spectrum of II is a methyl doublet at τ 8.78 (CH_3-CH-O-), which is replaced by a methyl singlet =C(CH_3)-O- at τ 8.30 when II is dehydrated with phosphorus oxychloride–pyridine. These data define the unit B in II and I. The remaining unassigned atoms (C_3H_5) of I are found in its n.m.r. spectrum as a single doubly deshielded proton at characteristically low field, τ 5.02 (X-CH-Y), and two overlapping aliphatic methylene groups at τ 8.10 (C-CH_2-C and C-CH_2-C). The only reasonable arrangement of these groups and unit B is in unit C, and this is confirmed by spin decoupling[12] experiments.

Acknowledgment.—This investigation was supported in part by Public Health Service Research Grant No. AI-01278 from the National Institute of Allergy and Infectious Diseases. We also thank the Upjohn Co. for generous samples of streptolydigin and Dr. Ragnar Ryhage for the use of mass spectrometric facilities at the Karolinska Institutet, Stockholm.

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(13) Guggenheim Foundation Fellow, 1962.

(14) Allied Chemical Fellow, Roger Adams Summer Fellow, National Science Foundation Summer Fellow.

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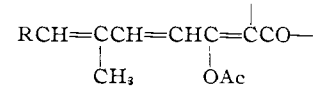
Streptolydigin. III. Chromophore and Structure

Sir:

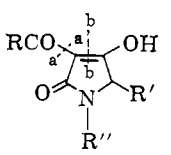
In the two preceding communications structures have been established for streptolic acid[1] and ydiginic acid[2]; these compounds account for all the carbon atoms and nitrogen functions of the antibiotic streptolydigin.[3] The present report establishes how these two halves are joined in the intact antibiotic and completes the structure (except for some stereochemical features) of streptolydigin.

Streptolydigin is an acidic enol (pK_a' 5.3 in 65% methanol,[3] positive ferric chloride and titanium trichloride tests,[3] green cupric salt) and the previously described oxidations of its sodium salt (with sodium periodate to give streptolic acid,[1] with ozone to give ydiginic acid[2]) occur at this function, just as the periodate oxidation of 2-methylcyclohexane-1,3-dione is reported to give glutaric and acetic acids,[4] and the periodate oxidation of 2,3-dihydroxy-2-cyclopentenone is reported to give α-ketoglutaric acid.[5] Acetylation of sodium streptolydigin with acetic anhydride–pyridine gives an enol acetate (carbonyl band, 1745 $cm.^{-1}$) whose ultraviolet spectrum λ_{max} 329 mμ (ϵ_{max} 30,000) accords well with that expected[6] for a trienone

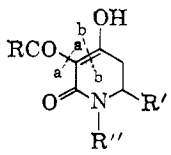


The complex ultraviolet spectrum of streptolydigin shifts markedly with variations in pH.[3] The simpler ultraviolet spectrum of octahydrostreptolydigin also displays shifts with pH: $\lambda\lambda_{max}$ 278 mμ (ϵ_{max} 16,200) and 246 (14,300) in 0.01 *N* ethanolic potassium hydroxide; $\lambda\lambda_{max}$ 281 and 246 mμ ($\epsilon\epsilon_{max}$ 13,000 and 12,700, respectively) at pH 8; λ_{max} 279 mμ (ϵ_{max} 10,500) in 0.01 *N* ethanolic sulfuric acid. This behavior is remarkable for enols in that the maximum near 280 mμ does not shift from acidic to basic solution, but is joined by a new maximum of approximately equal intensity near 240 mμ. It differs from that of simple β-diketones like acetylacetone (λ_{max} 274 mμ, ϵ_{max} 5700 in 0.01 *N* ethanolic H_2SO_4; λ_{max} 295 mμ, ϵ_{max} 20,000 in 0.01 *N* ethanolic KOH), which show a bathochromic shift in base, and is actually the reverse of the behavior of β-triketones like 2-acetyldimedone and triacetylmethane, which give two strong maxima in acid and only one in base (2-acetyldimedone: $\lambda\lambda_{max}$ 233 and 274 mμ, $\epsilon\epsilon_{max}$ 11,800 and 11,500, in 0.01 *N* ethanolic H_2SO_4; λ_{max} 275 mμ, ϵ_{max} 21,000, in 0.01 *N* ethanolic KOH).

The spectral behavior of octahydrostreptolydigin is, however, nearly exactly that of the chromophore A, exemplified by tenuazonic acid (A2)[7] and a synthetic model compound [A1. *Anal.* Found: C, 54.36; H, 5.90; N, 8.88; mol. wt. (mass spec.), 155], prepared from ethyl sarcosinate by the general route of Lacey.[8] The presence of the acylpyrrolidione (acyltetramic acid) chromophore in streptolydigin (A, R = $C_{17}H_{23}O_3$; R' = $CH(CH_3)CONHCH_3$; R'' = $C_6H_{11}O_2$) indicates that ydiginic acid[2] is an artifact of the ozonolysis of sodium streptolydigin or sodium octahydrostreptolydigin, a result of oxidative cleavage of bonds a–a and b–b, with imide formation from the newly formed incipient carboxyl group and the methyl amide function. In agreement with this deduction are the lack of imide absorption in the infrared spectra of streptolydigin itself and of other oxidation products obtained from the ozonolysis of sodium octahydrostreptolydigin. The other products (called diginic acids) still retain the open-chain N'-methyl-β-methylaspartamide structure, HOOC—CHCH—$CONHCH_3$, and will be discussed in the full paper.



A



B

A1, R = R'' = CH_3; R' = H
A2, R = CH_3; R' = CHC_2H_5; R'' = H
CH_3

B1, R = R'' = CH_3; R' = H

In principle, ydiginic acid could also arise from cleavage of an acylpiperidione (chromophore B) at bonds a–a and b–b. However, the spectral behavior of a synthetic model compound [B1. *Anal.* Found: C, 50.36; H, 5.41; N, 7.36 (sodium salt); mol. wt. (mass spec.), 169 (free acid)] is distinguished from that of octahydrostreptolydigin in that there is a definite bathochromic shift of the maximum near 280 mμ (from 274 mμ in acid to 286 mμ in base) and in that the model chromophore B1 gives only a single ultraviolet maximum at pH 8, while both octahydrostreptolydigin and the model compound A1 retain both maxima at pH 8. In agreement with these observations are pK_a data in water[9]: octahydrostreptolydigin has pK_a 3.25, compound A1 pK_a 3.50, compound B1 pK_a 7.12.

Elucidation of the chromophoric unit completes the structure of streptolydigin and assigns the antibiotic formula I.

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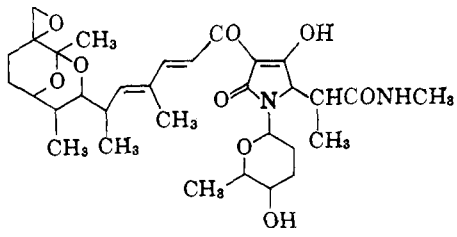
(9) We are indebted to Dr. H. Boaz, Eli Lilly and Co., for pK_a determinations.



I

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(10) Alfred P. Sloan Foundation Fellow.

(11) Roger Adams Fellow, Standard Oil of California Fellow, and Gillette-Toni Fellow.

(12) Allied Chemical Fellow, Roger Adams Summer Fellow, National Science Foundation Summer Fellow.

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Optical Rotatory Dispersion of Deoxyribonucleotides[1]

Sir:

The optical rotatory dispersion (ORD) of mononucleotides reveals considerable detail not evident in the corresponding absorption spectra. The magnitude of the peak and trough and the sign of the Cotton effect in the ultraviolet region vary significantly among the four deoxyribonucleotides (5′) we studied.

*d*AMP (A), *d*CMP (C), *d*GMP (G), and *d*TMP (T) exhibit only a *single* Cotton effect (between 220 and 380 mμ) with cross-overs (zero rotation) corresponding to their absorption maxima (Fig. 1 and 2),[2-5] as contrasted with the *multiple* Cotton effects of DNA.[6] (Our ORD results of DNA (above 190 mμ) show two peaks at 290 and 223 mμ, two troughs at 255 and 192 mμ, and cross-overs at about 273, 243, and 201 mμ, the magnitude of the second peak and trough being at least twice that of the first one. Although our findings differ in certain aspects from those of Fresco, *et al.*,[6] we agree with them that the multiple Cotton effects, especially the 201-mμ effect in our case, are highly sensitive to the secondary structure of DNA.)

The pyrimidine nucleotides (C and T) have a *positive* Cotton effect as does DNA near 273 mμ, but the purine ones (A and G) actually show a *negative* Cotton effect. Since the bases attached to the deoxyribose have little freedom of rotation and presumably have the same orientation relative to the sugar, this strikingly different rotatory behavior is perhaps related to the directions of the transition moments in the bases.[7,8] To what extent the $\pi-\pi^*$ transitions[7] and $n-\pi^*$ transitions[9] contribute to the Cotton effect still cannot be answered; indeed, a satisfactory explanation of these opposite Cotton effects is still lacking at present.[10]

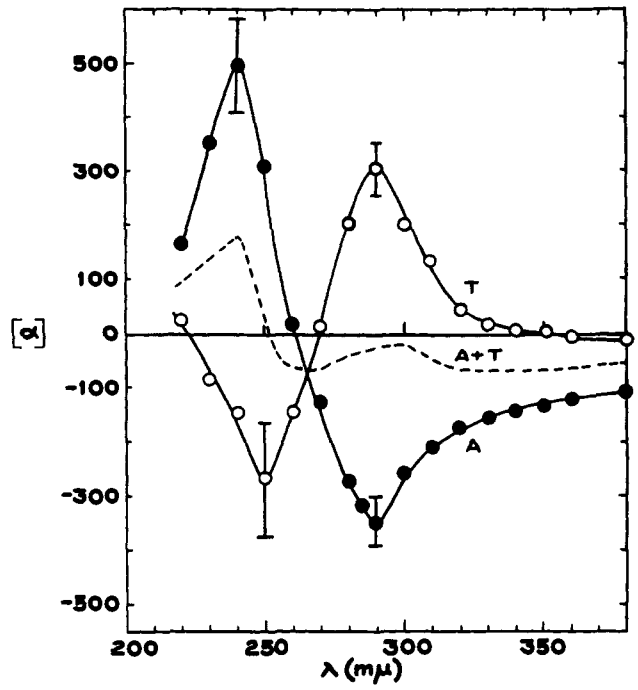

Fig. 1.—Ultraviolet rotatory dispersion of *d*AMP (A) and *d*TMP (T) in 0.15 *M* KF (pH 7.7–7.8). Broken line, $f_A[\alpha]_A + f_T[\alpha]_T$ (*f*'s are the mole fractions).

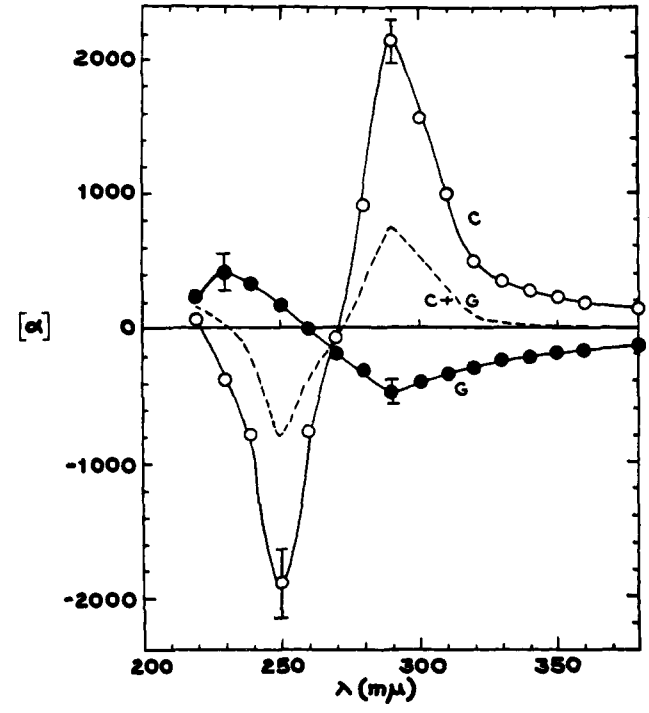

Fig. 2.—Ultraviolet rotatory dispersion of *d*CMP (C) and *d*GMP (G) in 0.15 *M* KF (pH 7.7–7.8). Broken line, $f_C[\alpha]_C + f_G[\alpha]_G$ (*f*'s are the mole fractions). Note that the ordinate scale is different from Fig. 1.

(1) This work was aided by grants from the U. S. Public Health Service (GM-K3-3441, GM-10880, and HE-06285).

(2) *d*CMP, *d*GMP, and *d*TMP were purchased from the California Corp. for Biochemical Research, and *d*AMP was from the Pabst Laboratories. All ORD measurements were made with a Rudolph manual spectropolarimeter (Model MSP-4); detail of the instrument calibration has been published elsewhere.[3] The concentrations of the solutions were so adjusted that the maximum absorbance was always less than 2. No concentration dependence of the rotations could be detected within normal experimental errors.

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(4) By assuming a gaussian curve for the circular dichroism and using the Kronig-Kramers transform,[5] the rotational strengths of *d*AMP, *d*CMP, *d*GMP, and *d*TMP were estimated to be approximately −3, +10, −4, and $+2 \times 10^{-40}$, respectively. These estimates were of course not unique in the absence of circular dichroism measurements and therefore should be viewed with reservation.

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