(IRA-400), the product was subjected to chromatography on carboxymethylcellulose (CMC). The free eicosapeptide *d*-sulfoxide was eluted from the column with 0.025 *M* ammonium acetate; ammonium acetate was removed by repeated lyophilization of the peptide; $[\alpha]^{21}D$ −43.1° (*c* 1.04, 10% acetic acid); single Pauly- and ninhydrin-positive spot on paper electrophoresis at pH 1.9, 3.5, 6.5, and 8.0; amino acid ratios in acid hydrolysate $Lys_{2.03}Glu_{3.16}Thr_{1.95}Ala_{5.04}Phe_{0.99}Arg_{0.96}His_{0.96}$-$Met_{0.73}Asp_{0.96}Ser_{2.95}$; amino acid ratios in AP-M digest $Lys_{2.34}Glu_{2.55}Thr_{2.16}Ala_{5.78}Phe_{1.26}Arg_{1.15}Gln_{0.80}His_{0.71}$-

O
↑
$Met_{0.74}Asp_{0.80}Ser_{2.65}$.[11]

The S-protein activating potency of a sample of this material has been recorded.[3,12] Reduction of the eicosapeptide *d*-sulfoxide with thioglycolic acid[3] gave, in quantitative yield, the crude eicosapeptide (S-peptide$_{1-20}$). For final purification this material was combined with S-protein and the ensuing RNAase-S′ purified by chromatography on Amberlite CG-50.[13] The highly active, partially synthetic enzyme was then dissociated into S-peptide$_{1-20}$ and S-protein[14] and the peptide separated from protein contaminants by chromatography on CMC. Synthetic S-peptide$_{1-20}$, thus purified, possessed S-protein activating potency identical with that of natural "S-peptide" (Figure 1); single Pauly-, chlorine-, and ninhydrin-positive spot on paper electrophoresis at pH 1.9, 3.5, and 6.5 with mobilities identical with "S-peptide"; amino acid ratios in AP-M digest $Lys_{1.98}Glu_{2.17}Thr_{2.00}Ala_{4.90}Phe_{1.03}Arg_{1.07}Gln_{1.07}$-$His_{0.95}Met_{0.91}Asp_{1.06}Ser_{3.10}$.

Acknowledgment. The skillful technical assistance of Miss Judy Montibeller and Mrs. Elaine Gleeson is gratefully acknowledged.

(11) The low recoveries of glutamine, histidine, methionine, aspartic acid, and serine in the enzymatic digest may be the result of some racemization. This point is under study, particularly since racemization has not been observed in our previous syntheses of similar peptides.[3]

(12) F. M. Finn and K. Hofmann, *J. Am. Chem. Soc.*, 87, 645 (1965).

(13) A. M. Crestfield, W. H. Stein, and S. Moore, *J. Biol. Chem.*, 238, 618 (1963).

(14) F. M. Richards and P. J. Vithayathil, *ibid.*, 234, 1459 (1959).

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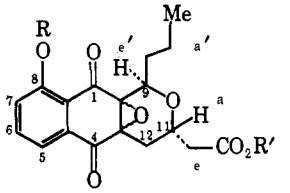
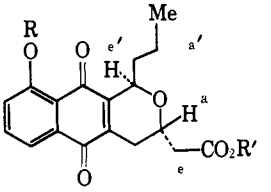
The Structure of Frenolicin

Sir:

In a program of study of microbial metabolites in these laboratories, Van Meter, *et al.*, isolated a pale yellow, crystalline antibiotic named frenolicin from a fermentation of *Streptomyces fradiae*.[1] We now report the characterization of frenolicin as the novel 1,4-naphthoquinone 2,3-epoxide (I)[2] and, in addition, describe the formation of deoxyfrenolicin (V), which exhibits significant inhibitory activity when tested *in vitro* against a variety of fungi and against an experimental ringworm infection in guinea pigs.



I, R = R′ = H
II, R = Ac, R′ = H
III, R = H, R′ = Me
IV, R = R′ = Me



V, R = R′ = H
VI, R = Ac, R′ = H
VII, R = H, R′ = Me

Frenolicin, $C_{18}H_{18}O_7$ (rather than $C_{13}H_{14}O_5$),[1] *m/e* 346, mp 161–162°, $[\alpha]^{25}D$ −37.7° (*c* 1.5, methanol), is a phenolic carboxylic acid (pK_a' = 10.0 and 5.6 in methanol–water, 1:1), ν_{max}^{KBr} 1710 and 1650 cm^{-1}. It forms a monoacetate (II), $C_{20}H_{20}O_8$, mp 161–163°, ν_{max}^{KBr} 1770 and 1700 cm^{-1}, and is converted with diazomethane to a methyl ester (III), $C_{19}H_{20}O_7$, mp 82–83°, ν_{max}^{KBr} 1735, 1705, and 1665 cm^{-1}. Treatment of frenolicin with methyl iodide in acetone in the presence of potassium carbonate gave O-methylfrenolicin methyl ester (IV), $C_{20}H_{22}O_7$, mp 109–110°, ν_{max}^{KBr} 1740 and 1695 cm^{-1}.

The ultraviolet absorption spectrum of frenolicin, in both neutral and basic media, λ_{max}^{MeOH} 234, 284 (sh), and 362 mμ (ε 18,300, 3460, and 5200), $\lambda_{max}^{0.01\ N\ NaOH}$ (in MeOH) 280 and 425 mμ (ε 6400 and 6150), indicated that it was most likely a derivative of β-hydrojuglone.[3] Alkaline potassium permanganate oxidation of O-methylfrenolicin methyl ester afforded a methoxyphthalonic acid (presumably 3-methoxy) identical with that obtained from a similar oxidation of 1,5-dimethoxynaphthalene.[4]

The 1,4-naphthoquinone oxide structure in frenolicin was suggested by the consumption of 2 moles of hydrogen (10% Pd–C in methanol) to give a colorless compound which was immediately air oxidized to the yellow-orange deoxyfrenolicin (V), $C_{18}H_{18}O_6$, mp 179–181°, ν_{max}^{KBr} 1725, 1665 (sh), 1640, and 1620 cm^{-1}, whose ultraviolet absorption, λ_{max}^{MeOH} 246, 274, and 420 mμ (ε 9070 11,400, and 4290), corresponds to that of eleutherin and isoeleutherin.[5] V was characterized as its monoacetate (VI), $C_{20}H_{20}O_7$, mp 180–182°, ν_{max}^{KBr} 1770, 1715, 1665, and 1590 cm^{-1}, and methyl ester (VII), $C_{19}H_{20}O_6$, mp 120°,

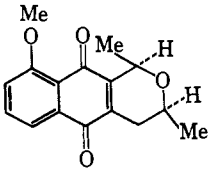
(1) J. C. Van Meter, M. Dann, and N. Bohonos, "Antibacterial Agents Annual–1960," Plenum Press, New York, N. Y., 1961, p 77.

(2) Symbols a′ and e′ denote pseudo-axial and pseudo-equatorial configurations of the bonds in question. All compounds reported here gave satisfactory elemental analyses. Nmr spectra were measured at 60 Mc in deuteriochloroform; shifts are expressed as δ values (parts per million) from tetramethylsilane as internal standard and coupling constants (*J*) are expressed in cycles per second. We thank the Organic Chemical Research Section of these laboratories for the elemental and spectral analyses and Dr. John Lancaster of the Stamford Laboratories for the spin-decoupling experiments.

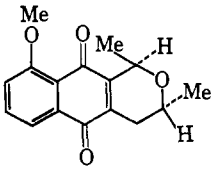
(3) R. H. Thomson, *J. Chem. Soc.*, 1737 (1950).

(4) C. A. Naylor, Jr., and J. H. Gardner, *J. Am. Chem. Soc.*, 53, 4109 (1931); W. H. Bentley, R. Robinson, and C. Weismann, *J. Chem. Soc.*, 91, 104 (1907).

(5) Eleutherin (i) and isoeleutherin (ii) were isolated from the tubers of *Eleutherine bulbosa* by H. Schmid, A. Ebnother, and Th. M. Meijer, *Helv. Chim. Acta.*, 33, 1751 (1950); H. Schmid and A. Ebnother, *ibid.*, 34, 1041 (1951). For a detailed nmr analysis of these two compounds see D. W. Cameron, D. G. I. Kingston, N. Sheppard, and Lord Todd, *J. Chem. Soc.*, 98 (1964).



i



ii

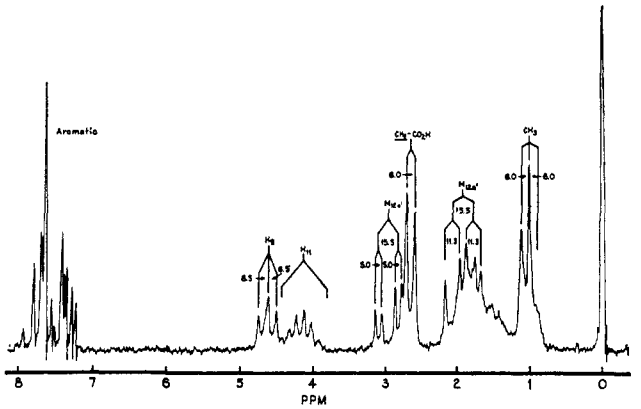


Figure 1. Nmr spectrum of frenolicin.

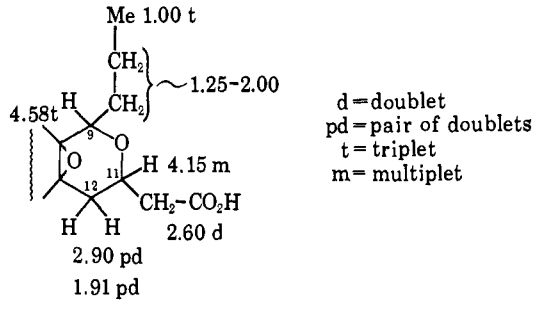
ν_{max}^{KBr} 1740, 1660 (sh), 1640, and 1614 cm^{-1}. As expected of a quinone, the yellow color of neutral solutions and the purple color in base were discharged with dithionite. Deoxyfrenolicin was also obtained by treatment of frenolicin with potassium iodide in refluxing acetic acid.[6]

The nmr spectrum of frenolicin (Figure 1) exhibits a primary C-methyl group with a three-proton triplet at δ 1.00 (J = 6.0 cps). This evidence, in conjunction with the loss of propyl (M − 43) as the strongest fragmentation ion in the mass spectra of frenolicin, O-methylfrenolicin methyl ester (IV), and deoxyfrenolicin methyl ester (VII), indicates the presence of the *n*-propyl grouping in frenolicin. The sharp two-proton doublet at 2.60 (J = 6.0 cps) in the nmr spectrum of frenolicin is assigned to two equivalent methylene protons deshielded by the carboxyl group and coupled with a tertiary proton, *i.e.*, $>CHCH_2COOH$.

A one-proton symmetrical triplet at δ 4.58 (J = 6.5 cps) implies the presence of a $-CH_2CHO-$ grouping. A pair of doublets at δ 2.90 (J = 5.0 and 15.5 cps) is attributed to the A portion of an ABX system where the A and B protons are nonequivalent geminal hydrogen atoms. The higher field signal of the B proton at δ 1.91 also appears as a pair of doublets (J = 11.3 and 15.5 cps). The X proton of the set was located by spin decoupling as a multiplet at δ 4.15. The latter proton was also shown to be coupled to the δ 2.60 methylene adjacent to the carboxyl group, establishing the grouping $-CH_2CH(CH_2COOH)O-$.

It remained to assemble the components that have been detailed above. To satisfy the molecular formula, the two fragments with unspecified oxygen atoms must be joined through an ether bridge, and a methylene group of the propyl side chain must be included in these fragments. An assembly of these portions with the established 1,4-naphthoquinone oxide system in a manner consistent with all foregoing data led to the partial structure VIII. This structure was further substantiated by the spin decoupling of the C-9 proton signal (δ 4.58, triplet to singlet) from an upfield methylene group at *ca.* δ 1.9, which must be part of the *n*-propyl side chain.

The only remaining structural feature to be clarified was the position of the phenolic hydroxyl group. Acylation or methylation of this group changes the nmr signal of the C-9 proton in frenolicin from a symmetrical triplet to a pair of doublets (δ 4.58, J = 3.0 and 10.0 cps) with no effect on the signal of the C-12 protons. This effect is apparently without analogy and is most likely related to the difference in magnetic anisotropy between the chelated carbonyl group of frenolicin and the nonchelated carbonyl of the derivatives. Confirmation of the nonequivalence of the methylene protons adjacent to the C-9 asymmetric carbon in O-methylfrenolicin methyl ester was obtained by spin-decoupling experiments which showed the C-9 proton signal to be collapsed to a doublet by irradiation at either of two different δ values in the methylene region (δ 1.62 and 2.12). On this basis the phenolic group was assigned to C-8 rather than to C-5.



VIII

Consideration of the coupling constants for the C-12 and C-11 protons in frenolicin ($J_{12,11}^{e'a}$ = 5.0 cps, $J_{12,11}^{a'a}$ = 11.3 cps) showed the C-11 proton to be axial.[7] Comparison of the coupling constants of the C-9, C-11, and C-12 protons in the nmr spectrum of deoxyfrenolicin methyl ester ($J_{12,12}^{gem}$ = 19.0, $J_{12,11}^{a'a}$ = 10.0, $J_{12,11}^{e'a}$ = 3.5, $J_{9,12}^{a'e'}$ = 2.0, $J_{9,12}^{e'e'} < 1$ cps) with the analogous protons in isoeleutherin[5] confirmed the assignment at C-11 and showed the C-9 proton to be pseudo-equatorial. Thus the relative stereochemistry at C-9 and C-11 is as shown in I.

(6) S. Bodforss, *Ber.*, **49**, 2801 (1916).

(7) M. Karplus, *J. Chem. Phys.*, 30, 11 (1959); H. Conroy, *Advan. Org. Chem.*, **2**, 311 (1960); C. N. Banwell and N. Sheppard, *Discussions Faraday Soc.*, **34**, 115 (1962).

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The Total Synthesis of (±)-Copaene and (±)-8-Isocopaene

Sir:

The tricyclic sesquiterpene copaene has been reported as a constituent of numerous essential oils,[1] although the structure of this material was in doubt for many years. Several structures containing a cyclopropane ring were proposed, criticized, and rejected before the correct formulation (**15**) was simultaneously ascertained by two groups of workers.[2] Since that time an unsuccessful

(1) See *inter alia* (a) Schimmel and Co., Berichte Schimmel, April 1914, p 48; (b) G. G. Henderson, W. M'Nab, and J. M. Robertson, *J. Chem. Soc.*, 3077 (1926); (c) L. H. Briggs and W. I. Taylor, *ibid.*, 1338 (1947); (d) L. H. Briggs and M. D. Sutherland, *J. Org. Chem.*, **13**, 1 (1948); (e) F. Vonasek, V. Herout, and F. Sorm, *Collection Czech. Chem. Commun.*, **25**, 919 (1960); (f) L. Westfelt, *Acta Chem. Scand.*, **18**, 572 (1964); (g) F. M. Couchman, A. R. Pinder, and N. H. Bromham, *Tetrahedron*, **20**, 2037 (1964).

(2) (a) P. DeMayo, R. E. Williams, G. Büchi, and S. H. Feairheller, *Tetrahedron*, **21**, 619 (1965); (b) V. H. Kapadia, B. A. Nagasampagi, V. G. Naik, and S. Dev, *ibid.*, **21**, 607 (1965).