Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.34; H, 3.05; N, 28.26; S, 32.35. Found: C, 36.50; H, 3.28; N, 28.51; S, 32.58.

4-Amino-7-chloro-1-methylimidazo[4,5-*d*]pyridazine (XVIII).—4,7-Dichloro-1-methylimidazo[4,5-*d*]pyridazine (V, R = CH_3) (24.1 g., 0.119 mole) was treated with a solution of 20 ml. of liquid ammonia in 200 ml. of ethanol in a stainless steel autoclave at 150° for five hours. After cooling, the product was isolated by suction filtration and washed with water to remove ammonium chloride. This material was dissolved in 150 ml. of hot dilute hydrochloric acid, decolorized with Norit, and precipitated by the addition of 20% aqueous potassium hydroxide. The product was thus obtained as 11.7 g. (53%) of almost colorless microcrystals, m.p. 273–275° dec.

Anal. Calcd. for $C_6H_6ClN_5$: C, 39.25; H, 3.29; Cl, 19.31; N, 38.15. Found: C, 39.30; H, 3.30; Cl, 19.15; N, 38.32.

4-Amino-1-methylimidazo[4,5-*d*]pyridazine (XIX) and 7-Amino-1-methyl-imidazo[4,5-*d*]pyridazine (XX).—Sixteen grams (0.087 mole) of 4-amino-7-chloro-1-methylimidazo-[4,5-*d*]pyridazine (XVIII) was hydrogenated at 30 p.s.i. (55–60°) over 2.0 g. of palladium-on-charcoal catalyst (5%) in 200 ml. of glacial acetic acid containing 7.14 g. of sodium acetate. After hydrogen uptake was complete (19 hours), the mixture was filtered and evaporated to dryness *in vacuo*. The residue was taken up in 150 ml. of water, neutralized with sodium bicarbonate, and evaporated to about 75 ml. *in vacuo*. After standing for two days at 4°, the precipitate was filtered with suction and washed with a little cold water. Recrystallization of this material from water with Norit gave 0.5 g. (3.9%) of **7-amino-1-methylimidazo[4,5-*d*]pyridazine (XX)** as colorless needles, m.p. 318–319° dec.

Anal. Calcd. for $C_6H_7N_5$: C, 48.31; H, 4.73; N, 46.96. Found: C, 47.73; H, 4.67; N, 46.83.

The original aqueous mother liquor plus washings from above was evaporated to dryness *in vacuo* to leave 30.4 g. of product mixed with the inorganic salts. This solid was ground to a fine powder, extracted with 1000 ml. of boiling absolute ethanol for 15 minutes on the steam-bath and filtered while hot. After standing at 4° overnight, the precipitate of colorless needles was filtered with suction and recrystallized from N,N-dimethylformamide to obtain **4-amino-1-methylimidazo[4,5-*d*]-pyridazine (XIX)** as colorless long needles (5.7 g., 44%), m.p. 295–296° dec.

Anal. Calcd. for $C_6H_7N_5$: C, 48.31; H, 4.73; N, 46.96. Found: C, 48.04; H, 4.74; N, 46.76.

The solubility characteristics of these two compounds (XIX and XX) were markedly different; *e.g.*, XIX was quite soluble in hot N,N-dimethylformamide or in cold water, while XX was practically insoluble in boiling N,N-dimethylformamide and only slightly soluble in cold water.

A comparison of the ultraviolet absorption spectra of XIX and XX with those of 9-methyl- and 7-methyladenine will be found in Table III.

NORTH CHICAGO, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY AND THE RESEARCH LABORATORIES OF PARKE, DAVIS AND CO.]

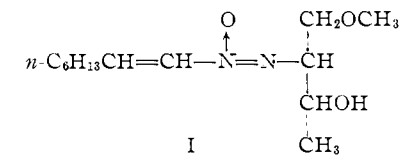
Elaiomycin.[1] An Aliphatic α,β-Unsaturated Azoxy Compound

BY CALVIN L. STEVENS, BERNARD T. GILLIS,[2,3] JAMES C. FRENCH[2] AND THEODORE H. HASKELL

RECEIVED JULY 1, 1958

The antibiotic Elaiomycin was shown to have the structure represented by formula I, containing the chemically unique aliphatic α,β-unsaturated azoxy group.

The antibiotic Elaiomycin was isolated from submerged culture filtrates of *Streptomyces hepaticus*.[4] The biological action and chemical structure of the antibiotic are unique. Elaiomycin, which was isolated as a distillable oil, has marked activity only against certain virulent and avirulent mammalian strains of tubercle bacilli[5] and is shown by this investigation to have the chemical structure I.



I

In a previous publication[6] I has been characterized as a stable, optically active oil, $C_{13}H_{26}N_2O_3$, which contained no ionizable groups in the *p*H range 2–10. The presence of one alkoxyl and two terminal methyl groups was indicated by analysis and compound I gave a positive iodoform test.

The hydrogen content of the molecular formula allowed only two double bonds and the ultraviolet absorption spectrum, with λ_{max} 237.5, ϵ 11,000, indicated the double bonds to be in conjugation.

The infrared spectrum[6] of I was relatively simple and had a weak absorption band at 6.03 μ, which was best interpreted as a carbon–carbon double bond. Any of the common functional groups which contain the carbon–oxygen or carbon–nitrogen double bond were excluded.[7] In addition, the infrared spectrum had a strong band at 2.92 μ which indicated an OH or possibly an NH group. The acetate derivative of I was prepared in 70% yield. This derivative II was shown to be a monoacetate, the infrared spectrum of which had no absorption in the 3 μ region. The absorption bands at 5.75 and 8.05 μ indicated an O-acetate and not an N-acetate and this evidence clearly showed Elaiomycin to have only one acylatable hydrogen incorporated in an hydroxyl group.

Elaiomycin absorbed two molar equivalents of hydrogen in alcohol solution in the presence of platinum catalyst to give a deoxydihydro product III.

(1) A preliminary communication appeared in THIS JOURNAL, **78**, 3229 (1956).

(2) Parke, Davis and Co. Fellow.

(3) Abstracted in part from the Doctoral thesis of Bernard T. Gillis, Wayne State University, April, 1956.

(4) L. E. Anderson, P. R. Burkholder, J. Ehrlich and H. S. Sun, *Antibiotics & Chemotherapy*, **6**, 100 (1956).

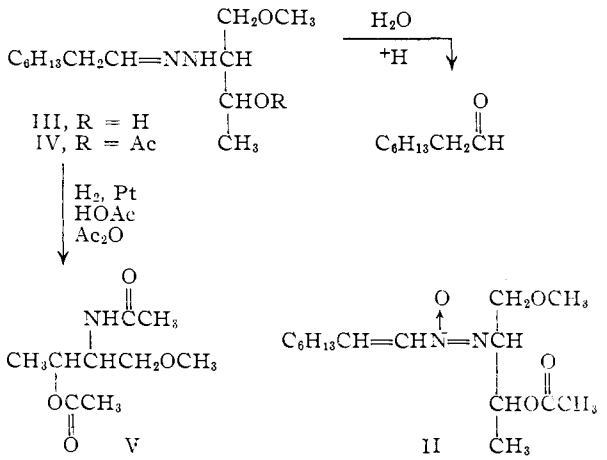
(5) The biologic studies of Elaiomycin have been reported by J. Ehrlich, L. E. Anderson, G. L. Coffey, W. H. Feldman, M. W. Fisher, A. B. Hillegas, A. G. Karlson, M. P. Kaudsen, J. K. Weston, A. S. Youmans and G. P. Youmans, *ibid.*, **4**, 338 (1954).

(6) The isolation and chemical characterization of Elaiomycin has been reported by T. H. Haskell, A Ryder and Q. R. Bartz, *ibid.*, **4**, 141 (1954). In this article the ultraviolet and the infrared spectra are reproduced and a discussion is presented of the homogeneity and purity of the product.

(7) An oxime of an aliphatic aldehyde or ketone was a possible exception. Heptaldehyde oxime had an absorption band, the intensity and position of which was similar to the 6.03 μ band of I.

This product had absorption in the 3 μ region of the infrared spectrum and the ultraviolet spectrum had λ_{max} 229, ϵ 5300. The rate of hydrogenation showed no change after the absorption of one equivalent. Of the two hydrogenations that occurred, the most significant one was that which caused the loss of a single atom of oxygen. One interpretation of this change would be the hydrogenolysis of the alcohol if it were, for example, in the allyl position to a double bond. In this interpretation the infrared absorption of III in the 3 μ region would be due to N–H formed from the reduction effected by the second equivalent of hydrogen. However, the acetate of I, which had λ_{max} 237.5, ϵ 11,000, also absorbed two equivalents of hydrogen under the same conditions to give a deoxydihydro *acetate* derivative IV, λ_{max} 230, ϵ 4700. These data prove that the hydrogenolysis did not involve the hydroxyl group. Rather the data strongly suggested the hydrogenolysis of an N-oxide. This N-oxide, along with the alcohol and the alkoxyl group, account for all of the oxygen atoms of the molecular formula.

The deoxydihydro derivative III of Elaiomycin and the corresponding acetate IV were basic as shown by the fact that they were immediately soluble in dilute acid solution. However, within a few seconds these products were hydrolyzed with the formation of the insoluble 1-octanal, which provided the carbon skeleton for the C_8-moiety.

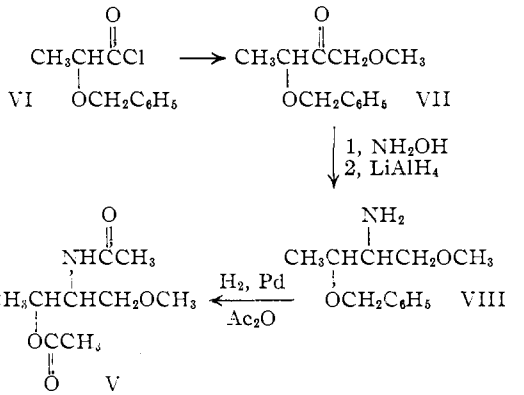


Elaiomycin absorbed four equivalents of hydrogen in acetic acid solution in the presence of platinum catalyst with the formation of *n*-octylamine and a C_5-amine, isolated as the crystalline acetate-amide V. The partially reduced derivative III absorbed two additional equivalents of hydrogen under these conditions to give the same mixture of amines. The fact that a molecule which has only two double bonds absorbs four equivalents of hydrogen requires two hydrogenolysis reactions. In addition to the N-oxide cleavage the formation of two amines showed that a nitrogen–nitrogen bond was cleaved and together with the N-oxide data clearly required an azoxy group in the original structure.

The structure of the C_5-moiety of Elaiomycin could be deduced from the following considerations. The fact that Elaiomycin is stable in mildly acidic aqueous solutions[6] means that no carbon atom in the C_5-moiety may be in the carbonyl state of oxidation; *i.e.*, a ketal, acetal, vinylamine or vinyl ether type of derivative would be hydrolyzed readily to the parent carbonyl compound. Therefore, the three functional groups, the alcohol, the alkoxyl and the azoxy group must be on different carbon atoms. Since the C_5-moiety must contain one terminal methyl group, these considerations eliminate alkoxyl groups higher than methoxyl and require the double bond be placed in the C_8-moiety.

Two pieces of evidence indicated that the OH group was attached to a carbon atom adjacent to that containing the nitrogen in the C_5-moiety. The extreme ease of dehydration of Elaiomycin accompanied by a shift of λ_{max} from 237.5 to 260 mμ suggested the hydroxyl group was in the β-position to the azoxy group. Further, after hydrogenation of Elaiomycin acetate to the deoxydihydro derivative IV, a migration of acetyl group from oxygen to nitrogen during an evaporative distillation was indicated by infrared spectra.

These considerations, together with the positive iodoform test for Elaiomycin, allowed the functional groups to be arranged only in the order $CH_3CHOHCH(N{=})CH_2OCH_3$. This arrangement was confirmed by the synthesis of a diastereoisomeric mixture of the acetate-amide V.



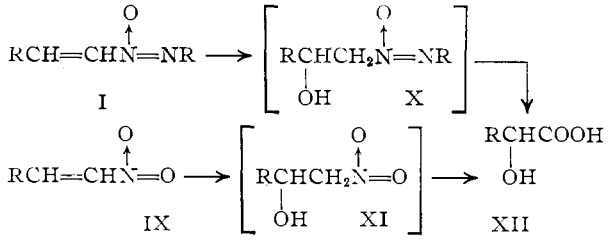
α-Benzyloxypropionyl chloride (VI) was prepared from the known acid in 93% yield and converted to the methoxymethyl ketone VII in 64% yield *via* the diazoketone. The oxime of VII was reduced with lithium aluminum hydride, debenzylated with a palladium-catalyzed hydrogenation and the product isolated as the acetylated acetate-amide V. The infrared spectrum of the synthetic V in chloroform solution was virtually identical with the spectrum of natural V taken in a similar solution. From the principles established by Curtin[8] and Cram,[9] the lithium aluminum hydride reduction of the oxime of VII would be expected to give a diastereoisomeric mixture in which the *threo* isomer would predominate.

The final step in the structural determination was the assignment of the position of the N-oxide oxygen. Acid hydrolysis of Elaiomycin gave 54% yield of racemic α-hydroxyoctanoic acid. An α,β-unsaturated nitro compound IX, which is the oxygen analog of I, is known to undergo the same reac-

(8) D. Y. Curtin, E. E. Harris and E. K. Meislich, THIS JOURNAL, **74**, 2901 (1952).

(9) D. J. Cram and F. A. Abd Elhafez, *ibid.*, **74**, 5828 (1952).

tion.[10] Each of these reactions is considered to first involve the acid-catalyzed addition of water to give the β-hydroxy compounds X and XI. Compound XI is a primary aliphatic nitro compound, of which the conversion to a carboxylic acid in acid solution is common.[11] By analogy, the conversion of X to a carboxylic acid in acid solution is interpreted to involve similar oxidation of the primary carbon adjacent to a quaternary nitrogen atom[12] and thus require the N-oxide oxygen to be attached to the nitrogen adjacent to the double bond in I.



The same conclusion results from an analysis of the spectral data. In Fig. 1 a comparison of the aromatic-aliphatic azoxy compounds[13] shows that

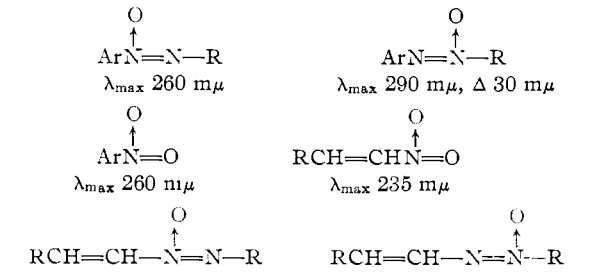


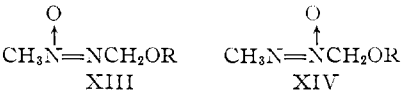
Fig. 1.—Ultraviolet absorption of nitro compounds and the corresponding azoxy compounds.

the aromatic nitro compound and its nitrogen analog have maximum absorption at about the same wave length (λ_{max} *ca.* 260 mμ) and that the isomeric azoxy compound, with longer conjugation, absorbs at significantly longer wave length (λ_{max} *ca.* 290 mμ, Δ 30 mμ). The fact that Elaiomycin has a maximum absorption at essentially the same wave length as the α,β-unsaturated nitro compound indicates that it is in fact the nitrogen analog. The isomeric α,β-unsaturated azoxy compound would be expected to have a longer conjugated system and absorb at a significantly longer wave length.

The isolation of racemic α-hydroxyoctanoic acid confirms the placement of the double bond in the C_8-moiety and with the assignment of the N-oxide oxygen to the nitrogen adjacent to this double bond completes the determination of the structure of Elaiomycin I.

The deoxydihydro derivative III and the corresponding acetate IV are formulated as the aliphatic hydrazones and not as the corresponding azo compounds because of spectral evidence. Compounds III and IV had λ_{max} of 229 and 230 mμ and ε of 5300 and 4700, respectively, which values were comparable to those of a model hydrazone,[14] $C_6H_{13}C(CH_3)=NNHCH_3$, λ_{max} 229, ε 5300. An aliphatic azo compound has been reported to absorb at higher wave length and lower intensity,[15] λ_{max} 350, ε *ca.* 10.

Macrozamin, an unsymmetrical aliphatic azoxy compound of plant origin, has been studied by Lythgoe and co-workers,[12] who regard structure XIII (R = primeverosyl) as very probably correct, although they indicated the desirability of verification of the position of the N-oxide by hydrolysis of an isomeric pair of primary azoxy compounds. The spectral analysis of Elaiomycin is considered by the present authors to provide sufficient evidence for the position of the N-oxide oxygen atom and thus Elaiomycin represents an unsymmetrical azoxy compound which upon hydrolysis is oxidized at the methylene carbon adjacent to the quaternary nitrogen. These data support structure XIII instead of structure XIV for Macrozamin. However, Elaiomycin is a derivative of a primary–secondary azoxy compound and the possibility that the isomeric azoxy compound will give the same products has not been eliminated.



The glucoside corresponding to Macrozamin has been isolated by two groups of workers.[16] Also, the antibiotic Hydroscopin A, which was reported in Japan,[17a] may be even more closely related to Elaiomycin although no structural work has been presented. Very recently a Japanese group[17b] also has reported the isolation of Elaiomycin.

Experimental

Elaiomycin Acetate (II).—To 1.65 g. of Elaiomycin, λ_{max} 237.5 mμ, ε 11,000, was added 10 ml. of cold redistilled acetic anhydride. One drop of 72% perchloric acid was added and the reaction mixture was stored at 5° for 9 hours. The light amber reaction mixture then was poured onto 50 ml. of ice-water and permitted to stand 12 hours at 5°. Sodium carbonate was added until the mixture was distinctly basic to litmus. The oily mixture was extracted three times with hexane and the combined hexane extracts were dried over sodium sulfate. After the hexane solution was concentrated, the residue was transferred to a Hickman still and distilled to yield 1.35 g. (70.3%) of Elaiomycin acetate (4-methoxy-3-(1-octenyl-*NON*-azoxy)-2-butanol acetate)[18] as a colorless oil, b.p. 84–90° (0.5 μ), λ_{max} 237.5, ε 11,000; $[\alpha]^{27}D$ +25.3° (3.08% in absolute alcohol).

Anal. Calcd. for $C_{15}H_{25}N_2O_4$: C, 59.97; H, 9.39. Found: C, 60.28; H, 9.43.

Treatment of the antibiotic with *p*-nitrobenzoyl chloride and pyridine gave only *p*-nitrobenzoic anhydride, the result of dehydration. Attempted benzoylation with benzoyl chloride and pyridine gave benzoic anhydride.

(10) R. L. Heath and J. D. Rose, *J. Chem. Soc.*, 85 (1947).

(11) H. B. Hass and E. F. Riley, *Chem. Revs.*, **32**, 395 (1943); W. E. Noland, *ibid.*, **55**, 151 (1955).

(12) B. W. Langley, B. Lythgoe and L. S. Rayner, *J. Chem. Soc.*, 4191 (1952).

(13) J. N. Brough, B. Lythgoe and P. Waterhouse, *ibid.*, 4069 (1954).

(14) Recently P. A. S. Smith and E. E. Most, Jr., *J. Org. Chem.*, **22**, 358 (1957), have reported aliphatic hydrazones from dimethylhydrazine.

(15) C. G. Overberger, M. T. O'Shaughnessy and H. Shalet, THIS JOURNAL, **71**, 2661 (1949); Lythgoe, *et al.*, ref. 12.

(16) N. V. Riggs, *Chemistry & Industry (London)*, 926 (1956); K. Nishida, A. Kobayashi and T. Nagahama, *Bull. Agri. Chem. Soc. Japan*, **19**, 77 (1955).

(17) (a) K. Nakazawa, *et al.*, *J. Antibiotics (Japan)*, **7**, 329 (1954); (b) S. Yamashita, *et al.*, *ibid.*, **10**, 224 (1957).

(18) In consultation with the Editors of "Chemical Abstracts," Elaiomycin was named 4-methoxy-3-(1-octenyl-*NON*-azoxy)-2-butanol; *cf.* the system adopted in ref. 13.

An ethereal solution of the antibiotic was saturated with dry hydrogen chloride gas and allowed to stand for 15 minutes at room temperature. Evaporation of the solvent gave an oil, λ_{max} 260 mμ.

Deoxydihydroelaiomycin (III).—Hydrogenation of 1.2825 g. (4.97 millimoles) of Elaiomycin which had been dissolved in 35 ml. of absolute ethanol and to which had been added 0.3 g. of prereduced platinum oxide resulted in the rapid uptake of 1.9 equivalents of hydrogen. Filtration of the solution and removal of the ethanol under vacuum left a colorless oil which was transferred to a Hickman still and distilled at 4 μ pressure. From the distillation was obtained 0.96 g. (79.3%) of deoxydihydroelaiomycin, b.p. 70–80° (4 μ); λ_{max} 229, ϵ 5300; $[\alpha]^{25}D$ +3.7° (5% in chloroform).

Anal. Calcd. for $C_{13}H_{23}N_2O_2$: C, 63.89; H, 11.55; N, 11.46. Found: C, 63.31; H, 11.44; N, 11.87.

Acid Hydrolysis of Deoxydihydroelaiomycin.—When 0.115 g. of Elaiomycin was placed in 8 ml. of 10% sulfuric acid, solution was effected, followed by cloudiness and finally appearance of an oil whose odor was that of an aldehyde. The mixture was shaken and made more acidic with additional sulfuric acid. Ether was added, and the oil was extracted. Evaporation of the ether and then addition of 2,4-dinitrophenylhydrazine solution caused an immediate heavy precipitate of a 2,4-dinitrophenylhydrazone. The solution was cooled to 0° and filtered to give a solid, m.p. 94–99°. Recrystallization from ethanol–water gave needles, m.p. 106–107°. The ultraviolet and infrared spectra of this compound were superimposable with octanal 2,4-dinitrophenylhydrazone and a mixture melting point determination with authentic octanal 2,4-dinitrophenylhydrazone was not depressed.

Anal. Calcd. for $C_{14}H_{20}N_4O_4$: C, 54.59; H, 6.53. Found: C, 54.39; H, 6.87.

Hydrogenation of Elaiomycin Acetate.—Elaiomycin acetate (0.85 g., 3.28 millimoles) was reduced in absolute ethanol with 0.2 g. of prereduced platinum oxide catalyst. The hydrogenation resulted in the rapid uptake of 2.15 equivalents of hydrogen. After filtration and concentration of the solution, the residual material was distilled from a Hickman still. The first fraction amounted to 0.1 g., b.p. 63–67° (0.5 μ), the infrared spectrum of which had strong absorptive bands at 5.73 and 8.03 μ indicating that the fraction consisted mainly of the deoxydihydroelaiomycin O-acetate (IV). The second fraction, b.p. 75–83° (0.5 μ), amounted to 0.2 g. The third fraction, b.p. 79–83°, amounted to 0.05 g. and the infrared spectrum, which had a weak band at 5.73 and a strong band at 6.1 μ, indicated the fraction consisted mainly of rearranged deoxydihydroelaiomycin N-acetate, the product of O to N acyl migration. The total yield was 0.35 g. (35%), λ_{max} 230 mμ, ϵ 4700.

Anal. Calcd. for $C_{15}H_{30}N_2O_3$: C, 62.46; H, 10.48; N, 9.71. Found: C, 62.42; H, 10.57; N, 9.53.

Two drops of the first fraction were immediately soluble in 3 *N* hydrochloric acid. After a few seconds the solution became turbid and treatment with dinitrophenylhydrazine reagent gave the derivative of 1-octanal, m.p. 106–107°, undepressed when mixed with an original sample.

Hydrogenolysis of Elaiomycin.—Hydrogenation of 1.1487 g. (4.4 millimoles) of Elaiomycin in glacial acetic acid with 0.3 g. of prereduced platinum oxide catalyst resulted in the uptake of four equivalents of hydrogen. Upon filtration of the catalyst, the solution was heated with 30 ml. of acetic anhydride at reflux for 3 hours, after which the acetic acid and excess acetic anhydride were removed under vacuum. The residue was distilled from a Hickman still; fraction 1, b.p. 85° (1.0 μ), 0.17 g., consisted of the acetate amide V with a small amount of unacetylated alcohol. Fraction 2, b.p. 85–90° (0.5 μ), 0.22 g., had approximately the same composition as fraction 1. Fraction 3, b.p. 90° (0.3 μ), 0.16 g., was crystalline acetate amide V, which was recrystallized from petroleum ether, m.p. 77–77.5°. The infrared spectrum showed an NH, ester, amide, monosubstituted amide, C-CH_3, and an ether band, $[\alpha]^{25}D$ −10.5° (5% in chloroform). Later 0.178 g. of pure acetate amide was isolated from the undistilled pot residue by extraction with water.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.18; H, 8.43; N, 6.89; OCH_3, 15.27. Found: C, 53.23; H, 8.52; N, 7.31; OCH_3, 14.90.

The pot residue that was insoluble in water was hydrolyzed with 20% hydrochloric acid for two hours at the reflux temperature. The excess acid was removed under reduced pressure and the resulting crude octylamine hydrochloride was dissolved in water and liberated with a 10% sodium hydroxide solution. The amine was extracted with ether, the ether was removed, and the residue was treated with an alcohol solution of ethyl oxalate. After 30 minutes on the steam-bath, the alcohol solution was concentrated, from which the crystalline oxamide derivative formed, m.p. 124–125°. A mixture melting point determination with authentic N,N′-di-(*n*-octyl)-oxamide[19] was undepressed.

After 2.26 g. of deoxydihydroelaiomycin had been reduced under the same conditions, the hydrogenation mixture was separated to give 0.7 g. (37%) of pure acetateamide V, m.p. 76–77°, and 0.6 g. (38%) of crude N-octylacetamide, which was converted to the crystalline N,N′-dioctyloxamide, m.p. 124–125°. The oxamide derivative was identical with the authentic sample.

Acid Hydrolysis of Elaiomycin.—After 2.75 g. (0.01065 mole) of Elaiomycin, 10 ml. of acetone and 10 ml. of 6 *N* hydrochloric acid were heated under reflux for 14 hours, the mixture was evaporated to 10 ml. under reduced pressure. The heavy oil and water mixture then was treated with 15 ml. of water and extracted three times with ether. The combined ether extracts were extracted with two 20-ml. portions of 4% sodium carbonate solution. The sodium carbonate solution was acidified with hydrochloric acid, after which an oil precipitated. The aqueous mixture containing the oil was extracted three times with ether and the combined ether extracts were dried over anhydrous sodium sulfate. Evaporation of the solution left a colorless oil which was placed under 0.2 mm. of pressure for 2 hours. The resultant solid residue was recrystallized from pentane. The first crop of crystals amounted to 0.5 g. and melted at 69.5–70.5°. The second crop amounted to 0.13 g. and melted at 67–68.5°. The mother liquor contained an additional 0.3 g. The total yield was 0.93 g. (54%) of α-hydroxyoctanoic acid; infrared bands at 2.86 and 5.81; pK_a' in 50% ethanol, 4.95. A mixture melting point determination with an authentic sample of α-hydroxyoctanoic acid, m.p. 69–69.5°, was not depressed, m.p. 68–69.5°. Titration of the acid showed a molecular weight of 156 (calcd. 160).

The authentic sample of racemic α-hydroxyoctanoic acid was prepared by acid hydrolysis of the cyanohydrin of heptaldehyde.

α-Benzyloxypropionyl Chloride.—To 327 g. (2.75 moles) of thionyl chloride was added with stirring 165.3 g. (0.917 mole) of O-benzyl-D,L-lactic acid.[20] The reaction was stirred overnight and then heated gently with stirring. The excess thionyl chloride was removed under reduced pressure and the remaining liquid distilled. The yield was 168 g. (93.3%) of α-benzyloxypropionyl chloride,[20] b.p. 78° (0.5 mm.), $n^{25}D$ 1.5080, d^{25} 1.1318. An anilide derivative[20] was prepared from this material and melted at 74.5–75°.

The Diazoketone of α-Benzyloxypropionyl Chloride.—To an ethereal solution of excess diazomethane made from 103 g. of wet N-nitroso-N-methylurea and 200 ml. of 50% potassium hydroxide, was added 49.66 g. (0.25 mole) of α-benzyloxypropionyl chloride over a period of 3.5 hours. The excess diazomethane and ether were removed by water aspirator to leave a light yellow oil, which was distilled at very low pressure. The yield was 46.9 g. (92%) of the diazo ketone, b.p. 55° (0.02 mm.), $n^{25}D$ 1.5082, d^{25} 1.085.

1-Methoxy-3-benzyloxybutanone-2.—The diazoketone (37.1 g., 0.1816 mole) in excess methanol and cooled to 0° was treated with 1 ml. of boron trifluoride-etherate.[21] Nitrogen was evolved smoothly during a period of 4.5 hours. The methanol was removed under reduced pressure and the residue was washed with saturated sodium carbonate, decolorized with charcoal and filtered. Removal of the ether and distillation yielded 26 g. (70%) of the methoxy benzyloxy ketone, b.p. 65° (1 μ), $n^{25}D$ 1.5005, d^{25} 1.078.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.20; H, 7.52.

(19) L. M. Rice, C. H. Grogan and E. E. Reid, THIS JOURNAL, **75**, 242 (1953).

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1-Methoxy-2-amino-3-benzyloxybutane.—The methoxy benzyloxy ketone (13.8 g., 0.066 mole) was treated with 7 g. (0.1 mole) of hydroxylamine hydrochloride, 4.1 g. (0.1 mole) of sodium hydroxide and sufficient ethanol to effect solution. The reaction was heated for 2 hours on a steam-bath, most of the ethanol was evaporated and the remainder of the reaction poured into water. The mixture was extracted with ether and the ether was evaporated and the resulting oil dried under vacuum and then dissolved in dry ether. To this ether solution was added 12.5 g. (0.33 mole) of lithium aluminum hydride. The reduction mixture was then heated with stirring for 7 hours, after which time the excess lithium aluminum hydride was destroyed with water. After a solution of Rochelle salt was added, the layers were separated and the aqueous layer was extracted four times with ether. The combined ether layers were dried over sodium sulfate and the ether was reduced in volume to 200 ml. and then extracted three times with 75-ml. portions of 3 *N* hydrochloric acid. The acid solutions were made basic with sodium hydroxide and the aqueous solution was then extracted three times with 300-ml. portions of ether. The ether solutions were dried over potassium hydroxide pellets, and the ether removed. The remaining material was distilled to yield 8.18 g. (59%) of the amine, b.p. 80° (0.4 mm.), $n^{25}D$ 1.5008, d^{25} 1.014, pK_a' (50% methanol) 8.3, mol. wt. by titration 208 (calcd. 209).

Anal. Calcd. for $C_{12}H_{19}NO_2$: C, 68.87; H, 9.15; N, 6.68. Found: C, 69.08; H, 8.95; N, 6.91.

1-Methoxy-2-acetamide-3-acetoxybutane.—The debenzylative acetylation of 1.56 g. (7.45 millimoles) of the above amine was carried out in acetic anhydride and acetic acid using 0.4 g. 10% palladium-on-charcoal catalyst. After uptake of 0.94 equivalent of hydrogen the reaction mixture was filtered and heated on the steam-bath for 2 hours. Distillation gave 1.01 g. (66%) of the synthetic acetate-amide, whose infrared spectrum had the same bands as those of the natural acetate-amide from Elaiomycin, b.p. 91° (0.1 μ), $n^{25}D$ 1.4543.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.18; H, 8.43; N, 6.89. Found: C, 53.28; H, 8.36; N, 7.08.

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[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND CHEMISTRY, UNIVERSITY OF WISCONSIN]

Separation and Preliminary Characterization of Oligomycins A, B and C[1]

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The antibiotic complex, oligomycin, has been separated into three biologically active components A, B and C. One crystalline preparation contained 67% A, 13% B and 20% C, another 20% A, 80% B and no C. The components were homogeneous and analyzed for the formulas: A, $C_{24}H_{40}O_6$; B, $C_{22}H_{36}O_6$; C, $C_{28}H_{46}O_6$. The oligomycins are neutral, unsaturated, optically active alcohols, soluble in many organic solvents but very insoluble in petroleum ether and water. Comparison of the physical, chemical and biological properties indicates that, while there are differences, the three antibiotics are closely related in structure.

In 1953 a crystalline antibiotic of marked antifungal potency was obtained from culture filtrates of a streptomyces similar to *Streptomyces diastatochromogenes*.[3] Two components were detected in the crystals by paper chromatography and the major one, called A, was obtained by column chromatography substantially free of the minor component. Many of the chemical, physical and biological properties of component A were determined. The antibiotic had no activity toward bacteria but was markedly active against certain fungi, particularly against the human pathogen, *Blastomyces dermatitidis*. Because of this limited activity, the antibiotic complex was named oligomycin.

Later work by Halliday[4] showed that the mycelium contained more oligomycin than the culture filtrate, and, if the *p*H of the medium was controlled, practically all of the antibiotic was retained in the mycelium. All subsequent isolations have been made from the mycelium. By means of selected cultures, improved media and control of aeration, Visser[5] obtained yields of from 1 to 1.3 g. per liter of oligomycin in the broth. Visser also worked out an isolation procedure for pilot plant fermentations and thereby obtained several hundred grams of crystalline oligomycin some of which has been used in this work.

Paper chromatograms showed the crystals contained a predominance of the A component but also revealed the presence of significant amounts of two other components, which later were designated oligomycins B and C. Details on the fermentation and isolation procedures will be published elsewhere. Marty[6] investigated the preparative separation of the three components and provided the basis for a partition chromatographic method which has proved very successful.

In the present study each of the three components has been obtained in sufficient quantity to permit preliminary characterization. Their homogeneity has been established by paper chromatography, constancy of rotations and melting point behavior. Analytical data on the recrystallized compounds and on several derivatives thereof indicate that the molecular formulas are A, $C_{24}H_{40}O_6$; B, $C_{22}H_{36}O_6$; and C, $C_{28}H_{46}O_6$. Oligomycins A and B both exist in polymorphic forms of distinctly different melting points. Although the three com-

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(2) Postdoctorate project associate on a Fulbright travel grant from the University of Delhi, India.

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