 © 1990 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 63, 3066—3072 (1990) [Vol. 63, No. 11

The Structures of Cytotoxic Diterpenes Containing Bromine from the Marine Red Alga *Laurencia obtusa* (Hudson) Lamouroux[1)]

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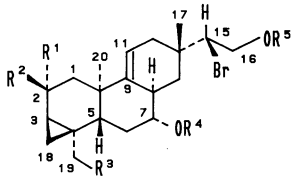
(Received April 19, 1990)

Four new brominated diterpenes possessing parguerane or isoparguerane skeleton have been isolated from the title alga collected in the Japanese waters along with several known parguerol, isoparguerol, and deoxyparguerol congeners. The structures of three new compounds were determined by the chemical correlation with the corresponding known compounds. The structural elucidation of the remaining one was carried out on the basis of a combination of chemical and spectroscopic means including 2D NMR technique. One of the new metabolites has proved to be cytotoxic. The cytotoxic properties of the several chemically derived compounds in addition to the known diterpenes are also described.

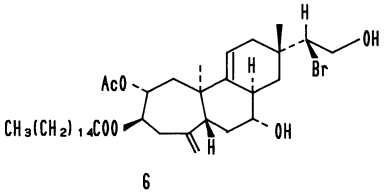
In connection with our search for bioactive metabolites from the marine organism in the Japanese waters, we have reported that the crude methanol extracts of the marine red alga *Laurencia obtusa* (Hudson) Lamouroux (Magire-sozo in Japanese) collected at Teuri Island, Hokkaido exhibit a significant level of cell growth inhibitory activity against P388 *in vitro* cell lines (ED_{50} 0.18 μg mL^{-1}).[2)] Careful separation of this extracts using the assay of cytotoxicity against P388 *in vitro* cell lines has led to the isolation of the active components which consists of new polyoxygenated squalenes.[2)] On the other hand, we have further reported the absolute stereostructure of deoxyparguerane derivative **5**, the same algal constituent, elucidated by means of X-ray crystallographic analysis.[3)] This successful result permits to assign the absolute configuration of other parguerane congeners. A group of parguerol and its derivatives has been first isolated as cytotoxic components from the sea hare *Aplysia dactylomela* collected near La Parguera, Puerto Rico by F. J. Schmitz et al.,[4)] and the absolute configuration of these compounds had not yet been resolved. Moreover, we have also reported the structural elucidation of brominated diterpene **6** with new A-seco-parguerane skeleton, which shows potential cell growth inhibitory activity against B16 *in vitro* cell lines.[5)] Further search for cytotoxic component from this algal extract has yielded a new cytotoxic diterpene **1** along with three inactive metabolites **2**, **3**, and **4** in addition to known congeners, **7**, **8**, **9**, and **10**.[4)] We wish to describe here the isolation and structural determination of the new diterpenes **1**, **2**, **3**, and **4** as well as the cytotoxic properties of these compounds against P388 and HeLa *in vitro* cell line together with those of several chemically derived compounds in addition to the known metabolites.

Results and Discussion

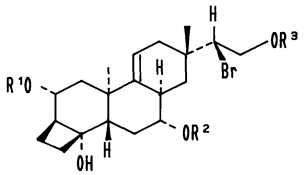
Specimens of *Laurencia obtusa* were collected off the coast of Teuri Island in Hokkaido in shallow waters (0 to −1.5 m). Methanol extracts of this alga were concentrated in vacuo and the resulting suspension was partitioned against diethyl ether. The ethereal fraction was treated by the usual method to give a neutral essential oil, which was fractionated by open column chromatography on silica gel with a step gradient to give five fractions of A [C_6H_6], B [C_6H_6–EtOAc (9:1)], C [C_6H_6–EtOAc (1:1)], D [EtOAc], and E [EtOAc–MeOH (9:1)]. The known triol **5** and tetraacetate **8** were isolated from the fraction E and B



1. R^1= R^3= OAc, R^2= R^4= H, R^5= Ac
3. R^1, R^2= =O, R^3 = OH, R^4= R^5= H
4. R^1= R^2= R^3= R^4= R^5= H
5. R^1= OH, R^2= R^3= R^4= R^5= H
7. R^1= OAc, R^2= R^3= R^4= R^5= H
8. R^1= R^3= OAc, R^2= H, R^4= R^5= Ac
9. R^1= R^3= OH, R^2= R^4= R^5= H



6



2. R^1= R^2= R^3= H
10. R^1= R^2= R^3= Ac

in 2% and 10% yield of the neutral oil, respectively. HPLC separation of the fraction C with the reverse-phase support gave the known compounds **6**, **7**, and **10** in 0.01%, 0.3%, and 2% yields, respectively. On the other hand, the known polar metabolite **9** was isolated from the aqueous fraction in 0.25% yield.

From the fraction rich in deoxyparguerol (**7**), compound **1** [a viscous oil, $[\alpha]_D$−37.8° (*c* 1.54, $CHCl_3$)] was obtained in 0.3% yield of the neutral oil. This compound was tentatively assigned the molecular formula of $C_{26}H_{37}O_7Br$ on the basis of high resolution mass spectral analysis (highest observed mass, M^+-H_2O) and showed very similar spectral properties to those of known parguerol 7,16,19-triacetate (**8**). The ^1H NMR and IR spectra of **1** revealed the presence of two primary, one secondary acetoxyl groups and one secondary hydroxyl group in the molecule [δ=2.07×2, 2.11 (3H each, s), 3.18 (1H, m), 3.78 (1H, d, *J*=12.0 Hz), 4.04 (1H, d, *J*=12.0 Hz), 4.26 (1H, d, *J*=9.0 Hz), 4.31 (1H, dd, *J*=9.0, 9.0 Hz), 5.35 (1H), ν_{max} 3500 cm^{-1}] instead of four acetoxyl groups in **8**. Acetylation of **1** with acetic anhydride and pyridine in the usual method gave a corresponding product whose spectral data including the optical rotation were identical with those of **8**. F. J. Schmitz et al. have reported that the unusual acetoxy-deshielded methine signals at δ= 5.2—5.4 in parguerane derivatives can be explained by placing these groups next to the cyclopropane ring.[4] Therefore, the remaining methine signal at δ=3.18 in **1** must be attributed to the proton at C-7, establishing the formula of 15-bromo-2,16,19-triacetoxy-7-hydroxy-9(11)-parguerene for **1**.

Compound **2** [a glassy solid, $[\alpha]_D$ +5.0° (*c* 0.46, CH_3OH)] was isolated from the aqueous fraction by use of HPLC (JASCO, Finepak SIL C18, 50% water in methanol) in 0.1% yield of the aqueous residue. Its IR and ^1H NMR spectra did not show the presence of any acetoxyl group. The presence of two tertiary methyls and trisubstituted double bond is evident in the ^1H and ^{13}C NMR spectra of **2** [δ=1.05, 1.27 (3H each, s), 5.46 (1H, br d, *J*=ca. 6 Hz), δ=22.5 (q), 24.9 (q), 117.2 (d), and 147.2 (s)]. Moreover, the ^1H NMR spectrum showed the presence of signals of a set of ABX pattern [δ=3.77 (1H, dd, *J*=9.5, 12.5 Hz), 4.02 (1H, dd, *J*=3.0, 12.5 Hz), 4.25 (1H, dd, *J*=3.0, 9.5 Hz)], signals at δ=3.14 (1H, ddd, *J*=7.0, 9.5, 9.5 Hz), 3.88 (1H, br d, *J*=ca. 4 Hz), and the absence of cyclopropane proton signals. By the above data as well as co-existence of isoparguerol 7,16-diacetate (**10**) in the same alga, it was surmised that the compound **2** was a deacetyl derivative of isoparguerol. Evidence of the structure **2** was provided by usual acetylation. Compound **2** was treated with acetic anhydride and pyridine to give a triacetate, which was found to be completely identical with **10** in all respects.

Moreover, HPLC separation of the aqueous fraction

Table 1. ^{13}C and ^1H NMR Chemical Shifts (ppm) of Compound **3** and C/H Correlations Observed in HMBC Experiments

C[a]	δC[b]	δH (Multiplicity, *J*/Hz)[c]	HMBC[d]
1	49.4	2.29 (d, *J*=14.5) Heq	H-3, 5, 20
		2.15 (d, *J*=14.5) Hax	
2	212.9		H-lax, leq, 18endo, 18exo
3	32.4	ca. 1.8 (m)	H-leq, 5, 18endo, 18exo, 19a, 19b
4	33.7		H-3, 5, 18endo, 18exo, 19a, 19b
5	47.1	ca. 1.8 (m)	H-lax, leq, 6ax, 6eq, 18endo, 18exo, 19a,19b, 20
6	34.1	2.37 (ddd, *J*=3.0, 4.5, 12.5) Heq	H-5
		1.94 (ddd, *J*=10.5, 12.5, 12.5) Hax	
7	77.6	3.16 (ddd, *J*=4.5, 9.5, 10.5)	H-5, 6ax, 6eq, 8, 14ax, 14eq
8	39.6	ca. 2.2 (m)	H-6ax, 6eq, 7, 11, 14ax, 14eq
9	143.8		H-12a, 12eq, 14eq, 20
10	45.6		H-lax, leq, 5, 11, 20
11	118.7	5.32 (ddd, *J*=1.5, 1.5, 6.0)	H-12ax, 12eq
12	40.1	2.48 (m) Heq	H-11, 14ax, 14eq, 15, 17
		1.81 (ddd, *J*=1.5, 2.5, 17.5) Hax	
13	36.4		H-11, 12ax, 12eq, 14ax, 14eq, 15, 16a, 16b, 17
14	39.3	ca. 2.3 (m) Heq	H-7, 12eq, 15, 17
		ca. 1.3 (m) Hax	
15	67.7	4.18 (dd, *J*=2.5, 9.5)	H-16a, 16b, 17
16	65.0	4.01 (dd, *J*=2.5, 12.5) Ha	H-15
		3.77 (dd, *J*=9.5, 12.5) Hb	
17	24.7	1.05 (s)	H-15
18	26.2	1.39 (dd, *J*=4.5, 10.5) Hexo	H-3, 5, 19a, 19b
		1.11 (dd, *J*=4.5, 5.0) Hendo	
19	66.7	3.74 (d, *J*=12.0) Ha	H-3, 5, 18endo, 18exo
		3.21 (d, *J*=12.0) Hb	
20	20.3	1.05 (s)	H-lax, leq, 5

a) The numbering system corresponds to that used for parguerane diterpenes. b) Measured at 67.8 MHz (CD_3OD, TMS=0). c) Measured at 270 MHz (CD_3OD, TMS=0). d) Measured at 270 MHz (CD_3OD, TMS=0).

afforded a ketone **3** (0.1% yield) [a glassy solid, $[\alpha]_D$ −41° (*c* 0.89, CH_3OH)]. Its ^1H NMR spectrum (Table 1) shows two tertiary methyl signals at δ=1.05 (6H, s), and an AB quartet due to the C-19 hydroxymethyl [δ=3.21 and 3.74 (1H each, d, J=12 Hz)]. In the ^1H NMR spectrum, compound **3** also exhibited signals nearly identical with those corresponding to the protons at C-7, C-11, C-15, and C-16 in the tetrol **9**, suggesting the presence of the same B-C ring including its substituents as that of **9** in **3**. The ^1H and ^{13}C NMR data of **3** were different from those of **9** in the lack of a proton signal at C-2 and, instead, in the presence of a carbonyl group [δ=212.9 (s)], corresponding to IR absorption at 1678 cm^{-1}. In ^{13}C NMR spectrum, the signal at δ=26.2 (t) was able to assign to C-18, because three bond coupling to the hydroxymethyl protons at C-19 was observed in ^1H-detected heteronuclear multiple-bond ^1H–^{13}C correlation (HMBC) spectrum[6] of **3**. The cyclopropane methylene proton signals at C-18 [δ=1.11 (1H, dd, J=4.5, 5.0 Hz), and 1.39 (1H, dd, J=4.5, 10.5 Hz)], which were related by ^{13}C–^1H COSY spectrum with one bond coupling to the same carbon absorption at δ=26.2, were shifted to very low field relative to those of **9** (δ=−0.01 and 0.83). Moreover, the presence of three-bond couplings from both H-18endo, and H-18exo to the carbonyl carbon were indicated in HMBC spectrum. The above data are consistent with the presence of a carbonyl group at C-2 in place of the hydroxyl group in **9** and hence, the structure of **3** can be represented as 15-bromo-7,16,19-trihydroxy-9(11)-pargueren-2-one. Reduction of **3** with $NaBH_4$ in C_2H_5OH gave two products which were separated by HPLC technique. The more polar product of them was found to be identical with the tetrol **9**, in all respects, confirming this ketone to be represented as the formula **3**. The structure of the less polar product was deduced to be a corresponding epoxide **21** by ^1H NMR analysis.

Minor component (0.08% yield) isolated from chromatographic fraction C was a diol **4** [mp 87—90°C, $[\alpha]_D$ −17.8° (*c* 1.71, $CHCl_3$)]. The highest mass ion peak observed in high resolution mass spectrum of **4** appeared at 366.1391, consistent with a formula of $C_{20}H_{29}O^{81}Br$. The presence of one or more hydroxyl groups and no acetate groups were indicated by IR and ^1H NMR data. Upon acetylation of **4** with acetic anhydride and pyridine, a corresponding product was obtained, IR and ^1H NMR spectra of which showed the presence of two acetyl groups [δ=2.09, 2.13 (3H each, s), ν_{max} 1740 cm^{-1}] and no hydroxyl groups, indicating that the highest mass ion peak observed for **4** itself corresponded to M^+-H_2O, thus yielding a formula of $C_{20}H_{31}O_2Br$ for **4**. The ^1H NMR spectrum of **4** (Table 2) was very similar to that of the triol **5**[3] with a few notable exceptions, and hence a structure similar to **5** could be inferred for **4**. The most obvious difference between the spectra of **4** and **5** was pointed

Table 2. ^1H NMR Data of Compound **4**[a]

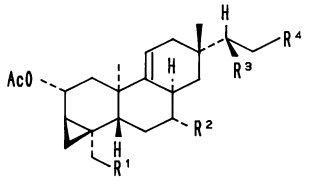
Position	δ	Multiplicity
1 ax	0.86	ddd, J=6.5, 13.2, 13.2
1 eq	1.81	br dd, J=6.5, 13.2
2 ax	2.00	dddd, J=6.5, 6.5, 13.2, 13.2
2 eq	1.62	br dd, J=6.5, 13.2
3	0.65	ddd, J=6.0, 6.5, 9.5
5	1.14	dd, J=3.5, 13.5
6 ax	1.61	ddd, J=11.0, 12.5, 13.5
6 eq	2.11	ddd, J=3.5, 4.5, 12.5
7	3.17	ddd, J=4.5, 9.5, 11.0
8	2.22	m
11	5.36	ddd, J=2.0, 2.0, 6.0
12 ax	1.82	ddd, J=2.0, 3.0, 17.5
12 eq	2.41	dddd, J=3.0, 4.5, 6.0, 17.5
14 ax	1.37	dd, J=12.0, 15.0
14 eq	2.24	ddd, J=3.0, 5.5, 15.0
15	4.30	dd, J=3.0, 9.0
16 a	3.83	dd, J=9.0, 12.5
16 b	3.93	dd, J=3.0, 12.5
17	1.06	s
18 endo	−0.03	dd, J=4.2, 6.0
18 exo	0.43	dd, J=4.2, 9.5
19, 20	0.98, 0.99	s

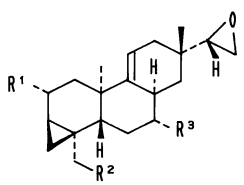
a) Measured at 270 MHz ($CDCl_3$, TMS=0). Assignments were carried out with the aid of ^1H–^1H COSY spectrum.

in **4**. The broad doublet signal at δ=4.29 (1H, br d, J=5.0 Hz) assigned to the proton at C-2 in **5** was not observed in that of **4**. Further, cyclopropane proton signals at δ=−0.03 (1H, dd, J=4.2, 6.0 Hz) and 0.43 (1H, dd, J=4.2, 9.5 Hz) in **4** were shifted upfield compared with those of **5** (δ=0.02 and 0.66). Also, the signal at δ=0.85 (1H, dd, J=6.0, 10.0 Hz) assigned to the proton at C-3 in **5** was replaced by the signal at δ=0.65 (1H, ddd, J=6.0, 6.5, 9.5 Hz) in **4**. Thus, the structure of the diol **4** was concluded to be 2-deoxy form of **5**. The ^1H–^1H COSY spectrum of **4** allowed a complete assignment of all proton resonances as shown in Table 2 and supported the structure **4** for this diol. Since the chemical shifts and coupling patterns in the ^1H NMR spectrum of **4** were nearly identical with those of the triol **5**, the stereostructure of this diol could be represented as the formula **4**.

We have previously reported that parguerol 7,16,19-triacetate (**8**) has cytotoxic property against P388 *in vitro* cell line but the triol **5** is inactive,[3] which prompts us to evaluate cell growth inhibitory activity of the compounds with modified functional groups, in addition to the above natural products. We have tried to prepare a debromo compound, epoxides, hydrolysis compounds, and a 15-hydroxy derivative, respectively, from the major metabolite **8**.

Irradiation of **8** in benzene containing tributyltin hydride and azobisisobutyronitrile with high pressure Mercury arc lamp at room temperature for 1 h gave a debromo product **11** whose structure was supported by





11. R^1= R^2= R^4= OAc R^3= H
12. R^1= R^2= R^3= OAc
13. R^1= R^2= OAc, R^3= Br, R^4= OH
14. R^1= R^2= OAc, R^3= OH
16. R^1= R^4= OH, R^2= OAc, R^3= Br
15. R^1= R^3= OAc, R^2= OH
17. R^1= OAc, R^2= R^4= OH, R^3= Br
18. R^1= OAc, R^2= R^3= OH
19. R^1= R^2= R^4= OH, R^3= Br
21. R^1= R^2= R^3= OH
20. R^1= R^2= OAc, R^3= R^4= OH

the spectral evidence [δ=4.06 (1H, ddd, J=7.0, 7.5, 10.8 Hz), 4.14 (1H, ddd, J=6.7, 8.2, 10.8 Hz), $-CH_2CH_2-OCOCH_3$].

Tetraacetate **8** was saponified with aqueous 10% Na_2CO_3 at room temperature to yield a mixture of hydrolysis products including epoxides. With a combination of silica-gel chromatography and HPLC using reverse-phase support, the mixture was separated into individual pure product **12**, **13**, **14**, **15**, **16**, **17**, **18**, and **19**. The structures of these compounds were established with comparison of the ^1H NMR spectrum of each other.

Preparation of C-15 hydroxy compound **20** was performed by the acid-catalyzed epoxide cleavage of the prepared epoxide **12**. The highest mass ion peak in the high resolution mass spectrum of **20** appeared at m/z 418.2382, consistent with a formula of $C_{24}H_{34}O_6$ (M^+—AcOH), thus supporting its structure.

Simultaneous epoxide formation with the saponification condition of **8** suggested the stereochemistry of the epoxides to be 15 *S*. Similarly, the configuration at C-15 of the compound **20** was presumed to be 15 *R* from the viewpoint of the employed reaction condition (CF_3COOH in benzene).

The results of cytotoxicity evaluation of compounds **1**—**20** against P388 and HeLa cell (*in vitro*) are given in Table 3. These data imply that an acetoxyl group at C-2 and a bromine at C-15 are indispensable and acetyl groups except for that at C-2 are not always essential for the appearance of cytotoxic activity.

Experimental

Melting points are uncorrected. The purity of each compound was always checked by TLC (silica gel) or HPLC (JASCO, Finepak SIL C18). The IR spectra were recorded on a JASCO A-102 and A-700 spectrophotometer. The ^1H and ^{13}C NMR spectra were measured on a JEOL JNM-GX 270 spectrometer, using tetramethylsilane or solvent peak as an internal reference. The low and high resolution mass spectra were obtained on JEOL JMS-D300, JMS-DX303, and JMS-HX110 spectrometer. Optical rotations were determined on a JASCO DIP-140 polarimeter. Silica gel (Merck, Kieselgel 60, 70—230 mesh) and polystyrene gel (Nippon Rensui, Diaion HP-20) were used for column chromatography and silica gel (Merck, Kieselgel 60 F_{254}) for thin-layer chromatography, respectively. HPLC using a UV detector (215 nm) was carried out on JASCO, Finepak SIL C18, and Megapak SIL C18 columns. Medium pressure column chromatography was performed using Wako RQ-2 column.

Initial Isolation Procedure. Specimens of the red alga *Laurencia obtusa* (Hudson) Lamouroux were collected in shallow waters (0 to −1.5 m) in August of 1985, off the coast of Teuri Island, Hokkaido.[7] Half dried alga (ca. 3 kg) was extracted with methanol (30 L) and the methanol solution was concentrated to 5 L in vacuo. The resulting aqueous suspension was partitioned against diethyl ether. The ether solution was washed with 0.5 M KOH to remove acidic material and dried over Na_2SO_4. Removal of the solvent gave a viscous neutral oil (73 g). The crude extracts exhibited potential cell growth inhibitory activity (ED_{50} 0.18 μg mL^{-1} for P388 *in vitro* cell line). On the other hand, the aqueous layer was passed over polystyrene gel and to remove ionic material, the polystyrene gel was washed with water and successively with methanol to obtain polar organic substance. Evaporation of the methanol left dark brown residue (19 g). The neutral portion was fractionated by column chromatography on silica gel with a step gradient (benzene–ethyl acetate) to give five fractions of A [C_6H_6, 14.5 g], B [C_6H_6–EtOAc (9:1), 17.3 g], C [C_6H_6–EtOAc (1:1), 21.8 g], D [EtOAc, 12.3 g], and E [EtOAc–MeOH (9:1), 4.3 g]. The triol **5** was isolated from the fraction E in 2% yield. Further separation of the fraction B by silica-gel chromatography gave the known tetraacetate **8** as the major component (10% of the neutral oil). The fraction C was chromatographed on ODS column (Wako, RQ-2) in a total of 4 runs. For each run, 15% and 5% water, 5% dichloromethane in methanol, and dichloromethane were used as eluent, in that order. The dichloromethane fraction was further separated on Finepak SIL C18 HPLC column with 15% water in methanol to give the known compound **6** (0.01% yield), and the fraction eluted

Table 3. Cytotoxic Activities (IC_{50}, μg mL^{-1}) of Compound **1**—**20** against P388 and HeLa Cell (*in vitro*)

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
HeLa	3.1	—[a]	—	—	—	25	0.3	6.3	—	6.3
P388	3.5	—	—	—	—	25	1.1	8.5	—	9.9
Compounds	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
HeLa	—	—	3.1	—	—	7.6	6.3	—	1.0	—
P388	—	—	2.3	—	—	3.1	7.3	—	1.8	—

a) Inactive.

with 5% water in methanol was further separated by HPLC (Finepak SIL C18, 40% water in methanol) to yield **7** (0.3%) and **10** (2%), respectively. As the known compound, further, the tetrol **9** was isolated (0.25%) from the aqueous residue with careful HPLC separation (Finepak SIL C18, 50% water in methanol). The structures of compounds **7**, **8**, and **10** were confirmed by the respective comparison of the spectral data with those of the authentic specimens. The ^{1}H NMR data of the tetrol **9** were different in part from those reported by F. J. Schmitz et al.[4] Most apparent differences were the chemical shifts of signals due to C-19 hydroxymethyl protons. In the ^{1}H NMR spectrum of **9** in $CDCl_3$ solution, these signals were observed at δ=2.98 and 3.89 (1H each, d, J=12 Hz), both of which were shifted to ca. 3.4 (2H) in more polar CD_3OD solution by a much greater value than other signals, indicating that the signals due to the C-19 hydroxymethyl group might be largely influenced with the employed solvent. Our confirmation of the structure **9** was established by the transformation of the tetrol **9** to the tetraacetate **8** under usual acetylation condition.

^{1}H NMR Data of 9: ($CDCl_3$, 270 MHz), δ=0.01 (1H, dd, J=5.0, 5.5 Hz), 0.77 (1H, dd, J=5.0, 10.0 Hz), 1.03, 1.23 (3H each, s), 2.98 (1H, d, J=12.0 Hz), 3.13 (1H, dd, J=4.4, 10.3, 10.3 Hz), 3.85 (1H, dd, J=9.5, 13.0 Hz), 3.89 (1H, d, J=12 Hz), 4.00 (1H, dd, J=2.5, 13.0 Hz), 4.25 (1H, dd, J=2.5, 9.5 Hz), 4.43 (1H, br d, J=4.0 Hz), and 5.33 (1H, br d, J=6.2 Hz); (CD_3OD, 270 MHz), δ=−0.01 (1H, dd, J=4.5, 6.0 Hz), 0.83 (1H, dd, J=4.5, 10.0 Hz), 1.03, 1.18 (3H each, s), 3.06 (1H, ddd, J=4.5, 10.0, 10.0 Hz), ca. 3.4 (2H), 3.77 (1H, dd, J=9.5, 12.5 Hz), 4.01 (1H, dd, J=2.5, 12.5 Hz), 4.23 (1H, dd, J=2.5, 9.5 Hz), 4.31 (1H, br d, J=4.5 Hz), and 5.39 (1H, br d, J=6.0 Hz).

Isolation of 1. Repeated purification with HPLC (Finepak SIL C18, 35% water in methanol) of the same fraction that yielded **6**, **7**, and **10** gave compound **1** in 0.3% yield: viscous oil; $[\alpha]_D$ −37.8° (c 1.54, $CHCl_3$); IR ν_{max} ($CHCl_3$), 3500, 1720, 1450, 1360, 1240, 1145, 1020, 970, and 945 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz), δ=0.16 (1H, dd, J=5.0, 6.0 Hz), 0.93 (1H, dd, J=5.0, 10.5 Hz), 1.08, 1.14, 2.07×2, 2.11 (3H each, s), 3.18 (1H, m), 3.78 (1H, d, J=12.0 Hz), 4.04 (1H, d, J=12.0 Hz), 4.26 (1H, d, J=9.0 Hz), 4.31 (1H, dd, J=9.0, 9.0 Hz), 4.56 (1H, d, J=9.0 Hz), and 5.35 (2H); ^{13}C NMR ($CDCl_3$, 67.8 MHz), CH_3: δ=19.8, 21.0, 21.2, 21.6, and 24.3, CH_2: δ=18.8, 33.5, 37.4, 38.0, 38.8, 66.1, and 69.8, CH: δ=22.1, 38.3, 45.8, 59.5, 68.3, 76.9, and 117.5, C: δ=20.8, 35.4, 36.5, 143.1, 170.6, 170.7, and 170.9; MS, m/z 524, 522, (0.1:0.1, M^+-H_2O), 497, 495, (0.1:0.1), 464, 462 (1.4:1.4), 454, 452 (0.1:0.1), 436, 434 (1.7:1.7), 404, 402 (4.3:4.2), 263 (20), 237 (19), 119 (15), 105 (16), and 43 (100); HR-MS, Found: m/z 524.1622. Calcd for $C_{26}H_{35}O_6{}^{81}Br$: (M^+-H_2O), 524.1597.

Acetylation of 1. Triacetate **1** (5 mg) was allowed to react with acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature for 16 h. To the reaction mixture was added a small amount of methanol to decompose an excess of acetic anhydride and the mixture was extracted into ether. The ether solution was washed with aqueous 10% $CuSO_4$, aqueous 5% $NaHCO_3$, and water. Dryness and evaporation gave a crude tetraacetate. Purification on chromatography over silica gel with 10% ethyl acetate in benzene yielded a pure product (5 mg). The spectral data of the tetraacetate were identical with those of compound **8**.

Isolation of 2 and 3. The aqueous material was separated by HPLC on ODS column using 50% water in methanol to yield **2** (0.1% yield) and **3** (0.1%) respectively, in addition to the known **9**. **2**: a glassy solid; $[\alpha]_D$ +5.0° (c 0.46, CH_3OH); IR ν_{max} (film), 3356, 1452, 1254, 1160, 1054, 1016, 951, and 754 cm^{-1}; ^{1}H NMR (CD_3OD, 270 MHz), δ=1.05, 1.27 (3H each, s), 3.14 (1H, ddd, J=7.0, 9.5, 9.5 Hz), 3.77 (1H, dd, J=9.5, 12.5 Hz), 3.88 (1H, br d, J=ca. 4 Hz), 4.02 (1H, dd, J=3.0, 12.5 Hz), 4.25 (1H, dd, J=3.0, 9.5 Hz), and 5.46 (1H, br d, J=ca. 6 Hz); ^{13}C NMR (CD_3OD, 67.8 MHz), CH_3: δ=22.5 and 24.9, CH_2: δ=16.9, 31.2, 35.4, 39.4, 40.3, 41.8, and 65.0, CH: δ=39.9, 46.9, 51.1, 68.1, 68.9, 78.1, and 117.2, C: δ=36.5, 37.6, 74.2, and 147.2; MS, m/z 401, 399 (13:14, M^+-CH_3), 383, 381 (22:24), 380, 378 (8.3:8.3), 365, 363 (24:27), 319, 317 (12:11), 299 (29), 281 (31), 271 (31), 255 (42), 237 (35), 227 (49), 199 (49), 185 (56), 159 (76), 157 (59), 145 (55), 143 (56), 131 (60), 107 (55), 105 (89), 91 (81), 55 (92), 43 (100), and 41 (96); HR-MS, Found: m/z 398.1287. Calcd for $C_{20}H_{29}O_3{}^{81}Br$: (M^+-H_2O), 398.1280. **3**: a glassy solid; $[\alpha]_D$ −41° (c 0.89, CH_3OH); IR ν_{max} (film), 3356, 1678, 1453, 1433, 1414, 1381, 1359, 1286, 1259, 1232, 1065, 1026, 941, 874, 800, 754, and 664 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; MS, m/z 396, 394 (2.1:2.2, M^+-H_2O), 378, 376 (1.6:1.4), 363, 361 (2.6:3.2), 315 (12), 270 (37), 209 (27), 183 (35), 157 (41), 145 (41), 119 (69), 118 (46), 107 (48), 105 (75), 91 (78), 82 (79), 55 (94), 43 (100), and 41 (98); HR-MS, Found: m/z 396.1119. Calcd for $C_{20}H_{27}O_3{}^{81}Br$: (M^+-H_2O), 396.1123.

Acetylation of 2. Tetrol **2** (10 mg) was acetylated in the same manner as described for **1**. Silica-gel chromatography with 10% ethyl acetate in benzene of the crude product yielded a pure triacetate (13 mg) which was identified as isoparguerol 7,16-diacetate with comparison of the spectral data.

Reduction of 3 with $NaBH_4$. To an ethanol solution (2 mL) of **3** (6 mg) was added $NaBH_4$ (5 mg), and the mixture was stirred at room temperature for 4 h under nitrogen atmosphere. Water was added to the mixture and the solution was concentrated under reduced pressure. The residue was dissolved in a small amount of methanol and the methanol solution was passed over ODS column, and then, the ODS column was washed with water to remove ionic material. Subsequently, methanol was made to flow through the column to obtain the products which showed two peaks with an intensity ratio of about 1:1 in HPLC analysis. Chromatography on Finepak SIL C18 column using 50% water in methanol gave two pure products. Spectral data of the more polar product (2.5 mg) was identical with those of the natural tetrol **9**. ^{1}H NMR data of the less polar product **21** was as follows: (CD_3OD, 270 MHz), δ=−0.01 (1H, dd, J=4.5, 6.0 Hz), 0.83 (1H, dd, J=4.5, 10.3 Hz), 0.94, 1.14 (3H each, s), 1.02 (1H, dd, J=6.0, 10.3 Hz), 2.54 (1H, dd, J=3.0, 5.0 Hz), 2.62 (1H, dd, J=4.5, 5.0 Hz), 2.76 (1H, dd, J=3.0, 4.5 Hz), 3.05 (1H, ddd, J=4.8, 10.5, 10.5 Hz), ca. 3.35 (2H), 4.29 (1H, br d, J=4.4 Hz), and 5.39 (1H, m).

Isolation of 4. A fraction rich in isoparguerol 7,16-diacetate (**10**) which was separated with chromatography on ODS column (RQ-2) of fraction C using 10% water in methanol, was repeatedly chromatographed with Finepak SIL C18 column (30% water in methanol) to give **4** as crystals: mp 87—90°C (CH_2Cl_2–diisopropyl ether); $[\alpha]_D$ −17.8° (c 1.71, $CHCl_3$); IR ν_{max} ($CHCl_3$), 3300, 1450, 1370, 1055, 1020, and 960 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR

($CHCl_3$, 67.8 MHz), CH_3: δ=17.9, 24.0, and 24.9, CH_2: δ=19.3, 21.4, 31.1, 35.1, 38.1, 39.1, and 64.4, CH: δ=19.2, 38.8, 46.4, 69.4, 77.4, and 117.1, C: δ=16.0, 35.3, 37.1, and 143.9; MS, *m/z* 366, 364 (1.5:1.5), 348, 346, (0.4:0.5), 333, 331 (0.6:0.7), 311, 309 (0.6:0.7), 266 (13), 251 (11), 105 (11), 71 (25), 57 (39), 55 (24), 44 (100), 43 (29), 41 (24), and 40 (40); HR-MS, Found: *m/z* 366.1391. Calcd for $C_{20}H_{29}O^{81}Br$: (M^+-H_2O), 366.1382.

Acetylation of 4. Sample of **4** (6 mg) was acetylated in the same manner as described for **1**. Workup in the usual manner and chromatographic purification on silica gel (10% ethyl acetate in benzene) afforded a pure acetate (7 mg): IR ν_{max} (CCl_4), 1740 and 1237 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz), δ=−0.02 (1H, dd, *J*=4.0, 6.0 Hz), 0.43 (1H, dd, *J*=4.0, 9.5 Hz), 0.65 (1H, ddd, *J*=6.0, 6.0, 9.5 Hz), 0.96, 1.00, 1.05, 2.09, 2.13 (3H each, s), 2.44 (1H, m), 2.56 (1H, m), 4.27 (1H, br d, *J*=8.5 Hz), 4.31 (1H, dd, *J*=8.5, 8.5 Hz), 4.42 (1H, ddd, *J*=4.5, 11.0, 11.0 Hz), 4.51 (1H, br d, *J*=8.5 Hz), and 5.40 (1H, br d, *J*=6.0 Hz).

Debromination of 8. Dry nitrogen gas was bubbled through a solution of **8** (50 mg) and azobisisobutyronitrile (2.5 mg) in dry benzene (5 mL) in Pyrex test tube for 15 min, and then tributyltin hydride (50 μL) was added. This stirred mixture was irradiated with high pressure Mercury arc lamp at room temperature for 1 h. The solvent was evaporated and the residue was dissolved in ether (5 mL). The ether solution was stirred with 2M-KF solution (1 mL) for 30 min, and successively washed with saturated salt solution. Dryness and evaporation of the solvent left a solid which was purified on silica-gel chromatography (10% ethyl acetate in benzene) to give a debromo compound **11** (35 mg): ^1H NMR ($CDCl_3$, 270 MHz), δ=0.17 (1H, dd, *J*=5.0, 5.5 Hz), 0.94, 1.15, 2.05, 2.06, 2.07, 2.09 (3H each, s), 2.28 (1H, ddd, *J*=4.0, 4.0, 12.0 Hz), 2.53 (1H, m), 3.70 (1H, d, *J*=12.0 Hz), 4.06 (1H, d, *J*=12.0 Hz), 4.06 (1H, ddd, *J*=7.0, 7.5, 10.8 Hz), 4.14 (1H, ddd, *J*=6.7, 8.2, 10.8 Hz), 4.41 (1H, ddd, *J*=4.5, 11.0, 11.0 Hz), 5.35 (1H, d, *J*=4.8 Hz), and 5.40 (1H, m); HR-MS, Found: *m/z* 444.2528. Calcd for $C_{26}H_{36}O_6$: (M^+−AcOH), 444.2512.

Epoxide Formation and Saponification of 8. A solution of parguerol 7,16,19-triacetate (**8**) (300 mg) in methanol containing aqueous 10% Na_2CO_3 was stirred at room temperature for 15 min under nitrogen atmosphere and then water was added to the solution. The mixture was extracted with $CHCl_3$ and the organic layer was washed with water and dried over Na_2SO_4. Evaporation of the solvent afforded a crude product, TLC analysis (silica gel, 50% ethyl acetate in benzene) of which indicated that the product consisted of two products, **12** and **13**. While, treatment of **8** (600 mg) for 4 h with the same reagent as described above afforded chiefly a mixture of saponification products, **14—19**. Separation of the combined products with a combination of silica-gel chromatography (ethyl acetate–benzene) and HPLC using reverse-phase material (JASCO, Finepak SIL C18, 40% water in methanol) yielded **12** (131 mg), **13** (183 mg), **14** (58 mg), **15** (47 mg), **16** (45 mg), **17** (90 mg), **18** (63 mg), and **19** (57 mg), respectively.

Compound 12: ^1H NMR ($CDCl_3$, 270 MHz), δ=0.17 (1H, dd, *J*=5.0, 5.5 Hz), 0.91, 1.12, 2.06, 2.07, 2.11 (3H each, s), 2.28 (1H, m), 2.52 (1H, dd, *J*=2.9, 4.8 Hz), ca. 2.60 (1H, m), 2.62 (1H, dd, *J*= 4.0, 4.8 Hz), 2.75 (1H, dd, *J*=2.9, 4.0 Hz), 3.69 (1H, d, *J*=12.0 Hz), 4.07 (1H, d, *J*=12.0 Hz), 4.42 (1H, ddd, *J*=4.8, 11.0, 11.0 Hz), 5.35 (1H, d, *J*=4.0 Hz), and 5.41 (1H, m); HR-MS, Found: *m/z* 400.2246. Calcd for $C_{24}H_{32}O_5$: (M^+−AcOH), 400.2250.

Compound 13: ^1H NMR ($CDCl_3$, 270 MHz), δ=0.16 (1H, dd, *J*=5.0, 5.5 Hz), 0.92 (1H, dd, *J*=5.0, 10.5 Hz), 1.04, 1.15, 2.07×2, 2.09 (3H each, s), 2.29 (1H, m), ca. 2.45 (2H), 3.65 (1H, d, *J*=12.0 Hz), ca. 3.85 (2H), 4.09 (1H, d, *J*=12.0 Hz), 4.26 (1H, dd, *J*=3.5, 8.5 Hz), 4.40 (1H, ddd, *J*=4.8, 11.0, 11.0 Hz), 5.36 (1H, d, *J*=4.5 Hz), and 5.42 (1H, br d, *J*=6.2 Hz); HR-MS, Found: *m/z* 480.1483. Calcd for $C_{24}H_{33}O_5{}^{79}Br$: (M^+−AcOH), 480.1512.

Compound 14: ^1H NMR ($CDCl_3$, 270 MHz), δ=0.16 (1H, dd, *J*=5.0, 5.5 Hz), 0.97, 1.10, 2.07×2 (3H each, s), ca. 2.22 (2H), 2.40 (1H,m), 2.51 (1H, dd, *J*=2.9, 4.8 Hz), 2.61 (1H, dd, *J*=4.0, 4.8 Hz), 2.75 (1H, dd, *J*=2.9, 4.0 Hz), 3.19 (1H, ddd, *J*=5.0, 11.0, 11.0 Hz), 3.75 (1H, d, *J*=12.0 Hz), 4.07 (1H, d, *J*=12.0 Hz), and ca. 5.35 (2H); HR-MS, Found: *m/z* 400.2262. Calcd for $C_{24}H_{32}O_5$: (M^+-H_2O), 400.2250.

Compound 15: ^1H NMR ($CDCl_3$, 270 MHz), δ=0.10 (1H, dd, *J*=5.0, 5.5 Hz), 0.84 (1H, dd, *J*=5.0, 10.5 Hz), 0.91, 1.11, 2.07, 2.10 (3H each, s), 2.43 (1H, m), 2.52 (1H, dd, *J*=2.9, 4.8 Hz), ca. 2.60 (1H, m), 2.62 (1H, dd, *J*=4.0, 4.8 Hz), 2.75 (1H, dd, *J*=2.9, 4.0 Hz), 3.24 (1H, br d, *J*=12.0 Hz), 3.62 (1H, br d, *J*=12.0 Hz), 4.43 (1H, ddd, *J*=4.8, 11.0, 11.0 Hz), 5.36 (1H, d, *J*=4.5 Hz), and 5.40 (1H, m); HR-MS, Found: *m/z* 358.2115. Calcd for $C_{22}H_{30}O_4$: (M^+−AcOH), 358.2144.

Compound 16: ^1H NMR ($CDCl_3$, 270 MHz), δ=0.10 (1H, dd, *J*=5.0, 5.5 Hz), 0.84 (1H, dd, *J*=5.0, 10.5 Hz), 1.04, 1.15, 2.07, 2.08 (3H each, s), ca. 2.45 (3H), 3.26 (1H, br d, *J*=12.0 Hz), 3.60 (1H, br d, *J*=12.0 Hz), ca. 3.85 (2H), 4.26 (1H, dd, *J*=3.5, 8.5 Hz), 4.42 (1H, ddd, *J*=4.8, 11.0, 11.0 Hz), 5.37 (1H, d, *J*=4.5 Hz), and 5.41 (1H, br d, *J*=4.5 Hz); HR-MS, Found: *m/z* 438.1396. Calcd for $C_{22}H_{31}O_4{}^{79}Br$: (M^+−AcOH), 438.1405.

Compound 17: ^1H NMR ($CDCl_3$, 270 MHz), δ=0.16 (1H, dd, *J*=5.0, 5.5 Hz), 0.92 (1H, dd, *J*=5.0, 10.5 Hz), 1.07, 1.14, 2.07×2 (3H each, s), ca. 2.25 (3H), 2.42 (1H, m), 3.18 (1H, m), 3.71 (1H, d, *J*=12.0 Hz), ca. 3.85 (2H), 4.09 (1H, d, *J*=12.0 Hz), 4.28 (1H, dd, *J*=3.5, 9.0 Hz), and 5.35 (2H); HR-MS, Found: *m/z* 482.1518. Calcd for $C_{24}H_{33}O_5{}^{81}Br$: (M^+-H_2O), 482.1419.

Compound 18: ^1H NMR ($CDCl_3$, 270 MHz), δ=0.09 (1H, dd, *J*=5.0, 5.5 Hz), 0.87 (1H, dd, *J*=5.0, 10.5 Hz), 0.96, 1.10, 2.07 (3H each, s), 2.21 (1H, m), ca. 2.40 (2H), 2.51 (1H, dd, *J*=2.9, 4.8 Hz), 2.61 (1H, dd, *J*=4.0, 4.8 Hz), 2.75 (1H, dd, *J*=2.9, 4.0 Hz), 3.19 (1H, ddd, *J*=4.5, 10.5, 10.5 Hz), 3.38 (1H, d, *J*=12.0 Hz), 3.57 (1H, d, *J*=12.0 Hz), and ca. 5.35 (2H); HR-MS, Found: *m/z* 358.2131. Calcd for $C_{22}H_{30}O_4$: (M^+−AcOH), 358.2131.

Compound 19: ^1H NMR ($CDCl_3$, 270 Hz), δ=0.09 (1H, dd, *J*=5.0, 5.5 Hz), 0.85 (1H, dd, *J*=5.0, 10.5 Hz), 1.07, 1.14, 2.07 (3H each, s), ca. 2.25 (2H), ca. 2.40 (2H), 3.18 (1H, ddd, *J*=4.5, 10.0, 10.0 Hz), 3.38 (1H, d, *J*=12.0 Hz), 3.55 (1H, d, *J*=12.0 Hz), ca. 3.90 (2H), 4.28 (1H, dd, *J*=3.5, 9.0 Hz), and ca. 5.35 (2H); HR-MS, Found: *m/z* 438.1381. Calcd for $C_{22}H_{31}O_4{}^{79}Br$: (M^+-H_2O), 438.1405.

Preparation of C-15 Hydroxy Compound 20. To an ice cooled trifluoroacetic acid (2 mL) was gradually added the prepared epoxide **12** (85 mg) in benzene (1 mL) and the mixture was stirred at 5 °C overnight. The reaction mixture was extracted with ether and ether solution was washed with water, aqueous 10% Na_2CO_3, and saturated salt solution. After removal of the solvent, the ester obtained was treated

with a mixture of methanol–H_2O containing a trace of aqueous 10% Na_2CO_3 to give a hydrolysis product **20** (31 mg): ^1H NMR ($CDCl_3$, 270 MHz), δ=0.17 (1H, dd, J=5.0, 5.5 Hz), 0.85, 1.14, 2.06×2, 2.11 (3H each, s), 2.61 (1H, m), ca. 3.55 (2H), 3.68 (1H, d, J=12.0 Hz), ca. 3.70 (1H, m), 4.07 (1H, d, J=12.0 Hz), 4.43 (1H, ddd, J=4.5, 10.0, 10.0 Hz), 5.35 (1H, d, J=4.8 Hz), and ca. 5.38 (1H, m); HR-MS, Found: m/z 418.2382. Calcd for $C_{24}H_{34}O_6$: (M^+—AcOH), 418.2356.

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