After 4 hr., 5.0 ml. of the reaction mixture was diluted with dichloromethane and washed with sodium bicarbonate solution. The organic layer was dried ($MgSO_4$); solvent was removed under reduced pressure. The resulting sirup,[27] from 5 ml. of ethyl ether and 5 ml. of pentane, furnished 15.9 mg. of IIc: m.p. 88–89°, m.m.p. (with authentic IIc) 87–89°.

The remaining portion of the reaction mixture showed on thin layer chromatography only IIIb and *p*-anisic acid.[28]

TABLE IV

Time, min.	Obsd. rotn., α_D, deg.	k, $\times 10^{-2}$, ln, min.
0	+14.68 (extrapolated)	..
5.0	14.23	0.55
10.0	13.82	0.53
15.0	13.38	0.54
20.0	13.00	0.53
30.0	12.19	0.54
40.0	11.40	0.55
50	10.71	0.55
60	10.07	0.54
120	6.85	0.53
195	4.20	0.51
240	2.96	0.51
300	1.75	0.50
360	0.85	0.50
420	0.180	0.49
480	−0.439	0.50
540	−0.88	0.52
1680 (∞)	−1.90	..

(27) Thin layer chromatography on silica gel G^{21} revealed two spots corresponding to IIc and IIIb.

(28) *p*-Anisic acid and other compounds with aromatic substituents were made more visible under ultraviolet light by the use of fluorescent zinc silicate in the silica gel G^{21} [*cf.* M. E. Tate and C. T. Bishop, *Can. J. Chem.*, **41**, 1801 (1963)].

Reaction of Ib with Water.—The mutarotation of 0.2105 g. (0.549 mmoles) of Ib in 5 ml. of anhydrous acetone diluted with 1.00 ml. of water in a 1.50-dm. tube at 20° was observed and the rate constants were calculated (see Table IV).

After 48 hr., the reaction mixture was concentrated under reduced pressure to dryness. The residue in dichloromethane was washed with saturated sodium bicarbonate solution, which on acidification gave 71.6 mg. (93.3%) of *p*-anisic acid. The dichloromethane solution was dried over sodium sulfate and evaporated under reduced pressure to a pale yellow sirup: 128.4 mg., $[\alpha]^{20}D$ −35.6° (*c* 0.79, dichloromethane). This sirup showed a definite hydroxyl band at 3500 cm.$^{-1}$ and two spots on thin layer chromatography (silica gel G^{21}) with 4:1 benzene–ethyl ether; the faster moving spot being identical with that of IIIb. A portion of the crude sirup (72 mg.) was methylated by stirring its methyl iodide (10 ml.) solution with 4.2 g. of silver carbonate for 18 hr. After filtration of the solids, the filtrate and dichloromethane washings were combined, washed with water, and dried with sodium sulfate. Removal of the solvents under reduced pressure afforded a yellow sirup: 69.8 mg., $[\alpha]^{20}D$ −31.9°[29] (*c* 0.7, dichloromethane), no absorption in the 3500-cm.$^{-1}$ region, two spots (thin layer chromatography, silica gel G^{21} with 4:1 benzene–ethyl ether and with 1:1 ethyl ether–heptane) corresponding to IIc and IIIb. The sirup in 5 ml. of ethyl ether and 20 ml. of pentane yielded a few needles when stored overnight in the refrigerator: m.p. (Kofler) 84–86°; m.m.p. (Kofler) (with IIc) 84–86°; infrared absorption spectrum identical with that of IIc.

Acknowledgment.—The authors gratefully acknowledge the elementa determ nations and n.m.r. spectra supplied by the Section on Microanalytical Services and Instrumentation, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, under the direction of Dr. William C. Alford and the aid of Dr. John D. Stevens in the interpretation of the n.m.r. spectra.

(29) This rotation corresponds to a 38% over-all yield of IIc from Ib assuming only the presence of IIc and IIIb.

Formation of 1,2-*O*-Isopropylidene-α-D-glucofuranose 5,6-Thionocarbonate by Rearrangement–Fragmentation of Bis(1,2-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose) Disulfide[1a]

W. M. DOANE, B. S. SHASHA, C. R. RUSSELL, AND C. E. RIST

Northern Regional Research Laboratory,[1b] *Peoria, Illinois*

Received August 3, 1964

Selective hydrolysis of bis(1,2:5,6-di-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose) disulfide (I) removed the 5,6-*O*-isopropylidene radical and gave bis(1,2-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose) disulfide (II) as an amorphous product. Dissolution of II in pyridine gave equimolar amounts of 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate (III), 1,2-*O*-isopropylidene-α-D-glucofuranose (VIII), carbon disulfide, and free sulfur. The structure of III is proved by its conversion into known compounds and by its independent synthesis.

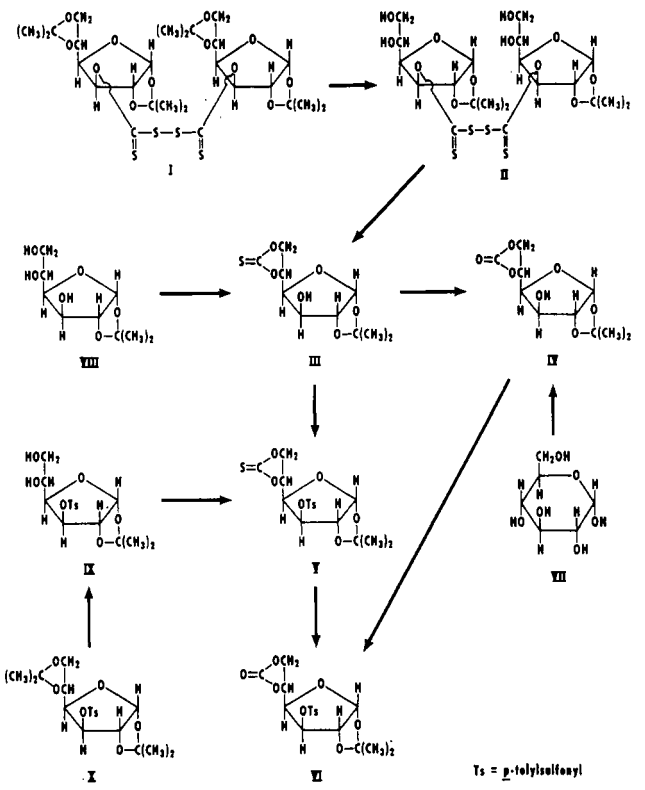
In elucidating the behavior of dithiocarbonate groups in starch *O*-(*S*-sodium dithiocarbonate) esters (starch xanthates) and the oxidatively cross-linked products thereof, starch bis(*O*-thiocarbonyl) disulfides (starch xanthides), model compounds of glucose, were prepared for study of their chemical and physical properties. Unexpectedly a rearrangement–fragmentation of one of these compounds, namely bis(1,2-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose) disulfide (II), formed nearly equimolar amounts of 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate (III) and 1,2-*O*-isopropylidene-α-D-glucofuranose (VIII). This report is concerned with the proof of structure of the cyclic thionocarbonate (III) (Scheme I).

The literature contains only a few reports of thionocarbonate derivatives of carbohydrate materials. Interestingly in each case, the thionocarbonate was an unexpected product. Thus Freudenberg and Wolf[2] reported that on long standing crystalline 2,3:5,6-di-*O*-isopropylidene-D-mannose 1-*O*-(*S*-methyl dithiocarbonate) gave bis(2,3:5,6-di-*O*-isopropylidene-D-mannose) 1-thionocarbonate. The structure of this com-

(1) (a) Presented before Division of Carbohydrate Chemistry, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964. (b) This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Article is not copyrighted.

(2) K. Freudenberg and A. Wolf, *Ber.*, **60**, 232 (1927).

SCHEME I



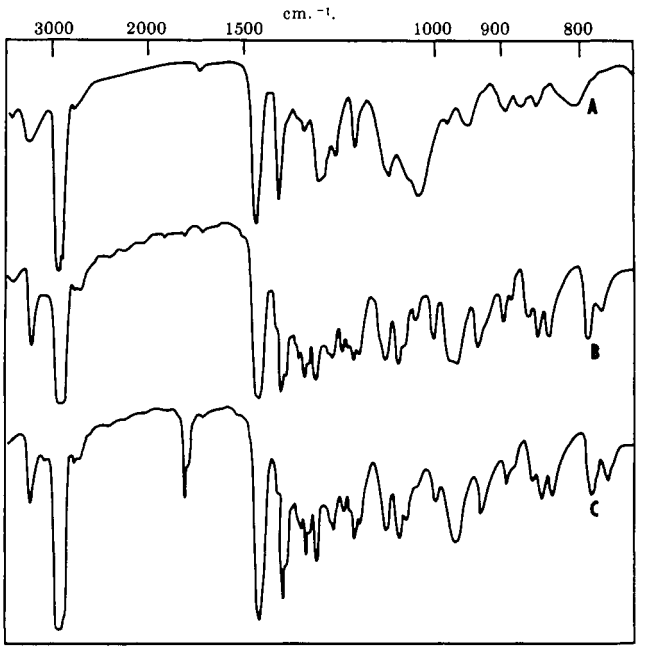


Figure 1.—Infrared spectra in Nujol: (A) bis(1,2-O-isopropylidene-3-O-thiocarbonyl-α-D-glucofuranose) disulfide (II); (B) 1,2-O-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate (III) isolated from pyridine treatment of II; (C) III as isolated from ethanol treatment of II, showing 1,2-O-isopropylidene-α-D-glucofuranose 5,6-carbonate (IV) as an impurity.

pound was not proved. Foster and Wolfrom[3] in attempts to prepare certain O-(S-alkyl dithiocarbonate) esters of methyl 3,4-O-isopropylidene-β-D-arabinopyranoside 2-O-(S-sodium dithiocarbonate) isolated in each case bis(methyl 3,4-O-isopropylidene-β-D-arabinopyranoside) 2-thionocarbonate but none of the expected product. More recently, Baker and Sachdev[4] treated the monosodium salt of 1,2:5,6-di-O-isopropylidene-D-mannitol with phenyl isothiocyanate in attempts to prepare the phenylthiourethan derivative. The expected product was not isolated but rather a mixture of a cyclic thionocarbonate and a cyclic carbonate.

In the present study bis(1,2:5,6-di-O-isopropylidene-3-O-thiocarbonyl-α-D-glucofuranose) disulfide (I) in acetone solution was treated with hydrochloric acid to remove the 5,6-O-isopropylidene grouping selectively. The hydrolysis was followed polarimetrically with a constant rotation reached in 90 min. Compound II, isolated from the hydrolysate as an amorphous solid in 60% yield, gave infrared absorption bands[5] characteristic for the xanthide grouping at 1025 and 1250 cm.$^{-1}$ (Figure 1, spectrum A). The spectra of I and II were similar except that II showed an additional absorption band at 3450 cm.$^{-1}$ characteristic of a free hydroxyl group. Unchanged starting material and mono- and di-O-isopropylidene-D-glucofuranose were identified after removal of II.

During attempts to crystallize II, an ethanol–water solution left standing for several hours at room temperature produced two types of crystals, which had m.p. 116° and 205–208°. The low-melting crystals were free sulfur. The high-melting large transparent needles, which were subsequently shown to be 1,2-O-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate (III), gave on analysis an empirical formula of $C_{10}H_{14}O_6S$, formula wt. 262. Molecular weight analysis by the Rast method gave 256 and 260. The infrared spectrum (Figure 1, spectrum C) lacked the characteristic "xanthide" absorption bands of II.

Treatment of III with excess acetic anhydride in pyridine solution gave an 84% yield of a crystalline compound whose empirical formula of $C_{12}H_{16}O_7S$ indicated substitution of only one acetyl group per mole of III. Similarly, when III was treated with excess p-toluenesulfonyl chloride in pyridine, a crystalline product V was obtained in 68% yield with an empirical formula of $C_{17}H_{20}O_8S_2$, which again indicated substitution of only one p-tolylsulfonyl group. The infrared spectra of the acetyl and p-tolylsulfonyl derivatives showed no characteristic absorption for a free hydroxyl group in the 3450-cm.$^{-1}$ region. Because the p-tolylsulfonyl group in V could not be replaced by iodine when reacted with sodium iodide in acetone, evidently the p-tolylsulfonyl group did not occupy position C-6 in the D-glucose unit.[6a] These experimental findings suggested that a possible structure of III might involve cyclization between C-6 and either C-3 or C-5.

Reaction of a solution of III in aqueous acetone with silver nitrate produced IV, a crystalline compound of empirical formula $C_{10}H_{14}O_7$, in an 83% yield. This compound was identified by its melting point, mixture melting point, and infrared spectrum as 1,2-O-isopropylidene-α-D-glucofuranose 5,6-carbonate. Similar treatment of the p-tolylsulfonyl derivative (V) produced 1,2-O-isopropylidene-3-O-p-tolylsulfonyl-α-D-glu-

(3) A. B. Foster and M. L. Wolfrom, *J. Am. Chem. Soc.*, **78**, 2493 (1956).
(4) B. R. Baker and H. S. Sachdev, *J. Org. Chem.*, **28**, 2135 (1963).
(5) M. L. Shankaranarayana and C. C. Patel, *Can. J. Chem.*, **39**, 1633 (1961).
(6) (a) R. S. Tipson, *Advan. Carbohydrate Chem.*, **8**, 181 (1953). (b) While this manuscript was being considered by the reviewers, Horton and Turner reported an alternate synthesis of the thionocarbonate III [*Tetrahedron Letters*, 2531 (1964)].

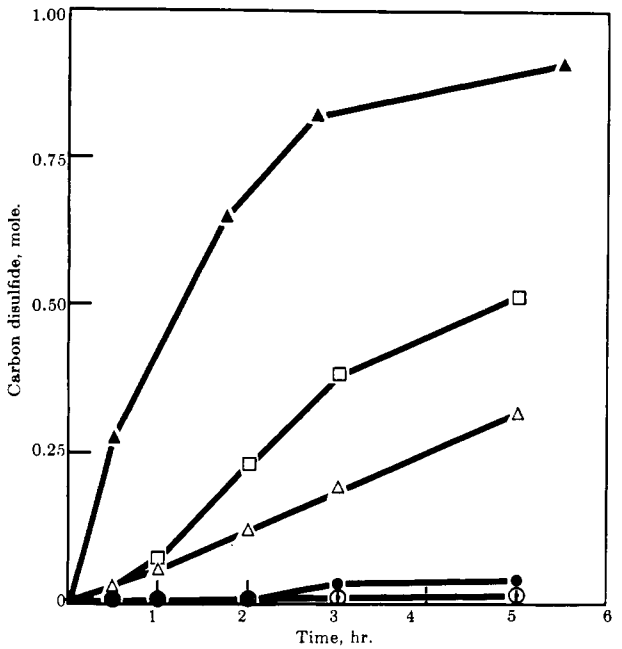


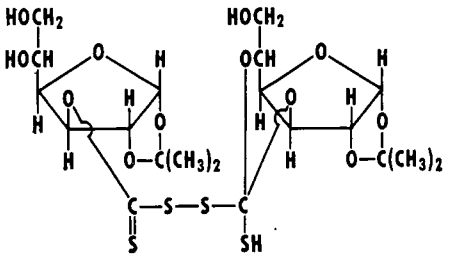
Figure 2.—Rate of carbon disulfide formation from rearrangement–fragmentation of 1.0 mole of bis(1,2-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose) disulfide (II) in various solvents: ▲, pyridine; □, 30% pyridine in ethanol; △, 10% pyridine in ethanol; ●, ethanol; and ○, 60% acetic acid in ethanol.

cofuranose 5,6-carbonate (VI), which was identified by its melting point and mixture melting point with an authentic sample.

The independent synthesis of both compounds III and V was achieved by procedures similar to those used for preparation of the analogous cyclic carbonate, which employ phosgene in the presence of an organic base. Thus, when suspensions of VIII and 1,2-*O*-isopropylidene-α-D-glucofuranose 3-*p*-toluenesulfonate (IX) in dioxane were treated with thiophosgene in the presence of pyridine, III and V were isolated in 48 and 36% yields, respectively.[6b] Foster and Wolfrom[3] previously used thiophosgene to prepare an intermolecular thionocarbonate derivative.

While attempting to prepare the trityl derivative of II in pyridine solution, none of the expected 6-trityl compound was formed. Instead, the rearrangement occurred giving, after 5 hr. at 25°, nearly equimolar amounts of III and VIII in theoretical yield. The rearrangement occurred more slowly in ethanol, isopropyl alcohol, and acetone, with the thionocarbonate still being formed after 72 hr. in ethanol. The longer times in these solvents apparently caused some hydrolysis of the thiocarbonyl to the carbonyl group since the analogous cyclic carbonate IV was present as an impurity in each case. Fractional recrystallization was only partially successful in separating a mixture of III and IV. The presence of IV was easily recognized by examination of the infrared spectrum at 1790 cm.$^{-1}$ (Figure 1, spectrum C). The pure thionocarbonate has no absorption in this region (Figure 1, spectrum B), but IV absorbs strongly. The intensity of absorption at 1790 cm.$^{-1}$ usually varied with different preparations as isolated from ethanol solutions. One preparation with a strong absorption contained 14% of the carbonate as indicated by the sulfur content. The possibility that some rearrangement of III had occurred to give a monothiocarbonate structure at C-5, C-6 with the sulfur atom bonded to either C-5 or C-6 was excluded, since treatment of III with barium hydroxide gave a sulfur-free product.

Although the precise mechanism of the rearrangement cannot be formulated at present, an ortho ester type structure (IIA) is proposed as an intermediate



IIA

analogous to that proposed for acetyl migration in 3-*O*-acetyl-1,2-*O*-isopropylidene-6-*O*-trityl-D-glucofuranose[7] and 3-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose.[8] Similarly, rearrangement of bis(1,2-*O*-isopropylidene-D-glucofuranose) 3-carbonate to 1,2-*O*-isopropylidene-D-glucofuranose 5,6-carbonate[7] was proposed as proceeding *via* an ortho ester intermediate. In support of such an intermediate, the rate of formation of III from II would be expected to be faster in the presence of base. As shown in Figure 2, after 5 hr. the reaction was less than 2% complete in the presence of acetic acid, 5% in ethanol, and up to 90% in pyridine. Dilution of the pyridine with ethanol considerably decreased the rate of carbon disulfide formation. It is proposed that the formation of IIA is the slow rate-determining step, with subsequent rearrangement and fragmentation occurring rapidly, since only 1 mole of III is formed from 1 mole of II.

Experimental

Melting points were determined in capillary tubes in a Büchi[9] melting point apparatus and were corrected. Optical rotations were measured at 5893 Å. with a Rudolph polarimeter. Molecular weights were determined by the Rast method in camphor or in a vapor pressure osmometer (Mechrolab, Inc., Mountain View, Calif.) in acetone as specified. Ultraviolet spectra were determined with a Beckman DU spectrophotometer. Infrared spectra were recorded by a Perkin-Elmer Infracord spectrophotometer with silver chloride optics as Nujol mulls or films as specified. Samples for sulfur analysis were combusted by the Schöniger flask technique and determined by the method of White.[10]

Bis(1,2:5,6-di-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose) Disulfide (I).—The procedure of Freudenberg and Wolf was modified slightly for the preparation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-*O*-(*S*-sodium dithiocarbonate). Metallic sodium (6 g.) was added to a solution of 20 g. of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose in 200 ml. of anhydrous ethyl ether. After standing for 12 hr. at 25° the solution was filtered and diluted with an additional 200 ml. of anhydrous ethyl ether. On addition of carbon disulfide (20 ml.) a yellow precipitate was obtained immediately. Subsequently most of the ether and excess of carbon disulfide were removed under reduced pressure. The precipitate was dissolved in 100 ml. of water and the pH adjusted to 7 with 0.5 *N* acetic acid. The product was oxidatively coupled by addition of 0.3 *M* iodine solution. The

(7) L. v. Vargha, *Ber.*, **67**, 1223 (1934).

(8) K. Josephson, *Ann.*, **472**, 217 (1929); H. Ohle, E. Euler, and R. Lichtenstein, *Ber.*, **62**, 2885 (1929).

(9) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

(10) D. C. White, *Mikrochim. Acta*, 807 (1962).

remaining ethyl ether evaporated on standing in the hood and the resulting crystalline product was washed twice with water and recrystallized from hot ethanol to yield 17 g. (66%) of colorless needles, m.p. 121–124°. A second recrystallization from hot hexane gave m.p. 129–129.5°, $[\alpha]^{20}D$ −50° (*c* 1.0, acetone). Danilov[11] reported m.p. 110–111° and gave no rotation.

Anal. Calcd. for $C_{26}H_{38}O_{12}S_4$: C, 46.6; H, 5.7; S, 19.1; mol. wt., 670. Found: C, 46.8; H, 5.8; S, 19.1; mol. wt., 679 (Mechrolab).

Bis(1,2-O-isopropylidene-3-O-thiocarbonyl-α-D-glucofuranose) Disulfide (II).—To a solution of 100 mg. of I in 8 ml. of acetone was added slowly with stirring 2 ml. of 5 *N* hydrochloric acid. The mixture was allowed to stand at 20° and the change in optical rotation with time was as follows.

Min.	5	15	30	60	90	120	195
α, deg.	−0.25	−0.18	−0.13	−0.06	−0.03	−0.03	−0.03

Since the optical rotation was constant after 90 min., the experiment was repeated on a larger scale. I (10 g.) in 800 ml. of acetone was treated with 200 ml. of 5 *N* hydrochloric acid, and, after this mixture stood 90 min., most of the acetone was removed by evaporation at 20° under reduced pressure until a slightly turbid solution resulted. Approximately 200 ml. of water was added and the suspension was extracted twice with ethyl ether. The ethyl ether extract was washed with water and dried over anhydrous magnesium sulfate. After filtration the filtrate was evaporated to dryness and the resulting yellow amorphous product was washed with hexane and dried to yield 5.3 g. (60%), $[\alpha]^{20}D$ −14° (*c* 1, methanol).

Anal. Calcd. for $C_{20}H_{30}O_{12}S_4$: C, 40.7; H, 5.1; S, 21.7; mol. wt., 590. Found: C, 41.9; H, 5.4; S, 21.0; mol. wt., 605 (Mechrolab).

1,2-O-Isopropylidene-α-D-glucofuranose 5,6-Thionocarbonate (III). A. From II.—One gram (1.7 mmoles) of II was dissolved in 2 ml. of pyridine; the solution was allowed to stand for 5 hr. at 25° and was then added to an excess of hexane. The precipitate was collected and extracted first with water and then with carbon disulfide, and the two extracts were saved for analysis. The remaining product was dried to constant weight to afford 393 mg. (1.5 mmoles) of III. Recrystallization from ethanol gave m.p. 206–208°, $[\alpha]^{20}D$ −16° (*c* 3, acetone). The infrared spectrum showed none of the characteristic "xanthide" absorption. The ultraviolet spectrum (ethanol) showed absorption maxima at 234 mμ (ε 15,700) and 287 mμ (ε 44).

Anal. Calcd. for $C_{10}H_{14}O_6S$: C, 45.8; H, 5.4; S, 12.2; mol. wt., 262. Found: C, 45.9; H, 5.4; S, 12.2; mol. wt., 256, 260 (Rast), and 261 (Mechrolab).

The aqueous extract was evaporated to give 379 mg. (1.7 mmoles) of VIII, m.p. 164, which was undepressed on admixture with an authentic sample. The carbon disulfide extract when evaporated to dryness gave free sulfur, 55 mg. (1.7 mmoles). In a duplicate experiment the pyridine solution was constantly swept by a slow stream of nitrogen that was passed into a measured volume (50 ml.) of carbon tetrachloride immersed in an ice–water bath. After 5 hr. an additional 50 ml. of carbon tetrachloride was added. The amount of carbon disulfide present, measured by the optical density at 318 mμ, was 1.5 mmoles. The molar extinction coefficient of a solution of carbon disulfide in carbon tetrachloride was determined from a known concentration to be ε 97 (Doran and Gillam[12] report ε 108). The concentration of carbon disulfide was confirmed by forming the piperidine–carbon disulfide complex and determining the absorption of the complex at 262 and 290 mμ.[13]

B. From 1,2-O-Isopropylidene-α-D-glucofuranose (VIII).—VIII (1 g.) was suspended in 20 ml. of freshly dried dioxane containing 1 ml. of thiophosgene (California Corp. for Biochemical Research, Los Angeles). The mixture was heated to 65° until a solution was obtained. Freshly dried pyridine (1 ml.) was slowly added with stirring, and heating was continued for 10 min. The dark red solution was evaporated and the resulting sirup was washed with water and carbon disulfide. The remaining dark-colored precipitate was crystallized from ethanol and gave 570 mg. (48%) of light brown plates, which changed to needles upon heating to 175–180° and melted at 205°. Recrystallization of the plates from ethanol gave colorless needles, m.p. 205°, unchanged in admixture with III as isolated from an ethanol solution. On admixture with pure thionocarbonate, the melting point was 205–206°. Infrared analysis showed the presence of IV as an impurity in this product.

3-O-Acetyl-1,2-O-isopropylidene-α-D-glucofuranose 5,6-Thionocarbonate.—A solution of 500 mg. of III in pyridine (5 ml.) was treated with acetic anhydride (2 ml.) for 20 hr. at 25°. The product was precipitated into ice–water and collected by centrifugation. The white precipitate was washed with water and crystallization from ethanol–water afforded 470 mg. (81%) of colorless needles, m.p. 144–146°.

Anal. Calcd. for $C_{12}H_{16}O_7S$: C, 47.4; H, 5.3; S, 10.5. Found: C, 47.0; H, 5.3; S, 10.4.

1,2-O-Isopropylidene-α-D-glucofuranose 5,6-Carbonate (IV). A. From III.—To a solution of 262 mg. (1 mmole) of III in 30 ml. acetone was added 360 mg. (2.1 mmoles) of silver nitrate. By addition of a few drops of water to dissolve the silver nitrate, the immediate formation of silver sulfide was observed. After 5 min. the suspension was centrifuged to remove most of the silver sulfide. The supernatant containing small amounts of colloidal silver sulfide was filtered, reduced to small volume (5 ml.), and again filtered. The clear filtrate was evaporated to dryness, and the white crystals formed were washed with water and dried to yield 203 mg. (83%), m.p. 226–226.5°.

Anal. Calcd. for $C_{10}H_{14}O_7$: C, 48.8; H, 5.7. Found: C, 48.8; H, 5.8.

B. From α-D-Glucose (VII).—Preparation of IV was achieved by treating overnight a suspension of VII in acetone with phosgene according to the procedure of Overend, Stacey, and Wiggins.[14] Both melting point and mixture melting point with the product prepared under A were 226–226.5°. The infrared spectra of A and B were identical.

1,2-O-Isopropylidene-α-D-glucofuranose 3-p-Toluenesulfonate (IX).—The preparation of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose 3-*p*-toluenesulfonate (X) was accomplished by modification of the method of Freudenberg and Ivers.[15] Hence, 5.2 g. of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose was dissolved in 25 ml. of pyridine and treated with 5 g. of *p*-toluenesulfonyl chloride for 3 hr. at 50–60°. After cooling, the oil which separated on addition of water soon solidified and was collected by filtration. The precipitate was crystallized from ethanol to afford 5.2 g. (64%) of needles, m.p. 122–122.5°. Freudenberg and Ivers reported m.p. 120–121°.

Anal. Calcd. for $C_{19}H_{26}O_8S$: C, 55.1; H, 6.3; S, 7.7. Found: C, 54.8; H, 6.3; S, 7.7.

The 5,6-O-isopropylidene group of X was selectively hydrolyzed on treatment with hot acetic acid solution. To a solution of 500 mg. of X in 10 ml. glacial acetic acid was added 2 ml. of water. The solution was refluxed on a steam bath for 45 min. during which time the observed rotation changed from −2.30 to −0.20°. The solution was evaporated to dryness and the last traces of water were removed as an azeotrope with benzene to afford a sirup IX which partially crystallized on long standing.

Anal. Calcd. for $C_{16}H_{22}O_8S$: C, 51.4; H, 5.9, S, 8.6. Found: C, 51.7; H, 6.1; S, 8.6.

1,2-O-Isopropylidene-3-O-(p-tolylsulfonyl)-α-D-glucofuranose 5,6-Thionocarbonate (V). A.—A solution of 200 mg. of III in 2 ml. of pyridine was treated with 1 g. of *p*-toluenesulfonyl chloride for 48 hr. at 25°. The product was precipitated into 40 ml. of water containing 3 ml. of glacial acetic acid. The precipitate was collected by centrifugation, washed with water, and recrystallized from an ethanol–water solution to yield 215 mg. (68%) of colorless needles, m.p. 154–156°.

Anal. Calcd. for $C_{17}H_{20}O_8S_2$: C, 49.0; H, 4.8; S, 15.4; mol. wt., 416. Found: C, 48.6; H, 4.8; S, 15.4; mol. wt., 402, 432 (Rast).

B. From IX.—A solution of 500 mg. of IX in 20 ml. of dry dioxane was treated with 0.7 ml. of thiophosgene for 10 min. at 60°. Pyridine (0.5 ml.) was added dropwise with stirring and heating was continued for an additional 10 min. The light red top layer was collected by decantation and was evaporated to dryness. The partially crystalline product was washed with water and recrystallized from ethanol. The yield of colorless needles was 200 mg. (36%), m.p. 153–156° (154–156° on admixture with the compound from A).

(11) S. N. Danilov, *Zh. Obshch. Khim.*, **19**, 826 (1949); *Chem. Abstr.*, **43**, 9439 (1949).

(12) W. Doran and A. E. Gillam, *J. Soc. Chem. Ind.* (London), **47**, 259T (1928).

(13) L. J. Brady, *Anal. Chem.*, **20**, 512 (1948).

(14) W. G. Overend, M. Stacey, and L. F. Wiggins, *J. Chem. Soc.*, 1358 (1949).

(15) K. Freudenberg and O. Ivers, *Ber.*, **55**, 929 (1922).

Anal. Calcd. for $C_{17}H_{20}O_8S_2$: C, 49.0; H, 4.8; S, 15.4. Found: C, 49.2; H, 4.9; S, 15.0.

1,2-*O*-Isopropylidene-3-*O*-(*p*-tolylsulfonyl)-α-D-glucofuranose 5,6-Carbonate (VI). A. From V.—To a solution of 100 mg. of V in 20 ml. of acetone was added 200 mg. of silver nitrate. As in IIIA, silver sulfide formed immediately upon addition of water. The solution was treated as described in IIIa to yield 79 mg. (83%) of a crystalline product, m.p. 100–103°. Haworth and Porter[16] report m.p. 103–105° for VI.

Anal. Calcd. for $C_{17}H_{20}O_9S$: C, 51.1; H, 5.0; S, 8.0. Found: C, 50.8; H, 5.1; S, 8.3.

B. From IV.—A solution of 100 mg. of IV in 2 ml. of pyridine was treated with 0.5 g. of *p*-toluenesulfonyl chloride as described in A for V. The crystalline product had m.p. 98–101° and 98–102° in admixture with the compound prepared in A above.

Barium Hydroxide Hydrolysis of III.—To a solution of 200 mg. of III in 4 ml. of acetone was added 4 ml. of barium hydroxide solution (2%) and the mixture was heated on a steam bath for 30 min. After cooling to room temperature, carbon dioxide was passed through the suspension to remove any excess barium hydroxide. Additional acetone (20 ml.) was added and the suspension was filtered. The clear filtrate was evaporated to give 150 mg. of a colorless sirup that contained no sulfur.

Acknowledgment.—We are grateful to Mrs. Clara McGrew, Mrs. Bonita Heaton, and Mrs. Anita Dirks for microanalysis and molecular weight determinations by vapor pressure osmometry.

(16) W. N. Haworth and C. R. Porter, *J. Chem. Soc.*, 2796 (1929).

A Natural Glycoside of Medicagenic Acid. An Alfalfa Blossom Saponin[1]

R. J. MORRIS AND E. W. HUSSEY

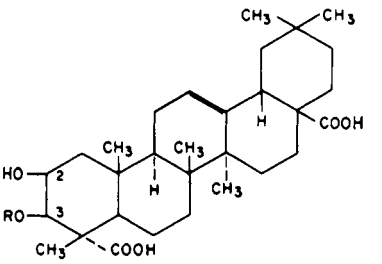
Department of Chemistry, University of Nevada, Reno, Nevada

Received March 23, 1964

The structural identification of a pure homogeneous saponin obtained from an alfalfa blossom concentrate has been completed. The crystalline triterpenoid aglycone was identified as medicagenic acid. Experimental evidence showed the probable structure of the saponin to be 2β-hydroxy-3β-(β-rhamnopyranosyl-β-glucopyranuronosyl-β-glucopyranosyloxy)-Δ^{12}-oleanene-23,28-dioic acid.

During recent years there has been interest in determining the relationship of saponin content in alfalfa and clover to a number of physiological and biological effects. Growth depressing effects,[2] ruminant bloat,[3,4] respiratory inhibition,[5,6] and the retarding of seed germination[7] are among the phenonena studied which may involve saponins.

Along with these pharmacological and biophysical studies, isolation and structural identity of the saponins and sapogenins found in alfalfa have been reported. From initial studies of some of these compounds,[8,9] the exact structure of medicagenic acid was deduced,[10] and some valuable information about total saponin content[11] accompanied by a characterization of individual sugars was obtained. Other investigators have recently reported chromatographic separations of these compounds.[12–15] These studies, although they have presented considerable detailed information about the individual sugars and aglycones found in saponins, have not been specifically concerned with purification and structural determination procedures applicable to individual saponins.



I, medicagenic acid, R = H
II, alfalfa root saponin, R = β-glucopyranosyloxy-
III, alfalfa blossom saponin, R = β-rhamnopyranosyl-β-glucopyranuronosyl-β-glucopyranosyloxy-

In a former communication, the isolation, purification, and structural identity of an alfalfa root saponin was reported.[16] The present work is an extension of alfalfa saponin studies and presents the isolation and structural identification of a more complex saponin. The sequence and individual identity of the hexoses in the structure were primarily determined by the use of controlled acid and enzymatic hydrolysis in conjunction with descending paper chromatography. Individual linkages to the aglycone as well as those between the hexoses were established by specific enzymatic hydrolysis. Evidence for the homogeneity of the sapogenin and saponin structures was provided by column separation followed by thin layer chromatography, while structural proof was obtained by the use of physical constants, elemental analysis, neutralization equivalent, and hydroxyl determinations, derivative studies, and infrared interpretations. Structural studies on this saponin showed glucose forming the primary attachment to medicagenic acid as observed earlier for an alfalfa root saponin.[16] It is postulated that this attachment is completed through the equatorial 3-hydroxyl

(1) Partial support for the research provided by a research grant (GM-06847-03) from the National Institutes of Health is gratefully acknowledged.

(2) G. C. Potter and F. A. Kummerov, *Science*, **120**, 224 (1954).

(3) I. L. Lindahl, *et al.*, "Alfalfa Saponins," U. S. Department of Agriculture, Technical Bulletin No. 1161, Agriculture Research Service, Washington, D. C., 1957.

(4) C. B. Coulson, *Nature*, **188**, 947 (1960).

(5) R. A. Shaw and H. D. Jackson, *Arch. Biochem. Biophys.*, **84**, 405 (1959).

(6) H. D. Jackson and R. A. Shaw, *ibid.*, **84**, 411 (1959).

(7) B. M. Michustin and A. N. Naumova, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 3 (1955).

(8) E. D. Walter, G. R. Van Atta, C. R. Thompson, and W. D. Maclay, *J. Am. Chem. Soc.*, **76**, 2271 (1954).

(9) A. L. Livingston, *J. Org. Chem.*, **24**, 1567 (1959).

(10) C. Djerassi, O. B. Thomas, A. L. Livingston, and C. R. Thompson, *J. Am. Chem. Soc.*, **79**, 5292 (1957).

(11) G. R. Van Atta, J. Guggolz, and C. R. Thompson, *J. Agr. Food Chem.*, **9**, No. 1, 77 (1961).

(12) C. B. Coulson, *J. Sci. Food Agr.*, **9**, 281 (1958).

(13) C. B. Coulson and T. Davies, *ibid.*, **13**, 53 (1962).

(14) W. A. Lourens and M. B. O'Donovan, *S. African J. Agr. Sci.*, **4**, 151 (1961).

(15) W. A. Lourens and M. B. O'Donovan, *ibid.*, **4**, 293 (1961).

(16) R. J. Morris, W. B. Dye, and P. S. Gisler, *J. Org. Chem.*, **26**, 1241 (1961).