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Chemical Constituents of *Gomphrena globosa.* II

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Abstract – One new sterol glucoside, gomphsterol β-D-glucoside **1** along with known compounds, β-sitosterol, stigmasterol, campesterol, stigmasterol-β-D-glucoside, friedelin, 3-*epi*-friedelinol, allantoin, and chrysoeriol-7-*O*-β-D-glucoside have been isolated from the aerial parts of *Gomphrena globosa* (Amaranthaceae). On the basis of spectroscopic (including 2D NMR) and chemical studies, the structure of **1** was elucidated as (22*E*,24*S*)-24-ethylcholesta-7,9(11),22-trien-3β-ol-3-*O*-β-D-glucopyranoside. Known compounds are reported for the first time from this plant species.

Keywords – *Gomphrena globosa*, Amaranthaceae, steroids, gomphsterol β-D-glucoside, triterpenoids, flavonoid

Introduction

Gomphrena globosa L. (Amaranthaceae), an annual herb of American origin, is cultivated as garden plant in the North Eastern Region of India for its beautiful pinkish leaves and stems as well as its use in traditional medicine for preparation of cough syrup and to stop local haemorrhage (Deb, 1983). Earlier investigation on the flowers (Hener *et al.*, 1992) and whole plant (Liu *et al.*, 1981) reported the presence of betalain, betacyanin, and flavone, and we reported earlier (Dinda *et al.*, 2004) the isolation of gomphrenoside, hopane-7β-ol, β-sitosterol-3-*O*-β-D-glucoside, and 1-triacontanol from the aerial parts of this plant. Our continued search for other chemical constituents has resulted in the isolation and characterization of one new sterol glucoside and eight known compounds. Herein we report the isolation and structure elucidation of these compounds.

Experimental

Plant material – The aerial parts of *Gomphrena globosa* L. in matured flowering stage were collected from the garden of Department of Chemistry, Tripura University and was identified by Dr. B. K. Dutta, taxonomist, Department of Life Sciences, Tripura University. A Voucher specimen of the flowering plant (# BD-01/05) has been deposited in the National Herbarium, Govt. of India, Shibpur, Howrah.

Extraction and isolation of phytochemicals – Aerial parts of *G. globosa* in matured flowering stage were collected. Flowers from the aerial parts were separated out and dried. The rest of the aerial parts was dried and crushed in to coarse powder. Both the dried flowering part (200 g) and the aerial parts containing leaves and stems (3 kg) were extracted separately with MeOH by percolation process. Both the MeOH extracts were concentrated in a rotavapour, dissolved separately in a little water and fractionated into benzene, chloroform and *n*-butanol soluble fractions by partition between water and benzene, water and chloroform, water and *n*-butanol, successively. Each fraction was concentrated and subjected to column chromatography (CC) with silica gel (60-120 mesh, Merck). The eluates of the column were monitored by thin layer chromatography (TLC) on silica gel G (Merck) coated glass plates in different solvent systems.

Phytochemicals from leaves and stems – The benzene fraction of the aerial parts on CC gave a residue of sterol mixture from C_6H_6-$CHCl_3$ (1 : 1) eluate. This residue on prep.TLC with $AgNO_3$-silica gel (1.5 : 8.5) gave campesterol (1.5 mg), $C_{28}H_{48}O$ (M^+ 400), mp 156 °C [lit. (Buckingham, and MacDonald, 1996) mp 157-158 °C], β-sitosterol (15 mg), $C_{29}H_{50}O$ (M^+ 414), mp 138 °C and stigmasterol (5 mg), $C_{29}H_{48}O$ (M^+ 412), mp 170 °C. All these sterols were identified by direct comparison (m.mp, *co*-TLC and GC analysis; RR_t, relative to cholesterol, 1.10 for campesterol; 1.16 for stigmasterol; 1.23 for sitosterol) with standard samples. GC experiments were performed with

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SE-30 column using N_2 gas and in the following temperature conditions: programing from 160 ° to 280 °C at 5 ° per min, injector and FID temperatures 280 °C and 300 °C, respectively.

The $CHCl_3$ fraction of the aerial parts on CC gave friedelin **3** (50 mg) from C_6H_6-$CHCl_3$ (1 : 1) eluate and 3-*epi*-friedelinol **4** (12 mg) from C_6H_6-$CHCl_3$ (2 : 3) eluate.

The *n*-BuOH fraction of the aerial parts on CC afforded stigmasterol-*β*-D-glucoside **2** (12 mg) from $CHCl_3$-EtOAc (3 : 1) eluate and gomphsterol *β*-D-glucoside **1** (70 mg) from $CHCl_3$-EtOAc (1 : 1) eluate.

Phytochemicals from flowers – Both C_6H_6 and $CHCl_3$ soluble fractions of the flowers on CC gave friedelin **3** (4 mg and 8 mg, respectively).

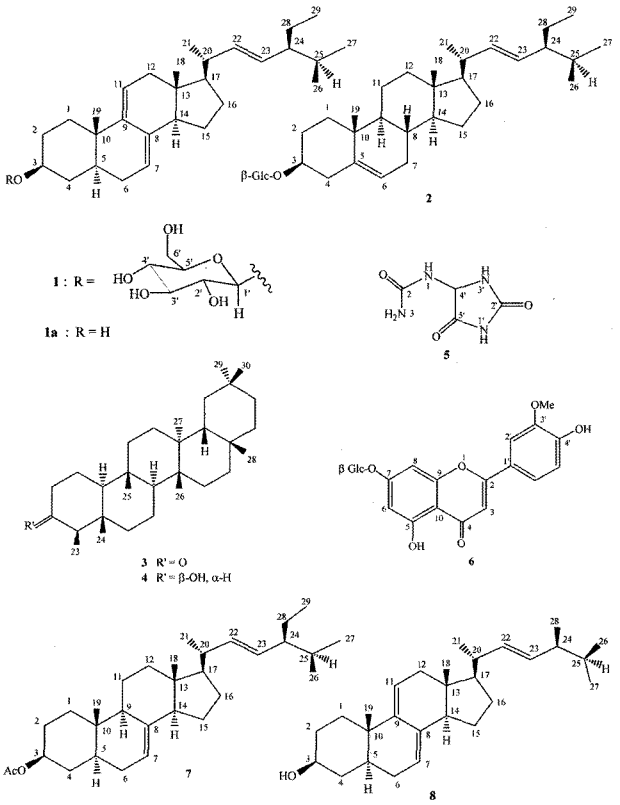
The *n*-BuOH fraction of the flowers on CC afforded allantoin **5** (20 mg) from $CHCl_3$-EtOAc (9 : 1) eluate and chrysoeriol-7-*O*-*β*-D-glucoside **6** (25 mg) from $CHCl_3$-EtOAc (4 : 1) eluate.

Characterization of phytochemicals

General – All the phytochemicals were characterized after meticulous purification to homogeneity on thin layer chromatography (TLC) in different solvent systems followed by crystallization and study of physical constant and spectroscopic data. In case of known compounds consultation of literature or direct comparison (m.mp and co-TLC) with respective authentic samples was carried out for their identification. Melting points were determined by open capillary method and are uncorrected. UV spectra were recorded on a Spectronic-21 spectrophotometer and IR spectra on a Shimadzu FT-IR spectrometer in KBr pellets. ^{1}H-, ^{13}C- and 2D- NMR spectra were taken on a Varian XL-400 spectrometer with 400 MHz for ^{1}H- and 100 MHz for ^{13}C- spectra using TMS as an internal reference (chemical shifts are expressed in δ, ppm); EI and FAB-MS were recorded on a Jeol JMS-700 Mstation mass spectrometer. NMR-DEPT experiments were carried out with flip angle θ of 45 °, 90 ° and 135 °.

Gomphsterol *β*-D-glucoside (1) – Amorphous solid, mp 265 °C. UV (MeOH) λ_{max} nm (log ε): 224 (3.36), 255 (3.07); IR(KBr) ν_{max} cm^{-1}: 3390, 1651, 1254, 1076, 972; ^{1}H- and ^{13}C- NMR (see Table 1); EI-MS *m/z* (rel. int.): 572 $[M]^+$(3), 410 [gomphsterol]$^+$(19), 392 (71), 271 (47), 253 (100), 229 (37), 213 (37), 139 (20); LR-FAB-MS (-ve): *m/z* (rel. int.): 571 $[M-H]^-$ (2), 409 [gomphsterol - H]$^-$ (13), 391 (13), 271 (16), 252 (36), 229 (13), 213 (61), 139 (100); *Anal.* Found: C, 73.31; H, 9.90. Calcd for $C_{35}H_{56}O_6$: C, 73.39; H, 9.85 %.

Stigmasterol-*β*-D-glucoside [(22*E*,24*S*)-24-ethylcholesta-5,22-dien-3*β*-ol-3-*O*-*β*-D-glucoside] (2) – Colorless crystals, mp 296 °C [lit. (Buckingham, 1994) 299 °C]; ^{1}H-NMR



(400 MHz,$CDCl_3$) δ: 3.45 (1H, m, H-3), 5.23 (1H, m, H-6), 0.69 (3H, s, H_3-18), 1.01 (3H, s, H_3-19), 1.02 (3H, d, J = 6.5 Hz, H_3-21), 5.02 (1H, dd, J = 14.5 and 6.5 Hz, H-22), 4.88 (1H, dd, J = 14.5 and 8.5 Hz, H-23), 0.81 (3H, d, J = 6.5 Hz, H_3-26), 0.85 (3H, d, J = 6.5 Hz, H_3-27), 0.78 (2H, m, H_2-28), 0.79 (3H, t, J = 7.0 Hz, H_3-29), 4.27 (1H, d, J = 8.0 Hz, H-1'), 3.10 (1H, dd, J = 9.0 and 8.0 Hz, H-2'), 3.29 (1H, dd, overlapped with dd of H-4', H-3'), 3.28 (1H, dd, overlapped with dd of H-3', H-4'), 3.15 (1H, m, H-5'), 3.61 (1H, dd, J = 12.0, and 4.5 Hz, H-6'), 3.71 (1H, dd, J = 12.0 and 2.5 Hz, H-6'); HR-FAB-MS (+ve) *m/z* (rel. int.): 597.4128 $[M + Na]^+$ (100%), calcd for $C_{35}H_{58}O_6Na$: 597.4131.

Friedelin (3) – Colorless crystals, mp 264 °C [lit.(Corey and Ursprung, 1956; Tanaka and Matsunaga, 1988) mp 265-266 °C]; IR(KBr) ν_{max} cm^{-1}: 1715, 1389, 1362; EI-MS *m/z* (rel. int.): 426 $[M]^+$ (79), 411 $[M-15]^+$ (48), 383 (31), 341 (20), 302 (51), 273 (80), 246 (47), 231 (68), 218 (70), 205 (100); HR-FAB-MS (+ve) *m/z* (rel. int.): 427.3945 $[M + H]^+$ (100%), calcd for $C_{30}H_{51}O$: 427.3940). It was identified by direct comparison with an authentic sample.

3-*epi*-Friedelinol (4) – Colorless needles, mp 281 °C [lit.(Sahu and Chakravarti, 1971) mp 283.5-285 °C]; IR (KBr) ν_{max} cm^{-1}: 3485, 1390, 1365; ^{13}C-NMR (100 MHz, $CDCl_3$) δ :17.5 (t, C-1), 35.2 (t, C-2), 72.7 (d, C-3), 49.2 (d, C-4), 37.1 (s, C-5), 41.7 (t, C-6), 15.8 (t, C-7), 53.2 (d,

C-8), 37.8 (s, C-9), 61.3 (d, C-10), 35.3 (t, C-11), 30.6 (t, C-12), 53.2 (s, C-13), 39.4 (s, C-14), 32.8 (t, C-15), 35.6 (t, C-16), 30.1 (s, C-17), 41.7 (d, C-18), 36.1 (t, C-19), 28.2 (s, C-20), 32.3 (t, C-21), 39.2 (t, C-22), 11.6 (q, C-23), 15.8 (q, C-24), 16.4 (q, C-25), 20.1 (q, C-26), 17.5 (q, C-27), 32.3 (q, C-28), 31.8 (q, C-29), 35.2 (q, C-30); EI-MS *m/z* (rel. int.): 428 [M^+, $C_{30}H_{52}O$] (47), 413 [M-Me]$^+$ (49), 410 (25), 304 (13), 275 (100), 257 (56), 207 (50). It was identified by direct comparison with an authentic sample.

Allantoin (5) – Colorless crystals, mp 242 °C [lit. (Rashed *et al.*, 2004) mp 243 °C]; IR (KBr) ν_{max} cm^{-1}: 3440, 3345, 3192, 3061, 1782, 1713 1658, 1603; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.88 (1H, d, J = 8.0 Hz, HN-1), 5.77 (2H, brs, H_2N-3), 10.52 (1H, s, HN-1'), 8.04 (1H, brs, HN-3'), 5.24 (1H, dd, J = 8.0 and 1.0 Hz, H-4'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 173.6 (s, $CONH_2$), 156.7 (s, C-2'), 62.4 (d, C-4'), 157.3 (s, C-5'); EI-MS *m/z* (rel. int.): 158 [M^+, $C_4H_6N_4O_3$] (9), 141 [M - NH_3]$^+$ (13), 130 [M - CO]$^+$ (86), 115 [M - CONH]$^+$ (33), 87 [130 - CONH]$^+$ (84), 70 [87 - NH_3]$^+$ (12), 60 [CON_2H_4]$^+$ (30), 44 [$CONH_2$]$^+$ (100). Physical and spectral data were in good agreement with literature values (Achari *et al.*, 1984; Rashed *et al.*, 2004).

Chrysoeriol-7-*O*-β-D-glucoside (6) – Pale yellowish needles, mp 238 °C, UV (MeOH) λ_{max} nm (log ε): 239 (4.49), 300sh (4.33), 345 (4.38); IR (KBr) ν_{max} cm^{-1}: 3390, 1657,1609, 1572, 1502, 812; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.97 (1H, s, H-3), 12.95 (1H, s, HO-5), 6.43 (1H, d, J = 2.0 Hz, H-6), 6.85 (1H, d, J = 2.0 Hz, H-8), 6.98 (1H, d, J = 2.0 Hz, H-2'), 3.88 (3H, s, MeO-3'), 10.02 (1H, brs, HO-4'), 6.93 (1H, d, J = 9.0 Hz, H-5'), 7.58 (1H, dd, J = 9.0 and 2.0 Hz, H-6'), 5.05 (1H, d, J = 8.0 Hz, H-1"), 3.24 (1H, dd, J = 9.0 and 8.0 Hz, H-2"), 3.30 (1H, hides inside the H_2O peak present as impurity in DMSO, H-3"), 3.43(1H, dd, J = 9.0 and 8.5 Hz, H-4"), 3.16 (1H, m, H-5"), 3.46 (1H, dd, J = 12.5 and 2.5 Hz, H-6"), 3.70 (1H, dd, J = 12.5 and 4.5 Hz, H-6"); ^{13}C -NMR (100 MHz, DMSO-d_6) δ: 164.2 (s, C-2), 103.4 (d, C-3), 182.1(s, C-4), 161.1 (s, C-5), 99.5 (d, C-6), 163.0(s, C-7), 95.0 (d, C-8), 156.9 (s, C-9 or C-8a), 105.3 (s, C-10, or C-4a), 121.3 (s, C-1'), 110.3 (d, C-2'), 150.9 (s, C-3'), 148.1 (s, C-4'), 115.8 (d, C-5'), 120.5 (d, C-6'), 56.0 (q, OMe), 100.0 (d, C-1"), 73.1 (d, C-2"), 77.3 (d, C-3"), 69.6 (d, C-4"), 76.5 (d, C-5"), 60.6 (t, C-6"); EI-MS *m/z* (rel. int.): 462 [M]$^+$ (1), 300 [M - glucosyl + H]$^+$(100), 272 [300 - CO]$^+$ (14), 257 (29), 229 (21), 153 (37), 148 (21); HR-FAB-MS (+ve) *m/z* (rel. int.): 463.1244 [M + H]$^+$ (100%), calcd for $C_{22}H_{23}O_{11}$, 463.1241. Physical and spectral data were in good agreement with those reported in the literature (Skaitsa *et al.*, 2000).

Acid hydrolysis of 1 – A solution of **1** (40 mg) in 2 *N* aqueous methanolic HCl (10 mL) was reflux for 2 hr. The reaction mixture was evaporated to a residue in a rotavapour. The residue was dissolved in a little H_2O and extracted with $CHCl_3$ (20 mL × 3). The aqueous layer was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated and tested for sugar. D-glucose was detected by TLC [R_f 0.45 in *n*-BuOH-pyridine-H_2O, 6 : 4 : 3] and optical rotation study (positive specific rotation). The combined $CHCl_3$ extract was concentrated and column purified to get gomphsterol **1a** (20 mg).

Gomphsterol 1a – (20 mg). White amorphous powder, mp 176 °C, UV (MeOH) λ_{max} nm (log ε) : 224 (3.92), 256 (3.62); IR (KBr) ν_{max} cm^{-1} : 3380, 1650, 970; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.56 (1H, m, H-3), 5.10 (1H, m, H-7), 5.30 (1H, m, H-11), 0.50 (3H, s, H_3-18), 0.80 (3H, s, H_3-19), 0.96 (3H, d, J = 6.5 Hz, H_3-21), 5.08 (1H, dd, J = 14.5 and 6.5 Hz, H-22), 4.96 (1H, dd, J = 14.5 and 8.5 Hz, H-23), 0.79 (3H, d, J = 7.0 Hz, H_3-26), 0.86 (3H, d, J = 7.0 Hz, H_3-27), 0.80 (2H, m, H_2-28), 0.75 (3H, t, J = 7.0 Hz H_3-29); ^{13}C- NMR (100 MHz, $CDCl_3$) δ: 36.6 (t, C-1), 29.5 (t, C-2), 77.3 (d, C-3), 39.6 (t, C-4), 40.7 (d, C-5), 29.3 (t, C-6), 117.2 (d, C-7), 138.2 (s, C-8), 140.2 (s, C-9), 36.6 (s, C-10), 121.8 (d, C-11), 39.4 (t, C-12), 43.2 (s, C-13), 50.0 (d, C-14), 22.8 (t, C-15), 28.8 (t, C-16), 55.9 (d, C-17), 11.8 (q, C-18), 19.6 (q, C-19), 40.3 (d, C-20), 21.2 (q, C-21), 138.0 (d, C-22), 129.2 (d, C-23), 51.1 (d, C-24), 31.7 (d, C-25),18.8 (q, C-26), 21.0 (q, C-27), 25.2 (t, C-28), 12.1 (q, C-29). *Anal.* Found : C, 84.72; H, 11.23. Calcd for $C_{29}H_{46}O$: C, 84.81; H, 11.29%.

Results and Discussion

Gomphsterol-β-D-glucoside **1**, responded positive Liebermann Burchard test for steroid. The positive FAB-mass spectrum showed a quasi-molecular ion peak at *m/z* 573 [M + H]$^+$ as well as its elemental analysis suggested its molecular formula $C_{35}H_{56}O_6$. The UV spectrum in MeOH showed absorption maxima at 224 (log ε, 3.36) and 255 nm (3.07) indicating the presence of unsaturation system in the molecule. The IR spectrum in KBr exhibited absorption bands for hydroxyl (3390 cm^{-1}) and olefinic (1651 cm^{-1}) functions. The *trans* nature of one olefinic function was indicated by the presence of IR band at 972 cm^{-1} (Williams and Fleming, 1994). The ^{1}H-, ^{13}C-NMR spectral data and HMBC correlation data (see Table 1) suggested its $\Delta^{7,9(11),22}$-C_{29} sterol glucoside structure **1** (Akihisa, 1989). The chemical shifts of H_3-18 and H_3-19 at δ 0.49 and 0.79 were very similar to reported values at δ 0.54 and 0.81 for [(22*E*,24*S*)-24-ethylcholesta-7,22-dien-3β-yl

acetate], spinasteryl acetate **7** (Rubinstein *et al.*, 1976). The chemical shift values for all ring carbons, C-1 to C-17, and the two angular methyl groups (C-18 and C-19) of **1** were very close to the reported values for (22*E*,24*R*)-methylcholesta-7,9(11)-22-trien-3β-ol **8** (Akihisa, 1989). The EI-MS of the compound recorded mass ions at *m/z* 410 [M – glucosyl unit + H]$^+$, 392 [M – glucose]$^+$, 271 [M – glucosyl unit-side chain + H]$^+$, 253[M-glucose-side chain]$^+$ (base peak), 229 [M – (glucosyl unit + ring D-fission)]$^+$, corroborating its C_{29}- sterol glucoside structure **1** with two double bonds in the steroid nucleus and an unsaturated side chain ($C_{10}H_{19}$) (Ravi *et al.*, 1978). Its negative FAB-MS recorded a base peak at *m/z* 139 supporting the presence of side chain of formula $C_{10}H_{19}$. The presence of hexose sugar was indicated from its positive FAB-MS, which showed mass ions at *m/z* 181 [hexose + H]$^+$ and 164 [hexosyl + H]$^+$. The compound on hydrolysis with 2 *N* aqueous methanolic HCl afforded gomphsterol **1a**, $C_{29}H_{46}O$ (M^+ 410), mp 176 °C and D-glucose. The shielded chemical resonance of C-29 carbon at δ 12.1 and of C-26 carbon at δ 18.8 in ^{13}C NMR spectrum and large vicinal proton coupling constant, $J_{H\text{-}23,H\text{-}24}$ = 8.5 Hz in ^1H NMR spectrum of **1a** indicated that the ethyl side chain may have β-configuration (Akihisa, 1989; Holland *et al.*, 1978).On the basis of the foregoing spectral and chemical studies, the structure of the glucoside was assigned as (22*E*,24*S*)-24-ethylcholesta-7,9(11),-22-trien-3β-ol-3-O-β-D-glucopyranoside **1**. To the best of our knowledge it is a new natural product and its aglycone, gomphsterol **1a** is also a new sterol.

Known sterol glucoside, stigmasterol-β-D-glucoside **2** (Buckingham, 1994), was also isolated from *n*-butanol soluble fraction of methanolic extract of the aerial parts and characterized from its spectral data (see Experimental). The genus *Gomphrena* is taxonomically important as both Δ^5 and Δ^7 steroids have been isolated.

The benzene soluble fraction of the methanolic extract of the aerial parts afforded β-sitosterol, as major component along with stigmasterol, and a trace of campesterol.

The chloroform soluble fraction of the methanolic extract of the aerial parts afforded friedelin **3**, and 3-*epi*-friedelinol **4**. Both these triterpenoids were identified by comparison of their spectral data (see Experimental) with literature (Corey and Ursprung, 1956; Sahu and Chakravarti, 1971; Tanaka and Matsunaga, 1988).

Both benzene and chloroform soluble fractions of methanolic extract of *G globosa* flowers afforded friedelin **3**. The *n*-butanol soluble fraction gave allantoin **5**, and chrysoeriol-7-*O*-β-D-glucoside **6**, identified by comparison of their physical and spectral data (see Experimental) with literature (Skaitsa *et al.*, 2000). All these known compounds are reported for the first time from *G globosa*.

Table 1. ^1H- ,^{13}C- and HMBC correlation NMR data of compound **1** in $CDCl_3$: CD_3OD, 9 : 1 (400 MHz for ^1H and 100 MHz for ^{13}C).

position C	δ_H	δ_C	HMBC (H → C)
1	1.21 m 1.26 m	37.0 t	C-2, C-3, C-4, C-10
2	1.83 m, 1.86 m	29.3 t	C-1, C-3
3	3.60 m	78.5 d	C-1', C-1
4	1.98 m, 2.18 m	39.4 t	C-3, C-5
5	1.96 m	40.0 d	
6	1.71 dd (13.5, 1.5) 1.79 dd (13.5, 5.5)	29.5 t	C-5, C-7, C-8
7	5.09 m	117.2 d	C-5, C-14
8	–	138.0 s	
9	–	140.1 s	
10	–	36.0 s	
11	5.31 m	122.1 d	C-9, C-10, C-12
12	2.22 dd (13.5, 1.5) 2.34 dd (13.5, 5.5)	38.6 t	C-10, C-11
13	–	43.1 s	
14		51.1 d	
15		22.9 t	
16		28.8 t	
17	1.22 m	56.0 d	C-16, C-20
18	0.49 s	11.7 q	C-12, C-13, C-17
19	0.79 s	19.2 q	C-1, C-5, C-9, C-10
20	1.95 m	40.3 d	C-17, C-21, C-22, C-23
21	0.96 d (6.5)	20.9 q	C-17, C-20, C-22
22	5.08 dd (15.0, 6.5)	138.2 d	C-20, C-21, C-24
23	4.98 dd (15.0, 8.5)	129.3 d	C-22, C-24
24	1.75 m	51.1 d	C-23, C-25
25	1.48 m	31.7 d	C-24, C-26, C-27
26	0.78 d (6.8)	18.8 q	C-25, C-27, C-28
27	0.87d (6.8)	20.9 q	C-25, C-26, C-28
28	0.80 m	25.3 t	C-24, C-29
29	0.74 t (7.0)	12.7 q	C-24, C-28
1'	4.37 d (8.0)	100.9 d	C-3, C-2', C-3', C-5'
2'	3.21 dd (9.0, 8.0)	73.4 d	C-3' , C-4'
3'	3.43 dd (9.0, 9.0)	75.5 d	C-4'
4'	3.37 [a]	70.0 d	C-3'
5'	3.28 ddd (9.0, 4.5, 2.5)	76.2 d	C-4'
6'	3.72 dd (12.5, 4.5) 3.80 dd (12.5, 2.5)	61.8 t	C-4'

All assignments are based on ^1H-^1H COSY and ^1H-^{13}C COSY spectra.

[a] Overlapped with the methanol peak; Multiplicities of carbons were determined by DEPT spectra. Coupling constants in Hertz (Hz) are given in parentheses.

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