

Structures and Absolute Configurations of New Lobane Diterpenoids
from the Okinawan Soft Coral *Sinularia flexibilis*

Toshiyuki HAMADA, Takenori KUSUMI, Midori O. ISHITSUKA, and Hiroshi KAKISAWA*

Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki 305

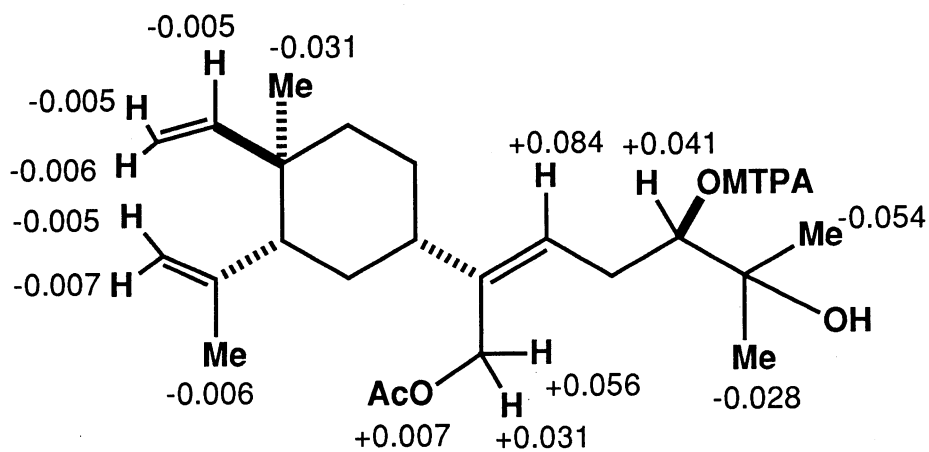
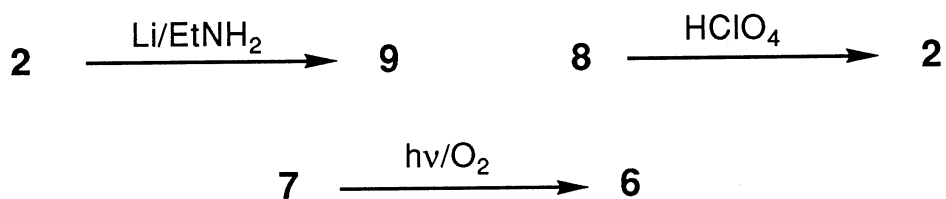
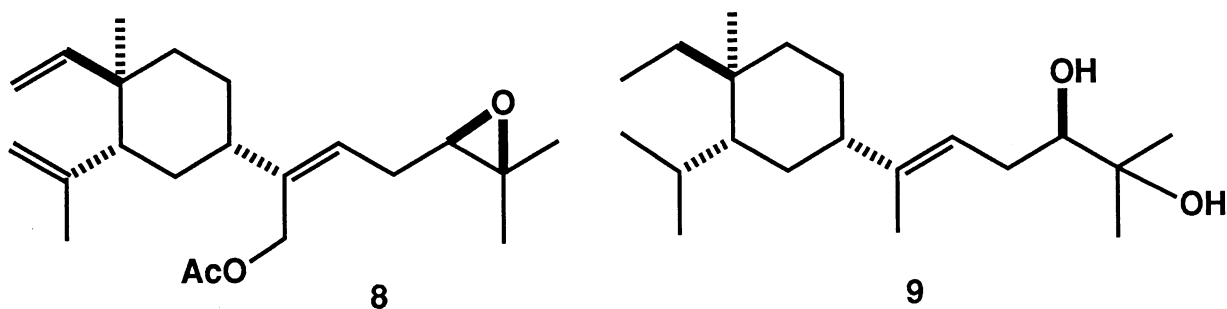
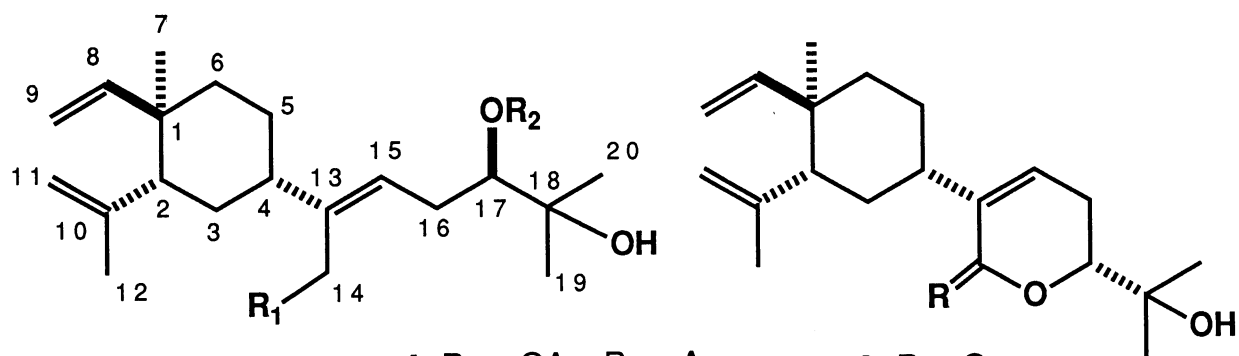
Three new diterpenoids possessing lobane skeleton have been isolated from the Okinawan soft coral of *Sinularia flexibilis*. Their structures and absolute configurations have been established by chemical transformations as well as spectroscopic analysis.

A growing number of recent investigations on marine natural products have revealed that marine organisms are rich in biologically active compounds, many of which have unique structures never found in terrestrial organisms.¹⁾ In the course of our works on physiologically significant substances from marine resources, we isolated new diterpenoids possessing lobane skeleton from the Okinawan soft coral *Sinularia flexibilis*. This report deals with the structures of the diterpenoids. Chemical correlation of these compounds, which confirmed the absolute configurations of them, are also reported.

Chromatographic separation of the acetone extract of the soft coral of *Sinularia flexibilis* collected at Okinawa Island, afforded three new diterpenes, **1**, **6**, and **8**, together with lobatriene (**7**), the absolute configuration of which has been recently elucidated.²⁾

Lobatrientriol (**1**),³⁾ C₂₀H₃₄O₃, a colorless oil, [α]_D²⁵ +15.0° (c 0.20, CHCl₃), exhibited the IR absorption at 3375 cm⁻¹ due to hydroxyl group. The ¹H and ¹³C NMR spectra showed that three olefin groups are present; vinyl [δ_C 150.2 (d), 110.0 (t); δ_H 5.81 (dd, *J* = 11, 17 Hz), 4.90 (d, *J* = 17 Hz), 4.88 (d, *J* = 11 Hz)], exomethylene [δ_C 147.6 (s), 112.2 (t); δ_H 4.81 (brs), 4.58 (brs)], and trisubstituted olefin [δ_C 147.1 (s), 124.3 (d); δ_H 5.46 (dd, *J* = 6.4, 9.8 Hz)]. This fact in combination with the molecular formula suggested the occurrence of one ring. The presence of the vinyl and exomethylene groups together with a tertiary methyl [δ_H 1.00 (s)] and an olefinic methyl [δ_H 1.69 (s)] which is long-range coupled with the exomethylene protons is reminiscent of 3-isopropenyl-4-methyl-4-vinylcyclohexan-1-yl group that is included in lobatriene (**7**), and eventually the presence of the moiety was confirmed by the H,H and H,C-COSY spectra. There are the signals of three oxygen-bearing carbons [δ_C 76.6 (d), 73.1 (s), 59.2 (t)] in the ¹³C NMR spectrum. The triplet carbon was correlated (H,C COSY) with the methylene protons appearing at δ 4.27 (1H, d, *J* = 11 Hz) and 3.94 (1H, d, *J* = 11 Hz), and thus the methylene group must be adjacent to the trisubstituted olefin. Also the proton network =CH-CH₂-CH(OH)- was suggested by the H,H COSY spectrum. The occurrence of (CH₃)₂C(OH)- group was inferred by the ¹H signals at δ 1.23 (3H, s) and 1.19 (3H, s) and the ¹³C signal at δ 73.1 (s), 26.6 (q), 23.6 (q). These data suggested structure **1** for lobatrientriol.

The relative stereochemistry of substituents on the cyclohexane was deduced from the coupling patterns of H-2 [δ 2.01 (dd, *J* = 4.2, 14 Hz)] and H-4 [δ 2.09 (brt, *J* = 11 Hz)] and the NOEs; H-5 α $\leftrightarrow$ Me-7, H-



3 α →Me-7. The relative configuration of H-17 was established by the chemical correlation (*vide infra*).

From the less polar fraction, the monoacetate (**2**), C₂₂H₃₆O₄, was isolated. Its NMR characters extremely resemble those of **1** except that an acetyl group [δ_{H} 2.05 (s)] is incorporated in **2** and H₂-14 of **1** (δ_{H} 4.27, 3.94) shifts downfield (δ_{H} 4.74, 4.53) in **2**. Therefore, **2** was supposed to be the acetate of **1**, the 14-OH of which is acetylated. The physical properties of **2** are coincident with those of loba-8,10,13(15)-triene-14,17,18-triol 14-acetate, which has been isolated by Australian workers from *Lobaphytum* species.⁴⁾ The stereochemistry at C-17, however, has not been assigned. Therefore, modified Mosher's method⁵⁾ was applied using (*R*) and (*S*)-MTPA esters (**3**), which were prepared by treating **2** with (+) and (-)-MTPA chlorides in pyridine. The results (shown in **3a**) led to the assignment of *R*-configuration at C-17. To confirm this, **2** was reduced with lithium in ethylamine, producing a product. The chromatographic behaviors, ¹H NMR, ¹³C NMR spectrum, and $[\alpha]_{\text{D}}$ of this product are the same as those of **9**,⁶⁾ which was also obtained by the similar reduction of lobatriene (**7**). These results established the stereochemistry as well as the absolute configuration of **2** as shown in its structure.

The monoacetate **2** was saponified (KOH/MeOH) to give the triol **1**, which proved to be identical (TLC, NMR, $[\alpha]_{\text{D}}$) with lobatrientriol. Furthermore, acetylation of **2** gave the diacetate **4** that was also obtained by acetylation of **1**. These experiments consequently established the absolute configuration of lobatrientriol (**1**). The absolute stereochemical features of **1** and **2** thus determined are corresponding well to that of **5**,⁴⁾ which was found in the present soft coral.

Acetoxylobaoxide (**8**),⁷⁾ C₂₂H₃₄O₃, a colorless oil, $[\alpha]_{\text{D}}^{25}$ -3.3° (c 0.06, CHCl₃) seemed to be closely related with **2** as judged from its NMR characters. The presence of a trisubstituted oxirane was easily recognized from the ¹H signal at δ 2.74 (1H, t, *J* = 6.0 Hz) and ¹³C signal at δ 63.7 (d) and 58.6 (s). The epoxide group was cleaved by treatment of **8** with perchloric acid (50% in H₂O). The product was identified (TLC, NMR, $[\alpha]_{\text{D}}$) with **2**, and, therefore, the structure (**8**) of acetoxylobaoxide was confirmed.

Lobatrienolide (**6**),⁸⁾ C₂₀H₃₀O₃, a colorless oil, $[\alpha]_{\text{D}}^{25}$ +89.3° (c 0.63, CHCl₃), exhibited intense absorption bands of a conjugated ester (1710, 1635 cm⁻¹) and hydroxyl (3440 cm⁻¹) groups. The ¹H and ¹³C NMR spectra revealed the presence of an ester (δ_{C} 164.7), vinyl (δ_{H} 5.81, 4.89, 4.90; δ_{C} 150.0, 110.1), exomethylene (δ_{H} 4.81, 4.57; δ_{C} 147.4, 112.3), trisubstituted olefin (δ_{H} 6.61; δ_{C} 137.1, 136.7) conjugated with the ester carbonyl, oxygen-bearing methine (δ_{H} 4.12; δ_{C} 83.4), olefinic methyl (δ_{H} 1.70; δ_{C} 24.9), and three quaternary methyls (δ_{H} 1.00, 1.23, 1.31; δ_{C} 16.7, 24.6, 24.6). The NMR characters of **6** are, as a whole, very similar to those of lobatriene (**7**). The presence of a carbonyl carbon signal in **6** in place of one methylene carbon of **7** implicated structure **6** for lobatrienolide. Indeed, photooxidation (benzene-CH₂Cl₂, methylene blue, fluorescent lamp,⁹⁾ 62 h) of lobatriene (**7**) afforded (5% yield) the product, whose physical data including optical rotation were identical with those of natural **6**. These facts established the structure and absolute configuration of lobatrienolide.

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References

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- 2) T. Kusumi, T. Hamada, M. O. Ishitsuka, and H. Kakisawa, *J. Org. Chem.*, in press.
- 3) ¹H-NMR (500 MHz, CDCl₃): δ 1.00 (1H, s, H-7), 1.19 (3H, s, H-19), 1.23 (3H, s, H-20), 1.46 (3H, m, H-5 α , H-6), 1.57 (2H, m, H-3), 1.62 (1H, m, H-5 β), 1.69 (3H, s, H-12), 2.01 (1H, dd, *J* = 4.2, 14

- Hz; H-2), 2.09 (1H, brt, $J = 11$ Hz, H-4), 2.20 (1H, brdd, $J = 14$, 6.4 Hz, H-16), 2.32 (1H, dt, $J = 14$, 10 Hz, H-16), 3.37 (1H, br d, $J = 9.8$ Hz; H-17), 3.94 (1H, dd, $J = 4.8$, 11 Hz; H-14), 4.27 (1H, br d, $J = 11$ Hz; H-14), 4.58 (1H, brs, H-11), 4.81 (1H, br s, H-11), 4.88 (1H, d, $J = 11$ Hz; H-9), 4.90 (1H, d, $J = 17$ Hz; H-9), 5.46 (1H, dd, $J = 6.4$, 9.8 Hz; H-15), 5.81 (1H, dd, $J = 11$, 17 Hz; H-8); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 16.8 (q; C-7), 23.6 (q; C-19), 24.8 (q; C-12), 26.6 (q; C-20), 27.3 (t; C-5), 30.3 (t; C-16), 33.4 (t; C-3), 39.8 (t; C-6), 40.0 (s; C-1), 45.3 (d; C-4), 52.9 (d; C-2), 59.2 (t; C-14), 73.1 (s; C-18), 76.6 (d; C-17), 110.0 (t; C-9), 112.2 (t; C-11), 124.3 (d; C-15), 147.1 (s; C-13), 147.6 (s; C-10), 150.2 (d; C-8). MS, m/z 187, 247, 286, 304 ($\text{M}^+ - \text{H}_2\text{O}$).
- 4) R. W. Dunlop and R. J. Wells, *Aust. J. Chem.*, **32**, 1345 (1979).
- 5) I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1991).
- 6) $[\alpha]_{\text{D}}^{25} +20.0^\circ$ (c 0.10, CHCl_3); IR (film) 3400, 1465, 1380 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 0.75 (3H, d, $J = 7.0$ Hz; H-11), 0.79 (3H, t, $J = 7.0$ Hz; H-9), 0.82 (3H, s, H-7), 0.89 (3H, d, $J = 7.0$ Hz; H-12), 1.14 (1H, dd, $J = 3.0$, 12 Hz; H-2), 1.18 (3H, s, H-19), 1.24 (3H, s, H-20), 1.28 (2H, m, H-6), 1.39 (2H, m, H-3), 1.47 (2H, m, H-5), 1.65 (3H, s, H-14), 1.83 (1H, tt, $J = 4.0$, 12 Hz; H-4), 1.90 (1H, sept, $J = 7.0$ Hz; H-10), 2.20 (2H, t, $J = 7$ Hz; H-16), 3.41 (1H, m, H-17), 5.24 (1H, t, $J = 7.0$ Hz; H-15); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 7.7, 15.0, 18.6, 20.2, 23.8, 24.6, 25.3 (seven q), 26.7 (d), 27.2 (t), 27.6 (t), 30.4 (t), 34.6 (t), 36.4 (s), 38.3 (t), 48.2 (d), 72.7 (s), 77.8 (d), 118.4 (d), 145.0 (s); MS, m/z 231, 249, 292 ($\text{M}^+ - \text{H}_2\text{O}$).
- 7) $[\alpha]_{\text{D}}^{25} -3.3^\circ$ (c 0.06, CHCl_3); IR (film) 1740 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 1.00 (1H, s, H-7), 1.29 (3H, s, H-19), 1.31 (3H, s, H-20), 1.42-1.50 (2H, m), 1.50-1.63 (4H, m), 1.69 (3H, s, H-12), 2.00 (1H, dd, $J = 6.2$, 16 Hz), 2.05 (3H, s, H-22), 2.32 (1H, ddd, $J = 14$, 7.4, 6.0 Hz; H-16), 2.38 (1H, ddd, $J = 14$, 7.4, 6.0 Hz; H-16), 2.74 (1H, t, $J = 6.0$ Hz, H-17), 4.57 (1H, br s, H-11), 4.58 (1H, d, $J = 12$ Hz; H-14), 4.65 (1H, d, $J = 12$ Hz; H-14), 4.81 (1H, d, $J = 1.0$ Hz, H-11), 4.89 (1H, d, $J = 11$ Hz; H-9), 4.90 (1H, d, $J = 17$ Hz; H-9), 5.56 (1H, t, $J = 7.4$ Hz; H-15), 5.80 (1H, dd, $J = 11$, 17 Hz; H-8); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 16.7 (q; C-7), 18.6 (q), 21.1 (q; C-22), 24.6 (q), 24.7 (q), 27.2 (t; C-5), 28.0 (t; C-16), 33.3 (t; C-3), 39.8 (s; C-1), 40.0 (t; C-6), 44.0 (d; C-4), 52.6 (d; C-2), 58.6 (s; H-18), 61.3 (t; C-14), 63.7 (d; C-17), 110.0 (t; C-9), 112.3 (t; C-11), 124.6 (d; C-15), 140.9 (s; C-13), 147.6 (s; C-10), 150.1 (d; C-8), 171.1 (s; C-21); MS, m/z 257, 271, 286, 346 (M^+).
- 8) $[\alpha]_{\text{D}}^{25} +89.3^\circ$ (c 0.63, CHCl_3); IR (film) 3440, 1710, 1635, 1370 cm^{-1} ; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 1.00 (3H, s, H-7), 1.23 (3H, s, H-19), 1.31 (3H, s, H-20), 1.36 (1H, dq, $J = 3.4$, 13 Hz; H-5 α), 1.42 (1H, td, $J = 3.3$, 13 Hz; H-6 α), 1.52-1.62 (m), 1.67 (1H, td, $J = 3.8$, 13 Hz; H-5 β), 1.70 (3H, s, H-12), 2.08 (1H, m, H-2), 2.32 (1H, ddd, $J = 3.7$, 6.8, 18 Hz; H-16), 2.48 (1H, ddt, $J = 13$, 18, 2.0 Hz; H-16), 4.12 (1H, dd, $J = 3.7$, 13 Hz; H-17), 4.57 (1H, br s, H-11), 4.81 (1H, br s, H-11), 4.89 (1H, d, $J = 11$ Hz; H-9), 4.90 (1H, d, $J = 17$ Hz; H-9), 5.81 (1H, dd, $J = 11$, 17 Hz; H-8), 6.61 (1H, dt, $J = 6.8$, 1.5 Hz; H-15); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 16.7 (q; C-7), 24.6 (q), 24.6 (q), 24.9 (q), 25.9 (q), 27.9 (t; C-5), 32.6 (t; C-3), 38.6 (d; C-4), 39.7 (s; C-1), 39.8 (t; C-6), 52.5 (d; C-2), 71.0 (s; C-18), 83.4 (d; C-17), 110.1 (t; C-9), 112.3 (t; C-11), 136.7 (s; C-13), 137.1 (d; C-15), 147.4 (s; C-10), 150.0 (d; C-8), 164.7 (s; C-14); MS, m/z 257, 285, 303, 318 (M^+).
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