Studies on the Chemical Constitution of Agar-agar. XV.[1]

Exhaustive Mercaptolyses of Agar-agar[2]

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Agar-agar, a gel-forming polysaccharide extracted from certain species of red sea weeds, has long been known to produce D-galactose on acid-hydrolysis. C. Araki, one of the present authors, revealed that 3,6-anhydro-L-galactose besides D-galactose is a-constitutional monosaccharide, on the bases that he isolated agarobiose[3] (4-D-galactopyranosyl-3,6-anhydro-L-galactose) from partial hydrolysis products of agar, and that he could obtain 2-methyl 3,6-anhydro-L-galactose dimethylacetal[4] from methylated agar as well as 3,6-anhydro-L-galactose dimethylacetal[5] from agar itself by the methanolysis method. Jones and Peat were of the opinion that 3,6-anhydro-L-galactose might arise secondarily from L-galactose-6-sulfate during some chemical operations[6]. Further evidence, however, which excludes the possibility of the latter opinion, is now made available by the mer-

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[1] Part XIV: C. Araki, *J. Chem. Soc. Japan*, **65**, 725 (1944). Works on this subject are reviewed by C. Araki in "A Summary of Organic Chemical Investigations on Agar-agar", Memoirs of the Faculty of Industrial Arts, Kyoto Technical Univ., **2**(B), 17 (1953), written in English.

[2] Read at the Ordinary Meeting of the Kinki Branch of the Chemical Society of Japan in September, 1950.

[3] C. Araki, *J. Chem. Soc. Japan*, **65**, 533, 627 (1944).

[4] C. Araki, *J. Chem. Soc. Japan*, **61**, 775 (1940).

[5] C. Araki, *J. Chem. Soc. Japan*, **65**, 725 (1944).

[6] W. G. M. Jones and S. Peat, *J. Chem. Soc.*, **1942**, 225.

captolysis of agar.

Mercaptolysis[7], substantially hydrolysis followed by simultaneous mercaptalation, has proved in a few cases[8] to be successful in isolating aldose components as their crystalline mercaptals. This has led the authors to apply the procedure to establish the constitutional sugars of agar.

Mercaptolysis of agar was accomplished by dissolving it in concentrated hydrochloric acid with ice-cooling and by stirring the solution with addition of ethylmercaptan for forty eight hours. Neutralization of the reaction mixture followed by evaporation produced well-known crystalline D-galactose diethylmercaptal, which was also identified by oxidation to mucic acid and acetylation to characteristic pentaacetate.

A filtered solution from crystals mentioned above was then extracted with ether in Soxhlet's percolator. Ether extracts yielded crystalline 3,6-anhydro-L-galactose diethylmercaptal, and small amounts of crystalline DL-galactose diethylmercaptal and of xylose diethylmercaptal-containing sirup. 3,6-Anhydro-L-galactose diethylmercaptal, which gave crystalline tri-*p*-nitrobenzoate, was identified by conversion to free sugar and then to crystalline diphenylhydrazone[5] and phenylosazone[5]. Further confirmative evidence was provided by comparison with the authentic specimen prepared for the first time. DL-Galactose diethylmercaptal, also a new compound, giving crystalline pentaacetate, was confirmed by its optical inactivity, its oxidation to mucic acid and by the preparation of an authentic sample.

As the residue obtained by evaporation of the solution, from which the monosaccharide derivatives mentioned above had been removed, seemed to comprise oligosaccharide diethylmercaptals, it was submitted to a second mercaptolysis for forty eight hours to yield monosaccharide units. Crystalline diethylmercaptals of D-, 3,6-anhydro-L- and of DL-galactose were isolated again. The non-crystalline residue of the second mercaptolysis products was further effected by the third mercaptolysis, the same products being isolated in a crystalline state. In the last two cases, no xylose derivative could be found. Thus repeated exhaustive mercaptolyses of agar furnished crystalline specimens of diethylmercaptals of D-galactose, 3,6-anhydro-L-galactose and of DL-galactose in yields of 30.2 %, 24.4 % and 1.0 % to agar employed, respectively.

It may not be a matter of surprise that such an acid sensitive sugar as 3,6-anhydro-L-galactose was out of perfect degradation in the presence of a strong mineral acid, inasmuch as 2-desoxy-aldohexose, which has been accepted as an acid-sensitive sugar equal to 3,6-anhydro-aldohexose, affords its stable mercaptal[9] under the similar condition. With regard to DL-galactose, one of the authors reported that he could not find it in the hydrolysate of agar extrated from *Gelidium amansii*[10], but afterwards it was isolated from an agarous substance of *Camphaephora Hypraeides*, J. Ag[11]. As commercial agar, employed in this investigation, is generally prepared by extracting a mixture of *Gelidium amansii* and several other species of sea weeds, it may be possible to assume that DL-galactose derivative isolated above arises from other species than *Gelidium amansii.* Xylose was also detected[12] in the hydrolysis products of commercial agar, but further evidence will be necessary for its conclusive identification.

From these results it is concluded that 3,6-anhydro-L-galactose as well as D-galactose is a chief constitutional sugar in the agar molecule, just as one of the authors has stated. Moreover, it may be of interest that derivatives of D-galactose and 3,6-anhydro-L-galactose can be isolated approximately in equal yield. This suggests that these two sugars are involved in almost equal amounts in the molecule of agar. But it would be still difficult to conclude that these two sugars comprise the main bulk of the molecule, as the exhaustive experimental conditions employed above have made it impossible to deal with the whole material quantitatively.

Experimental

Unless otherwise stated, concentration and evaporation of the solution were carried out under reduced pressure below 40°. Analyses for the thioethoxyl group are according to the method of the present authors[13]. All m.ps. are uncorrected.

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7) M. L. Wolfrom, L. W. Geoges and J. C. Sowden, *J. Am. Chem. Soc.*, **60**, 1026 (1938); M. L. Wolfrom, D. R. Myers and E. N. Lassettre, ibid., **61**, 2172 (1939).

8) I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, *J. Am. Chem. Soc.*, **68**, 2120 (1946); **69**, 1052 (1947); P.W. Kent, *Nature*, **166**, 442 (1950)·

9) W. G. Overend, M. Stacey and J. Stanek, *J. Chem. Soc.*, **1949**, 2481; W. G. Overend, F. Shafizadeh and M. Stacey, *J. Chem. Soc.*, **1950**, 671.

10) C. Araki, *J. Chem. Soc. Japan*, **58**, 1214 (1937).

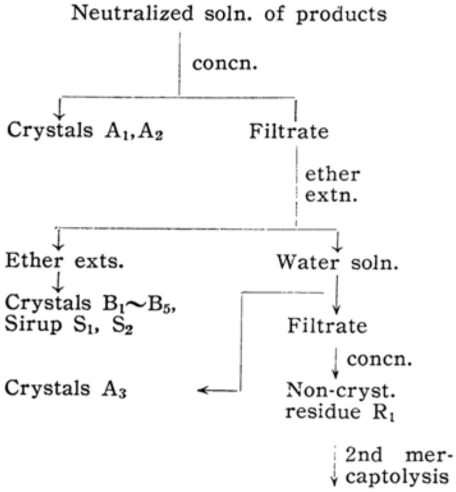
11) C. Araki, Collection of Treatises in Commemoration of the 45th Anniversary of Kyoto Technical College, pp 69 (1948).

12) S. Hirase and C. Araki, Memoirs of the Faculty of Industrial Arts, Kyoto Technical Univ., **1**, 19 (1952).

13) The method will be published elsewhere.

I. Mercaptolysis of agar-agar.—Commercial powdered agar (60.0 g., dry weight 49.1 g.), placed in a three-necked flask fitted with a mechanical stirrer, dropping funnel and calcium chloride tube, was dissolved in concentrated hydrochloric acid (*d* 1.20, 200 g.) by stirring and ice-cooling; the agar dissolved gradually in 40 mins. into a deep brown solution. Ethylmercaptan (80 g.) was then added from the dropping funnel with vigorous stirring in three portions so that the temperature did not exceed 10°. After addition, the stirring was continued for 48 hrs., maintaining the temperature at 10~12°. The reaction mixture was neutralized by pouring it on excess lead carbonate suspended in water (1 l.). Precipitates of the excess lead carbonate and lead chloride formed were removed by filtration and washing with cold water (1 l.). The combined filtrate and washings were saturated with hydrogen sulfide, the precipitate of lead sulfide being removed by filtration. The filtrate was then neutralized with silver carbonate after removal of hydrogen sulfide in vacuo, and filtered before and after a second treatment with hydrogen sulfide. The solution thus obtained was operated as shown in the accompanying diagram and as described below.

Operations for Mercaptolysis Products of Agar-agar.



II. Isolation and confirmation of D-galactose diethylmercaptal.—The neutralized solution obtained above was concentrated until it gave rise to crystalline magma. The crystals (A_1) were filtered, washed with cold water and dried; yield 7.25 g., m.p. 139~140°, $[\alpha]_D^{15}-4.3°$ in water. The second crop of the crystals (A_2) was obtained by concentrating the combined filtrate and washings mentioned above to about 200 cc., and allowing it to stand overnight in an ice-box; yield 1.95 g., m.p. 140~141°, $[\alpha]_D^{15}-4.6°$ in water. Crystals A_1 and A_2 were combined and recrystallized from alcohol. Pure D-galactose diethylmercaptal was obtained as prismatic needles, m.p. 141~142°, $[\alpha]_D^{15}-4.65°$ in water (*c* 0.680), $-3.99°$ in pyridine (*c* 1,003). Its m.p. was not depressed on admixture with an authentic sample (m.p. 142°)[14].

Anal. Found: C, 41.65; H, 7.99; SC_2H_5, 42.75. Calcd. for $C_{10}H_{22}O_5S_2$: C, 41.93; H, 7.74; SC_2H_5, 42.68 %.

Oxidation of the crystals mentioned above with nitric acid (*d* 1.15) yielded mucic acid, m.p. 212°, not depressed on admixture with an authentic specimen (m.p. 214°).

Pentaacetyl D-galactose diethylmercaptal[15]: m.p. 76~77°, $[\alpha]_D^{18}+11.31°$ in chloroform (*c* 2.210).

III. Isolation and confirmation of 3,6-anhydro-L-galactose diethylmercaptal.

a) Isolation:—Combined filtrate and washings from A_2, described in **II**, were concentrated to about 150 cc., and extracted with ether in a Soxhlet's percolator.

The first extract (20 hrs.) on cooling gave crystals (B_1, 6.95 g.), m. p. 102~5°, $[\alpha]_D^{12}+11.0°$ in water. The ethereal filtrate from B_1 on evaporation furnished further crops of crystals (B_2, 1.17 g.), m.p. 102~5°, $[\alpha]_D^{12}+10.8°$ in water. The mother liquor from B_2 was concentrated to a sirup (S_1, 1.20 g.), $[\alpha]_D^{12}-1.6°$ in water.

The second extract (30 hrs.) afforded crystals (B_3, 0.58 g.), m.p. 109~116°, $[\alpha]_D^{12}+1.7°$ in water, filtrate from which on evaporation gave further crystals (B_4, 0.22 g.), m.p. 102~5°, $[\alpha]_D^{12}+13.3°$ in water. Mother liquor from B_4 was concentrated to a sirup (S_2, 0.45 g.), $[\alpha]_D^{12}-6.1°$ in water.

The third extract (70 hrs.) gave crystals (B_5, 0.75 g.), m.p. 115~121°, $[\alpha]_D^{12}-2.5°$ in water. Ethereal filtrate from B_5 left nothing on evaporation.

The crystals B_1, B_2 and B_4, showing the same properties, were combined and recrystallized from ethyl acetate (40 cc.), yielding almost pure 3,6-anhydro-L-galactose diethylmercaptal (5.20 g.), m.p. 104~7°, $[\alpha]_D^{25}+12.1°$ in water. An additional product (2.61 g., m.p. 103~6°, $[\alpha]_D^{25}+11.8°$ in water), was recovered by evaporating the mother liquor. A pure substance was obtained after several recrystallizations from ethyl acetate containing a small amount of petroleum ether (b. p. 40~70°), forming silky needles with m.p. 110~111° and $[\alpha]_D^{14}+14.09°$ in water (c 1.636), $-21.11°$ in ethanol (c 1.041) and $-26.25°$ in pyridine (c 1.638). Its m.p. was not depressed on admixture with the authentic specimen, the preparation of which is described in **III—d.** It was readily soluble in water, methanol, ethanol and pyridine, moderately soluble in ether, acetone, ethyl acetate and chloroform, and practically insoluble in benzene and petroleum ether. It gave a Seliwanoff's ketose reaction.

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14) E. Fischer, *Ber.*, **27**, 678 (1894); R. M. Hann, W. D. Maclay and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1270 (1939).

15) M. L. Wolfrom, *J. Am. Chem. Soc.*, **52**, 2467 (1930).

Anal. Found: C, 44.48; H, 7.68; SC_2H_5, 45.65. Calcd. for $C_{10}H_{20}O_4S_2$: C 44.75; H, 7.51; SC_2H_5, 45.55 %.

b) Tri-*p*-nitrobenzoyl 3,6-anhydro-L-galactose diethylmercaptal:—To a solution of 3,6-anhydro-L-galactose diethylmercaptal (0.50 g.), isolated above, in pyridine (10 cc.) was added *p*-nitrobenzoyl chloride (1.20 g.). The reaction mixture, after being allowed to stand 2 days, was treated in the usual manner. Recrystallization of the product from ethanol containing a small amount of ethyl acetate furnished colorless fine needles of tri-*p*-nitrobenzoate (0.50 g.), m.p. 140~142°, $[\alpha]_D^{6}+18.48°$ in chloroform (c 0.974).

Anal. Found: N, 5.64; S, 8.91. Calcd. for $C_{31}H_{29}O_{13}N_3S_2$: N, 5.86; S, 8.96 %.

c) 3,6-Anhydro-L-galactose:—To a solution of 3,6-anhydro-L-galactose diethylmercaptal (5.32g.) in water (50 cc.), held in a three-necked flask equipped with a mechanical stirrer, finely powdered lead carbonate (15 g.) and a solution of mercuric chloride (11 g.) in water (200 cc.) were added. Rapid stirring was maintained for 3 hrs. at room temperature. The reaction mixture was then filtered, a small amount of lead carbonate being placed in a filter flask. The filtrate was concentrated to about 20 cc. in the presence of lead carbonate. Unaffected mercaptal and excess mercuric chloride were removed by exhaustive extraction with ether until nothing came into the extract. The remaining water solution was concentrated to dryness by repeatedly adding absolute ethanol and distilling it off. The residue was dissolved in absolute ethanol, insoluble lead compounds were removed by filtration, and the filtrate was concentrated to a glassy solid of 3,6-anhydro-L-galactose (2.90 g.), $[\alpha]_D^{25}-39.4°$ (initial) in water (c 1.75), −25.2° (24 hrs., constant). It reduced cool Fehling's solution, showed strong Seliwanoff's reaction, and restored the color to Schiff's reagent.

*Diphenylhydrazone*5): m.p. 154°, $[\alpha]_D^{13}-34.31°$ (initial) in methanol (c 1.370), −24.09° (24 hrs.).

*Phenylosazone*5): m.p. 217°, $[\alpha]_D^{15}-75.38°$ (initial) in pyridin-methanol (2:3) (c 0.738), −53.48° (24 hrs.).

d) 3,6-Anhydro-L-galactose diethylmercaptal from corresponding dimethylacetal:—A solution of 3,6-anhydro-L-galactose dimethylacetal (2.50 g.) in concentrated hydrochloric acid (2.5 g.) was shaken for 1 hr. with ethylmercaptan (1.6 g.) with ice-cooling. Small pieces of ice were added to induce crystallization, and separated crystals of the mercaptal were filtered, washed with small portions of ice-water, and dried over potassium hydroxide in vacuo; yield 2.37 g., m.p. 105~8°. Several recrystallizations from ethyl acetate containing a small amount of petroleum ether (b. p. 40~70°) gave silky needles, m.p. 110~111°, $[\alpha]_D^{15}+14.36°$ in water (c 1.253), −21.03° in ethanol (C 1.617) and −26.32° in pyridin (C 1.140).

Anal. Found: C, 44.49; H, 7.73; SC_2H_5, 45.43. Calcd. for $C_{10}H_{20}O_4S_2$: C, 44.75; H, 7.51; SC_2H_5, 45.55 %.

IV. Isolation and confirmation of DL-galactose diethylmercaptal.

a) Isolation:—Crystals B_3 and B_5, described in **III-a,** were considered to be mixtures from their ill-defined m.ps. These were combined and divided into two components by fractional crystallizations from acetone. From the less soluble fraction, D-galactose diethylmercaptal (0.48 g.) was obtained, m.p. alone and mixed m.p. 139~140°, $[\alpha]_D^{12}-4.40°$ in water. DL-Galactose diethylmercaptal was accumulated in the more soluble fraction. The crude crystals (0.55 g.), m.p. 123~5°, were purified by further recrystallizations from ethanol, forming prismatic needles, m.p. 127°, $[\alpha]_D^{15}\pm 0°$ in water (c 0.676) and pyridine (c 0.711). Its m.p. was not depressed on admixture with an authentic specimen, of which preparation is described in **IV-c.**

Anal. Found; C, 41.71; H, 7.84; SC_2H_5, 42.75. Calcd. for $C_{10}H_{22}O_5S_2$: C, 41.93; H, 7.74; SC_2H_5, 42.68 %.

The substance gave mucic acid on oxidation with nitric acid (d 1.15), m.p. 212°, undepressed when mixed with an authentic sample (m.p. 214°).

b) Pentaacetyl DL-galactose diethylmercaptal:—The crystals (0.20 g.) obtained above were acetylated with pyridine and acetic anhydride in the usual way. The product was recrystallized by dropping water to its methanolic solution, forming prisms (0.28 g.), m.p. 112~113°, $[\alpha]_D^{17}\pm 0°$ in chloroform (c 0.954).

Anal. Found: C, 48.17; H, 6.71; SC_2H_5, 24.51; CH_3CO, 43.85. Calcd. for $C_{20}H_{32}O_{10}S_2$: C, 48.37; H, 6.50; SC_2H_5, 24.61; CH_3CO, 43.33 %.

c) DL-Galactose diethylmercaptal from dulcitol:—Dulcitol (5.0 g.) was oxidized with 3 % hydrogen peroxide according to literature16) on the subject. The reaction product, however, was converted to diethylmercaptal, without direct isolation of DL-galactose, by the action of concentrated hydrochloric acid (2 g.) and ethylmercaptan (2 g.). The mercaptal obtained was purified by recrystallizations from ethanol, yielding prismatic needles (0.80 g.), m.p. 125~127°.

Anal. Found: C, 41.72; H, 7.95; SC_2H_5, 42.43. Calcd. for $C_{10}H_{22}O_5S_2$; C, 41.93; H, 7.74; SC_2H_5, 42.68 %.

V. Detection of xylose diethylmercaptal.

A small portion of each of sirups S_1 and S_2, the separation of which is described in **III-a,** was treated with mercuric chloride and mercuric oxide to yield free sugar. Both of resulting products were shown to be mixtures of xylose and 3,6-anhydro-L-galactose by paper chromatography. Hence, S_1 and S_2 were combined and extracted with cold benzene. Extract on solvent removal gave a colorless sirup (1.40 g.), $[\alpha]_D^{17}-7.7°$ in water, SC_2H_5 46.89 (calcd. for $C_9H_{20}O_4S_2$: 47.64 %). An attempt to crystallize xylose diethylmercaptal or its acetate was unsuccessful.

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16) C. Neuberg and J. Wohlgemuth, *Z. physiol. Chem.*, **36**, 219 (1902).

VI. Second mercaptolysis.

The residual water solution from ether extraction, described in **III-a,** was diluted with water to 200 cc. after the removal of ether in vacuo, and allowed to stand in an ice-box for a week. A small amount of crystals of D-galactose diethylmercaptal was deposited; yield 0.90 g., m.p. and mixed m.p. 137~139°, $[\alpha]_D^{15} -4.9°$ in water. The filtrate was concentrated to a sirup, which was dried by repeated additions of absolute methanol followed by evaporation, yielding an amorphous solid (R_1); yield 29.0 g., $[\alpha]_D^{13} -7.5°$ in water, SC_2H_5 22.62 % (hexobiose and hexotriose diethylmercaptals require SC_2H_5 27.2 and 22.6 %). It did not reduce a hot Fehling's solution, the observation excluding the possibility of the existence of ketose components.

R_1 was dissolved in concentrated hydrochloric acid (120 g.) and stirred with ethylmercaptan (40 g.) at 10~12° for 48 hrs. The reaction products were treated quite in the same manner as before; the yields of crystalline diethylmercaptals of D-galactose, 3,6-anhydro-L-galactose and of DL-galactose were 8.09 g., 7.29 g. and 0.23 g., respectively. No sirup containing xylose derivative could be found. The residue (R_2), corresponding to R_1 in the first series, was obtained as amorphous solid (12.7 g.), $[\alpha]_D^{21} -6.8°$ in water, SC_2H_5 22.10 %. This did not reduce a hot Fehling's solution.

VII. Third mercaptolysis.

R_2 obtained above was again submitted to mercaptolysis with concentrated hydrochloric acid (60 g.) and ethyl mercaptan (30 g.) at 10~12° for 48 hrs. The procedure was essentially the same as in the preceding two cases, except for being carried out on a smaller scale. The yields of crystalline dietylmercaptals of D-galactose, 3,6-anhydro-L-galactose and of DL-galactose isolated herein were 4.88 g., 4.22 g. and a trace, making the total yield 23.55 g., 19.85 g. and 0.78 g., respectively. No xylose derivative was found in this case. The residue (R_3), corresponding to R_1 in the first series, was brown a sirup (3.1 g.), $[\alpha]_D^{24} -1.7°$ in water, SC_2H_5, 21.05 %.

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