p-**Phenylphenacyl Ester of the** (*levo*)-**Acid.**—The ester, prepared by the procedures described above, was crystallized from methanol to give colorless needles, m.p. 165°. It was identical (mixed melting point and infrared spectrum) to the *p*-phenylphenacyl ester (m.p. 165°) of the dihydroxy acid isolated above from neogermbudine.

Anal. Calcd. for $C_{19}H_{20}O_5$: C, 69.5; H, 6.16. Found: C, 69.3; H, 6.32.

(*racemic*)-**High-melting Isomer of** α,β-**Dihydroxy-**α-**methylbutyric Acid** (**m.p. 110°**) **by Hydroxylation of Tiglic Acid.**—A solution of tiglic acid (50 g.) in acetic acid (600 cc.) containing 1.5 cc. of concentrated sulfuric acid was treated on the steam-bath with 30% hydrogen peroxide (200 cc. added portionwise over a period of 4 hours). After an additional hour of heating the warm mixture was made alkaline with sodium hydroxide and digested for a half hour at 100° to ensure saponification of any acetylated hydroxy acid. The resulting solution was adjusted to *p*H 2 with hydrochloric acid, evaporated to dryness and the residue extracted with acetone. The acetone extract was filtered from the inorganic salts and evaporated to give 62 g. of crude acid, m.p. 72–88°, which after several recrystallizations from acetone–ether gave colorless crystalline (*racemic*)-high-melting α,β-dihydroxy-α-methylbutyric acid; yield 40 g. (60%), m.p. 110–111°.

Anal. Calcd. for $C_5H_{10}O_4$: C, 44.8; H, 7.54. Found: C, 45.3; H, 7.63.

Attempts to resolve this racemic acid, by way of the brucine salt, were unsuccessful.

Dicyclohexylamine Salt.—Treatment of this racemic dihydroxy acid in ether with dicyclohexylamine gave the crystalline salt, m.p. 170–171°, which was identical (mixed melting point and infrared spectrum) to the dicyclohexylamine salt (m.p. 170–171°) of the non-volatile acid isolated above from germbudine, veratetrine and desacetylneoprotoveratrine.

p-**Phenylphenacyl Ester.**—The ester of this synthetic dihydroxy acid, prepared in the usual way, was recrystallized several times from methanol. It melted at 119–120° and had an infrared spectrum identical to that of the *p*-phenylphenacyl ester (m.p. 90–91°) of the non-volatile acid isolated from germbudine, veratetrine and desacetylneoprotoveratrine.

MONTREAL, CANADA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

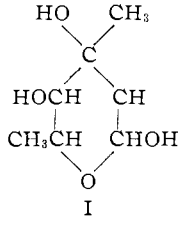
Magnamycin. IV. Mycaminose, an Aminosugar from Magnamycin

BY F. A. HOCHSTEIN AND PETER P. REGNA

RECEIVED JANUARY 22, 1955

Mycaminose, $C_8H_{17}NO_4$, is a dimethylamino sugar isolated from the acid hydrolysis products of the Magnamycin antibiotics. Mycaminose is a reducing sugar, which contains one C-methyl group, and yields two triacetates on acetylation. Periodate oxidation has yielded formic acid and a new sugar $C_7H_{15}NO_3$. Further oxidation forms acetaldehyde, but no formaldehyde or acetic acid. Mycaminose liberates dimethylamine very rapidly with base, indicating it to be a β-dimethylaminoaldehyde. The seven-carbon sugar, by contrast, loses dimethylamine only slowly. These observations are compatible with only one structure, II, for mycaminose.

When the antibiotics Magnamycin[1] or Magnamycin B are subjected to acid hydrolysis,[2,3] two unusual sugars may be isolated. One of these, mycarose, has been shown to have the structure I.[4] The second, mycaminose, is here shown to have the structure II.



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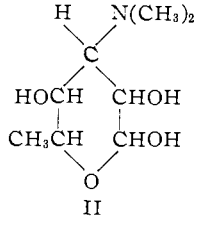


II

Mycaminose, $C_8H_{17}NO_4$, contains a dimethylamino group, and one C-methyl group.[3] It is therefore, a dimethylaminomethylpentose. Mycaminose reduces Fehling solution readily, loses dimethylamine rapidly in alkaline solution, and gives a slow positive iodoform test. Methylation in methanol–hydrochloric acid yields a methyl derivative, which no longer reduces Fehling solution, nor does it readily lose dimethylamine in alkaline solution. The presence of an aldehyde group in mycaminose is evident from the fact that periodate oxidation yields formic acid and a seven-carbon sugar. This aldehyde function must be present as a cyclic hemiacetal, since the infrared spectrum (Fig. 1) shows no carbonyl absorption. Further, the acetylation of mycaminose has yielded two isomeric triacetates,[3] as would be expected of a cyclic hemiacetal, and the hydrogenation of mycaminose to the alcohol can be effected only under forcing conditions.

The oxidation of mycaminose with one equivalent of periodate results in the formation of formic acid and of a new dimethylamino sugar, $C_7H_{15}NO_3$, both in excellent yield. The new sugar differs from mycaminose in that it reduces Fehling solution only slowly. Like mycaminose, it contains one C-methyl group, and the infrared spectrum shows no free carbonyl group. Further periodate oxidation of mycaminose, or of the derived seven carbon sugar proceeds more slowly, as would be expected for cleavage of a $HOC-CN(CH_3)_2$ bond.[5] Mycaminose eventually consumes up to four equivalents of periodate, and both it and the derived methyltetrose yield acetaldehyde, but no formaldehyde, and no acetic acid.

The formation of *formic acid*, and of a dimethylaminomethyltetrose on partial periodate oxidation, together with the qualitative tests, indicates that mycaminose must have the terminal group CHOH-CHO common to C.1 and C.2 of formulas III and IV.

(1) Magnamycin is Chas. Pfizer & Co.'s registered trade name for the antibiotic carbomycin.

(2) R. L. Wagner, F. A. Hochstein, K. Murai, N. Messina and P. P. Regna, THIS JOURNAL, **75**, 4684 (1953).

(3) F. A. Hochstein and K. Murai, *ibid.*, **76**, 5080 (1954).

(4) P. P. Regna, F. A. Hochstein, R. L. Wagner and R. B. Woodward, *ibid.*, **75**, 4625 (1953).

(5) E. H. Flynn, M. V. Sigal, P. F. Wiley and K. Gerzon, *ibid.*, **76**, 3121 (1954). See especially p. 3124.

	1	CHO	1	CHO			
CHO	2	CHOH	2	CHOH		CHO	
$CHN(CH_3)_2$	3	$CHN(CH_3)_2$	3	CHOH		CHOH	
CHOH	4	CHOH	4	$CHN(CH_3)_2$		$CHN(CH_3)_2$	
CHOH	5	CHOH	5	CHOH		CHOH	
CH_3	6	CH_3	6	CH_3		CH_3	
IIIa		III		IV		IVa	

The further oxidation to acetaldehyde and the positive iodoform test compels the assignment of the group CH_3CHOH- common to C.5 and C.6 of these same formulas. The failure to form formaldehyde in the periodate oxidation excludes the presence of terminal $-CH_2OH$ groups and hence, any branched chain structure. Mycaminose must therefore have structure III or IV, and the new dimethylaminomethyltetrose must have structure IIIa or IVa.

The remaining problem then was to determine the position of the dimethylamino group, which must be at either C.3 or C.4. The fact that mycaminose gives a normal rapid Fehling test, while the seven-carbon sugar does not, suggested the dimethylamino group to be on C.3 of mycaminose, β- to the aldehyde group and therefore on C.2 of the derived sugar.

The instability of β-aminocarbonyl compounds to alkali is well known. It is apparent, therefore, that if mycaminose has the β-aminoaldehyde structure III, it should be expected to lose dimethylamine much more rapidly than its derived dimethylaminomethyltetrose which must possess the α-aminoaldehyde structure IIIa. Conversely, if mycaminose has the γ-aminoaldehyde structure IV, the corresponding dimethylaminomethyltetrose, IVa must be a β-aminoaldehyde and would be expected to lose dimethylamine more rapidly than mycaminose.[6] The rate of loss of dimethylamine by mycaminose, by the C_7 sugar and by a model α-aminosugar, glucosamine was studied in 3 *N* alkali at 50°. Under these conditions, mycaminose loses half its dimethylamine in $1/2$ hour, while the methyltetrose and glucosamine show half-lives of the order of 20 hours. It was also noted qualitatively that methylmycaminose, in which the enolization necessary for β-elimination is effectively blocked, shows no appreciable loss of dimethylamine in 3 *N* alkali at 100° after one hour.

It is clear then that mycaminose must have a β-aminoaldehyde structure, and that the structure of mycaminose is III ≡ II.

We have assigned a pyranose ring structure II solely by analogy to the aminosugar pikrocinin (desosamine).[5,7,8] This sugar, which has been isolated from the antibiotics pikromycin[7] and erthromycin,[5,8] is entirely analogous to mycaminose except that it lacks the hydroxyl group at C.4. It can possess no furanose ring and must therefore have a pyranose structure.[9] The general similarity of mycaminose and of pikrocinin is entirely in accordance with this assignment.[10]

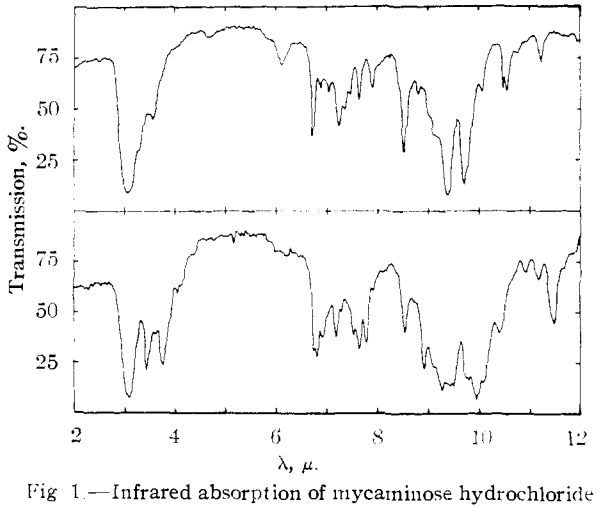


Fig. 1.—Infrared absorption of mycaminose hydrochloride hydrate (upper curve), and of the seven-carbon sugar hydrochloride (lower curve), both in potassium bromide pellets.

(6) In the improbable circumstance that the dimethylamino group was attached at C. 5, both sugars would be expected to lose dimethylamine at comparable rates. Further the seven-carbon sugar could not then exist in a furanose form, and would show aldehyde carbonyl absorption in the infrared.

Experimental

Mycaminose.—Mycaminose hydrochloride $C_8H_{17}NO_4 \cdot HCl \cdot H_2O$, m.p. 115–116°, $[\alpha]^{25}D$ +31 (*c* 1, in water, 24 hours) was prepared by the strong acid hydrolysis of Magnamycin as described in reference 3. Mycaminose shows no ultraviolet absorption peak above 220 mμ. The infrared spectrum is shown in Fig. 1. Mycaminose free base was isolated in low yield by the repeated extraction of a cold (0°) alkaline (*p*H 10.5) solution of the hydrochloride with large volumes of ethyl acetate. The acetate extract was concentrated to dryness and distilled slowly at 150°, 0.02 mm. to yield a colorless glass, soluble in chloroform, insoluble in benzene. It was not further investigated.

Mycaminose appears to react with phenylhydrazine, but a pure product was not isolated.

Methylmycaminoside.—One gram of mycaminose hydrochloride was dissolved in 35 ml. of anhydrous methanol and saturated with hydrochloric acid at 25°. After 72 hours at room temperature, the solution was concentrated to an oil *in vacuo*, dissolved in 25 ml. of water, immediately adjusted to *p*H 10.5 and extracted with five 25-ml. portions of ethyl acetate. The ethyl acetate extracts were combined, concentrated to a small volume and distilled at 100°. 0.2 mm. to yield 0.5 g. of colorless oil which crystallized rapidly, m.p. 65–75°.

Anal. Calcd. for $C_9H_{19}NO_4$: methoxyl, 15.12; N, 6.83. Found: methoxyl, 14.62; N, 6.47.

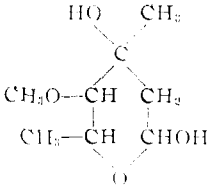
The product, which is very soluble in water and chloroform, was recrystallized six times from hot 50–60° ligroin and the flat needles sublimed at 100°, 0.3 mm., to yield 50

(7) H. Brockmann, H. B. Konig and R. Oster, *Ber.*, **87**, 856 (1954).

(8) R. K. Clark, Jr., *Antibiotics and Chemotherapy*, **3**, 663 (1953).

(9) No structure in this paper is intended to carry any stereochemical significance.

(10) It is of interest that the antibiotics pikromycin, erythromycin and carbomycin, which possess such similar amino sugars, also have similar microbiological spectra. If we may carry the analogy further, the desoxy sugar, cladinose, from erythromycin (see ref. 5 for a partial structure) may have the structure i, analogous to mycarose (I), which is isolated from Magnamycin. Structure i differs from mycarose isovalerate by the replacement of the 4-isovaleryl ester group by an O-methyl group.



i

mg. of a pure single isomer, m.p 126–126.5°, $[\alpha]^{25}D$ −39° (*c* 1, in water, 0.5 and 5 hours).

Anal. Calcd. for $C_9H_{19}NO_4$: C, 52.66; H, 9.33; N, 6.83; methoxyl, 15.12. Found: C, 52.63; H, 9.33; N, 6.61; methoxyl, 14.98.

Titration in aqueous solution showed an equivalent weight of 208 (calcd. 205.3), $pK_b = 8.2$.

A portion of the mixed isomers, m.p. 65–75°, gave a negative Fehling test, and evolved no detectable odor of dimethylamine after 1 hour at 100° in 3 *N* sodium hydroxide. (Mycaminose evolves dimethylamine instantly under these conditions.) Methylmycaminoside is not attacked by 0.01 *M* periodate at *p*H 4.5 during 72 hours.

Hydrogenation of Mycaminose.—Five grams of mycaminose hydrochloride was dissolved in 100 ml. of methanol and hydrogenated at 100°, 2000 lb. pressure for 6 hours over 20 g. of Raney nickel catalyst. The solution was filtered from catalyst, concentrated to dryness, washed with isopropyl alcohol and crystallized from ethanol–acetone to yield 2.5 g. of a pure mycaminitol, m.p. 135.5–136.5°, $[\alpha]^{25}D$ +8.6° (*c* 1, in water).

Anal. Calcd. for $C_9H_{19}NO_4$·HCl: C, 41.83; H, 8.78; N, 6.10. Found: C, 41.67; H, 8.61; N, 6.15.

The infrared spectrum of this compound shows a general similarity to that of mycaminose hydrochloride, except that it lacks the strong ether band of mycaminose at 8.5 μ, and it is devoid of aldehyde C–H absorption at 3.7 μ.

Rate of Deaminolysis of Mycaminose and Other Sugars.—One millimole (246 mg.) of mycaminose hydrochloride monohydrate was dissolved in 5 ml. of water, in a 25-ml. volumetric flask and heated to 50° in a bath thermostated to ±1°. At zero time, 20.0 ml. of preheated 3.97 *N* sodium hydroxide was added and mixed. The resulting solution was 3.14 *N* in sodium hydroxide. At appropriate intervals (30 minutes to 24 hours), 5-ml. aliquots (0.2 mmole) of the reaction mixture were removed, cooled rapidly to 20°, and the volatile components immediately distilled to dryness *in vacuo*, into an efficient Dry Ice trap. This operation required 5 minutes. The distillate was titrated with 0.05 *N* hydrochloric acid to determine evolved amine. "Blanks" containing 0.02 to 0.2 mmole of dimethylamine consistently gave an 85–90% recovery under these conditions of distillation and titration. Mycaminose yielded 0.40–0.45 equivalent of dimethylamine in 30 minutes, a maximum of 0.80 equivalent in 4.5 hours, and lessser amounts thereafter. Under identical conditions, the seven-carbon sugar yielded 0.05–0.06 equivalent of dimethylamine at 1 hour, 0.45 equivalent at 20 hours and a little more at 48 hours. Glucosamine, under identical conditions yielded 0.05 equivalent of ammonia at 1 hour, 0.35 equivalent at 24 hours, and decreasing amounts thereafter. Ammonia "blanks" gave a 75–80% recovery of ammonia under these conditions.

Periodate Oxidation of Mycaminose.

A. To Formic Acid and the Seven-carbon Sugar.—In a preliminary experiment. 50 mg. of mycaminose hydrochloride in 10 ml. of water at *p*H 3–4 consumed 2.5 equivalents of periodic acid in 20 hours, 3.5 equivalents in 48 hours. At the end of this time, one equivalent of formic acid was found by titration.

For a preparative experiment, 0.492 g. of mycaminose hydrochloride hydrate (2 mmoles) and 0.460 g. (2 mmoles) of potassium periodate were dissolved in 100 ml. of water and allowed to stand at room temperature for 48 hours. The solution was concentrated to dryness *in vacuo* and the distillate condensed in a −80° trap. The distillate was titrated with sodium hydroxide (1.50 meq. required) and concentrated to dryness to yield 0.103 g. of solid sodium formate (equivalent weight 68.5). This salt was converted to the *p*-bromophenacyl ester, and separated from unreacted *p*-bromophenacyl bromide and *p*-bromophenacyl alcohol by chromatography with benzene on a Florisil column. The purified ester melted at 100–101°. mixed melting point not depressed by admixture with an authentic preparation of *p*-bromophenacyl formate.[11] The infrared spectrum of this specimen was identical to that of an authentic sample. Paper chromatographic examination showed no evidence of the acetate, or of other esters.

The non-volatile residue from the distillation of formic acid was extracted with anhydrous ethanol, and the insoluble potassium iodate and periodate salts removed by filtration. On concentration to small volume and addition of ether, 0.394 g. of a crystalline hydrochloride salt was obtained. After recrystallization from methanol–ether, then from ethanol–ether, the pure compound was dried for 3 hours at 56° for analysis; m.p. 178.5–179.5° dec., $[\alpha]^{25}D$ −33° (*c* 5, in water, 24 hours).

Anal. Calcd. for $C_7H_{15}NO_3$·HCl: C, 42.53; H, 8.16; N, 7.09; Cl, 17.94; C-methyl(1), 7.60. Found: C, 42.25; H, 8.27; N, 7.05; Cl, 17.77; C-methyl, 7.71.

This compound gives a positive test with Fehling solution only after prolonged heating. The infrared spectrum (Fig. 1) resembles that of mycaminose in a general way; it shows no carbonyl absorption.

B. To Acetaldehyde.—Following a published quantitative procedure for the isolation of acetaldehyde from periodate oxidation,[12] mycaminose was oxidized in bicarbonate buffered solution. Acetaldehyde was liberated slowly (0.22 equivalent in 4 hours). The reaction mixture contained no formaldehyde after this period, as evidenced by its failure to yield the dimedone derivative. In our hands, 0.02 molar equivalent of formaldehyde could be detected readily in the reaction mixture.

In a preparative experiment, 500 mg. of the dimethylaminomethyltetrose hydrochloride was dissolved in 100 ml. of water containing 0.502 g. (1 equivalent) of periodic acid. The solution was heated under reflux for one hour, cooled, and the iodic acid neutralized with standard sodium hydroxide. The solution was evaporated to dryness *in vacuo*, and the distillate collected in a −80° cold trap. An acid solution of 2,4-dinitrophenylhydrazine was added, and the solution left to stand overnight. Crude acetaldehyde dinitrophenylhydrazone, 0.39 g. was separated by filtration, and purified by chromatography on an alumina column. The purified compound, m.p. 167–168.5°, showed no depression of the melting point when mixed with authentic acetaldehyde dinitrophenylhydrazone. The identification was confirmed by comparison of infrared absorption spectra, and by paper chromatography.

When a similar oxidation was carried out at 25° overnight, 0.10 g. of acetaldehyde dinitrophenylhydrazone was recovered.

Acknowledgments.—We are indebted to Mr. G. Hess for the spectral measurements and titration data in this paper, and to Mr. T. Toolan for the microanalyses. We are grateful too to Dr. R. B. Woodward for several helpful suggestions. We should like to acknowledge the capable assistance of Mrs. R. Paradies and of Messrs. J. Catania and M. Noseworthy.

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(11) J. F. Grove and P. J. Suter, *J. Chem. Soc.*, 3350 (1950).

(12) L. A. Shinn and B. H. Nicolet, *J. Biol. Chem.*, **138**, 91 (1941).