# Clerodane and *ent*-Kaurane Diterpene Glycosyl and Glycoside Derivatives from the Leaves of *Casearia sylvestris*

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Five new clerodane diterpene glycosyl derivatives, casearíasides A–E (**1**–**5**, resp.) and three new *ent*-kaurane diterpene glucosides, sylvestrisides C–E (**6**–**8**, resp.) were isolated from the leaves of *Casearia sylvestris*. Their structures were determined on the basis of chemical and spectroscopic analyses.

**Introduction.** – *Casearia sylvestris* SWARTZ (Flacourtiaceae) is a Brazilian and Paraguayan folk-medicinal plant called 'Guaçatonga' or 'Chá de Bugre' and used to treat snakebite, trauma, ulceration, obesity, and cough [1–5]. A number of clerodane diterpenes were reported from the leaves of *C. sylvestris*, some of which possess antitumoral, trypanocidal, and DNA-modifying bioactivities [6–12]. Our continued investigation of this species has led to the identification of five new clerodane diterpene glycosyl derivatives, casearíasides A–E[1]) (**1**–**5**, resp.), and three *ent*-kaurane[2]) diterpene glucosides, sylvestrisides C–E (**6**–**8**, resp.) (*Fig. 1*). Here, we report the isolation and structure elucidation of these eight new glycosyl and glycoside derivatives.

**Results and Discussion.** – The AcOEt-soluble portion of the MeOH extract of the powdered leaves of *C. sylvestris* was fractionated and purified by repeated column chromatography (silica gel), followed by prep. reversed-phase HPLC, to yield compounds **1**–**8**.

Casearíaside A[1]) (**1**) was obtained as a colorless amorphous powder. The molecular formula of **1** was determined to be $C_{26}H_{38}O_{10}$ by HR-ESI-MS (pos.) at *m*/*z* 533.2368 ($[M+Na]^+$, $C_{26}H_{38}NaO_{10}^+$). In the $^{13}$C-NMR spectrum (*Table 1*), 26 C-atom signals were observed, including six signals attributable to a $\beta$-glucopyranosyl unit. The absolute configuration of the glucose was determined to be D by GC analysis of its acetylated thiazolidine derivative obtained after acid hydrolysis and derivatization with L-cysteine methyl ester. The $^1$H-NMR data (*Table 2*) showed the resonance of an anomeric H-atom at $\delta$(H) 6.70 (*d*, $J = 7.8$ Hz) and resonances typical for a tricyclic clerodane-diterpenoid skeleton [13–15]: two tertiary Me ($\delta$(H) 0.98 and 1.66) and a secondary Me group ($\delta$(H) 0.99 (*d*, $J = 6.0$ Hz)), and two low-field olefinic H-atoms at

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

[2]) The index name (*Chem. Abstr.*) of '*ent*-kaurane' is 'kaurane'; for such systematic names, see *Exper. Part.*

Table 1. *$^{13}$C-NMR and DEPT Data* ((D$_5$)pyridine) *of Compounds* **1**–**5**[1]). $\delta$ in ppm.

| | **1**[a]) | **2**[b]) | **3**[a]) | **4**[a]) | **5**[b]) |
|---|---|---|---|---|---|
| C(1) | 28.8 (*t*) | 17.6 (*t*) | 17.3 (*t*) | 17.7 (*t*) | 17.9 (*t*) |
| C(2) | 68.8 (*d*) | 27.8 (*t*) | 24.5 (*t*) | 35.7 (*t*) | 27.7 (*t*) |
| C(3) | 143.2 (*d*) | 139.7 (*d*) | 141.2 (*d*) | 81.0 (*d*) | 139.6 (*d*) |
| C(4) | 142.0 (*s*) | 142.3 (*s*) | 138.5 (*s*) | 158.3 (*s*) | 143.1 (*s*) |
| C(5) | 39.0 (*s*) | 39.0 (*s*) | 40.7 (*s*) | 40.6 (*s*) | 39.3 (*s*) |
| C(6) | 35.7 (*t*) | 36.2 (*t*) | 35.5 (*t*) | 38.7 (*t*) | 38.1 (*t*) |
| C(7) | 27.5 (*t*) | 23.2 (*t*) | 28.4 (*t*) | 27.7 (*t*) | 27.7 (*t*) |
| C(8) | 36.4 (*d*) | 45.4 (*d*) | 38.2 (*d*) | 37.0 (*d*) | 36.6 (*d*) |
| C(9) | 38.8 (*s*) | 38.4 (*s*) | 37.0 (*s*) | 39.7 (*s*) | 38.3 (*s*) |
| C(10) | 45.9 (*d*) | 47.5 (*d*) | 45.7 (*d*) | 49.4 (*d*) | 47.0 (*d*) |
| C(11) | 36.1 (*t*) | 36.2 (*t*) | 37.2 (*t*) | 32.9 (*t*) | 36.2 (*t*) |
| C(12) | 22.6 (*t*) | 23.2 (*t*) | 22.4 (*t*) | 22.5 (*t*) | 22.4 (*t*) |
| C(13) | 172.3 (*s*) | 172.9 (*s*) | 172.8 (*s*) | 172.7 (*s*) | 142.1 (*s*) |
| C(14) | 115.3 (*d*) | 115.5 (*d*) | 115.2 (*d*) | 115.1 (*d*) | 125.9 (*d*) |
| C(15) | 174.7 (*s*) | 175.0 (*s*) | 174.8 (*s*) | 174.8 (*s*) | 58.8 (*t*) |
| C(16) | 73.8 (*t*) | 74.0 (*t*) | 73.8 (*t*) | 73.8 (*t*) | 66.4 (*t*) |
| C(17) | 16.3 (*q*) | 64.0 (*t*) | 16.2 (*q*) | 16.4 (*q*) | 16.4 (*q*) |
| C(18) | 165.9 (*s*) | 166.0 (*s*) | 166.9 (*s*) | 110.9 (*t*) | 165.7 (*s*) |
| Me(19) | 21.1 (*q*) | 21.6 (*q*) | 33.9 (*q*) | 22.6 (*q*) | 21.2 (*q*) |
| Me(20) | 18.7 (*q*) | 19.7 (*q*) | 18.3 (*q*) | 18.6 (*q*) | 18.7 (*q*) |
| Glc: | | | | | |
| C(1′) | 96.1 (*d*) | 96.1 (*d*) | 96.0 (*d*) | 102.9 (*d*) | 95.9 (*d*) |
| C(2′) | 74.5 (*d*) | 74.8 (*d*) | 74.4 (*d*) | 75.8 (*d*) | 74.5 (*d*) |
| C(3′) | 79.0 (*d*) | 79.2 (*d*) | 79.0 (*d*) | 79.1 (*d*) | 78.8 (*d*) |
| C(4′) | 71.4 (*d*) | 71.6 (*d*) | 71.5 (*d*) | 72.3 (*d*) | 71.3 (*d*) |
| C(5′) | 80.0 (*d*) | 80.0 (*d*) | 78.0 (*d*) | 77.5 (*d*) | 79.8 (*d*) |
| C(6′) | 62.4 (*t*) | 62.7 (*t*) | 68.5 (*t*) | 69.2 (*t*) | 62.4 (*t*) |
| Api: | | | | | |
| C(1″) | | | 111.4 (*d*) | 111.3 (*d*) | |
| C(2″) | | | 78.2 (*d*) | 78.2 (*d*) | |
| C(3″) | | | 80.8 (*s*) | 80.8 (*s*) | |
| C(4″) | | | 75.4 (*t*) | 75.3 (*t*) | |
| C(5″) | | | 66.0 (*t*) | 66.0 (*t*) | |

[a]) Measured at 150 MHz. [b]) Measured at 100 MHz.

$\delta$(H) 7.55 and 6.29 corresponding to an $\alpha,\beta$-unsaturated ester and a 3-substituted but-2-eno-4-lactone, respectively. The 3-substituted butenolactone was also supported by the characteristic resonances at $\delta$(C) 174.7 (*s*, C(15)), 115.3 (*d*, C(14)), 172.3 (*s*, C(13)), and 73.8 (*t*, C(16)). The absorption bands in the IR spectrum at 1717 and 1634 cm$^{-1}$ confirmed the presence of an $\alpha,\beta$-unsaturated lactone and an $\alpha,\beta$-unsaturated ester, respectively. Compared to the spectroscopic data of clerodermic acid (=(4a*R*,5*S*,6-*R*,8a*R*)-5-[2-(2,5-dihydro-5-oxofuran-3-yl)ethyl]-3,4,4a,5,6,7,8,8a-octahydro-5,6,8a-trimethylnaphthalene-1-carboxylic acid) [15], the main difference was an additional OH group in the aglycone moiety of **1**. The OH group was located at C(2) based on the HMBC cross-peaks of H–C(2) ($\delta$(H) 4.80 (*t*, $J$ = 8.4 Hz))/C(1), C(3), and C(4)

Table 2. *$^1$H-NMR Data* ((D$_5$)pyridine) *of Compounds* **1**–**5**[1]). $\delta$ in ppm, $J$ in Hz.

| | **1**[a]) | **2**[b]) | **3**[a]) | **4**[a]) | **5**[b]) |
|---|---|---|---|---|---|
| $CH_2$(1) | 2.03 (*q*, *J* = 12), 2.42–2.44 (*m*) | 1.35–1.38 (*m*), 1.54–1.58 (*m*) | 1.26–1.27 (*m*), 1.95–2.00 (*m*) | 1.60–1.64 (*m*), 2.20 (*t*, *J* = 12.6) | 1.41–1.44 (*m*), 1.69–1.72 (*m*) |
| H–C(2) or $CH_2$(2) | 4.80 (*t*, *J* = 8.4) | 1.90–1.92 (*m*), 2.16–2.20 (*m*) | 2.26–2.30 (*m*), 2.36–2.38 (*m*) | 1.65–1.67 (*m*), 1.77(*dt*, *J* = 3.6, 2.6) | 2.06–2.08 (*m*), 2.07–2.09 (*m*) |
| H–C(3) | 7.55 (br. *s*) | 6.82 (br. *s*) | 7.06 (br. *s*) | 4.95 (br. *s*) | 6.76 (br. *s*) |
| $CH_2$(6) | 1.74 (*dd*, *J* = 13.2, 13.8), 1.88 (*t*, *J* = 13.2) | 1.70–1.74 (*m*), 1.90–1.96 (*m*) | 1.65–1.70 (*m*), 1.90–1.95 (*m*) | 1.72–1.74 (*m*), 1.97 (*d*, *J* = 9) | 1.53–1.56 (*m*), 1.57–1.59 (*m*) |
| $CH_2$(7) | 1.54–1.60 (*m*), 1.62–1.66 (*m*) | 1.97–2.00 (*m*), 2.01–2.02 (*m*) | 1.53–1.59 (*m*), 1.61–1.64 (*m*) | 1.60–1.63 (*m*), 1.72–1.74 (*m*) | 1.20–1.25 (*m*), 1.38–1.41 (*m*) |
| H–C(8) | 1.60–1.64 (*m*) | 1.65–1.67 (*m*) | 1.60–1.62 (*m*) | 1.59–1.64 (*m*) | 1.40–1.42 (*m*) |
| H–C(10) | 1.51 (*d*, *J* = 12.6) | 1.16–1.18 (*m*) | 1.60–1.63 (*m*) | 1.31 (*d*, *J* = 12) | 1.19–1.22 (*m*) |
| $CH_2$(11) | 1.38 (*dd*, *J* = 12.6, 13.2), 2.87 (*d*, *J* = 12.6) | 1.13–1.16 (*m*), 2.70 (*d*, *J* = 12.8) | 1.41–1.42 (*m*), 3.31 (*d*, *J* = 13.8) | 1.72–1.76 (*m*), 2.65 (*d*, *J* = 13.2) | 0.98 (*t*, *J* = 12), 2.56 (*d*, *J* = 13.2) |
| $CH_2$(12) | 2.33 (*dd*, *J* = 13.8, 14.4), 2.49 (*dd*, *J* = 14.4, 13.8) | 2.12–2.14 (*m*), 2.37 (*t*, *J* = 13.2) | 2.40–2.45 (*m*), 2.40–2.45 (*m*) | 2.24 (*t*, *J* = 12), 2.38 (*dd*, *J* = 12.6, 14.2) | 2.06–2.08 (*m*), 2.13 (*dt*, *J* = 4.4, 12) |
| H–C(14) or $CH_2$(14) | 6.29 (*s*) | 6.03 (*s*) | 6.32 (*s*) | 6.26 (*s*) | 6.28 (*t*, *J* = 6.4) |
| $CH_2$(15) | | | | | 4.63 (*d*, *J* = 6.4) |
| $CH_2$(16) | 5.12 (*d*, *J* = 9.6) | 4.86 (br. *s*) | 5.13 (br. *s*) | 5.07 (br. *s*) | 4.45 (br. *s*) |
| Me(17) or $CH_2$(17) | 0.99 (*d*, *J* = 6.0) | 3.51 (*dd*, *J* = 7.6, 10), 3.97 (*dd*, *J* = 4.4, 10) | 0.89 (*d*, *J* = 6.0) | 0.99 (*d*, *J* = 6.6) | 0.74 (*d*, *J* = 6.4) |
| $CH_2$(18) | | | | 5.24 (br. *s*), 5.56 (br. *s*) | |
| Me(19) | 1.66 (*s*) | 1.38 (*s*) | 1.58 (*s*) | 1.70 (*s*) | 1.33 (*s*) |
| Me(20) | 0.98 (br. *s*) | 0.82 (*s*) | 1.00 (*s*) | 0.96 (*s*) | 0.61 (*s*) |
| Glc: | | | | | |
| H–C(1′) | 6.70 (*d*, *J* = 7.8) | 6.40 (*d*, *J* = 7.6) | 6.68 (*d*, *J* = 7.8) | 5.15 (*d*, *J* = 7.8) | 6.39 (*d*, *J* = 8.0) |
| H–C(2′) | 4.51(*t*, *J* = 8.4) | 4.25(*t*, *J* = 8.0) | 4.50–4.52 (*m*) | 4.25–4.30 (*m*) | 4.26–4.30 (*m*) |
| H–C(3′) | 4.63–4.68 (*m*) | 4.32–4.35 (*m*) | 4.60–4.62 (*m*) | 4.48 (*t*, *J* = 8.4) | 4.38–4.39 (*m*) |
| H–C(4′) | 4.65–4.70 (*m*) | 4.35–4.40 (*m*) | 4.51–4.55 (*m*) | 4.26–4.30 (*m*) | 4.39–4.42 (*m*) |
| H–C(5′) | 4.37–4.39 (*m*) | 4.07–4.10 (*m*) | 4.48–4.50 (*m*) | 4.31–4.34 (*m*) | 4.09–4.10 (*m*) |
| $CH_2$(6′) | 4.65–4.70 (*m*), 4.77 (br. *d*, *J* = 12) | 4.38–4.41 (*m*), 4.47 (br. *d*, *J* = 12) | 4.50–4.52 (*m*), 4.91 (br. *d*, *J* = 11.4) | 4.39 (*dd*, *J* = 7.6, 10.8), 4.95 (br. *d*, *J* = 11.4) | 4.40–4.42 (*m*), 4.49 (br. *d*, *J* = 11.4) |
| Api: | | | | | |
| H–C(1″) | | | 5.90 (*d*, *J* = 2.4) | 6.07 (*d*, *J* = 2.4) | |
| H–C(2″) | | | 4.97 (br. *s*) | 5.03 (br. *s*) | |
| H–C(3″) | | | | | |
| $CH_2$(4″) | | | 4.60 (*d*, *J* = 9.6), 4.82 (*d*, *J* = 9.6) | 4.63 (*dd*, *J* = 1.8, 9), 4.84 (*dd*, *J* = 1.8, 9) | |
| $CH_2$(5″) | | | 4.21 (br. *s*) | 4.46 (br. *s*) | |

[a]) Measured at 600 MHz. [b]) Measured at 400 MHz.

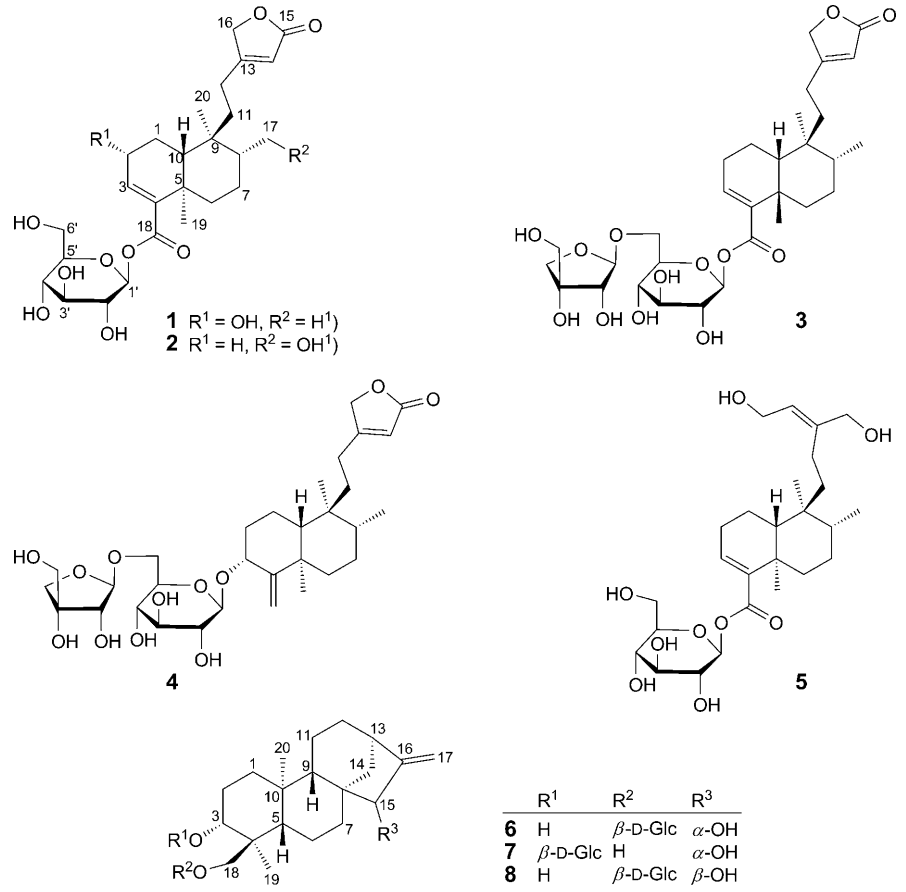


Fig. 1. *Compounds* **1**–**8** *isolated from* Casearia sylvestris SWARTZ

(*Fig. 2*). The $\alpha$-orientation of OH–C(2) was evident from the ROESY correlations H–C(10)/H–C(2) and H–C(8) (*Fig. 2*). The *trans-AB* ring junction was also confirmed by the chemical shift of the angular Me(19) group ($\delta$(C) 21.1) in analogs [16–18]. Thus, the aglycone of **1** was identified as (2$\alpha$)-2-hydroxyclerodermic acid. The $\beta$-glucopyranosyl unit was located at the carboxylic group owing to HMBCs between the anomeric H-atom at $\delta$(H) 6.70 and the C=O group at $\delta$(C) 165.9 (*Fig. 2*). Moreover, the resonance of the anomeric C-atom at $\delta$(C) 96.1 was characteristic of an ester-linked glycosylation [19]. Based on the above evidences, **1** was characterized as (2$\alpha$)-2-hydroxycleroda-3,13-diene-15,18-dioic acid 15,16-lactone 18-$\beta$-D-glucopyranosyl ester.

Caseariaside B[1]) (**2**) was isolated as a colorless amorphous powder. Its HR-ESI-MS analysis (*m*/*z* 533.2367 ([$M$ + Na]$^+$, $C_{26}H_{38}NaO_{10}^+$)) led to the same molecular formula $C_{26}H_{38}O_{10}$ as that of **1**. When the $^1$H- and $^{13}$C-NMR data of **2** were compared with those of **1**, the resonances for CH(2)–O and a secondary Me(17) group were absent in **2**, showing instead a $CH_2$ ($\delta$(H) 1.90–1.92 and 2.16–2.20 ($CH_2$(2)); $\delta$(C) 27.8 (C(2))) and

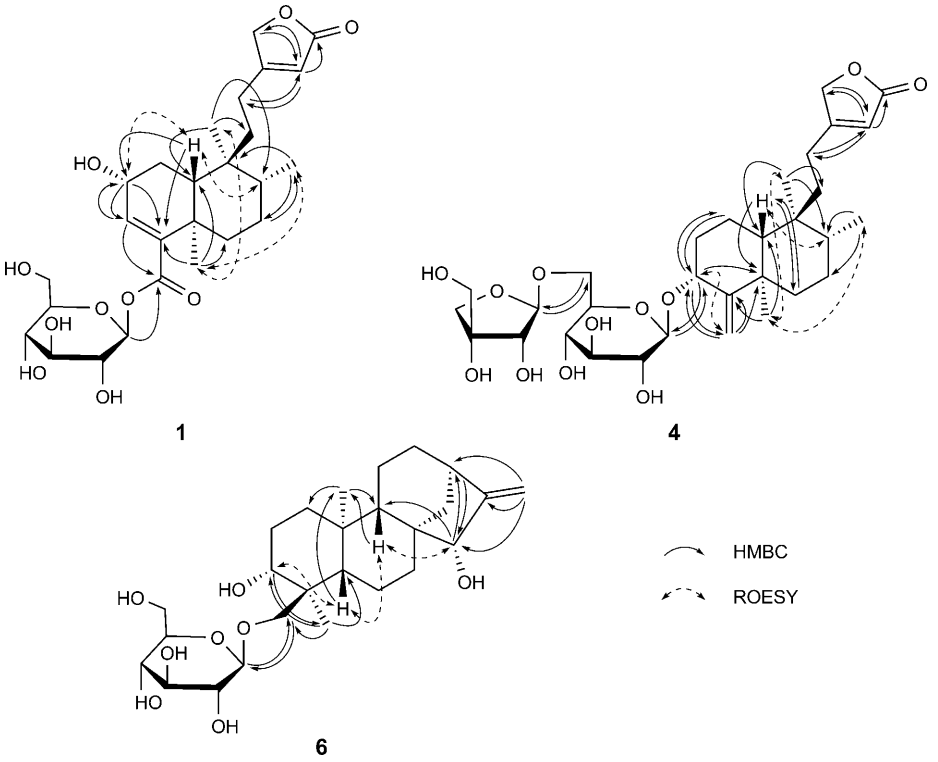


Fig. 2. *Key HMBCs and ROESY correlations of compounds* **1**, **4**, *and* **6**

a $CH_2-O$ group ($\delta$(H) 3.51 and 3.97 ($CH_2$(17)); $\delta$(C) 64.0 (C(17))). Also, the HMBCs of the $CH_2-O$ H-atoms with C(20) ($\delta$(C) 21.6) and C(6) ($\delta$(C) 36.2) verified the above deduction. Therefore, **2** was assigned as 17-hydroxycleroda-3,13-diene-15,18-dioic acid 15,16-lactone 18-$\beta$-D-glucopyranosyl ester.

Casearíaside C[1]) (**3**) was obtained as a colorless amorphous powder. The HR-ESI-MS (*m*/*z* 665.2576 ($[M+K]^+$)) established the molecular formula as $C_{31}H_{46}O_{13}$. The NMR data were very similar to those of the *cis*-fused clerodane diterpene marrubiagenine, except for the resonances assigned to two sugar units [13]. Evaluation of coupling constants and chemical shifts including the anomeric H- and C-atoms allowed the identification of the two sugar units as $\beta$-D-glucopyranosyl ($\delta$(H) 6.68 ($J=$ 7.8 Hz, H–C(1′)); $\delta$(C) 96.0 (C(1′))) and $\beta$-apiofuranosyl units ($\delta$(H) 5.90 ($J=2.4$ Hz, H–C(1″)); $\delta$(C) 111.4 (C(1″))) [20][21]. The D configuration of glucose was confirmed in a similar manner to that of **1**. The glucose unit was located at C(18) as in **1** due to the HMBC H–C(1′)/C(18). The HMBC H–C(1″) ($\delta$(H) 5.90 ($J=2.4$ Hz))/C(6′) ($\delta$(C) 68.5) determined that the apiofuranosyl moiety was linked to C(6′) of the glucopyranosyl unit. The *cis-AB* ring junction was further verified by the characteristic chemical shift of the angular Me(19) group ($\delta$(C) 33.9) [13][14]. Thus, **3** was characterized as *cis*-cleroda-3,13-diene-15,18-dioic acid 15,16-lactone 18-(6-*O*-$\beta$-apiofuranosyl-$\beta$-D-glucopyranosyl) ester.

Casearíaside D[1]) (**4**) was obtained as a colorless amorphous powder. Its molecular formula $C_{31}H_{48}O_{12}$ was determined by HR-ESI-MS (*m/z* 635.3046 ($[M+Na]^+$, $C_{31}H_{48}NaO_{12}^+$). Due to similarities in the NMR data with those of **3** and **1**, **4** was predicted to be a clerodane type diterpene with a 6-*O*-*β*-apiofuranosyl-*β*-D-glucopyranosyl unit. However, the resonances for the *α*,*β*-unsaturated carbonyl group C(18)=O and the adjacent olefinic bond were absent in **4**, showing instead signals for an exocyclic olefinic $CH_2$ (*δ*(H) 5.24 and 5.56 (2 br. *s*, each 1 H−C(18)); *δ*(C) 110.9 (C(18)) and 158.3 (C(4))) and a CH−O group (*δ*(H) 4.95 (br. *s*, H−C(3)); *δ*(C) 81.0 (C(3))). In the HMBC experiment (*Fig. 2*), the CH−O H-atom H−C(3) (*δ*(H) 4.95) correlated with C(1) (*δ*(C) 17.7) and C(5) (*δ*(C) 40.6); the two cyclic-$CH_2$ H-atoms correlated with C(3) (*δ*(C) 81.0), C(4) (*δ*(C) 158.3) and C(5) (*δ*(C) 40.6); the anomeric H−C(1′) of the *β*-D-glucopyranose moiety (*δ*(H) 5.15, $J=7.8$ Hz)) correlated with C(3) (*δ*(C) 81.0); the anomeric H−C(1″) of the *β*-apiofuranose unit (*δ*(H) 6.07 ($J=2.4$ Hz)) correlated with C(6′) of the *β*-D-glucopyranose unit. These HMBCs revealed that the 6-*O*-*β*-apiofuranosyl-*β*-D-glucopyranosyl unit and the exocyclic olefinic $CH_2$ group were located at C(3) and C(4), respectively. The *α*-orientation of the sugar unit at C(3) was determined due to a ROESY correlation H−C(3)/$H_a$−C(18) and the absence of a correlation H−C(3)/Me(19) (*Fig. 2*). Therefore, **4** was assigned as (3*α*)-3-[(6-*O*-*β*-apiofuranosyl-*β*-D-glucopyranosyl)oxy]cleroda-4(18),13-diene-15-oic acid 15,16-lactone.

Casearíaside E[1]) (**5**) was obtained as a colorless amorphous powder. Its molecular formula was demonstrated to be $C_{26}H_{42}O_9$ by HR-ESI-MS analyses. The $^1$H- and $^{13}$C-NMR data of **5** (*Tables 2* and *1*) were similar to those of **1**, except for the lack of the resonances of a CH(2)−O and a C(15)=O group in **5**, presenting instead a $CH_2$(2) (*δ*(C) 27.7) and a $CH_2$(15)−O group (*δ*(C) 58.8) as substitutes. The NMR data including HMBCs further indicated that a 2-substituted but-2-ene-1,4-diol system was present in the side chain. Interestingly, the C(13)=C(14) bond possessed an (*E*) configuration as determined by the ROESY correlation H−C(14)/$CH_2$(16). Moreover, the chemical shifts of C(15) (*δ*(C) 58.8) and C(16) (*δ*(C) 66.4) in **5** are distinct from those observed in analogous compounds possessing a (*Z*) configuration [20][22]. As a result of the above data, the structure of **5** was elucidated as (13*E*)-15,16-dihydroxycleroda-3,13-dien-18-oic acid 18-*β*-D-glucopyranosyl ester.

Sylvestriside C (**6**) was obtained as a colorless amorphous powder and had the molecular formula $C_{26}H_{42}O_8$ as calculated from the HR-ESI-MS (*m/z* 505.2781 ($[M+Na]^+$)). In the $^{13}$C-NMR spectrum (*Table 3*), 26 resonances were observed, of which six were attributable to a *β*-D-glucopyranosyl unit. The rest of the C-atom resonances corresponded to an exocyclic $CH_2$ (*δ*(C) 161.7 and 108.3), two CH−O (*δ*(C) 83.3 and 72.7), a $CH_2$−O (*δ*(C) 75.2) in the lowfield region, two Me, seven $CH_2$, and three CH groups, and to three quaternary C-atoms in the upfield region. The $^1$H-NMR spectral data exhibited resonances for an anomeric H-atom at *δ*(H) 4.84 (*d*, $J=8$ Hz), an exocyclic olefinic $CH_2$ group at *δ*(H) 5.48 and 5.21 (br. *s*, each 1 H), a $CH_2$−O group at *δ*(H) 4.43 and 3.54 (*d*, $J=10.4$ Hz, each 1 H), an CH−O group at *δ*(H) 4.03 (br. *s*), an allylic H-atom at *δ*(H) 2.74 (br. *s*, 1 H) characteristic for H−C(13), and two tertiary Me groups at *δ*(H) 0.95 and 1.04. According to the characteristic chemical shifts of the allylic H-atom at *δ*(H) 2.74 (br. *s*, H−C(13)) and the quaternary C-atom at *δ*(C) 39.8 (C(10)) [23][24], **6** was identified as a tetracyclic *ent*-kaurane[2]) diterpene glucoside.

The *ent* configuration was inferred from the negative optical rotation value ($[\alpha]_D^{20} = -47.9$). The oxygenations at C(3), C(18), and C(15) were deduced from the HMBCs Me(19)/C(3) and C(18), H–C(3)/C(18) and C(19); $CH_2$(18)/C(3) and C(19), H–C(15)/C(9), C(13), C(14), and C(17), H–C(13)/C(15), and $CH_2$(17)/C(15) (*Fig. 2*). The location of the $\beta$-D-glucopyranosyl unit at C(18) was determined by the HMBC of the anomeric H–C(1′) ($\delta$(H) 4.84) with $CH_2$(18)–O ($\delta$(C) 75.2) and the $CH_2$–O ($\delta$(H) 3.54 and 4.43) with the anomeric C(1′) ($\delta$(C) 106.0). The ROESY correlations H–C(3)/H–C(5), H–C(5)/H–C(9), and H–C(9)/H–C(15) determined the $\alpha$-orientations of OH–C(3) and OH–C(15) (*Fig. 2*). Thus, the structure of **6** was assigned as (3$\alpha$,4$\beta$,15$\alpha$)-18-($\beta$-D-glucopyranosyloxy)-*ent*-kaur-16-ene-3,15-diol[2]).

Sylvestriside D (**7**) was obtained as a colorless amorphous powder. The calculated molecular formula $C_{26}H_{42}O_8$ was the same as that of **6** based on the HR-ESI-MS (*m*/*z* 505.2780 ($[M+Na]^+$)). Its spectroscopic data (*Table 3*) was coincident with that of **6**, except for a significantly downfield shift of C(3) ($\Delta\delta = 7.4$) and an upfield shift of C(18) ($\Delta\delta = -11.0$) revealing that the $\beta$-D-glucopyranosyloxy unit was linked at C(3). This deduction was further confirmed by the HMBC between the anomeric H–C(1′) ($\delta$(H) 5.17 (*d*, *J* = 7.2 Hz)) and C(3) ($\delta$(C) 80.1). Thus, the structure of **7** was determined as (3$\alpha$,4$\beta$,15$\alpha$)-3-($\beta$-D-glucopyranosyloxy)-*ent*-kaur-16-ene-15,18-diol[2]).

Sylvestriside E (**8**) was obtained as a colorless amorphous powder. Its molecular formula was demonstrated to be $C_{26}H_{42}O_8$ by HR-ESI-MS (*m*/*z* 521.2519 ($[M+K]^+$)), similar to those of **6** and **7**. The spectroscopic data were similar to those of **6**. The main difference was the relative configuration of OH–C(15). The ROESY correlations (*Fig. 3*) $H_{exo}$–C(14)/H–C(13) and H–C(15) established the $\beta$-orientation of OH–C(15). Therefore, compound **8** was elucidated as (3$\alpha$,4$\beta$,15$\beta$)-18-($\beta$-D-glucopyranosyloxy)-*ent*-kaur-16-ene-3,15-diol[2]).

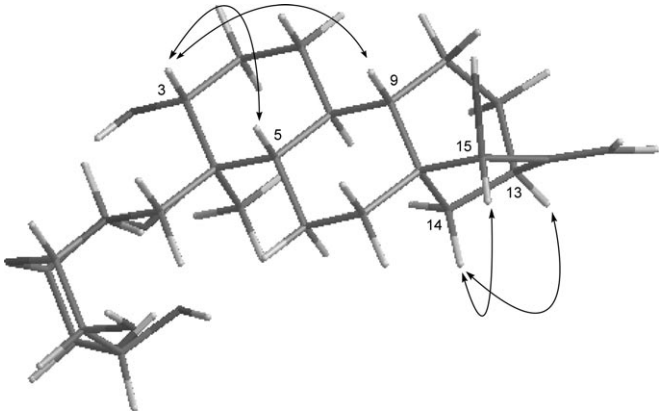


Fig. 3. *Key ROESY correlations of compound* **8**

The *in vitro* cytotoxicity of compounds **1**–**8** was evaluated against four solid-tumor cell lines: malignant melanoma (SK-MEL), oral epidermal carcinoma (KB), breast ductal carcinoma (BT549), and ovary carcinoma (SK-OV3) cells by the Neutral Red assay [25]. None of the compounds showed cytotoxicity up to 10 μg/ml. Compounds **1**–**8** were also found inactive *in vitro* against *Candida albicans*, *Candida glabrata*, *Candida*

Table 3. *$^1$H- and $^{13}$C-NMR Data* ((D$_5$)pyridine) *of Compounds* **6**–**8**. $\delta$ in ppm, $J$ in Hz.

| | **6**[a]) | | **7**[b]) | | **8**[b]) | |
|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| $CH_2$(1) | 0.71 (*t*, *J* = 10.4), 1.74 (*d*, *J* = 12.8) | 39.3 (*t*) | 1.05 (*t*, *J* = 13.8), 2.05 (*d*, *J* = 13.8) | 39.1 (*t*) | 0.61 (*t*, *J* = 12.0), 1.65–1.68 (*m*) | 38.7 (*t*) |
| $CH_2$(2) | 1.86–1.90 (*m*), 1.92–1.94 (*m*) | 28.0 (*t*) | 2.24–2.28 (*m*), 2.28–2.30 (*m*) | 24.5 (*t*) | 1.80–1.81 (*m*), 1.91–1.93 (*m*) | 27.8 (*t*) |
| H–C(3) | 4.20–4.23 (*m*) | 72.7 (*d*) | 4.43–4.45 (*m*) | 80.1 (*d*) | 4.12–4.13 (*m*) | 72.3 (*d*) |
| C(4) | | 43.6 (*s*) | | 43.3 (*s*) | | 43.3 (*s*) |
| H–C(5) | 1.60 (*d*, *J* = 11.6) | 48.0 (*d*) | 1.98 (*d*, *J* = 12.0) | 47.1 (*d*) | 1.76 (*d*, *J* = 8.4) | 46.8 (*d*) |
| $CH_2$(6) | 1.75–1.77 (*m*), 1.77–1.80 (*m*) | 20.2 (*t*) | 1.67–1.70 (*m*), 2.16–2.20 (*m*) | 19.6 (*t*) | 1.35–1.37 (*m*), 1.66–1.69 (*m*) | 20.3 (*t*) |
| $CH_2$(7) | 2.04–2.05 (*m*), 2.05–2.07 (*m*) | 36.1 (*t*) | 2.36–2.37 (*m*), 2.37–2.39 (*m*) | 36.1 (*t*) | 1.49–1.52 (*m*), 1.60–1.63 (*m*) | 34.3 (*t*) |
| C(8) | | 48.5 (*s*) | | 48.4 (*s*) | | 46.3 (*s*) |
| H–C(9) | 0.94–0.96 (*m*) | 55.0 (*d*) | 1.41–1.44 (*m*) | 55.1 (*d*) | 1.65–1.68 (*m*) | 47.2 (*d*) |
| C(10) | | 39.8 (*s*) | | 39.4 (*s*) | | 38.9 (*s*) |
| $CH_2$(11) | 1.44–1.45 (*m*), 1.45–1.47 (*m*) | 19.0 (*t*) | 1.78–1.80 (*m*), 1.80–1.81 (*m*) | 18.9 (*t*) | 1.43–1.45 (*m*), 1.91–1.94 (*m*) | 18.4 (*t*) |
| $CH_2$(12) | 1.42–1.44 (*m*), 1.44–1.48 (*m*) | 33.7 (*t*) | 1.78–1.79 (*m*), 1.79–1.81 (*m*) | 33.6 (*t*) | 1.31–1.34 (*m*), 2.27 (*t*, *J* = 12.0) | 39.1 (*t*) |
| H–C(13) | 2.74 (br. *s*) | 43.4 (*d*) | 3.05 (br. *s*) | 43.1 (*d*) | 2.65 (br. *s*) | 40.9 (*d*) |
| $CH_2$(14) | 1.63–1.65 (*m*), 1.90–1.92 (*m*) | 37.2 (*t*) | 1.95–1.96 (*m*), 2.18–2.21 (*m*) | 37.1 (*t*) | 0.97–0.98 (*m*), 1.91–1.94 (*m*) | 37.0 (*t*) |
| H–C(15) | 4.03 (br. *s*) | 83.3 (*d*) | 4.42 (br. *s*) | 83.1 (*d*) | 4.07 (br. *s*) | 82.3 (*d*) |
| C(16) | | 161.7 (*s*) | | 161.6 (*s*) | | 160.0 (*s*) |
| $CH_2$(17) | 5.21 (br. *s*), 5.48 (br. *s*) | 108.3 (*t*) | 5.47 (br. *s*), 5.75 (br. *s*) | 108.1 (*t*) | 5.11 (br. *s*), 5.51 (br. *s*) | 104.6 (*t*) |
| $CH_2$(18) | 3.54 (*d*, *J* = 10.4), 4.43 (*d*, *J* = 10.4) | 75.2 (*t*) | 3.88 (*d*, *J* = 10.8), 4.97 (*d*, *J* = 10.8) | 64.2 (*t*) | 3.50 (*d*, *J* = 9.6), 4.48 (*d*, *J* = 9.6) | 75.3 (*t*) |
| Me(19) | 0.95 (*s*) | 13.6 (*q*) | 1.12 (*s*) | 13.9 (*q*) | 0.96 (*s*) | 13.5 (*q*) |
| Me(20) | 1.04 (*s*) | 19.0 (*q*) | 1.33 (*s*) | 18.8 (*q*) | 1.02 (*s*) | 18.7 (*q*) |
| Glc: | | | | | | |
| H–C(1′) | 4.84 (*d*, *J* = 8.0) | 106.0 (*d*) | 5.17 (*d*, *J* = 7.2) | 104.1 (*d*) | 4.89 (*d*, *J* = 7.2) | 106.0 (*d*) |
| H–C(2′) | 4.04–4.08 (*m*) | 75.6 (*d*) | 4.32–4.37 (*m*) | 75.3 (*d*) | 4.10–4.15 (*m*) | 75.1 (*d*) |
| H–C(3′) | 3.98–4.04 (*m*) | 78.9 (*d*) | 4.35–4.39 (*m*) | 78.1 (*d*) | 4.08–4.12 (*m*) | 78.8 (*d*) |
| H–C(4′) | 4.15–4.19 (*m*) | 72.5 (*d*) | 4.25–4.28 (*m*) | 73.4 (*d*) | 4.31–4.33 (*m*) | 72.0 (*d*) |
| H–C(5′) | 4.22–4.26 (*m*) | 79.1 (*d*) | 4.54–4.56 (*m*) | 79.1 (*d*) | 4.31–4.33 (*m*) | 78.8 (*d*) |
| $CH_2$(6′) | 4.32 (*dd*, *J* = 12.0, 6.4), 4.55 (*dd*, *J* = 12.0, 2.4) | 63.4 (*t*) | 4.40–4.43 (*m*), 4.45–4.51 (*m*) | 64.2 (*t*) | 4.45–4.49 (*m*), 4.64 (*d*, *J* = 12.0) | 62.9 (*t*) |

[a]) Measured at 400 and 100 MHz, resp. [b]) Measured at 600 and 150 MHz, resp.

*krusei*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium intracellulare*, by using methods previously described [26].

This work is supported by the *United States Department of Agriculture Specific Cooperative Research* (Agreement No. 58-6408-6-067) and the *FDA/CFSAN Grant* entitled 'Science Based Authentication of Dietary Supplements' No. 2 U01 FD 002071–07.

## Experimental Part

*General.* Column chromatography (CC): silica gel ($SiO_2$; 70–230 and 200–300 mesh; *Merck*, Darmstadt, Germany). TLC: $SiO_2$ $GF_{254}$; detection by UV light and visualization by spraying with vanillin/$H_2SO_4$ followed by heating. Semiprep. HPLC: *Waters-LC-II* system equipped with a UV detector at 210 nm; *Phenomenex-Gemini-C18-ODS* (5 μm) column (10 × 250 mm; $t_R$ in min). Optical rotations: *Rudolph-Research-AutoPol-IV* polarimeter. UV Spectra: *Hewlett-Packard-8453* UV/VIS spectrometer; $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *Bruker-Tensor-27* FT-IR and *MIRacle* ATR-FT (attenuated total reflection *Fourier* transform) IR spectrometers; $\tilde{\nu}$ in $cm^{-1}$. $^1H$- and $^{13}C$-NMR Spectra: *American-Varian-Mercury-plus-400* ($^1H$ 400 MHz, $^{13}C$ 100 MHz) and *-600* ($^1H$ 600 MHz, $^{13}C$ 150 MHz) NMR spectrometers; $\delta$ in ppm rel. to $Me_4Si$ as internal standard, *J* in Hz. HR-ESI-MS: *Agilent-1100-SL* mass spectrometer; in *m*/*z* (rel. %).

*Plant Material.* The leaves of *Casearia sylvestris* were purchased from *Raintree Nutrition Inc.* (Carson City, NV 89701, USA), and were identified by TLC and HPLC analyses with the authenticated sample offered by Dr. *Rainer W. Bussmann*, Missouri Botanical Garden. Voucher specimens (#3247) were deposited with the National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, USA.

*Extraction and Isolation*. The dry powdered plant material of *C. sylvestris* (3 kg) was extracted by percolation with MeOH (4 × 4 l). The pooled MeOH solns. were concentrated to give a residue (342 g, 11.3%). The MeOH extract was partitioned between $H_2O$ (2 l) and petroleum ether (3 × 2 l) and then between $H_2O$ and AcOEt (3 × 2 l). The AcOEt layer afforded a waxy extract residue (207 g), which was further separated into *Fractions 1–9* by CC ($SiO_2$ (2500 g), 120 × 8 cm, gradient petroleum ether/AcOEt 3:1, 1:1, 1:4, and 1:10, and AcOEt/MeOH 8:1, 4:1, 1:1, 1:2, and 1:5). The residue (2.5 g) of *Fr. 6* was subjected to CC ($SiO_2$ (80 g), 60 × 6 cm, AcOEt): *Fr. 6.1–6.12.* Compound **7** (4.4 mg, $t_R$ 11.9) was obtained from *Fr. 6.6* by reversed-phase HPLC (MeOH/$H_2O$ 70:30, flow rate 5.0 ml/min). *Fr. 6.9* (80.2 mg) was separated by reversed-phase HPLC (MeOH/$H_2O$ 73:27, flow rate 5.0 ml/min): **2** (5.6 mg, $t_R$ 5.5), **1** (2.1 mg, $t_R$ 10.9), **3** (2.8 mg, $t_R$ 20.0), and **4** (3.0 mg, $t_R$ 35.3). *Fr. 6.12* (30.8 mg) was separated by reversed-phase HPLC (MeOH/$H_2O$ 70:30, flow rate 6.0 ml/min): **6** (6.6 mg, $t_R$ 15.5) and **8** (4.6 mg, $t_R$ 20.0). Compound **5** (7.7 mg, $t_R$ 6.8) was obtained from *Fr. 6.13* by reversed-phase HPLC (MeOH/$H_2O$ 66:34, flow rate 6.0 ml/min).

*Acid Hydrolysis and Determination of the Absolute Configuration of Glucose* [27]. Each compound **1**–**8** (1.0 mg) was hydrolyzed with 1N HCl (2 ml) for 3 h at 95°. The mixture was cooled, neutralized, and partitioned between AcOEt (2 ml) and $H_2O$ (2 ml). The aq. layer gave the sugar residue after drying. This residue was dissolved in pyridine (1 ml), and 0.1M L-cysteine methyl ester hydrochloride in pyridine (2 ml) was added. The mixture was heated at 60° for 1 h. An equal volume of $Ac_2O$ was added and heating continued for another 1 h. The acetylated thiazolidine derivatives were subjected to GC analysis (*ThermoQuest Trace 2000* GC; *Phenomenex ZB-5* column (30 m × 0.25 mm, 0.25 μm); carrier gas He; injection temp. 250°, detection temp. 280°; column temp., 150° (1 min), 20°/min to 300° (30 min). The D configuration of glucose was confirmed by the same retention time ($t_R$ = 13.59) of its acetylated thiazolidine derivative as that of the standard D-glucose (*Sigma-Aldrich*) prepared in a similar manner.

*Casearia side A* (= *(3*R*,4a*R*,5*S*,6*R*,8a*R*)-5-[2-(2,5-Dihydro-5-oxofuran-3-yl)ethyl]-3,4,4a,5,6,7,8,8a-octahydro-3-hydroxy-5,6,8a-trimethylnaphthalene-1-carboxylic Acid β-D-Glucopyranosyl Ester*; **1**): Colorless amorphous powder. $[\alpha]_D^{20} = -44.3$ ($c$ = 0.28, MeOH). UV (MeOH): 209 (3.24), 220 (sh). IR (KBr): 3308, 2924, 1717, 1634, 1226, 1059, 1013. $^1H$- and $^{13}C$-NMR ((D$_5$)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS (pos.): 533.2368 ($[M + Na]^+$, $C_{26}H_{38}NaO_{10}^+$; calc. 533.2363).

*Casearia side B* (= *(4a*R,*5*R,*6*R,*8a*R*)-5-[2-(2,5-Dihydro-5-oxofuran-3-yl)ethyl]-3,4,4a,5,6,7,8,8a-octahydro-6-(hydroxymethyl)-5,8a-dimethylnaphthalene-1-carboxylic Acid β-*D*-Glucopyranosyl Ester*; **2**): Yellow amorphous powder. $[\alpha]_D^{20} = -22.9$ ($c = 0.11$, MeOH). UV (MeOH): 202 (3.23), 220 (sh). IR (KBr): 3353, 2927, 1723, 1634, 1223, 1019. $^1$H- and $^{13}$C-NMR ((D$_5$)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS (pos.): 533.2367 ($[M+Na]^+$, $C_{26}H_{38}NaO_{10}^+$; calc. 533.2363).

*Casearia side C* (= *(4a*R,*5*S,*6*R,*8a*S*)-5-[2-(2,5-Dihydro-5-oxofuran-3-yl)ethyl]-3,4,4a,5,6,7,8,8a-octahydro-5,6,8a-trimethylnaphthalene-1-carboxylic Acid (6-*O*-*D*-Apio-β-*D*-furanosyl-β-*D*-glucopyranosyl) Ester*[3]); **3**): Colorless amorphous powder. $[\alpha]_D^{20} = -50.0$ ($c = 0.14$, MeOH). UV (MeOH): 201 (3.09), 220 (sh). IR (KBr): 3346, 2919, 1720, 1635, 1012. $^1$H- and $^{13}$C-NMR ((D$_5$)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS (pos.): 665.2576 ($[M+K]^+$, $C_{31}H_{46}KO_{13}^+$; calc. 665.2575).

*Casearia side D* (= *4-[2-{(1*S,*2*R,*4a*R,*6*R,*8a*R*)-6-[(6-*O*-*D*-Apio-β-*D*-furanosyl-β-*D*-glucopyranosyl)-oxy]decahydro-1,2,4a-trimethyl-5-methylenenaphthalen-1-yl}ethyl}furan-2(5*H*)-one*[3]); **4**): Colorless amorphous powder. $[\alpha]_D^{20} = -28.3$ ($c = 0.21$, MeOH). UV (MeOH): 202 (3.25), 220 (sh). IR (KBr): 3358, 2924, 1741, 1634, 1012. $^1$H- and $^{13}$C-NMR ((D$_5$)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS (pos.): 635.3146 ($[M+Na]^+$, $C_{31}H_{48}NaO_{12}^+$; calc. 635.3043).

*Casearia side E* (= *(4a*R,*5*S,*6*R,*8a*R*)-3,4,4a,5,6,7,8,8a-Octahydro-5-[(3*E*)-5-hydroxy-3-(hydroxymethyl)pent-3-en-1-yl]-5,6,8a-trimethylnaphthalene-1-carboxylic Acid β-*D*-Glucopyranosyl Ester*; **5**): Colorless amorphous powder. $[\alpha]_D^{20} = -68.1$ ($c = 0.39$, MeOH). UV (MeOH): 204 (3.13), 220 (sh). IR (KBr): 3353, 2927, 1684, 1220, 1017. $^1$H- and $^{13}$C-NMR ((D$_5$)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS (pos.): 521.2729 ($[M+Na]^+$, $C_{26}H_{42}NaO_9^+$; calc. 521.2727).

*Sylvestriside C* (= *(3α,4β,15α)-3,15-Dihydroxykaur-16-en-18-yl β-*D*-Glucopyranoside*; **6**): Colorless amorphous powder. $[\alpha]_D^{20} = -47.9$ ($c = 0.38$, MeOH). IR (KBr): 3368, 2926, 1386, 1077, 1020, 902. $^1$H- and $^{13}$C-NMR ((D$_5$)pyridine): *Table 3*. HR-ESI-MS (pos.): 505.2781 ($[M+Na]^+$, $C_{26}H_{42}NaO_8^+$; calc. 505.2777), 987.5662 ($[2M+Na]^+$, $C_{52}H_{84}NaO_{16}^+$; calc. 987.5657).

*Sylvestriside D* (= *(3α,4β,15α)-15,18-Dihydroxykaur-16-en-3-yl β-*D*-Glucopyranoside*; **7**): Colorless amorphous powder. $[\alpha]_D^{20} = -40.9$ ($c = 0.22$, MeOH). IR (KBr): 3338, 2929, 1305, 1076, 1035, 825. $^1$H- and $^{13}$C-NMR ((D$_5$)pyridine): *Table 3*. HR-ESI-MS (pos.): 505.2780 ($[M+Na]^+$, $C_{26}H_{42}NaO_8^+$; calc. 505.2777), 987.5681 ($[2M+Na]^+$, $C_{52}H_{84}NaO_{16}^+$; calc. 987.5657).

*Sylvestriside E* (= *(3α,4β,15β)-3,15-Dihydroxykaur-16-en-18-yl β-*D*-Glucopyranoside*; **8**): Colorless amorphous powder. $[\alpha]_D^{20} = -31.3$ ($c = 0.23$, MeOH). IR (KBr): 3329, 2928, 1305, 1076, 1037, 886. $^1$H- and $^{13}$C-NMR ((D$_5$)pyridine): *Table 3*. HR-ESI-MS (pos.): 521.2519 ($[M+K]^+$, $C_{26}H_{42}KO_8^+$; calc. 521.2517).

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*Received January 28, 2009*