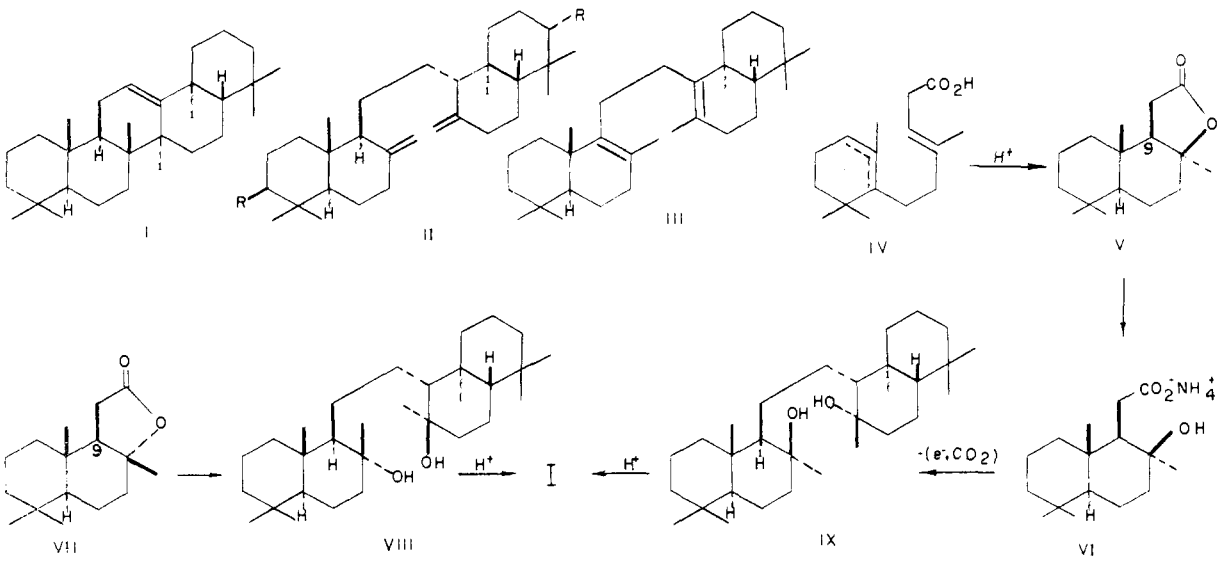

cyclization,[6] *without rearrangement*, has not yet been detected in nature. In addition, numerous attempts to effect the synthesis of pentacyclosqualene by acid-catalyzed cyclization of squalene have failed.[7]

We outline here a total synthesis of this substance, heretofore obtained only by partial synthesis from α-onoceradienediol[8] (II, R = OH), and, incidentally, the synthesis of both α-onoceradiene (II, R = H)[8] and β-onoceradiene (III).[8,9]

Acid-catalyzed cyclization of either α- or β-monocyclohomofarnesic acid (IV) leads to a mixture of lactones from which the (±)-lactone V has been isolated.[10,11] Hydrolysis of the (−)-lactone in methanolic potassium hydroxide followed by careful acidification and ammonolysis gave the ammonium salt VI in high yield. Oxidative electrolytic coupling of VI in refluxing methanol at a smooth platinum anode[12] gave after chromatography the diol IX (m.p. 185–186.5°, [α]D +42°; found: C, 80.58; H, 12.29). Cyclization of IX with perchloric acid in benzene-acetic acid yielded (+)-pentacyclosqualene, I (m.p. 275–276.5° (sealed capillary), [α]D +86°) identical in all respects with "γ-onocerene" prepared from α-onoceradienediol (II, R = OH).[8] Dehydration of the diol IX with phosphorus oxychloride–pyridine gave β-onoceradiene (m.p. 159–161°, [α]D +140°) identical with an authentic sample.

Similarly, the (+)-lactone VII led to the diol VIII (m.p. 270-271°, [α]D +13°; found: C, 80.41; H, 12.34) which on cyclization also gave (+)-pentacyclosqualene (I). α-Onoceradiene (II, R = H; m.p. 199.5–201°, [α]D +25°) was obtained from VIII by $POCl_3$ dehydration.[13]

It is a pleasure to thank Drs. D. H. R. Barton, G. Lucius and M. Stoll for gifts of materials and Dr. Sherlock Swann, Jr., and Mr. W. Garrison for valuable aid with the electrolytic experiments.

(6) Following the same stereocourse which leads to the triterpenes cited in ref. 2.

(7) See L. Ruzicka, *Experientia*, **9**, 359 (1953).

(8) D. H. R. Barton and K. Overton, *J. Chem. Soc.*, 2639 (1955).

(9) J. D. Cocker and T. G. Halsall, *ibid.*, 4262 (1956); K. Schaffner, R. Viterbo, D. Arigoni and O. Jeger, *Helv. Chim. Acta*, **39**, 174 (1956).

(10) G. Lucius, *Angew. Chemie*, **68**, 257 (1956); G. Lucius, private communication.

(11) Mild acid treatment of the (+)-*trans*-lactone VII obtained from sclareol served as a convenient source of the epimeric *cis*-lactone V; *cf.* L. Ruzicka and M. Janot, *Helv. Chim. Acta*, **14**, 645 (1931), and M. Stoll and M. Hinder, *Helv. Chim. Acta*, **36**, 1995 (1953). The lactone of L. Ruzicka, C. F. Seidel and L. L. Engel, *Helv. Chim. Acta*, **25**, 621 (1942), was shown by its characteristic infrared spectrum to correspond to the third lactone of Lucius[10] and is probably the C_8 epimer of VII since it can be prepared from *either* V or VII by acid-catalyzed isomerization. Further data on the sterochemistry and interconversions of these lactones will be reported later.

(12) The best yields of coupled material were obtained with high current density, refluxing methanol, and high salt concentrations. Ammonia was added periodically to inhibit lactonization.

(13) The dehydration products of the two epimeric diols IX and VII confirm, respectively, the diaxial and diequatorial orientations of the hydroxyl groups; *cf.* D. H. R. Barton, A. Campos-Neves and R. C. Cookson, *J. Chem. Soc.*, 3500 (1956).

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MACROLIDE ANTIBIOTICS. VI. A STANDARD OF ABSOLUTE CONFIGURATION AMONG MACROLIDE ANTIBIOTICS

Sir:

As pointed out in a recent review,[1] the macrolide group of antibiotics represents a therapeutically and biogenetically important class of natural products. Knowledge concerning the absolute configuration of these substances is limited to a single center (C-10) of magnamycin[1] and this is of no direct applicability to the other antibiotics of this class since magnamycin follows the "acetate" rather than "propionate" pattern so common among macrolide antibiotics. Attempts to use rotation rules such as Hudson's lactone rule for the determination of the absolute configuration in the erythromycin series[2] have led to ambiguous results. We should now like to report certain reactions with neomethymycin (Ia)[3] which establish the absolute

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

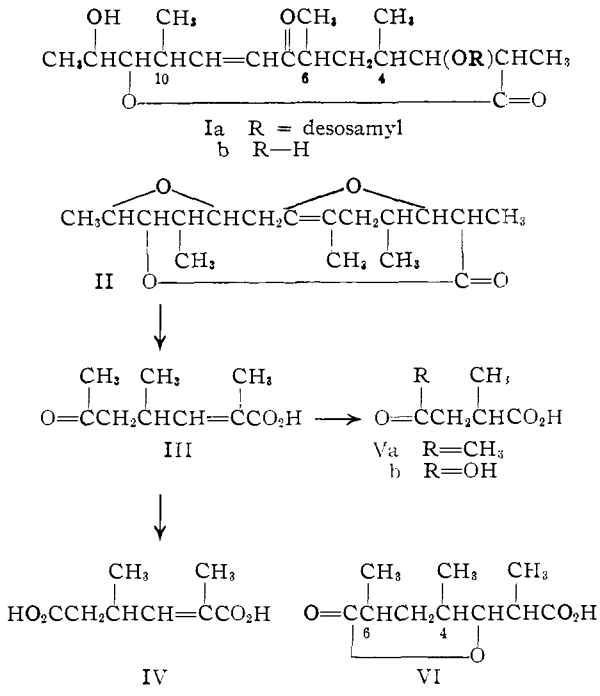
(2) K. Gerzon, E. H. Flynn, M. V. Sigal, P. F. Wiley, R. Monahan and U. C. Quarck, THIS JOURNAL, **78**, 6396 (1956), footnote 21.

(3) Paper V, C. Djerassi and O. Halpern, *ibid.*, **79**, 2022 (1957)

configurations of C-4 and C-6 of neomethymycin (Ia) as well as of methymycin,[4] pikromycin[5,6] and narbomycin[7] (see ref. 15).

When neomethymycin (Ia)[3,8] was boiled for 10 min. with 5 *N* sulfuric acid, extracted with ether and the *unwashed* ether extracts dried and distilled at 200° and 40 mm., there was isolated in over 70% yield the cyclic enol ether II[9] (m.p. 168–170° (from dilute methanol), $[\alpha]_D$ +124.5° (dioxane),[10] no high u.v. maximum, $\lambda_{max}^{CHCl_3}$ 5.75 μ (lactone) and 5.88 μ (enol ether) but no hydroxyl absorption, negative iodoform test; found for $C_{17}H_{26}O_4$: C, 69.15; H, 9.03; O, 22.22, OCH_3, 0.0; (5)C-CH_3, 23.84).[11] Ozonolysis of II at −80° in methylene chloride solution, decomposition of the ozonide with boiling water, saponification with 5% sodium hydroxide solution and purification of the acid fraction by silica gel chromatography and distillation yielded 6-oxo-2,4-dimethyl-2-heptenoic acid (III) (b.p. 100° at 0.001 mm., $[\alpha]_D$ +46° (chloroform), 11 $\lambda_{max}^{CHCl_3}$ 5.80, 5.88 and 6.02 (m) μ and broad "acid absorption" in 3.4 μ region, positive iodoform test; found for $C_9H_{14}O_3$: C, 62.96; H, 8.37) which formed a bright yellow 2,4-dinitrophenylhydrazone (m.p. 142–144°; found for $C_{15}H_{18}N_4O_6$: C, 51.46; H, 5.57; N, 16.05; neut. equiv., 340). Brief warming of the keto acid III with sodium hypoiodite solution in aqueous dioxane gave iodoform and crystalline 1,3-dimethyl 1-butene-1,4-dioic acid (IV), which may be a mixture of *cis-trans* isomers since it could not be obtained sharp melting after distillation at 120° and 0.001 mm. and recrystallization from ether–hexane (m.p. 130–155°, $[\alpha]_D$ +41° (chloroform), λ_{max}^{ETOH} 215 mμ, log ϵ 4.05; found for $C_8H_{12}O_4$: C, 55.60; H, 7.09; neut. equiv., 88). Ozonolysis of the unsaturated keto acid III followed by permanganate oxidation produced α-methyllevulinic acid (Va), $[\alpha]_D$ −5.6° (chloroform), which was characterized as the yellow 2,4-dinitrophenylhydrazone (m.p. 194–196° with sublimation from 165°; found for $C_{12}H_{14}N_4O_6$: C, 46.69; H, 4.64; N, 17.94; neut. equiv., 306). Hypoiodite oxidation of α-methyllevulinic acid (Va) furnished iodoform and (−)-methylsuccinic acid (Vb) (m.p. 111.5–113°, $[\alpha]_D$ −17.5° (*c* 0.97 in ethanol), $[\alpha]_D$ −9° (*c*, 1.0 in H_2O) found for $C_5H_8O_4$; C, 45.32; H, 6.03; neut. equiv., 65) identified by infrared comparison with an authentic sample kindly provided by Dr. E. J. Eisenbraun.[12] By proceeding without isolation of intermediates, a 20% over-all yield of (−)-methylsuccinic acid (Vb) could be realized from the enol ether II.

Since (−)-methylsuccinic acid has been related with L-glyceraldehyde,[12,13] the absolute configuration of C-4 in neomethymycin (Ia) has been established. In view of the fact that neomethymycin[3] as well as methymycin,[4] pikromycin,[14] and narbomycin[14] have been transformed into the identical lactonic acid VI, which still retains C-4 and C-6 of the parent compounds, and since the acid VI has been converted[14] into meso-α,α'-dimethylglutaric acid, the absolute configuration of C-4 and C-6[15] in all four antibiotics has been related experimentally to L-glyceraldehyde.



We are indebted to the Squibb Institute for Medical Research and the National Heart Institute (grant No. H-2574) of the National Institutes of Health, U. S. Public Health Service for financial assistance. The microanalyses were performed by

(4) Papers III and IV, C. Djerassi and J. A. Zderic, THIS JOURNAL, **78**, 2907, 6390 (1956).

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

(6) H. Brockmann and R. Oster, *Ber.*, **90**, 605 (1957).

(7) R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, R. Reusser and H. Zähner, *Helv. Chim. Acta*, **38**, 935 (1955).

(8) In our earlier communication (ref. 3) bearing on the structure proof of neomethymycin, we neglected to state that the methyl group was placed at C-10 rather than the equally plausible C-11 position because successive treatment of dihydroneomethynolide (Ib with double bond reduced) with lithium aluminum hydride, periodate oxidation, steam distillation of acetaldehyde and finally treatment of the residue with sodium hypoiodite did *not* yield any iodoform.

(9) The same product was obtained when neomethynolide (Ib) or cycloneomethynolide (VIa in ref. 3) was shaken with acidified ether and then distilled at 40 mm.

(10) The substance showed a positive plain dispersion curve (*cf.* C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 55 (1957))—in contrast to the three starting materials—thus demonstrating the disappearance of the ketone function.

(11) Single positive Cotton-effect curve in methanol solution (peak $[\alpha]_{314}$ + 560°).

(12) E. J. Eisenbraun and S. M. McElvain, THIS JOURNAL, **77**, 3383 (1955); see also E. Berner and R. Leonardson, *Ann.*, **538**, 1 (1939).

(13) For a summary of Fredga's work see J. A. Mills and W. Klyne in W. Klyne's "Progress in Stereochemistry," Academic Press, Inc., New York, N. Y., 1954, Vol. I, p. 203.

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

(15) Provided pikromycin and narbomycin possess a hydroxyl group at C-3. If the hydroxyl group is located at C-5—as has been suggested as a preferred structural alternative for pikromycin (ref. 5, 6)—then it should be noted that C-4 and C-6 as marked in formula VI correspond to C-2 and C-4 of pikromycin and since the sterochemical convention is then changed, the absolute configuration of these two centers in pikromycin would correspond to the D-series. This point will obviously not be settled until the constitution of pikromycin (and narbomycin) has been established unambiguously at which time it will be known which carbon atoms of the antibiotics correspond to those of the lactone acid VI. In any event, the latter represents the standard of absolute configuration for this portion of these macrolide antibiotics.

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POLYNUCLEOTIDES. I. MOLECULAR PROPERTIES AND CONFIGURATIONS OF POLYRIBOADENYLIC ACID IN SOLUTION[1]

Sir:

We wish to report the discovery of two configurations of polyriboadenylic acid (poly-A): one a flexible, randomly coiled, molecularly dispersed form and the other a rigid, presumably helical form composed of variable numbers of poly-A molecules.

Using polynucleotide phosphorylase from *Azotobacter vinelandii*,[2] samples of poly-A were prepared at various enzyme–substrate ratios and collected at different extents of reaction in order to provide products covering a wide molecular weight range. These samples in solution above *p*H 6.5 in water or above *p*H 5.7 in 0.15 *M* salt, exhibited a characteristic absorption spectrum having a maximum at 257 mμ ($E_{1\%} \cong 295$), were not birefringent, and displayed sedimentation constants, s^0, and intrinsic viscosities, $[\eta]$, that depended on molecular weight in the manner shown at the left side of Fig. 1. The molecular weights were calculated from the Flory–Mandelkern equation[3] using 2.3×10^6 for $\Phi^{1/3}/P$ and 0.55 for partial specific volume. The slopes, 0.45 and 0.65, respectively, are typical of randomly coiled chains: we conclude that this is the configuration under these conditions.

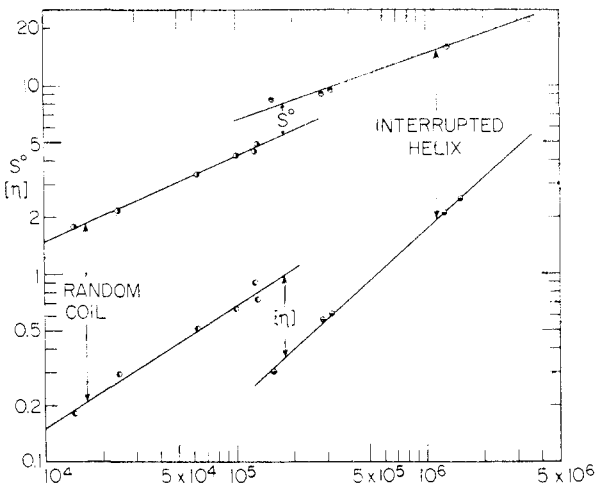

Fig. 1.—Molecular weight dependence of sedimentation constant, S^0_{20} in svedbergs and intrinsic viscosity $[\eta]_{25}$, in dl./g., of the two configurations of poly-A. For the curves on the left, the solvent was 0.15 *M* NaCl, 0.015 *M* citrate, *p*H 7.1; for those on the right it was 0.15 *M* acetate, *p*H 4.9. The open circle (○) represents measurements at *p*H 3.7 with no added salt present.

However, below *p*H 5 in 0.15 *M* salt and below *p*H 6.5 in water the poly-A samples displayed a different spectrum having a maximum at 252 mμ ($E_{1\%} \cong 255$) and had values of s^0 and $[\eta]$ that depended on the concentration and the temperature at which the transition had been brought about by lowering the *p*H.

By varying the concentration of poly-A (0.2 to 1.5 g./100 cc. for the sample having a molecular weight of 126,000) prior to lowering the *p*H to 4.9 (acetate buffer of ionic strength 0.15) the solutions were found to exhibit systematic variations in s^0 and $[\eta]$ that were clearly indicative of the formation of stable aggregates. The weights of these aggregates increased with the concentration of poly-A at which the transition was induced. Calculating the molecular weights (or particle weights) as before, it was found that the s^0 and $[\eta]$ varied with weight as shown in the right side of Fig. 2. The linear relations that result and the values of the slopes, 0.36 and 0.92, respectively, indicate that the species are homologous in structure and that the differences in weight are the result of different extents of linear growth. Unlike the randomly coiled configuration, this form does not show a marked increase in specific viscosity upon removal of salt. This and the behavior in flow birefringence lead to the conclusion that the structure is relatively rigid and rod-like, but not quite to the extent found for deoxyribonucleic acid (DNA). The stability of this structure appears to result from the lowering of the electrostatic energy brought about by titrating about half of the adenine groups. The failure to find spectra intermediate in character between the two described further suggests that the transition is abrupt and complete and that practically all the chromophoric groups (adenine) undergo the same change in environment. Moreover, at *p*H 4.9 in 0.15 *M* acetate, the transition to the randomly coiled form occurs in a very narrow temperature range near 75°, only a few degrees below the transition temperature observed for DNA. This coöperative behavior indicates a highly ordered arrangement of secondary bonds for the acid-stable form.

These observations are compatible with a structure in which poly-A molecules are associated through hydrogen-bonded base-pairing in a double stranded helix,[4] each strand of which has gaps where one poly-A molecule ends and another begins. This would resemble the interrupted helical model once proposed for DNA,[5] but the gaps in our case may be larger. The increased rotational freedom at the gaps would account for the smaller space filling properties exhibited by this configuration

(1) This investigation was supported by a research grant (C-2170) from the National Cancer Institute, Public Health Service.

(2) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955); M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Biochim. Biophys. Acta*, **20**, 169 (1956). We are very grateful to Professor Ochoa for making available to us unpublished information on the purification of the enzyme.

(3) P. J. Flory and L. Mandelkern, *J. Chem. Phys.*, **20**, 212 (1952).

(4) A two-stranded structure of this type has been proposed by F. H. C. Crick, D. Davies, A. Rich and J. D. Watson and is discussed by J. D. Watson in "The Chemical Basis of Heredity," W. D. McElroy and B. Glass, editors, the Johns Hopkins Press, Baltimore, 1957 and by A. Rich in the *Proc. Acad. Sci., New York*, in press. It is not necessary that the detailed atomic arrangements of the helical configuration in solution be the same as that which may ultimately be established in the solid state.

(5) C. A. Dekker and H. Schachman, *Proc. Natl. Acad. Sci.*, **40**, 894 (1954).