

## Two Novel Nortriterpenoids from *Gomphostemma parviflorum*

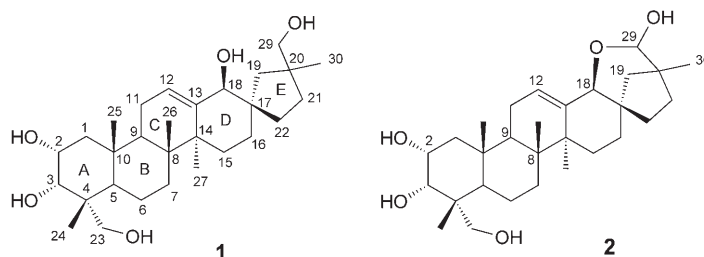
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Two new nortriterpenoids, gomphoparvin A (**1**) and B (**2**), together with five known compounds, were isolated from the AcOEt-soluble MeOH extract of *Gomphostemma parviflorum* (whole plant). Their structures and relative configurations were elucidated mainly on the basis of 1D- and 2D-NMR as well as MS analyses.

**Introduction.** – *Gomphostemma parviflorum* (Labiatae) is usually found in the shade of tropical green-forest, and is distributed in Xishuangbanna, Yunnan Province, China [1]. It belongs to only few rain-forest species of the genus Labiatae, and no work has been reported on its chemical constituents so far [2].

Herein, we report the isolation and structural elucidation of two new nortriterpenoids, gomphoparvins A (**1**) and B (**2**), from the title plant, which were obtained together with five known compounds, including ergosta-7,22-diene-3,5,6,9-tetrol [3], ergosta-7,22-dien-3-ol [4], asperphenamate [5],  $\beta$ -sitosterol [6], and daucosterol [7].



**Results and Discussion.** – The MeOH extract of the whole plant of *G. parviflorum* was divided into fractions soluble in petroleum ether, AcOEt, and BuOH. All compounds were isolated from the AcOEt-soluble extract upon chromatographic purification on silica gel, *Sephadex LH-20*, and *RP-18* gel.

Gomphoparvin A (**1**) was obtained as a colorless powder. The HR-FAB mass spectrum (negative mode) indicated the molecular formula  $C_{29}H_{48}O_5$  ( $m/z$  475.3421 ( $[M - H]^-$ ; calc. 475.3423), in accord with six degrees of unsaturation. In the  $^1H$ -NMR spectrum of **1** (Table 1), the *singlets* at  $\delta(H)$  1.09, 1.15, 1.07, 1.06, 1.29 (3 H each) indicated five Me groups at quaternary C-atoms: Me(24), Me(25), Me(26), Me(27), and Me(30)). Two hydroxymethyl groups at quaternary C-atoms were suggested from

the HMQC cross-peaks at  $\delta(\text{H})$  4.21, 3.74 ( $2d$ ,  $J = 12.4$  Hz, 1 H each) ( $\delta(\text{C})$  66.6 ( $t$ , C(23)), and from  $\delta(\text{H})$  3.86, 3.78 ( $2d$ ,  $J = 10.2$  Hz, 1 H each) ( $\delta(\text{C})$  71.3 ( $t$ , C(29))). An olefinic signal at  $\delta(\text{H})$  6.26 (*br. s*, 1 H) indicated the presence of a trisubstituted C=C bond at C(12)/C(13), including the signals at  $\delta(\text{C})$  118.4 ( $d$ ) and 142.9 ( $s$ ) in the  $^{13}\text{C}$ -NMR (DEPT) spectrum (Table I). Moreover, the signals of three oxygenated methines were clearly distinguished at  $\delta(\text{H})$  4.25–4.28 ( $m$ , 1 H), 4.21 ( $d$ ,  $J = 5.5$  Hz,

Table 1.  $^1\text{H}$ -,  $^{13}\text{C}$ -, and Selected 2D-NMR Data of **1**. Recorded at 500/125 MHz, resp., in ( $\text{D}_5$ )pyridine;  $\delta$  in ppm,  $J$  in Hz. Arbitrary atom numbering. Asterisks (\*) indicate masked signals.

| Atom                 | $\delta(\text{H})$                                      | $\delta(\text{C})$ | HMBC                     | NOESY          |
|----------------------|---|--------------------|--------------------------|----------------|
| CH <sub>2</sub> (1)  | 2.36 ( $dd$ , $J = 11.9, 3.8$ ),<br>1.34–1.37 ( $m$ )   | 48.2 ( $t$ )       | 2, 3, 9, 10, 25          |                |
| H–C(2)               | 4.25–4.28 ( $m$ )                                       | 69.0 ( $d$ )       | 3                        | 24, 25         |
| H–C(3)               | 4.21 ( $d$ , $J = 5.5$ )                                | 78.3 ( $d$ )       | 2, 4, 5, 24              |                |
| C(4)                 |   | 44.2 ( $s$ )       | –                        |                |
| H–C(5)               | 1.83 ( $d$ , $J = 11.6$ )                               | 48.1 ( $d$ )       | 4, 6, 23, 24, 25         | 9, 23          |
| CH <sub>2</sub> (6)  | 1.76–1.78 ( $m$ ),<br>1.48–1.51 ( $m$ )                 | 18.5 ( $t$ )       |                          |                |
| CH <sub>2</sub> (7)  | 1.72–1.75 ( $m$ ),<br>1.44–1.46 ( $m$ )                 | 34.1 ( $t$ )       | 6                        |                |
| C(8)                 |   | 39.9 ( $s$ )       |                          |                |
| H–C(9)               | 1.74 ( <i>br. s</i> )                                   | 47.9 ( $d$ )       | 10, 25                   | 5, 27          |
| C(10)                |   | 38.3 ( $s$ )       |                          |                |
| CH <sub>2</sub> (11) | 2.06–2.08 ( $m$ )                                       | 23.4 ( $t$ )       |                          |                |
| H–C(12)              | 6.26 ( <i>br. s</i> )                                   | 118.4 ( $d$ )      | 11                       | 11             |
| C(13)                |   | 142.9 ( $s$ )      |                          |                |
| C(14)                |   | 43.7 ( $s$ )       |                          |                |
| CH <sub>2</sub> (15) | 1.48–1.51 ( $m$ ),<br>0.99–1.00 ( $m$ )                 | 27.8 ( $t$ )       |                          |                |
| CH <sub>2</sub> (16) | 2.07–2.09 ( $m$ ),<br>1.48–1.51 ( $m$ )                 | 29.0 ( $t$ )       | 18                       |                |
| C(17)                |   | 50.5 ( $s$ )       |                          |                |
| H–C(18)              | 4.25–4.28*  | 75.4 ( $d$ )       | 13                       | 16, 19, 27     |
| CH <sub>2</sub> (19) | 2.58 ( $d$ , $J = 13.1$ )<br>1.31–1.33*                 | 48.6 ( $t$ )       | 17, 18, 20<br>21, 29, 30 | 16, 18, 27, 29 |
| C(20)                |   | 44.7 ( $s$ )       |                          |                |
| CH <sub>2</sub> (21) | 2.21–2.23 ( $m$ ),<br>1.51–1.53 ( $m$ )                 | 37.6 ( $t$ )       | 20, 29                   |                |
| CH <sub>2</sub> (22) | 2.06–2.09 ( $m$ ),<br>1.47–1.48 ( $m$ )                 | 29.0 ( $t$ )       |                          |                |
| CH <sub>2</sub> (23) | 4.21 ( $d$ , $J = 12.4$ ),<br>3.74 ( $d$ , $J = 12.4$ ) | 66.6 ( $t$ )       | 3, 4, 5, 24              |                |
| Me(24)               | 1.09 ( $s$ )  | 14.5 ( $q$ )       | 3, 4, 23                 | 2, 25          |
| Me(25)               | 1.15 ( $s$ )  | 18.0 ( $q$ )       | 1                        | 24             |
| Me(26)               | 1.07 ( $s$ )  | 18.0 ( $q$ )       | 9                        | 25             |
| Me(27)               | 1.06 ( $s$ )  | 23.2 ( $q$ )       | 13, 14                   | 16             |
| CH <sub>2</sub> (29) | 3.86 ( $d$ , $J = 10.2$ ),<br>3.78 ( $d$ , $J = 10.2$ ) | 71.3 ( $t$ )       | 19, 20, 21, 30           | 18             |
| Me(30)               | 1.29 ( $s$ )  | 26.5 ( $q$ )       | 19, 29                   |                |

1 H), and 4.25–4.28 (overlapped, 1 H). A total of 29 signals were found in the  $^{13}\text{C}$ -NMR spectrum due to 29 C-atoms. From these data, **1** was assigned the principal structure of an olean-12-ene nortriterpenoid. Careful comparison of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectroscopic data of **1** with those of notohamosin B [8] showed that they had the same carbon skeleton [8].

On the basis of HMQC and HMBC experiments (Table 1, Fig. 1), the correlations of the signal at  $\delta(\text{H})$  6.26 with those at  $\delta(\text{C})$  47.9 (*d*, C(9)), 43.7 (*s*, C(14)), and 75.4 (*d*, C(18)) showed that the trisubstituted C=C bond was located in 12-position, which was further confirmed by correlations between Me(27) and C(13). The two hydroxymethyl groups were attached at C(20) and C(4), based on the correlations of  $\delta(\text{H})$  4.21 and 3.74 with  $\delta(\text{C})$  44.2 (*s*, C(4)), and of  $\delta(\text{H})$  3.86 and 3.78 with  $\delta(\text{C})$  44.7 (*s*, C(20)). The remaining three OH groups were placed at C(2), C(3), and C(18), according to HMQC and HMBC data.

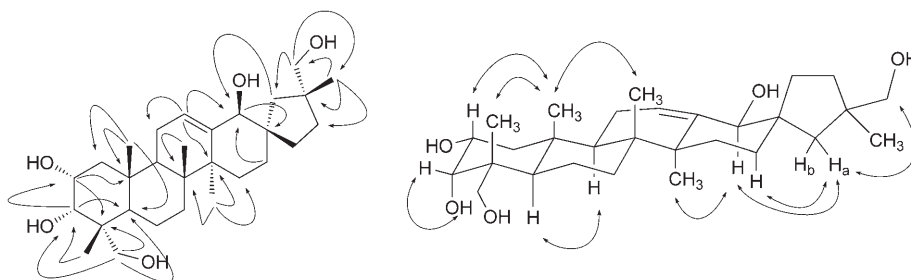


Fig. 1. Key HMBC ( $\rightarrow$ ) and NOESY ( $\leftrightarrow$ ) correlations for **1**

The relative configurations at C(2), C(3), C(5), C(8), C(9), C(10), C(14), C(17), and C(20) were determined by NOESY experiments (Fig. 1). The 2-OH group should be  $\alpha$ -orientated considering the NOESY correlations of H–C(2) with both Me(25) and Me(24). The coupling constant ( $J = 5.5$  Hz) between H–C(2) and H–C(3) revealed a  $3\alpha$ -OH group. The NOESY correlations of H–C(5)/H–C(9) and H–C(9)/Me(27) indicated  $\alpha$ -orientation of H–C(5), H–C(9), and Me(27). The relative configuration at C(17) was determined from the NOESY cross-peaks between H–C(16) and H–C(19), and between H–C(18) and H–C(19). From the NOESY spectrum, the correlations between  $\delta(\text{H})$  4.25–4.28 (H–C(18)) and  $\delta(\text{H})$  2.58 indicated that the signal resonating at  $\delta(\text{H})$  2.58 ( $\text{H}_a$ –C(19)) was  $\alpha$ -orientated; the key NOESY correlations between  $\text{H}_a$ –C(19) and  $\text{CH}_2(29)$ –OH could also be observed (Fig. 1), which suggested that the  $\text{CH}_2\text{OH}$  group was also in  $\alpha$ -position. All of these data were similar to those reported in the literature [9]. Thus, the structure of **1** was determined as (2 $\alpha$ ,3 $\alpha$ ,17*R*,18 $\beta$ )-19(18  $\rightarrow$  17)-abeo-28-norolean-12-ene-2,3,18,23,29-pentaol<sup>1)</sup>, and the compound was named *gomphoparvin A*.

Gomphoparvin B (**2**), obtained as a colorless powder, had the molecular formula  $\text{C}_{29}\text{H}_{46}\text{O}_5$ , based on HR-FAB-MS (negative mode) analysis ( $m/z$  473.3228 ( $[M - \text{H}]^-$ ; calc. 473.3267). Thus, **2** had one degree of unsaturation more than **1**. A total of 29 signals were observed in the  $^{13}\text{C}$ -NMR spectrum (Table 2) due to 29 C-atoms. Five Me

<sup>1)</sup> For systematic names, see *Exper. Part*.

groups, one hemi-acetal, and one hydroxymethyl group at a quaternary C-atoms were recognized from the  $^1\text{H-NMR}$  spectrum (*Table 2*), which suggested that **2** was also a nortriterpenoid. By comparing the NMR data of **2** with those of **1**, the hemi-acetal group was readily recognized from the signals at  $\delta(\text{H})$  5.14 (*s*, H–C(29)) and  $\delta(\text{C})$  100.0 (*d*, C(29)), one H-atom missing compared to the  $\text{CH}_2(29)$  group in **1**. The additional *F*-ring was formed by linking C(18) at  $\delta(\text{C})$  74.7 with C(29) *via* an oxygen bridge, which was confirmed by HMBC cross correlations between  $\delta(\text{H})$  5.14 (H–C(29)) and  $\delta(\text{C})$  74.7 (C(18)). The atoms C(2) and C(3) were found to carry OH groups, as inferred from the corresponding  $^{13}\text{C-NMR}$  data relative to those of **1**.

Table 2.  $^1\text{H}$ -,  $^{13}\text{C}$ -, and Selected 2D-NMR Data of **2**. Recorded at 500/125 MHz, resp., in ( $\text{D}_5$ )pyridine;  $\delta$  in ppm, *J* in Hz. Arbitrary atom numbering. Asterisks (\*) indicate masked signals.

| Atom              | $\delta(\text{H})$  | $\delta(\text{C})$ | HMBC           | NOESY          |
|-------------------|---|--------------------|----------------|----------------|
| $\text{CH}_2(1)$  | 2.33–2.35 ( <i>m</i> ),<br>1.35–1.38 ( <i>m</i> )                         | 48.2 ( <i>t</i> )  | 10, 25         |                |
| H–C(2)            | 4.25–4.28 ( <i>m</i> )  | 69.0 ( <i>d</i> )  | 3              | 24, 25         |
| H–C(3)            | 4.19 ( <i>d</i> , <i>J</i> = 1.9)   | 78.3 ( <i>d</i> )  | 2, 4, 5, 24    |                |
| C(4)              |   | 43.7 ( <i>s</i> )  |                |                |
| H–C(5)            | 1.79–1.81 ( <i>m</i> )  | 48.1 ( <i>d</i> )  | 3, 23, 24, 25  | 9, 23          |
| $\text{CH}_2(6)$  | 1.74–1.76 ( <i>m</i> ),<br>1.45–1.47 ( <i>m</i> )                         | 18.5 ( <i>t</i> )  |                |                |
| $\text{CH}_2(7)$  | 1.59–1.61 ( <i>m</i> ),<br>1.26–1.28 ( <i>m</i> )                         | 34.0 ( <i>t</i> )  | 6              |                |
| C(8)              |   | 39.8 ( <i>s</i> )  |                |                |
| H–C(9)            | 1.71–1.72 ( <i>m</i> )  | 47.9 ( <i>d</i> )  | 25, 26         | 5, 27          |
| C(10)             |   | 38.3 ( <i>s</i> )  |                |                |
| $\text{CH}_2(11)$ | 2.01–2.03 ( <i>m</i> )  | 23.4 ( <i>t</i> )  |                | 12             |
| H–C(12)           | 6.07 ( <i>br. s</i> )   | 119.3 ( <i>d</i> ) | 11             |                |
| C(13)             |   | 139.0 ( <i>s</i> ) |                |                |
| C(14)             |   | 44.5 ( <i>s</i> )  |                |                |
| $\text{CH}_2(15)$ | 1.59–1.61 ( <i>m</i> ),<br>0.98–1.99 ( <i>m</i> )                         | 27.8 ( <i>t</i> )  |                |                |
| $\text{CH}_2(16)$ | 1.88–1.90 ( <i>m</i> )  | 28.9 ( <i>t</i> )  | 17, 18         | 18, 27         |
| C(17)             |   | 46.8 ( <i>s</i> )  |                |                |
| H–C(18)           | 4.63 ( <i>br. s</i> )   | 74.7 ( <i>d</i> )  | 13             | 16, 19, 27, 29 |
| $\text{CH}_2(19)$ | 2.24 ( <i>d</i> , <i>J</i> = 10.6),<br>1.16*                              | 44.4 ( <i>t</i> )  | 18, 20, 21, 29 | 18, 27         |
| C(20)             |   | 44.6 ( <i>s</i> )  |                |                |
| $\text{CH}_2(21)$ | 1.79–1.82 ( <i>m</i> )  | 36.1 ( <i>t</i> )  |                |                |
| $\text{CH}_2(22)$ | 1.64–1.65 ( <i>m</i> ),<br>1.23–1.26 ( <i>m</i> )                         | 31.4 ( <i>t</i> )  | 29             | 16             |
| $\text{CH}_2(23)$ | 4.20 ( <i>d</i> , <i>J</i> = 10.4),<br>3.72 ( <i>d</i> , <i>J</i> = 10.4) | 66.6 ( <i>t</i> )  | 3, 4, 5, 24    | 5              |
| Me(24)            | 1.08 ( <i>s</i> )   | 14.5 ( <i>q</i> )  | 3, 23          | 2, 25          |
| Me(25)            | 1.14 ( <i>s</i> )   | 18.0 ( <i>q</i> )  | 1              | 2, 24, 26      |
| Me(26)            | 1.02 ( <i>s</i> )   | 17.9 ( <i>q</i> )  | 9              | 25             |
| Me(27)            | 0.97 ( <i>s</i> )   | 22.6 ( <i>q</i> )  |                | 16             |
| H–C(29)           | 5.14 ( <i>s</i> )   | 100.0 ( <i>d</i> ) | 18, 20, 21, 30 | 30             |
| Me(30)            | 1.20 ( <i>s</i> )   | 22.9 ( <i>q</i> )  | 19, 29         | 29             |

The relative configurations at the stereogenic centers of **2** were determined to be the same as those in compound **1**, based on NOESY experiments (Fig. 2). HMBC Correlations were found between H–C(23) and C(3), C(4), C(5), and C(24) (Table 2, Fig. 2); and NOESY correlations were observed for H–C(24)/H–C(2) and H–C(24)/H–C(25). The NOE for H–C(18)/Me(27) indicated that the H-atom at C(18) was  $\alpha$ -orientated. Furthermore, NOE correlations of H–C(18)/H<sub>a</sub>–C(19), H–C(18)/H–C(29), and H<sub>a</sub>–C(19)/H–C(29) were observed. Thus, the structure of **2** was elucidated as (2 $\alpha$ ,3 $\alpha$ ,17*R*)-18,29-epoxy-19(18  $\rightarrow$  17)-*abeo*-28-norolean-12-ene-2,3,23-triol<sup>1</sup>), and the compound was named *gomphoparvin B*.

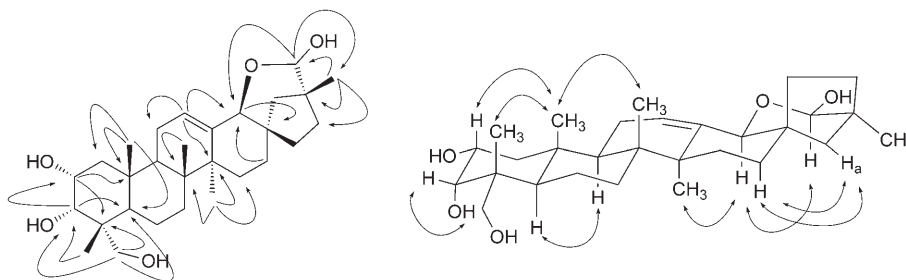


Fig. 2. Key HMBC ( $\rightarrow$ ) and NOESY ( $\leftrightarrow$ ) correlations for **2**

The five known compounds, ergosta-7,22-diene-3,5,6,9-tetrol [3], ergosta-7,22-dien-3-ol [4], asperphenamate [5],  $\beta$ -sitosterol [6], and daucosterol [7] were identified by co-TLC with authentic samples and by comparison of their spectroscopic data with those reported in the literature.

This work was financially supported by the *Key Scientific and Technological Project of Yunnan* (2001KY13) and the *National 863 Program* (2004AA2Z3321) to Y. F. W. The authors are grateful to Prof. Y.-L. Xu and the analytical group of the Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, for recording IR, NMR, and MS spectra.

### Experimental Part

**General.** Column chromatography (CC) was performed on silica gel (200–300 mesh; *Qingdao*) and *Sephadex LH-20* (*Amersham, GE healthcare Bio-sciences*). TLC: precoated silica-gel plates (*Qingdao*). Optical rotations: *Horiba SEAP-300 sensitive* polarimeter. IR Spectra: *BIO-rad FTS* IR spectrophotometer; in  $\text{cm}^{-1}$ . NMR Spectra: *Bruker AM-400* or *DRX-500* spectrometers; in  $\text{CDCl}_3$  soln.;  $\delta$  in ppm,  $J$  in Hz. HR-EI-MS: *VG AutoSpec-3000* mass spectrometer; in  $m/z$  (rel. %).

**Plant Material.** The aerial parts of *Gomphostemma parviflorum* were collected in Xishuangbanna (Yunnan, P. R. China) in July 2004. The plant was identified by Prof. *Xi-Wen Li*. A voucher specimen (No. 2004070819) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, P. R. China.

**Extraction and Isolation.** The air-dried, powdered aerial parts of *G. parviflorum* (5.0 kg) were extracted with 70% aq. MeOH at reflux ( $3 \times 30$  l) for 4, 3, and 3 h, resp. The solvent was removed *in vacuo*, the residue was suspended in  $\text{H}_2\text{O}$ , and then extracted with petroleum ether (PE), AcOEt, and BuOH. The AcOEt-soluble part (98 g) was subjected to CC ( $\text{SiO}_2$ ;  $\text{CHCl}_3/\text{MeOH}$  1:0  $\rightarrow$  0:1) to afford six fractions (*Fr. 1–6*). The chlorophylls in *Fr. 3* were removed by CC (*MCI* gel;  $\text{EtOH}/\text{H}_2\text{O}$  4:1), and the resulting pre-purified residue was repeatedly subjected to CC, first on  $\text{SiO}_2$  ( $\text{CHCl}_3/\text{acetone}$  7:3), then on *Sephadex LH-20* (MeOH), and finally on *RP-18* ( $\text{MeOH}/\text{H}_2\text{O}$  3:7  $\rightarrow$  8:2), which afforded **1**

(9 mg), **2** (27 mg), ergosta-7,22-diene-3,5,6,9-tetrol (10 mg), ergosta-7,22-dien-3-ol (15 mg), and asperphenamate (21 mg). *Fr.* 2 was repeatedly subjected to CC (SiO<sub>2</sub>; PE/acetone), which gave, after recrystallization,  $\beta$ -sitosterol (102 mg). Daucosterol (65 mg) was obtained by precipitation from *Fr.* 5.

*Gomphoparvin A* (= (2 $\alpha$ ,3 $\alpha$ ,17R,18 $\beta$ )-19(18  $\rightarrow$  17)-abeo-28-Norolean-12-ene-2,3,18,23,29-pentaol; **1**<sup>2</sup>). Colorless powder.  $[\alpha]_D^{20} = -7.94$  ( $c = 0.63$ , MeOH). IR (KBr): 3430, 2931, 1629, 1451, 1388, 1048. <sup>1</sup>H- and <sup>13</sup>C-NMR: see *Table 1*. FAB-MS (neg.): 475 (100,  $[M - H]^-$ ), 443 (10,  $[M - H - CH_2OH]^-$ ), 325 (5), 80 (4). HR-FAB-MS (neg.): 475.3421 ( $[M - H]^-$ , C<sub>29</sub>H<sub>47</sub>O<sub>5</sub><sup>-</sup>; calc. 475.3423).

*Gomphoparvin B* (= (2 $\alpha$ ,3 $\alpha$ ,17R)-18,29-Epoxy-19(18  $\rightarrow$  17)-abeo-28-norolean-12-ene-2,3,23-triol; **2**<sup>3</sup>). Colorless powder.  $[\alpha]_D^{20} = +46.13$  ( $c = 1.0$ , MeOH). IR (KBr): 3406, 2944, 2914, 1630, 1448, 1374, 1306, 1048, 1021, 977. <sup>1</sup>H- and <sup>13</sup>C-NMR: see *Table 2*. FAB-MS (neg.): 473 (100,  $[M - H]^-$ ), 441 (9,  $[M - H - CH_2OH]^-$ ). HR-FAB-MS (neg.): 473.3228 ( $[M - H]^-$ , C<sub>29</sub>H<sub>45</sub>O<sub>5</sub><sup>-</sup>; calc. 473.3267).

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*Received March 30, 2007*

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- <sup>2</sup>) Systematic name: *rel*-(1S,2R,4aS,4bR,6aR,7R,8S,9R,10aR)-3,4,4a,4b,5,6,6a,7,8,9,10,10a,10b,11-tetra-decahydro-3',7-bis(hydroxymethyl)-3',4a,4b,7,10a-pentamethyl-1H-spiro[chrysen-2,1'-cyclopentane]-1,8,9-triol.
  - <sup>3</sup>) Systematic name: *rel*-(1R,4S,5R,8R,9R,10S,11R,13S,16E,18S)-9-(hydroxymethyl)-4,5,9,13,21-pentamethyl-19-oxapentacyclo[19.2.1.0<sup>1,18</sup>.0<sup>4,17</sup>.0<sup>8,13</sup>]tetracos-16-ene-10,11,20-triol.