be readily crystallized and characterized. These were best prepared by treating a solution of the sugar derivative in glacial acetic acid with slightly more than the calculated amount of isonicotinylhydrazide dissolved in a minimum amount of water, and warming the mixture in a water bath for 1 hr. The hydrazones of N-carbobenzyloxymycosamine and N-benzoylmycosamine[4] separated on cooling and could be recrystallized from warm methanol.

N-Carbobenzyloxymycosamine isonicotinylhydrazone, m.p. 199–203° dec.

Anal. Calcd. for $C_{20}H_{24}N_4O_6$ (416.42): C, 57.68; H, 5.81; N, 13.45. Found: C, 57.59; H, 5.79; N, 13.28.

N-Benzoylmycosamine isonicotinylhydrazone, m.p. 212–213° dec.

Anal. Calcd. for $C_{19}H_{22}N_4O_5$ (386.40): C, 59.06; H, 5.74; N, 14.50. Found: C, 59.09; H, 5.50; N, 14.45.

Nystatin. IV. The Stereochemistry of Mycosamine

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It has been shown that mycosamine, a 3-amino-3,6-dideoxy-D-aldohexose,[1] corresponds configurationally to D-mannose. Degradation of N-benzoyl-1-deoxymycosaminol (VI), obtained in three steps from mycosamine diethyl mercaptal (II), to N-benzoyl-D-allothreonine (VIIa), established the L-configuration for C-2 and C-3. The D-configuration of C-4 followed from the finding that the 1-phenylflavazole derivative of mycosamine (XIIIa) was the enantiomorph of that of L-rhamnose (XIIIb).

In preceding papers of this series[1,2] it has been shown that mycosamine, the amino sugar occurring in nystatin and several other polyenic antibiotics, is a 3-amino-3,6-dideoxy-D-aldohexose. In the present paper we give an account of the degradative reactions which proved that mycosamine corresponds configurationally to D-mannose, and thus furnished the basis for its recently reported synthesis from D-glucose.[3]

Our scheme for determining the configuration of C-2 and C-3 entailed conversion of carbon atom 1 to a methyl group, protection of the amino group by acylation followed by scission of the chain between C-4 and C-5 with periodate and oxidation of the resulting α-acylamino-β-hydroxy-*n*-butyraldehyde to the corresponding acid, which on hydrolysis was then expected to yield one of the stereoisomers of threonine or allothreonine. This sequence was originally carried out[4] with N-acetylmycosamine as the starting material, and a very small amount of crystalline amino acid, which appeared to be D-allothreonine, was obtained as the end product. In view of the minute yield, and also of the fact that intermediates were amorphous and of doubtful purity, it was decided to repeat this sequence in the N-benzoyl series. This change also promised to do away with the final hydrolysis step in the above scheme, as the optically active N-benzoylthreonines and N-benzoylallothreonines, in contrast to the N-acetyl derivatives, are crystalline, well characterized compounds.

The degradative scheme in its original form envisaged the preparation of a suitable mercaptal of N-benzoylmycosamine which was then to be desulfurized with Raney nickel to the N-benzoyl-1-deoxo compound. However, no pure mercaptals could be isolated from the mixtures resulting from the treatment of N-benzoylmycosamine with ethyl or isopropyl mercaptan, or ethylene dithiol, the reaction apparently leading preponderantly to β-thioglycosides (*cf.* Experimental). In contrast, the crystalline diethylmercaptal hydrochloride (II) of mycosamine itself could be readily obtained from the hydrochloride of the base (I) and separated from the accompanying, likewise crystalline, α- and β-thioglycosides (IIIa and b). The mercaptal was benzoylated with benzoyl chloride in pyridine and the resulting amorphous product directly subjected to Raney nickel desulfurization. There was obtained a crystalline product, m.p. 82–85°, showing approximately the composition of the desired 2,4,5,N-tetrabenzoyl-1-deoxymycosaminol (IV). However, further examination of this material showed that it was a mixture of true IV, m.p. 137–138°, and of a tribenzoate (V), m.p. 95–97°, which differed from IV, as will be shown below, by the lack of the 2-benzoyloxy group.

The tetrabenzoyl derivative IV could be O-debenzoylated with sodium methoxide in methanol to give in good yield N-benzoyl-1-deoxymycosaminol (VI), m.p. 120–121°, which consumed, as expected, one molar equivalent of periodate. In the preparative experiment the mixture from the oxidation was freed of acetaldehyde by sweeping with nitrogen, the aldehydic four-carbon fragment extracted with chloroform and the residue from the latter treated in carbonate–bicarbonate buffer with sodium hypoiodite, according to the procedure of Willstätter and Schudel,[5] until one molar equivalent of the oxidizing reagent was consumed. The crystalline acid, obtained in 45% over-all yield (from VI), was identified as N-benzoyl-D-allothreonine (VIIa) by comparison (m.p., $[\alpha]_D$ and infrared spectrum) with an authentic specimen. Carbon atoms 2 and 3 of mycosamine have therefore the L-configuration as in D-mannose and D-talose.

Unexpectedly, some nonacidic crystalline material, which was shown to be N-benzoyl-D-allothreonine methyl ester (VIIb), was also produced in the last oxidation step. The source of the O-methyl group was a small amount of methanol which had been used for transferring the amorphous aldehyde to the vessel in which the oxidation with sodium hypoiodite was carried out. Although only a few minutes elapsed before the solution was diluted with water, this must have sufficed to transform part of the aldehyde into the methyl hemiacetal which was then oxidized to the methyl ester.

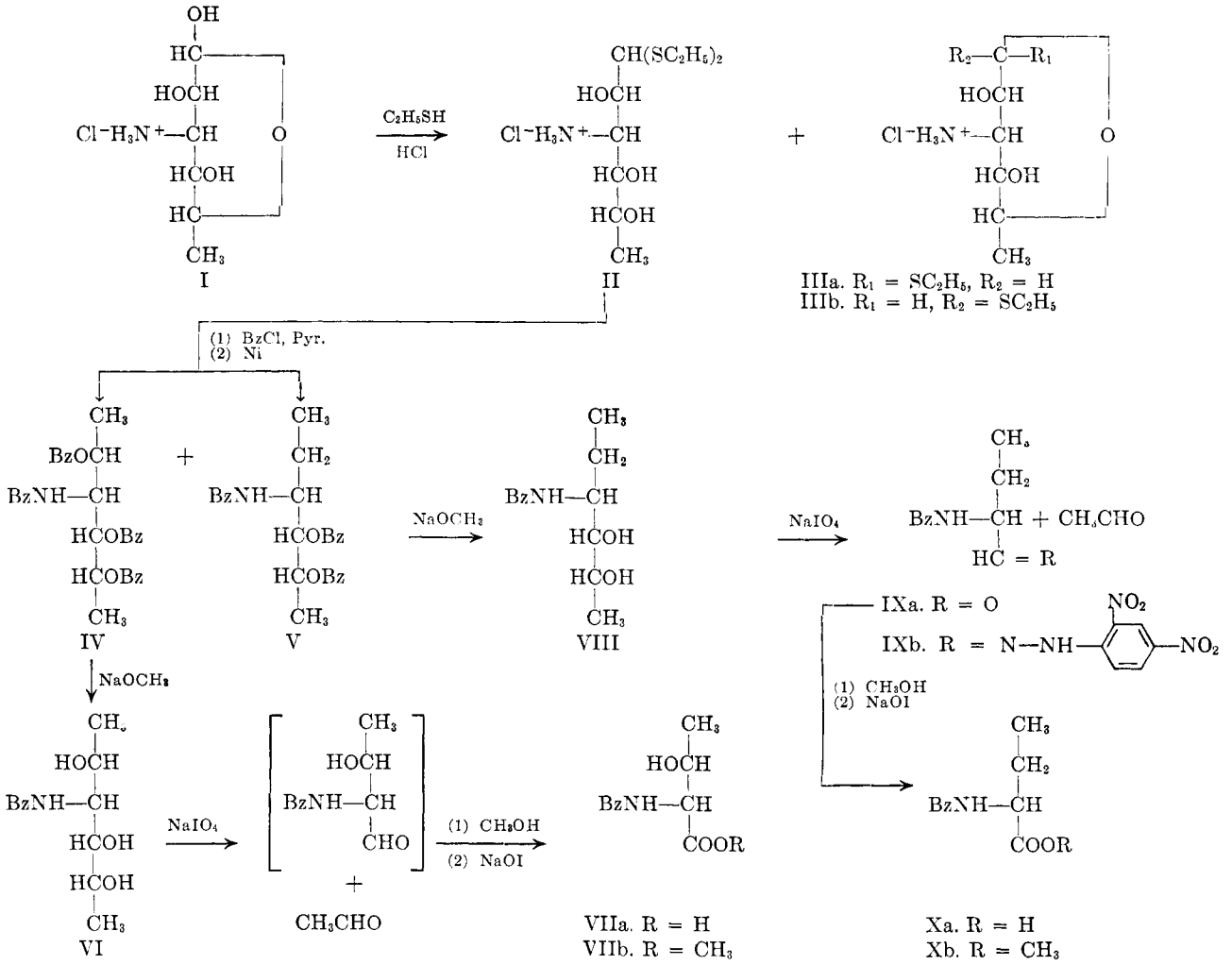
(1) D. R. Walters, J. D. Dutcher, and O. Wintersteiner, *J. Am. Chem. Soc.*, **79**, 5076 (1957).

(2) J. Dutcher, D. R. Walters, and O. Wintersteiner, *J. Org. Chem.*, **28**, 995 (1963).

(3) M. H. von Saltza, J. Reid, J. D. Dutcher, and O. Wintersteiner, *ibid.*, **83**, 2785 (1961).

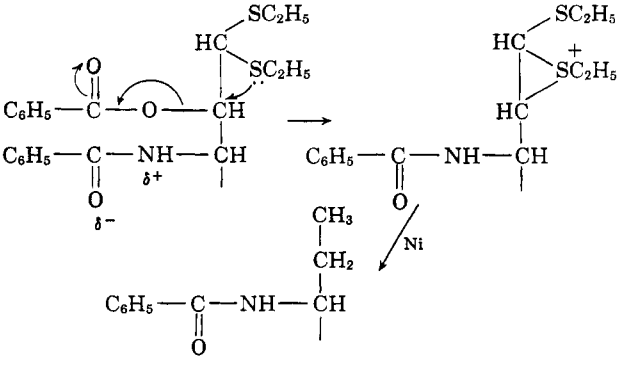
(4) In these laboratories by Dr. D. R. Walters.

(5) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918); R. W. Jeanloz and E. Forchielli, *J. Biol. Chem.*, **188**, 361 (1951).



The structure of the tribenzoyl derivative V, formed along with IV on Raney nickel reduction of the benzoylated mercaptal, was ascertained in an analogous way by subjecting its amorphous O-debenzoylation product VIII to the same two-step oxidation procedure. In this case the aldehyde (IXa) resulting from the periodate oxidation was crystalline. Oxidation of the aldehyde by the Willstätter–Schudel procedure gave D-2-benzamido-*n*-butyric acid (Xa), and, since prior to this step the aldehyde had been deliberately allowed to remain in contact with methanol for some time, also the methyl ester (Xb), which could, however, not be obtained in crystalline form. To substantiate the explanation given above for the formation of the ester VIIb, another sample of the aldehyde IXa was dissolved in pure methanol, and the extinction at 300 mμ of the solution was measured at short intervals. It was observed that the shoulder which the absorption curve shows at this wave length (aldehyde C = O superimposed on high amide band with maximum at 229 mμ) became obliterated within ten minutes.

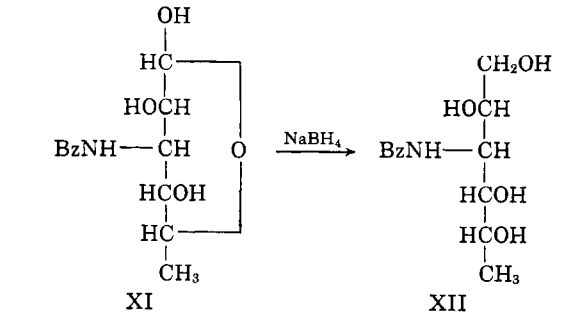
There is to our knowledge no precedent in sugar chemistry for the facile elimination of the 2-benzoyloxy group in the Raney nickel desulfurization of tetrabenzoylmycosamine diethylmercaptal. A reasonable mechanism would be the one pictured below, involving nucleophilic displacement of the 2-benzoyloxy group by an electron pair of the sulfur atom in one of the ethylmercapto groups (with the inductive effect of the neighboring benzamido group aiding in the formation of the new S—C bond), and subsequent replacement, in the resulting sulfonium ion, of all S-linkages at C-1 and C-2 by hydrogen. The nucleophilic substitution reaction postulated here is analogous to the well documented[6] neighboring group effect of the thioether group in aliphatic β-chloro sulfides and cyclic *trans*-2-chloro 1-thioethers on the hydrolytic removal of the chlorine, which likewise involves the intermediary formation of sulfonium ions.



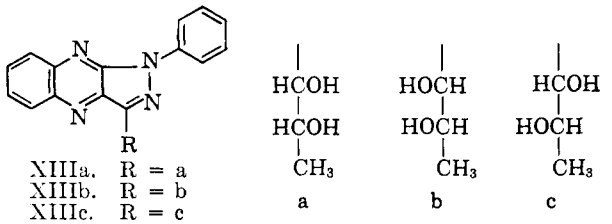
In the experimental part we also describe the sodium borohydride reduction of N-benzoylmycosamine (XI) to the N-benzoylmycosaminol (XII), which was prepared in connection with an attempt to convert it to

(6) *Cf.* H. Böhme and K. Sell, *Chem. Ber.*, **81**, 123 (1948); P. D. Bartlett and C. G. Swain, *J. Am. Chem. Soc.*, **71**, 1406 (1949); H. L. Goering and K. L. Hower, *ibid.*, **79**, 6542 (1957).

the 1-deoxo compound VI by the well known procedure involving, in principle, the formation of the 1-tosylate, replacement of the tosyloxy group by iodine, and reductive removal of the latter. All the intermediates along this route were obtained in amorphous form only, as was the final product, although it had the analytical composition and spectral properties required for VI.



XI XII



XIIIa. R = a
XIIIb. R = b
XIIIc. R = c

a b c

The configuration of carbon atom 4 in mycosamine could be deduced as being D from the rotation of the osazone hydrochloride, and was rigidly established by converting the hydrochloride (I) to the 1-phenylflavazole derivative XIIIa by the procedure of Ohle and Liebig.[7] Although the yield was only 1%, it could be shown that the recrystallized material corresponded in regard to melting point (213–215°) and paper chromatographic behavior to its enantiomorph XIIIb from L-rhamnose[7] and not to the diastereoisomer XIIIc which we prepared from L-fucose, and hence that the configuration of this carbon atom is D.

Experimental

All melting points are uncorrected and were determined using a capillary melting point apparatus. The infrared spectra were measured on a Perkin–Elmer double-beam spectrophotometer, Model 21. A few pertinent absorption bands have been reported with intensities designated (w), (m), and (s) meaning weak, medium, and strong, respectively.

A Cary recording spectrophotometer, Model 11M, was used for the ultraviolet measurements. The optical rotations reported were obtained with a Rudolph high precision polarimeter, no. 80. Woelm neutral alumina, activity grade I, was employed unless otherwise stated. Whatman no. 1 chromatographic paper was used for all paper chromatograms.

Reaction of Mycosamine Hydrochloride with Ethanethiol and Hydrochloric Acid. Mycosamine Diethylmercaptal Hydrochloride (II) and α- and β-Ethylthiomycosaminide Hydrochlorides (IIIa and IIIb).—Ethanethiol (12.5 ml.) which had been cooled to 0° was added to a solution of mycosamine hydrochloride (1.72 g.) in fuming hydrochloric acid (20 ml.)[8] at −6°. The reaction mixture was vigorously stirred at this temperature for 22 hr. While stirring and refrigeration were continued, powdered lead carbonate (*ca.* 70 g.) and cold ethanol (50 ml.) were gradually added until the mixture was neutralized. The insoluble lead salts were then filtered off and washed five times with 40-ml. portions of ethanol. The combined filtrate and washings were evaporated *in vacuo* to a thick sirup (2.18 g.).

When this product was examined on paper chromatograms developed in 1-butanol–ethanol–water (40:11:20) and sprayed with ninhydrin, three main spots were seen with R_f values of 0.44, 0.51, and 0.68. This material was dissolved with warming in absolute ethanol (10 ml.), and 781 mg. of white needle-like crystals which formed were filtered off. This product was recrystallized from absolute ethanol to give ethylthio-β-mycosaminide hydrochloride (IIIb), m.p. 275° dec.; $[\alpha]^{23}D$ −61.7° (*c*, 1.1 in water); R_f 0.44.

Anal. Calcd. for $C_8H_{18}O_3NSCl$ (243.7): C, 39.42; H, 7.44; N, 5.75; S, 13.15; Cl, 14.55. Found: C, 39.47; H, 7.47; N, 5.61; S, 13.52; Cl, 14.56.

The amorphous material (1.4 g.) recovered from the mother liquor of the β-thioglycoside was dissolved in 1-butanol–ethanol–water (40:11:20) (7 ml.). A cellulose partition column[9] (2.8 × 33 cm.) was prepared by slurrying Whatman standard-grade cellulose powder in acetone. The column was washed with 1-butanol–ethanol–water (40:11:20) (500 ml.) which solvent mixture was also used for applying the material to the column and for developing the chromatogram. The effluent was collected in 5-ml. portions, and the progress of elution of the three compounds corresponding to the above R_f values was followed by examining appropriate fractions on paper chromatograms. The eluted material was distributed over three main chromatographic bands: A (fractions 61–69, 794 mg., R_f 0.68), B (fractions 70–76, 340 mg., R_f 0.51), and C (fractions 77–81, 80 mg., R_f 0.44). Bands B and C contained the α- and β-thioglycosides, respectively. The crystalline material from band A, after recrystallization from ethyl acetate, yielded mycosamine diethylmercaptal hydrochloride (II) (676 mg.) which melted at 111.5–113°; $[\alpha]^{25}D$ +2.5° (*c*, 1.0 in water).

Anal. Calcd. for $C_{10}H_{24}O_3NS_2Cl$ (305.9): C, 39.26; H, 7.91; N, 4.58; S, 20.96; Cl, 11.60. Found: C, 39.51; H, 7.87; N, 4.72; S, 20.70; Cl, 11.70.

Crystalline ethylthio-α-mycosaminide hydrochloride (IIIa) (170 mg.) was obtained from an ethanol–ethyl acetate solution of band B and after recrystallization from the same solvent mixture melted at 247° dec.; $[\alpha]^{24}D$ +166° (*c*, 0.9 in water).

Anal. Calcd. for $C_8H_{18}O_3NSCl$ (243.7): C, 39.42; H, 7.44; N, 5.75; S, 13.15; Cl, 14.55. Found: C, 39.70; H, 7.40; N, 5.86; S, 13.37; Cl, 14.47.

From band C additional crystalline β-thioglycoside (60 mg., m.p. 275° dec.) was obtained. The mercaptal and the α- and β-thioglycosides were recovered in yields of 30%, 9% and 40%, respectively. When the β-thioglycoside was subjected to the treatment with ethyl mercaptan under the same conditions for 4 days, a similar ratio of products was obtained, indicating that this ratio represents an equilibrium.

Attempts to separate the mercaptal and the α-thioglycoside by fractional crystallization did not afford these compounds in chromatographically and analytically pure form.

Benzoylation of Mycosamine Diethylmercaptal Hydrochloride and Subsequent Raney Nickel Desulfurization of the Resultant Polybenzoate. 2,4,5,N-Tetrabenzoyl-1-deoxymycosaminol (IV), and 4,5,N-Tribenzoyl-1,2-dideoxymycosaminol (V).—Mycosamine diethylmercaptal hydrochloride (6.182 g.) was dissolved in dry pyridine (75 ml.) and the solution cooled to 0°. A solution of benzoyl chloride (17.0 g.) in dry pyridine (15 ml.) was added gradually with stirring to the cooled reaction mixture over a period of 45 min.

The reaction mixture was then allowed to stand at room temperature for 24 hr. After addition of ice water and standing, the mixture was evaporated *in vacuo* to one-half volume, and water (75 ml.) and chloroform (200 ml.) were added. The chloroform layer was washed successively with water, 2 *N* sulfuric acid, saturated sodium bicarbonate and water, dried, and the solvent removed *in vacuo*. The resulting amorphous product weighed 15 g.; Found: N, 1.90; S, 8.03; mol. wt. (Rast), 662. Calcd. for $C_{39}H_{39}O_7NS_2$ (668.8): N, 2.04; S, 9.34. $\lambda_{max}^{CHCl_3}$ 2.85 (w), 5.80 (s), 6.00 (s), 6.63 (s), and 9.01 (s) μ.

A solution of the benzoylated diethylmercaptal of mycosamine (13.0 g.) in absolute ethanol (250 ml.), to which approximately 130 g. of Raney nickel (Davison Chemical Co., Cincinnati, Ohio), as measured by wet volume, had been added, was boiled

(7) H. Ohle and R. Liebig, *Ber.*, **75**, 1536 (1942).

(8) The fuming hydrochloric acid was prepared by bubbling hydrogen chloride gas through concentrated hydrochloric acid, cooled to −3°, for 1 hr. Fuming hydrochloric acid has been reported to increase the rate of formation of glucosamine diethylmercaptal from glucosamine. M. W. Whitehouse, R. W. Kent, and C. A. Pasternak, *J. Chem. Soc.*, 2315 (1954).

(9) L. Hough and M. I. Taha, *ibid.*, 2042 (1956).

with stirring for 4.5 hr. The catalyst was filtered off and washed with hot ethanol (2 l.), and the colorless combined washings and filtrate were freed from solvent *in vacuo*. The sirupy residue (9.0 g.) was dissolved in ether (30 ml.), and hexane (60 ml.) was added. The resulting crystalline material (prisms, 7.0 g., m.p. 75–80°) was collected, and the mother liquor was taken to dryness. The residue was dissolved in a few milliliters of ether, from which on standing, rosettes of needles (90 mg., m.p. 134–135°) were deposited. Recrystallization from an ethyl acetate–ether–hexane mixture (4 ml.) yielded 44 mg. of 2,4,5,N-tetrabenzoyl-1-deoxymycosaminol (IV), m.p. 137–138.5°; $[\alpha]^{25}D$ +59° (*c*, 1.0 in ethanol); $\lambda_{max}^{methanol}$ 229 mμ (48,300), 268 mμ (sh.) (3,100), 273 mμ (3,190), and 280 mμ (sh.) (2,370); λ_{max}^{Nujol} 3.00 (m), 5.81 (s), 5.89 (s), 6.02 (s), 6.58 (s), 7.88 (s), 9.00 (s) μ.

Anal. Calcd. for $C_{34}H_{31}O_7N$ (565.6): C, 72.20; H, 5.52; N, 2.48. Found: C, 72.64; H, 5.37; N, 2.73; mol. wt. (Rast), 571.

The first crop of crystals was recrystallized from ether and yielded material (5.0 g., m.p. 82–85°) which could not be further purified by fractional crystallization, although it was subsequently shown to be a mixture. A 3.0-g. portion of this material dissolved in benzene–petroleum ether 1:1 (45 ml.) was adsorbed on a column (5.0 × 35 cm.) of Merck acid-washed alumina. The column was washed successively with the following solvent mixtures: benzene–petroleum ether 1:1 (600 ml.); benzene (600 ml.); 2% chloroform in benzene (500 ml.); 5% chloroform in benzene (5000 ml.); 15% chloroform in benzene (1000 ml.); and 50% chloroform in benzene (7000 ml.).

The chloroform–benzene (1:1) eluted a single band containing the bulk (*ca.* 3 g.) of the material on the column. The first four fractions (70 ml. each) of this band were combined (253 mg., dry weight) and yielded from ether a crystalline product which after recrystallization from the same solvent and drying at 64°/1 mm. melted at 95–97° (140 mg.) and was identified as 4,5,N-tribenzoyl-1,2-dideoxymycosaminol (V) by analysis and the degradation sequence described further below, $[\alpha]^{23}D$ +68.7 (*c*, 1.0 in ethanol); $\lambda_{max}^{methanol}$ 228 mμ (33,900), 267 mμ (sh.) (2,220), 274 mμ (2,260), 280 mμ (sh.) (1,600); λ_{max}^{Nujol} 3.09 (m), 5.84 (s), 6.08 (s), 6.44 (s), 7.80 (s), 8.97 (s) μ.

Anal. Calcd. for $C_{27}H_{27}O_5N$ (445.5): C, 72.79; H, 6.11; N, 3.14. Found: C, 72.84; H, 5.89; N, 3.39.

Additional small amounts of the tetrabenzoate IV were recovered from the last few benzene–chloroform 1:1 eluates. Attempts to devise a more efficient method for separating IV and V were abandoned when it was found that the N-benzoyl derivatives lent themselves more readily to chromatographic separation (see below). From the amounts of the O-debenzoylated products recovered, it is estimated that IV and V were formed in the reductive desulfurization reaction in the molar ratio 3:7.

O-Debenzoylation of Mixture of IV and V. N-Benzoyl-1-deoxymycosaminol (VI) and N-Benzoyl-1,2-dideoxymycosaminol (VIII).—To a solution of the crystalline mixture of IV and V (m.p. 82–85°, 3.47 g.) in anhydrous methanol (250 ml.), sodium methoxide in methanol (4.4 ml., 1.009 *N*) was added with stirring. The solution was refluxed for 4 hr., while protected from atmospheric moisture, and then freed from most of the solvent *in vacuo*. After the addition of water (50 ml.) the mixture was extracted with ether–petroleum ether (1:3) to remove methyl benzoate. The pH was adjusted to 6.0 by adding Dowex 50 (H^+) resin in portions with stirring. The resin was filtered off, and the aqueous solution was extracted twice with chloroform. The chloroform extract was taken to dryness, leaving a sirupy residue weighing 1.1 g. The aqueous phase was then extracted six times with 50-ml. portions of ethyl acetate. Evaporation *in vacuo* of the combined ethyl acetate phases left 690 mg. of a partially crystalline residue. Recrystallization from the same solvent yielded N-benzoyl-1-deoxymycosaminol (VI), 275 mg., m.p. 120–121°. However, the analyses of repeatedly recrystallized specimens from this and other runs were consistently 0.5% too high for both carbon and hydrogen. It proved necessary to resort to chromatographic purification with neutral alumina, activity V,[10] to secure a product which gave a correct analysis. This preparation, eluted from the column with methanol–ethyl acetate (1:9), melted at 120–121° and had $[\alpha]^{23}D$ +38° (*c*, 0.5 in ethanol); λ_{max}^{alc} 226 mμ (11,000); λ_{max}^{chlf} 6.09 (s), 5.69 (s) μ.

The analytical sample was dried at 100°/1 mm.

Anal. Calcd. for $C_{13}H_{19}O_4N$ (253.3): C, 61.64; H, 7.56; N, 5.53. Found: C, 61.46; H, 7.60; N, 5.51.

The compound consumed 1.03 and 1.06 molar equivalents of sodium metaperiodate in 40 min. and 3 hr., respectively.

(10) H. Brockmann and H. Schodder, *Ber.*, **74A**, 73 (1941).

When pure tetrabenzoyl-1-deoxymycosaminol (IV) (m.p. 137–138.5°) was O-debenzoylated in the same manner, VI (m.p. 120–121°) was obtained in 70% yield. On benzoylation, VI reverted to IV (m.p. 137.5–139°, yield 78%). The residue from the chloroform extract of the methanolysis products was combined with the mother liquor material from crude VI (together 1.5 g.) and the resulting material, dissolved in ether–benzene (1:9), was absorbed on an alumina column (activity IV, 3.0 × 35 cm.). The solvent series, benzene, ether, ethyl acetate, and methanol with increasing concentrations of each in the succeeding solvent starting with ether–benzene (1:9), was used for elution. The eluates were collected in 150-ml. portions. Ether alone eluted band A (fractions 11–13, 760 mg.), methanol–ethyl acetate (1:100) eluted band B (fractions 30–35, 385 mg.) and methanol–ethyl acetate (1:3) eluted band C (fractions 43–48, 220 mg.). Band C yielded crystalline N-benzoyl-1-deoxymycosaminol. Band A was an amorphous product, shown by subsequent oxidative degradation to be N-benzoyl-1,2-dideoxymycosaminol. Band B, apparently a chromatographic artifact, had an infrared spectrum identical with that of material from band A; λ_{max}^{chlf} 2.92 (m), 6.11 (s), 6.61 (s), 9.28 (m), 9.64 (m) μ. Rebenzoylation of bands A and B gave in each case tribenzoyl-1,2-dideoxymycosaminol (m.p. 95–97°, yield 30–35%) identical in infrared spectra and melting point undepressed in mixture with V.

Oxidative Degradation of N-Benzoyl-1-deoxymycosaminol (VI) to N-Benzoyl-D-allothreonine (VIIa).—A solution of N-benzoyl-1-deoxymycosaminol (76.3 mg., 0.301 mmole) in 0.08 *M* sodium acetate buffer (3.68 ml.), pH 4.8, to which a solution of sodium metaperiodate (88.2 mg., 0.412 mmole) in 1.6 ml. of water had been added, was allowed to stand at 0° in the dark for 1 hr. Titration of an aliquot (0.2 ml.) showed that 0.97 molar equivalents of periodate had been consumed. Ethylene glycol (0.1 mmole) was added, and after an interval, a stream of nitrogen gas was passed through the solution at 0° for 1 hr. The pH was adjusted to 6.7, whereupon the solution was taken *in vacuo* to near dryness. The residue was extracted five times with 10-ml. portions of chloroform. The combined chloroform phase was dried over anhydrous sodium sulfate and freed from the solvent *in vacuo*. The colorless amorphous residue (61 mg.) gave positive Schiff's and 2,4-dinitrophenylhydrazine tests. It was transferred with methanol (1 ml.) to a cooled mixture (0°) of 0.2 *M* sodium carbonate (7.5 ml.) and 0.2 *M* sodium bicarbonate (7.5 ml.). Cold 0.05 *N* aqueous iodine solution (30.0 ml.) and water (4.0 ml.) were added, and the reaction mixture was kept in the dark at 0° for 4.5 hr. Titration of an acidified aliquot with standard thiosulfate solution and simultaneous determination of the reagent blank showed that exactly 1.0 mole of iodine had been consumed per mole of aldehyde.

The reaction mixture was adjusted to pH 7.0, and after the addition of 0.5 *N* sodium thiosulfate in slight excess of the amount needed for discharging the iodine color, was extracted three times with ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield 17 mg. of a crystalline product. It was recrystallized from ethyl acetate to give N-benzoyl-D-allothreonine methyl ester (VIIb), m.p. 122–123°; $[\alpha]^{23}D$ −8.3° (*c*, 0.3 in water), identical (mixed melting point, infrared spectrum) with an authentic specimen {m.p. 121–122°, $[\alpha]^{23}D$ −7.1° (*c*, 2.0 in water)} prepared from the N-acylamino acid with diazomethane.

The remaining aqueous phase of the reaction mixture was adjusted to pH 2.0 and extracted six times with ethyl acetate. The combined extracts were dried and freed from solvent *in vacuo* to yield a brownish yellow solid (51 mg.) which was dissolved in water (0.3 ml.). On chilling, the solution deposited crystals of N-benzoyl-D-allothreonine (VIIa), 20 mg., m.p. 128–130° (unchanged on recrystallization); $[\alpha]^{23}D$ −14.9° (*c*, 1.4 in water); λ_{max}^{Nujol} 2.94 (m), 3.15 (s), 5.69 (s), 6.12 (s), 6.53 (s), 8.25 (s), 9.56 (s); lit.[11]: m.p. 127–128°; $[\alpha]D$ −17° (in water).

Anal. Calcd. for $C_{11}H_{13}O_4N$ (223.2): C, 59.18; H, 5.87; N, 6.28. Found: C, 59.23; H, 5.83; N, 6.31.

Additional crops raised the total yield to 45%. Comparison with an authentic specimen of the acid {m.p. 128–130°, $[\alpha]^{24}D$ −18.3° (*c*, 1.9 in water)}, prepared by the methods of Greenstein and Levintow[12] and of Sorensen and Anderson,[13] by infrared

(11) H. D. West and H. E. Carter, *J. Biol. Chem.*, **122**, 611 (1938).

(12) J. P. Greenstein and L. Levintow, *J. Am. Chem. Soc.*, **72**, 2812 (1950).

(13) S. P. L. Sorensen and A. C. Anderson, *Z. Physiol. Chem.*, **56**, 250 (1908).

measurements (Nujol) and an undepressed mixed melting point confirmed the identity. The spectrum was markedly different from that of N-benzoyl-D-threonine[14] (m.p. 145–146°) obtained by benzoylation of D-threonine (California Corporation for Biochemical Research).

Oxidative Degradation of N-Benzoyl-1,2-dideoxymycosaminol (VIII) to N-Benzoyl-D-α-amino-n-butyric Acid (Xa).—Amorphous N-benzoyl-1,2-dideoxymycosaminol (VIII, 240 mg., 1.01 mmole) was oxidized with sodium periodate as described above for N-benzoyl-1-deoxymycosaminol. The uptake of periodate after 1.25 hr. was 0.93 mole per mole of VIII. The reaction mixture was worked up as described above, except that chloroform was used for the extraction of the aldehydic degradation product. The combined chloroform extracts on evaporation left 186 mg. of a crystalline residue. Since attempts to purify this product by recrystallization were unsuccessful, it was sublimed twice at 50° (0.005 mm.). The resulting crystals of the (hitherto undescribed) D-α-benzamido-*n*-butyraldehyde (IXa) melted at 82–83° and gave a strong positive Schiff's test; λ_{max}^{Nujol} 3.04 (m), 5.78 (m), 6.09 (s), 6.54 (s) μ.

Anal. Calcd. for $C_{11}H_{13}O_2N$ (191.2): C, 69.09; H, 6.85; N, 7.33. Found: C, 69.45; H, 7.13; N, 7.44.

The 2,4-dinitrophenylhydrazone (IXb) was prepared; it melted at 208–209°, $\lambda_{max}^{methanol}$ 226 mμ (23,600), 259 mμ (12,700), 273 mμ (sh.) (11,400), 355 mμ (22,400).

Anal. Calcd. for $C_{17}H_{17}O_5N_5$ (371.4): N, 18.86. Found: N, 18.87.

The ultraviolet absorption at 300 mμ of a methanolic solution of the aldehyde (IXa) was measured at intervals. Values for $E_{1\,cm.}^{1\%}$ were 1.60, 0.52, 0.30, 0.28 and 0.27 at 3, 7, 12, 22 and 32 min. respectively.

The remainder of the solution, which contained 92 mg. (0.48 mmole) of the aldehyde, was then used for the Willstätter–Schudel oxidation in the manner described for the aldehyde from VI (25 ml. of carbonate–bicarbonate buffer, 50 ml. of 0.05 *N* iodine solution). The mixture was allowed to stand in the refrigerator for 16 hr., by which time 0.50 mmole of iodine had been consumed. The pH of the solution was adjusted to 7.0, excess sodium thiosulfate solution was added, and the aqueous reaction mixture was extracted three times with ethyl acetate. The combined extracts left, on evaporation of the solvent, 51 mg. of an amorphous product which could not be crystallized. It was obviously methyl D-α-benzamidobutyrate (Xb), as the infrared spectrum was identical with that of a likewise amorphous reference sample prepared by methylating N-benzoyl-D-α-aminobutyric acid with diazomethane.

Anal. Calcd. for $C_{12}H_{15}O_3N$ (221.3): OCH_3, 14.06. Found: OCH_3, 14.63.

Another portion of the aldehyde IXa (94 mg.) was transferred into the sodium hypoiodite oxidation mixture with dioxane instead of methanol and oxidized in the same manner. Excess sodium thiosulfate was added, the reaction mixture was brought to pH 7 and extracted three times with ethyl acetate. Evaporation of the ethyl acetate gave an amorphous product (33 mg.). This fraction was not further characterized. The aqueous reaction mixture was then adjusted to pH 2.0 and extracted five times with ethyl acetate. After the ethyl acetate was dried and evaporated, the residue (52 mg.), which was in part crystalline, yielded on recrystallization from ethyl acetate–ether and then from warm ethyl acetate 15 mg. of D-α-benzamidobutyric acid (Xa), m.p. 109–111°; $[\alpha]^{25}D$ −4.3° (*c*, 1.0 in ethanol). It was identified by comparison (mixed melting point, infrared) with an authentic sample (m.p. 110.5–111.5°, $[\alpha]^{23}D$ −4.1°) prepared by benzoylation of D-α-amino-*n*-butyric acid (California Corp. for Biochemical Research). Both specimens probably represent a polymorphic modification of the preparation described by Fischer and Mouneyrat[15] who reported the melting point as 120–121°.

1-Phenylflavazole Derivative (XIIIa) and Phenylosazone Hydrochloride of Mycosamine.—A solution of mycosamine hydrochloride (200 mg.) in water (10 ml.), *o*-phenylenediamine dihydrochloride (181 mg.), 50% acetic acid (0.48 ml.), and 5.5 *N* hydrochloric acid (0.35 ml.) was heated with stirring just below reflux temperature while carbon dioxide was slowly bubbled through the mixture. After 5 min., freshly distilled phenylhydrazine (540 mg.) was added. The reaction mixture was heated as described for 48 hr. and then cooled to 0° for several hours. The resulting precipitate was removed together with the solution by decantation from a tarry product adhering to the vessel. The tarry material was washed with water and ether and then dissolved in chloroform (15 ml.), and this solution was extracted successively with 5% hydrochloric acid, saturated sodium bicarbonate and water, dried and evaporated to a 1-ml. volume. The precipitate in the decanted portion was collected by centrifugation and work up similarly.

Paper chromatograms indicated that both chloroform extracts contained a flavazole and this technique could be used to purify the product. Accordingly, the chloroform solutions were streaked on fifteen sheets (each 9 in. wide) of Whatman no. 1 chromatographic paper. The sheets were dipped in a chloroform–propylene glycol mixture (3:1), and the chloroform was allowed to evaporate. The chromatograms were then developed in toluene saturated with propylene glycol. Other solvent systems for the paper chromatographic purification of flavazoles have been reported.[16] A yellow fluorescent band (R_f 0.5) was cut out and eluted with hot chloroform. The chloroform was extracted three times with water, dried and evaporated to yield a crystalline residue which was recrystallized from aqueous ethanol to give yellow crystals (2 mg., m.p. 213–215°). Sublimation of the material at 160° (2–3 μ) raised the melting point only slightly, m.p. 214–216°, $[\alpha]^{22}D$ −42.5 (*c*, 0.8 in pyridine), R_f 0.52.

That this compound had the structure and stereochemistry shown in formula XIIIa followed from the identity of its properties, except the sign of rotation, with those of the 1-phenylflavazole derivative of L-rhamnose (XIIIb), m.p. 215–217°; $[\alpha]^{23}D$ +40° (*c*, 1.2 in pyridine), R_f 0.51, which was prepared according to Ohle and Liebig.[7] These authors report m.p. 211°, $[\alpha]D$ + 43.8 (in pyridine). The constants of XIIIb permitted differentiation from the 1-phenylflavazole derivative of L-fucose (XIIIc), m.p. 199–200.5°, $[\alpha]^{23}D$ +36° (*c*, 1.4 in pyridine), R_f 0.58, the preparation of which is described further below. From the 5% hydrochloric acid wash of the chloroform solution containing the precipitate from the reaction mixture, another crystalline product (10 mg.) identified as mycosamine osazone hydrochloride was recovered, m.p. 218.5–220° dec. sealed tube; $[\alpha]^{23}D$ −120° (initial) → −110° (4 hr.), (*c*, 1.3 in pyridine–methanol–water; 4:3:3); λ_{max}^{alc} 254, 310, 397 mμ (19,000; 9,250; 22,100, respectively).

Anal. Calcd. for $C_{18}H_{24}O_2N_5Cl$ (377.9): C, 57.21; H, 6.40. Found: C, 57.36; H, 6.48.

The ninhydrin reaction was positive. Comparison of the specific rotation of this osazone with the specific rotations[17] in the same solvent mixture of the phenylosazone hydrochloride of 3-deoxy-3-amino-D-glucose and also of the phenylosazone of D-galactose (where carbon 4 has the L-configuration), respectively, $[\alpha]^{25}D$ −82° → −72°, and $[\alpha]^{24}D$ +53° → +30°, likewise indicated that carbon 4 in mycosamine has the D-configuration.

1-Phenylflavazole Derivative of L-Fucose (XIIIc).—L-Fucose (0.82 g.) was dissolved in water (50 ml.), and *o*-phenylenediamine hydrochloride (0.905 g.), 5.5 *N* hydrochloric acid (2.68 ml.), 50% acetic acid (2.4 ml.) and phenylhydrazine (2.7 g.) were added. Carbon dioxide was bubbled slowly through the reaction mixture which was stirred and heated (bath temperature 115°) for 24 hr. After cooling to 10°, the precipitate which had formed was filtered off and washed with cold ethanol (60 ml.). It was triturated with more cold ethanol, filtered and washed again. This material was then crystallized from hot ethanol and the resulting yellow product, 688 mg., m.p. 196–200°, subjected to sublimation *in vacuo* at 170° (1–2 μ), followed by recrystallization from pyridine–water (1:1), m.p. 199–200.5°; $[\alpha]^{23}D$ +36° (*c*, 1.4 in pyridine), $\lambda_{max}^{95\%\ alc}$ 268, 333, 409 mμ (42,600; 10,200; 3720, respectively).

Anal. Calcd. for $C_{18}H_{16}O_2N_4$ (320.3): C, 67.48; H, 5.03; N, 17.49. Found: C, 67.32; H, 4.80; N, 17.40.

N-Benzoylmycosamine (XI).—To a suspension of nystatin (58 g., 0.058 mole) in methanol (600 ml.) was added 2.5 *N* aqueous sulfuric acid (700 ml.), and the resultant solution was heated to gentle refluxing. After approximately 15 min. the condenser was turned so as to permit the methanol to distill slowly. Two hours later, when the bulk of the methanol had been removed, the aqueous solution was allowed to cool and then filtered to remove the dark, tarry solids which had formed. The filtrate was extracted four times with 200-ml. portions of 1-butanol which removed most of the pigment. The aqueous phase was then freed of butanol by concentration *in vacuo*. The remaining aqueous

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solution was treated with solid sodium carbonate to bring the pH to 7.9. After further buffering the solution by the addition of sodium bicarbonate (3.0 g.), benzoyl chloride (9.0 g., 0.064 mole) was added in small portions over a 60-hr. period at room temperature while stirring vigorously. During this period a pH of approximately 8 was maintained by the addition of sodium carbonate as needed.

The supernatant solution was decanted from the gummy solid which had formed. This gummy residue was washed with benzene, and the major portion of it which was soluble in warm water was recombined with the aqueous supernatant. After acidification to pH 3.5, the aqueous solution was extracted six times with 300-ml. volumes of ether. It was then readjusted to pH 6 and subjected to a continuous extraction with ethyl acetate.

The white, powdery, crystalline material (5.83 g., m.p. 171–175°) recovered was recrystallized three times from absolute ethanol and dried for 10 hr. at 100° (1 mm.) to yield XI, m.p. 179–181°; $[\alpha]^{22}D$ −58.9° (*c*, 1.5 in water); λ_{max}^{alc} 226 mμ (11,500); λ_{max}^{Nujol} 2.90 (sh., m), 3.00 (s), 6.13 (s), 6.53 (s) μ.

Anal. Calcd. for $C_{13}H_{17}O_5N$ (267.3): C, 58.42; H, 6.41; N, 5.24. Found: C, 58.59; H, 6.39; N, 5.60.

This compound reduced Fehling's solution and consumed 0.93 mole of periodate in two hours.

N-Benzoylmycosaminol (XII).—To a solution of N-benzoylmycosamine (5.25 g.) in water (330 ml.), prepared by heating at 60° and then quickly recooling to room temperature, a solution of sodium borohydride (1.82 g.) in water (45 ml.) was added with stirring over a 10-min. period. The reaction mixture was kept at room temperature for 5.5 hr., and the pH was then adjusted to 5 with 10% acetic acid. This solution was passed through a column (4 × 30 cm.) of MB-3 ion exchange resin.[18]

A large volume of water (5 l.) was used to elute the product from the column, and the resulting solution was brought to dryness *in vacuo*. Any boric acid remaining was removed from the residue by repeated addition and evaporation of absolute methanol. Crystallization of the residue (4.2 g.) from methanol–ether yielded XII, m.p. 125.5–127°; $[\alpha]^{24}D$ +39° (*c*, 1.5 in water).

Anal. Calcd. for $C_{13}H_{19}O_5N$ (269.3): C, 57.98; H, 7.11; N, 5.20. Found: C, 58.11; H, 7.16; N, 4.97.

β-Ethylthio-N-benzoylmycosaminide.—N-Benzoylmycosamine (300 mg., m.p. 176–179°) was dissolved in fuming hydrochloric acid (2 ml.) at 0°, and after the addition of ethanethiol (1.4 ml.), the reaction mixture was stirred vigorously at 0° for 16 hr. Lead carbonate was added until the supernatant was neutral. The solids were removed by filtration and washed with ethanol. Evaporation of the filtrate and washings gave a residue (*ca.* 350 mg.), the methanolic solution of which on chilling deposited crystals. These were recrystallized from methanol, 50 mg., m.p. 248–250° dec.; $[\alpha]^{23}D$ −57° (*c*, 0.7 in water).

Anal. Calcd. for $C_{15}H_{21}O_4NS$ (311.4): S, 10.30; N, 4.49. Found: S, 10.09; N, 4.42.

No other crystalline products could be isolated from the original mother liquor.

Isopropylthio-N-benzoylmycosaminide.—This compound was prepared from N-benzoylmycosamine (300 mg., m.p. 176–179°) and propanethiol-2 in the same way as described above for the ethyl thioglycoside. The crude partially crystalline product was recrystallized from methanol and then ethanol; 100 mg., m.p. 230.5–232°, $[\alpha]D$ −99° (*c*, 0.5 in methanol).

Anal. Calcd. for $C_{16}H_{23}O_4NS$ (325.4): C, 59.05; H, 7.12; S, 9.86. Found: C, 59.33; H, 7.35; S, 10.15.

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(18) A mixed bed resin obtained from Rohm & Haas Co., Philadelphia, Pa.

The Mechanism of Cyclic Anhydride Formation from Mono-*O*-tolylsulfonyl Tetritols[1,2]

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Under mild basic conditions, displacement of a tolylsulfonate anion from a tolylsulfonyl ester having hydroxyl groups both α and γ to the ester function proceeds preferentially by direct attack of the γ hydroxyl group with formation of a tetrahydrofuran ring. That an epoxide, which could be formed by a displacement involving the α hydroxyl group, is not an intermediate in the formation of the tetrahydrofuran ring is demonstrated by the fact that 1,2-epoxy-4-butanol gives only 1,2,4 butanetriol upon treatment with dilute base. 2-*O*-Tolylsulfonyl-L-erythritol gives only L-threitol and 2-*O*-tolylsulfonyl-D-threitol gives only erythritol. The synthesis of a number of derivatives of the tetritols is described.

The base-catalyzed displacement of *p*-tolylsulfonate anions from *p*-tolylsulfonyl esters by suitably situated hydroxyl groups in the same molecule leads to the formation of cyclic ethers. It has been demonstrated that tetrahydrofuran derivatives[3,5] as well as epoxides[3,4] can be formed directly and that 1,2-epoxides can undergo rearrangements to form tetrahydrofuran derivatives when a hydroxyl group is present at C-5 in a suitable steric position.[3,4]

The present study was designed to determine whether, in a system where there are free hydroxyl groups both α and γ to a tolylsulfonyl ester, the base-catalyzed displacement would preferentially involve the α hydroxyl group, and whether a 1,2-epoxide can undergo rearrangement with a C-4 hydroxyl group. To this end the 1- and 2-tolylsulfonyl esters of erythritol and threitol, and 2-*O*-*p*-tolylsulfonyl-1,2,4-butanetriol were prepared and their base catalyzed reactions studied.

1-*O*-Tolylsulfonyl-D-erythritol (I) was prepared by monotolylsulfonation followed by acid-catalyzed hydrolysis of 1,3-*O*-ethylidene-L-erythritol (II). The product contained approximately 35% of erythritol (III) as well as the desired monotolylsulfonyl ester, as could be demonstrated by paper chromatography. Attempts to purify it by paper column chromatography[6] gave a product which was contaminated by *p*-toluensulfonic acid and which, when attempts were made to isolate it from the column eluate, was largely decomposed to erythritol. The *p*-tolylsulfonyl ester component I which was present in the hydrolysate in ap-

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(6) Chromax-column, LKB-Produkter, Stockholm, Sweden.