Chemical Constituents from *Cynoglossum gansuense*

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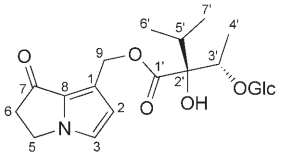
Two new alkaloids, *i.e.*, (2,3-dihydro-1-oxo-1*H*-pyrrolo[1,2-*a*]pyrrol-7-yl)methyl (2*S**,3*S**)-3-[(β-D-glucopyranosyl)oxy]-2-hydroxy-2-(1-methylethyl)butanoate (**1**) and 1,2-dihydro-8-methoxy-2-oxoquinoline-4-carboxylic acid (**2**), were isolated from the alcoholic extract of the whole plant of *Cynoglossum gansuense*, together with twelve known compounds Their structures were characterized by means of spectroscopic methods, especially by ^{1}H-, ^{13}C-, and 2D-NMR, as well as by HR-MS experiments and comparison with literature data.

Introduction. – The genus *Cynoglossum* (Boraginaceae) consists of *ca*. 60 species distributed throughout the world. Among them, *C. divaricatum*, *C. amabile*, and *C. zeylanicum* have been used as important folk medicines [1][2]. Herein, we report the first phytochemical study on *C. gansuens.* From the alcoholic extract of the whole plant of *C. gansuense* Y. L. LIU, we isolated two novel alkaloids, compounds **1** and **2**, along with the following twelve known compounds[1]): kaempferol-3-*O*-β-D-glucopyranoside [3], eugenylglucoside [4–7], lupa-12,20(29)-dien-3β-ol [8][9], 18α*H*-ursa-12,20(30)-dien-3α-ol [8][10], *trans*-phytol [11][12], lariciresinol-9-*O*-β-D-glucoside [13–15], 9-hydroxyguaiamonoepoxylignan-9-*O*-β-D-glucoside[2]) [13][14], stigmast-5-ene-3β,7α-diol [16], stigmast-4-en-3-one [16], stigmastane-3,6-dione [16][17], β-sitosterol [16][18], and β-sitosterol-3-(6′-glyceryl)-D-glucopyranoside [15][17].



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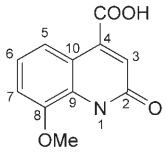
Glc = β-D-glucopyranosyl



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[1]) The (semisystematic) names reported in the literature are given.

[2]) Maybe optical isomer of lariciresinol-9-*O*-β-D-glucoside; see *Exper. Part.*

Results and Discussion. – Compound **1**, obtained as an optically active, yellowish oil ($[\alpha]_D^{29} = -20.0$ ($c = 0.5$, MeOH), had the molecular formula $C_{21}H_{31}NO_{10}$, as determined by HR-ESI-MS (m/z 480.1836 ($[M + Na]^+$; calc. 480.1840). Its UV spectrum showed bands at 202 and 287 nm, and the IR spectrum displayed absorption bands for OH (3371), Me_2CH (2929), and C=O (1731, 1689 cm^{-1}) moieties.

In the NMR spectra of **1** (*Table 1*), the signals at δ(H) 4.23 (d, $J = 8.0$ Hz) and δ(C) 105.60 (d), 69.89 (d), 74.10 (d), 76.50 (d), 76.84 (d), and 61.02 (t) indicated a β-glucopyranosyl (Glc) moiety [19], D-configuration being assumed on biogenetic grounds. The ^{1}H- and ^{13}C-NMR (DEPT) spectra indicated five quaternary C-atoms, nine CH, four CH_2, and three Me groups. In the ^{1}H-NMR spectrum, there were typical signals for three Me groups at δ(H) 1.21 (d, $J = 6.8$), 0.80 (d, $J = 6.8$), and 0.74 (d, $J = 6.8$ Hz), as well as signals for an olefin at δ(H) 7.24, 6.49 ($2d$, $J = 2.4$ Hz each, 2×1 H). In the ^{13}C-NMR spectrum, there were resonances for two C=O functions [δ(C) 189.84 (α,β-unsaturated C=O group); 172.81 (ester C=O group)], two C=C bonds [δ(C) 129.4, 123.4, 117.4, 116.6]; and eight oxygenated C-atoms [δ(C) 82.6, 82.5, 76.8, 76.5, 74.1, 69.9, 61.0, 57.6]. The ^{1}H- and ^{13}C-NMR data of **1** were fully assigned by means of ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Fig. 1*).

The HMBC correlations of both H–C(5)[3]) (δ(H) 4.26) and H–C(6) (δ(H) 2.92) with C(7) (δ(C) 189.84), between H–C(2) (δ(H) 6.49) and both C(1) (δ(C) 117.38)

Table 1. *^{1}H-, ^{13}C-, and 2D-NMR Data of* **1**. At 400/100 MHz, resp.; δ in ppm, J in Hz. Arbitrary atom numbering. Asterisks (*) mark overlapping signals.

Position	δ(H)	δ(C)	COSY	HMBC
1		117.38		2, 3, 9
2	6.49 (d, $J = 2.4$)	116.64	3	3, 9
3	7.24 (d, $J = 2.4$)	123.40	2	2
5	4.26 (t, $J = 6.0$)	42.13	6	6
6	2.92 (t, $J = 6.0$)	39.30	5	5
7		189.84		5, 6
8		129.40		2, 3, 9
9	5.13 (s)	57.61		2
1′		172.81		9
2′		82.55		3′, 5′, 6′, 7′
3′	3.76 (q, $J = 6.8$)	82.49	4′	4′, 1″
4′	1.21 (d, $J = 6.8$)	16.98	3′	3′
5′	2.03–2.06 (m)	31.27	6′, 7′	6′, 7′
6′	0.80 (d, $J = 6.8$)	15.86	5′	5′, 7′
7′	0.74 (d, $J = 6.8$)	17.67	5′	5′, 6′
1″	4.23 (d, $J = 8.0$)	105.60		3′
2″	2.90–3.10 (m)*	69.89		
3″	2.90–3.10 (m)*	74.10		
4″	2.90–3.10 (m)*	76.50		
5″	2.90–3.10 (m)*	76.84		
6″	3.62 (dd, $J = 3.2, 6.0$)	61.02		
	3.41 (dd, $J = 3.2, 6.0$)			

[3]) Arbitrary atom numbering. For systematic names of **1** and **2**, see *Exper. Part.*

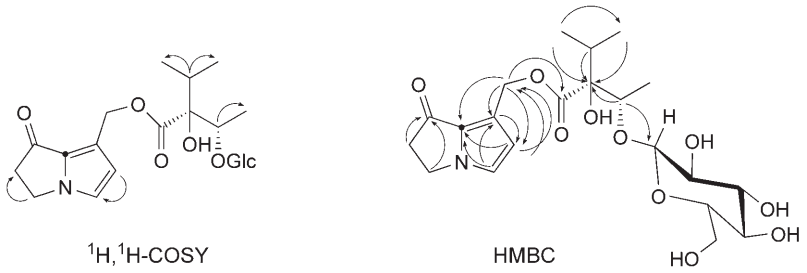

Fig. 1. *Selected COSY and HMBC correlations of* **1**

and C(3) (δ(C) 123.40), and between H–C(3) (δ(H) 7.24) and both C(1) (δ(C) 117.38) and C(2) (δ(C) 116.64) suggested a partly hydrated pyrrolizine framework. Furthermore, HMBC correlations between H–C(9) (δ(H) 5.13) and C(1) (δ(C) 117.38), C(1′) (δ(C) 172.81), C(2) (δ(C) 116.64), and C(8) (δ(C) 129.40), as well as of H–C(3′) (δ(H) 3.76) and H–C(5′) (δ(H) 2.04) with C(2′) (δ(C) 116.64) indicated that an oxygenated CH_2 group (CH_2(9)) was connected to the pyrrolizine unit at C(1), whereas C(3′) and C(5′) were attached to C(2′). The 3′-attachment of the GlcO moiety in **1** was derived from HMBC correlations between H–C(3′) (δ(H) 3.76) and the anomeric C(1″) resonance (δ(C) 105.60), as well as from a correlation between H–C(1″) (δ(H) 4.23) and C(3′) (δ(C) 82.49). In comparison with a structurally related compound previously reported in the literature [20], we found that C(3′) was shifted downfield by *ca*. 12 ppm, which further confirmed that the Glc unit was connected to the 3′-O-atom of the aglycone.

From the above data, the structure of compound **1** was, thus, elucidated as (2,3-dihydro-1-oxo-1*H*-pyrrolo[1,2-*a*]pyrrol-7-yl)methyl (2*S**,3*S**)-3-[(β-D-glucopyranosyl)-oxy]-2-hydroxy-2-(1-methylethyl)butanoate.

Compound **2**, isolated as a yellowish amorphous powder, showed the $[M+1]^+$ peak at *m*/*z* 219 by EI-MS, indicating the molecular formula $C_8H_{11}NO_4$. The IR spectrum (KBr) showed absorption bands for OH (3354), COOH (1708), and aromatic (1510 cm^{-1}) moieties.

The ^{1}H- and ^{13}C-NMR (DEPT) spectra of **2** (*Table 2*) showed one MeO group, four CH moieties, and six quaternary C-atoms. In the ^{1}H-NMR spectrum, the MeO group appeared at δ(H) 3.99 (*s*), together with olefinic signals at δ(H) 6.64 (*s*, 1 H), 7.61 (*dd*, J = 2.8, 7.2, 1 H), 7.31 (br. *d*, J = 7.2), and 7.28 (br. *d*, J = 7.2 Hz). In the ^{13}C-NMR spectrum, there were resonances for two C=O functions [δ(C) 176.9 (COOH), 163.5 (lactam C=O)], four C=C bonds [δ(C) 109.8, 138.2, 115.0, 124.1, 111.8, 148.7, 130.1, 128.0], and one oxygenated C-atom [δ(C) 56.4]. By comparison of the NMR-spectroscopic data of **1** with those of related compounds [21], a structure based on a quinolin-2-one was inferred. The HMBC correlations (*Fig. 2*) between the MeO H-atoms (δ(H) 3.99) and C(8) (δ(C) 148.7), and of both H–C(5) (δ(H) 7.61) and H–C(3) (δ(H) 6.64) with C(12) (δ(C) 176.9) showed that the COOH and MeO groups were attached at C(8) and C(4), respectively.

From above data, the structure of compound **2** was, thus, elucidated as 1,2-dihydro-8-methoxy-2-oxoquinoline-4-carboxylic acid.

Table 2. *^{1}H-, ^{13}C-, and HMBC Data of* **2**. At 400/100 MHz, resp.; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	δ(H)	δ(C)	HMBC
2		163.5	3
3	6.64 (*s*)	109.8	
4		138.2	3
5	7.61 (*dd*, J = 2.8, 7.2)	115.0	3, 7, 6
6	7.31 (br. *d*, J = 7.2)	124.1	7, 5
7	7.28 (br. *d*, J = 7.2)	111.8	5, 6
8		148.7	5, 6, 7, 11
9		130.1	3, 6
10		128.0	5, 7
MeO	3.99 (*s*)	56.4	
COOH		176.9	3, 5

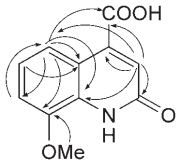

Fig. 2. *Selected HMBC* (H → C) *correlations of* **2**

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Experimental Part

General. TLC: silica gel GF_{254} (10 – 40 μm; *Qingdao Marine Chemical Factory*). Column chromatography (CC): silica gel (200 – 300 mesh; *Qingdao*) or *HPD-100* resin (*Hebei*, China). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Analytic Jena* apparatus; λ_{max} (log ε) in nm. IR spectra: *Nicolet NEXUS-670 FT-IR* spectrophotometer; in cm^{-1}. NMR Spectra: *Varian INOVA-400* spectrometer; in $CDCl_3$ or (D_6)DMSO soln.; δ in ppm rel. to Me_4Si, J in Hz. HR-ESI-MS: *Bruker APEX-II* mass spectrometer; in m/z.

Plant Material. *C. gansuense* Y. L. LIU was collected in Weiyuan County, Gansu Province, P. R. China, and identified by adjunct Prof. *Ji Ma* (Faculty of Pharmacy, First Military Medical University of PLA, Guangzhou, P. R. China). A voucher specimen (No. 2001-03) was deposited at the Key Laboratory for Natural Medicine of Gansu Province.

Extraction and Isolation. The air-dried, ground whole plant of *C. gansuens* (2.5 kg) was extracted with 95% EtOH at r.t. (7 × 24 h). The solvent was removed under reduced pressure, the residue (160 g) was suspended in H_2O (1.0 l), extracted with petroleum ether (b.p. 60 – 90°; 8 × 1 l), AcOEt (4 × 750 ml), and BuOH (12 × 1 l).

The PE-soluble fraction (64 g) was subjected to CC (SiO_2; PE/AcOEt 40 : 1 → 1 : 1) to afford five main fractions (*Fr. A – E*). *Fr. B* was further separated into three subfractions (*Fr. B1 – B3*) by CC (SiO_2; petroleum ether/$CHCl_2$ 5 : 1). *Fr. B1* was further purified by CC (SiO_2; petroleum ether/AcOEt 10 : 1) to afford *trans*-phytol (6 mg). *Fr. B2* was repeatedly subjected to CC (SiO_2; cyclohexane/Et_2O 25 : 1) to afford crude stigmast-4-en-3-one, which was purified by CC (SiO_2; $CHCl_3$/AcOEt 30 : 1; 8 mg of pure product), together with a mixture of lupa-12,20(29)-dien-3β-ol and 18αH-ursa-12,20(30)-dien-3α-ol, which was further purified by CC (SiO_2; petroleum ether/AcOEt 10 : 1) to afford 8 mg of the mixed products. *Fr. C* was subjected to CC (SiO_2; PE/acetone 15 : 1) to provide crude stigmast-5-ene-3β,7α-diol

and β-sitosterol. They were purified by CC (SiO_2; PE/acetone 10:1 and petroleum ether/AcOEt 18:1, resp.) to afford 7 and 15 mg of the pure compounds, resp. *Fr. D* was repeatedly chromatographed (SiO_2; $CHCl_3$/AcOEt 15:1) to afford stigmastan-3,6-dione (11 mg).

The AcOEt-soluble original fraction (6 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 10:1) to afford crude β-sitosterol-3-(6′-glyceryl)-D-glucopyranoside, which was further purified by CC (SiO_2; AcOEt/MeOH 15:1) to yield the pure compound (10 mg).

The BuOH-soluble fraction and *Fr. E* (32 g) from the PE-soluble extract were combined and further separated by CC (SiO_2; $CHCl_3$/MeOH 8:1) into five fractions (*Fr. F1–F5*). *Fr. F1* was further chromatographed (SiO_2; $CHCl_3$/MeOH 6:1) to yield crude **2**, which was repeatedly subjected to CC (SiO_2; AcOEt/MeOH 10:1) to afford 12 mg of pure material. *Fr. F2* was subjected to CC (SiO_2; AcOEt/MeOH 15:1) to provide crude **1**, which was further purified by CC (SiO_2; $CHCl_3$/MeOH 10:1) to afford 15 mg of pure material. *Fr. F3* was subjected to CC (SiO_2; AcOEt/MeOH 12:1) to give crude eugenylglucoside, which was further purified by CC (SiO_2; $CHCl_3$/MeOH 15:1) to give 12 mg of the pure compound. *Fr. F4* was chromatographed (SiO_2; AcOEt/MeOH 12:1) to afford crude lariciresinol-9-*O*-β-D-glucoside, which was further purified by CC (SiO_2; $CHCl_3$/AcOEt/MeOH 6:4:1) to yield 30 mg of the pure material. *Fr. F5* was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 100:15:1) to give a mixture of 9-hydroxyguaiamonoepoxylignan-9-*O*-β-D-glucoside and kaempferol-3-*O*-β-D-glucopyranoside, the former of which was purified by CC (AcOEt/MeOH 10:1) to yield 20 mg of material. The latter (residual fraction after CC) was re-subjected to CC (SiO_2; $CHCl_3$/MeOH 4:1) to yield 5 mg of the pure material.

*(2,3-Dihydro-1-oxo-1*H*-pyrrolo[1,2-*a*]pyrrol-7-yl)methyl (2*S**,3*S**)-3-[(β-*D*-Glucopyranosyl)oxy]-2-hydroxy-2-(1-methylethyl)butanoate* (**1**). Yellowish oil. $[\alpha]_D^{20} = -20$ ($c = 0.5$, MeOH). UV (MeOH): 202 (4.57), 287 (4.17). IR (KBr): 3371, 2929, 1731, 1689, 1384, 1332, 1234, 1157, 1074, 1025, 763. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 480.1836 ($[M + Na]^+$, $C_{21}H_{31}NaNO_{10}^+$; calc. 480.1840).

1,2-Dihydro-8-methoxy-2-oxoquinoline-4-carboxylic Acid (**2**). Yellowish, amorphous powder. IR (KBr): 3354, 2951, 2916, 2847, 1708, 1570, 1510, 1427, 1368, 1312, 1270, 1069, 868, 736. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 219 ($[M+1]^+$, $C_8H_{12}NO_4^+$).

Kaempferol-3-O-β-D-*glucopyranoside*. Yellow needles (MeOH). M.p. 231–233°. IR (KBr): 3406, 1745, 1654, 1608, 1498, 1362, 1209, 1179, 1073. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.03 (*d*, $J = 8.4$, 2 H); 6.87 (*dd*, $J = 2.1$, 8.7, 2 H); 6.41 (*d*, $J = 1.6$, 1 H); 6.19 (*d*, $J = 1.2$); 5.44 (*d*, $J = 7.2$, 1 H); 3.71 (*dd*, $J = 2.1$, 11.7, 1 H); 3.50 (*dd*, $J = 5.4$, 11.7, 1 H); 3.40 (*dd*, $J = 7.2$, 10.0, 1 H); 3.40–3.25 (*m*, 2 H); 3.20 (*ddd*, $J = 2.1$, 5.1, 11.7, 1 H). ^{13}C-NMR (100 MHz, (D_6)DMSO): 177.4; 165.1; 161.2; 156.5; 156.2; 133.2; 130.9; 120.9; 115.2; 103.8; 101.0; 99.0; 93.8; 77.5; 76.5; 74.2; 69.9; 60.8. EI-MS: 286 (100, $[M - Glc]^+$), 229 (11), 184, 153 (7), 121 (26).

Eugenylglucoside. Colorless powder. M.p. 130–131°. IR (KBr): 3382, 1514, 1266, 1225, 1077, 1031. ^{1}H-NMR (400 MHz, (D_6)DMSO): 6.97 (*d*, $J = 8.7$, 1 H); 6.78 (*d*, $J = 2.0$, 1 H); 6.64 (*dd*, $J = 2.0$, 8.2, 1 H); 5.91 (*ddt*, $J = 6.7$, 10.1, 16.8, 1 H); 5.04 (*dd*, $J = 1.9$, 17.0, 1 H); 5.01 (*dd*, $J = 1.9$, 9.8, 1 H); 4.82 (*d*, $J = 7.4$, 1 H); 3.72 (*s*, MeO); 3.64 (*dd*, $J = 1.8$, 11.8, 1 H); 3.42 (*dd*, $J = 5.2$, 11.8, 1 H); 3.27 (*d*, $J = 6.7$, 1 H). ^{13}C-NMR (100 MHz, (D_6)DMSO): 148.9; 144.9; 137.8; 133.5; 120.3; 115.6; 115.4; 113.0; 100.3; 76.9; 76.7; 73.2; 69.7; 60.6; 55.7; 39.0. EI-MS: 326 (M^+), 164 (100, $[M - Glc]^+$), 149, 133, 121, 103, 91, 73, 57.

Lupa-12,20(29)-dien-3β-ol. Colorless powder. IR (KBr): 3450, 2980, 1635, 1450, 1380, 1270, 1100, 1080, 1030, 770. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.12 (*t*, $J = 3.5$, 1 H); 4.67 (br. *d*, $J = 2.0$, 1 H); 4.56 (br. *d*, $J = 2.0$, 1 H); 3.25 (*dd*, $J = 5$, 12, 1 H); 1.68 (*s*, Me); 1.05 (*s*, Me); 0.96 (*s*, 2 Me); 0.82 (*s*, Me); 0.78 (*s*, Me); 0.75 (*s*, Me). ^{13}C-NMR (100 MHz, $CDCl_3$): 150.9; 145.2; 121.7; 109.3; 78.8; 55.3; 50.4; 48.2; 47.9; 43.0; 42.8; 40.8; 40.0; 38.8; 38.7; 37.1; 35.5; 34.2; 27.4; 27.4; 20.9; 18.3; 29.8; 28.0; 19.3; 18.0; 16.1; 15.9; 15.4; 14.5. EI-MS: 424 (5, M^+), 216 (24), 201 (18), 190 (20), 189 (95), 175 (58).

*18α*H-*Ursa-12,20(30)-dien-3α-ol*. Colorless needles. M.p. 163–165°. IR (KBr): 3395, 2905, 1590, 1460, 1390, 1025, 870. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.20 (*m*, 1 H); 4.65 (br. *s*, 2 H); 3.42 (*dd*, $J = 2.5$, 5.0, 1 H); 2.20 (*d*, $J = 5.5$, 1 H); 1.30 (*s*, Me); 1.13 (*s*, Me); 0.97 (*s*, Me); 0.90 (*d*, $J = 6.0$, Me); 0.83 (*s*, Me); 0.76 (br. *s*, 2 Me). ^{13}C-NMR (100 MHz, $CDCl_3$): 156.9; 139.6; 124.5; 105.9; 79.0; 55.4; 50.5; 48.7; 42.0; 40.9; 39.4; 38.9; 38.8; 38.3; 37.1; 34.5; 34.1; 28.0; 27.4; 26.6; 25.6; 25.5; 21.4; 19.5; 18.3; 16.8; 15.9; 15.4; 14.8. EI-MS: 424 (23, M^+), 216 (85), 207 (73), 202 (23), 189 (33), 187 (74), 95 (100).

trans-*Phytol*. Colorless oil. IR (KBr): 3340, 1668. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.40 (*t*, *J* = 6.8, 1 H); 4.15 (*d*, *J* = 6.8, 2 H); 1.98 (*t*, *J* = 6.8, 2 H); 1.66 (*s*, Me). ^{13}C-NMR (100 MHz, $CDCl_3$): 140.3; 123.1; 59.4; 39.9; 16.1. EI-MS (70 eV): 296 (M^+), 278 ($[M - H_2O]^+$), 71 (100).

Lariciresinol 9-O-β-Glucoside. Colorless, amorphous powder. $[\alpha]_D^{29} = -38.8$ (*c* = 0.50, MeOH). UV (MeOH): 231 (3.97), 283 (3.57). IR (KBr): 3400, 2920, 1650, 1500, 1440, 1355, 1265, 1150, 1070, 1025, 820. ^{1}H-NMR (400 MHz, (D_6)DMSO): 6.85 (br. *s*, 1 H); 6.77 (br. *s*, 1 H); 6.70 (br. *s*, 2 H); 6.65 (*d*, *J* = 8.0, 1 H); 6.54 (*d*, *J* = 8.0, 1 H); 5.08 (*d*, *J* = 4.8, 1 H); 4.98 (*d*, *J* = 5.2, 1 H); 4.19 (*t*, *J* = 7.2, 1 H); 4.13 (*dd*, *J* = 11.2, 7.2, 1 H); 4.04 (*dd*, *J* = 7.6, 7.2, 1 H); 4.00 (*dd*, *J* = 7.6, 7.2, 1 H); 3.75 (*s*, 2 MeO); 3.18 (*dd*, *J* = 12.4, 4.8, 1 H); 2.95–2.98 (*m*, 1 H); 2.82–2.89 (*m*, 1 H); 2.56 (*t*, *J* = 12.4, 1 H). ^{13}C-NMR (100 MHz, (D_6)DMSO): 147.5; 147.4; 145.7; 144.5; 134.0; 131.8; 120.8; 118.4; 115.3; 115.0; 113.0; 110.3; 103.2; 81.2; 76.6; 76.5; 73.3; 71.4; 69.8; 66.2; 60.8; 55.4; 49.8; 41.9; 32.0. FAB-MS: 545 ($[M + Na]^+$), 522 (M^+), 359 ($[M - Glc]^+$), 237, 219.

9-Hydroxyguaiamonoepoxylignan-9-O-β-D-glucoside. Colorless syrup. $[\alpha]_D^{29} = -4.1$ (*c* = 0.4, MeOH). The IR, UV, NMR, and MS data were basically identical with those of lariciresinol 9-*O*-*β*-glucoside. The relative configuration could not be unequivocally established.

Stigmast-5-ene-3β,7α-diol. Colorless needles (acetone). M.p. 202–204°. IR (KBr): 3605, 3400, 2950, 2935, 2860, 1665, 1464, 1380, 1228, 1192, 1111, 1057, 1010, 952, 928, 892. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.62 (*d*, *J* = 4.8, 1 H); 3.86 (*m*, 1 H); 3.59 (*m*, 1 H); 1.01 (s, 1 H); 0.94 (*d*, *J* = 6.6, 1 H); 0.86 (*t*, *J* = 7.2, 1 H); 0.83 (*d*, *J* = 6.6, 1 H); 0.81 (*d*, *J* = 6.6, 1 H); 0.70 (*s*, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 143.9; 123.9; 71.4; 63.4; 55.7; 49.4; 45.8; 42.3; 42.2; 42.0; 39.2; 37.5; 37.4; 37.0; 36.0; 33.9; 31.4; 29.1; 28.1; 26.0; 25.9; 23.1; 20.8; 19.8; 19.2; 18.7; 18.3; 12.0; 11.6. EI-MS: 430 (3, M^+), 412 (100).

Stigmast-4-en-3-one. Colorless needles (hexane). M.p. 87–89°. UV ($CHCl_3$): 246 (4.20). IR (KBr): 2926, 2856, 1677, 1621, 1469, 1392. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.74 (*d*, *J* = 2.0, 1 H); 1.19 (*s*, 1 H); 0.93 (*d*, *J* = 6.6, 1 H); 0.85 (*t*, *J* = 7.2, 1 H); 0.84 (*d*, *J* = 6.8, 1 H); 0.82 (*d*, *J* = 6.8, 1 H); 0.72 (*s*, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 199.5; 171.6; 123.7; 56.0; 55.9; 53.8; 45.8; 42.4; 39.6; 38.6; 36.0; 35.8; 35.7; 34.1; 33.9; 32.8; 32.1; 29.11; 28.1; 26.0; 24.1; 23.1; 21.0; 19.8; 19.2; 18.7; 17.4; 12.0; 11.9. EI-MS: 412 (100, M^+), 370 (8), 289 (12), 271 (10), 229 (31), 147 (29), 124 (93).

Stigmastane-3,6-dione. Colorless crystals (acetone). M.p. 202–204°. IR (KBr): 1707, 1461, 1425, 1384, 1259, 1239. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.96 (*s*, 1 H); 0.93 (*d*, *J* = 6.4, 1 H); 0.85 (*t*, *J* = 6.9, 1 H), 0.84 (*d*, *J* = 6.0, 1 H); 0.82 (*d*, *J* = 6.6, 1 H); 0.70 (*s*, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 211.2; 209.0; 57.5; 56.6; 56.0; 53.5; 46.6; 45.8; 43.0; 41.2; 39.4; 38.1; 38.1; 37.4; 37.0; 36.0; 33.8; 29.1; 28.1; 26.0; 24.0; 23.0; 21.6; 19.8; 19.0; 18.7; 12.5; 12.0; 11.9. EI-MS: 428 (100, M^+), 287, 245, 231, 217, 175, 149, 137, 123, 109, 95, 81, 69, 55, 43.

β-Sitosterol. Colorless needles (acetone). M.p. 139–140°. The TLC and the IR data were identical with those of an authentic sample.

β-Sitosterol-3-(6′-glyceryl)-D-glucopyranoside. Colorless wax. IR (KBr): 3417, 2927, 2854, 1740, 1466, 1379, 1174, 1083, 1021, 723. The ^{1}H- and ^{13}C-NMR data were identical to those reported. in [15][17].

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