Tetrahedron, 1958, Vol. 3, pp. 255–268. Pergamon Press Ltd., London

MACROLIDE ANTIBIOTICS—VII*

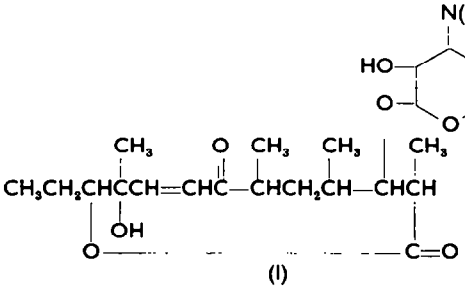
THE STRUCTURE OF NEOMETHYMYCIN†

CARL DJERASSI and O. HALPERN‡
Department of Chemistry, Wayne State University, Detroit, Michigan, U.S.A.

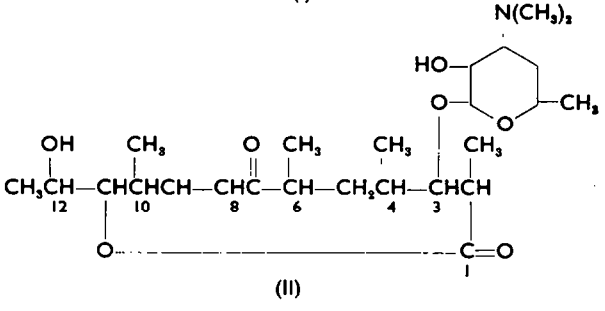
(*Received* 29 *January* 1958)

Abstract—Degradation experiments are described which establish rigorously structure (II) for neomethymycin ($C_{28}H_{43}NO_7$). This antibiotic belongs, therefore, to the macrolide group and differs from methymycin (I) only in the location of one hydroxyl group, which results in marked changes in chemical behavior. The two antibiotics appear to have the same absolute configuration at the relevant asymmetric centers as inferred by the isolation of a common degradation product and the general similarity of certain rotatory dispersion curves. The position of the hydroxyl group at C-12 in neomethymycin is noteworthy from a biogenetic standpoint.

THE term "macrolide"[1] encompasses a group of therapeutically and biogenetically important antibiotics characterized by a lactone moiety of medium or large ring size. Methymycin[2] was the first antibiotic of this class for which a complete structure (I) could be presented[3–6] and from its mother liquors§ there has now been obtained



(I)



(II)

* Paper VI, C. Djerassi and O. Halpern, *J. Amer. Chem. Soc.* **79**, 3926 (1957).

† For preliminary communication see C. Djerassi and O. Halpern, *J. Amer. Chem. Soc.* **79**, 2022 (1957)

‡ Postdoctorate research fellow, 1956–1957.

§ Kindly supplied by Dr. J. Vandeputte (Squibb Institute for Medical Research, New Brunswick, New Jersey), who first encountered the chloroform solvate of a second antibiotic in these mother liquors.

[1] R. B. Woodward, *Festschrift Arthur Stoll* pp. 524–544. Birkhäuser, Basel (1957); *Angew. Chem.* **69**, 50 (1957).

[2] M. N. Donin, J. Pagano, J. D. Dutcher and C. M. McKee, *Antibiotics Annual* 1953–1954 p. 179. Medical Encyclopedia Inc., New York.

[3] C. Djerassi, A. Bowers and H. N. Khastgir, *J. Amer. Chem. Soc.* **78**, 1729 (1956).

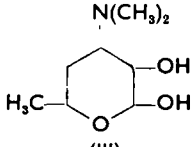
[4] C. Djerassi, A. Bowers, R. Hodges and B. Riniker, *J. Amer. Chem. Soc.* **78**, 1733 (1956).

[5] C. Djerassi and J. A. Zderic, *J. Amer. Chem. Soc.* **78**, 2907 (1956).

[6] C. Djerassi and J. A. Zderic, *J. Amer. Chem. Soc.* **78**, 6390 (1956).

another antibiotic for which we propose the name "neomethymycin" because of its close structural and stereochemical relationship to methymycin (I). On the basis of degradation experiments described in detail in this paper, neomethymycin is assigned formula (II).

Neomethymycin was first isolated in pure form by chromatographic separation from methymycin (I), since it proved to be slightly more polar. Subsequently, it was found that the bulk of the material could be separated quite readily as the methylene dichloride solvate and analysis showed it to be isomeric ($C_{25}H_{43}NO_7$) with methymycin (I)[2] and pikromycin.[7] Functional group analysis indicated the presence of at least six C-methyl and two N-methyl groups and the close similarity of the ultraviolet (λ_{max}^{EtOH} 227·5 mμ, log ε 4·10) and infrared [$\lambda_{max}^{CHCl_3}$ 2·93 μ (hydroxyl), 5·76 μ (lactone), 5·90 μ (unsaturated ketone) and 6·10 μ (double bond)] spectra suggested that the same chromophores were present in methymycin (I) and neomethymycin. Hydrolysis with hydrochloric acid[4] led in good yield (as the hydrochloride) to the amino-sugar desosamine (III),[8,9] which had already been isolated earlier from the antibiotics pikromycin,[7,9] erythromycin,[8] narbomycin[10] and methymycin.[4] Consequently, the difference between methymycin (I) and neomethymycin had to reside in the aglycone portion of the molecule and main attention, therefore, was paid to the isolation of this aglycone, named "neomethynolide", and to the determination of its structure.



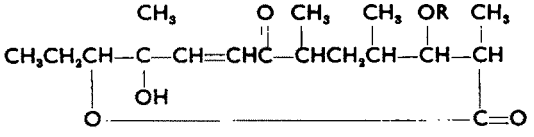
(III)

Careful hydrolysis of neomethymycin with sulfuric acid, under conditions[6] that in the case of methymycin (I) led to methynolide (IV), furnished the desired crystalline neomethynolide accompanied by an isomer, *cyclo*neomethynolide, which formed an integral part of the structure proof. Both substances possessed the same empirical formula ($C_{17}H_{28}O_5$) as methynolide (IV)[5,6] and spectral examination showed that neomethynolide rather than *cyclo*neomethynolide (*vide infra*) represented the true aglycone of neomethymycin (II). Thus it exhibited the same ultraviolet and infrared maxima (in the 2–6·2 μ region) as the parent antibiotic, and neither it nor neomethymycin were oxidized by periodic acid. In marked contrast to methynolide (IV), which forms only a monoacetate (V), neomethynolide upon acetylation led to a diacetate, indicating the absence of a tertiary hydroxyl group.

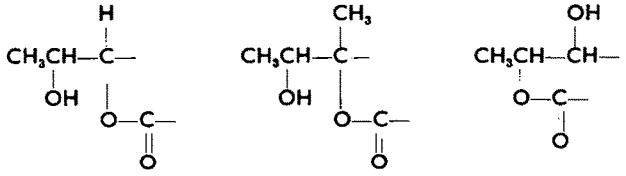
While neomethynolide—just like methynolide (IV)—was resistant to periodic acid, lithium aluminum hydride reduction followed by treatment with periodic acid resulted in the consumption of one equivalent of reagent and the formation of acetaldehyde. It should be noted that similar treatment of methynolide (IV)[5,6] or tetrahydrokromycin (XXVIII)[11] (a transformation product of pikromycin[7]) yielded propionaldehyde rather then acetaldehyde, thus defining one major point of structural difference

[7] H. Brockmann and W. Henkel, *Chem. Ber.* **84**, 284 (1951).
[8] R. K. Clarke, *Antibiot. & Chemother.* **3**, 663 (1953); E. H. Flynn, M. V. Sigal, P. F. Wiley and K. Gerzon, *J. Amer. Chem. Soc.* **76**, 3121 (1954).
[9] H. Brockmann, H. B. König and R. Oster, *Chem. Ber.* **87**, 856 (1954).
[10] 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).
[11] R. Anliker and K. Gubler, *Helv. Chim. Acta* **40**, 119 (1957).

between neomethymycin and these other macrolide antibiotics. The resistance of neomethynolide towards periodic acid, the lability of its lithium aluminum hydride reduction product when exposed to the same reagent and the isolation of acetaldehyde are only compatible with partial structures (VI), (VII) or (VIII), which were supported by the positive iodoform test exhibited by neomethynolide.*

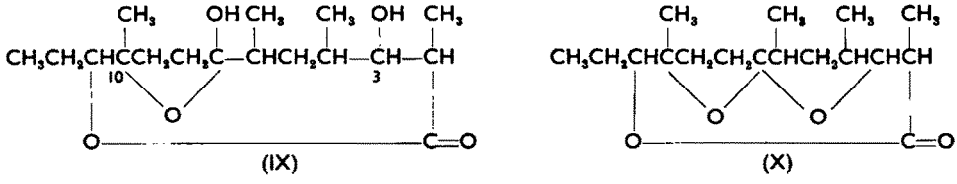


(IV, R=H) (V, R=Ac)



(VI) (VII) (VIII)

Hydrogenation of neomethynolide with palladized charcoal led to dihydroneomethynolide [subsequently shown to be (XXVI)] in which the double bond of the $\alpha\beta$-unsaturated ketone grouping had been reduced. This was demonstrated readily by the disappearance of the high-intensity ultraviolet absorption band of neomethynolide at 227·5 mμ as well as of the infrared bands at 5·90 and 6·10 μ associated with that chromophore. The corresponding derivative in the methymycin (I) series, dihydromethynolide[6] [(IV) with double bond reduced] appears to exist almost exclusively in the hemiketal form (IX)†, since it exhibits[6] a plain rotatory dispersion curve‡ and only a single infrared carbonyl band associated with the lactone group. Further, dihydromethynolide was found[6] to be extremely sensitive to acid, which transformed it almost instantaneously to the *spiro*ketal (X).



(IX) (X)

With these observations in the methymycin (I) series in mind, it should be noted that dihydroneomethynolide (XXVI) shows (Fig. 2) an anomalous rotatory dispersion curve, which can only be attributed[12] to a keto group, exhibits two infrared carbonyl

* Since the $\alpha\beta$-unsaturated ketone system of methymycin (I)—which in this paper has also been shown to be present in neomethymycin (II)—is capable of undergoing a reverse aldol condensation in an alkaline medium[4,5,6] with generation of a methyl ketone, which could then exhibit a positive iodoform reaction, it was necessary to exclude this possibility. This was done by examining dihydroneomethynolide (XXVI) which still yielded iodoform when treated with hypoiodite.

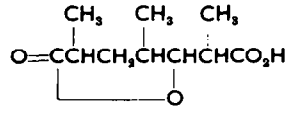
† The reason that the hydroxyl group at C-10 rather than at C-3 is assumed to be involved in hemiketal formation is due to the observation that dihydromethymycin [(I) with double bond reduced] also exhibits a plain rotatory dispersion curve[6] and in this substance only the C-10 hydroxyl group is available for cyclization to a hemiketal [(IX) with desosamine attached at C-3].

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

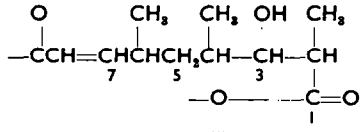
[12] See C. Djerassi, *Bull. Soc. Chim. Fr.* 741 (1957).

bands [$\lambda_{max}^{CHCl_3}$ 5·77 μ (lactone) and 5·85 μ (saturated ketone)] and most importantly is completely stable to acid. It is obvious, therefore, that the secondary hydroxyl group of neomethymycin, which gives rise to acetaldehyde, is not situated favorably with respect to the carbonyl center for hemiketal formation (in contrast to the tertiary hydroxyl group at C-10 of methymycin) and that this has to be attributed either to conformational or structural differences. If we assume that the rest of the molecule (C-1 to C-10) of methynolide (IV) and neomethynolide [subsequently shown to be (XIX)] is identical, then partial structure (VIII) could tentatively be excluded, since it should be able to participate in hemiketal or *spiro*ketal formation. The second alternative (VII) could be eliminated quite definitely because the nonvolatile residue from the lithium aluminum hydride–periodic acid reaction sequence on dihydroneomethynolide* did not give a positive iodoform test. If partial structure (VII) had been present, then a methyl ketone would have been formed which should have responded to hypoiodite as has in fact been observed in the pikromycin series.[11] From the above evidence, the partial formulation (VI) can be put forward provisionally for one terminal of the neomethymycin molecule and decisive support for it will be discussed below in connection with *cyclo*neomethynolide.

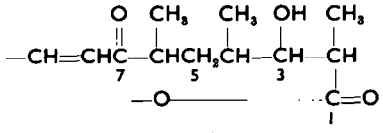
The analytical and spectral results suggested a close similarity between methynolide (IV) and neomethynolide and this supposition was strengthened greatly when neomethynolide was subject to ozonolysis,† whereupon the lactonic acid (XI) was isolated readily. This acid had been obtained earlier by permanganate oxidation of pikromycin,[13] narbomycin[13] and methymycin[5,6] and is known to arise from the first seven carbon atoms involved in the lactone ring. Its formation from neomethymycin implies that one of the partial structures (XII), (XIII), (XIV) or (XV) must be present in neomethymycin.



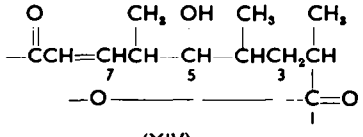
(XI)



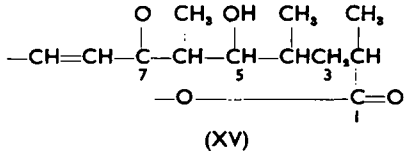
(XII)



(XIII)



(XIV)



(XV)

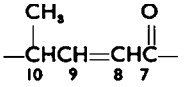
The lactonic acid (XI) and the partial structure (VI) are connected to each other, since they represent the two terminal points of the lactone ring; together they account

* The reaction was carried out with dihydroneomethynolide rather than neomethynolide in order to avoid any ambiguities arising from possible reverse aldolization.

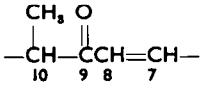
† This represents a marked improvement in yield over the earlier permanganate procedure[6,13] and consequently was also repeated with methynolide (IV), as reported in the experimental section.

[13] R. Anliker, D. Dvornik, K. Gubler, H. Heusser and V. Prelog, *Helv. Chim. Acta* **39**, 1785 (1956).

for thirteen of the seventeen carbon atoms of neomethynolide as well as for four of the five C-methyl groups. The position of the ultraviolet absorption maximum (227·5 mμ) of neomethymycin and neomethynolide precludes the location of the fifth C-methyl group on the double bond, from which it follows that the remaining four carbon atoms must be contained in unit (XVI) or (XVII).* In order to explain the ready formation of the lactonic acid (XI) by ozonolysis, one of the carboxyl groups of (XI) must have been formed by scission of the $\alpha\beta$-unsaturated ketone moiety, which, therefore, must be attached in one of the alternative manners indicated by expressions (XII)–(XV). It should be noted that the first two structures, (XII) and (XIII), bear the second hydroxyl group at C-3, which position has been established unequivocally as the site of substitution in methymycin (I).[5,6] The possibility that the hydroxyl group of neomethymycin is located at C-5 [(XIV) or (XV)]—a position preferred though not proved rigorously for pikromycin[11,14,15]—cannot be discarded with the evidence presented so far, although its stability under the acid cleavage conditions used for the preparation of neomethynolide would speak in favor of (XII) or (XIII).†



(XVI)



(XVII)

All these outstanding points could be settled by examining the second product of the acid cleavage of neomethymycin (II), which accompanied neomethynolide (XIX). This second substance, now named *cyclo*neomethynolide, was isomeric ($C_{17}H_{28}O_5$) with neomethynolide, but otherwise differed greatly from it. In contrast to neomethymycin (II) and neomethynolide (XIX), *cyclo*neomethynolide did not exhibit any ultraviolet absorption at 227·5 mμ and lacked the infrared bands at 5·90 and 6·10 μ typical of the unsaturated carbonyl system. The presence of a saturated ketone could be demonstrated by its anomalous rotatory dispersion with a negative single Cotton effect curve (Fig. 2) and the infrared band at 5·83 μ (in addition to the lactone band at 5·73 μ). The destruction of the unsaturated carbonyl chromophore was apparently caused by acid-catalyzed addition‡ of one of the hydroxyl groups, since acetylation of *cyclo*neomethynolide [subsequently shown to be (XXII)] yielded a mono-acetate, which did not exhibit any more hydroxyl absorption in the infrared region. Under the same conditions, neomethynolide (XIX) formed a diacetate (XX). Oxidation of *cyclo*neomethynolide with chromium trioxide–acetone reagent[16] afforded in excellent yield dehydro*cyclo*neomethynolide (XXIV) in which the free hydroxyl group had been converted into a ketone function. Alkaline hydrolysis of dehydro*cyclo*neomethynolide (XXIV) followed by basification furnished 63 per cent of carbon dioxide, which meant that the new carbonyl group, and hence the hydroxyl group from which it was derived, had to be located β to the lactone carboxyl function. These results

* As indicated by the numbering system, unit (XVI) can only be combined with (XIII) or (XV), while (XVII) can only be combined with (XII) or (XIV).

† It was appreciated, however, that steric factors in the medium-sized lactone ring might in certain circumstances place such an argument on tenuous grounds.

‡ The acid-catalyzed addition of methanol to the double bond of methymycin (I) has already been described earlier.[6]

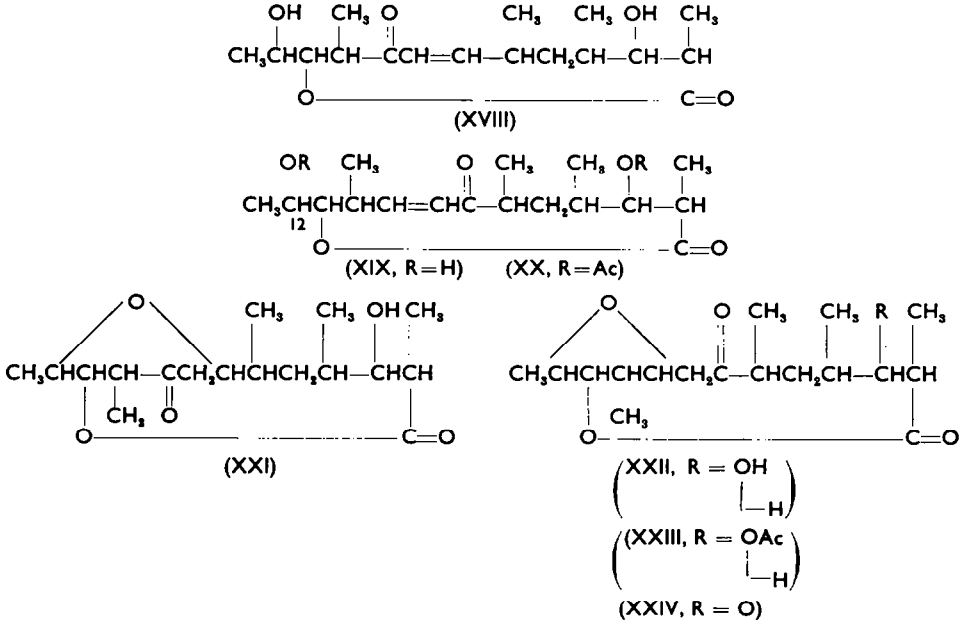
[14] H. Brockmann and R. Oster, *Chem. Ber.* **90**, 605 (1957).

[15] R. Anliker and K. Gubler, *Helv. Chim. Acta* **40**, 1768 (1957).

[16] See K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).

rule out partial structures (XIV) and (XV) and prove that one of the hydroxyl groups of neomethymycin (II) and its transformation products must be located at C-3 rather than at C-5.

With the elimination of (XIV) and (XV), there remain only two possible structures [(XVIII) or (XIX)] for neomethynolide that can be constructed by combination of (VI), (XVI) or (XVII) and (XII) or (XIII). In order to differentiate between these two structural alternatives [(XVIII) or (XIX)], it will be necessary to consider again the behavior of *cyclo*neomethynolide (XXII). The latter must contain a new oxide ring, produced by addition to the double bond of the hydroxyl group present in (VI) or (VIII). Tentative reasons for preferring (VI) have been outlined above and this has now been confirmed fully by the observation that *cyclo*neomethynolide (XXII) did not yield any iodoform upon treatment with hypoiodite. If the hydroxyl group of (VIII) had been involved in the ether linkage, then alkaline hypoiodite would have resulted in opening of the lactone ring and formation of iodoform. Further supporting evidence for the location of the ether linkage was adduced when it was observed that *cyclo*neomethynolide—in contrast to neomethynolide (XIX)—did not react with periodic acid after lithium aluminum hydride reduction. On the basis of structure (XVIII) for neomethynolide, *cyclo*neomethynolide would have to be (XXI), while if (XIX) were the correct structure, *cyclo*neomethynolide would have to be formulated as (XXII). Clearly the latter is preferable, since it involves a five- rather than seven-membered oxide ring.



Two additional arguments in favor of structure (XIX) for neomethynolide can be presented. Base treatment of (XVIII) should lead, at least in part, to a cross-conjugated dienone by β-elimination and such a dienone would not be expected to absorb much above 255 mμ.[17] On the other hand, β-elimination of (XIX) would afford a linearly

[17] E. A. Braude and J. A. Coles, *J. Chem. Soc.* 2012 (1950).

conjugated dienone with appreciably higher absorption and, while no pure product was isolated, spectrophotometric examination of an ethanolic solution of neomethynolide (XIX) containing some sodium ethoxide showed the loss of the 227·5 mμ peak and the concomitant appearance of a new absorption maximum at 280 mμ. This is precisely the region where a dienone acid of structure (XXV) would be expected to absorb.

Further, the rotatory dispersion curve (Fig. 1) of neomethymycin (II) shows a general similarity to that of methymycin (I)[6] and this would hardly be expected of a structure based on (XVIII) where the entire environment is changed around the

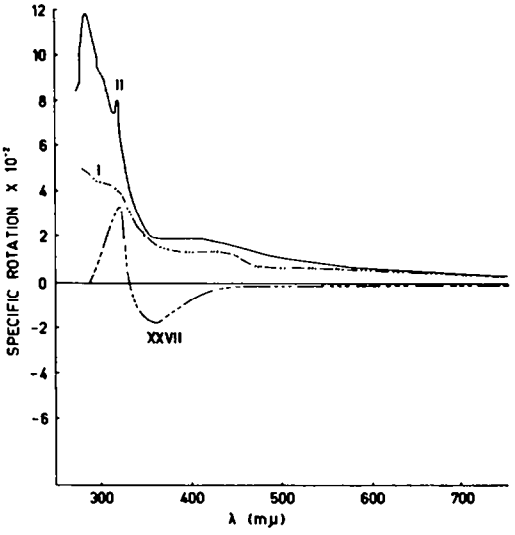


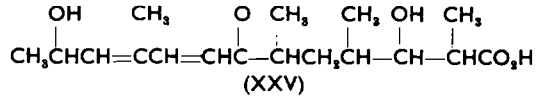
FIG. 1. Optical rotatory dispersion curves (dioxan solution) of methymycin (I), neomethy-mycin (II) and pikromycin (XXVII).

carbonyl chromophore, which would be chiefly responsible[12] for the characteristic rotatory dispersion features.

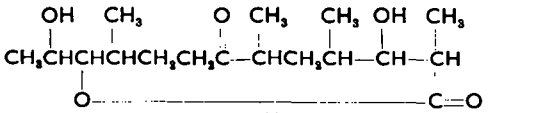
With the establishment of structure (XIX) for neomethynolide, there remains only the placement of the desosamine fragment (III) in order to arrive at a complete expression for the antibiotic itself. The glycosidic linkage could only involve the hydroxyl groups at either C-3 or C-12 and the general similarity of methymycin (I) and neomethymycin, carrying over even to their antibiotic activities,* would suggest that the sugar is attached at C-3 as has already been established[5,6] for methymycin (I). In fact, the lithium aluminum hydride–periodic acid sequence—described above for neomethynolide (XIX) and dihydroneomethynolide (XXVI)—was applied to neomethymycin itself and also yielded acetaldehyde. The conditions were such that it was rather unlikely that a glycosidic linkage could have been split but for further confirmation, neomethymycin was dissolved in warm sodium hydroxide solution and the resulting hydroxy acid was treated with sodium metaperiodate, which again produced acetaldehyde. The formation of acetaldehyde under these circumstances

* The microbiological assay, carried out at the Squibb Institute for Medical Research, indicated virtually identical activity for methymycin (I) and neomethymycin (II) except with *Bacillus subtilis* where the latter was effective at one-fourth the minimum inhibitory concentration of methymycin (I).

would only be possible if the hydroxyl group at C-12 were free, and neomethymycin can therefore be assigned structure (II).



(XXV)



(XXVI)

The general similarity of the rotatory dispersion curves (Fig. 1) of methymycin (I) and neomethymycin (II) coupled with the isolation of the same lactonic acid (XI)

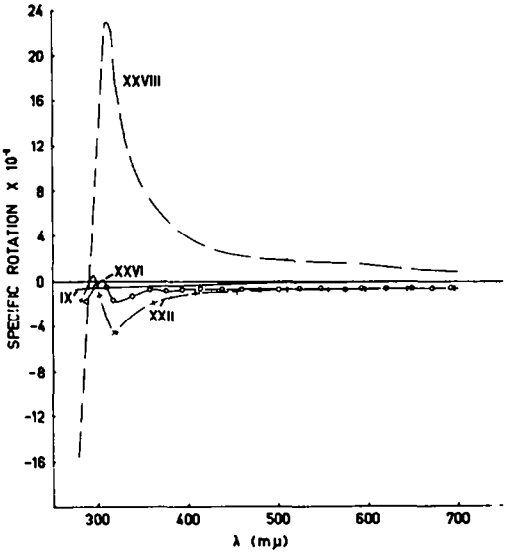
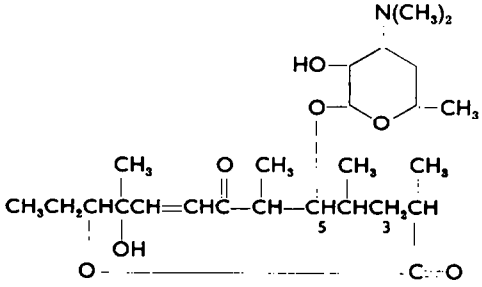


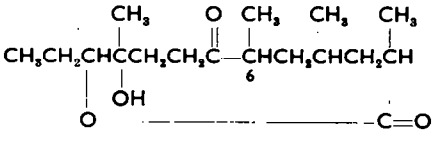
FIG. 2. Optical rotatory dispersion curves (dioxan solution) of dihydromethynolide (IX), *cyclo*neomethynolide (XXII), dihydroneomethynolide (XXVI) and tetrahydrokromycin (XXVIII).

from either antibiotic suggests that the two possess substantially the same conformation as well as identical absolute configurations at the relevant asymmetric centers. It is interesting to note that pikromycin,[7] which is isomeric with methymycin (I) and neomethymycin (II), exhibits a rotatory dispersion curve (Fig. 1) that is completely different yet yields the same lactonic acid (XI) upon oxidation.[13] On the basis of reported degradations[11,14,15] pikromycin can only have structures (XXVII) or (I) and, if the latter is correct, then pikromycin must be epimeric at C-3 with methymycin (I) and neomethymycin (II). Since this change alone is not likely to be responsible for the major rotatory dispersion difference between the antibiotics, which is further demonstrated by comparing the curves (Fig. 2) of dihydromethynolide (IX), dihydroneomethynolide (XXVI) and tetrahydrokromycin (XXVIII),[11] this implies that methymycin (I) and pikromycin must differ in absolute configuration at C-10 or C-11

or both, or that the conformation of the respective rings is different.* This latter possibility is not so far fetched, considering the subtle trans-annular factors that may control conformation in a medium-sized (twelve-)-membered lactone ring and in fact may even apply if pikromycin possesses structure (XXVII). If pikromycin differs from methymycin (I) only in the configuration at C-3, then it should be possible to interconvert these two antibiotics and attempts along those lines are currently in progress.



(XXVII)



(XXVIII)

The structure elucidation of antibiotics of this group is not only of intrinsic interest, but as the number of secure structure determinations among macrolide antibiotics increases—aside from methymycin (I) and neomethymycin (II) these include so far only magnamycin,[1] erythromycin[18] and most probably erythromycin B[19]—the statistical validity of biogenetic speculations[1,20] is strengthened. From that standpoint, neomethymycin (II) is important, since hydroxylation at C-12 represents an "extra" position in the sense discussed by Woodward.[1]

EXPERIMENTAL†

Isolation of neomethymycin (II). The first pure sample was secured by chromatographing the mother liquors from the original methymycin (I) isolation[2] on fifty times its weight of alumina, which was deactivated with 3 per cent of its weight of 10% aqueous acetic acid. The material was placed on the column in ether solution and eluted with ether containing 0·5 and then 1 per cent of methanol. Methymycin (I) appeared first followed by mixtures and gradually pure neomethymycin was obtained, which crystallized readily from ether–hexane, m.p. 156–158°, $[\alpha]_D$ +93°, λ_{max}^{EtOH} 227·5 mμ (log ε 4·10), $\lambda_{max}^{CHCl_3}$ 2·93, 5·76, 5·90 and 6·10 μ, rotary dispersion (Fig. 1) in dioxan (*c*, 0965) : $[\alpha]_{700}$ +58°, $[\alpha]_{589}$ +74°, $[\alpha]_{400-355}$ +202° (infl), $[\alpha]_{320}$ +809°, $[\alpha]_{317·5}$ +736°, $[\alpha]_{285}$ +1190°, $[\alpha]_{275}$ +840°. *Anal.* Calcd. for $C_{25}H_{43}NO_7$: C, 63·94; H, 9·23; N, 2·98

* In contrast to dihydromethynolide (IX), tetrahydrokromycin (XXVIII) does not exist as a hemiketal (see rotatory dispersion (Fig. 2) as well as infrared spectrum[11] and this is undoubtedly due to conformational differences. In fact, the absolute configuration of C-6 in tetrahydrokromycin (XXVIII) may not be identical with that existing in pikromycin, since hydrogenation of a 5–6 double bond is involved in the production of (XXVIII).

† Melting points were determined on the Kofler block. Unless noted otherwise, all rotations were measured in chloroform solution. We are indebted to Mrs. Dolores Phillips for the infrared and ultraviolet spectra and to Mr. Joseph F. Alicino (Squibb Institute for Medical Research) for the microanalyses.

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

[19] P. F. Wiley, M. V. Sigal, O. Weaver, R. Monahan and K. Gerzon, *J. Amer. Chem. Soc.* **79**, 6070 (1957).

[20] A. J. Birch, *Progress in the Chemistry of Organic Natural Products* (Edited by L. Zechmeister) Vol. XIV, p. 202. Springer, Vienna (1957).

2 N-CH_3, 6·4; 6 C-CH_3, 19·2; mol. wt., 469·6. Found: C, 63·75; H, 9·04; N, 3·07; N-CH_3, 5·90; C-CH_3, 16·76; OCH_3, 0·0 per cent; mol. wt. (perchloric acid titration), 472.

Neomethymycin solvates rather readily and when crystallized from aqueous acetone it yielded 1 cm long crystals of the acetone solvate, m.p. 156–158° with loss of solvent near 100°. A very characteristic solvate is that formed with methylene dichloride, and this serves very satisfactorily for the preparative isolation of neomethymycin from methymycin mother liquors by the following simple procedure. The crude antibiotic concentrate was dissolved in warm methanol and evaporated to a foam on the steam bath *in vacuo*, and methylene dichloride was added while still hot. The mixture was swirled until all dissolved and upon cooling colorless crystals of the methylene dichloride solvate separated. These were filtered off and treated twice more by the above scheme, and the combined mother liquors were then subjected to chromatography to recover methymycin. The neomethymycin–methylene dichloride solvate formed large hexagonal plates, m.p. 154–156°, with gas given off near 135–140°, $[\alpha]_D$ +66° (EtOH). *Anal.* Calcd. for $C_{25}H_{43}NO_7 \cdot CH_2Cl_2$: C, 56·32; H, 8·18; Cl, 12·79; mol. wt., 554·5. Found: C, 57·05; H, 8·02; Cl, 11·95 per cent; mol. wt. (perchloric acid titration), 545.

Hydrogenation of neomethymycin (II). The hydrogenation of 225 mg of neomethymycin–methylene dichloride solvate was conducted at 30° and atmospheric pressure in 5 ml of ethanol with 40 mg of 5% palladized charcoal catalyst. Hydrogen consumption stopped after 1·1 equivalents had been consumed, the catalyst was filtered off, the filtrate evaporated to dryness and the residue was crystallized from hexane–methylene dichloride. The resulting *dihydroneomethymycin–methylene dichloride* solvate (75 per cent yield) had no definite melting point, solvent being lost near 100°, $[\alpha]_D$ +3°. *Anal.* Calcd. for $C_{25}H_{45}NO_7 \cdot CH_2Cl_2$: Cl, 12·74. Found: Cl, 12·90 per cent. *Anal.* Calcd. for $C_{25}H_{45}NO_7$: C, 63·66; H, 9·62; N, 2·97. Found after drying at 100° and high vacuum to constant weight: C, 63·83; H, 9·60; N, 3·02 per cent; loss in weight: 15·23.

Isolation of desosamine (III). A solution of 200 mg of neomethymycin (II) in 5 ml of 5 N hydrochloric acid was heated on the steam bath for 15 min and extracted with chloroform, and the aqueous solution was evaporated to dryness *in vacuo*. The crude crystalline desosamine hydrochloride (95 mg) was recrystallized three times from methanol–acetone, whereupon it melted at 186–188° (dec.), undepressed upon admixture with desosamine hydrochloride obtained[4] from methymycin (I). The infrared spectra of both specimens in potassium bromide pellets were identical.

Isolation of acetaldehyde. Neomethymycin (II) did not consume any reagent when left for 4 hr at room temperature with 0·1 N aqueous solution of periodic acid, thus showing that the glycol system was blocked.

A solution of 300 mg of neomethymycin (II) and 400 mg of lithium aluminum hydride in 25 ml of dry ether was heated under reflux for 18 hr and the excess of reagent decomposed with ethyl acetate. After addition of a few milliliters of a saturated aqueous solution of sodium sulfate, the mixture was dried over anhydrous sodium sulfate and filtered, and the inorganic salts extracted thoroughly with chloroform. The combined filtrate and extracts were evaporated to dryness, benzene was added and the evaporation was repeated. The gummy residue (240 mg) exhibited only a trace of carbonyl band in the infrared region and to its aqueous solution was added 25 ml of

0·1 N periodic acid solution. The uptake of reagent stopped within 15 min (1·2 equivalents) and the solution was steam distilled into excess of 2,4-dinitrophenylhydrazine dissolved in sulfuric acid. The precipitate (125 mg) was collected and recrystallized from aqueous ethanol to yield 55 mg of yellow needles of acetaldehyde 2,4-dinitrophenylhydrazone, m.p. 166–168°. Identity was established by mixture melting-point determination, infrared spectra, comparison with an authentic sample and paper-chromatographic analysis in two systems* in which acetaldehyde and propionaldehyde 2,4-dinitrophenylhydrazones are separated easily. *Anal.* Calcd. for $C_8H_8N_4O_4$: C, 42·86; H, 3·60; N, 24·99. Found: C, 43·28; N, 3·65; N, 24·32 per cent.

Acetaldehyde was also isolated when the lithium aluminum hydride reduction product was oxidized with a 0·2 N aqueous solution of sodium metaperiodate.

Alternatively, a sample (ca. 200 mg) of neomethymycin (II), dihydroneomethymycin or dihydroneomethynolide (XXVI) was dissolved in 5 ml each of ethanol and of 2 N aqueous sodium hydroxide, warmed for 30 min at 70° and set aside at 25° for 2 hr; the pH was adjusted to 6·8 with hydrochloric acid and 25 ml of 0·1 N sodium metaperiodate solution were added. After standing overnight, the mixture was subjected to steam distillation and 30–54 per cent of acetaldehyde 2,4-dinitrophenylhydrazone was isolated.

Sulfuric acid cleavage of neomethymycin (II). A solution of 200 mg of neomethymycin in 10 ml of 5 N sulfuric acid was boiled for 10 min, cooled rapidly, saturated with ammonium sulfate and extracted with ether. Evaporation of the ether solution left a colorless oil, which could be crystallized from *wet* ether to furnish up to 35 per cent of *neomethynolide hydrate*, m.p. 95–110°. The mother liquors were dissolved in benzene–ether (1 : 1) and passed through a short column of alumina deactivated with acetic acid. Elution with benzene–ether mixtures and ether furnished nearly 60 per cent of cyclo*neomethynolide* as an oil, while remaining traces of neomethynolide were only removed with ether containing 2 per cent of methanol. The separation of the two cleavage products by chromatography was quite sharp and was utilized also in larger scale experiments.

Neomethynolide (XIX). Recrystallization of neomethynolide hydrate from wet ether–hexane or aqueous acetone yielded long, slender needles with a variable melting point range between 90–120°, $[\alpha]_D$ + 99°. *Anal.* Calcd. for $C_{17}H_{28}O_5 \cdot H_2O$: C, 61·79; H, 9·15; 5 C-CH_3, 22·75; H_2O, 5·45. Found: C, 62·65; H, 9·08; C-CH_3, 21·74 per cent. After drying at 100° in high vacuum: loss in weight, 5·63; C, 65·81; H, 8·99 per cent.

The substance could be rendered anhydrous by sublimation of the hydrate at 130–140° at 0·005 mm or by co-distillation with benzene followed by crystallization from benzene–hexane mixture, m.p. 186–187°, $[\alpha]_D$ +108°, λ_{max}^{EtOH} 227·5 mμ (log ε 4·10), $\lambda_{max}^{CHCl_3}$ 2·93, 5·75, 5·90 and 6·10 μ. *Anal.* Calcd. for $C_{17}H_{28}O_5$: C, 65·36; H, 9·03. Found: C, 65·61; H, 9·02 per cent.

Neomethynolide diacetate (XX). This was obtained as colorless needles by acetylating neomethynolide hydrate with acetic anhydride–pyridine overnight at room temperature and recrystallizing from aqueous acetone, m.p. 199–201°, depressed to 160–190° upon admixture with methynolide mono-acetate (V), $[\alpha]_D$ +84°, $\lambda_{max}^{CHCl_3}$ 5·71, 5·88 and 6·08 μ. *Anal.* Calcd. for $C_{21}H_{32}O_7$: C, 63·61; H, 8·14; acetyl, 21·71. Found: C, 63·90; N, 8·04; acetyl, 22·29 per cent.

* We are indebted to Dr. K. Murai (Research Laboratories, Chas. Pfizer and Co.) for this analysis.

Neomethynolide did not react with periodic acid, but when it was first reduced with lithium aluminum hydride as described above (except that tetrahydrofuran was used as solvent instead of ether) for neomethymycin (II) and then treated with an aqueous solution of periodic acid, 0·9 equivalents were consumed in ca. 30 min. Steam distillation into 2,4-dinitrophenylhydrazine solution and recrystallization furnished 30 per cent of pure acetaldehyde 2,4-dinitrophenylhydrazone with m.p. 168–169°. The infrared spectrum showed the characteristic band at 11·40 μ (chloroform), which is not present in propionaldehyde 2,4-dinitrophenylhydrazone. *Anal.* Calcd. for $C_8H_8N_4O_4$: C, 42·86; H, 3·60; N, 24·99. Found: C, 43·02; H, 3·75; N, 25·27 per cent.

In order to examine the possibility of eliminating the hydroxyl group involved in lactone formation, a solution of 200 mg of neomethynolide (XIX) in 5 ml of ethanol was diluted with 5 ml of 4 N aqueous sodium hydroxide solution and left at room temperature. Immediate ultraviolet spectroscopic examination showed the presence of the 227·5 mμ peak, but within 2 hr this had disappeared and a maximum at 280 mμ (log ε 3·23) was noted. After 18 hr at room temperature, no ultraviolet selective absorption was left, possibly owing to alkaline cleavage. In a second experiment, a solution of 188 mg of neomethynolide (XIX) in 10 ml of absolute ethanol containing 2·5 equivalents of sodium was left at room temperature and the ultraviolet absorption was examined at 2 hr intervals. The intensity of the 227·5 mμ maximum was reduced as the 280 mμ band started to appear and after standing overnight only a maximum at 280 mμ was noticeable. Its low extinction (log ε 3·34) indicated, however, that formation of the dienone acid (XXV) had to compete with other reactions involving the unsaturated carbonyl chromophore and no pure product was isolated from this mixture.

Dihydroneomethynolide (XXVI). Quantitative micro-hydrogenation of neomethynolide (XIX) in acetic acid–ethanol (2 : 1) at 0° with platinum oxide resulted in the uptake of 1·93 molar equivalents of hydrogen. Consequently in a preparative experiment with 165 mg of neomethynolide (XIX), the hydrogenation was carried out in absolute ethanol with 100 mg of 5% palladized charcoal catalyst and hydrogen consumption corresponding to one equivalent stopped after 15 min. Filtration of the catalyst, evaporation of the filtrate to dryness and recrystallization from ether–hexane gave in nearly quantitative yield heavy prisms of dihydroneomethynolide (XXVI), which exhibited m.p. 134–136°, $[\alpha]_D$ −43° (dioxane), $\lambda_{max}^{CHCl_3}$ 2·80, 2·90, 5·77 and 5·85 μ, rotary dispersion (Fig. 2) in dioxan (*c*, 0·023) : $[\alpha]_{700}$ −61°, $[\alpha]_{589}$ −61°, $[\alpha]_{400-350}$ −70° (infl), $[\alpha]_{320}$ −180°, $[\alpha]_{307·5}$ +17°, $[\alpha]_{285}$ −200°. *Anal.* Calcd. for $C_{17}H_{30}O_5$: C, 64·94; H, 9·62. Found: C, 64·67; H, 9·43 per cent.

The rotation of a dioxan solution of the substance did not change for 24 hr in the presence of 2 drops of concentrated sulfuric acid and the product was recovered unchanged upon sublimation at 130–140° and 0·01 mm.

A 120 mg sample of dihydroneomethynolide (XXVI) was reduced with lithium aluminum hydride in tetrahydrofuran for 24 hr and the crude total reduction product was dissolved in 25 ml of purified dioxan and treated with a solution of 112·5 mg of sodium metaperiodate in 5 ml of water. After the mixture had been standing for 30 min, the acetaldehyde was removed by steam distillation, and the solution was cooled and titrated against a blank that had been put through the same steps except for the absence of dihydroneomethynolide; 0·76 molar equivalents of reagent had been

consumed. The solution was then saturated with sodium chloride, extracted with ether continuously overnight and then evaporated to dryness. The residual oil was taken up in 5 ml of dioxan, concentrated to 2 ml, mixed with 5 ml of 10% sodium hydroxide solution and treated with a solution of 1 g of iodine and 2 g of potassium iodide in 8 ml of water. After being warmed for 2 min at 60°, the solution was decolorized by the addition of a few drops of 10% sodium hydroxide solution, poured into water and set aside. No iodoform was obtained, thus excluding definitely the location of a methyl group at C-11 as in (VII).

Ozonolysis of neomethynolide (XIX). Ozone was passed at −80° through a solution of 250 mg of neomethynolide (XIX) in 20 ml of ethyl acetate until a permanent blue color was obtained (ca. 30 min). The resulting solution was added dropwise to a mixture of 25 ml of 10% sodium hydroxide and 8 ml of 30% hydrogen peroxide, stirred at room temperature for 1 hr and then heated until all ethyl acetate had been removed. The aqueous solution was acidified with concentrated hydrochloric acid, set aside for 15 min and then saturated with sodium chloride. Ether extraction afforded an oil still containing acetic acid and the latter was removed by distillation *in vacuo* on a steam-bath. Three recrystallizations of the residue from ether–hexane afforded 40 mg of analytically pure lactonic acid (XI), m.p. 125–126°, $[\alpha]_D$ +42°, +43°. Its infrared spectrum in a potassium bromide pellet was identical with that of a specimen derived[6] from methymycin (I). *Anal.* Calcd. for $C_{10}H_{16}O_4$: C, 59·98; H, 8·05; mol. wt., 200·2. Found: C, 59·77; H, 7·78 per cent; neut. equiv., 195 (immediate titration), 97 (back titration after standing in excess of base).

Similar treatment* of methynolide (IV) (0·5 g) in 40 ml of ethyl acetate at −70°, followed by decomposition of the ozonide with alkaline hydrogen peroxide (1 hr at 25°, then sodium hydroxide pellets were added and the mixture was boiled for 1 hr) and continuous ether extraction gave 0·45 g of viscous oil. Careful crystallization from ether–hexane provided 200 mg of the lactonic acid (XI), m.p. 123–127°.

cyclo*Neomethynolide* (XXII). The substance, obtained above in the sulfuric acid cleavage of neomethymycin (II), could not be crystallized, but its homogeneity was established by repeated chromatography and conversion of the first and last eluates to the crystalline mono-acetate (XXIII). An analytical sample of *cyclo*neomethynolide (XXII) was prepared by taking a middle chromatogram fraction and distilling it at 140°/0·01 mm $[\alpha]_D$ −40°, no selective high ultraviolet absorption, $\lambda_{max}^{CHCl_3}$ 2·90, 5·72, 5·83 and sharp band at 9·01 μ [absent in both (XIX and XXVI)], rotary dispersion (Fig. 2) in dioxan (*c*, 0·127) : $[\alpha]_{700}$ −45°, $[\alpha]_{589}$ −48°, $[\alpha]_{320}$ −460°, $[\alpha]_{295}$ +51°, $[\alpha]_{280}$ −196°. *Anal.* Calcd. for $C_{17}H_{28}O_5$: C, 65·36; H, 9·03. Found: C, 65·48; H, 9·00 per cent.

Reduction with lithium aluminum hydride followed by treatment with aqueous periodic acid did not result in any appreciable consumption of reagent over a 6 hr period.

Acetylation with acetic anhydride–pyridine and recrystallization from aqueous methanol afforded cyclo*neomethynolide 3-acetate* (XXIII) as colorless prisms, m.p. 194° (with crystal change and sublimation from 175°), $[\alpha]_D$ +1°, $\lambda_{max}^{CHCl_3}$ 5·69, 5·83 and 8·05 μ, but no hydroxyl absorption. *Anal.* Calcd. for $C_{19}H_{30}O_6$: C, 64·38; H, 8·53; acetyl, 12·13. Found: C, 64·64; H, 8·16; acetyl, 11·93 per cent.

Attempted micro-hydrogenation in ethanol solution with either palladium–charcoal or platinum oxide did not result in any hydrogen uptake.

* This experiment was carried out by Mr. J. Kutney.

Dehydrocycloneomethynolide (XXIV). Oxidation of 87 mg of *cyclo*neomethynolide (XXII) in acetone solution at 5–10° with one equivalent of chromium trioxide–sulfuric acid solution[16] was complete in 2–3 min, whereupon the green solution was poured into a saturated solution of ammonium sulfate and extracted continuously with ether. The ether-soluble material was filtered in benzene solution through a very short column of alumina deactivated with acetic acid and the eluted product was recrystallized from light petroleum ether to yield 67 mg of heavy prisms, m.p. 122–124° (with sublimation), $[\alpha]_D$ +60°. *Anal.* Calcd. for $C_{17}H_{26}O_5$: C, 65·78; H, 8·44. Found: C, 65·94; H, 8·30 per cent.

A 64 mg sample of this ketone was subjected to base treatment, followed by acidification exactly as described earlier[6] for dehydromethynolide and yielded 5·77 mg (63·5 per cent) of carbon dioxide.*

Iodoform tests. The following procedure was used in determining the presence of a potential methyl ketone function. A few milligrams of substance, dissolved in 0·5 ml of purified dioxan. was mixed with 0·25 ml of 10% aqueous sodium hydroxide and to it was added dropwise a solution of 1 g of iodine and 2 g of potassium iodide in 8 ml of water until a deep red-brown color remained. After the solution had been heated for 1 min at 60°, 2 drops of 10% aqueous sodium hydroxide was added to remove excess of iodine, and the solution was cooled and diluted with much water. The resulting precipitate of iodoform was sublimed at 100°/40 mm, whereupon the yellow crystals melted at 124° (dec.). Under those conditions, neomethynolide (XIX) and dihydroneomethynolide (XXVI) yielded iodoform, while *cyclo*neomethynolide (XXII) and methynolide (IV) did not respond.

Rotatory dispersion of pikromycin (XXVII). Rotary dispersion (Fig. 1) in dioxan solution (ca. 0·125) on sample provided by Prof. H. Brockmann (University of Göttingen): $[\alpha]_{700}$ −14°, $[\alpha]_{589}$ −15°, $[\alpha]_{360}$ −171°, $[\alpha]_{320}$ +333°, $[\alpha]_{285}$ 0°.

Rotatory dispersion of tetrahydrokromycin (XXVIII). Rotary dispersion (Fig. 2) in dioxan solution (ca. 0·095) on sample provided by Dr. R. Anliker (E.T.H., Zürich): $[\alpha]_{700}$ +39°, $[\alpha]_{589}$ +102°, $[\alpha]_{312·5}$ +2300°, $[\alpha]_{280}$ −1560°.

Acknowledgement—Grateful acknowledgement is made to the Squibb Institute for Medical Research for a generous supply of antibiotic and for fellowship support.

* We are indebted to Mr. Joseph F. Alicino for this determination.