NPC Natural Product Communications

2016
Vol. 11
No. 5
583 - 584

Sesquiterpene Lactones from *Vernonia nigritiana*

Andrea Esposito[a], Nicola Malafronte[a], Rokia Sanogo[b], Antonio Vassallo[c,*], Massimiliano D'Ambola[d] and Lorella Severino[d]

[a]*Dipartimento di Farmacia, Università degli Studi di Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy*

[b]*Departement Medicine Traditionelle (DMT), INRSP, B.P. 1746, Bamako, Mali*

[c]*Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy*

[d]*Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, via Delpino 1, 80137 Napoli, Italy*

antonio.vassallo@unibas.it

Received: December 22nd, 2015; Accepted: February 25th, 2016

Two new sesquiterpenes, 8α-(4-hydroxymethacryloyl)-14-acetoxy-salonitenolide (**1**) and 8α-(2-hydroxymethyl 2-butenoyl)-14-acetoxy-salonitenolide (**2**), together with five known sesquiterpenes were isolated from the leaves of *Vernonia nigritiana* Oliv. & Hiern. Their structural characterization was obtained on the basis of extensive NMR spectroscopic and mass spectrometric studies.

Keywords: *Vernonia nigritiana*, Asteraceae, Germacranes, Sesquiterpene lactones.

Vernonia nigritiana Oliv. & Hiern. (Asteraceae) is an annual herb widely distributed in West Africa, where it is traditionally used against skin inflammations, infections, rheumatism, fever, headache and digestive insufficiency [1a]. Previous studies on some *Vernonia* species revealed anti-inflammatory and anti-cancer properties for their extracts or constituents [1a,b]. Phytochemical studies showed the presence of stigmastane glycosides [1c], alkaloids, terpenoids, flavonoids, phenolic derivatives, sucrose esters and coumarins [1a,b]. Members of the genus *Vernonia* are good sources of highly oxygenated sesquiterpene lactones, belonging to the germacranolides family, such as glaucolides, hirsutinolides, and cadinanolides [2a]. As part of an ongoing program to search for bioactive compounds from the family Asteraceae [1a], we have carried out a chemical study of the leaves of *V. nigritiana.* Because no information about the constituents of the apolar extract of *V. nigritiana* was available, we studied it for the presence of sesquiterpene lactones, isolating, along with five such known compounds, two new members of this family (**1-2**).

Compound **1** was assigned the molecular formula $C_{21}H_{28}O_8$ from ^{13}C, ^{13}C DEPT NMR, and HRESIMS data. The ESI-MS in positive ion mode showed the $[M+H]^+$ ion peak at *m/z* 409, and fragments at *m/z* 349 $[M+H-60]^+$) corresponding to the loss of an acetyl group, and *m/z* 263 $[M+H-60-86]^+$ corresponding to the subsequent loss of esterified groups. The ^{13}C NMR spectra indicated that **1** contained two CH_3, three CH_2, and two CH carbons, as well as three hydroxymethylenes, two hydroxymethines, six sp2 carbons, and three ester functionalities. The features of the NMR spectra suggested a germacrane skeleton with two tetra-substituted double bonds at $\Delta^{1(10)}$ and $\Delta^{4(5)}$. From two pairs of doublets and one singlet at δ_H 4.73 (1H, br d, J = 13.5 Hz), 4.59 (1H, d, J = 13.5 Hz), 4.13 (1H, br d, J = 14.0 Hz), 4.11 (1H, br d, J = 14.0 Hz), and 4.31 (2H, s) three hydroxymethyl groups were evident. The presence of a 4-hydroxymethacryloyl group at C-8 was deduced from the signals at δ_H 6.30 (s) and 5.95 (s) for H_2-3' and δ_H 4.31 (br s) for H-4', while the presence of an acetyl moiety was deduced from the signals at δ_H 2.09 (s). Results obtained from 1D TOCSY and COSY experiments established the correlations of all protons in compound **1**, showing the sequences H-1—H-3, H-5—H-9 and H-5—H-13.

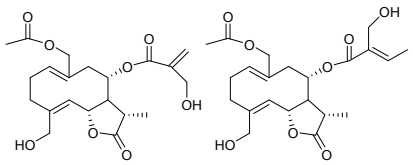

Figure 1: Compounds **1** and **2**.

The ^{13}C NMR spectrum was assigned on the basis of a HSQC experiment. The location of the 4-hydroxymethacryloyl group, the acetyl group, the hydroxymethylene moiety, and the lactone ring were confirmed by the key peaks observed in the HMBC spectrum. The signal of H-1 correlated with C-19, C-3, C-14; H-5 with C-15, C-3, C-6, C-7; H-8 with C-10, C-1', C-11; and H-6 with C-11, C-4, and C-8. 1D ROESY measurements supported the proposed structure and allowed the relative stereochemistry at C-8, C-6, and C-11 [2b]. Irradiation of H-6 affected H-8, H-11 and H-3β signals, while irradiation of H-7 influenced the H-13 and H-9α signals. Consequently, compound **1** was established as 8α-(4-hydroxy-methacryloyl)-14-acethoxy-salonitenolide.

The ^{13}C NMR spectrum of compound **2** showed signals for 22 carbons, including three ester groups. The HRESIMS of **2** showed a quasi-molecular ion at *m/z* 423.2011 $[M+H]^+$. This information, along with the ^{13}C NMR spectra, which sorted the 22 carbons into three methyls, six methylenes, seven methines, and six quaternary carbons, allowed the determination of eight double bond equivalents, two of which was a ring. 1D TOCSY and COSY spectra suggested the presence in the molecule of three spin systems attributable to C-1—C-3, C-5—C-9, and C-3'—C-5'. Also, for this compound, the features of the NMR data suggested a germacrane ring similar to that of compound **1** except for the acyl moiety linked at C-8. This was deduced from the position and pattern of the H-8 signal at δ_H 5.56 (br ddd 11.0, 9.4 and 2.0), and was characterized by the presence of a methyl doublet at δ_H 1.92, which was coupled with a quintet of a methine group (δ_H 6.96), and a hydroxymethyl moiety (δ_H 4.25). The chemical shift and the pattern of this signal, according to HSQC results, suggested the presence of a

2-hydroxymethyl 2-butenoyl group [2c]. The HMBC spectrum showed correlations between the hydroxymethyl signal at δ_H 4.73 and C-1, C-9 and C-1'; between the signal at δ_H 4.91 and C-3, C-7, C-15: between the signal at δ_H 5.51 and C-10, C-6, C-9, C-1'; and between the signal at δ_H 2.84 and C-12, C-13, C-9, C-5, locating the lactone group at C-6, C-7, the acetyl group at C-14, and the 2-hydroxymethyl 2-butenoyl group at C-8. The relative stereochemistry of **2** was determined by 1D ROESY experiments and comparison with literature data [2c,d]. Significant correlations were observed between H-8 and H-6, and H-11 showing that the Me-13 group and 2-hydroxymethyl 2-butenoyl group at C-8 were in the α position. The structure established for compound **2** is 8α-(2-hydroxymethyl 2-butenoyl)-14-acetoxy-salonitenolide.

The five known compounds were identified as glaucolide A [2c], glaucolide G [2c], 8α-(4-hydroxytigloyloxy)-hirsutinolide [2c] vernolide-B [2d], and vernolide-A [2d], by NMR and MS analyses.

Experimental

General: Optical rotations, Rudolph Research Analytical Autopol IV polarimeter; NMR, Bruker DRX-600 spectrometer; ESI-MS, Finnigan LC-Q Advantage Termoquest spectrometer, equipped with Xcalibur software. HR-ESIMS were acquired in positive and negative ion mode on a Q-TOF premier spectrometer [3a,b].

***Plant materials*:** The leaves of *V. nigritiana* were collected in 2012 from the Bougouni, Sikasso region of Mali, near Bandiagara,. The plant material was identified by Prof. Rokia Sanogo of DMT, where a voucher specimen was deposited (voucher number 1396).

***Extraction and isolation*:** Briefly, the leaves of *V. nigritiana* (300 g) were dried at 40°C, powdered and extracted with light petroleum (2.0 g), chloroform (5.7 g), chloroform/ methanol (9:1 v/v) (2.4 g) and methanol (9.0 g) by extensive maceration (3 times x 2 L). Part of the $CHCl_3$ extract (4.0 g) was separated by silica gel column chromatography (CC) eluting with $CHCl_3$, followed by increasing concentrations of MeOH (between 1% and 100%). Fractions of 25 mL were collected, analyzed by TLC and grouped into 9 fractions (I-IX). Fraction VII was subjected to RP-HPLC with MeOH/H_2O (65:35 v/v) as eluent to give glaucolide G (2.5 mg, t_R 37 min) and glaucolide A (2 mg, t_R 41 min). Fraction VIII was subjected to RP-HPLC with MeOH/H_2O (7:3 v/v) as eluent to give 8α-(4-hydroxytigloyloxy)-hirsutinolide (2 mg, t_R 41 min) [3b]. Part of the $CHCl_3$/MeOH (9:1) extract (2.0 g) was separated by Sephadex LH-20 with MeOH as eluent. Fractions of 10 mL were collected, analyzed by TLC and grouped into 10 fractions (A–L). Fraction D (500 mg) was separated by silica gel CC eluting with $CHCl_3$ followed by increasing concentrations of MeOH (between 1% and 100%). Fractions of 5 mL were collected, analyzed by TLC and grouped into 14 fractions. Fraction 11 (43 mg) was purified by RP-HPLC using MeOH/H_2O (58:32) to give compound **1** (1.4 mg, t_R 5 min). Fraction 12 (26 mg) was purified by RP-HPLC using MeOH/H_2O (1:1) to give vernolide-A (0.8 mg, t_R 8 min) and vernolide-C (1 mg, t_R 12 min). Fraction 13 (72 mg) was purified by RP-HPLC using MeOH/H_2O (1:1) to give compound **2** (1.8 mg, t_R 15 min).

Compound 1

$[\alpha]_D^{25}$: +26.2 (*c* 0.13, MeOH).
^{1}H NMR (600 MHz, CD_3OD): 1.32 (3H, d, *J* = 6.2 Hz, Me-13), 2.09 (3H, s, COMe), 2.11 (1H, ddd, *J* = 12.0,11.6,6.4 Hz, H-3), 2.27 (1H, m, H-2), 2.32 (1H, m, H-2), 2.37 (1H, br dd, *J* = 12.6,10.5 Hz, H-9), 2.58 (1H, ddd, *J* = 11.0,4.4,2.4 Hz, H-3), 2.82 (1H, m, H-7), 2.87 (1H, m, H-11), 2.95 (1H, br d, *J* = 10.5 Hz, H-9), 4.11 (1H, br d, *J* =14.0 Hz, H-15), 4.13 (1H, br d, *J* = 14.0 Hz, H-15), 4.31 (1H, s, H-4'), 4.59 (1H, d, *J* = 13.5 Hz, H-14), 4.73 (1H, d, *J* = 13.5 Hz, H-14), 4.91 (1H, br d, *J* = 9.0 Hz, H-5), 5.17 (1H, dd, *J* = 10.0,5.0 Hz, H-1), 5.34 (1H, dd, *J* = 9.0,8.7 Hz, H-6), 5.51 (1H, br ddd, *J* = 11.0,8.4,2.4 Hz, H-8), 5.95 (1H, s, H-3'), 6.30 (1H, s, H-3').
^{13}C NMR (600 MHz, CD_3OD): 10.5 (C-13), 21.0 (COMe), 27.7 (C-2), 35.9 (C-3), 41.0 (C-11), 45.2 (C-9), 54.0 (C-7), 61.4 (C-15), 61.6 (C-4'), 63.0 (C-14), 73.0 (C-8), 77.4 (C-6), 125.9 (C-3'), 130.3 (C-5'), 132.7 (C-10), 135.0 (C-1), 140.0 (C-4), 141.0 (C-2'), 165.9 (C-1'), 172.0 (COMe), 181.0 (C-12).
ESIMS *m/z*: 409 $[M + H]^+$
HRESIMS *m/z*: 409.1854 $[M + H]^+$ (calcd for $C_{21}H_{28}O_8$: 408.1784).

Compound 2

$[\alpha]_D^{25}$: +33.4 (*c* 0.11, MeOH).
^{1}H NMR (600 MHz, CD_3OD): 1.34 (3H, d, *J* = 6.5 Hz, Me-13), 1.92 (1H, d, *J* = 7.0 Hz, H-4'), 2.07 (3H, s, COMe), 2.11 (1H, ddd, *J* = 12.0,11.6,6.4 Hz, H-3), 2.27 (1H, m, H-2), 2.32 (1H, m, H-2), 2.38 (1H, br dd, *J* = 12.6,10.5 Hz, H-9), 2.58 (1H, ddd, *J* = 11.0,4.4,2.4 Hz, H-3), 2.84 (1H, m, H-7), 2.88 (1H, m, H-11), 2.95 (1H, br d, *J* = 10.5 Hz, H-9), 4.13 (1H, br d, *J* = 14.0 Hz, H-15), 4.14 (1H, br d, *J* = 14.0 Hz, H-15), 4.25 (2H, br s, H_2-5'), 4.60 (1H, d, *J* = 13.5 Hz, H-14), 4.73 (1H, d, *J* = 13.5 Hz, H-14), 4.93 (1H, br d, *J* = 9.0 Hz, H-5), 5.17 (1H, dd, *J* =10.0,5.2 Hz, H-1), 5.34 (1H, dd, *J* = 9.0,8.7 Hz, H-6), 5.56 (1H, ddd, *J* = 11.0,9.4,2.0 Hz, H-8), 6.96 (1H, q, *J* = 7.0 Hz, H-3').
^{13}C NMR (600 MHz, CD_3OD): 10.5 (C-13), 15.0 (C-4'), 21.0 (COMe), 27.7 (C-2), 35.9 (C-3), 41.0 (C-11), 45.2 (C-9), 54.0 (C-7), 59.2 (C-5'), 61.4 (C-15), 63.0 (C-14), 73.0 (C-8), 77.4 (C-6), 130.3 (C-5), 132.7 (C-10), 133.0 (C-2'), 135.0 (C-1), 140.0 (C-4), 142.2 (C-3'), 167.0 (C-1'), 172.0 (COMe), 181.0 (C-12).
ESIMS *m/z*: 423 $[M + H]^+$
HRESIMS *m/z*: 423.2011 $[M + H]^+$ (calcd for $C_{22}H_{30}O_8$: 422.1941).

References

[1] (a) Vassallo A, De Tommasi N, Merfort I, Sanogo R, Severino L, Pelin M, Della Loggia R, Tubaro A, Sosa S. (**2013**) Steroids with anti-inflammatory activity from *Vernonia nigritiana* Oliv. & Hiern. *Phytochemistry*, ***96***, 288–298; (b) Bruno M, Bancheva S, Rosselli S, Maggio A. (**2013**) Sesquiterpenoids in subtribe Centaureinae (Cass.) Dumort (tribe Cardueae, Asteraceae): Distribution, ^{13}C NMR spectral data and biological properties. *Phytochemistry*, ***95***, 19-93; (c) Liu J, Ma S, Yu S, Lv H, Li Y, Wu X, Liu Y. (**2010**) Seven new vernocuminosides from the stem bark of *Vernonia cumingiana* Benth. *Carbohydrate Research*, ***345***, 1156–1162.

[2] (a) Costa FB, Terfloth L, Gasteiger J. (**2005**) Sesquiterpene lactone-based classification of three Asteraceae tribes: a study based on self-organizing neural networks applied to chemosystematics. *Phytochemistry*, ***66***, 345–353; (b) Lazari D, Garcia B, Skaltsa H, Pedro JR, Harvala C. (**1998**) Sesquiterpene lactones from *Onopordon laconicum* and *O. sibthorpianum*. *Phytochemistry*, ***47***, 415–422; (c) Youn UJ, Miklossy G, Chai X, Wongwiwatthananukit S, Toyama O, Songsak T, Turkson J, Chang LC. (**2014**) Bioactive sesquiterpene lactones and other compounds isolated from *Vernonia cinerea. Fitoterapia*, ***93***, 194–200; (d) Kuo YH, Kuo YJ, Yu AS, Wu MD, Ong CW, Yang Kuo LM, Huang JT, Chen CF, Li SY. (**2003**) Two novel sesquiterpene lactones, cytotoxic vernolide-A and -B, from *Vernonia cinerea. Chemical & Pharmaceutical Bulletin*, ***51***, 425–426.

[3] (a) Cioffi G, D'Auria M, Braca A, Mendez J, Castillo A, Morelli I, De Simone F, De Tommasi N. (**2002**) Antioxidant and free-radical scavenging activity of constituents of the leaves of *Tachigalia paniculata*. *Journal of Natural Products*, ***65***, 1526-1529; (b) Dal Piaz F, Vassallo A, Lepore L, Tosco A, Bader A, De Tommasi N. (**2009**) Sesterterpenes as tubulin tyrosine ligase inhibitors. First insight of structure-activity relationships and discovery of new lead. *Journal of Medicinal Chemistry*, ***52***, 3814-3828.