Reaction of Protoadamantene-4-d (1-4-d) with Trifluoroacetic Acid. Protoadamantene-4-*d* (1-4-*d*) (100 mg, 0.75 mmol) was stirred in 10 g of trifluoroacetic acid at 25 °C for 6 h. The solution was then poured into 200 mL of 20% methanolic potassium hydroxide and stirred for 12 h. The bulk of the methanol was removed by rotary evaporation, and the residue was poured into 1 L of water and extracted with ether. The ether extracts were combined, washed with water, and dried ($MgSO_4$). Rotary evaporation of the ether left a white solid. GLC of the crude product on column B at 150 °C showed one peak, identified as 2-adamantanol by coinjection with authentic material. The crude product was then purified by recrystallization from hexane at −78 °C, giving 108 mg (0.71 mmol, 95%) of 2-adamantanol: mp (sealed tube) 258–260 °C. The shift-enhanced ^{2}H NMR spectrum (15 mg of substrate + 70 mg of $Pr(fod)_3$ in 400 μL of $CHCl_3$) consisted of a single signal at δ −8.7, distinct from and intermediate between the chemical shifts established for 2-adamantanol-2-*d* (δ −20.3) and the four 2-adamantanols-4-*d* (δ −2.0 to −7.3)[22] under these conditions. Oxidation of this product with Jones reagent[22] and mass spectral analysis of the ketone (M^+s at *m/e* 151 and 150) showed the material to consist of 85.8% monodeuterated and 14.2% undeuterated compound, corresponding to the isotopic composition of the starting olefin (1-4-*d*).

Reaction of Protoadamantene (1) with Trifluoroacetic Acid-d. A 100 mg (0.75 mmol) sample of protoadamantene (1) was stirred in 10 g of trifluoroacetic acid-*d* at 25 °C for 1 h. The solution was then poured into 200 mL of 20% methanolic KOH and stirred for 12 h. Workup as described above for the addition of trifluoroacetic acid to protoadamantene-4-*d* furnished 110 mg (0.72 mmol, 96%) of 2-adamantanol-4-*d*: mp (sealed tube) 258–260 °C. The ^{2}H NMR spectrum (15 mg of substrate + 70 mg of $Pr(fod)_3$ in 400 μL of $CHCl_3$) showed 4 signals corresponding[22] to the following product composition: 11-OH (δ −7.27), 68%; 12-OH (δ −4.13), 17%; 13-OH (δ −2.70), 9%; 14-OH (δ −1.95), 6%. Jones oxidation[22] of this product and mass spectral analysis of the ketone (M^+s at *m/e* 151 and 150) showed the material to consist of 96% monodeuterated and 4% undeuterated compound.

Repetition of the addition reaction over 6 h and identical analysis gave the same results.

Kinetics of the Addition of Trifluoroacetic Acid to Protoadamantene (1). Protoadamantene (100 mg, 0.75 mmol) was dissolved in 100 μL of dry methylene chloride and 10 μL of decane in a stoppered 20-mL test tube. This solution was placed in a constant temperature bath at 25 °C, and after 0.5 h 10 g of trifluoroacetic acid (preequilibrated at 25 °C) was added and the solution was shaken for 10 s. Five samples of approximately 1 mL were then withdrawn from the solution at approximately 10-s intervals and quenched by injection into 150-mL portions of ice water. The reaction time for each sample was carefully noted. Each of the product mixtures was then extracted with ether (3 × 50 mL), and the extracts were washed with saturated aqueous $NaHCO_3$ and dried ($MgSO_4$). The dried extracts were analyzed for remaining protoadamantene by GLC on column A by using the decane peak as internal integration standard. Each analysis was run at least in triplicate. Linear least-squares first-order kinetic analysis gave a rate constant $k_1 = 5.3 \times 10^{-2}$ s^{-1}, correlation coefficient 0.997.

Kinetics of the Addition of Trifluoroacetic Acid to Cyclohexene and Cycloheptene. Cyclohexene (300 μL, 0.243 g, 2.9 mmol) was dissolved in 50 μL of chloroform in a 20-mL test tube in a constant-temperature bath at 25.2 °C. After 30 min 10.0 g of trifluoroacetic acid (preequilibrated to the same temperature) was added, and the solution was shaken for 10 s. A 0.5-mL sample of this solution was transferred to a 5-mm NMR tube, and the ^{1}H NMR spectrum was recorded at 6 measured times over a 90-min duration on the A-60A spectrometer with the sample temperature maintained at 25.2 °C. The progress of the reaction was followed by comparative integration of the vinylic proton and chloroform peaks. Linear least-squares first-order kinetic treatment gave $k_1 = 1.29 \times 10^{-4}$ s^{-1}, correlation coefficient 0.996.

The same measurements with cycloheptene yielded $k_1 = 4.87 \times 10^{-4}$ s^{-1}, correlation coefficient 0.997.

Kinetics of the Trifluoroacetolysis of exo-4-Protoadamantyl Trifluoroacetate (4-OTFA). *exo*-4-Protoadamantyl trifluoroacetate (151 mg, 0.61 mmol) was dissolved in a solution of 50 mg of 1-adamantyl trifluoroacetate (internal integration reference) in 100 μL of methylene chloride. This solution was brought to 25.2 °C in a constant temperature bath, 10 g of trifluoroacetic acid was added, and the solution was shaken for 10 s. Six samples of approximately 1 mL were then withdrawn from the solution at approximately 2-min intervals (including a zero-time sample) and quenched at measured times by injection into 150 mL of ice water. Each of the quenched samples was extracted with methylene chloride (3 × 10 mL), and the extracts were washed with saturated aqueous $NaHCO_3$ and dried ($MgSO_4$). The bulk of the methylene chloride was then removed from each solution by careful distillation through a fractionating column down to a volume of ca. 0.5 mL. The residual solution was dissolved in acetone-d_6 and analyzed by ^{19}F NMR, integrating the *exo*-4-protoadamantyl trifluoroacetate peak at δ 0.25 against the 1-adamantyl trifluoroacetate peak at δ 0.01. First-order kinetic analysis gave $k_1 = 7.40 \times 10^{-4}$ s^{-1}, correlation coefficient 0.979.

Short-Term Reaction of Protoadamantene (1) with Trifluoroacetic Acid. Protoadamantene (100 mg, 0.75 mmol) was stirred in 10 g of trifluoroacetic acid at 25 °C for 5.0 min. The solution was then poured into 300 mL of ice water and worked up in the same manner as in the longer term reaction above, giving 108 mg of a yellow oil as crude product. The ^{19}F NMR spectrum of this material consisted of two signals in the ratio of 99:1, the major signal at δ 0.55 having the chemical shift of 2-adamantyl trifluoroacetate, with the minor signal at δ 0.25 having that of *exo*-4-protoadamantyl trifluoroacetate.

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Structure and Biosynthesis of Setomimycin. A Novel 9,9′-Bianthryl Antibiotic

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Abstract: The structure of an antibacterial and antitumor antibiotic setomimycin, produced by *Streptomyces pseudovenezuelae* AM-2947, was determined to be a unique substituted 9,9′-bianthryl 1 by means of various new ^{13}C NMR techniques including ^{13}C {^{1}H} selective decoupling, ^{13}C {^{1}H} selective population transfer, and ^{13}C {^{1}H} NOE experiments. The biosynthetic studies were also carried out by labeling with [1-^{13}C]- and [1,2-$^{13}C_2$]sodium acetates. The labeling pattern was determined by the ^{13}C–^{13}C coupling constants with the aid of ^{13}C {^{13}C} homonuclear decoupling experiments, which allowed for the elucidation that **1** is derived from two nonaketide metabolites via decarboxylation at the terminals.

Setomimycin, produced by *Streptomyces pseudovenezuelae* AM-2947, shows antimicrobial activities against Gram positive bacteria including *Mycobacteria* as well as antitumor activity against solid Sarcoma 180 in mice.[2] Described in this paper are

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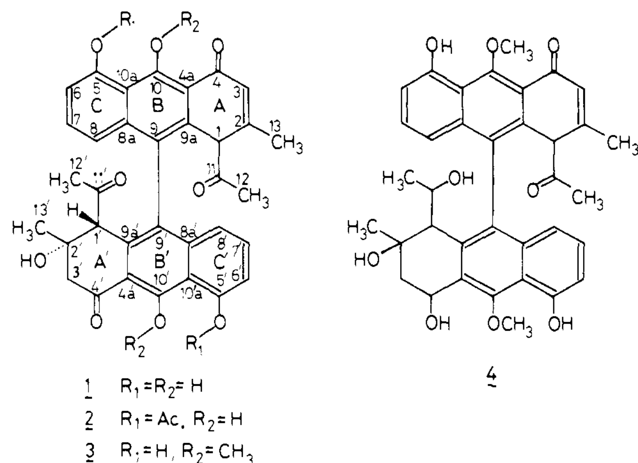


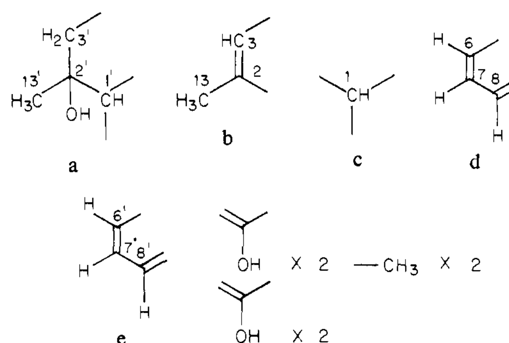
Figure 1. Structures of setomimycin (1), O-diacetylsetomimycin (2), O-dimethylsetomimycin (3), and tetrahydro-O-dimethylsetomimycin (4).

the structure of this novel 9,9′-bianthryl antibiotic as shown in Figure 1, first to be reported from *Actinomycetes*, which has been elucidated by application of various modern ^{13}C NMR techniques, especially useful to characterize nonprotonated carbons, and the biosynthetic studies carried out by the ^{13}C labeling method, which enabled us to show the incorporation pattern and also to confirm the assignment of the ^{13}C NMR spectrum and the proposed structure as well.

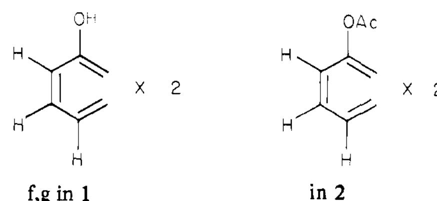
Structure Determination

Setomimycin (**1**), $C_{34}H_{28}O_9$, gave O-diacetylsetomimycin (**2**), $C_{38}H_{32}O_{11}$, by brief acetylation[2] and treatment of **1** with ethereal diazomethane provided O-dimethylsetomimycin (**3**), $C_{36}H_{32}O_9$, mp 239–240 °C, EIMS, m/z 608 (M^+). In the ^1H NMR spectrum of **1**, no first-order couplings were observed on the signals in the region of 1–5 ppm, which included four methyl singlets (δ_H 1.23, 1.41, 1.69 and 2.00), a D_2O exchangeable broad singlet (δ_H 2.98), and two methine singlets (δ_H 3.70 br, and δ_H 4.00), except for a pair of AB type methylene doublets (δ_H 2.65 br, J = 18 Hz and δ_H 3.15 br, J = 18 Hz). However, decoupling experiments evidenced two W-type long-range couplings between the methylene proton (δ_H 2.65, H-3′ eq) and a methine proton (δ_H 3.70, H-1′) and between the other methylene proton (δ_H 3.15, H-3′ax) and a methyl signal (δ_H 1.23, H-13′), and the pivotal carbon of these couplings was assigned to a tertiary carbinol (δ_C 71.7, C-2′) since it was the only nonprotonated sp^3 carbon in **1**. An allylic coupling was also detected between a methyl signal (δ_H 2.00, H-13) and an olefinic proton (δ_H 6.28 br s, H-3). In the aromatic proton region, two ABC spin systems were observed, i.e., a triplet (δ_H 7.26, J = 8 Hz, H-7′) coupled with a doublet (δ_H 6.28, H-8′) and with another doublet (δ_H 6.88, H-6′), and likewise, the other triplet (δ_H 7.40, J = 8 Hz, H-7) coupled with two doublets (δ_H 6.66, H-8, and δ_H 6.98, H-6). In addition, two pairs of phenolic hydroxyl signals were shown at δ_H 9.94, 10.04 and δ_H 16.30, 16.34, while the former two signals disappeared in the spectra of **2**,[2] and the latter pair was lacking in the spectra of **3**. These NMR data suggested the following structural units.

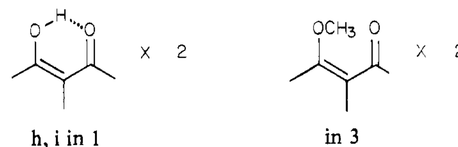
The ^{13}C NMR spectrum of **1** evidently displayed pairs of signals suggesting a dimeric structure except those due to the structural units **a** and **b**. If this is the case, the isolated methine group (δ_H 4.00, H-1), unit **c**, may be attached to C-2 of the unit **b** since dehydration of the unit **a** of a conceivable precursor would give rise to the unit **b**, and this extension was in fact confirmed by a 27% $^{13}C\{^1H\}$ NOE enhancement of an olefinic carbon signal (δ_C 158.0, C-2) by irradiation at the H-1 methine proton.



Phenolic hydroxyl groups were suggested on C-5 and C-5′ adjacent to the units **d** and **e**, respectively, because long-range C-H couplings ($^3J_{CCOH}$ = 7.4 Hz) observed on the C-6 and C-6′ signals (δ_C 112.4, coincided) disappeared by D_2O addition. The same couplings observed on **3** suggested again hydroxyl groups on C-5 and C-5′ of **3** rather than methoxyl groups. This argument was further supported by downfield shifts (0.5–0.6 ppm) of the H-8 and H-8′ signals of **1** to δ_H 7.20 and δ_H 6.72, respectively, by acetylation to **2**.[4]

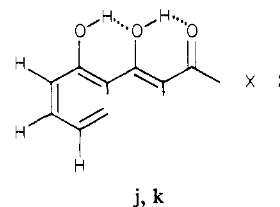


By comparison of the ^{13}C NMR spectra of **1** and **3**, distinct upfield shifts due to the loss of hydrogen bondings were observed on two carbonyl signals (δ_C 190.8 and 203.3) and on two phenolic carbon signals (δ_C 164.4 and 166.3),[5] on the other hand, the nonprotonated carbon signals at δ_C 108.4 and 109.2, attributable to the C-2 carbons of the enol forms of 1,3-diketone systems,[6] were conversely shifted downfield. These observations are consistent with the following structural units.



The infrared absorption shifts of the aromatic carbonyl groups also supported this assignment, i.e., two bands (1650 and 1630 cm^{-1}) of **1** shifted to 1690 and 1660 cm^{-1} in **3**, while the other two bands (1725 and 1710 cm^{-1}) remained unchanged.

Since the methoxyl groups of **3** were not located on C-5 and C-5′ as discussed above and since two nonprotonated carbon signals at δ_C 112.8 and 113.2 of **1** were attributable to sp^2 carbons flanked by oxygenated sp^2 carbons,[7] the partial structures of **f** and **g** were further extended as follows by connecting them with the units **h** and **i**.



(1) (a) Tokyo Institute of Technology, (b) Kitasato University, (c) The Kitasato Institute.

(2) Ōmura, S.; Tanaka, H.; Iwai, Y.; Nishigaki, K.; Awaya, J.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1978**, *31*, 1091.
(3) Seto, H.; Sasaki, T.; Yonehara, H.; Uzawa, J. *Tetrahedron Lett.* **1978**, 502.
(4) Durley, R. G.; MacMillan, J.; Simpson, T. J.; Glen, A. T.; Yurner, W. B. *J. Chem. Soc., Perkin Trans. 1*, **1975**, 163.
(5) Deshmukh, P. V.; Kakinuma, K.; Ameel, J. J.; Rinehart, K. L., Jr.; Wiley, P. F.; Li, L. H. *J. Am. Chem. Soc.* **1976**, *98*, 870.
(6) (a) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 289; (b) Kakinuma, K.; Milavetz, B. I.; Rinehart, K. L, Jr. *J. Org. Chem.* **1976**, *41*, 1358.
(7) Reference 6a, pp 183 and 196.

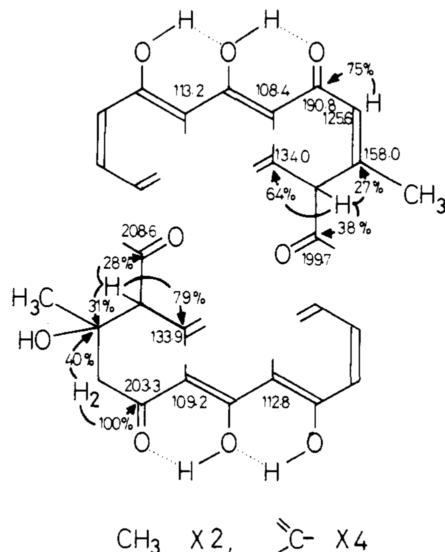


Figure 2. Summarized results of ^{13}C {^1H} NOE experiments.

Proximity of the two kinds of phenolic hydroxyl groups was supported by the upfield shifts of the hydroxyl proton signals of **1** to δ_H 15.24 and 15.36 in **2**.

Further connection of the units **j** and **k** with the units **a** and **b** was provided by the ^{13}C {^1H} NOE experiments summarized in Figure 2.[3]

The two remaining methyl groups were assigned to acetyl groups by the ^{13}C {^1H} selective population transfer experiments.[8,9] Thus, irradiation either at δ_H 1.38 or δ_H 1.44 around the methyl proton resonance (δ_H 1.41) caused the signal alteration of a carbonyl carbon at δ_C 199.7, as shown in Figure 3. Likewise, another acetyl carbonyl carbon was assigned to a signal at δ_C 208.6 by focusing on the methyl signal at δ_H 1.69. It is worthwhile to note that these methyl chemical shifts were rather unusual for acetyl groups.

One of the acetyl groups was chemically determined, i.e., $NaBH_4$ reduction of **3** gave a complicated mixture of products, from which tetrahydro-*O*-dimethylsetomimycin (**4**), $C_{36}H_{36}O_9$, EIMS, *m/z* 594 ($M^+ - H_2O$), was isolated. The structure of **4** was tentatively assigned as shown in Figure 1, based on the ^1H NMR data.[10] A methyl signal of **4** was observed as a doublet at δ_H 0.84 (J = 6.8 Hz) and the corresponding carbinol proton appeared as a broad quartet at δ_H 4.78 (J = 6.8 Hz).

The remaining nonprotonated sp^2 carbons were finally clarified by the low power ^{13}C {^1H} selective decoupling experiments.[9] The fact that irradiation at H-8′ (δ_H 6.27) affected singal sharpening of the C-8a′ doublet and collapsing of the C-9′ triplet into a doublet as shown in Figure 4 is evidence that C-8a′ (δ_C 138.5) and C-9′ (δ_C 124.1) were located at the two bond and three bond positions, respectively, from H-8′. Furthermore, irradiation at H-8′ sharpened the C-10a′ signal (δ_C 112.8) and simultaneous irradiation at H-3 decoupled the C-4a signal (δ_C 108.4). Also shown are the results obtained by irradiation at the H-1 and H-1′ methine protons. Similarly, C-8a (δ_C 136.9) and C-9 (δ_C 125.0) were decoupled by irradiation at H-8 (δ_H 6.66). Two phenolic arbons (C-5 and C-5′) were also clearly differenciated by decoupling of the H-7 and H-7′ proton signals. It is significant that the C-9′ triplet was collapsed into a doublet by irradiation either at H-1′ or at H-8′, which allowed us to assign the C-9′ location of the three bond position from both H-1′ and H-8′, resulting in a substituted hydroanthracene structure as shown in Figure 4. Since the other substituted hydroanthracene unit was similarly deduced, the resulting two hydroanthracene units of **1** must be assembled at C-9 and C-9′ to give ultimately a dimeric bianthryl structure for **1** as shown in Figure 1.

This 9,9′-bianthryl structure of **1** rationalized the aforementioned anomalous chemical shifts of the acetyl methyl protons by using the shielding effects of the aromatic rings; it is interesting that the rotation around the axis through C-9 and C-9′ seems strongly restricted, which may contribute the high optical rotation, $[\alpha]^{24}_D$ +502° (*c* 1.0, $CHCl_3$).[2]

So far, a series of 2,2′-bianthryl antibiotic pigments are known from *Streptomyces shiodaensis* as julimycins and julichromes,[11] e.g., julichrome $Q_{5,6}$ (**5**) in Figure 5, whose basic carbon skeletons seem quite similar to that of **1**, but none of the monomeric forms (Q_1 through Q_9) of julichromes are identical with those of **1**. Though the stereochemistry of **1** has not yet been determined, the relative stereochemistry of the ring A′ of **1** is identical with that of **5** evidenced by the dual W-type proton long-range couplings, as mentioned above. Furthermore, it is worth noting that the chromophores of **1** resemble chromomycinone (**6**) and olivin (**7**), chromophores of the clinically important aureolic acid group of antitumor antibiotics,[12] which are also active against Gram positive bacteria but not against Gram negative just like **1**. Since **6** and **7** are supposed to be responsible for association of their parent antibiotics with double helical DNA,[13,14] an analogous mechanism may be likely for the biological activities of **1**.

Biosynthesis

The biosynthesis of **1** is likely to be similar to those of julichromes, though the biosynthesis of the latter has not been reported except for minor speculations.[15] The carbon skeleton of **1** appears to be constructed by the oxidative coupling of substituted hydroanthracene monomers either prior to or after dehydration and the hydroanthracene precursor may well be derived from polyacetate (polymalonate), but the *odd* carbon number of the monomeric skeletons implies loss of one carbon atom from a conceivable polyacetate monomer containing *even* carbon atoms probably by decarboxylation via path A or B in Figure 6. Since the $^{13}C-^{13}C$ double labeling technique has been well established as a useful tool in studying the carbon–carbon bond cleavages by, for example, decarboxylation, fragmentation, or rearrangement during metabolism,[16] this method appeared to be suitable to clarify the actual pathway for **1**. In this case, the C-2 carbon atoms derived from decarboxylated acetate units of [1,2-$^{13}C_2$]-acetate fed would show up as singlet signals in the ^{13}C NMR spectrum by the loss of $^{13}C-^{13}C$ coupling whereas carbon atoms incorporated as intact acetate molecule should have coupling with adjacent carbon. In addition, these $^{13}C-^{13}C$ couplings may further provide firm support for the assigned structure since acceptable coupling constants must be observed between the appropriate carbons.

Feeding experiments were thus undertaken by adding either [1-^{13}C]- or [1,2-$^{13}C_2$]sodium acetate into the growing culture of *Streptomyces pseudovenezuelae* AM-2947, and the resulting ^{13}C-enriched antibiotic was extracted from the culture and purified by repeated preparative layer chromatography. The ^{13}C NMR spectra of the labeled **1** thus obtained are shown in Figure 7. The fact that **1** labeled from [1-^{13}C]sodium acetate showed 16 enriched signals with minute natural abundance peaks clearly suggested that **1** is in fact biosynthesized from two nonaketides each containing necessarily nine carboxyl derived carbon atoms accompanied by the loss of each one of the carboxyl carbons. The site of decarboxylation was then deduced by the [1,2-$^{13}C_2$]sodium acetate labeling. The ^{13}C NMR spectrum of **1** enriched by the doubly labeled acetate distinctly displayed two enriched singlets assigned to the acetyl methyl groups (C-12 and C-12′), whose enrichments were demonstrated by their peak heights, ca. three to four times higher than those of the central natural abundance peaks of two triplets due to the other methyl groups (C-13 and C-13′). All carbons except the abovementioned C-12 and C-12′ methyl groups appeared as triplets, whose couplings were confirmed by the extensive ^{13}C {^{13}C} homonuclear decoupling experiments. The total ^{13}C NMR assignment and the $^{13}C-^{13}C$ coupling constants thus obtained are listed in Table I.

(8) Jakobsen, H. J.; Bildsøe, H. *J. Magn. Reson.* **1977**, *26*, 183.
(9) Uzawa, J.; Uramoto, M. *Org. Magn. Reson.* **1979**, *12*, 612.
(10) Although the structure in which the C-11 acetyl carbonyl group is reduced cannot be ruled out, it is less likely because significant upfield shift of the C-13′ methyl proton signal, δ_H 0.70 in **4**, suggests considerable structural changes around ring A′.

(11) Tsuji, N.; Nagashima, K. *Tetrahedron* **1971**, *27*, 1557.
(12) Remers, W. A. "The Chemistry of Antitumor Antibiotics"; John Wiley and Sons: New York, 1979; Vol. 1, pp 133–175.
(13) Behr, W.; Honikel, K.; Hartmann, G. *Eur. J. Biochem.* **1969**, *9*, 82.
(14) Nayak, R.; Sirsi, M.; Podder, S. K. *Biochem. Biophys. Acta* **1975**, *378*, 195.
(15) Tsuji, N.; Nagasima, K. *Tetrahedron* **1970**, *26*, 5201.
(16) Tanabe, M. "Biosynthesis", A Specialist Periodical Report; The Chemical Society: London, 1976; Vol. 4, p 214.

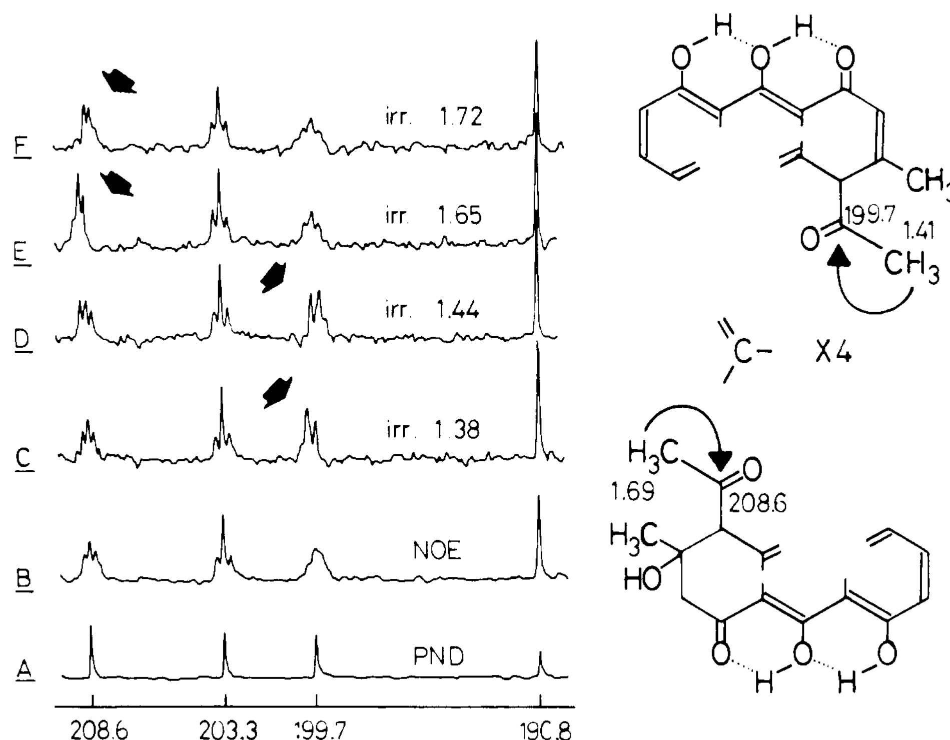


Figure 3. $^{13}C\{^1H\}$ selective population transfer results on the carbonyl signals of 1. (A) Normal proton noise decoupled spectrum. (B) Gated decoupled NOE spectrum. (C–F) Spectra obtained by irradiation at the respective ^1H NMR frequencies.

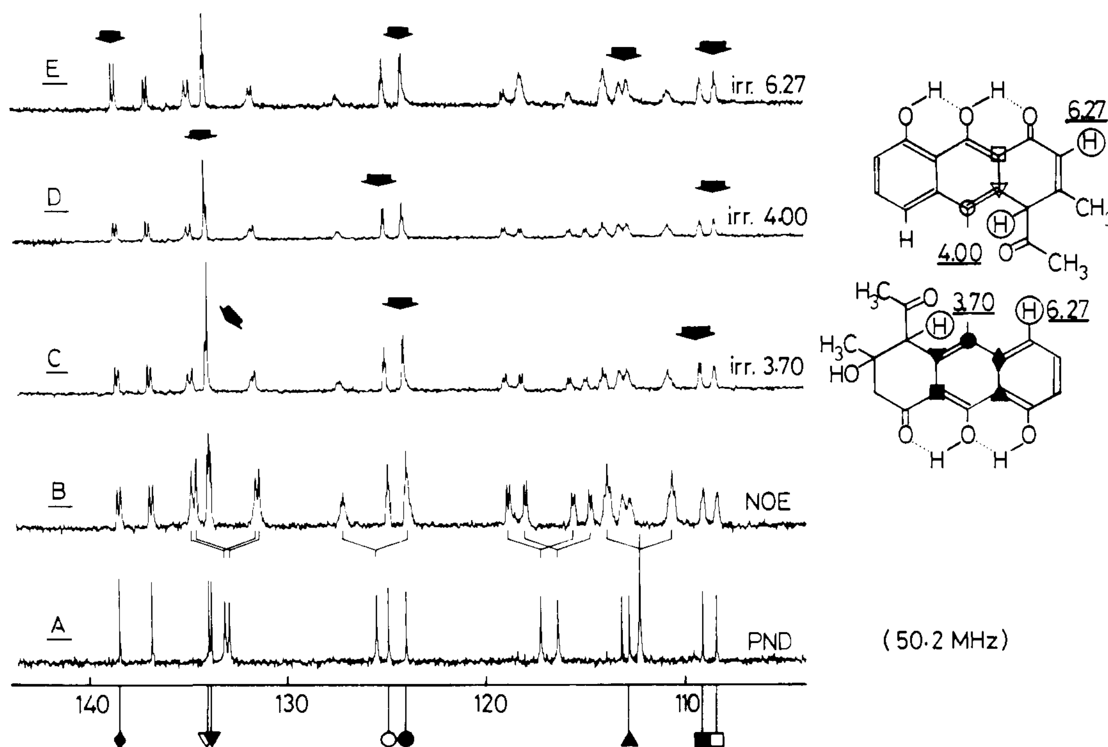


Figure 4. $^{13}C\{^1H\}$ low power selective decoupling experiments on 1 at 50.2 MHz. Marked signals represent the respective carbons in the partial structures. (A) Proton noise decoupled spectrum. (B) Gated decoupled NOE spectrum. (C–E) Spectra obtained by irradiation at the respective proton signals.

As a result, the biosynthetic pattern of 1 (and presumably of 5 as well) is clearly demonstrated to be path A in Figure 6, which includes decarboxylation of the terminal acetate units of two nonaketides formed in such a way that the clean elongations start from the C-13 and C-13′ methyl groups, as well as probable oxidative coupling of the resulting two phenolic intermediates at the sterically less favored C-9 and C-9′ sites.

Experimental Section

IR spectra were recorded on a Hitachi Model 260-10 spectrophotometer. UV–visible spectral data were obtained on a Shimadzu UV-200 double beam spectrophotometer. Proton and Carbon magnetic resonance spectra were recorded on JEOL PS-100, FX-100, FX-200 and/or a Varian XL-200 spectrometers, using deuteriochloroform as solvent. The chemical shifts are reported as δ downfield from tetramethylsilane as internal standard. High resolution mass spectral data were obtained on a Hitachi M-80 spectrometer. The melting points were determined on a Yazawa BY-1 apparatus and were uncorrected. The ^{13}C labeled compounds were purchased from Merck, Sharp and Dohme, Canada Ltd.

***O*-Dimethylsetomimycin (3).** Excess ethereal diazomethane was added to 140 mg of 1 and the mixture was gently stirred for 4 h at room temperature. The mixture was then evaporated under reduced pressure to give 146 mg of yellow powder, which was purified by preparative layer

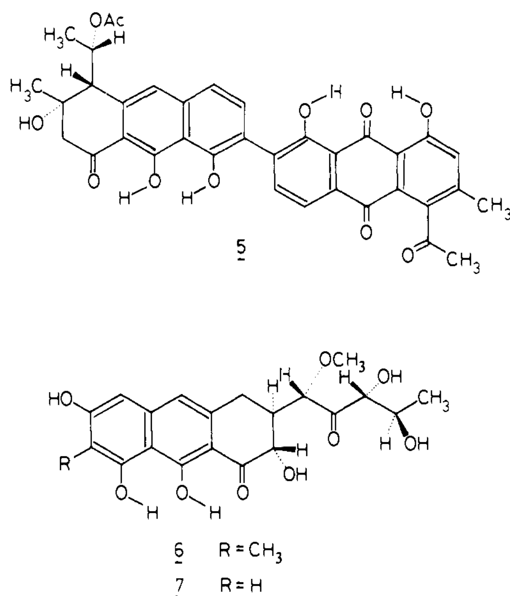


Figure 5. Structures of julichrome $Q_{5,6}$ (5), chromomycinone (6), and olivin (7).

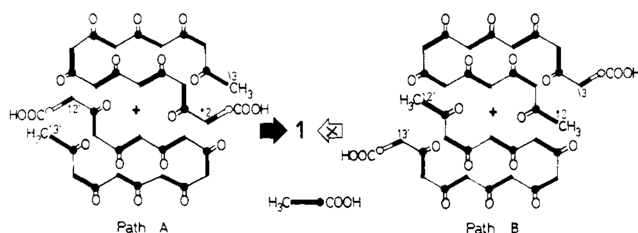


Figure 6. Possible biosynthetic patterns of setomimycin (1).

Table I. The ^{13}C-NMR Assignment of 1 and the Results of Feeding Experiments

carbon no.[a]	δ_C[b]	J_{C-C}, Hz[c]	carbon no.[a]	δ_C[b]	J_{C-C}, Hz[c]
1	60.7	31.6	1'	60.5	37.7
2	158.0*	41.8	2'	71.7*	38.9
3	125.6	56.3	3'	47.1	40.3
4	190.8*	56.3	4'	203.3*	40.3
5	158.2*	62.3	5'	158.5*	62.3
6	112.4	56.3	6'	112.4	56.3
7	133.1*	56.2	7'	133.3*	56.3
8	116.5	58.6	8'	117.3	58.7
9	125.0	67.0	9'	124.1	67.0
10	164.6*	65.9	10'	166.3*	64.7
11	199.7*	31.6	11'	208.6*	37.7
12	28.1	E.S.[d]	12'	33.8	E.S.[d]
13	22.9	41.8	13'	29.1	38.9
4a	108.4	65.9	4a'	109.2	64.7
8a	136.9*	58.6	8a'	138.5*	58.4
9a	134.0*	67.0	9a'	133.9*	67.0
10a	113.2	62.1	10a'	112.8	62.3

[a] The carbon numbering is as shown in Figure 1. [b] The signals marked asterisk were labeled by [1-^{13}C]sodium acetate. [c] The coupling constants were obtained from the [1,2-$^{13}C_2$]sodium acetate feeding. [d] Enriched carbon signal appeared as singlet.

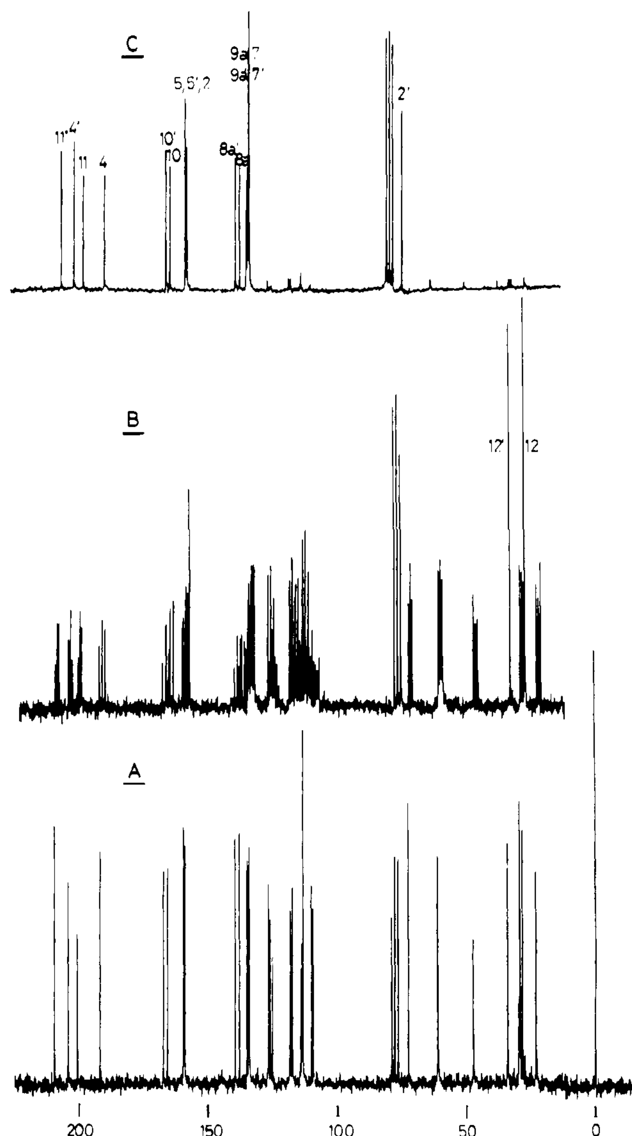


Figure 7. Comparison of the ^{13}C NMR spectra of labeled setomimycin (1). (A) Natural abundance spectrum. (B) Spectrum of the labeled 1 by [1,2-$^{13}C_2$]sodium acetate. (C) Spectrum of **1** labeled by [1-^{13}C]-sodium acetate. Spectral width of the spectrum C is different from that of the other two spectra.

chromatography on silica gel 60 F_{254} (Merck, 0.5-mm thickness), using benzene–acetone (2:1) as solvent. The main band was scraped and extracted with ethyl acetate. The filtered extract was removed of solvent to afford 60 mg of **3**, which was recrystallized from acetone–*n*-hexane to give 37 mg of yellowish needles, $C_{36}H_{32}O_9$, mp 239–240 °C; HRMS, obsd, *m/z* 608.2067, calcd for $C_{36}H_{32}O_9$ (M^+), 608.2044; IR (KBr) $\delta_{C=O}$ 1720, 1710, 1690, 1660 cm^{-1}; UV (EtOH) λ_{max} 226 nm (ϵ 44 700), 264 (44 800), 338 (9800); ^{1}H NMR (100 MHz) δ 1.23 (3 H, s), 1.31 (3 H, s), 1.66 (3 H, s), 1.95 (3 H, s), 2.54 (1 H, br s), 2.63 (1 H d, J = 18 Hz), 3.07 (1 H d, J = 18 Hz), 3.79 (1 H, s), 3.87 (1 H, s), 4.23 (3 H, s), 4.31 (3 H, s), 6.18 (1 H, s), 6.22 (1 H d, J = 8 Hz), 6.66 (1 H d, J = 8 Hz), 6.90 (1 H d, J = 8 Hz), 7.01 (1 H d, J = 8 Hz), 7.15 (1 H t, J = 8 Hz), 7.39 (1 H t, J = 8 Hz), 10.05 (1 H, br s), 10.23 (1 H, br s); ^{13}C NMR δ 22.2 (q), 28.7 (q), 29.5 (q), 33.6 (q), 50.1 (t), 60.1 (d), 61.3 (d), 64.9 (q), 65.7 (q), 71.5 (s), 112.4 (d), 112.6 (d), 116.6 (s), 116.9 (2 × C, s and d), 117.5 (d), 119.2 (s), 119.7 (s), 128.7 (d), 130.0 (s), 130.4 (s), 131.6 (2 × C, d), 135.7 (s), 136.0 (s), 136.4 (s), 137.3 (s), 152.3 (s), 156.7 (2 × C, s), 159.4 (s), 160.4 (s), 184.4 (s), 195.3 (s), 200.6 (s), 208.4 (s). Anal. Calcd for $C_{36}H_{32}O_9 \cdot C_3H_6O$: C, 70.26; H, 5.75. Found: C, 70.42; H, 5.61.

Tetrahydro-*O*-dimethylsetomimycin (4). To a solution of 90 mg of **3** in 20 mL of isopropyl alcohol was added 60 mg of $NaBH_4$ and the mixture was stirred for 4 h at room temperature. To the reaction we then added 5 mL of methanol and 2 mL of 1 N HCl, and the mixture was stirred for 5 min more and then diluted with 200 mL of water. After removal of isopropyl alcohol under reduced pressure, the mixture was extracted with ethyl acetate and the extract was washed with saturated aqueous $NaHCO_3$ solution and brine and then dryed over anhydrous $MgSO_4$. Filtration and evaporation of solvent under reduced pressure gave 92 mg of brownish residue, which was purified by flash chromatography[17] on 16 g of silica gel 60 (Merck, 230–400 mesh), using *n*-hexane–ethyl acetate (1:2). Appropriate homogeneous fractions, judged by thin layer chromatography on silica gel 60 F_{254} (Merck, 0.25-mm thickness), employing *n*-hexane–ethyl acetate (1:2) as solvent, were combined and evaporated to dryness under reduced pressure to give 8 mg of **4**, $C_{36}H_{36}O_9$; HRMS, obsd, *m/z* 594.2244, calcd for $C_{36}H_{34}O_8$ (M^+

(17) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

$- H_2O$), 594.2252; IR ($CHCl_3$) $\delta_{C=O}$ 1730, 1665 cm^{-1}; ^1H NMR (200 MHz) δ 0.70 (3 H, s), 0.84 (3 H, d, J = 6.8 Hz), 1.50 (3 H, s), 1.69 (1 H, br d, J = 14 Hz), 1.87 (3 H, s), 2.18 (1 H, br s), 2.35 (1 H, dd, J = 14 and 3.5 Hz), 4.15 (3 H, s), 4.31 (3 H, s), 4.47 (1 H, br s), 6.51 (1 H, d, J = 8 Hz), 6.63 (1 H, d, J = 8 Hz), 6.96 (2 H, d, J = 8 Hz), 7.18 1 H, t, J = 8 Hz), 7.24 (1 H, t, J = 8 Hz), 9.61 (1 H, br s), 10.16 (1 H, br s).

Feeding Experiments. A loopful culture of *Streptomyces pseudovenezuelae* strain AM-2947 on an agar slant was transferred into a 500-mL Sakaguchi Flask containing 100 mL of a medium (2% glycerol, 2% soybean meal, 0.3% NaCl and distilled water, pH 7.0) and incubated for 2 days at 27 °C to give a seed culture. One hundred test tubes (2 cm × 19 cm) each containing 10 mL of a production medium (1% glycerol, 1% soybeam meal, 0.3% NaCl and distilled water, pH 7.0) were inoculated with 0.5-mL aliquots of the seed culture and incubated at 27 °C with shaking. After 16 h, 0.5-mL portions of aqueous 2% [1-^{13}C]sodium acetate (90 atom % enriched) or 0.6% [1,2-$^{13}C_2$]sodium acetate (90 atom % enriched) solution was aseptically added to the cultures, which were incubated for an additional 2 days. Combined culture broth (1 L each) was extracted with ethyl acetate (1 volume) at pH 2.0 and the extract was evaporated to dryness under reduced pressure to give a crude powder. The crude sample was then purified by preparative layer chromatography on silica gel 60 F_{254} (Merck, 2-mm thickness), using chloroform–methanol (10:1) as solvent. The appropriate setomimycin band was scraped and extracted with chloroform–methanol (2:1). The extract was evaporated under reduced pressure to give a reddish orange residue, which was rechromatographed by preparative layer chromatography, using benzene–ethyl acetate (1:3) as solvent. The setomimycin band was again scraped, extracted with ethyl acetate, and stripped of solvent in vacuo to give 21.2 mg and 27.9 mg of setomimycin enriched from [1-^{13}C]- and [1,2-$^{13}C_2$]sodium acetate, respectively.

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Supplementary Material Available: ^{13}C NMR spectra of **1** and **3**, ^{13}C {^{1}H} low power decoupling spectra and ^{13}C {^{13}C} homonuclear decoupling spectra of the enriched **1** (5 pages). Ordering information is given on any current masthead page.

Stereocontrolled Total Synthesis of an α-Methylene Guaianolide in the 4,5-Epoxyosmitopsin Family

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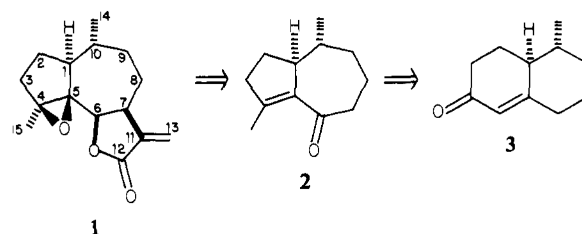
Abstract: Guaianolide **13** has been prepared in 19 steps and 7.9% overall yield from 1,3-cyclohexanedione. Two of the six chiral centers in racemic hydroazulene lactone **13** were introduced stereoselectively (4.5:1.0 ratio of stereoisomers), and the other four were introduced with virtually complete and remarkable stereocontrol. X-ray analysis of guaianolide **13** revealed *trans*-hydroazulene and *cis*-lactone ring fusions, and ^{1}H NMR showed an unusual chemical shift for one proton at δ 2.1. Decoupling experiments on α-methylene guaianolide **1** indicated that this characteristic downfield absorption is due quite unexpectedly to the C-8β hydrogen, which is situated close to the oxygen atom and in the plane of the 4β,5β-epoxide ring. Synthetic α-methylene guaianolide **1**, which shows significant antischistosomal activity, is the C-10 epimer of the structure reported for natural 4,5-epoxyosmitopsin.

Many hydroazulenic lactones have been isolated from plants and have been shown to possess high antitumor,[1] allergenic,[2] antischistosomal,[3] antihelminthic,[4] antifeedant,[5] contraceptive,[6] and root growth stimulatory and inhibitory[7] activities. Because of their high biological activity[8] and because they are available from natural sources often only in small quantities, some of these sesquiterpenes have been prepared in the laboratory. Although total syntheses of some *pseudoguaianolides* have recently been reported,[9] all of the published *guaianolide* hydroazulene syntheses have involved structural modifications of related naturally occurring decalin sesquiterpenes.[10] We recently reported the total synthesis and characterization of two stereoisomeric hydroazulenones,[11] and we record here the culmination of that project leading to the first, highly stereocontrolled, total synthesis of an α-methylene guaianolide which, although not itself a natural product, is structurally similar to natural 4,5-epoxyosmitopsin and which has some surprising NMR characteristics useful in assigning hydroazulene ring junction stereochemistry in guaianolides like **1** having a 4,5-epoxide group. Retrosynthetic analysis suggested octalone **3** as a precursor to hydroazulenone **2** which itself would be an intermediate for preparation of epoxyguaianolide **1**.



Preparation of Octalone 3. Because pure octalone **3** was needed on at least a 10-g scale to initiate the multistep synthesis of

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(1) (a) Hartwell, J. L.; Abbott, B. J. *Adv. Pharmacol. Chemother.* **1969**, *7*, 117. (b) Kupchan, S. M. In "Recent Advances in Phytochemistry"; Runeckles, V. C., Ed.; Plenum Press: New York, 1975; Vol. 9. (c) Lee, K.-H.; Wu, Y.-S.; Hall, I.-H. *J. Med. Chem.* **1977**, *20*, 911. (d) Ogura, M.; Cordell, G. A.; Farnsworth, N. R. *Phytochem.* **1978**, *17*, 957.

(2) (a) Mitchell, J. C. In "Recent Advances in Phytochemistry"; Runeckles, V. C., Ed.; Plenum Press: New York, 1975; Vol. 9, pp 119–139. (b) Bleumink, E.; Mitchell, J. C.; Geissman, T. A.; Towers, G. H. N. *Contact Dermatitis* **1976**, *2*, 81.

(3) (a) Garcia, M.; Silva, A. J. R.; Baker, P. M.; Gilbert, B.; Rabi, J. A. *Phytochem.* **1976**, *15*, 331. (b) Vichnewski, W.; Sarti, S. J.; Gilbert, B.; Herz, W. *Phytochem.* **1976**, *15*, 191. (c) Macaira, L. A.; Garcia, M.; Rabi, J. A. *J. Org. Chem.* **1977**, *42*, 4207.

(4) Sanchez-Viesca, F.; Romo, J. *Tetrahedron* **1963**, *19*, 1285.

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