Studies on the Constituents of Brazilian Propolis. II[1)]

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Seven new *p*-coumaric acid derivatives along with seventeen known compounds, including four flavonoids, one prenylated phenolic acid, four diterpenoic acids, one lignan, two *p*-coumaric acid esters and five cinnamic acid derivatives, were isolated from the ethyl acetate soluble fraction of 75% ethanol extract of Brazilian propolis. New compounds were elucidated as (*E*)-2,3-dihydroconiferyl *p*-coumarate, (*E*)-3-{2,3-dihydro-2-[2-[(*E*)-*p*-coumaroyloxy]-1-methylethyl]-5-benzofuranyl}-2-propenoic acid, (*E*)-4-(2,3-dihydrocinnamoyloxy)cinnamic acid, (*E*)-3-(2,2-dimethyl-3,4-dihydro-3-hydroxy-2*H*-1-benzopyran-6-yl)-2-propenoic acid, (*E*)-3-[2,3-dihydro-2-(1-methylethenyl)-5-benzofuranyl]-2-propenoic acid, (*E*)-3-[2,3-dihydro-2-(1-methylethenyl)-7-prenyl-5-benzofuranyl]-2-propenoic acid and (*E*)-3-{3-[(*E*)-4-(2,3-dihydrocinnamoyloxy)-3-methyl-2-butenyl]-4-hydroxy-5-prenylphenyl}-2-propenoic acid, on the basis of spectral evidence and chemical reaction. Five compounds: dihydrokaempferol (aromadendrin), 6-methoxykaempferol, 4-hydroxy-3-prenylbenzoic acid, plicatin B and capillartemisin A, were isolated from propolis for the first time.

Key words propolis; *Apis mellifera*; *p*-coumaric acid derivative; diterpenoic acid; flavonoid; Brazil

Propolis (bee glue) is a resinous hive product gathered by honeybees (*Apis mellifera*) from the buds and bark of certain trees and plants. We previously reported on the isolation and characterization of prenylcinnamic acid derivatives from Brazilian propolis.[1)] In this paper, we describe the isolation and structural elucidation of 24 constituents, including seven new p-coumaric acid derivatives from Brazilian propolis.

Results and Discussion

Propolis, which was obtained from the state of Minas Gerais in Brazil, was extracted with 75% ethanol at room temperature. The extract was processed by the method described in the Experimental section. Twenty-four compounds were isolated from the ethyl acetate soluble fraction of the 75% ethanol extract of Brazilian propolis.

Compounds **1**—**4** were known flavonoids. They were identified as naringenin[2,3)] (**1**), sakuranetin[3)] (**2**), dihydrokaempferol (aromadendrin)[3,4)] (**3**) and 6-methoxykaempferol[5)] (**4**). Compound **5** was a known phenolic compound. It was identified as 4-hydroxy-3-prenylbenzoic acid[6)] (**5**). Compounds **6**—**9** were known diterpenoic acids. They were identified as 15-acetoxyisocupressic acid[7)] (**6**), agathic acid 15-methyl ester[8)] (**7**), communic acid[7)] (**8**) and dehydroabietic acid[9)] (**9**). Compound **10** was a lignan of a trimeric coniferyl alcohol. It was identified as 1-(4-hydroxy-3-methoxyphenyl)-1,2-bis{4-[(*E*)-3-acetoxypropen-1-yl]-2-methoxyphenoxy}-propan-3-ol acetate[10)] (**10**). Compounds **11** and **12** were known *p*-coumaric acid esters. They were identified as benzyl *p*-coumarate (**11**) and phenethyl *p*-coumarate (**12**). Compounds **13**—**17** were known cinnamic acid derivatives. They were identified as cinnamic acid (**13**), ferulic acid (**14**), 3-(2,2-dimethy-2*H*-1-benzopyran-6-yl)-2-propenoic acid[11)] (**15**), plicatin B[12)] (**16**) and capillartemisin A[13)] (**17**). These known compounds were identified by comparison of spectral data and specific optical rotation with values in the literature and/or authentic samples. This is the first report of the isolation of **3**, **4**, **5**, **16** and **17** from propolis.

Compound **18** was obtained as an amorphous powder and afforded a $[M+H]^+$ ion peak at *m*/*z* 329 ($C_{19}H_{21}O_5$) in the positive FAB-MS. The IR spectrum of **18** showed a signal at 1688 cm^{-1}. The ^{1}H-NMR spectrum displayed signals due to a dihydroconiferyl group [δ 1.98 (2H, dt, J=7.5, 6.5 Hz), 2.66 (2H, t, J=7.5 Hz), 3.83 (3H, s), 4.16 (2H, t, J=6.5 Hz), 6.64 (1H, dd, J=8.5, 1.5 Hz), 6.71 (1H, d, J=8.5 Hz), 6.78 (1H, d, J=1.5 Hz)] except for those of the *p*-coumaric acid moiety. Assignment of the signals in the ^{1}H- and ^{13}C-NMR spectra was confirmed based on ^{1}H–^{1}H shift correlation spectroscopy (COSY) and ^{1}H–^{13}C COSY spectral data. In the heteronuclear multiple-bond correlation (HMBC) spectrum, the oxygenated methylene signal δ 4.16 (H-9′) showed correlation with the carboxyl carbon signal at δ 168.6 (C-9). Therefore, the structure of **18** was deduced to be (*E*)-2,3-dihydroconiferyl *p*-coumarate.

Compound **19** was obtained as an amorphous powder and afforded a $[M]^+$ ion peak at *m*/*z* 394 ($C_{23}H_{22}O_6$) in the positive FAB-MS. The IR spectrum of **19** showed signals at 1688 and 1682 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra, and the ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectral data revealed signals due to a *p*-coumaric acid moiety and agreed with those of **18**. The ^{1}H-NMR and ^{1}H–^{1}H COSY spectrum of **19** exhibited one methyl [δ 1.05 (3H, d, J=7.0 Hz)], one methine [δ 2.23 (1H, m)], one methylene [δ 3.11 (1H, dd, J=16.0, 8.0 Hz) and 3.35 (1H, dd, J=16.0, 4.4 Hz)], one oxygenated methylene [δ 4.22 (2H, m)], one oxygenated methine [δ 4.85 (1H, m)], two olefins [δ 6.30 (1H, d, J=16.0 Hz), 7.56 (1H, d, J=16.0 Hz) and δ 6.33 (1H, d, J=16.0 Hz), 7.61 (1H, d, J=16.0 Hz)], one aromatic ABX system [δ 6.74 (1H, d, J=8.5 Hz), 7.32 (1H, br d, J=8.5 Hz), 7.47 (1 H, br s)] and one aromatic A_2B_2 system [δ 6.81 (2H, d, J=8.5 Hz), 7.46 (2H, d, J=8.5 Hz)]. In the HMBC spectrum, the signal at δ 4.22 (H-12) showed correlation with the carboxyl carbon signals at δ 169.2 (C-9′), so the ester is made with carboxylic acid of *p*-coumaric acid. Other long-range correlations, depicted in Fig. 1 by arrows, indicated the presence of a *p*-coumaric acid moiety and a dihydrobenzofuran ring. Consequently, the structure of **19** was deduced to be (*E*)-3-{2,3-dihydro-2-[2-[(*E*)-*p*-coumaroyloxy]-1-methylethyl]-5-benzofuranyl}-2-propenoic acid. The configurations of C-2 and C-11 were not determined.

Compound **20** was obtained as an amorphous powder and

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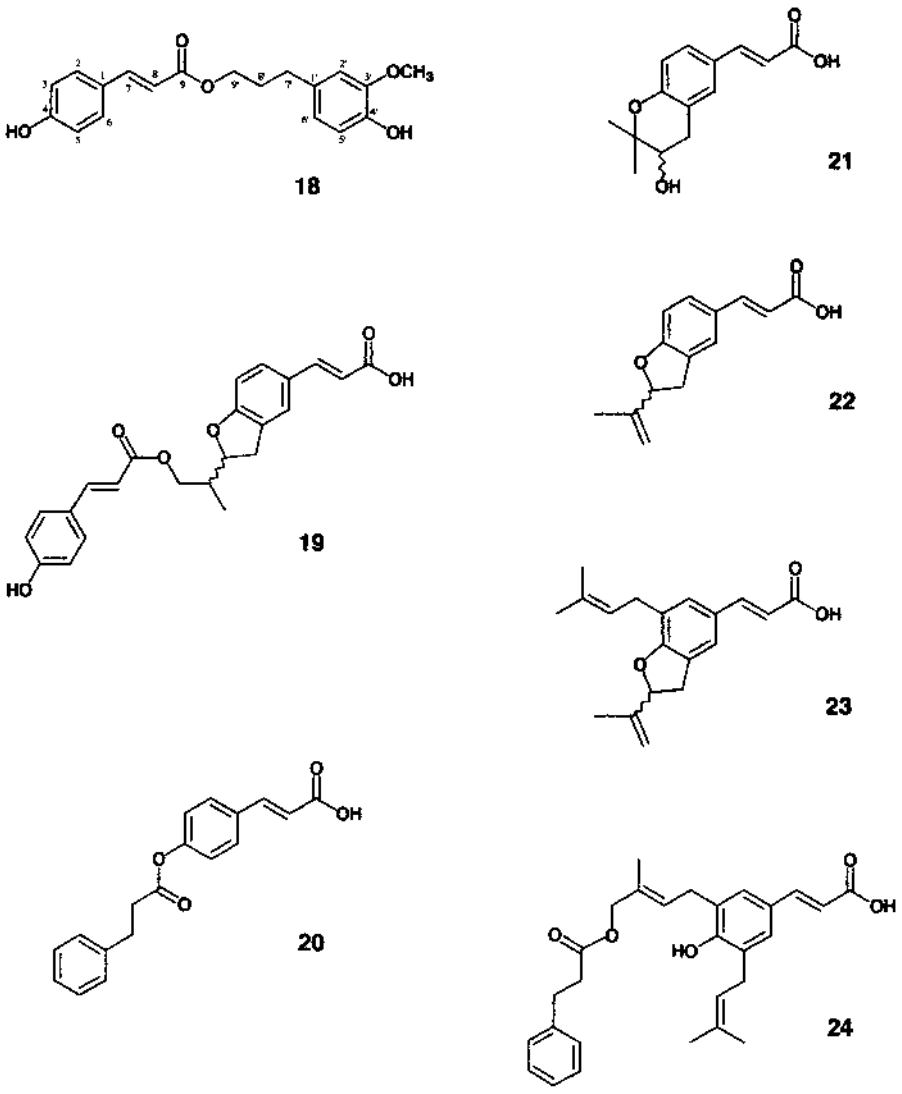

Chart 1

afforded a $[M+H]^+$ ion peak at *m/z* 297 ($C_{18}H_{17}O_4$) in the positive FAB-MS. The IR spectra of **20** showed signals at 1757 and 1686 cm^{-1}. The ^1H- and ^{13}C-NMR and the ^1H–^1H and ^1H–^{13}C COSY spectral data showed the presence of one dihydrocinnamoyl group and one *p*-coumaric acid moiety. In the HMBC spectrum, the signal at δ 2.90 (H-8′) showed a correlation with the carbon signals at δ 139.9 which was assigned to C-1′ of the dihydrocinnamoyl group, not the *p*-oxybenzene group. The possibility of a dihydro-*p*-coumaric acid moiety was denied. This structure was determined by comparison with the data of *p*-coumaric acid and (*E*)-3-prenyl-4-(2,3-dihydrocinnamoyloxy)cinnamic acid,[1,14] which attached a prenyl group to **20**. Thus, the structure of **20** was concluded to be (*E*)-4-(2,3-dihydrocinnamoyloxy)cinnamic acid.

Compound **21** was obtained as an amorphous powder and afforded a $[M]^+$ ion peak at *m/z* 248 ($C_{14}H_{16}O_4$) in the positive FAB-MS. The IR spectrum of **21** showed a signal at 1682 cm^{-1}. The ^1H-NMR spectrum indicated the presence of two tertiary methyls [δ 1.26 (3H, s), 1.34 (3H, s)], one methylene [δ 2.75 (1H, dd, *J*=16.5, 7.0 Hz), 3.04 (1H, dd, *J*=16.5, 5.0 Hz)], one hydroxy methine [δ 3.78 (1H, dd, *J*=7.0, 5.0 Hz)], one propenoic acid group [δ 6.30 (1H, d, *J*=16.0 Hz), δ 7.58 (1H, d, *J*=16.0 Hz)] and one aromatic ABX system [δ 6.76 (1H, d, *J*=8.0 Hz), 7.32 (1H, br s), 7.34 (1H, br d, *J*=8.0 Hz)]. In the HMBC spectrum, the signal at δ 3.78 (H-3) showed a correlation with two tertiary methyl carbon signals at δ 25.9 (C-12) and 21.4 (C-13), and with one aromatic carbon at δ 121.8 (C-4a). In addition, the aromatic proton signal at δ 7.32 (H-5) correlated with the methylene carbon signal at δ 31.9 (C-4) and 146.4 (C-9). Therefore, the position of the hydroxyl group in **21** was determined to be C-3. Other long-range correlations indicated the presence of a dihydrobenzopyran ring (Fig. 2). This structure was also supported by comparison with the data of (*E*)-3-(2,2-dimethyl-3,4-dihydro-3-hydroxy-8-prenyl-2*H*-1-benzopyran-6-yl)-2-propenoic acid.[1,8] Consequently, the structure of **21** was deduced to be (*E*)-3-(2,2-dimethyl-3,4-dihydro-3-hydroxy-2*H*-1-benzopyran-6-yl)-2-propenoic acid.

Compound **22** was obtained as an amorphous powder and afforded a $[M+H]^+$ ion peak at *m/z* 231 ($C_{14}H_{15}O_3$) in the positive FAB-MS. The IR spectrum of **22** showed a signal at 1682 cm^{-1}. Based on the ^1H- and ^{13}C-NMR spectra, **22** was determined to be a cinnamic acid derivative. The ^1H-NMR spectrum of **22** revealed one oxygenated methine [δ 4.35 (1H, dd, *J*=8.0, 4.5 Hz)], one methylene [δ 2.78 (1H, dd, *J*=13.5, 8.0 Hz), 2.91 (1H, dd, *J*=13.5, 4.5 Hz)], and a set of

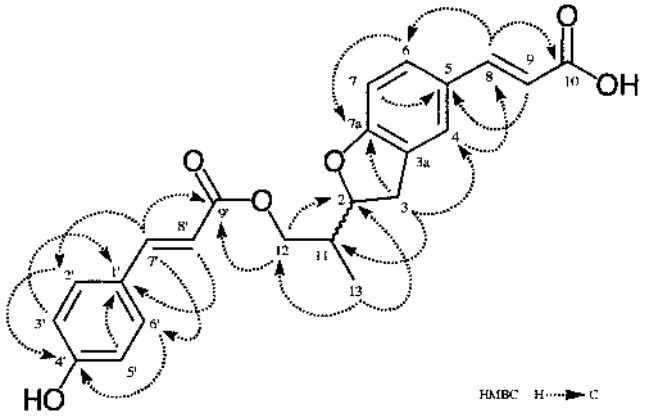

Fig. 1. HMBC Correlation of **19**

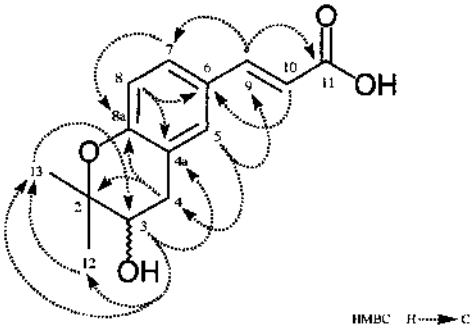

Fig. 2. HMBC Correlation of **21**

isopropenyl signals [δ 1.79 (3H, s), 4.77 (1H, s), 4.85 (1H, s)]. In the HMBC spectrum, the signal at δ 4.35 (H-2) showed correlation with the carbon signals at δ 18.1 (C-13), 111.3 (C-12) and 127.6 (C-3a). The aromatic proton signal at δ 7.34 (H-4) correlated with the methylene carbon signal at δ 38.1 (C-3) (Fig. 3).

Methylation of **22** with diazomethane furnished the methyl ester (**22a**). Compound **22a** afforded a $[M+H]^+$ ion peak at *m*/*z* 245 ($C_{15}H_{17}O_3$) in the positive FAB-MS. The IR spectrum, 1688 cm^{-1}, suggested the ester of **22**. **22a** was in agreement with the NMR data of the (*E*)-3-[2,3-dihydro-2-(1-methylethenyl)-5-benzofuranyl]-2-propenoic acid methyl ester which was previously isolated from *Baccharis linearis*.[11] Based on these facts, the structure of **22** was deduced to be (*E*)-3-[2,3-dihydro-2-(1-methylethenyl)-5-benzofuranyl]-2-propenoic acid.

Compound **23** was obtained as an amorphous powder and afforded a $[M+H]^+$ ion peak at *m*/*z* 299 ($C_{19}H_{23}O_3$) which was 68 mass units larger than that of **22** in the positive FAB-MS. The IR spectrum of **23** showed a signal at 1682 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra exhibited signals similar to those of **22**. The ^{1}H-NMR spectrum revealed a set of prenyl signals [δ 1.73 (3H, s), 1.78 (3H, s), 3.36 (2H, d, J=7.0 Hz), 5.33 (1H, br t)] and two aromatic signals [δ 7.11 (1H, br s), 7.25 (1H, br s)]. The structure of **23** was determined by analysis of its ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectrum and HMBC spectrum, and by comparison with the data of **22** and **22a**.[11] In the HMBC spectrum of **23**, the proton signal at δ 7.68 (H-8) showed correlation with the aromatic carbon signals at δ 128.9 (C-6) and 129.8 (C-4). So, the prenyl moiety is attached at the 7 position. Other long-range correlations

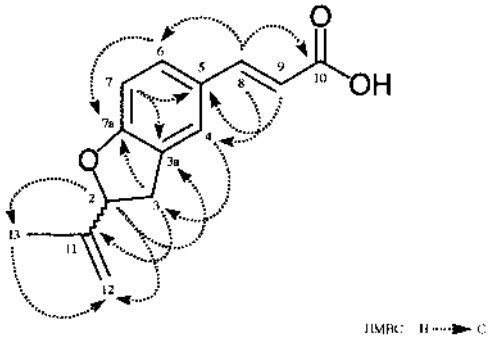

Fig. 3. HMBC Correlation of **22**

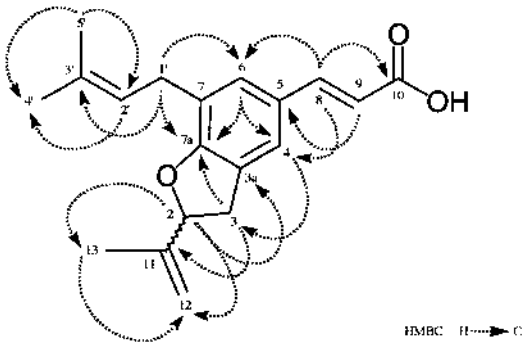

Fig. 4. HMBC Correlation of **23**

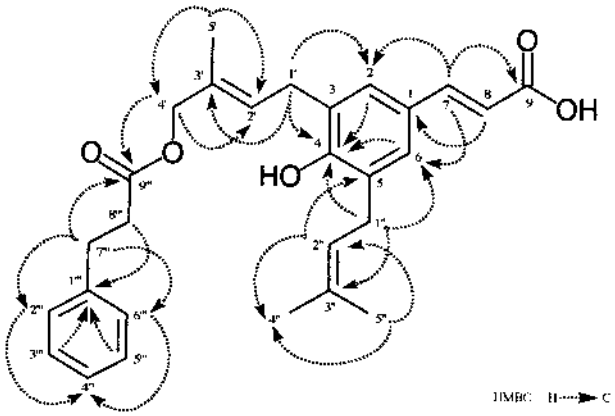

Fig. 5. HMBC Correlation of **24**

are depicted in Fig. 4. Consequently, the structure of **23** was concluded to be (*E*)-3-[2,3-dihydro-2-(1-methylethenyl)-7-prenyl-5-benzofuranyl]-2-propenoic acid.

Compound **24** was obtained as an amorphous powder and afforded a $[M-H]^+$ ion peak at *m*/*z* 447 ($C_{28}H_{31}O_5$) in the positive FAB-MS. The IR spectrum of **24** showed signals at 1720 and 1686 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra exhibited signals similar to those of capillartemisin A[13] (**17**), except for the presence of a dihydrocinnamoyl moiety.[1,14] Capillartemisin A is the *E*-hydroxyisoprenyl type, while capillartemisin B[13] is the *Z* type. So, **24** is the *E* type. In the HMBC spectrum, the oxygenated methylene proton signal at δ 4.52 (H-4′) showed a correlation with the carboxyl carbon signal at δ 172.8 (C-9‴). Other long-range correlations, depicted in Fig. 5 by arrows, supported the presence of a dihydrocinnamoyl group and capillartemisin A. Thus, the structure of **24** was deduced to be (*E*)-3-{4-hydroxy-3-[(*E*)-4-

(2,3-dihydrocinnamoyloxy)-3-methyl-2-butenyl]-5-prenylphenyl}-2-propenoic acid.

In this study, we found that the isolated compounds **1**, **2**, **9**, **15**, **16** and the methyl ester of **22** were contained in different *Baccharis* species (Compositae)[11,16] which have been widely used as a folk medicine in Brazil. The genus *Baccharis* is widespread in the tropical South American zone, and has many known constituents, including phenolic compounds, flavonoids and diterpenes. Thus, the phytochemical investigation suggested that *Baccharis* species are a significant sources of propolis.

Experimental

Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. UV spectra were measured on a Beckman DU 640 spectrophotometer. IR spectra were measured on a JASCO FT/IR-230 fourier transform IR spectrometer. FAB-MS spectra were taken on a JEOL JMS-SX102 spectrometer. ^{1}H- and ^{13}C-NMR were recorded on a JEOL GSX-500 (500 and 125.65 MHz, respectively). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectra were recorded with standard JEOL software. HPLC was run on JASCO system 880 and JASCO system 987 instruments. Reversed-phase HPLC was carried out on a column of Deverosil ODS-15/30 and Deverosil ODS-5 (Nomura Chemical, Ltd.). Detection was by UV absorption at 280 or 205 nm.

Propolis Material Brazilian propolis glue, which was obtained from the state of Minas Gerais in Brazil, was supplied by Dai Ki Kenko-kan Co., Ltd.

Extraction and Isolation Propolis (450 g) was extracted with 75% EtOH (1 l) at room temperature for 24 h. After filtration, concentration of the 75% EtOH extract (150 g) under reduced pressure gave a crude material (29.5 g). It was partitioned with EtOAc–H_2O to yield an EtOAc extract (21.8 g), a H_2O extract (6.3 g) and a residue (1.4 g). The EtOAc extract (5.0 g) was subjected to silica gel column chromatography with a *n*-hexane–$CHCl_3$–MeOH gradient system to give eight fractions [fr. 1 *n*-hexane eluate (5.8 mg); fr. 2 *n*-hexane : $CHCl_3$=1 : 1 eluate (5.8 mg); fr. 3 $CHCl_3$ eluate (1197.8 mg); fr. 4 $CHCl_3$: MeOH=9 : 1 eluate (2354.3 mg); fr. 5 $CHCl_3$: MeOH=8 : 2 eluate (295.6 mg); fr. 6 $CHCl_3$: MeOH=1 : 1 eluate (350.4 mg); fr. 7 MeOH eluate (295.9 mg); fr. 8 MeOH+1%AcOH eluate (669.4 mg)].

Fraction 3 [$CHCl_3$ eluate] (1197.8 mg) was repeatedly rechromatographed by preparative HPLC [ODS: 2%AcOH in CH_3CN–H_2O, 1%AcOH in MeOH–H_2O (UV 280 nm) or CH_3CN–H_2O, MeOH–H_2O (UV 205 nm)] to furnish compounds **2** (2.5 mg), **6** (3.0 mg), **7** (3.7 mg), **8** (12.5 mg), **9** (4.6 mg), **10** (4.1 mg), **11** (2.1 mg), **12** (2.5 mg), **16** (3.1 mg) and **23** (5.9 mg). Fraction 4 [$CHCl_3$: MeOH=9 : 1 eluate] (2.0 g) was subjected to reversed-phase chromatography by preparative HPLC, using a gradient solvent of 2% AcOH in CH_3CN–H_2O (2 : 8) to (8 : 2), to give 76 fractions. These were rechromatographed by preparative HPLC [ODS: 2%AcOH in CH_3CN–H_2O and 1%AcOH in MeOH–H_2O (UV 280 nm)] to give compounds **1** (1.1 mg), **3** (2.6 mg), **4** (2.6 mg), **5** (3.1 mg), **13** (1.0 mg), **14** (2.7 mg), **15** (14.5 mg), **17** (3.9 mg), **18** (1.8 mg), **19** (4.1 mg), **20** (1.7 mg), **21** (4.8 mg), **22** (3.0 mg), **23** (7.0 mg) and **24** (2.1 mg). The known compounds were identified by comparison of spectral data and/or specific optical rotation with reported values and/or authentic samples and chemical reactions.

Compound 18 Amorphous powder. FAB-MS *m/z*: 329 $[M+H]^+$. UV λ_{max}^{MeOH} nm (log ε): 224 (4.36), 290 (4.24), 311 (4.26). IR (KBr): 1688 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.98 (2H, dt, *J*=7.5, 6.5 Hz, H-8′), 2.66 (2H, t, *J*=7.5 Hz, H-7′), 3.83 (3H, s, OMe), 4.16 (2H, t, *J*=6.5 Hz, H-9′), 6.33 (1H, d, *J*=16.0 Hz, H-8), 6.64 (1H, dd, *J*=8.5, 1.5 Hz, H-6′), 6.71 (1H, d, *J*=8.5 Hz, H-5′), 6.78 (1H, d, *J*=1.5 Hz, H-2′), 6.81(2H, d, *J*=8.5 Hz, H-3, 5), 7.46 (2H, d, *J*=8.5 Hz, H-2, 6), 7.58 (1H, d, *J*=16.0 Hz, H-7). ^{13}C-NMR (CD_3OD) δ: 31.8 (C-8′), 32.7 (C-7′), 56.4 (OMe), 64.6 (C-9′), 113.2 (C-2′), 115.2 (C-5′), 115.8 (C-8), 116.8 (C-3, 5), 121.9 (C-6′), 127.2 (C-1), 131.2 (C-2, 6), 133.4 (C-1′), 145.7 (C-4′), 146.5 (C-7), 148.9 (C-3′), 161.3 (C-4), 168.6 (C-9).

Compound 19 Amorphous powder. FAB-MS *m/z*: 394 $[M]^+$. UV λ_{max}^{MeOH} nm (log ε): 211 (4.43), 226 (4.38), 311 (4.59). IR (KBr): 1688, 1682 cm^{-1}. $[\alpha]_D^{25}$ −16.7° (*c*=0.22, MeOH). ^{1}H-NMR (CD_3OD) δ: 1.05 (3H, d, *J*=7.0 Hz, H-13), 2.23 (1H, m, H-11), 3.11 (1H, dd, *J*=16.0, 8.0 Hz, Ha-3), 3.35 (1H, dd, *J*=16.0, 4.5 Hz, Hb-3), 4.22 (2H, m, H-12), 4.85 (1H, m, H-2), 6.30 (1H, d, *J*=16.0 Hz, H-9), 6.33 (1H, d, *J*=16.0 Hz, H-8′), 6.74 (1H, d, *J*=8.5 Hz, H-7), 6.81 (2H, d, *J*=8.5 Hz, H-3′, 5′), 7.32 (1H, br d, *J*=8.5 Hz, H-6), 7.46 (2H, d, *J*=8.5 Hz, H-2′, 6′), 7.47 (1H, br s, H-4), 7.56 (1H, d, *J*=16.0 Hz, H-8), 7.61 (1H, d, *J*=16.0 Hz, H-7′). ^{13}C-NMR (CD_3OD) δ: 11.7 (C-13), 33.5 (C-3), 39.4 (C-11), 67.1 (C-12), 85.8 (C-2), 110.2 (C-7), 115.0 (C-8′), 116.0 (C-9), 116.8 (C-3′, 5′), 125.5 (C-4), 127.1 (C-1′), 128.9 (C-5), 129.5 (C-3a), 130.6 (C-6), 131.2 (C-2′, 6′), 145.9 (C-8), 146.7 (C-7′), 161.3 (C-4′), 163.2 (C-7a), 169.2 (C-9′), 172.0 (C-10).

Compound 20 Amorphous powder. FAB-MS *m/z*: 297 $[M+H]^+$. UV λ_{max}^{MeOH} nm (log ε): 205 (4.26), 272 (4.11). IR (KBr): 1757, 1686 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.90 (2H, t, *J*=7.5 Hz, H-8′), 3.08 (2H, t, *J*=7.5 Hz, H-7′), 6.40 (1H, d, *J*=16.0 Hz, H-8), 7.05 (2H, d, *J*=7.5 Hz, H-3, 5), 7.24—7.34 (5H, m, H-2′, 3′, 4′, 5′, 6′), 7.54 (2H, d, *J*=7.5 Hz, H-2, 6), 7.71 (1H, d, *J*=16.0 Hz, H-9). ^{13}C-NMR ($CDCl_3$) δ: 30.9 (C-7′), 36.0 (C-8′), 117.0 (C-8), 122.2 (C-3, 5), 126.5 (C-4′), 128.4 (C-2′, 6′), 128.6 (C-3′, 5′), 129.4 (C-2, 6), 131.9 (C-1), 139.9 (C-1′), 147.0 (C-7), 152.0 (C-8), 171.1 (C-9), 172.3 (C-9′).

Compound 21 Amorphous powder. FAB-MS *m/z*: 248 $[M]^+$. UV λ_{max}^{MeOH} nm (log ε): 202 (4.19), 212 (4.14), 225 (4.06), 294 (4.17), 302 (4.16). IR (KBr): 1682 cm^{-1}. $[\alpha]_D^{25}$ +1.35° (*c*=0.22, MeOH). ^{1}H-NMR (CD_3OD) δ: 1.26 (3H, s, H-13), 1.34 (3H, s, H-12), 2.75 (1H, dd, *J*=16.5, 7.0 Hz, Ha-4), 3.04 (1H, dd, *J*=16.5, 5.0 Hz, Hb-4), 3.78 (1H, dd, *J*=7.0, 5.0 Hz, H-3), 6.30 (1H, d, *J*=16.0 Hz, H-10), 6.76 (1H, d, *J*=8.0 Hz, H-8), 7.32 (1H, br s, H-5), 7.34 (1H, br d, *J*=8.0 Hz, H-7), 7.58 (1H, d, *J*=16.0 Hz, H-9). ^{13}C-NMR (CD_3 OD) δ: 21.4 (C-13), 25.9 (C-12), 31.9 (C-4), 70.1 (C-3), 78.8 (C-2), 116.3 (C-10), 118.6 (C-8), 121.8 (C-4a), 128.1 (C-6), 128.5 (C-7), 131.5 (C-5), 146.4 (C-9), 156.7 (C-8a), 171.0 (C-11).

Compound 22 Amorphous powder. FAB-MS *m/z*: 231 $[M+H]^+$. UV λ_{max}^{MeOH} nm (log ε): 203 (4.30), 231 (4.09), 312 (4.15). IR (KBr): 1682 cm^{-1}. $[\alpha]_D^{25}$ −0.74° (*c*=0.14, MeOH). ^{1}H-NMR (CD_3OD) δ: 1.79 (3H, s, H-13), 2.78 (1H, dd, *J*=13.5, 8.0 Hz, Ha-3), 2.91 (1H, dd, *J*=13.5, 4.5 Hz, Hb-3), 4.35 (1H, dd, *J*=8.0, 4.5 Hz, H-2), 4.77 (1H, s, Ha-12), 4.85 (1H, s, Hb-12), 6.27 (1H, d, *J*=16.0 Hz, H-9), 6.79 (1H, d, *J*=8.5 Hz, H-7), 7.31 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 7.34 (1H, d, *J*=2.0 Hz, H-4), 7.57 (1H, d, *J*=16.0 Hz, H-8). ^{13}C-NMR (CD_3OD) δ: 18.1 (C-13), 38.1 (C-3), 76.4 (C-2), 111.3 (C-12), 115.7 (C-9), 116.7 (C-7), 127.2 (C-5), 127.6 (C-3a), 129.1 (C-6), 132.8 (C-4), 146.8 (C-8), 148.7 (C-11), 159.4 (C-7a), 171.4 (C-10).

Diazomethane Methylation of Compound 22 A solution of **22** (2.8 mg) in MeOH (1 ml) was methylated with an excess of CH_2N_2–Et_2O until the yellow color persisted. The solvent was removed under reduced pressure to furnish **22a** (2.8 mg).

Methyl ester of **22** (**22a**) Amorphous powder. FAB-MS *m/z*: 245 $[M+H]^+$. UV λ_{max}^{MeOH} nm (log ε): 203 (4.30), 231 (4.09), 312 (4.15). IR (KBr): 1688 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.82 (3H, s, H-13), 2.78 (1H, dd, *J*=14.0, 8.5 Hz, Ha-3), 2.97 (1H, dd, *J*=14.0, 4.0 Hz, Hb-3), 3.79 (OMe), 4.35 (1H, dd, *J*=8.5, 4.0 Hz, H-2), 4.84 (1H, s, Ha-12), 4.95 (1H, s, Hb-12), 6.32 (1H, d, *J*=16.0 Hz, H-9), 6.87 (1H, d, *J*=8.5 Hz, H-7), 7.37 (1H, d, *J*=2.0 Hz, H-4), 7.40 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 7.64 (1H, d, *J*=16.0 Hz, H-8). ^{13}C-NMR ($CDCl_3$) δ: 18.1 (C-13), 37.0 (C-3), 51.6 (OMe), 75.3 (C-2), 110.6 (C-12), 115.4 (C-9), 117.0 (C-7), 127.0 (C-5), 127.7 (C-3a), 128.5 (C-6), 130.9 (C-4), 144.6 (C-8), 147.1 (C-11), 159.4 (C-7a), 167.7 (C-10).

Compound 23 Amorphous powder. FAB-MS *m/z*: 299 $[M+H]^+$. UV λ_{max}^{MeOH} nm (log ε): 218 (4.16), 232 (4.11), 303 (4.14). IR (KBr): 1682 cm^{-1}. $[\alpha]_D^{25}$ +2.25° (*c*=0.37, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.73 (3H, s, H-5′), 1.78 (3H, s, H-4′), 1.82 (3H, s, H-13), 2.81 (1H, d, *J*=12.5 Hz, Ha-3), 2.97 (1H, dd, *J*=12.5, 8.5 Hz, Hb-3), 3.36 (2H, d, *J*=7.0 Hz, H-1′), 4.40 (1H, d, *J*=8.5 Hz, H-2), 4.90 (1H, s, Ha-12), 5.01 (1H, s, Hb-12), 5.33 (1H, br t, H-2′), 6.26 (1H, d, *J*=16.0 Hz, H-9), 7.11 (1H, br s, H-4), 7.25 (1H, br s, H-6), 7.68 (1H, d, *J*=16.0 Hz, H-8). ^{13}C-NMR ($CDCl_3$) δ: 17.8 (C-5′), 18.1 (C-13), 25.8 (C-4′), 28.7 (C-1′), 38.1 (C-3), 78.1 (C-2), 111.4 (C-12), 113.8 (C-9), 121.9 (C-2′), 125.8 (C-3a), 126.0 (C-5), 128.9 (C-6), 129.8 (C-4), 130.2 (C-7), 133.4 (C-3′), 146.3 (C-11), 147.3 (C-8), 156.5 (C-7a), 171.9 (C-10).

Compound 24 Amorphous powder. FAB-MS *m/z*: 447 $[M-H]^+$. UV λ_{max}^{MeOH} nm (log ε): 204 (4.26), 208 (4.25), 232 (4.06), 305 (4.05). IR (KBr): 1720, 1686 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.73 (3H, s, H-5′), 1.79 (6H, s, H-4″,5″), 2.67 (2H, t, *J*=7.5 Hz, H-8‴), 2.96 (2H, t, *J*=7.5 Hz, H-7‴), 3.36 (4H, br s, H-1′, 1″), 4.52 (2H, s, H-4′), 5.29 (1H, br t, H-2″), 5.60 (1H, br t, H-2′), 6.31 (1H, d, *J*=16.0 Hz, H-8), 7.18—7.20 (5H, m, H-2, 6, 2‴, 4‴, 6‴), 7.25 (2H, m, H-3‴, 5‴), 7.68 (1H, d, *J*=16.0 Hz, H-7). ^{13}C-NMR ($CDCl_3$) δ: 14.0 (C-5′), 17.9 (C-5″), 25.8 (C-4″), 28.5 (C-1′), 30.1 (C-1″), 31.0 (C-7‴), 35.9 (C-8‴), 69.8 (C-4′), 114.5 (C-8), 121.0 (C-2″), 126.2 (C-1, 2′, 4‴), 127.0—128.6 (C-2, 3, 5, 6), 128.3 (C-2‴, 6‴), 128.5 (C-3‴, 5‴), 132.3 (C-3′), 136.1 (C-3″), 140.4 (C-1‴), 146.9 (C-7), 155.2 (C-4), 171.0 (C-9), 172.8 (C-9‴).

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References and Note

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