Anal. Calcd. for $C_5H_6N_2O_4$: C, 37.93; H, 3.88; N, 17.72; mol. wt., 158. Found: C, 38.20; H, 4.03; N, 17.95; mol. wt. (electr. titr.), 160.

Ammonium N-(Oximinoacetyl)-3-aminoacrylate (VI).—A mixture of 1.62 g. of N-(oximinoacetyl)-3-aminoacrylic acid (V) and 81 ml. of water was stirred, and the mixture was adjusted to pH 6.0 by dropwise addition of 0.6 *N* ammonia solution. The clear solution that resulted was concentrated under reduced pressure until crystals appeared. Refrigeration and filtration gave 0.43 g., m.p. 145° dec. A second crop of 1.65 g., m.p. 143° dec., was obtained by evaporating the filtrate to dryness under reduced pressure. The ultraviolet spectrum and analytical data were obtained on the first crop of crystals. The ultraviolet spectrum in ethanol had maxima at 233 mμ (ε 14,850) and 285 mμ (ε 13,200).

Anal. Calcd. for $C_5H_9N_3O_4$: C, 34.29; H, 5.18; N, 24.00. Found: C, 34.88; H, 5.16; N, 21.92.

These highly unsatisfactory analyses were not improved by extensive efforts to purify the compound.

3-(Oximinoacetamido)acrylamide (U-15,774, II).—A solution of 1.75 g. (0.01 mole) of ammonium N-(oximinoacetyl)-3-aminoacrylate (VI) and 2.16 g. (0.011 mole) of N,N′-dicyclohexylcarbodiimide in 100 ml. of anhydrous dimethylformamide was allowed to stand at room temperature for 2 days. The crystalline precipitate was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure at 30°. The residue was triturated with 10 ml. of methanol. The mixture was refrigerated and filtered, and the filter cake was washed with 6 ml. of cold methanol. The filtrate and washings were mixed with 10 g. of silica, and the mixture was evaporated to dryness. The residue was placed on the top of a column of 150 g. of silica packed in 1-propanol in a 29-mm.-diameter column. The column was eluted with 1-propanol, and fifty 20-ml. fractions were collected. They were analyzed by ultraviolet spectra and thin layer chromatography. Fractions 8–28 were combined and evaporated to dryness under reduced pressure. The residue weighed 621 mg. and contained about 65% II by ultraviolet analysis.

The material from this run was combined with the material from three similar runs, two being the same size and one being one-half the size of this one. The total material, 1.97 g., was dissolved in 200 ml. of methanol and deposited on 20 g. of silica as above. The silica was put on top of 250 g. of silica packed in 1-propanol in a 29-mm.-diameter column. The column was eluted with 1-propanol collecting sixty 20-ml. fractions. After analysis of the fractions by ultraviolet spectra and thin layer chromatography, fractions 14–25 were combined, and fractions 26–60 were combined. The latter pool appeared to contain almost pure II. Concentration of the second pool under reduced pressure gave 225 mg. of residue. The residue was dissolved in 20 ml. of methanol, and the solution was concentrated under reduced pressure to about 3 ml. Refrigeration and filtration of the residue gave a filter cake which was washed with two 1-ml. portions of cold methanol. The solid thus obtained was recrystallized from water, dissolving below 70°, and from 80% methanol containing a trace of sulfur dioxide to yield 29 mg., m.p. 210° dec. The ultraviolet spectrum in ethanol had maxima at 223 mμ (ε 15,750) and 285 mμ (ε 15,200). The infrared spectrum was identical with that of natural 3-(oximinoacetamido)-acrylamide (II). Paper chromatography indicated no impurity.

Anal. Calcd. for $C_5H_7N_3O_3$: C, 38.22; H, 4.49; N, 26.75. Found: C, 38.30; H, 4.68; N, 26.12.

The first pool (fractions 14–25) was worked up in the same fashion except using more methanol for recrystallization and replacing the recrystallization from water with one from 80% methanol containing sulfur dioxide. There was obtained 67 mg. of product, m.p. 198° dec. Thin layer chromatography using silica gel with a 9:1 ethanol–methanol system showed only II to be present.

Acid Hydrolysis of U-22,956 (III).—U-22,956 (500 mg.) was dissolved in 20 ml. of 4 *N* hydrochloric acid, and the solution was boiled for 2 hr. The cooled reaction mixture was extracted with four 50-ml. portions of ether. The combined extracts were dried over magnesium sulfate, filtered, and evaporated to dryness under reduced pressure to give fumaric acid identified by infrared spectra and by comparison of its sublimation point with that of an authentic sample.

Concentration of the ether-extracted aqueous solution and crystallization of the residue from methanol–ether gave 100 mg. of a product whose infrared spectrum was identical with that of a mixture of hydroxylamine hydrochloride and ammonium chloride.

γ-Aminobutyric Acid from U-22,956 (III).—A mixture of 376 mg. of U-22,956, 50 mg. of PtO_2, and 30 ml. of ethanol was hydrogenated at atmospheric pressure. The hydrogen consumption after 4 hr. was equivalent to 4 moles/mole of U-22,956. The catalyst was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue crystallized upon refrigeration and was recrystallized from methanol–ether. The product was identified as γ-aminobutyric acid by its infrared spectrum, paper chromatography, and color tests.

Acknowledgment.—We wish to thank Dr. George Slomp and his staff for analyses, titrations, and spectrophotometric data, and Mr. Nelson J. Major for technical assistance.

Anisomycin. I. Determination of the Structure and Stereochemistry of Anisomycin

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The structure of the antibiotic anisomycin has been shown to be 2-*p*-methoxyphenylmethyl-3-acetoxy-4-hydroxypyrrolidine with the substituents on the pyrrolidine ring *trans* with respect to adjacent substituents, as shown in structure I.

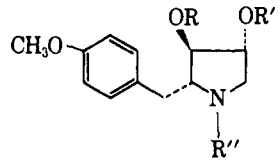
The antibiotic anisomycin[1] has been isolated from cultures of various *Streptomyces* species. It possesses good activity against certain pathogenic protozoa, notably *Trichomonas vaginalis* and *Endamoeba histolytica*,[2] and has been used for the treatment of amebic dysentary.[3] The present report establishes the structure of anisomycin as 2-*p*-methoxyphenylmethyl-3-acetoxy-4-hydroxypyrrolidine with the relative stereochemical configuration shown in structure I.

Published data[1] are in agreement with the molecular formula $C_{14}H_{19}NO_4$ for I. In addition, analyses show that I possesses a methoxyl group, an acetyl group, a C-methyl group, and two active hydrogens. The nitrogen must be present as an amine since titration of I gives a pK_a value of 7.9. The infrared absorption spectra of I indicates the presence of a hydroxyl group (2.82 μ), an ester (5.78 and 8.05 μ), and an aromatic ring (6.22 μ). The presence of an aromatic ring system was

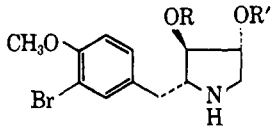
(1) B. A. Sobin and F. W. Tanner, Jr., *J. Am. Chem. Soc.*, **76**, 4053 (1954).

(2) J. E. Lynch, A. R. English, H. Bauck, and H. Deligianis, *Antibiot. Chemotherapy*, **4**, 844 (1954).

(3) A. B. Cué and J. G. H. Diaz, *Rev. Invest. Bol. Univ. Guadalajara*, **1**, 94, (1961); G. G. Miranda and E. Urbina, *ibid.*, **1**, 95 (1961); J. A. Portilla, *ibid.*, **1**, 95 (1961); A. A. Plata, H. B. Zapata, and V. A. Munoz, *ibid.*, **1**, 96 (1961).



I, R = $COCH_3$; R′, R″ = H
II, R, R′, R″ = H
III, R, R″ = $COCH_3$; R′ = H
IV, R, R′, R″ = $COCH_3$
V, R, R′ = H; R″ = $COCH_3$
IX, R, R″ = $COCH_3$; R′ = CH_3
X, R, R″ = H; R′ = CH_3



VIa, R = $COCH_3$
b, R = H

substantiated by the ultraviolet absorption spectrum of I which had maxima at 224 and 277 mμ and which is very similar to that of *p*-anisyl alcohol.

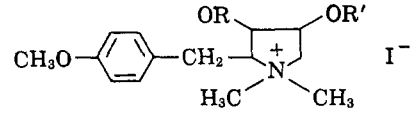
The ester group of I was defined by the following reactions. Hydrolysis of I with either acid or base provides deacetylanisomycin (II) and acetic acid. Both I and II may be acetylated by means of acetic anhydride and pyridine to give the identical compound, diacetylanisomycin (IV). If I is dissolved in acetic anhydride, a crystalline product precipitates which is N-acetylanisomycin (III). Basic hydrolysis of III provided the N-acetyl isomer V of anisomycin.

Positive identification of *p*-methoxyphenyl group in the molecules was readily demonstrated by oxidative degradation of I to anisic acid by means of potassium permanganate. The action of bromine on I in chloroform solution led to monobromoanisomycin (VIa) which was likewise oxidized by permanganate to give 3-bromoanisic acid. It appeared that the *p*-methoxyphenyl group was attached to a unit containing an acetoxy group, an amine, a hydroxyl group, and an additional C_5H_7 fragment. Since there was no addition of bromine to this fragment and also since I does not absorb hydrogen in the presence of platinum catalysts, the presence of a double bond was unlikely. The molecular formula requires the presence of a second ring in the molecule. The nature of this ring was demonstrated through zinc dust distillation of I which provided a pyrrolic material. The compound could not be purified for satisfactory analysis but it did give positive test with pyrrole reagents and its infrared spectrum was virtually identical with that of a sample of 2-anisylpyrrole which was prepared from 2-anisoylpyrrole.

Deacetylanisomycin (II) was oxidized with sodium periodate in borax–boric acid buffer to give anisaldehyde (25%), identified as its 2,4-dinitrophenylhydrazone, formaldehyde (37%), identified as its dimedon derivative, ammonia (40%), identified as the chloroplatinate salt, and a volatile acid (1.19 equiv.), identified as formic acid as its toluide. The conditions used for the periodate oxidation suggested that deacetylanisomycin might be a glycol. Furthermore, the isolation of anisaldehyde from the periodate oxidation and the isolation of anisic acid from the permanganate oxidation strongly suggested that anisomycin had a benzylic hydroxyl group. This was disproved by other experiments. It was hoped that controlled oxidation of II would produce a dialdehyde, which would establish the diol structure. However, oxidation of anisomycin (I) or deacetylanisomycin (II) at various pH ranges caused complete degradation to anisaldehyde. Although the oxidations slowed appreciably when 1 molecular equiv. of periodic acid had been consumed, it was still not possible to isolate any intermediate degradation products.

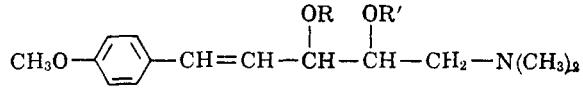
In order to determine whether anisomycin contained a glycol or a benzylic alcohol, several tests were carried out. Various reductions of anisomycin were attempted to remove any reactive alcohol functions. Hydrogenation of the antibiotic I and its diacetyl derivative IV over palladium did not cause loss of a hydroxyl function. Zinc reductions in acetic acid, under vigorous conditions, gave acetylated derivatives of anisomycin (IV and V) as the only products. These results excluded the presence of a benzylic alcohol. Colorimetric tests,[4] intended to show the presence of a *cis*- or *trans*-glycol, were not convincing.

Treatment of I with methyl iodide in the presence of base provided N-methylanisomycin methiodide (VII). In a similar manner the corresponding deacetyl compound VIII could be made from II. Both VII and VIII yielded the same diacetate XIV on treatment with acetic anhydride and pyridine. Reaction of VII or VIII with strong caustic solution resulted in a Hofmann elimination reaction to provide 1-(*p*-methoxyphenyl)-3,4-dihydroxy-5-dimethylamino-1-pentene (XIII). Both hydroxyl groups were still present in the molecule since treatment of XIII with acetic anhydride and pyridine gave the corresponding diacetyl compound XIV. The ultraviolet spectrum of XIII showed a peak at 262 and shoulders at 292 and 303 mμ. These values are quite similar to those reported for anethole,[5] indicating that the double bond is in conjugation with the *p*-methoxyphenyl ring. The location of the hydroxyl groups in the olefin XIII was established by glycol cleavage with lead tetraacetate to give *p*-methoxycinnamaldehyde.



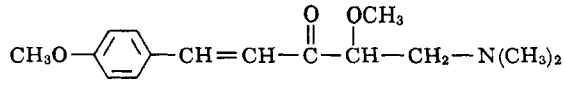
VII, R = $COCH_3$; R′ = H
VIII, R, R′ = H

XI, R = H; R′ = CH_3
XII, R, R′ = $COCH_3$



XIII, R, R′ = H
XIV, R, R′ = $COCH_3$

XV, R = H; R′ = CH_3



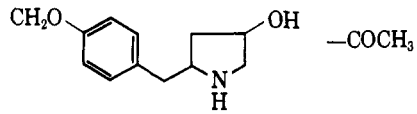
XVI

N-Acetylanisomycin (III) with methyl iodide in the presence of silver oxide provided O-methyl-N-acetylanisomycin (IX) as indicated by the disappearance of hy-

(4) R. Criegee, B. Marchand, and H. Wannowius, *Ann.*, **550**, 99 (1942).
(5) T. W. Campbell, S. Linden, S. Godshalls, and W. S. Young, *J. Am. Chem. Soc.*, **69**, 880 (1947).

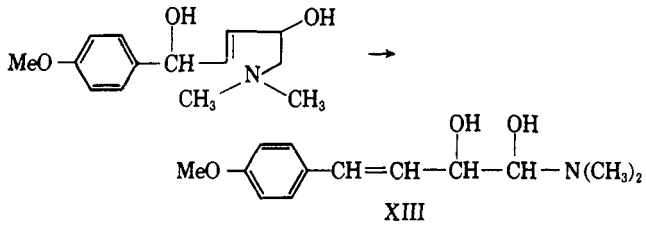
droxyl absorption in the infrared spectra and by methoxyl analysis of IX. Basic hydrolysis of IX removed the acetyl groups to give the O-methyl ether (X). Reaction of X with methyl iodide in the presence of base provided the corresponding N-methyl methiodide (XI) which without isolation underwent a Hofmann elimination in strong sodium hydroxide solution to provide 1-(*p*-methoxyphenyl)-3-hydroxy-4-methoxy-5-dimethylamino-1-pentene (XV). The position of the hydroxyl group was established by mild oxidation of the allylic alcohol XV with manganese dioxide. The resulting ketone XVI was shown to be α,β unsaturated by its infrared absorption at 5.93 μ and the ultraviolet spectrum of XVI exhibited a maximum at 331 mμ indicating conjugation of the carbonyl with the double bond and the *p*-methoxyphenyl ring. The ultraviolet spectrum of XVI was similar to that of 1-*p*-methoxyphenyl-5-dimethylamino-1-penten-3-one hydrochloride (λ_{max} 323 mμ), prepared by a Mannich reaction of anisylideneacetone, formaldehyde, and dimethylamine hydrochloride.[6]

On the basis of these experiments a partial structure for anisomycin (XVII) can be written. The acetoxy group must be located on either the benzylic carbon, or at C-3 of the pyrrolidine ring.[7]



XVII

The glycol structure is suggested by the Hofmann degradation product XIII. However, it can be argued that XIII was produced from a benzylic alcohol *via* an allylic rearrangement, which would be in accord with the results of oxidation of the antibiotic.



XIII

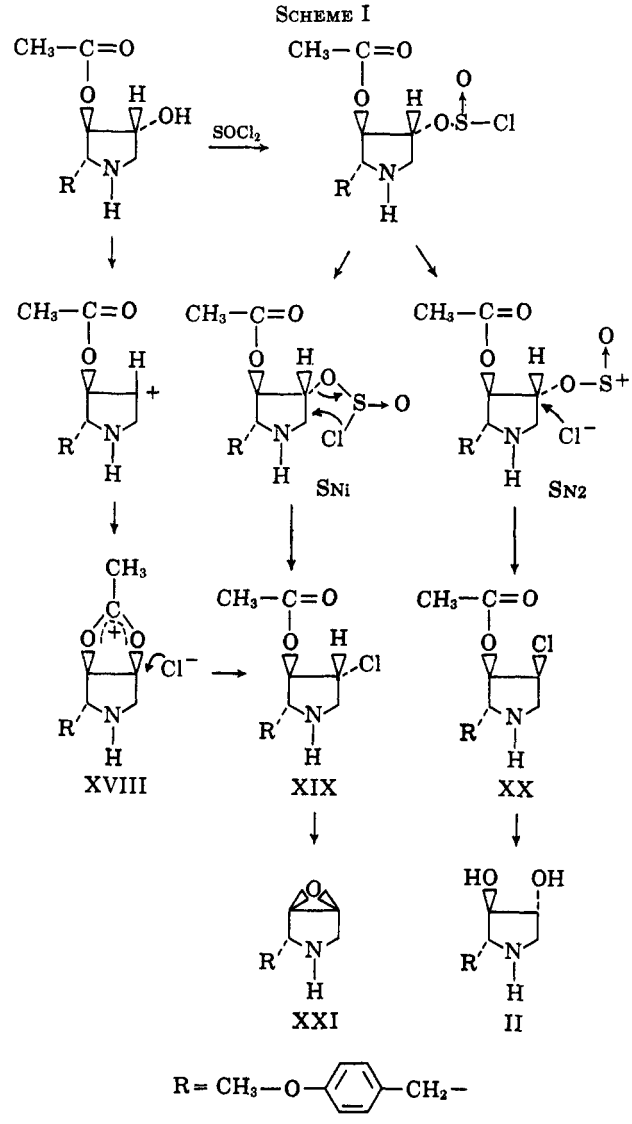
The action of phosphorus pentachloride on I in chloroform solution gave the *trans*-chloro compound (XIX) in excellent yield. Usually, the reaction of an optically active alcohol with phosphorus pentachloride is accompanied by inversion,[8] but this was not the case with anisomycin as was demonstrated by further studies. Apparently, therefore, this reaction involves neighboring-group participation of the acetoxy function at C-3, which stabilizes the intermediate carbonium ion (XVIII) and allows retention of configuration.

(6) H. B. Nisbet, *J. Chem. Soc.*, 1237 (1938).

(7) Mass spectrometry eliminated the possibility of a benzylic alcohol. One would expect facile cleavage at the benzyl carbon atom [K. Bieman, *Angew. Chem., Intern. Ed. Engl.*, **1**, 98 (1962)] and, if a hydroxyl group were present at the benzyl position, an ion of *m/e* 137 should result. Such a peak was not found, but instead there was a peak at *m/e* 121 assignable to the *p*-methoxybenzyl ion or more properly the methoxytropylium ion [P. N. Rylander, S. Meyerson, and H. Grubb, *J. Am. Chem. Soc.*, **79**, 842 (1957)]. We wish to thank Professor K. Bieman for the determination of this spectrum.

(8) J. Kenyon, A. G. Lipscomb, and H. Phillips, *J. Chem. Soc.*, 2275 (1931).

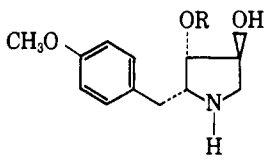
SCHEME I



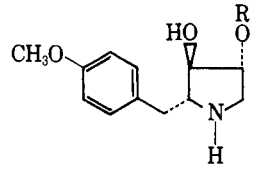
When anisomycin was treated with thionyl chloride in chloroform solution, the crystalline chlorosulfite was obtained, which decomposed on warming to produce sulfur dioxide and a mixture of the *cis*- (XX) and *trans*-chloro (XIX) compounds. Attempts to carry out this reaction in the presence of small amounts of pyridine gave only tarry products. Thermal decomposition of the chlorosulfite must involve both SNi and SN2 mechanisms. The ratio of *cis*- to *trans*-chloro compounds in the reaction products varied with changes in the experimental conditions. Alcoholic potassium hydroxide solution rapidly converted XIX to the epoxide XXI in excellent yield, but under the same conditions the *cis*-chloro compound XX was unchanged and could be separated from the epoxide XXI by partition chromatography. More vigorous treatment of XX with potassium hydroxide produced deacetylanisomycin (II) with inversion of configuration at C-4, but the epoxide survived this more vigorous treatment and was separated from the more water-soluble deacetyl compound II by countercurrent distribution (Scheme I). In this manner it was possible to estimate the relative proportions of the *cis*- and *trans*-chloro isomers obtained from the chlorosulfinate.

The most convenient preparation of the epoxide was *via* the phosphorus pentachloride reaction, which

without purification was converted into the crude epoxide in 90% yield. Formation of the epoxide from XIX proved that XIX is the *trans*-chloroacetoxy compound and that anisomycin has a glycol structure. However, it throws no light on the stereochemistry of anisomycin. This information was obtained by hydrolysis of the epoxide. Treatment of XXI with aqueous acids or alkalis produced some deacetylanisomycin (II) as demonstrated by paper chromatography, but we were unable to isolate it from the reaction mixtures. Reaction of the epoxide XXI with acetic acid gave the compound XXIIa in 86% yield, isomeric with anisomycin (I). Since cleavage of the epoxide must produce a *trans* product, we expected to obtain a mixture of two isomers, XXIIa and XXIIb. Only one product was

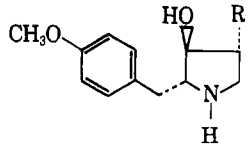


XXIIb, R = CH_3CO



XXIIa

obtained; therefore, some factor must be operating which inhibits nucleophilic attack at one or other of the chemically equivalent carbon atoms of the epoxide. This can only be steric hindrance of the C-3 atom due to the *p*-methoxyphenylmethyl substituent at C-2, which must therefore be *trans* to the epoxide ring. Further confirmation was obtained by reaction of the epoxide VIII with various amines, alkoxides, and mercaptans. In each case, only one product was obtained from each reaction.



XXIII, R = $(CH_3)_2N$–, $(Et)_2N$–, morpholino, piperidino, N^1-methylpiperazino

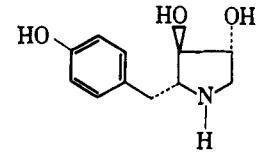
XXIV, R = OCH_3, OC_2H_5, OC_3H_7, OC_4H_9, $OCH_2CH_2OCH_3$, $OCH_2CH_2N(CH_3)_2$

XXV, R = SCH_3, SC_2H_5, SC_3H_7, SC_4H_9, $SCH_2C_6H_5$, SC_6H_5

In anisomycin the acetoxy group is at C-3 and the stereochemistry of this oxygen function does not change during the production of the epoxide. Therefore, since the *p*-methoxyphenylmethyl substituent is *trans* to the epoxide, it must also be *trans* to the acetoxyl of anisomycin.

Treatment of the epoxide XXI with sodium methoxide yielded 2-*p*-methoxyphenylmethyl-3-hydroxy-4-*trans*-methoxypyrrolidine (XXIV, R = OCH_3). This material was identical with the methoxy derivative X, which was prepared by methylating the silver salt of N-acetylanisomycin (III) followed by hydrolysis of the acetyl groups.

Hydrolysis of XXIIa with sodium hydroxide solution gave a product identical with deacetylanisomycin (II). Furthermore, hydrolysis of the methoxy derivative XXIV (R = OCH_3) by hydrobromic acid solution, afforded 2-*p*-hydroxyphenylmethyl-3,4-dihydroxypyrrolidine (XXVI), identical with the product obtained by a similar hydrolysis of anisomycin.



XXVI

No inversions would be expected to take place in the course of these hydrolyses. We know that XXIIa and XXIV (R = OCH_3) must be derivatives of a *trans*-1,2-diol because they are obtained by ring opening of the epoxide XXI. Thus, it follows that anisomycin must be a *trans*-glycol derivative with the relative stereochemical configuration shown in structure I.

Experimental[9]

Anisomycin (2-*p*-methoxyphenylmethyl-3-acetoxy-4-hydroxypyrrolidine, I) may be recrystallized from ethyl acetate, water, toluene, or the higher alcohols. It is obtained as colorless crystals, m.p. 141.6–142.2°, $[\alpha]^{23}D$ −30°, λ_{max} 224 mμ (ε 10,800) and 277 mμ (ε 1600).[10]

Anal. Calcd. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28; CH_3O, 11.70; CH_3CO, 16.22; CH_3, 5.67; neut. equiv., 265.3. Found: C, 63.51; H, 7.21; N, 5.22; CH_3O, 11.70; CH_3CO, 16.32; CH_3, 5.33; neut. equiv., 268 (pK_a = 7.9); active H, 1.9.

The hydrochloride of anisomycin was obtained by treating a cooled ethyl acetate solution of anisomycin with enough concentrated hydrochloric acid to precipitate the salt. The product was recrystallized from a mixture of ethyl acetate and ethanol and was obtained as colorless crystals, m.p. 187–188°.

Anal. Calcd. for $C_{14}H_{20}ClNO_4$: C, 55.72; H, 6.68; N, 4.64; CH_3O, 10.28. Found: C, 55.93; H, 6.78; N, 4.61; CH_3O, 10.26.

Deacetylanisomycin (II).—A mixture of anisomycin (5 g.) and 125 ml. of 1 *N* sodium hydroxide was boiled under reflux for 2 hr. and filtered while hot. Long colorless needles crystallized from the filtrate. These were filtered, washed with cold water, and dried. The product weighed 3.2 g. and had m.p. 176–178°; $[\alpha]^{25}D$ −20°; λ_{max}^{KBr} 3.0, 3.1, 6.22 μ.

Anal. Calcd. for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68; N, 6.27; CH_3O, 13.90; neut. equiv., 223.3. Found: C, 64.43; H, 7.69; N, 6.21; CH_3O, 14.01; neut. equiv., 228 (pK_a = 9.2).

The filtrate from the recovery of deacetylanisomycin was made strongly acid with sulfuric acid and distilled. A portion of the distillate was adjusted to pH 6–7 and *p*-bromophenacyl bromide was added. Methanol was added to obtain a homogeneous solution, and the solution was adjusted to a slightly acid pH with hydrochloric acid. The mixture was boiled under reflux for several hours, and then analyzed by means of paper chromatography, in a descending system with cyclohexane as the solvent; the presence of *p*-bromophenacyl acetate was demonstrated.

The hydrochloride salt of II was prepared by dissolving the base in 10% ethanolic hydrochloric acid and adding ether. The resulting precipitate was collected and dried and recrystallized from a mixture of ethanol and ethyl acetate, m.p. 224–226°.

Anal. Calcd. for $C_{12}H_{18}ClNO_3$: C, 55.49; H, 6.98. Found: C, 55.27; H, 7.03.

Acid hydrolysis of anisomycin (I) was accomplished by boiling with 2 *N* HCl for 3 hr. and evaporating the solution to dryness. The residue was recrystallized from ethanol–ethyl acetate as needles of deacetylanisomycin hydrochloride, m.p. 224–226°.

N-Acetylanisomycin (III).—Anisomycin (4.89 g.) was added in several portions to 150 ml. of acetic anhydride and the suspension

(9) Infrared spectra were determined in chloroform solution unless otherwise stated. Ultraviolet absorption spectra were determined in methanol. Optical rotations were measured at 1% concentration in methanol. Paper chromatograms were carried out with benzene–diethylamine (90:10) for eluent on papers which had been saturated with formamide–methanol (40:60), blotted, and allowed to evaporate dry. Samples on paper were easily located with an acid permanganate spray.

(10) Anisyl alcohol: λ_{max} 226 mμ (ε 11,000), 275 (2100), and 282 (1530).

was stirred at room temperature for 24 hr. The antibiotic slowly dissolved and III precipitated. The product was filtered and washed with ether; it weighed 4.26 g. (72%) and had m.p. 186–188°; $[\alpha]^{27}D$ +41°; λ_{max}^{KBr} 3.07, 5.74, 6.22 (broad), 8.05 μ. An analytical sample was prepared by recrystallization from ethanol (m.p. 186–188°).

Anal. Calcd. for $C_{16}H_{21}NO_5$: C, 62.52; H, 6.88. Found: C, 62.45; H, 7.07.

N,O-Diacetylanisomycin (IV).—Anisomycin (10 g.) was warmed on a steam bath for 2 hr. with 20 ml. of acetic anhydride and 60 ml. of pyridine. The reaction mixture was poured into ice–water and the suspension was adjusted to pH 10 with sodium hydroxide. The product was extracted with ether. The ether extract was washed successively with 1 *N* sulfuric acid, water, 5% sodium bicarbonate, and more water, and then dried over anhydrous sodium sulfate. Concentration of the ether extract gave an oil that was crystallized from ether to give 3.0 g. (23%) of IV: m.p. 85–87°; $[\alpha]^{25}D$ +92°; λ_{max} 5.76, 6.12, 8.05 μ.

Anal. Calcd. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01; CH_3O, 8.88; CH_3CO, 36.96. Found: C, 61.85; H, 6.64; N, 3.92; CH_3O, 8.92; CH_3CO, 36.65.

IV could also be prepared by acetylating deacetylanisomycin (II) in acetic anhydride and pyridine. The product gave no depression of melting point, and the infrared spectra were identical.

N-Acetyldeacetylanisomycin (V).—N-Acetylanisomycin (13.2 g.) was suspended in 150 ml. of methanol. Concentrated ammonium hydroxide (50 ml.) was added and the suspension was heated to the boiling point to give a clear solution. Concentrated ammonium hydroxide (30 ml.) was added and the solution was allowed to stand at 25° for 16 hr. Evaporation *in vacuo* gave an oil that was crystallized from ethyl acetate and ether and filtered to give 8.2 g. (72%) of V: m.p. 143–144.5°; λ_{max}^{KBr} 2.98, 3.18, 6.25 (broad) μ.

Anal. Calcd. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22. Found: C, 63.33; H, 6.94.

Bromination of Anisomycin.—A solution of I (5 g.) in 100 ml. of chloroform was treated with 100 ml. of 10% bromine in chloroform solution. A dark oil separated which was crystallized by trituration with ether and recrystallized from ethyl acetate. The crude product was recrystallized again from acetonitrile to provide 2.2 g. (36.5%) of the hydrobromide of bromoanisomycin (VIa), m.p. 195–198.5°.

Anal. Calcd. for $C_{14}H_{19}Br_2NO_4$: C, 39.55; H, 4.50; neut. equiv., 425. Found: C, 39.28; H, 4.27; neut. equiv., 422.

The mother liquors of VIa slowly deposited a hydrobromide of bromodeacetylanisomycin (VIb). It was recrystallized from acetonitrile, m.p. 250–255°.

Anal. Calcd. for $C_{12}H_{17}Br_2NO_3$: C, 37.71; H, 4.48; neut. equiv., 383. Found: C, 37.31; H, 4.82; neut. equiv., 391.

The hydrobromide of VIa was converted to VIa by means of dilute sodium hydroxide and the free base was recrystallized from ethyl acetate, m.p. 135–137.5°.

Anal. Calcd. for $C_{14}H_{18}BrNO_4$: C, 48.85; H, 5.27; Br, 23.22. Found: C, 48.86, H, 5.30; Br, 22.91.

Potassium Permanganate Oxidation of Anisomycin.—To a stirred suspension of 1 g. of I in 10 ml. of 1 *N* sodium hydroxide was added a solution of 3.52 g. of potassium permanganate in 400 ml. of water over a period of 6 hr. The solution was extracted with three 100-ml. portions of ether. Concentration of the combined ether extracts resulted in a gum which gave anisaldehyde phenylhydrazone (55 mg., m.p. 121–122°) when it was treated with phenylhydrazine in ethanol. The aqueous layer from the ether extraction was acidified with hydrochloric acid and anisic acid (219 mg., 38%, m.p. 183–184°) separated. Anisic acid and anisaldehyde phenylhydrazone were identified by comparison of melting point, and mixture melting point, and infrared spectra with authentic samples.

VIa (100 mg.) was oxidized with potassium permanganate as above. 3-Bromoanisic acid (28 mg., 46.5%) was recovered and its identity was determined by comparison with an authentic sample.

Zinc Dust Distillation of Anisomycin.—A mixture of anisomycin (25 g.) and 50 g. of zinc dust was heated in a sand bath at temperatures up to 300° at a pressure of 20 mm. The distillate (12.7 g.) was collected and steam distilled from a basic aqueous solution. The steam distillate (1.7 l.) was extracted with ether and the ether extract dried over anhydrous potassium carbonate. The ether was removed *in vacuo* and the residual oil was distilled, yield 0.9 g., m.p. 118–128° (2 mm.). The material was difficult to purify but it behaved like a pyrrole in that it gave red colors with acidic dimethylaminobenzaldehyde solution and with isatin in acetic acid.

Anal. Calcd. for $C_{12}H_{13}NO$: C, 76.97; H, 7.00. Found: C, 76.6 ± 0.5; H, 7.8 ± 0.5.

Synthesis of 2-Anisylpyrrole.—Magnesium (15 g.) was placed in a flask with 200 ml. of dry ether, and ethyl bromide (48.5 ml.) was added slowly to prepare ethylmagnesium bromide. Pyrrole (40.6 g.) dissolved in dry ether (80 ml.) was added dropwise and the mixture was warmed on a steam bath for 2 hr. The reaction mixture was cooled while anisoyl chloride (104 g.) dissolved in dry ether (500 ml.) was introduced. The mixture was warmed for 3.5 hr. and allowed to stand overnight. Ammonium chloride (10% aqueous solution) was added, the product was extracted with ether, and the ether extract was dried over potassium carbonate. Evaporation of the ether extract and crystallization of the residue from dilute alcohol gave crude **2-anisoylpyrrole** (32 g., 26%, m.p. 105–114°). Recrystallization from dilute alcohol gave an analytical sample, m.p. 112–115°.

Anal. Calcd. for $C_{12}H_{11}NO_2$: C, 71.62; H, 5.51. Found: C, 71.58; H, 5.69.

2-Anisoylpyrrole (6.2 g.) was mixed with 190 ml. of ethanol and copper chromite catalyst (8.7 g.) and reduced with hydrogen at 3000 p.s.i. and 160° for 1 hr. The catalyst was filtered, the ethanol was distilled, and the residue was vacuum distilled at 140–145° (2.5 mm.). Crude 2-anisylpyrrole (6.05 g.) was recovered which was redistilled to obtain a purified sample.

Anal. Calcd. for $C_{12}H_{13}NO$: C, 75.97; H, 7.00. Found: C, 76.51; H, 7.22.

The infrared spectrum of the most highly purified sample of the pyrrole obtained by the zinc dust distillation of anisomycin was virtually identical with that of the synthetic 2-anisylpyrrole.

Sodium Periodate Oxidation of Deacetylanisomycin (II).—A solution of deacetylanisomycin (875 mg.) dissolved in 350 ml. of pH 7.4 borax–boric acid buffer was mixed with 350 ml. of a 4.3% solution of sodium metaperiodate. After a day at room temperature, a 200-ml. sample of the reaction mixture was adjusted to pH 3 with acetic acid and treated with 200 ml. of saturated dimedon solution. The solution was refrigerated for 2 days, and the dimedon derivative of formaldehyde (121 mg., 37.5%, m.p. 191–192°) was recovered. This was identified by infrared comparison with a sample of authentic material.

The remainder of the reaction mixture was placed in a flask equipped with an exit tube leading into 0.01 *N* hydrochloric acid. Saturated potassium carbonate solution was added to the flask, and the mixture was aerated for 2.5 hr. The titrable base which was collected in an aliquot of the hydrochloric acid solution amounted to a 40% yield. The remainder of the acid solution was evaporated and the base was identified as ammonia by preparing ammonium chloroplatinate from the residue and comparing it with an authentic sample.

In a parallel experiment 130 mg. of II in 50 ml. of buffer solution was mixed with 50 ml. of 4.3% sodium metaperiodate. After 1 day at room temperature, the solution was extracted with ether several times and the combined ether extract was evaporated. The addition of 2,4-dinitrophenylhydrazine reagent in ethanol to the residue yielded 44 mg. (24%) of anisaldehyde 2,4-dinitrophenylhydrazone, m.p. 245–248° dec., identified by comparison with an authentic sample.

A solution of 2.25 g. of II and 15 g. of sodium metaperiodate in 500 ml. of 0.1 *N* sodium hydroxide was left at room temperature for 1 day. A portion (292 ml.) of the reaction mixture was acidified with phosphoric acid and distilled until a total of 215 ml. of distillate was obtained. An aliquot equivalent to 5.85 mmoles of II contained 6.94 moles of titrable acid; this corresponds to 1.19 equiv. of acid/mole of II. The remainder of the distillate was neutralized with sodium hydroxide and evaporated. The residue was treated with hydrochloric acid and toluidine to yield the toluide of formic acid (m.p. 52–53°).

Deacetylanisomycin (223 mg., 0.001 mole) was dissolved in 30.0 ml. of borate buffer, pH 6.8, and 10 ml. of 0.444 *M* periodic acid was added. Samples (2 ml.) of the reaction mixture were taken at intervals of 5 min. for the first 0.5 hr., then at 10-min. intervals for the next hour, and then at intervals of 30 min. Each aliquot was immediately mixed with 7.5 ml. of saturated sodium bicarbonate solution and 15 ml. of 0.1 *N* As_2O_3 solution and titrated with 1.035 *N* iodine solution. In this fashion we were able to measure the rate at which periodic acid was consumed (Table I).

TABLE I

Amount, mole	Time consumed, min.
0.003	5
0.005	10
0.008	30
0.010	40
0.015	95
0.020	150
0.030	255

Although the rate of oxidation slowed after 0.01 mole of HIO_4 had been consumed, the only aldehyde isolated from the reaction mixture was anisaldehyde, characterized as 2,4-dinitrophenylhydrazone derivative.

Similar studies at lower pH ranges showed that periodic acid was consumed at a steady, but much slower rate than at pH 6.8; anisaldehyde and anisic acid were the only degradation products isolated from the reaction mixtures.

Attempted Zinc Reduction of Anisomycin.—Zinc dust (2.0 g.) was added to a solution of anisomycin (2.0 g.) in acetic acid, and the mixture was stirred and heated at reflux temperature for 16 hr. The hot mixture was filtered and evaporated to a gum. Paper chromatography of the gum showed three major components, R_f 0.42, 0.55, and 0.83. One of these components (R_f 0.42) was basic and later shown to be unchanged anisomycin. The gum was purified by partition chromatography on acid-washed Super-Cel (hexane–benzene–methanol–water, 1:2:1:1). A neutral fraction, R_f 0.83, was collected and further purified by molecular distillation (210° at 2×10^{-3} mm.). It was identified as diacetylanisomycin (IV, 748 mg., m.p. and m.m.p. 85–87°).

Anisomycin (122 mg., R_f 0.42) was isolated from the partition column and was characterized by comparison of infrared and ultraviolet spectra, and melting point and mixture melting point with an authentic sample.

N-Acetylanisomycin (III) was also obtained from the column (407 mg., R_f 0.55, m.p. and m.m.p. 186–188°).

N-Methylanisomycin Methiodide (VII).—A suspension of anisomycin (20 g.), anhydrous potassium carbonate (20 g.), and methyl iodide (120 g.) in 500 ml. of acetone was stirred at reflux temperature for 5 hr. using a Dry Ice condenser for cooling. The reaction mixture was filtered hot and the precipitate was washed with hot acetone. The filtrate and washings were combined and evaporated to dryness *in vacuo*. The resulting oil was crystallized from methanol–ethyl acetate to provide 26 g. (82%) of methiodide, m.p. 166–168°. Several recrystallizations from methanol–carbon tetrachloride gave an analytical sample, m.p. 168–169°, $[\alpha]^{25}D$ −44°.

Anal. Calcd. for $C_{16}H_{24}INO_4$: C, 45.61; H, 5.74; I, 30.13; N, 3.33; CH_3O, 7.37; CH_3N, 6.89. Found: C, 45.63; H, 5.78; I, 30.10; N, 2.95; CH_3O, 7.20; CH_3N, 7.17.

N-Methyldeacetylanisomycin Methiodide (VIII).—Deacetylanisomycin (22 g.), anhydrous potassium carbonate (23 g.), methyl iodide (100 ml.), and 500 ml. of acetone were treated as in the previously described experiment. The product was crystallized from methanol–ethyl acetate, yield 23.9 g., 64%, m.p. 170–173°. Recrystallization from methanol–carbon tetrachloride gave analytically pure methiodide salt, m.p. 172.5–173.5°, $[\alpha]^{26}D$ −13.9°.

Anal. Calcd. for $C_{14}H_{22}INO_3$: C, 44.34; H, 5.85; I, 33.47; N, 3.69; CH_3O, 9.19. Found: C, 44.17; H, 5.85; I, 33.60; N, 3.51; CH_3O, 8.26.

Acetylation of N-Methyldeacetylanisomycin Methiodide.—A solution of 1 g. of N-methyldeacetylanisomycin methiodide in 10 ml. of pyridine and 10 ml. of acetic anhydride was left at room temperature for 16 hr. Evaporation *in vacuo* provided 1.1 g. of oil that was crystallized from acetone–cyclohexane to give 960 mg. (78%) of XII, m.p. 180–181°. Recrystallization from the same solvent mixture provided an analytical sample, m.p. 180–181°, $[\alpha]^{25}D$ −25.4°.

Anal. Calcd. for $C_{18}H_{26}INO_5$: C, 46.66; H, 5.66; I, 27.39; N, 3.02. Found: C, 46.35; H, 5.75; I, 27.02; N, 2.82.

VII (1 g.) under the conditions described above yielded 710 mg. (65%) of XII after recrystallization from acetone–cyclohexane, m.p. 179–180°. The sample was shown to be identical with the one above by infrared spectra comparison and mixture melting point determination.

Reaction of N-Methylanisomycin Methiodide VII with Base.—N-Methylanisomycin methiodide (5 g.) in 100 ml. of 1 *N* sodium hydroxide solution was refluxed and stirred for 1 hr. The solution was cooled and extracted with ether, and the ether was dried over anhydrous sodium sulfate. Evaporation of the ether *in vacuo* gave an oil which was crystallized from acetone and cyclohexane to provide 2.86 g. (96%) of product, m.p. 93.5–94°. An analytical sample was prepared by recrystallization from methylene chloride–cyclohexane to give XIII: m.p. 92.5–93.5°; λ_{max} 262 mμ (ε 18,100), 292 (sh) (4360), 303 (sh) (2340); $[\alpha]^{25}D$ −39.3°.

Anal. Calcd. for $C_{14}H_{21}NO_3$: C, 66.90; H, 8.42; N, 5.57; neut. equiv., 251. Found: C, 66.65; H, 8.26; N, 5.50; neut. equiv., 240 (pK_a = 9.15).

Reaction of N-Methyldeacetylanisomycin Methiodide (XII) with Base.—A solution of 10 g. of N-methyldeacetylanisomycin methiodide in 100 ml. of water was treated with 20 ml. of 50% sodium hydroxide solution. The solution was refluxed for 3 hr., cooled to room temperature, and extracted with five 50-ml. portions of ethyl acetate. The ethyl acetate was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo* to provide 5.85 g. of crude product that was crystallized from acetone–cyclohexane to give 5.77 g. (87%) of XIII, m.p. 91.5–92.5°. The compound was shown to be identical by a mixture melting point determination and comparison of their infrared spectra with the product isolated in the previous experiment.

Acetylation of XIII.—A solution of 500 mg. of XIII, 10 ml. of pyridine, and 10 ml. of acetic anhydride was allowed to stand at room temperature for 24 hr. The excess reagent was decomposed in ice–water, and the resulting suspension was extracted repeatedly with ether. Evaporation of the ether gave 520 mg. of an oil which was crystallized from acetone–cyclohexane to provide 330 mg. (50%) of diacetate (XIV), m.p. 87.5–88.5°. An analytical sample was prepared by recrystallization from ether–cyclohexane: m.p. 88–89°; $[\alpha]^{25}D$ +17°; λ_{max}^{KBr} 5.77 (sh), 5.82, and 623 μ.

Anal. Calcd. for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found: C, 64.31; H, 7.64; N, 4.45.

Lead Tetraacetate Oxidation of XIII.—A solution of 3 g. of XIII and 50 ml. of acetic acid was stirred and 5.5 g. of lead tetraacetate was added. After 30 min., 120 ml. of water was added, and the resulting solution was saturated with hydrogen sulfide. The lead sulfide was filtered and the filtrate was extracted repeatedly with chloroform. The chloroform was dried over anhydrous sodium sulfate and evaporated to provide 1.09 g. of an oil which was distilled *in vacuo*, b.p. 123° at 1 mm., to give 440 mg. of crystalline material that was recrystallized from ethanol–water. The product (210 mg.), m.p. 53–55°, was identified as *p*-methoxycinnamaldehyde[11] by a mixture melting point determination and comparison of their infrared spectra. A semicarbazone of the aldehyde was prepared in the usual manner, m.p. 217–218°, lit.[11] m.p. 199°. A mixture melting point determination and comparison of the infrared spectra showed it to be identical with the semicarbazone of *p*-methoxycinnamaldehyde.

The aqueous layer after chloroform extraction was treated with picric acid and after several days 270 mg. of a picrate salt crystallized, m.p. 206–207° dec. The material was identical with the picrate salt of XIII, m.p. and m.m.p. 210–211° dec., and by comparison of their infrared spectra.

Anal. Calcd. for $C_{20}H_{24}N_4O_{10}$: C, 50.00; H, 5.04; N, 11.66. Found: C, 50.26; H, 5.25; N, 12.01.

O-Methyl-N-acetylanisomycin (IX).—A suspension of N-acetylanisomycin (8.2 g.), 33 ml. of acetone, 40 ml. of methyl iodide, and 7.7 g. of silver oxide was stirred and refluxed for 2 hr. Methyl iodide (10 ml.) and 3 g. of silver oxide were added and the refluxing was continued. Additional methyl iodide and silver oxide were added at 2-hr. intervals three more times and finally the suspension was stirred and refluxed for a period of 16 hr. Filtration of the suspension gave a clear solution which was evaporated to dryness *in vacuo* to provide 8.2 g. of oil which was dissolved in ether and concentrated to 30 ml. After several days in the refrigerator, the product (5.5 g., 64%) crystallized, m.p. 71.5–73°. An analytical sample was prepared by recrystallization from ether.

Anal. Calcd. for $C_{17}H_{23}NO_5$: C, 63.53; H, 7.21; N, 4.36; CH_3O, 19.31. Found: C, 63.74; H, 7.32; N, 4.12; CH_3O, 19.51.

O-Methyldeacetylanisomycin (X).—O-Methyl-N-acetylanisomycin (IX, 5.5 g.) was stirred and refluxed in 150 ml. of 1 *N*

(11) M. Scholtz and A. Wiedemann, *Chem. Ber.*, **36**, 845 (1903).

potassium hydroxide solution for 16 hr. The solution was filtered hot, and the product was allowed to crystallize from the filtrate. The precipitate was recrystallized from ethyl acetate to yield 3.5 g. (86%) of IX, m.p. 156–157.5°, $[\alpha]^{25}D$ −42°.

Anal. Calcd. for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; CH_3O, 26.16. Found: C, 65.93; H, 7.93; CH_3O, 25.95.

Preparation of 1-*p*-Methoxyphenyl-3-hydroxy-4-methoxy-5-dimethylaminopent-1-ene (XV).—A suspension of 16 g. of O-methyldeacetylanisomycin (X) and 16 g. of anhydrous potassium carbonate in 50 ml. of methyl iodide and 500 ml. of acetone was stirred and refluxed for 1 hr. Methyl iodide (10 ml.) was added at 1-hr. intervals over 4 hr. The suspension was cooled, filtered, and washed with acetone. Evaporation of the filtrate *in vacuo* yielded an oil (XI) which without isolation was stirred and refluxed in 250 ml. of 10% sodium hydroxide solution for 16 hr. The cooled solution provided 15.2 g. of XV, m.p. 87–89°, which was recrystallized from ethyl acetate–hexane to provide 13.9 g., m.p. 88–89°, $[\alpha]^{25}D$ −42.3°.

Anal. Calcd. for $C_{15}H_{23}NO_3$: C, 67.89; H, 8.74; N, 5.28; CH_3O, 23.40. Found: C, 68.16; H, 8.80; N, 5.37; CH_3O, 23.31.

A picrate salt of XV was prepared in ethanol and recrystallized from methanol, m.p. 151–152°.

Anal. Calcd. for $C_{21}H_{26}N_4O_{10}$: C, 51.01; H, 5.30; N, 11.33. Found: C, 50.97; H, 5.36; N, 11.15.

Manganese Dioxide Oxidation of XV.—A solution of XV (2 g.) in 100 ml. of chloroform was stirred with 10 g. of MnO_2[12] for 3 days. Filtration and evaporation of the solvent left 1.63 g. of an oil that was dissolved in methanol and treated with picric acid. Filtration gave crude XVI as the picrate salt (930 mg., m.p. 163–173°). Two recrystallizations from methanol provided an analytically pure sample, m.p. 172–173°.

Anal. Calcd. for $C_{21}H_{24}N_4O_{10}$: C, 51.22; H, 4.91; N, 11.38; CH_3O, 12.63. Found: C, 50.84; H, 5.10; N, 11.18; CH_3O, 12.65.

From the picric acid mother liquor was obtained 764 mg. of a second picrate, m.p. 148–151°. Recrystallization from methanol gave a sample, m.p. 151–152°, which was shown to be identical with the picrate salt of XV by mixture melting point determination and comparison of infrared spectra.

A solution of XVI picrate salt (600 mg.) in 50 ml. of methanol was passed through a column of neutral chromatographic grade alumina. Elution with 1:1 chloroform–ethanol gave, after evaporation, 207 mg. of an oil which was dissolved in 10 ml. of ether and treated with hydrogen chloride gas. Filtration gave 130 mg. of XVI as the hydrochloride salt, m.p. 165–166°, $[\alpha]^{24}D$ −15.7°, λ_{max} 237 mμ (ε 8500) and 331 (24,800), infrared λ_{max} 5.93 μ.

Anal. Calcd. for $C_{15}H_{22}ClNO_3$: C, 60.09; H, 7.40; N, 4.67; CH_3O, 20.70. Found: C, 59.83; H, 7.53; N, 4.66; CH_3O, 20.96.

2-*p*-Methoxyphenylmethyl-3-acetoxy-4-*trans*-chloropyrrolidine Hydrochloride (XIX).—Anisomycin (5.0 g.) was dissolved in 50 ml. of chloroform. Phosphorus pentachloride (4.0 g.) was added in portions, and a vigorous exothermic reaction occurred with much foaming. The mixture was stirred for 2 min., then evaporated to dryness *in vacuo* to produce an orange foam, which was purified by chromatography on acid-washed Florisil. Elution with chloroform gave a trace of yellow gum which was discarded. Elution with 2% methanol in chloroform gave a colorless gum which was crystallized from acetone–hexane as white needles: 1.33 g., 22%; m.p. 170–171.5°; R_f 0.93; infrared λ_{max}^{KBr} 3.0, 3.5, 3.8 (broad), 4.1, 5.73, 6.2, 6.3, 6.6 μ; ultraviolet λ_{max} 225 mμ (ε 11,600), 276 (1620), 282 (1450).

Anal. Calcd. for $C_{14}H_{19}Cl_2NO_3$: C, 52.5; H, 5.98; Cl, 22.06; N, 4.38. Found: C, 52.17; H, 6.07; Cl, 22.13; N, 4.31.

2-*p*-Methoxyphenylmethylpyrrolidine 3,4-Epoxide (XXI).—2-*p*-Methoxyphenylmethyl-3-acetoxy-4-*trans*-chloropyrrolidine (XIX, 3.0 g.) was dissolved in 20 ml. of 10% KOH in ethanol. A vigorous exothermic reaction occurred which subsided in 2 min. The mixture was stirred until cold, diluted with 60 ml. of water, and extracted with chloroform. The dried chloroform solution was evaporated to an oil which rapidly crystallized (1.4 g., 73%, m.p. 78°). An analytical sample was prepared by sublimation *in vacuo:* m.p. 78–79°; R_f 0.75; infrared $\lambda_{max}^{CHCl_3}$ 3.45, 6.20, 6.30, 6.65, 8.1 (broad), 8.5 μ; ultraviolet λ_{max} 225 mμ (ε 11,200), 275 (1630), 282 (1410).

Anal. Calcd. for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.28; H, 7.46; N, 6.79.

For preparative purposes, the epoxide was prepared without purification of the intermediate chloro compound. The epoxide so obtained was sufficiently pure for synthetic purposes.

Anisomycin (100 g.) was dissolved in 800 ml. of chloroform in a 12-l. flask fitted with an efficient stirrer. Phosphorus pentachloride (80 g.) was added in portions to control the reaction, and the resultant orange solution was evaporated to dryness under reduced pressure to give an amorphous solid. This solid was dissolved in 300 ml. of ethanol, and then 500 ml. of a 20% KOH–ethanol solution was added. A vigorous reaction occurred, and the solution was stirred until cool, diluted to 2 l. with water, and extracted several times with chloroform. The chloroform extract was washed with water, dried, and evaporated to yield 68–79 g. (80–100%) of crude epoxide (calcd. 77 g.), m.p. 62–65°. One recrystallization from hexane gave pure epoxide, 66–70 g. (86–91%), m.p. 77–78°.

Reaction of Anisomycin with Thionyl Chloride.—Thionyl chloride (28 ml.) was added dropwise with stirring to a solution of anisomycin (100 g.) in chloroform (500 ml.). The temperature of the reaction mixture was kept below 35° throughout the addition. After 1.5 min., a crystalline solid separated from the solution. The mixture was stirred for 15 min., then the temperature was raised to 62°, and the mixture was boiled vigorously for 10 min., or until evolution of sulfur dioxide ceased. Evaporation of the chloroform gave the crude chloro compound as an amorphous solid (122 g.), R_f, 0.92.

A sample of the crude chloro compound (28 g.) was purified by adsorption chromatography on acid-washed Florisil. The column was developed by gradient elution with chloroform–methanol mixtures. Two crystalline fractions were obtained; the second fraction was recrystallized from acetone–hexane mixture (6.3 g., m.p. 170–171°) and was identical with 2-*p*-methoxyphenylmethyl-3-acetoxy-4-*trans*-chloropyrrolidine hydrochloride (XIX) made from anisomycin and phosphorus pentachloride. The first fraction was recrystallized from acetone–ether mixture (8.7 g., m.p. 153–156°) and appeared to be 2-*p*-methoxyphenylmethyl-3-acetoxy-4-*cis*-chloropyrrolidine hydrochloride (XX).

Anal. Calcd. for $C_{14}H_{19}Cl_2NO_3$: C, 52.5; H, 5.98; Cl, 22.06; N, 4.38. Found: C, 52.38; H, 5.72; Cl, 22.03; N, 4.44.

Treatment of the first fraction (6.3 g., m.p. 170–171°) with ethanolic KOH, as described above, gave 2-*p*-methoxyphenylmethylpyrrolidine 3,4-epoxide (XXI, 4.1 g., 90%).

Reaction of 2-*p*-Methoxyphenylmethyl-3-acetoxy-4-*cis*-chloropyrrolidine (XX) with Potassium Hydroxide.—The *cis*-chloro compound (500 mg.) was dissolved in 5 ml. of 20% KOH in ethanol, and the solution was warmed on a steam bath for 20 min. Evaporation of the mixture gave a crude solid, which was dissolved in water and extracted several times with 1-butanol, and the butanol extract was washed with water and evaporated to dryness. Crystallization of the residual solid from acetone gave 150 mg. of deacetylanisomycin (II, 43%), m.p. and m.m.p. 170–172°.

Reactions of Mixtures of Crude Chloro Compounds XIX and XX Obtained from the Chlorosulfinate of Anisomycin. A.—A mixture of the *cis*- (XX) and *trans*-chloro (XIX) compounds (20.0 g.) was dissolved in 100 ml. of 10% KOH in ethanol. A vigorous reaction occurred, the temperature of the mixture rose to 68°, and deacetylanisomycin (II) crystallized from solution. The mixture was boiled on a steam bath for 10 min., cooled, poured into water, and extracted with several volumes of chloroform, and the extract was washed, dried over anhydrous sodium sulfate, and evaporated to a solid. Paper chromatography showed that this solid contained the epoxide XXI and deacetylanisomycin (II). Countercurrent distribution of the solid in a system composed of hexane–benzene–methanol–water (1:2:1:1) separated the deacetylanisomycin (II, 5.3 g., 34%), m.p. 170–173°, from the epoxide XXI (6.2 g., 42%), m.p. 73–75°. Deacetylanisomycin migrated with the more polar solvent.

B.—A mixture of the *cis*- (XIX) and *trans*-chloro (XX) compounds (20.0 g.) obtained by thermal decomposition of the chlorosulfite of anisomycin, was dissolved in 100 ml. of ethanol containing 3.5 g. of potassium hydroxide. The mixture was stirred for 5 min., then cooled, diluted with water, and extracted with chloroform. Examination of the residue obtained by evaporation of the chloroform, showed the presence of the epoxide XXI and a chloro compound. The mixture was purified by partition chromatography on acid-washed Super-Cel. Hexane–benzene–

(12) O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

methanol–water (1:2:1:1) were used for the solvent system. The less polar fraction was collected, dissolved in methanol, and saturated with hydrogen chloride gas and 2-*p*-methoxyphenylmethyl-3-acetoxy-4-*cis*-chloropyrrolidine hydrochloride (XX) crystallized out (4.2 g.), m.p. and m.m.p. 152–156°. The epoxide XXI was collected from a more polar fraction and was crystallized from hexane (6.1 g., m.p. 76°).

Anisomycin was treated with thionyl chloride using various experimental conditions, and the resultant mixture of *cis*- and *trans*-chloro compounds was treated with 10% KOH in ethanol (method a). Yields of deacetylanisomycin and the epoxide were determined by countercurrent distribution. The following examples show the variations of the yields of products with the conditions used for the preparation of the mixture of chloro compounds.

Anisomycin was treated with thionyl chloride at 0° for 1 hr., then the mixture was heated at 60° until evolution of SO_2 had ceased. The chloro compounds were converted into the epoxide (9%) and deacetylanisomycin (86%) (method a). In a similar reaction carried out at 30° for 15 min. followed by heating to 60° for 10 min. the yield of the epoxide was 42% and deacetylanisomycin, 51%. When the reaction was carried out without external cooling the temperature of the mixture rose to 52° (reaction time 9 min.); yield of epoxide 50%, deacetylanisomycin 46%. In a similar reaction, the temperature of the mixture rose to 39° (reaction time 11.0 min.); yield of epoxide 18%, deacetylanisomycin 76%.

2-*p*-Methoxyphenylmethyl-3-*trans*-hydroxy-4-acetoxypyrrolidine (XXIIa).—A solution of the epoxide XXI (6.0 g.) in acetic acid (100 ml.) was boiled under reflux for 1.5 hr., then the excess acid was distilled under reduced pressure. The residue was dissolved in dilute hydrochloric acid, and the solution was extracted with chloroform which was discarded. Potassium hydroxide solution was used to adjust the acidic solution to pH 8.5, and the mixture was extracted with chloroform. Evaporation of the washed and dried (Na_2SO_4) extract gave 6.4 g. (86%) of white crystals, m.p. 141–145°. Recrystallization from ethyl acetate–hexane mixtures raised the melting point to 155–156.5° (5.45 g., 73%): R_f 0.45; infrared λ_{max}^{KBr} 305, 3.45, 3.75, 5.75, 6.20, 6.30, 6.60 μ; ultraviolet λ_{max} 225 mμ (ϵ 10,900), 276 (1380), 283 (1170).

Anal. Calcd. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.36. Found: C, 63.48; H, 7.25; N, 5.28.

Reaction of 2-*p*-Methoxyphenylmethylpyrrolidine 3,4-Epoxide with Amines 2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-morpholinopyrrolidine.—The epoxide XXI (1.0 g.) was added to a solution of morpholine (10 ml.) in methanol (10 ml.), and the mixture was gently refluxed for 16 hr. and evaporated *in vacuo* to a yellow gum. Trituration of the gum with acetone produced crystals (576 mg., 40%), m.p. 141–143°, which were recrystallized from acetone–hexane as white needles, m.p. 143–144.5°, of 2-*p*-methoxyphenylmethyl-3-hydroxy-4-*trans*-morpholinopyrrolidine.

Anal. Calcd. for $C_{16}H_{24}N_2O_3$: C, 65.72; H, 8.27; N, 9.58. Found: C, 65.72; H, 8.64; N, 9.47.

In a similar fashion, the following amines were prepared.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-piperidinopyrrolidine was isolated as white needles, m.p. 136–137° (39%), from acetone–hexane mixtures.

Anal. Calcd. for $C_{17}H_{26}N_2O_2$: C, 70.31; H, 9.02; N, 9.65. Found: C, 70.22; H, 9.00; N, 9.74.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-4′-methylpiperazinopyrrolidine was isolated as white needles, m.p. 162–163° (34%), from acetone–hexane mixtures.

Anal. Calcd. for $C_{17}H_{27}N_3O_2$: C, 66.85; H, 8.91; N, 13.76. Found: C, 66.87; H, 9.08; N, 13.94.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-N′,N′-dimethylaminopyrrolidine was prepared by heating the epoxide (10 g.) with dimethylamine (11 ml.) in methanol (50 ml.) in a pressure bottle at 60° for 16 hr. It was isolated as white needles of the di-*p*-toluenesulfonate, m.p. 255–256° (21.0 g., 72%), from methanol–ethyl acetate.

Anal. Calcd. for $C_{14}H_{22}N_2O_2 \cdot 2C_7H_8SO_3$: C, 56.56; H, 6.44; N, 4.71; S, 10.89. Found: C, 56.67; H, 6.63; N, 4.87; S, 11.17.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-N′,N′-diethylaminopyrrolidine was prepared from the epoxide XXI (10 g.) and diethylamine (10 ml.) in methanol (50 ml.). The mixture was heated in a pressure bottle at 80° for 16 hr., and the product was isolated by evaporation of the solvent and crystallization of the residual gum as the dihydrochloride salt (9.1 g., 53%), m.p. 182–185°, from ethyl acetate.

Anal. Calcd. for $C_{16}H_{26}N_2O_2 \cdot 2HCl$: C, 54.6; H, 8.04; Cl, 20.2; N, 8.00. Found: C, 54.4; H, 8.11; Cl, 20.5; N, 8.16.

Reaction of 2-*p*-Methoxyphenylmethylpyrrolidine 3,4-Epoxide (XXI) with Alkoxides. 2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-methoxypyrrolidine (X).—The epoxide (10 g.) was dissolved in a solution of sodium (5 g.) in dry methanol, and the mixture was refluxed for 3 hr. and then evaporated to dryness *in vacuo*. The crude product was dissolved in 2 *N* hydrochloric acid solution, which was extracted with chloroform, and the chloroform was discarded. Sodium hydroxide solution was then added to the aqueous layer to pH 9.5, the solution was extracted with chloroform, and the extract was washed, dried over anhydrous sodium sulfate, and evaporated to yield 8.8 g. (76%) of the crude ether, m.p. 146–148°. This material was purified by recrystallization from acetone–hexane mixtures as white prisms: 7.3 g. (63%); m.p. 158°; $[\alpha]^{25}D$ −42°; R_f 0.52; infrared λ_{max}^{KBr} 3.05, 3.45, 3.75, 6.20, 6.30, 6.60 μ, identical with the spectrum of the sample obtained by hydrolysis of O-methyl-N-acetylanisomycin (IX).

Anal. Calcd. for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.87; H, 8.09; N, 5.84.

In a similar fashion, the following ethers were prepared.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-ethoxypyrrolidine was isolated as white needles, m.p. 115–118° (66%), from acetone–hexane mixture: R_f 0.6; infrared λ_{max}^{KBr} 3.05, 3.45, 3.66 (broad), 6.18, 6.30, 6.60 μ.

Anal. Calcd. for $C_{16}H_{25}NO_3$: C, 68.78; H, 9.02; N, 5.01. Found: C, 68.33; H, 8.56; N, 5.14.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-*n*-propoxypyrrolidine was isolated as white needles, m.p. 139–140° (38%), from acetone–hexane: R_f 0.82; infrared λ_{max}^{KBr} 3.05, 3.45 (broad), 3.70 (broad), 6.20, 6.30, 6.60 μ.

Anal. Calcd. for $C_{17}H_{27}NO_3$: C, 67.89; H, 8.74; N. 5.28. Found: C, 68.08; H, 8.78; N, 4.96.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-*n*-butoxypyrrolidine was isolated as white needles, m.p. 115–118° (88%), from acetone–hexane: R_f 0.91; infrared λ_{max}^{KBr} 3.06, 3.45 (broad), 3.70 (broad), 6.20, 6.30, 6.60 μ.

Anal. Calcd. for $C_{16}H_{25}NO_3$: C, 68.78; H, 9.02; N, 5.01. Found: C, 68.33; H, 8.56; N, 5.14.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-2′-methoxyethoxypyrrolidine was isolated as white needles, m.p. 102–103° (43%), from ethyl acetate–hexane: R_f 0.3; infrared λ_{max}^{KBr} 3.05, 3.50, 3.70 (broad), 6.20, 6.30, 6.65 μ.

Anal. Calcd. for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98. Found: C, 63.83; H, 8.26; N, 5.14.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-2′-dimethylaminoethoxypyrrolidine was isolated as white needles, m.p. 130–131° (57%), from ethyl acetate–hexane: R_f 0.22; infrared λ_{max}^{KBr} 2.95 (broad), 3.05, 3.5 (broad), 6.20, 6.30, 6.63 μ.

Anal. Calcd. for $C_{16}H_{26}N_2O_3$: C, 65.28; H, 8.90; N, 9.52. Found: C, 64.57; H, 7.88; N, 9.46.

Reaction of 2-*p*-Methoxyphenylmethylpyrrolidine 3,4-Epoxide (XXI) with Mercaptans. 2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-methylthiopyrrolidine.—To a solution of 4.7 g. (5.25 ml.) of methyl mercaptan in 100 ml. of 10% aqueous sodium hydroxide was added 10.0 g. of the epoxide. The reaction vessel was equipped with a water-cooled reflux condenser topped with a Dry Ice condenser to prevent excessive loss of the methyl mercaptan, and the mixture was refluxed gently for 2 hr. Chloroform extraction of the cooled reaction mixture gave a yellow solution which was washed with water, dried over anhydrous sodium sulfate, and evaporated to a gum, which crystallized when triturated with ether (10.5 g., 85%). This material was purified by recrystallization from ethanol–water mixture, followed by recrystallization from chloroform–hexane: 8.0 g. (64%); m.p. 149.5–150°; R_f 0.60; infrared λ_{max}^{KBr} 3.30 (broad), 3.45, 3.55, 3.75, 6.20, 6.30, 6.63 μ.

Anal. Calcd. for $C_{13}H_{19}NO_2S$: C, 61.7; H, 7.58; N, 5.53; S, 12.64. Found: C, 61.4; H, 7.63; N, 5.40; S, 12.60.

In a similar fashion the following sulfides were prepared.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-ethylthiopyrrolidine was isolated as white crystals from chloroform–hexane: m.p. 119–120° (49% yield); R_f 0.85; infrared λ_{max}^{KBr} 3.07, 3.45 (broad), 3.75 (broad), 6.20, 6.30, 6.62 μ.

Anal. Calcd. for $C_{14}H_{21}NO_2S$: C, 62.90; H, 7.92; N, 5.24; S, 11.97. Found: C, 62.67; H, 7.78; N, 5.02; S, 11.67.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-propylthiopyrrolidine was isolated as needles from chloroform–hexane: m.p. 123–124° (35% yield); R_f 0.95; infrared λ_{max}^{KBr} 3.05, 3.30 (broad), 3.45 (broad), 3.75 (broad), 6.20, 6.30, 6.62 μ.

Anal. Calcd. for $C_{15}H_{23}NO_2S$: C, 63.05; H, 8.25; N, 4.98; S, 11.77. Found: C, 63.49; H, 8.25; N, 5.22; S, 11.37.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-*n*-butylthiopyrrolidine was isolated as white needles from chloroform–hexane: m.p. 100–101° (78% yield); R_f 0.95; infrared λ_{max}^{KBr}3. 05, 3.42 (broad), 3.75 (broad), 6.18, 6.30, 6.60 μ.

Anal. Calcd. for $C_{16}H_{25}NO_2S$: C, 65.06; H, 8.53; N, 4.74; S, 10.83. Found: C, 65.33; H, 8.51; N, 4.60; S, 10.64.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-phenylthiopyrrolidine was isolated as needles from chloroform–hexane: m.p. 106–108° (56% yield); R_f0. 98; infrared λ_{max}^{KBr} 3.05, 3.12, 3.3 (broad), 3.45 (broad), 3.8 (broad), 6.20, 6.30, 6.62 μ.

Anal. Calcd. for $C_{18}H_{20}NO_2S$: C, 68.55; H, 6.71; N, 4.44; S, 10.15. Found: C, 68.85; H, 6.58; N, 4.13; S, 10.10.

2-*p*-Methoxyphenylmethyl-3-hydroxy-4-benzylthiopyrrolidine was isolated as white needles from chloroform–hexane: m.p. 122° (45% yield); R_f 0.98; infrared λ_{max}^{KBr} 2.95 (broad), 3.05, 3.30 (broad), 3.45 (broad), 3.75 (broad), 6.20, 6.30, 6.61, 6.70 μ.

Anal. Calcd. for $C_{19}H_{23}NO_2S$: C, 69.28; H, 7.04; N, 4.25; S, 9.71. Found: C, 69.38; H, 6.97; N, 4.24; S, 9.92.

Hydrolysis of 2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-acetoxypyrrolidine (XXIIa).—The acetoxy XXIIa (1.0 g.) was suspended in 25 ml. of 2 *N* sodium hydroxide solution. The mixture was warmed to dissolve the acetoxy compound, then refluxed for 2 hr. White needles, m.p. 172–173°, separated from the cooled reaction mixture; the yield was 500 mg. (60%). The needles were recrystallized twice from acetone: m.p. 172.5–173.5°, mixture melting point with an authentic sample of deacetylanisomycin (II) gave no depression; $[\alpha]^{25}D$ −20°, identical with II; infrared λ_{max}^{KBr} 3.0, 3.1, 6.22 μ, identical with the spectrum of deacetylanisomycin (II).

Hydrolysis of 2-*p*-Methoxyphenylmethyl-3-hydroxy-4-*trans*-methoxypyrrolidine.—The methoxy compound XXIV (R = OCH_3), 500 mg., was heated for 5 hr. with boiling 48% hydrobromic acid. Evaporation of the reaction mixture gave a brown solid which was dissolved in hot 2-propanol, treated with activated charcoal, filtered, and allowed to crystallize. 2-*p*-Hydroxyphenylmethyl-3,4-*trans*-dihydroxypyrrolidine hydrobromide was obtained (390 mg., 63%), m.p. 201–204°, identical with material obtained by a similar hydrolysis of anisomycin (I): infrared λ_{max}^{KBr} 2.96, 3.04, 6.23, 6.29, 6.34 μ.

Constituents of *Mammea americana* L. IV. The Structure of Mammeigin[1,2]

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Mammeigin, $C_{25}H_{24}O_5$, a new 4-phenylcoumarin isolated from the seed oil of *Mammea americana* L., is shown to be 5-hydroxy-6-isovaleryl-8,8-dimethyl-4-phenyl-2H,8H-benzo[1,2-*b*:3,4-*b'*]dipyran-2-one. This structural assignment is based not only on spectroscopic evidence, but also on a chemical interrelation of mammeigin with mammeisin (4-phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenylcoumarin). The isolation of mammeol, a diterpene alcohol, is also recorded.

Chemical examination of various parts of the mamey tree (*Mammea americana* L., family *Guttiferae*) received its impetus over a century ago in the report of de Grosourdy[4] on its insecticidal activity. A number of studies of this property of mamey seed extracts[5–8] culminated in the isolation[9] of a crystalline active principle, mammein, for which structure 1 was advanced[10] on the basis of degradative and spectroscopic[10,11] as well as synthetic evidence.[12] Subsequently, a second toxic[13] coumarin isolated from the fruit peelings was shown[14] to possess structure **2**, 4-phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenylcoumarin, later named mammeisin.[15] Recent papers from this laboratory have dealt with the constitution of mamey wax,[2b] and with the isolation of 2-hydroxyxanthone[2a]; the present article details the isolation and proof of structure of still another mamey oil constituent, mammeigin (**3**) (see Figure 1).

When mamey oil, from which the wax had been removed by precipitation with acetone,[2b] was chromatographed on aluminum oxide, a yellow semisolid material was eluted with a benzene–Skellysolve B (9:1) mixture. After an extensive series of recrystallizations from ether–hexane mixtures, a small amount of mammeigin, yellow needles, m.p. 144–146°, was obtained which gave microanalytical data for $C_{25}H_{24-26}O_5$. The phenolic hydroxyl group in mammeigin (**3**) was evident from the infrared absorption band at 3400 cm.$^{-1}$ (potassium bromide), the characteristic shift of the ultraviolet maxima in the presence of added alkali, and from the positive reaction (green color) with ferric chloride. The general similarity of the infrared and ultraviolet spectra of mammeigin to those of mammeisin,[14] in particular, the infrared bands at 773 and 708 cm.$^{-1}$ indicative of a monosubstituted phenyl group, allowed the supposition that we were dealing with a new phenolic 4-phenylcoumarin. This supposition received strong support from phytochemical considerations, since four of the six previously known

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(3) This article is based on a portion of a thesis presented by W. H. M. to the Department of Chemistry, The Ohio State University, inpartial fulfillment of the requirements for the Ph.D. degree, June 1964. Preliminary communication of this work has been made.[2c]

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