Anal. Calcd. for $C_{24}H_{27}N$: C, 87.49; H, 6.56. Found: C, 87.39; H, 6.66.

Compound XII by Reduction of Phenylmesitylacetic Acid *p*-Toluide with Lithium Aluminum Hydride.—A solution of 3.43 g. (0.01 mole) of the amide in 50 ml. of anhydrous tetrahydrofuran was reduced with 3.80 g. (0.10 mole) of lithium aluminum hydride. From this reaction 3.43 g. of crude product melting at 128–160° was obtained. Three recrystallizations from acetone–water gave 2.93 g. (89%) of white solid melting at 153–154°. The infrared curve of this compound was identical to that obtained for the product from catalytic reduction. A mixture melting point was not depressed. Lithium aluminum hydride reduction of the amide could not be effected in ethyl ether solution.

Anal. Calcd. for $C_{24}H_{27}N$: C, 87.49; H, 6.56. Found: C, 87.49; H, 6.68.

Preparation of 1,2-Dimesityl-2-phenyl-N-*p*-tolylvinylamine (XI).—Lithium metal (1.39 g., 0.20 g. at.), cut into small pieces under ether and nitrogen and weighed into a beaker of ether, was placed in a 500-ml. three-necked flask equipped with a stirrer, condenser, drying tube, dropping funnel and nitrogen inlet tube and covered with 50 ml. of ether. A solution of 19.91 g. (0.1 mole) of bromomesitylene in 30 ml. of ether was then added dropwise over a 1-hr. period to the vigorously stirred lithium–ether suspension. The resulting suspension was stirred and refluxed for 24 hr. Ether was added occasionally to compensate for losses through evaporation. At the end of this period, a solution of 3.25 g. (0.01 mole) of mesitylphenylketene-*p*-tolylimine, m.p. 106–108°, in 50 ml. of dry ether was added dropwise over a 1-hr. period to the stirred lithium aryl suspension at the reflux temperature while a slow stream of nitrogen was passed through the mixture. The resulting suspension was refluxed an additional hour and then stirred at room temperature for two days after the addition of 100 ml. of ether. Decomposition was effected by the dropwise addition of 100 ml. of cold water. The ether layer was removed, the aqueous layer extracted twice with 100-ml. portions of ether and the combined ether extracts washed with cold water, dried over anhydrous magnesium sulfate, filtered and finally evaporated to dryness under vacuum. The resulting pale yellow residue was washed thoroughly with pentane and then recrystallized from isopropyl alcohol. The yield of white platelets melting at 195–196.5° was 2.72 g. (61%).

Anal. Calcd. for $C_{33}H_{35}N$: C, 88.94; H, 7.92. Found: C, 88.62; H, 8.26.

A mixture consisting of 1.00 g. of the vinylamine XI, 50 ml. of methanol, 1.00 ml. of 20% sodium hydroxide solution and 5 ml. of 30% hydrogen peroxide was stirred at room temperature for two days and then refluxed for 5 hr. The vinylamine was recovered (77%) unchanged. A mixture of 158 mg. of the vinylamine, 5 ml. of acetic anhydride and 2 ml. of dry pyridine was allowed to stand for two days at room temperature and was then refluxed for 1 hour from which 140 mg. of XI was recovered.

A mixture of 187 mg. of the compound melting at 191–192°, 20.0 ml. of acetone and 5 ml. of concd. HCl was refluxed for two days. Evaporation of the mixture to dryness under vacuum gave 170 mg. of the starting material.

Preparation of 2,2-Diphenyl-N-methyl-N-phenylvinylamine.—A homogeneous solution of 9.81 g. (0.05 mole) of diphenylacetaldehyde, 10.72 g. (0.1 mole) of N-methylaniline and 100 ml. of dry benzene was refluxed in a 200-ml. flask equipped with a Dean–Stark water separator, a condenser and a drying tube. The theoretical volume of water, 0.90 ml., had separated after 10 hr. The resultant pale yellow solution was evaporated to dryness *in vacuo* and the residual liquid distilled *in vacuo*. Three fractions were collected. The first boiled at 40–60° (0.3 mm.) and amounted to 5.11 g. of unreacted methylaniline. The second fraction, b.p. 100–150° (0.3 mm.), 2.80 g., was not identified but probably contained a small amount of the vinylamine. The third fraction, b.p. 150–160° (0.3 mm.), 8.53 g., was the vinylamine. Refractionation of the latter pale yellow viscous oil gave 6.55 g. (46%) of a pale yellow extremely viscous oil boiling at 173–176° (0.5–0.6 mm.).

Anal. Calcd. for $C_{21}H_{19}N$: C, 88.38; H, 6.71. Found: C, 88.18; H, 6.88.

The ultraviolet absorption of the following compounds was determined in absolute ethanol using a Cary automatic recording ultraviolet spectrophotometer.

Compound	Maxima, mμ	Extinction (ε)
1. 2,2-Diphenyl-N-methyl-N-phenylvinylamine (XIII)	337	21,400
2. 2-Phenyl-2-mesityl-N-*p*-tolylvinylamine (X)	334	27,500
3. 2-Phenyl-1,2-dimesityl-N-*p*-tolylvinylamine (XI)	332	15,000

Acknowledgment.—The authors are indebted to Dr. J. M. Vandenbelt, Mr. R. B. Scott and their associates at Parke, Davis and Co. for the infrared and ultraviolet data reported in this paper.

DETROIT 2, MICHIGAN

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES, ELI LILLY AND COMPANY]

Erythromycin. X.[1] Structure of Erythromycin

BY PAUL F. WILEY, KOERT GERZON, EDWIN H. FLYNN, MAX V. SIGAL, JR., OLLIDENE WEAVER, U. CAROL QUARCK, ROBERT R. CHAUVETTE AND ROSMARIE MONAHAN

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Evidence for the complete structure of erythromycin is presented.

Previous papers in this series[2] have reported that the antibiotic, erythromycin, is a polyhydroxyketolactone having the molecular formula $C_{37}H_{67}NO_{13}$. In the molecule there are an aminosugar, desosamine and a nitrogen free sugar, cladinose. The structures of desosamine[2a,3] and cladinose[2c] and of dihydroerythronolide,[2d] the reduced aglycone portion of erythromycin, have been published. For the complete elucidation of the erythromycin structure there remains to be established the positions of the sugar moieties, the position of the ketonic carbonyl and the size of the lactone ring. The solutions of these problems are described in this paper, and I is shown to be the structure of erythromycin.

Oxidation of erythromycin N-oxide[2a] with sodium periodate followed by mild alkaline hydrolysis gave rise to a steam volatile product VI. This

(1) Previous paper in this series: "Erythromycin. IX. Degradative Studies of Erythromycin B," K. Gerzon, R. Monahan, O. Weaver, M. V. Sigal, Jr., and P. F. Wiley, THIS JOURNAL, **78**, 6412 (1956).

(2) (a) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, *ibid.*, **76**, 3121 (1954); (b) M. V. Sigal, Jr., P. F. Wiley, E. H. Flynn, U. C. Quarck and O. Weaver, *ibid.*, **78**, 388 (1956); (c) P. F. Wiley and O. Weaver, *ibid.*, **78**, 808 (1956); (d) K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monahan and U. C. Quarck, *ibid.*, **78**, 6396 (1956).

(3) R. K. Clark, *Antibiotics and Chemotherapy*, **3**, 663 (1953).

compound reacted slowly with 2,4-dinitrophenylhydrazine to give 2,3-pentanedione bis-(2,4-dinitrophenylhydrazone) (VII). Compound VI could not be 2,3-pentanedione since it did not form a precipitate in the presence of a nickel salt and hydroxylamine but did so after oxidation with ferric chloride. Such behavior is indicative of an α-hydroxyketone. The survival of this ketone in the presence of periodate must be due to its occurrence as an ester of the potential carboxyl function in the parent molecule so that it occurs in an oxidizable form as a hydroxyketone after alkaline hydrolysis. Although the hydroxyketone VI may be either 3-hydroxy-2-pentanone or the isomeric 2-hydroxy-3-pentanone, the original ester linkage must be at C-3 of the hydroxyketone as shown in V. The evidence for this is the previously reported[2d] isolation of propionaldehyde and acetic acid by periodate oxidation of the acid formed by alkaline hydrolysis of dihydroerythronolide (IV). The isolation of the hydroxyketone VI is proof that the lactone ring in erythromycin is the same as that in dihydroerythronolide, namely, terminating at C-13,[4] and in conjunction with earlier reports[2b,d] establishes that the only change in the aglycone portion of erythromycin in the series I → IV is reduction of the ketonic carbonyl. Since the structure of IV has been established,[2d] the carbon skeleton and the positions of the oxygen substituents in the aglycone portions of erythromycin are now determined.

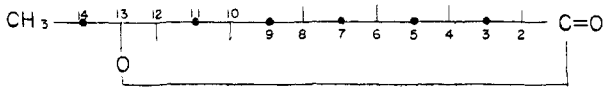
The placement of the ketonic carbonyl in erythromycin has been established by degradation of the acid hydrolysis product, erythralosamine,[2a,b] for which is proposed structure VIII. Evidence for this structure will be discussed in connection with the position of desosamine. Oxidation of erythralosamine with chromic oxide formed a crystalline compound having the composition $C_{29}H_{51}NO_{11}$ (IX). Although the structure of this compound has not been fully established, such knowledge is not necessary for the validity of the information deduced from the dilactones X and XI. Acid hydrolysis of IX formed a neutral crystalline material having the molecular formula $C_{13}H_{18}O_4$ (X). The infrared spectrum of this compound was consistent with the presence of a γ-lactone (band at 5.67 μ) and a δ-lactone conjugated with a carbon–carbon double bond (band at 5.83 μ). The ultraviolet absorption (λ 209 mμ, ϵ 13,000) also indicates an α,β-unsaturated lactone. Catalytic reduction of the dilactone X occurred readily forming a saturated neutral compound XI with the composition $C_{13}H_{20}O_4$. Analytical data indicated the presence of four CH_3C– groups. The existence of two lactone rings in this compound was shown by its ready base hydrolysis, consuming two equivalents of base in the process, with reversion to the starting material XI after acidification. The ultraviolet absorption of this material showed that the α,β-unsaturation had disappeared. The longer wave length infrared absorption of X had shifted down until a single broad carbonyl band (5.66–5.78 μ) appeared with a slight peak at 5.72 μ. This was interpreted as confirmatory evidence for the presence of the olefinic group in the six-membered lactone portion of X.

The principal evidence for the structure of the δ-lactone portion of the lactone XI is the isolation of the diol XV by degradation of XI. Reduction of the C_{13}-dilactone XI with lithium aluminum hydride formed a liquid which was not characterized but whose infrared spectrum was consistent with the structure XII. Treatment of this with sodium metaperiodate followed by sodium borohydride reduction of the resulting mixture (presumably XIII and XIV) gave a mixture from which only one product was isolated. The liquid product was characterized as its bis-(3,5-dinitrobenzoate). The analysis and infrared and ultraviolet spectra of this derivative were compatible with the formulation 2,4-dimethyl-1,5-pentanediol bis-(3,5-dinitrobenzoate) (XVI). This compound was identical with a synthetic sample of the ester XVI as shown by identical melting points, infrared spectra and X-ray diffraction patterns. The synthetic ester was derived from mixed *meso*- and DL-α,α'-dimethylglutaric acid. Comparison with ester obtained from *meso*-α,α'-dimethylglutaric anhydride showed identity in all respects except melting points. These findings show that the diol XV has been isolated from the C_{13}-dilactone XI evidently as a mixture of stereoisomers. Attempts to isolate a second diol corresponding to the ketoalcohol XIII were unsuccessful.

Additional evidence for the structure of the δ-lactone portion of the C_{13}-dilactone XI was furnished by isolation of the 2,4-dinitrophenylhydrazone XX of the aldehyde acid XVIII. Alkaline hydrolysis of the C_{13}-dilactone XI followed by periodate oxidation gave two carbonyl containing compounds (XVIII and XIX) which were isolated as their 2,4-dinitrophenylhydrazones (XX and XXI). Analytical data obtained from the first of these 2,4-dinitrophenylhydrazones (XX) indicated that it was derived from α,γ-dimethylglutaraldehydic acid (XVIII). The infrared spectrum and X-ray diffraction pattern of the derivative XX were identical with those of the 2,4-dinitrophenylhydrazone of α,γ-dimethylglutaraldehydic acid prepared from *meso*-α,α'-dimethylglutaric anhydride. However, there is some difference in melting points of the two compounds which may be due to substantial alteration in melting point arising from small amounts of impurities as most of the evidence indicates that the two 2,4-dinitrophenylhydrazones are the same.

The structure of the γ-lactone portion of the C_{13}-dilactone XI is shown by the isolation of α-methyllevulinic acid from XI. The second 2,4-dinitrophenylhydrazone XXI gave analytical data which were consistent with formulation of its structure as α-methyllevulinic acid 2,4-dinitrophenylhydrazone. The melting point and X-ray diffraction pattern of XXI were identical with those of synthetic (−)-α-methyllevulinic acid 2,4-dinitrophenylhydrazone. The infrared spectrum was identical with that of synthetic DL-α-methyllevulinic acid. However, the optical rotation of various samples of the 2,4-dinitrophenylhydrazone XXI derived from

(4) The numbering used in this discussion is indicated in the schematic formula



the C_{13}-dilactone XI varied and none were as high as that of the resolved synthetic XXI. Hypoiodite oxidation of the mixture containing the acids XVIII and XIX gave rise to an acid which was converted to its bis-(*p*-bromophenacyl) ester XXIII. Comparison of this by the usual analytical and physical methods with an authentic sample of DL-α-methylsuccinic acid bis-(*p*-bromophenacyl) ester showed that the two were identical.[5] These data are indicative of formation of α-methyllevulinic acid during the oxidation of the hydrolyzed C_{13}-dilactone XI but as a mixture of D- and L-isomers arising from racemization at some point in the reaction series leading from erythromycin.

The synthetic compounds used for comparison were prepared by the routes shown in Fig. 4.

The degradative products obtained from the C_{13}-dilactone XI coupled with analytical and physical data derived from the lactone proved that it has structure XI. This in turn leaves only structure X for the preceding lactone. These lactones contain one of the two vicinal oxygen pairs present in the lactone ring of erythromycin, and this is necessarily the one at C-5–C-6, since the C-12–C-15 portion of erythronolide could not correspond to either the γ-lactone or the δ-lactone segments of lactone X. The double bond conjugated with the δ-lactone carbonyl in X must arise from hydroxyl at the original C-3. Since this is true, then the six-membered lactone portion of X represents the C-1–C-5 part of the original erythromycin molecule, and the five-membered lactone represents C-6–C-9. There is no oxygen substituent in the C_{13}-dilactone X which could have been derived from the ketonic carbonyl by the conditions used other than the one at C-9. The isolation of the hydroxyketone VI indicates that hydroxyl groups are present at C-11 and C-12. Previous evidence[2d] shows the absence of oxygen at C-10 so that the ketonic carbonyl cannot be at a carbon beyond C-9 and consequently must be at this position. Furthermore the regularly alternating methyl substituents in the C_{13}-dilactone X are additional proof that the C_9 moiety of dihydroerythronolide must fit into the aglycone as previously reported[2d] instead of in the alternative position mentioned. In summary, these findings unequivocally establish the structure of the aglycone portion of erythromycin to be as shown in I.

A number of possible positions for cladinose can be eliminated from a consideration of the oxygen substituents in the erythromycin lactone ring. As mentioned previously, the lactone ring terminates at C-13, and hydroxyl groups are at C-11 and C-12. The ketone function is at C-9, leaving only C-3, C-5 and C-6 as possible sites for cladinose. It has been pointed out previously[2b] that desosamine is attached to a carbon atom adjacent to one having a hydroxyl group. Since there are only two such possibilities, namely, C-11–C-12 and C-5–C-6, and the former has been eliminated, of necessity then desosamine is at C-5 or C-6 and cladinose may be at the remaining carbon of this pair or at C-3. The formation of a ketal upon acid treatment of erythromycin[2b] would be evidence that hydroxyl was attached to C-5 or C-6 and consequently proof of such reaction would place cladinose. Very mild acid treatment of erythromycin, *p*H 2.5 for a few minutes or *p*H 4.0 for several hours, formed anhydroerythromycin (XXX) which has the molecular formula $C_{37}H_{65}NO_{12}$ indicating loss of one mole of water either by spiroketal or olefin formation. The infrared spectrum of this compound shows the disappearance of one carbonyl group, and ultraviolet absorption studies made it clear that this was the ketonic carbonyl. A strong band appeared in the infrared at 11.04 μ which is probably due to the two oxygen containing rings involved in the spiroketal. This compound was very resistant to alkaline hydrolysis in agreement with the properties of similar spiroketals obtained from methymycin.[6] There was no evidence for an olefin in the infrared spectrum. In addition, the loss of water to form an olefinic hemiketal would make it necessary that the molecule should have four active hydrogens rather than the three found. Anhydroerythromycin N-oxide, obtained by oxidation of anhydroerythromycin, gave a negative tetranitromethane test for carbon–carbon double bonds. These facts indicate that the water loss was due to spiroketal rather than olefin formation. Periodate oxidation of anhydroerythromycin, which was done on the N-oxide to avoid the complications introduced by the basic tertiary nitrogen, revealed the absence of vicinal hydroxyl groups. Therefore the hydroxyl group at C-12 must take part in the spiroketal formation. The one at C-11 would be most unlikely to enter into such reaction because of the extremely strained ring system which would result. The second hydroxyl participating in the spiroketal formation would then be the one existing at either C-5 or C-6 since a hydroxyl at C-3 would be too distant to participate in this reaction. In this case cladinose could be at neither C-5 nor C-6 and therefore must be at C-3.

Erythralosamine (VIII) has been discussed in some detail previously.[2a,b] It has been shown to be derived from erythromycin by hydrolytic cleavage with loss of cladinose and two moles of water. The lactone carbonyl is still present, but the ketonic group has disappeared. There are present only two hydroxyl groups; one is in desosamine which is still present. Anhydroerythromycin (XXX) is very easily convertible to erythralosamine which is similar to XXX in being resistant to alkaline hydrolysis. The isolation of the C_{13}-dilactone X by degradation of erythralosamine shows that there has been no rearrangement of carbon or oxygen functions in the C-1–C-9 portion of the lactone ring of erythralosamine. These facts are consistent with the structure VIII proposed. Although lactone VIII is not easily hydrolyzed by alkali, vigorous hydrolyzing conditions led to a complete breakdown of the molecule with elimination of dimethylamine.[2a] This can only result from elimination of the desosamine by alkali, this being followed by decomposition of the desosamine. In order for desosamine to be eliminated, it must be in a position in which an adjacent hydrogen could be activated by one of the carbonyl functions. Only the C-5 position would fulfil this requirement. This could occur by alkaline

(5) We wish to thank Mr. W. N. Cannon for supplying the DL-α-methylsuccinic acid.

(6) C. Djerassi and J. A. Zderic, THIS JOURNAL, **78**, 6390 (1956).

elimination of the hydroxyl group at C-3 to give an α,β-unsaturated lactone. In such a system hydrogen at C-4 would be activated, and desosamine, if at C-5, could be eliminated. The great stability of the lactone ring to alkali is undoubtedly an important factor in this reaction. Because of this the activating effect of the lactone ring remains long enough for the elimination to occur. These considerations and the fact that desosamine is at either C-5 or C-6 are sufficient evidence to prove that desosamine is at C-5 and complete the proof that erythromycin has structure I. This assignment of the desosamine position is in agreement with findings on methymycin[6] and pikromycin[7] in that the oxygen attached to desosamine is secondary rather than tertiary.

A tentative assignment of the configuration of the asymmetric carbon atoms C-2, C-3, C-4, C-8, C-9, C-10 and C-13 in IV has been made.[2d] The corresponding carbon atoms in I, II and III would undoubtedly have the same configurations. In the formulas written in the present publication, no implications as to stereochemistry are intended beyond those found in the previous publication.[2d]

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 and Miss Ann VanCamp for physical chemical data.

Experimental[8]

2,3-Pentanedione Bis-(2,4-dinitrophenylhydrazone) (VII) from Periodate Oxidation of Erythromycin N-Oxide.—Erythromycin N-oxide (10 g., 2 mmoles) was dissolved in 100 ml. of methanol, and 100 ml. of 0.02 *M* sodium periodate was added. The solution was allowed to stand at room temperature overnight and then evaporated *in vacuo* to remove the methanol. The aqueous residue was extracted with four 50-ml. portions of chloroform. Evaporation of the combined chloroform extracts to dryness gave a residue which weighed 1.34 g. This residue was suspended in 200 ml. of 0.05 *N* sodium hydroxide solution, and the suspension was steam distilled until 100 ml. of distillate was collected.

To a 15-ml. aliquot of the distillate, 1 ml. of a 20% hydroxylamine hydrochloride solution, 3 ml. of a 20% sodium acetate solution and 1 ml. of a 10% nickel chloride solution was added. No precipitate formed on standing.

A 20-ml. aliquot of the distillate was mixed with 15 ml. of a 10% ferric chloride solution, and the mixture was distilled. Fifteen milliliters of distillate was collected and treated with hydroxylamine hydrochloride, sodium acetate and nickel chloride as described above. An orange precipitate formed.

To a third aliquot (50 ml.), 400 ml. of Brady reagent was added. The initial material which precipitated was removed, and a crystalline product which melted at 274–276° formed on standing. The X-ray diffraction pattern of this material was identical with the pattern obtained from an authentic sample of 2,3-pentanedione bis-(2,4-dinitrophenylhydrazone).

Chromic Oxide Oxidation of Erythralosamine to IX.—Fifteen grams of erythralosamine was dissolved in 150 ml. of glacial acetic acid, and a solution of 4.0 g. of chromic oxide in 100 ml. of 50% aqueous acetic acid was added dropwise with stirring at 45° over a period of 1 hr. After the reaction mixture had stood overnight, it was diluted with 800 ml. of water, adjusted to *p*H 7.5 with 20% sodium hydroxide solution and extracted with one 400-ml. portion of chloroform followed by three 300-ml. portions. The combined chloroform extracts were washed with 200 ml. of 2.5% sodium carbonate solution, then with 200 ml. of water and evaporated to dryness. The residue was triturated with 300 ml. of hot petroleum ether (60–70°). The insoluble material (7.8 g.) was crystallized from 50% methanol to give 1.15 g. of product melting at 200–210°. Another recrystallization from chloroform raised the melting point to 208–215°. Titration in 66% dimethylformamide showed a pK'_a of 8.0.

Anal. Calcd. for $C_{29}H_{51}NO_{11}$: C, 59.05; H, 8.73; N, 2.37; mol. wt., 590. Found: C, 59.14; H, 8.67; N, 2.37; mol. wt., 574 (elect. titr.).

Another sample was purified by two recrystallizations from acetonitrile, m.p. 219–221°.

Anal. Found: C, 59.06; H, 8.75; N, 2.32; mol. wt., 592 (X-ray).

Unsaturated C_{13}-Dilactone (X).—Five hundred milligrams of the erythralosamine oxidation product IV was dissolved in 30 ml. of 10% aqueous hydrochloric acid, and the solution was heated under reflux for 4 hr. This was followed by extraction of the cooled solution with four 50-ml. portions of chloroform. The combined chloroform extracts were washed with water, and then evaporated to dryness under reduced pressure leaving a crystalline residue. After this residue had been recrystallized twice from isopropyl ether, it melted at 132–134°. The infrared spectrum had absorption bands at 5.67 and 5.83 μ. There was absorption in the ultraviolet spectrum at 209 mμ, ϵ 13,000.

Anal. Calcd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61; CH_3C (4), 25.1; mol. wt., 238.3. Found: C, 65.43, 65.27; H, 7.76, 7.50; CH_3C, 19.1; mol. wt., 254.6 (ebull. in acetone).

Saturated C_{13} Dilactone (XI).—One gram of the unsaturated C_{13}-dilactone X was dissolved in 50 ml. of methanol, and the solution was shaken under hydrogen at 33 p.s.i. in the presence of platinum oxide for 20 hr. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. Crystallization of the residue twice from isopropyl ether gave 270 mg. of product melting at 128–130°. There was only end absorption in the ultraviolet. The infrared spectrum had a broad band at 5.66–5.78 μ with a slight maximum at 5.72.

Anal. Calcd. for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39; CH_3C (4), 24.9; mol. wt., 240.3. Found: C, 65.17, 65.04; H, 8.39, 8.41; CH_3C, 21.1; mol. wt., 241.8 (X-ray).

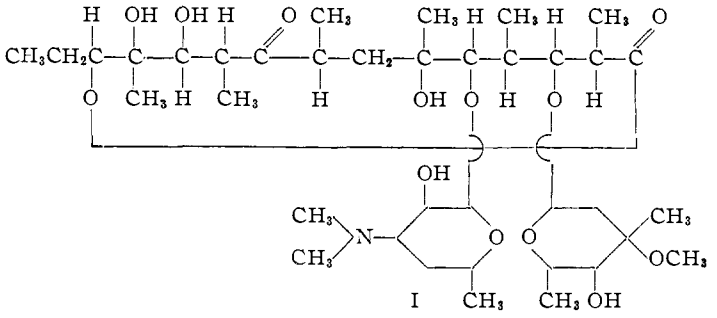
Alkaline Hydrolysis of C_{13}-Dilactone XI and Reconversion to Lactone.—A suspension of 240 mg. (1.0 mmole) of C_{13}-dilactone XI in 5.0 ml. of 1.0 *N* sodium hydroxide solution was prepared, and the mixture was heated on the steam-bath for 3 hr. After the solution cooled, it was diluted to 250 ml. with water and titrated to *p*H 7.5 with 1.0 *N* hydrochloric acid. The consumption of base in the hydrolysis was 1.2 mmoles. The solution was then diluted with an equal volume of 1.0 *N* hydrochloric acid. No benzene or chloroform extractable material was present (*i.e.*, dilactone). The solution was then diluted with an equal volume of concentrated hydrochloric acid and allowed to stand for 48 hr. at room temperature. Extraction with chloroform gave a gum which crystallized from isopropyl ether giving a product melting at 117–122°. An X-ray diffraction pattern showed that this product was identical with the C_{13}-dilactone XI.

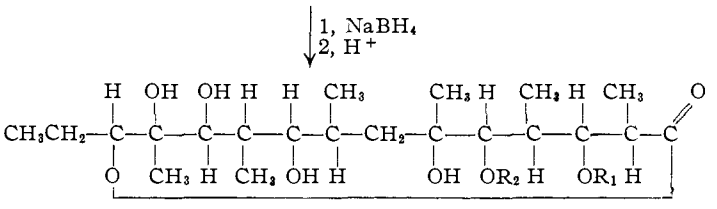
2,4-Dimethyl-1,5-pentanediol Bis-(3,5-dinitrobenzoate) (XVI) from C_{13}-Dilactone XI. (a) **Reduction of C_{13}-Dilactone XI.**—One and forty-five hundredths grams (6.0 mmoles) of C_{13}-dilactone XI was placed in the thimble of a Soxhlet extractor. This was extracted using 800 ml. of boiling ether containing 4.5 g. (120 mmoles) of lithium aluminum hydride until the lactone had disappeared. The ether solution was cooled and water (4.75 ml.) was added slowly followed by 3.56 ml. of 20% sodium hydroxide solution and 16.6 ml. of water. The solid was removed by filtration, and the filtrate was washed with three 100-ml. portions of ether which were added to the filtrate. The combined ether solution was washed with saturated sodium chloride solution, filtered and evaporated under reduced pressure. The yield of residual oil weighed 1.54 g. The infrared spectrum showed the presence of hydroxyl groups but carbonyl groups were absent.

(b) **Periodate Oxidation.**—The residue from the reduction (1.54 g., 6 mmoles) was dissolved in 60 ml. of water, and 1.33 g. (6.2 mmoles) of sodium metaperiodate was added.

(7) R. Anliker and K. Gubler, *Helv. Chim. Acta*, **40**, 119 (1957).

(8) Melting points were determined on a Kofler micro melting point apparatus unless indicated otherwise. The capillary melting points are uncorrected.





II, R_1 = cladinosyl, R_2 = desosaminyl $\xrightarrow{H^+}$ III, R_1 = H, R_2 = desosaminyl $\xrightarrow{H^+}$ IV, $R_1 = R_2 = H$

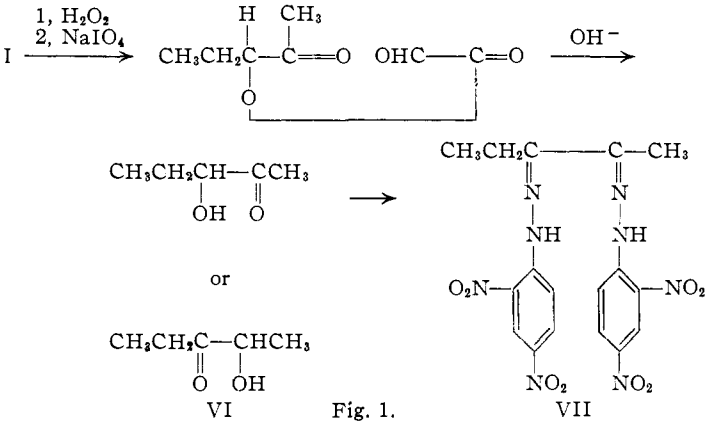
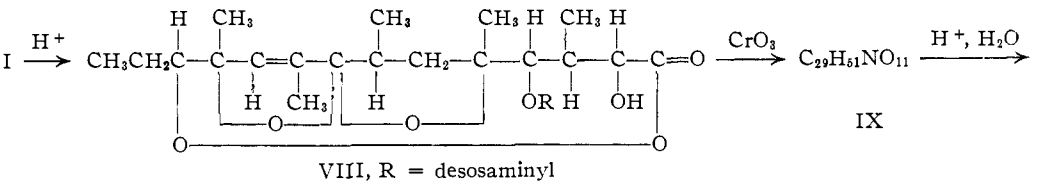
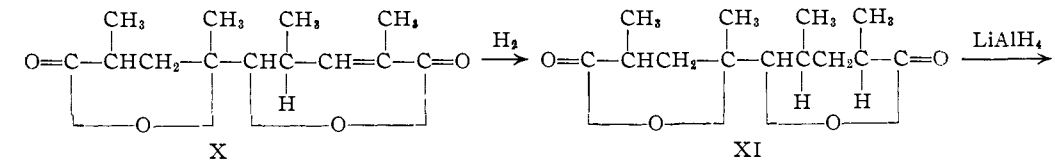


Fig. 1.



VIII, R = desosaminyl



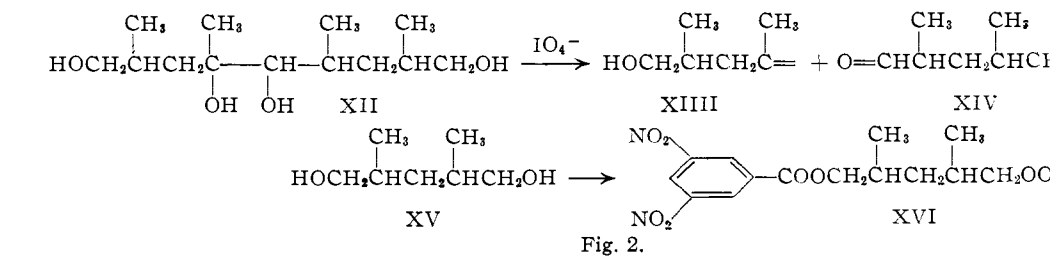


Fig. 2.

anol, and 0.17 g. (4.6 mmoles) of sodium borohydride was added. After the reaction mixture had stood for 4 hr., it was adjusted to *p*H 3.5 with concentrated hydrochloric acid and extracted continuously overnight with ether. The ether extract was dried over magnesium sulfate, filtered and evaporated to dryness under reduced pressure. The residual liquid weighed 1.15 g. Its infrared spectrum showed strong absorption at 3.0 μ but none in the carbonyl region.

(d) **Preparation of Ester (XVI).**—This was prepared according to the procedure of Brewster and Ciotti.[9] One-half gram of the sodium borohydride reduction product was used and 1.74 g. (8.2 mmoles) of 3,5-dinitrobenzoic acid and 3.13 g. (16.4 mmoles) of *p*-toluenesulfonyl chloride were used. After the product had been crystallized from alcohol, it weighed 0.7 g. and melted at 128–135° (cap.). Two more recrystallizations from ethanol gave a melting point of 128–130° (cap.). The infrared spectrum was identical with that of both synthetic *meso* and mixed *meso*-DL-2,4-dimethyl-1,5-pentanediol bis-(3,5-dinitrobenzoate) as were the X-ray diffraction patterns. There was no depression in melting point when this product was mixed with synthetic mixed *meso*-DL. The product was not optically active.

Anal. Calcd. for $C_{21}H_{20}N_4O_{12}$: C, 48.50; H, 3.88; N, 10.78. Found: C, 48.48; H, 4.00; N, 10.96.

Periodate Oxidation of Alkali Hydrolyzed C_{13}-Dilactone XI. (a) **α,γ-Dimethylglutaraldehydic Acid 2,4-Dinitrophenylhydrazone (XX).**—One-half gram (2.0 mmoles) of the C_{13}-dilactone XI was suspended in 10 ml. of 1.0 *N* sodium hydroxide solution, and the mixture was heated for 4 hr. on the steam-bath. The resulting solution was diluted to 20 ml. with water and titrated to *p*H 8.0 with 1.0 *N* hydrochloric acid. A total of 4.13 mmoles of base was neutralized by the hydrolysis. This solution was filtered. To the filtrate was added a solution of 430 mg. (2.0 mmoles) of sodium metaperiodate in 5 ml. of water dropwise with stirring over a period of 30 minutes. After the oxidation mixture had stood for 1 hr. at room temperature, 500 mg. of barium chloride was added. The barium iodate which precipitated was removed by filtration, and the filtrate was added to 450 ml. of Brady reagent. The crystalline precipitate, which had formed after the solution had stood for 2 hr. at room temperature, it was extracted continuously overnight with ether. The ether extract was dried over magnesium sulfate, filtered and evaporated to dryness. The residue was a liquid.

(c) **Sodium Borohydride Reduction.**—The product from periodate oxidation was dissolved in 50 ml. of 50% meth-

(9) J. H. Brewster and C. J. Ciotti, Abstracts of Papers of September, 1955, Meeting of the American Chemical Society, p. 60-O.

stood overnight, weighed 87 mg. and melted at 85–90°. One recrystallization from chloroform–petroleum ether raised the melting point to 102–105°. The remaining product was purified by chromatography on 2.0 g. of silica gel using a 1:1 chloroform–benzene solvent mixture. The main fraction, which moved as a solvent band, weighed 30 mg. and melted at 108–110° after recrystallization from chloroform–petroleum ether. The infrared spectra by mull and in chloroform solution were identical with those of synthetic α,γ-dimethylglutaraldehydic acid 2,4-dinitrophenylhydrazone obtained from *meso*-α,β-dimethylglutaric acid. The X-ray diffraction patterns were also identical.

Anal. Calcd. for $C_{13}H_{16}N_4O_6$: C, 48.15; H, 4.97; N, 17.28. Found: C, 48.86; H, 5.26; N, 16.94.

(b) **α-Methyllevulinic Acid 2,4-Dinitrophenylhydrazone (XXI).**—The filtrate from the above 87 mg. was extracted with five 100-ml. portions of reagent grade ether. The combined ether extracts were washed with water and concentrated to dryness. The oily residue crystallized on cooling and weighed 510 mg. This material was dissolved in 15 ml. of chloroform and 50 ml. of benzene was added. The resulting solution was placed on a column containing 20 g. of silica gel which had been packed with the same solvent mixture. Development of the chromatogram with this solvent combination gave two major fractions, the first (60 mg.) being identified as acetaldehyde 2,4-dinitrophenylhydrazone by analysis and X-ray diffraction. The second fraction, collected in several portions and combined, weighed about 80 mg. Crystallization from chloroform–petroleum ether gave a melting point of 183–186°. The infrared spectrum was identical with that of synthetic DL-α-methyllevulinic acid 2,4-dinitrophenylhydrazone. The X-ray diffraction pattern differed from that of DL- but was identical with that of synthetic (−)-α-

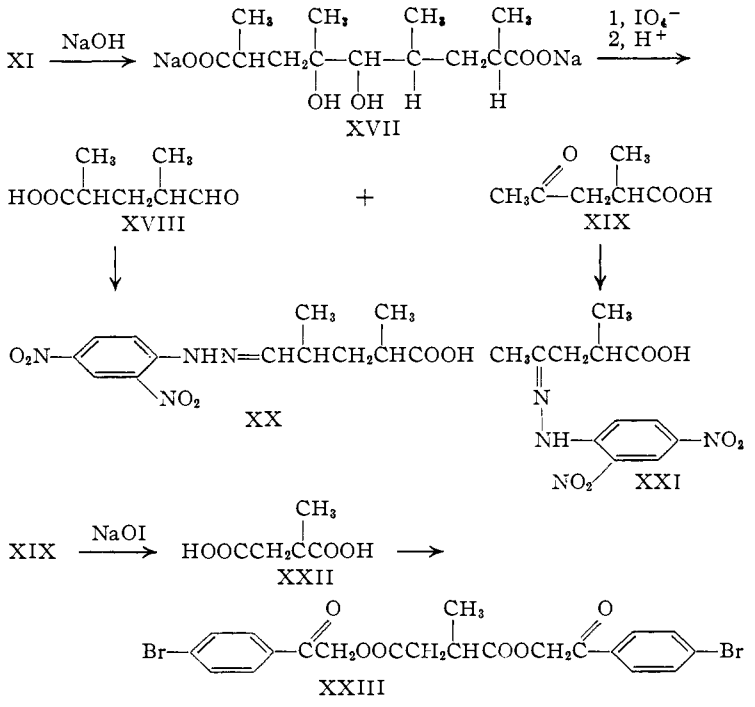


Fig. 3.

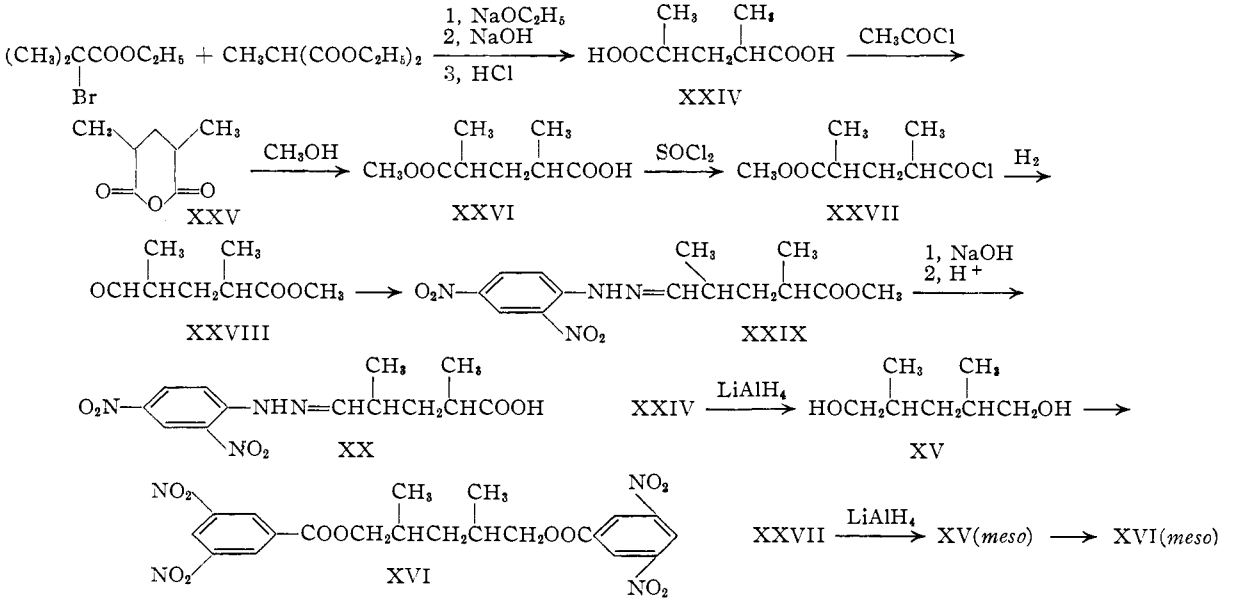


Fig. 4.

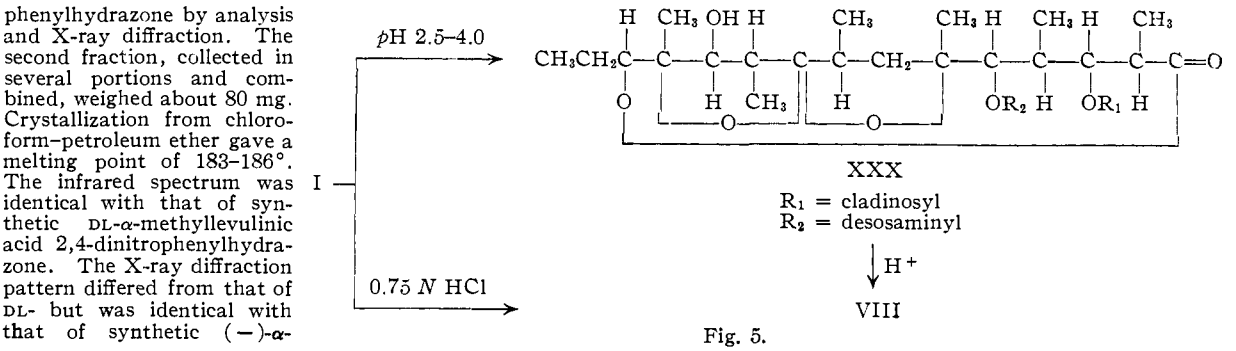


Fig. 5.

methyllevulinic acid 2,4-dinitrophenylhydrazone. After two additional crystallizations from ethanol, the product melted at 190–193°.

Anal. Calcd. for $C_{12}H_{14}N_4O_6$: C, 46.45; H, 4.55; N, 18.06. Found: C, 46.26; H, 4.69; N, 17.88.

DL-α-Methylsuccinic Acid Bis-(*p*-bromophenacyl Ester) (XXIII) from C_{13}-Dilactone XI.—C_{13}-Dilactone XI (1.25 g., 5.2 mmoles) was used, and the experiment was run essentially as in the preceding experiment up to the point at which Brady reagent was added. In this case the reaction mixture was acidified with 1.0 *N* hydrochloric acid and extracted continuously with ether for 16 hr. The ether extract was dried over magnesium sulfate, filtered and evaporated to dryness under reduced pressure. The residual oil weighed 1.12 g.

The oil was dissolved in 30 ml. of 6% sodium hydroxide solution, and 50 ml. of potassium iodide–iodine solution containing 8.6 g. of potassium iodide and 4.3 g. of iodine was added dropwise over a period of 20 minutes. After the solution had stood overnight, the iodoform was removed by filtration. The filtrate was acidified with 6 *N* hydrochloric acid, and sodium bisulfite was added until the color was discharged. Continuous extraction with ether overnight followed by concentration of the extract gave a residual oil weighing 0.91 g. A solution of the residue in methanol was adjusted to *p*H 8.1 with 65 ml. of 1.0 *N* sodium hydroxide solution, and the mixture was evaporated to dryness. Ten milliliters of water was added to the residue, and the solution was adjusted to *p*H 6.8 by the addition of 5 ml. of 1.0 *N* sodium hydroxide solution. To this solution was added 30 ml. of ethanol and 2.0 g. of *p*-bromophenacyl bromide, and the mixture was boiled for 2 hr. Cooling the reaction mixture gave 1.0 g. of amorphous solid. After one crystallization from ethanol, the product weighed 0.4 g. and melted at 120–125°. A mixed melting point with the corresponding ester of synthetic DL-α-methylsuccinic acid was not depressed, and the infrared spectra of the two compounds were identical.

Anal. Calcd. for $C_{21}H_{18}O_6Br_2$: C, 47.93; H, 3.45; Br, 30.83. Found: C, 48.28; H, 4.01; Br, 30.37.

Anhydroerythromycin (XXX).—Five grams of erythromycin was suspended in 100 ml. of water. Acetic acid was added slowly until the solid had dissolved and the *p*H was stationary at 4.0. The solution was allowed to stand at room temperature for 48 hr. and neutralized with sodium bicarbonate. Extraction with three 25-ml. portions of chloroform and evaporation of the extracts gave 5.4 g. of residue, m.p. 110–120° (cap.). One gram of this was crystallized by dissolving it in 60 ml. of water at 0°, filtering and warming slowly to 55°. A second crystallization using 30 ml. of water gave a product melting at 142–150° (cap.). Before this material was dried, it was crystalline but loss of solvent destroyed the crystallinity. The infrared spectrum showed absorption at 2.90 μ, at 5.83 μ and at 11.04 μ. The ultraviolet spectrum was transparent in the carbonyl region.

Anal. Calcd. for $C_{37}H_{65}NO_{12}$: C, 62.07; H, 9.15; N, 1.96; CH_3C (10), 20.8; active H, 3.0; mol. wt., 718. Found: C, 62.28, 62.25; H, 9.37, 9.24; N, 1.95. 2.02; CH_3C–, 15.7; active H, 3.12, 3.23; mol. wt., 768 (elect. titr.).

A similar product was obtained by adjusting to *p*H 2.5 with concentrated hydrochloric acid and neutralizing after thirty minutes.

Anhydroerythromycin Methiodide.—One-half gram of anhydroerythromycin was dissolved in 25 ml. of dry ether, and 1 ml. of methyl iodide was added. The solid which precipitated after 18 hr. standing weighed 0.42 g. and melted at 209–217° (cap.). Two recrystallizations from a mixture of absolute alcohol–dry ether gave a product melting at 224–229° dec. (cap.). The infrared spectrum showed strong hydroxyl absorption at 2.90, carbonyl absorption at 5.86 and a band at 11.06 μ.

Anal. Calcd. for $C_{38}H_{68}INO_{12}$: C, 53.19; H, 8.00; I, 14.79. Found: C, 53.26, 53.44; H, 8.04, 8.30; I, 14.14.

Anhydroerythromycin N-Oxide.—Two grams of anhydroerythromycin was dissolved in 50 ml. of methanol. Forty milliliters of water and 10 ml. of 30% hydrogen peroxide were added, and the solution was allowed to stand for three days. The methanol was removed by evaporation under reduced pressure. The residue was extracted with one 40-ml. portion and three 20-ml. portions of chloroform. Evaporation of the combined extracts gave 1.95 g. of product melting at 166–175° (cap.). This was purified by solution in chloroform and precipitation with petroleum ether (60–70°). An amorphous product melting at 180° (cap.) was obtained. The ultraviolet absorption spectrum had only end absorption. The infrared spectrum had bands at 2.85, 5.84 and 11.06 μ. The pK'_a in 66% dimethylformamide was 5.4.

Anal. Calcd. for $C_{37}H_{65}NO_{13}$: C, 60.71; H, 8.96; N, 1.92; mol. wt., 732. Found: C, 60.54, 60.48; H, 9.07, 9.18; N, 2.12, 1.99; mol. wt., 777 (X-ray).

The same product was obtained by treating erythromycin N-oxide with acid under the conditions used to obtain anhydroerythromycin.

This compound gave a negative tetranitromethane test for carbon–carbon double bonds.

Alkaline Hydrolysis of Anhydroerythromycin.—One gram of anhydroerythromycin was dissolved in 25 ml. of alcohol, and 25 ml. of 2.0 *N* sodium hydroxide solution was added. The solution was refluxed for 5 hr. The alcohol was removed by evaporation under reduced pressure. Concentrated hydrochloric acid was added until the *p*H was 7.0. Extraction with three 10-ml. portions of chloroform and evaporation gave 0.7 g. of product which gave an infrared spectrum identical to that of starting material, and titration indicated the absence of a carboxyl group.

It was found that treatment with boiling 1.0 *N* sodium hydroxide solution for 48 hr. did give a large amount of hydrolysis.

Periodate Titration of Anhydroerythromycin N-Oxide.—This was run three times using approximately 130 mg. of anhydroerythromycin N-oxide. This material was dissolved in 50 ml. of methanol, and 40 ml. of water and 10 ml. of 0.1 *M* sodium periodate were added. Ten-milliliter aliquots were titrated using 0.01 *M* arsenite solution. In no case was there any consumption of periodate over a period of 24 hr.

Erythralosamine N-Oxide from Anhydroerythromycin N-Oxide.—This was run exactly as described in the methanolysis of erythromycin.[2a] From 2.0 g. of anhydroerythromycin N-oxide, 0.19 g. of the erythralosamine N-oxide melting at 195° was obtained. The melting point and X-ray diffraction pattern were identical to those of erythralosamine N-oxide.[2a]

Diethyl 2-Carbethoxy-2,4-dimethylglutarate.—This was run according to the general procedure of Auwers[10] but with considerable modification.

Sodium (15 g.) was added to 300 ml. of absolute alcohol, and after reaction was complete, the excess alcohol was distilled into a 3-necked one-liter round-bottomed flask in which sodium ethoxide was prepared by adding 10.8 g. (0.47 atom) of sodium. After all the sodium had reacted, the solution was heated to boiling, stirred rapidly and 82 g. (0.47 mole) of diethyl methylmalonate was added in about two minutes. Ethyl α-bromoisobutyrate (92 g., 0.47 mole) was added immediately. The solution deposited sodium bromide rapidly. Stirring was continued and the mixture was refluxed for 2 hr. The solution gave a neutral reaction with moist litmus paper. Most of the ethanol was removed under reduced pressure, 200 ml. of water was added and the resulting organic layer was separated. The aqueous layer was washed once with ether, and the ether was combined with the organic fraction. Distillation was carried out at 0.7 mm. The fraction boiling at 124–126° was redistilled to yield 75.0 g. (55%), b.p. 123–125° at 0.6 mm.

Anal. Calcd. for $C_{14}H_{24}O_6$: C, 58.31; H, 8.39. Found: C, 58.19; H, 8.38.

α,α′-Dimethylglutaric Acid (XXIV).—Five hundred milliliters of 2 *N* sodium hydroxide solution was added to 75 g. (0.26 mole) of diethyl 2-carbethoxy-2,4-dimethylglutarate in 400 ml. of ethanol. After the solution had stood at room temperature for 48 hr., 500 ml. of concentrated hydrochloric acid was added, and the reaction solution was heated under reflux for 24 hr. The cooled solution was extracted with one 300-ml. portion and three 100-ml. portions of chloroform. The combined extracts were dried, and the chloroform was removed. The residue was crystallized from 200 ml. of petroleum ether at 5° to give 24 g. (58%) of a mixture of stereoisomers of α,α′-dimethylglutaric acid of melting point 104–106°. Titration in water showed pK'_a values of 4.2 and 5.4.

(10) K. Auwers, *Ber.*, **24**, 1929 (1891).

Anal. Calcd. for $C_7H_{12}O_4$: C, 52.49; H, 7.55. Found: C, 52.60; H, 7.47.

Meso-α,α'-dimethylglutaric Anhydride (XXV).—This was done by a modification of Auwers' procedure.[11]

Forty-one grams (0.26 mole) of α,α'-dimethylglutaric acid was suspended in 115 g. of acetyl chloride, and the mixture was allowed to stand at room temperature for 20 hr. The resulting solution was heated under reflux for 1 hr., and excess acetyl chloride was removed under reduced pressure. The residual gum was dissolved in 100 ml. of benzene, and 290 ml. of petroleum ether was added. The crystalline product which separated weighed 20.1 g. (54%), m.p. 76–82°. Two additional recrystallizations in a similar manner gave 14 g. of *meso*-α,α'-dimethylglutaric anhydride, m.p. 95–95.5°.

Anal. Calcd. for $C_7H_{10}O_3$: C, 59.14; H, 7.09. Found: C, 59.49; H, 7.16.

Monomethyl α,α'-Dimethylglutarate (XXVI).—A mixture of 19.5 g. (0.14 mole) of *meso*-α,α'-dimethylglutaric anhydride and 10 ml. of methanol was refluxed for 3 hr. The reaction mixture was allowed to stand overnight at room temperature and distilled at 0.3 mm. The fraction boiling at 110–112° was collected. The yield was 17.0 g. (71%).

Anal. Calcd. for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.00; H, 8.09.

Methyl 2-Methyl-4-(chloroformyl)-valerate (XXVII).—Twenty milliliters of thionyl chloride was added to 17 g. (0.098 mole) of monomethyl α,α'-dimethylglutarate, and the mixture was allowed to stand at room temperature for 1 hr. Reaction was completed by heating on the steam-bath for 2 hr. Distillation of the mixture gave a product boiling at 68–70° at 0.4 mm.

Anal. Calcd. for $C_8H_{13}O_3Cl$: C, 49.88; H, 6.80. Found: C, 49.60; H, 7.01.

Methyl α,γ-Dimethylglutaraldehydate (XXVIII).—Methyl 2-methyl-4-(chloroformyl)-valerate (5.0 g., 26 mmoles) was dissolved in 250 ml. of dry xylene. Seven hundred milligrams of 5% palladium on barium sulfate catalyst and 0.06 ml. of quinoline–sulfur catalyst poison[12] were added. Efficient stirring was maintained, hydrogen was bubbled through the mixture, then the vessel was placed in an oil-bath at 135°. Exit gases were trapped in 0.1 *N* sodium hydroxide solution. After 4 hr. at 135°, 23 meq. (88% of theory) of base had been neutralized by the evolved hydrogen chloride. The reaction mixture was cooled, filtered and the xylene was removed at 8 mm. The remainder of the liquid distilled at 50° at 0.35 mm.

Anal. Calcd. for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.06; H, 9.23.

Titration indicated the presence of a small amount of free acid.

α,γ-Dimethylglutaraldehydic Acid 2,4-Dinitrophenylhydrazone (XVI).—A portion of the methyl α,γ-dimethylglutaraldehydate was converted to its 2,4-dinitrophenylhydrazone by treatment with Brady reagent. The product was recrystallized from alcohol. It was then dissolved in ethanol, and an equal volume of 0.1 *N* sodium hydroxide solution was added. After the solution had stood at room temperature for 2 hr., it was acidified with 1 *N* hydrochloric acid, then diluted to turbidity with water. The crystals which formed were removed by filtration and recrystallized twice from ethanol and twice from chloroform–petroleum ether. The product melted at 125–132°. The infrared spectrum of this compound in a mineral oil mull and in chloroform and its X-ray diffraction pattern were identical with those of the 2,4-dinitrophenylhydrazone XVI obtained from the C_{13}-dilactone XI.

2,4-Dimethyl-1,5-pentanediol Bis-(3,5-dinitrobenzoate) (XXIII).—A solution of 1.58 g. (10 mmoles) of α,α'-dimethylglutaric acid in 350 ml. of dry ether was added to a well-stirred mixture of 7.6 g. (200 mmoles) of lithium aluminum hydride in 1 l. of dry ether. The mixture was stirred and refluxed for 1 hr. Eight milliliters of water was added slowly to the cooled solution followed by 6 ml. of 20% sodium hydroxide solution and more water (28 ml.). The solids were removed by filtration and washed with three 100-ml. portions of dry ether. The combined ether solutions were washed with 32 ml. of saturated sodium chloride solution, and the ether was removed by evaporation under reduced pressure. The residue weighed 1.08 g.

This was converted to its bis-(3,5-dinitrobenzoate) using the procedure of Brewster and Ciotti.[9] The product melted at 128–132° (cap.) after two recrystallizations from alcohol. This was identical with material from the C_{13}-dilactone XI as shown by identical infrared curves and X-ray diffraction patterns and by no depression in mixed melting point.

Anal. Calcd. for $C_{21}H_{20}N_4O_{12}$: C, 48.50; H, 3.88; N, 10.78. Found: C, 48.35; H, 3.62; N, 10.99.

The bis-(3,5-dinitrobenzoate) of the *meso* compound was obtained in the same fashion except using methyl 2-methyl-4-(chloroformyl)-valerate (XXVII) as the starting material. The product was recrystallized twice from ethanol, m.p. 121° (cap.). The infrared spectrum and X-ray diffraction pattern were identical with the mixed *meso*-DL-derivative and with material derived from the C_{13}-dilactone XI.

Anal. Calcd. for $C_{21}H_{20}N_4O_{12}$: C, 48.50; H, 3.88; N, 10.78. Found: C, 48.61; H, 4.02; N, 10.94.

DL-α-Methyllevulinic Acid 2,4-Dinitrophenylhydrazone.—The acid was prepared according to the procedure of Chakravarti[13] in 27% over-all yield, b.p. 130° at 12 mm.; $[\alpha]^{25}D$ 1.4395.

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.58; H, 7.99.

One hundred and sixty-four milligrams (1.26 mmoles) of DL-α-methyllevulinic acid was dissolved in 250 ml. of Brady reagent. After the solution had stood for several hours at room temperature, the crystalline precipitate was removed by filtration, washed with cold water and air-dried, m.p. 190–194°. The infrared spectrum was identical with that of α-methyllevulinic acid 2,4-dinitrophenylhydrazone (XV) derived from C_{13}-dilactone XI, but the X-ray diffraction patterns differed.

Anal. Calcd. for $C_{12}H_{14}N_4O_6$: C, 46.45; H, 4.55; N, 18.06. Found: C, 46.79; H, 4.74; N, 18.16.

(−)-α-Methyllevulinic Acid 2,4-Dinitrophenylhydrazone (XV). (a) ***d*-Dimethyl Tartrate.**—*d*-Tartaric acid was esterified according to the procedure of Gonzalez R.[14] The yield of ester boiling at 160–168° at 17 mm. was 30%.

(b) **D-Tartramidic Acid Hydrazide.**—This was done by a modification of Nerdel and Henkel's procedure.[15] The separation of *d*-tartramide and *d*-methyl tartramidate was achieved by continuous extraction of the mixture with acetonitrile rather than by the unsatisfactory method used by Nerdel and Henkel. The D-methyl tartramidate crystallized from the acetonitrile.

(c) **(+)-α-Methyllevulinic Acid D-Tartramazone.**—*d*-Tartramidic acid hydrazide (11.4 g., 70 mmoles) was suspended in a solution of 9 g. (69 mmoles) of DL-α-methyllevulinic acid in 150 ml. of pyridine. The reaction mixture was allowed to stand at room temperature for several days with occasional stirring. The solid was removed by filtration, and the filtrate was evaporated under reduced pressure. The residual oil was dissolved in methanol from which the product crystallized in several crops. The different crops were combined and recrystallized from ethanol to give 4.02 g. (21%) of product melting at 179–180.5°; $[\alpha]^{31}D$ +84.3° (*c* 0.866, water).

Anal. Calcd. for $C_{10}H_{17}N_3O_6$: C, 43.63; H, 6.23; N, 15.27. Found: C, 43.64; H, 6.42; N, 15.19.

(d) **(+)-α-Methyllevulinic Acid (XIII).**—A solution of 2.55 g. (9.3 mmoles) of (+)-2-methyllevulinic acid *d*-tartramazone in 64 ml. of 25% by volume sulfuric acid was heated on the steam-bath for 15 minutes. The cooled solution was extracted with ether. The ether extracts were combined, dried over magnesium sulfate and evaporated to

TABLE I

Fraction	Wt., mg.	$[\alpha]^{26}D$	*c*
1	50	+19.5	1.945, ethanol
2	140	+19.7	1.925, ethanol
3	30	+21.8	1.19, acetic acid

(11) K. Auwers, *Ann.*, **285**, 332 (1895).

(12) E. B. Hershberg and J. Cason in "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 629.

(13) I. R. N. Chakravarti, *J. Indian Chem. Soc.*, **20**, 173 (1943).

(14) E. Gonzalez R., *Ciencia (Mex.)*, **8**, 175 (1947); *C. A.*, **43**, 127 (1949).

(15) F. Nerdel and E. Henkel, *Ber.*, **85**, 1138 (1952).

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_{13}-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

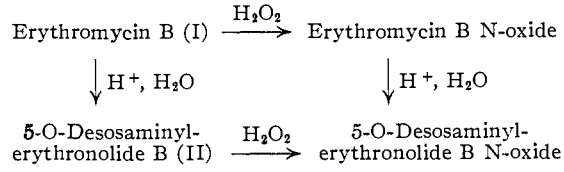
RECEIVED MAY 29, 1957

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 $C_{29}H_{55}NO_8$ 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).