide, and its osazone and dimethyl acetal were identical with the corresponding authentic samples obtained naturally.

Agarobiose is a disaccharide which has been obtained from agar by a partial acid hydrolysis.[1] The structure has been dientified as 3, 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose (I) on the basis of chemical evidence.[2,3] The idsaccharide was later isolated in the forms of diethyl thioacetal

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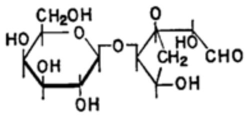
1) C. Araki, *Nippon Kwagaku Kwaisi (J. Chem. Soc. Japan)*, **65**, 533 (1944).

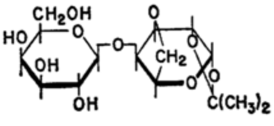
2) C. Araki, *ibid.*, **65**, 627 (1944).

3) S. Hirase and C. Araki, This Bulletin, **27**, 105 (1954).

and dimethyl acetal from agar[3,4] and the polysaccharide of the red seaweed *Gloiopeltis furcata*.[5] The present paper will deal with the synthesis of the disaccharide.



(I)



(IV)

The starting compound, 1, 2-*O*-isopropylidene-3, 6-anhydro-L-galactopyranose (II), prepared by the condensation of 3, 6-anhydro-L-galactose (III) with acetone in a manner previously reported,[6] was allowed to condense with tetra-*O*-acetyl-α-D-galactopyranosyl bromide in chloroform in the presence of silver oxide and anhydrous calcium sulfate. The deacetylation of the product with sodium methoxide and subsequent purification on a cellulose-powder column gave crystalline 1, 2-*O*-isopropylidene-3, 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose (IV). The removal of the *O*-isopropylidene group with dilute sulfuric acid yielded 3, 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose (I) in an amorphous form. This disaccharide was then converted to the crystalline phenylosazone. Furthermore, IV was treated with dilute methanolic hydrogen chloride to give the dimethyl acetal of I. These derivatives were identical with those prepared from natural agarobiose.

Experimental

General Procedure. Solutions were evaporated under reduced pressure at about 40°C. Paper chromatography was performed on Toyo Filter Paper No. 51 using a 1-butanol - acetic acid - water (4 : 1 : 2 v/v) mixture. The chromatographic spray reagent used was *o*-aminophenol in ethanol acidified with phosphoric acid.[7]

1, 2-*O*-Isopropylidene-3, 6-anhydro-L-galactopyranose (II). III (4.5 g), obtained by the hydrolysis of the dimethyl acetal,[8] was shaken in a mixture of acetone (300 m*l*) and concentrated sulfuric acid (3 m*l*) with anhydrous cupric sulfate (30 g) at room temperature for 5 hr. The mixture was then filtered, neutralized with sodium carbonate, and re-filtered. The filtrate was evaporated to a syrup, which was then dissolved in water (30 m*l*). An insoluble oil was removed by filtration with charcoal, and the filtrate was repeatedly extracted with chloroform. The evaporation of the extracts gave a mobile syrup (3.0 g) which crystallized on standing. The product was recrystallized twice from ether-hexane (1 : 1 v/v); yield 1.2 g; mp 91—92°C, $[\alpha]_D^{20}$ −26.8° (*c* 1.17, water). The values reported for the enantiomorph are mp 92°C and $[\alpha]_D^{14}$ +26.94° (water).[6]

Found: C, 53.28; H, 6.89%. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98%.

1, 2-*O*-Isopropylidene-3, 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactopyranose (IV). A mixture of II (3.0 g), silver oxide (7.5 g), Drierite[9] (15 g), and some pieces of glass beads was shaken in chloroform (60 m*l*) for 1 hr. Tetra-*O*-acetyl-α-D-galactopyranosyl bromide[10] (6.2 g) was then added, and the resulting mixture was shaken at room temperature for 24 hr. The mixture was filtered, and the filtrate was evaporated to dryness. The residue (7 g) was dissolved in a mixture of absolute methanol (50 m*l*) and a 0.1 N sodium methoxide solution (5 m*l*), and left at room temperature for 3 hr. The neutralization of the solution with carbon dioxide and subsequent evaporation afforded a syrup, which was then taken up in water (20 m*l*) and washed with chloroform (recovery of II: 1.2 g). The aqueous layer of the extraction was evaporated to a syrup (4.9 g), which was then repeatedly extracted with boiling acetone. The syrup (3.0 g) obtained on the evaporation of the extract showed several spots on a paper chromatogram. This syrup was chromatographed on a cellulose-powder column (4×35 cm) using a 1-butanol - water (6 : 1 v/v) mixture. Fractions of 14 m*l* were collected and monitored by paper chromatography. A compound with R_f 0.49 was obtained as a syrup (1.8 g). Crystallization was effected by dissolving it in acetone (5 m*l*), and by adding ethyl acetate (5 m*l*) and then ether (5 m*l*); yield 1.3 g, mp 173—175°C. Two recrystallizations from an acetone - ethyl acetate (1 : 2 v/v) mixture gave IV in prisms; mp 175—177°C, $[\alpha]_D^{16}$ −24.8° (*c* 1.29, water), $[\alpha]_D^{16}$ −35.0° (*c* 1.03, methanol).

Found: C, 49.62; H, 6.52%. Calcd for $C_{15}H_{24}O_{10}$: C, 49.45; H, 6.64%.

Agarobiose (I). The compound IV (0.20 g) was heated with 0.02 N sulfuric acid (10 m*l*) at 100°C. The optical rotation of the solution changed from $[\alpha]_D$ −25.5° to a constant value of $[\alpha]_D$ −18.0° within 45 min. The solution was then neutralized with barium carbonate and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in methanol, filtered, and then evaporated to give a colorless, amorphous solid (I) (0.15 g), $[\alpha]_D^{15}$ −15.9° (*c* 0.82, water). It reduced a Fehling solution at room temperature and restored the color to a Schiff reagent.

Phenylosazone: mp 220—221°C, $[\alpha]_D^{16}$ −136.2° (5 min)→−109.2° (44 hr, *c* 0.42, pyridine-methanol (2 : 3 v/v)). (For the reported physical constants, see Refs. 3 and 4.) The melting point was not depressed on admixture with an authentic sample of natural origin.

Found: C, 57.88; H, 5.74; N, 11.11%. Calcd for $C_{24}H_{30}O_8N_4$: C, 57.36; H, 6.02; N, 11.15%.

Agarobiose Dimethyl Acetal. The compound IV (0.30 g) was refluxed with 0.5% methanolic hydrogen

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4) C. Araki and S. Hirase, *ibid.*, **27**, 109 (1954).

5) S. Hirase, C. Araki and T. Ito, *ibid.*, **31**, 428 (1958).

6) C. Araki and K. Arai, *Nippon Kwagaku Kwaisi (J. Chem. Soc. Japan)*, **63**, 1720 (1942).

7) S. Hirase, C. Araki and S. Nakanishi, This Bulletin, **26**, 183 (1953).

8) C. Araki, *Nippon Kwagaku Kwaisi (J. Chem. Soc. Japan)*, **65**, 725 (1944).

9) Anhydrous calcium sulfate of the W. A. Hammond Drierite Co., Ohio, U. S. A.

10) W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 1852 (1942).

chloride (15 m*l*) for 30 min. The solution was neutralized with silver carbonate, filtered, and evaporated to a syrup, which was then crystallized from a methanol-acetone (1 : 2 v/v) mixture; yield 0.22 g, mp 163—165°C, $[\alpha]_D^{16}$ $-29.4°$ (*c* 1.17, water). (For the reported physical constants, see Refs. 4 and 5.) The melting point was not depressed on admixture with an authentic sample of natural origin.

Found: C, 42.25; H, 7.11; OCH_3, 16.58%. Calcd for $C_{14}H_{26}O_{11}$: C, 45.40; H, 7.08; OCH_3, 16.76%.

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