in route II preparations indicates that at least two competing reactions are involved.

The products from these reactions were isolated by fractional crystallization and identified by elemental analyses and by comparing their ORD and nmr spectra with those reported earlier.[7] The ORD spectra were measured on a Cary 60 ORD using concentrations in the range of 15–20 mg of sample for 10 ml of H_2O and a 1.0-cm path length in the spectral range 600–380 mμ. A tenfold dilution and a path length of 0.10 cm were used in the range 300–185 mμ. Nmr spectra were taken on a Varian A-60[14] at a concentration of about 0.005 g/0.1 cc of D_2O at the probe ambient temperature (≈35°). Scans were taken after the solutions had stood at room temperature for 1–2 hr, and the spectra showed that all exchangeable hydrogens were lost. An external standard of sodium tetramethylsilane was used.

Acknowledgment. Stimulating discussions with J. C. Little, L. I. Peterson, and W. L. Dilling of the Edgar C. Britton Research Laboratory and with Professor Dean Cooke of the University of Michigan are gratefully acknowledged. One of the authors (C. F. L.) is also grateful to the National Institutes of Health for financial support (GM 10372) in the course of this work.

(14) The solutions were prepared in a microcell; scans were on the "Dog" mode of the time-averaging attachment by the Dow Chemical Co., Midland, Mich.

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Received August 5, 1966

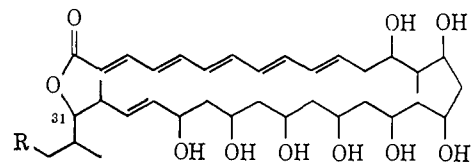
The Mycoticins, Polyene Macrolides from *Streptomyces ruber*[1]

Sir:

Mycoticin is a yellow, crystalline neutral metabolite of *Streptomyces ruber* exhibiting notable activity against pathogenic fungi. Its isolation was first reported in 1954 by Burke, *et al.*,[2] who suggested the formula $C_{18}H_{30}O_5$ and provided a brief chemical and physical characterization.

We wish to summarize here the results of a structural investigation on this pigment showing it to be a mixture of two polyene, polyhydroxy macrocyclic lactones,[3] mycoticin A, $C_{36}H_{58}O_{10}$ (Ia), and the homolog, mycoticin B, $C_{37}H_{60}O_{10}$ (Ib).

After repeated recrystallization from methanol, mycoticin[4] is obtained as fine yellow needles, mp 221–222°, which rapidly decompose in air and light;[5]



I a, R = H
b, R = CH_3

$[\alpha]^{22}D$ +63.4° (0.48%, dioxane); infrared peaks (KBr) at 3400 (broad OH), 1695 (C=O), 1610 (C=C), 1570 (conjugated C=C), and 1010 cm^{-1}. Mycoticin shows between three and four C-methyl groups in Kuhn–Roth analysis and gives negative Zeisel and ferric chloride tests and also negative reactions for aldehyde or ketone, vicinal hydroxyl groups (periodate), and primary alcohol (trityl chloride in pyridine). Presence of a pentaene system conjugated to a carbonyl group is shown by the ultraviolet absorption spectrum, exhibiting broad bands at λ_{max}^{EtOH} 262 ($E_{1\,cm}^{1\%}$ 79) and 364 mμ ($E_{1\,cm}^{1\%}$ 948), in good agreement with the absorption expected for conjugated ester–pentaene chromophores.[6] Likewise, reduction of the carbonyl group in mycoticin with lithium aluminum hydride yields a product showing a pattern of peaks at 303, 317, 328, and 349 mμ which is characteristic of conjugated pentaene systems such as dodeca-2,4,6,8-pentaene[7] and tetrahydrocicutol.[8] Further confirmation of the location of the double bond system adjacent to the carbonyl group is found in the results of the sequence: ozonolysis of mycoticin, catalytic hydrogenation, and then saponification, whereby glycolic acid is formed.

The presence of an isolated double bond in the molecule is indicated by the consumption of a sixth mole of hydrogen on catalytic hydrogenation to give dodecahydromycoticin,[9] mp 138.2–138.8°, showing a carbonyl peak in the infrared at 1725 cm^{-1}. Dodecahydromycoticin is unusually resistant toward hydrolysis, requiring long refluxing in 10% aqueous methanolic sodium hydroxide for cleavage. Neutralization of this hydrolyzed solution and treatment with *p*-bromo- or *p*-chlorophenacyl halides produces the corresponding *p*-halophenacyl esters in yields greater than 70%. The elemental analyses of these derivatives (and earlier negative Zeisel tests) reveal that no alcoholic fragment is lost during saponification, and, thus, that mycoticin is a lactone and not an ester.

Acetylation of mycoticin with acetic anhydride in pyridine yields an octaacetate, mp 155.5–156°, which can be reconverted to mycoticin by mild alkaline hydrolysis. The nmr spectrum of the acetate shows 12 olefinic protons (complex multiplet at τ 2.3–4.5), 9 *H*COCOR protons (diffuse multiplet at τ 4.5–5.7), 24 acetoxy protons (closely spaced peaks centered at τ 7.9), and 12 C–CH_3 protons (a broad peak at τ 9.05), confirming the presence of six double bonds, eight hydroxyl groups, and four methyl groups in the parent pigment. Hydrogenation of mycoticin acetate produces a perhydro derivative exhibiting nmr peaks at τ 4.7–5.7 (9 H), 8.0 (24 H), and 9.15 (12 H) with no

(1) Taken in part from the doctoral dissertation of D. J. McCaustland, Yale University, 1960.

(2) R. C. Burke, J. H. Swartz, S. S. Chapman, and W. Huang, *J. Invest. Dermatol.*, 23, 163 (1954).

(3) The mycoticins, sharing structural features in common with other macrolides such as filipin and fungichromin, represent the first members of this class of antibiotics in which the polyene section of the carbon chain is conjugated to the lactone carbonyl group.

(4) In this discussion, "mycoticin" refers to the mixture of Ia and Ib isolated from the mycelium of *S. ruber* (ATCC 3348).

(5) Satisfactory carbon and hydrogen analyses were obtained for mycoticin, the dodecahydro derivative, the octaacetate, and the *p*-halophenacyl ester derivatives assuming the parent pigment to be a 1:1 mixture of Ia and Ib.

(6) K. Hirayama, *J. Am. Chem. Soc.*, 77, 383 (1955).

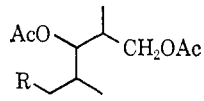
(7) P. Nayler and M. C. Whiting, *J. Chem. Soc.*, 3037 (1955).

(8) E. F. L. T. Anet, B. Lythgoe, M. H. Silk, and S. Trippett, *ibid.*, 309 (1953).

(9) Although it was not possible to separate mycoticin into pure Ia and Ib, the polytrimethylsilyl ether of the dodecahydro derivative yielded two closely spaced vpc components in equal amounts.

resonance in the olefinic region. This evidence shows that in the acetate there are no C-methyl groups attached to double bonds.

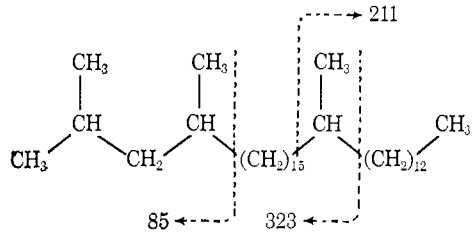
Ozonolysis of mycoticin using a reductive work-up produces a mixture of hydroxy aldehydes which, on steam distillation, yields 2,4-dimethyl-2-pentenal and 2,4-dimethyl-2-hexenal. The same products, identified by independent synthesis, may be obtained by similar treatment of mycoticin acetate. If, however, the octaacetate is ozonized in tetrahydrofuran and then reduced with lithium aluminum hydride, a diol is obtained, mp 83–85°, which on acetylation forms a 1:1 mixture (separable by vpc) of the acetates, IIa and IIb. The structures of these derivatives were estab-



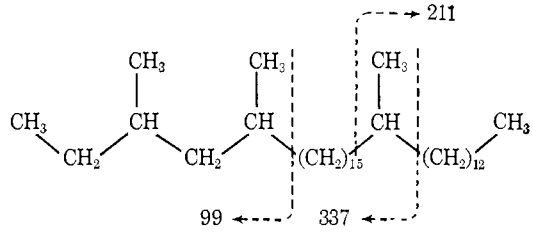
II a, R = H
b, R = CH_3

lished by spectroscopic comparison with authentic diacetates independently synthesized.[10]

The manner in which the seven- and eight-carbon fragments corresponding to IIa and IIb are incorporated in the mycoticins and the crucial information on the complete carbon skeleton in each of the macrolides were provided by mass spectrometric studies.[11] Dodecahydromycoticin was reduced by lithium aluminum hydride to a polyol which was tosylated and then further reduced by lithium aluminum hydride. After chromatography on alumina, the hydrocarbon fraction was hydrogenated and then separated by vpc. Two components were obtained in essentially equal amounts and their mass spectra determined.[12]



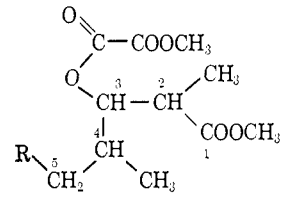
IIIa



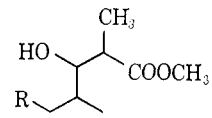
IIIb

The salient features of these spectra[14] included the following: peaks at *m/e* 211 for both components, indicating a common C_{15} fragment; peaks at *m/e* 85 and 323 for IIIa, showing loss of C_6 and C_{23} units; and peaks at *m/e* 99 and 337 for IIIb, showing a corresponding loss of C_7 and C_{24} fragments. Taken with the chemical evidence derived from ozonolysis, the mass spectral data permit unambiguous assignment of structures IIIa, $C_{36}H_{74}$, and IIIb, $C_{37}H_{76}$, to the hydrocarbon backbones in the two macrolides.

The striking resistance toward hydrolysis shown by dodecahydromycoticin suggests that the lactone oxygen is derived from a hydroxyl group located in a relatively hindered position of the molecule, and on this basis, structure I was considered to be a likely possibility. Proof that the lactone oxygen is, in fact, at C-31 was obtained from ozonolysis studies on mycoticin acetate. Oxidation of the ethyl acetate ozonolysis solution with 30% hydrogen peroxide followed by esterification with diazomethane yielded a mixture of IVa and IVb which could not be separated by thin-layer chromatography. The nmr spectrum of this mixture shows, in



IV a, R = H
b, R = CH_3



V a, R = H
b, R = CH_3

addition to methylene (at C-5) and C–CH_3 resonances, peaks at τ 4.9 (multiplet, 1 H, C-3), 6.13 and 6.35 (singlets, 6 H, two OCH_3 groups), 7.2 (multiplet, 1 H, C-2), 8.0 (multiplet, 1 H, C-4). The positions and general appearance of these peaks are in complete agreement with the nmr spectrum exhibited by an equimolar mixture of synthetic IVa and IVb prepared independently by acylation of the esters Va and Vb with methoxalyl chloride in pyridine. Location of the lactone oxygen at any of the other eight hydroxylated sites would have yielded an ozonolysis product containing the O–$COCH_3$ residue. No trace of acetyl methyl resonance was found in the spectrum.

The above physical and chemical evidence plus the results of other degradative studies shortly to be reported in detail are all in accord with structures Ia and Ib for the mycoticins.

Acknowledgments. We wish to acknowledge support of this work by Grants E-4798 and AI 04798 from the National Institutes of Health. The authors thank Dr. R. C. Burke for valuable assistance in the microbiological phases of this work.

(10) Synthetic samples of IIa and IIb were prepared by the reactions of isobutyraldehyde and α-methylbutyraldehyde with ethyl β-bromopropionate followed by reduction and acetylation.

(11) We are grateful to Professor K. Biemann for generous assistance in obtaining and interpreting the mass spectra of these hydrocarbons.

(12) This general method for determining the carbon skeletons of macrolides was first employed by Cope in investigations on fungichromin.[13]

(13) A. C. Cope, R. K. Bly, E. P. Burrows, O. J. Ceder, E. Ciganek, B. T. Gillis, R. F. Porter, and H. E. Johnson, *J. Am. Chem. Soc.*, **84**, 2170 (1962).

(14) Formation of M − 2 peaks at *m/e* 504 and 518 in lieu of parent peaks is in accord with related observations on fungichromin.[13,15]

(15) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 81.

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Received November 9, 1966