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Determination of the absolute configuration at the two cyclopropane moieties of plakoside A, an immunosuppressive marine galactosphingolipid

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Abstract—The absolute configuration of plakoside A {(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} was determined as 11*S*,12*R*,11‴*S*,12‴*R* by its degradation to two cyclopropane-containing fatty acids followed by their derivatization with a chiral reagent and subsequent HPLC analysis of the resulting derivatives. © 2002 Elsevier Science Ltd. All rights reserved.

In 1997, plakoside A (**1**), $[\alpha]_D^{25}=+7$ (MeOH), was isolated by Fattorusso and co-workers as an immunosuppressive metabolite of the Caribbean sponge, *Plakortis simplex*.[1] It is a glycosphingolipid with a prenylated D-galactose moiety and cyclopropane-containing alkyl chains. 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, remained unknown, although the *cis* stereochemistry of the ring substituents was suggested by its detailed ^{1}H NMR analysis.[1]

Due to the unique structure and bioactivity of **1**, its synthesis attracted 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**, $[\alpha]_D=+10.4$ (MeOH), and found its spectroscopic data identical to those reported for natural plakoside A.[2] They therefore claimed their synthetic product to be the natural product itself.[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**, $[\alpha]_D^{22}=+8.9$ (MeOH), and found its spectroscopic data also identical to those of natural plakoside A.[3] Subsequently, (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*)-**1**, $[\alpha]_D^{22}=+10.5$ (MeOH), was also prepared by us, which was spectroscopically indistinguishable from the natural **1**.[4] Accordingly, even after the synthesis of two diastereoisomers of **1**, we were unable to solve the stereochemical problem concerning the cyclopropane moieties.

In order to solve this stereochemical problem, we decided to reassume degradation studies on natural plakoside A (**1**), and one of us (Fattorusso) reisolated 5 mg of **1**. 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 (Akasaka and Ohrui).[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 HPLC analysis at −50°C to clarify their stereochemistry. In this letter we report our results, which establish the absolute configuration of plakoside A as (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.

Keywords: cyclopropanes; immunosuppressive compounds; marine metabolites; sphingolipids; sponges; stereochemistry.

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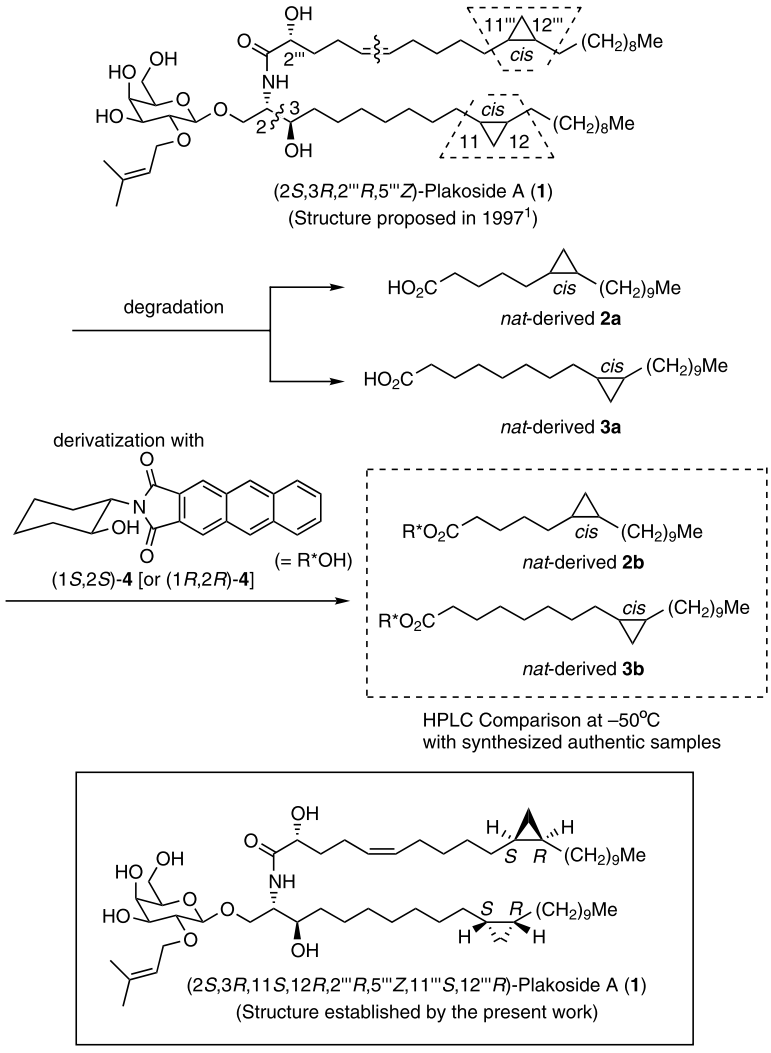
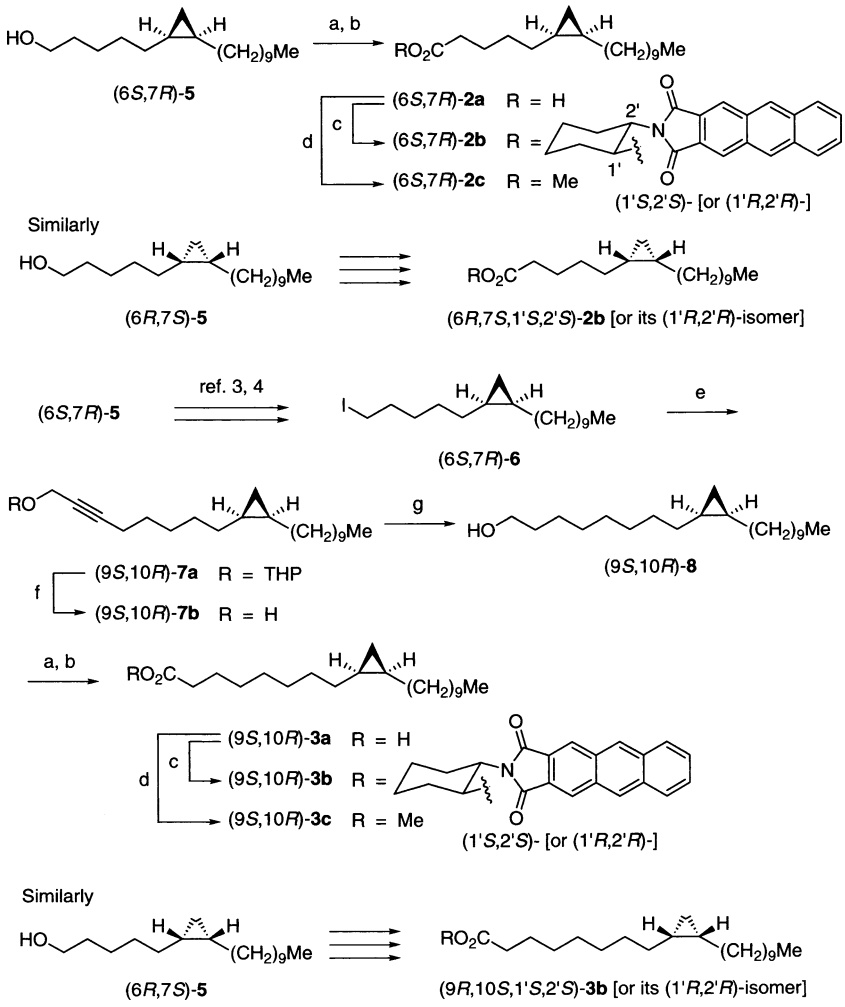


Scheme 1. Strategy adopted for the clarification of the absolute configuration of plakoside A.

Syntheses of the authentic samples of **2b** and **3b** are summarized in Scheme 2. The known alcohol (6*S*,7*R*)-**5**[4] was oxidized under the Swern conditions, and the resulting aldehyde was further oxidized with sodium chlorite to give acid (6*S*,7*R*)-**2a**. Treatment of this acid **2a** with (1*S*,2*S*)- or (1*R*,2*R*)-**4** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide 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. For the synthesis of the acid (9*S*,10*R*)-**3a** with a longer side-chain 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**. Corresponding free alcohol (9*S*,10*R*)-**7b** was reduced with diimide, and the resulting saturated alcohol (9*S*,10*R*)-**8** was oxidized to give 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.

Fig. 1 shows the separation of the standard sample mixture of **2b** and that of **3b** 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 cleanly separated. Similarly, (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 ascertained in the present case, too.[5]

We then carried out the degradation of the natural plakoside A after preliminary examination employing the synthetic material (Scheme 3). It soon became clear that the conventional hydrolysis of the amide linkage of **1** was very difficult to achieve. An indirect and milder method for the cleavage of **1** was therefore needed.[6] Accordingly, plakoside A (**1**) was first acetylated to give the known pentaacetate (**9**).[1] This acetate **9** was treated with sodium nitrite in acetic anhydride and acetic acid to give initially the *N*-nitroso compound, which decomposed into a cleaved mixture of the upper chain part with a double bond at C-5‴ and the lower chain part



Scheme 2. Synthesis of the authentic samples **2b** and **3b**. *Reagents and conditions*: (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%).

with 2,3-diacetoxy groups instead of 2-amido-3-acetoxy groups.[6,7] This mixture was hydrolyzed with potassium hydroxide and the products containing the unsaturated upper chain part and the 2,3-dihydroxylated lower chain part were oxidized with Lemieux–von Rudloff reagent (sodium periodate and potassium permanganate) to furnish a mixture of the desired degradation products **2a** and **3a**. A part of the mixture was methylated with diazomethane to give a mixture containing **2c** and **3c**, which were identified with authentic **2c** and **3c** by GC–MS analysis. The major part of the 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 (see Fig. 1). Plakoside A therefore possesses 11*S*,12*R*,11‴*S*,12‴*R* configuration. It should be mentioned that another cyclopropane-containing natural product, PHYLPA, a specific inhibitor of DNA poly-

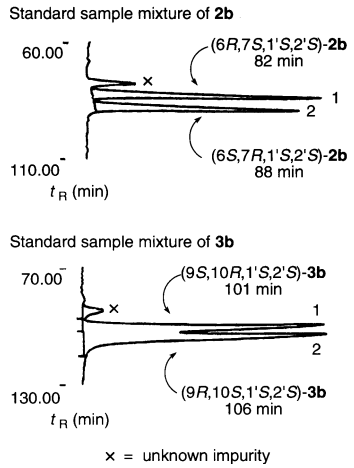
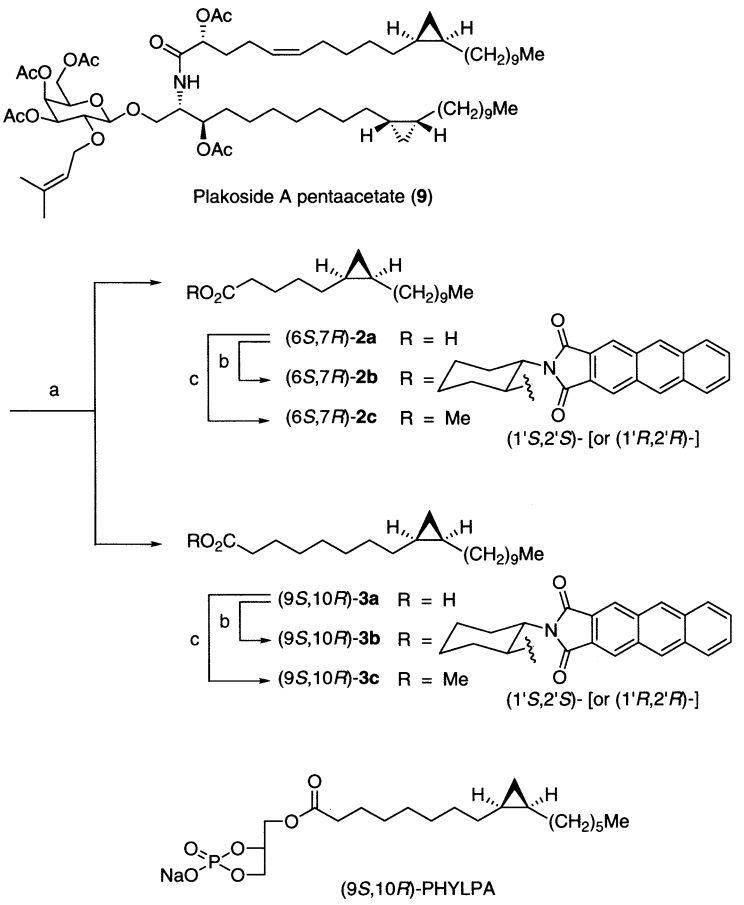


Figure 1. HPLC separation of the derivatized acids. Conditions: Reversed-phase column (Develosil C-30-UG-3, 3 μm; 4.6 mm×150 mm, Nomura Chemical Co., Aichi, Japan); column temp., −50°C; detection, fluorometry by monitoring the fluorescence intensity at 462 nm (excitation at 298 nm); mobile phase for **2b**, MeCN/THF/*n*-hexane (200:150:25) at the flow rate of 0.1 ml/min, for **3b**, MeCN/THF/*n*-hexane (175:175:10) at the flow rate of 0.2 ml/min.



Scheme 3. Degradation of plakoside A pentaacetate and the structure of PHYLPA. *Reagents and conditions*: (a) i. $NaNO_2$, Ac_2O, AcOH, $CHCl_3$, 0°C–room temp.; ii. KOH, EtOH, heat; iii. $NaIO_4$, $KMnO_4$, *t*-BuOH, H_2O; iv. dil. HCl (1.1 mg of a mixture of **2a** and **3a** starting from 2.0 mg of **9**); (b) (1*S*,2*S*)- or (1*R*,2*R*)-**4**, *N*,*N*-4-dimethylaminopyridine, EDC, toluene, MeCN, room temp., >10 h; (c) CH_2N_2, Et_2O.

merase α produced by a true slime mold *Physarum polycephalum*, also possesses 9*S*,10*R* configuration.[8]

In conclusion, the 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**[5] was extremely useful in solving the present problem.

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