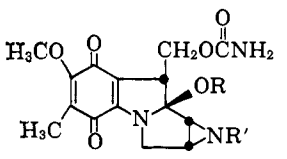
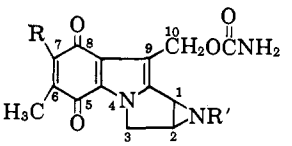
Aziridinomitosenes: A New Class of Antibiotics Related to the Mitomycins

Sir:

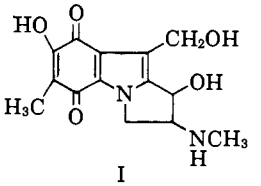
During the investigation of the structures of the mitomycin antibiotics[1] we found that hydrogenation of these substances under a wide variety of conditions was generally disappointing, producing in most cases intractable mixtures of degradation products. However, when mitomycin B[2] (VI, R = H, R′ = CH_3) or N-methylmitomycin A[1a] (VI, R = R′ = CH_3) was hydrogenated over a platinum catalyst at atmospheric pressure in N,N-dimethylformamide (DMF) solution, 1 mole of hydrogen was rapidly absorbed. When the nearly colorless solution was allowed to reoxidize and then was evaporated under reduced pressure, trituration of the brown residue with ethanol, followed by recrystallization from DMF and from pyridine, produced a new compound in yields up to 40%, as orange to brick red rectangular plates showing no definite melting point but loss of birefringence at 230°, $[\alpha]^{25}D$ +36.4 ± 5.1° (*c* 0.99, dimethyl sulfoxide). *Anal.* Calcd. for $C_{16}H_{17}N_3O_5$ (331.32): C, 58.00; H, 5.18; N, 12.69; O, 24.15. Found: C, 58.63, 58.47; H, 5.47, 5.40; N, 12.61; O (direct), 25.00. Hydrolysis of this substance with 6 *N* hydrochloric acid gave the known degradation product I. Since I can be obtained directly from mitomycin B under the same hydrolysis conditions, the skeleton of the new molecule and the location of its substituents had evidently not been altered in any unexpected fashion from those of the starting material. The ultraviolet absorption spectrum of the compound was identical with that of *apo*-mitomycin B[1a]; its infrared spectrum was similar to that of *apo*-mitomycin B below 9 μ, but showed a strong band



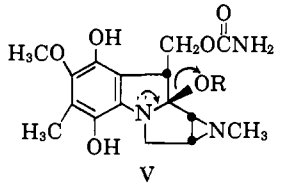
VI



II, R = -OCH_3; R′ = -CH_3
III, R = -OCH_3; R′ = -H
IV, R = -NH_2; R′ = -CH_3



I



V

at 8.34 μ, suggesting that an aziridine ring was still present.[3-7] We therefore formulate the new compound as 7-methoxy-1,2-(N-methylaziridino)mitosene (II).[8]

The proton magnetic resonance spectrum of II is fully consistent with the assigned structure. Comparison with the p.m.r. spectrum of *apo*-mitomycin A[9] showed that the only changes in nonexchangeable protons besides the additional N-methyl group at 2.5 δ were an upfield displacement of approximately 1.5 δ (from 4.75 to 3.25 δ) for the resonance of the protons at positions 1 and 2, a similar upfield displacement (from 5.75 to 4.25 δ) of the resonance due to the two protons on position 3, with a small diminution of the splitting of the latter two-proton peak. Both the upfield displacements were about two-thirds of the magnitude of the corresponding displacements observed on comparing the spectrum of mitomycin A itself with that of *apo*-mitomycin A; evidently the steric and inductive effects of the aromatization of ring B serve to offset, in part, the upfield displacements due to fusion of the aziridine onto the A ring.[10]

When mitomycin A was hydrogenated in DMF as described above, the aziridinomitosene III was obtained as orange needles, dec. pt. 205–250°, but in substantially poorer yield.[11] Hydrogenation of porfiromycin[12] under the same conditions gave no product, but addition of a few drops of acetic acid to the reduced solution before reoxidation enabled us to obtain a small amount of IV, which was, however, more conveniently prepared in 70% yield by ammonolysis of II. Compound IV formed purple needles from acetone; its new chromophore has λ_{max} 248, 308, and 525 mμ (in methanol). Infrared and p.m.r. spectra of IV confirm the assigned structure.

Addition of either triethylamine or phenol to DMF solutions of reduced mitomycin B prevented the formation of II and led to recovery of starting material instead. The formation of II (or of its hydroquinone) thus probably occurred subsequent to the reduction, at either the hydro- or semiquinone stage. Electron spin resonance and electrolytic reduction studies[13] indicated that mitomycin B semiquinone was quite stable, whereas DMF solutions of mitomycin B hydroquinone, when allowed to stand with efficient exclusion of oxygen, developed the ultraviolet spectrum of *apo*-mitomycin B hydroquinone. We believe, therefore, that reduction of mitomycin B to its hydroquinone releases the free electron pair of N-4 from its involvement in resonance with the quinone carbonyl group, facilitating elimination of the group at 9a as shown in formula V. The process is inhibited by triethylamine, either through ionization of other groups in the molecule or by retarding the protonation of the departing anion, and

(1) (a) J. S. Webb, *et al.*, *J. Am. Chem. Soc.*, **84**, 3185 (1962); (b) *ibid.*, **84**, 3186 (1962); (c) we are indebted to Dr. N. Bohonos and his collaborators for supplies of these antibiotics.

(2) T. Hata, *et al.*, *J. Antibiotics* (Tokyo), **9A**, 141 (1956).

(3) Fused epoxide rings have bands in the same area,[6,7] and the following fused aziridine compounds exhibit similar absorptions: mitomycin B, 8.34 μ; N-methylmitomycin A, 8.25 μ; porfiromycin, 8.18 μ; N-methylcyclopentenimine,[4] 8.27 μ; and N-butylcyclohexenimine,[5] 8.35 μ. These bands are absent from the spectra of derivatives of these compounds in which the aziridine ring has been opened.

(4) We are indebted to Mr. C. F Beck for preparing this compound.

(5) O. E. Paris and P. E. Fanta, *J. Am. Chem. Soc.*, **74**, 3007 (1952).

(6) "Technique of Organic Chemistry," Vol. IX, A. Weissberger, Ed., Interscience Publishers, New York, N. Y., 1956, pp. 437–440.

(7) N. B. Colthup, to be published.

(8) (a) The suggested nomenclature in this series is described in ref. 1a; (b) *cf.* J. B. Patrick and J. S. Webb, South African Patent Application 62/4542 (filed Oct. 29, 1962; accepted March 21, 1963).

(9) *Apo*-mitomycin B would obviously be a preferable compound for comparison, but difficulties in purification and separation of stereoisomers prevented its use for this purpose.

(10) It is interesting, although by no means unprecedented, that the two nonequivalent C-3 protons happen to have very nearly identical chemical shifts, and that their geminal coupling constant is not observable.

(11) In spite of considerable effort, no procedure could be found which would produce III in yields comparable to those obtained in the preparation of II, and careful chromatography of the mixture (for which we are indebted to Mr. C. Pidacks and his staff) was generally necessary in order to obtain any of the desired product. This is one of a number of unexpected phenomena in this series which depend on the nature of the substituent on the aziridine nitrogen atom. A discussion of these phenomena will be published later.

(12) R. R. Herr, *et al.*, "Antimicrobial Agents Annual," 1960 Plenum Press, New York, N. Y., 1961, p. 23.

(13) We are indebted to W. G. Hodgson and W. Jura for these experiments.

also by phenol, through protonation of the nitrogen atom. Although a small amount of II has been shown to form in dimethyl sulfoxide solutions of mitomycin B on several months' standing, the reduction–reoxidation remains the only practical method of preparation.

Bioassays[14] of II *in vitro* showed that it was a potent antibiotic with as broad a spectrum of antibacterial activity as the mitomycins themselves. In mice the compound was active both by oral and subcutaneous routes against infections of *Staphylococcus aureus* (var. Smith) and *Streptococcus pyogenes* C-203; in the latter infection the ED_{50} (subcutaneous) is 0.5 mg./kg., equivalent to the parent antibiotic and 128 times as active as tetracycline under the same conditions.

The oral activity of the mitomycins is surprising in view of their susceptibility to acid degradation. The fact that II is orally active suggests that the biologically active species is really II itself (or its hydroquinone)[15] or a related indolohydroquinone. Inconsistent with this hypothesis, however, is the fact that IV does not show the same high activity as II or III.

(14) We are indebted to A. C. Dornbush and the late M. Hauck for *in vitro* testing, and to G. Redin and E. Ewald for *in vivo* testing.

(15) The recent evidence of Schwartz, *et al.*,[16] that a biological reduction may serve to activate mitomycin C *in vivo* reinforces this concept, inasmuch as II is formed *via* the hydroquinone V. The hydroquinone of II would seem to fit all presently known requirements for the "active species."

(16) H. S. Schwartz, J. E. Sodergren, and F. S. Philips, *Science*, **142**, 1181 (1963). NOTE ADDED IN PROOF.—Prof. W. Szybalski has kindly informed us in advance of p iblication (Iyer and Szybalski, *ibid.*, in press) that he postulates, on the basis of biochemical evidence, substantially the same mechanism of action for the mitomycins as the one we propose here.

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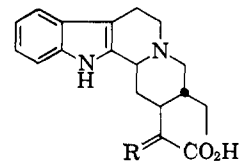
A Novel C–D Ring Cleavage of Dihydrocorynantheine Derivatives[1]

Sir:

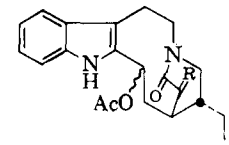
Indole alkaloids bearing an oxygen function at C-3 have been the subjects of several recent investigations. Representatives of this new class of alkaloids include burnamicine,[2] echitamine,[3] and vobasine.[4] We wish to report a novel C–D ring cleavage of dihydrocorynantheine derivatives which simultaneously introduces an acetoxy group at C-3. This reaction may provide a useful synthetic entry to this new group of indole alkaloids oxygenated at C-3.

The action of hot acetic anhydride containing sodium acetate converts the dihydrocorynantheine derivatives Ia and Ib to the corresponding acetoxylactams, IIa, m.p. 176–177°, and IIb, m.p. 194–195°, obtained in approximately 50% yield.

The structures IIa and IIb are supported by the following observations. The ultraviolet spectrum of

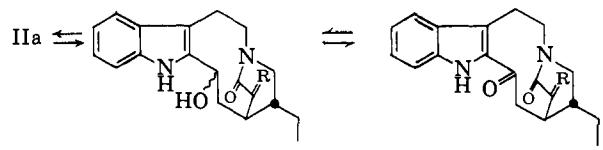


Ia, R = CH_3OCH
b, R = H_2



IIa, R = CH_3OCH
b, R = H_2

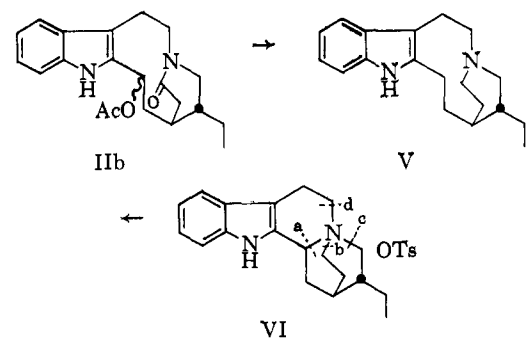
IIa is virtually identical with that of dihydrocorynantheine, whereas IIb shows normal indole absorption. The infrared spectrum of IIa shows absorption at 1720, 1665, and 1608 $cm.^{-1}$ whereas IIb absorbs at 1720 and 1640 $cm.^{-1}$. The n.m.r. spectra of both IIa and IIb indicate an acetate group. Saponification of IIa yielded an alcohol, III, m.p. 149–150°, which was acetylated with acetic acid and sodium acetate to regenerate the acetate IIa. Oxidation of III with manganese dioxide gave in 37% yield the ketone IV, m.p. 240° dec. The ketone IV reverted to the alcohol, III, obtained in 96% yield, by the action of sodium borohydride.



III, R = CH_3OCH IV, R = CH_3OCH

The ultraviolet spectrum of the ketone shows 2-acylindole absorption combined with the α-methoxymethylenecarbonyl chromophore: λ_{max}^{EtOH} 313 mμ (ε 12,700), 238 (25,400), and 217 (26,900). The infrared spectrum shows absorption at 1670, 1640, and 1610 $cm.^{-1}$. Similarly, compound IIb yields a noncrystalline alcohol upon saponification and the alcohol affords a 2-acylindole upon oxidation with manganese dioxide.

Compelling structural evidence was obtained by lithium aluminum hydride reduction of the acetoxylactam, IIb. The product obtained from the acetoxylactam, IIb, by the action of lithium aluminum hydride in refluxing tetrahydrofuran is the oxygen-free base, V, m.p. 178.5–179°. The same compound is obtained from the lithium aluminum hydride reduction of the known quaternary ammonium salt VI.[5] The two samples showed identical infrared spectra, melting points, and mixture melting points.



IIb V VI

In principle, lithium aluminum hydride reduction of the quaternary ammonium salt VI could have broken any one of the four bonds indicated by the dotted lines a, b, c, and d. Cleavage of bond b would have pro-

(1) The authors gratefully acknowledge financial support from the National Science Foundation (Grant GP-252) and the National Institutes of Health (Grant NB 03232-03).

(2) M. F. Bartlett and W. I. Taylor, *J. Am. Chem. Soc.*, **85**, 1203 (1963).

(3) J. A. Hamilton, T. M. Hamor, H. M. Robertson, and G. A. Sim, *J. Chem. Soc.*, 5061 (1962).

(4) U. Renner, D. A. Prins, A. L. Burlingame, and K. Bieman, *Helv. Chim. Acta*, **46**, 2186 (1963); M. P. Cava, S. K. Talapatra, J. A. Weisbach, B. Douglas, and G. O. Dudek, *Tetrahedron Letters*, **No. 2**, 53 (1963).

(5) E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, **81**, 1474 (1959).