Studies on the Chemical Constitution of Agar-agar. XVI[1]. *Isolation of Crystalline Agarobiose Diethylmercaptal by Mercaptolysis of Agar-agar*[2]

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In Part XV the present authors reported the isolation of crystalline diethylmercaptals of D-galactose, 3,6-anhydro-L-galactose and of DL-galactose, and the non-crystalline residue (designated as R_I) from the products of the first forty eight hours mercaptolysis of agar. They suggested, moreover, that R_I, which upon further mercaptolysis produced monosaccharide derivatives above mentioned, would comprise some oligosaccharide diethylmercaptals. On the other hand, one of the authors could isolate agarobiose (4-D-galactopyranosyl 3,6-anhydro-L-galactose) from the partial hydrolysis products of agar[3]. Hence, in a search for agarobiose diethylmercaptal, R_I has been investigated.

R_I was shown to be divided into the *n*-butanol-soluble fraction and the insoluble one. Acetylation of the former produced a crystalline compound, which proved to be hexaacetyl agarobiose diethylmercaptal (I) for the reasons described later. Upon saponification, the acetate regenerated crystalline agarobiose diethylmercaptal (II).

It appears probable that the prolonged mercaptolysis described above has led to the further cleavage of agarobiose units once produced into monosaccharide units. In order to obtain the agarobiose derivative in a better yield, the partial mercaptolysis has been carried out under the milder condition, namely, for five hours at 3~5°. The reaction mixture gave crude agarobiose diethylmercaptal by the solvent fractionation in a yield of 46.2% of agar employed. This result and the isolation of D-galactose diethylmercaptal and of 3,6-anhydro-L-galactose diethylmercaptal in almost equimolar proportion[1] demonstrate that agarobiose units extend over approximately half, at least, of the molecule of agar.

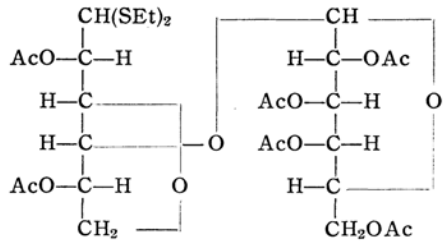
The assignment of the structures of agarobiose diethylmercaptal (II) and its hexaacetate (I) to the compounds isolated as above mentioned arises for the following reasons. Mercaptolysis of II yielded D-galactose diethylmercaptal (III) and 3,6-anhydro-L-galactose diethylmercaptal (IV), indicating that II was a disaccharide diethylmercaptal, the component sugars of which were D-galactose and 3,6-anhydro-L-galactose. Demercaptalation of II regenerated the parent free sugar, the phenylosazone of which was found to have the same characteristics as agarobiose phenyosazone described by one of the authors before.[3] On the other hand, methylation of II with dimethyl sulfate and sodium hydroxide solution followed by subsequent demercaptalation with mercuric chloride afforded crystalline hexamethyl *aldehydo*-agarobiose (VI), which was then oxidized to hexamethyl agarobionic acid (VII). Methyl ester of VII showed no increase of the methoxyl content on further methylation with methyl iodide and silver oxide. When VII was submitted to the hydrolysis with N-sulfuric acid, there were produced 2,3,4,6-tetramethyl D-galactose (VIII) and crystalline 2,5-dimethyl 3,6-anhydro-L-galactonic acid (IX). The former was identified as its crystalline anilide. The latter was identified by its properties and also by its conversion to the crystalline amide[3]. The identification of these two cleavage fragments enables the statement to be made that the structure of hexamethyl agarobionic acid (VII) is represented by that, in which C_1 of 2,3,4,6-tetramethyl D-galactose (VIII) is united glycosidically to C_4 of 2,5-dimethyl 3,6-anhydro-L-galactonic acid (IX). This agrees well with the assignment of the structures of agarobiose diethylmercaptal and its hexaacetate to the compounds under question.

Experimental

Evaporation and concentration were carried out under reduced pressure below 40°. The specific rotation was measured in the aqueous solution unless otherwise stated. The thioethoxyl content was determined by the method of these authors[4]. The yield, the specific rotation and the thioethoxyl content of the ash-containing sample are calculated into the case of the ash-free one. All melting points are uncorrected.

Mercaptolysis of agar. a) for 48 hrs.:— Agar (60.0 g., dry weight 49.1 g.) was mercaptolysed

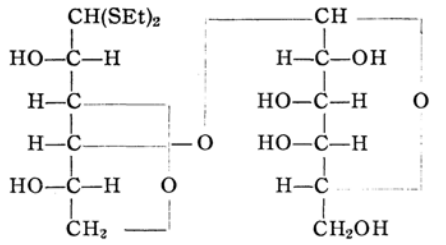
1) Part XV: C. Araki and S. Hirase, This Bulletin, **26**, 463 (1953).
2) Read at 5th (1952) and 6th (1953) Annual Meetings of the Chemical Society of Japan.
3) C. Araki, *J. Chem. Soc. Japan*, **65**, 533, 627 (1944).
4) The method will be published elsewhere.



(I)

Hexaacetyl agarobiose diethylmercaptal

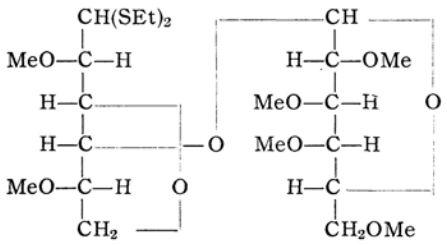
NH_3 (MeOH)



(II)

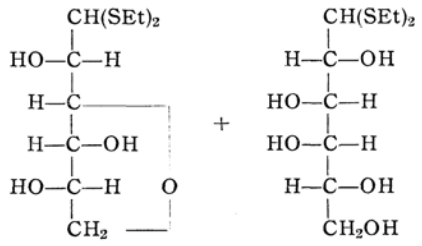
Agarobiose diethylmercaptal

Me_2SO_4 (NaOH)



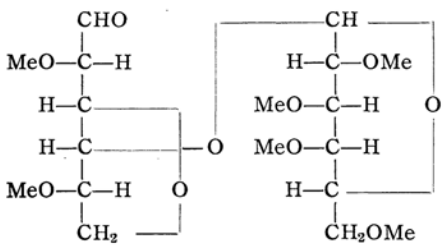
(V)

EtSH (HCl)



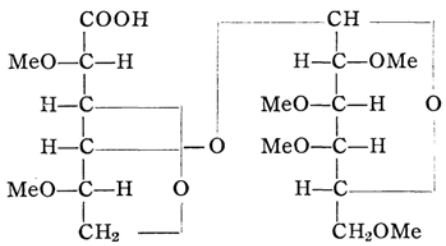
(IV) (III)

H_2O ($HgCl_2$)



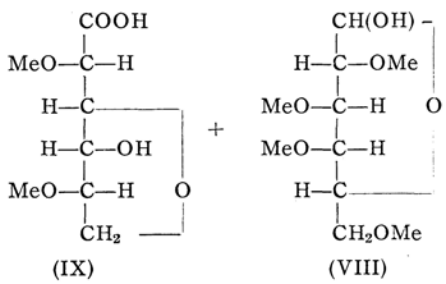
(VI)

O ($KMnO_4$)



(VII)

H_2O (H_2SO_4)



(IX) (VIII)

Me: $-CH_3$

Et: $-C_2H_5$

Ac: $-COCH_3$

exactly in the same manner as described in Part XV[1]. The non-crystalline residue, designated as R_1, was obtained as a hygroscopic amorphous powder (ash 2.86%); yield 29.1 g., $[\alpha]_D^7$ −8.0° (*c* 2.62), SC_2H_5 23.08%.

This was dissolved in absolute methanol (50 cc.) and *n*-butanol (500 cc.) was added with vigorous shaking. The insoluble precipitate was filtered, washed first with *n*-butanol and then with ethyl acetate, and dried, when a hygroscopic white powder (ash 6.40%) was obtained; yield 5.9 g., $[\alpha]_D^{25}$ −18.6° (*c* 2.09), SC_2H_5 15.82%.

The combined filtrate and the butanol washings, in which agarobiose diethylmercaptal was contained, were concentrated with occasional additions of water to a sirup, which was dried by repeatedly adding absolute methanol and subsequent distilling off, forming an amorphous powder (ash 2.00%); yield 23.2 g., $[\alpha]_D^{25}$ −7.1° (*c* 2.12), SC_2H_5 25.17%.

b) for 5 hrs.:—Agar (60.0 g., dry weight 49.1 g.) was mercaptolysed for 5 hrs. at 3~5° (ice-cooling) by the reaction with concentrated hydrochloric acid (*d* 1.20, 200 g.) and ethylmercaptan (80 g.). The solution of the reaction products, from which inorganic substances had been removed in the same manner as described before[1], was concentrated to about 200 cc. and allowed to stand overnight in an ice-box. Since no crystals were deposited, the solution was concentrated to 150 cc., and extracted with ether for continuous 140 hrs. in a Soxhlet's apparatus. From ether extracts, D-galactose diethylmercaptal (1.04 g.) and 3,6-anhydro-L-galactose diethylmercaptal (0.78 g.) were obtained in a crystalline state.

The remaining water solution was then concentrated to a sirup, which was dissolved in absolute methanol (500 cc.). The precipitate formed was filtered, washed and dried, a hygroscopic white powder (ash 9.08%) being obtained; yield 5.4 g., $[\alpha]_D^{25}$ −26.8° (*c* 0.96), SC_2H_5 6.18%. The combined filtrate and the washings were evaporated to dryness, and the residue was redissolved in absolute methanol (50 cc.). To this solution was added *n*-butanol (500 cc.) with shaking. After overnight standing it was treated in the same way as in the case of *a*. The butanol-insoluble precipitate was obtained in the form of a hygroscopic white powder (ash 2.46%); yield 10.1 g., $[\alpha]_D^{25}$ −24.0° (*c* 1.18), SC_2H_5 13.17%. The butanol-soluble fraction, being considered as crude agarobiose diethylmercaptal from its specific rotation and its thioethoxyl content, was obtained in the form of a hygroscopic amorphous powder (ash 0.68%) with a pale yellow color; yield 29.8 g. (46.2% of the agar employed, when calculated as its parent free sugar), $[\alpha]_D^{25}$ −8.8° (*c* 1.21) and −46.9° in pyridine (*c* 0.86), SC_2H_5 25.71% (agarobiose diethylmercaptal requires SC_2H_5 28.39%).

Hexaacetyl agarobiose diethylmercaptal (I). *a*) The butanol-soluble fraction (12.0 g.) of 48 hrs. mercaptolysis products was acetylated with pyridine (100 cc.) and acetic anhydride (100 cc.) for 2 days at room temperature. The reaction product (16.0 g.), isolated by the solvent removal of the chloroform extract in the usual way, was dissolved in methanol (25 cc.), and water (5 cc.) was added to give a turbidity. Deposited crystals were filtered, washed with 50% aqueous methanol and dried; yield 7.6 g., m.p. 96~99°. An additional specimen (1.4 g.) was obtained from the mother liquor, m.p. 96~99°. Pure hexaacetyl agarobiose diethylmercaptal was obtained after recrystallization twice from methanol-water (7:3) as long needles; m.p. 101~103.5°, $[\alpha]_D^{25}$ −11.8° in chloroform (*c* 1.02), −22.1° in benzene (*c* 1.27) and −16.7° in ethanol (*c* 1.38). *Anal.* Found: C, 49.05; H, 6.13; CH_3CO, 37.71; SC_2H_5, 18.45, M.W. (Rast' method), 688. Calcd. for $C_{28}H_{42}O_{18}S_2$: C, 49.25; H, 6.20; CH_3CO, 37.82; SC_2H_5, 17.94%; M.W., 682.8.

b) The butanol-soluble fraction (14.9 g.) of the 5 hrs. mercaptolysate was acetylated in the same manner as above mentioned. The yield of crude crystals (m.p. 96~99°) of the hexaacetate was 17.0 g. (71.7% of the theoretical amount).

Agarobiose diethylmercaptal (II). A solution of hexaacetyl agarobiose diethylmercaptal (10.0 g.) in absolute methanol (100 cc.) was saturated with dry ammonia under ice-cooling, and allowed to stand overnight. The solution was evaporated to a sirup, which was then dissolved in ethyl acetate (50 cc.). White crystals separated immediately; yield 5.3 g. (83.2% of the theoretical amount), m.p. 168~170°. The recrystallization was repeated twice from absolute ethanol, giving pure agarobiose diethylmercaptal, m.p. 171~172°, $[\alpha]_D^{25}$ −8.47° (*c* 3.07), −20.9° in methanol (*c* 1.39) and −51.7° in pyridine (*c* 1.47). It is readily soluble in water, methanol, pyridine and hot ethanol. *Anal.* Found: C, 44.38; H, 6.95; SC_2H_5, 28.49. Calcd. for $C_{16}H_{30}O_9S_2$: C, 44.65; H, 7.02; SC_2H_5, 28.39%.

Agarobiose. Agarobiose diethylmercaptal (5.0 g.), mercuric chloride (7.5 g.) and excess lead carbonate were treated in the same way as in the demercaptalation of 3,6-anhydro-L-galactose diethylmercaptal[1]. A solution of the resulting sirup (3.4 g.) in absolute methanol (10 cc.) was poured into absolute ethanol (50 cc.) with stirring, forming the precipitate of agarobiose, which was obtained as a hygroscopic white powder after filtration and drying; yield 2.0 g., $[\alpha]_D^{27}$ −21.5° (initial, *c* 1.16)→−16.4° (equilibrium). It reduced a cold Fehling's solution, showed a strong Seliwanoff's reaction, and restored the color to a Schiff's reagent.

Phenylosazone[3]: Yellow fine needles, m.p. 221~222° (decomp.), $[\alpha]_D^{15}$ −135.5° (initial)→−110.8° (equilibrium) in pyridine-ethanol (2:3) (*c* 0.406).

Mercaptolysis of agarobiose diethylmercaptal. The mercaptolysis of agarobiose diethylmercaptal (2.0 g.) was accomplished by dissolving it in concentrated hydrochloric acid (*d* 1.20, 5 cc.) and shaking with an addition of ethylmercaptan (3 cc.) at 10~12° for 24 hrs. Successive treatments of the reaction mixture with lead carbonate, hydrogen sulfide, silver carbonate and again hydrogen sulfide removed inorganic ions. The water solution (about 50 cc.) thus obtained upon concentration gave crystals of D-galactose diethyl-

mercaptal (III) (0.53 g.), m.p. alone or on admixture with an authentic specimen, 140~142°, $[\alpha]_D^{10}$ −4.5° (*c* 0.85).

The mother liquor was further evaporated to dryness, from which 3,6-anhydro-L-galactose diethylmercaptal (IV) (0.50 g.) was obtained by the repeated extraction with cold ethyl acetate followed by evaporation, m.p. and mixed m.p. 109~110°, $[\alpha]_D^{10}$ +14.1° (*c* 0.78).

The residue of the extraction was proved to be unaffected agarobiose diethylmercaptal by its m.p. and its specific rotation.

Hexamethyl agarobiose diethylmercaptal (V). Hexaacetyl agarobiose diethylmercaptal (20.0 g.) was methylated in a 80% acetone solution three times by the reaction with dimethyl sulfate and a 30% sodium hydroxide solution in the usual way. The methylated product distilled at 188~191°/0.040 mmHg. (bath temperature 240~260°), giving a viscous liquid; yield 10.6 g. (71% of the theory), n_D^{25} 1.4952, $[\alpha]_D^{10}$ −17.5° in chloroform (*c* 1.15), −12.5° in ethanol (*c* 1.12). It is hardly soluble in cold water, but readily soluble in organic solvents. *Anal.* Found: C, 51.11; H, 7.96; SC_2H_5, 23.05. Calcd. for $C_{22}H_{42}O_9S_2$: C, 51.33; H, 8.23; SC_2H_5, 23.20%.

Hexamethyl *aldehydo*-agarobiose (VI). According to the procedure of Wolfrom and others[5], hexamethyl agarobiose diethylmercaptal (4.8 g.) was demercaptalated by the action of mercuric chloride (10 g.) in an acetone solution containing water for 6 hrs. at room temperature in the presence of cadmium carbonate. The product was a brown sirup, which was then dissolved in water, a brown oily precipitate being removed by decantation. The aqueous solution, after being decolorized with charcoal, was concentrated to dryness. The resulting colorless sirup (3.4 g.) crystallized almost completely. Recrystallization of the crude crystals from dry ether gave fine needles of the *aldehydo*-sugar; yield 2.6 g. (67% of the theoretical amount), m.p. 92~93°, $[\alpha]_D^{10}$ −4.0° (initial)→−9.33° (equilibrium) in water (*c* 0.75), −9.77° (initial)→−53.71° (equilibrium after 2 hrs.) in chloroform (*c* 1.024). It reduced a hot Fehling's solution, decolorized a neutral permanganate solution, and restored the color to a Schiff's solution. *Anal.* Found: C, 52.68; H, 7.78; OCH_3, 45.31. Calcd. for $C_{18}H_{32}O_{10}$: C, 52.93; H, 7.90; OCH_3, 45.58%.

Hexamethyl agarobionic acid (VII). To a solution of the *aldehydo*-sugar (8.0 g.) in water (50 cc.) was dropped a solution of potassium permanganate (2.2 g.) in 0.1N potassium hydroxide solution under stirring at room temperature. The oxidation proceeded immediately. The precipitate of manganese oxide was removed by filtration and the potassium ion was exchanged by a cation resin Amberlite IR-120. The solution was then neutralized exactly with 0.1N barium hydroxide solution, and concentrated to dryness. The residue, from which the unaffected *aldehydo*-sugar was extracted with ether, was dissolved in water. The treatment of the solution with a cation resin Amberlite IR-120 followed by subsequent evaporation gave hexamethyl agarobionic acid as a glassy solid; yield 4.5 g. (54% of the theoretical amount), $[\alpha]_D^{10}$ −25.5° (*c* 0.55). *Anal.* Found: OCH_3, 43.55; Neut. Equiv., 423. Calcd. for $C_{18}H_{32}O_{11}$: OCH_3, 43.86%; Neut. Equiv. 424.

Hexamethyl methyl agarobionate. The free acid (3.8 g.) above mentioned was esterified by the reaction with diazomethane in an ethereal solution, yielding a viscous liquid with a pale yellow color; yield 3.6 g., b.p. 161~163°/0.030 mmHg., n_D^{25} 1.4681, $[\alpha]_D^{14}$ −23.4° (*c* 1.03), −31.1° in chloroform (*c* 1.16). *Anal.* Found: C, 49.99; H, 7.78; OCH_3, 47.21; Sapn. Equiv., 436. Calcd. for $C_{19}H_{34}O_{11}$: C, 49.76; H, 7.47; OCH_3, 47.37%; Sapn. Equiv., 438.

This was methylated twice with methyl iodide and silver oxide in the usual way; b.p. 160~161°/0.027 mmHg., $[\alpha]_D^{17}$ −23.0° (*c* 2.73), OCH_3 found 47.25%.

The reaction with ammonia or phenylhydrazine failed to give any crystalline derivative.

Hydrolysis of hexamethyl agarobionic acid. Hexamethyl agarobionic acid (1.1 g.) was hydrolysed in N-sulfuric acid (50 cc.) in a boiling water bath. The reaction was followed polarimetrically: $[\alpha]_D$ −22.8° (initial); −15.9° (0.5 hr.); −13.6° (1 hr.); +0.05° (3 hrs.); +15.9° (8 hrs.); +27.6° (14 hrs.) +30.5° (24, 32 and 40 hrs., constant). The solution was neutralized with barium carbonate, filtered and evaporated. From the resulting sirup, 2,3,4,6-tetramethyl D-galactose was extracted with boiling ether, leaving barium 2,5-dimethyl 3,6-anhydro-L-galactonate.

a) 2,3,4,6-Tetramethyl D-galactose (VIII)—The ether extract upon concentration gave a colorless sirup of 2,3,4,6-tetramethyl D-galactose; yield 0.51 g., $[\alpha]_D^{10}$ +113.0° (*c* 0.46), OCH_3 found 52.35% ($C_3H_8O_2(OCH_3)_4$ requires OCH_3 52.55%).

Anilide: m.p. alone or when mixed with an authentic specimen, 192°, $[\alpha]_D^{10}$ −79.2° (initial)→+41.7° (equilibrium) in acetone (*c* 0.48).

b) 2,5-Dimethyl 3,6-anhydro-L-galactonic acid (IX)—Barium 2,5-dimethyl 3,6-anhydro-L-galactonate, described above, was converted to the free acid by means of a cation exchange resin Amberlite IR-120. The resulting product crystallized completely; yield 0.41 g., m.p. 159~160°. Recrystallization from ethyl acetate gave a pure substance[3]; m.p. 161°, $[\alpha]_D^{7}$ −58.6° (*c* 0.44). *Anal.* Found: C, 46.38; H, 6.63; OCH_3, 29.97; Neut. Equiv., 205. Calcd. for $C_8H_{14}O_6$: C, 46.58; H, 6.85; OCH_3, 30.12%; Neut. Equiv., 206.

Amide: The free acid (0.12 g.) above mentioned was esterified with diazomethane in an ethereal solution. The methyl ester (0.12 g.), obtained as a colorless sirup with $[\alpha]_D^{15}$ −39.3° (*c* 2.52), was then converted to the corresponding amide in the usual way[3]; flakes, m.p. 172°, $[\alpha]_D^{15}$ −67.0° (*c* 0.63). *Anal.* Found: C, 46.74; H, 7.39. Calcd. for $C_8H_{15}O_5N$: C, 46.80; H, 7.37%.

The authors wish to express their thanks

5) M. L. Wolfrom et al., *J. Am. Chem. Soc.*, **51**, 2188 (1929); **52**, 2464, 3619 (1930); **53**, 4379 (1931); **56**, 880, 985 (1934).

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