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Note

Anhydrous mercaptolysis of agar: an efficient preparation of 3,6-anhydro-L-galactose diethyl dithioacetal

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Abstract

Mercaptolysis of agar in a 2:1 mixture of 0.5 M ethanethiolic HCl and 0.5 M methanolic HCl (v/v) at 60 °C for 12 h liberated component sugars almost quantitatively as their dithioacetals without any accompanying methyl glycosides. Simple extraction procedures afforded 3,6-anhydro-L-galactose diethyl dithioacetal in 86% yield. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

3,6-Anhydrogalactose is one of the major constituents of such red algal galactans as agar, porphyran, and carrageenan [1], but residues of this sugar in galactans are readily decomposed during acid hydrolysis or methanolysis, as commonly used to liberate component sugars from polysaccharides. Authentic 3,6-anhydrogalactose as an analytical standard is not commercially available. Colorimetric determination of 3,6-anhydrogalactose in galactans has used the resorcinol method of Yaphe and Arsenault [2] or the thymol method of Matsuhiro and Zanlungo [3], wherein a standard solution of the sugar was prepared by demercaptolysis of 3,6-anhydro-L-galactose diethyl dithioacetal (**1**) with mercuric chloride or by acid hydrolysis of synthetic methyl 3,6-anhydro-α-D-galactopyranoside [4].

Araki and Hirase reported the mercaptolysis of agar, by dissolving agar in concentrated hydrochloric acid and stirring the mixture with occasional additions of ethanethiol, and obtained **1** and D-galactose diethyl dithioacetal (**2**) [5], but the method was time consuming and required complicated manipulations, and gave only 25% of the theoretical amount of **1**.

We have effected almost quantitative liberation of the component 3,6-anhydrogalactose

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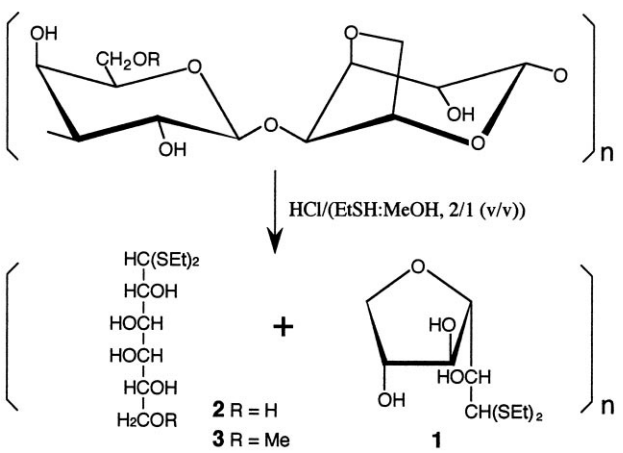
from galactans by an anhydrous mercaptolysis procedure and describe here an efficient and simple method for the preparation of **1** by the anhydrous mercaptolysis of agar.

2. Results and discussion

Agar was mercaptolyzed in 0.5 M HCl–EtSH, but the mercaptolyzate was brownish because of slight decomposition of component sugars. We added MeOH to the solvent system and found that mercaptolysis occurred preferentially in a 2:1 (v/v) mixture of 0.5 M HCl–EtSH and 0.5 M HCl–MeOH without any formation of brownish products or methyl glycosides. After 12 h of anhydrous mercaptolysis at 60 °C, the agar was mercaptolyzed almost completely to give component monosaccharide diethyl dithioacetals (Scheme 1). It should be noted that, although the solvent system contained MeOH in 2:1 (v/v) ratio of EtSH to MeOH, no methyl glycosides were detected throughout the mercaptolysis.

Most of the **2** could be removed as a precipitate from the mercaptolyzate by addition of 5 volumes of Et_2O, and all of **1** was found together with small amounts of **2** and 6-*O*-methyl-D-galactose diethyl dithioacetal (**3**, R_f 0.56) in the filtrate. Removal of most of the **2** at this stage was essential for the isolation of **1** since the following Et_2O extraction and crystallization could not eliminate **2** and **3** completely. After neutralization of the filtrate followed by evaporation of the organic solvents, compound **1** in the residual aqueous solution was extracted with Et_2O and the combined extract was evaporated to dryness to obtain 714 mg of almost pure **1** (yield 86%). Finally, 543 mg of homogeneous **1** was obtained as crystalline silky needles from 1.0 g of agar (yield 65%). It is advisable in the crystallization of **1** for the additions of Et_2O and petroleum ether to be performed stepwise, and the resultant crystals to be collected each time and checked for purity by thin-layer chromatography (TLC), since a small amount of **3** is apt to crystallize initially.



Scheme 1.

Agarose can also be used as a good starting material for the preparation of **1** under the same conditions as just described. 3,6-Anhydro-D-galactose diethyl dithioacetal can be obtained from carrageenan by the present method. In order to find the most appropriate conditions throughout the procedure, it is advisable to check the behavior of component sugar dithioacetals in each step by TLC.

3. Experimental

Materials.—Reagents used in this study were of highest grade unless otherwise noted. The following were purchased from commercial sources: liquefied HCl gas (> 99.7%), Tsurumi Soda Co.; agar powder (44.6% anhydrogalactose as determined by the resorcinol method [2], 49.3% galactose by the anthrone method [7]), AcOH, 1-butanol, Et_2O, EtOAc, EtSH (first grade), MeOH, and petroleum ether (bp 30–60 °C), Wako Pure Chemical Industries; D-galactose and 6-*O*-methyl-D-galactose, Sigma Chemical Co.; Silica Gel 60 precoated TLC plates, E. Merck. Compounds **1**, **2**, and **3** were prepared from agar according to Araki and Hirase [5]. Solutions of HCl in EtSH and HCl in MeOH were prepared by passing dry HCl gas slowly into EtSH and MeOH, respectively.

Thin-layer chromatography.—Small amounts of the mercaptolyzates were applied to a TLC plate and developed with 2:1:1 (v/v/v) 1-butanol–AcOH–water. Plates were sprayed with diphenylamine reagent [6] and heated for 20–30 min at 120 °C to reveal sugar dithioacetals.

Preparation of ethanethiolic HCl.—Anhydrous ethanethiolic HCl was prepared by passing dry HCl gas slowly into EtSH at room temperature. The maximum concentration of HCl in EtSH at room temperature was found to be ~0.8 M. A solution of 0.5 M HCl in EtSH at 5 °C could be used for at least one month.

Anhydrous mercaptolysis of agar.—Agar (1.0 g) contained in a bottle with a Teflon-lined screw cap was suspended in a mixture of 20 mL of 0.5 M HCl in EtSH and 10 mL of 0.5 M HCl in MeOH and the suspension was heated for 12 h at 60 °C with occasional shaking. During the initial stage of heating, the suspended agar gradually dissolved and an almost clear solution was obtained after 2–3 h. After 12 h of heating, the agar was mercaptolyzed almost completely to give component monosaccharide dithioacetals. No methyl glycosides were detected throughout mercaptolysis.

Isolation of 3,6-anhydro-L-galactose diethyl dithioacetal (**1**) *from the mercaptolyzate.*—To the mercaptolyzate (30 mL) was added 5 vol of Et_2O, and the mixture was cooled in iced water overnight. A copious precipitate that formed (compound **2**, R_f 0.53) was removed by filtration with a glass microfiber filter. The filtrate was neutralized by the addition of 16 mL of 1 M $NaHCO_3$ and then the organic solvents were removed with a rotary evaporator. The residual aqueous solution (~15 mL) was extracted four times with 3 vol of Et_2O and the combined extract was evaporated to dryness to afford 714 mg of almost pure **1**.

Compound **1** was readily soluble in water, MeOH, and EtOH, moderately soluble in EtOAc, somewhat less soluble in Et_2O, and practically insoluble in petroleum ether. Compound **1** was crystallized essentially according to the method of Araki and Hirase [5]; it was dissolved with warming in a small volume of EtOAc and the warm solution was clarified by filtration. Diethyl ether was added to the solution to initiate crystallization and then petroleum ether was added in the final stage of crystallization to afford finally 543 mg (65%) of homogeneous **1** as silky needles from 1.0 g of agar; mp 109.7 °C (Ref [5] mp 110–111 °C); R_f 0.61; $[\alpha]_D^{25}$ +10.6°; ^{1}H NMR (400 MHz, D_2O): δ 1.25 (t, 3 H, $J_{H,H'}$ 7.33 Hz, CH_3 of SEt), 1.26 (t, 3 H, $J_{H,H'}$ 7.33 Hz, $(CH_3)'$ of SEt), 2.68–2.82 (m, 4 H, CH_2 of SEt), 3.83 (dd, 1 H, $J_{5,6'}$ 2.9 Hz, H-6′), 3.97 (dd, 1 H, $J_{2,3}$ 4.9 Hz, H-2), 4.02 (dd, 1 H, $J_{6,6'}$ 9.8 Hz, H-6), 4.07 (d, 1 H, $J_{1,2}$ 5.4 Hz, H-1), 4.12 (dd, 1 H, $J_{4,5}$ 2.6 Hz, H-4), 4.15 (dd, 1 H, $J_{3,4}$ 4.4 Hz, H-3), 4.25 (ddd, 1 H, $J_{5,6}$ 4.9 Hz, H-5); EI MS of Me_3Si derivative (70 eV): m/z 394 (11%, $[M-Me_3SiOH]^+$), 349 (11, $[M-CH(SEt)_2]^+$), 333 (14, $[M-Me_3SiOH-SEt]^+$), 259 (83, $[M-CH(SEt)_2-Me_3SiOH]^+$), 247 (9, $[M-CH(SEt)_2-Me_3SiOCH]^+$), 243 (26, $[M-(Me_3SiOH)_2-SEt]^+$), 217 (44, $[(Me_3SiO)_2C_3H_3]^+$), 191 (8, $[(Me_3SiO)_2CH]^+$), 189 (2), 176 (22), 169 (16), 161 (12), 157 (31), 147 (42), 135 (100, $[HC(SEt)_2]^+$), 133 (27), 131 (16), 129 (31), 117 (21), 115 (21), 103 (78, $[Me_3SiOCH_2]^+$). Anal. Calcd for $C_{10}H_{20}O_4S_2$: C, 44.75; H, 7.51; S, 23.89. Found: C, 44.72; H, 7.49; S, 23.96.

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