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Structures of the Irieols, New Dibromoditerpenoids of a Unique Skeletal Class from the Marine Red Alga *Laurencia irieii*

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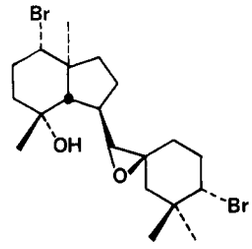
The structures of seven new dibromoditerpenoids of a unique structure class are described; they are natural products from the red seaweed *Laurencia irieii*. The structures of the previously reported irieol A (**1**) and iriediol (**2**) have been further refined and their absolute stereochemistries determined by a combination of chemical and spectral methods. The structures of irieol (**14**), irieol D (**18**), irieol E (**16**), irieol F (**21**), and irieol G (**22**) were determined by their oxidative cleavage to 4(*S*)-bromo-3,3-dimethylcyclohexanone (**6**) and an aldehyde **12**, which was further converted to an aldehyde identical with that obtained from the previously described sesquiterpenoid opositol. The structures of the closely related compounds irieol B (**23**) and irieol C (**24**) have been assigned based upon ^1H and ^{13}C NMR and mass spectral fragmentation data. The conformational equilibrium of 4(*S*)-bromo-3,3-dimethylcyclohexanone (**6**) has been determined by temperature-dependent circular dichroism experiments.

As a consequence of our long-term interest in the natural products chemistry of the unusually prolific red seaweed *Laurencia* (Rhodomelaceae, Rhodophyta), we have observed several unrecorded species of this genus from the Gulf of California (Sea of Cortez), Mexico.[2] One such new *Laurencia* sp., for which we have suggested the name *L. irieii*,[3] was found to be an unusually rich source for novel diterpenoids, in contrast to the more typically found sesquiterpenoids and nonterpenoid C_{15} acetylene-containing derivatives.[4] It should be noted, however, that a less common number of *Laurencia* species have been shown to contain the labdane-derived diterpenoids aplysin-20[5] and concinndiol,[6] as well as the ring-contracted analogue neoconcinndiol hydroperoxide.[7] Also, our recent studies of *L. obtusa* have yielded a new diterpenoid, obtusadiol, which possesses an unprecedented bicyclic skeleton.[8]

Collections of *L. irieii* were obtained from several locales, the major collections being made in the extreme northern Gulf near Puerto Peñasco, Mexico. One collection, made approximately 300 miles to the south (Bahia de Concepcion), contained identical compounds but in slightly different ratios. In all cases, the algae were mildly air-dried and the chloroform–methanol extracts purified by silica gel column chromatography. Individual components were then obtained by rechromatography using thick-layer techniques or by high-pressure liquid chromatography (LC).

Irieol A and Iriediol. The structures of irieol A and iriediol were determined by X-ray crystallographic methods, and these results were reported in an earlier communication.[9] Irieol A (**1**) was obtained in crystalline form, mp 142–144 °C, $[\alpha]^{25}_D$ 0° (*c* 1.01, $CHCl_3$), upon benzene elution of the silica gel column. Low-resolution mass spectral analysis of **1** indicated an elemental composition of $C_{20}H_{30}Br_2O$ for the $M^+ - H_2O$ fragment. Infrared absorption at 3450 cm^{-1} revealed that irieol A was an alcohol, which was further shown to be tertiary by its failure to acetylate under mild conditions (Ac_2O–pyridine, room temperature). The ^1H NMR spectrum of **1** (see Table I) substantiated the structure assignment determined earlier by X-ray crystallography. It is of interest to point out that the C-14 bromine atom in **1** is in an axial orientation in the crystal. This conformation is also adopted in solution, as is reflected in the ^1H NMR spectrum of **1** by the small couplings noted for the C-14 methine proton (δ 4.36, broad singlet).

Iriediol (**2**) was crystallized from column chromatography fractions eluted with benzene–ether (9:1). The mass spectrum of **2** also exhibited an $M^+ - H_2O$ fragment which analyzed for $C_{20}H_{30}Br_2O$. Treatment of **2** with acetic anhydride–pyridine at 25 °C gave the monoacetate **3**. Oxidation of **2** with chromium trioxide–pyridine yielded the cyclopentanone **4** ($\nu_{C=O}$



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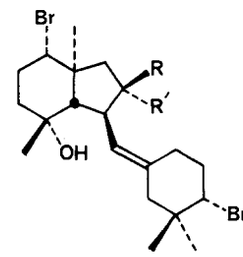

2, R'=OH, R=H
3, R'=OAc, R=H
4, R'=R=(=O)

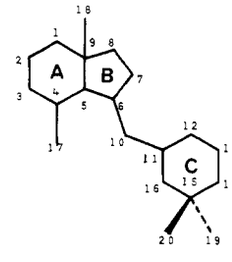

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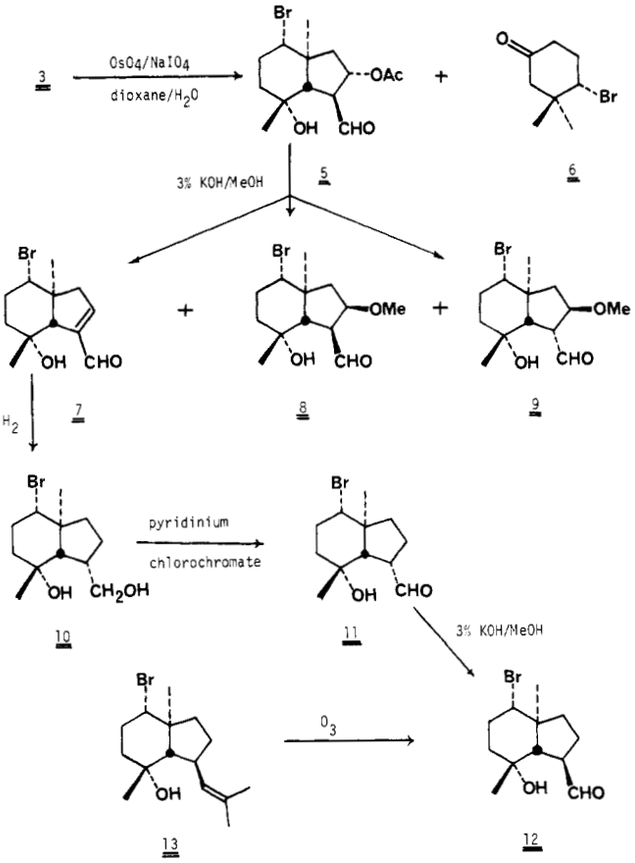
Table I. ^{1}H NMR Assignments for the Irieols and Derivatives[a]

proton at carbon no.	compounds													
	1	2	3	4	14	16	17	18	19	21	22	23	24	25
1	3.97 dd (12, 5)	3.91 dd (12, 4)	3.82 dd (12, 4)	4.14 dd (12, 4)	3.86 dd (12, 5)	3.93[b] dd (12,4)	3.91[b] dd (12, 4)	3.91[b] dd (12, 4)	4.00[b] dd (12, 4)	3.97[b] dd (12, 4)	3.94[b] dd (12, 4)	3.82[b] dd (12, 4)	3.93[b] dd (12, 4)	3.92[b] dd (12, 4)
2 (axial)		2.34 dddd (14, 12, 12, 4)	2.34 dddd (14, 12, 12, 4)		2.29 dddd (14, 12, 12, 4)									
6		3.06 ddd (10, 10, 4)	3.18 ddd (10, 10, 4)	3.44 dd (10, 10)	3.06 dddd (10, 10, 8, 5)		2.69 ddd (10, 8, 4)		4.17 dd (12, 8)	2.56 dd (12, 8)	2.98 dd (10, 10)	2.46 m		
7		3.84 m	4.75 m					5.36 ddd (12, 8, 4)	5.48 ddd (12, 8, 2)	4.64 ddd (12, 8, 4)	4.64 ddd (12, 8, 4)	4.35 ddd (12, 8, 4)		5.22 ddd (12, 8, 4)
10	2.61 d (10)	5.05 d (10)	5.03 d (10)	4.83 d (10)	5.06 d (10)	3.63 bs	5.11 bs	3.82 d (10)		3.72 bs	5.41 bs			
14	4.36 bs	4.14 dd (9, 4)	4.09 dd (9, 4)	4.20 dd (12, 4)	4.05 dd (10, 4)	4.00[b] dd (12, 4)	3.93[b] dd (12, 4)	3.98[b] dd (12, 4)	4.07[b] dd (12, 4)	4.08[b] dd (12, 4)	3.94[b] dd (12, 4)	3.89[b] dd (12, 4)	3.96[b] dd (12, 4)	3.97[b] dd (12, 4)
CH_3-17	1.27	1.41	1.34	1.32	1.16	1.27	1.36	1.44	1.30	1.34	1.34	1.20	1.27	1.34
CH_3-18	1.24	1.20	1.19	1.23	1.16	1.23	1.25	1.26	1.28	1.27	1.27	1.20	1.20	1.20
CH_3-19	1.20	1.07	1.05	1.06	1.04	1.19	1.17	1.20	1.07	1.19	1.20	1.16	1.16	1.20
CH_3-20	1.09	0.93	0.95	0.93	0.91	1.05	1.04	1.05	0.98	1.08	1.05	1.03	1.00	1.00
misc.			1.98 (OAc)				2.14 (OAc)	2.07 (OAc)	1.98 (OAc)		2.18 (OAc)			2.05 (OAc)
									3.43 (OH)					

[a] Chemical shifts are in parts per million (δ) relative to internal Me_4Si. Spectra were recorded at 220 MHz in $CDCl_3$ solution, and coupling constants (hertz) are in parentheses. [b] Assignments may be reversed.

Scheme I



1740 cm^{-1}), thus confirming the placement of a secondary hydroxyl in a five-membered ring.[10] While X-ray analysis indicated that the C-14 bromine substituent is axial in the crystal, in analogy to irieol A, the ^{1}H NMR spectrum illustrated bands which suggested a conformational equilibrium in solution. The C-14 methine proton was observed as a broadened four line pattern at δ 4.14 with $J \cong 9$ and 4 Hz. These coupling constants are between those expected for an axial proton (12 and 4 Hz) and an equatorial proton (4 and 4 Hz) and suggest that the equilibrium is shifted toward an equatorial bromine. The A ring stereochemistry of iriediol, as well as several other irieols, was predicted by the presence of a complex ^{1}H NMR signal (for iriediol a dddd at δ 2.34 with J = 14, 12, 12, and 4 Hz), which characterizes rigid *cis*-1,4-cyclohexane bromohydrin systems with an axial hydroxyl group. The signal in question arises from the deshielded C-2 axial proton, and this band has been observed and analyzed by decoupling studies for the related compounds oppositol,[11] bromosphaerol,[12] and 1(*S*)-bromo-4(*R*)-hydroxy-(−)-δ-selin-7-ene.[13]

Attempts to compare the absolute configurations of iriediol and irieol A by X-ray crystallographic techniques led to puzzling results. Irieol A was firmly established to have the absolute stereochemistry as drawn in **1**, whereas iriediol was initially determined to be enantiomeric at each comparable center.[9] However, an unequivocal assignment of **2** was not made since the crystallographic differences between Friedel pairs were small. Another puzzle existed with irieol A, which, despite belonging to a chiral crystallographic space group, fortuitously failed to show a measurable rotation at the sodium D line.

To solve these problems and to rigorously establish the absolute configuration of iriediol, both compounds were degraded to the aldehyde **12** (Scheme I), which was also produced from the sesquiterpenoid oppositol (**13**). The absolute configuration of oppositol had been firmly established earlier by X-ray crystallography.[14] Treatment of iriediol acetate (**3**)

Table II. ^{1}H NMR Assignments for Irieol Degradation Products [a]

proton at carbon no.	compounds								
	7	8	9	10	11	12	15	5	20
1	4.30 dd (12, 5)	3.94 dd (12, 4)	4.08 dd (12, 4)	3.68 dd (12, 4)	3.90 dd (12, 4)	4.00 dd (12, 4)	4.02 dd (12, 4)	3.93 dd (12, 4)	4.06 dd (12, 4)
2 (axial)					2.43 dddd (14, 12, 12, 4)	2.35 dddd (14, 12, 12, 4)	2.34 dddd (14, 12, 12, 4)	2.34 dddd (14, 12, 12, 4)	
6		3.06 dd (10, 4)	3.27 m	2.64 m	3.16 m	3.08 m	3.00 m	3.22 ddd (10, 4, 4)	3.50 ddd (10, 8, 4)
7	7.08 dd (4, 4)	4.27 m	4.27 m					4.91 ddd (8, 4, 4)	5.50 ddd (8, 8, 6)
10	9.72 s	9.93 s	9.66 d (4)	4.06 d (14) 4.14 dd (14, 4)	9.76 bs	9.64 bs (4)	9.64 d	10.00 d (4)	9.67 d (6)
CH_3-17	1.44	1.27	1.11	1.38	1.27	1.21	1.25	1.36	1.18
CH_3-18	1.21	1.05	1.03	1.32	1.09	1.09	1.16	1.02	1.04
misc.		3.32 (OCH_3)	3.20 (OCH_3)					2.04 (OAc)	2.00 (OAc)

[a] Chemical shifts are in parts per million (δ) relative to internal Me_4Si. Spectra were recorded at 220 MHz in $CDCl_3$ solution, and coupling constants (hertz) are in parentheses.

Table III. ^{13}C NMR Shift Assignments for the Irieols and Derivatives [a]

carbon	compounds								
	1 [b]	2	3	14	16	18	21	24	25
1 [c]	66.1	65.8	65.9	66.1	65.2	65.2	65.1	65.8	65.3
2	30.9	30.8	30.8	31.2	31.0	30.6	30.6	31.0	30.8
3	41.1	42.9	42.9	40.6	41.5	44.0	44.0	40.7	39.7
4	71.3	71.5	71.2	71.7	72.1	71.7	71.7	72.1	71.7
5	59.7	59.8	59.8	60.9	56.3	54.1	53.9	60.2	57.7
6	*d*	47.2	43.7	35.7	37.9	41.1	42.1	32.5	36.4
7	*d*	78.2	79.7	28.0	20.6	75.6	75.7	28.0	73.5
8	43.0	50.6	48.2	42.9	43.6	44.9	46.7	43.1	43.3
9	*d*	46.9	46.9	48.0	47.0	47.4	45.3	47.4	45.4
10	64.3	129.4	128.3	132.4	78.3	78.6	80.3	52.0	49.1
11	67.9	134.7	135.0	131.9	75.0	75.3	75.7	73.1	72.6
12	*d*	33.8	33.5	33.6	36.4	35.4	38.3	38.3	38.4
13	*d*	28.4	27.7	29.2	30.1	30.2	30.2	30.4	30.3
14 [c]	64.1	64.5	63.9	64.9	64.4	64.2	64.0	64.8	64.3
15	*d*	38.4	38.3	38.3	37.1	36.6	36.5	36.6	36.6
16	*d*	48.9	48.2	48.7	45.8	50.2	50.9	51.3	51.3
17	31.8	31.2	31.0	31.2	32.8	32.7	32.8	31.8	32.4
18	16.2	17.9	17.3	16.4	16.7	18.1	18.0	16.4	17.7
19	24.5	23.1	23.8	23.0	22.9	22.9	23.0	22.8	23.0
20	30.8	29.6	29.1	29.6	31.7	31.4	31.5	31.8	32.1

[a] Chemical shifts are in parts per million (δ) relative to internal Me_4Si (20 MHz, $CDCl_3$ solution), and assignments have been made based upon off-resonance multiplicities. [b] No off-resonance data available. [c] Assignments may be reversed. [d] Others signals at 40.0, 37.1, 36.1, 34.9, 29.4, 25.7, and 24.8 ppm were observed which were not assigned.

with osmium tetroxide–sodium periodate in aqueous dioxane gave a crystalline aldehyde **5** and 4-bromo-3,3-dimethylcyclohexanone (**6**), whose structures were secured by ^{1}H and ^{13}C NMR, IR, and mass spectral analyses (Tables II and IV). Aldehyde **5**, upon treatment with 3% potassium hydroxide in methanol, gave the α,β-unsaturated aldehyde **7** (major product) and two epimeric methoxide addition products (**8** and **9**). Catalytic hydrogenation (platinum oxide in ether) of **7** yielded the primary alcohol **10**,[15] which was in turn oxidized with pyridinium chlorochromate in methylene chloride to the aldehyde **11**. Ozonation of oppositol gave the aldehyde **12**, which was highly comparable but not identical with **11**, as recognized by the ^{1}H NMR aldehyde proton bands for each compound. For **11** the aldehyde proton appears at δ 9.76 as a broad singlet, whereas for **12** the aldehyde proton was observed at δ 9.64 as a clean doublet, J = 4 Hz. Since these data suggested **11** and **12** were epimeric at the potentially epimerizable center C-6, both aldehydes were treated with dilute potassium hydroxide in methanol. Treatment of **12** at 60 °C

Table IV. ^{13}C NMR Shift Assignments for Oppositol and Irieol Degradation Products [a]

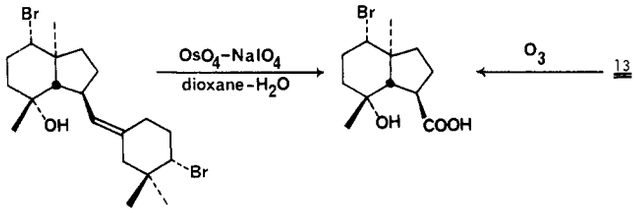
carbon	compounds				
	13	12	7	5	20
1	63.3	63.0	61.4	62.5	62.2
2	31.2	31.0	31.0	30.9	30.8
3	40.4	41.1	41.3	41.8	41.9
4	71.6	71.0	69.6	70.0	69.9
5	60.7	56.8	60.9	58.6	55.0
6	36.4	49.9	150.4	57.6	52.2
7	28.5	22.2	156.4	75.3	73.2
8	42.0	42.2	47.4	47.8	48.7
9	47.7	48.2	52.7	*b*	46.8
10	132.0	203.6	189.6	201.5	199.9
17	30.6	31.3	31.9	31.9	31.2
18	16.3	16.2	15.5	17.4	17.5

[a] Chemical shifts are in parts per million (δ) relative to internal Me_4Si ($CDCl_3$ solution), and assignments have been made based upon off-resonance multiplicities. [b] Band not observed.

for 2 h gave no reaction; however, **11** was quantitatively converted to **12** at 25 °C, indicating that the more thermodynamically stable configuration is the "β" oriented aldehyde. Compound **12** produced from oppositol (**13**) showed $[\alpha]^{25}_D$ +6.26° (*c* 8.71, $CHCl_3$), and **12** produced from iriediol showed the lesser value of $[\alpha]^{25}_D$ +2.16° (*c* 0.47, $CHCl_3$), thus providing evidence that iriediol has the same absolute stereochemistry as oppositol and irieol A but also indicating that iriediol may be partially racemized.

The concept of utilizing the cleavage product 4-bromo-3,3-dimethylcyclohexanone (**6**), which contains a single asymmetric center at C-4, to assess the absolute stereochemistry of iriediol was explored. The circular dichroism behavior of the chiral ketone was measured, and at 25 °C **6** exhibited a positive Cotton effect (Figure 1). At −196 °C the magnitude of this effect greatly increased, indicating a shift in a conformational equilibrium to a more stable conformer. Although **6** is a chiral cyclohexanone, the chiral center at C-4 lies in the vertical plane defining the Octant Rule[16] and cannot contribute to the sign of the Cotton effect. The positive contribution arises from the C-3 *gem*-dimethyl group which by the Octant Rule definition must be located in the upper left positive quadrant. Since it is unknown whether bromine is more stable in the axial or equatorial orientation, the optical properties of **6** cannot be used to determine its absolute configuration. However, since the absolute stereochemistry at C-4 has been established as *S* based upon relating iriediol (**2**) to oppositol (**13**), the conformational equilibrium of this ketone can be calculated from the temperature dependent CD data. Following calculations reported by Moscowitz et al.,[17] the equilibrium mixture of **6** at 25 °C is found to be 76% equatorial bromine and 24% the axial conformer. It is interesting to note that the conformational equilibrium of the closely related compound 4-bromocyclohexanone is reported[18] to consist of 63% axial and 37% equatorial bromine conformers.

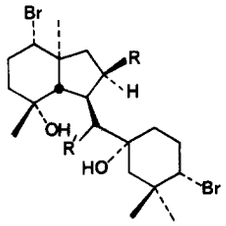
Irieol. Inspection of the nonpolar silica gel chromatography fractions obtained with petroleum ether–benzene (1:1) elution gave the monohydroxylated diterpenoid irieol (**14**), an oil, in

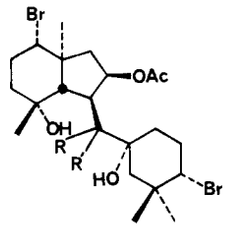


14 15

0.01% (dry plant) yield. The mass spectrum of irieol showed an $M^+ - H_2O$ fragment at *m/e* 428/430/432 for $C_{20}H_{30}Br_2$. The off-resonance decoupled ^{13}C NMR spectrum of **14** confirmed the molecular formula of $C_{20}H_{32}Br_2O$ as well as suggested a relatedness to **2** (see Table III). Infrared spectrophotometry showed only hydroxyl absorption (3450 cm^{-1}). Attempted acetylation under standard conditions gave no reaction, which allowed irieol to be assigned as a tertiary alcohol consistent with ^{13}C NMR studies. The ^1H NMR spectrum of irieol (Table I) exhibited comparable bands to **2** except those associated with the five-ring hydroxyl. Oxidative degradation of **14** with osmium tetroxide–sodium periodate in dioxane, followed by further Jones oxidation, gave the crystalline acid **15** which was shown to be identical with that obtained from oxidative ozonation of oppositol: mp 162–163 °C; $[\alpha]^{25}_D$ −5.43° (*c* 0.35, $CHCl_3$). In addition to **15**, 4(*S*)-bromo-3,3-dimethylcyclohexanone (**6**) was isolated, thus establishing the structure of irieol as drawn including absolute stereochemistry.

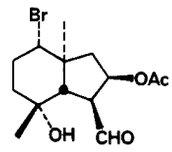
Irieol D, E, F, and G. The polar silica gel fractions (diethyl ether elution) yielded a complex mixture which migrated as one spot on TLC (1:1 hexane–ether). Separation of this mixture by LC (μ-porasil) yielded four new irieol diterpenoids, irieol G, E, D, and F (in order of elution). Irieol E (**16**), an amorphous solid, showed intense hydroxyl absorption in the





16 R=H, R'=OH (irieol E)
17 R=H, R'=OAc
21 R=OH, R'=OH (irieol F)
22 R=OH, R'=OAc (irieol G)
23 R=OH, R'=H (irieol B)
24 R=H, R'=H (irieol C)
25 R=OAc, R'=H

18 R=OH, R'=H (irieol D)
19 R=R'=(=O)



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infrared. Acetylation with acetic anhydride–pyridine gave the monoacetate **17**, which revealed the presence of one secondary hydroxyl group in the molecule. The ^{13}C NMR spectrum of **16** indicated the presence of two additional tertiary hydroxyl-bearing carbons at 72.1 and 75.0 ppm, respectively. Treatment of **16** with periodic acid in diethyl ether gave the aldehyde **12** and the bromo ketone **6**, which were identical, including rotations, with those obtained from iriediol (**2**). Since periodic acid cleaves 1,2-diols, irieol E must have a C-10, C-11 diol structure with the absolute stereochemistry shown.

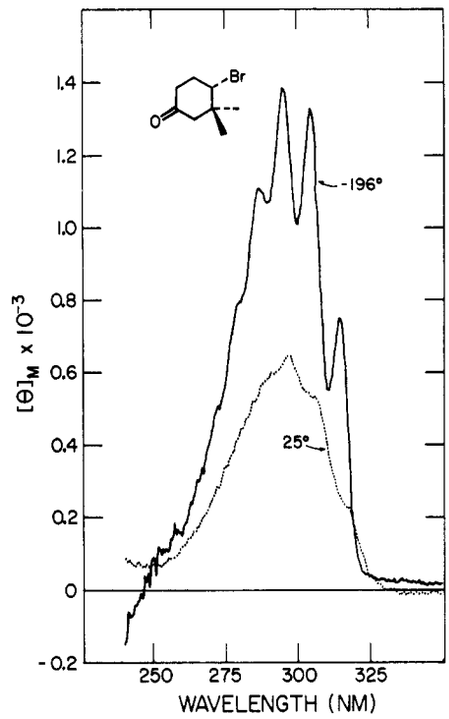


Figure 1. Circular dichroism spectrum of 4(*S*)-bromo-3,3-dimethylcyclohexanone (**6**) at 25 °C and at −196 °C as a glass in EPA.[17]

The only centers not rigorously defined stereochemically are at C-10 and C-11. Since the side chain at ring C is expected to adopt the equatorial position as in irieol A, an axial hydroxyl at C-11 is suggested. The C-10 α-hydroxyl proton, seen in the ^{1}H NMR spectrum at δ 3.63 for **16** and at δ 5.11 for its acetate **17**, is a singlet, which indicates that the proton on C-10 is orthogonal to the proton at C-6. In **17** the C-6 proton is visualized as a ddd at δ 2.69 with couplings of 10, 8, and 4 Hz, consistent with a dihedral angle of 90° to the C-10 proton. However, this information does not allow an assignment of the stereochemistry at C-10.

Irieol D (**18**), an amorphous solid, also showed an $M^+ - H_2O$ peak as the largest mass fragment in its mass spectrum, which analyzed for $C_{22}H_{34}Br_2O_4$. Jones oxidation of irieol D gave the crystalline ketone acetate **19**, the infrared characteristics of which ($\nu_{C=O}$ 1710 cm^{-1}) precluded the five-membered ring ketone as obtained from iriediol (**2**). Oxidative cleavage of **18** with periodic acid in diethyl ether gave the familiar ketone **6**, as well as a new aldehyde **20** which was isomeric but not identical with **5**. Hydrolysis of the acetate **20** with 3% potassium hydroxide in methanol resulted in β-elimination to yield the α,β-unsaturated aldehyde **7** (Scheme I), which was identical, including rotation, with that obtained from iriediol. Since the aldehyde **20** could be converted to **7**, the centers at C-1, C-4, C-5, and C-19 must be identical. Therefore, the variation between aldehydes **5** and **20** just lie at either C-6 or C-7. Examination of the ^{1}H NMR spectrum of **20** (Table II) showed the C-6 proton as an eight line pattern (δ 3.50) with couplings of 10, 8, and 4 Hz. In contrast, aldehyde **5**, the structure of which is secure from the X-ray work, showed an eight line pattern at δ 3.22 with coupling constants of 10, 4, and 4 Hz. From examination of molecular models and considerations of dihedral angles,[16] the 10-Hz coupling in both **5** and **20** must be derived from the trans vicinal methine proton (C-5–C-6) coupling. Since C-6 in **20** is identical with **5**, the variation in **20** must reflect the alternative stereochemistry at C-7. These data define the relative and absolute stereochemistry of irieol D (**18**), except for the hydroxyl-bearing centers at C-10 and C-11. The C-11 hydroxyl group is predicted as axial, in analogy to irieol E (**16**), and this assignment is supported by the comparable ^{13}C NMR values for this center (Table III). The stereochemistry of the C-10 hydroxyl cannot be assigned based upon these data.

Irieol F (**21**), a crystalline solid, was shown to have the molecular composition $C_{20}H_{34}Br_2O_4$ by elemental and mass spectral analyses. The ^{13}C NMR spectrum of **21** showed two secondary hydroxyl-bearing carbons (80.3 and 75.6 ppm) and two tertiary hydroxyl-bearing carbons (71.7 and 75.7 ppm), thus confirming that irieol F is a tetraol. Treatment of **21** with periodic acid in diethyl ether yielded the α,β-unsaturated aldehyde **7** and the bromo ketone **6**, both of which were identical in all respects with the aldehyde and ketone produced from **2** and **18**. A comparison of the ^{1}H NMR characteristics of irieol F with those of iriediol showed that the C-7 hydroxyl was inverted (Table I). Analysis of the C-6 proton band showed the vicinal C-5–C-6 coupling of 12 Hz, which confirmed that irieol F possessed the same C-6 stereochemistry as iriediol. As in **16** and **18**, the C-11 hydroxyl in **21** is proposed as axial based upon X-ray evidence from irieol A and ^{13}C NMR comparisons (Table III). As in the prior metabolites, the stereochemistry of the C-10 hydroxyl could not be determined by these data.

Irieol G (**22**), an amorphous solid, was foreseen to be related to irieol F (**21**) by acetylation at C-10. Mild basic hydrolysis converted **22** to **21**.

Irieol B and C. Investigation of the silica gel column fractions obtained from diethyl ether–benzene (1:1) elution yielded two compounds which were inseparable by further chromatographic procedures. Treatment of this mixture with acetic anhydride–pyridine served to acetylate one of the compounds, and the mixture was subsequently purified by repeated crystallization of the acetate. Crystalline irieol B acetate (**25**) analyzed for $C_{22}H_{36}Br_2O_4$ by combustion analysis and was reconverted to the natural product, irieol B (**23**), by mild alkaline hydrolysis. Comparison of the combined spectral data for irieol B (**23**) and irieol C (**24**) with the other irieols clearly showed their similarities. However, the lack of C-10 hydroxylation in both metabolites prevented the use of the previously utilized cleavage reactions to obtain smaller fragments. The noncrystalline component of the above mixture, irieol C (**24**), which was isolated as a glass, analyzed for $C_{20}H_{34}Br_2O_2$ by combustion analysis. The failure of irieol C to acetylate under mild conditions indicated that both hydroxyl groups were tertiary. In order to securely assign the structures of both **23** and **24**, a complete comparison of both ^{1}H and ^{13}C NMR spectral data was made with all the irieols (Tables I and III). Of particular importance were the readily assignable ^{13}C values obtained for **23–25** (Table III).

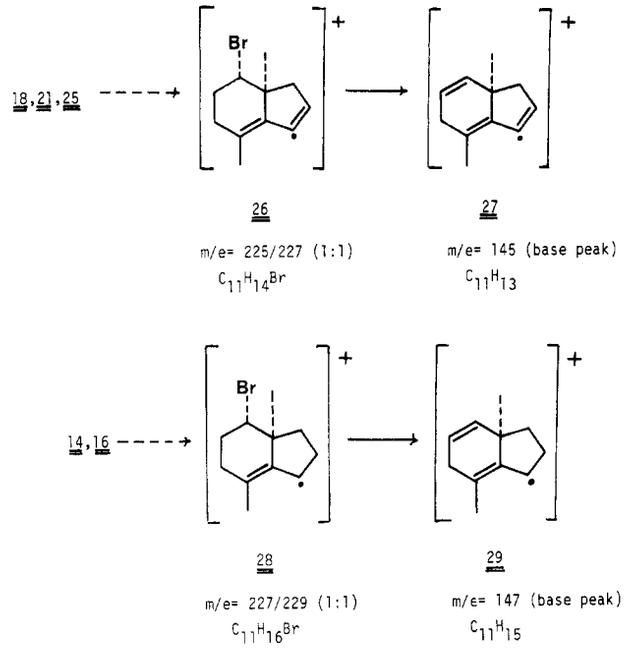
^{13}C NMR Study of the Irieols and Derivatives. ^{13}C NMR data for the irieols, derivatives, and degradation products are tabulated in Tables III and IV, respectively. Assignments of the chemical shifts of the A ring carbons (C-1–C-5 and C-9) were made based upon comparisons of the ^{13}C NMR values for oppositol (**13**) and the aldehydes **5**, **7**, **12**, and **20** (Table IV), all of which are structurally analogous. Off-resonance decoupling experiments with oppositol indicate that the 63.3 ppm doublet is the C-1, bromine-bearing methine carbon. Carbons 2 and 3 are assigned at 31.2 (t) and 40.4 (t) ppm since these bands are also observed in the degradation products in Table IV. The singlet at 71.6 ppm is assigned in oppositol to C-4, consistent with the other irieol degradation products. In oppositol the C-9 singlet appears at 47.7 ppm while C-5 has a chemical shift of 60.7 ppm. C-5 can be distinguished from C-6 based upon the deshielding effects observed in comparing oppositol (**13**) to the aldehyde **7**. In the B ring of oppositol only C-7 and C-8 must be distinguished. Of the two remaining off-resonance triplets, the highest field band, 28.5 ppm, was assigned to C-7. This leaves the neopentyl carbon, C-8, assigned to the triplet at 42.0 ppm. This assignment is substantiated by the C-8 shift to 47.4 ppm in the aldehyde **7** and to 48.7 ppm in **20**. The most deshielded methyl group in oppositol (30.6 ppm) is assigned to the C-4 bearing methyl, which leaves the 16.3 ppm quartet assigned to the bridgehead quaternary methyl (C-18). Analysis of the ^{13}C NMR spectrum for the bromo ketone **6** yields the following assignments: C-1, 208.7 (s); C-2, 51.9 (t); C-3, 40.5 (s); C-4, 61.9 (d); C-5, 32.5 (t); C-6, 38.7 (t) ppm; and two methyl quartets at 25.9 and 28.2 ppm.

Having as a basis the complete ^{13}C NMR analysis of oppositol, several closely related compounds (**5**, **7**, **12**, and **20**), and the ketone **6**, the assignments for the irieols became feasible.

Inspection of Table III shows that the irieols and their acetates all have similar chemical shifts for the A ring carbons, C-1 through C-5, C-9, C-17, and C-18; however, a distinct shift in C-17 is observed with a change in C-7 functionality. The chemical shifts of the B ring carbons C-6, C-7, and C-8 vary consistently with changes in functionality at C-10 and C-7. When C-10 is hydroxylated as in **16**, **17**, and **18**, its chemical shift varies from 78.3 to 80.3 ppm, while C-11 has a chemical shift between 75.0 and 75.7 ppm. The remainder of the C ring carbons are assigned for the irieols based upon analogy to the bromo ketone **6**.

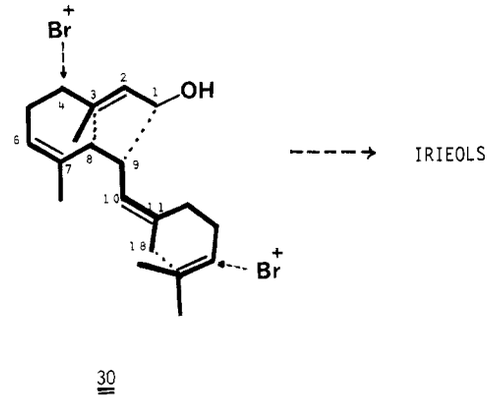
Inspection of the ^{13}C NMR bands for irieol B acetate (**25**) and irieol C (**24**) reveals their close similarities to the other irieol derivatives. The only obvious variations are at C-6, C-10, and C-11, which illustrate the lack of hydroxylation at C-10 and the resultant neighboring group effects.

Mass Spectral Fragmentation of the Irieols. Analysis of high-resolution mass spectral data obtained for the irieols revealed several consistencies. None of the irieols show parent ions, but rather an $M^+ - H_2O$ fragment due to dehydration involving the C-4 hydroxyl group. The major diagnostic skeletal fragmentation apparently consists of cleavage of the C ring at the C-6–C-10 bond. Irieol D (**18**), irieol F (**21**), and irieol B acetate (**25**) exhibit a common base peak at *m/e* 145, which analyzes for $C_{11}H_{13}$ (anal 145.1011, calcd 145.1017) and



which we propose is accounted for by ion **27.** The brominated ion **26** can also be observed in the mass spectra of the irieols. For the irieols which lack functionality at C-7 (**14, 16,** and **24**), the fragments **28** ($C_{11}H_{16}Br$) and **29** ($C_{11}H_{15}$) are prominent. In the mass spectrum of irieol C (**24**) the fragment **29** represents the base peak. Such fragmentation data is useful in substantiating the assignment of **23** and **24** to the irieol family of diterpenoids.

Biogenesis of the Irieols. The irieols are regular diterpenoid structures which can be rationalized as being produced from geranylgeraniol (**30**) via successive bromonium ion in-



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duced cyclizations. If geranylgeraniol is proposed as a direct precursor, it must follow that several double-bond migrations, $\Delta^{2,3} \rightarrow \Delta^{3,4}$, $\Delta^{6,7} \rightarrow \Delta^{7,8}$, and $\Delta^{10,11} \rightarrow \Delta^{11,18}$, must occur prior to bromonium ion induced cyclization. The occurrence of the closely related but sesquiterpenoid alcohol oppositol in *Laurencia* suggests that similar pathways exist for cyclization of C-15 and C-20 precursors.

Experimental Section

^1H NMR spectra were recorded on a Varian HR-220 spectrometer, ^{13}C NMR spectra on a Varian CFT-20 spectrometer, and infrared spectra on a Perkin-Elmer Model 137 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter, using a 10 cm microcell. Low-resolution mass spectra were obtained on a Hewlett-Packard 5930A mass spectrometer, and high-resolution mass spectra were obtained through the Departments of Chemistry, UCLA and Iowa State University.

Collection, Extraction, and Chromatographic Separation. *Laurencia irieii* was collected in August 1973 (Puerto Peñasco, Mexico), air-dried, and ground within 1 week to a 1 mm particle size (Wiley mill). Soxhlet extraction of the dried alga (1 kg) with chloroform–methanol (1:1) gave, after solvent evaporation, a dark green oil (35 g). The crude extract (30 g) was applied to a column containing 300 g of silica gel (Grace, grade 62). The column was eluted with a solvent gradient system of increasing polarity from petroleum ether to benzene to diethyl ether.

Irieol (14). Two fractions which were eluted with petroleum ether–benzene (1:1) contained **14.** These fractions were combined (300 mg) and rechromatographed (preparative silica gel TLC, 20% diethyl ether–petroleum ether) to yield a pure sample of irieol (55 mg, 0.0055% dry weight basis): oil; $[\alpha]^{23}_D$ −23.1° (*c* 1.34, $CHCl_3$); IR ($CHCl_3$); 3600, 3010 cm^{-1}; mass spectrum, *m/e* (relative intensity) 432 (1.5), 430 (3), 428 (1.5) ($M^+ - H_2O$) for $C_{20}H_{32}Br_2$.

Irieol A (1). One fraction eluted with benzene deposited crystals upon solvent evaporation. Crystallization from chloroform–diethyl ether gave 20 mg of 1 (0.0020% dry weight): mp 142–144 °C; $[\alpha]^{25}_D$ 0° (*c* 1.01, $CHCl_3$); IR ($CHCl_3$) 3600, 1205 cm^{-1}; mass spectrum, *m/e* (relative intensity) 448 (1), 446 (2), 444 (1) for $C_{20}H_{30}Br_2O$ ($M^+ - H_2O$).

Iriediol (2). Diethyl ether–benzene (1:20) elution gave five fractions which deposited crystals. Crystallization from chloroform gave **2** (400 mg, 0.04% dry weight): mp 103–105 °C dec; $[\alpha]^{23}_D$ −18.3° (*c* 2.5, $CHCl_3$); IR ($CHCl_3$) 3700, 3010 cm^{-1}; mass spectrum, *m/e* (relative intensity) 448 (3), 446 (6), 444 (3) for $C_{20}H_{30}Br_2O$ ($M^+ - H_2O$).

Irieol B (23) and C (24). Irieol B and C (**23** and **24**) were obtained as a 1:1 mixture (1.5 g, 0.15% dry weight) in fractions obtained upon diethyl ether–benzene (1:1) elution. Thin-layer chromatography showed that **23** and **24** migrated as a single spot. Treatment of this mixture with acetic anhydride–pyridine overnight at room temperature gave a crystalline material after normal workup. Recrystallization from diethyl ether–petroleum ether (1:5) gave pure irieol B acetate (**25**). Repeated crystallization gave pure samples of irieol C (**24**) in the diethyl ether–petroleum ether mother liquor. Saponification of **25** (3% KOH–MeOH) gave irieol B (**23**) as an amorphous solid: IR ($CHCl_3$) 3500 cm^{-1}; mass spectrum, *m/e* (relative intensity) 466 (1), 464 (2), 462 (1) for $C_{20}H_{30}Br_2O_2$ ($M^+ - H_2O$). For irieol B acetate (**25**): mp 127–129 °C; $[\alpha]^{25}_D$ 37.8° (*c* 2.6, $CHCl_3$); IR ($CHCl_3$) 3400, 1735, 1250 cm^{-1}. Mass measurement observed *m/e* 491.0618; $C_{21}H_{31}Br_2O_3$ ($M^+ - H_2O - CH_3$) requires *m/e* 491.0620. Anal. Found: C, 50.16; H, 6.91. $C_{22}H_{36}Br_2O_4$ requires C, 50.42, and H, 6.87. For irieol C (**24**): amorphous solid; mp 110–112 °C; $[\alpha]^{25}_D$ 34.2° (*c* 2.7, $CHCl_3$); IR ($CHCl_3$) 3500 cm^{-1}; mass spectrum, *m/e* (relative intensity) 432 (0.6), 430 (1.2), 428 (0.6) for $C_{20}H_{30}Br_2$ ($M^+ - 2\ H_2O$). Anal. Found: C, 51.34; H, 7.53. $C_{20}H_{34}Br_2O_2$ requires C, 51.54, and H, 7.29.

Irieol D (18), E (16), F (21), and G (22). Diethyl ether elution of the silica gel column gave a complex mixture which consisted of **16, 18, 21,** and **22** (3 g, 4:2:3:1). This mixture migrated as a single component on a silica gel thin-layer plate. Subjection to LC (2 ft μ-porasil, 7% diethyl ether–dichloromethane) separation, however, gave pure samples of each diterpenoid, eluted in the order irieol G (**22**), E (**16**), D (**18**), and F (**21**).

Irieol E (16): amorphous solid; $[\alpha]^{25}_D$ −30.4° (*c* 3.22, $CHCl_3$); IR ($CHCl_3$) 3600, 3400 cm^{-1}; mass spectrum, *m/e* 462/464/466 (1:2:1) for $C_{20}H_{32}Br_2O_2$ ($M^+ - H_2O$).

Irieol D (18): amorphous solid; $[\alpha]^{25}_D$ −30.0° (*c* 4.02, $CHCl_3$); IR ($CHCl_3$) 3500, 1735 cm^{-1}. High-resolution mass measurement observed *m/e* 522.0815; $C_{22}H_{34}Br_2O_4$ requires *m/e* 522.0803 ($M^+ - H_2O$).

Irieol F (21): crystalline solid; mp 147–148 °C; $[\alpha]^{25}_D$ −27.0° (*c* 3.38, $CHCl_3$); IR ($CHCl_3$) 3400 cm^{-1}. Anal. Found: C, 48.07; H, 7.01. $C_{20}H_{34}Br_2O_4$ requires C, 48.21, and H, 6.88.

Irieol G (22): amorphous solid; $[\alpha]^{25}_D$ −11.3° (*c* 1.79, $CHCl_3$); IR ($CHCl_3$) 3600, 1735 cm^{-1}; mass spectrum, *m/e* 520/522/524 (1:2:1) for $C_{22}H_{34}Br_2O_4$ ($M^+ - H_2O$).

Oxidation of Iriediol (2) to the Ketone 4. 2 (20 mg) was dissolved in pyridine (2 mL) and maintained at 0 °C. Cornforth reagent (CrO_3–pyridine–water) was added dropwise with stirring until a red color persisted. After stirring at 0 °C for 30 min, the reaction mixture was diluted with diethyl ether (25 mL) and poured onto ice. The ether was washed with 5% HCl (5 × 25 mL), water (25 mL), and saturated $NaHCO_3$ (25 mL) and dried ($MgSO_4$). Evaporation of the ether gave an oil which was chromatographed over silica gel (0.5 g) to give the

cyclopentanone 4 (12 mg): oil; IR 3400, 1740 cm^{-1}; mass spectrum, *m/e* (relative intensity) 464 (7), 462 (13) 460 (7) for $C_{20}H_{30}Br_2O_2$.

Oxidative Cleavage of Iriediol Acetate (3). A catalytic amount of crystalline OsO_4 (~5 mg) was added to a stirred solution containing iriediol acetate (50 mg, 0.099 mmol), dioxane (4 mL), and water (1 mL). When the solution became dark brown, in about 30 min, $NaIO_4$ (42 mg) was added in three portions over a 15-min period. After stirring for 3 h at 25 °C, the mixture was pale yellow and a fine white precipitate was present. The reaction mixture was extracted with ether (3 × 50 mL), and the ether extract washed with water (2 × 50 mL) and dried ($MgSO_4$). Removal of the ether in vacuo gave a black oily residue which was chromatographed over silica gel to yield the bromo ketone **6** (6.8 mg, 34%) and the crystalline aldehyde **5** (11.9 mg, 36%). For **6**: oil; $[\alpha]^{23}_D$ +31.9° (*c* 0.68, $CHCl_3$); IR (film) 2920, 1715, 1460, 1440, 1420, 1385, 1360, 1340, 1320, 1280, 1235, 1225, 1180, 1150, 1110, 1000, 960, 910, 880, 840, 780, 750, 680 cm^{-1}. High-resolution mass measurement, *m/e* 204.0149; $C_8H_{13}BrO$ requires *m/e* 204.0150. For **5**: $[\alpha]^{23}_D$ −26.6° (*c* 1.19, $CHCl_3$); IR ($CHCl_3$) 3600, 3450, 2700, 1730, 1710 cm^{-1}; mass spectrum, *m/e* 332/334 (1:1) for $C_{14}H_{21}BrO_4$.

Aldehydes 7, 8, and 9. To a stirred solution of the aldehyde **6** (6 mg, 0.018 mmol) in MeOH (1 mL) was added 1 mL of 6% KOH–MeOH dropwise at 25 °C. After 1 h, ether was added and the ether was washed with 5% HCl (2 × 25 mL) followed by water (25 mL). The ether layer was dried and the volume reduced in vacuo to yield a mixture of two components (TLC), one of which was UV-active. TLC separation (1:1 diethyl ether–petroleum ether) gave the α,β-unsaturated aldehyde **7** (3.7 mg, 77%) and the methoxide addition products **8** and **9** as a mixture (1.0 mg, 18%). For **7**: oil; $[\alpha]^{23}_D$ +16.2° (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) 3600, 3450, 3100, 2700, 1670; mass spectrum, *m/e* 272/274 (1:1) for $C_{12}H_{17}BrO_2$. For **8** and **9**: IR ($CHCl_3$) 3600, 3450, 2700, 1710, 1100 cm^{-1}; mass spectrum, *m/e* 304/306 (1:1) for $C_{13}H_{21}BrO_3$.

Catalytic Hydrogenation of the α,β-Unsaturated Aldehyde 7. The aldehyde **7** (20 mg, 0.073 mmol) in diethyl ether was added to a 50-mL Erlenmeyer suction flask containing a catalytic amount of 10% Pd/C (10 mg) and a stirring bar. The flask was fitted with a septum and balloon. The reaction vessel was purged with hydrogen, and then the balloon was filled. After stirring at 25 °C for 12 h, the hydrogen was removed, the solution filtered, and the ether evaporated to give, without purification, the primary alcohol **10** (14 mg, 69%): IR ($CHCl_3$) 3650, 3450 cm^{-1}; mass spectrum, *m/e* 276/278 (1:1) for $C_{12}H_{21}BrO_2$.

Oxidation of the Primary Alcohol 10. To a solution of the primary alcohol **10** (10 mg, 0.036 mmol) in CH_2Cl_2 (2 mL) was added 1 equiv of pyridinium chlorochromate with stirring at 25 °C. After 3 h, the reaction mixture was diluted with CH_2Cl_2 (25 mL) and filtered through a silica gel column to yield the epimeric aldehyde **11** (7.5 mg, 76%): IR ($CHCl_3$) 3100, 2515, 1715 cm^{-1}; mass spectrum, *m/e* 274/276 (1:1) for $C_{12}H_{19}BrO_2$.

Epimerization of the Aldehyde 11. To the aldehyde **11** (5 mg, 0.018 mmol) in MeOH (1 mL) was added 6% methanolic potassium hydroxide (1 mL) dropwise with stirring at 25 °C. After 1 h, the reaction mixture was diluted with diethyl ether (50 mL) and washed with 5% HCl (2 × 25 mL) and water (2 × 25 mL). The ether was dried ($MgSO_4$) and the volume reduced in vacuo to yield, without purification, a pure sample of **12** (4.7 mg, 97%) ($[\alpha]^{25}_D$ +2.16° (*c* 0.47, $CHCl_3$); IR (film) 3100, 2815, 2515, 1715, 1450, 1360, 1320, 1220, 1195, 1180, 1110, 1020, 990, 960, 935, 925, 890, 780, 715 cm^{-1}; mass spectrum, *m/e* 274/276 (1:1) for $C_{12}H_{19}BrO_2$), which was identical with that produced by ozonation of oppositol (**13**).[14]

Oxidative Cleavage of Irieol A (1). To a solution of irieol A (**1**) (10.1 mg, 0.022 mmol) in diethyl ether (2 mL) was added saturated H_5IO_6 in diethyl ether (1 mL) with stirring at 25 °C. HIO_4 began to precipitate after 2 h, and after 8 h the reaction was filtered. Evaporation of the ether gave an oil which was purifed by TLC to give the aldehyde **12** (1.5 mg, 20%), $[\alpha]^{25}_D$ +6.01° (*c* 0.15, $CHCl_3$), which was identical in all respects with that produced from **13** and **2**. The ketone **6** could not be isolated.

Oxidative Cleavage of Irieol (14). A catalytic amount of crystalline OsO_4 (1 mg) was added to a stirred solution containing **14** (12 mg, 0.026 mmol), dioxane (2 mL), and water (0.5 mL). The solution became dark brown within 30 min, at which time $NaIO_4$ (11 mg, 0.052 mmol) was added. When the solution became pale yellow, in about 3 h, it was extracted with diethyl ether (3 × 25 mL) and the combined ether extracts were washed with brine (2 × 25 mL). The ether layer was dried ($MgSO_4$) and evaporated to yield a black residue which was stored in chloroform for 24 h at 25 °C. Preparative layer chromatography gave the crystalline acid **15** (3.5 mg, 46%) (mp 160–162 °C; $[\alpha]^{23}_D$ −7.69° (*c* 0.39, $CHCl_3$); IR ($CHCl_3$) 3200, 1730 cm^{-1}; mass spectrum, *m/e* 290/292 (1:1) for $C_{12}H_{19}BrO_3$), which was identical with a sample produced by oxidative ozonation of **13**, $[\alpha]^{25}_D$ −5.43° (*c* 0.35, $CHCl_3$). In addition, the bromo ketone **6** was obtained and found to be identical with that produced from **2**.

Oxidative Cleavage of Irieol E (16). Irieol E (**16**) (250 mg, 0.52 mmol) was dissolved in 10 mL of diethyl ether, and excess (10 mL) saturated H_5IO_6 in diethyl ether was added dropwise with stirring. After 2 h, when considerable amounts of HIO_4 had precipitated, the reaction mixture was filtered and the ether evaporated to give a viscous oil. Preparative layer chromatography (silica) gave pure samples of the aldehyde **12**, $[\alpha]^{25}_D$ +6.26° (*c* 8.71, $CHCl_3$) (87.1 mg, 61.3%), and the ketone **6**, (84.8 mg, 80%) $[\alpha]^{25}_D$ +36.4° (*c* 8.48, $CHCl_3$), both of which were shown to be identical with samples produced from **2**.

Jones Oxidation of Ireiol D (18). **18** (35 mg) was dissolved in acetone and maintained at −5 °C. Jones reagent[19] was added dropwise until a red color persisted, at which time the reaction was diluted with diethyl ether (20 mL) and poured onto ice. The ether layer was washed with water (2 × 25 mL) and saturated $NaHCO_3$ (2 × 25 mL). The ether was dried ($MgSO_4$) and evaporated to yield a crystalline residue. Trituration with hexane gave pure crystalline **19** (20 mg): mp 215–217 °C; IR 3400, 1740, 1705 cm^{-1}. High-resolution mass measurement, *m/e* 520.0859; $C_{22}H_{34}Br_2O_4$ ($M^+ - H_2O$) requires *m/e* 520.0824.

Oxidative Cleavage of Irieol D (18). Irieol D (**18**) 40 mg. 0.007 mmol) was dissolved in diethyl ether (2 mL), and excess (2 mL) saturated H_5IO_6 in diethyl ether was added dropwise with stirring. A white precipitate was immediately visible, and the reaction was allowed to proceed for 1 h. The reaction mixture was then filtered and the ether evaporated to give a semisolid residue. Trituration with hexane left the pure crystalline aldehyde **20** (16.5 mg, 64.5%): mp 109–111 °C; $[\alpha]^{23}_D$ +22.4° (*c* 1.65, $CHCl_3$); IR ($CHCl_3$) 3500, 2700, 1710, 1730 cm^{-1}; mass spectrum, *m/e* 332/334 (1:1) for $C_{14}H_{21}BrO_4$. Preparative layer chromatography (silica) of the hexane phase gave the bromo ketone **6** (10.2 mg, 65.0%), $[\alpha]^{25}_D$ +22.6° (*c* 1.02, $CHCl_3$), which was identical with that produced from **2**.

Oxidative Cleavage of Irieol F (21). **21** (50 mg) was dissolved in diethyl ether (15 mL), and excess (5 mL) saturated H_5IO_6 in diethyl ether was added with stirring. After 2 h, the mixture was filtered and the ether evaporated to give an oil. Preparative layer chromatography gave the α,β-unsaturated aldehyde **7**, $[\alpha]^{25}_D$ +38.4° (*c* 0.56, $CHCl_3$), and the ketone **6**, which were identical with samples produced from **2**.

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Registry No.—**1**, 58844-61-4; **2**, 55708-74-2; **3**, 67506-09-6; **4**, 67506-10-9; **5**, 67506-27-8; **6**, 67506-29-0; **7**, 67506-21-2; **8**, 67506-22-3; **9**, 67529-78-6; **10**, 67506-23-4; **11**, 67506-24-5; **12**, 67506-25-6; **13**, 50906-92-0; **14**, 67506-11-0; **15**, 67506-26-7; **16**, 67506-12-1; **17**, 67506-13-2; **18**, 67506-14-3; **19**, 67506-15-4; **20**, 67506-28-9; **21**, 67506-16-5; **22**, 67506-17-6; **23**, 67506-18-7; **24**, 67506-19-8; **25**, 67506-20-1.

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Synthesis of γ-Lactone Ring Fused to Steroidal Ring D of Salamander Alkaloids

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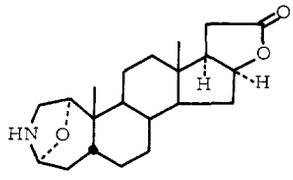
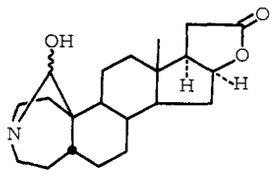
Synthesis of steroids with a γ-lactone ring fused to ring D is described with a view to total synthesis of the salamander alkaloids such as samandaridine (**1**) and cycloneosamandaridine (**2**) and its revised structure **3**. Reformatsky reaction of 3β,16β-diacetoxy-5-androsten-17-one (**4**) with methyl bromoacetate gave 17α-substituted ester **6a**. Dehydration and catalytic hydrogenation of **6b** afforded 17β-substituted ester **10**, which was cyclized to give 3β-acetoxy-16β-hydroxy-5α-pregnan-21-oic acid γ-lactone (**11**). The same product was obtained from 16α-acetoxy ketone **12**. In this case, cyclization of hydroxy carboxylic acid **17b** proceeded under more vigorous conditions of high temperature and strong acid catalysis.

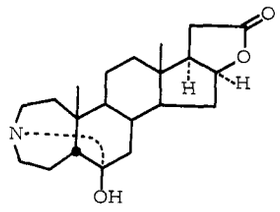
The biologically active salamander alkaloids[1] consist of steroidal ring systems which are distinguished by a nitrogen-containing ring A and substituents on ring D. Previous synthetic studies have been concerned with the construction of the ring A. However, little effort has been devoted to the introduction of ring D substituents. Conversion of samandarone to samandaridine (**1**) by Habermehl[2] and our



1



2



3

transformation of the 17-oxo function to the 16 position in the total synthesis of samandarone[3] are rare instances. The five-membered lactone ring fused to ring D of samandaridine (**1**) has been derived from a steroid which contains an oxygen function at the 16 position, but not from the normal 17-oxo steroid. Our purpose of synthesis and confirmation of the proposed structure of cycloneosamandaridine (**2**)[4] required stereospecific construction of this β-oriented γ-lactone ring. In this paper we wish to report the preparation of 3β-acetoxy-16β-hydroxy-5α-pregnan-21-oic acid γ-lactone (**11**) from both 3β,16β-diacetoxy-5-androsten-17-one (**4**)[5] and 3β,16α-diacetoxy-5α-androstan-17-one (**12**).[6] The results mentioned in this paper were useful for our synthesis of structure **2**, earlier assigned to cycloneosamandaridine, and will also be applicable to the synthesis of the newly proposed structure **3**.[7]

Reformatsky reaction of 16α- and 16β-acetoxy-17-oxo steroids with methyl bromoacetate was considered to be a suitable method for preparation of the two carbon unit attached to the 17 position.[8] The use of both C-16 isomers seemed indispensable for proving the stereochemistry of the fused γ-lactone ring at the late stage of our sequence, as it would be difficult to determine the configuration at position 17 by spectroscopic means before and after the lactonization.

Reformatsky reaction of **4**, which was obtained from dehydroepiandrosterone,[5] with methyl bromoacetate afforded two glycols. The less polar glycol **5a** was easily converted to an acetonide **7** and a diacetate **5b**. The NMR spectrum of **5a** exhibited a one-proton singlet at 3.23 ppm, while in **5b** this signal was observed in the lower field at 4.47 ppm. The most likely explanation for **5a** is methoxycarbonylmethylation from the less hindered 16α side after the regioisomerization of **4**, with the configuration of the vicinal hydroxyl groups having a β cis relationship. The more polar glycol **6a** showed a triplet at 3.90 ppm which was attributed to the 16α hydrogen. This compound was readily converted to an acetonide **8** and a diacetate **6b**, wherein the resonance attributed to the 16α hydrogen shifted downfield to 4.90 ppm (quartet). These facts clearly showed the glycol **6a** to be the desired 17α-substituted derivative.

Dehydration of **6b** was achieved to afford an unsaturated ester **9**. Although the details of the geometrical isomerism are not clearly known, **9** consisted of one isomer and showed a one-proton doublet at 5.66 ppm which was attributed to an olefinic hydrogen adjacent to the carboxyl group. Catalytic hydrogenation of **9** gave **10**. It is reasonable to assume that the stereochemistry is 5α as is usually the case in steroid chemistry.[9] Hydrogenolysis at the 17 position must have proceeded by means of the less hindered α-site attack. This is further confirmed by the next stage of the sequence where the lactonization between the 16β-hydroxyl group and the γ-carboxylic group proceeded quite readily. Thus, **10** was hydro-

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