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Phytochemistry 66 (2005) 2381–2387
PHYTOCHEMISTRY
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Phenolic and terpenoid compounds from *Chione venosa* (SW.) URBAN var. *venosa* (Bois Bandé)

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Received 31 March 2005; received in revised form 30 June 2005
Available online 2 September 2005

Dedicated to Univ. Prof. Dr. Wolfgang Kubelka on the occasion of his 70th birthday

Abstract

The Caribbean island of Grenada furnishes the popular aphrodisiac drug Bois Bandé, which consists of the stem bark and the roots of *Chione venosa* (SW.) URBAN var. *venosa* (Rubiaceae), a native tree growing in the islands' rain forest. The phytochemical investigation of dichloromethane and methanolic-aqueous extracts of the bark and the roots yielded three acetophenone derivatives described for the first time in plants – *ortho*-hydroxy-acetophenone-azine (**1**), acetophenone-2-*O*-[β-D-apiofuranosyl-(1″ → 6′)-*O*-β-D-glucopyranoside] (**2**) and acetophenone-2-*O*-β-D-glucopyranoside (**3**) – along with five known compounds, α-morroniside (**4**), sweroside (**5**), diderroside (**6**), daucosterol (**7**) and β-sitosterol (**8**). Their structures were elucidated by 1D and 2D NMR analysis, UV–Vis and ESI–MS.

Keywords: *Chione venosa* (SW.) URBAN var. *venosa*; Rubiaceae; Bois Bandé; Aphrodisiac; Spectroscopic analysis; Acetophenones; Iridoids; Triterpenes

1. Introduction

A Caribbean aphrodisiac known under the vernacular name Bois Bandé enjoys great popularity with the locals of the West Indies and also gains increasing significance in Europe due to the growing number of respective remedies available as "Bois Bandé" on the Internet and due to tourism. In the different West Indian islands different species are collected (Howard, 1989) and offered for sale at the local markets. The Caribbean island of Grenada furnishes the Bois Bandé which consists of the stem bark and the roots of a native tree growing in the islands' rain forest. Contrary to Grenadian sources (Groome, 1970) the drug does not stem from Roupala montana AUBL. (Proteaceae) but *Chione venosa* (SW.) URBAN var. *venosa* (Rubiaceae). Despite the strong belief of the Grenadian locals in the efficacy of this bark there exist warnings that too extensive consumption or high dosages of the drug may cause unwanted side-effects in the urogenital tract. Thus the phytochemical composition of this species is of interest, because no investigations about its compounds have been carried out so far. As *Chione* is considered a monotypic genus having four varieties, the chemical study of one variety of the sole species may also contribute to the question of the genus' taxonomic position within the family Rubiaceae, which is considered to be a very complex taxon (Robbrecht, 1993). Even though a taxonomic

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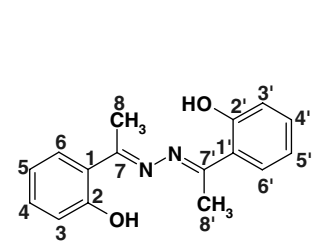
0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.07.002

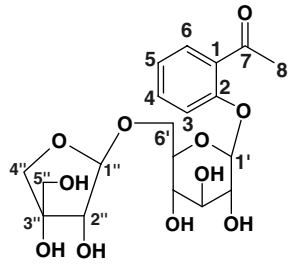
revision and phylogenetic study of this genus was recently published (Taylor, 2003a,b) the tribal placement of *Chione* is still under discussion.

2. Results and discussion

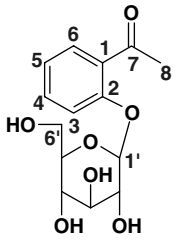
For the first time three *ortho*-hydroxy-acetophenone derivatives were detected in plants (**1**–**3**), one of them, compound (**2**), is new. In addition, three iridoid glycosides (**4**–**6**) already known from other genera as well as two widely distributed triterpenes (**7**, **8**) were isolated from the bark and root of *Chione venosa* (SW.) URBAN var. *venosa*. The structures of all eight compounds were elucidated.



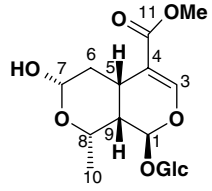
1 (*o*-Hydroxy-acetophenone-azine)



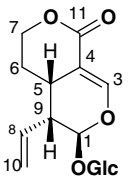
2



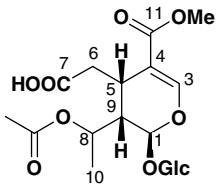
3



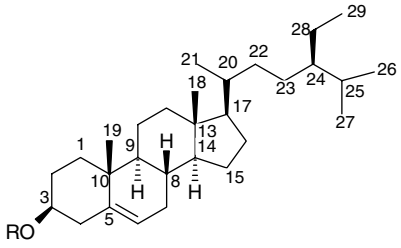
4 (α-Morroniside)



5 (Sweroside)



6 (Diderroside)



7: R=Glucose (Daucosterol)
8: R=H (β-Sitosterol)

Compound **1** was isolated from the root and emerged when we scrutinised the drug for potentially included alkaloids. This procedure required the moistening of the crude drug with ammonia, after 20 min dichloromethane was used for extraction. The organic layer was purified by extraction with aqueous acid. Subsequent alkalinisation with ammonia, re-extraction with dichloromethane and CC on silica gel yielded yellow needles of compound **1**. The other compounds resulted from the 40% methanolic-aqueous extract of the stem bark after separation on a column of Sephadex-LH-20 (**3**, **7**, **8**) and semipreparative HPLC on RP8 (**2**, **4**–**6**), respectively. The five substances which are known to be present in other plants were identified by comparing their spectroscopic data with literature values: α-morroniside (**4**) (Inouye et al., 1973; Gross and Sticher, 1986; Otsuka and Kijima, 2001), sweroside (**5**) (Ma et al., 1994; Machida et al., 1995), diderroside (**6**) (Adeoye and Waigh, 1983; Cardona Zuleta et al., 2003) and daucosterol (**7**) (Misra and Tiwari, 1973; Paulo et al., 2000). β-Sitosterol (**8**) was identified by co-chromatography with a reference substance.

The occurrence of eight carbons and four aromatic protons in the NMR spectra of compound **1** compared with the molecular weight of 268 amu led to the conclusion that **1** is a symmetric molecule containing two OH-groups and two nitrogens. The combination of the information from the one- and two-dimensional NMR experiments and mass spectra (HR–MS) revealed an *ortho*-hydroxy-acetophenone azine structure for **1**. The recorded ^{1}H and ^{13}C NMR data correlate well with the literature (Meléndez et al., 1985; Höpfl and Farfán, 1998). This compound is known as a synthetic analytical agent (Anschütz and Scholl, 1911; Ried and Nyiondi-Bonguen, 1973), which forms boron chelates (Höpfl and Farfán, 1998) and complexes with divalent transition metal ions (El-Sayed et al., 2002). Furthermore, **1**

is discussed as a substance useful in dielectric and photosemiconductors technologies (Ammar et al., 2002), but its isolation from a plant is new. As compound **1** was not detected in freshly prepared dichloromethane extracts the presumption arises that it might be an artefact generated from acetophenone during the above mentioned extraction procedure.

Compound **2** has the molecular formula $C_{19}H_{26}O_{11}$, as deduced from the HR–MS. The ^{1}H and ^{13}C NMR experiments indicated the presence of a 1,2-disubstituted aromatic ring with one acetyl group in addition to a β-D-apiofuranosyl-(1″ → 6′)-*O*-β-D-glucopyranosyl unit. The chemical shifts of the aromatic carbons and protons indicated the substitution in *ortho*- or *meta*-position. The 1,2-disubstitution was deduced from the ^{1}H–^{1}H COSY spectrum: the protons H-4 and H-5 showed two big coupling constants originating from the two vicinal protons, respectively, whereas H-3 and H-6 had only one big coupling constant. To prove the absolute configuration of both sugars a technique was applied published by Reznicek et al. (1993). This procedure required the chemical reaction of compound **2** with R-(−)-2-butanol after its enzymatic hydrolysis to yield the corresponding diastereomeric 2-butyl-glycosides. These derivatives as well as those of the reference sugars were separated by GC–MS after trimethylsilylation and showed D configuration for both, the glucose and the apiose. The NMR data of the anomeric centers suggested β-configurations for both sugar moieties. The complete relative stereochemistry was assigned by selective 1D TOCSY and NOE experiments. The chemical shifts of the carbohydrate unit were in perfect accordance with the data reported for glycosides with identical glycosylation pattern isolated from *Salvia officinalis* (Wang et al., 1998; Lu and Foo, 2000) and *Canthium berberidifolium* (Kanchanapoom et al., 2002). Compound **2** is new and has not yet been reported in literature.

The NMR spectra of compound **3** resembled to those of **2**, its mass spectrum led to the molecular formula $C_{14}H_{18}O_7$. The mass difference of 132 amu and the missing ^{1}H and ^{13}C NMR resonances of the apiose moiety suggested that compound **3** is an acetophenone-2-*O*-β-D-glucopyranoside. The assignment was complicated by overlapping signals of the protons H-3′ and H-5′ at δ 3.45 and very similar shifts for the associated carbons at δ 78.6. The glucopyranosyl unit was confirmed by GC–MS analysis after acid hydrolysis and trimethylsilylation using glucose as reference substance. Acetophenones substituted in position 4 are described frequently in plants (Ushima and Furuya, 1989; Shao et al., 1996) whereas the presented glycosides with substitution only in position 2 are new from natural sources. In 1899 *ortho*-acetophenone was detected in the volatile oil of the wood and bark of a plant identified as *Chione glabra* (Dunstan and Henry, 1899). The determination of this species is questionable, because the genus *Chione* is represented by only one species, *Chione venosa*, and the name *Chione glabra* DC stands synonymously for *Chione venosa* (SW.) URBAN (Taylor, 2003a). As the investigated specimens were reported to have been collected from the island of Grenada, we presume that the plant material studied in 1899 originated from *Chione venosa* (SW.) URBAN var. *venosa*. Dunstan and Henry speculated about the presence of *ortho*-acetophenone and possible derivatives which may contribute to the faecal odour of the fresh wood. According to literature *ortho*-acetophenone contributes to the odour of various plants, such as cacao (Marion et al., 1967; Flament et al., 1967), coffee (Stoll et al., 1967) and tomatoes (Viani et al., 1969).

The genus *Chione* has been subject to taxonomic studies for years and its assignment to a specific tribe is still under discussion. De Candolle placed this genus in the subtribe Guettardeae, Hooker established a more restrictive group, the tribe Chiococceae, and suggested inclusion of *Chione*. Later, when more morphological and molecular data were acquired, several authors concluded that *Chione* should be excluded from the Chiococceae, others claimed their maintenance in this tribe (Taylor, 2003a). Based on molecular sequence data (DNA sequences from the chloroplast *trnL-F* region) Rova et al. (2002) affiliated *Chione* with the subfamily Cinchonoideae but stated that it remained of uncertain tribal position. The results of our study, the isolation of the iridoids diderroside, sweroside and α-morroniside, indicate a close relationship of *Chione* to the Cinchonoideae, as these compounds are also found in the genera *Nauclea* and *Adina*, both Cinchonoideae (Inouye et al., 1988).

Ours is the first study of the chemical composition of *Chione venosa* (SW.) URBAN var. *venosa*. It has revealed the presence of three acetophenones hitherto unknown in plants, three iridoids and two well-known triterpenes. These results not only enhance the knowledge of a traditionally used medicinal plant but also contribute to the intra-familiar classification of the Rubiaceae through the detection of iridoids, which seem to be of taxonomic interest.

3. Experimental

3.1. General experimental procedures

NMR-spectra were recorded on a Varian Unity Inova 400 NMR spectrometer at 297 K. Sample tubes: 5 mm diameter (Kontes Glass Company, The Gerresheimer Group). Dual probe head with shielded *z*-gradients or broadband probe (400 MHz). Internal standard: TMS. Solvents: acetone-d_6, methanol-d_4 and chloroform-*d*. HMBC experiments were optimised for a long-range

coupling constant of 8 Hz. Before NOE experiments were performed, dissolved oxygen was removed by bubbling Ar through the solution. Assignments marked with an asterisk are interchangeable.

HR–MS was performed on a PE-SCIEX Qstar QTOF mass spectrometer using the ionspray source in the negative ESI mode, exact mass calibration with quercitrin $m/z = 447.0927$ (100%), $[M-H]^-$. Ionisation of compound **1** required the positive ESI mode, exact mass calibration with quinine $m/z = 325.1916$ (100%), $[M+H]^+$.

For IR spectra a solution of the respective compound in methanol was dropped on a silicon plate (13 × 1 mm, polished optically, Korth Kristalle GmbH) leaving a slight film. Spectra were recorded with a Perkin–Elmer System 2000GC IR (software Spectrum for Windows 1.30); resolution: 4 cm^{-1}; J-stop resolution: 7.77 cm^{-1}; apodization: strong; gain: 1; OPD velocity: 2 cm/s; interferogram: bi-directional double sided; phase correction: self 64; number of scans: 1; scan range: 5200–370 cm^{-1}; interval: 1.0 cm^{-1}.

Optical rotation was determined with a Perkin–Elmer Polarimeter 341 and photomultiplier 1P28A at 20 °C.

UV spectra were recorded on line in methanol–water by DAD detection during the HPLC runs.

Analytical TLC was performed on silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm). The spots were visualised under UV 254 nm and by spraying anisaldehyde-sulphuric acid (Dequeker, 1964) followed by heating at 150 °C for 5 min.

CC was carried out on silica gel (Merck, Kieselgel 60, 0.40–0.063 mm) and Sephadex®-LH-20 (Pharmacia Biotech). The columns for semipreparative and analytical HPLC were SRD Polyprep® C8 (12 μm; 250 × 16 mm) and LiChrospher® 100 RP8 (5 μm; 250 × 4 mm) with a guard column LiChrospher® 100 RP8 (5 μm; 4 × 4 mm), respectively. The Perkin–Elmer HPLC apparatus consisted of two pumps (Series 10), a DAD (LC-235) and the Perkin–Elmer Omega® software. Varying methanol–water mixtures (v/v) with a flow rate of 1.0 ml/min and 10.0 ml/min, respectively, were employed at room temperature as mobile phases. The water was adjusted to pH 3 by addition of TFA. The elution was initially isocratic and then a linear gradient with a flow rate of 1%/min was applied. System 1: 0–20 min MeOH–H_2O (15:85) isocratic, within 35 min (20–55 min) up to MeOH–H_2O (50:50); system 2: 0–30 min MeOH–H_2O (5:95) isocratic, within 45 min (30–75 min) up to MeOH–H_2O (50:50); system 3: 0–30 min MeOH–H_2O (11:89) isocratic, within 39 min (30–69 min) up to MeOH–H_2O (50:50). Detection was performed at 210 and 240 nm.

GC–MS was performed on a Shimadzu QP2010 GC–MS using a WCOT Phenomenex Zebron ZB-5 column (0.25 mm × 60 m; 0.25 μm); temperature gradient: 100 °C to 270 °C, rate: 3 °C/min; injector: 270 °C; interface: 270 °C; ion source: 250 °C; carrier gas: He 5.0 at a flow rate of 2 ml/min; split ratio: 1:10; vacuum: 4.0×10^{-4} Pa; scan: 40–500 amu/0.5 sec. Before analysis 1–2 mg of compound **2** and the reference sugars were dissolved in 1 ml water and subjected to enzymatic hydrolysis with β-glucuronidase for 5 h at 37 °C. After evaporation to dryness 450 μl R-(−)-2-butanol and 50 μl concentrated HCl were added, the mixture was kept for 15 h at 100 °C. The dried residue was redissolved in 100 μl pyridine (dried) and trimethylsilylated by addition of 40 μl hexamethyl-disilazane and 50 μl trimethyl-chlorosilane, 1 μl of this solution was injected.

3.2. Plant material

Samples of Bois Bandé (cut dried stem bark and root) were purchased from local collectors in Grenada in 1998. The plant material was microscopically identified by comparison with authentic bark and root samples of *Chione venosa* (SW.) URBAN var. *venosa*. Voucher specimens are deposited at the herbarium of the Institute of Pharmacognosy, University of Vienna.

3.3. Extraction and isolation

Dried roots (7 g) of *Chione venosa* (SW.) URBAN var. *venosa* were pulverised and moistened with 2 ml of concentrated NH_3 over a period of 20 min. The extraction was performed by shaking with CH_2Cl_2 (4 × 140 ml) at room temperature for 20 min. The volume of the combined extracts was reduced to 100 ml under vacuum and subsequently extracted with 5% HCl (v/v, 5 × 110 ml). After adjustment with concentrated NH_3 to pH 8 the combined aqueous fractions were extracted with CH_2Cl_2 (4 × 300 ml) yielding long yellow crystal needles after evaporation of the organic solvent. They were further purified by CC on silica gel using CH_2Cl_2 (30 ml) as eluent and gave 5.1 mg of compound **1**.

Dried stem bark (50 g) of *Chione venosa* (SW.) URBAN var. *venosa* was pulverised and extracted with 40% MeOH (v/v, 500 ml) at room temperature for one week. Concentration of the MeOH extract in vacuo was performed by addition of *n*-BuOH to remove the water and yielded 12 g extract which was split into two parts. 2 g were redissolved in *iso*-PrOH and separated on Sephadex®-LH-20 (30 g-dry weight) with *iso*-PrOH (750 ml) into seven fractions (I–VII). Fractions I, IV and VI were further purified by CC on silica gel using $CHCl_3$ and $CHCl_3$–MeOH mixtures with increasing polarity: $CHCl_3$ (10 ml), $CHCl_3$–MeOH (95:5, 20 ml), $CHCl_3$–MeOH (9:1, 70 ml), $CHCl_3$–MeOH (85:15, 20 ml), $CHCl_3$–MeOH (8:2, 10 ml), $CHCl_3$–MeOH (75:25, 10 ml), $CHCl_3$–MeOH (1:1, 10 ml). 3.0 mg of compound **8** crystallised from fraction I/$CHCl_3$. Fraction IV/$CHCl_3$–MeOH (85:15) yielded 4.7 mg of compound **7** and fraction VI/$CHCl_3$–MeOH (9:1) was

further purified by CC on silica gel running $CHCl_3$–MeOH (9:1, 20 ml) as mobile phase. This purification process resulted in the isolation of 8.0 mg of crystallised compound **3**. The second part of the MeOH extract (10 g) was subjected to semipreparative HPLC on RP8 and gave two fractions employing system 1 (fraction 1 $R_t = 30$–33 min and fraction 2 $R_t = 38$–41 min). Further separation of fraction 1 on RP8 furnished the compounds **2** (2.6 mg) and **4** (3.2 mg) running system 2; fraction 2 afforded the compounds **5** (2.0 mg) and **6** (2.7 mg) running system 3.

*3.3.1. o-Hydroxy-acetophenone-azine (**1**)*

Yellow needles from CH_2Cl_2, isolated in 0.073% yield, mp 197–199 °C. TLC R_f: 0.7 (mobile phase: CH_2Cl_2); detection: yellow at day light; orange and yellow fluorescence under $UV_{254\,nm}$ and $UV_{366\,nm}$, respectively; black with Dragendorff reagent. UV λ_{max} (MeOH 70%) nm: 220, 240 (sh), 295, 360. IR ν_{max} cm^{-1}: 1607 (s), 1562 (m), 1494 (m), 1440 (m), 1363 (m), 1300 (s), 1248 (s), 1160 (w), 837 (s), 755 (s). ^1H NMR (acetone-d_6) δ: 2.59 (6H, *s*, H-8/8′), 6.90–6.96 (2H, *m*, H-5/5′), 6.94 (2H, *d*, J = ~ 7.5 Hz, H-3/3′), 7.37 (2H, *td*, J = 7.7, 1.4 Hz, H-4/4′), 7.78 (2H, *dd*, J = 7.9, 1.2 Hz, H-6/6′), 13.01 (2H, *s*, OH-2/2′); ^{13}C NMR (acetone-d_6) δ: 14.9 (C-8/8′), 118.4 (C-3/3′), 119.7 (C-5/5′), 120.0 (C-1/1′), 130.3 (C-6/6′), 133.6 (C-4/4′), 161.5 (C-2/2′), 169.3 (C-7/7′); HR–MS (positive) *m/z:* 269.1273 (100%) $[M+H]^+$ (calculated for $C_{16}H_{17}N_2O_2$: 269.1290).

*3.3.2. Acetophenone-2-O-[β-D-apiofuranosyl-1″ → 6′-O-β-D-glucopyranoside] (**2**)*

Colourless oil isolated in 0.003% yield, $[\alpha]_D^{20}$ −45° ($CHCl_3$; c 0.214). TLC R_f: 0.5 (mobile phase: $CHCl_3$–MeOH–1% formic acid 7:3:0.3); detection: light brown with anisaldehyde-sulphuric acid reagent. UV λ_{max} (MeOH 8%) nm: 210, 250, 300. IR ν_{max} cm^{-1}: 1677 (s), 1599 (m), 1477 (m), 1452 (s), 1381 (m), 1296 (s), 1260 (s), 1076 (s), 993 (w), 897 (w), 820 (w), 766 (w), 738 (m). ^1H NMR (methanol-d_4) δ: 2.70 (3H, *s*, H-8), 3.39 (1H, *t*, *J* = 8.5 Hz, H-4′), 3.49 (1H, *t*, *J* = ~ 9 Hz, H-3′), 3.55 (1H, *t*, *J* = ~ 8 Hz, H-2′), 3.57–3.61 (1H, *m*, H-5″), 3.60–3.66 (1H, *m*, H-5′), 3.64 (1H, *d*, *J* = 9.1 Hz, H-$6'_a$), 3.77 (1H, *d*, *J* = 9.6 Hz, H-$4''_a$), 3.93 (1H, *d*, *J* = 2.5 Hz, H-2″), 3.98 (1H, *d*, *J* = 9.6 Hz, H-$4''_b$), 4.04 (1H, *d*, *J* = 9.1 Hz, H-$6'_b$), 4.99 (1H, *d*, *J* = 2.5 Hz, H-1″), 5.04 (1H, *d*, *J* = 7.5 Hz, H-1′), 7.12 (1H, *td*, *J* = 7.6, 0.9 Hz, H-5), 7.34 (1H, *d*, *J* = 8.2 Hz, H-3), 7.56 (1H, *td*, *J* = 7.6, 1.8 Hz, H-4), 7.68 (1H, *dd*, *J* = 7.8, 1.8 Hz, H-6); ^{13}C NMR (methanol-d_4) δ: 32.5 (C-8), 65.8 (C-5″), 69.1 (C-6′), 71.8 (C-4′), 75.2 (C-2′), 75.3 (C-4″), 77.5 (C-5′), 78.3 (C-2″), 78.6 (C-3′), 80.8 (C-3″), 102.9 (C-1′), 111.3 (C-1″), 117.9 (C-3), 123.7 (C-5), 130.8 (C-1), 131.1 (C-6), 135.4 (C-4), 158.4 (C-2), 202.8 (C-7). HR–MS (negative) *m/z*: 429.1508 (20%) $[M-H]^-$ (calculated for $C_{19}H_{25}O_{11}$: 429.1396).

*3.3.3. Acetophenone-2-O-β-D-glucopyranoside (**3**)*

White crystals isolated in 0.016% yield, $[\alpha]_D^{20}$ −66° (MeOH; c 0.114). TLC R_f: 0.6 (mobile phase: $CHCl_3$–MeOH 8:2); detection: brown with anisaldehyde-sulphuric acid reagent, positive reaction with Dragendorff reagent. IR ν_{max} cm^{-1}: 1671 (s), 1598 (m), 1485 (m), 1453 (m), 1421 (w), 1361 (m), 1296 (m), 1234 (s), 1075 (s), 1046 (s), 897 (w), 836 (w), 765 (m). ^1H NMR (methanol-d_4) δ: 2.67 (3H, *s*, H-8), 3.40 (1H, *t*, *J* = ~ 9 Hz, H-4′), 3.44–3.51 (2H, *m*, H-3′, H-5′), 3.53 (1H, *t*, *J* = ~ 9 Hz, H-2′), 3.70 (1H, *dd*, *J* = 12.1, 5.5 Hz, H-$6'_a$), 3.89 (1H, *dd*, *J* = 12.1, 2.1 Hz, H-$6'_b$), 5.06 (1H, *d*, *J* = 7.5 Hz, H-1′), 7.09 (1H, *td*, *J* = 7.9, 0.9 Hz, H-5), 7.31 (1H, *d*, *J* = 8.0 Hz, H-3), 7.51 (1H, *td*, *J* = 7.9, 1.8 Hz, H-4), 7.66 (1H, *dd*, *J* = 7.7, 1.7 Hz, H-6); ^{13}C NMR (methanol-d_4) δ: 32.5 (C-8), 62.8 (C-6′), 71.5 (C-4′), 75.2 (C-2′), 78.6 (C-3′, C-5′), 102.7 (C-1′), 117.6 (C-3), 123.6 (C-5), 130.6 (C-1), 131.1 (C-6), 135.3 (C-4), 158.4 (C-2), 202.9 (C-7). ESI–MS (negative) *m/z*: 297 (50%) $[M-H]^-$ ($C_{14}H_{18}O_7$: 298).

*3.3.4. α-Morroniside (**4**)*

Colourless oil isolated in 0.006% yield. TLC R_f: 0.6 (mobile phase: $CHCl_3$–MeOH–1% formic acid 7:3:0.3); detection: violet with anisaldehyde-sulphuric acid reagent. UV λ_{max} (MeOH 5%) nm: 240. HR–MS (negative) *m/z*: 405.1408 (20%) $[M-H]^-$ (calculated for $C_{17}H_{25}O_{11}$: 405.1396).

*3.3.5. Sweroside (**5**)*

Colourless oil isolated in 0.004% yield. TLC R_f: 0.6 (mobile phase: $CHCl_3$–MeOH–1% formic acid 7:3:0.3); detection: green-yellow with anisaldehyde-sulphuric acid reagent. UV λ_{max} (MeOH 10%) nm: 245. HR–MS (negative) *m/z*: 357.1123 (10%) $[M-H]^-$ (calculated for $C_{16}H_{21}O_9$: 357.1185).

*3.3.6. Diderroside (**6**)*

Colourless oil isolated in 0.006% yield. TLC R_f: 0.3 (mobile phase: $CHCl_3$–MeOH–1% formic acid 7:3:0.3); detection: petrol with anisaldehyde-sulphuric acid reagent. UV λ_{max} (MeOH 5%) nm: 235. IR ν_{max} cm^{-1}: 1709 (s), 1638 (m), 1575 (s), 1440 (w), 1403 (s), 1266 (s), 1193 (w), 1161 (w), 1077 (s), 1044 (m). ^1H NMR (methanol-d_4) δ: 1.38 (3H, *d*, *J* = 6.4 Hz, H-10), 2.00 (3H, *s*, CH_3COO-8), 2.18–2.23 (1H, *m*, H-9_β), 2.38–2.45 (1H, *m*, H-6_a), 2.59–2.66 (1H, *m*, H-6_b), 3.20 (1H, *t*, *J* = 8.3 Hz, H-2′), 3.28 (1H, *t*, *J* = ~ 7.5 Hz, H-4′), 3.28–3.33 (1H, *m*, H-5′), 3.31–3.38 (1H, *m*, H-5_β), 3.37 (1H, *t*, *J* = ~ 8.0 Hz, H-3′), 3.67 (1H, *d* br, *J* = 12.0 Hz, H-$6'_a$), 3.67 (3H, *s*, OCH_3-11), 3.88 (1H, *d*, *J* = 12.0 Hz, H-$6'_b$), 4.69 (1H, *d*, *J* = 7.9 Hz, H-1′), 5.22 (1H, quint., *J* = ~ 6 Hz, H-8), 5.78 (1H, *d*, *J* = 6.4 Hz, H-1_α), 7.43 (1H, *s*, H-3); ^{13}C NMR (methanol-d_4) δ: 19.7 (C-10), 21.8 (CH_3COO-8), 30.4 (C-5), 37.9 (C-6), 44.7 (C-9), 51.9 (OCH_3-11), 63.1 (C-6′), 70.7 (C-8),

71.8 (C-4′), 75.1 (C-2′), 78.2 (C-3′), 78.6 (C-5′), 97.2 (C-1), 100.5 (C-1′), 111.9 (C-4), 154.0 (C-3), 169.1 (C-11), 172.7 (CH_3COO-8), C-7 was not detectable. HR–MS (negative) *m/z*: 463.1483 $[M-H]^-$ (calculated for $C_{19}H_{27}O_{13}$: 463.1451).

3.3.7. Daucosterol (7)

White crystals isolated in 0.056% yield, $[\alpha]_D^{20}$ −29° ($CHCl_3$–MeOH 1:1; c 0.058). TLC R_f: 0.7 (mobile phase: $CHCl_3$–MeOH 8:2); detection: violet with anisaldehyde-sulphuric acid reagent. IR ν_{max} cm^{-1}: 1667 (m), 1598 (m), 1484 (w), 1452 (m), 1362 (w), 1295 (m), 1233 (m), 1074 (s). ^1H NMR (chloroform-d: methanol-d_4 1:1) δ: 0.70 (3 H, s, H-18), 0.83* (3H, *d*, $J = \sim 6.5$ Hz, H-26), 0.85* (3H, *d*, $J = \sim 6.5$ Hz, H-27), 0.86 (3H, *t*, $J = 8.3$ Hz, H-29), 0.90–0.99 (1H, *m*, H-9), 0.91–0.98 (1H, *m*, H-24), 0.94 (3H, *d*, $J = 6.4$ Hz, H-21), 0.97–1.07 (1H, *m*, H-14), 1.00–1.07 (1H, *m*, H-22$_a$), 1.03 (3H, *s*, H-19), 1.04–1.13 (1H, *m*, H-15$_a$), 1.09 (1H, *t*, $J = \sim 13$ Hz, H-1$_\alpha$), 1.09–1.17 (1H, *m*, H-17), 1.15–1.22 (2H, *m*, H-23), 1.19 (1H, *t*, $J = \sim 11.5$ Hz, H-12$_\alpha$), 1.21–1.32 (2H, *m*, H-28), 1.24–1.32 (1H, *m*, H-16$_a$), 1.32–1.39 (1H, *m*, H-22$_b$), 1.34–1.42 (1H, *m*, H-20), 1.43–1.50 (1H, *m*, H-8), 1.45–1.55 (2H, *m*, H-11), 1.53–1.59 (1H, *m*, H-7$_a$), 1.57–1.64 (1H, *m*, H-15$_b$), 1.57–1.66 (1H, *m*, H-2$_a$), 1.64–1.72 (1H, *m*, H-25), 1.83–1.90 (1H, *m*, H-16$_b$), 1.88 (1H, *d*, $J = \sim 13$ Hz, H-1$_\beta$), 1.90–1.97 (1H, *m*, H-2$_b$), 1.95–2.03 (1H, *m*, H-7$_b$), 2.00–2.06 (1H, *m*, H-12$_\beta$), 2.24–2.32 (1H, *m*, H-4$_a$), 2.39–2.45 (1H, *m*, H-4$_b$), 3.22 (1H, *t*, $J = 8.3$ Hz, H-2′), 3.27–3.31 (1H, *m*, H-5′), 3.35–3.42 (1H, *m*, H-4′), 3.38–3.44 (1H, *m*, H-3′), 3.57–3.65 (1H, *m*, H-3), 3.73 (1H, *dd*, $J = 12.0$, 5.1 Hz, H-6′$_a$), 3.86 (1H, *dd*, $J = 12.0$, 2.6 Hz, H-6′$_b$), 4.41 (1H, *d*, $J = 7.8$ Hz, H-1′), 5.36–5.39 (1H, *m*, H-6); ^{13}C NMR (chloroform-d_3: methanol-d_4 1:1) δ: 11.1 (C-18, C-29), 18.0 (C-21), 18.1* (C-26), 18.5 (C-19), 18.9* (C-27), 20.5 (C-11), 22.5 (C-28), 23.7 (C-15), 25.4 (C-23), 27.7 (C-16), 28.6 (C-25), 29.0 (C-2), 31.4 (C-7, C-8), 33.4 (C-22), 35.6 (C-20), 36.2 (C-10), 36.8 (C-1), 38.1 (C-4), 39.3 (C-12), 41.8 (C-13), 45.4 (C-24), 49.7 (C-9), 55.5 (C-17), 56.3 (C-14), 61.1 (C-6′), 69.7 (C-4′), 73.1 (C-2′), 75.6 (C-5′), 76.1 (C-3′), 78.4 (C-3), 100.6 (C-1′), 121.4 (C-6), 139.9 (C-5).

Acknowledgements

We thank Telfor Bedeau, nature guide in Grenada, for guiding us to the drug-furnishing trees.

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