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PHYTOCHEMICAL INVESTIGATIONS OF *Vernonia galamensis* SEEDS

Ashebir Fiseha,[1] Mekuria Tadesse,[2] Tesfaye Bekele,[3] and Belete Bedemo[4]*

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Vernonia galamensis *is a new potential industrial oil seed crop for semiarid areas with very high content of naturally epoxidized vernolic acid (80% of the oil). Chemical investigation of the seeds of* Vernonia galamensis *afforded two compounds, which are vernolic acid derivatives. Their structures were established by various spectroscopic techniques: IR, UV, NMR, and 2D NMR spectra (COSY, HSQC, and HMBC), and by comparison with literature data for the known compound methylvernolate. The* cis-*(12*S*,13*R*)-(3-methylpentyl) vernolate (**1**) and the* cis-*(12*S*,13*R*)-(2,3-propanediol) vernolate (**2**) were isolated and characterized.*

Keywords: *Vernonia galamensis*, Asteraceae, vernolic acid, methyl vernolate, vernolic acid derivatives, oil seed crop, extraction, fractionation.

The genus *Vernonia* is one of the largest groups in the family Asteraceae and includes more than 1000 species distributed widely in tropical and subtropical regions of Africa, Asia, and America [1]. Studies of most *Vernonia* species indicate the presence of many metabolites, mainly sesquiterpene lactones, flavonoids, *Vernonia* oil, and vernolic acid [2–11]. The species *V. galamensis* is an industrial oil seed crop originating in Ethiopia, which combines all the possible merits for semiarid tropics and subtropics [1, 12]. The seeds of *V. galamensis* (Cass.) Less. contain 40% epoxy oil (trivernolin), which, when hydrolyzed, yields different fatty acids with a very high content (80%) of vernolic acid [4, 12–14].

Several *Vernonia* species have been found to be of various economic importance as potential sources of *Vernonia* oil and vernolic acid, as traditional medicine and food, and possessing antitumor and antiviral activities [1, 3, 15]. The oil obtained from *V. galamensis* is already epoxidized in nature by enzymatic action and may be able to fill some industrial market niches [16]. The presence of the epoxy group and the low viscosity and polymerizing characteristics of this oil make it especially valuable as a solvent in industrial coatings and paints [17, 18].

Successive fractionation of 30 g of thick oil of the CH_2Cl_2 extract and 25 g of MeOH extract of the seed by flash chromatography resulted in the isolation and characterization of two vernolic acid derivatives, *cis*-(12*S*,13*R*)-(3-methylpentyl) vernolate (**1**) and *cis*-(12*S*,13*R*)-(2,3-propanediol) vernolate (**2**). They were identified and characterized by their IR, PMR, ^{13}C NMR, COSY, HSQC, and HMBC spectral data and by comparison to reported data [18, 19].

Compound **1** was obtained from *n*-hexane–CH_2Cl_2 (98:2) as a colorless oil with R_f 0.30.

Compound **2** was obtained from $CHCl_3$–EtOAc (20:80) as a yellow gummy residue with R_f 0.28.

IR spectrum of compound **1** (KBr, ν, cm^{-1}): 3010 (=C-H), 2928 (C-H), and 1732 (C=O).

PMR spectrum of compound **1** (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.49 (1H, m, H-9), 5.41 (1H, m, H-10), 4.10 (2H, t, J = 7.6, H-1′), 2.90 (2H, m, H-12, H-13), 2.35 (1H, m, H-11b), 2.18 (1H, m, H-11a), 2.27 (2H, t, J = 6.8, H-2), 2.05 (2H, m, H-8), 1.68 (1H, m, H-3′), 1.61 (2H, m, H-3), 1.52 (4H, m, H-14 and H-2′), 1.35–1.26 (16H, m, H_2-4, H_2-5, H_2-6, H_2-7, H_2-15, H_2-16, H_2-17, H_2-4′), 0.93–0.89 (9H, m, H_3-18, H_3-5′, H_3-6′).

1) Addis Ababa University, Department of Chemistry, P. O. Box 1176, Addis Ababa, Ethiopia; 2) Ethiopia Agriculture Research Institute, Essential Oils Research Center, P. O. Box 3395 Addis Ababa, Ethiopia; 3) Ethiopia Agriculture Research Institute, Essential Oils Research Center, P. O. Box 3395, Addis Ababa, Ethiopia; 4) Bahir Dar University, Department of Chemistry, P. O. Box 79, Bahir Dar, Ethiopia, fax: (+251) 058 220 20 25, e-mail:beleteb1996@yahoo.com. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 588–590, September–October, 2010. Original article submitted July 18, 2009.

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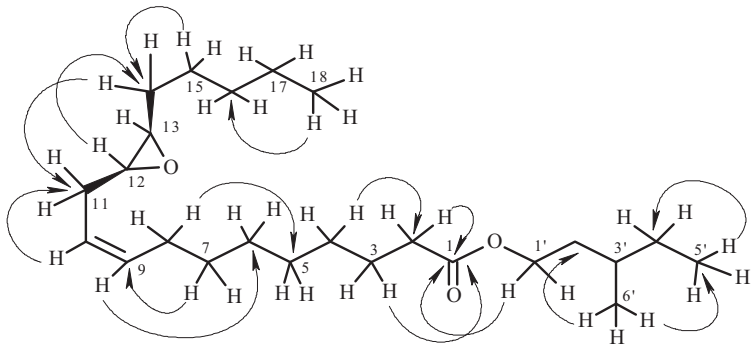

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Fig. 1. Selected HMBC correlations of compound **1**.

^{13}C NMR spectrum of compound **1** (100 MHz, $CDCl_3$, δ): 173.5 (C-1), 123.9 (C-10) 132.2 (C-9), 62.6 (C-1′), 56.9 (C-12), 56.3 (C-13), 37.3–13.8 (C-2 to C-8, C-11, C14 to C-18, C-2′ to C-6′).

In addition, DEPT experiments showed 24 carbon signals, comprising one quaternary (carbonyl carbon), five methine (including one aliphatic, two olefinic, two oxygenated), fifteen methylene (one oxygenated and fourteen aliphatic), and three methyl carbon atoms.

The epoxide carbon atoms have chemical shifts of around 56.9 (*cis*) and 58.5 ppm (*trans*), but these may appear as two different signals through the long-range influence of a carbonyl group or the omega methyl function [18]. In addition, most of the epoxy carbons have signals for protons attached to epoxidized carbons at δ 2.70 ppm for *cis* and δ 2.45 ppm for *trans* [18].

On the basis of these spectra data and comparison with methylvernolate [17, 18], compound **1** was deduced to have the structure shown in Fig. 1.

Moreover, the above prediction was supported by two-dimensional NMR (COSY, HSQC, and HMBC) spectra data of the compound.

The COSY spectrum of compound **1** ($CDCl_3$, δ, ppm, J/Hz) showed strong correlations of H-2 at δ 2.27 with H-3 at 1.61, H-8 at 2.05 with H-9 at 5.49, H-10 at 5.41 with H-9 at 5.49, H-10 at 5.41 with H-11b at 2.35, H-11a at 2.18 with H-10 at 5.41, H-11b at 2.35 with H-11a at 2.18, H-12 at 2.90 with H-11a at 2.18, H-12 at 2.90 with H-11b at 2.35, H-13 at 2.90 with H-12 at 2.90, H-13 at 2.90 with H-14 at 1.52, H-1′ at 4.10 with H-2′ at 1.52, and H-2′ at 1.52 with H-3′ at 1.68.

The HSQC spectrum of compound **1** ($CDCl_3$, δ, ppm, J/Hz) showed a proton signal at 5.49 attached to C-9 (132.3), at 5.41 to C-10 (123.9), two protons at 4.10 to C-1′ (62.6), and two protons at 2.90 to C-12 and C-13 (56.9 and 56.3), respectively. In addition, two protons at 2.35 and 2.18 are attached to C-11 (26.2), hence they are diastereotopic protons. Two protons at 2.27 are attached to C-2 (34.2), two protons at 2.05 to C-8 (25.9), a proton at 1.68 to C-12 (34.1), and two protons at 1.61 to C-3 (24.8).

The spectra data of compound **2** showed the presence of epoxy, olefinic, and carbonyl functional groups that are similar to functional groups of methylvernolate and compound **1**. However, in compound **2** there is additional functional group.

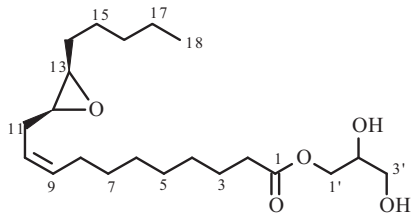
IR spectrum of compound **2** (KBr, ν, cm^{-1}): 3454 (C-H), 3010 (=CH), 2928 (C-H), and 1732 (C=O).

PMR spectrum of compound **2** (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.14 (2H, d, H-1′), 3.92 (1H, m, H-2′), 3.78 (1H, d, H-3′a), 3.66 (1H, d, H-3′b), 5.42 (1H, m, H-10), and 5.49 (1H, m, H-9), 2.90 (2H, m, H-13), 2.35 (1H, m, 11b), 2.18 (1H, m, H-11a), 2.27 (2H, t, J = 6.8, H-2), 2.05 (2H, m, H-8), 1.61 (2H, q, H-3).

^{13}C NMR spectrum of compound **2** (100 MHz, $CDCl_3$, δ, ppm): 65.1 (C-1′), 70.2 (C-2′), 63.4 (C-3′), 174.2 (C-1), 123.8 (C-10), 132.6 (C-9), 57.4 (C-12), 56.7 (C-13).

The DEPT experiments showed a total of 21 carbon signals, comprising one quaternary (carbonyl carbon), five methine (including two olefinic and three oxygenated), fourteen methylene (two oxygenated and twelve aliphatic), and one methyl carbon atom.

Based on the spectra data and comparison with the reported data for the known compound methylvernolate, compound **2** was deduced to have the structure **2**.



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EXPERIMENTAL

General. The IR spectrum was measured with a Perkin–Elmer BX infrared spectrometer in the range of 4000–400 cm^{-1}, the NMR spectra were recorded on a Bruker Advance spectrometer in $CDCl_3$, 400 MHz for the PMR spectrum and 100 MHz for the ^{13}C NMR spectrum, with TMS as an internal standard. Silica gel with fluorescent indicator 254 nm on aluminum cards with a layer thickness of 0.2 mm was used for TLC and silica gel 60 (Merck), particle size 0.063–0.200 mm, was used for column chromatography.

Plant Material. The seeds of *V. galamensis* were provided by Ethiopian Agricultural Research Institute, Essential Oils Research Center, which were collected from Meki and Bulbula regions around Zeway, Ethiopia, in 2005. A voucher specimen is deposited at the National Herbarium, Department of Biology, Addis Ababa University, Ethiopia.

Extraction and Isolation. Dried and grounded seeds of *V. galamensis* (400 g) were extracted for 36 h using 900 mL of CH_2Cl_2 followed by extraction using 850 mL of MeOH for 48 h. The CH_2Cl_2 extract was freed of solvent to give 90 g of thick oil, and that of MeOH extract was concentrated to yield 43 g.

A portion of the CH_2Cl_2 extract (30 g) was placed on a column packed with silica gel (130 g) using *n*-hexane. Successive elution using *n*-hexane with increasing polarity of CH_2Cl_2 and MeOH resulted in 12 fractions: fr.1 (100% *n*-hexane, 100 mL), fr.2 (2% CH_2Cl_2, 50 mL), fr.3 (5% CH_2Cl_2, 50 mL), fr.4 (15% CH_2Cl_2, 50 mL), fr.5 (30% CH_2Cl_2, 100 mL), fr.6 (50% CH_2Cl_2, 100 mL), fr.7 (100% CH_2Cl_2, 100 mL), fr.8 (10% MeOH–CH_2Cl_2, 50 mL), fr.9 (20% MeOH–CH_2Cl_2, 50 mL), fr.10 (40% MeOH–CH_2Cl_2, 75 mL), fr. 11 (50% MeOH–CH_2Cl_2, 50 mL), and fr. 12 (100% MeOH, 100 mL).

TLC of fractions 2–4 showed the presence of similar compounds, so the fractions were combined. The combined fraction was then loaded onto a column chromatograph and eluted with C_6H_{14}–CH_2Cl_2 with increasing polarity to yield five fractions. The second fraction (which was eluted using 2:98 of CH_2Cl_2–*n*-hexane) was passed through a column chromatograph using CH_2Cl_2–*n*-hexane solvent mixture, which resulted in 12 fractions. Fractions 3–5, which were eluted by 2% CH_2Cl_2–*n*-hexane, were then combined on the basis of TLC analysis. TLC application of this combined fraction gave a single spot, which was used for spectral analysis. The compound is *cis*-(12*S*,13*R*)-(3-methylpentyl)-vernolate (**1**).

The MeOH extract (25 g) was subjected to column chromatography and successive elution using $CHCl_3$ with increasing polarity of EtOAc and MeOH, resulting in 16 fractions. Fractions 4–5 adsorbed on silica gel and loaded onto a column was eluted with chloroform with increasing polarity of EtOAc, which resulted in five fractions. The 80% EtOAc–$CHCl_3$ fraction gave (*cis*-(12*S*,13*R*)-(2,3-propanediol)vernolate (**2**).

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