dryness under reduced pressure. The residual oil was distilled at 5 mm. to give three fractions.

Fraction three was analyzed.

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.29; H, 7.89.

(e) (−)-α-Methyllevulinic Acid 2,4-Dinitrophenylhydrazone (XV).—A solution of 200 mg. (0.73 mmoles) of (+)-α-methyllevulinic acid *d*-tartramazone in 5 ml. of 25% sulfuric acid by volume was heated on the steam-bath for a few minutes. The cooled reaction mixture was extracted with chloroform which had been washed with sodium bisulfite solution. After the combined chloroform extracts had been dried over magnesium sulfate, the chloroform was removed by evaporation under reduced pressure. The residual oil, 45 mg. (47%), was added to 120 ml. of Brady reagent, and the solution was allowed to stand overnight at room temperature. The resulting crystalline precipitate was removed by filtration, washed with water and air dried, yield 100 mg., m.p. 187–191°. After two recrystallizations from absolute ethanol the optical rotation was $[\alpha]^{25}D$ −44.5° (*c* 1.19, acetic acid). The X-ray diffraction pattern was identical with that of a derivative obtained from C_{12}-dilactone XI.

Anal. Calcd. for $C_{12}H_{14}N_4O_6$: C, 46.45; H, 4.55; N, 18.06. Found: C, 46.76; H, 4.61; N, 17.85.

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Erythromycin. XI.[1] Structure of Erythromycin B[2]

BY PAUL F. WILEY, MAX V. SIGAL, JR., OLLIDENE WEAVER, ROSMARIE MONAHAN AND KOERT GERZON

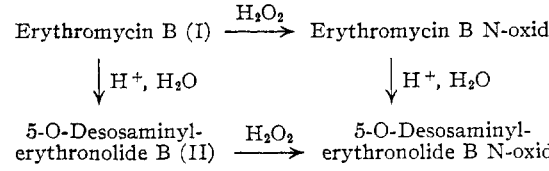
RECEIVED MAY 29, 1957

A structure for erythromycin B is proposed on the basis of physical data, degradation and analogy to erythromycin.

In a previous report[3] from this Laboratory the isolation and characterization of a second crystalline antibiotic, erythromycin B, from *Streptomyces erythreus* was described. The molecular formula $C_{37}H_{71}NO_{12}$ has been proposed,[4] and the presence of the sugars desosamine[5] (IX) and cladinose[6] (X) has been demonstrated.[4] Erythromycin B and erythromycin, the structure of which has been reported recently,[1] have very similar properties,[3,4] the principal difference being the greater acid stability of the former. From a consideration of the results published in these reports and the studies described in this paper, structure I is proposed for erythromycin B.

Repeated recrystallization of erythromycin B from acetone resulted in a product having a melting point of 198°, somewhat higher than previously reported.[3] The pure antibiotic gave analytical data in agreement with the molecular formula $C_{37}H_{67}NO_{12}$ (erythromycin, $C_{37}H_{67}NO_{13}$). Erythromycin B contains a single basic group having a pK'_a of 8.8 in 66% dimethylformamide solution. A molecular weight of 730, quite consistent with the theoretical value of 718 calculated for the above formula, was indicated by titration. Ultraviolet absorption occurs at 289 mμ, ε 36.4. The infrared absorption curve[3] is quite similar to that of erythromycin[7] but having somewhat less absorption in the hydroxyl region. As in erythromycin there are two peaks in the carbonyl region, at 5.80 and 5.90 μ and intense absorption at 8.5–10.0 μ.

Erythromycin B is hydrolyzed readily with 1.0 *N* sodium hydroxide to an amino acid. Although this compound was not obtained pure, most of the physical and analytical data derived from it are consistent with hydrolysis of the lactone ring and loss of water adjacent to the ketone carbonyl. Mild acid hydrolysis of erythromycin B formed cladinose and an amorphous compound[8] II having the molecular formula $C_{29}H_{53}NO_9$. This compound was oxidized to an N-oxide which was identical with the N-oxide derived by acid hydrolysis of erythromycin B N-oxide.



The ultraviolet absorption of the N-oxide of the hydrolytic product II occurs at 285 mμ, ε 37, showing the retention of the ketonic carbonyl. This is in contrast to erythromycin which forms a spiroketal readily in the presence of acid.[1,9]

The reduction of erythromycin B with sodium borohydride followed by mild acid hydrolysis of the intermediate dihydroerythromycin B yielded 5-O-desosaminyldihydroerythronolide B (III). The lactone III no longer contains cladinose and the ketone has been reduced. The latter fact is shown by its transparency to ultraviolet light in the ketone region and the presence of only one carbonyl band in the infrared absorption. That the remaining carbonyl is part of the lactone system is shown by hydrolysis of III to an amino acid.

(1) Previous paper in this series: "Erythromycin. X. Structure of Erythromycin", P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., O. Weaver, U. C. Quarck, R. R. Chauvette and R. Monahan, THIS JOURNAL, **79**, 6062 (1957).

(2) A preliminary report of this work was published as a Communication to the Editor: see K. Gerzon, R. Monahan, O. Weaver, M. V. Sigal, Jr., and P. F. Wiley, *ibid.*, **78**, 6412 (1956).

(3) C. W. Pettinga, W. M. Stark and F. R. Van Abeele, *ibid.*, **76**, 569 (1954).

(4) R. K. Clark, Jr., and M. Taterka, *Antibiotics and Chemotherapy*, **5**, 206 (1955).

(5) R. K. Clark, Jr., *ibid.*, **3**, 663 (1953).

(6) P. F. Wiley and O. Weaver, THIS JOURNAL, **78**, 808 (1956).

(7) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, *ibid.*, **76**, 3121 (1954).

(8) A higher melting compound for which the formula $C_{29}H_{55}NO_9$ was proposed has been isolated by Clark, *et al.* (reference footnote 4).

(9) M. V. Sigal, Jr., P. F. Wiley, K. Gerzon, E. H. Flynn, U. C. Quarck and O. Weaver THIS JOURNAL, **78**, 388 (1956).

The previously mentioned amino acid obtained by hydrolysis of erythromycin B was treated with sodium metaperiodate, but no propionaldehyde was isolated. Quantitative oxidation of erythromycin B and the basic lactone III with periodate gave erratic results presumed to be due to the presence of the tertiary amino group. Consequently these compounds were oxidized to the corresponding N-oxides neither of which reacted with periodate demonstrating the lack of vicinal hydroxyl groups in both compounds.

The basic lactone III was hydrolyzed with 1.0 *N* hydrochloric acid to give, in addition to desosamine, dihydroerythronolide B[10] (IV). The molecular formula for this product, $C_{21}H_{40}O_7$, is consistent with a simple hydrolytic cleavage of III with removal of desosamine. The lactone group was still present as demonstrated by infrared absorption at 5.92 μ. In contrast to the compounds containing desosamine, one mole of sodium periodate was consumed by IV. The product obtained by periodate oxidation was not definitely characterized. However, the increased carbonyl absorption in both the ultraviolet and infrared spectra and the positive iodoform test were consistent with the structure V. Oxidation of V with peroxytrifluoroacetic acid led to a semisolid compound which was not characterized but the structure of which was presumably VI. In agreement with this was the transparency to ultraviolet light in the aldehyde–ketone region. Saponification of the ester-acid VI at *p*H 12.0 gave the known *meso*-α,α'-dimethyl-β-hydroxyglutaric acid (VIII) which has already been isolated from dihydroerythronolide.[11] A second compound (VII) having the molecular formula $C_{12}H_{26}O_4$ was also isolated. This compound has at least four CH_3C groups and its infrared absorption indicates that hydroxyl groups are present but carbonyl groups are absent. Compound VII does not react with sodium metaperiodate and gives a negative iodoform test. These data are consistent with the structure VII.

Isolation of the C_7-dicarboxylic acid VIII and the C_{12}-tetrol VII and demonstration of a methyl ketone function in V account for the 21 carbon atoms of the lactone IV.

Of the seven oxygen atoms in dihydroerythronolide B two must be in the lactone ring. The remaining five are present as hydroxyl groups, only two of which can be adjacent as demonstrated by the results of periodate oxidations of the N-oxides of I and III and similar oxidation of IV. The isolation of the C_7-dicarboxylic acid VIII shows the positions of these adjacent hydroxyl groups to be C-5 and C-6.[12] That one hydroxyl group must be at C-3 is shown by isolation of the acid VIII. The remaining two hydroxyl groups cannot be adjacent and must be at C-9 and/or higher numbered carbon atoms as this is required by lack of periodate oxidation of the tetrol VII. Also the presence of four CH_3CO groups in VII and its negative iodoform reaction precludes oxygen at C-14 and C-15 in IV. Failure to isolate propionaldehyde by hydrolysis of erythromycin B followed by periodate oxidation makes it necessary to assign the two remaining hydroxyl groups and the oxygen of the lactone ring to C-9, C-11 and C-13. The isolation of the C_7-dicarboxylic acid VIII definitely establishes the arrangement of seven of the carbon atoms in IV starting with the lactone carbonyl, and the arrangement of two more is known from the positive iodoform test on V. On the basis of these facts and the probable structure of the tetrol VII and in view of the common biogenesis and close similarity in properties of erythromycin B and erythromycin, the structure IV is indicated for dihydroerythronolide B. This structure would also be consistent with the fact that erythromycin B does not form a spiroketal under acid catalysis for which an hydroxyl group at C-12 appears necessary.

Although there is no direct evidence for the position of the ketonic carbonyl function, there is considerable indirect evidence. The absence of β-keto-ester properties precludes C-3 as a site, and C-6 is eliminated due to the methyl substituent. This leaves as possibilities C-5, C-9, C-11 and C-13, and of these C-9 must be considered most likely in analogy to erythromycin.

Periodate oxidation of the N-oxides of I and III and of the neutral lactone IV indicates that desosamine must be on one of the two adjacent oxygen atoms at C-5 and C-6 with C-5 being probable, again in analogy to erythromycin. Likewise, cladinose is most probably at C-3 as in erythromycin.

All of this evidence points to I as the structure for erythromycin B.

The configurations of the asymmetric carbon atoms in erythromycin B and in the tetrol VII have been drawn on an arbitrary basis. The two carbon atoms, C-2 and C-4 in I, must correspond to the two methyl substituted carbons in the *meso*-acid VIII and must, therefore, have identical configurations. Furthermore, the close correspondence in the optical rotation values (Table I) for the degradation products of erythromycin with those of the similar compounds from erythromycin B points to identical configurations of corresponding asymmetric carbon atoms in the two molecules.

TABLE I

Compound	$[\alpha]^{25}D$	Concn.
5-O-Desosaminyldihydroerythronolide	−2.0	1, CH_3OH
5-O-Desosaminyldihydroerythronolide B	−1.7	1, CH_3OH
Dihydroerythronolide	+9.5	2, CH_3OH
Dihydroerythronolide B	+6.0	1, CH_3OH

Acknowledgments.—The authors are grateful to Messrs. W. L. Brown, H. L. Hunter, G. M. Maciak and Miss Gloria Beckmann for microanalyses; and to Drs. H. E. Boaz and R. R. Pfeiffer and Messrs. P. W. Landis and L. G. Howard for physical chemical data.

Experimental[13]

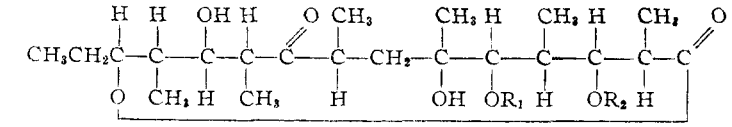
Erythromycin B. Physical Properties and Analysis.—Two grams of erythromycin B was recrystallized five times

(10) This should be named 3,5,6,9,11-pentahydroxy-2,4,6,8,10,12-hexamethylpentadecan-13-olide in agreement with our previous nomenclature for erythronolide.[11]

(11) K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monahan and U. C. Quarck, THIS JOURNAL, **78**, 6396 (1956).

(12) For the numbering system used see reference footnote 1.

(13) Melting points are uncorrected.

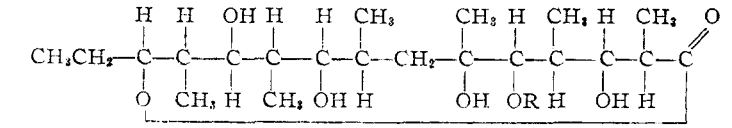


$\xrightarrow{H^+, H_2O}$

I → II

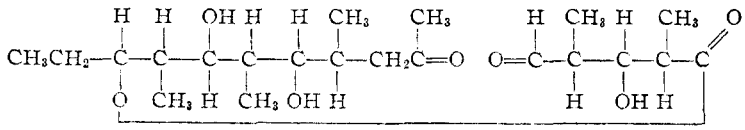
R_1 = desosaminyl, R_2 = cladinosyl → R_1 = desosaminyl, R_2 = H

1, $NaBH_4$
2, H^+, CH_3OH



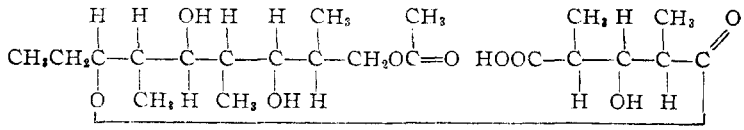
III, R = desosaminyl $\xrightarrow{H^+, H_2O}$ IV, R = H

$NaIO_4$



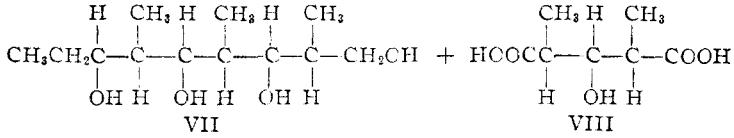
V

F_3CCOOH

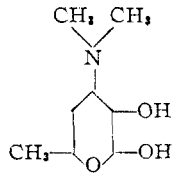


VI

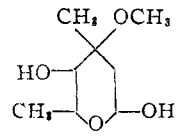
OH^-, H_2O



VII + VIII



Desosamine
IX



Cladinose
X

from acetone. The final product melted at 198°. Titration in 66% dimethylformamide showed a pK'_a of 8.8, apparent molecular weight, 730. The ultraviolet spectrum showed a peak at 289 mμ, ε 36.4. The infrared absorption curve was identical with the one previously reported.[3]

Anal. Calcd. for $C_{37}H_{67}NO_{12}$: C, 61.90; H, 9.41; N, 1.95; C-CH_3 (10), 20.9; OCH_3 (1), 4.3. Found: C, 62.09, 62.07; H, 9.46, 9.67; N, 1.99; C-CH_3, 14.61; OCH_3, 4.79.

Erythromycin B N-Oxide.—Four grams of erythromycin B was dissolved in 100 ml. of methanol. Eighty milliliters of water and 20 ml. of 30% hydrogen peroxide were added. The solution was allowed to stand at room temperature for one day. The methanol was removed by evaporation under reduced pressure and the solid product was removed by filtration. This was dried and recrystallized from 25 ml. of 95% acetone. The yield of material melting at 191–193° was 3.6 grams. Titration in 66% dimethylformamide showed a pK'_a of 5.4. The ultraviolet absorption showed a peak at 285 mμ, ε 31. Absorption occurred in the infrared in the hydroxyl region and at 5.84 and 5.94 μ.

Anal. Calcd. for $C_{37}H_{67}NO_{13}$: C, 60.58; H, 9.20; N, 1.91. Found: C, 60.79, 60.60; H, 9.44, 9.42; N, 1.98.

One hundred and nine milligrams (0.15 mmole) of the N-oxide was dissolved in 100 ml. of 50% methanol which was 0.01 molar in sodium metaperiodate. Titration of 10-ml. aliquots with arsenite showed 0.1 mole equivalent uptake of periodate in 24 hr.

Alkaline Hydrolysis of Erythromycin B and Periodate Oxidation of the Product.—A solution of one gram of erythromycin B in 50 ml. of 50% alcoholic 1.0 *N* sodium hydroxide was refluxed for 2.5 hr. The alcohol was removed by evaporation under reduced pressure. The remaining aqueous solution was extracted with three 10-ml. portions of chloroform. The combined chloroform extracts were evaporated to dryness. The residue was dissolved in 15 ml. of water, and the solution was adjusted to *p*H 7.0 with dilute hydrochloric acid. The neutral solution was evaporated to dryness under reduced pressure. The residue was extracted with two 10-ml. portions of boiling dry acetone filtering each extract. Evaporation of the acetone extracts gave a water-soluble solid melting at 110–120°. Titration in 66% dimethylformamide showed pK'_a's at 6.6 and 9.0 with an apparent molecular weight of 726. Absorption maxima in the ultraviolet occurred at 229 mμ, ε 4050 and 280 mμ, ε 1190. The infrared absorption spectra showed pronounced absorption at 2.90 μ and at 6.36 μ with very little absorption at 5.80–5.90 μ.

Anal. Calcd. for $C_{37}H_{67}NO_{12}$: C, 61.76; H, 9.41; N, 1.95. Found: C, 61.70; H, 9.51; N, 2.19.

One-half gram (1.4 mmoles) of this product was dissolved in 50 ml. of water. The solution was adjusted to *p*H 6.5 with sulfuric acid, and 0.6 g. (2.8 mmoles) of sodium metaperiodate was added. Dry nitrogen was bubbled through this solution into 150 ml. of Brady reagent for 3 hr. No precipitate was obtained.

5-O-Desosaminylerythronolide B (II).—This was run according to the procedure of Hasbrouck and Garven.[14] A solution of 10 g. of erythromycin B in 130 ml. of 0.35 *N* hydrochloric acid was allowed to stand at room temperature for 68 hr. The reaction mixture was poured into a solution of 6.6 g. of sodium bicarbonate in 165 ml. of water. The product was removed by extraction with five 35-ml. portions of chloroform. The chloroform was removed from the combined extracts by evaporation under reduced pressure leaving a solid residue. The product was purified by solution in a mixture of 1:1 petroleum ether (60–70°)-cyclohexane. This was done twice giving a yield of 6.6 g. (85%) melting at 79–83°, $[\alpha]^{28}D$ −52.5° (*c* 2, ethanol). Titration in 66% dimethylformamide showed a pK'_a of 8.3 with an apparent molecular weight of 582 (calcd. 560). Absorption occurred in the infrared at 2.90–2.95 μ and at 5.90 μ with a shoulder at 5.85 μ.

Anal. Calcd. for $C_{29}H_{53}NO_9$: C, 62.14; H, 9.56; N, 2.51. Found: C, 62.26, 62.40; H, 9.56, 9.73; N, 2.33, 2.39.

About one gram of the above product was dissolved in 10 ml. of 5% hydrochloric acid and the solution was evapo-

(14) R. B. Hasbrouck and F. C. Garven, *Antibiotics and Chemotherapy*, **3**, 1040 (1953).

rated to dryness under reduced pressure. The residue was dissolved in alcohol and precipitated with ether, then dissolved in acetone and again precipitated with ether. This gave an amorphous product melting at 130–145°.

Anal. Calcd. for $C_{29}H_{53}NO_9 \cdot HCl$: C, 58.41; H, 9.15; Cl, 5.96. Found: C, 58.45; H, 9.14; Cl, 6.00.

5-O-Desosaminylerythronolide B N-Oxide. (a) From Erythromycin B N-Oxide.—One gram of erythromycin B N-oxide was dissolved in 13 ml. of 0.35 *N* hydrochloric acid, and the solution was allowed to stand at room temperature for 68 hr. It was then poured into a solution of 1.0 g. of sodium bicarbonate in 25 ml. of water. The aqueous solution was extracted with five 5-ml. portions of chloroform, and the combined extracts were evaporated to dryness. The product was crystallized by dissolving it in chloroform and adding petroleum ether (60–70°) and cooling. This was repeated twice. It was necessary to transfer the crystals rapidly to a vacuum desiccator and dry. The final product melted at 167–173°. The ultraviolet spectrum showed a peak at 285 mμ, ε 37. The infrared spectrum had bands at 2.95 and 5.85 μ with a shoulder at 5.81 μ.

Anal. Calcd. for $C_{29}H_{53}NO_{10}$: C, 60.50; H, 9.28; N, 2.43. Found: C, 60.63; H, 9.39; N, 2.59.

(b) From 5-O-Desosaminylerythronolide B.—Two grams of 5-O-desosaminylerythronolide (II) was dissolved in 50 ml. of methanol. A mixture of 40 ml. of water and 10 ml. of 30% hydrogen peroxide was added. After the solution had stood overnight at room temperature, the methanol was removed by evaporation under reduced pressure. The residual solution was extracted with four 5-ml. portions of chloroform, and the combined extracts were evaporated to dryness. This was then recrystallized as above giving a product melting at 169–174° and having an infrared spectrum identical to that of the product in part (a).

5-O-Desosaminyldihydroerythronolide B (III).—A solution of 14.3 g. (0.04 mole) of erythromycin B in 50 ml. of methanol was added slowly with stirring to a filtered solution of 4.0 g. (0.12 mole) of sodium borohydride in 50 ml. of methanol. The reaction mixture was maintained at 0° for 18 hr. and then at room temperature for 3 hr. The resulting solution containing dihydroerythromycin B was added to an ice-cooled methanolic hydrogen chloride solution (prepared by adding 75 ml. of acetyl chloride to 1750 ml. of methanol). After the solution had stood at room temperature for 36 hr., most of the methanol was removed by evaporation under reduced pressure. The residue was dissolved in 250 ml. of chloroform, and the resulting solution was washed three times with 50-ml. portions of saturated sodium carbonate solution and twice with water. The combined washings were extracted with 100 ml. of chloroform which was added to the main chloroform layer. This solution was extracted with four 30-ml. portions of 1.0 *N* hydrochloric acid following each acid extraction by washing with 100 ml. of water. The combined aqueous layers were extracted with 100 ml. of chloroform and filtered. The filtrate was neutralized with 5% sodium hydroxide solution and then made basic with 10% sodium carbonate solution. The product was extracted with four 100-ml. portions of chloroform. The combined extracts were washed with saturated sodium chloride solution and dried over magnesium sulfate. The residue obtained after removal of the chloroform was crystallized from 25 ml. of ether. The yield of material melting at 207–208° was 7.4 g. (67%). The infrared spectrum showed strong absorption at 2.98 and 5.92 μ. The ultraviolet spectrum was transparent between 220 and 400 mμ.

Anal. Calcd. for $C_{29}H_{55}NO_9$: C, 62.00; H, 9.67; N, 2.49; C-CH_3 (6), 21.4; mol. wt., 561.7. Found: C, 62.21; H, 9.76; N, 2.62; C-CH_3, 18.0; mol. wt., 548 (electrometric titration, pK'_a = 8.2 in 66% dimethylformamide); $[\alpha]^{25}D$ 1.7 (*c* 1, methanol).

5-O-Desosaminyldihydroerythronolide B N-Oxide.—5-O-Desosaminyldihydroerythronolide B (2 g.) was dissolved in 125 ml. of methanol, and 125 ml. of 5% hydrogen peroxide was added. The solution was allowed to stand at room temperature for 24 hr. The methanol was removed by evaporation under reduced pressure, and the residue was extracted with four 25-ml. portions of chloroform. The combined chloroform extracts were filtered and dried over magnesium sulfate. The solution was filtered and concentrated under reduced pressure. The melting point of the amorphous product was 170–173°.

Anal. Calcd. for $C_{29}H_{55}NO_{10}$: C, 60.25; H, 9.59; N, 2.42. Found: C, 59.45; H, 9.36; N, 2.46.

One hundred and forty-four mg. (0.25 mmole) of the N-oxide was dissolved in 25 ml. of methanol, and 5 ml. of 0.1 *M* sodium metaperiodate solution was added. The solution was diluted to 50 ml. with distilled water. Titration of 10-ml. aliquots of this solution showed less than one mole per cent. of the N-oxide was oxidized over a period of 24 hr.

Alkaline Hydrolysis of 5-O-Desosaminyldihydroerythronolide B.—A solution of 200 mg. of 5-O-desosaminyldihydroerythronolide B in 10 ml. of 1.0 *N* 50% ethanolic sodium hydroxide solution was allowed to stand overnight at room temperature. The alcohol was removed by evaporation under reduced pressure. The residue was extracted with three 5-ml. portions of chloroform. The aqueous layer was adjusted to *p*H 7.0 with hydrochloric acid and evaporated to dryness under reduced pressure. The residue was extracted with three 5-ml. portions of dry boiling acetone filtering each extract. Evaporation of the combined extracts gave 160 mg. of an amorphous product melting at 60–70°. Titration in 66% dimethylformamide showed pK'_a's of 6.3 and 8.6 with an apparent molecular weight of 547. The ultraviolet spectrum showed only end absorption. There was strong absorption in the infrared at 2.95 and 6.40 μ with weak absorption at 5.9 μ.

Dihydroerythronolide B (IV).—A two-phase system consisting of 400 ml. of toluene and 350 ml. of 1.0 *N* hydrochloric acid was heated under reflux and stirred efficiently to produce a well dispersed emulsion. 5-O-Desosaminyldihydroerythronolide B (8.75 g.) was added to the emulsion, and heating and stirring were continued for 4 hr. The aqueous layer was removed and extracted with 100 ml. of toluene which was added to the original toluene solution. The combined toluene solutions were washed twice with 50-ml. portions of 1.0 *N* hydrochloric acid, and the acid washings were heated for 2 hr. with 400 ml. of toluene. This toluene solution was added to the previously obtained one and the combined solution washed with 5% sodium carbonate solution. The toluene solution was dried by distilling a portion of it, and the remainder was concentrated under reduced pressure. The residue was dissolved in anhydrous ether, and the solution was filtered quickly and cooled at 0°. The yield of crystalline material melting at 182°, $[\alpha]^{25}D$ +6.0° (*c* 1, methanol), was 2.6 g. (41%). The infrared showed strong absorption at 2.94 and 5.92 μ.

Anal. Calcd. for $C_{21}H_{40}O_7$: C, 62.35; H, 9.97; C-CH_3 (7), 26.0; mol. wt., 404.5. Found: C, 62.69; H, 10.01; C-CH_3, 19.9; mol. wt., 410.3 (ebull. in acetone).

Periodate Oxidation of Dihydroerythronolide B (IV).—Ten milliliters of 0.1 *M* sodium metaperiodate solution was added to a solution of 101 mg. (0.25 mmole) of dihydroerythronolide B in 50 ml. of methanol. The resulting solution was diluted to 100 ml. with distilled water. Ten-ml. aliquots were removed at intervals and titrated with 0.01 *M* sodium arsenite solution. In 3 hr. 0.68 molar equivalents of periodate had been consumed and 1.08 molar equivalents in 24 hr.

Degradation of Dihydroerythronolide B with Sodium Metaperiodate.—A solution of 1.5 g. (7.0 mmoles) of sodium metaperiodate in 100 ml. of water was added to a solution of 2.4 g. (6.0 mmoles) of dihydroerythronolide B in a mixture of 100 ml. of methanol and 50 ml. of water. After the mixture had stood at room temperature for 24 hr. in the dark, the methanol was removed by evaporation under reduced pressure at room temperature. The aqueous residue was extracted with five 250-ml. portions of chloroform. The combined chloroform extracts were washed with saturated sodium chloride solution, filtered and dried over magnesium sulfate. The drying agent was removed, and the filtrate was evaporated under reduced pressure. There was obtained a sirupy residue (2.3 g.) which contained the aldehyde–ketone V as shown by the ultraviolet maximum at 278–280 mμ, ε 70 ± 10.

Trifluoroacetic anhydride (3.4 ml., 24 mmoles) was added dropwise to a stirred solution of 0.8 g. of 90% hydrogen peroxide in 20 ml. of methylene chloride,[15] the temperature being kept between 5 and 9°. The solution of oxidizing agent was added to a stirred suspension of 29 g. (204 mmoles) of anhydrous disodium hydrogen phosphate in 100 ml. of

(15) W. D. Emmons and G. B. Lucas, THIS JOURNAL, **77**, 2287 (1955).

methylene chloride containing 2.3 g. (6.0 mmoles) of the above residue V. During the addition the mixture boiled gently and refluxing was continued for an additional hour. After the solution had cooled to room temperature, the liquid layer was removed by careful decantation, and the salts were extracted five times with methylene chloride. The extracts were combined with the decanted solution and washed with saturated sodium chloride solution, filtered and dried over magnesium sulfate. The drying agent was removed by filtration, and the chloroform was removed by evaporation under reduced pressure leaving a semi-solid residue consisting of the acid–ester VI. This residue showed strong infrared absorption in the carbonyl region. The ultraviolet spectrum, however, was transparent between 210 and 400 mμ. Electrometric titration showed the presence of a carboxyl group. Addition of alkali followed by back titration indicated that two equivalents of ester groups had been hydrolyzed.

The ester–acid VI was stirred with 7 ml. of methanol and a solution of 0.55 g. of sodium hydroxide in 30 ml. of water (*p*H 11.8). After 1 hr. additional alkali (5.0 ml. of water containing 370 mg. of sodium hydroxide) was added. After the solution had stood for 4 hr. (*p*H 11.7) most of the methanol was removed by evaporation under reduced pressure, and the *p*H was adjusted to 9.7 by addition of 8.15 ml. of 1.0 *N* hydrochloric acid. The methanol was removed by evaporation, and the residue was extracted continuously overnight with ether.

The ether extract was concentrated and dried over magnesium sulfate. The drying agent was removed by filtration, and the ether was removed from the filtrate by evaporation under reduced pressure. One hundred and fifty milligrams of a crystalline residue of the C_{12}-tetrol VII was obtained, m.p. 146–148°, $[\alpha]^{25}D$ −1.6° (*c* 1, methanol).

Anal. Calcd. for $C_{12}H_{26}O_4$: C, 61.50; H, 11.18; C-CH_3 (4), 25.68; mol. wt., 234. Found: C, 61.64; H, 11.30; C-CH_3, 21.62; mol. wt., 230.7 (ebull. in acetone).

This product gave a negative iodoform reaction and was not oxidized by periodate using the conditions mentioned previously. The infrared spectrum showed strong absorption in the hydroxyl region but none in the carbonyl region.

The aqueous layer was adjusted carefully to *p*H 2.00 using 5.2 ml. of 1.0 *N* hydrochloric acid. The solution was filtered, and the volume of the filtrate was reduced to 30 ml. by evaporation. It was then extracted continuously overnight with ether. The extract was dried twice over magnesium sulfate, filtered and concentrated. The light yellow oily residue crystallized after standing overnight in the refrigerator. The crystals were removed and washed with a little ice-cold ether. The X-ray powder diagram was identical with that of a sample of *meso*-α,α′-dimethyl-β-hydroxyglutaric acid previously isolated from erythromycin.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Erythromycin. XII.[1] The Isolation, Properties and Partial Structure of Erythromycin C

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The isolation of a third crystalline antibiotic, erythromycin C, from *Streptomyces erythreus* is described. Some of the physical, chemical and biological properties of the antibiotic are discussed and a partial structure proposed.

The isolation of erythromycin and erythromycin B from fermentation broths of *Streptomyces erythreus* has been reported.[2,3] Paper chromatography of some of the fractions obtained in the isolation of erythromycin B indicated the presence of additional antibiotic activity. As a result of this evidence, fermentation broths from certain strains of *Streptomyces erythreus* were investigated in search of a new antibiotic. Examination of these fermentation broths by paper chromatography showed three zones of antibiotic activity (Fig. 1). The least mobile of the zones in a methanol, acetone, water (19:6:75) system is erythromycin B; erythromycin C is the fastest moving component and erythromycin has intermediate mobility. After the presence of erythromycin C in appreciable quantity in a crude fermentation broth of *Streptomyces erythreus* had been shown by paper chromatographic examination, the broth was adjusted to *p*H 9.75 and was extracted with chloroform. The chloroform extract, which contained all three antibiotic substances, was concentrated under reduced pressure to approximately one-tenth of the original volume and allowed to stand overnight in the refrigerator. The crystalline precipitate which formed was found by paper chromatography to contain only erythromycin and erythromycin B activity. The mother liquor was concentrated to dryness under reduced pressure, and the residue was fractionated by Craig countercurrent distribution in order to separate the remaining erythromycin from erythromycin C. A 60-tube, all glass apparatus was used with a solvent system prepared by mixing twenty parts of methyl isobutyl ketone, one part of acetone and twenty parts of 0.1 *N* phosphate buffer at *p*H 6.5. A typical distribution curve is shown in Fig. 2. The fractions shown to contain erythromycin C by paper chromatography were concentrated under reduced pressure until only the aqueous phase remained. This solution was adjusted to *p*H 9.75 and extracted with chloroform. The erythromycin C was crystallized from the concentrated chloroform solution.

It was also possible to separate erythromycin and erythromycin C by chromatography on a cellulose column. A mixture of the two antibiotics in acetone solution was adsorbed on a small amount of cellulose powder and dried. The powder was then packed in a thin band at the top of a previously prepared cellulose column. Development of the column with dilute ammonium hydroxide solution saturated with methyl isobutyl ketone gave at first fractions containing no antibiotic activity followed by fractions containing erythromycin C. The

(1) Previous paper in this series: "Erythromycin. XI. Structure of Erythromycin B," P. F. Wiley, M. V. Sigal, Jr., O. Weaver, R. Monahan and K. Gerzon, THIS JOURNAL, **79**, 6070 (1957).

(2) J. M. McGuire, R. L. Bunch, R. C. Anderson, H. E. Boaz, E. H. Flynn, H. M. Powell and J. W. Smith, *Antibiotics and Chemotherapy*, **2**, 281 (1952).

(3) C. W. Pettinga, W. M. Stark and F. R. Van Abeele, THIS JOURNAL, **76**, 569 (1954).