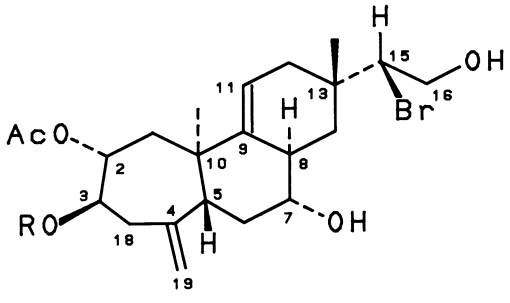
CHEMISTRY LETTERS, pp. 277-280, 1990.

A New Cytotoxic Diterpene from the Marine Red Alga Laurencia obtusa (Hudson) Lamouroux[1)]

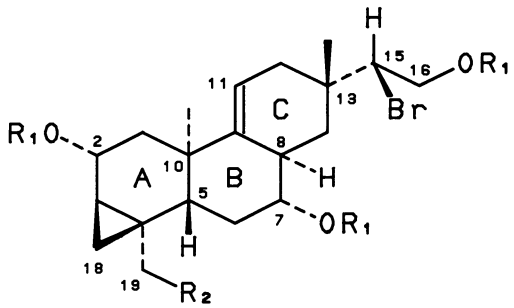
Satoshi TAKEDA, Takeshi MATSUMOTO,† Kanki KOMIYAMA,††
Etsuro KUROSAWA, and Teruaki SUZUKI*
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060
†Department of Bioscience and Technology, School of Engineering,
Hokkaido Tokai University, Sapporo 005
††The Kitazato Institute, 9-1, 5-chome, Shirogane, Minato-ku, Tokyo 108

A new cytotoxic diterpene has been isolated from the title alga. This compound possesses a new, A-seco-parguerane skeleton and a hexadecanoic acid ester group. The structure was established by spectroscopic means, including two-dimensional NMR methods.

We have recently reported[2)] that the cytotoxic diterpene, parguerol triacetate (2),[3)] was isolated as the major metabolite along with an inactive triol (3), from the marine red alga Laurencia obtusa (Hudson) Lamouroux collected at Teuri Island, Hokkaido, and the absolute configuration of the triol was established as 3 on the basis of X-ray crystallographic study.



1 R=CO(CH$_2$)$_{14}$CH$_3$



2 R$_1$=Ac, R$_2$=OAc
3 R$_1$=H, R$_2$=H

The isolation of both the active and inactive metabolites prompted us to investigate remaining neutral fractions of this alga and several new cytotoxic or inactive diterpenes were isolated. One of the active metabolites proved to be a compound with new framework (0.01% of yield). We wish to describe here the structural determination of the cytotoxic compound 1 with the new carbocyclic skeleton.[4)]

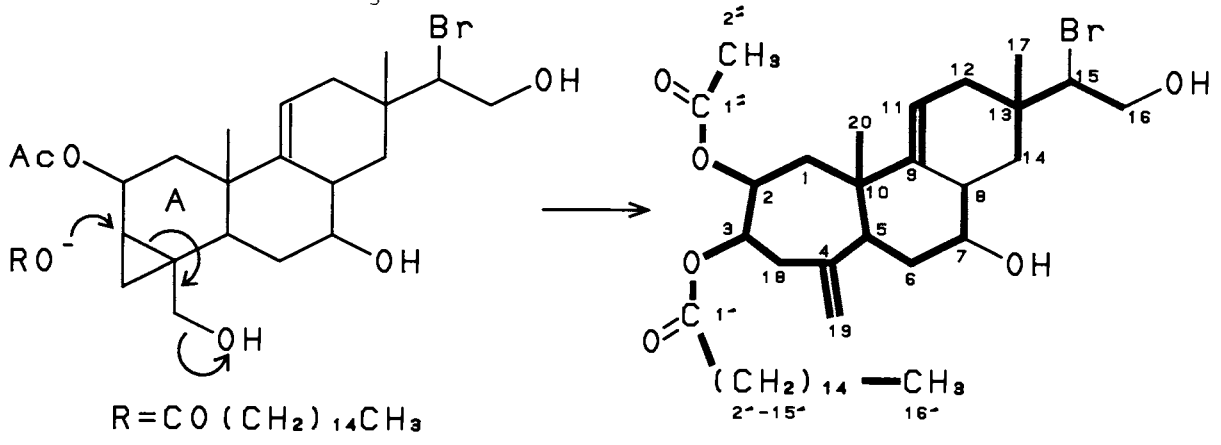
2-Acetoxy-15-bromo-7,16-dihydroxy-3-palmitoxy-neoparguera-4(19),9(11)-diene (1), viscous oil, $[\alpha]_D$ -27.2° (c 2.40, $CHCl_3$), had a molecular formula of $C_{38}H_{63}O_6Br$ [HR-MS: obsd 678.3642, calcd for $C_{38}H_{61}O_5{}^{81}Br$ (M^+-H_2O) 678.3682]. The IR spectrum of 1 showed the presence of hydroxyl and carbonyl groups (3400, 1738 cm^{-1}). The ^1H and ^{13}C NMR data of 1 (see Table 1) confirmed the presence of one acetate, two tertiary methyl groups, one trisubstituted double bond, and one exo-methylene group. The presence of a palmitoxyl group was shown by a fragment ion peak at m/z 239 (relative intensity; 27.6), consistent with a formula of $C_{16}H_{31}O$ (HR-MS: obsd 239.2386, calcd 239.2375) in the mass spectrum and by signals at δ 14.1, 22.7, 31.9, 29.1-29.7, 25.0, and 34.5 due to the acyloxyl group with straight carbon framework in the ^{13}C NMR spectrum.[5)] These functional groups account for four of seven degrees of unsaturation implied by the molecular formula and establish that 1 must have three rings. Compound 1 exhibited NMR signals nearly identical with those corresponding to protons at C-7, C-11, C-15, and C-16 of 3 (see Table 1 and reference 2), suggesting that the same B-C ring moiety including its substituents as that of triol 3 is present in 1.

The most obvious differences in the spectra of 1 and 3 are the absence of cyclopropane signals and the presence of signals due to an exo-methylene group and palmitoxyl moiety in the spectra of 1. Logical and biogenetically reasonable explanation for the above results would be formation of 1 from parguerol (4)[3)] through cleavage of the cyclopropane ring, as proposed in Fig. 1. The ^1H-detected heteronuclear multiple-bond ^1H-^{13}C correlation (HMBC)[6)] spectrum coupled with ^1H-^1H and ^{13}C-^1H COSY spectra allowed complete assignment of all proton- and carbon-resonances (Table 1), and confirmed the carbon-carbon and carbon-oxygen connectivities shown by bold lines in Fig. 1.

Table 1. ^{13}C and ^{1}H NMR chemical shifts (ppm) of compound 1 and C/H correlations observed in HMBC experiments

C[a]	δC[b]	δH (multiplicity, J/Hz)[c]	HMBC[d]
1	40.4	1.57 (dd, J=10.5,15.5) Hα	H-2,20
		2.07 (d, J=15.5) Hβ	
2	70.1	5.27 (dd, J=10.5,10.5)	H-1α,1β,3,18α,18β
3	76.1	4.84 (ddd, J=7.0,10,5,10,5)	H-1α,1β,2,18α,18β
4	143.4		H-5,18α,18β,19a,19b
5	46.4	1.98 (dd, J=2.5,10.5)	H-1β,6eq,6ax,18α,18β,19a,19b
6	35.2	1.80 (ddd, J=2.5,6.5,12.5) Heq	
		1.88 (ddd, J=10.5,10.5,12.5) Hax	
7	76.8	3.29 (ddd, J=6.5,9.5,10.5)	H-6eq,6ax
8	38.6	ca.2.26 (m)	H-6eq,6ax,7
9	140.3		H-1α,1β,5,12eq,12ax
10	40.4		H-1α,1β,2,6eq,6ax,11,20
11	117.2	5.46 (br d, J=6.0)	H-12eq,12ax
12	38.8	2.46 (m) Heq	H-11,17
		1.93 (ddd, J=2.5,3.5,17.5) Hax	
13	35.4		H-11,15,17
14	38.6	2.29 (ddd, J=3.0,6.0,12.5) Heq	H-12eq,15,17
		1.43 (dd, J=10.5,12.5) Hax	
15	69.1	4.27 (dd, J=3.0,9.0)	H-12ax,14ax,16b,17
16	64.3	3.92 (dd, J=3.0,12.5) Ha	H-15
		3.84 (dd, J=9.0,12.5) Hb	
17	24.8	1.09 (3H, s)	H-15
18	42.7	2.78 (dd, J=7.0,13.0) Hα	H-19a,19b
		2.11 (dd, J=10.5,13.0) Hβ	
19	114.5	5.11 (br s) Ha	H-18α,18β
		4.80 (br s) Hb	
20	21.7	0.97 (3H, s)	H-1α
1'	172.9		H-3,2',3'
2'	34.5	2.23 (2H, hex)	
3'	25.0	ca.1.55 (2H, m)	H-2'
4'	29.1		H-2',3'
∿13'	∿29.7	ca.1.20∿	
14'	31.9	1.35 (24H)	H-16'
15'	22.7		H-16'
16'	14.1	0.68 (3H, t, J=6.5)	
1"	169.7		H-2,2"
2"	21.1	2.30 (3H, s)	

a) The numbering system corresponds to that used for parguerane diterpenes.[3] b) Measured at 100 MHz ($CDCl_3$, TMS=0). c) Measured at 400 MHz ($CDCl_3$, TMS=0). d) Measured at 270 MHz ($CDCl_3$, TMS=0).



4 1

Fig. 1. Biogenesis and connectivities of carbon-carbon and carbon-oxygen bonds in 1 by HMBC.

The relative stereochemistry of 1 at C-2, C-3 and C-5 positions was determined by the extensive NOE difference spectrum experiments, and the results are depicted in Fig. 2. The NOEs and coupling constants (Table 1) are in good accord with the conformation (Fig. 2).

The absolute configurations at chiral centers of 1 are assumed to be the same as those of 3, since 1 is considered to be biogenetically related to 3 (Fig. 1).

Compound 1 was cytotoxic against B16 cell in vitro with the IC_{50} values of 0.78 μg/ml.

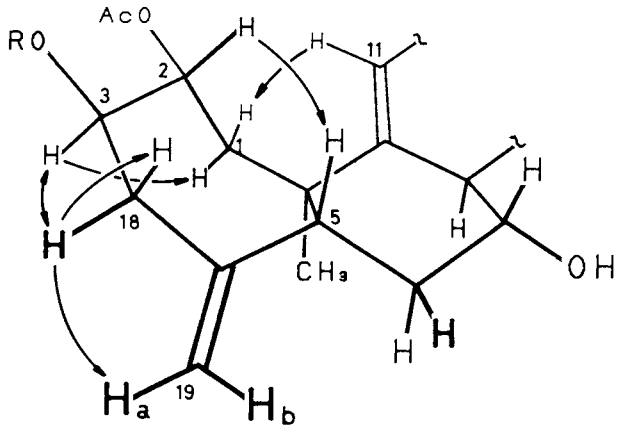


Fig. 2. NOEs (◀—— and ——▶) and conformation of A ring in 1.

References

1) Part 76 of "Constituents of marine Plants." Part 75; S. Takeda, Y. Iimura, K. Tanaka, E. Kurosawa, and T. Suzuki, Chem. Lett., 1990, 155.

2) T. Suzuki, S. Takeda, N. Hayama, I. Tanaka, and K. Komiyama, Chem. Lett., 1989, 969.

3) F. J. Schmitz, D. P. Michaud, and P. G. Schmidt, J. Am. Chem. Soc., 104, 6415 (1982).

4) We propose the name neoparguerane for the new parent hydrocarbon corresponding to 1.

5) E. Pretsch, T. Clerc, J. Seible, and W. Simon, "Tabellen zur Strukturaufklärung organischer Verbindungen," Springer, Berlin (1981), p.C35.

6) A. Bax and M. F. Summers, J. Am. Chem. Soc., 108, 2093 (1986); A. Bax, A. Aszalos, Z. Dinya, and K. Sudo, ibid., 108, 8056 (1986).

(Received November 8, 1989)