and **26** obtained were contaminated with 20% of the corresponding (*Z*)-1-vinyl iodide as evidenced by the ^1H and ^{13}C NMR spectra.

(26) A vinyl iodide incorporating the triethylsilyloxy functionality such as **25** has been utilized successfully in the synthesis of prostaglandin congeners in this laboratory.

(27) C. E. Castro, E. J. Gaughan, and D. C. Owlsey, *J. Org. Chem.*, **31**, 4071 (1966).

(28) The numbers in the parentheses represent the chemical shifts of the corresponding 16-epimer. The two peaks of those carbons are approximately of equal height.

Plakortin, an Antibiotic from *Plakortis halichondrioides*

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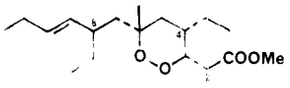
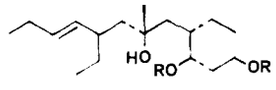
The Caribbean sponge *Plakortis halichondrioides* contains a lipid-soluble antibiotic, plakortin. The structure of plakortin (1) was deduced from spectroscopic data and by chemical degradation. Plakortin (1) was shown to be a cyclic peroxide. A related ketone (**12**) was isolated and the structure deduced from spectroscopic data.

Although there have been several large compilations of data recording in the in vitro antimicrobial activity of marine sponges,[2] relatively few of the metabolites responsible for antimicrobial activity have been isolated and identified.[3] Antimicrobial screening of crude extracts of some Caribbean sponges revealed that the crude ethanol extract of *Plakortis halichondrioides* (Wilson) inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial activity was associated with the major metabolite of the sponge, which was named plakortin. In this paper, we wish to describe the structural elucidation of plakortin (1).



1



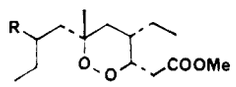
2 R = H
3 R = Ac

Plakortis halichondrioides (Wilson) was collected using SCUBA (−10 m) at Hookers Reef, Panama. The ether-soluble portion of an ethanol extract of the sponge was chromatographed on Florisil to obtain plakortin (1) (5.7% dry weight). Plakortin (1) had the molecular formula $C_{18}H_{32}O_4$. The infrared spectrum of plakortin (1) indicated the presence of an ester group (1735 cm^{-1}) and the absence of other carbonyl or hydroxyl groups. The ^{13}C NMR spectrum contained a carbonyl signal at δ 171.9 (s), a methoxyl signal at 51.5 (q), two signals for carbon atoms bearing oxygen at 81.0 (s) and 78.8 (d), and two signals at 134.4 (d) and 131.5 (d) due to a disubstituted olefin. The ^1H NMR spectrum confirmed the presence of a trans-disubstituted olefin [δ 5.38 (dt, 1 H, J = 15, 6, 6 Hz) and 5.10 (dd, 1 H, J = 15, 9 Hz)] and a methyl ester [δ 3.70 (s, 3 H)]. We therefore concluded that plakortin (1) was the methyl ester of a carboxylic acid containing a cyclic peroxide and a trans-disubstituted olefin.

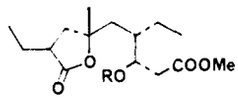
The ^1H spectrum also contained four additional methyl signals at δ 1.37 (s, 3 H), 0.97 (t, 3 H, J = 7 Hz), 0.90 (t, 3 H, J = 7 Hz), and 0.80 (t, 3 H, J = 7 Hz) and a signal assigned to the proton at C-3 at 4.49 (m, 1 H, J = 9.5, 6, 3.5 Hz) which was coupled to two mutually coupled signals at 3.05 (dd, 1 H, J = 15.5, 9.5 Hz) and 2.35 (dd, 1 H, J = 15.5, 3.5 Hz) and a third signal at 2.18 (m, 1 H). Since each of the triplet methyl signals must be adjacent to a methylene group, the structure of plakortin (1) could be solved by determining the position of the olefinic bond in the chain, its relationship to the peroxide ring, and the size of the peroxide ring.

The presence of the peroxide ring was confirmed by reduction of plakortin (1) with lithium aluminum hydride in dry ether at 0 °C to obtain the triol **2**. On acetylation with acetic anhydride in pyridine, the triol gave a diacetate **3**. By comparison of the ^1H NMR spectra of the triol **2** and the diacetate **3**, we deduced that the triol contained a primary alcohol, derived from reduction of the methyl ester, together with secondary and tertiary alcohols resulting from reduction of the cyclic peroxide ring.

Ozonolysis of plakortin (1), followed by addition of dimethyl sulfide to the ozonide, gave a mixture of an acid **5** and an aldehyde **4** which rapidly autoxidized to the acid **5**. The acid **5**, $C_{15}H_{26}O_6$, had lost a three-carbon fragment and contained only two methyl triplets at δ 0.97 and 0.92 in the ^1H NMR spectrum. Esterification of the acid **5** with diazomethane, followed by hydrogenation of the corresponding diester **6** over 10% palladium on charcoal, resulted in the formation on the γ-lactone **7** (IR 1765 cm^{-1}). The secondary alcohol function-

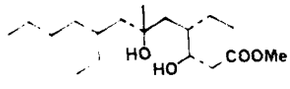


4 R = CHO
5 R = COOH
6 R = COOMe

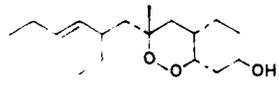


7 R = H
8 R = Ac

ality of the lactone **7** was acetylated with acetic anhydride in pyridine to obtain the corresponding acetate **8**. Hydrogenation of plakortin (1) under identical conditions resulted in the formation of a dihydroxy ester (**9**) which did not cyclize to a lactone, indicating that the ester which had resulted from cleavage of the olefin was involved in γ-lactone formation with the oxygen on the fully substituted carbon atom. Since the olefinic proton at δ 5.10 in plakortin (1) was coupled to only one nonolefinic proton, there must be an alkyl group at C-8.



9



10

Reduction of plakortin (1) with lithium tri-*tert*-butoxyaluminum hydride in refluxing ether resulted in reduction of the ester group, but not the peroxide bond, to obtain a primary alcohol **10**. The mutually coupled signals at δ 2.35 and 3.05 in the ^1H NMR spectrum of plakortin (1) were absent from the ^1H NMR spectrum of the alcohol **10**, suggesting that these signals were due to a methylene group situated between the

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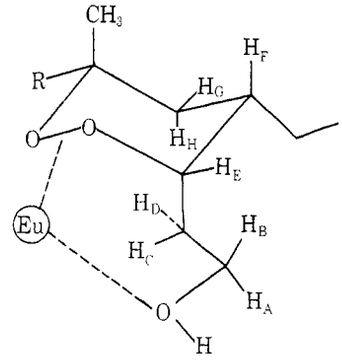
Table I. Chemical Shifts (δ), Eu(fod)$_3$-Induced Chemical Shifts ($\Delta\delta$), and Calculated and Measured (Using Dreiding Model) Eu–hydrogen Distances for Selected Hydrogen Atoms in the ^{1}H NMR Spectrum of Alcohol 10



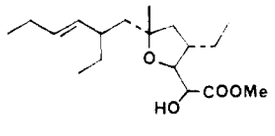
	δ, ppm	$\Delta\delta$, ppm	r_{calcd}, Å	r_{meas}, Å
H_A	3.84	*a*		
H_B	3.84	*a*		
H_C	~2.3	7.66	5.5	5.4
H_D	~1.8	4.83	6.4	6.2
H_E	4.11	5.77	6.0	6.0
H_F	2.2	3.13	7.4	7.8
H_G	~1.5	2.28	8.2	8.4
H_H	~1.5	3.71	7.0	7.3
CH_3-ax	1.39	2.28	8.2	7.8
CH_3-eq				6.6

[a] Variation of chemical shift with concentration of Eu(fod)$_3$ added was not linear.

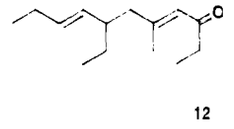
carboxylic ester and the carbon bearing the peroxide functionality. Since the proton signal at δ 4.49 in **1** was coupled to three other protons, there must be a side chain at C-4. A lanthanide-induced shift (LIS) study on the alcohol **10** (see below) clearly showed the presence of a six-membered peroxide ring. Thus both alkyl side chains at C-4 and C-8 must be ethyl groups, allowing the structure **1** to be drawn.

Some stereochemical information could be obtained from interpretation of the LIS data. The protons at C-5 could be resolved into two signals with coupling constants of 13 and 12 Hz and 13 and 4 Hz, respectively. These coupling constants are typical of methylene protons in a six-membered ring which are coupled to a single axial proton. The coupling between the protons at C-3 and C-4 (6 Hz) was best observed in the spectrum of plakortin (**1**) and indicated an equatorial proton at C-3. A semiquantitative analysis of the LIS data (Table I) allowed an assignment of a europium ion position and confirmed these stereochemical assignments. The relative stereochemistry at C-8 has not been determined.

On treatment with sodium methoxide in methanol, plakortin (**1**) underwent an interesting rearrangement to an isomeric ether **11**. The ether **11** contained a hydroxyl group



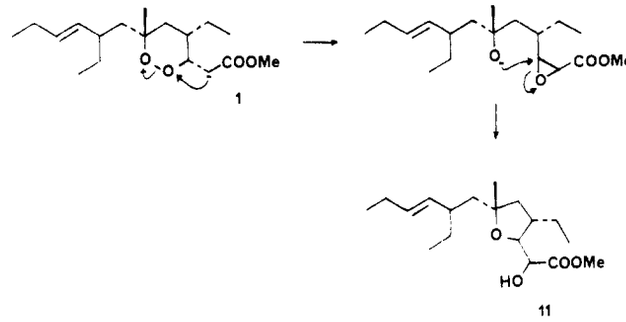
11



12

(IR 3550 cm^{-1}) which was shown to be at C-2. The ^{1}H NMR spectrum of **11** contained a signal at δ 4.34 (d, 1 H, J = 2.5 Hz) which was shifted to δ 5.10 on acetylation; this signal was assigned to the C-2 proton of an α-hydroxy ester. A spin-decoupling study on the ether **11** revealed that the α-hydroxy proton at C-2 was coupled to a proton at δ 3.80, which was in turn coupled to a single proton at δ 2.32 by a 10-Hz coupling constant. The proton at 2.32 ppm was in turn coupled to two

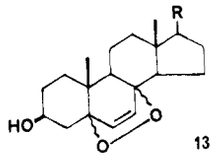
Scheme I. A Mechanism for the Rearrangement of Plakortin (1) to Alcohol 11



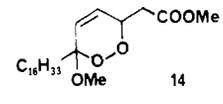
mutually coupled methylene protons at 1.91 and 1.36 ppm. A mechanism for the rearrangement is suggested in Scheme I.

The sponge contained a ketone **12** as a minor metabolite. The structure of ketone **12**, $C_{14}H_{24}O$, was assigned on the basis of its spectral data. The ultraviolet [λ_{max} (MeOH) 237 nm (ϵ 18 900)] and infrared (1690 cm^{-1}) spectra indicated the presence of an α,β-unsaturated ketone. The ^{1}H NMR spectrum contained four methyl signals at δ 2.10 (s, 3 H), 1.07 (t, 3 H, J = 7 Hz), 0.95 (t, 3 H, J = 7 Hz), and 0.85 (t, 3 H, J = 7 Hz), a methylene quartet at 2.44, and three olefinic protons at 6.00 (s, 1 H), 5.39 (dt, 1 H, J = 15, 6, 6 Hz), and 5.07 (dd, 1 H, J = 15, 9 Hz). The methyl triplet at δ 1.07 and the methylene quartet at δ 2.44 suggest the presence of an ethyl ketone, while the singlets at δ 6.00 and 2.10 are due to a proton at C-4 and a methyl group at C-5 which lie cis to the carbonyl group. All other features of the ^{1}H NMR spectrum and the ^{13}C NMR spectrum are consistent with the remaining portion of the ketone **12** being identical with the eight-carbon side chain in plakortin (**1**).

Cyclic peroxides of steroids having the general structure **13** have been found in many sponges.[4] Since the steroidal peroxides were found as mixtures of α and β peroxide isomers in



13



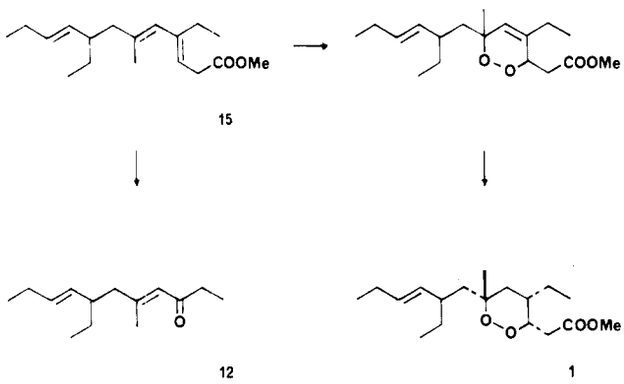
14

the same 85:15 ratio that was obtained by photooxidation of ergosterol,[5] it has been suggested that the cyclic peroxides were autoxidation products of steroidal 5,7-dienes. The cyclic peroxide chondrillin (**14**) was shown to be optically active and must therefore be formed by an enzyme-mediated addition of oxygen to the corresponding diene.[6] Since plakortin (**1**) was also optically active and was not a mixture of diastereoisomers, it must be assumed that plakortin (**1**) was formed by enzyme-mediated reactions. The isolation of the ketone **12** as a minor product has led to the suggestion that both the ketone **12** and plakortin (**1**) might be derived from a common 1,3-diene intermediate **15** (Scheme II). The carbon skeleton of plakortin (**1**) has not previously been described.

Experimental Section

Melting points were measured on a Fisher-Johns apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian HR-220 and CFT-20 instruments, respectively. Infrared and ultraviolet spectra were recorded on Perkin-Elmer Model 136 and 124 spectrophotometers, respectively. Optical rotations were measured on a Perkin-Elmer 141 polarimeter, using a 10-cm cell thermostated at 20 °C. Low-resolution mass spectra were recorded on a Hewlett-Packard 5930-A mass spectrometer. High-resolution mass measurements were obtained from the Analytical Facility at California Institute of Technology. All solvents used were either spectral grade or redistilled from glass prior to use.

Scheme II. Both Plakortin (1) and the Ketone 12 Can Be Derived from a Common Intermediate (15)



Extraction and Chromatography. *Plakortis halichondrioides* (Wilson) was collected by hand, using SCUBA (−10 m), at Hookers Reef, San Blas, Panama (9° 33′ 35″ N, 79° 41′ W) and stored in ethanol for ~1 yr. The sponge (49 g dry weight) was homogenized in ethanol and filtered. The solid was exhaustively extracted with ethanol in a Soxhlet extractor, and the combined ethanol extracts were evaporated to a gum. The organic material was partitioned between water and ether to obtain a crude ether extract (6.9 g). The ether extract (5.1 g) was chromatographed on a Florisil column using a sequence of solvents of increasing polarity from hexane through ether and ethyl acetate to methanol. A fraction eluted with ether was further purified by LC on μ-Porasil using 4% ether in hexane to obtain the ketone **12** (90 mg, 0.25% dry weight). Fractions eluted with 5–20% ethyl acetate in ether gave plakortin (**1**; 2.08 g, 5.2% dry weight), which was essentially pure but which could be further purified by LC on μ-Porasil using 7% ether in hexane.

Plakortin (Methyl 4,8-diethyl-6-methyl-3,6-peroxy-9-dodecenoate, 1): $[\alpha]_D^{20}$ +189° (*c* 2.9, $CHCl_3$); IR (CCl_4) 1735, 1470, 1450, 1390, 1000, 975 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (t, 3 H, *J* = 7 Hz), 0.90 (t, 3 H, *J* = 7 Hz), 0.97 (t, 3 H, *J* = 7 Hz), 1.37 (s, 3 H), 1.55 (dd, 1 H, *J* = 13, 4 Hz), 2.05 (m, 3 H), 2.18 (m, 1 H), 2.35 (dd, 1 H, *J* = 15.5, 3.5 Hz), 3.05 (dd, 1 H, *J* = 15.5, 9.5 Hz), 3.70 (s, 3 H), 4.49 (m, 1 H, *J* = 9.5, 6, 3.5 Hz), 5.10 (dd, 1 H, *J* = 15, 9 Hz), 5.38 (dt, 1 H, *J* = 15, 6, 6 Hz); ^{13}C NMR ($CDCl_3$) δ 11.0 (q), 11.5 (q), 13.9 (q), 21.3 (q), 25.2 (t), 29.5 (t), 29.9 (t), 31.4 (t), 34.9 (d), 36.0 (t), 40.2 (d), 46.5 (t), 51.5 (q), 78.8 (d), 81.0 (s), 131.5 (d), 134.4 (d), 171.9 (s); high-resolution mass measurement 312.228, $C_{18}H_{32}O_4$ requires 312.230.

7-Ethyl-5-methyl-4,8-undecadien-3-one (12): $[\alpha]_D^{20}$ +17° (*c* 1.4, $CHCl_3$); UV (MeOH) 237 nm (ε 18 900); IR (CCl_4) 1690, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, 3 H, *J* = 7 Hz), 0.95 (t, 3 H, *J* = 7 Hz), 1.07 (t, 3 H, *J* = 7 Hz), 2.10 (s, 3 H), 2.44 (q, 2 H, *J* = 7 Hz), 5.07 (dd, 1 H, *J* = 15, 9 Hz), 5.39 (dt, 1 H, *J* = 15, 6, 6 Hz), 6.00 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 8.2 (q), 11.6 (q), 14.1 (q), 19.6 (q), 25.6 (t), 28.0 (t), 37.5 (t), 42.6 (d), 47.4 (t), 124.4 (d), 132.1 (d), 132.7 (d), 156.9 (s), 201.6 (s); high-resolution mass measurement 208.181, $C_{14}H_{24}O$ requires 208.183.

Reduction of Plakortin (1) with Lithium Aluminum Hydride. Lithium aluminum hydride (20 mg, 0.53 mmol) was added to a stirred solution of plakortin (**1**; 30 mg, 0.096 mmol) in dry ether at 0 °C. After stirring for 15 min, the excess reagent was destroyed with ethyl acetate and the product was partitioned between ether and dilute hydrochloric acid. The ether extract was dried over anhydrous sodium sulfate and the solvent evaporated to yield a crude alcohol mixture (23 mg). The mixture of alcohols was separated on a silica gel plate to obtain the triol **2** (15 mg, 55% theoretical) and the alcohol **10** (2 mg). The triol **2** gave the following spectral data: IR (CCl_4) 3225, 1470, 1390, 975, 880 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (t, 3 H, *J* = 7 Hz), 0.91 (t, 3 H, *J* = 7 Hz), 0.98 (t, 3 H, *J* = 7 Hz), 1.19 (s, 3 H), 3.85 (m, 3 H), 5.22 (dd, 1 H, *J* = 15, 9 Hz), 5.55 (dt, 1 H, *J* = 15, 6, 6 Hz).

A portion of the triol (10 mg, 0.035 mmol) was dissolved in a mixture of acetic anhydride (1 mL) and pyridine (2 mL) and the solution was allowed to stand overnight at room temperature. Evaporation of the reagents in vacuo gave a residue which was partitioned between ether and water. The ether extract was dried over sodium sulfate and the solvent evaporated to give a residue (11 mg) which was purified by LC on μ-Porasil using 40% ether in hexane as eluent to obtain the diacetate **3** (9 mg, 70% theoretical): IR (CCl_4) 3450, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 (t, 3 H, *J* = 7 Hz), 0.93 (t, 3 H, *J* = 7 Hz), 0.98 (t, 3 H, *J* = 7 Hz), 1.11 (s, 3 H), 2.00 (s, 3 H), 2.04 (s, 3 H), 4.09 (m, 2 H), 5.23 (m, 2 H), 5.50 (dt, 1 H, *J* = 15, 6, 6 Hz); high-resolution mass measurement 370.273, $C_{21}H_{38}O_5$ requires 370.272.

Ozonolysis of Plakortin (1). A stream of ozone in oxygen was bubbled into a solution of plakortin (**1**; 20 mg, 0.064 mmol) in dichloromethane (5 mL) at −78 °C until a blue-colored solution was obtained. Excess ozone was removed in a stream of dry nitrogen. Dimethyl sulfide (0.2 mL) was added, and the solution was allowed to warm to room temperature. After 30 min, the solvents were removed in vacuo to obtain a yellow oil. Chromatography of the product on a silica gel plate using 1:1 hexane–ether gave the aldehyde **4** (9 mg, 46% theoretical) and the acid **5** (7 mg, 36% theoretical). On standing overnight, the aldehyde **4** oxidized to the acid **5**. A solution of diazomethane solution in ether was added to a solution of the acid **5** in ether until the solution remained yellow. Evaporation of the solvent in vacuo gave the methyl ester **6**. In a subsequent experiment, plakortin (**1**; 120 mg, 0.38 mmol) was converted into the ester **6** (115 mg, 0.37 mmol) in 96% yield.

Acid 5: IR (CCl_4) 2665 (br), 1740, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3 H, *J* = 7 Hz), 0.97 (t, 3 H, *J* = 7 Hz), 1.39 (s, 3 H), 2.04 (dd, 1 H, *J* = 15, 10 Hz), 2.23 (m, 1 H), 2.42 (dd, 1 H, *J* = 15.5, 3.5 Hz), 2.50 (m, 1 H), 3.02 (dd, 1 H, *J* = 15.5, 9.5 Hz), 3.40 (s, 3 H), 4.52 (m, 1 H, *J* = 9.5, 6, 3.5 Hz).

Ester 6: ^1H NMR ($CDCl_3$) 0.86 (t, 3 H, *J* = 7 Hz), 0.91 (t, 3 H, *J* = 7 Hz), 1.30 (s, 3 H), 2.01 (dd, 1 H, *J* = 15, 10 Hz), 2.18 (m, 1 H), 2.37 (dd, 1 H, *J* = 15, 3 Hz), 2.50 (m, 1 H), 3.03 (dd, *J* = 15, 9 Hz), 3.67 (s, 3 H), 3.70 (s, 3 H), 4.50 (m, 1 H).

Hydrogenation of Ester 6. Palladium on charcoal catalyst (10%, 10 mg) was added to a solution of the ester **6** (115 mg, 0.37 mmol), and the solution was stirred under an atmosphere of hydrogen overnight. The catalyst was removed by filtration and the solvent evaporated to obtain the lactone **7** as a pale yellow oil (115 mg): IR (CCl_4) 3200, 1765, 1730 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 11.7 (q), 12.0 (q), 23.5 (t), 24 3 (t), 27.6 (q), 37.8 (t), 39.9 (t), 40.3 (t), 40.6 (d), 42.2 (d), 51.8 (q), 69.1 (d), 84.6 (s), 173.5 (s), 173.6 (s). A portion of the lactone **7** (12 mg) was dissolved in a mixture of acetic anhydride (0.5 mL) and pyridine (1.0 mL), and the solution was allowed to stand overnight. The solvents were removed in vacuo, and the residue was partitioned between ether and water. The organic material was purified by LC on μ-Porasil, using 40% ether in hexane as eluant, to obtain the lactone acetate **8** (7 mg, 56% theoretical): IR (CCl_4) 1765, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (t, 6 H, *J* = 7 Hz), 1.41 (s, 3 H), 2.02 (s, 3 H), 2.26 (dd, 1 H, *J* = 13, 10 Hz), 2.54 (m, 2 H), 2.67 (m, 1 H), 3.67 (s, 3H), 5.41 (m, 1H); high-resolution mass measurement 328.186, $C_{17}H_{28}O_6$ requires 328.188.

Reduction of Plakortin (1) with Lithium Tri-*tert*-butoxyaluminum Hydride. Lithium tri-*tert*-butoxyaluminum hydride (100 mg, 0.39 mmol) was added to a solution of plakortin (**1**; 50 mg, 0.06 mmol) in ether (10 mL), and the solution was boiled under reflux for 2 h. The excess reagent was destroyed by addition of water, and the reaction product was partitioned between ether and dilute hydrochloric acid. The ether extract was dried over anhydrous sodium sulfate and the solvent evaporated to give a colorless oil (48 mg). The oil was purified by LC on μ-Porasil, using 40% ether in hexane as eluant, to obtain the alcohol **10** (38 mg, 85% theoretical): IR (CCl_4) 3310, 1470, 1388, 975 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.79 (t, 3 H, *J* = 7 Hz), 0.87 (t, 3 H, *J* = 7 Hz), 0.98 (t, 3 H, *J* = 7 Hz), 1.39 (s, 3 H), 1.45 (t, 1 H, *J* = 14 Hz), 1.52 (dd, 1 H, *J* = 14, 5 Hz), 2.04 (m, 5 H), 2.20 (m, 1 H), 3.84 (t, 2 H, *J* = 6 Hz), 4.11 (m, 1 H), 5.09 (dd, 1 H, *J* = 15, 9 Hz), 5.36 (dt, 1 H, *J* = 15, 6, 6 Hz).

Lanthanide-Induced Shift Experiment. A solution of the alcohol **10** (7 mg) in deuteriochloroform (500 μL) was prepared. NMR spectra (220 MHz) were recorded after each addition (5 μL) of a solution of $Eu(fod)_3$ (27 mg) in deuteriochloroform (58 μL). The induced shifts (Δδ) were deduced by plotting the chemical shift of each proton signal against the quantity of reagent added. The induced shifts are summarized in Table I.

Treatment of Plakortin (1) with Sodium Methoxide. Plakortin (**1**; 25 mg, 0.08 mmol) was added to a 1 N solution of sodium methoxide in methanol (10 mL), and the solution was allowed to stand at room temperature for 3 h. The base was neutralized by addition of dry ice and the solvent evaporated. The ether-soluble material (22 mg) was essentially one compound which was purified by LC to obtain the alcohol **11** (14 mg, 56% theoretical): IR (CCl_4) 3550, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 (t, 3 H, *J* = 7 Hz), 0.86 (t, 3 H, *J* = 7 Hz), 0.98 (t, 3 H, *J* = 7 Hz), 1.14 (s, 3 H), 1.36 (m, 1 H), 1.68 (m, 1 H), 1.91 (m, 1 H), 2.02 (m, 3 H), 2.32 (m, 1 H), 3.77 (s, 3 H), 3.80 (dd, 1 H, *J* = 10, 2.5 Hz), 4.34 (d, 1 H, *J* = 2.5 Hz), 5.14 (dd, 1 H, *J* = 15, 9 Hz), 5.34 (dt, 1 H, *J* = 15, 6, 6 Hz); ^{13}C NMR ($CDCl_3$) 12.6, 13.6, 13.9, 25.2, 25.6, 27.4, 29.7, 40.8, 41.6, 44.7, 47.9, 52.1, 71.9, 83.3, 84.8, 131.3, 134.3, 172.7.

Acetylation of Alcohol 11. A solution of the alcohol **11** (8 mg) in acetic anhydride (0.2 mL) and pyridine (0.3 mL) was allowed to stand at room temperature for 18 h. The solvent was removed in vacuo to obtain the corresponding acetate: ^1H NMR ($CDCl_3$) δ 0.82 (t, 3 H, *J* = 7 Hz), 0.92 (t, 3 H, *J* = 7 Hz), 0.97 (t, 3 H, *J* = 7 Hz), 1.10 (s, 3 H),

2.15 (s, 3 H), 3.71 (s, 3 H), 3.81 (dd, 1 H, J = 10, 2.5 Mz) 5.10 (d, 1 H, J = 2.5 Hz), 5.15 (m, 1 H), 5.35 (m, 1 H).

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Registry No.—1, 66940-35-0; 2, 66940-36-1; 3, 66940-37-2; 4, 66940-38-3; 5, 66940-39-4; 6, 66940-40-7; 7, 66940-41-8; 8, 66940-42-9; 10, 66940-43-0; 11, 66940-44-1; 11 acetate, 66940-45-2; 12, 66984-56-3.

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Biosynthesis of the Anthracycline Antibiotics Nogalamycin and Steffimycin B

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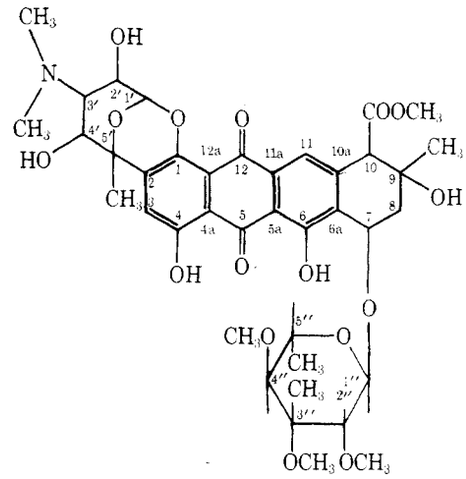
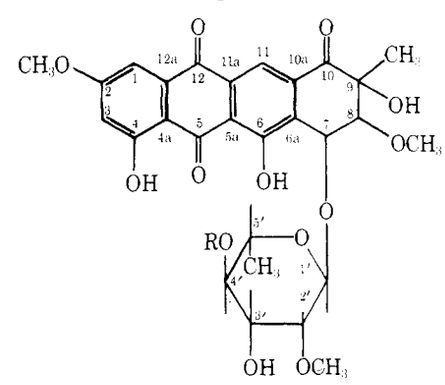
It has been shown that the aglycones of nogalamycin (1) and steffimycin B (3) arise from ten acetate units starting with the methyl groups at C-9. The neutral sugars are derived from glucose, while CH_3O and CH_3N methyl groups come from methionine.

The antibiotic nogalamycin (1) has been of interest as an antitumor agent for a number of years.[1] Some of its conversion products are even more active in this respect, and their antitumor properties are being extensively investigated.[2] Furthermore, 1 is a member of the anthracycline antibiotic family of which one member, adriamycin, is widely used in cancer chemotherapy.[3] Steffimycin (2) and steffimycin B (3) are also anthracycline antibiotics although they are only very modestly active as antitumor agents. However, the steffimycins are members of a subgroup of three anthracyclines whose structures differ markedly from the other anthracyclines. For these reasons it was felt that it would be worthwhile to investigate the biosynthesis of 1, 2, and 3 and compare their biosynthesis with those of daunomycin[4] and ϵ-pyrromycinone[5] which have already been reported. In the case of daunomycin only biosynthesis of the aglycone was established, but in the present work the biosynthesis of the sugars was also studied.

The procedure utilized to study the biosynthetic pathways of 1 and 3 was addition of ^{13}C-labeled compounds which might logically be expected to act as antibiotic precursors to fermentations of *Streptomyces nogalater*, UC-2783, and *Streptomyces elgreteus*, UC-5453, grown on minimal media. The ^{13}C-enriched 1 and 3 formed by *S. nogalater* and *S. elgreteus*, respectively, was isolated, and the positions of the ^{13}C-enriched carbon atoms established by ^{13}C NMR spectra. As a result of previous work[4,5] and current concepts of biosynthesis, it seemed very probable that both aglycones would be built completely from acetate units. For example, it has been shown[4] that the aglycone of daunomycin arises through a polyketide intermediate derived from acetate and one unit of propionate with loss of the terminal carboxyl group. Ollis and co-workers[5] have proposed a similar biosynthetic pathway for ϵ-pyrromycinone, the aglycone of rutilantin. A common biosynthetic pathway for formation of hydroxyanthraquinones by fungi is the condensation of ten acetate units.[6] Accordingly, *S. nogalater* and *S. elgreteus* fermentations in appropriate carbon-poor media were enriched with $CH_3{}^{13}COONa$ and $^{13}CH_3COONa$ to give 1 and 3 labeled with



1



2, R = H
3, R = CH_3

^{13}C. Isolation of the products was carried out, and ^{13}C NMR spectra were obtained to establish the positions of the carbon atoms enriched with ^{13}C. Similar procedures were used, but

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