against Gram-positive bacteria and no activity against Gram-negative organisms. Compounds B, C, D and E were highly active *in vitro* against Gram-positive and selected Gram-negative bacteria. When administered subcutaneously or orally, at sublethal doses, B, C, D and E protected mice infected with *Staphylococcus aureus* Smith and *Streptococcus pyogenes* C-203. Compounds D and E were also active in mice against transplanted mammary adenocarcinoma (72j). Compound D was effective against leptospirosis in the chick embryo test. Antibiotics B, C, D and E showed considerable toxicity in these tests. The D component was the least toxic.

Compound $A_2$ crystallized in red-purple needles, melting at 124–126°. Calculated for $C_{16}H_{19}N_3O_6$: C, 55.0; H, 5.5; N, 12.0; O, 27.5; mol. wt., 349; 4.3 for 1 $CH_3$; active H, 0.289 for 1, 0.578 for 2; found: C, 54.8; H, 5.7; N, 11.8; O, 27.2; mol. wt., 346 ± 7; OMe, 4.6; NMe, 2.6; CMe, 3.1; active H, 0.37. $\lambda_{max}^{MeOH}$: 218 mμ ($E_{1\,cm}^{1\%}$ 448), 323 mμ ($E_{1\,cm}^{1\%}$ 278), 530 mμ ($E_{1\,cm}^{1\%}$ 34); $\lambda_{max}^{KBr}$: 2.88, 3.11, 3.17, 3.42, 5.83, 6.06, 6.15, 6.35, 6.79, 6.99, 7.13, 7.34, 7.55, 7.66, 7.77, 7.95, 8.23, 8.31, 8.60, 8.79, 8.90, 9.08, 9.30 (sh), 9.51, 9.74, 10.00, 10.16, 10.41, 10.69, 11.11, 11.34, 11.61, 11.89, 12.28, 12.71, 12.81 (sh), 13.22, 13.40 (sh), 14.12, 14.77 μ. The n.m.r. spectrum in $CDCl_3$ indicated a total of 18 to 19 protons, 2 or 3 of which were "active" and readily exchanged with deuterium in the presence of $CD_3OD$. The spectrum further indicated the following functional groups: 1 OMe, 1 NMe, 1 CMe, which is in essential agreement with the data given above. One active hydrogen atom was present as NH, the remaining could not be unambiguously assigned. These data differentiated the compound from any previously reported. It is named mitiromycin A to indicate the relationship with the mitomycins and porfiromycin.

Compound B crystallized in purple needles with m.p. 159–160°. Calculated for $C_{16}H_{19}N_3O_6$: C, 55.0; H, 5.5; N, 12.0; O, 27.5; mol. wt., 349; OMe, 8.5 for 2 $CH_3$; CMe, 4.3 for 1 $CH_3$; active H, 0.86 for 3; found: C, 54.7; H, 5.8; N, 11.8; O, 27.9; mol. wt., 366 ± 10; OMe, 8.4; NMe, none; CMe, 4.0; active H, 0.93; $[\alpha]^{25}D$ −143°, (*C* 0.107 in methanol); $\lambda_{max}^{MeOH}$: 212 mμ ($E_{1\,cm}^{1\%}$ 500), 319 mμ ($E_{1\,cm}^{1\%}$ 295), 515 mμ ($E_{1\,cm}^{1\%}$ 35); $\lambda_{max}^{KBr}$: 2.93, 3.03, 3.39, 5.85, 6.08, 6.31, 6.87, 7.08, 7.27, 7.43, 7.67, 8.15, 8.48, 8.98, 9.31, 10.01, 10.42, 11.18, 11.64, 12.02, 12.63, 13.14, 14.25 μ. Although the $\lambda_{max}$ in the ultraviolet–visible region were similar to those reported for mitomycin A,[2] the specific absorptions of our B component were considerably higher than those published for mitomycin A. The "mitomycin A-like" compound[3] differed appreciably from our B antibiotic in elemental analyses. Recently a sample of mitomycin A[4] made available to us was found to be identical with our B component by paper chromatography, ultraviolet and infrared absorption.

Compound C crystallized in dark purple-blue needles which decomposed without melting when heated: Calculated for $C_{16}H_{19}N_3O_6$: C, 55.0; H, 5.5; N, 12.0; O, 27.5; mol. wt., 349; 4.3 for 1 $CH_3$; active H, 0.87 for 3; found: C, 55.3; H, 5.6; N, 11.9; O, 27.7; mol. wt. 326 ± 6, OMe, 4.3; NMe, 3.2; CMe, 3.4; active H, 0.88; $[\alpha]^{25}D$ −835°, (*C* 0.012 in methanol); $\lambda_{max}^{MeOH}$: 218 mμ ($E_{1\,cm}^{1\%}$ 480), 320 mμ ($E_{1\,cm}^{1\%}$ 290), 540 mμ ($E_{1\,cm}^{1\%}$ 41.5); $\lambda_{max}^{KBr}$: 2.91, 3.03, 3.12, 3.38, 5.76, 5.90, 6.03, 6.18, 6.38, 6.92, 7.07, 7.36, 7.51, 7.70, 7.94, 8.09, 8.32, 8.52, 8.65, 8.80, 9.02, 9.20, 9.34, 9.61, 10.03, 10.18, 10.52, 10.92, 11.39, 11.82, 12.24, 12.79, 13.06, 13.30, 14.12 μ. These infrared maxima were in good agreement with those published for mitomycin B,[2] but the elemental analyses and specific absorptions in the ultraviolet–visible region of our C compound were appreciably different. Mitomycin B was not available for comparison.

Pigments D and E were also isolated as purple crystalline compounds. Direct comparison of the chemical and physical properties, including paper chromatographic mobilities, of pigment D with those of porfiromycin[5] proved them to be identical. Similarly, crystalline pigment E was compared directly with mitomycin C[3,4] and found to be identical by paper chromatography, ultraviolet and infrared absorption and by X-ray powder diffraction.

When compound B was treated with aqueous ammonium carbonate, a crystalline product was obtained with characteristics corresponding to compound E (mitomycin C).[6]

The above compounds were reduced readily with sodium hydrosulfite and reoxidized in air. This suggests the possibility of a quinoid structure.

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(2) T. Hata, Y. Sano, R. Sugawara, A. Matsumae, K. Kanamori, T. Shima and T. Hoshi, *J. Antib.*, Tokyo, Ser. A, **9**, 141 (1956).

(3) S. Wakaki, H. Marumo, K. Tomioka, G. Shimizu, E. Kato, H. Kamada, S. Kudo and Y. Fujimoto, *Antibiotics & Chemotherapy*, **8**, 228 (1958).

(4) We wish to thank Dr. T. Hata, Kitasato Institute, Tokyo, and Kyowa Fermentation Industry Ltd., Tokyo, Japan, for the samples of mitomycin A and C.

(5) R. R. Herr, M. E. Bergy, T. E. Eble and H. K. Jahnke, "Antimicrobial Agents Annual 1960," Plenum Press, New York, N. Y., 1961, p. 23. We wish to thank Dr. G. M. Savage of the Upjohn Company for the sample of porfiromycin.

(6) J. Patrick, *et al.*, of our laboratories, independently observed a similar conversion when pigment B was treated with aqueous ammonia.

D. V. LEFEMINE
BIOCHEMICAL RESEARCH SECTION M. DANN
F. BARBATSCHI
W. K. HAUSMANN
LEDERLE LABORATORIES DIVISION V. ZBINOVSKY
P. MONNIKENDAM
AMERICAN CYANAMID COMPANY J. ADAM
PEARL RIVER, NEW YORK N. BOHONOS

RECEIVED JUNE 25, 1962

## THE STRUCTURES OF MITOMYCINS A, B AND C AND PORFIROMYCIN—PART I

*Sir:*

On the basis of chemical studies and spectrophotometric data we have concluded that mitomycins A, B and C and porfiromycin all have the common

structure I[1] differing only in minor substituents (Table A). These antibiotics are the first members of a new and unusual structural type, representing the first naturally occurring examples of an aziridine,[2] the pyrrolo[1,2-a]indole ring system, an aminobenzoquinone and a pyrrolizine elaborated by a microörganism.

Mitomycins A and B[3] have received little attention, but mitomycin C[4] has been tested widely in cancer chemotherapy,[5] although only one brief account of a partial structure study has appeared.[6] The activity,[7a] isolation,[7b] evaluation,[7c] and assay[7d] of porfiromycin have been reported recently but no information as to its structure[7e] has been available.

We have studied a group of four antibiotics which were isolated by Lefemine, *et al.*,[8] of these Laboratories, from three soil isolates of *Streptomyces verticillatus*. These compounds were later recognized as mitomycins A, B and C and porfiromycin.[9] Most of the present investigation was carried out on mitomycin A (I-A), which was correlated with the other three antibiotics as follows. Reaction of I-A with methanolic ammonia replaced the quinone methoxyl at C-7 (6.0 $\tau$, $CDCl_3$) by an amino group, producing mitomycin C (I-C). Similarly N-methylmitomycin A (I-E) (made by treatment of I-A with $CH_3I$ and $NaHCO_3$ in aqueous dimethylformamide) reacted with methanolic ammonia to give porfiromycin (I-D). Although mitomycin B (I-B) has not yet been obtained directly from or converted to any of the other three antibiotics, it has been degraded to products identical with those obtained directly from N-methylmitomycin A (I-E) and from I-D as described below.

I, MITOSANE

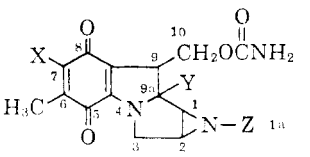
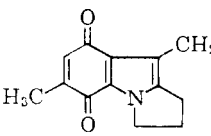


TABLE A

| | Compound | X | Y | Z |
|---|---|---|---|---|
| I-A | Mitomycin A | $H_3CO$ | $OCH_3$ | H |
| I-B | Mitomycin B | $H_3CO$ | OH | $CH_3$ |
| I-C | Mitomycin C | $H_2N$ | $OCH_3$ | H |
| I-D | Porfiromycin | $H_2N$ | $OCH_3$ | $CH_3$ |
| I-E | N-Methyl I-A | $H_3CO$ | $OCH_3$ | $CH_3$ |

The chromophore of I-A and I-B [$\lambda_{max}^{MeOH}$ 218 m$\mu$ ($\epsilon$ 17,400), 320 m$\mu$ ($\epsilon$ 10,400), 520 m$\mu$ ($\epsilon$ 1,400)], was identified by its similarity to the spectrum of 2-dimethylamino-5-methoxybenzoquinone,[10] m. p. 165–168°, [$\lambda_{max}^{MeOH}$ 218 m$\mu$ ($\epsilon$ 18,500), 305 m$\mu$ ($\epsilon$ 13,900), 490 m$\mu$ ($\epsilon$ 3,900)]. Likewise the chromophore of I-C and I-D [$\lambda_{max}^{MeOH}$ 217 m$\mu$ ($\epsilon$ 24,600), 360 m$\mu$ ($\epsilon$ 23,000), 555 m$\mu$ ($\epsilon$ 209)] was similar to that of 2,5-bis-dimethylaminobenzoquinone,[11] [$\lambda_{max}^{MeOH}$ 222 m$\mu$ ($\epsilon$ 24,000), 365 m$\mu$ ($\epsilon$ 21,400), 513 m$\mu$ ($\epsilon$ 407)]. Furthermore, I-A in 1 *N* NaOH was converted to a product (not isolated) with $\lambda_{max}^{0.1\ N\ NaOH}$ 323–330 (doublet) ($\epsilon$ 24,500) which was nearly identical spectrally with 2,5-dihydroxy-*p*-xyloquinone,[12] [$\lambda_{max}^{0.1\ N\ NaOH}$ 332 ($\epsilon$ 26,300)].

In 0.1 *N* HCl at 25° for a few hours I-A underwent a marked change forming one mole of methanol and a new product, *apo*-mitomycin A (Table B II-A),[13] $C_{15}H_{17}N_3O_6$, golden plates from dimethylformamide–water, m.p. 180–200° dec., $[\alpha]_{6907}^{23}$ −10° (3.3% 0.1 *N* HCl), containing one C-methyl, one O-methyl, and one amino group.[14] The methanol formed did not come from hydrolysis of the quinone methoxyl group, as indicated by the continued presence of the 6.0 $\tau$ peak in the n.m.r. spectrum of II-A ($D_6$ dimethyl sulfoxide). The chromophore of II-A [$\lambda_{max}^{CH_3OH}$ 232 m$\mu$ ($\epsilon$ 20,700), 285 m$\mu$ ($\epsilon$ 14,400), 346 m$\mu$ ($\epsilon$ 3,620), 430 m$\mu$ ($\epsilon$ 1,200)] was unaltered by short term pH changes, but hydrolysis in 0.1 *N* NaOH at 25° liberated one mole of methanol resulting in a new product (II-F),[15] $C_{14}H_{17}N_3O_6$, purple needles from dimethylformamide–water, $[\alpha]_{6907}^{30}$ + 14.2° (1% 0.1 *N* HCl) which was an indicator [$\lambda_{max}^{0.1\ N\ HCl}$ 235 m$\mu$ ($\epsilon$ 21,700), 294 m$\mu$ ($\epsilon$ 15,900), 346 m$\mu$ ($\epsilon$ 3,920), 460 ($\epsilon$ 1,050), $\lambda_{max}^{0.1\ N\ NaOH}$ 254 m$\mu$ ($\epsilon$ 19,200), 312 m$\mu$ ($\epsilon$ 11,900), 560 m$\mu$ ($\epsilon$ 1,220)], a characteristic of 2-hydroxyquinones. When oxidized with $KMnO_4$ in alkali II-F showed spectral changes typical of the Hooker oxidation[16] of a 3-alkyl-2-hydroxy-1,4-naphthoquinone. An n.m.r. peak (8.1–8.2 $\tau$, unsplit) likewise indicated the presence of a methyl group



2,3-dihydro-9-hydroxymethyl-6-methyl-1*H* pyrrolo[1,2-a]indole-5,8-dione, carbamate.

(1) The systematic names of these compounds are cumbersome. For example, mitomycin A is 1,1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)-6,8a-dimethoxy-5-methylazirino[2′,3′-3,4]pyrrolo[1,2-a]indole 4,7-dione carbamate. We propose the trivial name MITOSANE for structure I (where X = Y = Z = H) common to all four antibiotics. Thus mitomycin A (I-A) becomes 7,9a-dimethoxymitosane.

(2) W. R. Roderick, *J. Chem. Ed.*, **39**, 2 (1962).

(3) T. Hata, *et al.*, *J. Antibiotics* (Ser. A), **9**, 141 (1956).

(4) S. Wakaki, *et al.*, *Antibiotics & Chemotherapy*, **8**, 228 (1958).

(5) R. Jones, Jr., *et al.*, "Fourth National Cancer Conference Proceedings, 1960," J. B. Lippincott, Philadelphia, Pa., 1961, p. 175.

(6) S. Wakaki, *Cancer Chemotherapy Reports*, **18**, 79 (1961).

(7) (a) C. DeBoer, *et al.*, P. Gray, B. Tabenkin and S. G. Bradley, "Antimicrobial Agents Annual 1960," Plenum Press, New York, N. Y., 1961, pp. 17–22. (b) R. R. Herr, *et al.*, *ibid.*, pp. 23–26. (c) C. Lewis, *et al.*, *ibid.*, pp. 27–36. (d) L. J. Hanka, *ibid.*, pp. 37–39. (e) Discussion, *ibid.*, p. 40.

(8) D. Lefemine, *et al.*, *J. Am. Chem. Soc.*, **84**, 3184 (1962), to whom we are indebted for the supplies of antibiotics used during this investigation.

(9) Because no authentic sample of mitomycin B has been available, it has not been compared directly with I-B.

(10) Prepared from 2-dimethylamino-5-hydroxybenzoquinone [F. Kehrmann, *Ber.*, **23**, 897 (1890)] by methylation with diazomethane.

(11) R. Baltzly and E. Lorz, *J. Am. Chem. Soc.*, **70**, 861 (1948).

(12) F. Fichter, *Ann.*, **361**, 363 (1908).

(13) (a) This product seems to be a mixture of *cis*- and *trans*-isomers which are difficult to separate. It is always accompanied by a more soluble isomer which is probably the 1-amino-2-hydroxy isomer. We propose the trivial name MITOSENE for the structure common to this group of compounds, *viz.*,

(b) All compounds gave satisfactory analyses.

(14) Under the same conditions N-methyl I-A produced a homologous product (II-B), identical by infrared and X-ray comparisons with the product obtained by similar treatment of I-B. I-B and I-A are thus related.

(15) Also I-C in 0.1 *N* HCl at 25° for 24 hours produced a product identical with II-F. I-B on hydrolysis (0.1 *N* HCl at room temp. for 5 hours then in 0.1 *N* NaOH) gave the homologous product II-J, $C_{15}H_{19}N_3O_6$, blue, hydrated crystals, $[\alpha]_{6907}^{23}$ +16° (1% 0.1 *N* HCl), which was identical with that obtained by hydrolysis of I-D in 0.1 *N* HCl for 24 hours at 25°.

(16) L. F. Fieser, *et al.*, *J. Am. Chem. Soc.*, **58**, 1223 (1936).

on the quinone ring (no adjacent proton) in both I-A and II-A.

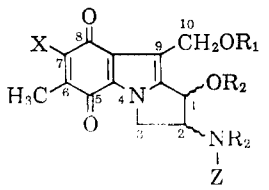
II 

TABLE B

| | X | Z | $R_1$ | $R_2$ |
|---|---|---|---|---|
| II-A | $H_3CO$ | H | $CONH_2$ | H |
| II-B | $H_3CO$ | $CH_3$ | $CONH_2$ | H |
| II-F | HO | H | $CONH_2$ | H |
| II-G | $H_3CO$ | H | $CONH_2$ | $COCH_3$ |
| II-H | $H_3CCO_2$ | H | $CONH_2$ | $COCH_3$ |
| II-J | HO | $CH_3$ | $CONH_2$ | H |
| II-K | $H_3CCO_2$ | $CH_3$ | $CONH_2$ | $COCH_3$ |
| II-L | HO | H | H | H |
| II-M | $H_3CCO_2$ | H | $COCH_3$ | $COCH_3$ |

The foregoing data suffice to establish the interrelations and chromophores of the antibiotics in this series; the following communication[17] presents additional data sufficient to prove the total structures.

(17) J. S. Webb, D. B. Cosulich, J. H. Mowat, J. B. Patrick, R. W. Broschard, W. E. Meyer, R. P. Williams, C. F. Wolf, W. Fulmor, C. Pidacks and J. E. Lancaster, *J. Am. Chem. Soc.*, **84**, 3187 (1962).

JOHN S. WEBB
DONNA B. COSULICH
JOHN H. MOWAT
JAMES B. PATRICK
ROBERT W. BROSCHARD
WALTER E. MEYER
RICHARD P. WILLIAMS
CARL F. WOLF
WILLIAM FULMOR
CHARLES PIDACKS

ORGANIC CHEMISTRY SECTION
LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

JOHN E. LANCASTER

RESEARCH SERVICE DEPARTMENT
CENTRAL RESEARCH DIVISION
AMERICAN CYANAMID COMPANY
STAMFORD, CONNECTICUT

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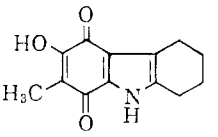
## THE STRUCTURES OF MITOMYCINS A, B, AND C AND PORFIROMYCIN—PART II

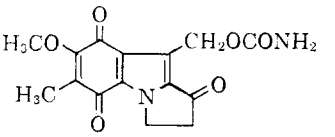
*Sir:*

In the preceding communication[1] we report the interrelations and chromophores of the mitomycins and porfiromycin. In the determination of the rest of the skeletal structure and the arrangement of functional groups, the reactions described below were of major significance.

*apo*-Mitomycin A (II-A)[2] formed a diacetyl derivative (II-G) m.p. 244–246.5° dec., with carbonyl bands at 6.44 $\mu$ (amide II) and 5.75 $\mu$ (alkyl acetate) suggesting the presence in II-A of alkyl $NH_2$ and OH, neither of which was present in I-A. One of the two remaining nitrogen atoms and both unassigned oxygen atoms in II-A and II-F were part of a $—OCONH_2$ group: hydrolysis of either II-A or II-F (and even I-A) in 6 *N* HCl at 25° produced one mole each of $CO_2$ and $NH_4^+$ and a new compound (II-L) [$C_{13}H_{14}N_2O_5$; $[\alpha]^{25}{}_{6907}$ + 26 ± 5° (1% 0.1 *N* HCl); tetraacetyl derivative (II-M) m.p. 225–230°], but in strong base neither $NH_3$ nor $CO_3^=$ were formed from II-A and II-F until after brief acidification.[3] The band at 5.8 $\mu$, absent in II-L but present in I-A, -B, -C and -D; II-A, -B, -F and -J, was attributed to a carbamate carbonyl.[4] By exclusion the remaining nitrogen atom in II-A must have been the original nitrogen of the aminobenzoquinone in I-A; in II-A it was neither hydrolyzable nor basic; there remained the possibility that it was heterocyclic. In fact the ultraviolet spectrum of 5,6,7,8-tetrahydro-3-hydroxy-2-methyl-1,4-carbazoledione[5] (III) [$\lambda_{max}^{0.1\ N\ HCl}$ 237 m$\mu$ ($\epsilon$ 20,200), 293 m$\mu$ ($\epsilon$ 19,000), 370 m$\mu$ ($\epsilon$ 4,330), 510 m$\mu$ ($\epsilon$ 1,390), $\lambda_{max}^{0.1\ N\ NaOH}$ 246 m$\mu$ ($\epsilon$ 24,500), 306 m$\mu$ ($\epsilon$ 12,500), 365 m$\mu$ ($\epsilon$ 4,620), 595 m$\mu$ ($\epsilon$ 1,610)], was nearly identical with that of II-F.

The arrangement of the groups (—OH, $—NH_2$, $—CH_2OCONH_2$) on the skeleton of II-A was established by treatment of it with $HNO_2$ to produce a compound (IV) $C_{15}H_{14}N_2O_6$, which contained a new carbonyl group, $\lambda_{max}^{KBr}$ 5.77 $\mu$, was optically inactive, and lacked the $NH_2$ and OH groups of IIA. The ultraviolet spectrum of IV [$\lambda_{max}^{CH_3OH}$ 280 m$\mu$ ($\epsilon$ 41,400)] indicated probable conjugation of the new carbonyl with the indoloquinone chromophore, but was *unlike* that of ethyl 5-hydroxy-2,6-dimethyl-4,7-dioxo-3-indolecarboxylate[6]; therefore, the new carbonyl probably was not at the 3-indole position and hence must be at 2-. The n.m.r. spectrum of IV ($D_6$-dimethyl sulfoxide) displayed two widely separated triplets characteristic of an $A_2X_2$ pattern not shown by II-A. This suggested the presence of the moiety $Y—CH_2—CH_2—CO—$ at the 2-indole position. Y had to be the indole nitrogen: the $—CH_2OCONH_2$ group in IV must be at the 3- rather than 1-indole position since strong acid hydrolysis of IV produced no formaldehyde, and acid permanganate oxidation of IV yielded $\beta$-alanine, identified by paper chromatography[7]


III


IV

(1) J. S. Webb, D. B. Cosulich, J. H. Mowat, J. B. Patrick, R. W. Broschard, W. E. Meyer, R. P. Williams, C. F. Wolf, W. Fulmor, C. Pidacks and J. E. Lancaster, *J. Am. Chem. Soc.*, **84**, 3185 (1962).

(2) All formula rubrics refer to Tables A and B in the preceding Communication.

(3) T. W. J. Taylor and W. Baker, "Sidgwick's Organic Chemistry of Nitrogen," Oxford University Press, London, England, 1942, p. 272.

(4) S. Pinchas and D. Ben-Ishai, *J. Am. Chem. Soc.*, **79**, 4099 (1957).

(5) Prepared by Dr. W. Remers of these Laboratories, unpublished data.

(6) H. Teuber and G. Thaler, *Ber.*, **91**, 2253 (1958).

(7) E. D. Moffat and R. I. Lytle, *Anal. Chem.*, **31**, 926 (1959).