Marine Natural Products: Parguerol, Deoxyparguerol, and Isoparguerol. New Brominated Diterpenes with Modified Pimarane Skeletons from the Sea Hare *Aplysia dactylomela*[1,2]

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Abstract: Five new brominated diterpenes, **8**, **16**, **17**, **19**, and **21**, have been isolated from extracts of the sea hare *Aplysia dactylomela* collected near La Parguera, Puerto Rico. The compounds possess new carbocyclic skeletons which are modifications of the known pimarane framework. Three of the compounds, parguerol (**8**), parguerol 16-acetate (**16**), and deoxyparguerol (**17**), contain a cyclopropane ring bridging positions 3 and 4 of the pimarane skeleton. The remaining two, isoparguerol (**19**) and isoparguerol 16-acetate (**21**), have an ethano group bridging C-3 and C-4 of the pimarane skeleton to form a cyclobutane ring. All five compounds are cytotoxic. Extensive ^{1}H NMR decoupling studies and some degradative experiments were used to establish the structures, and NOE experiments and CD data were employed to establish stereochemical details. Also isolated were several known or minor variants of known algal metabolites: 2,3,5-tribromo-*N*-methylindole (**1**), elatol (**2a**), two chamigrenes (**5a** and **6a**), allolaurinterol acetate (**3**), and isoobtusol acetate (**4**).

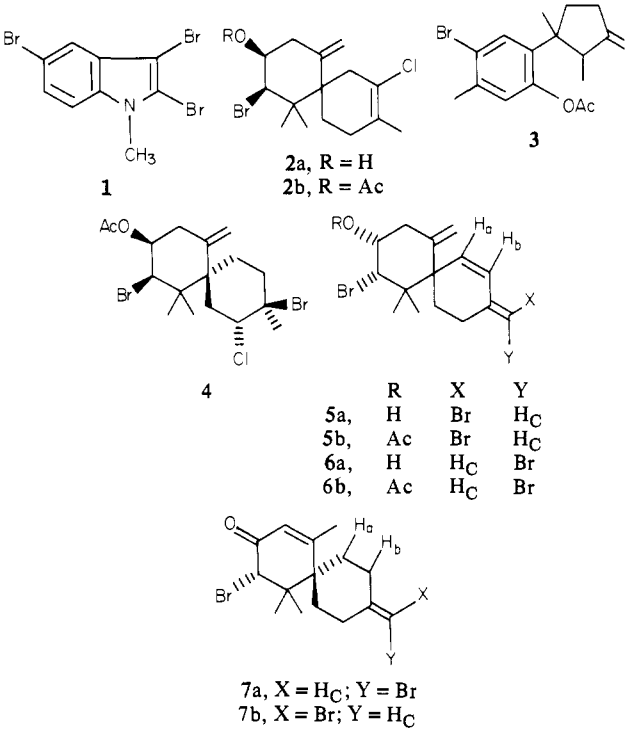
Since 1963 when Yamamura and Hirata first reported[3] brominated sesquiterpenes from the sea hare *Aplysia kurodai*, nearly 100 new secondary metabolites have been isolated from opisthobranch molluscs.[4] These soft-bodied animals have attracted attention because of the toxicity[5] of some species and because they have proven to be a rich source of novel metabolites which they accumulate from dietary sources,[6] primarily algae, but also occasionally sopnges.[5] Our attention was drawn to a Caribbean sea hare *Aplysia dactylomela* because of a report[7] that extracts thereof exhibited cytotoxicity and in vivo tumor inhibition. In a detailed study of extracts of this sea hare collected at Bimini, Bahamas, we have isoated over a period of several years 16 new compounds,[1] three of which show significant levels of cytotoxicity and one of which shows drug metabolism inhibition in mice.[8] Since *A. dactylomela* yielded such an interesting array of metabolites and because the content of sea hare digestive glands is diet dependent,[5,6] we decided to analyze specimens of this species collected from another location. We report here on metabolites obtained from *A. dactylomela* collected near La Parguera, Puerto Rico. The most significant discovery has been the isolation of a group of brominated diterpenes with new carbocyclic skeletons, some containing a cyclopropane and others a cyclobutane ring. The new compounds are most closely related to the pimarane diterpenes. Interestingly, our work to date indicates that the complex mixtures obtained from *A. dactylomela* from Puerto Rico and Bimini, Bahamas, do not have any common components.

Results and Discussion

Specimens of *A. dactylomela* were collected by hand in shallow waters (0 to −1 m) near La Parguera, Puerto Rico, and the digestive glands were removed and stored in isopropyl alcohol. The methylene chloride soluble material from a concentrated chloroform–methanol (2:1) extract of homogenized glands was partitioned between aqueous methanol and a succession of organic solvents according to the procedure of Kupchan[9] to give hexane, carbon tetrachloride, and chloroform soluble fractions. The last two fractions exhibited significant levels of cytotoxicity [ED_{50} (PS) 2.4 and 1.1 μg/mL, respectively].[10]

From the carbon tetrachloride fraction the known algal metabolites **1**,[11] **2a**[12] (elatol), and **5a**[13a,b] (see Chart I) were obtained by open-column and high-pressure liquid chromatography (HPLC). Elatol (**2a**), previously reported[12] as an oil, crystallized. Elatol acetate (**2b**) prepared by routine acetylation exhibited a melting point identical with reported values[12] but gave a lower optical rotation. Also isolated were allolaurinterol acetate (**3**) and isoobtusol acetate (**4**) (Chart I), whose parent alcohols had previously been isolated from algae.[14,15] In the case of **4** an authentic

Chart I

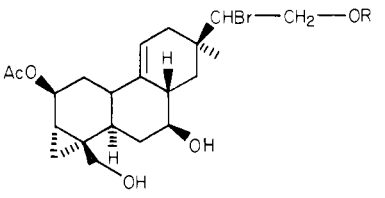


† University of Oklahoma.
‡ Oklahoma Medical Research Foundation.

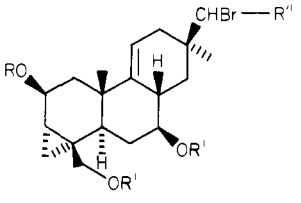
(1) Preliminary report: Schmitz, F. J.; Gopichand, Y.; Michaud, D. P.; Prasad, R. S.; Remaley, S.; Hossain, M. B.; Rahman, A.; Sengupta, P. K.; van der Helm, D. *Pure Appl. Chem.* **1981**, *51*, 853.
(2) Taken from the Ph.D. Thesis of D. P. Michaud, University of Oklahoma, 1980.
(3) Yamamura, S.; Hirata, Y. *Tetrahedron* **1963**, *19*, 1485.
(4) Michaud, D. P. Ph.D. Dissertation, University of Oklahoma, Norman, OK, 1980.
(5) See, for example: Scheuer, P. J. *Isr. J. Chem.* **1977**, *16*, 52.
(6) Stallard, M. O.; Faulkner, D. J. *Comp. Biochem. Physiol. B* **1974**, *49B*, 25; **1974**, *49B*, 37.
(7) Sigel, M. M.; Welham, L. L.; Lichter, W.; Dudek, L. E.; Gargus, J. L.; Lucas, A. H. *Food–Drugs Sea, Proc. Conf. Drugs Sea 2nd* **1969**, 281.
(8) Kaul, P. N.; Kulkarni, S. K. *J. Pharm. Sci.* **1978**, *67*, 1293. Kaul, P. N.; Kulkarni, S. K.; Schmitz, F. J.; Hollenbeak, K. H. "Food-Drugs from the Sea, Myth or Reality"; Kaul, P. N., Sindermann, C., Eds.; University of Oklahoma Press: Norman, OK, 1978; p 99.
(9) Kupchan, M. S.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178.

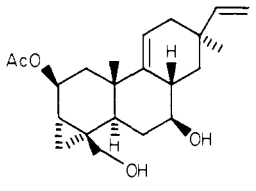
0002-7863/82/1504-6415$01.25/0

Chart II



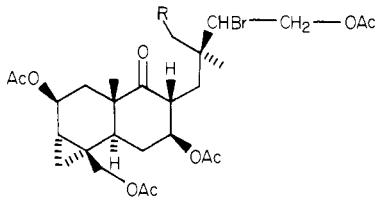
8, R = H
16, R = Ac





	R	R′	R″
9,	Ac	Ac	CH_2OAc
10,	Ac	Bz	CH_2OBz
12,	H	H	CH_2OH
13,	H	H	CHO

7



14, R = $CH(OMe)_2$
15, R = CO_2H

Table I. Physical Properties and Formulas of New Diterpenes

compd	mp, °C	[α], deg (concn in $CHCl_3$)	formula
8		−40.0 (0.03)	$C_{22}H_{33}BrO_5$
16	121–123	−40.5 (0.40)	$C_{24}H_{35}BrO_6$
17		−35.8 (0.62)	$C_{22}H_{33}BrO_4$
19	139–141	+3.6 (0.14)	$C_{22}H_{33}BrO_5$
21	180–182	−18.8 (0.09)	$C_{24}H_{35}BrO_6$

sample[16] was obtained for reference, while for **3** published spectral properties of synthetic material were available for comparison. A new chamigrene derivative, **6a** (Chart I), a stereoisomer of **5a**, was also separated. Upon standing at room temperature pure **5a** and **6a** each isomerize to give mixtures of the two compounds. Isomers **5a** and **6a** exhibit nearly identical ^1H NMR signals for

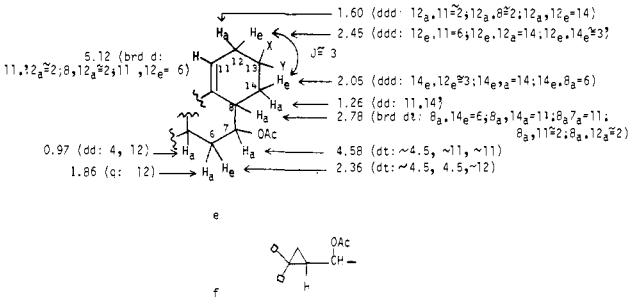


Figure 1. Partial structures e (from ^1H NMR data for **9** in C_6D_6 at 360 MHz) and f.

their quaternary methyl groups, CHOH, CHBr, and the exocyclic methylene protons (see Experimental Section). The 3(15)*E* configuration in **6a** is supported by the fact that the chemical shifts of the H_a, H_b, and H_c protons in **6a** differ from those in the **5a** [3(15)*Z* isomer] in the same manner as do the corresponding pairs of protons in the 3(15)*E* and *Z* isomeric ketones **7a** and **7b**.[13a,c]

The cytotoxic chloroform-soluble material from the solvent partitioning scheme was fractionated first by Sephadex LH-20 chromatography and then by chromatography over silica gel with open columns and HPLC. This led to isolation of the group of related monobromo diterpenes **8**, **16**, **17**, **19**, and **21** (Charts II and IV), whose physical properties are listed in Table I. The major component **8**, named parguerol,[17,18] was assigned the molecular formula $C_{22}H_{33}O_5Br$ on the basis of combustion data and high-resolution mass spectral analysis (highest observed mass, $M^+ - 18$). The infrared spectrum of **8** indicated the presence of hydroxyl and acetate groups. ^1H NMR data (see Table II) confirmed the presence of one acetate methyl group, two quaternary methyl groups (1.06 and 1.13 ppm), one trisubstituted double bond, and one trisubstituted cyclopropane ring, partial structure a (see H_3, H-18exo, 18-endo). ^{13}C NMR data confirmed that **8** has one trisubstituted double bond and no carbonyl groups other than that of the acetate. These groups account for three of the six degrees of unsaturation implied by the molecular formula and establish that **8** must have three rings in addition to the cyclopropane moiety a.

Acetylation of **8** yielded **9**, the IR and ^1H NMR of which showed that no hydroxyl groups were present and that three acetate groups had been added. Benzoylation under standard conditions yielded the noncrystalline tribenzoate **10**. Hence **8** possesses three primary and/or secondary hydroxyl groups, and these together with the acetate group of the natural product account for all of the oxygen atoms, thereby excluding the possibility of any ether functionality.

The three primary/secondary alcohol groups were assigned to partial structures b, c, and d on the basis of comparison of the

(10) Gueran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, No. 2. Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations in μg/mL of test material in the growth medium that causes 50% inhibition of cell growth. "Active" materials display an $ED_{50} \leq 20$ μg/mL. PS(P388) refers to in vitro lymphocytic leukemia.

(11) Carter, G. T.; Rinehart, K. L.; Li, L. H.; Kuentzel, S. L.; Connor, J. L. *Tetrahedron Lett.* **1978**, 4479.

(12) (a) Sims, J. J.; Lin, G. H. Y.; Wing, R. M. *Tetrahedron Lett.* **1974**, 3487. (b) For $[\alpha]_D$ see footnote 3 in ref 15. (c) For ^{13}C NMR, see: Sims, J. J.; Rose, A. F.; Izac, R. R. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. II, Chapter 5.

(13) (a) Suzuki, M.; Kurosawa, E. *Tetrahedron Lett.* **1978**, 4805. (b) Suzuki, M.; Furushahi, A.; Hashiba, N.; Kurosawa, E. *Tetrahedron Lett.* **1979**, 879; (c) [$\Delta\delta_{6a-5a}$ H_a = −0.15, H_b = −0.41, H_c = +0.21; $\Delta\delta_{7a-7b}$, H_a = −0.20, H_b = −0.41, H_c = +0.26].

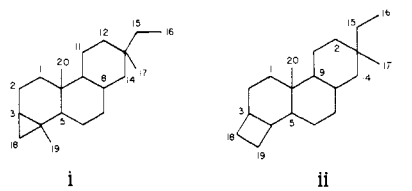
(14) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. *Aust. J. Chem.* **1976**, *29*, 2533.

(15) Gonzalez, A. G.; Darias, J.; Diaz, A.; Fourneron, J. D.; Martin, J. D.; Perez, C. *Tetrahedron Lett.* **1976**, 3051. Compound III in this paper is isoobtusol, although this name is not used. The name isoobtusol is used in Martin and Darius (Martin, J. D.; Darias, J. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. 1, Chapter 3).

(16) We thank Drs. A. G. Gonzalez and J. D. Martin for an authentic sample.

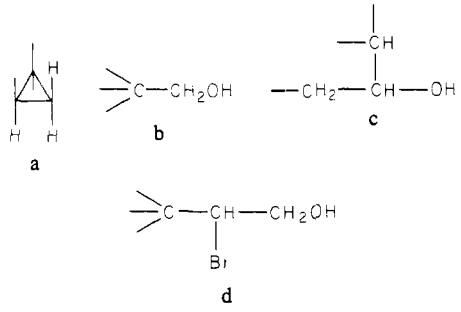
(17) This name is derived from the town of La Parguera, Puerto Rico, the area where the specimens of *Aplysia dactylomela* were collected and also the site of the marine laboratory of the University of Puerto Rico, Mayaguez. This name was selected because both parts of the binomial *Aplysia dactylomela* have already been incorporated into the names of other metabolites and because the sea hare is most likely not the ultimate source of this alcohol and its derivatives.

(18) We suggest the names parguerane and isoparguerane for the new carbon skeletons i and ii corresponding to **8** and **19**, respectively.



i ii

(19) In C_6D_6 the protons of the acetoxybromoethyl side chain are superimposed on the broad triplet due to the >CHOAc absorbing at 4.58 ppm, but this caused no difficulty in interpretation.



^{1}H NMR data of parguerol (**8**) and its peracetate **9** in $CDCl_3$. Partial structure b was evident from a pair of mutually coupled doublets (J = 12 Hz; see H-19α,β, Table II) that shifted downfield in the spectrum of acetate **9**. Partial structure c was assigned on the basis of a doubled triplet methine signal (3.12 ppm) that shifted to 4.41 ppm in the acetylated product **9**. Partial structure d was inferred from the ABX pattern exhibited by the protons assigned as H-14 and H-16α,β (see Table II).[19] The larger chemical shift noted for the geminally coupled protons of the ABX multiplet upon acetylation (see H-16α,β, Table II) indicated a primary hydroxyl group with a neighboring secondary bromine instead of the converse.

Partial structure e, which represents the major portion of the carbocyclic skeleton in **8**, was deduced from extensive normal as well as difference[20] decoupling studies of acetate **9** in $CDCl_3$ and C_6D_6; see Figure 1 and Table II. The coupling between H-12e and H-14e was assigned to a W coupling across a quaternary carbon because H-12ax was definitely not coupled to either H-14e or H-14a nor was H-14a coupled to H-12a or H-12e. The 11–12-Hz couplings for protons on carbons 5–8 corresponded to diaxial couplings in a six-membered ring, suggesting that the unclosed portion of partial structure e was part of a cyclohexane ring. Resolution enhancement[21] of the spectrum taken at 360 MHz in $CDCl_3$ was required to see definitively the coupling of H-11 to H-8, H-12a and the homoallylic coupling H-12a to H-8 (see parital structure e).

Evidence that provided a basis for a logical way in which to combine all the partial structures was provided by several microscale degradation reactions. Deacetylation of **8** with lithium aluminum hydride yielded the tetraol **12** whose ^{1}H NMR spectrum was nearly identical with that of **8** except that a new multiplet for a >C*H*OH was evident at 4.40 ppm (d, J = 5.5 Hz), while the acetate methyl signal and one of the two coincident doublets at 5.35 ppm observed in the spectrum of **8** were missing. This hydrolysis confirmed that the two-proton doublet at 5.35 ppm in the $CDCl_3$ spectrum is due to superposition of signals from an olefinic proton and an acetate-deshielded methine proton. Manganese dioxide oxidation of the tetraol **12** gave a product, **13**, that exhibited IR absorption corresponding to a saturated aldehyde (1722 cm^{-1}). In the ^{1}H NMR spectrum of **13**, the AB portion of the hydroxybromoethyl group in **8** was missing, while the proton assigned to CHBr (4.18 ppm, d, J = 4 Hz) was coupled to the aldehyde proton (9.56 ppm, d, J = 4 Hz). Thus, the hydroxybromoethyl arrangement was confirmed. Absence of any IR absorption corresponding to an α,β-unsaturated ketone in the product from MnO_2 oxidation of **12** indicated that none of the alcohol groups, or the precursor acetate in **8**, were allylic to the double bond. Hence the unusually far downfield position, 5.25 ppm, of the acetoxy-deshielded methine proton (>CHOAc) in **8** could best be explained by placing this group next to the cyclopropane ring to give a cyclopropyl carbinyl acetate arrangement (partial structure f, Figure 1).

Reduction of **8** with Zn gave a mixture of products of which one, **11**, exhibited an ABX pattern in its ^{1}H NMR spectrum [4.89, 2 H, d, J = 12 Hz, and d, J = 17 Hz; 5.73 ppm, J = 12, 17 Hz] characteristic of the vinyl group expected from elimination of Br and OH from the hydroxybromoethyl group. Irradiation of the δ 5.73 signal in **11** sharpened slightly the quaternary methyl signal at 1.05 ppm in addition to collapsing the overlapping doublets at 4.89 ppm. Sharpening of the methyl signal was ascribed to removal of a small W coupling between the olefinic and methyl group protons in **11** and provided evidence for assigning methyl and $CHBrCH_2OH$ groups to positions X and Y in partial structure e. The carbocyclic skeleton of the resulting partial structure has geminally situated methyl and ethyl substituents as found in ring C of the pimarane and rosane diterpenes.[22] Using the pimarane skeleton as a basic framework, it is possible to accommodate readily all of the spectrally deduced structural elements of parguerol by assuming that one of the ring A geminal dimethyl groups of the pimarane skeleton is bonded to C-3 in parguerol to form a cyclopropane ring. Thus structure **8** was proposed for parguerol, and spectral evidence confirming the ring A portion of **8** was obtained from the seco degradation products **14** and **15** (see below).

The relative stereochemical features of **8** were resolved by additional ^{1}H NMR data. NOE effects were observed for both the bromomethine (H-15) and the C-10 methyl (H-20) signals when the H-8 signal (2.78 ppm) was saturated in a difference NOE experiment[20] on acetate **9** in C_6D_6. This established the relative stereochemistry shown in **8** for C-8, C-10, and C-13. The β orientation of the hydroxyl group at C-7 follows from the large J values for H_7 (~11 Hz), which indicate that this methine proton is axial. The H-3 signal, though not discernibly coupled with H_2, is W coupled to one of the protons at C-1 (1.89 ppm resonance in $CDCl_3$; see discussion of **14** below). This requires that both H_1 and H_3 be equatorial and hence the cyclopropane ring must be α-oriented. The angular methyl group at C-10 in **9** (identified by the difference NOE experiment) experienced an induced downfield shift of 2.27 ppm compared to 0.34 ppm for the other quaternary methyl group with a 0.75:1 mol ratio of Eu(fod)$_3$:**9** in CCl_4, providing further evidence for a β-C-19 stereochemistry. An axial orientation of the acetoxy group at C-2 follows from the small couplings noted for the C-2 proton; see Table II.

Ozonolysis of **8** in dichloromethane–methanol followed by reductive workup with dimethyl sulfide[23] yielded the acetal **14**. Jones' oxidation of **14** yielded the acid **15**. In the ^{1}H NMR spectrum of acetal **14** *in benzene-d_6*, all three cyclopropane proton signals could be seen clearly and one of them, 0.98 ppm (H-3), was broader than the other two (H-18endo, H-18exo). Decoupling experiments established that H-3 was coupled (very small J) to a broadened doublet (J = 16 Hz), 2.02 ppm, H-1e. The only further coupling observed for H-1e was to H-1a while the latter was also coupled (5 Hz) to H-2. The H-1 to H-3 coupling must thus be due to a W coupling of equatorially oriented protons as are present in the ring-A structure of **14**. Hence this portion of structure **8**, which was postulated to a large extent on biogenetic grounds, was confirmed. The limited multiplicity of the H-1a and H-1e signals also confirmed the presence of a quaternary carbon at C-10. A molecular model of **8** reveals that H_2 forms a dihedral angle of approximately 90° with H-1e and H-3, and this can account for the lack of coupling between H-2 and the H-1e and H-3 protons.

In the ^{1}H NMR spectrum of acetal **14** in C_6D_6, the signal for H-5 was clearly observable at 0.88 ppm as a double doublet with a small coupling (3 Hz) to H-6eq and a large coupling (14 Hz) to H-6ax (1.98 ppm). Both H-6a and H-6e were coupled to H-7. The presence of a diaxial coupling in the H-5 signal confirms an A/B trans ring juncture for **8**.

Acetal **14** displays a positive Cotton effect (+6375°). In contrast, secogorgosterol diacetate, whose absolute configuration is shown in formula **22** (Chart IV), gives rise to a strong negative

(20) See, for example: Kuo, M.; Gibbons, W. A. *J. Biol. Chem.* **1979**, *254*, 6278. Hall, L. D.; Saunders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703 and references cited therein.

(21) See, for example: De Marco, A.; Wurthrich, K. *J. Magn. Reson.* **1976**, *24*, 201.

(22) Devon, T. K.; Scott, A. I. "Handbook of Naturally Occurring Compounds"; Academic Press: New York, 1972, Vol. II.

(23) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, 1969; Vol. 2, p 156. Ibid., 1975; Vol. 5, p 260.

Table II. ^{1}H NMR Chemical Shifts[a] and Selected Multiplicities for 8, 9, 14, and 16

	8		9				14			16	
proton	δ ($CDCl_3$)	m (J, Hz)	δ ($CDCl_3$)	m (J, Hz)	δ (C_6D_6)	m (J, Hz)	δ ($CDCl_3$)	δ (C_6D_6)	m (J, Hz)	δ ($CDCl_3$)	m (J, Hz)
1ax			1.29	dd (4, 16)[b]	0.88		1.42	1.28	d (~16, 5)		
1eq			1.94	br d (16)[b]	1.69		1.96	2.02	br d (~16)	1.94	
2	5.35	br d (5)	5.36	br d (5)	5.34	br d (5)	5.32	5.30	br d (5)	5.36	br d (6)
3	1.04		1.13		1.01	dd (~6, 11)	1.16	0.98	dd (7, 11)	1.04	
5					0.97	dd (4, 12)		0.88	dd (3, 14)		
6ax			1.76	ddd (10, 11, 13)	1.86	q (~12)		1.98	*e*		
6eq			2.29	dt (4, 4, 12)	2.36	dt (4.5, 4.5, 13)	2.48	2.40	ddd (3, 5, 13)		
7	3.12	dt (5, 10, 10)	4.41	dt (5, 11, 11)	4.58	dt (~4.5, 11, 11)[c]	4.42	4.56	dt (5, ~11.5, 11.5)	3.15	dt (5, 10, 10)
8			2.59	complex t = 10	2.78	dt (~6, 11, 11)[d]	3.07	3.12	*f*		
11	5.35	d (5)	5.40	br d (2, 2.5, 6)[b]	5.12	br d (~6)	4.54	4.57	t (5.5)	5.35	br d (6.5)
12ax			1.82	br d (~2, ~2.5, 18)[b]	1.60	br d (~2, ~2, 14)	1.75	1.92	d (5.5)		
12eq			2.47	complex d = 18	2.45	v br d (3, 6, ~14)	1.75	1.92		2.45	
14ax			1.32		1.26	dd (11, 14)	1.21	1.45	d (14)	1.38	
14eq			2.02		2.05	ddd (3, 6, 14)	2.52	2.74	dd (8, 14)	2.34	
15	4.26	dd (3, 9)	4.49	dd (2, ~10)	4.52		4.24	4.42	dd (4, 8)	4.60	
16α	3.84	dd (9, 12)	4.31	t (~10, ~10)	4.21	dd (9.5, 11)	4.42	4.67	dd (8, 12)	4.23	
16β	3.95	dd (3, 12)	4.27	dd (2, ~10)	4.52		4.57	4.77	dd (4, 12)	4.26	
17	1.06		1.05		0.91		0.96	1.09		1.06	
18endo	0.09	t (5.4)	0.17	t (5.5)	−0.25	t (~6)	0.20	−0.36	t (~6)	0.08	t (~6)
18exo	0.87	dd (5, 10)	0.93	dd (5, 10)	0.56	dd (~6, 11)	0.98	0.49	dd (~5.5, 11)	0.87	dd (~5, 11)
19α	3.38	d (12)	3.71	d (12)	3.56	d (12)	3.72	3.56	d (12)	3.42	d (13)
19β	3.52	d (12)	4.04	d (12)	3.85	d (12)	4.10	3.76	d (12)	3.48	d (13)
20	1.13		1.16		1.20		1.38	1.26		1.13	
OAc	2.07		2.08		1.64		2.06	1.61		2.07	
OAc			2.08		1.71		2.09	1.65		2.11	
OAc			2.10		1.73		2.11	1.74			
OAc			2.14		1.77		2.13	1.84			

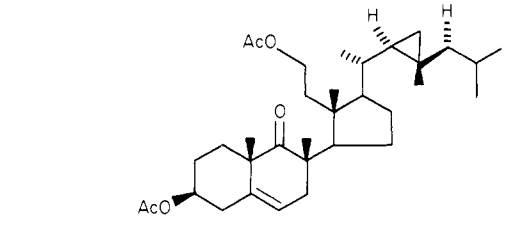
[a] 270 MHz except for 9 (360 MHz), (normal and resolution enhanced). [b] *J*'s obtained by resolution enhancement and decoupling with resolution enhancement. [c] Signal overlapped with H-16$_{\alpha,\beta}$; *J*'s determined by decoupling experiments and comparison to spectrum of 9 in $CDCl_3$. [d] Very broad triplet, J = 11 Hz; 6-Hz J obtained from decoupling experiments. H-8 is also coupled (very small *J*'s) to H-11 and H-12$_{ax}$. [e] Overlapped by H-1$_{eq}$ and H-12 signals. [f] Overlapped by OCH_3 signal.

Cotton effect (−10 000°).[24] Hence, **14** would appear to be enantiomeric with **22**, and we tentatively assign to **8** an absolute configuration that is enantiomeric to that implied by the formula drawn in this paper. Since there are significant structural differences in the decalin portions of **14** and **22**, the CD data cannot be taken as an unambiguous proof of absolute configuration.

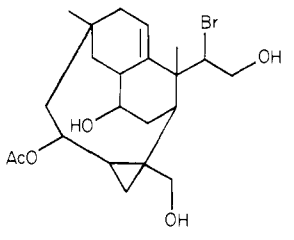
The computer-assisted structure elucidation program CONGEN[25] was used to help predict all possible structures by using the molecular fragments deduced from spectral and chemical analysis of parguerol. In addition to **8**, the program generated two other possible gross structures, **23** and **24** (Chart III; stereochemistry was not considered and an isoprenoid skeleton was assumed). Both **23** and **24** make less sense biogenetically than **8**. Structure **23** was ruled out on the basis that the observed ^{1}H NMR multiplicities for a number of the protons do not match those expected from a model of **23**. Structure **24** was ruled out because the allylic methine proton (corresponding to H-8 in structure **8**) would be equatorial and irradiation of this proton could not produce NOE effects on both the methyl and >CH(Br) group signals as was observed for **8**.

Trace quantities of the acetate derivative **16** were also isolated from *Aplysia*. Low-resolution mass spectral data, *m/e* 480 and 482, for **16** were consistent with the formula $C_{24}H_{33}BrO_5$, and infrared data indicated the presence of hydroxyl and acetate groups. The ^{1}H NMR spectrum of **16** was nearly identical with that of parguerol (**8**) except for the presence of an additional acetate methyl group signal at 2.11 ppm and a downfield shift of the signals corresponding to the C-16 protons to 4.23 and 4.26 ppm. Hence it was surmised that **16** was parguerol 16-acetate, $C_{24}H_{35}BrO_6$, and that the *m/e* ions of 480 and 482 corresponded to $M^+ - H_2O$. Acetylation of **16** afforded a product whose ^{1}H NMR spectrum was superiposable with that of parguerol peracetate **9**, thus confirming structure **16** for parguerol 16-acetate.

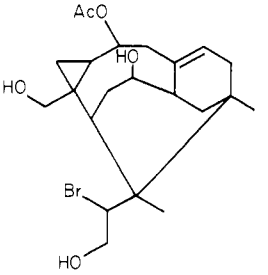
Chart III



22



23



24

(24) Spraggins, R. L. Ph.D. Dissertation, University of Oklahoma, Norman, OK, 1970.

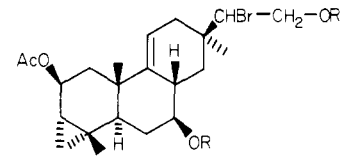
(25) See, for example: Cheer, C. J.; Smith, D. H.; Djerassi, C. *Tetrahedron* **1976**, *32*, 1807.

Table III. ^{1}H NMR Chemical Shifts[a] and Selected Multiplicities for **17**–**21**

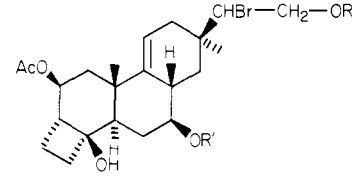
	17		**18**		**19**		**20**			**21**
proton	δ ($CDCl_3$)	m (*J*, Hz)	δ ($CDCl_3$)	m (*J*, Hz)	δ ($CDCl_3$)	m (*J*, Hz)	δ ($CDCl_3$)	δ (C_6D_6)	m (*J*, Hz)	δ ($CDCl_3$)
1ax	1.21	dd (6, 15)	1.26		1.65		1.88	1.29	dd (~4, 15.5)	
1eq	1.89	d (15)	1.91	d (15)			1.68	1.82	d (~15.5)	
2	5.29	br d (6)	5.28	br d (5)	4.96	br d (5)	4.95	4.88	d (~4)	4.97
3	0.79	dd (6, 11)	0.78	dd (6, 10)			2.48	2.11	br t (~10.4)	2.54
5	1.06		1.14					1.10	dd (~16, ~5)[b]	
6ax	1.68		1.70	q (12)			1.85	1.88		1.78
6eq	2.12		2.16	dt (4, 4, 13)						2.38
7	3.16	dt (5, 10, 10)	4.38	dt (5, 11, 11)	3.29	dt (6, 12, 12)	4.50	4.58	m	3.27
8	2.22		2.56	v br t (11)			2.60	2.75	br t (11, 11)	2.45
11	5.34	br d (6)	5.36	br d (6.7)	5.44	br d (6)	5.48	5.15	d (6)	5.44
12ax	1.79		1.82	br d (2, 4, 18)			1.82	1.62	v br d (17)	
12eq	2.40	v br d (18)	2.43	v br dd (~7, 18)	2.45	v br d (16)	2.48	2.45	v br d (17)	
14ax	1.35		1.33				1.35	1.25		
14eq	2.25		2.02	ddd (4, 6, 15)				2.05	br d (14, 6, 4)[b]	
15	4.27	dd (4, 9)	4.53		4.29	dd (3, 10)	4.49	4.50		4.28
16α	3.83		4.28		3.84	dd (10, 12)	4.25	4.24	dd (10, 13)	4.60
16β	3.90		4.28		3.94	dd (3, 12)	4.30	4.50		4.28
17	1.04		1.02		1.08		1.05	0.88		1.09
18endo	0.01	t (5.6)	0.01	t (5)				0.72	quint (10.4)	
18exo	0.66	dd (5, 11)	0.65	dd (4, 10)				1.35	quint (~10)	
19α	1.02		1.00					1.10	quart (10–11)[b]	
19β	1.02		1.00					1.41	quart (10)	
20	1.12		1.12		1.21		1.21	1.26		1.22
OAc	2.05		2.08		2.03		2.02	1.61		2.03
OAc			2.10				2.05	1.66		2.11
OAc			2.14				2.11	1.69		

[a] 270 MHz. [b] *J*'s obtained from decoupled or decoupling difference spectra.

Chart IV



17, R = H
18, R = Ac



	R	R′
19,	H	H
20,	Ac	Ac
21,	Ac	H

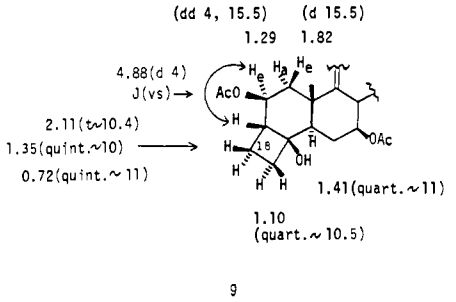
Another minor component isolated from chromatographic fractions containing parguerol was deoxyparguerol, **17** (Chart IV). The highest mass ions in the low-resolution mass spectrum of **17** appeared at *m/e* 422 and 424, consistent with a formula of $C_{22}H_{31}BrO_3$. However, upon acetylation (see below) a product was obtained having the formula $C_{26}H_{37}BrO_6$ (high-resolution mass spectrum), indicating that the highest mass ions observed for deoxyparguerol itself corresponded to $M^+ - H_2O$, thus yielding a formula of $C_{22}H_{33}BrO_4$ for **17**. The presence of one or more hydroxyl groups and one acetate group was indicated by IR and ^{1}H NMR data. As can be seen from Table III, the ^{1}H NMR spectrum of **17** was very similar to that of **8** with a few notable exceptions, and hence a structure similar to **8** could be inferred. The spectrum of **17** contains one more quaternary methyl signal (1.02 ppm) than is found in the spectrum of **8**, and at the same time the AB quartet due to the C-19 hydroxymethyl of **8** is missing in the spectrum of **17**. Also all three cyclopropane proton signals were shifted upfield in **17** relative to **8**. These data are consistent with the presence of a methyl group in **17** in place of the hydroxymethylene group in **8**. Acetylation of **17** and its acetate derivative **18** (see below) confirmed that the remainder of the structure was identical with that of parguerol.

Acetylation of deoxyparguerol (**17**) yielded the triacetate **18** having the formula $C_{26}H_{37}BrO_6$ (high-resolution mass spectrum); ^{1}H NMR, see Table III. No hydroxyl absorption was evident in the IR spectrum of **18** and hence it was clear that deoxyparguerol itself contained two hydroxyl groups and thus had the formula $C_{22}H_{33}BrO_4$. In agreement with structures **17** and **18**, the ^{1}H NMR signals corresponding to protons at C-7 and C-16 were shifted from 3.16, 3.82, and 3.90 ppm in deoxyparguerol (**17**) to 4.38 and 4.28 ppm (2 H), respectively, in **18**. Since the chemical shifts of the C-17 and C-20 protons of deoxyparguerol (**17**) were nearly identical with those of parguerol (**8**), the same stereochemistry was inferred.

HPLC separation of fractions rich in parguerol yielded an isomeric diterpene, isoparguerol, **19**. The highest mass ions observed in the field desorption mass spectrum of **19** were at *m/e* 438 and 440, corresponding to a formula of $C_{22}H_{31}BrO_4$. Data described below for the acetate derivative of isoparguerol, namely **20**, revealed that these ions corresponded to $M^+ - H_2O$, and hence the formula for **19** was $C_{22}H_{33}BrO_5$. The infrared spectrum contained absorptions corresponding to hydroxyl and acetate groups (3360, 1710, and 1250 cm^{-1}). The ^{1}H NMR spectrum (Table III) revealed that only one acetate group was present, while two quaternary methyl signals were evident at 1.08 and 1.21 ppm, similar to the singlet signals of **8**, **16**, and **17**. Isoparguerol (**19**) also exhibited signals nearly identical with those in parguerol (**8**) corresponding to protons at C-11, C-7, C-15, and C-16 (see Tables II and III). Thus a close similarity between isoparguerol (**19**) and parguerol (**8**) was inferred, with rings B and C and their substituents being identical.

The most obvious differences in the spectra of **19** and **8** were the absence in the spectrum of **19** of any cyclopropane hydrogen signals and of the AB quartet signals due to the hydroxymethylene group. Also, the slightly broadened doublet signal at ~5.3–5.4 ppm assigned to H-2 in **8**, **16**, and **17** was shifted to 4.96 ppm in **19**. This shift was consistent with the loss of the cyclopropylcarbinyl feature.

Acetylation of **19**, a monoacetate, yielded a triacetate product **20** which still retained hydroxyl absorption in the infrared (3500



g

Figure 2. Partial structure g based on ^1H NMR data for **20** in C_6D_6 at 270 MHz.

cm^{-1}), revealing the presence of a tertiary hydroxyl group in **19**. This demonstrated that isoparguerol (**19**) must contain five oxygens (1 OAc, 3 OH's) rather than the four suggested by its mass spectrum (see above). The highest peaks observed in the mass spectrum of **20** occurred at *m/e* 480 and 482, corresponding to $M^+ - AcOH$.

A logical explanation that would account for loss of the cyclopropane and hydroxymethylene groups with concomitant formation of a tertiary alcohol group would be rearrangement of the cyclopropylcarbinyl primary alcohol group to a *tertiary* tertiary cyclobutyl alcohol moiety, as is proposed in the structures **19** and **20**. This hypothesis was confirmed by extensive ^1H NMR decoupling studies on **20** in C_6D_6 (Table III) which resulted in the proton chemical shift and coupling assignments outlined on partial structure g, Figure 2. The reference point in this spin system was the H-2 proton signal at 4.88 ppm. Irradiation of this signal removed a 4-Hz coupling from a partially obscured double doublet at 1.29 ppm (H-1a) and also sharpened a doublet at 1.82 ppm (J = 16 Hz, H-1e). The two latter signals were confirmed to be coupled geminally to each other. Irradiation of the H-2 signal (4.88 ppm) also sharpened slightly the broadened triplet signal at 2.11 ppm, thus locating the H-3 signal. Confirmation of the sequence of proton signals for H-3, H-18, and H-19 (partial structure g, Figure 2) was aided greatly by difference decoupling since the 1.35 and 1.41 ppm multiplets overlapped each other to some extent. C-19 was confirmed to be bonded on one side to a quaternary carbon since the H-19 protons were shown to be coupled only to each other and to the H-18 protons.

Isoparguerol (**19**) is assigned the same relative configuration as parguerol (**8**) because in their ^1H NMR spectra both compounds exhibit identical multiplicities and nearly the same chemical shifts for their H-7, H-8, H-11, and H-17 protons (Tables II and III). Since the C-20 protons (1.21 ppm) of isoparguerol (**19**) resonate downfield relative to those of parguerol (**8**), the C-4 tertiary hydroxyl group is assumed to be cis to C-20. Observation of a small W coupling between H-3 and H-1e also support this relative configuration of the cyclobutane ring.

Also isolated in small quantities was isoparguerol 16-acetate (**21**). The highest ions observed in the low-resolution mass spectrum of **21** occured at *m/e* 480–482 which corresponded to $M^+ - H_2O$ from a formula of $C_{24}H_{35}BrO_6$. The infrared spectrum of **21** showed hydroxyl and acetate absorptions, and the ^1H NMR spectrum contained signals for two acetate methyl and two quaternary methyl groups (1.09 and 1.22 ppm). The spectra of **21** and **19** were otherwise virtually identical except that the signals for H-15 and H-16 in **21** were shifted downfield to positions similar to those observed for the acetate **20**. This suggested that **21** was isoparguerol 16-acetate, and indeed, acetylation of **21** gave a product whose ^1H NMR spectrum was identical with that of isoparguerol peracetate **20**.

The mass spectra of parguerol (**8**), deoxyparguerol (**17**), and their acetates are characterized by losses of H_2O, HOAc, Br, and CH_3. No reverse Diels–Alder fragmentation of the type that might be expected[26] for a $\Delta^{9(11)}$-pimarene was observed. The mass spectra of isoparguerol (**19**) and isoparguerol 16-acetate (**21**) both contained ions of significant intensity due to loss of 28 amu (corresponding to ethylene) from M^+ and a variety of other ions. Loss of ethlene can be attributed to cleavage of the cyclobutane ring and provides substantiation for that structural feature. Illustrative sets of these ions in the spectra of **19** and **20** that are not observed in the spectra of **8** and **9** are outlined in Chart V.

Chart V

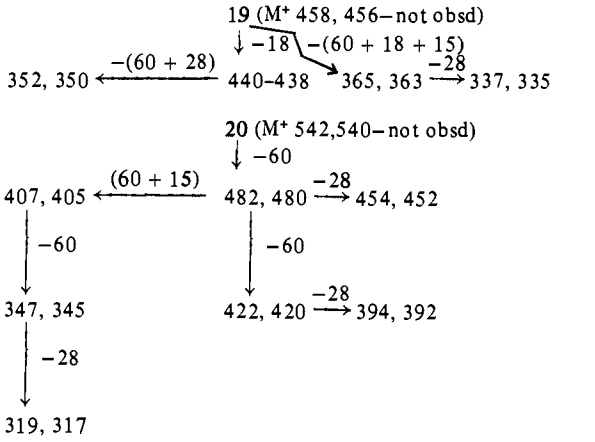


Table IV. Cytotoxicity Data for *Aplysia* Metabolites

compd	ED_{50} (PS)[10]	compd	ED_{50} (PS)[10]
1	47	8	3.8
2a	26	**16**	4.3
3	40	17	0.38
4	40	19	4.6
5a	50	**21**	0.52
6a	40		

The parguerols possess modified pimarane skeletons. The presence of cyclopropane rings in pimaranes is novel, although a cyclopropane ring bridging C-4 and C-5 is found in the devadarene skeleton.[22] The cyclobutane ring of isoparguerol has no parallel in known pimaranes to our knowledge. The parguerols also differ from most pimaranes in having oxygenation at C-2 and C-7 and unsaturation in the 9(11) position. Oxygenation in other pimaranes is generally at C-3 and unsaturation generally occurs at C-7, C-8(14), or C-15, although at least one instance of 9-(11)-unsaturation in pimaranes is known.[26a]

All of the metabolites isolated from *Aplysia* are assumed to be of algal origin. Compounds **1**, **2a**, **3**, **4a**, and the free alcohol of **5** are known algal metabolites. Since our preliminary publication of this work, deoxyparguerol 16-acetate has been isolated[27] from the alga *Laurencia obtusa* collected from Kimmeridge, U.K.

The results of cytotoxicity evaluation of most of the pure compounds described in this paper are given in Table IV. The parguerol/isoparguerol-type diterpenes are the most active of all the compounds isolated.

Experimental Section

Melting points ae uncorrected. Solvents were distilled prior to use. Infrared spectra were taken on a Perkin-Elmer 298 instrument and UV spectra were taken on Perkin-Elmer Lamda 3, Varian Superscan 3, or Cary 118 spectrophotometers. ^1H NMR spectra were taken on Varian XL-100, Bruker 270, and Bruker HXS-360 MHz instruments in the solvents specified; signals are reported in parts per million (δ) downfield from internal tetramethylsilane.

^{13}C spectra were obtained at 47.1 kHz on a JEOL FX-100 NMR spectrometer at the Colorado State University Regional NMR Center. Mass spectra were taken on CEC (Du Pont, Monrovia, CA) 110 (high resolution) and Hewlett-Packard 5985B spectrometers (low resolution). A Perkin-Elmer 141 polarimeter was used for obtaining optical rotations. Column chromatographic adsorbent used was Brinkmann silica gel 60 (230–400 mesh). Centrifugal chromatography was carried out with a Model CLC-5 centrifugal chromatograph, NSI Hitachi Scientific Instruments, with Merck silica gel 60 (230–240 mesh). An Altex Li-

(26) (a) Jeffries, P. R.; Rajajckak, T. *Aust. J. Chem.* **1973**, *26*, 1973. (b) Bohlmann, F.; Weickgenannt, G.; Zdero, C. *Chem. Ber.* **1973**, *106*, 826.

(27) Higgs, M. D.; Faulkner, D. J. *Phytochemistry* **1982**, *21*, 789.

Chrosorb Si60 5-μm preparative (10 mm × 25 cm) column (silica gel) was used for HPLC separations using a differential refractometer detector.

Initial Partitioning Procedure. Specimens of the sea hare *Aplysta dactylomela* were collected at 0 to −1 M in December of 1977, off the coast of La Parguera, Puerto Rico. Excised digestive glands (8 kg wet wt from 300 specimens) that had been stored in 70% 2-propanol for about 1 month were homogenized in a Waring blender with chloroform–methanol (2:1), and the resulting suspension was filtered through cheesecloth. The chloroform–methanol extract was concentrated under reduced pressure and then diluted with a little water, and the resulting aqueous suspension was partitioned against methylene chloride (2 L). The methylene chloride solution was evaporated to give 314.0 g of extract, designated as fraction A. Further partitioning of the aqueous suspension with 1-butanol (1 L) produced 21.59 g of butanol solubles, fraction B. The remaining aqueous suspension was then lyophilized, and the solids obtained were washed with methanol. The methanol-soluble portion, fraction C, amounted to 62.3 g, while the insoluble salts, fraction D, accounted for 71.7 g. The 2-propanol solution that was used as a preservative for the glands was first concentrated and then partitioned in the same manner to give parallel fractions A, 15.9 g; B, 11.9 g; C, 107.2 g; and D, 120.0 g.

A portion of fraction A (308 g) was dissolved in 1 L of 10% aqueous methanol and partitioned against 2 L of hexane to give 232.52 g of hexane solubles, fraction E. Water (125 mL) was added to the aqueous methanol solution which was then partitioned against 1.5 L of carbon tetrachloride to give 16.44 g of CCl_4 solubles, fraction F. The aqueous methanol solution was further diluted with water (260 mL) and partitioned with 1.5 L of chloroform to give 6.83 g of chloroform solubles, fraction G. The remaining aqueous methanol solution was concentrated and lyophilized, affording fraction H, 3.61 g.

Isolation of 2,3,5-Tribromo-*N*-methylindole (1). A portion (1.14 g) of fraction F was chromatographed over silica gel 60 PF-254 with chloroform as eluent. The first 50-mL fraction collected contained an off-white solid. Recrystallization from hexane yielded pure 2,3,5-tribromo-*N*-methylindole (**1**) as white needles, 32.4 mg (0.0004%); mp 124–124.8 °C (lit.[11] mp 120–122 °C); IR (KBr) 2930, 1488, 1448, 1345 cm^{-1}; UV (EtOH) λ_{max} 292 nm (log ϵ_{max} 4.01), with inflections at 300 and 284 nm; 100-MHz ^1H NMR ($CDCl_3$) δ 3.73 (s, 3 H, NCH_3), 7.10 (d, 1 H, J = 8 Hz, H-7), 7.29 (dd, 1 H, J = 2, 8 Hz, H-6), 7.60 (d, 1 H, J = 2 Hz, H-4); mass spectrum (70 eV), m/e (relative intensity) 371 (30), 369 (100) base peak, 367 (97), 365 (37), 356 (3), 354 (10), 352 (10), 350 (3), 290 (4), 289 (4), 288 (10), 287 (6), 286 (5), 285 (3), 275 (1), 273 (3), 271 (1), 249 (3), 247 (6), 245 (3), 209 (9), 208 (4), 207 (9), 206 (3), 194 (13), 192 (13), 128 (18), and 87 (11); high-resolution mass measurement M^+ 364.80523, $C_9H_6NBr_3$[79] requires 364.80502.

Isolation of Elatol (2a). Another portion (2.13 g) of fraction F was chromatographed over silica gel with chloroform (50-mL fractions) to give in fractions 3–8 a yellow oil (883 mg). Rechromatography of this oil on silica gel with 5% ethyl acetate in hexane gave little further resolution (688 mg in main fractions), but a third chromatography over silica gel with a step gradient (hexane containing 1 and 2% ethyl acetate, respectively) with extensive elution at each step gave in the latter fractions 284 mg of crude elatol as a pale yellow oil that slowly crystallized. Recrystallization from methanol gave elatol as an off-white sticky mass of crystals, 123 mg (0.0015%); mp 62–66 °C; $[\alpha]_D$ +55.2° (*c* 3.68, $CHCl_3$); [lit. $[\alpha]_D$ +83.5° (*c* 0.365 MeOH);[12b] NMR identical with reported values, ^1H[12a] and ^{13}C (±0.1 ppm).[12c]

Isolation of 3 (Allolaurinterol Acetate), 5a, and 6a. The mother liquor (1.5 g) from recrystallization of a batch of elatol was chromatographed on TLC mesh silica gel in a CLC-5 centrifugal chromatograph with 100 mL each of 2.5%, 5%, and 10% ethyl acetate in hexane followed by 200 mL of 20% ethyl acetate–hexane. Ater the first 150 mL of eluent had been collected, 8-mL fractions were taken. Fractions 2–5 (105 mg) were concentrated and rechromatographed on an Altex LiChrosorb Si60 5μm HPLC column with 5% ethyl acetate–hexane to give allolaurinterol acetate (**3**) as colorless needles: 17.7 mg (0.0002%), R_v = 30 mL; mp 80–82 °C $[\alpha]_D$ +41° (*c* 0.2, $CHCl_3$) (lit.[14] mp 86.6–89.1 °C, $[\alpha]_D$ +48.2°); IR (neat) 1763, 1656 cm^{-1}; UV (EtOH) λ_{max} 208 (7900), 268 (600), and 276 nm (600); 100-MHz ^1H NMR ($CDCl_3$) δ 0.70 (d, 3 H, J = 7.5 Hz, $CHCH_3$), 1.13 (s, 3 H, CH_3), 2.33 (s, 3 H, $OCOCH_3$), 2.36 (s, 3 H, $ArCH_3$), 2.77 (q, 1 H, J = 7.5 Hz, $CHCH_3$), 4.90 (br s, 1 H, $C{=}CH_2$), 4.98 (br s, 1 H, $C{=}CH_2$), 6.95 (s, 1 H, ArH), 7.39 (s, 1 H, ArH); mass spectrum (70 eV), m/e (relative intensity) 338 (19), 336 (22), 323 (29), 321 (32), 296 (17), 295 (11), 294 (16), 293 (9), 282 (14), 281 (98), 280 (20), 279 (100) base peak, 267 (17), 265 (21), 239 (22), 237 (23), 215 (16), 214 (22), 213 (16), 212 (13), 201 (25), 200 (26), 199 (33), 145 (46), 115 (63), 91 (72), 86 (79), and 77 (63).

Fractions 9–11 (1.12 g) of the above CLC-5 centrifugal chromatography were combined to give a pale yellow oil. A portion (85 mg) of this oil was chromatographed by HPLC on silica gel (Altex LiChrosorb Si60 5μm) with 10% ethyl acetate in hexane to give **6a** (21 mg, 0.003%, R_v = 40.2 mL), elatol (**2a**) (47 mg, R_v = 45.0 mL), and the chamigrene derivative **5a** (10 mg, 0.0001%, R_v = 60.6 mL).

For **5a**: $[\alpha]_D$ −4.8° (*c* 0.77, $CHCl_3$) (lit.[13] $[\alpha]_D$ −4°); ^1H NMR as in ref 13.

For **6a**: $[\alpha]_D$ −33.3° (*c* 1.47, $CHCl_3$); UV (EtOH) λ_{max} 247 nm (16 000); IR (neat) 3550, 3460, 3060, 3030, 1638, 1460, 1425, 1348, 1200 cm^{-1}; 100-MHz ^1H NMR ($CDCl_3$) δ 1.01 (s, 3 H, CH_3), 1.24 (s, 3 H, CH_3), 1.5–3.0 (m, 7 H), 4.20 (m, 1 H, >C*H*OH), 4.66 (d, 1 H, J = 3 Hz, >CHBr), 4.84 (s, 1 H, $C{=}CH_2$), 5.12 (s, 1 H, $C{=}CH_2$), 5.95 (br d, 1 H, J = 11 Hz, H_b), 6.13 (s, 1 H, C=CHBr), 6.24 (d, 1 H, J = 10 Hz, H_a); mass spectrum (70 eV), m/e (relative intensity) 379 (2), 378 (12), 377 (4), 376 (25), 374 (13), 297 (22), 295 (20), 279 (36), 277 (33), 253 (8), 251 (8), 241 (39), 239 (38), 237 (9), 235 (8), 213 (17), 211 (20), 198 (11) , 197 (16), 183 (11), 173 (15), 171 (24), 169 (14), 167 (15), 149 (33), 133 (38), 117 (29), 115 (35), 107 (11), 105 (18), 91 (57), 85 (100) base peak, 83 (63), and 69 (52).

Acetylation of 2a (Elatol), 5a, and 6a. A mixture of **2a**, **5a**, and **6a** (32 mg) was allowed to react with pyridine (1 mL) and acetic anhydride (2 mL) overnight at room temperature. The mixture was poured over crushed ice and extracted into chloroform. The chloroform solution was washed with 1 N sulfuric acid and dried over sodium sulfate. The acetates were separated by HPLC on an Altex LiChrosorb Si60 column with 15% ethyl acetate in hexane as the solvent to give **6b** (13.9 mg, R_v = 17.6 mL), elatol acetate (**2b**) (15.2 mg, R_v = 19.2 mL), and **5a** (2.7 mg, R_v = 21.6 mL).

For elatol acetate (**2b**): mp 155–156 °C; $[\alpha]_D$ +83.5° (*c* 1.46, $CHCl_3$) [lit.[12b] mp 157–158 °C; $[\alpha]_D$ +125° (*c* 0.014, MeOH)]; IR (neat) 3090, 1743, 1680, 1643, 1245, cm^{-1}; 100-MHz ^1H NMR ($CDCl_3$) δ 1.03 (s, 3 H, CH_3), 1.08 (s, 3 H, CH_3), 1.68 (s, 3 H, $C{=}C{-}CH_3$), 1.5–2.8 (m, 8 H), 2.06 (s, 3 H, $OCOCH_3$), 4.52 (d, 1 H, J = 3 Hz, >CHBr), 4.76 (s, 1 H, $C{=}CH_2$), 5.00 (s, 1 H, $C{=}CH_2$), 5.26 (dd, 1 H, J = 3, 7 Hz, >CHOAc); mass spectrum (70 eV), m/e (relative intensity) 283 (6), 237 (38), 236 (20), 235 (100) base peak, 209 (14), 207 (30), 199 (68), 193 (14), 179 (22), 159 (53), 157 (67), 155 (16), 153 (29), 145 (56), 143 (31), 141(31), 133 (42), 119 (45), 117 (40), 115 (61), 107 (63), 105 (68), 93 (50), 91 (89), 85 (86), 77 (63).

Acetate **5b**: mp 115–117 °C $[\alpha]_D$ −25° (*c* 0.18, $CHCl_3$) (lit.[13] mp 113–114 °C; $[\alpha]_D$ −25°).

Acetate **6b**: mp 121–123 °C; $[\alpha]_D$ −39.3° (*c*, 1.37, $CHCl_3$); IR (neat) 3060, 3040, 1745, 1642, 1375, and 1245 cm^{-1}; 100-MHz ^1H NMR ($CDCl_3$) δ 1.01 (s, 3 H, CH_3), 1.20 (s, 3 H, CH_3), 2.07 (s, 3 H, $OCOCH_3$), 1.5–3.0 (m, 6 H), 4.55 (d, 1 H, J = 3 Hz, >CHBr), 4.80 (br s, 1 H, $C{=}CH_2$), 5.00 (br s, 1 H, $C{=}CH_2$), 5.30 (dd, 1 H, J = 4, 7 Hz, >CHOAc), 5.88 (br d, 1 H, J = 11 Hz, H_b), 6.10 (br s, 1 H, C=CHBr), 6.23 (d, 1 H, J = 11 Hz, H_a); mass spectrum (70 eV), m/e (relative intensity) 420 (2), 418 (4), 416 (2), 360 (2), 358 (3), 356 (2), 339 (4), 337 (4), 280 (17), 279 (92), 278 (32), 277 (100) base peak, 251 (3), 249 (3), 237 (17), 235 (19), 223 (4), 221 (5), 199 (11), 198 (17), 197 (21), 183 (15), 173 (25), 171 (37), 169 (23), 155 (30), 141 (26), 129 (35), 128 (35), 115 (66), 105 (29), 91 (95), 85 (45).

Isolation of Isoobtusol Acetate (4). A portion (130.4 g) of fraction E was chromatographed over Silicar CC-7 in a total of 11 runs. For each run, approximately 1 L each of hexane, chloroform, ethyl acetate, and methanol were used, in that order. After removal of solvent, the followign combined fractions were obtained: hexane, 1.9 g; chloroform, 57.6 g; ethyl acetate, 35.7 g; and methanol, 22.4 g. Part of the chloroform fraction (44.3 g) was chromatographed on Sephadex LH-20 ($CHCl_3$–MeOH; 1:1), collecting 60-mL fractions. Fractions 15–17 (1.73 g) were combined and filtered to give isoobtusol acetate (**4**), 86 mg (0.001%); after crystallization from benzene–hexane, mp 170–173 °C; $[\alpha]_D$ +57.0° (*c* 0.405, $CHCl_3$); authentic sample[16] mp 167–170 °C, $[\alpha]_D$ +51.0° (*c* 0.514, $CHCl_3$); IR (KBr) 3085, 1738, 1640, 1234 cm^{-1}; 270-MHz ^1H NMR ($CDCl_3$) δ 1.12 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3), 1.82 (dq, 1 H, J = 14, 3, 3, 3 Hz, CH_2CH_2), 1.86 (dq, 1 H, J = 14, 3, 3, 3 Hz, CH_2CH_2), 1.92 (s, 3 H, $>CBrCH_3$), 2.11 (s, 3 H, $OCOCH_3$), 2.08 (m, 1 H, CH_2CH_2), 2.26 (m, 1 H, CH_2CH_2), 2.40 (dd, 1 H, J = 12.4, 4.6 Hz, CH_2CHOAc), 2.83 (dd, 1 H, J = 16, 4.2 Hz, CH_2CHCl), 2.96 (t, 1 H, J = 12.2 Hz, CH_2CHOAc), 3.18 (br d, 1 H, J = 15.6 Hz, CH_2CHCl), 4.46 (m, 2 H, CHBr and CHCl), 4.83 (dt, 1 H, J = 12.2, 4.4, 4.4 Hz, C*H*OAc), 4.99 (s, 1 H, $C{=}CH_2$), 5.21 (s, 1 H, $C{=}CH_2$); 25-MHz ^{13}C NMR ($CDCl_3$) 21.0 (q), 24.5 (q), 25.0 (q), 25.5 (t), 33.0 (q), 33.2 (t), 33.9 (t), 35.1 (t), 43.6 (s), 44.2 (s), 65.0 (d), 66.2 (d), 70.9 (s), 71.4 (d), 114.5 (t), 146.7 (s), 169.8 (s); mass spectrum (70 eV), (relative intensity) no molecular ion was observed, m/e 398 (1), 396 (2), 394 (0.8), 341 (2), 339 (2), 320 (5), 319 (20), 318 (16), 317 (100) base peak, 316 (18), 315 (62), 314 (3), 281 (13), 279 (14), 237 (21), 235 (20), 200 (12), 199 (36), 173 (11), 171 (26), 159 (13), 157 (44), 145 (23), 143 (30), 133 (17), 131 (17), 129 (24), 117 (22), 115 (25), 107 (42), 105

(61), 93 (44), 91 (77), 85 (56), 79 (46), and 77 (46).

Isolation of Parguerol (8) and Deoxyparguerol (17). A portion (5.5 g) of the chloroform extract, fraction G, was chromatographed on Sephadex LH-20, using chloroform–methanol (1:1) and collecting 60-mL fractions. Fractions 13 and 14 were combined (0.94 g) and chromatographed on silica gel TLC mesh type H (62 g), using 4% methanol in chloroform and collecting 25-mL fractions to yield parguerol (**8**) (184 mg, 0.0023%) as a pale yellow glass in fractions 16–18. Fractions 5–7 from this silica gel chromatography were combined (275 mg) and rechromatographed on thin-layer mesh silica gel with 3% methanol in chloroform. Fractions 11–16 of this chromatography were combined to give deoxyparguerol (**17**) (76 mg, 0.001%) as a glassy solid. All attempts to crystallize either parguerol or deoxyparguerol failed.

Parguerol (**8**) has $[\alpha]_D$ −40.0° (*c* 0.03, $CHCl_3$); IR (neat) 3380 (br), 3060, 1725, 1250 cm^{-1}; ^1H NMR, see Table II (not cited in Table II: 1.00–1.50 (m, 4 H), 1.70–2.10 (m, 4 H), 2.15–2.60 (m, 6 H)); 25-MHz ^{13}C NMR ($CDCl_3$) 18.3, 20.3, 21.6, 21.8, 23.9, 24.6, 33.4, 35.3, 36.6, 37.4, 38.2, 39.0, 45.8., 64.4, 68.2, 68.6, 68.8, 76.8, 117.4, 142.9, 170.5 ppm; mass spectrum (70 eV), (relative intensity) no molecular ion was observed, *m/e* 394 (2), 392 (2), 365 (4), 363 (5), 362 (4), 360 (4), 350 (8), 349 (22), 348 (8), 347 (35), 345 (11), 319 (4), 317 (4), 303 (3), 301 (3), 299 (10), 281 (32), 263 (24), 255 (10), 253 (11), 237 (37), 157 (41), 145 (46), 131 (50), 129 (45), 119 (68), 105 (100) base peak, 93 (64), 91 (93), 81 (53), 79 (68), and 55 (64); high-resolution mass spectrum for $C_{22}H_{31}Br^{81}O_4$ ($M^+ - H_2O$) calcd, 440.13864; obsd, 440.13898.

Anal. Calcd for $C_{22}H_{33}BrO_5$: C, 57.77; H, 7.27; Br, 17.47. Found: C, 58.04; H, 7.01; Br, 16.72.

Deoxyparguerol (**17**) has $[\alpha]_D$ −35.8° (*c* 0.62, $CHCl_3$); IR (neat) 3360, 3055, 1725, 1455, 1380, and 1250 cm^{-1}; ^1H NMR, see Table II; mass spectrum (70 eV), *m/e* (relative intensity) 424 (3), 422 (3), 364 (8), 362 (8), 349 (5), 347 (6), 331 (4), 329 (4), 325 (5), 322 (4), 321 (2), 320 (5), 284 (15), 283 (58), 265 (31), 255 (10), 240 (13), 239 (51), 197 (23), 185 (20), 183 (28), 171 (24), 169 (26), 157 (49), 145 (57), 131 (56), 121 (41), 119 (64), 105 (100) base peak, 93 (78), 91 (92), 81 (66), 79 (60), 55 (46).

Acetylation of Parguerol (8). Parguerol (19 mg) was allowed to react with pyridine (1 mL) and acetic anhdyride (2 mL) at room temperature for 24 h. After the usual workup [see acetylation of elatol (**2a**)], 24 mg of parguerol acetate (**9**) was obtained: IR (neat) 3020, 1745, and 1240 cm^{-1}; ^1H NMR in $CDCl_3$ and C_6D_6, see Table II; 270-MHz ^1H NMR (CCl_4) δ 0.17 (t, 1 H, *J* = 6 Hz, H-18endo), 0.94 (dd, 1 H, *J* = 5, 10.7 Hz, H-18exo), 1.06 (s, 3 H, H-17), 1.00–1.40 (m, 4 H), 1.12 (s, 3 H, H-20), 1.76 (m, 2 H, H-6ax and H-12ax), 1.89 (d, 1 H, *J* = 15.4 Hz, H-leq), 1.97 (m, 1 H, H-14eq), 2.02, 2.03, 2.06, 2.08 (each s, 3 H, $OCOCH_3$), 2.25 (dt, 1 H, *J* = 4, 4, 12 Hz, H-6eq), 2.45 (br d, 1 H, *J* = 17.4 Hz, H-12eq), 2.62 (br t, 1 H, *J* = 11, 12 Hz, H-8), 3.74 (d, 1 H, *J* = 12 Hz, H-19), 3.96 (d, 1 H, *J*= 12 Hz, H-19), 4.16 (m, 2 H, H-16), 4.31 (dt, 1 H, *J* = 5, 11, 11 Hz, H-7), 4.47 (dd, 1 H, *J* = 3, 11 Hz, H-15), 5.27 (d, 1 H, *J* = 5 Hz, H-2), 5.38 (d, 1 H, *J* = 6 Hz, H-11); 25-MHz ^{13}C NMR ($CDCl_3$) 18.9, 19.7, 20.7, 21.0, 21.2, 21.3, 21.7, 22.0, 24.2, 29.6, 35.1, 35.4, 36.4, 37.4, 38.6, 45.6 (d), 58.9 (d), 65.8 (t), 68.2 (d), 69.5 (t), 78.3 (d), 118.1 (d), 142.0 (s), 170.2 (s), 170.4 (s), 170.4 (s), 170.7 (s); mass spectrum (70 eV), *m/e* (relative intensity) 464 (10), 462 (10), 436 (8), 434 (6), 406 (6), 405 (6), 404 (22), 403 (6), 402 (21), 389 (6), 387 (6), 376 (3), 374 (3), 361 (4), 359 (3), 329 (12), 327 (12), 323 (22), 264 (26), 263 (100) base peak, 237 (66), 235 (36), 221 (64), 193 (33), 169 (22), 157 (21), 131 (26), 105 (37), 93 (21), 91 (33).

Parguerol Tribenzoate (10). Parguerol (**8**, 5 mg) was added to a solution of pyridine (1 mL) and freshly distilled benzoyl chloride (2 drops), and the mixture was allowed to react at room temperature overnight. After the usual workup [see acetylation of elatol (**2a**)], the product was chromatographed by preparative TLC with 20% ethyl acetate in hexane to give parguerol tribenzoate (**10**): 2.8 mg, R_f 0.33; IR (film) 3065, 1725, 1604, 1585, 1450, and 1385 cm^{-1}; 270-MHz ^1H NMR ($CDCl_3$) δ 0.27 (t, 1 H, *J* = 5.7 Hz, H-18endo), 1.04 (dd, 1 H, *J* = 5.4, 10.8 Hz, H-18exo), 1.08 (s, 3 H, H-17), 1.28 (s, 3 H, H-20), 1.15–1.55 (m, 4 H), 1.80–2.05 (m, 3 H), 2.04 (s, 3 H, $OCOCH_3$), 2.18 (br d, 1 H, *J* = 15 Hz, H-14eq), 2.54 (m, 2 H, H-6eq and H-12eq), 2.92 (br t, 1 H, *J* = 11 Hz, H-8), 3.90 (d, 1 H, *J* = 12 Hz, H-19), 4.36 (d, 1 H, *J* = 12 Hz, H-19), 4.46 (m, 2 H, H-15 and H-16), 4.69 (dt, 1 H, *J* = 4, 11, 11 Hz, H-7), 4.83 (dd, 1 H, *J* = 3, 12 Hz, H-16), 5.40 (d, 1 H, *J* = 5 Hz, H-2), 5.46 (d, 1 H, *J* = 6 Hz, H-11), 7.35–8.10 (m, 15 H, Ar H); mass spectrum (70 eV), *m/e* (relative intensity) 329 (1), 327 (1), 264 (4), 263 (15), 237 (9), 221 (6), 106 (8), 105 (100) base peak, 77 (19).

Deoxyparguerol Acetate (18). Deoxyparguerol (**17**, 20 mg) was allowed to react with acetic anhydride (2 mL) and pyridine (1 mL) overnight at room temperature. Workup in the usual manner gave **18** in quantitative yield: IR (neat) 3055, 3020, 2955, °2870, 1737, 1365, 1240, 1020, 750 cm^{-1}; ^1H NMR, see Table II; 25-MHz ^{13}C NMR ($CDCl_3$) 17.2 (s), 19.5 (d), 21.1, 21.4, 21.7, 23.2, 23.6, 24.2, 30.7, 35.2, 35.4, 37.0, 37.5, 38.1, 38.7, 46.4 (d), 59.0 (d), 66.0 t), 69.2 (d), 78.7 (d), 117.9 (d), 142.4 (s), 170.5 (s); mass spectrum (70 eV), *m/e* (relative intensity) 467 (26), 466 (84), 465 (26), 464 (97), 422 (3), 420 (4), 407 (8), 406 (19), 405 (8), 404 (19), 399 (20), 391 (11), 389 (10), 331 (17), 329 (19), 325 (62), 299 (16), 283 (18), 266 (17), 265 (80), 240 (23), 239 (100) base peak, 223 (38), 209 (32), 197 (33), 183 (39), 169 (39), 157 (51), 145 (62), 131 (50), 119 (31), 105 (58), 91 (57), 81 (24), 79 (34), 55 (20); high-resolution mass measurement (M^+ – HOAc) 464.1544, $C_{24}H_{33}O_4Br^{79}$ requires 464.1562.

Ozonolysis of Parguerol Acetate. Parguerol acetate (**9**, 24 mg) was dissolved in 8 mL of methylene chloride–methanol (1:1). The solution was cooled in a dry ice–acetone bath, and ozone was bubbled in at a rate of 4 mL/s. When a persistent pale blue color appeared, the gas flow was discontinued and the reaction vessel was kept cold for 0.5 h. Dimethyl sulfide (4 drops) was added[23] and the flask was slowly allowed to come to room temperature. The solution was washed with water, dried over sodium sulfate, and evaporated to give 21 mg of crude **14**. Chromatography gave pure **14**: IR (film) 1700–1750 (vs), 1450, 1365, and 1250 cm^{-1}; 270-MHz ^1H NMR ($CDCl_3$) δ 0.20 (t, 1 H, *J* = 6 Hz, H-18endo), 0.96 (s, 3 H, H-17), 0.90–1.60 (m, 5 H), 1.37 (s, 3 H, H-20), 1.85–2.05 (m, 2 H), 1.75 (m, 2 H, H-12), 2.06, 2.09, 2.11, 2.13 (each, s, 3 H, $OCOCH_3$), 2.50 (m, 2 H, H-6eq and H-14eq), 3.07 (dd, 1 H, *J* = 8, 11 Hz, H-8), 3.32 (s, 3 H, OCH_3), 3.34 (s, 3 H, OCH_3), 3.72 (d, 1 H, *J* = 12 Hz, H-19), 4.10 (d, 1 H, *J* = 12 Hz, H-19), 4.24 (dd, 1 H, *J* = 3, 9 Hz, H-15), 4.50 (m, 4 H, H-7, H-11, H-16), 5.32 (d, 1 H, *J* = 5 Hz, H-2); ^1H NMR in C_6D_6, see Table II; mass spectrum (70 eV), *m/e* (relative intensity) 497 (2), 495 (2), 428 (100), base peak, 415 (21), 368 (22), 353 (14)8 337 (17), 308 (25), 295 (29), 293 (31), 261 (44), 233 (45), 162 (56), 105 (70), 95 (53), 91 (78), 79 (49).

Oxidation of the Keto Acetal 14. Jones' reagent (5 drops) was added dropwise to a solution of the keto acetal **14** (21 mg) in acetone (1 mL) until an orange color persisted. The resulting acid was dissolved in chloroform, washed with water, and dried to give **15** as an oil: 6 mg; IR (neat) 3400 (br), 3020, 1700–1750 (s, with most intense maximum 1720–1750), 1450, 1370, and 1240 cm^{-1}; 270-MHz ^1H NMR (benzene-d_6) δ −0.36 (t, 1 H, *J* = 6 Hz, H-18endo), 0.52 (dd, 1 H, *J* = 6, 11.3 Hz, H-18exo), 0.88 (s, 3 H, H-17), 0.80–2.10 (m, 8 H), 1.26 (s, 3 H, H-20), 1.65, 1.67, 1.70, 1.89 (eac, s, 3 H, $OCOCH_3$), 2.35–2.65 (m, 2 H), 3.04 (dd, 1 H *J* = 8, 11 Hz, H-8), 3.56 (d, 1 H, *J* = 12 Hz, H-19), 3.73 (d, 1 H, *J* = 12 Hz, H-19), 4.45 (m, 2 H, H-7 and H-16), 4.84 (dd, 1 H, *J* = 3, 12.5 Hz, H-16), 5.09 (br d, 1 H, *J* = 9 Hz, H-15), 5.29 (d, 1 H, *J* = 5.7 Hz, H-2); mass spectrum (70 eV), *m/e* (relative intensity) 491 (19), 448 (10), 431 (63), 417 (13), 389 (30), 388 (31), 375 (56), 370 (32), 266 (42), 199 (48), 119 (49), 105 (100) base peak, 91 (68), 79 (52).

Conversion of Parguerol (8) to 12 and 13. A saturated solution (3 mL) of lithium aluminum hydride in THF was added to 1 mL of THF containing 10.5 mg of parguerol (**8**) at room temperature and the reaction was stirred for 5 min. The reaction was quenched with water, and the product was extracted into chloroform and then purified by preparative TLC (10% methanol in chloroform solvent) to yield tetraol **12** (2.7 mg, R_f 0.13–0.29); IR not taken; 270-MHz ^1H NMR ($CDCl_3$) δ 0.02 (t, 1 H, *J* = 5.5 Hz, H-18endo), 0.80 (dd, *J* = 5, 10 Hz, H-18exo), 1.04 (s, 3 H, CH_3), 1.20 (s, 3 H, CH_3), 2.43 (v br d, *J* = 17 Hz, H-12eq), 3.15 (dt, *J* = 5, 10, 10 Hz, H-7), 3.20 (d, 1 H, *J* = 12 Hz, H-19), 3.68 (d, 1 H, *J* = 12 Hz, H-19), 3.85 (dd, 1 H, *J* = 9, 12 Hz, H-16), 3.95 (dd, 1 H, *J* = 3, 12 Hz, H-16), 4.30 (dd, 1 H, 3, 9 Hz, H-15), 4.40 (d, 1 H, *J* = 5.5 Hz, H-2), 5.35 (br d, 1 H, *J* = 5 Hz, H-11).

Tetraol **12** was stirred in a few milliliters of acetone at room temperature with 25.6 mg of activated manganese dioxide[28] for 1 h. The mixture was filtered and the product was purified by HPLC on an Altex LiChrosorb Si60 column with 15% 2-propanol in chloroform as solvent. The oxidation product (2.3 mg) had the following spectral properties: IR (film) 3360, 2925, 2855, 1722, 1600, *J* 1378, 1015, 750 cm^{-1}; 270-MHz ^1H NMR ($CDCl_3$) δ 0.08 (t, 1 H, *J* = 6 Hz, H-18endo), 0.90 (dd, 1 H, *J* = 5, 10 Hz, H-18exo), 1.21 (s, 3 H, CH_3), 1.28 (s, 3 H, CH_3), 1.1–2.7 (m, 14 H), 3.20 (m, 1 H, H-7), 3.38 (d, 1 H, *J* = 12 Hz, H-19), 3.64 (d, 1 H, *J* = 12 Hz, H-19), 4.18 (d, 1 H, *J* = 4 Hz, H-15), 4.44 (d, 1 H, *J* = 4 Hz, H-2), 5.43 (d, 1 H, *J* = 5 Hz, H-11), 9.56 (d, 1 H, *J* = 4 Hz, H-16).

Reduction of 8 with Zinc–Acetic Acid To Give 11. Zinc (87.4 mg) and glacial acetic acid (2 drops) were added to 5 mg of parguerol (**8**) dissolved in tetrahydrofuran. After the mixture had been heated under reflux for 1 h, starting material was recovered by dilution with water and extraction. The procedure was repeated with 5 drops of acetic acid and, after 1 h of reflux, the mixture was stirred further at room temperature for 5 h. Preparative TLC of the product mixture with 10% 2-propanol

(28) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, 1967; Vol. 1, p 637.

in chloroform separated a band containing three compounds (1.7 mg total, R_f's ranging from 0.36 to 0.59) from the remaining starting material. Separation by HPLC of this mixture of three compounds on Partisil PXS (10/250 mm) with 10% 2-propanol in chloroform as the solvent system yielded one compound (**11**) (0.2 mg) that had the following spectral properties: 270-MHz ^{1}H NMR ($CDCl_3$) δ 0.08 (m, 1 H, H-18endo), 0.86 (m, 1 H, H-18exo), 1.02 (s, 3 H, H-20), 1.05 (s, 3 H, H-17), 2.08 (s, 3 H, $OCOCH_3$), 4.89 (d, 1 H, J = 17 Hz, -C=CH_2), 4.89 (d, 1 H, J= 12 Hz, -CH=CH_2), 5.73 (dd, 1 H, J = 12, 18 Hz, -CH=CH_2). Lack of sufficient material prevented complete spectral characterization.

Isolation of Isoparguerol (19), Parguerol 16-Acetate (16), and Isoparguerol 16-Acetate (21). A fraction (1 g) rich in parguerol (**8**) which was obtained from a Sephadex LH-20 chromatography (see isolation of parguerol (**8**) above) was chromatographed on silica gel 60 (100 g, 230–400 mesh) with 10% methanol in chloroform. The first fraction (150 mL), A, contained 661 mg of material and the second (50 mL), B, 309 mg. Filtration of fraction B through 1.6 g of neutral alumina (activity II) with chloroform removed most of the green pigmentation and gave crude parguerol. A portion of this crude parguerol (160 mg) was chromatographed on an Altex LiChrosorb Si60 HPLC 5μm column (10 × 250 mm) with 10% 2-propanol in chloroform as eluent to give parguerol (**8**) (150 mg, 0.002%, R_v = 34.8 mL) and a minor component, isoparguerol (6.8 mg, 0.0001%, R_v = 33.2 mL). Fraction A (661 mg) from the above silica gel chromatography was rechromatographed over silica gel, using 3–5% methanol in chloroform and collecting 100-mL fractions. Fractions 3 and 4 (245 mg) were combined, passed through neutral alumina, and separated by HPLC on a 10-μm Bondapak C_{18} reverse-phase column (7.8 × 300 mm) with 20% aqueous methanol. Two fractions of interest were collected: fraction IV (67 mg, R_v = 19.2 mL) and fraction V (35 mg, R_v = 20.8 mL). Fraction IV was further purified by preparative TLC with 4% methanol in chloroform to give parguerol 16-acetate (**16**) (22 mg, 0.0003%, R_f 0.13–0.33). Fraction V (32 mg) was rechromatographed on HPLC (5μm LiChrosorb Si60, 10 × 250 mm) with 5% 2-propanol in chloroform to give isoparguerol 16-acetate (**21**) (4.9 mg, 0.00006%, R_v = 32 mL).

Isoparguerol (**19**): $[\alpha]_D$ +3.6° (c 0.14, $CHCl_3$); mp 139–141 °C; IR (neat) 3360, 1710, 1375, 1365, and 1250 cm^{-1}; 270-MHz ^{1}H NMR ($CDCl_3$) (partial data in Table III) δ 1.08 (s, 3 H, H-17), 1.21 (s, 3 H, H-20), 1.00–2.60 (m, 18 H), 2.03 (s, 3 H, $OCOCH_3$), 3.29 (dt, 1 H, J = 6.5, 11.6 Hz, H-7), 3.84 (dd, 1 H, J = 9, 11 Hz, H-16), 3.94 (br d, 1 H, J = 12 Hz, H-16), 4.29 (dd, 1 H, J = 4, 10 Hz, H-15), 4.96 (d, 1 H, J = 4.8 Hz, H-2), 5.44 (d, 1 H, J = 6 Hz, H-11); mass spectrum (70 eV), m/e (relative intensity) 440 (4), 438 (4), 422 (1), 420 (1), 394 (2), 392 (2), 383 (3), 381 (3), 380 (2), 378 (2), 365 (15), 363 (16), 362 (6), 360 (5), 352 (30), 350 (28), 347 (36), 345 (35), 337 (8), 335 (8), 319 (12), 317 (14), 299 (14), 281 (30), 271 (42), 237 (36), 227 (50), 197 (29), 185 (36), 183 (32), 159 (59), 157 (55), 145 (53), 143 (62), 133 (42), 131 (66), 119 (79), 105 (100) base peak, 91 (81), 79 (55), 55 (49).

Parguerol 16-acetate (**16**): mp 121–123 °C $[\alpha]_D$ −40.5° (c 0.40, $CHCl_3$); IR (neat) 3400, 3060, 1742, 1726, 1450, 1380, 1368, 1245 cm^{-1}; ^{1}H NMR (see Table II for partial data) δ 0.95–1.50 (m, 4 H), 1.70–2.10 (m, 5 H), 2.20–2.55 (m, 4 H); mass spectrum (70 eV), m/e (relative intensity) 482 (2), 480 (3), 436 (4), 434 (4), 422 (6), 420 (5), 407 (3), 405 (3), 404 (5), 402 (6), 391 (10), 389 (15), 341 (13), 331 (11), 329 (19), 327 (9), 323 (14), 281 (37), 263 (71), 255 (25), 237 (79), 221 (43), 157 (58), 145 (64), 131 (64), 119 (77), 105 (100) base peak, 91 (100) base peak, 79 (58).

Isoparguerol 16-acetate (**21**): mp 180–182 °C; $[\alpha]_D$ −18.8° (c 0.09, $CHCl_3$); IR ($CHCl_3$) 3600, 1725, 1250 cm^{-1}; 270-MHz ^{1}H NMR ($CDCl_3$) (partial data in Table III) δ 1.09 (s, 3 H, H-17), 1.22 (s, 3 H, H-20), 1.10–2.10 (m, 13 H), 2.03, 2.11 (ea s, 3 H, $OCOCH_3$, 2.20–2.60 (m, 4 H), 3.27 (dt, 1 H, $w_{1/2}$ = 22.7 Hz, H-7), 4.28 (m, 2 H, H-15 and H-16), 4.60 (m, 1 H, H-16), 4.97 (d, 1 H, J = 4 Hz, H-2), 5.44 (d, 1 H, J = 6.3 Hz, H-11); mass spectrum (70 eV), m/e (relative intensity) 482 (3), 480 (3), 454 (4), 452 (4), 440 (3), 438 (3), 425 (2), 423 (3), 422 (2), 420 (2), 412 (4), 410 (4), 407 (13), 405 (15), 394 (8), 392 (7), 379 (5), 377 (4), 365 (4), 363 (3), 347 (57), 345 (58), 319 (19), 317 (26), 281 (18), 271 (17), 265 (33), 255 (41), 253 (51), 239 (28), 237 (37), 227 (90), 157 (65), 145 (68), 143 (72), 131 (78), 119 (100) base peak, 105 (97), 91 (93), 79 (49), 55 (53).

Isoparguerol Triacetate (20). Isoparguerol (**19**, 1.5 mg) was acetylated with acetic anhydride (0.2 mL) and pyridine (0.1 mL) overnight at room temperature. Workup in the usual manner (see acetylation of elatol (**2a**)) and chromatography of the crude product gave pure isoparguerol triacetate (**20**): IR (neat) 3500, 1735, 1240 cm^{-1}; ^{1}H NMR, see Table III; mass spectrum (70 eV), m/e (relative intensity) 482 (7), 480 (7), 454 (11), 452 (11), 423 (7), 422 (20), 421 (7), 420 (21), 407 (12), 405 (13), 404 (5), 402 (7), 394 (18), 392 (18), 347 (56), 345 (57), 333 (11), 331 (9), 319 (16), 317 (21), 281 (34), 271 (22), 265 (25), 263 (27), 255 (36), 253 (50), 237 (35), 227 (100) base peak, 211 (39), 209 (36), 185 (44), 157 (71), 143 (70), 131 (70), 119 (75), 105 (81), 91 (62), 69 (48), 61 (56).

Acetylation of Parguerol 16-Acetate (16) to Parguerol Peracetate (9). A 1-mg sample of **16** was acetylated in the manner described for **8**. TLC analysis indicated that the product consisted of one component with an R_f identical with that of parguerol acetate (**9**). The 270-MHz ^{1}H NMR spectrum ($CDCl_3$) of this product was identical with that of **9** prepared from **8**.

Acetylation of Isoparguerol 16-Acetate (21) to Isoparguerol Peracetate (20). A 1-mg sample of **21** was acetylated in the same manner as described for **8**. TLC analysis of the product indicated complete conversion of **21** to a single new product with an R_f identical with that of isoparguerol acetate (**20**) prepared from **19**. The 270-MHz ^{1}H NMR spectra of the two samples of **20** were the same.

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