glycinamide. Dry ammonia was bubbled into a stirred suspension of 3.54 g of the S-benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl nitrated resin in 150 ml of anhydrous methanol at 0° for 1.5–2 hr (until the solution was saturated with ammonia). The reaction mixture was stirred overnight at 0–4°.[25] The methanol and ammonia were removed under aspirator vacuum. Dimethylformamide (200 ml) was added to the dry residue and the suspension was stirred vigorously for 3 hr. The resin was filtered off and washed three times with dimethylformamide.

The solvent was removed from the combined filtrate and washings on a rotary evaporator at below 40°. The residue was dissolved in 20 ml of dimethylformamide and 15 ml of distilled water was added gradually with stirring. The turbid solution was allowed to stand in the refrigerator overnight. The precipitate was filtered off, washed with ethanol, and dried *in vacuo* over KOH pellets; yield, 197 mg, mp 234–236°.

A sample was prepared for analysis by reprecipitating twice from a dimethylformamide–water mixture. The compound was filtered off, washed with ethanol, and dried *in vacuo*, over KOH pellets; mp 238–240°, $[\alpha]^{21}D$ −37.3° (*c* 0.6, dimethylformamide).

Anal. Calcd for $C_{64}H_{85}O_{12}N_{11}S_2$: C, 60.8; H, 6.78; N, 12.2. Found: C, 60.9; H, 6.78; N, 11.9.

Deamino-oxytocin. S-Benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (155 mg) was dissolved in 125 ml of stirred boiling liquid ammonia (distilled from sodium in an all-glass apparatus). A fresh sodium stick was introduced and withdrawn when the blue color pervaded the solution. The sodium stick was momentarily introduced intermittently until the blue color persisted for 30 sec. The ammonia was removed by lyophilization at the water pump and the residue was dissolved in 300 ml of 0.03% trifluoroacetic acid. The pH of the solution was adjusted to 8.0 with ammonium hydroxide and an excess of 0.1 *N* potassium ferricyanide (4.5 ml) was added to the stirred solution. After 15 min AG 3-X4 resin (chloride form) was added and stirring was continued for 15 min to remove ferrocyanide and excess ferricyanide ions. The resin was removed by filtration, and the solution was lyophilized.

The lyophilized residue was dissolved in 20 ml of the upper phase of the solvent system 1-butanol–benzene–3.5% aqueous acetic acid (containing 1.5% pyridine) (1:1:2) and applied to a Sephadex G-25 (100–200 mesh) column (2.16 × 110 cm) that had been equilibrated with the lower phase. The column was eluted with the upper phase and 96 fractions of 9.5 ml each were collected. The chromatogram obtained by plotting the Folin–Lowry color values[26] of the fractions showed a single peak with a maximum at fraction 52 (R_f 0.19; lit.[9] 0.19). The fractions corresponding to this peak were pooled, twice the volume of water was added, and the resulting mixture was concentrated under reduced pressure and lyophilized; yield, 49 mg (41%).

The lyophilized powder was dissolved in 6 ml of 0.2 *N* acetic acid and subjected to gel filtration[21] on a Sephadex G-25 (200–270 mesh) column (2.82 × 64 cm) that had been equilibrated with 0.2 *N* acetic acid. The column was eluted with 0.2 *N* acetic acid and 120 fractions of 4.9 ml each were collected. A plot of the Folin–Lowry color values of the various fractions showed a single symmetrical peak with a maximum at fraction 65. The fractions corresponding to this peak were pooled and lyophilized to give a white powder; yield, 39.4 mg; $[\alpha]^{25}D$ −95.1° (*c* 0.5, 1 *N* acetic acid), lit. $[\alpha]^{20}D$ −88.3° (*c* 0.5, 1 *N* acetic acid)[9] and $[\alpha]^{21}D$ −107° (*c* 0.5, 1 *N* acetic acid).[7]

This lyophilized powder (25 mg) was dissolved in 0.8 ml of water in a water bath at 80–90°. The solution was filtered through a sintered-glass funnel and was allowed to stand at room temperature for 5 hr. Crystals began to form within 1 hr and the bulk of the deamino-oxytocin had crystallized out after 5 hr. The solution was allowed to stand overnight in the refrigerator. The deamino-oxytocin crystals were filtered off, washed with water, and dried *in vacuo*; yield, 14.8 mg; mp 182–183°, uncorrected (Fisher-Johns melting point apparatus; electrically heated aluminum block), lit.[9] mp 179° (modified Kofler block, corrected) and 178–184° (capillary, corrected); $[\alpha]^{21}D$ −103.3° (*c* 0.46, 1 *N* acetic acid), lit.[9] $[\alpha]^{20}D$ −90.4° (*c* 0.5, 1 *N* acetic acid).

Acknowledgments. The authors are indebted to the following: Mr. Joseph Albert for the elemental analyses; Mr. Roger Sebbane for the amino acid analyses; and Miss Margitta Wahrenburg and Mrs. Jessie Lawrence for the bioassays, under the direction of Dr. W. Y. Chan.

(25) Ammonolysis conditions as described to us by Dr. Maurice Manning in a personal communication.

(26) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

The Structure of Frenolicin[1]

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Abstract: Evidence is put forward which describes the structure and stereochemistry of frenolicin as I.

The isolation and preliminary characterization of frenolicin, a pale yellow crystalline antibiotic from *Streptomyces fradiae*, were described by Van Meter, Dann, and Bohonos.[2] They suggested the molecular formula to be $C_{13}H_{14}O_5$ and indicated the presence of two acidic functions assigned to a phenolic or enolic grouping and a carboxylic acid. In addition, they observed that frenolicin readily absorbed 2 mol of hydrogen and contained a C-methyl group. We wish to report in detail work which has led to the structure and relative stereochemistry of frenolicin as that formulated by the novel naphthoquinone epoxide I.[3]

Repetition of the elemental analyses gave figures more indicative of the formula $C_{18}H_{18}O_7$, rather than that previously proposed,[2] and the molecular weight (346) required by this formula was confirmed by mass spec-

(1) A portion of this work was reported in preliminary form: G. A. Ellestad, H. A. Whaley, and E. L. Patterson, *J. Am. Chem. Soc.*, **88**, 4109 (1966).

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

(3) Symbols a′ and e′ denote pseudo-axial and pseudo-equatorial configurations of the bonds in question.

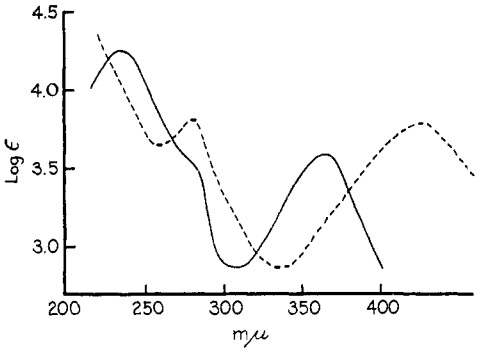
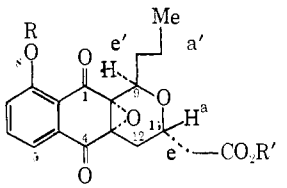
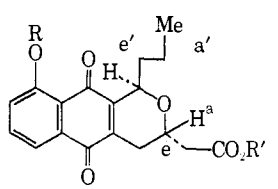


Figure 1. Ultraviolet spectrum of frenolicin: ——, in methanol; ----, in methanolic sodium hydroxide.

tral analysis. Confirmation that frenolicin was a phenolic carboxylic acid[2] (pK_a' = 10.0 and 5.2 in methanol–water, 1:1) was obtained by the formation of a monoacetate (II), ν_{max} 1770 and 1710 cm^{-1}, and with diazomethane a methyl ester (III), ν_{max} 1735, 1710, and



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

1650 cm^{-1}. The infrared absorption at 1710 and 1650 cm^{-1} in the methyl ester indicated the presence of two other carbonyl groups one of which was hydrogen bonded to the phenolic hydroxyl group. O-Methylfrenolicin methyl ester (IV) could be obtained (ν_{max} 1740 and 1695 cm^{-1}) by reaction with methyl iodide in the presence of potassium carbonate. This left two oxygen atoms to be accounted for and their inertness in the above reactions suggested they were ethereal in nature.

The ultraviolet spectrum of frenolicin in both neutral and basic media (Figure 1) showed a striking resemblance to that of β-hydrojuglone[4] suggesting that this moiety was present in frenolicin. Supporting evidence for this portion of the carbon skeleton was obtained by potassium permanganate oxidation of O-methylfrenolicin methyl ester which provided a methoxyphthalonic acid (presumably 3-methoxy) identical with an authentic specimen obtained from a similar oxidation of 1,5-dimethoxynaphthalene.[5]

The remarkable susceptibility of frenolicin to reduction with a variety of reagents, despite the apparent lack of any olefinic groups, to give deoxyfrenolicin (V), $C_{18}H_{18}O_6$, suggested the 1,4-naphthoquinone epoxide structure. Frenolicin consumed 2 mol of hydrogen (10% Pd–C in methanol) to give a colorless compound which was immediately air oxidized to the yellow-orange quinone V, whose ultraviolet spectrum λ_{max} 246, 274, and 420 mμ (ε 9070, 11,400, and 4290) corresponded to that of juglone.[6] Deoxyfrenolicin was characterized as its monoacetate (VI) and methyl ester (VII). Frenolicin could be reduced with sodium hydrosulfite at room temperature to a colorless hydroquinone which in turn was slowly air oxidized to V.[7] In addition, reduction occurred to give V directly with potassium iodide in refluxing acetic acid[8] and surprisingly with 30% hydrobromic acid in acetic acid at room temperature overnight; indeed this latter reagent seemed to be the method of choice for preparing deoxyfrenolicin.

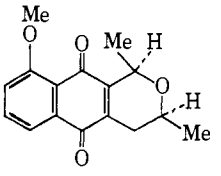
It remained to account for the residual eight carbon atoms which must include the C-methyl group, the carboxylic acid, and the remaining ethereal oxygen atom. Since frenolicin does not contain an alkoxy group, this last oxygen must be incorporated in a ring. The only positions available for attachment are at 2 and 3 of the naphthoquinone oxide system and comparison of the ultraviolet spectrum of deoxyfrenolicin with that of the eleutherins[9] indicated that the heterocyclic ring is joined in a similar manner.

The nmr spectrum of frenolicin was particularly useful in delineating these remaining structural details as was mass spectral analysis. The spectrum of frenolicin (Figure 2) showed a three-proton triplet at δ 1.00 (J = 6.0 cps) attributed to a primary C-methyl group. The strongest fragmentation ions in the mass spectra of frenolicin, O-methylfrenolicin methyl ester, and deoxyfrenolicin methyl ester (see below) resulted from the loss of 43 mass units strongly supporting the presence of an n-propyl grouping in frenolicin. The two-proton doublet at δ 2.60 (J = 6.0 cps) is assigned to two equivalent methylene protons deshielded by the carboxyl group and coupled with a tertiary proton, *i.e.*, $CHCH_2CO_2H$.

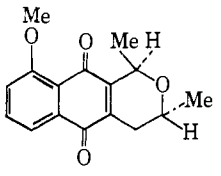
The one-proton symmetrical triplet at δ 4.58 (J = 6.5 cps) suggests a tertiary proton on a carbon bearing an oxygen atom and adjacent to a methylene group. Spin-decoupling experiments (δ 4.58 triplet to a singlet) showed that the adjacent methylene group with resonance at *ca.* δ 1.9 must be part of the n-propyl grouping and that there is little or no difference in chemical shift between these two geminal protons.

A pair of doublets at δ 2.90 (J = 5.0 and 15.0 cps) was shown to be coupled with an upfield pair at δ 1.91 (J = 11.0 and 15.0 cps) by irradiation of the signal at δ 1.91 which collapsed the pattern at δ 2.90 to a doublet. These resonances are assigned to the geminal protons of a CH_2 group adjacent to a tertiary hydrogen whose signal was located as a multiplet at δ 4.15 by spin decoupling with either of the two frequencies of

(4) R. H. Thomson, *J. Chem. Soc.*, 1737 (1950).
(5) 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).
(6) See R. A. Morton, "Biochemistry of Quinones," Academic Press Inc., New York, N. Y., 1965, p 49.
(7) M. Tishler, L. F. Fieser, and N. L. Wendler, *J. Am. Chem. Soc.*, 62, 2866 (1940).
(8) S. Bodforss, *Ber.*, 49, 2801 (1916); M. M. Shemyakin, D. A. Bochvar, and L. A. Shchukina, *Zh. Obshch. Khim.*, 22, 439 (1952).
(9) Elethrein (i) and isoeleuterin (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).



i



ii

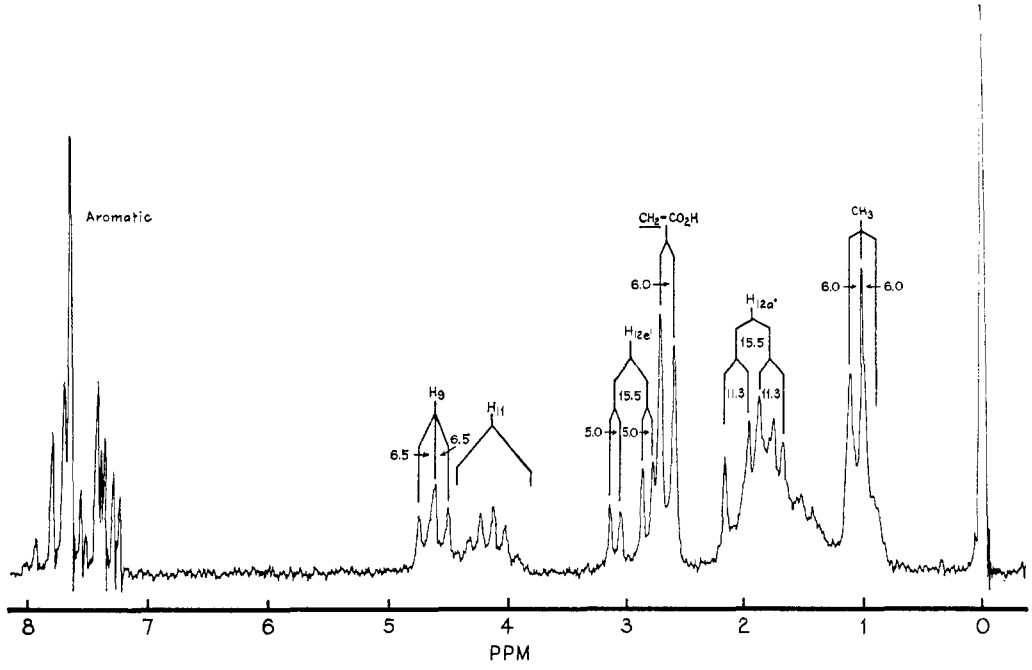


Figure 2. Nmr spectrum of frenolicin.

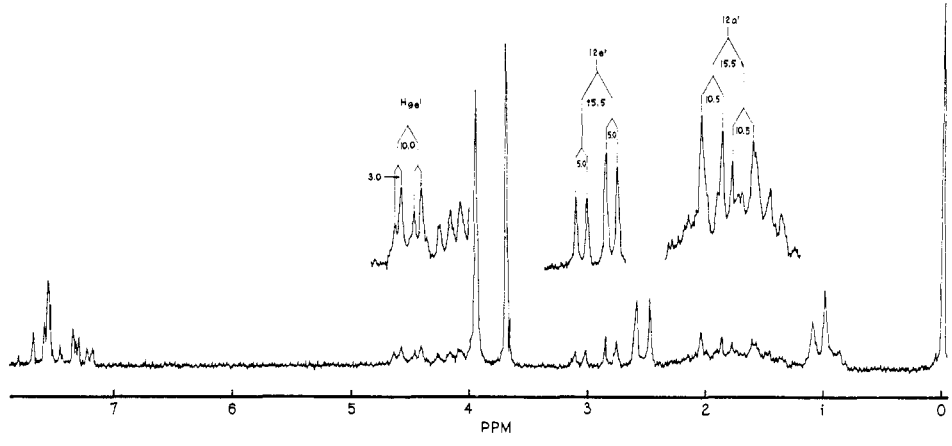


Figure 3. Nmr spectrum of O-methylfrenolicin methyl ester.

the neighboring geminal hydrogens. In addition, decoupling experiments indicated the X proton of the ABX system to be the same hydrogen that was splitting the CH_2 protons at δ 2.60 adjacent to the carboxyl group. Hence, the $CH_2CH(CH_2CO_2H)O$ grouping is indicated in frenolicin. It is significant that irradiation of either the signal at δ 2.90 or the 2.60 doublet perturbed the δ 4.15 multiplet without any effect on the symmetrical triplet at δ 4.58.

A combination of these fragments with the established 1,4-naphthoquinone epoxide system consistent with the foregoing arguments led to the complete structure for frenolicin as that shown in I with the exception of the placement of the phenolic hydroxyl group. This latter function was placed at C-8 (rather than at C-5) on the basis of an unusual change in the nmr pattern of the C-9 proton on going from frenolicin to acetylfrenolicin or O-methylfrenolicin methyl ester (Figure 3). Acetylation or methylation of the phenolic hydroxyl group changed the nmr signal of the C-9 proton from a symmetrical triplet to a pair of doublets (δ 4.58, peak separations of 3 and 10 cps) with no effect on the signal of the C-12 protons. The nonequivalence of the methylene protons adjacent to the C-9 asymmetric carbon in O-methylfrenolicin methyl ester was confirmed by spin decoupling which showed the C-9 signal to be perturbed by irradiation over a range of ~35 cps (δ 1.60–2.10) in the methylene region of the spectrum. Whether this is a result of a change in the magnetic anisotropy of the carbonyl group due to the depolarization upon removal of the hydrogen bonding or a change in the steric interaction between the carbonyl and the propyl group, or a combination of both, it seems evident that the geminal protons adjacent to the C-9 proton are subject to different shielding effects in the unchelated derivatives of frenolicin than in frenolicin itself.

From the magnitudes of the vicinal coupling constants[10] between the C-12 methylene group and the adjacent tertiary proton at C-11, it can be shown that the doublet of doublets at 2.90 ($J_{11,12\text{-a,e}'}$ = 5.0 cps)

(10) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2871 (1963), and references therein; H. Conroy, *Advan. Org. Chem.*, **2**, 311 (1960); C. N. Banwell and N. Sheppard, *Discussions Faraday Soc.*, **34**, 115 (1962).

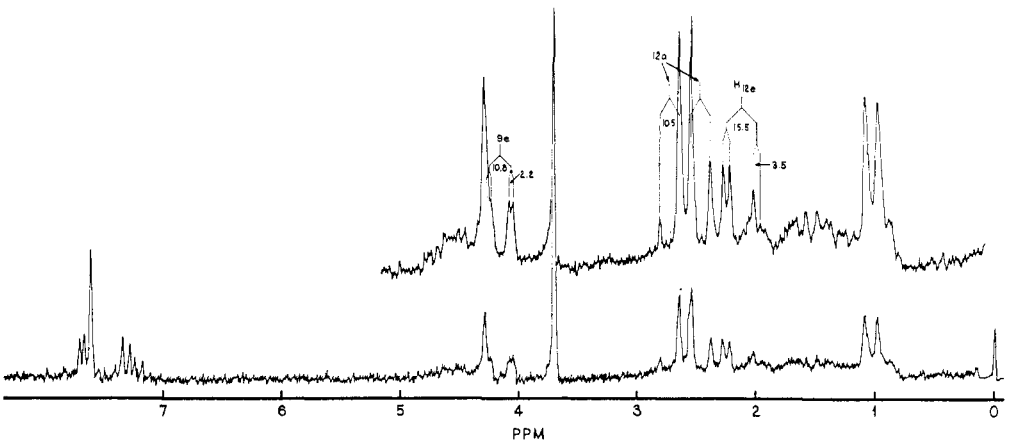
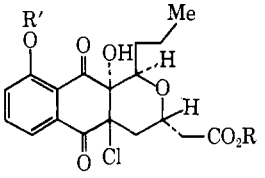


Figure 4. Nmr spectrum of frenolicin chlorohydrin methyl ester.

is associated with the pseudo-equatorial[11] C–H bond and the corresponding upfield signal at δ 1.91 ($J_{11,12\text{-a}',a}$ = 11.3 cps) with the pseudo-axial C–H bond. Also the C-11 proton is shown to have the axial configuration from the same data. The configuration of the C-9 proton relative to the C-11 hydrogen could be determined by taking advantage of the homoallylic couplings between the C-12 geminal protons and the C-9 hydrogen in deoxyfrenolicin. These long-range proton–proton couplings have been previously discussed with stereochemical implications in regard to eleutherin and isoeleutherin,[12] and comparison of the coupling constants of the C-9, C-11, and C-12 protons in the nmr spectrum of deoxyfrenolicin methyl ester ($J_{12,12\text{-}gem}$ = 19.0, $J_{12,11\text{-a}',a}$ = 10.0, $J_{12,11\text{-e}',a}$ = 3.5, $J_{9,12\text{-a}',e'}$ = 2.0, $J_{9,12\text{-e}',e'} < 1$ cps) with the corresponding signals in the nmr spectra of the eleutherins confirmed the axial assignment at C-11 and showed the C-9 proton to be pseudo-equatorial.

Treatment of frenolicin with hydrochloric acid in acetic acid, dioxane, or by solution in concentrated hydrochloric acid gave a chlorohydrin which with diazomethane gave a methyl ester identical with that prepared from frenolicin in methanolic hydrochloric acid and from frenolicin methyl ester and pyridine hydrochloride. Structures for the chlorohydrin and its methyl ester can be formulated with some certainty as VIII and IX, respectively, on the basis of the following arguments. Attack of the chloride ion at C-3, as opposed to C-2, seems favored on steric grounds because of the propyl group at C-9. Electronic considerations would also favor epoxide ring opening at C-3 because the electronegative oxygen atom at C-9 would tend to inhibit developing carbonium ion character at C-2 in the transition state.[13] The more polarized C-1 carbonyl group would discourage attack at C-2 for the same reason. It was hoped to obtain additional evidence for C-3 opening by formation of a fluorohydrin and observe the splitting pattern of the fluorine signal in the nmr spectrum. However, frenolicin was inert to hydrofluoric acid and boron trifluoride etherate. The ring opening is certainly *trans*-diaxial, as expected,[13] since reclosure can be effected easily with sodium methoxide as well as with potassium carbonate in methanol.



VIII, R = R′ = H
IX, R = Me; R′ = H
X, R = R′ = Me

Significant changes occur on passing from the nmr spectrum of frenolicin to that of the chlorohydrin (Figure 4), notably in the chemical shifts and coupling constants of the ring-C hydrogens. These differences reflect the change in magnetic field effects which accompany the formation of the chlorohydrin and the corresponding conformational changes on going from the half-chair to the chair form. The C-11 proton frequency is at δ 4.60, some 25 cps downfield from its position in frenolicin suggesting a *cis*-1–3-diaxial relation with the C-3 chlorine atom.[14] The C-9 proton signal is found slightly upfield at δ 4.20 and is a doublet of doublets (J = 2.2 and 10.8 cps). The propyl side chain is now truly axial and the CH_2 group adjacent to the C-9 hydrogen experiences a different shielding effect from the C-1 carbonyl group than in frenolicin. The slight upfield shift is probably due to the adjacent axial hydroxyl group which is known to cause shielding of adjacent equatorial protons.[14a] The C-12 axial hydrogen is observed at δ 2.60 ($J_{11,12\text{-a},a}$ = 10.5 cps) while the corresponding equatorial proton is upfield at δ 2.20 ($J_{11,12\text{-a},e}$ = 3.5 cps).[15] Although this is a striking reversal of the equatorial protons absorbing at

(11) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 239.

(12) D. W. Cameron, D. G. I. Kingston, N. Sheppard, and A. Todd, *J. Chem. Soc.*, 98 (1964).

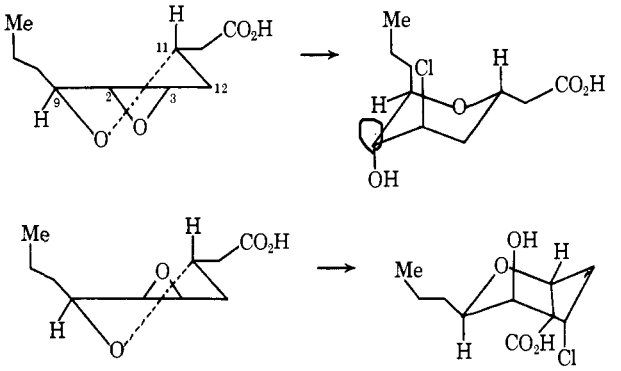
(13) R. E. Parker and N. S. Isaacs, *Chem. Rev.*, 59, 746 (1959).

(14) (a) K. Tori and T. Komeno, *Tetrahedron*, 21, 309 (1965); (b) S. G. Levine, N. H. Eudy, and E. C. Farthing, *Tetrahedron Letters*, 1517 (1963); (c) M. Tomoeda, M. Inuzuka, T. Furuta, and T. Takahashi, *Tetrahedron Letters*, 1233 (1964).

(15) Although the magnitude of the vicinal coupling constants between the C-12 and C-11 protons in the chlorohydrin are strongly suggestive of dihedral angles of 180 and 60° indicating a normal chair form, it can be argued that these values do not unequivocally rule out the possibility of the heterocycle ring existing in a boat form, *i.e.*, angles of ~5 and 115° which in certain situations might give similar J values to those recorded above. However, the 1–3 diaxial interaction between the C-11 and C-3 substituents just described negates this latter alternative.

lower field than their axial counterparts,[16] it is not without ample precedent.[17] The upfield shift of the C-12 equatorial hydrogen can be related to the introduction of the electronegative axial chlorine on the adjacent C-3 carbon atom as can the dramatic low-field shift of the C-12 axial proton.[14a] In addition, the 1-3-diaxial interaction of the C-12 axial proton with the C-2 hydroxyl group must make a significant contribution to the paramagnetic shift of this hydrogen.[14]

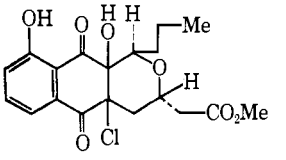
As already mentioned above, the C-9 and C-11 protons in frenolicin are pseudo-equatorial and axial, respectively. Based on epoxide opening at C-3 with inversion, it can be seen from Dreiding models and the drawings below that only when the oxiran ring and the C-11 hydrogen are *trans* to each other in frenolicin can the latter proton remain axial in the chlorohydrin.[18]



While this evidence is by no means unequivocal it nevertheless allows us to make a tentative assignment of the relative stereochemistry of the epoxide ring as shown in I.

One facet of the chemistry of the chlorohydrin remained to be resolved. The chlorohydrin methyl ester upon reaction with hot pyridine, gave unchanged chlorohydrin, frenolicin methyl ester, and an isochlorohydrin XI. Frenolicin isochlorohydrin methyl ester also gave frenolicin methyl ester upon treatment with sodium methoxide as the only identifiable product. The ultraviolet spectrum of XI indicated the β-hydrojuglone chromophore to be intact, and both chlorohydrins with zinc and acetic acid gave, after chromatography over silica gel, deoxyfrenolicin methyl ester. The stability of the isomeric chlorohydrin to hot pyridine suggests a *cis* structure and supporting evidence is found in the chemical shift of the C-2 hydroxyl proton in the nmr spectra of the two isomers. In frenolicin chlorohydrin methyl ester the C-2 hydroxyl proton is observed at δ 4.3, whereas in the isochlorohydrin it appears at δ 7.2 implying that in the latter compound the hydroxyl hydrogen is hydrogen bonded to the adjacent chlorine, consistent with the *cis* nature of the two substituents.

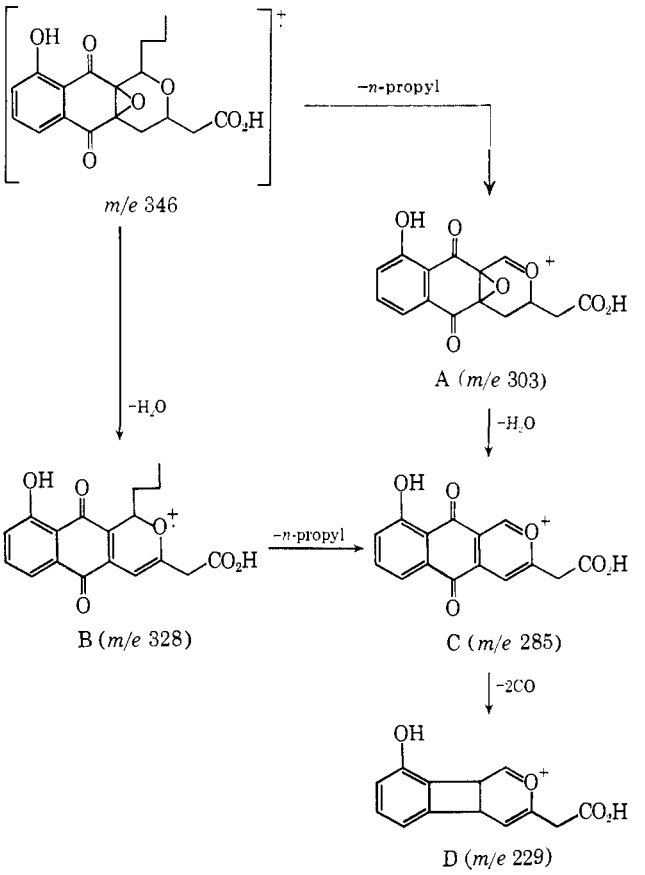
Two possible pathways merit consideration for



XI

obtaining a *cis*-chlorohydrin from IX. The β-hydroxy ketone system allows a retroaldol recondensation-type reaction to take place which could theoretically produce all four chlorohydrins. The *trans* pair would close to epoxides, however, and one might expect to isolate both frenolicin and its isomer from such a reaction. The fact that frenolicin methyl ester is the only identifiable product from XI on treatment with methoxide suggests that epimerization at C-2 only is occurring and hence tends to preclude this first possibility. Also, a stronger base than pyridine is usually necessary to effect a retroaldol reaction. A more likely explanation is that an acyloin-type equilibrium[19] is involved with the C-1 carbonyl group. The pyridine is probably not able to completely break up the very strong chelation between the phenolic hydroxyl group and the C-1 carbonyl and hence this internal protonation acts as the driving force for the equilibration to take place. Supporting evidence for this alternative pathway is found from the reaction of the O-methyl methyl ester of the *trans*-chlorohydrin (X) with hot pyridine whereupon only frenolicin methyl ester is obtained indicating that protonation of the C-1 carbonyl group by the phenolic hydroxyl is an

Scheme I



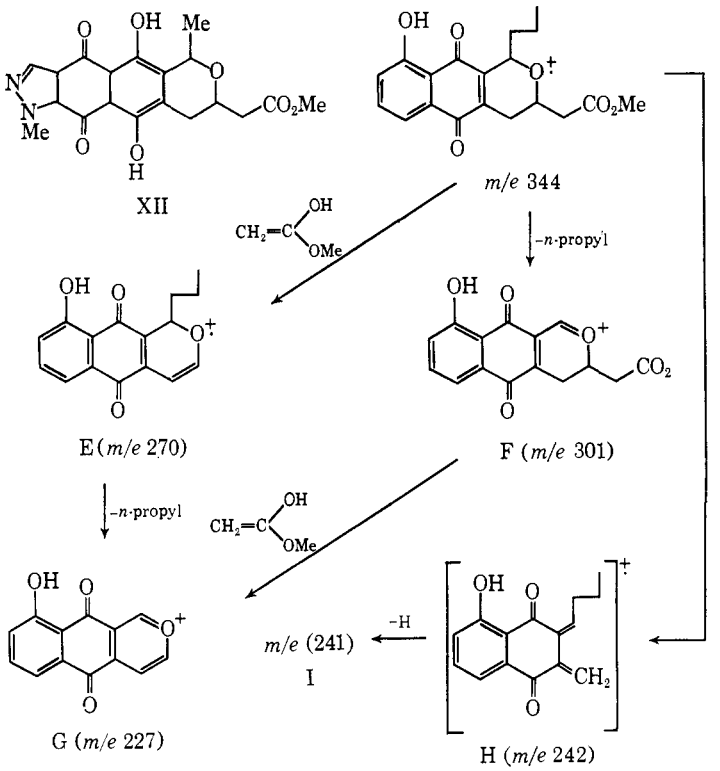
(16) L. M. Jackman, "Nuclear Magnetic Resonance Spectroscopy," Pergamon Press Inc., New York, N. Y., 1959, pp 116–117.

(17) See C. W. Shopee, T. E. Bellas, R. E. Lack, and S. Sternhell, *J. Chem. Soc.*, 2483 (1965); E. R. H. Jones and D. A. Wilson, *ibid.*, 2933 (1965); J. R. Hanson, *ibid.*, 5036 (1965); N. S. Bhacca, J. E. Gurst, and D. H. Williams, *J. Am. Chem. Soc.*, **87**, 302 (1965); K. L. Williamson and W. S. Johnson, *ibid.*, **83**, 4623 (1961).

(18) For a related discussion on the stereochemistry of the 3-methoxycyclohexene oxides, see R. U. Lemieux, R. K. Kullnig, and R. Y. Moir, *ibid.*, 80, 2237 (1958).

(19) N. L. Wendler, D. Taub, and R. W. Walker, *Tetrahedron*, **11**, 163 (1960).

Scheme II

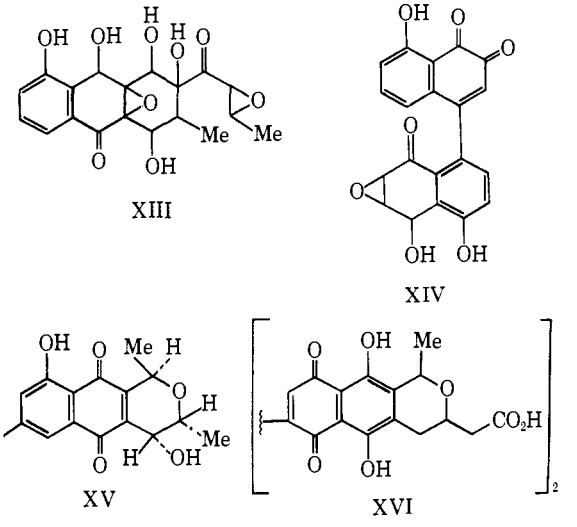


essential prerequisite for this isomerization to take place. In order to relate frenolicin chlorohydrin methyl ester to the O-methyl derivative X, the former was methylated with methyl iodide and potassium carbonate in refluxing acetone which instead of X gave O-methylfrenolicin methyl ester. The chlorohydrins were, however, shown to be the same except for the O-methyl group by their almost identical nmr spectra.

The mass spectra of frenolicin and O-methylfrenolicin methyl ester were characterized by strong molecular ions and a number of abundant fragments, a few of which can be assigned plausible structures. Both compounds gave essentially the same fragmentations with regard to the most abundant peaks except that in the O-methyl methyl ester these ions were 28 mass units higher. As mentioned above, the strongest fragmentation is that of M − 43 (A) (due to the loss of the *n*-propyl group) which in turn loses water to form ion C. The presence of a small peak at *m/e* 328 signifies the loss of water (M − 18) to give ion B which by loss of the propyl group also gives C. The *m/e* 229 (D) species most likely arises from the *m/e* 285 peak by extrusion of two CO's. All but the last fragmentation are substantiated by the appropriate metastable ions (see Scheme I).

The mass spectrum of deoxyfrenolicin methyl ester is very similar to that recently published for the fragmentation of XII, a derivative of actinorhodin.[20] The loss of the $CH_2CO_2CH_3$ grouping by a McLafferty rearrangement to give the ion *m/e* 270 followed by loss of the propyl group to give ion G, or the reverse, by loss of propyl to give ion F with loss of the ester side chain to give ion G, appear to be major fragmentations due to the obvious stability of the *m/e* 227 ion. It is significant that the ester side chain is not lost by a McLafferty rearrangement in the spectrum of frenolicin or its O-methyl methyl ester and is apparently due to the controlling influence of the epoxide which loses water instead. That the loss of water is due to the epoxide ring is substantiated by the fact that deoxyfrenolicin methyl ester does not lose water in any of its major fragmentations. As in the decomposition of the actinorhodin derivative, fragments at 242 and 241 are observed due to loss of $CH_3O_2CCH_2CHO$ and hydrogen, respectively.

To our knowledge, frenolicin is the first 1,4-naphthoquinone 2,3-epoxide found in nature although cervicarcin (XIII),[21] a metabolite produced by *Streptomyces ogaensis*, is closely related as is mycochrysone (XIV),[22] isolated from an unnamed discomycete. In addition to eleutherin and isoeleutherin, frenolicin is biogenetically related to quinone A (XV), a degradation



(20) H. Brockmann, A. Zeeck, K. van der Merwe, and W. Müller, *Ann.*, **698**, 209 (1966).

(21) S. Marumo, K. Saski, and S. Suzuki, *J. Am. Chem. Soc.*, **86**, 4507 (1964).

(22) G. Read and L. C. Vining, *Chem. Ind.* (London), 1239 (1963).

product from protoaphin-$f_{b'}$,[23] and actinorhodin (XVI), from *Streptomyces coelicolor*.[20]

Aside from its weak antibacterial activity, the most notable property of frenolicin is its ability to cause skin irritation if strict cleanliness is not observed. During the course of the structure elucidation, however, it was observed that deoxyfrenolicin (V) possessed significant antifungal activity *in vitro* against a variety of fungi and *in vivo* against some experimental ringworm infections in guinea pigs. In addition, V showed a marked increase in antibacterial activity *in vitro* over frenolicin.

Experimental Section[24]

Frenolicin Methyl Ester (III). A solution of 0.40 g of frenolicin in ether was treated with an excess of an ethereal solution of diazomethane and allowed to stand for about 10 min. The solution was washed with 10% sodium bicarbonate, then water, and dried. Evaporation of the solvent under vacuum gave a gum which, when chromatographed over acid-washed silica gel and eluted with benzene–chloroform (7:3), afforded 0.10 g of crystalline product. Recrystallization from hexane–benzene gave the analytical sample: mp 82–83°; $[\alpha]^{25}D$ −31.7° (*c* 0.987, MeOH); ν_{max}^{KBr} 3400, 3100, 1735, 1705, and 1665, 1610 and 1585 cm^{-1}; λ_{max}^{MeOH} 233, 250 sh, 285 sh, and 362 mμ (ε 14,440, 7550, 2880, and 4680); $\lambda_{max}^{0.1NNaOH\ in\ MeOH}$ 216. 249, 280, and 425 mμ (ε 23,400, 7550, 5400, and 5400).

Anal. Calcd for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59. Found: C, 63.67; H, 5.40.

Acetylfrenolicin (II). Frenolicin (1.0 g) was treated with 5 ml of acetic anhydride in 20 ml of pyridine. This solution was held at room temperature for 1 hr then heated 10 min on a steam bath. After cooling the reaction mixture was poured into acidic ice water and allowed to stand overnight. Crude acetylfrenolicin was extracted into chloroform and obtained as a residue on evaporation of the solvent. This residue was dissolved in carbon tetrachloride and chromatographed on 20 g of silica gel. Acetylfrenolicin was eluted with chloroform–carbon tetrachloride (1:1) and crystallized on evaporation of the solvent. It was recrystallized from *n*-butyl chloride to give colorless needles: mp 161.5–163.5°; $[\alpha]^{25}D$ +10.3° (*c* 1.06, MeOH); ν_{max}^{KBr} 1770, 1700, and 1600 cm^{-1}; λ_{max}^{MeOH} 226, 260 sh, and 315 mμ (ε 19,000, 5430, and 1990).

Anal. Calcd for $C_{20}H_{20}O_8$: C, 61.85; H, 5.19. Found: C, 61.98; H, 5.32.

O-Methylfrenolicin Methyl Ester (IV). Frenolicin (2.0 g) was dissolved in 25 ml of acetone contained in a 50-ml, round-bottomed flask. Five grams of anhydrous potassium carbonate and 10 ml of methyl iodide were added to the reaction mixture, and this mixture was refluxed for 4 hr. After cooling the reaction mixture was filtered, and the filtrate was evaporated to dryness *in vacuo*. The crude product was dissolved in about 30 ml of hot methanol, cooled, and seeded to yield 1.85 g of pale yellow blades: mp 109–110°; $[\alpha]^{25}D$ +39.6° (*c* 1.06, MeOH); ν_{max}^{KBr} 1742, 1695, and 1587 cm^{-1}; λ_{max}^{MeOH} 229 and 347 mμ (ε 17,300 and 4980).

Anal. Calcd for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 64.39; H, 6.01.

Permanganate Oxidation of O-Methylfrenolicin Methyl Ester. O-Methylfrenolicin methyl ester (0.50 g) was treated with 5 ml of 1 *N* sodium hydroxide on a steam bath until completely dissolved (15 min). A solution of 5 g of potassium permanganate in 20 ml of water was added and heated on a steam bath 15 min, then left at room temperature overnight. An additional 1 g of potassium permanganate was added and the resulting mixture heated on the steam bath for an additional 3 hr. The mixture was filtered while hot and washed with hot water. The combined filtrate and wash was adjusted to pH 3 with hydrochloric acid and extracted with ether in a continuous process. Evaporation of the ether gave 0.18 g of crude powder which crystallized from water to give 38 mg of colorless prisms, mp 193–195° (gas evolution). This product was identified as 3-methoxyphthalonic acid by comparison with authentic material prepared by alkaline permanganate oxidation of 1,5-dimethoxynaphthalene.

Anal. Calcd for $C_{10}H_8O_6$: C, 53.58; H, 3.60. Found: C, 53.31; H, 3.78.

Deoxyfrenolicin (Hydrogenation of Frenolicin) (V). A solution of 2.0 g of frenolicin in 40 ml of methanol was hydrogenated at atmospheric pressure over 0.20 g of 10% palladium–charcoal. After 3 hr, slightly more than 2 mol of hydrogen had been consumed. Upon exposure of the colorless solution to the atmosphere, the color changed immediately to a greenish yellow. The catalyst was removed and the filtrate evaporated to a dark green oil which partially crystallized upon standing. The crude product was taken up in a minimum quantity of benzene with a trace of methanol and chromatographed on acid-washed silica gel. Elution with benzene afforded an orange-red band which, when concentrated to a small volume, gave 0.24 g of orange-yellow crystals, mp 179–181°. Continued elution with benzene–chloroform (1:3) gave another 0.77 g of orange-yellow crystals, mp 179–181°.

Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found: C, 65.68; H, 5.84.

Deoxyfrenolicin. Potassium Iodide Reduction of Frenolicin (V). A solution of 1.78 g of frenolicin in 100 ml of glacial acetic acid was refluxed with 2.2 g of potassium iodide for 45 min. The reaction mixture was poured into water and the yellow precipitate collected by filtration and dried to give 1.47 g of crude product. Recrystallization from benzene gave yellow needles: mp 179–181°; $[\alpha]^{25}D$ +112° (*c* 1.07, MeOH); ν_{max}^{KBr} 3010, 1725, 1665, 1650, 1625, and 1585 cm^{-1}; λ_{max}^{MeOH} 246, 274, and 415 mμ (ε 9070, 11,400, and 4290); $\lambda_{max}^{0.1NNaOH\ in\ MeOH}$ 276 and 515 mμ (ε 12,880 and 5275). Deoxyfrenolicin obtained in this fashion was shown to be identical with that obtained from the hydrogenation of frenolicin by mixture melting point, infrared, and ultraviolet absorption spectra.

Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found: C, 65.84; H, 5.70.

Deoxyfrenolicin (V) with Hydrobromic Acid. A solution of 0.5 g of frenolicin in 10 ml of acetic acid was treated with 2 ml of 30% hydrobromic acid in acetic acid and allowed to stand overnight at room temperature. The solution was poured into ice water and the orange-yellow precipitate filtered. Recrystallization from benzene gave deoxyfrenolicin by melting point, mixture melting point, and infrared spectrum in almost quantitative yield.

Acetyldeoxyfrenolicin (VI). Deoxyfrenolicin (0.20 g) was left in a solution containing 2 ml of acetic anhydride and 4 ml of pyridine overnight at room temperature. The solution was poured onto a mixture of ice and 10% hydrochloric acid which was extracted with chloroform. The chloroform extract was washed with water, dried, and concentrated to a crystalline residue. Recrystallization from benzene gave 0.12 g of pale yellow needles: mp 180–182° with some prior softening at about 175°; $[\alpha]^{25}D$ +58° (*c* 1.00, EtOH); ν_{max}^{KBr} 3050, 1770, 1715, and 1665 cm^{-1}; λ_{max}^{EtOH} 240, 250, 263, 270, and 340 mμ (ε 14,500, 13,200, 12,100, 12,300, and 12,980).

Anal. Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.86; H, 5.86.

Deoxyfrenolicin Methyl Ester (VII). Deoxyfrenolicin (0.85 g) in a solution of methanol–ether was treated with an excess of ethereal diazomethane. After washing with 10% sodium bicarbonate and water, the solution was dried and concentrated to a crystalline mass. Recrystallization from hexane–benzene (5:1) afforded 0.65 mg of yellow crystals. A second recrystallization gave the analytical sample: mp 119–120°; $[\alpha]^{25}D$ +105° (*c* 1.01, $CHCl_3$); ν_{max}^{KBr} 1740, 1660, 1614, and 1580 cm^{-1}; λ_{max}^{MeOH} 245, 274, and 415 mμ (ε 9175, 11,250, and 4130).

Anal. Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 65.95; H, 6.08.

Frenolicin Chlorohydrin (VIII). Frenolicin (5 g) in 150 ml of concentrated hydrochloric acid and 150 ml of acetic acid was heated on the steam bath for 1 hr. The solution was concentrated to dryness and the residue slurried with water and filtered. Crystallization from benzene–hexane gave 4.2 g of chlorohydrin, mp 165–170°. Recrystallization from benzene–hexane gave the analytical sample: mp 174–175°; ν_{max}^{KBr} 1712 br and 1667 cm^{-1}; λ_{max}^{MeOH} 234, 270 sh, and 352 (ε 20,800, 4950, and 5940).

Anal. Calcd for $C_{18}H_{19}O_7Cl$: C, 56.50; H, 4.97; Cl, 9.28. Found: C, 56.49; H, 5.04; Cl, 9.81.

Treatment of frenolicin chlorohydrin with an ethereal solution of

(23) D. W. Cameron, R. I. T. Cromartie, D. G. I. Kingston, and A. Todd, *J. Chem. Soc.*, 51 (1964).

(24) Nmr spectra were recorded with a Varian A-60 in $CDCl_3$; shifts are expressed as δ values (parts per million) from tetramethylsilane as internal standard and coupling constants (*J*) are expressed in cycles per second. The spin decoupling was done on a Varian DP-60 equipped with a Varian integrator–decoupler. We thank W. J. Fulmor's group for the spectral analyses, Dr. John Lancaster of the Stamford Laboratories for the spin-decoupling experiments, L. M. Brancone and associates for the elemental analyses, and Dr. J. Karliner for some of the mass spectra which were obtained on a direct inlet MS9 (AEI). All melting points are uncorrected.

diazomethane gave frenolicin chlorohydrin methyl ester as shown by melting point, mixture melting point, and infrared spectroscopy (see below).

Deoxyfrenolicin Methyl Ester from Frenolicin Chlorohydrin Methyl Ester. A solution of 0.2 g of frenolicin chlorohydrin methyl ester in 10 ml of acetic acid was refluxed for 15 min with 0.48 g of zinc dust. The solution was allowed to cool, filtered, and concentrated. Chloroform was added and the solution was again filtered and concentrated to a dark brown gum. Chromatography over silica gel and elution with benzene–chloroform gave methyl deoxyfrenolicin by melting point, mixture melting point, and infrared comparison with an authentic sample. Frenolicin isochlorohydrin methyl ester was treated in the same manner as above to give deoxyfrenolicin methyl ester by melting point, mixture melting point, and infrared comparison.

Frenolicin Chlorohydrin Methyl Ester (IX). A solution of 3.0 g of frenolicin in 125 ml of methanol was saturated with hydrochloric acid and allowed to stand at room temperature overnight. The methanol was removed *in vacuo* and the residue crystallized from benzene–hexane to give 2.3 g of frenolicin chlorohydrin methyl ester with mp 125–127°. A second crop yielded 864 mg with mp 124–125°; $[\alpha]^{25}D$ +118° (*c* 1.00, MeOH); ν_{max}^{KBr} 1727 and 1664 cm^{-1}; λ_{max}^{MeOH} 234, 280 sh, and 355 mμ (ε 18,400, 3560, and 5540). The mass spectrum exhibited a molecular ion at *m/e* 396 in accord with the molecular formulation $C_{19}H_{21}O_7Cl$.

Frenolicin Isochlorohydrin Methyl Ester (XI). Frenolicin chlorohydrin methyl ester (2.30 g) in 50 ml of pyridine was heated on a steam bath for 45 min. The solution was poured into a mixture of cracked ice and 2 *N* hydrochloric acid and then extracted with chloroform. The chloroform extract was washed with 2 *N* hydrochloric acid and water, dried, and concentrated to a gum which from benzene gave 628 mg of frenolicin isochlorohydrin methyl ester: mp 181–183°; $[\alpha]^{25}D$ +19.2° (*c* 1.04, MeOH); ν_{max}^{KBr} 1712 and 1667 cm^{-1}; λ_{max}^{MeOH} 234, 280 sh, and 351 mμ (ε 18,800, 3960, and 5150). High-resolution mass spectral analysis gave $C_{19}H_{21}O_7Cl$. The mother liquors were chromatographed over silica gel. Elution with benzene gave first 133 mg of frenolicin methyl ester by melting point, mixture melting point, and infrared comparison. Further elution with benzene provided material with mp 75–100° and finally 324 mg of starting material, mp 125–127°.

O-Methylfrenolicin Methyl Ester from IX with Potassium Carbonate and Methyl Iodide. Frenolicin chlorohydrin methyl ester (300 mg) was methylated with methyl iodide and potassium carbonate in refluxing acetone as described above for frenolicin to give O-methylfrenolicin methyl ester by mixture melting point with an authentic sample, and infrared spectrum.

Frenolicin Methyl Ester from Frenolicin Chlorohydrin Methyl Ester with Sodium Methoxide. The chlorohydrin IX (0.2 g) in 20 ml of methanol was treated with 3 equiv of sodium methoxide and the solution allowed to stand overnight at room temperature. The solution was then acidified with hydrochloric acid and extracted with chloroform; the extract was washed with water, dried, and concentrated to a gum which crystallized from chloroform–hexane to give 110 mg of frenolicin methyl ester by mixture melting point with an authentic sample and infrared spectrum. In a similar manner 200 mg of the isochlorohydrin methyl ester gave 23 mg of frenolicin methyl ester as the only identifiable product.

O-Methylfrenolicin Methyl Ester Chlorohydrin (X). O-Methylfrenolicin methyl ester (0.5 g) in methanol was treated with 0.5 g of pyridine hydrochloride and the solution refluxed for 2 hr. The methanol was removed *in vacuo;* the residue was taken up in methylene chloride, washed with water, dried, and concentrated to a gum, which, when allowed to stand overnight in benzene–hexane, gave 327 mg of X, mp 143–145°. Recrystallization from the same solvents gave the analytical sample: mp 143–145°; ν_{max}^{KBr} 1739, 1709 br, and 1587 cm^{-1}; λ_{max}^{MeOH} 232 and 348 mμ (ε 17,500 and 4550); $[\alpha]^{25}D$ +131.8° (*c* 1.148, MeOH).

Anal. Calcd for $C_{20}H_{23}O_7Cl$: C, 58.47; H, 5.64; Cl, 8.63. Found: C, 58.26; H, 5.87; Cl, 8.93.

O-Methylfrenolicin Methyl Ester from X with Pyridine. The O-methyl methyl ester X (0.29 g) was dissolved in pyridine (15 ml); the solution was heated on the steam bath for 45 min. The solution was cooled, diluted with methylene chloride, washed several times with 1 *N* hydrochloric acid and water, and dried. Concentration to dryness gave a gum which was chromatographed over silica gel. Elution with benzene gave 106 mg of O-methylfrenolicin methyl ester.

Frenolicin from VIII and Potassium Carbonate. A solution of 200 mg of VIII in 20 ml of methanol was treated with 100 mg of potassium carbonate and gently refluxed for 1 hr. The methanol was removed under vacuum and the remaining gum was taken up in water and acidified to congo red with 1 *N* hydrochloric acid. Extraction with methylene chloride, followed by washing with water, and evaporation of the solvent gave a pale yellow gum. Crystallization from benzene–hexane gave 100 mg of frenolicin by melting point, mixture melting point, and infrared spectrum.