Determination of the Absolute Configuration at the Two Cyclopropane Moieties of Plakoside A, an Immunosuppressive Marine Galactosphingolipid[‡]

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Plakoside A (**1**) {(2*S*,3*R*,11*R**,12*S**)-2-[(2‴*R*,5‴*Z*,11‴*R**,12‴*S**)-2‴-hydroxy-11‴,12‴-methylene-5‴-docosenamido]-1-*O*-[2′-*O*-(3′′-methyl-2′′-butenyl)-β-D-galactopyranosyl]-11,12-methylene-1,3-docosanediol} is a prenylated galactosphingolipid isolated as an immunosuppressant from the marine sponge *Plakortis simplex*. The absolute configuration of plakoside A was determined as 11*S*,12*R*,11‴*S*,12‴*R* by its degradation to two cyclopropane-containing fatty acids **2a** and **3a** followed by their derivatization with a chiral reagent **4** and subsequent HPLC analysis of the resulting derivatives **2b** and **3b**.

Introduction

In 1997, plakoside A (**1**, Scheme 1) was isolated by Fattorusso and co-workers as an immunosuppressive metabolite of the Caribbean sponge *Plakortis simplex*.[1] It is a structurally unique glycosphingolipid with a prenylated D-galactose moiety and cyclopropane-containing alkyl chains, and shows strong immunosuppressive activity without cytotoxicity. Its 2*S*,3*R*,2‴*R* stereochemistry was proposed on the basis of the CD measurements of its degradation products.[1] The absolute configuration at the two cyclopropane moieties of **1**, however, has remained unknown, although the *cis* stereochemistry of the ring substituents was suggested by detailed ^{1}H NMR analysis of **1**.[1]

Due to the unique structure and bioactivity of **1** together with the scarcity of the natural **1** (5 mg of **1** could be isolated from 57 g of the dried sponge[1]), its synthesis attracted the attention of chemists, and two independent syntheses of **1** have been reported.[2–4] In 2000, Nicolaou et al. accomplished the synthesis of (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*)-**1**, and found its spectroscopic data to be identical to those reported for natural plakoside A.[2] They therefore claimed their synthetic product to be the natural product.[2] In 2001, we first reported the synthesis of (2*S*,3*R*,11*S*,12*R*,2‴*R*,5‴*Z*,11‴*S*,12‴*R*)-**1**, and found that its spectroscopic data are also identical to those of natural plakoside A.[3] Subsequently in the same year, we also prepared (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*)-**1**, which was spectroscopically indistinguishable from the natural **1**.[4] Even after the synthesis of the two diastereoisomers of **1**, we were unable to solve the stereochemical problem concerning the cyclopropane moieties. There have been a number of reported examples where two diastereoisomers with separated stereogenic centers show indistinguishable spectroscopic data.[4]

In order to solve this stereochemical problem, we decided to resume degradation studies on natural plakoside A (**1**), and one of us (E. F.) reisolated 5 mg of **1** from the Caribbean sponge. Scheme 1 summarizes our plan for the clarification of the absolute configuration of **1**. Two cyclopropane-containing fatty acids, *nat*-derived **2a** and **3a**, are to be obtained by degradation of **1**. These two acids, **2a** and **3a**, give esters **2b** and **3b** upon treatment with (1*S*,2*S*)- or (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanol (**4**), which is known to be a chiral and fluorescent derivatizing reagent developed by two of us (K. A. and H. O.).[5] Authentic samples of **2b** and **3b** with known absolute configuration at their respective cyclopropane moiety are to be synthesized and compared with *nat*-derived **2b** and **3b** by

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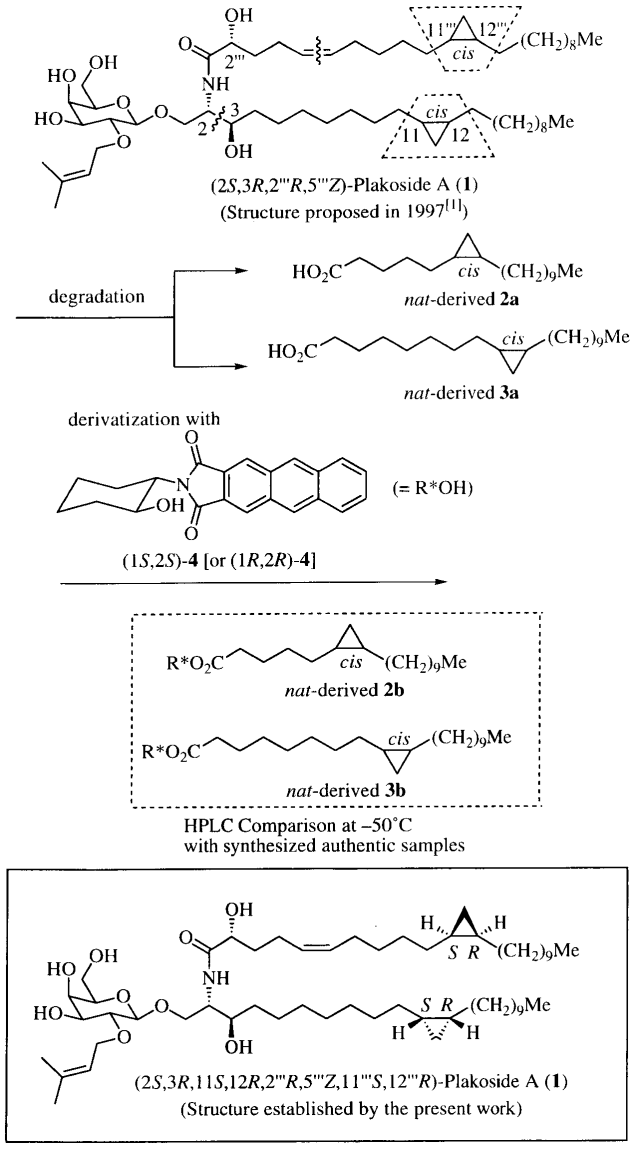


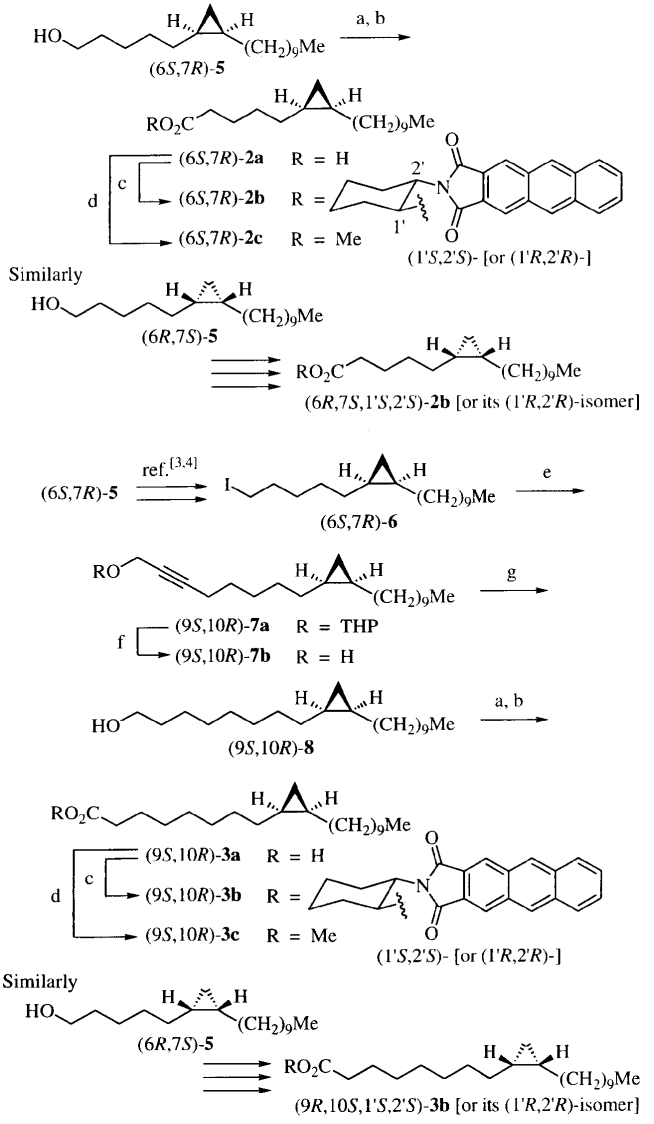
Scheme 1. Strategy adopted for the clarification of the absolute configuration of plakoside A

HPLC analysis at −50 °C to clarify their stereochemistry. In this paper we report in detail our results, which have been published as a preliminary communication.[6] In short, the structure, including the absolute configuration of plakoside A, is (2*S*,3*R*,11*S*,12*R*,2′′′*R*,5′′′*Z*,11′′′*S*,12′′′*R*)-**1**. This means that Nicolaou's synthetic **1** is not the natural product, but its diastereoisomer.

Results and Discussion

Synthesis, Derivatization and HPLC Analysis of the Authentic Samples of the Cyclopropane-Containing Fatty Acids 2a and 3a

Scheme 2 summarizes our synthesis of the authentic samples of **2a** and **3a** and their derivatization to **2b** and **3b**. The known alcohol (6*S*,7*R*)-**5**[4] was oxidized under Swern conditions, and the resulting aldehyde was further oxidized with sodium chlorite to give the acid, (6*S*,7*R*)-**2a**. Treatment of **2a** with (1*S*,2*S*)- or (1*R*,2*R*)-**4** in the presence of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC) gave (6*S*,7*R*,1′*S*,2′*S*)- or (6*S*,7*R*,1′*R*,2′*R*)-**2b**. The methyl ester (6*S*,7*R*)-**2c** was also prepared from (6*S*,7*R*)-**2a** by methylation with diazomethane. Similarly, (6*R*,7*S*)-**5** yielded (6*R*,7*S*)-**2a** and its derivatives.



Scheme 2. Synthesis of the authentic samples of **2b** and **3b**; reagents: (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (b) $NaClO_2$, 34.5% H_2O_2, MeCN, H_2O [72% for (6*S*,7*R*)-**2a**, 73% for (6*R*,7*S*)-**2a**, 73% for (9*S*,10*R*)-**3a**, 75% for (9*R*,10*S*)-**3a**; two steps]; (c) (1*S*,2*S*)- or (1*R*,2*R*)-**4**, *N*,*N*-4-dimethylaminopyridine, EDC, toluene, MeCN, room temp., >10 h; (d) CH_2N_2, Et_2O; (e) HC≡CCH_2OTHP, *n*BuLi, THF, HMPA (89%); (f) *p*TsOH·H_2O, MeOH, CH_2Cl_2 (95%); (g) 80% N_2H_4·H_2O, EtOH, 34.5% H_2O_2, (76%)

For the synthesis of acid (9*S*,10*R*)-**3a** with a side-chain longer than that of **2a**, the known iodide (6*S*,7*R*)-**6**[4] was homologated by treatment with the lithium salt of propargyl alcohol tetrahydropyranyl (THP) ether to furnish (9*S*,10*R*)-**7a**. The corresponding free alcohol (9*S*,7*R*)-**7b** was reduced with diimide, and the resulting saturated alcohol (9*S*,10*R*)-**8** was oxidized to give the carboxylic acid

(9*S*,10*R*)-**3a**. Its derivatization with (1*S*,2*S*)- or (1*R*,2*R*)-**4** afforded (9*S*,10*R*,1′*S*,2′*S*)- or (9*S*,10*R*,1′*R*,2′*R*)-**3b**. The methyl ester (9*S*,10*R*)-**3c** was also prepared. Similarly, (6*R*,7*S*)-**6** gave (9*R*,10*S*,1′*S*,2′*S*)-**3b** and its (1′*R*,2′*R*)-isomer.

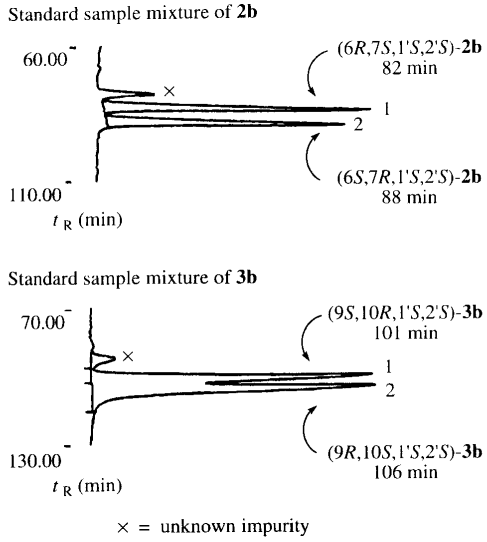


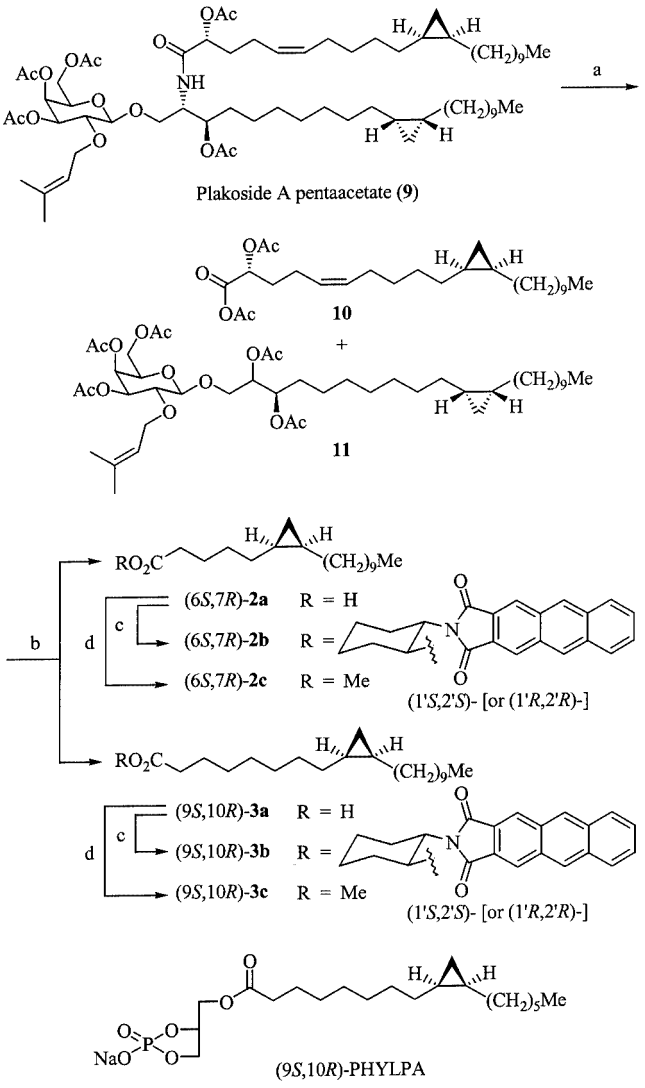
Figure 1. HPLC separation of the derivatized acids (for detailed analytical conditions, see Exp. Sect.)

Figure 1 shows the separation of a standard mixture of the diastereoisomers of **2b** and **3b**, respectively, by reversed-phase HPLC at a column temperature of −50 °C. At this temperature, the two diastereoisomers (6*R*,7*S*,1′*S*,2′*S*)-**2b** and (6*S*,7*R*,1′*S*,2′*S*)-**2b** could be separated cleanly. In the same manner, (9*S*,10*R*,1′*S*,2′*S*)-**3b** could be separated from (9*R*,10*S*,1′*S*,2′*S*)-**3b**. The usefulness of the derivatizing reagent **4** for the determination of the absolute configuration at stereogenic centers far separated from a functional group such as carbonyl was also ascertained in the present case.[5,7]

Degradation of Plakoside A to Cyclopropane-Containing Fatty Acids 2a and 3a, and Their Derivatization followed by HPLC Analysis

Due to the scarcity (5 mg) of the reisolated plakoside A (**1**), preliminary degradation experiments were executed with synthetic **1**. It soon became clear that hydrolysis of the amide linkage of **1** was very difficult to achieve. The amide bond could not be cleaved under conventional alkaline conditions, while acidic conditions cleaved the cyclopropane rings more rapidly than the amide linkage. It was therefore obvious that an indirect and mild method for the cleavage of the amide bond of **1** was needed.

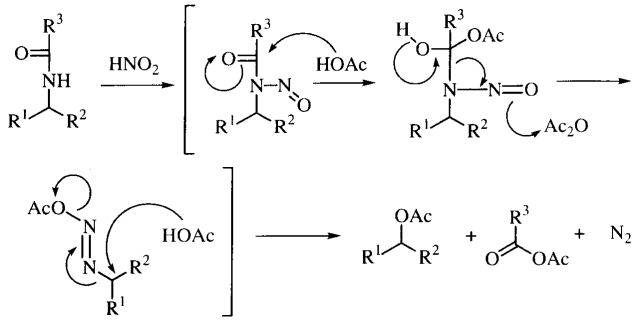
It occurred to us that the cleavage might be realized via *N*-nitrosation at the amide nitrogen,[8,9] since one of us (K. M.) used this method in his diterpenoid synthesis in 1971 to convert *N*-acetamide to acetate.[8] To examine this possibility, natural plakoside A (**1**) was first acetylated to give



Scheme 3. Degradation of plakoside A pentaacetate and the structure of PHYLPA; reagents: (a) $NaNO_2$, Ac_2O, AcOH, $CHCl_3$, 0 °C → room temp.; (b) i) KOH, EtOH; ii) $KMnO_4$, $NaIO_4$, *t*BuOH, H_2O; iii) dil. HCl; (c) (1*S*,2*S*)- or (1*R*,2*R*)-**4**, *N,N*-4-dimethylaminopyridine, EDC, toluene, MeCN, room temp., >10 h; (d) CH_2N_2, Et_2O

the known pentaacetate **9** (Scheme 3).[1] This acetate (2.0 mg) was treated with sodium nitrite in acetic anhydride and acetic acid to give the corresponding *N*-nitroso compound, which decomposed into a cleaved mixture of the upper-chain part **10** with a double bond at C-5′′′ and the lower-chain part **11** with 2,3-diacetoxy groups instead of 2-amido-3-acetoxy groups. A plausible mechanism for this reaction is shown in Scheme 4. When *N*-(1-ethyl)propylbenzamide was subjected to the same reaction conditions, acetic benzoic anhydride could be detected by IR spectroscopy as one of the products (see Exp. Sect.).

The resulting mixture of **10** and **11** was hydrolyzed with potassium hydroxide, and the product containing the unsaturated upper-chain part and the 2,3-dihydroxylated lower-chain part was oxidized with Lemieux−von Rudloff reagent (potassium permanganate and sodium periodate) to furnish



Scheme 4. A plausible mechanism for the degradation reaction

a mixture of the desired degradation products **2a** and **3a** (1.1 mg).

A small portion of this mixture of **2a** and **3a** was methylated with diazomethane to give a mixture of methyl esters **2c** and **3c**, which could be identified when compared to authentic **2c** and **3c** by GC-MS analysis. The major portion of the acid mixture was then derivatized with **4** to give a mixture containing **2b** and **3b**, whose HPLC analysis definitely proved that they were (6*S*,7*R*)-**2b** and (9*S*,10*R*)-**3b**. The mixture could be analyzed directly without preliminary separation of **2b** from **3b**, because the retention times of these two derivatives were different enough to allow their exact analysis even as a mixture (see Figure 1). Plakoside A therefore possesses the 11*S*,12*R*,11′′′*S*,12′′′*R* configuration.

Conclusion

The hitherto unknown absolute configuration of plakoside A (**1**) was determined as 2*S*,3*R*,11*S*,12*R*,2′′′*R*,5′′′*Z*,11′′′*S*,12′′′*R*. Akasaka and Ohrui's chiral and fluorescent derivatizing reagent **4** was extremely useful in solving the present stereochemical problem. It should be mentioned that another cyclopropane-containing natural product, PHYLPA, a specific inhibitor of DNA polymerase α produced by a true slime mold *Physarum polycephalum*, also possesses the 9*S*,10*R* configuration.[10]

The immunosuppressive activity of synthetic plakoside A [(2*S*,3*R*,11*S*,12*R*,2′′′*R*,5′′′*Z*,11′′′*S*,12′′′*R*)-**1**] and its diastereoisomer [(2*S*,3*R*,11*R*,12*S*,2′′′*R*,5′′′*Z*,11′′′*R*,12′′′*S*)-**1**] is now being examined in Italy, and the result will be reported in due course.

Experimental Section

General: IR: Jasco FT/IR-460 Plus. ^{1}H NMR: Jeol JNM-LA400 (400 MHz) and Jeol JNM-LA500 (500 MHz) (TMS at δ = 0.00 ppm or $CHCl_3$ at δ = 7.26 ppm as an internal standard). ^{13}C NMR: Jeol JNM-LA400 (100 MHz) and Jeol JNM-LA500 (126 MHz) ($CHCl_3$ at δ = 77.0 ppm as an internal standard). Optical rotation: Jasco DIP-1000. GC-MS: Shimadzu GCMS-QP5050A. MS: Jeol JMS-SX102A and Hitachi M-80B. Column chromatography: Merck Kieselgel 60 Art 1.07734. TLC: 0.25 mm Merck silica gel plates (60F−254).

Analytical HPLC Instruments: The HPLC pump used was Jasco PU-980 equipped with Rheodyne 7125 sample injector with a 20 μL sample loop. The fluorescence detector was Jasco FP-920 with a 5 μL flow cell. The integrator was Chromatocorder 12 (System Instrument, Tokyo, Japan). Cryocool CC100-II was used to control the column temperature.

(9*S*,10*R*)-9,10-Methylene-1-tetrahydropyranyloxyicos-2-yne [(9*S*,10*R*)-7a]: A solution of *n*BuLi (1.56 M in *n*-hexane, 0.82 mL, 1.28 mmol) was added to a stirred solution of 1-tetrahydropyranyloxy-2-propyne (267 mg, 1.90 mmol) in dry THF (5 mL) at −78 °C under argon. The mixture was stirred at −20 °C for 1 h. A solution of (6*S*,7*R*)-**6** (312 mg, 0.825 mmol) in dry THF (5 mL) and HMPA (5 mL) were added dropwise to the resulting solution at −78 °C. The stirred mixture was allowed to warm to room temperature over a period of 10 h. After quenching with saturated aqueous NH_4Cl solution, the mixture was extracted with diethyl ether. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (20 g; hexane/ethyl acetate, 150:1) to give 288 mg (89%) of (9*S*,10*R*)-**7a** as a colorless oil, n_D^{26} = 1.4721. $[\alpha]_D^{26}$ = −0.19 (*c* = 0.59, $CHCl_3$). IR (film): ṽ = 3060 (w, C−H), 2220 (w, C≡C), 1120 (s, C−O), 1025 (s, C−O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = −0.33 (ddd, *J* = 5.2, 5.2, 4.0 Hz, 1 H, 21-H_a), 0.53−0.59 (m, 1 H, 21-H_b), 0.60−0.68 (m, 2 H, 9-, 10-H), 0.88 (t, *J* = 6.4 Hz, 3 H, 20-H_3), 1.15−1.42 (m, 24 H, 6-, 7-, 8-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-H_2), 1.46−1.67 (m, 6 H, 5-, 4′-, 5′-H_2), 1.70−1.90 (m, 2 H, 3′-H_2), 2.22 (ddt, *J* = 7.2, 7.2, 2.2 Hz, 4-H_2), 3.49−3.56 (m, 1 H, 6′-H_a), 3.84 (ddd, *J* = 12.0, 9.0, 3.2 Hz, 1 H, 6′-H_b), 4.20 (dt, *J* = 15.1, 2.2 Hz, 1 H, 1-H_a), 4.29 (dt, *J* = 15.1, 2.2 Hz, 1 H, 1-H_b), 4.81 (t, *J* = 3.4 Hz, 1 H, 2′-H) ppm. $C_{26}H_{46}O_2$ (390.6): calcd. C 79.94, H 11.87; found C 79.93, H 12.08.

(9*R*,10*S*)-9,10-Methylene-1-tetrahydropyranyloxyicos-2-yne [(9*R*,10*S*)-7a]: In the same manner as described above, 1.18 g of (6*R*,7*S*)-**6** was converted into 1.06 g (86%) of (9*R*,10*S*)-**7a** as a colorless oil, n_D^{26} = 1.4724. $[\alpha]_D^{26}$ = +0.31 (*c* = 0.60, $CHCl_3$). Its IR and ^{1}H NMR spectra were identical with those of (9*S*,10*R*)-**7a**. $C_{26}H_{46}O_2$ (390.6): calcd. C 79.94, H 11.87; found C 80.00, H 11.84.

(9*S*,10*R*)-9,10-Methyleneicos-2-yn-1-ol [(9*S*,10*R*)-7b]: A catalytic amount of *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O, 4 mg) was added to a stirred solution of (9*S*,10*R*)-**7a** (150 mg, 0.38 mmol) in MeOH (4 mL) and CH_2Cl_2 (2 mL) at room temperature. The mixture was stirred at room temperature for two days. It was then poured into a saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (6 g, hexane/ethyl acetate, 50:1) to give 112 mg (95%) of (9*S*,10*R*)-**7b** as a colorless oil, n_D^{25} = 1.4709. $[\alpha]_D^{25}$ = +0.64 (*c* = 1.09, $CHCl_3$). IR (film): ṽ = 3330 (s, O−H), 3060 (w, C−H), 2225 (w, C≡C), 1015 (s, C−O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = −0.33 (ddd, *J* = 5.1, 5.1, 3.9 Hz, 1 H, 21-H_a), 0.53−0.59 (m, 1 H, 21-H_b), 0.61−0.67 (m, 2 H, 9-, 10-H), 0.88 (t, *J* = 6.8 Hz, 3 H, 20-H_3), 1.06−1.56 (m, 27 H, 5-, 6-, 7-, 8-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-H_2, OH), 2.22 (ddt, *J* = 7.1, 7.1, 2.2 Hz, 2 H, 4-H_2), 4.25 (dt, *J* = 6.1, 2.2 Hz, 2 H, 1-H_2) ppm. $C_{21}H_{38}O$ (306.5): calcd. C 82.28, H 12.50; found C 82.24, H 12.75.

(9*R*,10*S*)-9,10-Methyleneicos-2-yn-1-ol [(9*R*,10*S*)-7b]: In the same manner as described above, 373 mg of (9*R*,10*S*)-**7a** was converted

into 259 mg (88%) of (9*R*,10*S*)-**7b** as a colorless oil, n_D^{24} = 1.4713. $[\alpha]_D^{25}$ = −0.85 (*c* = 2.87, $CHCl_3$). Its IR and ^1H NMR spectra were identical with those of (9*S*,10*R*)-**7b**. $C_{21}H_{38}O$ (306.5): calcd. C 82.28, H 12.50; found C 82.27, H 12.71.

(9*S*,10*R*)-9,10-Methyleneicosan-1-ol [(9*S*,10*R*)-8]: A 34.5% aqueous H_2O_2 solution (5.0 mL) was added dropwise to a stirred solution of (9*S*,10*R*)-**7b** (286 mg, 0.933 mmol) in 99% EtOH (20 mL) and 80% aqueous hydrazine monohydrate (5.0 mL) at room temperature. The mixture was stirred at room temperature for two days. It was then poured into water and extracted with ethyl acetate. The combined organic layers were washed with a saturated aqueous $FeSO_4$ solution, water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (6 g, hexane/ethyl acetate, 100:3) to give 220 mg (76%) of (9*S*,10*R*)-**8** as a colorless solid, m.p. 34.5−36.0 °C. $[\alpha]_D^{25}$ = −0.011 (*c* = 2.61, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3330 (s, O−H), 3070 (w, C−H), 1060 (m, C−O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = −0.34 (ddd, *J* = 5.1, 5.1, 4.2 Hz, 1 H, 21-H_a), 0.52−0.59 (m, 1 H, 21-H_b), 0.60−0.69 (m, 2 H, 9-, 10-H), 0.88 (t, *J* = 6.8 Hz, 3 H, 20-H_3), 1.06−1.40 (m, 31 H, 3-, 4-, 5-, 6-, 7-, 8-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-H_2, OH), 1.51−1.61 (m, 2 H, 2-H_2), 3.64 (br. s, 2 H, 1-H_2) ppm. $C_{21}H_{42}O$ (310.6): calcd. C 81.22, H 13.63; found C 81.47, H 13.29.

(9*R*,10*S*)-9,10-Methyleneicosan-1-ol [(9*R*,10*S*)-8]: In the same manner as described above, 286 mg of (9*R*,10*S*)-**7b** was converted into 220 mg (76%) of (9*R*,10*S*)-**8** as a colorless solid, m.p. 35.0−36.5 °C. $[\alpha]_D^{25}$ = +0.019 (*c* = 2.31, $CHCl_3$). Its IR and ^1H NMR spectra were identical with those of (9*S*,10*R*)-**8**. $C_{21}H_{42}O$ (310.6): calcd. C 81.22, H 13.63; found C 80.94, H 13.55.

(9*S*,10*R*)-9,10-Methyleneicosanoic acid [(9*S*,10*R*)-3a]: A solution of dimethyl sulfoxide (DMSO, 0.54 mL, 7.6 mmol) in dry CH_2Cl_2 (3.0 mL) was added to a stirred solution of oxalyl chloride (0.30 mL, 3.5 mmol) in dry CH_2Cl_2 (3.0 mL) at −65 °C under argon. After stirring for 20 min at that temperature, a solution of (9*S*,10*R*)-**8** (200 mg, 0.644 mmol) in dry CH_2Cl_2 (3.0 mL) was added dropwise to the reaction mixture. The resulting white suspension was stirred at −40 °C for 40 min. Triethylamine (2.1 mL, 15 mmol) was then added to the mixture and the resulting white suspension was allowed to warm to 0 °C over a period of 1 h with stirring. The reaction was quenched by the addition of water and the resulting mixture was extracted with Et_2O. The combined organic layers were washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (6 g, hexane/ethyl acetate, 50:1) to give 175 mg of (9*S*,10*R*)-aldehyde as a colorless oil. This was immediately employed in the next step without further purification.

A solution of sodium chlorite (3.20 g, 35.4 mmol) in H_2O (20 mL) was added dropwise to a stirred solution of (9*S*,10*R*)-aldehyde (175 mg, 0.567 mmol), $NaH_2PO_4{\cdot}2H_2O$ (393 mg, 2.51 mmol) and 34.5% aqueous H_2O_2 (2.0 mL) in MeCN (10 mL) and H_2O (4 mL) at 0 °C. The mixture was stirred at room temperature for 24 h and then extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (6 g, hexane/ethyl acetate, 100:3) to give 153 mg (73%, 2 steps) of (9*S*,10*R*)-**3a** as a colorless powder. An analytical sample for GC-MS was treated with a solution of diazomethane in diethyl ether to give its methyl ester (9*S*,10*R*)-**3c**.

(9*S*,10*R*)-9,10-Methyleneicosanal: IR (film): $\tilde{\nu}$ = 3060 (w, C−H), 2710 (m, CHO), 1730 (s, C=O) cm^{-1}.

(9*S*,10*R*)-3a: M.p. 33.0−34.0 °C. $[\alpha]_D^{25}$ = +0.25 (*c* = 0.58, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3400−2500 (br. m, COOH), 3060 (w, C−H), 1715 (s, C=O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = −0.34 (ddd, *J* = 5.2, 5.2, 4.1 Hz, 1 H, 21-H_a), 0.52−0.59 (m, 1 H, 21-H_b), 0.59−0.69 (m, 2 H, 9-, 10-H), 0.88 (t, *J* = 6.6 Hz, 3 H, 20-H_3), 1.04−1.36 (m, 29 H, 4-, 5-, 6-, 7-, 8-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-H_2, COOH), 1.58−1.71 (m, 2 H, 3-H_2), 2.36 (t, *J* = 7.1 Hz, 2 H, 2-H_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 10.9, 14.1, 15.7, 15.8, 22.7, 24.7, 28.66, 28.72, 29.1, 29.28, 29.37, 29.41, 29.65, 29.68, 29.70, 29.73, 30.1, 30.2, 31.9, 34.1, 180.4 ppm. MS (EI): *m*/*z* (%) = 29 (28), 41 (74), 55 (100), 69 (76), 83 (67), 97 (57), 111 (32), 125 (15), 139 (13), 153 (7), 306 (23), 324 (2). HRMS: calcd. 324.3028; found 324.3015 [M^+]. $C_{21}H_{40}O_2$ (324.5): calcd. C 77.72, H 12.42; found C 77.61, H 12.48.

Methyl (9*S*,10*R*)-9,10-Methyleneicosanoate [(9*S*,10*R*)-3c]: GC-MS [Column: DB-5®, 0.25 mm × 30 m; temp: 100 °C (2 min) + 5 °C/min to 250 °C; Carrier gas: He, 100 kPa] t_R = 30.07 [*m*/*z* (%) = 69 (100), 74 (68), 83 (66), 97 (53), 111 (23), 123 (12), 139 (10), 153 (6), 166 (4), 180 (3), 194 (2), 208 (4), 222 (5), 235 (2), 249 (3), 264 (6), 277 (2) 288 (1), 306 (10), 338 (1)]. HRMS ($C_{22}H_{42}O_2$): calcd. 338.3185; found 338.3185 [M^+].

(9*R*,10*S*)-9,10-Methyleneicosanoic Acid [(9*R*,10*S*)-3a]: In the same manner as described above, 227 mg of (9*R*,10*S*)-**8** was converted into 158 mg (75%, 2 steps) of (9*R*,10*S*)-**3a** as a colorless powder, m.p. 33.5−34.0 °C. $[\alpha]_D^{25}$ = −0.37 (*c* = 0.58, $CHCl_3$). Its IR, ^1H NMR, ^{13}C NMR and mass spectra were identical to those of (9*S*,10*R*)-**3a**. HRMS: calcd. 324.3028; found 324.3026 [M^+]. $C_{21}H_{40}O_2$ (324.5): calcd. C 77.72, H 12.42; found C 77.87, H 12.37.

(6*S*,7*R*)-6,7-Methyleneheptadecanoic Acid [(6*S*,7*R*)-2a]: In the same manner, 54.8 mg of (6*S*,7*R*)-**5** was converted into 41.3 mg (72%, 2 steps) of (6*S*,7*R*)-**2a** as a colorless solid. An analytical sample for GC-MS was treated with a solution of diazomethane in diethyl ether to give its methyl ester (6*S*,7*R*)-**2c**.

[(6*S*,7*R*)-2a]: M.p. ca. 29 °C. $[\alpha]_D^{25}$ = +0.53 (*c* = 0.35, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3300−2500 (br. s, COOH), 3060 (w, C−H), 1710 (s, C=O) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = −0.32 (ddd, *J* = 5.2, 5.2, 4.6 Hz, 1 H, 18-H_a), 0.55−0.60 (m, 1 H, 18-H_b), 0.61−0.69 (m, 2 H, 6-, 7-H), 0.88 (t, *J* = 7.0 Hz, 3 H, 17-H_3), 1.08−1.49 (m, 23 H, 4-, 5-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-H_2, COOH), 1.64−1.71 (m, 2 H, 3-H_2), 2.36 (t, *J* = 7.6 Hz, 2 H, 2-H_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.9, 14.1, 15.4, 15.7, 22.7, 24.6, 28.25, 28.67, 29.34, 29.57, 29.63, 29.67, 29.69, 30.16, 31.9, 34.0, 179.9 ppm. MS (EI): *m*/*z* (%) = 29 (28), 41 (80), 55 (100), 69 (78), 83 (73), 97 (57), 111 (36), 114 (23), 137 (10), 151 (7), 222 (8), 264 (23), 282 (3). HRMS: calcd. 282.2559; found 282.2540 [M^+]. $C_{18}H_{34}O_2$ (282.5): calcd. C 76.54, H 12.13; found C 76.26, H 11.91.

Methyl (6*S*,7*R*)-6,7-Methyleneheptadecanoate [(6*S*,7*R*)-2c]: GC-MS [Column: DB-5®, 0.25 mm × 30 m; temp: 100 °C (2 min) + 5 °C/min to 250 °C; Carrier gas: He, 100 kPa] t_R = 24.75 [*m*/*z* (%) = 69 (87), 74 (100), 83 (63), 97 (48), 110 (29), 128 (13), 137 (8), 152 (5), 166 (3), 180 (6), 193 (3), 207 (2), 222 (6), 235 (3), 247 (2), 264 (7), 278 (1) 296 (2)]. HRMS ($C_{19}H_{36}O_2$): calcd. 296.2715; found 296.2724 [M^+].

(6*R*,7*S*)-6,7-Methyleneheptadecanoic acid [(6*R*,7*S*)-2a]: In the same manner, 59.5 mg of (6*R*,7*S*)-**5** was converted into 45.4 mg (73%, 2 steps) of (6*R*,7*S*)-**2a** as a colorless solid, m.p. ca. 29 °C. $[\alpha]_D^{25}$ = −0.37 (*c* = 0.52, $CHCl_3$). Its IR, ^1H NMR, ^{13}C NMR and mass spectra were identical with those of (6*S*,7*R*)-**2a**. HRMS: calcd.

282.2559; found 282.2551 [M^+]. $C_{18}H_{34}O_2$ (282.5): calcd. C 76.54, H 12.13; found C 76.74, H 12.29.

Degradation of Plakoside A to Carboxylic Acids 2a and 3a: Sodium nitrite (63.5 mg, 0.92 mmol) was added to a stirred solution of plakoside A pentaacetate (**9**, 2.0 mg, 1.7 μmol) in $CHCl_3$ (1 mL), AcOH (1 mL) and Ac_2O (2 mL) at 0 °C. The mixture turned green, and a yellow gas was evolved. After stirring for 1 h at room temperature, the solvent was removed in vacuo and the residue was dissolved in diethyl ether. The mixture was washed with water, saturated aqueous $NaHCO_3$ solution, and brine, dried with $MgSO_4$, and concentrated in vacuo. A 5% KOH solution in EtOH (3 mL) was then added to a stirred solution of the residue in EtOH (5 mL) at room temperature. The mixture was stirred for 16 h at room temperature and then refluxed for 6 h. The resulting mixture was concentrated in vacuo. The residue was dissolved in *t*BuOH (7 mL) and water (7 mL). Sodium periodate (484 mg, 2.26 mmol) and potassium permanganate (105 mg, 0.66 mmol) were added to the mixture at room temperature. The mixture was stirred at 50 °C for 12 h. It was then acidified with aqueous 1 N HCl and extracted with $CHCl_3$. The combined organic layers were washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (0.5 g, hexane/ethyl acetate, 20:5) to give 1.1 mg of a mixture of **2a** and **3a** as a colorless oil. A small portion of it was treated with a solution of diazomethane in diethyl ether to give a mixture of their methyl esters, **2c** and **3c**.

Degraded Carboxylic Acids: ^{1}H NMR (400 MHz, $CDCl_3$): δ = −0.28−−0.35 [m, 1 H, 18-H_a (**2a**), 1 H, 21-H_a (**3a**)], 0.54−0.69 [m, 3 H, 6-, 7-H, 18-H_b (**2a**), 3 H, 9-, 10-H, 21-H_b (**3a**)], 0.88 [t, *J* = 6.7 Hz, 3 H, 17-H_3 (**2a**), 3 H, 20-H_3 (**3a**)], 1.08−1.71 [m, 27 H, 3-, 4-, 5-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-H_2, COOH (**2a**), 31 H, 3-, 4-, 5-, 6-, 7-, 8-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-H_2, COOH (**3a**)], 2.35 [t, *J* = 7.7 Hz, 2 H, 2-H_2 (**2a**), 2 H, 2-H_2 (**3a**)] ppm.

Methyl Esters: GC-MS [Column: DB-5®, 0.25 mm × 30 m; temp: 100 °C (2 min) + 5 °C/min to 250 °C; Carrier gas: He, 100 kPa] t_R = 24.68 [*m*/*z* (%) = 69 (75), 74 (100), 83 (54), 96 (45), 110 (26), 123 (12), 128 (11), 137 (8), 152 (5), 166 (4), 180 (6), 193 (3), 207 (3), 222 (6), 235 (2), 246 (3), 264 (6), 277 (1) 296 (2)], 30.01 [*m*/*z* (%) = 69 (100), 74 (73), 83 (63), 97 (47), 111 (23), 123 (13), 139 (10), 153 (6), 166 (4), 180 (3), 194 (3), 208 (4), 222 (5), 235 (3), 249 (2), 264 (5), 277 (3) 288 (2), 306 (12), 338 (2)].

Cleavage of the Amide Bond of *N*-(1-Ethyl)propylbenzamide: Sodium nitrite (901 mg, 13.1 mmol) was added to a stirred solution of *N*-(1-ethyl)propylbenzamide (101 mg, 0.528 mmol) in $CHCl_3$ (2 mL), AcOH (6 mL) and Ac_2O (12 mL) at 0 °C. The mixture turned green and a yellow gas was evolved. After stirring for 2 h at room temperature, the mixture was diluted with diethyl ether. The resulting mixture was then filtered to remove sodium acetate, and the filtrate was concentrated in vacuo at 45 °C with addition of dry toluene to facilitate the removal of acetic acid. The evaporation was interrupted when ca. 0.3 mL of the residue remained. Its IR spectrum showed the presence of acetic benzoic anhydride [$\tilde{\nu}$ = 1810, 1735 cm^{-1}] rather than acetic anhydride [$\tilde{\nu}$ = 1825, 1755 cm^{-1}]. After complete evaporation, the residue showed peaks in its IR spectrum at $\tilde{\nu}$ = 1790, 1720 cm^{-1}, indicating the presence of benzoic anhydride. The mechanism shown in Scheme 4 for this reaction demands the formation of acetic benzoic anhydride. ^{13}C NMR spectroscopy could not be used to verify the formation of acetic benzoic anhydride, since there are no significant differences between the chemical shift values of the carbonyl carbons of acetic benzoic anhydride (δ_c = 161.73, 166.01 ppm) and those of acetic anhydride (δ_c = 165.97 ppm) or benzoic anhydride (δ_c = 161.83 ppm).

Sample Preparation Procedure for Analytical HPLC: More than 5 equivalents (to carboxylic acid **2a** or **3a**) of (1*S*,2*S*)- or (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanol (**4**) and also 5 equivalents (to **2a** or **3a**) of *N*,*N*-4-dimethylaminopyridine were added to the sample solution in a mixture of toluene and acetonitrile (1:1). After addition of an excess amount (over the reagent) of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC), the mixture was kept at room temperature for more than 10 h, and an aliquot was then loaded onto a silica gel TLC (10 cm length, Silica gel 60 F_{254}, Art-5744, Merck) and developed with *n*-hexane/ethyl acetate (4:1, v/v). The target spot detected by fluorescence was collected, packed in a Pasteur pipette, and eluted with ethyl acetate/ethanol (4:1, v/v). After evaporation of the solvent with a N_2 gas stream, the residue was dissolved in methanol and used for an HPLC analysis.

HPLC Separation: The acid derivatives **2b** and **3b** were separated on a reversed-phase column (Develosil C-30-UG-3, 3 μm, 4.6 mm I.D. × 150 mm, Nomura Chemical Co., Aichi, Japan). The detection was carried out by monitoring the fluorescence intensity at 462 nm (excitation at 298 nm). For **2b**, the separation was performed with a mixture of acetonitrile/THF/*n*-hexane (200:150:25) at the rate of 0.1 mL/min (condition A). For **3b**, the separation was performed with a mixture of acetonitrile/THF/*n*-hexane (175:175:10) at a rate of 0.2 mL/min (condition B). In both cases, the column temperature was kept at −50 °C. The temperature of the sample solution was gradually raised to room temperature by using a loop, and detection was carried out at room temperature.

HPLC Analysis of 2b Derived from Natural Plakoside A: The authentic *cis*-(6*S*,7*R*)- and (6*R*,7*S*)-acid esters **2b** of (1*S*,2*S*)-**4** gave peaks at 88 min and 82 min, respectively. The (1*S*,2*S*)-**2b** of the acid derived from the natural product gave the peak at 88 min as a major one, while a small peak was also detected at 82 min. To confirm the absolute configuration and enantiomeric purity of the acid **2a**, the (1*R*,2*R*)-**4** derivative of the acid **2a** was also analyzed; it gave a single peak at 82 min (no peak at 88 min). Therefore, the peak at 82 min in the previous (1*S*,2*S*)-labeling experiment was ascribed to the one derived from an achiral acid, and the absolute configuration of the acid **2a** derived from the natural product was determined to be (6*S*,7*R*), and further the sample was free from its enantiomer. The (6*S*,7*R*)-acid derived by oxidation of the synthetic sample with $NaIO_4$/$KMnO_4$ was also analyzed. By labeling with (1*S*,2*S*)-**4**, it gave a main peak at 88 min and small peaks at 81−83 min, while it gave a main peak at 82 min and no peak at 88 min by labeling with (1*R*,2*R*)-**4**. The above results unequivocally showed that the absolute configuration of the acid derived from the natural product was (6*S*,7*R*).

HPLC Analysis of 3c Derived from Natural Plakoside A: The (1*S*,2*S*)-**4** ester of the authentic (9*S*,10*R*)-**3a** and its enantiomer gave peaks at 101 min and 106 min, respectively. The (1*S*,2*S*)-**4** ester of the acid derived from the natural product gave a major peak at 101 min and a smaller but significant peak at 106 min. However, the (1*R*,2*R*)-**4** ester of the acid gave a major peak at 106 min and no peak at 101 min. Again, the peak at 106 min in the (1*S*,2*S*)-**4** labeling could be ascribed to the one by an achiral acid generated by the oxidation. These results showed that the absolute configura-

tion of the acid derived from the natural product was (9*S*,10*R*) and the acid was free from its enantiomer.

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