Tetrahedron, 1958, Vol. 4, pp. 369-381. Pergamon Press Ltd., London

MACROLIDE ANTIBIOTICS—VIII*

THE ABSOLUTE CONFIGURATION OF CERTAIN CENTERS IN NEOMETHYMYCIN, ERYTHROMYCIN AND RELATED ANTIBIOTICS

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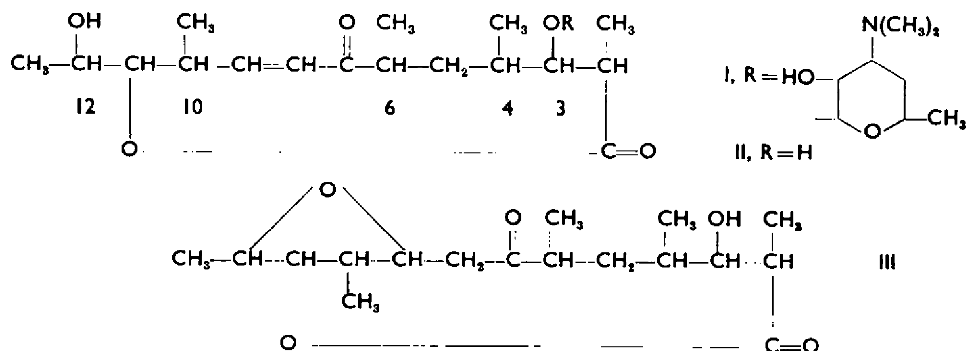
Abstract—Neomethymycin (I) has been transformed into "anhydro*cyclo*neomethynolide" (V) which could be degraded *via* (−)-α-methyllevulinic acid to (−)-α-methylsuccinic acid, thus establishing the absolute configuration of positions 4 and 6 in the lactonic acid (XIII). This acid is a common degradation product of methymycin, neomethymycin, pikromycin and narbomycin and there is thus available a standard of absolute configuration for these macrolide antibiotics. By a different sequence of reactions, erythromycin (XXIII) has been converted into (+)-α-methyllevulinic acid and (+)-α-methylsuccinic acid. Coupled with earlier results reported in the literature, it is now possible to assign absolute configurations to positions 10 (R) and 8 (R) of erythromycin and this applies most likely also to positions 4 (R) and 2 (S). Attention is called to the biogenetic significance of these observations.

RECENTLY, there has been presented evidence from this laboratory[1,2] which established structure (I) for the antibiotic neomethymycin. One of the key reactions was the sulfuric acid cleavage of neomethymycin (I) to the aglycone neomethynolide (II) and the latter's internal Michael-type addition product, *cyclo*neomethynolide (III). Incidental to these studies, it was observed that when the total sulfuric acid reaction mixture was extracted with ether and the *unwashed* ether extract dried and distilled, there was produced in over 70% yield a new substance, "anhydro*cyclo*neomethynolide". The structure elucidation[3] of anhydro*cyclo*neomethynolide has opened the way to the determination of the absolute configuration of certain asymmetric centers common to several 12-membered macrolide antibiotics and the present communication deals with a description of these experiments as well as with the assignment of absolute configuration to certain centers in the 14-membered macrolide erythromycin (XXIII).



* Part VII: C. Djerassi and O. Halpern, *Tetrahedron* 3, 255 (1958).
[1] C. Djerassi and O. Halpern, *J. Amer. Chem. Soc.* 79, 2022 (1957).
[2] C. Djerassi and O. Halpern, *Tetrahedron* 3, 255 (1958).
[3] C. Djerassi and O. Halpern, *J. Amer. Chem. Soc.* 79, 3926 (1957).

The analytical composition ($C_{17}H_{26}O_4$) of anhydro*cyclo*neomethynolide indicated the loss of water as compared ($C_{17}H_{28}O_5$) to neomethynolide (II) or *cyclo*neomethynolide (III). A relationship to *cyclo*neomethynolide (III) rather than neomethynolide (II) was already indicated by the absence of the ultraviolet and infrared absorption bands associated with the latter's α:β-unsaturated carbonyl chromophore. Furthermore, it was observed that anhydro*cyclo*neomethynolide (subsequently shown to be V) could also be produced from neomethynolide (II) or *cyclo*neomethynolide (III) by solution in acidified ether followed by distillation at 40 mm. Since the ether ring of *cyclo*neomethynolide (III)—formed[2] by acid—catalysed Michael addition of the C-12 hydroxyl group of neomethynolide (II) to the double bond of the α:β-unsaturated ketone moiety—was found[2] to be quite stable, it seemed safe to assume that such an ether ring was also present in anhydro*cyclo*neomethynolide (V). In accordance with this assumption was the experimental observation that anhydro*cyclo*neomethynolide (V), just like *cyclo*neomethynolide (III), did not give any iodoform under conditions where neomethynolide (II) responded readily.

Anhydro*cyclo*neomethynolide did not exhibit any infrared hydroxyl absorption but did possess bands at 5·75 and 5·88 μ. The former was clearly due to the lactone ring and the latter was initially believed to be due to a carbonyl group until it was observed that anhydro*cyclo*neomethynolide exhibited only a positive plain* rotatory dispersion curve in contrast to the anomalous curves[2] of its three precursors I, II and III. Since the anomalous Cotton effect* curves were associated with the presence of a carbonyl group,[4] this indicated that the 5·88 μ band was due to another grouping. The most likely possibility was an enol ether function,† produced by addition of the C-3 hydroxyl group to the carbonyl group at C-7 followed by dehydration, and support for this assumption was adduced by the observation that the 5·88 μ band was not affected by reduction with lithium aluminum hydride.‡

Barring skeletal rearrangement, a cyclic ether and a cyclic enol ether grouping can be derived from neomethynolide (II) in only two ways, leading to structure (IV) or (V) for anhydro*cyclo*neomethynolide. A secure differentiation between these two alternatives seemed only possible by ozonolysis and identification of the cleavage fragments.

The ozonization was conducted at —80° in methylene dichloride, the ozonide decomposed with boiling water and the total product saponified in order to cleave the two ester linkages of the intermediate (VI). Chromatography of the resulting acid mixture on silica gel afforded two acids, the less polar of which was identified (*vide infra*) as 6-oxo-2:4-dimethyl-2-heptenoic acid (VII). No degradation experiments were performed on the more polar product, but its analysis as well as that of its ethyl ester (VIIIb) were compatible with 3:5-dimethyl-4-hydroxytetrahydrofuran-2-acetic acid (VIIIa). These two fragments could only arise from an intermediate such as (VI), from which it follows that anhydro*cyclo*neomethynolide must possess structure (V) rather than the alternative (IV).

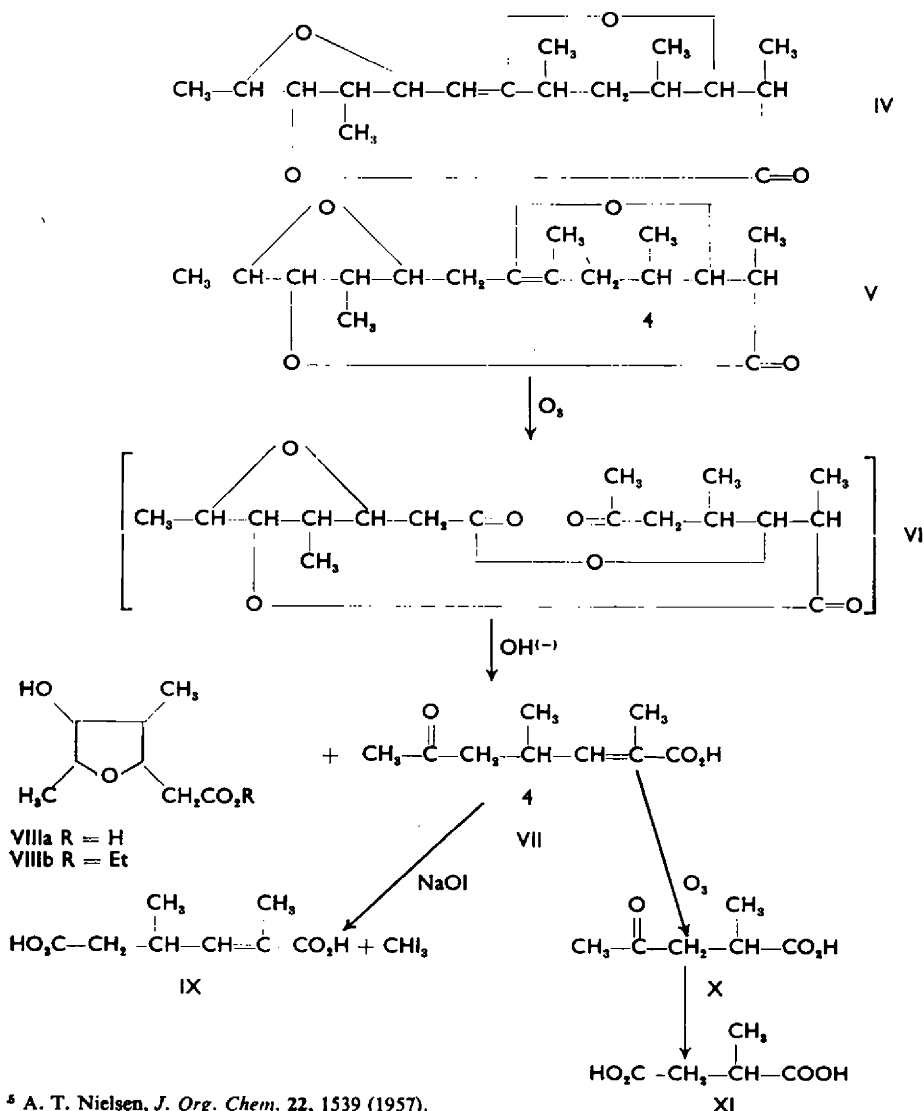
* For nomenclature in rotatory dispersion work see C. Djerassi and W. Klyne, *Proc. Chem. Soc.* 55 (1957).

† Enol ethers usually absorb in the 6 μ region [F. E. Bader, *Helv. Chim. Acta* **36**, 215 (1953)] but under certain conditions may be displaced to shorter wavelength [G. Stork, *J. Amer. Chem. Soc.* **74**, 768 (1952)].

‡ For pertinent references see J. Rudinger and M. Ferles, *Lithium Aluminum Hydride*. Czechoslovak Acad. Science, Praha (1956) and N. G. Gaylord, *Reduction with Complex Metal Hydrides*. Interscience, New York (1956).

[4] C. Djerassi, *Bull. Soc. Chim. Fr.* 741 (1957).

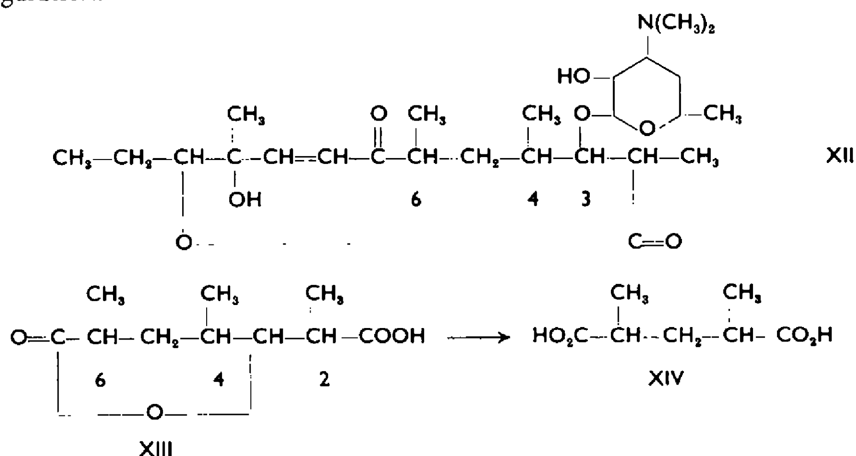
The structure of (+)-6-oxo-2:4-dimethyl-2-heptenoic acid (VII) was derived from the following considerations. The analytical composition and infrared absorption bands were consistent with formulation (VII) and the presence of a saturated carbonyl group was further demonstrated by the single, positive Cotton effect curve of this acid as well as by the formation of a yellow 2:4-dinitrophenylhydrazone. Chemical confirmation for a methyl ketone function was provided by hypoiodite oxidation of (VII) yielding iodoform and a crystalline, unsaturated dibasic acid (IX), the ultra-violet absorption[5] of which indicated that the double bond had to be in conjugation with one of the carboxyl functions. Ozonolysis of the unsaturated keto acid (VII) followed by permanganate oxidation provided (—)-α-methyllevulinic acid (X), characterized as the (+)-2:4-dinitrophenylhydrazone, and hypoiodite oxidation furnished iodoform together with (—)-α-methylsuccinic acid (XI). Since (—)-α-methylsuccinic



[5] A. T. Nielsen, *J. Org. Chem.* 22, 1539 (1957).

acid has been related[6–8] to L-glyceraldehyde, the absolute configuration of its asymmetric center corresponds to that of C-4 in neomethymycin (I) and can be defined unambiguously as (S) according to the new convention.[9]

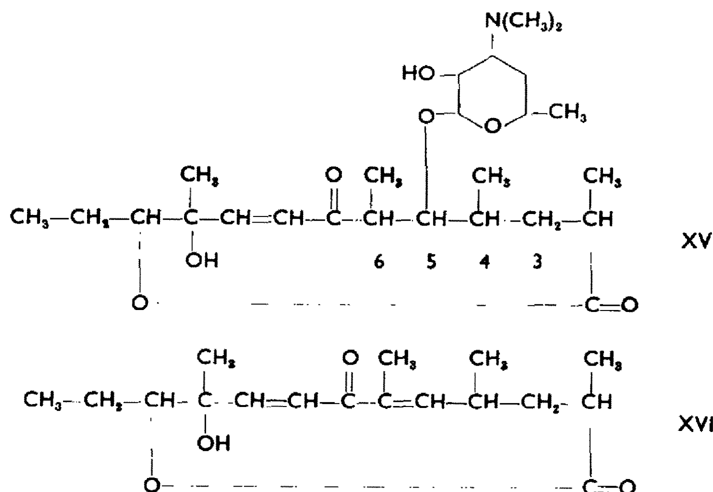
Ozonolysis[1,2] of neomethynolide (II) affords the lactonic acid (XIII) which has already been degraded[10] to *meso*-α:α'-dimethylglutaric acid (XIV). Thus with the relative configuration of C-4 and C-6 established as *cis* through (XIV), an absolute configuration of (R) can be assigned to C-6 in the structures I, II, III and XIII. Furthermore, the antibiotic methymycin (XII) has been shown[11] to differ from neomethymycin (I) only at positions 10 and 12. The steric identity of all asymmetric centers between C-1 and C-7 of neomethymycin (I) and methymycin (XII) is demonstrated by the isolation of the same lactonic acid (XIII) from both antibiotics. Consequently, C-4 and C-6 of methymycin (XII) also possess the 4S and 6R configurations.



Very pertinent to the present work is the report[10] that pikromycin[12] and narbomycin[13] also yield the same (+)-lactonic acid (XIII) upon oxidation. This observation establishes the carbon skeleton of these two antibiotics between C-1 and C-7 but does *not* prove the position of the desosamine-bearing hydroxyl group which could be at C-3 (see I and XII) or at C-5 (*e.g.* XV). Indeed, pikromycin,[12] which is isomeric with neomethymycin (I)[1,2] and methymycin (XII),[11] has been assigned[14–16] tentatively structure (XV) with the glycosidic linkage at C-5, since acid hydrolysis leads to kromycin (XVI), whose constitution is established.[15] It was pointed out,[14] however, that kromycin (XVI) could also have arisen from a structure such as (XII) by assuming

[6] A. Fredga, *Arkiv. Kemi. Min. Geol.* **24A**, No. 32 (1947).
[7] J. A. Mills and W. Klyne in W. Klyne's, *Progress in Stereochemistry* Vol. I, p. 203. Academic Press New York (1954).
[8] E. J. Eisenbraun and S. M. McElvain, *J. Amer. Chem. Soc.* **77**, 3383 (1955).
[9] R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia* **12**, 81 (1956).
[10] R. Anliker, D. Dvornik, K. Gubler, H. Heusser and V. Prelog, *Helv. Chim. Acta* **39**, 1785 (1956).
[11] C. Djerassi and J. A. Zderic, *J. Amer. Chem. Soc.* **78**, 2907, 6390 (1956).
[12] H. Brockmann and W. Henkel, *Chem. Ber.* **84**, 284 (1951).
[13] R. Corbaz, L. Ettlinger, E. Gaumann, W. Keller, P. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, R. Reusser and H. Zahner, *Helv. Chim. Acta* **38**, 935 (1955).
[14] R. Anliker and K. Gubler, *Helv. Chim. Acta* **40**, 119 (1957).
[15] R. Anliker and K. Gubler, *Helv. Chim. Acta* **40**, 1768 (1957).
[16] H. Brockmann and R. Oster, *Chem. Ber.* **90**, 605 (1957).

a *trans*-annular hydride shift in the medium-sized 12-membered lactone ring. If pikromycin possesses the structure (XII) already proved[11] for methymycin, then the two antibiotics would differ in the stereochemistry at C-3,* which would be responsible for the ease of elimination of the hydroxyl group of pikromycin and the stability of that of methymycin in the acid cleavage of the respective antibiotics.



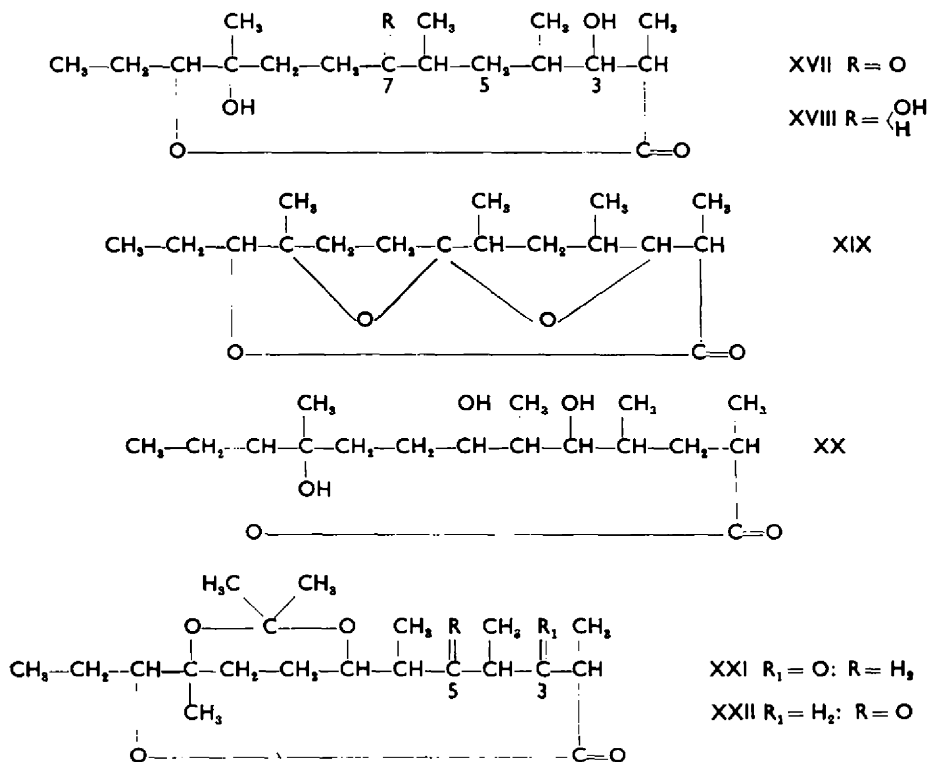
In an attempt to interconvert methymycin and pikromycin through a common intermediate, advantage was taken of the observation of Anliker and Gubler[15] that tetrahydropikronolide (XVIII or XX, depending upon whether pikromycin is XII or XV) upon oxidation with chromium trioxide in acetone solution leads to an acetonide in which the original glycosidically-bound hydroxyl group has been converted into a ketone (XXI or XXII). Consequently if, pikromycin and methymycin are simply epimeric at C-3, it might be possible to convert both antibiotics to the same acetonide-ketone (XXI). When dihydromethynolide (XVII)[11] was reduced with sodium borohydride and the resulting tetrahydromethynolide (XVIII) oxidized† under the conditions employed by Anliker and Gubler[15] with tetrahydropikronolide, there was isolated in good yield the known[11] spiroketal (XIX) rather than any ketonic acetonide, demonstrating that in the tetrahydromethynolide series (XVIII) oxidation occurs first at C-7 followed by immediate ring closure to the spiroketal (XIX) in the acid oxidation medium.

This failure at interconversion can be ascribed to three alternatives: (a) pikromycin possesses structure (XV) rather than (XII); (b) pikromycin possesses structure (XII) but isomerization at C-3 imposes on the molecule a completely different conformation with consequent differing behavior in the oxidation; (c) a difference in conformation—assuming that pikromycin and methymycin are epimeric at C-3—might also result in an alternate steric course in the sodium borohydride reduction of dihydromethynolide (XVII) and hence tetrahydromethynolide and tetrahydropikronolide, though both possessing structure (XVIII), may differ in the stereochemistry at C-7 and C-3.

*And conceivably also at C-10 and C-11.

† Unpublished experiment by Dr. Michael Cais in this laboratory.

Such a difference at C-3 and C-7 could alter the confirmation of the medium-sized lactone ring to such an extent that the oxidation of the two epimers might proceed in completely different directions.



The important conclusion to be drawn is that if pikromycin does indeed possess structure (XV), carbon atoms 4 and 6 must correspond to C-4 and C-2 of the common degradation product (XIII). Since the same enantiomorph of the lactonic acid (XIII) was obtained from both methymycin (XII) and pikromycin (XV), the latter must have the opposite configuration (R) at C-4 as compared to methymycin (XII) and neomethymycin (I). In fact, the considerable likeliness exists that this antipodal relationship applies also to the other two asymmetric centers (C-2 and C-6) of these two groups of antibiotics which are represented in the common degradation product (XIII). Further discussion as to the possible biogenetic significance of this stereochemical point will be deferred until the end of this paper, but it is obvious that a definite assignment of absolute configuration to pikromycin will have to await unambiguous location of the sugar portion, a statement which applies with equal force to narbomycin.[13] Once this structural point has been settled, then an assignment of absolute configuration can be made immediately to pikromycin and narbomycin—in the manner done above for neomethymycin (I) and methymycin (XII)—since the absolute configuration of the lactonic acid (XIII) at positions 4 and 6 has been settled rigorously in the present investigation.

From a therapeutic standpoint, the most important member of the macrolide group* of antibiotics is erythromycin, whose structure (XXIII) has been elucidated in a series of outstanding investigations emanating from the Lilly Laboratories.[17,18] The same group[17] also assigned tentatively absolute configurations‡ to several asymmetric centers of erythromycin (XXIII) by the use of rotational rules, which however suffer from an inherent degree of uncertainty.† Consequently, it was felt essential to confirm these assignments as far as possible by classical chemical correlations leading eventually to L- or D-glyceraldehyde and this has now been possible through the kind co-operation of Dr. K. Gerzon and his colleagues of the Eli Lilly Research Laboratories.[17]

The multi-step conversion of erythromycin (XXIII) to the unsaturated dilactone (XXIV) has already been recorded.[18] The latter still retains carbon atoms 4, 6 and 8 of the parent antibiotic and as has already been recognised by the Lilly group,[18] this substance represents a nearly ideal substrate for the establishment, by chemical means, of the absolute configuration of one or more centers. In fact, by carrying out substantially the earlier recorded[18] degradations, except that we employed milder and non-racemizing conditions, it was possible to transform the derived saturated lactone (XXV)[18] into the required reference compound, α-methylsuccinic acid.

In order to establish that the C-8 center of the lactone (XXV) is unaffected by mild basic conditions required to open the ring, a specimen was warmed with sodium bicarbonate solution for a few minutes which was shown to result in lactone opening to the extent of over 80%. Acidification of the solution regenerated the lactone (XXV) with unchanged rotation. The reaction was now repeated on a larger scale and the solution of the dibasic acid (XXVI) was oxidised with potassium metaperiodate and the oxidation mixture treated with 2:4-dinitrophenylhydrazine. After two recrystallizations, there was obtained pure (—)-α-methyllevulinic acid 2:4-dinitrophenylhydrazone corresponding to (+)-α-methyllevulinic acid (XXVII). Comparison was effected with a sample of synthetic material which was resolved by the earlier recorded procedure[18] except that a product of considerably superior optical purity was obtained. As indicated already above in the degradation of (—)-α-methyllevulinic acid to (—)-α-methylsuccinic acid, the sign of rotation does not change in these two acids and, therefore C-8 of erythromycin corresponds to the asymmetric center of (+)-α-methyllevulinic acid, which in turn is related *via* (+)-α-methylsuccinic acid to D-glyceraldehyde. According to the new convention,[8] C-8 of erythromycin (XXIII) has the (R) configuration.‡

Gerzon *et al.*[17] have shown definitely that C-8 and C-10 of erythromycin (XXIII) must possess the opposite stereochemistry since these two centers are contained in the degradation product (—)-2:4-dimethylpentane-1:3:5-triol, which was still optically

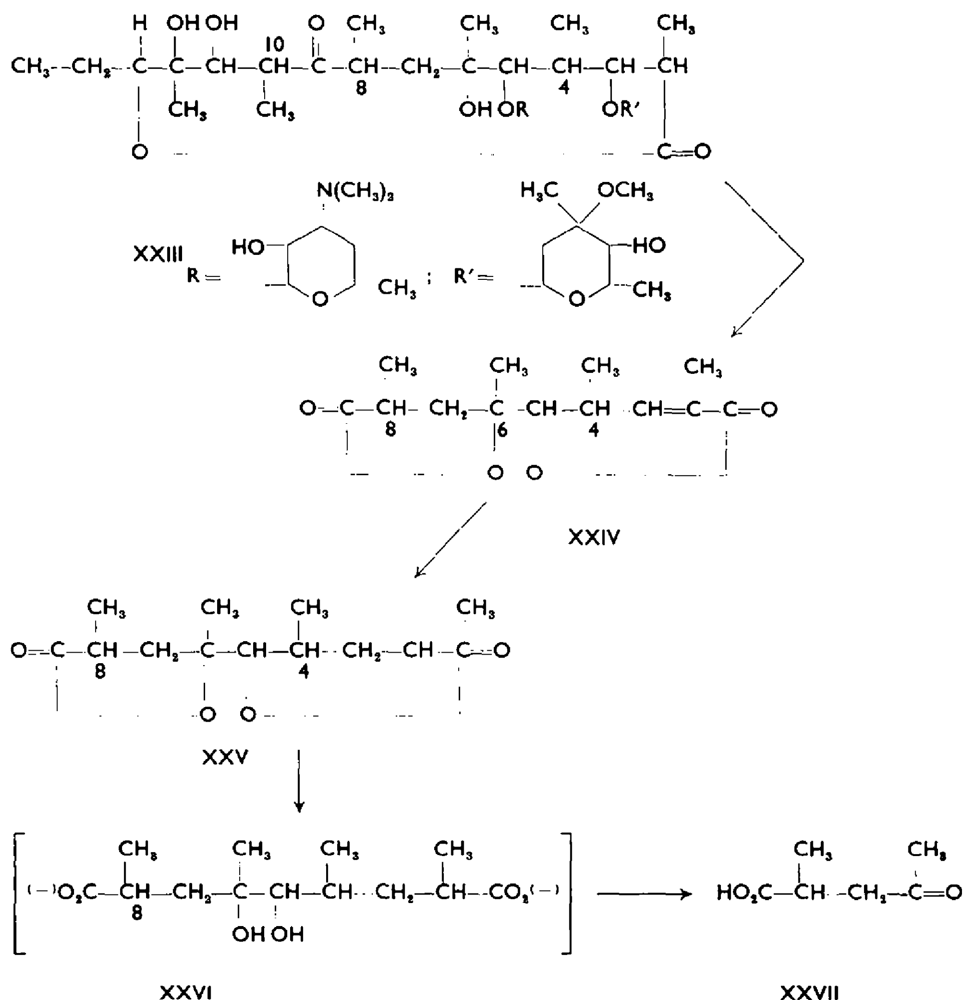
* This term—suggested by R. B. Woodward, *Festschrift Arthur Stoll* pp. 524–544. Birkhäuser, Basel (1957).—encompasses a group of antibiotics possessing a medium-sized or large-sized lactone ring.

† See footnote 21 in ref. 17.

‡ Gerzon *et al.*[17]—using the system of W. Klyne, *Chem. & Ind.* 1022 (1951)—have proposed the 2L, 3D, 4L, 8L, 9D, 10D configurations. The R and S notations[9] employed in our present paper (2S, 4R, 8R, 10R) do not alter the conclusions of Gerzon *et al.*

[17] K. Gerzon, E. H. Flynn, M. V. Sigal, P. F. Wiley, R. Monahan and U. C. Quarck, *J. Amer. Chem. Soc.* **78**, 6396 (1956).
[18] P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, O. Weaver, U. C. Quarck, R. R. Chauvette and R. Monahan, *J. Amer. Chem. Soc.* **79**, 6062 (1957).

active.* Since C-8 has been shown above to possess the (R) configuration, C-10 must be assigned the (R) notation (see footnote 18 on p. 375). By means of the hydrazide rotation rule,[19] Gerzon *et al.*[17] have presented strong circumstantial evidence that the configurations of C-8 and C-4 are identical. Consequently, if one accepts the (R) configuration for C-4, it follows* that C-2 must be (S).



The present chemical degradations have fully substantiated the earlier work[17] based on rotation rules, where it was pointed out that erythromycin (XXIII) has a "meso" character, the asymmetric centers between C-1 and C-8 possessing largely or exclusively the opposite configuration from those between C-10 and C-13 (see footnote‡ on p. 375). If one couples this observation with the possibility noted above that the C-1 to C-7 portions of neomethymycin (I) and pikromycin (XV) may be largely or

* Its *meso* isomer, on the other hand, was derived from the first five carbon atoms of erythromycin (XXIII) showing that C-2 and C-4 had to be assigned opposite (4R:2S) notations.

[19] P. Levene, *J. Biol. Chem.* 23, 145 (1915).

exclusively enantiomeric, then one is led to the interesting speculation that biogenetically the macrolide rings* may be constructed from two fragments joined together at a late stage rather than by successive fusions of C_2 or C_3 units. Extensive biochemical experimentation with labeled precursors would be necessary to establish this intriguing point of detail.

EXPERIMENTAL†

*Anhydro*cyclo*neomethynolide* (V). A solution of 8·0 g of neomethymycin (I) methylene dichloride solvate[2] was boiled for 10 min with 400 cc of 5 N H_2SO_4, cooled, saturated with ammonium sulfate and extracted with ether. The unwashed ether solution was dried over sodium sulfate, concentrated, seeded with neomethynolide (II) and chilled. After filtering 0·825 g of neomethynolide (II),[2] the remaining ether was evaporated and the residue was distilled at a bath temperature of 180–200° and 40 mm. The entire, colorless distillate (3·3 g) crystallized and after recrystallization from aqueous acetone or aqueous methanol afforded flat prisms, m.p. 168–170°, $[\alpha]_D$ +134° (chloroform), $[\alpha]_D$ +124·5° (dioxane), positive, plain dispersion curve rising to 780° at 270 mμ, $\lambda_{max}^{CHCl_3}$ 5·75 and 5·88 μ. The substance did not give any iodoform when treated with sodium hypoiodite under standard conditions[2] and did not exhibit any ultraviolet absorption maximum except for end absorption (log ε 3·78 at 220 mμ). Treatment of a small sample with lithium aluminum hydride in ether solution (24 hr refluxing) followed by decomposition with sodium sulfate yielded an oil which exhibited no more absorption at 5·75 μ but still retained the infrared band at 5·88 μ attributed to the enol ether. (Found: C, 69·15; H, 9·03; O, 22·22; C—CH_3, 23·84; OCH_3, 0·0; Calc. for $C_{17}H_{26}O_4$: C, 69·36; H, 8·90; O, 21·74; 5 C—CH_3, 25·52%).

When an ethereal solution of neomethynolide (II)[2] or *cyclo*neomethynolide (III)[2] was shaken with 5 N H_2SO_4, dried, evaporated and heated at 40 mm, there sublimed near 150° anhydro*cyclo*neomethynolide (V) in nearly quantitative yield.

*Ozonolysis of anhydro*cyclo*neomethynolide* (V). Ozone was passed for 45 min at −80° through a solution of anhydro*cyclo*neomethynolide (V, 2·0 g) in methylene dichloride (25 cc), the solution turning blue after about 35 min. The solvent was removed *in vacuo* at room temperature, the residue was heated under reflux for 1 hr with water (50 cc), followed by the addition of sodium hydroxide (10 g) dissolved in aqueous methanol (50 cc, 50%). After heating for another hour while distilling most of the methanol, the dark orange solution was cooled, saturated with sodium chloride and extracted with ether. The ether extract was concentrated to near dryness, dissolved in methanol (5 cc) and again heated under reflux (30 min) with sodium

* At the present time, this very circumstantial evidence applies only to those macrolide antibiotics which follow partially or wholly the "propionate pattern" (see reference 17). A. J. Birch in L. Zechmeister's, *Progress in the Chemistry of Organic Natural Products* Vol. XIV, p. 202. Springer, Vienna (1957); and A. J. Birch, R. J. English, R. A. Massy-Westropp, M. Slaytor and H. Smith, *J. Chem. Soc.* 365 (1958) has suggested that the extra methyl groups, which confer the "propionate pattern" upon these antibiotics, do not arise from the incorporation of biochemical equivalents of propionic acid but rather that the fundamental chain is constructed by the standard acetic acid scheme starting from acetate followed by introduction of the methyl groups from C_1 precursors. If the latter scheme obtains then it would be interesting to determine whether the unusual stereochemical features discussed above are somehow associated with this secondary methyl introduction step.

† Melting points were determined on the Kofler block. The microanalyses were performed in part by Mr. Joseph F. Alicino (Squibb Institute for Medical Research) and in part by the microanalytical staff of Eli Lilly and Co., where all X-ray diffraction patterns were obtained. We are greatly indebted to both groups for their valuable help.

hydroxide (5 cc, 10%), saturated with sodium chloride and ether extracted. The aqueous layers remaining from both ether extractions were combined, acidified with hydrochloric acid and the pale yellow solution was extracted continuously overnight with ether.

Evaporation of the dried ether solution and maintaining the residue (2·0 g of colored oil) for 30 min at 105° and 0·005 mm afforded 354 mg of a clear oil (VII). The non-volatile residue was dissolved in benzene solution and chromatographed on silica gel (100 g). Elution with benzene and benzene–ether (9 : 1) did not remove any material but when benzene–ether (7 : 3) was employed, a clear oil was obtained which was distilled at 105° and 0·005 mm; yield 231 mg (total yield 585 mg, 51%). The analytical sample of (+)-6-*oxo*-2:4-*dimethyl-2-heptenoic acid* (VII) was redistilled at 100° and 0·001 mm, $[\alpha]_D$ +46° (chloroform), single positive Cotton effect in dioxane (*c*, 0·123: $[\alpha]_{700}$ +18°, $[\alpha]_{589}$ +30°, $[\alpha]_{315}$ +560°, $[\alpha]_{277\cdot5}$ −1180°; the infrared absorption spectrum (chloroform solution) showed the broad band in the 3–3·5 μ region typical of acids as well as bands at 5·80, 5·88 and 6·02 μ (m). (Found: C, 62·96; H, 8·37; Calc. for $C_9H_{14}O_3$: C, 63·51; H, 8·29%).

The bright yellow 2:4-*dinitrophenylhydrazone* of (VII) was recrystallized from aqueous methanol whereupon it exhibited m.p. 142–144°. (Found: C, 51·46; H, 5·57; N, 16·05; neut. equiv., 340. Calc. for $C_{15}H_{18}N_4O_6$: C, 51·42; H, 5·18; N, 15·99%; mol. wt., 350·3).

Further elution of the silica gel column with ether–methanol (9 : 1) afforded 3:5-*dimethyl-4-hydroxytetrahydrofuran-2-acetic acid* (VIIIa), which was distilled at 140° and 0·005 mm. (Found: C, 54·39; H, 8·30; C—CH_3, 18·31; neut. equiv., 176. Calc. for $C_8H_{14}O_4$: C, 55·16; H, 8·10; 2 C—CH_3, 17·2% mol. wt., 174·2).

Treatment of a sample of the acid (VIIIa) at room temperature for 30 min with an ethereal solution of diazoethane and distillation of the crude neutral fraction at 100° and 0·005 mm afforded the *ethyl ester* (VIIIb). (Found: C, 59·08; H, 8·87; OEt, 21·87; Calc. for $C_{10}H_{18}O_4$: C, 59·38; H, 8·97; OEt, 22·28%).

(+)-1:3-*Dimethyl*-1-*butene*-1:4-*dioic acid* (IX). To (+)-6-oxo-2:4-dimethyl-2-heptenoic acid (VII, 135 mg) in pure dioxane (1 cc) was added sodium hydroxide solution (1 cc, 10%) followed by the dropwise addition of a solution of potassium iodide (2 g) and iodine (1 g) in water (8 cc) until a brown color persisted. After warming at 60° for 2 min, the mixture was decolorized by the addition of a few drops of 10 per cent sodium hydroxide and the iodoform was precipitated by addition of water (5 cc). The iodoform was collected, the filtrate was saturated with sodium chloride and extracted continuously overnight with ether (extract discarded). The aqueous solution was then acidified, a small amount of sodium bisulfite was added and the ether extraction was repeated. Evaporation of the dried ether extract and distillation at 120° and 0·001 mm yielded a clear distillate which was recrystallized twice from ether–hexane without achieving a sharp melting point. It is conceivable that the acid (IX) is a mixture of *cis* and *trans* isomers; yield, 26 mg, m.p. 130–155°, $[\alpha]_D$ +41° ($CHCl_3$), λ_{max}^{EtOH} 215 mμ, log ε 4·05. (Found: C, 55·60; H, 7·09; neut. equiv., 88; Calc. for $C_8H_{12}O_4$: C, 55·80; H, 7·03% neut. equiv. 86).

(−)-α-*Methyllevulinic acid* (X). A solution of (+)-6-oxo-2:4-dimethyl-2-heptenoic acid (VII, 80 mg) in methylene dichloride (10 cc) was ozonized at −80° until a permanent blue color was obtained (ca. 15 min). After removal of the solvent *in vacuo* and

heating of the residue for 1 hr with water (20 cc), the mixture was cooled and treated dropwise with an aqueous solution of potassium permanganate (3%) until the violet color persisted (ca. 10 min). The color was discharged with sodium bisulfite, 3 drops conc hydrochloric acid was added and after saturation with sodium chloride, the solution was extracted continuously overnight with ether. Evaporation of the solvent and distillation at 100° and 1 mm provided (—)-*α-methyllevulinic acid* (X, 19 mg) which was directly transformed into the (+)-2:4-*dinitrophenylhydrazone*. Recrystallization from aqueous methanol and from ethanol gave yellow crystals, m.p. 191–193·5° (dec),* $[\alpha]_D$ +81° (acetic acid); the infrared spectrum and the X-ray diffraction pattern were identical with those of a synthetic specimen of the antipode described below. (Found: C, 46·69, 46·05; H, 4·64, 4·75; N, 17·94; neut. equiv., 306; Calc. for $C_{12}H_{14}N_4O_6$: C, 46·45; H, 4·55; N, 18·06% mol. wt. 310·3).

(—)-*α-Methylsuccinic acid* (XI). Treatment of the total (—)-α-methyllevulinic acid obtained from the ozonolysis of (VII) with sodium hypoiodite afforded iodoform (30%) and (—)-α-methylsuccinic acid (50%), m.p. 104–105°, $[\alpha]_D$ —9° (water).

The following procedure without isolation of intermediates led to (—)-α-methylsuccinic acid (20%) based on anhydro*cyclo*neomethynolide (V): The ozonolysis was performed on anhydro*cyclo*neomethynolide (V, 600 mg), in methylene dichloride (20 cc) at —80° for 25 min, the solvent was removed and the residue was heated under reflux for 2 hr with water (25 cc). Upon cooling sodium hydroxide (1·5 g) was added, heating was continued for 1 hr, followed by the dropwise addition at room temperature of potassium iodide (4 g) and iodine (2 g) in water (16 cc). Iodoform and other neutral components were removed by ether extraction (after saturation with sodium chloride), sodium bisulfite was added to the aqueous residue which was acidified (congo red) with conc HCl and then subjected to continuous ether extraction. The residue (409 mg) after evaporation of the ether was dissolved in water (20 cc) and heated on the steam bath for 30 min with chromium trioxide (2 g) and conc sulfuric acid (5 cc). Hydrazine hydrate was added to the cooled solution until it turned blue and it was then extracted continuously overnight with ether after prior saturation with ammonium sulfate. The extract was concentrated to dryness, heated for 5 min on the steam bath with water (1 cc) again evaporated to dryness and then sublimed at 100° and 0·001 mm, furnishing (—)-α-methylsuccinic acid (26·6 mg). The residue from the sublimation was once again oxidized with chromium trioxide (1 g) in conc sulfuric acid (2·5 cc) and water (10 cc) and after repeated sublimation afforded an additional 24·7 mg (XI). Two recrystallizations from benzene–hexane raised the m.p. to 111·5–113·5°, $[\alpha]_D$ —17·5° (ethanol), the infrared spectrum and X-ray diffraction pattern being identical with a sample of the (+)-antipode derived[8] from (+)-pulegone or obtained by resolution. (Found: C, 45·32; H, 6·03; neut. equiv., 65; Calc. for $C_5H_8O_4$: C, 45·45;. H, 6·10% mol. wt., 132·1.)

Resolution of dl-*α-methyllevulinic acid.* α-Methyllevulinic acid was synthesized[20] in 38% yield from ethyl α-bromopropionate and ethyl acetoacetate. The fraction with b.p. 123–129°/1·5 mm and n_D^{28} 1·4400–1·4428 was used for further work. (Found: C, 55·61; H, 7·52; neut. equiv., 129 (by electrometric titration in 66%DMF, *pKa'*, 7·3); Calc. for $C_6H_{10}O_3$: C, 55·37; H, 7·75% neut. equiv. 130.)

* In another experiment, the 2:4-dinitrophenylhydrazone exhibited m.p. 194–196° with sublimation from 165°, but this depended very much upon the rate of heating.

[20] See E. A. Braude and C. J. Timmons *J. Chem. Soc.* 3313 (1953).

The 2:4-dinitrophenylhydrazone was prepared with Brady's reagent and recrystallized from ethanol, m.p. 191–192° (dec). (Found: C, 46·36; H, 4·76; N, 18·32; Calc. for $C_{12}H_{14}N_4O_6$: C, 46·45; H, 4·55; N, 18·06%).

The resolution was performed with D-tartramidic acid hydrazide as reported earlier[18] and furnished a tartramazone with m.p. 176–177°, $[\alpha]_D^{28}$ +61·5° (*c*, 0·87 in water). This was cleaved with sulfuric acid and yielded, after continuous extraction with ether and distillation at a bath temperature of 120–140°/1 mm 51% *(+)-α-methyllevulinic acid*, $[\alpha]_D^{28}$ +11·1° (*c*, 3·9 in acetic acid). Treatment with Brady's reagent afforded the *(−)-2:4-dinitrophenylhydrazone*, m.p. 191–193° (dec) from ethanol, $[\alpha]_D^{25}$ −78·3° (*c*, 0·66 in acetic acid).

This synthetic (+)-α-methyllevulinic acid was converted into (+)-α-methylsuccinic acid in the following fashion: bromine (2·6 g) was added at 0° to a solution of sodium hydroxide (1·8 g) in water (21 cc) and this was followed by (+)-α-methyllevulinic acid (0·624 g) in water (3 cc). After stirring at room temperature for 2 hr, the mixture was let stand overnight, steam distilled for a few minutes and then extracted with chloroform (discarded). The residual aqueous solution was acidified to pH 2 with conc hydrochloric acid, saturated with sodium chloride and extracted continuously for 14 hr with ether. The ether solution was dried, evaporated and the solid residue was recrystallized from benzene–hexane to give *(+)-α-methylsuccinic acid* (103 mg), m.p. 105–106°. Two further recrystallizations from benzene raised the m.p. to 110–111°, $[\alpha]_D^{28}$ +16·5° (*c*, 0·60 in ethanol). Found: C, 45·12; H, 5·85; *pKa'* (water), 6·2 and 8·6; Calc. for $C_5H_8O_4$: C, 45·45; H, 6·10%).

Conversion of erythromycin (XXIII) *to (+)-α-methyllevulinic acid* (XXVII). The transformation of erythromycin (XXIII) to the saturated C_{13} lactone (XXV) has already been recorded.[18] No difficulty was encountered in repeating the reported[18] oxidation of erythralosamine[21] to the unsaturated C_{13} lactone (XXIV), m.p. 130–132°, $[\alpha]_D$ +199° (chloroform), but the yield in the hydrogenation of (XXIV, 600 mg) was raised to 88 per cent by conducting it for 1·5 hr at 23° and 756 mm with platinum oxide (150 mg) in methanol (25 cc). After trituration with *iso*propyl ether, the saturated lactone (XXV) exhibited m.p. 128–130°, $[\alpha]_D$ −71° (chloroform).

Since there existed evidence[18] that alkaline opening of the lactone (XXV) was associated with partial racemization, mild, non-racemizing conditions were examined as follows. A sample (69 mg) of the lactone was dissolved in 0·5 N Na_2CO_3 (5 cc) by warming on the steam bath for 4 min. The solution was diluted with water (60 cc) and titrated to pH 7 with standard hydrochloric acid, a blank being run simultaneously. Titration indicated formation of the dihydroxy dibasic acid (XXVI, 80%) and the solution was strongly acidified by addition of conc hydrochloric acid and left for 60 hr. Continuous extraction with chloroform for 5 hr, evaporation of the solvent and recrystallization from *iso*propyl ether provided the unchanged dilactone (XXV), m.p. and mixture m.p. 126–127°, $[\alpha]_D$ −78° (chloroform). Identity was further confirmed by coincidence of the infrared spectra.

In a preparative experiment, the dilactone (XXV, 771 mg) was dissolved in 0·5 N Na_2CO_3 (25 cc) by warming on the steam bath, the pH of the solution was adjusted to 7·6 by dropwise addition of 0·123 N HCl (11·4 cc) and potassium metaperiodate (830 mg) was added with stirring until the reagent had dissolved. After standing at

[21] E. H. Flynn, M. V. Sigal, P. F. Wiley and K. Gerzon, *J. Amer. Chem. Soc.* **76**, 3121 (1954).

room temperature overnight, the solution was acidified to pH 2 by addition of sulfuric acid (30%) and then extracted continuously with ether for 12 hr. The extract was dried and evaporated, leaving 902 mg of a light yellow oil.

A portion (592 mg) of this oil was treated with 8 cc of Brady's reagent (2 g of 2:4-dinitrophenylhydrazine, 30 cc of methanol, 10 cc of water and 4 cc of conc H_2SO_4) and after 30 min, the yellow solid (303 mg, m.p. 144–154°) was collected and recrystallized twice from ethanol. The resulting 2:4-dinitrophenylhydrazone (130 mg) exhibited m.p. 193–195°, $[\alpha]_D$ −79° (*c*, 0·92 in acetic acid) and did not give any depression in m.p. upon admixture with the above described synthetic resolved sample of the (—)-2:4-dinitrophenylhydrazone of (+)-α-methyllevulinic acid. The infrared spectrum (nujol mull) and the X-ray diffraction pattern were identical with those of the resolved material but differed significantly from those of *dl*-α-methyllevulinic acid 2:4-dinitrophenylhydrazone.

Since it has been demonstrated above with synthetic material that the (+)-antipode of α-methyllevulinic acid corresponds to (+)-α-methylsuccinic acid, the determination of the absolute configuration of C-8 and hence also of C-10 in erythromycin (XXIII) is completed.

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