Two New C_{13} *nor*-Isoprenoids from the Leaves of *Casearia sylvestris*

Wei WANG,[a] Xing-Cong LI,[a] Zulfiqar ALI,[a] and Ikhlas Ahmed KHAN*,[a,b]

[a] *National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi; and* [b] *Department of Pharmacognosy, School of Pharmacy, University of Mississippi; MS 38677, U.S.A.*

Received February 19, 2009; accepted March 20, 2009; published online March 24, 2009

Two new C_{13} *nor*-isoprene glycosides, (6*S*,9*S*)-6,9-dihydroxymegastiman-4-en-9-*O*-*β*-D-glucopyranoside (1) and (6*S*,9*S*)-6,9-dihydroxymegastiman-4-en-9-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (2) were isolated from the leaves of *Casearia sylvestris*, along with icariside B_5 (3), byzantionoside B (4), blumenol B (5), blumenol C (6) and loliolide (7). The structures of these compounds were determined on the basis of 1D and 2D NMR, MS and circular dichroism (CD) spectroscopic analyses, chemical methods and comparison with the literature data.

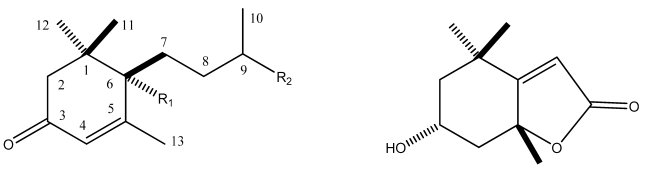
Key words *Casearia sylvestris*; Flacourtiaceae; C_{13} *nor*-isoprenoid glycoside

Casearia sylvestris (Flacourtiaceae) is a shrub or small tree widely distributed in tropical countries of South and Central America. It has a long history of use in Brazilian herbal medicine to treat snakebite, trauma, ulceration, fevers and diarrhea.[1—3] The leaves of *C. sylvestris* named "Chá de Bugre" were used in Brazil as appetite suppressant and weight loss products.[4,5] Many diterpenoids were reported from the *C. sylvestris* with antitumoral, trypanocidal and DNA-modifying bioactivities.[6—12]

In continuation of our program to search for new chemical and bio-marker of the dietary supplement, the present paper described the separation and structure elucidation of two new C_{13} *nor*-isoprene glycosides, (6*S*,9*S*)-6,9-dihydroxymegastiman-4-en-9-*O*-*β*-D-glucopyranoside (**1**) and (6*S*,9*S*)-6,9-dihydroxymegastiman-4-en-9-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**) from the leaves of *C. sylvestris*, together with four known C_{13} *nor*-isoprenoids, namely, icariside B_5 (**3**),[13] byzantionoside B (**4**),[14] blumenol B (**5**) and blumenol C (**6**),[15,16] and one known C_{11} *nor*-isoprene loliolide (**7**).[17] All these compounds were reported for the first time from the title plant. Their structures (Fig. 1) were determined on the basis of 1D and 2D NMR, MS and circular dichroism (CD) spectroscopic analyses, chemical methods and comparison with the literature data.

Compound **1** was obtained as a colorless amorphous powder, $[\alpha]_D^{20}$ +11.85 (c=0.81, MeOH). The HR-ESI-MS (m/z 411.2001, $[M+Na]^+$, Calcd 411.1995) established the molecular formula as $C_{19}H_{32}O_8$. The ^{13}C-NMR spectrum displayed 19 carbon resonances, including 6 carbon resonances [$\delta_{C\text{-}1'—C\text{-}6'}$102.9 (d), 75.8 (d), 78.9 (d), 72.6 (d), 79.2 (d), 63.6 (t)] ascribed to a *β*-glucopyranosyl unit. The ^{1}H-NMR spectroscopic data showed the resonances for two tertiary methyl groups at δ 1.19 and 1.23, one secondary methyl group at δ 1.26 (d, J=6.0 Hz), one vinyl methyl group at δ 2.12 and an olefinic proton at δ 6.01, together with the anomeric proton of a *β*-D-glucopyranosyl unit at δ 4.87 (d, J=8.0 Hz). These spectroscopic data were very similar to those of icariside B_5 (**3**)[13] which was obtained from the same subfraction at different retention time by preparative HPLC method. Furthermore, the same molecular weight and opposite optical rotation signs indicated **1** and **3** were isomers. The *β*-D-glucopyranosyl unit of **1** was located at C-9 due to the heteronuclear multiple bond connectivity (HMBC) correlation (Fig. 2) observed between the anomeric proton (δ 4.87) and C-9 (δ 75.5). The *S*-configuration at C-6 was confirmed by the fact that the CD spectrum (Fig. 3) showed a strong positive cotton effect at 220 nm and a strong negative cotton effect at 252 nm like that of **3**.[13,18] The chemical shift of C-9 (δ 75.5) in **1** was different from that of **3** (δ 76.9)[13] which has (6*S*,9*R*) configurations and was in agreement with that of byzantionoside B (**4**) (δ 75.0)[14] having *S*-configuration at C-9. So the absolute configurations at C-9 of **1** were identified as 9*S*. The D-glucose was identified by GC analysis of its acetylated thiazolidine derivative after acid hydrolysis. Based on the above evidence, the structure of **1** was assigned as (6*S*,9*S*)-6,9-dihydroxymegastiman-4-en-9-*O*-*β*-D-glucopyranoside.

Compound **2** was obtained as a colorless amorphous powder. The molecular formula, $C_{24}H_{40}O_{12}$, was determined by HR-ESI-MS (m/z: 543.2419 $[M+Na]^+$, Calcd for $C_{24}H_{40}O_{12}Na$: 543.2417). When its ^{1}H- and ^{13}C-NMR spectroscopic data were compared with those of **1** (Table 1), a set of additional signals of typical *β*-D-apiofuranosyl unit resonances [$\delta_{H\text{-}1''}$ 5.83; $\delta_{C\text{-}1''—C\text{-}5''}$ 111.3 (d), 78.1 (d), 80.8 (s), 75.3 (t), 65.9 (t)] were found.[19,20] The HMBC correlation between the anomeric proton ($\delta_{H\text{-}1''}$ 5.83) of apiofuranosyl unit



1 6*S*,9*S*; R_1 = OH, R_2 = *O*-*β*-D-glucopyranosyl
2 6*S*,9*S*; R_1 = OH, R_2 = *O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranosyl
3 6*S*,9*R*; R_1 = OH, R_2 = *O*-*β*-D-glucopyranosyl
4 6*R*,9*S*; R_1 = H, R_2 = *O*-*β*-D-glucopyranosyl
5 6*S*,9*R*; R_1 = OH, R_2 = OH
6 6*R*,9*R*; R_1 = H, R_2 = OH

Fig. 1. Structures of **1**—**7**

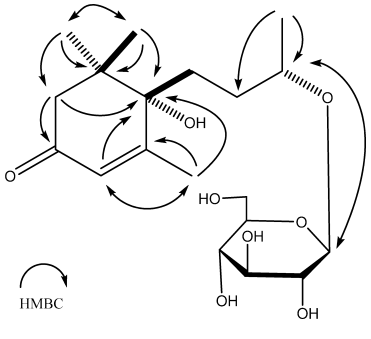
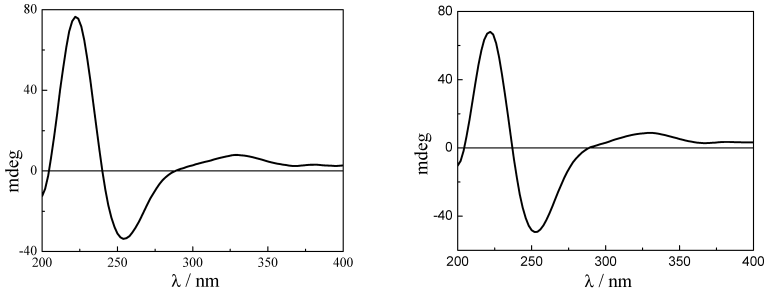


Fig. 2. Key HMBC Correlations for **1**

* To whom correspondence should be addressed. e-mail: ikhan@olemiss.edu



1 (6*S*) **2** (6*S*)

Fig. 3. CD Spectra of **1** and **2**

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** and **2** (Pyridine-d_5, δ in ppm, J in Hz)

No.	**1**		**2**	
	δ_H [a] (mult., J, Hz)	δ_C [b] (mult.)	δ_H [a] (mult., J, Hz)	δ_C [b] (mult.)
1		42.8 (s)		42.6 (s)
2	2.35 (1H, d, J=17.6 Hz)	51.3 (t)	2.41 (1H, d, J=17.6 Hz)	51.0 (t)
	2.75 (1H, d, J=17.6 Hz)		2.93 (1H, d, J=17.6 Hz)	
3		198.3 (s)		198.4 (s)
4	6.01 (1H, s)	126.9 (d)	6.08 (1H, s)	126.6 (d)
5		169.3 (s)		169.2 (s)
6		78.7 (s)		78.4 (s)
7	2.03 (1H, dt, J=4.8, 12.0 Hz)	35.3 (t)	2.20 (1H, m)	35.4 (t)
	2.43 (1H, dt, J=2.4, 12.8 Hz)		2.55 (1H, t, J=13.2 Hz)	
8	1.69 (1H, m)	33.8 (t)	1.63 (1H, m)	33.6 (t)
	2.01 (1H, m)		2.10 (1H, m)	
9	4.03 (1H, q, J=5.6 Hz)	75.5 (d)	4.04 (1H, m)	75.3 (d)
10	1.26 (3H, d, J=6.0 Hz)	20.8 (q)	1.21 (3H, d, J=6.0 Hz)	20.8 (q)
11	1.23 (3H, s)	24.3 (q)	1.26 (3H, s)	24.5 (q)
12	1.19 (3H, s)	24.7 (q)	1.23 (3H, s)	25.0 (q)
13	2.12 (3H, s)	22.2 (q)	2.14 (3H, s)	22.0 (q)
Glc-*O*-				
1′	4.87 (1H, d, J=8 Hz)	102.9 (d)	4.80 (1H, d, J=7.8 Hz)	102.6 (d)
2′	3.97 (1H, m)	75.8 (d)	3.91 (1H, t, J=8.4 Hz)	75.3 (d)
3′	3.92 (1H, m)	78.9 (d)	4.20 (1H, m)	78.8 (d)
4′	4.15 (1H, t, J=9.2 Hz)	72.6 (d)	4.04 (1H, m)	72.2 (d)
5′	4.22 (1H, t, J=8.8 Hz)	79.2 (d)	4.06 (1H, m)	77.6 (d)
6′	4.31 (1H, dd, J=5.6, 11.6 Hz)	63.6 (t)	4.20 (1H, m)	69.3 (t)
	4.53 (1H, dd, J=1.2, 11.6 Hz)		4.70 (1H, m)	
Api-*O*-				
1″			5.83 (1H, s)	111.3 (d)
2″			4.73 (1H, br s)	78.1 (d)
3″				80.8 (s)
4″			4.36 (1H, d, J=9.0 Hz)	75.3 (t)
			4.57 (1H, d, J=9.0 Hz)	
5″			4.16 (2H, br s)	65.9 (t)
6″				

a) 400 MHz, *b*) 100 MHz.

and C-6′ (δ 69.3) of glucopyranosyl unit confirmed the β-D-apiofuranosyl unit was connected with C-6′ of β-D-glucopyranosyl. The (6*S*,9*S*) configurations of **2** were determined in a similar manner as those of **1**. The D-apiose and D-glucose were also cofirmed by GC analysis after acid hydrolysis. Therefore the structure of **2** was assigned as (6*S*,9*S*)-6,9-dihydroxymegastiman-4-en-9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Experimental

General Procedures Optical rotations were measured on a Rudolph Research AutoPol IV polarimeter. CD spectra were measured on a Olis DSM 20 CD spectrophotometers. IR spectra were recorded on a Bruker Tensor 27 FT-IR and MIRacle ATRFT-IR spectrometers. UV spectra were obtained on a Hewlett-Packard 8453 UV/vis spectrometer. HR-ESI-MS data were obtained on an Agilent Series 1100 SL mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on American Varian Mercury plus 400 (^{1}H 400 MHz, ^{13}C 100 MHz) NMR spectrometers. HPLC separations were conducted on a Waters LC Module I with diode-array detector, using a Phenomenex Gemini C18 5μ ODS column (10×250 mm). Silica gel (70—230, 200—300 mesh, Merck, Darmstadt, Germany) was used for column chromatography and silica gel GF_{254} for TLC. Spots on the plate were observed under UV light and visualized by spraying with vanillin–H_2SO_4 followed by heating.

Plant Material The leaves of *Casearia sylvestris* were purchased from Raintree Nutrition Inc. (Carson City, NV 89701) and were identified by TLC and HPLC analyses with the authenticated sample offered by Dr. Rainer W.

Bussmann (Missouri Botanical Garden). Voucher specimens (#3247, 3812) were deposited at National Center for Natural Products Research, University of Mississippi, U.S.A.

Extraction and Isolation The dried and powdered plant material of *C. sylvestris* (3 kg) was extracted by percolation with MeOH (4×41×72 h). The MeOH solution was evaporated *in vacuo* to give a gummy residue (342 g). The MeOH extracts were partitioned between H_2O and EtOAc. The EtOAc layer afforded a waxy residue (207 g), which was further separated into nine fractions by column chromatography on silica gel (2500 g, 120×8 cm) with gradient elution of petroleum ether–EtOAc and $CHCl_3$–MeOH. Fraction 4 was subjected to HPLC over a Phenomenex Gemini C18 5μ octadecyl silica (ODS) column (10×250 mm, flow rate 6.0 ml/min) with MeOH–H_2O (30 : 70) as mobile phase to yield compounds **7** (20.8 mg, t_R=7.6 min) and **6** (10.4 mg, t_R=9.4 min) respectively. The residue (0.8 g) of fraction 5 was subjected to silica gel column chromatography (40 g, 60×6 cm) using EtOAc to give compound **5** (77.2 mg). The residue (2.5 g) of fraction 6 was subjected to silica gel column chromatography (80 g, 60×6 cm) eluted with EtOAc to yield 12 subfractions. Compound **4** (6.0 mg, t_R=6.8 min) was obtained from subfraction 1 by HPLC on a Phenomenex Gemini C18 5μ ODS column (10×250 mm, flow rate 7.0 ml/min) eluted with MeOH–H_2O (18 : 82). Subfraction 6 (80.2 mg) was chromatographed over HPLC on a Phenomenex Gemini C18 5μ ODS column (10×250 mm, flow rate 5.0 ml/min) with MeOH–H_2O (13 : 87) as mobile phase to obtain compounds **3** (7.5 mg, t_R=12.0 min) and **1** (16.2 mg, t_R=12.8 min) respectively. Subfraction 12 (90.2 mg) was chromatographed over the same ODS column (flow rate 6.0 ml/min) with MeOH–H_2O (12 : 88) as mobile phase to yield compound **2** (5.7 mg, t_R=11.3 min).

(6*S*,9*S*)-6,9-Dihydroxymegastiman-4-en-9-*O*-β-D-glucopyranoside (**1**): Colorless amorphous powder; $[\alpha]_D^{20}$ +11.85 (c=0.81, MeOH); UV (MeOH) λ_{max} (log ε): 241 (3.78) nm; CD (MeOH): +76.30 (220), −34.53 (252); IR (KBr) ν_{max}: 3371, 2968, 1648, 1548, 1374, 1305, 1074, 1035, 826 cm^{-1}; positive HR-ESI-MS m/z: 411.2001 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_8Na$: 411.1995); ^{1}H- and ^{13}C-NMR (pyridine-d_5, 400/100 MHz) spectroscopic data, see Table 1.

(6*S*,9*S*)-6,9-Dihydroxymegastiman-4-en-9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**2**): Colorless amorphous powder; $[\alpha]_D^{20}$ −32.98 (c=0.29, MeOH); UV (MeOH) λ_{max} (log ε): 240 (3.82) nm; CD (MeOH): +67.02 (220), −49.05 (252); IR (KBr) ν_{max}: 3360, 2927, 1646, 1549, 1366, 1306, 1017, 826 cm^{-1}; positive HR-ESI-MS m/z: 543.2419 $[M+Na]^+$ (Calcd for $C_{24}H_{40}O_{12}Na$: 543.2417); ^{1}H- and ^{13}C-NMR (pyridine-d_5, 400/100 MHz) spectroscopic data, see Table 1.

Acid Hydrolysis and Determination of Absolute Configuration of Sugars[21] Compounds (**1**, **2**) (1.0 mg) were hydrolyzed with 1 N HCl (2 ml) for 3 h at 95 °C. The reaction mixture was cooled, neutralized and partitioned between EtOAc (2 ml) and H_2O (2 ml). The aqueous layer obtained on acid hydrolysis gave the sugar residue after drying. The residue was dissolved in pyridine (0.1 ml) and 0.1 M L-cysteine methyl ester hydrochloride in pyridine (0.2 ml) was added. The mixture was heated at 60 °C for 1 h. An equal volume of Ac_2O was added with heating continued for another 1 h. Acetylated thiazolidine derivatives were subjected to GC analysis (Conditions: a ThermoQuest Trace 2000 GC; Column, Phenomenex ZB-5 column (30 m×0.25 mm×0.25 μm); carrier gas He; injection temperature 250 °C, detection temperature 280 °C; column temperature, 150 °C (1 min), 20 °C/min to 300 °C (30 min). The D configurations were confirmed for glucose and apiose by having the same retention time of their acetylated thiazolidine derivatives with those of the standard D-glucose (13.59 min), L-glucose (13.27 min) and D-apiose (9.84 min) (Sigma-Aldrich) prepared in a similar manner.

Acknowledgements This work was supported by the United States Department of Agriculture Specific Cooperative Research Agreement Number 58-6408-6-067 and the FDA/CFSAN grant entitled "Science Based Authentication of Dietary Supplements" Number 2 U01 FD 002071-07. We were grateful to Dr. Bharathi Avula for conducting the HR-ESI-MS spectra.

References

1) Lorenzi H., Matos F. J. A., "Plantas Medicinais Do Brasil: Nativas e Exóticas," Instituto Plantarum, São Paulo, 2002, pp. 220—221.
2) Tobias M. L., Oliveira F., de Oliveira K. P., Marques L. C., *Revista Eletronica de Farmacia*, **4**, 95—103 (2007).
3) Esteves I., Souza I. R., Rodrigues M., Cardoso L. G. V., Santos L. S., Sertie J. A. A., Perazzo F. F., Lima L. M., Schneedorf J. M., Bastos J. K., Carvalho J. C. T., *J. Ethnopharmacol.*, **101**, 191—196 (2005).
4) Cruz G. L., "Dicionario Das Plantas Uteis Do Brasil," 5th ed., Bertrand, 1995, pp. 599—600.
5) Oshima-Franco Y., Alves C. M. V., Andréo Filho N., Gerenutti M., Cintra A. C. O., Leite G. B., Rodrigues-Simioni L., Silva M. G., *J. Venom. Anim. Toxins incl. Trop. Dis.*, **11**, 465—478 (2005).
6) Itokawa H., Totsuka N., Takeya K., Watanabe K., Obala E., *Chem. Pharm. Bull.*, **36**, 1585—1586 (1988).
7) Itokawa H., Totsuka N., Morita H., Takeya K., Iitaka Y., Schenkel E. P., Motidome M., *Chem. Pharm. Bull.*, **38**, 3384—3388 (1990).
8) De Carvalho P. R. F., Furlan M., Young M. C. M., Kingston D. G. I., Bolzani V. D. S., *Phytochemistry*, **48**, 1659—1662 (1998).
9) Oberlies N. H., Burgess J. P., Navarro H. A., Pinos R. E., Fairchild C. R., Peterson R. W., Soejarto, D. D., Farnsworth N. R., Kinghorn A. D., Wani M. E., *J. Nat. Prod.*, **65**, 95—99 (2002).
10) Espindola L. S., Júnior J. R. V., de Mesquita M. L., Marquié P., de Paula J. E., Mambu L., Santana J., *Planta Med.*, **70**, 1093—1095 (2004).
11) Dos Santos A. G., Perez C. C., Tininis A. G., Bolzani V. D. S., Cavalheiro A. J., *Quim. Nova*, **30**, 1100—1103 (2007).
12) Wang W., Zhao J., Wang Y., Li X. C., Khan I. A., *Planta Med.*, **74**, 354 (2008).
13) Miyase T., Ueno A., Takizawa N., Kobayashi H., Oguchi H., *Chem. Pharm. Bull.*, **36**, 2475—2484 (1988).
14) Takeda Y., Zhang H., Masuda T., Honda G., Otsuka H., Sezik E., Yesilada E., Sun H., *Phytochemistry*, **44**, 1335—1337 (1997).
15) Bercht C. A. L., Samrah H. M., Lousberg R. J. J. C., Theuns H., Salemink C. A., *Phytochemistry*, **15**, 830—831 (1976).
16) Galbraith M. N., Horn D. H. S., *J. Chem. Soc., Chem. Commun.*, **3**, 113—114 (1972).
17) Kimura J., Maki N., *J. Nat. Prod.*, **65**, 57—58 (2002).
18) Buske A., Schmidt J., Porzel A., Adan G., *Eur. J. Org. Chem.*, **18**, 3537—3543 (2001).
19) Liva R. R. H., Kasai R., Yamasaki K., *Phytochemistry*, **60**, 339—343 (2002).
20) Li W., Wei K., Fu H., Koike K., *J. Nat. Prod.*, **70**, 1971—1976 (2007).
21) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **35**, 501—506 (1987).