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Cytotoxic polyisoprenes and glycosides of long-chain fatty alcohols from *Dimocarpus fumatus*

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Abstract

The ethanolic extract from the stem bark of *Dimocarpus fumatus*, showed in vitro cytotoxic activity against KB cells. Fractionation of the extract gave compounds belonging to different classes. The two major components have been identified as a benzoquinone, sargaquinone, and a chromene, sargaol. One sphingolipid, soyacerebroside I, two glycosides of sitosterol, and fatty acids were also identified. Besides these known compounds, two new glycosides of long-chain fatty alcohols have been identified as 1-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]hexadecanol and 1-*O*-[[α-L-arabinopyranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl] hexadecanol, and a mixture of three new diacylglycerylglucosides has been isolated. These structures were elucidated by analysis of 2D-NMR and mass spectra.

Keywords: *Dimocarpus fumatus*; Sapindaceae; Stem bark; Hexadecanol glycosides; Diacylglycerylglucoside; Benzoquinone; Chromene; Cytotoxicity

1. Introduction

Dimocarpus fumatus is a large tree found in Malaysia and Vietnam. Its leaves are paripennate with three or four pairs of leaflets, and the inflorescence is terminal, with few long branches, hairy, unisexual flowers (Yap, 1989). It was selected as part of a program aimed at finding new compounds with cytotoxic activity from plants collected in Malaysia. Primary chemical screening showed that no alkaloids, saponins and triterpenes were present, but we were able to isolate and elucidate the structure of two known polyisoprenes, soyacerebroside I, two glycosides of β-sitosterol, a mixture of three new diacylglycerylglucosides and two new glycosides of long-chain fatty alcohols.

2. Results and discussion

Dried and powdered stem bark of *D. fumatus* was extracted with boiling ethanol and the extract was solubilized in methanol and precipitated with acetone. The filtrate was evaporated to give an oily residue, which contained polyisoprenes **1** and **2**. The precipitate was dialyzed against water and purified by repeated silica gel column chromatography and preparative TLC to give soyacerebroside **3**, sterols **4** and **5**, diacylglycosylglycerol **6**, and two new glycosides of long-chain fatty alcohols **7** and **8**.

Compound **1**, sargaquinone, had a molecular formula of $C_{27}H_{38}O_2$ as deduced from the EI mass spectrum which displayed two peaks at *m*/*z* 394 $[M]^+$ and 396 $[M + 2H]^+$. The presence of a disubstituted quinone was supported by the observation of two *meta*-coupled aromatic protons in the ^1H NMR

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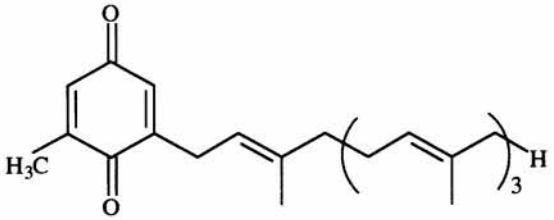

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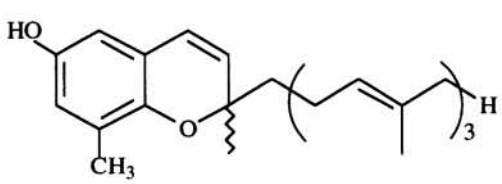

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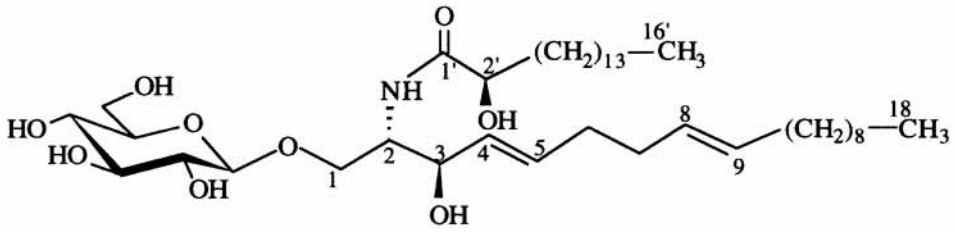

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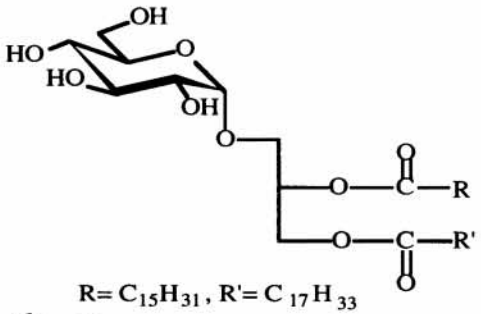
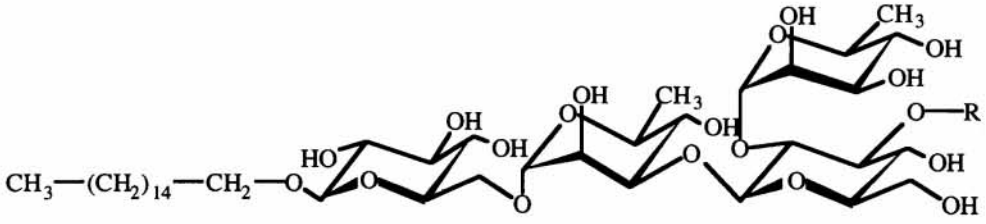


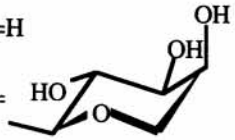
6a R= $C_{15}H_{31}$, R'= $C_{17}H_{33}$ or R= $C_{17}H_{33}$, R'= $C_{15}H_{31}$

6b R=R'= $C_{17}H_{33}$



7 R=H

8 R=



spectrum at δ 6.48 (dt, $J = 2.5$ and 2 Hz) and 6.55 (dq, $J = 2.5$ and 1 Hz), this latter signal coupling to one methyl at δ 2.08 (d, $J = 1$ Hz). The prenyl side-chain contained four isoprenyl units and was characterized as a (2′*E*,6′*E*,10′*E*,14′*E*) geranylgeranyl derivative. The first methylene in the chain showed a correlation in the COSY spectrum with the aromatic proton at δ 6.48. This and ^{13}C NMR data were in accordance with a 2-geranylgeranyl-6-methyl-1,4-benzoquinone structure (Numata et al., 1992; Numata et al., 1991; Rivera, Podesta, Norte, Cataldo, & Gonzalez, 1990). This compound is known as sargaquinone and has been previously isolated from the marine brown alga, *Sargassum tortile* (Chapmann & Hall, 1982–1997).

The mass spectrum of compound **2** ($[M]^+$ at *m*/*z* 394) indicated that it was an isomer of sargaquinone **1**. Its ^{1}H NMR spectrum showed signals for two *meta*-coupled aromatic protons at δ 6.33 and 6.49 (H-5 and H-8, d, $J = 2.8$ Hz), for two coupled olefinic protons (H-3 and H-4) at δ 5.61 and 6.27 (d, $J = 9.8$ Hz), for two methyls, one on an aromatic nucleus (H-11) at δ 2.17, and one on a quaternary carbon (H-12) at δ 1.38. ^{13}C NMR showed three quaternary carbons (C-2, C-7 and C-10) bearing oxygen functions. These signals and the occurrence of an EI mass fragment ion at *m*/*z* 175 $[M-C_{16}H_{27}]^+$ implied a chromenol structure for **2**. Signals for three olefinic protons and four vinylic methyl groups in the NMR spectra, suggested the presence of a *tris*-isoprenyl chain. Compound **2** was thus tris(isoprenyl) 2-chromenol or sargaol, also isolated from *S. tortile* (Numata et al., 1992). An optical rotation ($[\alpha]_D$ +2.54°, $CHCl_3$, *c* 0.55) was measured for compound **2**, but it was due to contamination and this was approved by recording a CD spectrum (Kikuchi et al., 1983). Sargaol from *D. fumatus* is thus, most probably, a racemic mixture as mentioned by Nakamura et al. (Numata et al., 1992) and this may be readily explained by the facile interconversion of **1** (achiral) and **2**.

NMR data for compound **3** indicated that it contained one sugar residue, an amide linkage and aliphatic long chains, suggesting a glycosphingolipid nature (Hung, Lee, Kim, & Kang, 1996). The sugar was identified as a β-D-glucose on the basis of ^{13}C NMR chemical shifts (δ 103.8, 77.1 (2C), 74.2, 70.8 and 62.1) and of the $J_{1'',2''}$ coupling constant of 7.7 Hz. Signals for two carbon atoms attached to oxygen were observed at δ 69 (CH_2OH) and 72.4 (CHOH), one methine attached to nitrogen appeared at δ 53.9 (CHNH), and an amide carbonyl signal was detected at δ 177.2 in the ^{13}C NMR spectrum. COSY and HOHAHA experiments demonstrated the presence of the partial structure of a sphingadienine moiety with double bonds at C-4 and C-8 (Hung et al., 1996). The 4,5 alkene bond was found to be *trans*, as evidenced by the large vicinal coupling constant ($J_{4,5} = 15$ Hz). The *trans* geometry of the double bonds was further demonstrated by the deshielding of the vicinal methylenes (C-6, C-7 and C-10) (Shibuya et al., 1990; Okuyama & Yamazaki, 1983). The positive FAB mass spectrum of **3** gave peaks at *m*/*z* 737 $[M + Na + H]^+$, 715 $[M + 2H]^+$ and 697 $[(M + 2H)-H_2O]^+$, indicating that its M_r was 713. Peaks at *m*/*z* 262 $[C_{18}H_{30}O]^+$ and 277 $[C_{18}H_{30}ON]^+$ were assigned to the 4(*E*), 8(*E*) octadeca-sphingadienine. In the ^{13}C NMR spectrum of **3**, another CHOH signal appeared at δ 72.6, whose corresponding proton at δ 4 (dd, $J = 8$ and 3.5 Hz) showed couplings with adjacent methylene protons (COSY) and amide carbonyl (HMBC). The amide acyl chain was deduced to be 2-hydroxypalmitic acid. The relative configurations of C-2, C-3 and C-2′ of **3** were established on the basis of the ^{13}C NMR spectral data in agreement with those published for 1-*O*-β-D-glucopyranosyl-2-*N*-2′*S*-hydroxypalmitoyl-2*S*,3*R*,4(*E*),8(*E*)-octadecasphingadienine (Mori & Kinsho, 1988). This compound is also known as soyacerebroside I and has been previously isolated from *Glycine max*, *Tetragonia tetragonoides* (Shibuya et al., 1990; Okuyama & Yamazaki, 1983) and *Prunus jamasakura* (Yoshioka, Etoh, Yagi, Sakata, & Ina, 1990).

Compounds **4** and **5** were identified as 3-*O*-β-D-glucopyranosyl β-sitosterol (daucosterol) and 6′-stearoyl-3-*O*-β-D-glucopyranosyl-β-sitosterol, respectively, by comparison of various data with those reported in the literature (Pei-Wu, Fukuyama, Rei, Jinxian, & Nakagawa, 1988).

Compound **6** appeared as a mixture of inseparable compounds displaying clusters of $[M]^+$ in the *m*/*z* 800–900 range. Its spectral data indicated the presence of one sugar unit and of one or more aliphatic unsaturated long chains. The sugar unit was identified as α-D-glucopyranose by ^{1}H and ^{13}C NMR and by the vicinal coupling constant $J_{1',2'} = 3.8$ Hz. An ABMXY system connected to oxygenated carbons (δ 64.8, 67.3 and 68.1) was observed in the NMR spectra, suggesting a glycerol moiety (Jung, Lee, & Kang, 1996). ROESY experiment showed a correlation between the anomeric proton of glucose and one methylene (δ 4.10 and 3.57) of the glycerol moiety. The two other hydroxyl groups of the glycerol unit were esterified by fatty acid chains characterized by carbonyls at δ 177.2. Other signals for protons in the chain were observed at δ 2.31 and 2.34 (t, $J = 7.5$ Hz) for protons α to the carbonyl, and at δ 5.33 for olefinic protons. The chains were identified by mass spectrometry as composed of palmitic acid, oleic acid and stearic acid with respective ion peaks at *m*/*z* 239, 262 and 264. Recording of a GC mass spectrum after transesterifica-

tion of the mixture proved that the major compound was palmitic acid. The positive FAB mass spectrum displayed a set of $[M + K + 2Li - 2H]^+$ at m/z 807.6, 819.9, 833.8 and 849.7 consistent with four compounds of M_r 756, 768, 782 and 798, respectively. Loss of one fatty acid chain led to only two fragment ions at m/z 500 $[M - RCOO]^+$ and 518 $[M - RCO]^+$, and indicated the presence in the substructure of one mono-unsaturated fatty acid chain corresponding to oleic acid. The MS/MS spectra of the ion peak $[500 + K + Li - 2H]^+$ at m/z 551.4 gave the four quasi-molecular peaks previously observed. The high mass peaks obtained in the EI mass spectrum at m/z 577 and 603 corresponded to the losses of a glucose unit $[M - OGlc]^+$. The first component **6a** possessed a M_r of 756 and it contained oleic and palmitic acids. The MS/MS of the quasi-molecular peak at m/z 807 $[M + K + 2Li - 2H]^+$ obtained by the proposal and showed ion peaks at m/z 551 $[M + K + 2Li - 2H - C_{16}H_{32}O_2]^+$ and 239 $[C_{15}H_{31}CO]^+$. Consequently, component **6a** was characterized as 1-*O*-oleyl-2-*O*-palmitoyl-3-*O*-α-D-glucopyranosylglycerol and/or 1-*O*-palmitoyl-2-*O*-oleyl-3-*O*-α-D-glucopyranosylglycerol. The second component **6b** with a M_r of 782 contained two oleic acids and its structure was deduced to be 1,2-*O*-dioleyl-3-*O*-α-D-glucopyranosylglycerol. Although glycosides of diacylglycerol are common metabolites of bacteria and important constituents of their membranes (Hauksson, Rilfors, Lindblom & Arvidson, 1995), their occurrence is rare in plants and we believe that compounds **6a** and **6b** are novel in the vegetable kingdom.

The positive FAB mass spectrum of compound **7** displayed a peak at m/z 881 $[M + Na]^+$ suggesting a M_r of 858 ($C_{40}H_{74}O_{19}$). Losses of 6-desoxyhexose and of a disaccharide consisting of an hexose and of a 6-desoxyhexose from the $[M + Na]^+$ led to fragment ions at m/z 759 and 573, respectively. The NMR spectral data of **7** exhibited signals for a glycosidic moiety with four sugar residues and for an aliphatic linear chain, which was characterized by an intense broad signal at δ 1.33 and by a three proton triplet at δ 0.89. A two proton resonance at δ 1.6 correlated in the COSY spectrum with two geminal protons at δ 3.55 and 3.85. There was no resonance for a carbonyl in the ^{13}C NMR spectrum and, therefore, the chain was assumed to correspond to a saturated linear alcohol. The four anomeric carbons at δ 102 (2C), 104.9 and 104.4 were attached to proton doublets at δ 5.22 (d, $J = 1.7$ Hz), 4.75 (d, $J = 1.4$ Hz), 4.59 (m, $W_{1/2} = 7.4$ Hz) and 4.22 (d, $J = 7.7$ Hz), respectively, in the HMQC spectrum. Two methyl carbons at δ 18 and 18.1 were assigned to the methyls of 6-desoxyhexoses, and two hydroxymethyls at δ 62.2 and 68.2 corresponded to two hexoses and/or pentoses. COSY and HOHAHA experiments allowed the full identification of the spin systems of two β-D-glucoses starting from the doublets at δ 4.22 and 4.59, and of two α-L-rhamnoses from the narrow doublets at δ 5.22 and 4.75. Sequencing of the sugar chain was achieved by observation of HMBC correlations between the CH_2O of the long-chain alcohol and H-1 of one glucose (δ_H 4.22), between the C-6 of this latter glucose (δ_C 68.2) and H-1 of the first rhamnose (δ_H 4.75), between C-3 of this rhamnose (δ_C 82.4) and H-1 of the second glucose (δ_H 4.59), and finally between C-2 of this second glucose (δ_C 79.2) and H-1 of the second rhamnose (δ_H 5.22). The alcohol was identified as hexadecanol by the FAB mass spectrum which showed a fragment at m/z 225 corresponding to $[C_{16}H_{33}]^+$. The structure of compound **7** was thus concluded to be 1-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl] hexadecanol.

Compound **8** exhibited an ion peak at m/z 1014 $[M + Na + H]^+$ suggesting a M_r of 990 ($C_{45}H_{82}O_{23}$), and a fragment ion at m/z 881 corresponding to the loss of a pentose, thus giving compound **7**. The ^1H and ^{13}C 1D- and 2D-NMR (COSY, HOHAHA, HMQC) allowed identification of all ^1H and ^{13}C signals of the four sugars present in **7** and of a fifth sugar system with an anomeric proton at δ 4.39 and identified as one α-L-arabinose. HMBC and ROESY experiments linked the arabinose residue to the second terminal glucose in position 3. Consequently, the structure of compound **8** was determined to be 1-*O*-[[α-L-arabinopyranosyl-(1 → 3)] α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl] hexadecanol.

Natural glycosides of straight-chain alcohols are rather rare and compounds **7** and **8** are among the most complex ones isolated so far (Miyase, Ueno, Takizawa, Kobayashi, & Oguchi, 1988; Yuda, Ohtani, Mizutani, Kasai, & Tanaka, 1990; Nishimura, Sasaki, Morota, Chin, & Mitsuhashi, 1990). Advances in structural elucidation and separation means will certainly allow to extend this family of compounds. It is also worth underlining that, unlike many plants from the Sapindaceae, *D. fumatus* does not contain saponins. However, it does contain a large variety of secondary metabolites, some of them being characteristic of the marine kingdom. To ascertain that no error was performed during the handling and transportation of extracts, the collection was carried out twice with the same results. Sargaquinone **1** and sargaol **2** showed cytotoxicity on P-388 cells at the 10 μg ml^{-1} level, accounting for some, but maybe not all, of the activity of the crude extract. These compounds were inactive on tubuline and topoisomerase-1.

3. Experimental

3.1. General

^1H and ^{13}C NMR were recorded at 300 and 75 MHz, respectively; 2D expts were performed using standard Bruker microprograms. For FABMS $CHCl_3$ or MeOH solns were added to glycerol or glycerol with LiCl, with *m*-NBA, or with *m*-NBA plus LiCl matrix. Preliminary cytotoxicity tests were performed on KB cells at ICSN; pure compounds were assayed on P-388 cell lines, tubuline and topoisomerase-1 at Rhône–Poulenc Vitry, according to Likhitwitayawuid, Angerhofer, Cordell, and Pezzuto (1993).

3.2. Plant material

Stem bark of *D. fumatus* (BL.) Leenh. was collected in the north of Malaya, in the Baling-Kedah province. A vaucher specimen is deposited under the code KL 4391 at the Forest Research Institute, Kejong, Malaysia, and at the Museum of Natural History of Paris, France.

3.3. Extraction and isolation

The EtOH extract (155 g) prepared from 3.7 kg of stem bark was solubilized in 275 ml of MeOH and poured into 1375 ml of Me_2CO. The ppt. was filtered and dried over KOH in vacuo (12 g). After evapn, the filtrate was again ppted by Et_2O to afford a second ppt. which was dried over P_2O_5 in vacuo (13 g). After evapn, the second filtrate gave an oily residue (125 g). The ppts were dissolved in H_2O and dialyzed against H_2O in seamless cellulose tubing. After 4 days, the content of the tubes was freeze-dried to afford 9.6 g of the mixt. of compounds (yield 6.2%). A part of the oily residue (3.5 g) was chromatographed on a silica gel column with a gradient of $CHCl_3$–MeOH (from 1:0 to 1:1). A mixt. of **1** and **2** was obtained and then chromatographed on silica gel CC eluted with CH_2Cl_2–hexane; frs 9–15 eluted with CH_2Cl_2–hexane (1:1) were purified by prep. TLC to give **1** (7 mg) and **2** (35 mg). The dialyzed ppt. (9 g) was suspended in $CHCl_3$–MeOH (3:2). The sol. fr. (6 g) was fractionated by vacuum liquid chromatography (VLC) with $CHCl_3$–MeOH–H_2O (15:10:1). The fr. containing the least polar compounds (987 mg) was chromatographed on a silica gel column eluted with a gradient of $CHCl_3$–MeOH (from 5:0 to 3:2). Compound **1** (37 mg) was collected in frs 4–14 eluting with $CHCl_3$. Compound **4** (4 mg) and a mixt. of free fatty acids (7 mg) in frs 45–60 eluting with $CHCl_3$–MeOH (49:1), were further purified on a silica gel column eluted with a gradient of CH_2Cl_2–MeOH (from 99:1 to 9:1). Compound **5** (7 mg) collected in frs 61–67 and **3** in frs 68–74 eluting with $CHCl_3$–MeOH (9:1), were purified by prep. TLC in CH_2Cl_2–MeOH (9:1) and silica gel CC, respectively. Compound **6** (5.5 mg), collected in frs 101–112 eluting with $CHCl_3$–MeOH (4:1), was purified by reverse-phase RP-18 CC eluted with a gradient of MeOH–H_2O (from 3:2 to 9:1). Frs 113–117, 118–123 and 124–157 eluting with $CHCl_3$–MeOH (7:3) contained compounds **7** (11 mg) and **8** (13 mg); final purifications were performed by reverse-phase silica gel CC and prep. TLC in $CHCl_3$–MeOH–H_2O (35:15:2).

3.4. Acid hydrolysis of glycosidic mixture

An aliquot of the dialyzed ppt. (580 mg) was purified by VLC with $CHCl_3$–MeO–H_2O (from 35:15:1 to 35:15:2). The polar fr. (60 mg) was dissolved in 4.4 ml of a mixt. (1:1) of 6.5% aq. $HClO_4$ and 0.02 N H_2SO_4, and heated at 140°C in a sealed tube for 2 h. After cooling, the ppt. obtained was filtered, rinsed with H_2O and dried in vacuo over P_2O_5. The acidic aq. layer was neutralized with 0.5 M KOH and freeze-dried. Sugars were identified by comparison with authentic samples as glucose and rhamnose by TLC in MeCOEt–*iso*-PrOH–Me_2CO–H_2O (20:10:7:6).

3.5. Sargaquinone **1**

Pale brown oil. EIMS *m/z*: 396.4 $[M+2H]^+$, 394.4 $[M]^+$, 379.4 $[M-15]^+$, 325.3 $[C_{22}H_{29}O_2]^+$, 257.3 $[C_{17}H_{21}O_2]^+$, 203.2 $[C_{15}H_{25}]^+$, 189.2 $[C_{12}H_{13}O_2]^+$, 17.1 $[C_{11}H_{11}O_2]^+$, 137.1 $[C_{10}H_{17}]^+$, 135.2 $[C_8H_7O_2]^+$, 123.2 $[C_7H_7O_2]^+$, 121.1 $[C_7H_5O_2]^+$, 107.1 $[C_6H_3O_2]^+$, 69.1 $[C_5H_9]^+$. ^1H NMR ($CDCl_3$): δ 1.60 (s, CH_3-18′, and CH_3-19′), 1.61 (s, CH_3-16′), 1.65 (s, CH_3-20′), 1.68 (s, CH_3-17′), 1.95–2.12 (m, H-4′, H-5′, H-8′, H-9′, H-12′, and H-13′), 2.08 (d, $J=1$ Hz, H-7), 3.14 (brd, $J=7$ Hz, H-1′), 5.10 (m, H-6′, H-10′, and H-14′), 5.16 (brt, $J=7$ Hz, H-2′), 6.48 (dt, $J=2.5$, 2 Hz, H-3), 6.55 (dq, $J=2.5$, 1 Hz, H-5). ^{13}C NMR ($CDCl_3$): δ 15.9 (18′ and 19′), 16.0 (7), 16.1 (20′), 17.6 (16′), 25.6 (17′), 26.4 (9′), 26.7 (13′), 27.5 (1′ and 5′), 39.6 (8′ and 12′), 39.7 (4′), 117.9 (2′), 123.8 (6′), 124.1 (10), 124.2 (14′), 131.2 (15′), 132.2 (5), 133.1 (3), 134.9 (11′), 135.4 (7′), 139.9 (3′), 145.8 (6), 148.4 (2), 187.9 (1 and 4).

3.6. Sargaol **2**

Pale brown oil. $[\alpha]_D^{21°C}$ +2.54° ($CHCl_3$, *c* 0.55). UV $\lambda_{max}^{CHCl_3}$ (nm): 256.5. EIMS *m/z*: 394 $[M]^+$, 379 $[M-15]^+$, 175.1 $[C_{11}H_{11}O_2]^+$, 137.1 $[C_{10}H_{17}]^+$. ^1H NMR ($CDCl_3$): δ 1.38 (s, CH_3-12), 1.60 (s, CH_3-15′ and CH_3-16′), 1.61 (s, CH_3-13′), 1.72 (s, CH_3-14′), 1.72 (t, $J=8$ Hz, H-1′), 1.97 (m, H-5′ and H-9′), 2.05 (m, H-2′, H-6′ and H-10′), 2.17 (s, CH_3-11), 5.13 (m, H-3′, H-7′, H-11′), 5.61 (d, $J=9.8$ Hz, H-3), 6.27 (d,

J = 9.8 Hz, H-4), 6.33 (d, J = 2.9 Hz, H-5), 6.49 (d, J = 2.8 Hz, H-8). ^{13}C NMR ($CDCl_3$): δ 15.4 (11), 15.9 (16′), 16.0 (15′), 17.7 (13′), 22.6 (2′), 25.7 (12), 25.8 (14′), 26.6 (10′), 26.7 (6′), 39.7 (5′ and 9′), 40.8 (1′), 77.8 (2), 110.3 (6), 117.0 (8), 121.4 (5), 122.8 (4), 124.1 (3′), 124.3 (7′), 124.4 (11′), 126.4 (9), 130.7 (3), 131.2 (12′), 134.9 (8′), 135.2 (4′), 144.9 (10), 148.7 (7).

3.7. Soyacerebroside I 3

$[\alpha]_D^{21°C}$ +3.35° (MeOH–$CHCl_3$, 3:2, c 0.358). (Mori and Kinsho (1988): $[\alpha]_D^{15.5°C}$ +5.4° (MeOH–$CHCl_3$, 3:2, c 0.648)). Positive ion FABMS (glycerol) m/z: 737 $[M + Na]^+$, 715 $[M + H]^+$, 697 $[M + H - H_2O]^+$, 552 $[M + H - Glc]^+$, 534 $[M + H - H_2O - Glc]^+$, 277 $[C_{18}H_{32}ON]^+$, 262 $[C_{18}H_{31}O]^+$, 207 $[C_{15}H_{27}]^+$. ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 0.90 (t, J = 6.7 Hz, CH_3-16′ and CH_3-18′), 1.28–1.31 (m, Hs-11-17 and Hs-5′-15′), 1.42 (m, Hs-4′), 1.58 (ddd, J = 14, 8, 4 Hz, H-3′a), 1.70 (dq, J = 14, 7 Hz, H-3′b), 1.97 (m, Hs-10), 2.06 (m, Hs-6), 2.07 (m, Hs-7), 3.19 (dd, J = 8.8, 7.7 Hz, glc-2″), 3.28–3.31 (m, glc-4″ and glc-5″), 3.36 (t, J = 9 Hz, glc-3″), 3.67 (dd, J = 12, 5.1 Hz, glc-6″a), 3.71 (dd, J = 10, 3.5 Hz, H-1a), 3.81 (dd, J = 12, 1.4 Hz, glc-6″b), 3.98 (ddd, J = 7.5, 5, 3.5 Hz, H-2), 4.00 (dd, J = 8, 3.5 Hz, H-2′), 4.10 (dd, J = 10, 5.5 Hz, H-1b), 4.12 (brt, J = 7.5 Hz, H-3), 4.27 (d, J = 7.7 Hz, glc-1″), 5.42 (tlike, J = 4 Hz, H-8 and H-9), 5.47 (dd, J = 15, 7 Hz, H-4), 5.73 (brdt, J = 15, 6 Hz, H-5), 7.5 (m NH). ^{13}C NMR ($CDCl_3$): δ 14.3 (16′ and 18), 23.2 (15′, 17 and 4′), 29.8–29.9–30.2–30.3 (11–15 and 5′–13′), 32.5 (16, 14′ and 5), 32.8 (10), 33.1 (7), 35.2 (3′), 53.9 (2), 62.1 (glc-6″), 69.0 (1), 70.8 (glc-4″), 72.4 (3), 72.6 (2′), 74.2 (glc-2″), 77.1 (glc-3″ and glc-5″), 103.8 (glc-1″), 129.8 (4), 130.0 (9), 131.6 (8), 134.2 (5), 177.2 (1′).

3.8. Daucosterol 4

^{1}H NMR ($CDCl_3$): δ 0.8–0.9 (s, CH_3-26, CH_3-27 and CH_3-29), 0.94 (d, J = 6.5 Hz, CH_3-21), 1.01 (s, CH_3-19), 1.27 (s, CH_3-20), 1.70 (s, CH_3-18), 2.28 (m, H-4), 2.40 (m, H-4), 3.28 (m, glc-2), 3.31 (m, glc-5), 3.40–3.50 (m, glc-3 and glc-4), 3.58 (m, H-3), 3.77 (dd, J = 11, 4.4 Hz, glc-6a), 3.86 (dd, J = 11, 3.3 Hz, glc-6b), 4.41 (d, J = 7.3 Hz, glc-1), 5.37 (brd, J = 4.5 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 12 (18 and 29), 18.5 (21), 18.7 (27), 19 (19), 19.5 (26), 20.8 (11), 22.8 (28), 24.5 (15), 28.0 (23), 28.7 (16), 28.9 (25), 31.6 (2, 7), 31.7 (8), 34.0 (22), 36.0 (20), 36.5 (10), 37.0 (1), 38.5 (12), 39.5 (4), 42.1 (13), 45.7 (24), 50.0 (9), 55.8 (17), 56.5 (14), 61.7 (glc-6), 70.0 (glc-4), 73.3 (glc-2), 75.4 (glc-5), 76.2 (glc-3), 78.9 (3), 100.9 (glc-1), 121.9 (6), 141.6 (5).

3.9. 6′-Stearoyl-3-O-β-D-glucopyranosyl-β-sitosterol 5

Positive ion FABMS (NBA) m/z: 866 $[M + Na + H]^+$, (NBA + LiCl) m/z: 851 $[M + Li + H]^+$ (NOE) m/z: 866 $[M + Na + H]^+$, 397 $[C_{29}H_{49}]^+$, 281 $[C_{18}H_{35}O_2 - 2H]^+$ (100), 267 $[C_{18}H_{35}O]^+$. ^{1}H NMR ($CDCl_3$): δ 0.61 (s, CH_3-18), 0.76 (d, J = 6.5 Hz, CH_3-26 and CH_3-27), 0.78 (t, J = 7 Hz, CH_3-29), 0.82 (d, J = 6.5 Hz, H-22), 0.89 (t, J = 7 Hz, CH_3-18″), 0.93 (s, CH_3-19), 1.18 (m, H-28), 1.22 (s, CH_3-20), 1.23 (brs, Hs-4″-17″), 1.53 (m, Hs-3″), 1.59 (m, H-25), 1.85 (m, H-4), 2.20 (brt, J = 13 Hz, H-2a), 2.26 (t, J = 6.5 Hz, Hs-2″), 2.30 (m, H-2b), 3.31 (m, glc-2′), 3.37 (m, glc-5′), 3.46 (m, H-3), 3.40–3.50 (m, glc-3′ and glc-4′), 4.20 (dd, J = 12, 1 Hz, glc-6′a), 4.30 (d, J = 8 Hz, glc-1′), 4.36 (dd, J = 12,4 Hz, glc-6′b), 5.28 (brd, J = 5 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 11.9 (18), 12.0 (29), 14.1 (18″), 18.8 (21), 19.0 (27), 19.3 (19), 19.8 (26), 21.1 (11), 22.7 (17″), 23.1 (28), 24.4 (15), 25.0 (3″), 26.1 (23), 28.2 (16), 29.2 (25), 29.2–29.7 (4″ to 16″), 31.9 (2, 7, 8), 34.0 (22), 34.2 (2″), 36.1 (20), 36.7 (10), 37.3 (1), 38.9 (12), 39.8 (4), 42.3 (13), 45.8 (24), 50.2 (9), 56.1 (17), 56.8 (14), 63.2 (glc-6′), 70.1 (glc-4′), 73.5 (glc-2′), 73.9 (glc-5′), 76.0 (glc-3′), 79.6 (3), 10 1.1 (glc-1′), 122.1 (6), 140.3 (5), 174.6 (1″).

3.10. Compound 6

^{1}H NMR (CD_3OD): δ 0.89 (t, J = 7 Hz, CH_3-16′ and CH_3-18′), 1.30 (m, Hs-15′ and 17′), 1.60 (m, –CH_2–), 2.02 (m, Hs-8″, Hs-11″), 2.31 (t, J = 7.5 Hz, Hs-2′), 2.34 (t, J = 7.5 Hz, Hs-2″), 2.93 (dd, J = 14, 9 Hz, glc-6‴a), 3.08 (t, J = 9.5 Hz, glc-4‴), 3.35 (dd, J = 14, 2 Hz, glc-6‴b), 3.40 (dd, J = 9.7, 3.8 Hz, glc-2‴), 3.57 (dd, J = 10.8, 6.3 Hz, H-3b), 3.63 (t, J = 9.5 Hz, glc-3‴), 4.07 (brdt, J = 9.5, 2 Hz, glc-5‴), 4.10 (dd, J = 10.8, 5.5 Hz, H-3a), 4.19 (dd, J = 12, 7 Hz, H-1a), 4.50 (dd, J = 12, 3 Hz, H-1b), 4.76 (d, J = 3.8 Hz, glc-1‴), 5.32 (m, H-2), 5.33 (m, H-9″ and H-10″). ^{13}C NMR (CD_3OD): δ 14.4 (16″ and 18″), 23.7 (15′, 17′ and 28), 26.0 (3′ and 3″), 30.3–30.8 (4′–13′ and 4″–15″), 33.1 (14′ and 16″), 35.0 (2′), 35.2 (2″), 64.8 (1), 67.3 (3, glc-6′), 68.1 (2), 69.9 (glc-4′), 71.8 (glc-5′), 73.5 (glc-2′), 75.0 (glc-3′), 100.0 (glc-1′), 177.2 (1′ and 1″).

3.11. Compound 7

$C_{40}H_{74}O_{19}$. $[\alpha]_D$ −37.2° (CH_3OH, c 0.492). Positive ion FABMS m/z: (glycerol) 881 $[M + Na]^+$, 735 $[M + Na - rha]^+$, (NBA) 881 $[M + Na]^+$, 735 $[M + Na - rha]^+$, 573 $[M + Na - rha - glc]^+$; EIMS m/z: 225 (100%) $[C_{16}H_{33}]^+$. ^{1}H NMR (CD_3OD): δ 0.89 (t, J = 6.5 Hz, CH_3-16), 1.28–1.29 (m, H-3–H-15), 1.6 (m, Hs-2), 3.55 (dd, J = 9.1, 2.7 Hz, H-1a), 3.85 (dd, J = 9.6, 2.9 Hz, H-1b). ^{13}C NMR (CD_3OD):

Table 1
^1H and ^{13}C NMR data of osidic moieties of glycosides **7** and **8** in CD_3OD

	7		**8**	
	δC	δH	δC	δH
β-D-Glucose				
1	104.9	4.59 d (4.4)	105	4.63 d (7)
2	79.2	3.46 m	79.7	3.59 m
3	78.7	3.46 m	86.4	3.63 m
4	71.1	3.38 dd (7, 6)	69.4	3.48 t (9)
5	77.4	3.24 ddd (6.6, 5, 2)	77.1	3.26 m
6	62.2	3.71 dd (14, 6)	62.1	3.72 dd (12, 5)
		3.79 dd (14, 2)		3.80 dd (12, 2.5)
α-L-Rhamnose				
1	102	5.22 d (1.7)	102.4	5.26 d (1.7)
2	72	3.90 dd (3.3, 2)	71.8	4.0 dd (3.1, 1.8)
3	71.9	3.76 dd (9.5, 3)	71.9	3.73 dd (10, 3)
4	74.4	3.35 t (9.5)	74.1	3.37 t (9.5)
5	69.8	4.17 dq (9.5, 6.2)	70.2	4.12 dq (9, 6)
6	18	1.26 d (6)	18	1.27 d (6)
β-D-Glucose				
1	104.4	4.22 d (7.7)	104.4	4.22 d (7.7)
2	75.1	3.16 t (8)	75.1	3.16 t (8)
3	78.1	3.33 dd (8, 6)	78.1	3.33 t (8.5)
4	71.6	3.26 t (7)	71.6	3.28 t (9)
5	76.8	3.39 ddd (7, 5.5, 1.5)	76.8	3.38 m
6	68.2	3.64 dd (11.3, 5.4)	67.6	3.62 dd (11, 5)
		3.95 dd (11.3, 1.4)		3.95 dd (11, 1.4)
α-L-Rhamnose (terminal)				
1	102	4.75 d (1.5)	102.1	4.75 d (1.5)
2	71.9	4.12 dd (3.2, 1.7)	71.9	4.13 dd (3, 1.5)
3	82.4	3.76 dd (9.5, 3)	82.4	3.78 dd (9, 3)
4	72.5	3.48 t (9.5)	74.3	3.50 t (9.5)
5	69.8	3.72 dq (9.5, 7)	69.8	3.71 m
6	18.1	1.25 d (7)	18.1	1.25 d (7)
α-L-Arabinose (terminal)				
1		–	105.4	4.39 d (7)
2		–	72.4	3.6 dd (10, 7)
3		–	72.5	3.53 m
4		–	69.8	3.82 brt (2)
5		–	68.2	3.61 dd (12, 1.5)
				3.91 dd (12, 2)

δ 14.4 (16), 71.0 (1); ^1H and ^{13}C NMR of osidic part: see Table 1.

3.12. Compound **8**

$[\alpha]_D$ −26.7° (CH_3OH, *c* 0.225). Positive FABMS (glycerol) *m/z*: 1014 $[M + H + Na]^+$, 881 $[M + Na - ara]^+$, (+LiCl) 998 $[M + H + Li]^+$. ^1H NMR (CD_3OD): δ 0.89 (t, $J = 6.5$ Hz, CH_3-16), 1.28–1.29 (m, H-3–H-15), 1.62 (qt, $J = 7.5$ Hz, Hs-2), 3.55 (m, H-1a), 3.85 (m, H-1b). ^{13}C NMR (CD_3OD): δ 14.4 (16), 23.7 (15), 33 (14), 30.4–30.9 (2–13); ^1H and ^{13}C NMR of osidic part: Table 1.

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