TABLE III

Amine	Yield, %	B.p., °C.	B.p., °C. (lit.)	Picrate M.p., °C.	Picrate M.p., °C. (lit.)	Formulas	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
m-Methylbenzyl	48	201	201–202[a] (755 mm.)	190	198 dec.[b]					
p-Methylbenzyl	62	200.5	198–200[c] (680 mm.)	205–215 dec.	205 dec.[c]					
p-Fluorobenzyl	60	183		203		$C_{13}H_{11}FN_4O_7$	44.07	44.42	3.13	3.15
o-Methylmercaptobenzyl	38	145 (4 mm.)		203–204		$C_{14}H_{14}N_4O_7S$	43.98	44.17	3.69	3.67
β-(*p*-Chlorophenyl)-ethyl	23	90 (5 mm.)	114–116[d] (15 mm.)	210		$C_{14}H_{13}ClN_4O_7$	43.70	43.78	3.41	3.51

[a] Ref. 19. [b] C. W. Shoppee, *J. Chem. Soc.*, 696 (1932). [c] K. G. Lewis, *ibid.*, 2250 (1950). [d] D. H. Hey and J. M. Williams, *ibid.*, 1527 (1951).

in 30% yield. On neutralization with sodium hydroxide, extraction with ether, and vacuum distillation, the free amine boiled at 113–115° (4 mm.), solidified to a white solid, and gave a picrate melting at 237° dec. (lit. 231° dec.[17]). This method was found to be superior to that recommended by Shoppee.[17]

The Gabriel synthesis for the preparation of *p*-bromobenzylamine[17] was modified in the last step by the use of hydrazine.[18] N-(*p*-Bromobenzyl)-phthalimide was converted to the *p*-bromobenzylamine, which distilled at 100° at 5 mm. (lit. 102° at 12 mm.[19]) in 72% yield.

2-Thenylamine (b.p. 56–58°(3 mm.)) and **5-chloro-2-thenylamine** (b.p. 76–79°(3 mm.)) were made by aminomethylation of thiophene and of 2-chlorothiophene.[20]

The method of Leonard and Blackford[21] was used with tests on the inhibition of growth of *A. niger* (ATCC 215–4247). Concentration of the candidate fungicide was measured in parts per million (p.p.m.). If no growth occurred at 250 p.p.m., tests were run at lower concentrations. Controls, which were run parallel with each test, measured the growth of the organism in the absence of a potential inhibitor and in separate plates in the presence of 25 and of 50 p.p.m. of the fungicide 2,2′-dihydroxy-5,5′-dichlorodiphenyl (G-4).

Tests against the gram-positive bacteria, *B. subtilis*, 9945, and the gram-negative bacteria *E. coli*, 4157, were run in a Difco Bacto-Agar medium containing 250 or less p.p.m. of the candidate bacteriocide. Complete inhibition of growth of the bacteria 72 hours after streaking the culture was taken as the criterion of inhibition. If no growth occurred at concentrations of 250 p.p.m., measurements were made at greater dilutions.

Acknowledgment.—The authors are indebted to Sara Bond, Margaret Skorvaga and Carol Quarck who assisted in the synthesis of compounds and to Barbara Bayless and Dorcas Clarke who performed the microbiological tests.

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DURHAM, N. C.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Erythromycin. VI. Degradation Studies[1]

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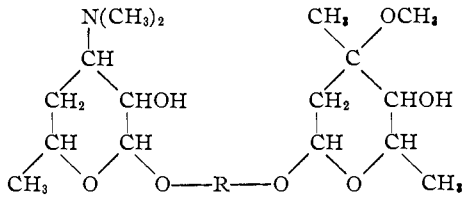
Erythromycin has been shown to be a bisglycoside of a twenty-one carbon polyhydroxy ketolactone. By reduction and hydrolysis of erythromycin, the aglycone, dihydroerythronolide, has been isolated and characterized. Other degradation products of erythromycin have been obtained and studied.

In the first paper[2] of this series, erythromycin was shown to be a glycoside containing the dimethylaminodeoxy sugar desosamine and the methoxydeoxy sugar cladinose.[3] The partial structure I was suggested.

The work reported in this paper is concerned with the aglycone portion [R(OH)$_2$] of I and evidence is presented that this is a twenty-one carbon polyhydroxy ketolactone, for which we propose the name erythronolide.

The presence of the free hydroxyl group vicinal to the dimethylamino group in the desosamine moiety of I has been inferred previously from the reduced pK'_a values of the monoacyl derivatives of I.[2,5] Direct evidence for this free hydroxyl group has been obtained now as a consequence of the isolation of methylamine as a product of the



I, R = $C_{21}H_{38}O_6$[4]

(1) Part of the work presented in this paper has been reported in a preliminary communication, P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., and U. C. Quarck, THIS JOURNAL, **77**, 3676 (1955).
(2) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, THIS JOURNAL, **76**, 3121 (1954).
(3) The structure of cladinose shown here has been reported recently; P. F. Wiley and O. Weaver, THIS JOURNAL, **77**, 3422 (1955).
(4) The composition of erythromycin was given in the first paper of this series (ref. 2) as $C_{37}H_{67-69}NO_{13}$ and the composition of R as $C_{21}H_{36-38}O_6$. On the basis of analytical data and chemical information presented in this paper, the correct empirical formula of erythromycin is $C_{37}H_{67}NO_{13}$ and R is $C_{21}H_{38}O_6$.
(5) H. W. Murphy, "Antibiotics Annual," 1953–1954, Medical Encyclopedia, Inc., New York, N. Y., 1954, pp. 500–513.

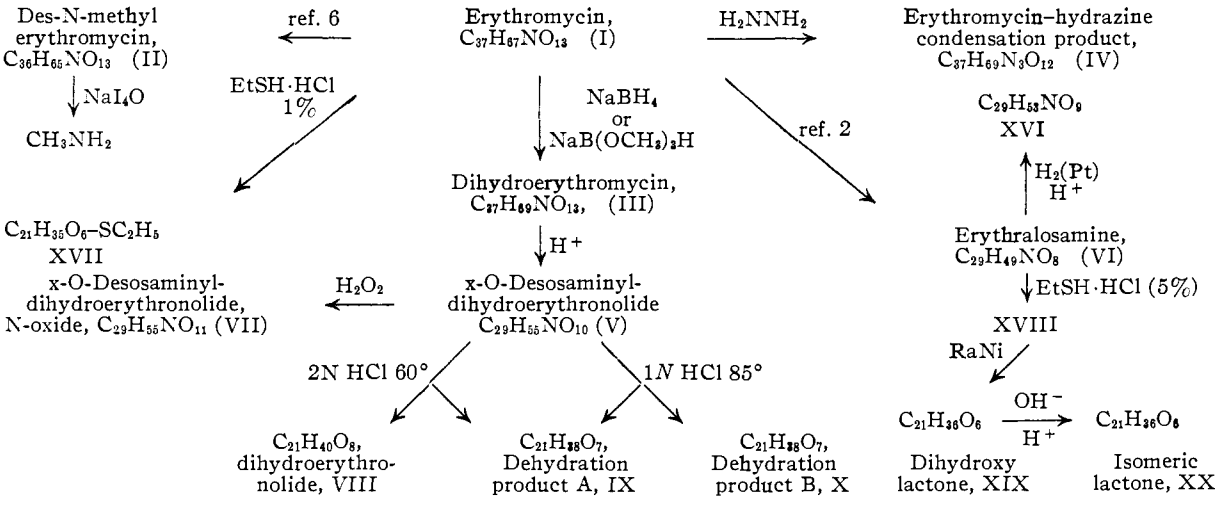
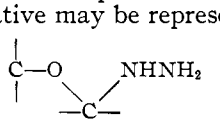


Fig. 1.

oxidation of des-N-methylerythromycin (II)[6] with sodium metaperiodate (Fig. 1).

The presence of a lactone group in I was indicated in a preceding paper.[2] Presumptive evidence for a ketone group was found in the fact that erythromycin absorbs in the ultraviolet at 278 mμ (ϵ 27) and that ultraviolet absorption characteristic of an α,β-unsaturated ketone appeared after treatment with dilute base.[2,7] Furthermore, the presence of absorption bands in the infrared spectrum at 5.78 and 5.91 μ is compatible with a ketolactone structure. Direct evidence for this ketolactone structure was provided by the reduction of erythromycin to dihydroerythromycin. When compound I was treated with sodium borohydride (or sodium trimethoxyborohydride) an amorphous reduction product containing boron was isolated. This product was converted to crystalline, boron-free dihydroerythromycin, $C_{37}H_{69}NO_{13}$ (III), by a brief acid treatment at *p*H 2.5. That the ketone group had been reduced was evidenced by the absence of the 278 mμ absorption band in the ultraviolet spectrum of III. Furthermore, the infrared spectrum contained only one carbonyl band at 5.84 μ, which was attributable to the lactone group.

Attempts to prepare the normal carbonyl derivatives of I were not successful. However, compound I did react with hydrazine to give a crystalline condensation product $C_{37}H_{69}N_3O_{12}$ (IV) that no longer exhibited the absorption in the ultraviolet which we have assigned to the ketonic carbonyl. The infrared spectrum indicated that this compound was neither a hydrazone nor a hydrazide. The carbonyl region of the spectrum contained a single band at 5.80 μ, consistent with a lactone structure. Titration of IV showed the presence of two ionizable groups with pK'_a values of 4.9 and 8.6. The partial structure of this hydrazine derivative may be represented as



(6) E. H. Flynn, H. W. Murphy and R. E. McMahon, THIS JOURNAL, **77**, 3104 (1955).

(7) N. R. Kuzel, J. M. Woodside, J. P. Comer and E. E. Kennedy, *Antibiotics and Chemotherapy*, **4**, 1234 (1954).

The reaction of dihydroerythromycin (III) with hydrogen chloride–methanol solution removed cladinose and gave a crystalline base with the composition $C_{29}H_{55}NO_{10}$ (V). Infrared and ultraviolet studies indicated V to be a substituted polyhydroxylactone. Since V differs from the acid degradation product of I, erythralosamine, $C_{29}H_{49}NO_8$ (VI),[2] by the elements of 2H + $2H_2O$, it appears that the carbonyl group of I is required for the loss of both molecules of water in the formation of VI. Compound VI underwent extensive decomposition in 1 *N* sodium hydroxide solution yielding dimethylamine, propionaldehyde and propionic acid.[2] None of these products were obtained when V was hydrolyzed under similar conditions. When V was treated with dilute hydrogen peroxide, the amine oxide VII was formed. Like erythromycin N-oxide,[2] VII reacted with one mole of sodium metaperiodate and, therefore, contains one pair of vicinal hydroxyl groups.

When V was subjected to acid hydrolysis under carefully controlled conditions, desosamine was removed and two neutral crystalline products were isolated. The first of these had the composition $C_{21}H_{40}O_8$ (VIII) and represents the aglycone portion of erythromycin in which the ketone group has been reduced to hydroxyl. It is, therefore, dihydroerythronolide. The precursor, V, of dihydroerythronolide, thus becomes x-O-desosaminyldihydroerythronolide. The second product was formed in smaller amounts and has the composition $C_{21}H_{38}O_7$ (IX). It has been termed dehydration product A. Purification of dihydroerythronolide and dehydration product A by recrystallization proved to be impractical because of mixed crystal formation. Consequently, dihydroerythronolide was purified by chromatography on acid-washed alumina. Dehydration product A (IX) was freed of dihydroerythronolide (VIII) by treatment with sodium metaperiodate; VIII was oxidized by this reagent while IX was not affected.

When V was subjected to acid hydrolysis under slightly more vigorous conditions than those employed in the preparation of dihydroerythronolide, dehydration product A was formed again, together with an isomeric compound termed dehydration product B (X).

Dihydroerythronolide yielded a mixture of IX and X after hydrolysis with acid under suitable conditions. Spectral studies on dihydroerythronolide (VIII) indicated it to be a polyhydroxylactone. With acetic anhydride in pyridine VIII reacted to form an anhydrodiacetate, $C_{25}H_{42}O_9$ (XI). The fact that VIII was recovered unchanged after treatment with platinum and hydrogen in acetic acid indicated the absence of a hemiacetal or lactol function. The C-methyl content was found to be 20.2%, suggesting the presence of six or more C-methyl groups. This high value is consistent with results obtained on erythromycin itself. Two moles of sodium metaperiodate were consumed by VIII, but no acid was formed in the reaction. Therefore, VIII contains two α-glycol groupings. Since x-O-desosaminyldihydroerythronolide contains only one α-glycol grouping, the desosamine moiety of V apparently is attached by a glycosidic linkage involving an oxygen of one of the α-glycol groupings in dihydroerythronolide.

The infrared spectrum of dehydration product A (IX) indicated this compound to be an unsaturated polyhydroxylactone. The ultraviolet spectrum of the product showed no maximum in the 220–400 mμ region. Catalytic reduction of IX yielded a crystalline substance XII having the composition $C_{21}H_{40}O_7$. Spectral studies indicated this latter compound to be a saturated polyhydroxylactone. Neither IX nor XII reacted with sodium metaperiodate. IX reacted with acetic anhydride in pyridine to give a triacetate, $C_{27}H_{44}O_{10}$ (XIII). Dehydration product A is formed apparently from dihydroerythronolide by the loss of one mole of water with the concurrent formation of a double bond and the loss of *both* periodate reactive centers. The relationship between dihydroerythronolide and dehydration product A is obscure.

Dehydration product B (X) had an ultraviolet absorption band at 289 mμ (ϵ 38) and had an infrared spectrum characteristic of a hydroxy ketolactone. It reacted with one mole of sodium metaperiodate. It is likely that one α-glycol group in dihydroerythronolide underwent a pinacol-type dehydration to give X.

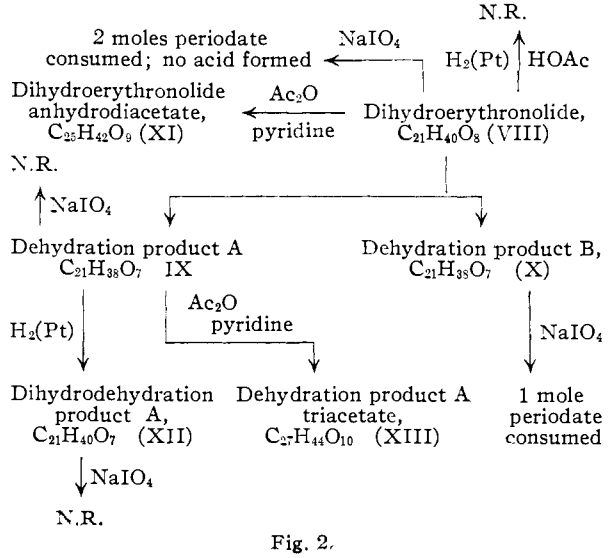


Fig. 2.

The reactions of dihydroerythronolide and of its dehydration products are summarized in the accompanying chart (Fig. 2).

Active hydrogen determinations on several of the compounds named above (*i.e.*, V, VIII, IX and X) were inconclusive because of the formation of insoluble products. Therefore, as a point of reference, we sought to establish with certainty the number of hydroxyl groups in erythralosamine. Erythralosamine (VI) (Fig. 3) was treated with ethyl chloroformate to form O,N-dicarboethoxydes-N-methylerythralosamine (XIV). Compound XIV also was obtained from O,N-dicarboethoxydes-N-methylerythromycin[2] (XV) by acid-catalyzed methanolysis. Active hydrogen determinations on XIV indicated one hydroxyl group.

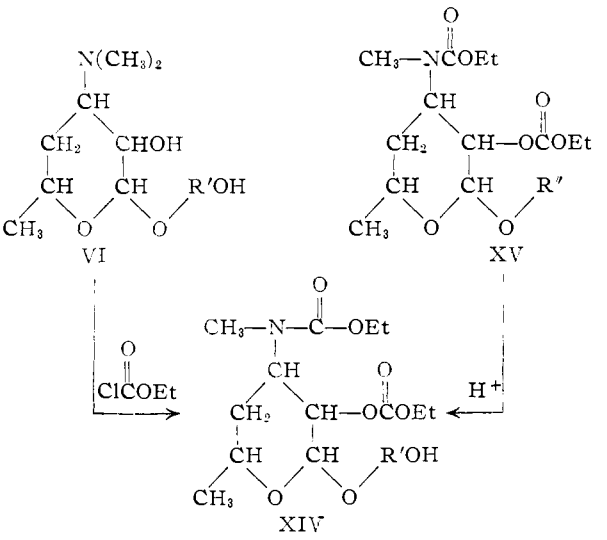


Fig. 3.

Erythralosoamine (VI), therefore, contains two hydroxyl groups; one is in the desosamine moiety and one is in the non-sugar moiety (R'). Of the four remaining oxygen functions in VI, two are present in the lactone group, and the other two probably are in the form of a ketal. The formation of a ketal group would account for the loss of one mole of water and the loss of the ketone carbonyl that occurs in passing from erythromycin to erythralosamine.[2] The loss of the second mole of water that occurs when VI is formed we now believe results in the formation of a double bond. Evidence for such a double bond and/or a ketal structure was provided by the presence of a pronounced absorption band at 11.0 μ in the infrared spectra of both erythralosamine and O,N-dicarboethoxydes-N-methylerythralosamine. The apparent relationship of the oxygen functions in VI, V and VIII are shown in Fig. 4.

Thus, six hydroxyl groups are assigned to compounds V and VIII; and this is consistent with the empirical formulas of these products. Furthermore, the composition $C_{21}H_{40}O_8$ of dihydroerythronolide (VIII) allows only one ring (*i.e.*, the lactone ring). Consequently erythronolide, the aglycone portion of erythromycin, contains only one ring (the lactone ring). It also contains a ketonic-carbonyl and five hydroxyls. In erythro-

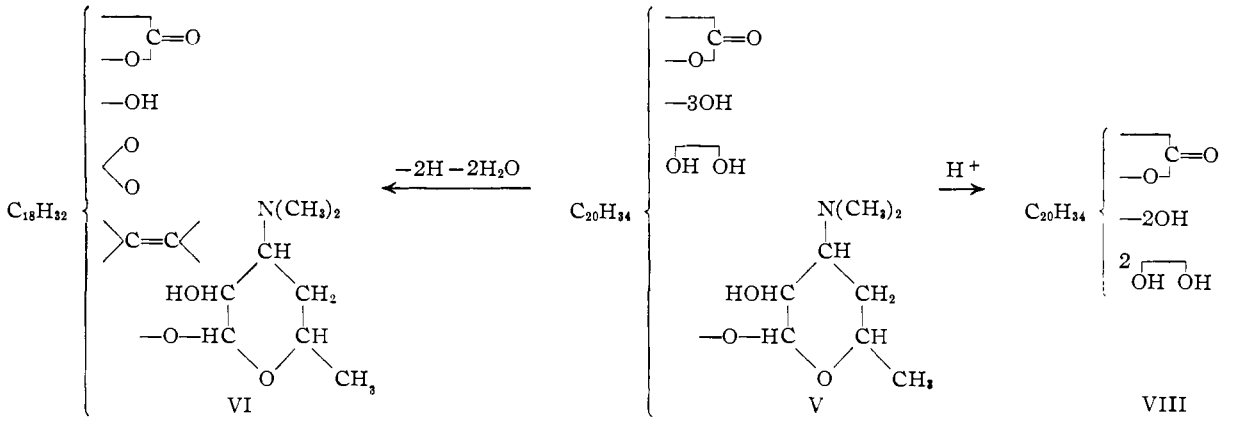


Fig. 4

mycin two of these five hydroxyls are involved in glycosidic linkages. Whereas the empirical formula of erythromycin (I) was given previously as $C_{37}H_{67-69}NO_{13}$,[2] the evidence presented in this paper (*i.e.*, the presence of a ketone group and the analytical data and interrelationships of compounds III, V, VI and VIII) is consistent only with the formula $C_{37}H_{67}NO_{13}$.

The reduction of erythralosamine (VI) in an acidic medium has been reported to yield a mixture of products.[2] The product most easily isolated had the composition $C_{29}H_{53}NO_9$ (XVI). Infrared and ultraviolet studies indicated this substance to be a polyhydroxylactone; there was no infrared absorption band at 11.0 μ as was present in VI.

Hasbrouck and Garven[8] have reported that treatment of erythralosamine with ethyl mercaptan and hydrogen chloride gave desosamine ethyl hemimercaptal and a sulfur-containing oil. By the use of ethyl mercaptan containing hydrogen chloride (1%), we have obtained from erythromycin a neutral crystalline hemimercaptal with the composition $C_{21}H_{35}O_6$-SC_2H_5 (XVII). When erythralosamine was treated with ethyl mercaptan and hydrogen chloride (5%), a neutral oil was obtained with the approximate composition $C_{21}H_{33}O_5$-SC_2H_5 (XVIII). A similar substance was isolated from erythromycin or the 0.005 *N* sodium hydroxide hydrolysis product[2] of erythromycin by the use of these same reagents. Desulfurization of XVIII with Raney nickel yielded a mixture of compounds from which a crystalline substance with the composition $C_{21}H_{36}O_6$ (XIX) was obtained. The two remaining products of the desulfurization of XVIII were apparently successive dehydration products of XIX. The infrared absorption spectrum of XIX had maxima at 2.93 and 5.85 μ; only end absorption was present in the ultraviolet. An active hydrogen determination indicated the presence of two hydroxyl groups. These data characterize XIX as a dihydroxylactone. Conclusive proof of the lactone nature of this compound was found in the fact that, when it was hydrolyzed with base, a new isomeric lactone (XX) was obtained after acidification.

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

Experimental[9]

Methylamine from the Reaction of Des-N-methylerythromycin and Periodate.—Des-N-methylerythromycin[6] (7.2 g., 0.02 *M*) was dissolved in 350 ml. of water by adjusting the *p*H to a stable value of 5.8 with 0.1 *N* hydrochloric acid, not allowing the acidity to drop below *p*H 5.0 during the time solution was taking place. The solution was diluted to one liter with water and 2 l. of ethanol was added. Sodium metaperiodate (4.28 g., 0.02 *M*) in one liter of water was added. After 20 hours in the dark at room temperature a faint test for periodate ion was obtained. The solution was concentrated *in vacuo* to remove alcohol, and a crystalline precipitate (3.4 g.) removed by filtration. The filtrate was extracted with four 50-ml. portions of chloroform, the combined chloroform extract was washed once with 50 ml. of water, dried, and the chloroform removed under reduced pressure. The white, amorphous residue was insoluble in dilute acid or base.

The aqueous solution which remained after chloroform extraction was adjusted to *p*H 11.0 with dilute sodium hydroxide. The alkaline solution was distilled at 25° at 4–8 mm., the distillate being collected through an ice-cooled condenser. The alkaline distillate was added to a solution of 0.25 g. of picryl chloride in 25 ml. of ethanol. The solution was heated on the steam-bath 30 minutes, then allowed to stand overnight. The crystalline precipitate was removed and proved to be primarily reagent containing some methyl picramide. The reaction mixture was concentrated to *ca.* 50 ml. *in vacuo* and the resulting precipitate was recrystallized from ethanol. The recrystallized material melted at 111–113°. The X-ray diffraction pattern was identical with one obtained from an authentic sample of methyl picramide which also melted at 111–113°.

Dihydroerythromycin (III).—A solution of 10 g. of erythromycin in 15 ml. of methanol was added slowly with stirring to a filtered solution of 3 g. of sodium borohydride in 30 ml. of methanol. During the addition and for 4 hours afterward the temperature of the mixture was maintained at 4°. The solution was then allowed to stand at room temperature for 2 days. Dry carbon dioxide gas was passed through the solution and the precipitated sodium salts were filtered off. The filtrate was then concentrated to dryness under reduced pressure at room temperature and the solid residue was taken up in 100 ml. of chloroform. The chloroform solution was washed with three 25-ml. portions of a 10% sodium bicarbonate solution and with two 25-ml. portions of water. The chloroform solution was filtered, dried

(9) Melting points were determined on a Kofler micro melting point apparatus unless indicated otherwise. Infrared spectra were obtained with a Beckman IR-2T spectrophotometer or a Baird double-beam recording spectrophotometer. Ultraviolet measurements were made with a Cary recording spectrophotometer.

over sodium sulfate, and the chloroform removed under reduced pressure. The white amorphous dihydro-product (9.5 g.) thus obtained contained boron (flame test) and did not crystallize. The infrared absorption spectrum of this material had only one band in the carbonyl region at 5.81 μ, while the ultraviolet spectrum was transparent between 220 and 400 mμ.

The crude amorphous reduction product (6.6 g.) was dissolved in dilute hydrochloric acid and the *p*H adjusted to 2.5. The solution was maintained at this *p*H at room temperature for 15 minutes and was subsequently neutralized with a 1 *N* sodium hydroxide solution. The *p*H of the solution was raised to 11.2 by means of a concentrated sodium carbonate solution and the organic material extracted with three 125-ml. portions of chloroform. The amorphous white powder obtained after removal of the chloroform gave a negative flame test for boron. Crystallization from an isopropyl alcohol–water mixture gave 2.0 g. of dihydroerythromycin (III). Crystallinity was rapidly lost on air-drying. A sample dried in vacuum at room temperature melted at 133–135°. Titration in 66% dimethylformamide showed a pK_a' of 8.6.

Anal. Calcd. for $C_{37}H_{69}NO_{13}$: C, 60.38; H, 9.45; N, 1.90; mol. wt., 735.9. Found: C, 60.36; H, 9.48; N, 1.88; mol. wt., 736 ± 15 (electrometric titration).

The infrared spectrum on the crystallized material showed strong absorption at 3.0 and 5.84 μ. The ultraviolet spectrum was transparent between 220 and 400 mμ.

Dihydroerythromycin was prepared also by the reduction of erythromycin (8.5 g.) with sodium trimethoxyborohydride (3.0 g.) in methanol at 4°.

Erythromycin–Hydrazine Condensation Product (IV).—Fifty grams of erythromycin was dissolved in 150 ml. of anhydrous methanol by gentle warming on the steam-bath. To this solution was added a solution of 12.5 g. of anhydrous hydrazine in 50 ml. of anhydrous methanol. The mixture was heated on the steam-bath for 24 hours with the exclusion of moisture from the air. The methanol and excess hydrazine were removed by evaporation under reduced pressure leaving an amorphous white solid which was crystallized from an isopropyl alcohol-water mixture. The yield of air-dried material, m.p. 135–136°, was 31 g. This material lost 7.5% of its weight when it was dried *in vacuo* at 100° for two hours. The X-ray diffraction pattern of the crystallized material was well defined; crystallinity was rapidly lost when the product was allowed to dry in the air.

The infrared spectrum had a strong band at 5.80 μ and a broad band of low intensity at 6.1–6.2 μ. There was only end absorption in the ultraviolet. Electrometric titration gave pK'_a values of 4.9 and 8.6. Analysis on material dried in a pig at 100° for two hours:

Anal. Calcd. for $C_{37}H_{69}N_3O_{12}$: C, 59.41; H, 9.30; N, 5.62; mol. wt., 747.9. Found: C, 59.25; H, 9.49; N, 5.49; mol. wt., 785 ± 20 (electrometric titration).

x-O-Desosaminyldihydroerythronolide (V).—Fifty grams of erythromycin was reduced with sodium borohydride and the amorphous dihydroerythromycin containing boron was isolated as described above. This product (49 g.) was dissolved in 2.5 liters of methanol containing 1% hydrogen chloride. The reaction mixture was allowed to stand at room temperature for 3 days and then was concentrated under reduced pressure to remove most of the methanol. The residue was taken up in 250 ml. of chloroform and the chloroform solution was poured slowly with efficient stirring into a flask containing 150 ml. of a saturated sodium chloride solution, 150 ml. of a saturated sodium bicarbonate solution and 250 ml. of a 20% sodium carbonate solution. The layers were separated and the aqueous layer was extracted with three 150-ml. portions of chloroform. The combined chloroform extracts were washed with 100 ml. of a saturated sodium bicarbonate solution and then with 100 ml. of a saturated sodium chloride solution. The chloroform solution was then extracted with 70 ml. of 1 *N* hydrochloric acid and washed with 70 ml. of water, the wash water being added to the acidic extract. The chloroform solution was extracted again with 35 ml. of 1 *N* hydrochloric acid and washed with 35 ml. of water. To the combined acidic extracts was added 12 g. of sodium bicarbonate and 5 g. of sodium carbonate and the basic solution was extracted with five 150-ml. portions of chloroform. The combined chloroform solutions were washed with 100 ml. of a saturated sodium chloride solution and dried over magnesium sulfate. The chloroform solution was concentrated under reduced pressure to a volume of about 75 ml. Approximately three times the volume of ether was added and the solution was allowed to stand at 0° for 2 hours. The crystalline x-O-desosaminyldihydroerythronolide (V) which precipitated weighed 32 g. and melted at 209–210°. After further recrystallization the melting point was 212–213°. The infrared spectrum showed strong absorption at 2.85 and 5.86 μ. The ultraviolet spectrum was transparent between 220 and 400 mμ. Titration in 66% dimethylformamide showed a pK'_a of 8.0.

Anal. Calcd. for $C_{29}H_{55}NO_{10}$: C, 60.29; H, 9.59; N, 2.42; C-CH_3 (7) 18.2; mol. wt., 577.7. Found: C, 60.39; H, 9.67; N, 2.35; C-CH_3, 16.34; mol. wt., 585 ± 10 (electrometric titration); $[\alpha]^{25}D$ −2° (*c* 1 in methanol); $[\alpha]^{25}D$ −5° (*c* 1 in pyridine).

x-O-Desosaminyldihydroerythronolide was also prepared without prior isolation of the sodium borohydride reduction product. After reduction of erythromycin, dry carbon dioxide was passed through the methanolic solution; the solution was filtered and the requisite amount of methanolic hydrogen chloride was added to the filtrate.

Alkaline Hydrolysis of x-O-Desosaminyldihydroerythronolide (V).—x-O-Desosaminyldihydroerythronolide (1.0 g.) was dissolved in 50 ml. of 1 *N* aqueous methanolic (1:1) sodium hydroxide and the solution was refluxed 16 hours. No volatile base was detected. The reaction mixture was then steam distilled at constant volume. The distillate contained no basic material and failed to give a precipitate with Brady reagent. Acidification of the reaction mixture followed by steam distillation gave no significant amounts of steam-volatile acid.

x-O-Desosaminyldihydroerythronolide N-Oxide (VII).—x-O-Desosaminyldihydroerythronolide (2.0 g.) was dissolved in 240 ml. of a solution of 2.5% hydrogen peroxide in aqueous methanol (1:1). The solution was allowed to stand for 24 hours at room temperature. The reaction mixture was concentrated under reduced pressure and then was extracted with chloroform. The chloroform extractable material weighed 1.8 g. After crystallization from chloroform–petroleum ether (Skellysolve B), 0.95 g. of product was obtained which melted at 208–210°. Recrystallization raised the melting point to 210–212°. The pK'_a of this material was 4.0 in water.

Anal. Calcd. for $C_{29}H_{55}NO_{11}$: C, 58.66; H, 9.33; N, 2.36. Found: C, 58.52; H, 9.37; N, 2.24.

Periodate Oxidation of x-O-Desosaminyldihydroerythronolide N-Oxide (VII).—x-O-Desosaminyldihydroerythronolide N-oxide (300 mg., 0.506 m*M*) was dissolved in 100 ml. of aqueous 0.01 *M* sodium metaperiodate. Ten-ml. aliquots were removed at intervals and the remaining periodate was determined by titration with 0.01 *M* arsenite in the usual manner. The consumption of periodate was essentially complete in about 3 hours; 0.89 mole equivalent of periodate was consumed.

Dihydroerythronolide (VIII) and Dehydration Product A (IX) from x-O-Desosaminyldihydroerythronolide (V).—Thirty-seven grams of x-O-desosaminyldihydroerythronolide was dissolved in 1100 ml. of 2 *N* hydrochloric acid. The acidic solution was stirred vigorously with 500 ml. of chloroform at 60° (reflux) for 24 hours. The mixture was cooled to room temperature, the layers were separated and the aqueous layer was extracted with two 200-ml. portions of chloroform. The combined chloroform extracts were washed with 40 ml. of a saturated sodium chloride solution, and the wash solution was added to the acidic layer. The entire procedure was repeated two more times on the acidic solution, using fresh chloroform each time, so that the hydrolysis was carried out over a period of 72 hours. The combined chloroform solutions from the three extractions were washed with 100 ml. of a saturated sodium bicarbonate solution and with 100 ml. of a saturated sodium chloride solution, and then dried over magnesium sulfate. The chloroform extract was concentrated under reduced pressure to a volume of about 150 ml. and then an equal volume of hot petroleum ether (Skellysolve B) was added. The crystalline product obtained weighed 14.1 g. (52% of theory) and melted at 200–202°. This material was a mixture consisting primarily of dihydroerythronolide (VIII) and containing some dehydration product A (IX). The components could not be separated by fractional crystallization.

The reaction product (10 g.) was chromatographed over

450 g. of alumina (acid-washed, Merck) in a column 3.4 cm. in diameter. The material was put on the column in 2.1 liters of a benzene–chloroform (3:1.25) solution. The column was eluted with 5 l. of a 3:1 benzene–chloroform solution, then with 2 l. of a 1:1 chloroform–benzene solution, then with 2 l. of a 3:1 chloroform–benzene solution, and then with 600 ml. of chloroform. Approximately 1 g. of dehydration product A (identical with that described in the following section) was obtained from the first 7 l. of eluate, and approximately another gram of material was recovered in the form of dihydroerythronolide–dehydration product A mixed crystals from the next 2.6 l. The column was then eluted with 1 l. of a chloroform–methanol (4:1) solution. The eluate was evaporated to dryness under reduced pressure and the solid residue was dissolved in 1 l. of chloroform. The chloroform solution was dried over magnesium sulfate and concentrated under reduced pressure to a volume of about 100 ml. An equal volume of hot petroleum ether (Skellysolve B) was added precipitating 7.3 g. of dihydroerythronolide (VIII), m.p. 185–187°, with prior sublimation. In the infrared spectrum there was a broad band at 2.75–2.90 μ and a strong band at 5.86 μ. The ultraviolet spectrum was transparent between 220 and 400 mμ. After mild basic hydrolysis, electrometric titration revealed a pK'_a of 4.3 in water.

Anal. Calcd. for $C_{21}H_{40}O_8$: C, 59.97; H, 9.59; C-CH_3 (6), 21.4; mol. wt., 420.5. Found: C, 60.04; H, 9.56; C-CH_3, 20.21; mol. wt., 405; $[\alpha]^{27}D$ +9.5° (*c* 2 in methanol).

The purity of the dihydroerythronolide obtained from the chromatography was ascertained by titration with sodium metaperiodate solution. In some cases the material obtained took up 1.98 moles of sodium metaperiodate, indicating a purity of 99%, but in other runs the product was less pure. The degree of separation of the dihydroerythronolide from the dehydration product A seemed to depend upon the length of time the material remained on the column.

Dehydration product A could be separated readily from dihydroerythronolide by dissolving a mixture of the two in an aqueous methanol solution of sodium metaperiodate. After the solution had stood for 10 hours at room temperature the methanol was removed and crystalline dehydration product A was obtained.

Dehydration Products A (IX) and B (X) from x-O-Desosaminyldihydroerythronolide (V).—Ten grams of x-O-desosaminyldihydroerythronolide was dissolved in 410 ml. of 1 *N* hydrochloric acid solution and added to an equal volume of toluene. Hydrolysis was effected by heating the two-phase system with vigorous refluxing and adequate stirring during a period of 18 hours. The reaction temperature under these conditions ranged between 82 and 85°. The layers were separated while still hot, the toluene layer was filtered by gravity and subsequently heated to boiling for a period of about 5 minutes. The toluene solution was then allowed to stand at room temperature for 24 hours. The crystalline dehydration product A (IX) was removed by filtration, washed with two 10-ml. portions of ethyl ether and air-dried. It weighed 1.6 g. and melted at 220–221° (prior sublimation at 200°); recrystallization from a chloroform–petroleum ether (Skellysolve B) mixture raised the melting point to 229–230° (prior sublimation at 200°).

The infrared spectrum contained strong absorption bands at 2.95 and 5.78 μ; a band of medium intensity was present at 6.1 μ. The ultraviolet spectrum was transparent in the 220–400 mμ region.

Anal. Calcd. for $C_{21}H_{38}O_7$: C, 62.66; H, 9.52; O, 27.83; C-CH_3 (5), 18.65, (6) 22.45; mol. wt., 402.5. Found: C, 62.53, 62.66; H, 9.53, 9.56; O, 28.23, 27.84; C-CH_3, 19.89; mol. wt., 406 (b.p. in methanol), 403.2 (X-ray crystallographic analysis); $[\alpha]^{27}D$ −17° (*c* 2, in methanol).

Concentration of the mother liquor from the crystallization of dehydration product A to a volume of about 75 ml. gave an additional 0.5 g. of dehydration product A.

The filtrate from the second crystallization was further concentrated to about 10 ml. and 50 ml. of anhydrous ether was added. The solution was then allowed to stand for 24 hours at room temperature and filtered. Two washings of the filter cake with 10-ml. portions of a 1:1 mixture of ether and petroleum ether (Skellysolve B) gave 700 mg. of white crystalline dehydration product B (X) melting at 185°. After recrystallization from a benzene–petroleum ether mixture (1:3) the melting point was 192–193°.

The infrared spectrum (mineral oil mull) showed absorption at 2.88, 5.78 and 5.95 μ. The ultraviolet spectrum contained an absorption maximum at 289 mμ (ϵ 35–40).

Anal. Calcd. for $C_{21}H_{38}O_7$: C, 62.66; H, 9.52; O, 27.83; C-CH_3 (5), 18.65, (6) 22.45; mol. wt., 402.5. Found: C, 62.34, 62.85; H, 9.31, 9.36; O, 27.95; C-CH_3, 20.36; mol. wt., 401.6 (X-ray crystallographic analysis).

A further amount of dehydration product B (325 mg.) was obtained from the mother liquor of the first ether crystallization.

From the mother liquor of crystallization of dehydration product B a yellow sirupy residue was obtained which could be distilled at a pressure of 0.1 mm. This product was not further investigated.

Dehydration Products A (IX) and B (X) from Dihydroerythronolide.—Five hundred milligrams of chromatographically purified dihydroerythronolide was hydrolyzed for 7.5 hours with 25 ml. of 1 *N* hydrochloric acid solution and 25 ml. of toluene under the conditions described for the preparation of dehydration products A and B directly from x-O-desosaminyldihydroerythronolide and the products were isolated in the fashion described. There was obtained 200 mg. of dehydration product A melting at 219–221° and 100 mg. of dehydration product B melting at 185–193°. Recrystallization from a chloroform–petroleum ether (Skellysolve B) solvent mixture raised the respective melting points to 222–225° and to 190–192°. The X-ray diffraction patterns and the infrared absorption spectra of the two recrystallized products were identical in all respects with those of the previously obtained samples. The X-ray diffraction pattern of dehydration product B in this case, however, showed a few faint lines derived from the pattern of dehydration product A.

Dihydroerythronolide Anhydrodiacetate (XI).—Dihydroerythronolide (2.0 g.) was dissolved in 20 ml. of anhydrous pyridine and 6 ml. of acetic anhydride was added. The reaction mixture was allowed to stand at room temperature for 24 hours and then concentrated to one-third of the original volume under reduced pressure at room temperature. The residue was poured onto 50 g. of cracked ice. The precipitate formed was separated from the supernatant liquid and dissolved in 150 ml. of benzene. The benzene solution was washed with 10 ml. of a 5% hydrochloric acid solution, with 20 ml. of a 5% sodium bicarbonate solution and with two 20-ml. portions of water. The benzene solution was dried over magnesium sulfate and evaporated to dryness under reduced pressure at room temperature. The solid residue obtained was dissolved in benzene and on the addition of petroleum ether (Skellysolve B) the anhydrodiacetate XI separated. It weighed 1.46 g. (80% of theory) and melted at 83–85°. After recrystallization from benzene–petroleum ether (Skellysolve B) the melting point was 84–85°. The infrared spectrum showed strong absorption at 5.76 and 8.02 μ. The ultraviolet spectrum was transparent between 220 and 400 mμ.

Anal. Calcd. for $C_{25}H_{42}O_9$: C, 61.70; H, 8.70; CH_3CO-(2), 17.7; active H, 3. Found: C, 61.42; H, 8.67; CH_3-CO, 14.9; active H, 2.7.

Attempted Reduction of Dihydroerythronolide.—Dihydroerythronolide (420 mg.) was subjected to reduction in glacial acetic acid at atmospheric pressure with hydrogen and Adams catalyst (100 mg.) during a period of 30 hours. No hydrogen was taken up by the solution beyond that of a blank run using the same amount of solvent and catalyst. The recovered material (300 mg.) was identified as unchanged dihydroerythronolide by means of the undepressed mixed melting point and the identity of X-ray diffraction patterns.

Periodate Oxidation of Dihydroerythronolide (VIII).—Dihydroerythronolide (420 mg., 1.00 m*M*) was dissolved in 100 ml. of a 40% aqueous methanol solution of 0.02 *M* sodium metaperiodate. Five-ml. aliquots were removed at intervals and the remaining periodate was determined by titration with 0.01 *M* arsenite in the usual manner. The consumption of periodate was essentially complete in about 2.5 hours; 1.8 mole equivalents was consumed. The remainder of the solution after oxidation was complete was titrated (electrometrically) with 0.1 *N* sodium hydroxide; no free acid could be detected.

Dihydrodehydration Product A (XII).—One gram of dehydration product A was reduced with hydrogen and Adams catalyst (50 mg.) at atmospheric pressure in glacial acetic

acid. One mole equivalent of hydrogen was taken up in about 20 minutes while the further slow uptake of hydrogen over a period of 24 hours paralleled the uptake of a solvent and catalyst blank. Recovery of the material followed by recrystallization from ethyl ether yielded 200 mg. of the crystalline dihydro product (XII) melting at 200°. The infrared spectrum showed the loss of the 6.1 μ ethylenic band present in the starting material. The ultraviolet spectrum was transparent between 220 and 400 mμ. The X-ray diffraction pattern differed from that of the starting material when both were crystallized from the same solvent.

Titration of a sample of dihydrodehydration product A with sodium metaperiodate revealed that none of the oxidant was utilized over a period of 24 hours.

Anal. Calcd. for $C_{21}H_{40}O_7$: C, 62.35; H, 9.97. Found: C, 62.49; H, 9.91.

A reduction of dehydration product A (400 mg.) with Adams catalyst and hydrogen at atmospheric pressure in absolute ethanol gave 250 mg. of the same dihydro product.

Dehydration Product A Triacetate (XIII).—The procedure used for the acetylation of dehydration product A (IX) was the same as that described for the preparation of the dihydroerythronolide anhydrodiacetate (XI). Acetylation of 1.0 g. of IX yielded a mixture of solid acetates from which 150 mg. (11%) of the more insoluble crystalline dehydration product A triacetate (XIII) was isolated by means of fractional recrystallization from a mixture of benzene and petroleum ether (Skellysolve B). The material melted at 250–252°. The infrared spectrum showed strong absorption at 5.75, 6.10 and 7.98 μ. The ultraviolet spectrum was transparent between 220 and 400 mμ.

Anal. Calcd. for $C_{27}H_{44}O_{10}$: C, 61.34; H, 8.39; CH_3CO-(3), 24.4. Found: C, 61.17; H, 8.25; CH_3CO-, 22.6.

Periodate Oxidation of Dehydration Product B (X).—Dehydration product B (400 mg., 0.99 m*M*) was dissolved in 100 ml. of a 40% aqueous methanol solution of 0.02 *M* sodium metaperiodate. Five-ml. aliquots were withdrawn at intervals and the remaining periodate was determined by titration with 0.01 *M* arsenite in the usual manner. The consumption of periodate was essentially complete in about 4 hours; 1.04 mole equivalents was consumed.

O,N-Dicarboethoxydes-N-methylerythralosamine (XIV). (a) **By Methanolysis of O,N-Dicarboethoxydes-N-methylerythromycin (XV).**—O,N-Dicarboethoxydes-N-methylerythromycin[2] (2.0 g.) was dissolved in 100 ml. of methanol containing 1% hydrogen chloride and allowed to stand for 24 hours. The solution was then poured into 50 ml. of a 6% sodium bicarbonate solution and the methanol removed by concentration under reduced pressure. The resulting suspension was extracted with three 50-ml. portions of chloroform. The chloroform-extractable material weighed 2.2 g. This residue was dissolved in 25 ml. of petroleum ether (Skellysolve B) and on standing solid crystalline O,N-dicarboethoxydes-N-methylerythralosamine (XIV) separated, yield 1.05 g., m.p. 170–172. After recrystallization from a mixture of benzene and petroleum ether (Skellysolve B) and then from methanol–water solution, the melting point was 175–177°.

The infrared spectrum contained hydroxyl absorption at 2.77 μ and carbonyl absorption at 5.73, 5.80 and 5.90 μ. A strong maximum was present at 11.0 μ; a similar band was found in the infrared spectrum of erythralosamine (VI), but was not present in the spectrum of O,N-dicarboethoxydes-N-methylerythromycin (XV) or in the spectrum of erythromycin (I).

Anal. Calcd. for $C_{34}H_{55}NO_{12}$: C, 60.96; H, 8.27; N, 2.09; active H, 1; EtO (2), 13.4. Found: C, 61.18, 60.71; H, 8.32, 8.24; N, 1.99, 2.07; active H, 0.82, 0.81; EtO, 14.52, 14.27.

(b) **By Acylation of Erythralosamine (VI).**—Erythralosamine (5.4 g.) was dissolved in 100 ml. of acetone and 7.0 g. of solid sodium bicarbonate was added. Ethyl chloroformate (5.5 g. in 60 ml. of acetone) was added dropwise over a period of 30 minutes to the above suspension with stirring. After 3 hours of additional stirring, the reaction mixture was filtered and taken to dryness under reduced pressure. The residue was dissolved in 25 ml. of benzene and 150 ml. of petroleum ether (Skellysolve B) was added. A crystalline product separated which weighed 1.35 g. and melted at 171–176°. Its X-ray diffraction pattern was identical with that of O,N-dicarboethoxydes-N-methylerythralosamine (XIV) obtained above.

Reduction of Erythralosamine (VI).—Erythralosamine (15.0 g.) was dissolved in 150 ml. of 0.5 *N* hydrochloric acid and reduced in the presence of platinum catalyst (1.0 g.) under a pressure of 40 lb. of hydrogen for a period of 24 hours. The solution was filtered, made basic with solid sodium carbonate and extracted with chloroform. The chloroform solution was evaporated to dryness under reduced pressure and the residue was dissolved in 150 ml. of petroleum ether (Skellysolve B). On standing 2.8 g. of crystalline material separated; part (1.5 g.) existed in the form of a fine white powder which was mechanically separated from the remainder which was present in the form of solid clumps. The fine white powder when crystallized from a benzene–petroleum ether (1:1) solvent mixture yielded 0.75 g. of reduction product XVI melting at 210–215°. Recrystallization from aqueous methanol raised the melting point to 218–220°. The infrared spectrum of XVI showed strong absorption at 2.87 and 5.84 μ. There was no band at 11.0 μ as was present in the infrared spectrum of erythralosamine. Titration in 66% dimethylformamide showed a pK'_a of 8.0.

Anal. Calcd. for $C_{29}H_{53}NO_9$: C, 62.23; H, 9.54; N, 2.50; mol. wt., 559.7. Found: C, 62.21, 62.04; H, 9.56, 9.55; N, 2.41; mol. wt., 550 (electrometric titration).

Mercaptanolysis of Erythralosamine (5% HCl).—Ten grams of erythralosamine was added to 200 ml. of dry ethyl mercaptan containing 5% dry hydrogen chloride. The resulting mixture was refrigerated overnight and allowed to stand at room temperature for four hours. The ethyl mercaptan was removed by evaporation at room temperature under reduced pressure. Fifty milliliters of water was added to the residue, and the mixture was extracted with four 80-ml. portions of ether. The combined ether extracts were concentrated under reduced pressure at room temperature. The residue was dissolved in 60 ml. of cyclohexane and the solution was washed with 30 ml. of water and dried over magnesium sulfate. The drying agent was filtered off and the solvent removed by evaporation. The residue was heated under a pressure of 0.2 mm. to a temperature of about 170° until all volatile material was removed. The residue (XVIII) was a viscous brown material weighing 5.6 g. The ultraviolet absorption spectrum showed a peak at 274 mμ, ϵ 1400. The infrared spectrum showed strong absorption at 2.92 and 5.82 μ.

Anal. Calcd. for $C_{23}H_{38}O_5S$: C, 64.76; H, 8.99; S, 7.52; mol. wt., 426.5. Found: C, 65.52; H, 9.02; S, 9.80; mol. wt., 435.4.

This experiment is illustrative of a number of runs. Analyses were very poor and not consistent but were similar to the above. Similar results were obtained by running the mercaptanolysis on erythromycin and the product of 0.005 *N* sodium hydroxide hydrolysis of erythromycin.[2]

Mercaptanolysis of Erythromycin (1% HCl).—Four grams of erythromycin was added to 75 ml. of dry ethyl mercaptan containing 1% dry hydrogen chloride. The experiment was run just as was the preceding one. The yield of viscous material was 1.34 g. This material was dissolved in the minimum amount of ethyl acetate, and the solution was cooled. There was obtained 0.32 g. of crystalline solid melting at 164–169°. The product XVII was recrystallized twice each alternately from acetone–water and ethyl acetate. The final melting point was 191–192° (capillary).

Anal. Calcd. for $C_{23}H_{40}O_6S$: C, 62.13; H, 9.06; S, 7.21. Found: C, 62.17; H, 9.01; S, 6.72.

Desulfurization of Erythralosamine-Ethyl Mercaptan-Hydrogen Chloride Product (XVIII). (a) $C_{21}H_{34}O_5$; Monodehydration Product of XIX.—Thirty-four and one-half grams of the product obtained by treatment of erythralosamine with ethyl mercaptan containing 5% hydrogen chloride was added to a mixture of 4 l. of 70% alcohol and 450 g. of Raney nickel. The mixture was stirred for three days. The nickel was filtered off and washed thoroughly with alcohol. The combined filtrate and washings were evaporated under reduced pressure until the alcohol was removed. The remaining mixture was extracted with four 200-ml. portions of ether. The combined ether extracts were dried over magnesium sulfate. This solution was filtered and the ether was removed by evaporation. The residue was a viscous oil weighing 20.8 g. This oil was distilled three times discarding each time a forerun and collecting the first time a broad fraction boiling at a bath temperature of 160–200° under a pressure of 0.4 mm. and finally a fraction boiling at a bath temperature of 155–165° under a pressure of

0.15 mm. The product was a soft, plastic, nearly colorless material. The infrared absorption spectrum showed absorption at 2.92 and 5.80 μ.

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.81; H, 9.35; C-CH_3 (6), 24.5; active H, 1; mol. wt., 366.5. Found: C, 69.05; H, 9.40; C-CH_3, 21.2; active H, 1.09, 0.90; mol. wt., 359.1.

(b) $C_{21}H_{36}O_6$.—The residues from the above distillation were combined and distilled. The fraction boiling at a bath temperature of 190–200° under a pressure of 0.03 mm. was retained. This distillate formed a brittle glassy solid upon cooling. Three and one-tenth grams of this distillate was dissolved in 30 ml. of dry ether. Cooling gave 420 mg. of crystalline solid melting at 196–201°. After two recrystallizations from ethyl acetate the product XIX melted at 215° (capillary). The infrared absorption spectrum showed absorption at 2.93 and 5.85 μ. Only end absorption was present in the ultraviolet.

Anal. Calcd. for $C_{21}H_{36}O_6$: C, 65.59; H, 9.44; O, 24.97; C-CH_3 (7), 27.3; active H, 2; mol. wt., 384.5. Found: C, 65.83; H, 9.47; O, 25.24; C-CH_3, 23.7; active H, 1.86; mol. wt., 384.7 (X-ray crystallographic analysis).

The same product was isolated from several undistilled desulfurization products by crystallization from ethyl acetate.

(c) **Distillation of Foreruns.**—The foreruns from the distillations in part (a) were fractionated twice. The first time the fraction boiling at 119–137° at 0.04 mm. was retained; the second time there was retained the fraction boiling at 130–134° at 0.05 mm., $n^{25}D$ 1.4896. The ultraviolet absorption spectrum showed maxima at 210 mμ, ϵ 5700 and 268 mμ, ϵ 1550. The infrared spectrum showed strong absorption at 5.80 μ.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78; mol. wt., 350.5. Found: C, 71.90; H, 9.07; mol. wt., 359.2; active H, 0; >C=C<, 2.4.

The above experimental results were obtained starting with the product from erythralosamine and ethyl mercaptan, but the same products were isolated starting with erythromycin or with the product of 0.005 *N* sodium hydroxide hydrolysis of erythromycin.

Isomerization of XIX.—Four hundred milligrams of XIX was refluxed for 16 hours in a mixture of 20 ml. of methanol and 20 ml. of 2 *N* sodium hydroxide solution. The cooled reaction mixture was acidified with 10% sulfuric acid. A precipitate formed and was removed by filtration. Recrystallization from ethyl acetate gave 70 mg. of the isomeric lactone XX melting at 185–186° (capillary). This depressed the melting point of starting material upon admixture. Further recrystallization from the same solvent caused no change in melting point. The infrared absorption spectrum showed absorption at 2.90 and 5.75 μ. Only end absorption was present in the ultraviolet region.

Anal. Calcd. for $C_{21}H_{36}O_6$: C, 65.59; H, 9.44; mol. wt., 384.5. Found: C, 65.65; H, 9.41; mol. wt., 370.

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF PARKE, DAVIS & CO.]

Some New Constituents of *Piscidia erythrina* L.

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A reinvestigation of the constituents of *P. erythrina* has led to the isolation of piscidic acid, rotenone and five apparently new aromatic substances. The characterization of the new substances is described.

The leguminaceous plant *Piscidia erythrina* L., commonly designated and commercially available as Jamaica Dogwood, has been the subject of numerous chemical investigations over the past 70 years. The interest in this species has arisen largely from reports of the analgesic and insecticidal properties of the root-bark as well as its toxicity to fish. The pharmacognosy of the plant and the older literature recently have been reviewed.[1] We now wish to present the results of some further studies on the constituents of this plant.

Among certain of the earlier reports[2] of isolation work on *P. erythrina*,[3] several highly oxygenated neutral and phenolic compounds, a glycoside, an alkaloid, sterols and waxes are very briefly described. A few more fully characterized substances have also been reported. Freer and Clover[4] obtained from an aqueous extract of the root bark an acid, m.p. 182–183°, which they named piscidic acid; this isolation has since been confirmed and the compound has been shown to be *p*-hydroxybenzyltartaric acid.[5] By ligroin extraction these workers isolated two neutral compounds, a substance $C_{21}H_{14}O_5(OCH_3)_2$, m.p. 201°, a substance $C_{20}H_{12}O_4(OCH_3)_2$, m.p. 216°, and a phenolic compound, m.p. 159°. In the most recent investigation, Russell and Kaczka[6] obtained two compounds by extraction of root material with petroleum ether, one of which was identified as rotenone; the other, a neutral substance $C_{21}H_{14}O_5(OCH_3)_2$, m.p. 203–204°, was named ichthynone. It seems quite probable that the substance, m.p. 201°, of Freer and Clover and ichthynone are in fact the same compound. Degradation studies on ichthynone[7] indicate that it is a chromenochromone of the dehydrorotenoid type. Piscidic acid also was isolated in this most recent study.[7]

In the present work, attention has been directed again to the rather highly oxygenated, aromatic compounds which appear to be characteristic of this plant. Although several previous investigators have used hydrocarbon solvents for the extraction of these constituents, thus avoiding the removal of large amounts of tannins, polysaccharides and pigments, we employed ethanol in order to ensure as complete an extraction of the resinoids as possible. The total extract, representing about

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(1) E. C. Auxence, *J. Econ. Botany*, **7**, 270 (1954).

(2) E. Hart, *Am. Chem. J.*, **5**, 39 (1883); P. W. Danckwortt and E. Schütte, *Arch. Pharm.*, **272**, 791 (1934); F. Hauschild, *ibid.*, **274**, 388 (1936).

(3) This plant also has been designated as *P. piscipula* Sarg. and *Ichthyomethia piscipula* A. Hitch.; the equivalence of these names appears to be generally accepted.

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