[Chem. Pharm. Bull. 34(11)4641—4652(1986)]

Marine Natural Products. XVI.[1] Structures of Five New Diterpenes from an Okinawan Soft Coral of *Xenia* sp. (Xeniidae)

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(Received June 12, 1986)

Two pairs of diastereomeric diterpenes, xeniolone (**1**) and isoxeniolone (**3**), and hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**), were isolated, together with their putative biogenetic precursor, germacrexeniolone (**20**), from an Okinawan soft coral of *Xenia* sp. (Xeniidae). On the basis of chemical and physicochemical evidence, the absolute stereostructures of these five diterpenes have been elucidated. It has also been shown that germacrexeniolone (**20**) is gradually air-oxidized to yield xeniolone (**1**) and isoxeniolone (**3**) upon standing, while in an aqueous acetone solution, it yields hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**).

Keywords——soft coral; *Xenia* sp.; Xeniidae; xeniolone; isoxeniolone; hydratoxeniolone; hydratoisoxeniolone; germacrexeniolone; diterpene diastereomer

In our continuing studies in search of bioactive marine natural products,[2] we isolated a pair of diastereomeric diterpenes named xeniolone (**1**) and isoxeniolone (**3**), from an Okinawan soft coral of *Xenia* sp. (Xeniidae), and reported their absolute stereostructures, having a perhydroazulene skeleton, in a recent communication.[3] Further investigation of the chemical constituents of the same soft coral has led us to the isolation of another pair of new diastereomeric diterpenes, named hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**), and a new diterpene, germacrexeniolone (**20**), having a germacrene-c carbon skeleton. This paper presents a full account of the structure elucidation of these five diterpenes (**1**, **3**, **16**, **18**, **20**). It is also demonstrated that germacrexeniolone (**20**) is gradually converted in the air to xeniolone (**1**), isoxeniolone (**3**), hydratoxeniolone (**16**), and hydratoisoxeniolone (**18**), depending upon the conditions.[4]

The acetone extract of the fresh soft coral collected in July at Zamami-jima in Okinawa Prefecture was partitioned into an ethyl acetate–water mixture. Chromatographic separation of the ethyl acetate-soluble portion provided a mixture of xeniolone (**1**) and isoxeniolone (**3**), which gave a single spot on a thin-layer chromatogram (TLC), and another mixture of hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**), which also gave a single spot on ordinary TLC. High-performance liquid chromatography (HPLC) with a reversed-phase column of the mixture of xeniolone (**1**) and isoxeniolone (**3**) effected the separation of **1** and **3**, in 2 and 1.5% yields, respectively, from the above ethyl acetate-soluble portion.

Xeniolone (**1**) is an optically active diterpene. The infrared (IR) spectrum of **1** showed absorption bands due to a hydroxyl group (3618, 3450 (br) cm^{-1}) and a carbonyl moiety (1707 cm^{-1}). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals ascribable to a trisubstituted double bond (δ 5.99, 1H, d, J = 3.0 Hz, 6-H), a terminal methylene moiety (δ 4.88, 4.85, both 1H, s, 18-H_2), a methyl group geminal to a hydroxyl

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group (δ 1.46, 3H, s, 4-CH_3), and three secondary methyl groups [δ 1.04, 3H, d, J=7.0 Hz, 11-CH_3; δ 0.88, 6H, d, J=6.5 Hz, 15-$(CH_3)_2$]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of xeniolone (**1**), together with the above evidence, has shown that **1** is a dicarbocyclic diterpene.

Isoxeniolone (**3**) is a diastereomeric isomer of xeniolone (**1**) and gave an IR spectrum very similar to that of **1**. Ordinary TLC of **3** developing with a hexane–ethyl acetate (1 : 1) mixture showed an *Rf* value (0.5) identical with that of **1**. The ^{1}H- and ^{13}C-NMR spectra of isoxeniolone (**3**) showed signals very similar to those of xeniolone (**1**) [δ 6.00, 1H, d, J=3.0 Hz, 6-H; δ 4.87, 4.83, both 1H, s, 18-H_2; δ 1.44, 3H, s, 4-CH_3; δ 1.03, 3H, d, J=7.0 Hz, 11-CH_3; δ 0.88, 0.87; both 3H, d, J=6.5 Hz, 15-$(CH_3)_2$].

Dehydration of xeniolone (**1**) and isoxeniolone (**3**) with 10% palladium–carbon in *m*-xylene in the presence of iodine,[5] provided an identical azulene ketone (**5**) in good yields.[6] Acetylation of **1** and **3** with acetic anhydride and pyridine in the presence of silver cyanide,[7,8] yielded the respective monoacetates (**2**, **4**). Sensitized photooxygenation[5,9] of these monoacetates (**2**, **4**) in an acetone–pyridine (10 : 1) mixture containing Rose Bengal, and sub-

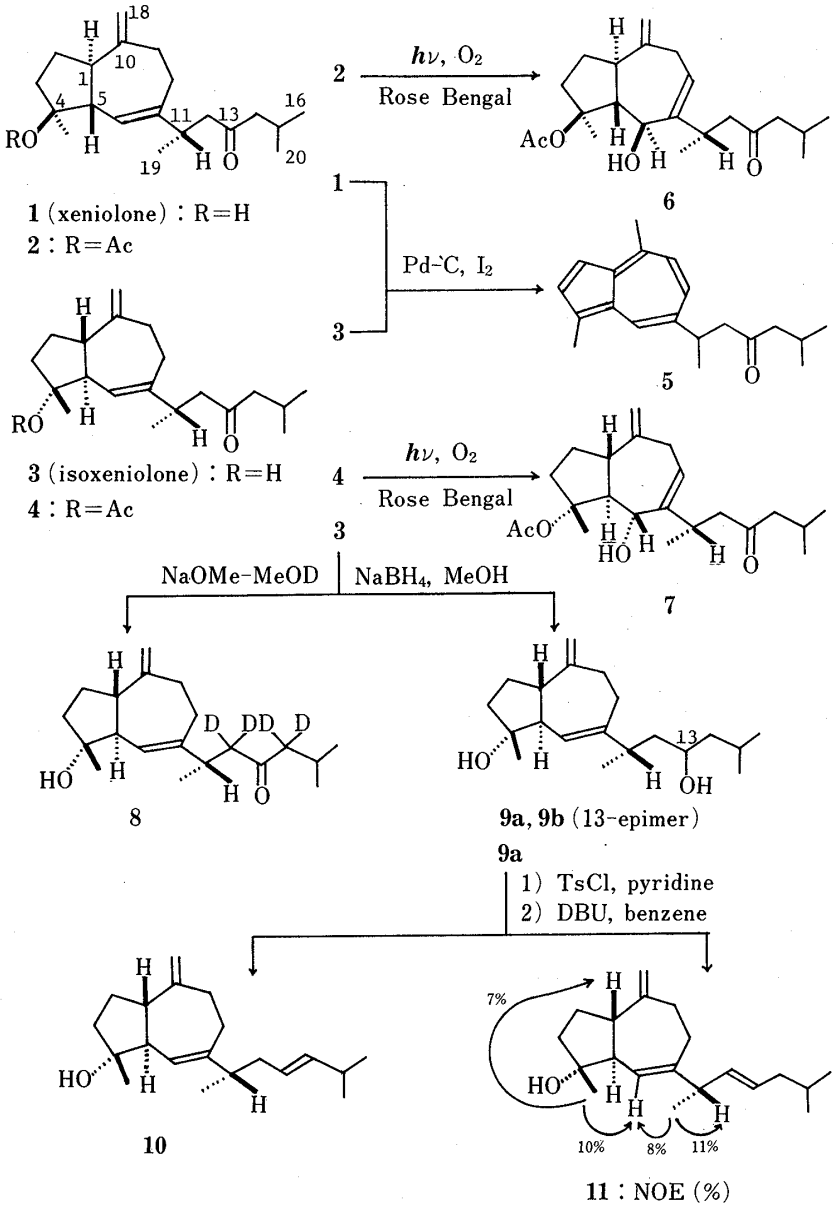

Chart 1

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sequent sodium sulfite reduction, furnished the allylic alcohols **6** (from **2**) and **7** (from **4**).

Treatment of isoxeniolone (**3**) with sodium methoxide in deuterium methoxide afforded a tetradeutero derivative (**8**), and mass spectrometric (MS) analysis of this has shown that **3** has a side chain containing a $-CH_2-CO-CH_2-CH(CH_3)_2$ moiety. Sodium borohydride reduction of **3** gave two diols (**9a**, **9b**). Tosylation of one of the diols (**9a**) followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) treatment yielded two isomeric trienols (**10**, **11**). In the ^{1}H-NMR spectrum of **11**, signals due to 1-H and 5-H were observed with a coupling of $J=$ 12 Hz, and the following nuclear Overhauser effects (NOEs) were observed: 1) 11% for the 11-H signal and 8% for the 6-H signal upon irradiation of 11-CH_3 and 2) 10% for 6-H and 7% for 1-H upon irradiation of 4-CH_3.

Based on the above evidence, it has been clarified that xeniolone (**1**) and isoxeniolone (**3**) are stereoisomeric diterpenes having a perhydroazulene skeleton. The relative configuration of the perhydroazulene moiety in isoxeniolone is expressed as shown in **3**.

Oxidation of xeniolone (**1**) and isoxeniolone (**3**) with osmium tetroxide in the presence of sodium periodate provided the nordiketo-alcohols **12** and **13**, respectively. The circular dichroism (CD) spectrum of **12** showed a negative Cotton curve, $[\theta]_{285}$ −5300 (neg. max.), due to the n→π* transition whereas **13** gave a positive CD curve, $[\theta]_{285}$ +6100 (pos. max.) (Fig. 1). Thus, the stereostructures of xeniolone (**1**) and isoxeniolone (**3**) can be formulated as shown, except for the common C-11 configuration. The identical C-11 configuration in **1** and **3** is further substantiated by the ready conversion from germacrexeniolone (**20**) to **1** and **3** (*vide infra*).

In order to determine the common C-11 configuration of xeniolone (**1**) and isoxeniolone (**3**), **1** and **3** were treated with methyl magnesium iodide and the resulting diol mixture (**14**)

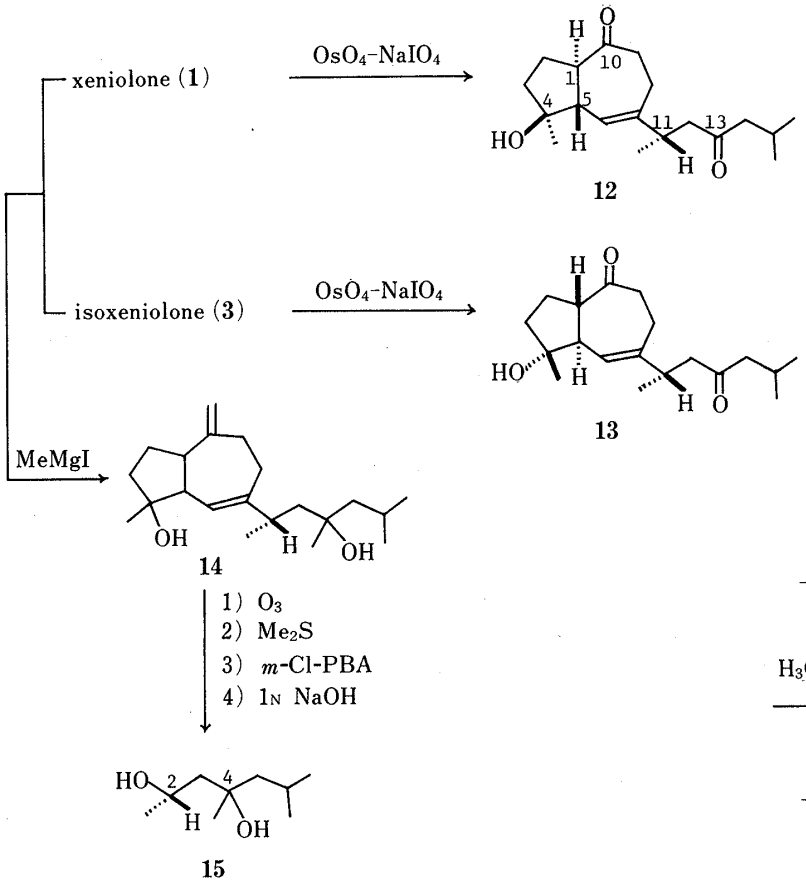

Chart 2

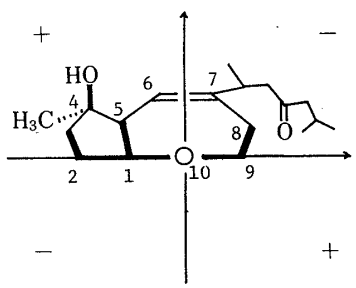

Fig. 1. The Octant Projection of **13**

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was subjected to successive reactions (ozone oxidation, Baeyer–Villiger oxidation, and alkaline hydrolysis) to yield a diol (**15**).[10] Application of Horeau's method[11] to **15** resulted in the recovery of α-phenylbutyric acid, $[\alpha]_D$ −1.9°. Thus, the 2*S* configuration in **15** has been clarified and consequently the absolute stereostructures of xeniolone (**1**) and isoxeniolone (**3**) have been determined as shown.

The mixture of hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**), which gave a single spot on TLC as mentioned above, was hardly separable and could not be separated by HPLC under various conditions so far tested. However, the separation was accomplished after acetylation. Thus, treatment of the mixture with acetic anhydride and pyridine in the presence of silver cyanide afforded a mixture of 4-*O*-acetates (again giving a single spot on TLC) which was subjected to reversed-phase HPLC to afford 4-*O*-acetylhydratoxeniolone (**17**) and 4-*O*-acetylhydratoisoxeniolone (**19**). Alkaline hydrolysis of **17** and **19** provided hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**), respectively.

Hydratoxeniolone (**16**) is an oily diterpene ketodiol. The ^{1}H-NMR spectrum of **16** showed signals due to an olefinic proton (δ 5.52, 1H, br d, *J*=*ca.* 3 Hz, 6-H), three secondary methyl groups [δ 0.91, 6H, d, *J*=7.0 Hz, 15-$(CH_3)_2$; δ 1.00, 3H, d, *J*=7.0 Hz, 11-CH_3], and two methyl groups geminal to a tertiary hydroxyl group (δ 1.20, 3H, s, 4-CH_3; δ 1.27, 3H, s, 10-CH_3). Hydratoisoxeniolone (**18**) is a diastereoisomer of hydratoxeniolone (**16**). The ^{1}H-NMR spectrum of **18** is very similar to that of **16**: 6-H at δ 5.52, br d, *J*=*ca.* 3.0 Hz; 4-CH_3 at δ 1.18, s; and 10-CH_3 at δ 1.25, s. It appeared that hydratoxeniolone (**16**) and hydrato-

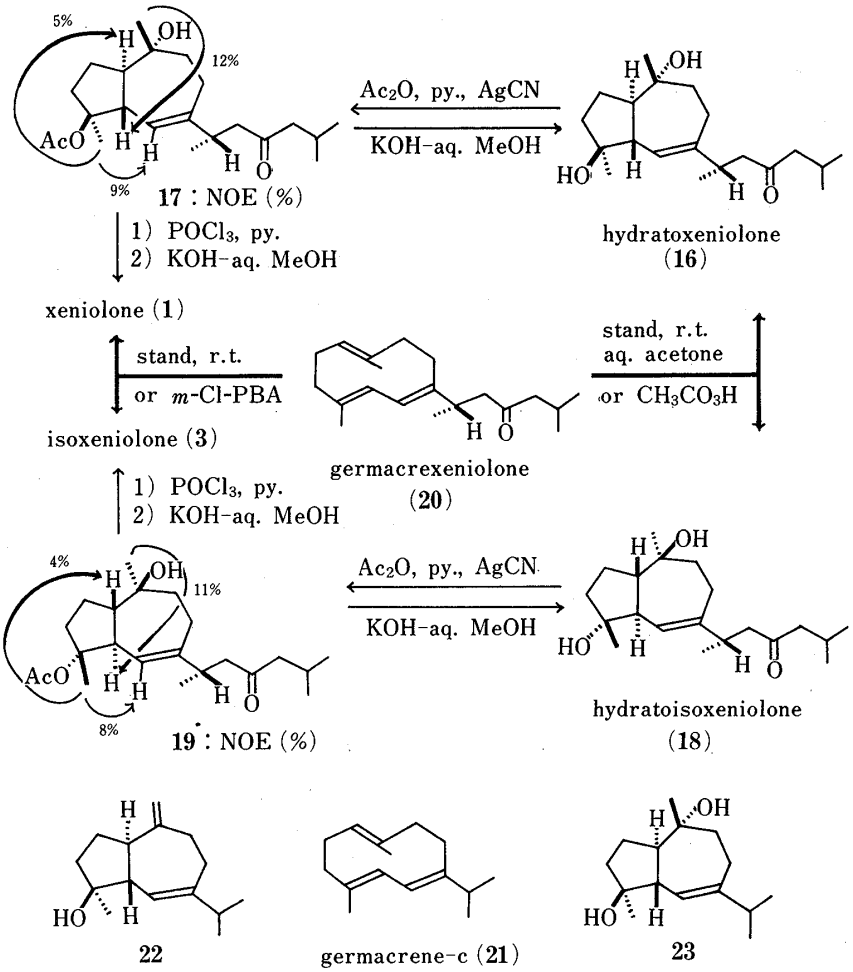

Chart 3

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isoxeniolone (**18**) are hydrated derivatives of xeniolone (**1**) and isoxeniolone (**3**), respectively, at their terminal methylene moiety at C-10.

In order to clarify the chemical correlation of these four compounds (**1**, **3**, **16**, and **18**), the following reactions were carried out. Thus, treatment of the above-mentioned 4-*O*-acetylhydratoxeniolone (**17**) and 4-*O*-acetylhydratoisoxeniolone (**19**) with phosphorus oxychloride in pyridine furnished 4-*O*-acetylxeniolone (**2**) and 4-*O*-acetylisoxeniolone (**4**), respectively. Alkaline hydrolysis of **2** and **4** provided xeniolone (**1**) and isoxeniolone (**3**). Therefore, it has been elucidated that hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**) are hydrated derivatives of xeniolone (**1**) and isoxeniolone (**3**), respectively, at the C-10, 18 bond.

The C-10 configurations of **16** and **18** have been determined by detailed NOE examinations of **17** and **19**. Irradiation of 4-CH_3 (δ 1.39) of **17** resulted in 5% NOE of 1-H (δ 1.86) and 9% NOE of 6-H (δ 5.55), whereas irradiation of 10-CH_3 (δ 1.25) resulted in 12% NOE of 5-H (δ 2.40). Similarly, irradiation of 4-CH_3 (δ 1.40) of **19** caused 4% and 8% increases of the signals due to 1-H (δ 1.85) and 6-H (δ 5.54). Irradiation of 10-CH_3 (δ 1.24) caused 11% increase of the 5-H signal (δ 2.39). Consequently, the absolute configurations of hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**) have been determined as shown. Here again, it has become clear that **16** and **18** are diastereomeric, having the common 11*S* configuration.

In our recent paper,[1] we reported the isolation of germacrene-c (**21**) and two racemic guaiane-type sesquiterpenes (**22**, **23**) from an Okinawan soft coral of *Xenia* sp. (Xen-83-ZM-1) which was collected at Zamami-jima and was closely related to the species investigated in this paper. It was shown there that guaiane-type sesquiterpenes (**22**, **23**) were secondarily formed from germacrene-c (**21**) in air. Since the hydroazulene skeletons of xeniolone (**1**) and isoxeniolone (**3**) are identical with or antipodal to the guaiane framework of **22** and those of hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**) are comparable to the guaiane framework of **23**, we looked for a germacrene-c type diterpenoid in the present *Xenia* sp. (Xen-83-ZM-2).[12]

Chromatographic separation of the above ethyl acetate-soluble portion (see Experimental) yielded such a diterpene, named germacrexeniolone (**20**). The ultraviolet (UV) spectrum of **20** showed an absorption maximum at 254 nm (ε 10000) which was comparable to the UV absorption of germacrene-c (**21**).[1]

Germacrexeniolone (**20**) obtained here was shown to be a single compound by TLC (silica gel, silver nitrate-impregnated silica gel), HPLC (ordinary phase, reversed-phase), and gas-liquid chromatography (GLC) (2% SE-30) examinations. However, the ^{1}H-NMR spectrum of **20** showed a complicated signal pattern comprising a pair of three-proton doublets assignable to three olefinic protons on the germacrene-c type skeleton of **20** [δ 6.25, 6.23, totally 1H, each d, J=9.5 Hz; δ 5.19, 5.15, totally 1H, each d, J=9.5 Hz; δ 4.95, 4.93, totally 1H, each dd-like, J=*ca.* 5, 2 Hz]. Furthermore, the ^{13}C-NMR spectrum of **20** (in deuterochloroform) showed signals due to five methyl groups (C-16, 17, 18, 19, 20), side chain carbons (C-11, 12, 13, 14, 15), six olefinic carbons (C-1, 4, 5, 6, 7, 10), and four methylene carbons (C-2, 3, 8, 9), each of which appeared as a double peak. Among these, signals due to the skeletal carbons of **20** coincided with carbon signals of germacrene-c (**21**)[1] except for the side-chain carbon signals. The ^{13}C-NMR spectra of germacrexeniolone (**20**) taken in d_6-benzene and d_5-pyridine also showed signal patterns of approximately a 1 : 1 mixture. It is noteworthy that the ^{13}C-NMR spectra of **20** taken in d_6-benzene gradually changed. When the measuring temperature was elevated (50 °C→70 °C→75 °C), the peak intensities of one of each pair of signals decreased and finally disappeared, leaving well-resolved signals of twenty carbons.

Therefore, it has been concluded that germacrexeniolone (**20**) is a diterpene counterpart of germacrene-c (**21**), and may exist in two conformers in solution at room temperature.

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As was shown in the conversion from germacrene-c (**21**) to two cyclization products (**22**, **23**),[1] prolonged exposure (for 7 d) of germacrexeniolone (**20**) at room temperature (25 °C) in air resulted in the formation of xeniolone (**1**) and isoxeniolone (**3**). The oxidative conversion from **20** to **1** and **3** was more readily effected by treatment of **20** with *m*-chloroperbenzoic acid (*m*-Cl-PBA). On the other hand, when germacrexeniolone (**20**) was left standing in an aqueous acetone solution, hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**) were formed, although the conversion proceeded much more slowly. Here again, the transformation of **20** to **16** and **18** was readily effected by peracetic acid oxidation of **20** in an acetone–phosphate buffer (pH 7.3) mixture. Thus, the absolute configuration of germacrexeniolone (**20**) has been determined and the diastereomeric correlation between xeniolone (**1**) and isoxeniolone (**3**) and between hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**) has been further substantiated.

Xeniolone (**1**) and isoxeniolone (**3**) were isolated as major diterpenes from the ethyl acetate-soluble portion of the soft coral and the chemical conversions from germacrexeniolone (**20**) to **1**, **3**, **16**, and **18** were shown to progress slowly. Therefore, it is considered that most of xeniolone (**1**), isoxeniolone (**3**), hydratoxeniolone (**16**), and hydratoisoxeniolone (**18**) are naturally occurring constituents. Since they are diastereomerically formed from **20** with retention of the 11*S* configuration of **20**, they may be separated chromatographically, in contrast to the isolation of **22** and **23** mostly in racemic forms as reported in our previous investigation.[1] In regard to the biogenetic pathway of these diterpenes, a scheme starting from geranylgeranyl pyrophosphate *via* germacrexeniolone (**20**) as shown in Chart 4 seems feasible.

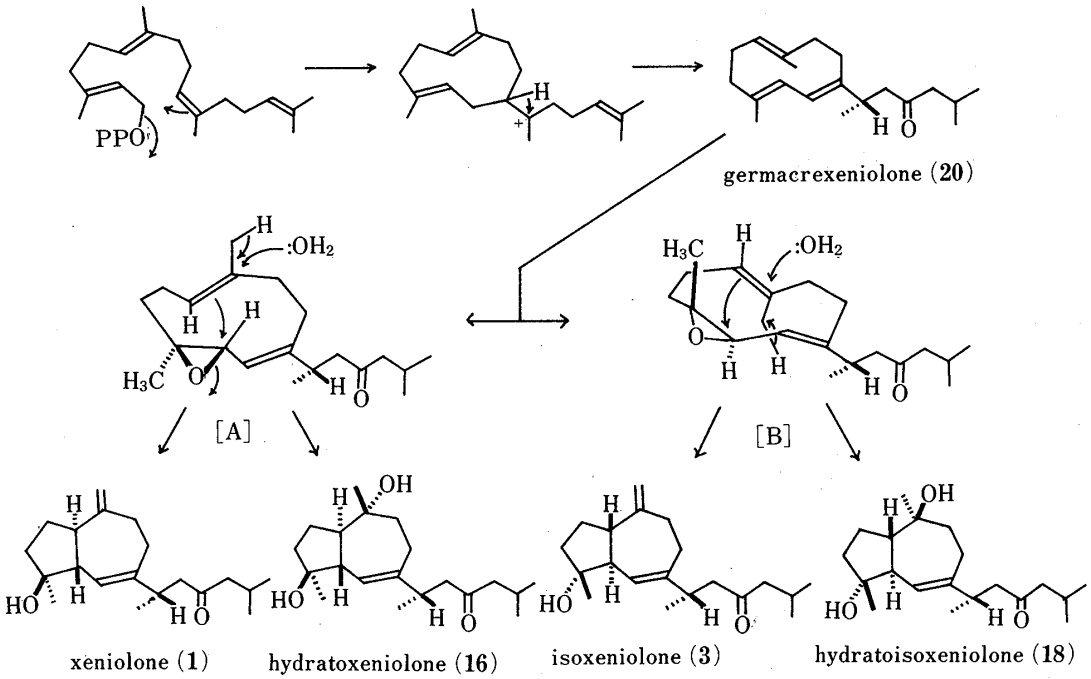

Chart 4

Some diterpenoids having a hydroazulene skeleton similar to those of the present diterpenes were isolated from various brown algae of Dictyotaceae[13a–h] and also from the sea hare *Aplysia depilans*,[14a,b] which is a predator of brown algae. Among them, pachydictyol[13a] and dictyotadiol[13c] were shown to have 11*R* configuration by X-ray analysis.

Experimental

The following instruments were used to obtain physical data: specific rotations, JASCO DIP-181 digital

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polarimeter (l=5 cm); UV spectra, Hitachi 330 spectrometer; IR spectra, Hitachi 260-30 IR spectrometer; CD spectra, JASCO J-500A spectropolarimeter and JASCO DP-501 data processor; mass and high-resolution MS, JEOL D-300 mass spectrometer; ^{1}H-NMR spectra, JEOL JNM FX-500S (500 MHz) or JEOL JNM FX-90Q (90 MHz) spectrometer; ^{13}C-NMR spectra, JEOL JNM FX-90Q (22.5 MHz) spectrometer. For chromatography, the following conditions were used: HPLC, Shimadzu LC-5A; column chromatography, Silica gel 60, 70—230 mesh (Merck); TLC: Silica gel 60 F_{254} (Merck, precoated); detection by spraying 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4 followed by heating.

Isolation of Xeniolone (1) and Isoxeniolone (3)——The soft coral (600 g) of *Xenia* sp. (Xen-83-ZM-2),[1,12] which was collected at Zamami-jima, Okinawa Prefecture, in July 1983 and preserved frozen, was cut finely and immersed in acetone. The acetone solution was concentrated under reduced pressure. The acetone extract was partitioned into an AcOEt–H_2O mixture. Removal of the solvent under reduced pressure furnished the AcOEt extract (27.5 g). A part of the extract (5 g) was subjected to column chromatography (SiO_2 300 g, hexane–AcOEt=3:1, $CHCl_3$–MeOH=40:1) to furnish a fraction containing germacrexeniolone (**20**) (1.6 g), a fraction containing xeniolone (**1**) and isoxeniolone (**3**) (1.2 g), and a fraction containing hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**) (1.2 g). The fraction of **1** and **3** was subjected to column chromatography (SiO_2 70 g, hexane–AcOEt=3:1) and subsequent HPLC (Cosmosil 5C_{18}, MeOH–H_2O=4:1) to furnish xeniolone (**1**) (106 mg) and isoxeniolone (**3**) (76 mg). Xeniolone (**1**), colorless oil, $[\alpha]_D^{22}$ −20° (c=1.0, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3618, 3450 (br), 1707, 1639. High-resolution MS: Found 304.239. Calcd for $C_{20}H_{32}O_2$ (M^+)=304.240. ^{1}H-NMR (500 MHz, d_5-pyridine) δ: 0.88 (6H, d, J=6.5 Hz, 15-(CH_3)$_2$), 1.04 (3H, d, J=7.0 Hz, 11-CH_3), 1.46 (3H, s, 4-CH_3), 2.42 (1H, br dd, J=*ca.* 12.0, 8.5 Hz, 1-H), 2.70 (1H, br d, J=*ca.* 12 Hz, 5-H), 2.83 (1H, m, 11-H), 4.85, 4.88 (both 1H, s, 18-H_2), 5.99 (1H, d, J=3.0 Hz, 6-H). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 47.0 (d, C-1), 80.4 (s, C-4), 54.9 (d, C-5), 123.6 (d, C-6), 146.8 (s, C-7), 153.4 (s, C-10), 38.9 (d, C-11), 49.0 (t, C-12), 210.1 (s, C-13), 52.3 (t, C-14), 24.4 (d, C-15), 22.6 (2C, q, C-16, 20), 24.1 (q, C-17), 106.7 (t, C-18), 19.5 (q, C-19), 40.0, 36.9, 30.1, 24.8 (each t, C-2, 3, 8, 9). MS m/z (%): 304 (M^+, 2), 286 ($M^+ - H_2O$, 13), 204 ($M^+ - H_2C$=C(OH)–CH_2–$CH(CH_3)_2$, 48), 146 (100). Isoxeniolone (**3**), colorless oil, $[\alpha]_D^{22}$ +31° (c=1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3608, 3408 (br), 1705, 1642. High-resolution MS: Found 304.240. Calcd for $C_{20}H_{32}O_2$ (M^+)=304.240. ^{1}H-NMR (500 MHz, d_5-pyridine) δ: 0.87, 0.88 (both 3H, d, J=6.5 Hz, 15-(CH_3)$_2$), 1.03 (3H, d, J=7.0 Hz, 11-CH_3), 1.44 (3H, s, 4-CH_3), 2.42 (1H, br dd, J=*ca.* 12.0, 8.5 Hz, 1-H), 2.69 (1H, br d, J=*ca.* 12.0 Hz, 5-H), 2.84 (1H, m, 11-H), 4.83, 4.87 (both 1H, s, 18-H_2), 6.00 (1H, d, J=3.0 Hz, 6-H). ^{13}C-NMR (22.5 MHz, $CDCl_3$), δc: 47.0 (d, C-1), 80.5 (s, C-4), 54.9 (d, C-5), 123.7 (d, C-6), 146.7 (s, C-7), 153.4 (s, C-10), 39.1 (d, C-11), 49.0 (t, C-12), 210.0 (s, C-13), 52.2 (t, C-14), 24.4 (d, C-15), 22.6 (2C, q, C-16, 20), 24.1 (q, C-17), 106.7 (t, C-18), 19.7 (q, C-19), 40.1, 36.8, 29.8, 24.8 (each t, C-2, 3, 8, 9). MS m/z (%): 304 (M^+, 3), 286 ($M^+ - H_2O$, 13), 204 (51), 146 (100).

Acetylation of Xeniolone (1) and Isoxeniolone (3)——1) A solution of **1** (30 mg) in pyridine (2 ml) was treated with AgCN (100 mg) and Ac_2O (1 ml), and the mixture was stirred under a nitrogen atmosphere at 90 °C for 10 h, then poured into ice-water. The whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract yielded a product which was purified by column chromatography (SiO_2 3 g, hexane–AcOEt=15:1) to furnish 4-*O*-acetylxeniolone (**2**) (27 mg). **2**, colorless oil, $[\alpha]_D^{29}$ +5° (c=2.6, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1722, 1710, 1639. High-resolution MS: Found 346.252. Calcd for $C_{22}H_{34}O_3$ (M^+)=346.251. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.90 (6H, d, J=6.5 Hz, 15-(CH_3)$_2$), 1.01 (3H, d, J=6.5 Hz, 11-CH_3), 1.43 (3H, s, 4-CH_3), 1.97 (3H, s), 4.70, 4.77 (both 1H, s, 18-H_2), 5.61 (1H, d, J=3.0 Hz, 6-H). MS m/z (%): 346 (M^+, 0.3), 286 (M^+ −AcOH, 49), 186 (286-side chain, 100). 2) A solution of **3** (30 mg) in pyridine (1.5 ml) was treated with AgCN (50 mg) and Ac_2O (0.7 ml) and the mixture was stirred under a nitrogen atmosphere at 90 °C for 10 h. Work-up of the reaction mixture followed by chromatographic purification as described above furnished 4-*O*-acetylisoxeniolone (**4**) (20 mg). **4**, colorless oil, $[\alpha]_D^{29}$ +11° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725, 1712, 1640. High-resolution MS: Found 346.252. Calcd for $C_{22}H_{34}O_3$ (M^+)=346.251. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.91 (6H, d, J=6.5 Hz, 15-(CH_3)$_2$), 1.01 (3H, d, J=6.5 Hz, 11-CH_3), 1.44 (3H, s, 4-CH_3), 1.98 (3H, s), 4.71, 4.78 (both 1H, s, 18-H_2), 5.62 (1H, d, J=3.0 Hz, 6-H). MS m/z (%): 346 (M^+, 0.2), 286 (49), 186 (100).

Photosensitized Oxygenation of 2 and 4——1) A solution of **2** (52 mg) in acetone–pyridine (10:1) (4 ml) was treated with Rose Bengal (10 mg), and the solution was irradiated externally with a 100 W high pressure Hg lamp at 0 °C for 1 h in a Pyrex tube, while bubbling oxygen. The reaction mixture was treated with aq. sat. Na_2SO_3 (1 ml) and stirred at room temperature (25 °C) for 1 h. The reaction mixture was then poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product which was purified by column chromatography (SiO_2 5 g, hexane–AcOEt=8:1) to furnish **6** (13 mg). **6**, colorless oil, $[\alpha]_D^{25}$ −47° (c=1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1704, 1638. High-resolution MS: Found 362.245. Calcd for $C_{22}H_{34}O_4$ (M^+)=362.245. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.91 (6H, d, J=6.5 Hz, 15-(CH_3)$_2$), 1.01 (3H, d, J=6.5 Hz, 11-CH_3), 1.65 (3H, s, 4-CH_3), 2.05 (3H, s), 2.31 (1H, m, 1-H), 2.87 (1H, m, 11-H), 3.15 (1H, dd, J=10.5, 10.5 Hz, 5-H), 4.22 (1H, d, J=10.5 Hz, 6-H), 4.64, 4.78 (both 1H, s, 18-H_2), 5.64 (1H, dd, J=9.5, 4.5 Hz, 8-H). MS m/z (%): 362 (M^+, 1), 344 ($M^+ - H_2O$, 6), 85 (100). 2) A solution of **4** (41 mg) in acetone–pyridine (10:1) (4 ml) was treated with Rose Bengal (10 mg) and photooxygenated as described above. Work-up of the reaction mixture followed by chromatographic purification as described above furnished **7** (13 mg). **7**, colorless oil, $[\alpha]_D^{25}$ −35° (c=0.6, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1704, 1640. High-resolution MS: Found 362.243. Calcd for $C_{22}H_{34}O_4$ (M^+)=362.245. ^{1}H-NMR (500 MHz, $CDCl_3$)

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δ: 0.88 (6H, d, J=6.5 Hz, 15-$(CH_3)_2$), 1.13 (3H, d, J=7.0 Hz, 11-CH_3), 1.67 (3H, s, 4-CH_3), 2.04 (3H, s), 2.31 (1H, m, 1-H), 2.79 (1H, m, 11-H), 3.05 (1H, dd, J=10.5, 10.5 Hz, 5-H), 4.41 (1H, d, J=10.5 Hz, 6-H), 4.62, 4.74 (both 1H, s, 18-H_2), 5.71 (1H, dd, J=9.5, 4.5 Hz, 8-H). MS m/z (%): 362 (M^+, 2), 344 (M^+-18, 10), 85 (100).

Dehydrogenation of Xeniolone (1) and Isoxeniolone (3) Giving 5——1) A solution of **1** (20 mg) in *m*-xylene (4 ml) was treated with 10% Pd–C (4 mg) and I_2 (2 mg), and the mixture was heated under reflux for 2 h, then filtered. Removal of the solvent under reduced pressure from the filtrate yielded a product which was purified by column chromatography (SiO_2 3 g, hexane–AcOEt=3:1) to furnish **5** (9 mg). **5**, blue oil,[6] UV λ_{max}^{MeOH} nm: (ε): 655 (300), 603 (350), 368 (3200), 352 (4600), 334 (4600), 305 (14000), 292 (31000), 286 (29000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1708. High-resolution MS: Found 282.200. Calcd for $C_{20}H_{26}O$ (M^+)=282.198. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.86, 0.87 (both 3H, d, J=6.5 Hz, 15-$(CH_3)_2$), 1.38 (3H, d, J=7.0 Hz, 11-CH_3), 2.68, 2.84 (both 3H, s, 1,4-CH_3), 3.48 (1H, d, J=7.0 Hz, 11-H), 7.00 (1H, d, J=10.5 Hz), 7.25 (1H, d, J=4.0 Hz), 7.41 (1H, dd, J=10.5, 2.0 Hz), 7.65 (1H, d, J=4.0 Hz), 8.18 (1H, d, J=2.0 Hz). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 209.3 (s), 144.7 (s), 140.5 (s), 137.3 (s), 136.4 (s), 136.2 (d), 135.3 (d), 133.4 (d), 125.7 (s), 125.1 (d), 113.2 (d), 52.7, 52.3 (both t, C-12, 14), 39.2 (d, C-11), 24.6 (d, C-15), 22.6 (2C, q, C-16, 20), 24.1 (q), 22.8 (q), 13.0 (q). MS m/z (%): 282 (M^+, 43), 183 (M^+ – side chain, 100). 2) A solution of **3** (25 mg) in *m*-xylene (4 ml) was treated with 10% Pd–C (5 mg) and I_2 (3 mg) and the whole mixture was heated under reflux for 2 h. Work-up of the reaction mixture followed by chromatographic purification as described above furnished **5** (13 mg). **5** obtained here was shown to be identical with the above authentic sample by TLC, UV, IR, ^{1}H-NMR, and MS comparisons.

Deuteration of Isoxeniolone (3) Giving 8——A solution of **3** (50 mg) in 1 N $NaOCH_3$–CH_3OD (2 ml) was left standing at room temperature (25 °C) for 12 h, then poured into ice-water, and the whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract yielded a product which was purified by column chromatography (SiO_2 5 g, hexane–AcOEt=2:1) to furnish **8** (44 mg). **8**, MS m/z (%): 308 (M^+, 20), 290 (M^+-H_2O, 9), 204 ($M^+-D_2C=C(OH)-CD_2-CH(CH_3)_2$, 46), 146 (100). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 153.5 (s), 146.9 (s), 123.7 (d), 106.8 (t), 80.5 (s), 55.0 (d), 47.1 (d), 40.2 (t), 38.9 (d), 36.9 (t), 30.1 (t), 24.9 (t), 24.3 (d), 24.2 (q), 22.6 (2C, q), 19.5 (q).

$NaBH_4$ Reduction of Isoxeniolone (3) Giving 9a and 9b——A solution of **3** (120 mg) in MeOH (30 ml) was treated with $NaBH_4$ (200 mg), and the mixture was stirred under a nitrogen atmosphere at room temperature (20 °C) for 1 h, then poured into ice-water. The whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product which was purified by column chromatography (SiO_2 12 g, hexane–AcOEt=4:1) and subsequently by HPLC (Cosmosil $5C_{18}$, MeOH–H_2O=10:1) to furnish **9a** (42 mg) and **9b** (30 mg). **9a**, colorless needles (from ether), mp 98—101 °C, $[\alpha]_D^{18}$ +24° (c=0.6, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3605, 3455 (br), 1636. High-resolution MS: Found 306.259. Calcd for $C_{20}H_{34}O_2$ (M^+)=306.256. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.90, 0.92 (both 3H, d, J=6.5 Hz), 1.00 (3H, d, J=7.0 Hz), 1.25 (3H, s), 3.72 (1H, m), 4.72, 4.79 (both 1H, s), 5.65 (1H, s). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 47.1 (d, C-1), 80.6 (s, C-4), 55.2 (d, C-5), 123.9 (d, C-6), 148.6 (s, C-7), 153.5 (s, C-10), 41.1 (d, C-11), 43.5 (t, C-12), 69.1 (d, C-13), 47.1 (t, C-14), 24.7 (d, C-15), 22.2, 23.7 (both q, C-16, 20), 24.3 (q, C-17), 106.9 (t, C-18), 19.9 (q, C-19), 24.9, 28.9, 36.9, 40.3 (each t, C-2, 3, 8, 9). MS m/z (%): 306 (M^+, 1), 288 (M^+-H_2O, 17), 270 (M^+-2H_2O, 3), 146 (100). **9b**, colorless oil, $[\alpha]_D^{22}$ +42° (c=0.6, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3602, 3450 (br), 1639. High-resolution MS: Found 306.256. Calcd for $C_{20}H_{34}O_2$ (M^+)=306.256. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.88, 0.90 (both 3H, d, J=6.5 Hz), 1.00 (3H, d, J=7.0 Hz), 1.25 (3H, s), 3.67 (1H, m), 4.73, 4.79 (both 1H, s), 5.64 (1H, br s). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 47.0 (d, C-1), 80.5 (s, C-4), 55.4 (d, C-5), 124.6 (d, C-6), 147.1 (s, C-7), 153.4 (s, C-10), 40.4 (d, C-11), 43.0 (t, C-12), 68.2 (d, C-13), 47.2 (t, C-14), 24.7 (d, C-15), 22.2, 23.5 (both q, C-16, 20), 24.3 (q, C-17), 106.9 (t, C-18), 20.2 (q, C-19), 24.9, 27.9, 36.9, 40.1 (each t, C-2, 3, 8, 9). MS m/z (%): 306 (M^+, 1), 288 (M^+-H_2O, 22), 270 (M^+-2H_2O, 4), 146 (100).

Dehydration of 9a Giving 10 and 11——1) A solution of **9a** (70 mg) in pyridine (3 ml) was treated with *p*-toluenesulfonyl chloride (TsCl) (200 mg), and the mixture was stirred at room temperature (25 °C) for 1 h, then poured into ice-water. The whole was extracted with AcOEt. The AcOEt extract was washed with aq. 5% HCl, aq. sat. $NaHCO_3$, and aq. sat. NaCl, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract yielded a product which was purified by column chromatography (SiO_2 7 g, benzene–AcOEt=4:1) to furnish the tosylate of **9a** (80 mg), colorless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3565 (br), 1649, 1611. ^{1}H-NMR (500 MHz, d_5-pyridine) δ: 0.82, 0.83 (both 3H, d, J=7.0 Hz), 0.95 (3H, d, J=7.0 Hz), 1.45 (3H, s), 2.24 (3H, s), 4.84 (1H, m), 4.88, 4.92 (both 1H, s), 5.96 (1H, d, J=3.0 Hz), 7.34 (2H, d, J=8.0 Hz), 8.08 (2H, d, J=8.0 Hz). MS m/z (%): 460 (M^+, 0.2), 164 (100). 2) A solution of the tosylate of **9a** (80 mg) in benzene (4 ml) was treated with DBU (12 ml), and the mixture was heated under reflux for 2 h, then poured into water. The whole was extracted with AcOEt. The AcOEt extract was washed successively with aq. 5% HCl, aq. sat. $NaHCO_3$, and aq. sat. NaCl, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product, which was purified by column chromatography (SiO_2 8 g, hexane–AcOEt=8:1) and HPLC (Cosmosil $5C_{18}$, MeOH–H_2O=6:1) to furnish **10** (24 mg) and **11** (6 mg). **10**, colorless oil, $[\alpha]_D^{18}$ +52° (c=1.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3605, 3455 (br), 1635. High-resolution MS: Found 288.247. Calcd for $C_{20}H_{32}O$ (M^+)=288.245. ^{1}H-NMR (500 MHz, d_5-pyridine) δ: 0.96, 0.97 (both 3H, d, J=6.5 Hz, 15-$(CH_3)_2$), 0.99 (3H, d, J=6.5 Hz, 11-CH_3), 1.46 (3H, s, 4-CH_3), 2.48 (1H, ddd-like, J=*ca.* 12, 8, 7 Hz, 1-H), 2.72 (1H, br d, J=*ca.* 12 Hz, 5-H), 4.85, 4.89 (both 1H, s, 18-H_2), 5.40 (2H, m, 13, 14-H), 5.95 (1H, d, J=3.0 Hz, 6-H).

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^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.28 (1H, dt, J=15.5, 7.5 Hz, 13-H), 5.36 (1H, dd, J=15.5, 6.0 Hz, 14-H). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 47.1 (d, C-1), 80.7 (s, C-4), 55.2 (d, C-5), 126.0 (d, C-6), 147.5 (s, C-7), 153.9 (s, C-10), 44.1 (d, C-11), 38.3 (t, C-12), 138.6 (d, C-13), 123.4 (d, C-14), 31.1 (d, C-15), 22.8, 23.2 (2C, q, C-16, 20), 24.2 (q, C-17), 106.5 (t, C-18), 19.2 (q, C-19), 24.9, 28.9, 36.8, 40.2 (each t, C-2, 3, 8, 9). MS m/z (%): 288 (M^+, 3), 270 ($M^+ - H_2O$, 3), 147 (100). **11**, colorless oil, $[\alpha]_D^{18}$ +27° (c=0.6, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3430 (br), 1635. High-resolution MS: Found 288.246. Calcd for $C_{20}H_{30}O$ (M^+)=288.245. ^{1}H-NMR (500 MHz, d_5-pyridine) δ: 0.87 (6H, d, J=6.5 Hz, 15-$(CH_3)_2$), 1.13 (3H, d, J=7.0 Hz, 11-CH_3), 1.46 (3H, s, 4-CH_3), 1.57 (1H, m, 15-H), 1.88 (1H, d, J=6.0 Hz, 14-H), 1.89 (1H, d, J=6.5 Hz, 14-H), 2.19 (1H, dd, J=13.5, 10.0 Hz), 2.29 (1H, dd, J=15.5, 10.0 Hz), 2.45 (1H, ddd-like, J=*ca.* 12, 8, 8 Hz), 2.60 (1H, dd, J=13.5, 10.0 Hz), 2.74 (1H, br d, J=*ca.* 12 Hz, 5-H), 2.85 (1H, m, 11-H), 4.85, 4.88 (both 1H, s, 18-H_2), 5.45 (2H, m, 12, 13-H), 6.04 (1H, d, J=2.5 Hz, 6-H). MS m/z (%): 288 (M^+, 5), 270 ($M^+ - H_2O$, 2), 119 (100).

Oxidation of Xeniolone (1) and Isoxeniolone (3) with OsO_4–$NaIO_4$——1) A solution of **1** (30 mg) in tetrahydrofuran (THF)–H_2O (5 : 8, 3 ml) was treated with 5 drops of aq. OsO_4 solution (OsO_4 10 mg, H_2O 10 ml) and $NaIO_4$ (50 mg), and the mixture was stirred at room temperature (25 °C) for 12 h, then poured into water. The whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. Na_2SO_3 and aq. sat. NaCl, then dried over $MgSO_4$. A product, obtained after removal of the solvent under reduced pressure from the AcOEt extract, was purified by column chromatography (SiO_2 3 g, hexane–AcOEt=4 : 1) to furnish **12** (12 mg). **12**, colorless oil, $[\alpha]_D^{25}$ −58° (c=0.7, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3598, 3480 (br), 1704. CD ($c=1.2\times10^{-2}$, MeOH): $[\theta]_{310}$ 0, $[\theta]_{285}$ −5300 (neg. max.), $[\theta]_{245}$ 0, $[\theta]_{225}$ +1900! High-resolution MS: Found 306.217. Calcd for $C_{19}H_{30}O_3$ (M^+)=306.219. ^{1}H-NMR (500 MHz, d_5-pyridine) δ: 0.89 (6H, d, J=7.0 Hz, 15-$(CH_3)_2$), 1.05 (3H, d, J=6.5 Hz, 11-CH_3), 1.39 (3H, s, 4-CH_3), 2.77 (1H, br d, J=*ca.* 12 Hz, 5-H), 2.86 (1H, m, 11-H), 3.07 (1H, m, 1-H), 6.06 (1H, s, 6-H). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 80.4 (s, C-4), 122.6 (d, C-6), 146.1 (s, C-7), 211.5 (s, C-10), 38.7 (d, C-11), 49.0 (t, C-12), 209.6 (s, C-13), 52.4 (t, C-14), 24.6 (d, C-15), 22.7 (2C, q, C-16, 20), 51.9, 53.0 (both d, C-1, 5), 19.3, 19.7 (both q, C-17, 19), 42.0, 39.4, 26.1, 22.2 (each t, C-2, 3, 8, 9). MS m/z (%): 306 (M^+, 3), 288 ($M^+ - H_2O$, 5), 206 (M^+ − side chain, 100). 2) Similarly, a solution of **3** (30 mg) in aq. THF (3 ml) was treated with 5 drops of aq. OsO_4 solution and $NaIO_4$ (50 mg) and the mixture was stirred at room temperature for 12 h. Work-up of the reaction mixture followed by chromatographic purification as described above furnished **13** (13 mg). **13**, colorless oil, $[\alpha]_D^{25}$ +40° (c=0.4, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3450 (br), 1701. CD ($c=3.6\times10^{-2}$, MeOH): $[\theta]_{320}$ 0, $[\theta]_{285}$ +6100 (pos. max.), $[\theta]_{238}$ 0, $[\theta]_{226}$ −850! High-resolution MS: Found 306.220. Calcd for $C_{19}H_{30}O_2$ (M^+)=306.219. ^{1}H-NMR (500 MHz, d_5-pyridine) δ: 0.90 (6H, d, J=6.5 Hz, 15-$(CH_3)_2$), 1.05 (3H, d, J=6.5 Hz, 11-CH_3), 1.40 (3H, s, 4-CH_3), 2.76 (1H, br d, J=12.5 Hz, 5-H), 2.87 (1H, m, 11-H), 3.07 (1H, m, 1-H), 6.06 (1H, s, 6-H). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 80.4 (s, C-4), 123.0 (d, C-6), 145.8 (s, C-7), 211.6 (s, C-10), 39.1 (d, C-11), 49.0 (t, C-12), 209.8 (s, C-13), 52.3 (t, C-14), 24.6 (d, C-15), 22.7 (2C, q, C-16, 20), 19.9, 19.3 (both q, C-17, 19), 52.9, 52.0 (both d, C-1, 5), 42.0, 39.4, 25.5, 22.3 (each t, C-2, 3, 8, 9). MS m/z (%): 306 (M^+, 2), 288 ($M^+ - H_2O$, 5), 206 (M^+ − side chain, 100).

Grignard Reaction of Xeniolone (1) and Isoxeniolone (3)——A Grignard reagent [prepared with Mg (300 mg), CH_3I (1.25 ml), THF (4 ml), and benzene (3 ml)] was treated with a solution of a mixture of **1** and **3** (500 mg) in benzene (4 ml). The reaction mixture was stirred under a nitrogen atmosphere at 40 °C for 30 min, then poured into ice-water, and the whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. A product, obtained after work-up of the AcOEt extract in the usual manner, was purified by column chromatography (SiO_2 60 g, hexane–AcOEt=5 : 1) to furnish a diol mixture (**14**) (297 mg). **14**, colorless oil. High-resolution MS: Found 320.269. Calcd for $C_{21}H_{36}O_2$=320.271. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.95 (6H, d, J=6.5 Hz, 15-$(CH_3)_2$), 1.02 (3H, d, J=7.0 Hz, 11-CH_3), 1.15 (3H, s, 13-CH_3), 1.25 (3H, s, 4-CH_3), 4.72, 4.78 (both 1H, s, 18-H_2), 5.69 (1H, br s, 6-H). MS m/z (%): 320 (M^+, 0.2), 302 ($M^+ - H_2O$, 6), 284 ($M^+ - 2H_2O$, 6), 146 (100).

Degradation of 14 Giving 15——A solution of **14** (297 mg) in hexane–AcOEt (6 : 1) (28 ml) was treated with pyridine (0.5 ml), and the mixture was bubbled with ozonized oxygen at −78 °C for 20 min. The reaction mixture was then treated with $(CH_3)_2S$ (7 ml), stirred at room temperature (25 °C) for 3 h, and poured into ice-water. The whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product (315 mg), which was dissolved in $CHCl_3$ (10 ml). The solution was treated with *m*-chloroperbenzoic acid (500 mg) and the mixture was stirred at room temperature for 60 h. The reaction mixture was treated with 1 N NaOH–aq. 80% MeOH (20 ml), stirred for a further 5 h, then poured into water. The whole was extracted with AcOEt. The AcOEt extract was washed with aq. dil. $NaHCO_3$ and aq. sat. NaCl, then dried over $MgSO_4$. A product, obtained after removal of the solvent under reduced pressure from the AcOEt extract, was purified by column chromatography (SiO_2 7 g, hexane–AcOEt=5 : 1) to furnish **15** (26 mg). **15**, colorless oil. Chemical ionization (CI)-MS (isobutane): 161 (M^++1, 10), 143 ($M^+ + 1 - H_2O$, 100). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.95, 1.01 (both 3H, d, J=6.0 Hz), 1.19 (3H, d, J=6.0 Hz), 1.25 (3H, s), 3.72 (1H, m).

Application of Horeau's Method to 15——A solution of **15** (26 mg) in pyridine (1 ml) was treated with α-phenylbutyric anhydride (62 μl), and the mixture was stirred under a nitrogen atmosphere at room temperature (25 °C) for 40 h, then partitioned into an AcOEt–aq. dil. $NaHCO_3$ mixture. Removal of the solvent under reduced pressure from the AcOEt phase gave a product, which was purified by column chromatography (SiO_2 4 g, hexane–

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AcOEt = 5 : 1) to furnish the α-phenylbutyl ester (13 mg). The aqueous phase was acidified with aq. dil. HCl and the whole aq. mixture was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. A product, obtained after removal of the solvent under reduced pressure, was purified by HPLC (Zorbax ODS, MeOH–H_2O = 8 : 1) to furnish α-phenylbutyric acid (51 mg). The α-phenylbutyl ester, colorless oil. High-resolution MS: Found 291.194. Calcd for $C_{18}H_{27}O_3$ ($M^+ - CH_3$) = 291.196. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.40 (1H, m), 5.11 (1H, m), 7.29 (total 5H, br s). MS *m/z* (%): 306 (M^+, 0.1), 291 ($M^+ - CH_3$, 1), 288 ($M^+ - H_2O$, 0.4), 119 (100). α-Phenylbutyric acid: $[\alpha]_D^{20}$ −1.9° (*c* = 4.2, benzene).

Isolation of Hydratoxeniolone (16) and Hydratoisoxeniolone (18)——A fraction containing **16** and **18** described above (0.7 g) was purified by column chromatography (SiO_2 70 g, $CHCl_3$–MeOH = 40 : 1) and HPLC (Cosmosil $5C_{18}$, MeOH–H_2O = 2 : 1) to provide a mixture of **16** and **18** (126 mg). The mixture was dissolved in pyridine (2 ml) and treated with AgCN (150 mg) and Ac_2O (2 ml). The reaction mixture was stirred under a nitrogen atmosphere at 90 °C for 24 h, and poured into ice-water, then the whole was worked up as described above for the acetylation of xeniolone (**1**). The product was purified by column chromatography (SiO_2 20 g, hexane–AcOEt = 10 : 1) and HPLC (Cosmosil $5C_{18}$, MeOH–H_2O = 2 : 1) to furnish **17** (21 mg) and **19** (20 mg). **17**, colorless oil, $[\alpha]_D^{22}$ +23° (*c* = 1.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3605, 3458 (br), 1723. High-resolution MS: Found 304.240. Calcd for $C_{20}H_{32}O_2$ ($M^+ - AcOH$) = 304.240. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.91 (6H, d, *J* = 7.0 Hz, 15-$(CH_3)_2$), 1.01 (3H, d, *J* = 7.0 Hz, 11-CH_3), 1.25 (3H, s, 10-CH_3), 1.39 (3H, s, 4-CH_3), 1.86 (1H, m, 1-H), 1.99 (3H, s), 2.40 (1H, br d, *J* = *ca.* 11.5 Hz, 5-H), 2.70 (1H, m, 11-H), 5.55 (1H, d, *J* = 3.0 Hz, 6-H). MS *m/z* (%): 304 ($M^+ - AcOH$, 10), 286 ($M^+ - AcOH - H_2O$, 63), 186 (100). **19**, colorless oil, $[\alpha]_D^{22}$ −16° (*c* = 1.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3460 (br), 1721. High-resolution MS: Found 304.240. Calcd for $C_{20}H_{32}O_2$ ($M^+ - AcOH$) = 304.240. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.90 (6H, d, *J* = 7.0 Hz), 1.00 (3H, d, *J* = 7.0 Hz), 1.24 (3H, s), 1.40 (3H, s), 1.85 (1H, m), 1.99 (3H, s), 2.39 (1H, br d, *J* = 11.5 Hz), 2.70 (1H, m), 5.54 (1H, d, *J* = 3.0 Hz). MS *m/z* (%): 304 ($M^+ - AcOH$, 8), 286 (304 − H_2O, 50), 57 (100). **17** (21 mg) was treated with a solution of NaOH (200 mg) in aq. 90% MeOH (15 ml), and the mixture was stirred at room temperature (25 °C) for 10 h, then poured into water. The whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract furnished hydratoxeniolone (**16**) (12 mg). **16**, colorless oil, $[\alpha]_D^{22}$ +8° (*c* = 0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3440 (br), 1703. High-resolution MS: Found 322.251. Calcd for $C_{20}H_{34}O_3$ (M^+) = 322.251. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.91 (6H, d, *J* = 7.0 Hz, 15-$(CH_3)_2$), 1.00 (3H, d, *J* = 7.0 Hz, 11-CH_3), 1.20 (3H, s, 4-CH_3), 1.27 (3H, s, 10-CH_3), 1.87 (1H, m, 1-H), 2.17 (1H, br d, *J* = 10.5 Hz, 5-H), 2.69 (1H, m, 11-H), 5.52 (1H, d, *J* = 3.0 Hz, 6-H). NOE: irradiation of 4-CH_3 caused 5% NOE for 1-H, 10% NOE for 6-H; irradiation of 10-CH_3 caused 4% NOE for 5-H. ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 80.1 (s, C-4), 123.6 (d, C-6), 147.0 (s, C-7), 75.1 (s, C-10), 38.9 (d, C-11), 49.1 (t, C-12), 210.0 (s, C-13), 52.3 (t, C-14), 24.5 (d, C-15), 22.7 (3C, q, C-16, 17, 20), 21.6 (q, C-18), 19.6 (q, C-19), 50.5, 50.7 (both d, C-1, 5), 42.5, 40.5, 25.3, 21.6 (each t, C-2, 3, 8, 9). MS *m/z* (%): 322 (M^+, 0.2), 304 ($M^+ - H_2O$, 0.4), 286 ($M^+ - 2H_2O$, 4), 146 (100). Alkaline hydrolysis of **19** (20 mg) in the same manner furnished hydratoisoxeniolone (**18**) (19 mg). **18**, colorless oil, $[\alpha]_D^{22}$ −4.7° (*c* = 1.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450 (br), 1705. High-resolution MS: Found 322.251. Calcd for $C_{20}H_{34}O_3$ (M^+) = 322.251. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.90 (6H, d, *J* = 7.0 Hz), 0.99 (3H, d, *J* = 7.0 Hz), 1.18 (3H, s), 1.25 (3H, s), 1.86 (1H, m), 2.16 (1H, br d, *J* = *ca.* 10.5 Hz), 2.68 (1H, m), 5.52 (1H, d, *J* = 3.0 Hz). Upon irradiation of 4-CH_3 (δ 1.18), 5% NOE for 1-H (δ 1.86) and 10% NOE for 6-H (δ 5.52) were observed, and upon irradiation of 10-CH_3 (δ 1.25), 6% NOE for 5-H (δ 2.16) was observed. ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 80.0 (s, C-4), 123.6 (d, C-6), 146.6 (s, C-7), 75.0 (s, C-10), 39.0 (d, C-11), 49.0 (t, C-12), 210.1 (s, C-13), 52.2 (t, C-14), 24.4 (d, C-15), 22.6 (2C, q, C-16, 20), 22.6, 21.4 (both q, C-17, 18), 19.6 (q, C-19), 50.4 (2C, d, C-1, 5), 42.5, 40.4, 24.9, 21.5 (each t, C-2, 3, 8, 9). MS *m/z* (%): 322 (M^+, 0.4), 304 ($M^+ - H_2O$, 6), 288 ($M^+ - 2H_2O$, 5), 146 (100).

Dehydration of 17 and 19 Giving 2 and 4——A solution of **17** (18 mg) in pyridine (3 ml) was treated with $POCl_3$ (3 drops), and the mixture was stirred under a nitrogen atmosphere at 0 °C for 2 h and then at room temperature (25 °C) for a further 7 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product, which was purified by column chromatography (SiO_2 2 g, hexane–AcOEt = 10 : 1) and HPLC (Zorbax ODS, MeOH–H_2O = 6 : 1) to furnish **2** (5 mg). Dehydration of **19** (17 mg) in the same manner furnished **4** (5 mg). **2** and **4** thus obtained were shown to be identical with the respective authentic samples by TLC (hexane–AcOEt = 2 : 1, *Rf* = 0.75), $[\alpha]_D$, and ^{1}H-NMR comparisons.

Alkaline Hydrolysis of 2 and 4 Giving Xeniolone (1) and Isoxeniolone (3)——**2** (5 mg) obtained above from **17** was dissolved in 1% KOH–aq. 90% MeOH (1.5 ml), and the mixture was stirred at room temperature for 22 h, then poured into water. The whole was extracted with AcOEt. The AcOEt extract was washed with aq. sat. NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product, which was purified by column chromatography (SiO_2 2 g, hexane–AcOEt = 10 : 1) to furnish xeniolone (**1**) (3 mg). Alkaline hydrolysis of **4** (5 mg), which was obtained from **19**, in the same manner furnished isoxeniolone (**3**) (4 mg). Xeniolone (**1**) and isoxeniolone (**3**) thus obtained were shown to be identical with the respective authentic samples by $[\alpha]_D$, IR, MS, ^{1}H-NMR and HPLC (Cosmosil $5C_{18}$) comparisons.

Isolation of Germacrexeniolone (20)——A fraction containing germacrexeniolone (**20**) described above (1.6 g) was purified by column chromatography (SiO_2 100 g, hexane–AcOEt = 50 : 1) and HPLC (Zorbax SIL, hexane–

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ether = 200 : 3) to furnish germacrexeniolone (**20**) (145 mg). **20**, colorless oil, $[\alpha]_D^{20}$ +33° (c = 1.4, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 254 (10000), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1707. High-resolution MS: Found 288.247. Calcd for $C_{20}H_{32}O$ (M^+) = 288.245. MS m/z (%): 288 (M^+, 20), 188 (100). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.25, 6.23 (total 1H, each d, J = 9.5 Hz), 5.19, 5.15 (total 1H, each d, J = 9.5 Hz), 4.95, 4.93 (total 1H, dd-like each, J = *ca.* 5, 2 Hz). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δc: 209.3 (s, C-13), 143.1, 142.4 (total 1C, s), 140.9 (s), 129.0 (d), 127.8 (s), 125.0 (d), 123.4, 122.6 (total 1C, d), 52.4 (t, C-14), 49.8, 49.5 (total 1C, t, C-12), 39.7 (t), 39.7, 39.3 (total 1C, t), 38.3, 36.8 (total 1C, d, C-11), 32.6, 31.0 (total 1C, t), 29.6, 27.5 (total 1C, t), 24.4 (d, C-15), 22.5 (2C, q, C-16, 20), 20.4, 19.9 (both q, C-17, 19), 16.4 (q, C-18).

Conversion from Germacrexeniolone (20) to Xeniolone (1) and Isoxeniolone (3)——1) Germacrexeniolone (**20**) (30 mg) was left standing in air at room temperature (25 °C) for 7 d. The resulting product was purified by column chromatography (SiO_2 3 g, hexane–AcOEt = 3 : 1) and HPLC (Zorbax ODS, MeOH–H_2O = 4 : 1) to furnish xeniolone (**1**) (5 mg) and isoxeniolone (**3**) (6 mg). 2) A solution of germacrexeniolone (**20**) (5 mg) in $CHCl_3$ (1 ml) was treated with *m*-Cl-PBA (4 mg), and the mixture was stirred at room temperature (20 °C) for 30 min, then poured into ice-water. The whole was extracted with AcOEt. The AcOEt extract was washed successively with aq. 15% Na_2SO_3, aq. sat. $NaHCO_3$, and brine, then dried over $MgSO_4$. The product, obtained after work-up of the extract in the usual manner, was purified by column chromatography and HPLC as described above to furnish xeniolone (**1**) (2 mg) and isoxeniolone (**3**) (2 mg). **1** and **3** thus obtained here were shown to be identical with the respective authentic samples by $[\alpha]_D$, IR, MS, ^{1}H-NMR (90 MHz, $CDCl_3$), and HPLC (Zorbax ODS) comparisons.

Conversion from Germacrexeniolone (20) to Hydratoxeniolone (16) and Hydratoisoxeniolone (18) and Their Monoacetates (17, 19)——1) A solution of germacrexeniolone (**20**) (100 mg) in aq. 90% acetone (2 ml) was stirred at room temperature (25 °C) for 40 d.[15] Removal of the solvent under reduced pressure from the reaction mixture gave a product, which was purified by column chromatography (SiO_2 10 g, $CHCl_3$–MeOH = 10 : 1) to afford a mixture of hydratoxeniolone (**16**) and hydratoisoxeniolone (**18**) (70 mg). The mixture was acetylated as described above for acetylation of **16** and **18** and the product was purified by HPLC (Cosmosil $5C_{18}$, MeOH–H_2O = 2 : 1) to furnish **17** (7 mg) and **19** (6 mg). 2) A solution of germacrexeniolone (**20**) (25 mg) in acetone (2 ml) was treated with CH_3COOOH (0.25 ml) and phosphate buffer (pH 7.3) (0.5 ml), and the mixture was stirred at room temperature (20 °C) for 1 h, then poured into water. The whole was extracted with AcOEt. A product, obtained after removal of the solvent under reduced pressure from the AcOEt extract, was purified by column chromatography (SiO_2 2 g, $CHCl_3$–MeOH = 40 : 1) to give a mixture of **16** and **18** (12 mg). The mixture was acetylated with Ac_2O (0.25 ml) and AgCN (30 mg) in pyridine (0.5 ml) under a nitrogen atmosphere with stirring at 90 °C for 10 h, then poured into ice-water. The whole was extracted with AcOEt. A product, obtained after work-up of the AcOEt extract in the usual manner, was purified by column chromatography (SiO_2 2 g, hexane–AcOEt = 5 : 1) and HPLC (Cosmosil $5C_{18}$, MeOH–H_2O = 2 : 1) to furnish **17** (3 mg) and **19** (3 mg). **17** and **19** thus obtained here were shown to be identical with the respective authentic samples by $[\alpha]_D$, IR, MS, ^{1}H-NMR (90 MHz, $CDCl_3$), and HPLC (Cosmosil $5C_{18}$) comparisons.

Acknowledgement The authors are grateful to the Ministry of Education, Science, and Culture of Japan for financial support (Grant No. 59470121). One of the authors (Z. Cui) is grateful to the State Education Commission, the People's Republic of China for giving him the opportunity to study at Osaka University.

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