Bicycloekasantalic Acid (XII).—The ester (XI, 7.74 g.) was saponified by refluxing with alcoholic potash (60 ml., 10%) for 2 hr. After working up, bicycloekasantalic acid (6.3 g.), b.p. 145–155°/0.8 mm. was crystallized from petroleum ether, m.p. 68°, $[\alpha]^{29}D$ −42.69° (c, 4.68). Characteristic peaks occurred at 2685, 1703 (—COOH), 1658, 880 cm.$^{-1}$ (>C=CH$_2$) in the infrared region.

Anal. Calcd. for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.01; H, 9.52.

Lactone (XIII).—Tricycloekasantalic acid (V, 25 g.) was stirred under reflux with sulfuric acid (650 ml., 15%) according to the method of Semmler.[4] The product was taken up in ether and washed free of acid with alkali. Removal of solvent and crystallization (ether–petroleum ether) yielded the lactone (20 g.), m.p. 103°, $[\alpha]^{28}D$ +2.8° (c, 5.0). Infrared peaks in (CS_2) at 1770 cm.$^{-1}$ (γ-lactone), doublet at 1381 and 1361 cm.$^{-1}$ (*gem*-dimethyl group).

Anal. Calcd. for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.00; H, 9.50.

Diol (XIV).—The lactone (XIII, 13 g.) was reduced with lithium aluminum hydride (4.09 g.) in ethereal solution. The reaction product crystallized from ether–benzene as white glistening plates, m.p. 112°, $[\alpha]^{28}D$ +1.94° (c, 5.00). Infrared peaks occurred at 3180, 1066, 1037, 979 cm.$^{-1}$ (—OH).

Anal. Calcd. for $C_{12}H_{22}O_2$: C, 72.68; H, 11.18. Found: C, 72.54; H, 11.20.

8-Camphenyl Ethanol (XV).—A mixture of the diol (XIV, 5.0 g.), fused sodium acetate (5.50 g.), and acetic anhydride (25 ml.) was refluxed for 4 hr. at 140°. The acetic anhydride was distilled, the residue diluted with water and warmed on the water bath for 15 min. After cooling, it was extracted with ether and washed with 5% sodium carbonate solution. Removal of solvent yielded the acetate (5.26 g.), b.p. 105–110°/0.5 mm., $n^{28}D$ 1.4749, $\alpha^{29}D$ −8.94°. Infrared peaks occurred at 1726, 1240 (—OCOCH$_3$), 842 cm.$^{-1}$ (>C=CH—).

Anal. Calcd. for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 75.13; H, 9.83.

Saponification of the above acetate (5.26 g.) with alcoholic potash (35 ml., 10%) yielded the alcohol (3.5 g.), b.p. 96–98°/2 mm., $n^{25}D$ 1.4963, $[\alpha]^{28}D$ −9.49° (c, 9.30). Infrared spectrum showed absorption at 1666, 842 (>C=CH—), doublet at 1382 and 1366 (*gem*-dimethyl) 3300, 1050 cm.$^{-1}$ (—OH).

Anal. Calcd. for $C_{12}H_{20}O$: C, 79.94; H, 11.18. Found: C, 79.56; H, 11.20.

Hydrogenation of XV.—The alcohol (XV, 0.2706 g.) was hydrogenated in acetic acid (20 ml.) solution in the presence of Adams catalyst (40 mg.) at 20° and 708.2 mm. The amount of hydrogen taken up (39.50 ml.) corresponded to one double bond.

Ozonolysis of XV.—The alcohol (XV, 3.40 g.) in chloroform (75 ml.) was ozonized to completion. The solvent was removed *in vacuo* and the residual ozonide decomposed with water (10 ml.) on a water bath for 3 hr. The volatile portion showed a negative test for formaldehyde and acetone. The nonvolatile portion (1.80 g.) was extracted with ether and the ether extract washed with sodium bicarbonate solution. The neutral portion (1.50 g.) was chromatographed over alumina (27 g., neutral, grade III). The fraction (0.21 g.) eluted with petroleum ether (b.p. 40–60°) had a strong camphoraceous odor, b.p. 108° (bath)/20 mm., $n^{22}D$ 1.4702. The infrared spectrum showed strong absorption at 1726 (five-membered ring ketone), a doublet at 1382 and 1366 cm.$^{-1}$ (*gem*-dimethyl).

Anal. Calcd. for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 79.29; H, 10.73.

Semicarbazone, melted at 208° (ethanol); mixed melting point was not lowered when admixed with the semicarbazone of an authentic sample of camphenilone.

Anal. Calcd. for $C_{10}H_{17}N_3O$: C, 61.51; H, 8.78; N, 21.52. Found: C, 61.70; H, 9.10; N, 21.40.

Permanganate Oxidation of (XV).—The alcohol (XV, 2.0 g.) was stirred with water (60 ml.) at room temperature and potassium permanganate (8.04 g.) was added to this in several portions during 4 hr. The filtrate was freed from neutral material and concentrated to about 15 ml. It was acidified, extracted with ether, and dried (sodium sulfate). Removal of solvent yielded 0.872 g. of the acid which was esterified with diazomethane. The methyl ester, b.p. 160° (bath)/0.7 mm. showed infrared peaks at 3445 (—OH) and 1725 cm.$^{-1}$ (—COOCH$_3$).

Anal. Calcd. for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 63.80; H, 8.60.

Periodate Oxidation.—The dihydroxy ester from the above experiment (0.38 g.) was dissolved in ethanol (6 ml.). Sodium metaperiodate solution (9%, 6 ml.) was added to this dropwise at room temperature during 30 min. with stirring. After addition the contents of the flask were stirred for another 30 min. The solution was filtered and extracted with ether. After removal of solvent, the residue was chromatographed over alumina (10 g., neutral, grade III). The fraction (50 mg.) eluted with petroleum ether (b.p. 40–60°) was identified as camphenilone by its infrared spectrum and semicarbazone, m.p. 208°.

Degradation Products of Hygromycin B[1]

PAUL F. WILEY,[2a] MAX V. SIGAL, JR., AND OLLIDENE WEAVER[2b]

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Indiana

Received February 28, 1962

The isolation of D-talose and hyosamine (N-methyl-2-deoxystreptamine) from the antibiotic, hygromycin B, and the structure of hyosamine are reported. A tentative structure is suggested for hygromycin B$_2$.

The isolation and some of the physical and chemical properties of the antibiotic, hygromycin B, have been reported.[3] It is an amorphous, polyhydroxy, dibasic compound having pK_a' values of 7.1 and 8.8 and is soluble in water, methanol, and ethanol but insoluble in most less polar solvents. There is one N-methyl group present, but O-methyl and C-methyl groups are absent. A molecular formula of $C_{15}H_{28}N_2O_{8-10}$ was sug-

(1) A preliminary report of a portion of this work has been published. See P. F. Wiley and M. V. Sigal, Jr., *J. Am. Chem. Soc.*, **80**, 1010 (1958).

(2) (a) Present address: Research Laboratories, The Upjohn Co., Kalamazoo, Michigan; (b) present address: Northern Regional Research Laboratories, Peoria, Illinois.

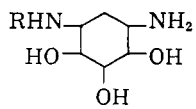
(3) R. L. Mann and W. W. Bromer, *J. Am. Chem. Soc.*, **80**, 2714 (1958).

gested. The infrared spectrum shows absorption in the 3-μ region indicative of OH and/or NH, but no bands suggestive of unsaturation are present.

The molecular formula of hygromycin B has not been definitely established due to failure to achieve complete purification of the antibiotic. However, analytical data and equivalent weight determinations derived from the crystalline *p*-hydroxyazobenzene-*p'*-sulfonic acid salt and from hygromycin B regenerated from this salt were most consistent with a molecular formula of $C_{15}H_{30}N_2O_{10}$.

Hygromycin B is easily degraded by acid. Preparation of the *p*-hydroxyazobenzene-*p'*-sulfonic acid salt gives rise to two degradation products designated hygromycin B_2 and hyosamine. Aqueous hydrolysis of hygromycin B at pH 1.0 for ten minutes on the steam bath forms hygromycin B_2. Hyosamine was also obtained by acidic hydrolysis, but somewhat more vigorous conditions were required. Both of these compounds are devoid of antibacterial activity. Hydrolysis of hygromycin B with boiling 0.5 *N* sulfuric acid for a short period gives a neutral compound as well as hyosamine.

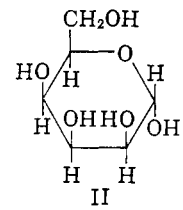
Analytical and physicochemical data derived from the picrate and the hydrochloride of hyosamine established that its molecular formula is $C_7H_{16}N_2O_3$. The infrared spectrum has bands indicative of OH/NH, but none attributable to unsaturated functional groups. Two basic groups are present, pK_a' values of 7.2 and 9.0, one of which was shown to be a methylamino group by analysis. Hyosamine dihydrochloride consumes four moles of periodate per mole in five to six hours with consumption of about one mole more in another eighteen hours. Such oxidation gives rise to at least two moles of formic acid but no formaldehyde. Ammonia is also formed. These data are consistent with Ia for the structure of hyosamine.



Ia. R = CH_3
Ib. R = H

Although several stereoisomers are possible for such a compound the all *trans* arrangement of the substituents seemed most reasonable by analogy to 2-deoxystreptamine (Ib)[4,5] derived from the antibiotics neomycin and kanamycin. Consequently, hyosamine was converted to N,N'-trimethylhyosamine by the Clarke–Eschweiler procedure,[6] and the product was compared as its dihydrochloride with the same salt of N,N'-tetramethyl-2-deoxystreptamine.[7] The melting points, infrared spectra, and X-ray diffraction patterns of the two compounds were identical, establishing that the structure of hyosamine is Ia (N-methyl-2-deoxystreptamine) with all substituents *trans*.

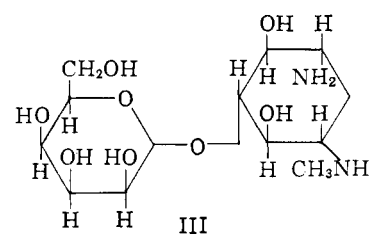
The neutral compound obtained from hygromycin B by acid hydrolysis has a molecular formula of $C_6H_{12}O_6$, m.p. 128–132°; $[\alpha]^{25}D$ +16.9° (*c* 1, H_2O) at equilibrium, and it is reducing. This compound forms a phenylosazone identical with D-galactose phenylosazone as shown by comparison of melting points and X-ray diffraction patterns, and no depression of mixture melting points. The collective data reported above are strongly indicative that the neutral compound is D-talose. Comparison of the product isolated from hygromycin B with synthetic α-D-talose[8] by means of mixture melting points, X-ray diffraction patterns, and paper chromatography showed that the two are identical. A similar comparison of the methylphenylhydrazones of the two compounds also showed identity. These data unequivocally establish that the neutral degradation product is D-talose (II), most probably in the α-form. Papergrams on an acid hydrolysate of hygromycin B indicated that D-talose was the only reducing sugar present.



II

Analysis of hygromycin B_2 as its sulfate and determination of its equivalent weight by potentiometric titration was consistent with a molecular formula of $C_{13}H_{26}N_2O_8$. Hygromycin B_2 has two nitrogen atoms, so it must contain hyosamine, and hydrolysis of hygromycin B_2 gives D-talose. Since these two compounds in combination minus water account for the total hygromycin B_2 molecule, it must be the hyosamine glycoside of D-talose attached at C-4, C-5, or C-6 of hyosamine.

No definitive evidence is available to establish the point at which D-talose is attached to hyosamine nor the configuration of C-1 of D-talose. However, theoretical considerations suggest that the attachment must be at C-5 as in III. The



III

(4) F. A. Kuehl, Jr., M. N. Bishop, and K. Folkers, *J. Am. Chem. Soc.*, **73**, 881(1951).

(5) H. E. Carter, J. R. Dyer, P. D. Shane, K. L. Rinehart, Jr., and M. Hichens, *ibid.*, **83**, 3723 (1961).

(6) H. T. Clarke, H. B. Gillespie, and S. Z. Weisshaus, *ibid.*, **55**, 4571 (1933).

(7) J. Daly, R. C. Durant, S. L. Freiss, G. F. Holland, H. Kny, and B. Witkop, *ibid.*, **82**, 5928 (1960).

(8) Supplied by General Biochemicals, Inc.

easy hydrolysis of hygromycin B would not be expected if the glycosidic linkage were adjacent to one of the nitrogen atoms.[9] Furthermore, if the glycosidic attachment were adjacent to a nitrogen atom, a greater difference in pK_a' values than actually exists would be expected between hydromycin B_2 and hyosamine.[10] These pK_a' values are essentially identical.

The remaining moiety of hygromycin B appears to be a $C_2H_5O_2$ residue which, on the basis of periodate oxidation studies, is attached to the talose moiety, but this C_2 fragment was not isolated or identified.

Experimental

p-Hydroxyazobenzene-p'-sulfonic Acid Salt of Hygromycin B.—A solution of 3.0 g. of hygromycin B in 30 ml. of water was filtered and added to a solution of 6.0 g. of p-hydroxyazobenzene-p'-sulfonic acid in 90 ml. of water. The mixture was refrigerated and filtered, wt. 3.0 g. Four recrystallizations from water gave a product which decomposed slowly starting at 180° but was not melted at 300°.

Anal. Calcd. for $C_{15}H_{30}N_6SO_{18}$: C, 49.05; H, 5.28; N, 8.81; S, 6.72; O, 30.14. Found: C, 49.39; H, 4.96; N, 8.85; S, 6.63; O, 30.65.

A large number of such experiments were run, and the analytical values obtained were usually similar to these, but in many cases the carbon values were higher or lower.

Hygromycin B.—Ten grams of the p-hydroxyazobenzene-p'-sulfonic acid salt of hygromycin B was dissolved in the minimum amount of water and passed over 100 ml. of IRA-400 resin (OH). The resin column was washed with water until the washings were neutral. The combined effluent and washings were freeze-dried. The residue was dissolved in methanol and precipitated by addition of ethanol. The precipitate was removed by filtration and dried under reduced pressure, wt. 2.1 g., m.p. 190–220° dec.

Anal. Calcd. for $C_{15}H_{30}N_2O_{10}$: C, 45.25; H, 7.56; N, 7.07; O, 40.22; mol. wt., 398.4. Found: C, 45.48; H, 7.29; N, 7.17; O, 39.28; mol. wt. (electr. titr.), 374. No consistent analytical data were obtained from a series of such experiments as this, but those analyses which were in agreement were consistent with the above formula.

Hygromycin B_2. (a) p-Hydroxyazobenzene-p'-sulfonic Acid Procedure.—The p-hydroxyazobenzene-p'-sulfonic acid salt of 200 g. of hygromycin B was prepared as above and recrystallized twice. The combined mother liquors were evaporated under a pressure of approximately 10 mm. until about one tenth of the original volume remained. This mixture was filtered, and the precipitate was recrystallized from water. Two crystalline fractions were obtained. The second of these was dissolved in water, and the solution was passed over sufficient IRA-400 resin (OH) to remove the sulfonic acid. The column was washed thoroughly with water, and the combined eluate and washings were evaporated to dryness under reduced pressure leaving 22 g. of residue.

One-half gram of this product was dissolved in aqueous ethanol by adding 3 ml. of ethanol followed by sufficient water to dissolve. Concentrated sulfuric acid was added until a precipitate formed. The mixture was refrigerated and filtered. The solid obtained was recrystallized twice from aqueous methanol, m.p. 220–225° dec. The infrared spectrum showed several bands at 2.85 to 3.10 μ and a band was present at 6.20 μ. The pK_a' values were 7.1 and 8.8.

Anal. Calcd. for $C_{13}H_{26}N_2O_8 \cdot H_2SO_4$: C, 35.78; H, 6.47; N, 6.42; S, 7.35; mol. wt., 436. Found: C, 35.55; H, 7.03; N, 6.57; S, 6.83; mol. wt. (electr. titr.), 472.

(b) **Acidic Hydrolysis Procedure.**—Five grams of hygromycin B was dissolved in 44.1 ml. of 1.0 *N* sulfuric acid. The resulting solution (pH 1.0) was heated in a boiling water bath for 10 min. The sulfate ion was removed by addition of a saturated solution of barium hydroxide. The mixture was treated with charcoal and filtered. The filtrate was evaporated to dryness under reduced pressure at about 50°. The residue was dissolved in 10 ml. of water, the solution was warmed, and 15 ml. of methanol was added. Refrigeration gave a precipitate which was removed by filtration. The filtrate was made strongly acid with concentrated sulfuric acid and refrigerated. The product obtained melted at 218–225°. Its X-ray diffraction pattern and infrared curve were identical with those of hygromycin B_2 sulfate.

Hyosamine. (a) Acidic Hydrolysis Procedure.—Fifty grams of hygromycin B was dissolved in 1 l. of 6 *N* hydrochloric acid, and the solution was heated under reflux for 18 hr. The cooled reaction mixture was filtered and extracted with six 500-ml. portions of ether. The aqueous solution was evaporated to dryness under reduced pressure. This was repeated three times. The residue was dissolved in 1500 ml. of water, and the solution was treated with charcoal. The filtrate from the charcoal treatment was added to a filtered solution of 115 g. of picric acid in 6 l. of water. Refrigeration gave 47 g. of crystalline picrate, m.p. 244° dec; yield 58%. A sample was recrystallized three times from water, m.p. 250° dec., pK_a's 7.1 and 8.9.

Anal. Calcd. for $C_{19}H_{22}N_8O_{17}$: C, 35.96; H, 3.50; N, 17.67; O, 43.03; CH_3N(1), 2.36; mol. wt., 634.4. Found: C, 36.19; H, 3.78; N, 17.40; O, 42.44; CH_3N, 2.35; mol. wt. (electr. titr.), 670.

Five grams of hyosamine dipicrate was dissolved in 140 ml. of nitrobenzene, and the solution was extracted with three 110-ml. portions of 1 *N* hydrochloric acid. The combined extracts were extracted with three 110-ml. portions of ether. The aqueous layer was evaporated to dryness under reduced pressure. The residue was dissolved in methanol, and the solution was evaporated to dryness under reduced pressure. This procedure was repeated twice. The final residue was dissolved in methanol, and the product was precipitated by addition of ether. There was obtained 1.4 g. of hyosamine dihydrochloride, m.p. 170–200° dec., $[\alpha]^{25}D$ +10.7° (*c* 1, H_2O), pK_a's 7.2 and 9.0. The infrared spectrum had bands in the 3.0-μ region. There was no significant ultraviolet absorption between 220 mμ and 400 mμ.

Anal. Calcd. for $C_7H_{16}N_2O_3 \cdot 2HCl$: C, 33.74; H, 7.28; N, 11.25; Cl, 28.46; CH_3N(1), 6.02; mol. wt., 249.4. Found: C, 33.54; H, 7.10; N, 10.94; Cl, 27.90; CH_3N, 5.66; mol. wt. (electr. titr.), 278.

(b) **p-Hydroxyazobenzene-p'-sulfonic Acid Procedure.**—The first crystalline salt obtained from the mother liquors of the crystallization of the p-hydroxyazobenzene-p'-sulfonic salt of hygromycin B was subjected to IRA-400 resin (OH) as described under hygromycin B_2. One-half gram of the basic material obtained was dissolved in water and treated with a solution of picric acid. The crystalline picrate obtained, m.p. 247° dec., had an X-ray diffraction pattern identical with the X-ray diffraction pattern of hyosamine dipicrate.

Periodate Titration of Hyosamine Dihydrochloride.—A solution of 29.5 mg. (0.118 mmole) of hyosamine dihydrochloride was dissolved in 100 ml. of 0.01 *M* sodium periodate solution. This was adjusted to pH 5.0 with sodim bicarbonate, and 10-ml. aliquots were titrated with 0.01 *M* sodium arsenite solution.

Formic Acid and Ammonia from Hyosamine.—A solution of 0.37 g. (1.5 mmoles) of hyosamine dihydrochloride in 10 ml. of water was passed over 50 ml. of IR-45, and the column was washed thoroughly with water. The effluent and wash-

(9) A. B. Foster, D. Horton, and M. Stacey, *J. Chem. Soc.*, 81 (1957).

(10) R. B. Woodward, *Angew. Chem.*, **69**, 50 (1957).

Time, hr.	Ml. of arsenite Sample	Blank	Moles/ mole
0	6.48	9.92	3.0
3/4	6.00	10.00	3.3
1 1/4	5.90	10.00	3.5
3	5.75	10.00	3.6
6	5.40	10.00	3.9
24	4.10	10.00	5.0

ings were combined and evaporated to dryness under reduced pressure. The residue was dissolved in a solution of 1.28 g. (6.0 mmoles) of sodium metaperiodate in 20 ml. of water. The solution was allowed to stand overnight and passed over 20 ml. of IR-120 (H). The column was washed with water which was added to the effluent. This aqueous solution was adjusted to pH 8.5 with saturated barium hydroxide solution, concentrated under reduced pressure, and adjusted to pH 2.0 with 1 *N* sulfuric acid. The mixture was centrifuged, and the supernatant was decanted and steam distilled until approximately 100 ml. of distillate had been collected. Titration of the distillate with 0.1 *N* sodium hydroxide solution showed that the distillate contained 2.9 mmoles of acid. The volatile acid was identified as formic acid by conversion to its barium salt and identification of this salt by its X-ray diffraction pattern.

The IR-120 column was eluted with 120 ml. of 2 *N* hydrochloric acid. The effluent was evaporated to dryness under reduced pressure. The residue was dissolved in water. The solution was made alkaline with sodium hydroxide and steam distilled into a solution of 0.84 g. (3.0 mmoles) of *p*-hydroxyazobenzene-*p'*-sulfonic acid in 30 ml. of water. The distillate was evaporated to dryness under reduced pressure, and the residue was crystallized from ethanol. The X-ray diffraction pattern of the product was identical with that of an authentic sample of ammonium *p*-hydroxyazobenzene-*p'*-sulfonate.

Attempted Isolation of Formaldehyde from Periodic Acid Oxidation of Hyosamine Dihydrochloride.—This was done by the procedure of Reeves.[11] No formaldehyde was found.

***N,N'*-Tetramethyl-2-deoxystreptamine from Hyosamine.**—A solution of 7.0 g. of hyosamine dihydrochloride in a mixture of 15 ml. of formic acid and 5.6 ml. of 37% formaldehyde was heated under reflux for 24 hr. Fifty milliliters of hydrochloric acid was added, and the solution was evaporated to dryness under reduced pressure. Crystallization from ethanol gave 5.0 g. of product, m.p. 270–273° dec. Recrystallization from the same solvent raised the melting point to 275° dec. This compound had no optical activity. The infrared spectrum showed absorption at 2.80 μ.

Anal. Calcd. for $C_{10}H_{22}N_2O_3 \cdot 2HCl$: C, 41.27; H, 8.31; N, 9.62; Cl, 24.37; $CH_3N(4)$, 20.60. Found: C, 41.19; H, 8.04; N, 9.41; Cl, 23.78; CH_3N, 18.13.

A sample of this was converted to its methiodide by refluxing in methanol and methyl iodide. Recrystallization from ethanol–water gave a product melting at 258° dec.

Anal. Calcd. for $C_{12}H_{28}I_2N_2O_3$: C, 28.70; H, 5.62; N, 5.58; O, 9.56; I, 50.55. Found: C, 28.82; H, 5.47; N, 5.53; O, 9.74; I, 49.55.

***N,N'*-Tetramethyl-2-deoxystreptamine from 2-Deoxystreptamine.**—This was prepared as was the same product from hyosamine. Purification required three recrystallizations from ethanol–water and one from ethanol, m.p. 271–273° dec. (lit.,[7] m.p. 277°). The infrared spectrum and the X-ray diffraction pattern of this material were identical with those of the material obtained from hyosamine.

Anal. Calcd. for $C_{10}H_{22}N_2O_3 \cdot 2HCl$: C, 41.27; H, 8.31; N, 9.62; $CH_3N(4)$, 20.60. Found: C, 41.52; H, 8.47; N, 9.41; CH_3N, 19.04.

D-Talose from Hygromycin B.—A solution of 4.0 g. of hygromycin B in 120 ml. of 0.5 *N* sulfuric acid was heated under reflux for 2.5 hr. The sulfuric acid was removed by addition of saturated barium hydroxide solution. The precipitate was removed by centrifugation, and the supernatant was passed over 110 ml. of IRC-120 (H) which was washed with 150 ml. of water. The combined effluent and washings were adjusted to pH 6.5 with saturated barium hydroxide solution, and the precipitate was removed by centrifugation. The supernatant was evaporated to dryness under reduced pressure leaving a brown residue, wt. 0.93g. The residue was triturated with 10 ml. of anhydrous methanol, and the mixture was filtered. The filtrate was allowed to evaporate to dryness at room temperature. This procedure was repeated. There was obtained 0.37 g. of crystalline product, m.p. 118–128°.

One half of this material was dissolved in 0.4 ml. of warm water. Cooling gave a precipitate which was removed by filtration. The filtrate was evaporated to dryness at room temperature. The residue was triturated with 5 ml. of anhydrous methanol, and the mixture was filtered. The filtrate was evaporated nearly to dryness at room temperature and filtered. The procedure was repeated to give the final product, m.p. 128–132° (lit., 133–134°,[12] 130–135°,[13] 127–129°,[14] $[\alpha]^{25}D$ +16.9° (*c* 1, H_2O) at equilibrium (lit., +20.8°,[12] +19.7°,[13] +20.6°[14]). The mixture melting point of this material with synthetic α-D-talose was not depressed, and R_f values on paper chromatography and X-ray diffraction patterns of the two compounds were the same.

Anal. Calcd. for $C_6H_{12}O_6$: C, 39.99; H, 6.72; mol. wt., 180. Found: C, 39.96; H, 6.92; mol. wt. (crystallographic), 178.

Preparation of the phenylosazone of natural D-talose by the usual procedure gave galactose phenylosazone, m.p. 190–195°. This was shown to be identical with galactose phenylosazone by a mixture melting point and X-ray diffraction patterns.

D-Talose Phenylmethylhydrazone.—A solution of 0.32 g. of D-talose from hygromycin B and 0.22 g. of phenylmethylhydrazone in 13 ml. of anhydrous methanol was heated under reflux for 0.5 hr. Refrigeration of this solution gave a crystalline product, wt. 0.22 g., m.p. 153–156°. Two recrystallizations from methanol raised the melting point to 155–156° (lit.,[13] 154°) undepressed upon admixture with an authentic sample. The X-ray diffraction patterns of the two samples were identical.

Anal. Calcd. for $C_{13}H_{20}N_2O_5$: C, 54.92; H, 7.07; N, 9.85. Found: C, 54.95; H, 7.12; N, 9.58.

Paper Chromatography of Hygromycin B Hydrolysate.—A solution of 1.0 g. of hygromycin B in 30 ml. of 0.5 *N* sulfuric acid was heated under reflux for 2.5 hr. This solution was chromatographed on paper using pyridine–ethyl acetate (2:5) saturated with water as the moving phase. The only reducing material found to be present had an R_f value identical with that of D-talose.

D-Talose from Hygromycin B_2.—Two grams of hygromycin B_2 was hydrolysed by the procedure used to obtain D-talose from hygromycin B. The sirup was converted to D-talose phenylmethylhydrazone, m.p. 153–155°, by the procedure reported above. The X-ray diffraction pattern of this compound was identical with that of an authentic sample.

Acknowledgment.—The authors wish to thank Dr. R. L. Mann for the acidic hydrolysis procedure which gave hygromycin B_2. We also wish to thank Mr. W. L. Brown and associates for the microanalyses, Dr. Harold Boaz and associates for titrations and spectra, Mr. Harold Bird for paper chromatography, and Dr. Ralph Pfeiffer and associates for X-ray data.

(11) R. E. Reeves, *J. Am. Chem. Soc.*, **63**, 1476 (1941).

(12) W. W. Pigman and H. S. Isbell, *J. Res. Natl. Bur. Std.*, **19**, 189 (1937).

(13) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **93**, 631 (1931).

(14) W. Bosshard, *Helv. Chim. Acta*, **18**, 482 (1935).