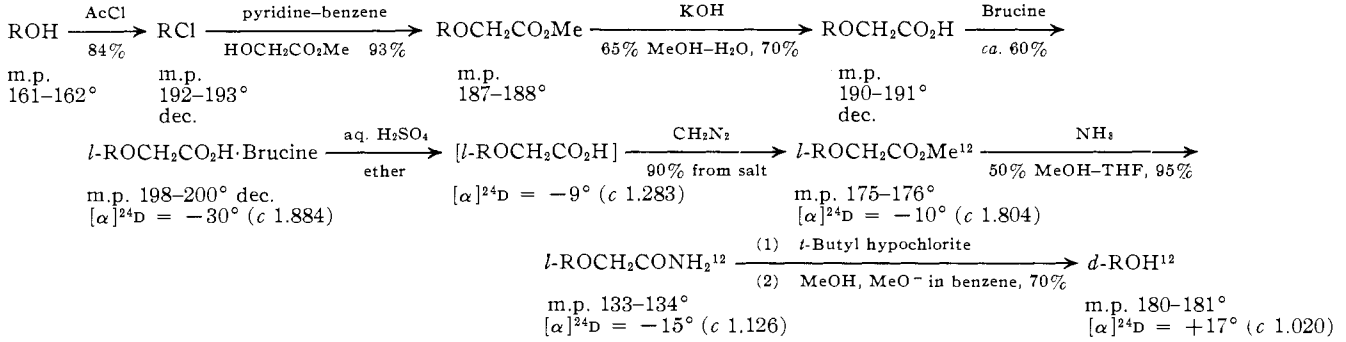
CHART I



carbon tetrachloride, rotation +0.07°). Wallis[6] reported for *l*-ROH $[\alpha]^{20}D = -5.4°$ (*c* 0.451 in carbon tetrachloride, rotation −0.05°). By fractional crystallization of this *d*-ROH from ether one can obtain *d*-ROH with $[\alpha]^{24}D = +17°$ (*c* 0.937), m.p. 180–181°, unchanged by three recrystallizations, and *d,l*-ROH, m.p. 161–162°, after crystallization from cyclohexane. Except for the possibility of anomalous racemates,[11] which are extremely rare, the evidence indicates that this alcohol is optically pure.

Our method of resolution is outlined in Chart I. Except as noted beyond, the reactions were carried out by standard methods with yields indicated beneath the arrows. Pilot experiments were carried out with both trityl- and *d,l*-phenylbiphenyl-α-naphthylmethyl derivatives. The acid was liberated from the brucine salt in the usual way[6] and the ether solution was treated immediately with diazomethane. Treatment of triphenylmethoxyacetamide according to the usual method for preparing methyl carbamates[13] (bromine in methanolic methoxide) afforded tritylmethyl ether in 85% yield. However, use of *t*-butyl hypochlorite by the procedure of Baumgarten[14] for N-haloamine rearrangement led to the Hofmann rearrangement. *l*-Amide (0.0029 mole) dissolved in 300 ml. of benzene was treated with 0.006 mole of *t*-butyl hypochlorite. Care was exercised to exclude water. To the solution (65°) was added methanolic sodium methoxide (0.009, g.-atom of sodium in 30 ml. methanol) and the mixture was refluxed for 2 hr. Sodium chloride was removed by filtration and the solvent was removed *in vacuo*. The residue was triturated with ether. The solution was filtered and concentrated, whereupon crystals formed, $[\alpha]^{24}D = +14.5°$ (*c* 2.046). The infrared spectrum of this material indicated that it was the alcohol contaminated with a carbonyl compound, probably the methylcarbamate.[15] One recrystallization from ether afforded 0.78 g. of *d*-ROH, $[\alpha]^{24}D = +17.2°$ (*c* 1.020), unchanged within experimental error by further recrystallizations.

Isolation of the alcohol from this last reaction gives assurance that the C–O bond at the asymmetric center has not broken during the reaction. Heterolysis of this bond to give either carbonium ion or carbanion would have resulted in formation of the ether (ROMe) or the hydrocarbon (R–H), respectively. Homolysis would not be expected to give alcohol. The evidence indicates that this procedure is satisfactory for resolution of triarylcarbinols in high optical yield and its generality is under investigation. The optical purity of our alcohol is being checked by isotopic dilution studies.

Wallis[6] converted *l*-$RSCH_2CO_2H$ to *d*-ROH with silver nitrate in aqueous acetone and *l*-$RSCH_2CO_2H$ to *l*-ROEt with silver nitrate in ethanol. Treatment of Wallis' *d*-ROH, $[\alpha]^{24}D = +10°$, for 1 week with excess of ethyl iodide and silver carbonate[16] yielded an oil, $[\alpha]^{24}D = -3.5°$ (*c* 1.20), whose infrared spectrum indicated that it was a mixture of ether and alcohol. Alcohol $[\alpha]^{24}D = +9.4°$ (*c* 15) was recovered from this mixture by crystallization from benzene–petroleum ether (b.p. 60–70°). This rotation shows that the alcohol was not racemized under the reaction conditions and, therefore, the *d*-ROH has the same configuration as *l*-ROEt. The active alcohol was converted *via* the potassium salt and methyl iodide to *l*-ROMe $[\alpha]^{24}D = -5.8°$ (*c* 1.72). Thus, the reactions of the thioacid with silver nitrate in aqueous and in alcoholic solution take the same stereochemical course. Our current working hypothesis is that these reactions proceed with net *retention* of configuration. This view is based on the reasonable assumption that the least soluble diastereomeric brucine salt of the thioacid (*l*-$RSCH_2CO_2H$) has the same configuration at the triarylmethyl carbon as that of the least soluble brucine salt of the oxygen acid (*l*-$ROCH_2CO_2H$). Experiments are in progress to establish this point by the method of quasiracemates[11] and to develop chemical means of stereochemical correlation for such compounds. For this purpose the thionbenzoate isomerization[9] and the nitrosoamide deamination[17] are being tested.

We are investigating other leaving groups and other triarylmethyl derivatives.

Acknowledgment.—The author expresses his appreciation to the following students of the graduate preresearch course who carried out several of the pilot experiments: L. DeBow, C. Elliger, J. Robbins, and N. Werstiuk.

(10) Unless otherwise stated, all rotations were taken in chloroform in a 2-dm. cell such that observed rotations were greater than ±0.2°.

(11) A. Fregda, *Tetrahedron*, **8**, 126 (1960); E. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 106–110.

(12) Satisfactory analysis was obtained for this material.

(13) E. S. Wallis and J. F. Lane, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 282.

(14) H. E. Baumgarten, J. E. Dirks, J. M. Petersen, and D. C. Wolf, *J. Am. Chem. Soc.*, **82**, 4422 (1960).

(15) In the *d,l*-series, use of amide–*t*-BuOCl–NaOMe in the ratio 1:1:1 gave a yellow oil whose infrared spectrum is consistent with the carbamate structure. Treatment of this material with excess methoxide in methanol gave ROH.

(16) K. Mislow, *J. Am. Chem. Soc.*, **73**, 4043 (1951).

(17) E. H. White and J. E. Stuber, *ibid.*, **85**, 2168 (1963).

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The Structure of Telomycin

Sir:

The acidic hydrolysis of the antibiotic Telomycin[1] yields alanine, aspartic acid, glycine, β-hydroxyleu-

(1) M. Misiek, O. B. Fardig, A. Gourevitch, D. L. Johnson, I. R. Hooper, and J. Lein, *Antibiot. Ann.*, 852 (1957–1958).

cine,[2] *cis*- and *trans*-3-hydroxyproline,[3] serine, *allo*-threonine, and threonine. Basic hydrolysis produces tryptophan and β-methyltryptophan.[4] Quantitative amino acid determinations by both the Moore–Stein technique and mass spectrometry show that only one of each amino acid is present. None of the amino acids was attacked by D-amino acid oxidase. Potentiometric titration indicated that telomycin has one free carboxyl function and one free amino group; molecular weight determinations averaged 1300. The molecular weight of Telomycin is calculated at 1273.3 on the basis of the eleven amino acids linked by ten peptide bonds and one lactone. The presence of a lactone is confirmed by the disappearance of the characteristic ester-type infrared band at 1745 cm.$^{-1}$, accompanied by a change in optical rotation [from $[\alpha]^{28}D$ −133° to $[\alpha]^{28}D$ −32°; (*c* 1, 2:1 methanol–water)] on treatment with 0.32 *N* barium hydroxide at ambient temperature. Potentiometric titration of the newly formed "telomycic acid" disclosed an additional carboxylic acid function (pK_a 3.6). Chromic acid oxidation[5] showed that threonine is protected in Telomycin but not in telomycic acid, thus establishing threonine as the O-terminus of the lactone. As expected, Telomycin travels to the cathode while telomycic acid migrates to the anode on electrophoresis at pH 6.4.

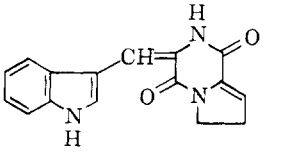
Although attempts to degrade Telomycin or telomycic acid to peptides by means of acidic or enzymatic partial hydrolysis were unpromising, partial basic hydrolysis was successful. Prolonged treatment with aqueous sodium hydroxide, followed by ion-exchange neutralization, gave (in addition to traces of amino acids) five water-soluble peptides and one water-insoluble hexapeptide.

The water-soluble peptides were separated by electrophoresis, yielding a pentapeptide (Asp, Ser, Thr, *allo*-Thr, Ala); a tetrapeptide (Asp, Ser, Threo, *allo*-Thr); two tripeptides (Asp, Ser, Thr) and (Thr, *allo*-Thr, Ala); and a dipeptide (Asp, Ser). The pentapeptide had aspartic acid as the N-terminal amino acid, as did Telomycin itself (Sanger DNFB method). Hydrazinolysis (Akabori) of the pentapeptide revealed alanine as C-terminal. These observations, together with p*K* studies, establish the N-terminal sequence of telomycin as β-Asp-Ser-Thr-*allo*-Thr-Ala.

The purified (thin-layer chromatography) water-insoluble hexapeptide contains both 3-hydroxyprolines, glycine, tryptophan, β-methyltryptophan, and β-hydroxyleucine; the N-terminal amino acid is glycine (Sanger DNFB). Since the C-terminal amino acid of telomycic acid is *cis*-C-hydroxyproline (Akabori), this must also be the position occupied in the hexapeptide.

The Edman technique confirmed glycine as N-terminal and revealed *trans*-3-hydroxyproline as the next amino acid in the hexapeptide. Partial hydrolysis (acidic) of the hexapeptide gave a tripeptide containing both tryptophans and β-hydroxyleucine (acidic and basic total hydrolyses). Edman degradation on this tripeptide showed β-hydroxyleucine to be N-terminal and hydrazinolysis produced only tryptophan.

Alkaline hydrolysis under nitrogen of either the hexapeptide or Telomycin gave indole-3-aldehyde and a yellow crystalline product, m.p. 280–283° dec. *Anal.* Calcd. for $C_{16}H_{13}O_2N_3$: C, 68.80; H, 4.69; N, 15.05; mol. wt., 279.29. Found: C, 68.71; H, 4.81; N, 15.02; mol. wt., 279.1 (mass number). The ultraviolet spectrum shows a band at 380 mμ (ε 16,000). Hydrogenation over rhodium–charcoal followed by acid hydrolysis gave proline and hydrotryptophan. These data are in good accord with the formulation of the yellow, crystalline product I.

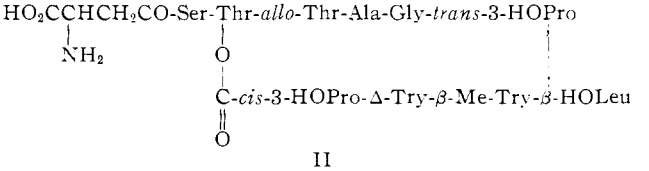


I

The ultraviolet spectra of Telomycin, telomycic acid, and the hexapeptide all have, in addition to tryptophan-type absorption, a band at 339 mμ (ε 22,000).

This ultraviolet chromophore can be accounted for by a dehydrotryptophan system. For example, methyl β-(3-indolyl)-α-benzamidoacrylate [prepared by the Erlenmeyer condensation of β-indolealdehyde and hippuric acid followed by methanol] has an absorption band at 341 mμ (ε 21,000).

All of the foregoing observations are compatible with the representation of Telomycin as structure II.



II

The formation of tryptophan from the dehydrotryptophan system on alkaline hydrolysis is surprising, and this type of reaction is being investigated further.

Acknowledgment.—This work was aided by a Grant from the Public Health Service, National Institutes of Health. We are also indebted to Bristol Laboratories, Syracuse, New York, for our supply of Telomycin, and to Prof. George Büchi for helpful discussions concerning the ultraviolet chromophore.

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(2) J. C. Sheehan, K. Maeda, A. K. Sen, and J. A. Stock, *J. Am. Chem. Soc.*, **84**, 1303 (1962).

(3) The *trans* configuration has been assigned to the "slow-moving" 3-hydroxyproline on the basis of the classically rigorous conversion of its precursor, 3-methoxy-L-proline, to L-methoxysuccinamide. J. C. Sheehan and J. G. Whitney, *ibid.*, in press.

(4) By electrophoresis and paper chromatography the β-methyltryptophan from Telomycin has been shown to correspond to synthetic "A" racemate kindly provided by Professor H. R. Snyder of the University of Illinois. *Cf.* H. R. Snyder and D. S. Matteson, *J. Am. Chem. Soc.*, **79**, 2217 (1957). The β-methyltryptophan structure was suggested by Professor Klaus Biemann (M.I.T.) on the basis of mass spectrometric measurements on the isolated amino acid furnished by our laboratory. *Cf.* K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co. Inc., New York, N. Y., 1962, p. 275.

(5) J. C. Sheehan, H. G. Zachau, and W. B. Lawson, *J. Am. Chem. Soc.*, **80**, 3349 (1958).

The Separation of Ketimine Isomers

Sir:

Earlier claims of the separation of the geometric isomers of aldimines and ketimines[1] have been disputed.[2] We have now succeeded in preparing and separating both forms of a number of ketimines derived from 2-amino-5-chlorobenzophenone.

These compounds are typified by the morpholinoethylimines, obtained by heating 2-amino-5-chloro-

(1) O. Anselmino, *Ber.*, **40**, 3465 (1907); W. Manchot and J. R. Furlong, *ibid.*, **42**, 3030 (1909); M. E. Taylor and T. L. Fletcher, *J. Am. Chem. Soc.*, **80**, 2246 (1958).

(2) V. De Gaouck and R. J. W. Le Fèvre, *J. Chem. Soc.*, 741 (1938); *ibid.*, 1392 (1939); D. Y. Curtin and J. W. Hausser, *J. Am. Chem. Soc.*, **83**, 3474 (1961).