[CONTRIBUTION FROM THE DIVISION OF ORGANIC CHEMISTRY, THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Ethyl Thioglycosides of D-Mannose and D-Galactose and a New Synthesis of Styracitol

BY JOSEF FRIED AND DORIS E. WALZ

In the course of recent work on the structure of mannosidostreptomycin[1,2] two isomeric ethyl thiohexosides were obtained which were not identical with any of those described in the literature. The present work, originally undertaken for the purpose of identifying these two substances, has afforded a method for the preparation of ethyl thio-D-mannopyranosides and D-galactosides, as well as a new synthesis for styracitol (1,5-anhydromannitol).

The alkyl thioglycosides, in contrast to the well-explored sugar mercaptals, have met with but occasional interest and their study has been confined to derivatives of glucose and fructose. Schneider and Sepp[3] were the first to synthesize an ethyl thioglucoside, later shown to be the α-furanoside,[4,5] by partial cleavage of glucose diethyl mercaptal with mercuric chloride. This substance has been found[6] to undergo an unusual sequence of reactions in the presence of 0.01 *N* hydrochloric acid. Under these conditions approximately one half of the material was converted into the β-furanoside and eventually into glucose and ethyl mercaptan, while the other half escaped the hydrolyzing effect of the acid by rearranging into the acid resistant pyranoid form. The ring shift resulted in the formation of ethyl α-thioglucopyranoside which was isolated and characterized as the tetraacetate. Ethyl α-thioglucopyranoside has also been prepared by the prolonged action of 22% hydrochloric acid on both glucose diethyl mercaptal and on an equimolecular mixture of glucose and ethyl mercaptan.[7,8] The fourth isomer, ethyl β-thioglucopyranoside, was prepared by Schneider and co-workers[9] according to the original Fischer method,[10] reaction of the potassium mercaptide with acetobromoglucose, and by the action of ethyl iodide on thioglucose.[11]

The formation of the two ethyl thiomannosides by mercaptolysis of mannosidostreptomycin[1,2] in the presence of concentrated hydrochloric acid suggested that simple glycosides might be mercaptolyzed in an analogous fashion. This was indeed found to be the case. Both α- and β-methyl D-mannopyranoside on treatment with ethyl mercaptan and concentrated hydrochloric acid for eighteen hours at room temperature yielded after acetylation ethyl tetraacetyl-1-thio-β-D-mannopyranoside, $[\alpha]^{25}D$ $-67°$ and m. p. 161–162°. Methyl β-mannopyranoside yielded in addition a small amount of the α-anomer ($[\alpha]^{25}D$ $+104°$; m. p. 107–108°). Better results from a preparative point of view were obtained when the above conditions were applied to the free sugar. Thus, when D-mannose was shaken with ethyl mercaptan and concentrated hydrochloric acid for eighteen hours at room temperature, and the resulting mixture after neutralization with sodium carbonate was evaporated to dryness *in vacuo*, the residue upon acetylation afforded a 32% yield of ethyl tetraacetyl-1-thio-β-D-mannopyranoside. Chromatography of the mother liquor yielded in addition a small amount of the α-anomer. Catalytic deacetylation with barium methylate afforded the free α- and β-ethyl thio-D-mannosides of which only the latter was obtained in crystalline form. Its properties are listed in Table I.

It is well known that the action of ethyl mercaptan and hydrochloric acid on D-mannose for a period of five minutes produces the diethyl mercaptal in 63% yield.[12] The absence of this substance from the products of the eighteen-hour reaction, as shown by careful chromatography of the mother liquor, after removal of the crystalline ethyl tetraacetyl-1-thio-β-D-mannopyranoside, indicated that the mercaptal is likely to be an intermediate in the formation of the thioglycosides.

The stability of the two ethyl thiomannosides toward strong acid suggested that they possess pyranoid ring structures. More definite proof, however, was sought by utilizing the demercaptalation reaction of Wolfrom and his collaborators,[13] using mercuric chloride and cadmium carbonate in methanol, in the hope of replacing the mercapto group of the more abundantly available β-anomer by methoxyl. In spite of the use of a large excess of mercuric chloride and a reaction time of twenty-four hours, the starting material was recovered unchanged. Conclusive evidence for the pyranose structure of ethyl 1-thio-β-D-mannoside was adduced by reductive desulfurization of the latter with Raney nickel, which afforded the 1,5-anhydrohexitol styracitol[14] in good yield. The 1,5-oxide structure of styracitol has been securely established by Zervas,[15] who succeeded in synthesizing this substance by catalytic reduction of tetra-

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(12) P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **74**, 695 (1927).
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(15) L. Zervas, *Ber.*, **63**, 1689 (1930).

TABLE I

Substance	M. p., °C.	$[\alpha]_D$ in H_2O[d]	$[M]_D$	2A	2B
Ethyl thio-α-D-mannopyranoside	Amorphous				
Ethyl thio-β-D-mannopyranoside	118–119	− 83°	−18600		
Ethyl thio-α-D-galactopyranoside	153.5–154	+320°	71500		
Ethyl thio-β-D-galactopyranoside	121.5–122.5	− 23.5°	− 5300	76800	66200
Ethyl thio-α-D-glucopyranoside	117[a]	+269°[a]	60200		
Ethyl thio-β-D-glucopyranoside	99–100[b]	− 55.1°[b]	−12300	72500	47900
Tetraacetates of		$[\alpha]_D$ in $CHCl_3$[d]			
Ethyl thio-α-D-mannopyranoside	107–108	+104°	40800		
Ethyl thio-β-D-mannopyranoside	161–162	− 67°	−26300	67000	14500
Ethyl thio-α-D-galactopyranoside	108–109	+218°	85400		
Ethyl thio-β-D-galactopyranoside	74–75	− 8.0°	− 3100	88500	82300
Ethyl thio-α-D-glucopyranoside	96–97.5[a]	+194°[c]	76000		
Ethyl thio-β-D-glucopyranoside	78–79[b]	− 25.6°[c]	−10030	86030	65970

[a] P. Brigl, K. Gronemeier and A. Schulz, *Ber.*, **72**, 1052 (1939). [b] W. Schneider, J. Sepp and O. Stiehler, *ibid.*, 51, 220 (1918). [c] E. Pacsu and E. J. Wilson, Jr., THIS JOURNAL, **61**, 1450 (1939). [d] The specific rotations were determined at temperatures ranging from 24 to 27°.

acetyl-2-hydroxyglucal, followed by deacetylation.

The structure of styracitol has been the subject of considerable controversy. Evidence has been presented by several groups of workers supporting either the 1,5-anhydromannitol[16,17] or the 1,5-anhydrosorbitol[18,19] structure. The present synthesis leaves little doubt that the mannitol structure, which is overwhelmingly favored by previous evidence, is the correct one.

In applying the conditions for the preparation of the ethyl thio-D-mannosides to D-galactose we obtained, after acetylation and chromatographic purification, two crystalline ethyl tetraacetyl-1-thio-D-galactosides. Their properties as well as those of the crystalline ethyl thiogalactosides obtained after deacetylation are listed in Table I. In the last two columns of this table are also given the 2A and 2B values calculated from the molecular rotations on the basis of Hudson's rules of isorotation.[20] For the sake of comparison, the values for the two anomeric ethyl thio-D-glucopyranosides and their tetraacetates have also been included. The agreement between the 2A values calculated from the molecular rotations of the two thiogalactosides (76800) and their tetraacetates (88500) and those for the thioglucopyranosides (72500) and their tetraacetates (86030) appears to be close enough to warrant the conclusion that the two ethyl thiogalactosides are anomeric and possess pyranoid structures. The 2A value for the tetraacetyl-thio-D-mannosides (67000) shows the deviation characteristic of all mannose derivatives.[21]

An attempt to prepare the ethyl thioglycosides of L-arabinose in an analogous manner yielded only arabinose diethyl mercaptal. This result demonstrates the influence of stereo configuration on the reactivity of the sugar mercaptals rendering difficult any prediction as to the general applicability of the above procedure.

(16) L. Zervas and I. Papadimitriou, *Ber.*, **73**, 174 (1940).

(17) R. C. Hockett and M. Conley, THIS JOURNAL, **66**, 464 (1944).

(18) Y. Asahina and H. Takimoto, *Ber.*, **64**, 1803 (1931).

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(20) C. S. Hudson, *ibid.*, **31**, 66 (1909).

(21) E. Pacsu, *ibid.*, **61**, 2669 (1939).

Experimental[22]

α- and β-Ethyl Tetraacetyl-1-thio-D-mannopyranosides.—D-Mannose (5.0 g.) was added to ice-cold concentrated hydrochloric acid (20 ml.) and ethyl mercaptan (20 ml.), and the mixture was shaken for sixteen hours at room temperature. At the end of this period the excess ethyl mercaptan and part of the hydrochloric acid were removed *in vacuo* at a bath temperature not exceeding 30°. The residual acid solution (10 ml.) was diluted with water (10 ml.) and alcohol (10 ml.) and alkalinized by the addition of solid sodium carbonate to a *p*H of 8. During the addition of the sodium carbonate it was necessary to add water and alcohol in order to prevent the mixture from solidifying. The solvents were then removed *in vacuo* and the resulting residue dried by distilling absolute alcohol (60 ml.) from it. The dried residue was then acetylated by shaking overnight with pyridine (40 ml.)–acetic anhydride (40 ml.), and the acetylation mixture was concentrated *in vacuo* to a sirup. The latter was partitioned between ice-water and chloroform and the chloroform layer was washed with dilute acid, sodium bicarbonate and water. The dried chloroform solution upon removal of the solvent *in vacuo* yielded a brown sirup (10.7 g.) which crystallized on standing at room temperature. Crystallization was complete after two days at 4° after which time the crystalline mass was digested with a small amount of cold absolute alcohol and filtered by suction. The crystals, after washing with cold alcohol (3.24 g.), melted at 157–158°. Recrystallization from absolute alcohol raised the melting point to 161–162°; $[\alpha]^{24}D$ −67° (*c*, 0.67 in chloroform).

Anal. Calcd. for $C_{18}H_{34}O_9S$: C, 48.98; H, 6.17; S, 8.16; O-acetyl, 43.84. Found: C, 49.19; H, 5.98; S, 8.41; O-acetyl, 44.7.

The sirupy mother liquor from which the bulk of the ethyl tetraacetyl-1-thio-β-D-mannopyranoside had been removed by filtration was concentrated *in vacuo* and the alcohol-free oily residue (7.44 g.) was dissolved in a mixture of benzene (75 ml.) and hexane (185 ml.) for chromatography on alumina (100 g.). A mixture of 2 parts of benzene and 5 parts of hexane eluted at first (720 ml.) oily material, which was followed by a series of crystalline fractions. A total of 1270 ml. of the above solvent mixture eluted approximately 150 mg. of crystals which after several recrystallizations from ether–hexane melted at 107–108°; $[\alpha]^{25}D$ +104° (*c*, 0.88 in chloroform).

(22) The melting points reported in this paper were taken in capillary tubes and have been corrected for stem exposure.

Anal. Calcd. for $C_{16}H_{24}O_9S$: C, 48.98; H, 6.17; S, 8.16. Found: C, 49.07; H, 5.98; S, 8.43.

Mercaptolysis of Methyl α-D-Mannopyranoside.—Methyl α-D-mannopyranoside (400 mg.) was shaken with concentrated hydrochloric acid (2 ml.) and ethyl mercaptan (5 ml.) for eighteen hours at room temperature. The mixture was cooled to 0°, neutralized with concentrated ammonia and evaporated to dryness *in vacuo*. The thoroughly dried residue was acetylated with pyridine (10 ml.)–acetic anhydride (10 ml.), and the acetylation mixture was worked up as in the preceding experiment. Chromatography on alumina (25 g.) from benzene (10 ml.)–hexane (15 ml.), yielded crystals (40 mg.), eluted by equal volumes of benzene and ether, which melted at 159.5–161° and were identified as ethyl tetraacetyl 1-thio-β-D-mannoside.

Mercaptolysis of Methyl β-D-Mannopyranoside.—Methyl β-D-mannopyranoside (190 mg.) was mercaptolyzed as described for the α-anomer. Acetylation of the crude product yielded a semicrystalline residue which when taken up in cold absolute alcohol (1.5 ml.) yielded crystalline ethyl tetraacetyl-1-thio-β-D-mannopyranoside. The alcoholic mother liquor was freed from solvent *in vacuo*, dissolved in benzene (4 ml.) and hexane (4 ml.) and chromatographed on acetic acid washed alumina (7 g.). A mixture of benzene (140 ml.) and hexane (140 ml.) eluted material which crystallized readily on seeding with ethyl tetraacetyl-1-thio-α-D-mannoside. After two recrystallizations the melting point remained constant at 107–108°, and showed no depression on admixture of ethyl tetraacetyl-1-thio-α-D-mannoside

Ethyl 1-Thio-β-D-mannopyranoside.—Ethyl tetraacetyl-1-thio-β-D-mannopyranoside (500 mg.) was suspended in dry methanol (75 ml.) and a 0.9 *N* solution of barium methylate (1 ml.) was added. The mixture was allowed to stand at room temperature for sixteen hours with occasional shaking during the first hour. Carbon dioxide was then passed through the solution for ten minutes and the methanol was removed *in vacuo*. The residue was taken up in water, the precipitate of barium carbonate centrifuged off and the solution evaporated to dryness *in vacuo*. Upon addition of a few drops of absolute alcohol the residue crystallized rapidly. Recrystallization from absolute alcohol yielded clusters of prismatic needles (145 mg.), which melted at 118–119°; $[\alpha]^{25}D$ −83° (*c*, 0.6 in water).

Anal. Calcd. for $C_8H_{16}O_5S$: C, 42.85; H, 7.19; S, 14.27. Found: C, 42.71; H, 6.98; S, 14.37.

Reductive Desulfurization of Ethyl Tetraacetyl-1-thio-β-D-mannopyranoside to Styracitol.—A solution of ethyl tetraacetyl-1-thio-β-D-mannopyranoside (300 mg.) in absolute alcohol (20 ml.) was refluxed with Raney nickel[23] (4 ml.) for four hours. The mixture was diluted with alcohol (20 ml.) and the Raney nickel removed by centrifugation and subsequent gravity filtration of the turbid supernate. The nickel was washed with fresh portions of hot alcohol and the combined filtrates were evaporated to dryness *in vacuo*. The sirupy residue was deacetylated in methanol (45 ml.) with 0.9 *N* barium methylate (0.6 ml.) at 4° for sixteen hours. After removal of the barium as described in the preceding experiment, the residue was dissolved in a small amount of absolute alcohol and was seeded with authentic styracitol.[24] On cooling, crystals (70 mg.) were obtained, which after two recrystallizations from methanol melted at 155–156° and had $[\alpha]^{27}D$ −48° (*c*, 1.1 in water). There was no depression in melting point on admixture of the above authentic sample of styracitol of m. p. 155–156° and $[\alpha]^{26}D$ −50° (*c*, 1.0 in water).

Anal. Calcd. for $C_6H_{10}O_5$: C, 43.94; H, 7.37. Found: C, 44.01; H, 7.44.

An additional amount of crystals (30 mg.) was obtained on concentration of the ethanolic mother liquor. The total yield of crude crystalline product was 100 mg. or 80%.

Attempted Replacement of the Ethylmercapto Group in Ethyl Tetraacetyl-1-thio-β-D-mannopyranoside by Methoxyl.—To a solution of ethyl tetraacetyl-1-thio-β-D-mannopyranoside (200 mg., 0.51 millimole) in absolute methanol (4 ml.) was added mercuric chloride (775 mg., 2.85 millimoles) and cadmium carbonate (575 mg.). The mixture was refluxed for eighteen hours, taken up in chloroform (20 ml.), filtered, and extracted with water to remove the mercuric chloride. The chloroform solution was dried over sodium sulfate and evaporated to dryness *in vacuo*. The crystalline residue proved to be unchanged starting material.

α- and β-Ethyl Tetraacetyl-1-thio-D-galactopyranosides.—D-Galactose (5.0 g.) was treated with concentrated hydrochloric acid (20 ml.) and ethyl mercaptan (20 ml.) exactly as described above for D-mannose. Upon acetylation with pyridine (40 ml.)–acetic anhydride (40 ml.) a brown sirup (7.7 g.) was obtained, which crystallized only to a small extent on long standing. A portion of the sirup (6.67 g.) was therefore chromatographed on alumina (100 g.) from a benzene (67 ml.)–hexane (167 ml.) solution. Elution of the column with benzene–hexane (2:5) yielded at first a considerable amount of oily material. After 600 ml. of the eluant had passed through the column the succeeding 900 ml. of effluent, collected in 13 fractions of 70 ml., yielded on evaporation crystals melting at 107–108°. Since no depression in melting point was observed when the first and the thirteenth fraction were mixed, all the fractions were combined and recrystallized from ether–hexane. The resulting material (287 mg.) melted at 108–109° and had $[\alpha]^{26}D$ +218° (*c*, 1.18 in chloroform).

Anal. Calcd. for $C_{16}H_{24}O_9S$: C, 48.98; H, 6.17; S, 8.16; acetyl, 43.84. Found: C, 49.02; H, 6.10; S, 8.40; acetyl, 43.0.

Subsequent elution of the column with the same solvent mixture gave two fractions (240 ml. each) from which a small amount of the above crystalline material (m. p. 103–104°) was obtained on seeding. Continued elution with 1900 ml. of benzene–hexane yielded upon evaporation of the solvents a second crystalline substance, which after several recrystallizations from ether–hexane melted at 74–75° (114 mg.); $[\alpha]^{26}D$ −8.0 (*c*, 2.1 in chloroform).

Anal. Calcd. for $C_{16}H_{24}O_9S$: C, 48.98; H, 6.17; S, 8.16; acetyl, 43.84. Found: C, 48.91; H, 6.23; S, 8.53; acetyl, 44.1.

Elution with benzene (320 ml.) afforded a small amount of crystals (5.2 mg.), which melted at 141.5–142°. The substance contained no sulfur and was probably penta-acetyl-β-D-galactopyranose, which has been reported[25] to melt at 142°.

Ethyl 1-Thio-α-D-galactopyranoside.—Ethyl tetraacetyl-1-thio-α-D-galactopyranoside (130 mg.) was deacetylated in dry methanol solution (21 ml.) with 0.9 *N* barium methylate (0.28 ml.) as described above for ethyl thio-β-D-mannopyranoside. The residue, from which the barium had been removed, crystallized upon addition of absolute alcohol. On recrystallization from this solvent fine needles (49 mg.) were obtained which melted at 153.5–154° and had $[\alpha]^{27}D$ +320° (*c*, 0.55 in water).

Anal. Calcd. for $C_8H_{16}O_5S$: C, 42.85; H, 7.19; S, 14.27. Found: C, 42.81; H, 6.94; S, 14.55.

Ethyl 1-Thio-β-D-galactopyranoside.—Ethyl tetraacetyl-1-thio-β-D-galactopyranoside (60 mg.) was deacetylated as described in the preceding experiment. Since the barium-free residue could not be induced to crystallize it was dissolved in ethanol (0.5 ml.)–benzene (1.5 ml.) and chromatographed on alumina (1 g.). A mixture of alcohol (1 part) and benzene (3 parts) eluted at first (26 ml.) amorphous material, which was discarded. All subsequent fractions obtained with this solvent mixture (165 ml.) and with equal volumes of benzene and alcohol (300 ml.) crystallized on rubbing with ethyl acetate. The

(23) Similar yields were obtained with both freshly prepared and six-months-old Raney nickel.

(24) The authors wish to thank Dr. John T. Sheehan for kindly supplying them with a sample of pure styracitol isolated from *Styrax obassia*.

(25) E. Erwig and W. Koenig, *Ber.*, **22**, 2207 (1889).

combined fractions were recrystallized from a minimum of alcohol and ethyl acetate and yielded 13 mg. of long thin needles which melted at 121.5–122.5° and had $[\alpha]^{26}D$ −23.5° (*c*, 0.89 in water).

Anal. Calcd. for $C_8H_{16}O_5S$: C, 42.85; H, 7.19; S, 14.27. Found: C, 42.58; H, 6.77; S, 13.50.

Reaction of L-Arabinose with Ethyl Mercaptan.—L-Arabinose (2 g.) was treated with ethyl mercaptan (8 ml.) and hydrochloric acid (8 ml.) as described for methyl α-D-mannopyranoside. The dried neutralized residue was acetylated with pyridine (20 ml.)-acetic anhydride (20 ml.), the crude acetylation product dissolved in absolute alcohol (3 ml.), and the solution was allowed to stand in the refrigerator overnight. The resulting crystals (2.35 g.) were filtered and washed with cold alcohol. After two recrystallizations from 95% alcohol the crystals melted at 79–80° $[\alpha]^{27}D$ −27.0° (*c*, 1.6 in chloroform). These data are in accord with those given for tetraacetyl-L-arabinose diethyl mercaptal by Wolfrom and Newlin.[26]

Anal. Calcd. for $C_{17}H_{28}O_8S_2$: C, 48.11; H, 6.65; S, 15.08. Found: C, 48.27; H, 6.65; S, 15.27.

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(26) M. L. Wolfrom and M. R. Newlin, THIS JOURNAL, **52**, 3619 (1930).

Summary

α- and β-ethyl tetraacetyl-1-thio-D-mannopyranosides have been prepared by the prolonged action of ethyl mercaptan and hydrochloric acid on both D-mannose and methyl β-D-mannopyranoside. Methyl α-D-mannopyranoside yielded only the β-anomer. Ethyl 1-thio-β-D-mannopyranoside has been obtained in crystalline form.

Styracitol has been prepared from ethyl tetraacetyl-1-thio-β-D-mannopyranoside by desulfurization with Raney nickel.

α- and β-ethyl 1-thio-D-galactopyranosides and their tetraacetates have been prepared in crystalline form.

The action of ethyl mercaptan and hydrochloric acid on L-arabinose for eighteen hours led only to the diethyl mercaptal.

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The Reaction of Simple Antigens with Purified Antibody[1]

BY ARTHUR B. PARDEE[2] AND LINUS PAULING

Many biological reactions, including the interaction of antibodies and antigens, the effect of enzymes on their substrates, and the self-reproducing behavior of genes, are characterized by a high degree of specificity. The great problem of the nature of the forces responsible for this biological specificity is being attacked vigorously in many ways at the present time. During the past six years we have carried out a series of investigations[1] on the reactions of antisera with simple substances, extending and refining the work of Landsteiner.[3] The results obtained provide strong support for the concept that biological specificity is due to a detailed complementariness in surface configuration of the molecules involved (antigen and antibody) and that the forces which contribute to specific attraction of two molecules—van der Waals electronic forces, hydrogen bond forces, etc.—are in general short-range forces, effective over distances of a few Ångström units. The conclusion has been reached that the surface approximation of antibody and the haptenic groups of antigens is to within about 1 Å.

Much of our work has consisted of studies of the precipitation of an antiserum by a simple polyhaptenic substance. The observation that certain simple substances containing two or more haptenic groups would form precipitates with the homologous antiserum was made by Landsteiner and van der Scheer.[4] It was suggested by Landsteiner that the forces between dye molecules which favor the formation of colloidal solutions, that is, of polymerized aggregates, are responsible for the ready precipitability of these substances, many of which are dyes. We, however, have presented evidence that the presence of two or more haptenic groups in each molecule, making the formation of a framework possible, is responsible for their precipitability.

The suggestion that it is polymerization of these simple precipitating antigens that gives them their precipitating power has been revived by Boyd and Behnke,[5] who reported that they had found one of the simple antigens used by us to be highly (11-fold) polymerized in saline solution, and who stated that accordingly the results of our earlier investigations might not justify the

(1) The Serological Properties of Simple Substances. XIV. For number XIII of this series see D. Pressman, J. H. Bryden, and L. Pauling, THIS JOURNAL, **70**, 1352 (1948).

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(3) See K. Landsteiner, "The Specificity of Serological Reactions," Harvard University Press, Cambridge, Massachusetts, 1945.

(4) K. Landsteiner and J. van der Scheer, *Proc. Soc. Exptl. Biol. Med.*, **29**, 747 (1932); *J. Exptl. Med.*, **56**, 399 (1932); **57**, 633 (1933); **67**, 79 (1938).

(5) W. C. Boyd and J. Behnke, *Science*, **100**, 13 (1944). In this preliminary note about their work these authors wrote that details would be published elsewhere; their detailed paper has not yet appeared.