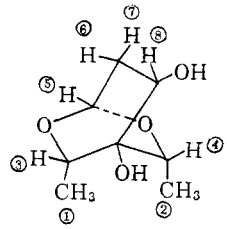
10.4) and 2.3 active hydrogens, but formed only mono acyl derivatives [mono-3,5-dinitrobenzoate, m.p. 167–168°. *Anal.* Calcd. for $C_{15}H_{16}N_2O_9$ (368): C, 48.91; H, 4.37; N, 7.60. Found: C, 49.1; H, 4.57; N, 7.80].

TABLE I

HIGH RESOLUTION N.M.R. DATA FOR A SATURATED D_2O SOLUTION OF THE $C_8H_{14}O_4$ COMPOUND (I)



Assignment[a]	Chemical shift[b]	Spectrum type	Coupling constants[e]
H_1, H_2	5.26, 5.28[c]	Two AX_3	$J_{13} = J_{24} = 6.7$
H_3, H_4	1.94, 2.17[c]		$J_{67} = 14.9$
H_5	1.50	X of ABXY	$J_{78} = 3.6$
H_6	3.97	AB of ABXY	$J_{68} = 9.8$
H_7	4.63[d]	($J_{XY} = 0$)	$J_{56} = 2.4$
H_8	2.18	Y of ABXY	$J_{57} = 1.8$

[a] Spectra were recorded at both 40 and 60 Mc. to confirm assignments. [b] Shifts are given in p.p.m. using an external benzene reference. Add 3.51 to convert to approximate τ values. [c] It is not possible to assign separately 1 and 2, or 3 and 4. [d] In assigning H_6 and H_7, H_6 was assumed to be the proton with the larger coupling to H_8 (near-zero dihedral angle). [e] J values are the absolute values, read from the first-order splittings, in c.p.s.

Structure I for the $C_8H_{14}O_4$ sugar followed from the transformations shown in Chart A. Reaction with one mole of periodate was rapid, without fragmentation and produced a dicarbonyl compound isolated[3] as a mono-2,4-dinitrophenylhydrazone (II) [m.p. 128–130°. *Anal.* Calcd. for $C_{14}H_{16}N_4O_7$: C, 47.77; H, 4.57; N, 15.9. Found: C, 47.8; H, 4.93; N, 16.2] and also as a bis-2,4-dinitrophenylhydrazone [m.p. 185–190°. *Anal.* Calcd. for $C_{20}H_{20}N_8O_{10}$ (532): C, 45.11; H, 3.78; N, 21.05. Found: C, 45.2; H, 4.10; N, 20.9]. Acid hydrolysis of II formed 1-(2,4-dinitrophenyl) pyrazole (III) [m.p. 107–108°, $\lambda_{Max.}^{CH_3OH}$ 303 mμ (ϵ = 11,700). *Anal.* Calcd. for $C_9H_6N_4O_4$ (234): C, 46.2; H, 2.56; N, 23.9. Found: C, 46.3; H, 2.78; N, 24.0], identified by comparison with an authentic sample prepared by heating excess 1,1,-3,3-tetramethoxypropane with 2,4-dinitrophenylhydrazine in dilute methanolic perchloric acid. Hence, one fragment of periodate oxidation with subsequent acid cleavage was malondialdehyde. No volatile carbonyl compound was detected during the conversion of II to III.

Without isolation the remaining five-carbon fragment was identified as the hitherto unreported[4] 2,4-dihydroxy-3-pentanone (IV) by the following method. After removal of III, the acid hydrolysate of II on treatment with periodate consumed one mole of the oxidant and produced one mole of acetaldehyde (estimated by bisulfite titration[5] and isolated as the 2,4-dinitrophenylhydrazone,[6] m.p. 136–150°) and 0.9 mole of lactic acid (estimated by base titration and identified *via* the *p*-bromophenacyl ester, m.p. 108–110°, by comparison with an authentic sample prepared from *dl*-lactic acid). The formation of these two fragments in high yield demonstrates that the periodate substrate must have been IV.

This sequence of reactions and compounds indicates that the compound $C_8H_{14}O_4$ has structure I, whose main features are completely compatible with the n.m.r. spectrum, presented in Table I.

It appears that this unique structure is actually a secondary reaction product resulting from acid catalyzed ring-closure of the natural "sugar." This postulate is supported by the observation that the crystalline anhydrosugar (I) is accompanied by a syrupy reducing sugar, presumably unchanged V.

(3) Prior to reaction with 2,4-dinitrophenylhydrazine it was necessary to remove the iodate ion present by means of its insoluble barium salt, because the iodate and the carbonyl reagent reacted to form tarry products.

(4) G. W. K. Cavill and D. H. Solomon, *J. Chem. Soc.*, 4426 (1955) describe the diacetate. G. Gál, *et al.*, *Magyar Kém. Folyóirat.*, **68**, 5 (1958) [*Chem. Abs.*, **52**, 18308i (1958)] report the oxime.

(5) E. J. Conway, "Microdiffusion Analysis," Crosby Lockwood and Son, Ltd., London, Eng., 4th Edn., 1957, p. 278.

(6) N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," Thomas Y. Crowell Co., New York, N. Y., 1947, p. 400.

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RECEIVED JUNE 13, 1962

ISOLATION AND CHARACTERIZATION OF MITIROMYCIN AND OTHER ANTIBIOTICS PRODUCED BY *STREPTOMYCES VERTICILLATUS*

Sir:

Three soil isolates of *Streptomyces verticillatus*, Lederle strains AA-849, AB-286 and AB-929 were found to produce mixtures of ethyl acetate extractable antibiotics which were active in bacterial mouse protection tests. When purified preparations made from these cultures were compared by paper chromatography, they appeared to contain identical groups of antibiotics. The solvent system used consisted of benzene, methanol, 0.01 *M* phosphate buffer pH 6.8 (20:1:1 by vol.). Bioautography with *Bacillus subtilis* indicated six antibiotics, A_1, A_2, B, C, D and E with R_f values 0.75, 0.65, 0.36, 0.15, 0.09 and 0.01, respectively.

The extracts from strains AA-849 and AB-929 were resolved into fractions by partition chromatography with mixtures of benzene, ethyl acetate, methanol and phosphate buffer on Celite[1] and silicic acid columns. Five purple pigments, corresponding to A_2, B, C, D and E, were isolated in crystalline form. They were recrystallized from ethyl acetate–petroleum ether or from methanol. *trans*-Cinnamide also was isolated from a number of fractions.

At the highest level tested (500 mcg./ml.), compound A_2 showed comparatively low activity

(1) Johns-Manville Company brand of diatomaceous earth.

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. λ_{max}^{MeOH}: 218 mμ ($E_{1\,cm}^{1\%}$ 448), 323 mμ ($E_{1\,cm}^{1\%}$ 278), 530 mμ ($E_{1\,cm}^{1\%}$ 34); λ_{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); λ_{max}^{MeOH}: 212 mμ ($E_{1\,cm}^{1\%}$ 500), 319 mμ ($E_{1\,cm}^{1\%}$ 295), 515 mμ ($E_{1\,cm}^{1\%}$ 35); λ_{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 λ_{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); λ_{max}^{MeOH}: 218 mμ ($E_{1\,cm}^{1\%}$ 480), 320 mμ ($E_{1\,cm}^{1\%}$ 290), 540 mμ ($E_{1\,cm}^{1\%}$ 41.5); λ_{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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(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.

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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