Other properties of homotropone also indicate a trend toward those of tropone. Thus the carbonyl stretching frequency of II in the infrared occurs at 1650 cm.$^{-1}$ compared with 1660 cm.$^{-1}$ for both eucarvone and cyclooctatrienone. Homotropone also has a higher boiling point than cyclooctatrienone and is twice as soluble in water as is the trienone (4.3 g./100 ml. as compared to 2.4 g./100 ml.).

In conclusion we believe that the above data indicate that in this particular system the effects of homoconjugation of the cyclopropyl ring are not insignificant. However when the systems I and II are compared with the tropylium cation and tropone, respectively, it is of course clear that this type of conjugation compares unfavorably with that possible with an ethylenic type double bond.

Acknowledgment.—We thank the Alfred P. Sloan Foundation and the Robert A. Welch Foundation for financial assistance.

(11) Celanese Corporation Fellow, University of Texas, 1962–1963.

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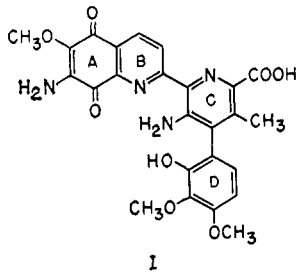
RECEIVED JULY 11, 1963

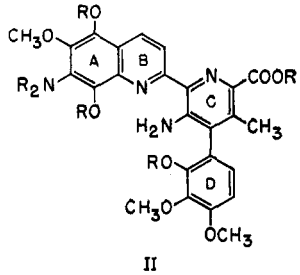
The Structure of Streptonigrin

Sir:

Streptonigrin, a metabolite of *Streptomyces flocculus*,[1] is an antibiotic which exhibits striking activity against a variety of animal tumors.[2,3] We wish to record our conclusion that streptonigrin possesses the unique structure I.



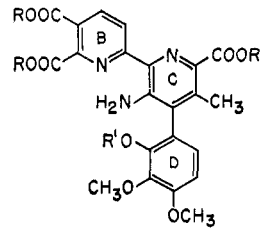
I



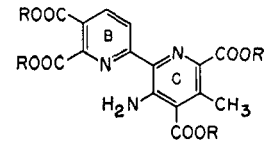
II

Streptonigrin is a monobasic acid and is readily susceptible to reversible two-electron reduction. Its composition, $C_{25}H_{22}O_8N_4$, only approximately determinable by elementary analyses,[1] was deduced exactly by mass spectrometric comparison of hexamethyldihydrostreptonigrin (II, R = CH_3), $C_{31}H_{36}O_8N_4$ (mol. wt.[4] 592), m.p. 185–186°, and hexadeuteriomethyldihydrostreptonigrin (II, R = CD_3), $C_{31}H_{18}D_{18}O_8N_4$ (mol. wt. 610), m.p. 185–186°, prepared by catalytic hydrogenation of streptonigrin followed by alkylation with, respectively, light and heavy dimethyl sulfate, in acetone in the presence of potassium carbonate. That the acidity of streptonigrin is associated with a carboxyl group was demonstrated by the observation that pentamethyldihydrostreptonigrin (II, COOH in place of COOR), $C_{30}H_{34}O_8N_4$ (mol. wt. 578), m.p. 215–216°, obtained by alkaline hydrolysis of the hexamethyldihydro derivative (II, R = CH_3), when volatilized directly into the ion source of the mass spectrometer, showed pyrolytic evolution of carbon dioxide (*m/e* 44), a small peak at *m/e* 578, and an intense peak at *m/e* 534–the molecular weight of the decarboxylation product (II, H in place of COOR).

Streptonigrin was oxidized by alkaline hydrogen peroxide to the *tribasic* streptonigric acid (III, R = R′ = H), $C_{22}H_{19}O_9N_3$, m.p. 210–215° dec. [tetramethyl derivative (III, R = R′ = CH_3), $C_{26}H_{27}O_9N_3$ (mol. wt. 525), m.p. 166–167°]. Oxidation of streptonigric acid with alkaline permanganate gave the *tetrabasic* streptonigrinic acid (IV, R = H), $C_{15}H_{11}O_8N_3$, m.p. >300°



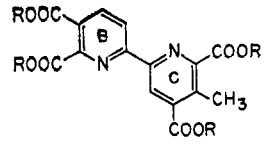
III



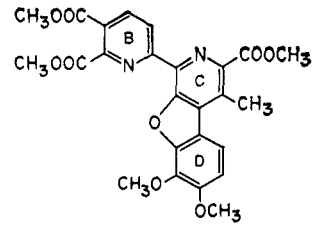
IV

[tetramethyl derivative (IV, R = CH_3), $C_{19}H_{19}O_8N_3$ (mol. wt. 417), m.p. 145–146°]. The empirical relationships among the members of this degradative series bespeak the presence in streptonigrin of methoxyaminoquinone, or methoxyhydroxyquinonimine, and hydroxydimethoxyphenyl systems as substituents in place of three of the hydrogen atoms of a $C_{12}H_{11}O_2N_3$ moiety. The latter is known to include a carboxyl group (*vide supra*), and further, nuclear magnetic resonance studies[5] demonstrated the presence in all of these compounds of a primary amino group and a methyl group attached to an aromatic ring [*e.g.*, in IV, R = CH_3: 7.42 τ (CH_3) and 2.16 τ (NH_2, lost on deuteration)]. The residual grouping of atoms, $C_{10}H_8N_2$, clearly suggests that streptonigrin is a *diazabiphenyl*, bearing amino, carboxyl, methyl, hydroxydimethoxyphenyl, and methoxyaminoquinone or methoxyhydroxyquinonimine substituents; it remains to verify that deduction, and choose among the 1,944,576 formulas (!), exclusive of tautomeric modifications, which can be constructed from the general hypothesis.

Tetramethyl streptonigrinate (IV, R = CH_3) was converted by nitric acid–ether to tetramethyldesaminostreptonigrinate (V, R = CH_3), $C_{19}H_{18}O_8N_2$ (mol. wt. 402), m.p. 143–144°.[6] The corresponding acid (V, R = H), m.p. 165° dec., obtained by alkaline hydrolysis, was decarboxylated over soda lime at 350° to 5-methyl-2,2′-bipyridyl, m.p. ~5°, identified by direct comparison (identical infrared, ultraviolet, and nuclear magnetic resonance spectra) with a synthetic sample, obtained by decarboxylation of the dicarboxylic acid from alkaline permanganate oxidation of 3-methyl-1,10-phenanthroline.[7] Streptonigrinic acid (IV, R = H) was oxidized by sodium hypochlorite to pyridine-2,3,6-tricarboxylic acid, m.p. 255–256°,[8] which was de-



V



VI

(1) K. V. Rao and W. P. Cullen, *Antibiot. Ann.*, 950 (1959–1960).

(2) J. J. Oleson, L. A. Calderella, K. J. Mjos, A. R. Reith, R. S. Thie, and I. Toplin, *Antibiot. Chemotherapy*, **11**, 158 (1961).

(3) W. L. Wilson, C. Labra, and E. Barrist, *ibid.*, **11**, 147 (1961).

(4) All molecular weights reported in this communication are experimental values determined by single-focus mass spectrometry. Because of the crucial importance of the composition of II (R = CH_3), its molecular weight was also determined using a double-focusing spectrometer: found, 592.2553; calcd., 592.2531.

(5) All nuclear magnetic resonance measurements were made on $CDCl_3$ solutions.

(6) *Cf.* K. N. Menon, W. H. Perkin, and R. Robinson, *J. Chem. Soc.*, 830 (1930), and O. Fischer and W. Boesler, *Ber.*, **45**, 1930 (1912), for other deaminations by nitric acid.

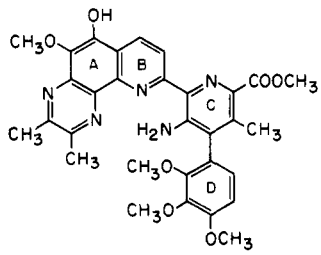
(7) F. H. Case, *J. Am. Chem. Soc.*, **70**, 3994 (1948).

(8) A. Eckert and S. Loria, *Monatsh. Chem.*, **38**, 241 (1917).

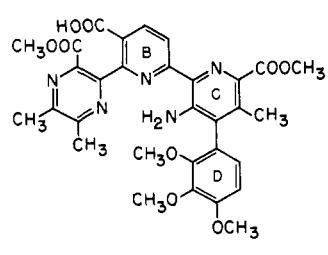
carboxylated very readily to pyridine-2,5-dicarboxylic acid, m.p. 256–258°,[9] identical with an authentic sample. When the product from hydrogenation of streptonigrinic acid (IV, R = H) over reduced platinum oxide in aqueous ethanolic hydrochloric acid was oxidized with alkaline permanganate, a crude acidic material was obtained from which, by distillation from soda lime, 3-amino-5-methylpyridine [mol. wt. 108; n.m.r. spectrum 7.83 τ (CH_3), 5.90 τ (NH_2), 3.20 τ (H at C-4), 2.08 τ (H at C-2); ultraviolet spectrum: 234, 300 mμ (MeOH)] was produced. Since streptonigrinic acid must contain two *adjacent* carboxyl groups (originating from destruction of a quinonoid ring) and two *adjacent* hydrogen atoms [n.m.r. spectrum of the ester (IV, R = CH_3): quartet, 1.06, 1.22, 1.64, 1.80 τ, the further degradations just detailed complete the proof of the structure (IV, R = H)].

When O-methylstreptonigric acid (III, R = H, R′ = CH_3), m.p. 205–207°, obtained by alkaline hydrolysis of the ester (III, R = R′ = CH_3), was oxidized by hot alkaline permanganate, 2,3,4-trimethoxybenzoic acid, m.p. 98–99°,[10] identical with an authentic sample, was obtained. The ester (III, R = R′ = CH_3) was converted by nitric acid–ether to the benzofuran derivative (VI), $C_{25}H_{22}O_9N_2$ (mol. wt. 494), m.p. 186–187°, whose formation demonstrates the contiguous positions of the amino and trimethoxyphenyl substituents. Further, the analogous O-trideuteriomethyl compound (III, R = CH_3, R′ = CD_3) *was converted by the same reagents to the identical benzofuran* (mol. wt. 494). The phenolic hydroxyl group of streptonigrin must therefore be in the 2 position of the phenyl ring D, and these facts complete the proof of the structure (III, R = R′ = H) for streptonigric acid.

It remains to choose among 12 possible nontautomeric arrangements of the quinonoid ring A. Streptonigrin cannot be a hydroxyquinonimine, since it gave a *non-acidic* derivative, $C_{27}H_{26}O_8N_4$ (mol. wt. 534), m.p. 230–232°, containing only *two* new O-methyl groups, even on long-continued treatment with dimethyl sulfate, acetone, and potassium carbonate. This dimethylstreptonigrin reacted readily with hydroxylamine to give a substance, m.p. 202–204°, which was reduced by sodium dithionite in aqueous alcohol to an easily oxidizable diamino compound. The latter was condensed with diacetyl to give the quinoxaline (VII), m.p. 260–262°, which was oxidized by potassium per-



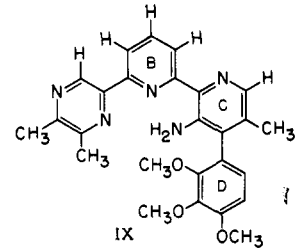
VII



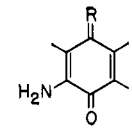
VIII

manganate in hot pyridine to the acid (VIII), m.p. 160–162° dec. [methyl ester, $C_{32}H_{33}O_9N_5$ (mol. wt. 631), m.p. 260–262°]. Hydrolysis with aqueous alcoholic potassium hydroxide gave the corresponding triacid, m.p. 193–195° dec., which was decarboxylated at 250° to the compound IX, $C_{26}H_{25}O_3N_5$ (mol. wt. 457), m.p. 192–194°. The nuclear magnetic resonance spectrum of IX possesses sharp singlets at 0.93 τ (H at C-2 in pyrazine ring), 1.98 (H at C-6 in pyridine ring C), and a typical ABC pattern ($J_{AB} \sim 2$ c.p.s., $J_{AC} = J_{BC} \sim 8$ c.p.s.)—1.35 + 1.38; 1.47 + 1.50; 1.72 +

(9) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **124**, 659 (1938).
(10) N. Rabjohn and A. Mendel, *J. Org. Chem.*, **21**, 218 (1956).



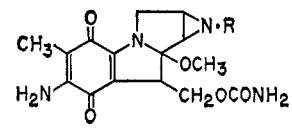
IX



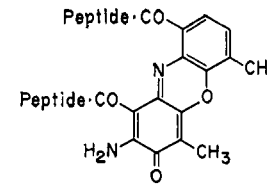
X

1.75; 1.85 + 1.88; 1.98 + 2.11 + 2.23[11]—for the three *adjacent* hydrogen atoms in the pyridine ring B. These observations complete the proof of the full structure I for streptonigrin.

The presence of the common structural unit X in the otherwise very differently constituted molecules of streptonigrin, mitomycin C (XI, R = H)[12] and porfiromycin (XI, R = CH_3),[12] and the actinomycins (XII),[13] taken with the observed loss of activity concomitant with replacement of the primary amino



XI



XII

group, is strongly suggestive of an intimate relationship between the aminoquinone structure X and the marked anticancer activity of all of these substances.[14]

Acknowledgment.—We wish to acknowledge the cooperation of Drs. James A. McCloskey, P. Bommer, Claude Wintner, and R. L. Wagner in making many physical measurements, to express appreciation to the National Institutes of Health for generous support, and to thank Mr. John L. Davenport for his unfailing interest and encouragement.

(11) *Cf.* spectra 3D-14 and 3E-14 in K. B. Wiberg and B. J. Nist, "The Interpretation of NMR Spectra," W. A. Benjamin, Inc., New York, N. Y., 1962, pp. 184, 246.
(12) *Cf.* J. S. Webb, *et al.*, *J. Am. Chem. Soc.*, **84**, 3185, 3187 (1962).
(13) *Cf.* H. Brockmann, *Fortschr. Chem. Org. Naturstoffe*, **18**, 1 (1960).
(14) *Cf.* L. D. Hamilton, W. Fuller, and E. Reich, *Nature*, **198**, 538 (1963).

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RECEIVED JULY 10, 1963

Studies of the Cycloheptatriene–Norcaradiene Isomerism in Some Novel Steroids

Sir:

We recently reported the reactions of steroidal alcohols with diethyl-[2-chloro-1,1,2-trifluoroethyl]-amine (I).[2] We have since examined closely the reactions of the fluoramine I with 19-hydroxyandrost-4-ene-3,17-dione (IIa).[3]

(1) This communication constitutes "Steroids CCXXXIX and Spectra and Stereochemistry VIII"; for "Steroids CCXXXVIII and Spectra and Stereochemistry VII," see A. D. Cross and I. T. Harrison, *J. Am. Chem. Soc.*, forthcoming publication.
(2) L. H. Knox, E. Velarde, S. Berger, D. Cuadriello, and A. D. Cross, *Tetrahedron Letters*, 1213 (1962).
(3) A. S. Meyer, *Experientia*, **11**, 99 (1955).