violet absorption spectrum of I in isoöctane showed the following maxima (log ϵ's in parentheses): 216 mμ (4.70); 256 mμ (4.36); 288 mμ (4.35); 395 mμ (2.77); with shoulders at 252 mμ (4.34) and 320 mμ (3.50).

The bisketene I in benzene solution reacted essentially instantaneously with oxygen, water, methanol and aniline. The bisketene–oxygen product was a yellow amorphous solid, m.p. 175–178° dec., insoluble in the common organic solvents. Its infrared spectrum showed absorption in the carbonyl region at 5.58 and 5.75 μ. The insoluble oxygen product (0.5 g.) upon prolonged boiling with dilute aqueous sodium hydroxide gave a solution from which, after acidification, crude 9,10-anthracenedicarboxylic acid (0.5 g.) separated, m.p. 328–332° dec. The infrared absorption spectrum of this acid was identical with that of pure 9,10-anthracenedicarboxylic acid, m.p. 341° dec. Part of the crude acid was converted to the dimethyl ester, m.p. 179–181° (lit.[4] value 180–181°).

With water I formed a mixture of *cis*- and *trans* - 9,10 - dihydroanthracene - 9,10 - dicarboxylic acid (IIb), m.p. 285° dec.,[5,6] identified by direct comparison with authentic IIb. Treatment of I with methanol gave a mixture of the *cis* and *trans* isomers of the dimethyl esters IIc, m.p. 137–155°.[5] *Anal.* Calcd. for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 73.12; H, 5.49. This IIc was indistinguishable from IIc prepared by methanolysis of IIa. A mixture of dianilides (IId), m.p. 220–248° identical with that prepared directly from IIa was obtained on treatment of I with aniline. A sharp melting IId, m.p. 254–255°, was obtained after several recrystallizations from ethanol. *Anal.* Calcd. for $C_{28}H_{22}O_2N_2$: C, 80.36; H, 5.30; N, 6.69. Found: C, 80.10: H, 5.50; N, 6.51.

(4) H. Beyer and H. Fritsch, *Ber*, **74**, 494 (1941).

(5) J. Mathieu, *Ann. chim.*, **20**, 215 (1945); *Compt. rend.*, **219**, 555 (1944).

(6) A. H. Beckett, *J. Chem. Soc.*, 4159 (1955).

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THE STRUCTURE OF THE ANTIBIOTIC NEOMETHYMYCIN

Sir:

We have recently[1] described experiments which demonstrate that the macrolide antibiotic methymycin[2] possesses structure Ia. From the fermentation mother liquors[3] it has been possible to isolate a second antibiotic ("neomethymycin")—isomeric with methymycin ($C_{25}H_{43}NO_7$)—for which the expression IIa has now been established.

Hydrochloric acid hydrolysis[4] of neomethymycin (IIa) (m.p. 156–158°, $[\alpha]D$ +93° (all rotations in $CHCl_3$), λ_{max}^{EtOH} 227.5 mμ, log ϵ 4.10, $\lambda_{max}^{CHCl_3}$ 2.93, 5.76, 5.90 and 6.10 μ; found for $C_{25}H_{43}NO_7$: C, 63.75; H, 9.04; N, 3.07; N-CH_3, 5.90; C-CH_3, 16.76; -OCH_3, 0.00; neut. equiv. (perchloric acid titration), 472), yielded desosamine hydrochloride,[4a] thus demonstrating that any structural difference between methymycin and neomethymycin must reside in the aglycone portion. Cleavage of neomethymycin with aqueous sulfuric acid[1] gave two products. The more polar one represented the authentic aglycone, neomethynolide (IIb) (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; found for $C_{17}H_{28}O_5$: 65.61; H, 9.02; C-CH_3, 21.74), as demonstrated by its analytical composition and retention of all pertinent ultraviolet and infrared bands associated with the lactone and unsaturated carbonyl chromophores of neomethymycin (IIa). Ozonolysis (decomposition with alkaline peroxide) of neomethynolide (IIb) afforded in good yield the lactonic acid III (m.p. 125–126°, $[\alpha]D$ +42°) already obtained earlier by permanganate oxidation of the antibiotics pikromycin,[5] narbomycin[5] and methymycin.[1] This establishes the carbon sequence from C-1 to C-7[6]; C-7 to C-9 can only be represented by $-\overset{9}{\underset{\|}{C}}(O)-\overset{8}{CH}=\overset{7}{CH}-$ or $-\overset{9}{CH}=\overset{8}{CH}-\overset{7}{\underset{\|}{C}}(O)-$ in order to explain the ready scission of the molecule at C-7 with formation of a carboxyl group.

In marked contrast to methynolide (Ib), neomethynolide (IIb) forms a diacetate (m.p. 199–201°, $[\alpha]D$ +84°; found for $C_{21}H_{32}O_7$: C, 63.90; H, 8.04; CH_3CO, 22.29), gives a positive iodoform test (as does dihydroneomethynolide[7]) and yields acetaldehyde (rather than propionaldehyde) upon successive treatment with lithium aluminum hydride and periodic acid.[8] These results require partial structures IV or V and together with the acid III account for four of the five C-methyl groups of neomethynolide (IIb). The remaining C-methyl group must be present as an additional $-\overset{CH_3}{\underset{|}{CH}}-$ grouping since location on the double bond is excluded by the ultraviolet absorption maximum.

The proper combination of these structural fragments follows from the structure of the second sulfuric acid cleavage product of neomethymycin (IIa) which has been named cycloneomethynolide (VIa). This substance (b.p. 140° (0.01 mm.), $[\alpha]D$ −39.5°, no high ultraviolet max., $\lambda_{max}^{CHCl_3}$

(1) C. Djerassi and J. A. Zderic, THIS JOURNAL, **78**, 2907, 6390 (1956).

(2) M. N. Donin, J. Pagano, J. D. Dutcher and C. M. McKee, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, N. Y., p. 179.

(3) Kindly supplied by Dr. J. Vandeputte (Squibb Institute for Medical Research) who first encountered the chloroform solvate of neomethymycin.

(4) C. Djerassi, A. Bowers, R. Hodges and B. Riniker, THIS JOURNAL, **78**, 1733 (1956).

(4a) R. K. Clarke, *Antibiotics and Chemotherapy*, **3**, 663 (1953); E. H. Flynn, M. V. Sigal, P. F. Wiley and K. Gerzon, THIS JOURNAL, **76**, 3121 (1954); H. Brockmann, H. B. König and R. Oster, *Chem. Ber.*, **87**, 856 (1954).

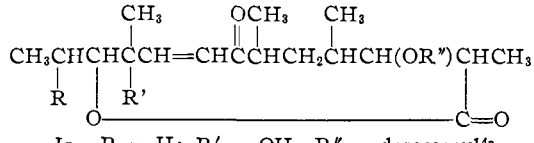
(5) R. Anliker, D. Dvornik, K. Gubler, H. Heusser and V. Prelog, *Helv. Chim. Acta*, **39**, 1785 (1956).

(6) The numbering system employed for erythromycin (K. Gerzon, E. H. Flynn, M. V. Sigal, P. F. Wiley, R. Monahan and U. C. Quarck, THIS JOURNAL, **78**, 6396 (1956)) seems suitable for all macrolide antibiotics and has been adopted in this paper.

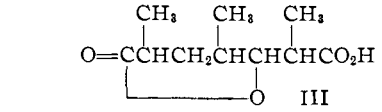
(7) Thus showing that this could not have been due to generation of a methyl ketone by reverse aldol condensation.

(8) Neomethynolide (IIb), like Ib, is not attacked by periodic acid showing that the lactone ring is involved with one of the two hydroxyl groups of the glycol moiety.

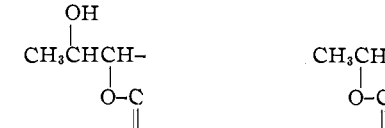
2.90, 5.73 and 5.83 μ, negative single Cotton effect in rotatory dispersion (typical of saturated ketone); found for $C_{17}H_{28}O_5$: C, 65.48; H, 9.00) is isomeric with neomethynolide (IIb) but lacks the unsaturated carbonyl chromophore. Since cycloneomethynolide (VIa) forms only a monoacetate (VIb) (m.p. 194° (subl.), $[\alpha]D$ +1°, $\lambda_{max}^{CHCl_3}$ 5.69, 5.83 and 8.05 μ (but *no* OH absorption); found for $C_{19}H_{30}O_6$: C, 64.64; H, 8.16; CH_3CO, 11.93), does not give a positive iodoform test and does not react with periodic acid after lithium aluminum hydride reaction, a new ether linkage must have been formed by addition of the C-12 hydroxyl group to the double bond of the α,β-unsaturated ketone. In conformity with this view, cycloneomethynolide (VIa) can be oxidized readily to the ketone VIc (m.p. 122–124°, $[\alpha]D$ +60°; found for $C_{17}H_{26}O_5$; C, 65.94; H, 8.30) which furnished 63% of CO_2 after treatment with alkali followed by acidification. These results are best rationalized by structure VIa for cycloneomethynolide from which it follows that neomethynolide must be IIb and neomethymycin IIa.[9] This structure represents a novel substitution pattern of considerable biogenetic interest[10] among macrolide antibiotics and explains the absence of spiroketal formation, so facile in the methymycin series (I). The details of these and related experiments as well as the conclusions to be drawn from rotatory dispersion measurements of various macrolide transformation products will be reported in a complete paper.



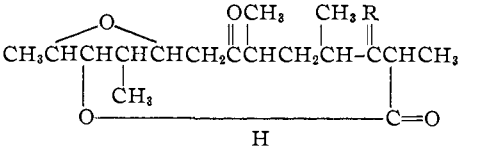
Ia, R = H; R′ = OH; R″ = desosamyl[4a]
Ib, R = R″ = H; R′ = OH
IIa, R = OH; R′ = H; R″ = desosamyl[4a]
IIb, R = OH; R′ = R″ = H



III



IV V



VIa. R = ⌊OH
b, R = H
c. R = O ⌊OAc

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(9) The glycosidic linkage is placed at C-3 (rather than C-12) because warming of neomethymycin or dihydroneomethymycin with alkali (opening of lactone ring) followed by sodium metaperiodate cleavage yielded acetaldehyde under conditions where the glycosidic linkage would not be expected to be affected.

(10) *Cf.* R. B. Woodward, "Festschrift Arthur Stoll," Birkhäuser, Basel, 1957, pp. 524–544; *Angew. Chem.*, **69**, 50 (1957).

FORMATION OF A THREE-STRANDED POLYNUCLEOTIDE MOLECULE

Sir:

It has been shown that molecules of the synthetic polyribonucleotides, polyadenylic acid (poly-A) and polyuridylic acid (poly-U), associate in aqueous solution,[1] and further demonstrated[2,3] that fibers drawn from mixtures of the acids produce an X-ray diffraction pattern similar to that of desoxyribose nucleic acid (DNA). This complex is a two-stranded helical molecule in which the poly-A and poly-U are intertwined.

A continuous variation study has been made of the complexing reaction in which the total concentration of the bases in solution is maintained constant while varying their ratio. The complexes which form have a lower optical density at 259 mμ than their uncombined constituents, and the minimum of optical density occurs where the largest amount of complex is formed.

Experiments were carried out with poly-A and poly-U prepared in our laboratory with polyribonucleotide phosphorylase from *E. coli* obtained by the method of Littauer and Kornberg[4] as well as with polymers kindly supplied by S. Ochoa.[5] The solutions contain 0.1 *M* sodium chloride and 0.01 *M* glycylglycine at *p*H 7.4. The open circles of Fig. 1 show the variation with composition of the optical density at 259 mμ. The position and sharpness of the minimum clearly shows formation of a very strong 1:1 complex.

In some experiments, it was noted that the points on the right side of Fig. 1 (open circles) fall below the straight line, producing a bowed appearance. The shape of the curve was such as to suggest the formation of a new relatively weak complex involving two poly-U strands for each poly-A strand.

Ultracentrifuge studies at low concentrations using ultraviolet optics showed that the sedimentation coefficient of the new complex (s_{20} = 17.3) was greater than that of the 1:1 complex (s_{20} = 12.6). (The values of s_{20} for this poly-A and poly-U were 8.0 and 3.2, respectively.)

The addition of divalent cations has been found to drive this reaction to completion. In solutions which are 0.01 *M* in $MgCl_2$ the shape of the optical density curve changes to that seen in the solid curve of Fig. 1. This curve has a minimum at 67% U, 33% A, *i.e.*, 2:1.

We have interpreted these results to mean that the original two-stranded molecule has taken on a third strand of poly-U which fills the helical groove in the (A + U) complex. We have built models to show that such a three-stranded complex is possible with divalent cations neutralizing the charges on

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(2) A. Rich and D. R. Davies, THIS JOURNAL, **78**, 3548 (1956).
(3) A. Rich, *Ann. N. Y. Acad. Sci.*, in press (1957).
(4) U. Z. Littauer and A. Kornberg, *J. Biol. Chem.*, in press.
(5) M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Science*, **122**, 907 (1955).