Mass spectra of streptolol and methyl octahydro-streptolate are in good accord with the structures assigned, containing peaks at M − 18 (m/e 290 and 324, respectively) from loss of water, and major peaks at 165 and 167, respectively, corresponding to fragmentation along line a–a, together with loss of water.

Structure I for streptolic acid is well accounted for biogenetically by a combination of the propionate and acetate pathways recently demonstrated for the macrolides erythromycin,[8] magnamycin,[9] and methymycin.[10]

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.

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(10) A. J. Birch, E. Pride, R. W. Rickards, P. J. Thomson, J. D. Dutcher, D. Perlman, and C. Djerassi, *Chem. Ind.* (London), 1245 (1960).

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

(12) Roger Adams Fellow, National Science Foundation Predoctoral Cooperative Fellow.
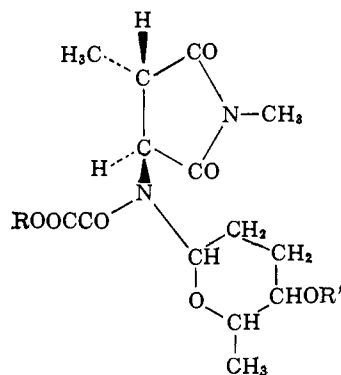
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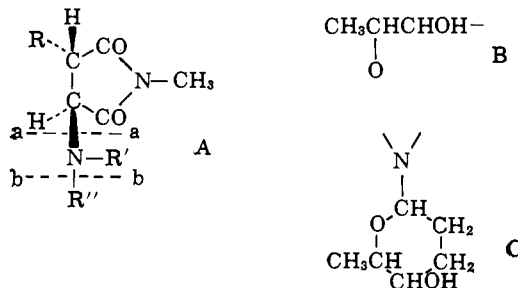

Streptolydigin. II. Ydiginic Acid

Sir:

We wish to present evidence which assigns structure I to ydiginic acid, $C_{14}H_{20}N_2O_7$, m.p. 97–103°, $[\alpha]^{25}D$ −37° (c 1.01, 95% EtOH) [*Anal.* Found: C, 51.05; H, 6.50], one of the principal water-soluble compounds obtained from ozonolysis of sodium streptolydigin[1] or of sodium octahydrostreptolydigin. Together, ydiginic acid (I) and streptolic acid (accompanying communication)[1] account for all of the carbon, oxygen, and nitrogen atoms of the antibiotic streptolydigin.[2]

I (ydiginic acid), R = R′ = H
II (methyl ydiginate), R = CH_3; R′ = H
III (methyl O-acetylydiginate), R = CH_3; R′ = $COCH_3$)

Hydrolysis of ydiginic acid in refluxing 4 N aqueous sodium hydroxide gives L-*threo*-β-methylaspartic acid,[3] $[\alpha]^{23}D$ +12.4° (c 1.01, 5 N HCl), $[\alpha]^{27}D$ −10° (c 1.08, H_2O), and methylamine, while hydrolysis of I in 4 N hydrochloric acid at 95° gives the corresponding N′-methylimide (IV), m.p. 80°, $[\alpha]^{26}D$ −152° (c 0.45, $CHCl_3$), identified by comparison of its infrared and

(1) K. L. Rinehart, Jr., J. R. Beck, W. W. Epstein, and L. D. Spicer, *J. Am. Chem. Soc.*, **85**, 4035 (1963).

(2) T. E. Eble, C. M. Large, W. H. De Vries, G. F. Crum, and J. W. Shell, *Antibiot. Ann.*, 893 (1955–1956).

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n.m.r. spectra with those of a synthetic sample prepared from commercial (±)-β-methylaspartic acid. The imide unit is shown to be present in ydiginic acid itself by comparison of its characteristic infrared (carbonyl bands at 1775 and 1700 cm.$^{-1}$)[4,5] and ultraviolet (apparent maximum at 203 mμ, ε ca. 10,000)[5] spectra to those of authentic samples of N-methylsuccinimide and IV.

IV, R = CH_3; R′ = R″ = H
V, R = CH_3; R′ = H; R″ = $COCH_3$
VI, R = H; R′ = H; R″ = $COCH_3$

The second nitrogen atom (the amino nitrogen of β-methylaspartic acid) in ydiginic acid is present as a tertiary amide, since it is nonbasic and the infrared spectrum of I contains an amide carbonyl band at 1665 cm.$^{-1}$ but no amide II or N–H stretching absorption.[6]

Acetylation of the methyl ester (II) of ydiginic acid with acetic anhydride and pyridine gives methyl O-acetylydiginate (III), $C_{17}H_{24}N_2O_8$, m.p. 276–279°, $[\alpha]^{31}D$ −25° (c 1.13, MeOH) [*Anal.* Found: C, 53.15; H, 6.55; N, 7.21]. The mass spectrum of III contains, *inter alia*, significant peaks at m/e 384 (M), 324 (M − HOOCCH₃), 297 (M − 87), 237 (M − 87 − HOOCCH₃), 141, and 126. The relative intensity of the last two peaks (126 >> 141) is in agreement with the tertiary amide assignment, since model secondary amides (R′ = H) like V and VI show stronger peaks corresponding to b–b fragmentation (141 and 127 in V and VI, respectively) than those corresponding to a–a cleavage (126 and 112 in V and VI, respectively). That the ion of mass 297 cannot have lost acetic acid is shown by the metastable ion[7] sequence 297 $\xrightarrow{m^* 189.1}$ 237, thus it must have lost the carbomethoxyl group and 28 additional mass units. Absence of a strong peak at m/e 74 or 88 shows[8] the 28 mass units can be neither –CH_2CH_2– nor –$CHCH_3$–, so they must be –CO–, and the 87-mass unit fragment must be CH_3OOCCO–. Since the only carbonyl group in methyl ydiginate besides the imide and carbomethoxyl units (from infrared considerations) is the amide noted above, an oxamate unit CH_3OOCCO-N< is assigned to II and III; thus, the peak at m/e 297 arises from b–b cleavage. In agreement with this assignment, ydiginic acid (I) has $pK_a \leq 1.70$, while the model compound N-cyclohexyloxamic acid[9] has pK_a 2.16 ± 0.03.[10]

The formation of III establishes in I an alcoholic function, which is shown to be secondary by the shift of a one-proton peak at τ 6.4 in the n.m.r. spectrum of II to τ 5.22 in the spectrum of III.[11] Also in the n.m.r.

(4) H. K. Hall, Jr., and R. Zbinden, *J. Am. Chem. Soc.*, **80**, 6428 (1958).

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

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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.

(12) W. A. Anderson and R. Freeman, *J. Chem. Phys.*, **37**, 85 (1962).

(13) Guggenheim Foundation Fellow, 1962.

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

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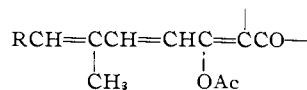
Streptolydigin. III. Chromophore and Structure

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.
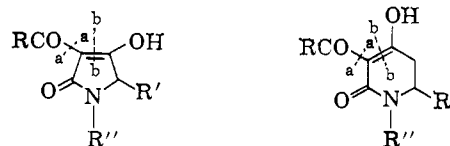
Streptolydigin is an acidic enol (pK_a' 5.3 in 65% methanol,[3] positive ferric chloride and titanium trichloride tests,[3] green cupric salt) and the previously described oxidations of its sodium salt (with sodium periodate to give streptolic acid,[1] with ozone to give ydiginic acid[2]) occur at this 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

$$RCH{=}CCH{=}CHC{=}CCO-$$
$$\overset{|}{CH_3} \quad \overset{|}{OAc}$$

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, re-

(1) K. L. Rinehart, Jr., J. R. Beck, W. W. Epstein, and L. D. Spicer, *J. Am. Chem. Soc.*, **85**, 4035 (1963).

(2) K. L. Rinehart, Jr., and D. B. Borders, *ibid.*, **85**, 4037 (1963).

(3) T. E. Eble, C. M. Large, W. H. DeVries, G. F. Crum, and J. W. Shell, *Antibiot. Ann.*, 893 (1955–1956).

(4) M. L. Wolfrom and J. M. Bobbitt, *J. Am. Chem. Soc.*, **78**, 2489 (1956).

(5) G. Hesse and K. Mix, *Chem. Ber.*, **92**, 2427 (1959).

(6) Octatrienal absorbs at 314 mμ, ϵ_{max} 37,000: A. Smakula, *Angew. Chem.*, **47**, 657 (1934).

spectively) 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 acylpyrrolidone (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.

A1, R = R'' = CH_3; R' = H
A2, R = CH_3; R' = CHC_2H_5; R'' = H   (with CH_3 below)

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.

(7) C. E. Stickings, *Biochem. J.*, **72**, 332 (1959).

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