corresponding diamine which reacted with formic acid to give II (calcd. for $C_9H_{10}N_2O$: C, 66.65; H, 6.22; N, 17.28. Found: C, 66.89; H, 5.99; N, 17.40). A mixed melting point of the isolated and synthetic compounds was not depressed. Therefore, I was a 5-hydroxybenzimidazole glycoside.

The available knowledge made it seem likely that the Factor III nucleoside, like α-ribazole,[6] is an α-D-ribofuranoside. For comparison, two methoxybenzimidazole ribofuranosides were synthesized. Reaction of the chloromercuri derivative of 5-methoxybenzimidazole (which probably was a mixture of 1-chloromercuri-5- and -6-methoxybenzimidazoles) with triacetyl-α-D-ribofuranosyl chloride followed by deacetylation gave two products which were purified by countercurrent distribution in a butanol–water system and separated as their picrates (III) m.p. 144–146° and (IV) 78–82° (calcd. for $C_{19}H_{19}N_5O_{12}$: C, 44.80; H, 3.76; N, 13.75. Found: (III) C, 44.74; H, 3.40; N, 13.88; (IV) C, 44.36; H, 3.94; N, 13.58). Regeneration of the bases by use of IR-45 resin gave from II a crystalline base (V), m.p. 162–164°, $[\alpha]^{22}D$ −33°, and from III a non-crystalline base (VI), $[\alpha]^{21}D$ −36°. The nearly equal $[\alpha]D$ values indicate the same glycosidic configuration and therefore a difference in the position of the methoxy group. The positions of these groups were determined by the method described above. From V was obtained 1-methyl-6-methoxybenzimidazole and from VI, 1-methyl-5-methoxybenzimidazole,[7] m.p. 110–113°. Therefore V was a 5-methoxybenzimidazole riboside and VI was the 6-methoxy isomer.

Demethylation of V with 48% hydrobromic acid gave a non-crystalline product (VII) which was purified by paper chromatography (R_f 0.47), $[\alpha]^{25}D$ about −59°. The ultraviolet absorption spectra of VII and I were nearly identical. If the assumption that I is a D-ribofuranoside is correct, it is the α-form and the synthetic compound VII is the β-form.

The similarities in the behavior of the natural hydroxy- and methoxybenzimidazole glycosides with that of the corresponding synthetic ribosides lend support to the postulate that I is an α-D-ribofuranoside. Studies are now in progress to obtain further evidence concerning structure.

(6) N. G. Brink and K. Folkers, THIS JOURNAL, **74**, 2856 (1952).
(7) A. M. Simonov and P. A. Uglov, *J. Gen. Chem.*, **21**, 884 (1951).

CONTRIBUTION FROM THE MERCK, SHARP & DOHME RESEARCH LABORATORIES DIVISION OF MERCK & CO., INC. RAHWAY, NEW JERSEY
CLIFFORD H. SHUNK FRANKLIN M. ROBINSON JAMES F. MCPHERSON MARJORIE M. GASSER KARL FOLKERS
RECEIVED JUNE 5, 1956

THE STRUCTURE OF ELAIOMYCIN, A TUBERCULOSTATIC ANTIBIOTIC

Sir:

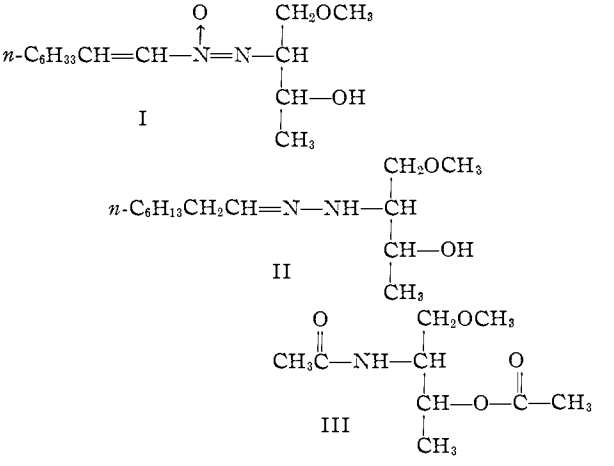
Structure I is proposed for the antibiotic Elaiomycin.[1] This antibiotic is chemically unique, since to our knowledge the chromophore represented by the *aliphatic α,β-unsaturated azoxy* group has not previously been reported.[2]

The previous characterization indicated I as an optically active, neutral oil, $C_{13}H_{26}N_2O_3$, with one alkoxyl and two terminal methyl groups. The hydrogen content allowed two double bonds and the ultraviolet spectrum (λ_{max} 237.5, ϵ = 11,000) showed them in conjugation.

The infrared spectrum of I indicated an –OH and/or possibly an –NH. The infrared spectrum of the monoacetate derivative, b.p. 84–90° (0.5 μ); λ_{max} 237.5, ϵ = 11,000; $[\alpha]^{27}D$ +25.3°, 3% in ethanol (calcd. for $C_{15}H_{28}N_2O_4$: C, 59.97; H, 9.39. Found: C, 60.28; H, 9.70), which had no absorption in the 3 μ region, indicated an acetate ester and thus confirmed the presence of the only acetylatable hydrogen as an –OH.

I and the acetate of I absorbed two moles of hydrogen (Pt, alcohol) to give II,[3] b.p. 70–80° (4 μ); λ_{max} 229, ϵ = 5300; $[\alpha]^{25}D$ +3.7°, 5% in chloroform (calcd. for $C_{13}H_{28}N_2O_2$: C, 63.89; H, 11.55; N, 11.46. Found: C, 63.31; H, 11.44; N, 10.80), and the acetate of II, b.p. 63–67° (0.5 μ); λ_{max} 230, ϵ = 4700 (calcd. for $C_{15}H_{30}N_2O_3$: C, 62.46; H, 10.48; N, 9.71. Found: C, 62.40; H, 10.60; N, 9.53).

The facile reductive loss of a single oxygen atom indicated an N-oxide, which, with the alcohol and alkoxyl groups, accounted for the three oxygen atoms. II and the acetate of II were easily hydrolyzed to *n*-octanol, providing the skeleton of the C_8 moiety.



The fact that in the acetic acid I absorbed four moles of hydrogen (Pt) or II absorbed two moles of hydrogen with the formation of two amines showed that the two fragments were joined in the original via the two nitrogen atoms and clearly indicated an azoxy group. The amines were identified as *n*-octylamine and a C_5 amine, isolated as the completely acetylated acetate-amide III, b.p. 85–90°

(1) T. H. Haskell, Q. Bartz, *et al.* [*Antibiotics and Chemotherapy*, **4**, 141, 338 (1954)] described the isolation, purification, spectra, chemical characterization, and biologic studies of Elaiomycin [4-methoxy-3-(1-octenyl-*NON*-azoxy)-2-butanol].

(2) Recently the plant poison, Macrozamin, has been shown by Lythgoe [*J. Chem. Soc.*, 2311 (1951)] to contain an asymmetrical aliphatic azoxy group. Hydroscopin A [*J. Antibiotics (Japan)*, **7**, 329 (1954)] may also be similar although no structural work has been reported.

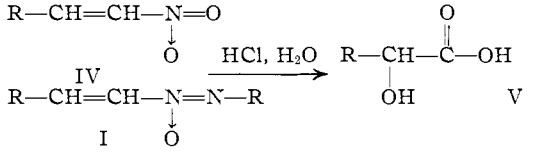
(3) II was formulated as an aliphatic hydrazone since the ultraviolet and infrared spectra differed from those of an azo compound but resembled those of a model compound C_6H_{13}—$C(CH_3)$=N—NH—CH_3 (λ_{max} 229, ϵ = 5300).

(0.3 μ); m.p. 77–77.5°; $[\alpha]_D$ −10.5°, 5% in chloroform (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, 15.27).

The extreme ease of dehydration of Elaiomycin accompanied by a shift of λ_{max} from 237.5 mμ to 260 mμ, along with infrared spectral evidence of an O to N acyl migration during the evaporative distillation of II-acetate, indicated the –OH to be on a carbon atom adjacent to that containing the nitrogen in the C_5 moiety.

Since only one C–CH_3 group could be in the C_5 moiety and since Elaiomycin gave a positive iodoform test, the C_5 fragment was constructed as CH_3—CHOH—CH(N=)CH_2OCH_3, and this structure was confirmed by the synthesis of a diastereoisomeric mixture of the acetate-amide III, the infrared spectrum of which was essentially identical to that of III.

The assignment of the N-oxide oxygen atom is based on aqueous acid hydrolysis of I to α-hydroxyoctanoic acid (V, R = n-C_6H_{13}) in 54% yield. We believe the carbon adjacent to the quaternary nitrogen to be oxidized[4] as is the oxygen analog IV under the same conditions.[5] Also, the ultraviolet curve of I (λ_{max} 237.5) is similar to that of IV[6] (λ_{max} 235) while the alternate structure, >C=C—N=N(→ O)–, would be expected to absorb at a longer wave length.[7] This work con-



clusively places the carbon to carbon double bond in the C_8 moiety, allows the assignment of the N-oxide oxygen to the nitrogen atom nearest the double bond, and completes that portion of the structure proof based on degradation experiments.

(4) J. N. Brough, B. Lythgoe and P. Waterhouse, *J. Chem. Soc.*, 4069 (1954).

(5) R. L. Heath and J. D. Rose, *ibid.*, 85 (1947).

(6) E. A. Braude, E. R. H. Jones and G. G. Rose, *ibid.*, 1104 (1947).

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

DEPARTMENT OF CHEMISTRY C. L. STEVENS
WAYNE UNIVERSITY B. T. GILLIS
PARKE, DAVIS AND COMPANY J. C. FRENCH
DETROIT 1, MICH. T. H. HASKELL

RECEIVED APRIL 20, 1956

CONVERSION OF ACETATE AND GLYOXYLATE TO MALATE[1]

Sir:

An enzyme has been obtained from acetate-grown *Escherichia coli*, strain E-26, which converts equimolar concentrations of acetate and glyoxylate to malic acid. This enzyme has been tentatively designated as *malate synthetase*.

Purification of malate synthetase by several ammonium sulfate precipitations, calcium phosphate gel and protamine sulfate treatments, resulted in a 50-fold purification with an approximate 30% yield (Table I). These preparations were found to be free of fumarase as measured by the sensitive spectrophotometric method of Racker.[2] Experiments conducted with these fumarase-free preparations revealed that for each μmole of acetyl phosphate and glyoxylate disappearing, one μmole of malate is formed (Table II). In addition to the substrates, transacetylase,[3] coenzyme A, magnesium ions and cysteine were added. In the absence of acetyl phosphate, coenzyme A, glyoxylate, transacetylase, malate synthetase or boiled enzyme controls, no malate is formed.

TABLE I

PURIFICATION OF MALATE SYNTHETASE

The complete assay system contained 10 μmoles of acetyl phosphate, 2 μmoles of cysteine, 10 μmoles of $MgCl_2$, 15 μg. of coenzyme A, 25 μmoles of potassium glyoxylate, 10 units of transacetylase, 30 μmoles of TRIS buffer, *p*H 8.0 and enzyme, final volume, 3.0 ml.; incubation time, 15 min.; temperature, 33°.

	Fraction	Total volume, ml.	Units[a]/ml.	Total units	Protein, mg./ml.	Specific activity, units/mg./protein	Recovery, %
1	Crude extract	50.0	21.6	1890	26.7	0.81	100
2	$(NH_4)_2SO_4$ fraction (0.35–0.70 saturation)	29.0	53.6	1555	27.9	1.92	82
3	Pr–SO_4 supernatant[b]	33.5	42.6	1426	11.7	3.64	76
4	Calcium phosphate gel[c] (supernatant)	68.0	10.7	728	0.52	20.58	38
5	$(NH_4)_2SO_4$ fraction (0.40–0.60 saturation)	33.0	17.9	581	0.47	37.90	31

[a] A unit is defined as the amount of enzyme required to catalyze the disappearance of 1 μmole of acetylphosphate per 15 min. under standard conditions. [b] 0.1 mg. protamine sulfate per mg. protein at *p*H 6.7. [c] 1.5 mg. $Ca_3(PO_4)_2$ gel added per mg. protein at *p*H 5.0.

TABLE II

STOICHIOMETRY OF THE MALATE-SYNTHETASE REACTION

The reaction mixture contained 15 μmoles of acetyl phosphate, 15 μmoles of potassium glyoxylate, 30 μmoles of TRIS buffer, *p*H 8.0, 20 μmoles of $MgCl_2$, 2 μmoles of cysteine, 25 μg. of coenzyme A, 10 units of transacetylase, and 260 μg. of enzyme; final volume, 2.0 ml.; temperature of reaction 33°; incubation time, 30 min.

Expt.	Acetyl phosphate, μmoles Initial	Final	Δ	Glyoxylate, μmoles Initial	Final	Δ	Malate formed, μmoles
1	15.00	7.72	7.28	15.00	6.45	8.55	7.56
2	15.00	7.84	7.16	15.00	6.85	8.15	7.33

Malate was routinely estimated by the fluorometric method of Hummell.[4] In large scale ex-

(1) A preliminary report of part of this investigation was presented at the national meetings of the Society of American Bacteriologists at Houston, Texas, and a summary published in *Bact. Proc.*, pp. 111 (1956).

(2) E. Racker, *Biochim. et Biophys. Acta*, **4**, 20 (1950).

(3) The source of transacetylase used in the assay system was a partially purified preparation from *E. coli*, American Type Culture Collection No. 4157, free of fumarase and malate synthetase enzymes.

(4) J. P. Hummell, *J. Biol. Chem.*, **180**, 1225 (1949).