several months to investigate the sterol content of the non-saponifiable fraction of a wide variety of tissues. Satisfactory results have been obtained by evaporating petroleum ether extracts of saponified tissues to dryness, dissolving the residue in a suitable solvent (chloroform) and applying the crude mixture directly to the column. Preliminary studies have produced good results with methyl esters of mixtures of bile acids isolated from bile. Both the 4-ft and 6-ft columns have given excellent service for many weeks; their actual lifetime has not been determined.

The authors gratefully acknowledge kind gifts of desmosterol from Dr. WILLIAM M. STOKES, Providence College, and Dr. T. R. BLOHM, the Wm. S. Merrell Company, and of 4,4-dimethyl-$\Delta^8$-cholestenol from Dr. K. BLOCH, Harvard University.

*Research and Development Division,* W. L. HOLMES
*Smith Kline and French Laboratories, Philadelphia, Pa. (U.S.A.)* E. STACK

1 R. K. BEERTHUIS AND J. H. RECOURT, *Nature*, 186 (1960) 372.
2 W. J. A. VANDEN HEUVEL, C. C. SWEELEY AND E. C. HORNING, *J. Am. Chem. Soc.*, 82 (1960) 3481.
3 E. O. A. HAAHTI, W. J. A. VANDEN HEUVEL AND E. C. HORNING, *J. Org. Chem.*, 2 (1961) 626.
4 S. R. LIPSKY AND R. A. LANDOWNE, *Anal. Chem.*, 33 (1961) 818.
5 H. J. O'NEILL AND L. L. GERSHBEIN, *Anal. Chem.*, 33 (1961) 182.
6 W. J. A. VANDEN HEUVEL, C. C. SWEELEY AND E. C. HORNING, *Biochem. Biophys. Research Communs.*, 3 (1960) 33.
7 J. SJOVALL, C. R. MELONI AND D. A. TURNER, *J. Lipid Research*, 2 (1961) 317.
8 J. W. FARQUHAR, W. INSULL, Jr., P. ROSEN, W. STOFFEL AND E. H. AHRENS, Jr., *Nutrition Revs.*, 17, No. 8, Part II.
9 E. C. HORNING, E. A. MOSCATELLI AND C. C. SWEELEY, *Chem. and Ind.*, (1959) 751.

Received July 31st, 1961

*Biochim. Biophys. Acta*, 56 (1962) 163–165

## Isolation of 6-deoxy-D-altrose from chemically reduced hygromycin

In a previous publication[1,2], we have reported that 5-keto-6-deoxy-D-arabohexose, a component of hygromycin A, arises from D-glucose probably without rearrangement of the carbon skeleton. In the course of this work, it was necessary to reduce the antibiotic, and isolate the sugar as its L-fucose diethylmercaptal derivative. However, one would also expect to find the other isomer of the reduction, namely 6-deoxy-D-altrose. We would now like to report the isolation and characterization of this compound.

Hygromycin A was reduced as previously described[3]. The reduced antibiotic was purified by charcoal-column chromatography[4]. It was then hydrolyzed by refluxing in 1.5 *N* $H_2SO_4$ for 2 h. After neutralization with $BaCO_3$, the filtrate was passed through Dowex-50 ($H^+$) and Dowex-1 ($CO_3^{2-}$) to remove any remaining ions, and concentrated *in vacuo*. Sugars were then purified by paper chromatography on Whatman No. 1 paper in an isopropanol–water solvent (9:1). Three methylpentoses were observed after application of the nitroprusside spray reagent[5]. One of these has previously been identified as L-fucose[3]. The second methylpentose was chromatographed along with an authentic sample of 6-deoxy-D-altrose (kindly supplied by Dr. V. GINSBURG, National Institutes of Health, Bethesda, Md., U.S.A.) as shown in Table I. The

equilibrium rotation of the isolated methylpentose was found to be $\alpha_D^{20}$ + 20.6; the $\alpha_D^{20}$ value previously reported for 6-deoxy-D-altrose is + 18.0. The methylpentose was further identified as 6-deoxy-D-altrose by reduction with $NaBH_4$ to 6-deoxy-D-altritol, m.p. 117–120°; the previously reported value is 116–119°[6]. (Found: C, 43.5 H, 8.6; Calcd. for $C_6H_{14}O_5$: C, 43.37; H, 8.43.)

TABLE I

$R_F$ VALUES OF SUGARS FROM REDUCED HYGROMYCIN A

Solvents were as follows: I, isopropanol–butanol–water (14:2:4); II, butanol–acetic acid–water (4:1:5); III, isopropanol–water (9:1); IV, ethyl acetate–pyridine–water (12:5:4); V, ethyl acetate–acetic acid–water (14:3:3); VI, phenol–water (4:1).

| | *I* | *II* | *III* | *IV* | *V* | *VI* |
|---|---|---|---|---|---|---|
| Unknown 1 | 0.45 | 0.36 | 0.33 | 0.47 | 0.25 | 0.62 |
| Fucose | 0.44 | 0.35 | 0.32 | 0.47 | 0.25 | 0.61 |
| Unknown 2 | 0.60 | 0.48 | 0.50 | 0.63 | 0.38 | 0.65 |
| 6-deoxyaltrose | 0.60 | 0.48 | 0.51 | 0.63 | 0.38 | 0.64 |
| Unknown 3 | 0.51 | 0.42 | 0.39 | 0.50 | 0.30 | 0.57 |

The third methylpentose, the $R_F$ values for which are also presented in Table I, has not yet been identified.

The relationship of fucose: 6-deoxy-D-altrose: unknown methylpentose in a hydrolysate of reduced hygromycin A, as determined by the anthrone[7] or dinitrosalicyclic acid[8] reagents was found to be 1: 0.54: 0.79. From 1 g of antibiotic, about 50 mg of 6-deoxy-D-altrose was isolated.

Chemically reduced hygromycin A was also treated with ethyl mercaptan. The recrystallized sugar mercaptals were then converted to free sugars with $HgCl_2$ and chromatographed on paper as previously described[2]. Both fucose and 6-deoxy-altrose were identified. The ratio of fucose to 6-deoxy-altrose was 27:1.

This work was supported by a grant (G-5029) from the National Science Foundation and a grant (E-1501) from the National Institutes of Health.

*Department of Biological Sciences,*
*Purdue University,*
*West Lafayette, Ind. (U.S.A.)*

A. D. ELBEIN*
HENRY KOFFLER
H. R. GARNER

1 A. D. ELBEIN, R. L. MANN, H. E. RENIS, W. M. STARK, H. KOFFLER AND H. R. GARNER, *Federation Proc.*, 19 (1960) 85.
2 A. D. ELBEIN, R. L. MANN, H. E. RENIS, W. M. STARK, H. KOFFLER AND H. R. GARNER, *J. Biol. Chem.*, 236 (1961) 289.
3 R. L. MANN AND D. O. WOOLF, *J. Am. Chem. Soc.*, 79 (1957) 120.
4 R. L. MANN, R. M. GALE AND F. R. VAN ABEELE, *Antibiotics and Chemotherapy*, 3 (1953) 1279.
5 D. M. WALDRON, *Nature*, 170 (1952) 461.
6 N. K. RICHTMEYER, L. C. STEWART AND C. S. HUDSON, *J. Am. Chem. Soc.*, 72 (1950) 4935.
7 F. A. LOOWUS, *Anal. Chem.*, 24 (1952) 219.
8 K. H. MEYER, G. NOELTING AND P. BERNFELD, *Helv. Chim. Acta.*, 31 (1948) 103.

Received July 20th, 1961

* Present Address: Rackham Arthritis Research Unit, University of Michigan, Ann Arbor, Mich. (U.S.A.).