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DITERPENOID FEEDING-DETERRENTS FROM *LAURENCIA SAITOI*

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Key Word Index—*Laurencia saitoi*; Rhodomelaceae; Ceramiales; red alga; diterpene; brominated compound; feeding-deterrent activity.

Abstract—Three new diterpenes and 14 known, brominated and non-brominated diterpenes have been isolated from the red alga *Laurencia saitoi* collected in 'Isoyake areas' in the Sea of Japan. Some of these diterpenes showed significant feeding-deterrent activity against young abalone (*Haliotis discus hannai*) and young sea urchins (*Strongylocentrotus nudus* and *S. intermedius*), thus suggesting that these metabolites provide a chemical defense against marine herbivorous animals in 'Isoyake areas'. © 1997 Elsevier Science Ltd. All right reserved

INTRODUCTION

In recent years, 'Isoyake areas' i.e. areas where macroalgae have disappeared and crustose coralline red algae now dominate extensive areas of the sublittoral rocky surfaces, have extended over a wide field along the coast of the Sea of Japan in Hokkaido. Many benthic invertebrates such as sea urchins and abalones are generally associated with such crustose coralline algal communities, and it is suggested that excessive grazing by herbivorous animals is one of the main factors needed to maintain 'Isoyake areas'. In previous papers [1, 2], we reported that the brown alga *Dictyopteris undulata* grew sympatrically with marine herbivores by virtue of containing feeding-deterrent compounds such as zonarol, isozonarol and chromazonarol.

In 'Isoyake areas' at Suttsu near Iwanai, western Hokkaido, *Laurencia saitoi* Perestenko, which passed under the name *L. obtusa* (Hudson) Lamouroux in Japan [3], grows sympatrically with the sea urchin *Strongylocentrotus nudus*, suggesting that this alga has a defense mechanism against marine herbivores as a result of producing some feeding-deterrent substances. We have investigated the feeding deterrents from this alga by using the cellulose plate method [2, 4] against the young abalone *Haliotis discus hannai*. This led to the isolation of three new and 14 known halogenated and non-halogenated diterpenoids. In this paper we describe the isolation and structures of these compounds as well as their defensive roles in this alga.

RESULTS AND DISCUSSION

L. saitoi collected off the coast of Suttsu in July 1991 was extracted with methanol, and the methanol solution was concentrated *in vacuo* and partitioned between ether and water. The ethereal solution was shaken with 5% potassium hydroxide solution to separate the acidic fraction, and then washed with water, dried and evaporated to leave the neutral fraction. The neutral fraction was found to display potent feeding-deterrent activity (Electivity index: $Ei = 0.85 \pm 0.04$), while the acidic fraction and the water-soluble fraction were inactive. A combination of column chromatography and TLC as well as HPLC of the neutral fraction led to the isolation of three new compounds, **11**, **15** and **16**, and 14 known compounds **1**–**10**, **12**–**14** and **17**. These compounds could be divided into three groups of compounds having parguerane (**1**–**8**) isoparguerane (**9**–**11**) and deoxyparguerane (**12**–**17**) skeleta [6] on the basis of the detailed analyses of the ^{1}H NMR data.

Parguerane-type compounds

One of two major metabolites, compound **7**, was identified as parguerol triacetate which had previously been isolated from this species collected at Teuri Island, Hokkaido [5], by comparison of the spectral

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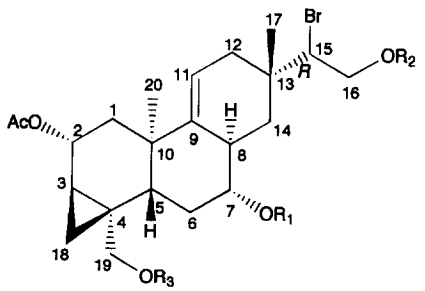
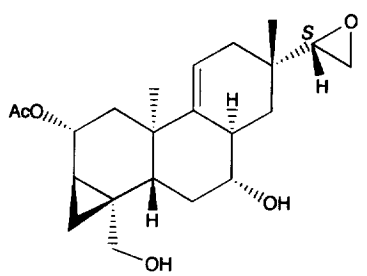


1: $R_1=R_2=R_3=H$
2: $R_1=Ac$, $R_2=R_3=H$
3: $R_2=Ac$, $R_1=R_3=H$
4: $R_3=Ac$, $R_1=R_2=H$
5: $R_1=R_2=Ac$, $R_3=H$
6: $R_2=R_3=Ac$, $R_1=H$
7: $R_1=R_2=R_3=Ac$



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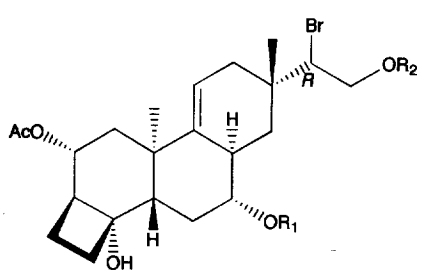
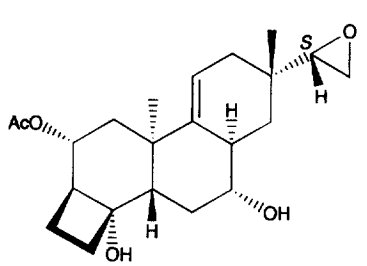


9: $R_1=R_2=H$
10: $R_1=R_2=Ac$



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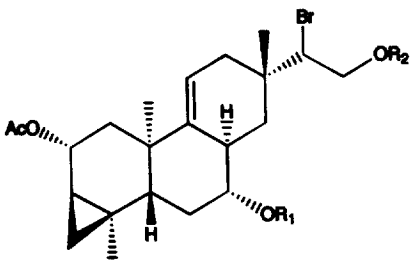
data with those of an authentic specimen. Compound **6** was identified as parguerol 16,19-diacetate also obtained from Teuri's species. Compounds **1** and **3** were found to be parguerol and parguerol 16-acetate, respectively, which had previously been isolated from the sea hare *Aplysia dactylomela*, by comparison of the spectral data with those reported in the literature [6]. Furthermore, compounds **2** and **4**, were deduced to be parguerol 7-acetate and parguerol 19-diacetate, respectively, which are partial hydrolysis products [5] of parguerol triacetate (**7**). On acetylation with acetic anhydride and pyridine both compounds **2** and **4** gave parguerol triacetate (**7**). In addition, compound **5** was identified as parguerol 7,16-diacetate, which had recently been isolated from *L. filiformis* collected at Bells Beach, Victoria, southern Australia [7], by independent spectral analyses and conversion to parguerol triacetate (**7**). Compound **8** was identified as a debrominated compound [5], which was obtained by treatment of **7** with sodium carbonate in methanol, by comparison of the spectral data with those of the authentic sample. Parguerol (**1**) and parguerol 16-acetate (**3**) have been found for the first time in marine alga and, furthermore, parguerol 7-acetate (**2**), parguerol 19-acetate (**4**) and compound **8** have been isolated for the first time as natural products.

Isoparguerane-type compounds

The other major metabolite, compound **10**, was identified as isoparguerol 7,16-diacetate also obtained from Teuri's *Laurencia* species [5]. Compound **9** was isoparguerol previously isolated from *A. dactylomela* [6] and now isolated for the first time from a marine alga. Compound **11**, $[\alpha]_D^{27}$ $-28.6°$ ($CHCl_3$), was analysed for $C_{22}H_{30}O_4$ (HR-EIMS). The ^1H and ^{13}C NMR data of **11** were very similar to those of isoparguerol (**9**). However, distinct differences were observed e.g., in the spectrum of **11** signals due to protons on a monosubstituted oxirane ring appeared at δ 2.77 (1H, *dd*, J = 3.9 and 2.9 Hz), 2.62 (1H, *dd*, J = 4.4 and 3.9 Hz) and 2.52 (1H, *dd*, J = 4.4 and 2.9 Hz) replaced those in **9** due to a bromomethine proton at δ 4.29 (1H, *dd*, J = 9.3 and 2.9 Hz) and hydroxymethyl protons at δ 3.94 (1H, *dd*, J = 11.7 and 2.9 Hz) and 3.84 (1H, *dd*, J = 11.7 and 9.3 Hz). As in the case of the transformation of **7** into **8** [5], treatment of isoparguerol (**9**) with sodium carbonate in methanol gave a debrominated compound which was identical with **11** in all respects.

Deoxyparguerane-type compounds

Compounds **12** was found to be deoxyparguerol which had also been obtained from *A. dactylomela* [6] and from Teuri's *Laurencia* [5]. Moreover, compound **13** was deoxyparguerol 16-acetate, which had previously been isolated from *L. obtusa* collected at Kimmeridge Bay, Dorset, by comparison of the spectral data with those reported in the literature [8]. Com-



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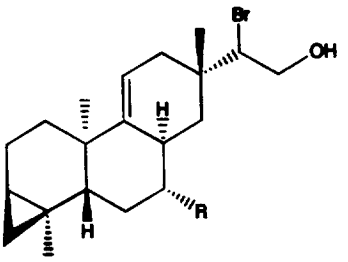
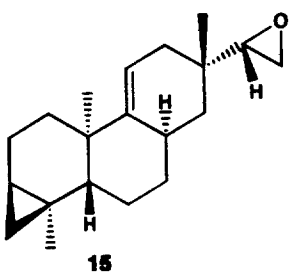
13: R_1=H, R_2=Ac



14: R=OH



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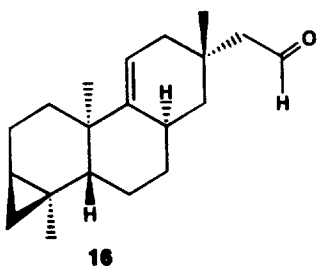

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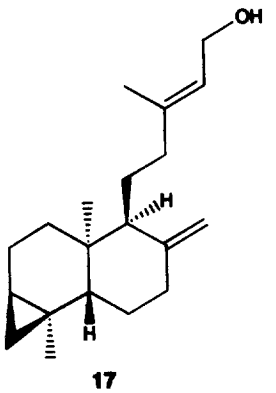

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pound **14** was identified as 2-deacetoxydeoxyparguerol which had previously been isolated from Teuri's *Laurencia* [5].

One of the three non-halogenated diterpenoids, compound **15**, $[\alpha]_D^{26}$ −37.6° ($CHCl_3$), was analysed for $C_{20}H_{30}O$ (HR-EIMS). The IR spectrum showed the absence of hydroxyl and carbonyl groups. The ^1H NMR spectrum (Table 1) revealed three characteristic signals at δ −0.03 (1H, *dd*, J = 5.9, 3.9 Hz), 0.40 (1H, *dd*, J = 9.3, 3.9 Hz) and 0.61 (1H, *ddd*, J = 9.3, 6.3, 5.9 Hz), which were typical to the cyclopropane protons of 2-deacetoxydeoxypargerane-type compound as observed in the ^1H NMR spectrum of 2-deacetoxydeoxyparguerol (**14**). Furthermore, the ^1H NMR spectrum displayed the absence of the hydroxyl group at C-7 and the presence of an isolated monosubstituted oxirane ring at δ 2.54 (1H, *dd*, J = 4.9, 2.9 Hz), 2.60 (1H, *dd*, J = 4.9, 3.9 Hz) and 2.78 (1H, *dd*, J = 3.9, 2.9 Hz) as in the case of compounds **8** and **11**. The ^1H and ^{13}C NMR spectra of **15** were very similar to those of 2-deacetoxy-7-dehydroxydeoxyparguerol recently isolated from *L. filiformis* [7], thus suggesting that the planar structure is represented by formula **15**. This was confirmed by HMBC experiments (Table 1).

The relative configuration was determined by the NOE difference spectra. As shown in Fig. 1, NOEs were observed between H-3/H_3-19, H-8/H_3-20 and H-8/H-15, proving that the relative configurations at C-3, C-4, C-5, C-8, C-10 and C-13 in **15** are the same as those of parguerol (**1**), deoxyparguerol (**12**) and their congeners. The co-occurrence of **15** with parguerol (**1**) and deoxyparguerol (**12**) in the same alga suggested that **15** had the same absolute configurations at C-3, C-4, C-5, C-8, C-10 and C-13. Based on the assumption that the oxirane rings in **8**, **11** and **15** are formed from the (15*R*)-bromohydrin [5] by intramolecular nucleophilic substitution reaction, the absolute configuration at C-15 was deduced to be *S*.

Compound **16**, $[\alpha]_D^{26}$ −13.0° ($CHCl_3$), was an isomer of compound **15**. The IR spectrum showed an absorption maximum at ν_{max} 1721 cm^{-1} due to a formyl group. The ^1H and ^{13}C NMR spectra were almost identical with those of **15** except for the signals of a formylmethyl group in **16** instead of those of the monosubstituted oxirane group in **15**. The planar structure **16** was confirmed by the HMBC spectrum (Table 2). The relative configuration was also determined by the NOE difference spectra. As shown in Fig. 1, NOEs were observed between H-3/H_3-19, H-8/H_3-20 and H_3-20/H-16(formyl proton), proving that **16** had the same configurations at C-3, C-4, C-5, C-8, C-10 and C-13 as those of **15**. The co-occurrence of **16** with **15** also suggested that **16** has the same absolute configuration.

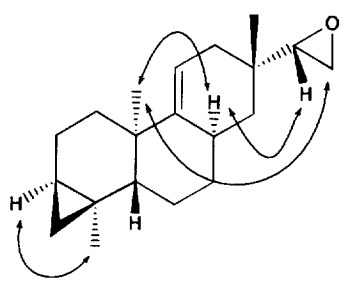
The structure of the third metabolite, compound **17**, was established by a combination of ^1H and ^{13}C NMR, ^1H–^1H COSY, HSQC, NOESY and HMBC spectroscopy. In the course of preparing this paper, we found the report on the isolation of preparguene from the Australian *L. filiformis* [7]. Comparison of the spectral data and $[\alpha]_D$ value of **17** with those of preparguerene showed that they were the same metabolite.

The feeding-deterrent activity of compounds **1**–**17** against the young abalone *Haliotis discus hannai* and the young sea urchins *Stronglyocentrotus nudus* and *S. intermedius* were tested by the cellulose plate method. The bioassay results against the young abalone *H. discus hannai* are summarized in Table 3. As shown in Table 3, the major metabolite, parguerol

Table 1. ^{13}C NMR (100 MHz, DEPT), ^{1}H NMR (400 MHz) and HMBC data* for **15**

C †	^{13}C δ	^{1}H δ	J (Hz)	Long range correlations
1	31.1	1.60	*m*	H_2-2, H-3, H-5, H_3-20
		0.90	*m*	
2	19.5	1.99	*m*	H_2-1, H_2-18
		1.82	*m*	
3	19.4	0.61	*ddd*, J = 9.3, 6.3, 5.9	H_2-1, H_2-2, H_2-18, H_3-19
4	16.4			H_2-2, H-5, H_2-18, H_3-19
5	49.9	1.13	*dd*, J = 12.3, 3.4	H_2-1, H_2-6, H_2-7, H_2-18, H_3-19, H_3-20
6	25.5	1.8	*m*	H-5, H_2-7
		1.6	*m*	
7	35.9	1.88	*m*	H-5, H_2-6, H_2-14
		0.97	*m*	
8	30.9	2.40	*m*	H_2-6, H_2-7, H-11, H_2-14
9	147.6			H_2-1, H_3-20
10	37.5			H_2-1, H_2-2, H-5, H_2-6, H-11, H_3-20
11	115.4	5.27	*m*	H_2-12
12	33.2	1.78	*br s*	H-11, H_3-17
13	31.5			H-11, H_2-14, H-15, H_2-16, H_3-17
14	42.6	1.78	*m*	H-15, H_3-17
		1.13	*m*	
15	57.0	2.78	*dd*, J = 3.9, 2.9	H_2-14, H_2-16, H_3-17
16	45.6	2.60	*dd*, J = 4.9, 3.9	H-15
		2.54	*dd*, J = 4.9, 2.9	
17	25.4	0.89	*s*	H_2-14, H-15
18	21.4	0.40	*dd*, J = 9.3, 3.9	H_2-2, H-5, H_3-19
		−0.03	*dd*, J = 5.9, 3.9	
19	24.1	0.98	*s*	H-3, H-5, H_2-18
20	18.3	0.92	*s*	H-5

* Measured in chloroform-d_1.
† Assignment was made with the aid of HSQC spectrum.



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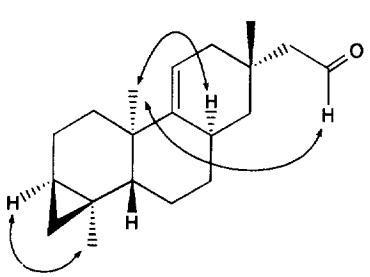

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Fig. 1. NOEs of compounds **15** and **16**.

triacetate (**7**) showed potent feeding-deterrent activity against the young abalone *H. discus hannai*. Deoxyparguerol 16-acetate (**13**) and 2-deacetoxydeoxyparguerol (**14**) also showed potent activity, while other compounds showed moderate or weak activity. Furthermore, compounds **7**, and **13** showed potent feeding-deterrent activity against the young sea urchin *S. nudus*, $Ei = 0.92 \pm 0.06$ and $Ei = 0.75 \pm 0.08$, and against that *S. intermedius*, $Ei = 0.97 \pm 0.04$ and $Ei = 0.96 \pm 0.08$, respectively.

The two most potent feeding deterrents, parguerol triacetate (**7**) and deoxyparguerol 16-acetate (**13**), were obtained in about 0.03 and 0.005% yield, respectively, based on the wet weight of alga. Therefore, each individual of *L. saitoi* contains sufficient amounts of diterpenoid feeding-deterrents, such as **7**, **13** and so on, to inhibit grazing by herbivorous animals. It appears that in 'Isoyake areas', *L. saitoi* grow sympatrically with marine herbivores such as sea urchins as a result of producing these diterpenoids.

EXPERIMENTAL

General. NMR: 400 MHz (^{1}H) and 100 MHz (^{13}C), $CDCl_3$, TMS as int. standard; Low and high resolution MS: 70 eV; CC: alumina (Merck, aluminium oxide 90, activity II–III) and silica gel (Merck, Kieselgel 60, 70–230 mesh); Prep. TLC: silica gel (Merck, Kieselgel 60 F_{254S}); HPLC: Megapak SIL-C_{18} (JASCO, reversed-phase) and Megapak SIL-CN (JASCO, normal phase).

Table 2. ^{13}C NMR (100 MHz, DEPT), ^{1}H NMR (400 MHz) and HMBC data* for **16**

C†	^{13}C δ	^{1}H δ	J (Hz)	Long range correlations
1	31.2	1.60	*m*	H_2-2, H-3, H-5, H_3-20
		0.90	*m*	
2	19.5	1.99	*m*	H_2-1, H_2-18
		1.81	*m*	
3	19.3	0.61	*ddd*, J = 9.3, 6.3, 5.9	H_2-1, H_2-2, H_2-18, H_3-19
4	16.3			H_2-2, H-5, H_2-18, H_3-19
5	50.1	1.13	*dd*, J = 12.3, 3.4	H_2-1, H_2-6, H_2-7, H_2-18, H_3-19, H_3-20
6	25.5	1.84	*m*	H-5, H_2-7
		1.55	*m*	
7	35.8	1.6	*m*	H-5, H_2-6, H_2-14
		0.9	*m*	
8	31.0	2.26	*m*	H_2-6, H_2-7, H-11, H_2-14
9	147.3			H_2-1, H_2-12, H_2-14, H_3-20
10	37.5			H_2-1, H_2-2, H-5, H_2-6, H-11, H_3-20
11	114.8	5.30	*m*	H_2-12
12	39.0	1.91	*br s*	H-11, H_2-14, H_2-15, H_3-17
13	31.7			H-8, H-11, H_2-12, H_2-14, H_2-15, H_3-17
14	43.7	1.72	*dd*, J = 14.5, 6.0	H_2-12, H_2-15, H_3-17
		1.02	*dd*, J = 14.5, 2.4	
15	50.2	2.29	*dd*, J = 14.7, 2.9	H_2-12, H-16, H_3-17
		2.17	*dd*, J = 14.7, 2.9	
16	204.0	9.83	*dd*, J = 2.9, 2.9	H_2-15
17	28.6	1.07	*s*	H_2-14, H_2-15
18	21.4	0.40	*dd*, J = 9.3, 3.9	H_2-2, H-3, H-5, H_3-19
		−0.03	*dd*, J = 5.9, 3.9	
19	24.1	0.97	*s*	H-5, H_2-18
20	18.0	0.95	*s*	H_2-1, H-5

* Measured in chloroform-d_1.

† Assignment was made with the aid of HSQC spectrum.

Table 3. Feeding-deterrent activity (Electivity index (*Ei*)) of compounds **1**–**17** against the young abalone *Haliotis discus hannai*

Compound	Electivity index (*Ei*)
1	0.22±0.29
2	0.11±0.12
3	0.11±0.07
4	−0.01±0.14
5	0.24±0.14
6	0.08±0.08
7	0.88±0.06
8	−0.05±0.15
9	−0.01±0.21
10	0.21±0.24
11	0.45±0.10
12	0.11±0.14
13	0.74±0.12
14	0.70±0.12
15	0.63±0.06
16	0.59±0.10
17	0.42±0.09

Bioassay. Bioassays were carried out by the cellulose plate method using cellulose TLC sheets [2, 4]. Circles of 200 mm in diameter were prepd from the cellulose TLC aluminum sheet (200 mm × 200 mm; Merck, No. 5552). Sixteen circles of 20 mm in diameter were drawn 8 mm from the edge of the circle and 10 mm apart. The sample and control zones were arranged alternatively. In assays with the young abalone *Haliotis discus hannai*, 7.5 μg of phosphatidylcholine (PC, Wako Chemicals), which is known to be a feeding stimulant for herbivores [9, 10], was dissolved in EtOH and absorbed on to the control zone. 75 μg of each fr. or each isolated compound was mixed with 7.5 μg of PC dissolved in EtOH and absorbed on to the sample zone. Each sheet was placed on the bottom of the cylindrical test aquarium (300 mm in diameter and 150 mm in depth) made of PVC plate, which was filled with 5 l of filtered seawater and the temp. maintained at 20°. Five young abalone (25–30 mm in shell length), which had been starved for a day, were then placed in the test aquarium, and the assays were conducted for 15 hr in the dark. In the assays with the young sea urchins *Strongylocentrotus nudus* and *S. intermedius*, 40 μg of PC was used for each control and sample zone. Ten young sea urchins (*ca* 30 mm in test diameter) which had been starved for two days were placed in the aquarium, and the assays were conducted for 15 hr in the dark. All assays were carried out in 12 replicates. After 15 hr the sheets were taken out of the aquarium and the feeding-deterrent activity was evaluated by comparing the number of biting traces left on the sample zone with that on

the control zone. Relative activity (electivity index: *Ei*) was defined by the following equation (*Pi*: average number of biting traces of the control (PC), *pi*: average number of biting traces of each sample) [11]. Significant differences ($p < 0.01$ or 0.05) of feeding-deterrent activity were assessed by the *t*-test.

$$Ei = \frac{Pi - pi}{Pi + pi}$$

Isolation of compounds. Laurencia saitoi was collected at Suttsu near Iwanai, western Hokkaido, in July 1991. Partially dried alga (900 g) was extracted with MeOH and the MeOH soln concentrated *in vacuo* and partitioned between Et_2O and H_2O. The ethereal soln was shaken with aq. 5% KOH, washed with H_2O, dried over anhydrous Na_2SO_4 and evapd to leave a neutral brown oil (20 g). The neutral fr. was found to show potent feeding-deterrent activity ($Ei = 0.85 \pm 0.04$), while the acidic fr. and the H_2O-soluble fr. were inactive. The neutral extract (10 g) was fractionated by CC over alumina with a step gradient (hexane, C_6H_6 and EtOAc). The potently active fr. ($Ei = 0.98 \pm 0.02$) eluted with C_6H_6 was then subjected to RP-HPLC (MeOH–H_2O (9:1)) followed by prep. TLC with C_6H_6 to give **15** (10 mg) and **16** (15 mg). Another potently active fr. ($Ei = 0.94 \pm 0.02$) eluted with C_6H_6–EtOAc (10:1) was submitted to repeated RP-HPLC (MeOH–H_2O, 17:3 and 4:1) followed by normal phase HPLC (hexane–*iso*-PrOH (20:1)) to yield **7** (510 mg) and **17** (28 mg). The moderately active fr. ($Ei = 0.78 \pm 0.09$) eluted with EtOAc was further submitted to repeated normal phase HPLC (hexane–*iso*-PrOH, 20:1 and 9:1) followed by prep. TLC with C_6H_6–EtOAc (1:1) to give **13** (84 mg), **10** (467 mg), **6** (80 mg) and **5** (97 mg). The other active fr. ($Ei = 0.65 \pm 0.07$) eluted with MeOH was submitted to RP-HPLC (MeOH–H_2O, 7:3) followed by a combination of normal phase HPLC (hexane–*iso*-PrOH, 7:3, 4:1, 9:1 and 20:1) and prep. TLC with EtOAc to afford **14** (26 mg), **1** (73 mg), **11** (14 mg), **8** (28 mg), **4** (112 mg), **9** (40 mg), **2** (30 mg), **12** (78 mg) and **3** (16 mg).

Parguerol-7-acetate (**2**). Oil; $[\alpha]_D^{26}$ −5.41° ($CHCl_3$; *c* 0.640); IR, ν_{max}^{neat} cm^{-1}: 3448, 1727, 1245, 1073, 1024, 954 and 755; ^1H NMR identical with that of the authentic sample [5].

Parguerol-19-acetate (**4**). Oil; $[\alpha]_D^{26}$ −17.1° ($CHCl_3$; *c* 0.810); IR, ν_{max}^{neat} cm^{-1}: 3412, 1727, 1246, 1056, 1024, 949 and 755; ^1H NMR identical with that of the authentic sample [5].

Compound **8**. Oil; $[\alpha]_D^{26}$ −49.9° ($CHCl_3$; *c* 1.11); IR, ν_{max}^{neat} cm^{-1}: 3400, 1725, 1248, 1075, 1048, 1020, 952 and 755; ^1H NMR identical with that of the authentic sample [5].

Compound **11**. Oil; $[\alpha]_D^{27}$ −28.6° ($CHCl_3$; *c* 0.970); IR ν_{max}^{neat} cm^{-1}: 3386, 1721, 1248, 1162, 1054, 1018, 949, 916, 858 and 754; ^1H NMR: δ 0.98 (3H, *s*; H_3-17), 1.17 (3H, *s*; H_3-20), 2.03 (3H, *s*; Ac), 2.77 (1H, *dd*, $J = 3.9$ and 2.9 Hz; H-15), 2.52 (1H, *dd*, $J = 4.4$ and 2.9 Hz; H_a-16), 2.62 (1H, *dd*, $J = 4.4$ and 3.9 Hz; H_b-16), 3.28 (1H, *m*; H-7), 4.95 (1H, *br d*, $J = 3.9$ Hz; H-2) and 5.43 (1H, *m*; H-11); ^{13}C NMR, CH_3: δ 21.5 (C20), 21.6 (Ac) and 25.8 (C17), CH_2: δ 15.6 (C18), 29.9 (C19), 32.6 (C12), 35.0 (C6), 37.9 (C1), 38.6 (C14) and 45.6 (C16), CH: δ 39.2 (C8), 45.0 (C3), 47.4 (C5), 56.9 (C15), 70.0 (C2), 77.2 (C7) and 118.2 (C11), C: δ 31.3 (C10), 36.2 (C13), 73.1 (C4), 144.8 (C9) and 170.5 (Ac); LR-EIMS *m/z* (rel. int.): 358 [32; M]$^+$, 283 (26), 239 (22), 143 (32), 131 (28), 119 (25), 105 (32), 93 (20), 91 (29), 81 (20), 79 (21), 69 (23), 55 (37), 43 (100) and 41 (36); HR-EIMS *m/z*: 358.2139. Calc. for $C_{22}H_{30}O_4$, 358.2144 [M].

Compound **15**. Oil; $[\alpha]_D^{26}$ −37.6° ($CHCl_3$; *c* 0.644); IR, ν_{max}^{neat} cm^{-1}: 3044, 1015, 912, 857, 807 and 755; ^1H and ^{13}C NMR: Table 1; LR-EIMS *m/z* (rel. int.): 286 [60; M]$^+$, 271 [54; M-CH_3]$^+$, 231 (69), 145 (50), 133 (50), 131 (55), 119 (64), 107 (55), 105 (87), 95 (54), 93 (59), 91 (82), 81 (67), 79 (55), 67 (51), 55 (82) and 41 (100); HR-EIMS *m/z*: 286.2303. Calcd for $C_{20}H_{30}O$, 286.2297 [M].

Compound **16**. Oil; $[\alpha]_D^{26}$ −13.0° ($CHCl_3$; *c* 1.54); IR ν_{max}^{neat} cm^{-1}: 3050, 2726, 1721, 1016, 989, 918, 867, 817 and 755; ^1H and ^{13}C NMR: Table 2; LR-EIMS *m/z* (rel. int.): 286 [28; M]$^+$, 271 [13; M-CH_3]$^+$, 243 (25), 242 (100), 231 (35), 227 (39), 187 (41), 133 (35), 105 (42), 95 (21), 93 (30), 91 (33), 81 (25), 79 (26), 67 (22), 55 (35) and 41 (47); HR-EIMS *m/z*: 286.2309 and 242.2031. Calcd for $C_{20}H_{30}O$, 286.2297 [M] and $C_{18}H_{26}$, 242.2034 [M-C_2H_4O].

Acetylation of **2** *to give parguerol triacetate* (**7**). Acetylation of **2** (2.9 mg) was carried out with Ac_2) (150 μl) and pyridine (150 μl) at room temp. for 12 hr in the usual manner. The products were purified by prep. TLC (hexane–EtOAc, 1:1) to give a triacetate (3.3 mg) which was identical with parguerol triacetate (**7**) in all respects.

Acetylation of **4** *to give parguerol triacetate* (**7**). Acetylation of **4** (4.3 mg) by the procedure just described and purification of the products by prep. TLC (hexane–EtOAc 1:1) gave a triacetate (5.0 mg) which was identical with parguerol triacetate (**7**) in all respects.

Alkali treatment of isoparguerol (**9**). A soln of **9** (6.0 mg) in Na_2CO_3 (20 mg) and MeOH (300 μl) was stirred at room temp. for 2 hr under a N_2 atmosphere and then H_2O was added. The reaction mixt. was extracted with Et_2O. The ethereal soln was washed with H_2O and dried over Na_2SO_4. Removal of the solvent gave an oily residue which was then subjected to prep. TLC (hexane–EtOAc, 1:1) to afford a debrominated product (3.0 mg); oil; $[\alpha]_D^{24}$ −30.6° ($CHCl_3$; *c* 0.450); IR and ^1H NMR coincident with those of **11**.

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