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INSECT ANTIFEEDANT ELEMANOLIDE LACTONES FROM *VERNONIA AMYGDALINA*

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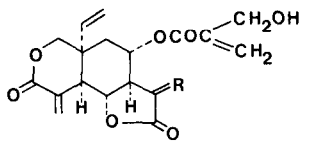
Key Word Index—*Vernonia amygdalina*; Compositae; insect antifeedant; elemanolide; 11,13-dihydrovernodalin.

Abstract—Chemical investigation of insect antifeedants from the bitter tasting leaves of *Vernonia amygdalina* by the application of semi-preparative reversed phase HPLC has led to the isolation and characterization of vernodalin, vernodalol and a new sesquiterpene lactone, 11,13-dihydrovernodalin. This new compound exhibited *in vitro* cytotoxicity and antifeedant activity against the African armyworm *Spodoptera exempta*. The structure of this new antifeedant was determined by spectroscopic data, and the assignment of the methyl group at the α-position on C-11 was based on the ^{1}H NMR long range coupling constants, as well as successive large *J* values.

INTRODUCTION

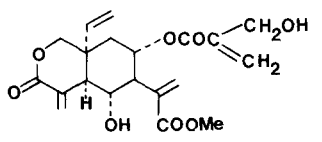
In our continuing search for naturally occurring insect antifeedants we have examined the bitter tasting leaves of *Vernonia amygdalina* (Compositae) which are used as a folk medicine to cure fever in East Africa [1]. The two elemanolide lactones, namely vernodalin (**1**) [2] and vernodalol (**2**) [3], were previously isolated from this tropical plant as cytotoxic principles. A number of elemanolide lactones have been isolated from *Vernonia* species [4]. Due to the interest in antitumor activity, much attention has been focused on the synthesis of vernolepin-type sesquiterpenoids [5–8].

This paper describes the isolation of insect antifeedant sesquiterpene lactones, mainly by semi-preparative HPLC, which resulted in the separation of vernodalin, vernodalol and a new antifeedant elemanolide lactone, 11,13-dihydrovernodalin.



1 R = CH_2

3 R = 



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RESULTS AND DISCUSSION

In addition to the two aforementioned known sesquiterpene lactones **1** and **2**, a new elemanolide lactone, 11,13-dihydrovernodalin (**3**), has been isolated from the ether extract of the dried leaves of the East African medicinal plant *V. amygdalina* as an antifeedant against the African armyworm *S. exempta* with a leaf-disk assay [9]. Interestingly, despite the lack of an exocyclic conjugated double bond on the γ-lactone moiety, this new sesquiterpene lactone **3** exhibited *in vitro* cytotoxicity which is almost comparable to vernodalin [2]. The isolation of the antifeedant sesquiterpene lactones was done through monitoring with the leaf disk assay.

After a preliminary separation of the crude extract by column chromatography (silica gel), the corresponding active fraction was submitted to HPLC for further purification on a reversed-phase semi-preparative column. The order of elution under the applied HPLC conditions was: vernodalin 20.6 min; 11,13-dihydrovernodalin 22.8 min; and vernodalol 26.5 min with a peak area ratio of 1 : 4 : 1, respectively. Fractions were collected and identification of each pure compound was based on spectroscopic data.

11,13-Dihydrovernodalin, colorless oil, gave the following spectroscopic results; CIMS 363 $[M+1]^+$ and EIMS showed prominent peaks at *m/z* (rel. int.) 278 (2.6), 260 (6.5), 248 (38), 230 (100) and 85 (67). Except for the ion at *m/z* 85 $[-CO-C(CH_2OH)=CH_2]^+$, each of the other fragment ions was 2 amu higher than the corresponding peak for vernodalin. The UV spectrum showed only end absorption. The IR spectrum displayed characteristic bands at 3400 (hydroxyl group), 1780 (γ-lactone moiety), 1730 (shoulder, δ-lactone moiety), 1720 (carboxyl group of hydroxymethacrylate side chain), 1690 (shoulder, conjugated double bond) and 1635 cm^{-1} (vinyl group).

The ^{1}H NMR signals are summarized in Table 1. Assignment of the methyl group at the α-position of C-11 of the γ-lactone moiety, rather than at C-4 of the δ-lactone ring, was based on the coupling constant patterns for 5-H (δ 2.90, $J_{5,14}$ = 1.8 Hz, *W*-type coupling), 7-H (δ 2.04) and 11-H (δ 2.64) with large *J* values. This was also supported

Table 1. ^{1}H NMR spectral data of 11,13-dihydrovernodalin in $CDCl_3$

Assignment	Chemical shift (δ)	Coupling constant (Hz)
H-1	1H 5.73	*dd*, J = 10.8, 17.7
H-2	1H 5.31	*dd*, J = 2, 17.7
	1H 5.29	*dd*, J = 2, 10.8
H-5	1H 2.90	*dddd*, J = 0.8, 1, 1.8, 11.0
H-6	1H 4.07	*dd*, J = 11, 11
H-7	1H 2.04	*ddd*, J = 11, 11, 12.1
H-8	1H 5.12	*ddd*, J = 4.6, 10.8, 11
H-9	1Hα 1.71	*dd*, J = 10.8, 14.2
	1Hβ 2.1	*dd*, J = 4.6, 14.2
H-11	1H 2.64	*dq*, J = 6.9, 12.1
H-13	3H 1.42	*d*, J = 6.9
H-14	1Hα 4.28	*dd*, J = 1.8, 12.1
	1Hβ 4.52	*d*, J = 12.1
H-15	1H 6.75	*dd*, J = 0.8, 1
	1H 5.94	*dd*, J = 1, 1
H-3′	2H 4.35	*br s*
H-4′	1H 6.27	*dd*, J = 0.8, 1
	1H 5.96	*dd*, J = 0.8, 1

Table 2. ^{13}C NMR spectral data of 11,13-dihydrovernodalin in $CDCl_3$

Carbon	δ	Carbon	δ
C-1	139.2	C-11	45.7
C-2	116.1	C-12	176.8
C-3	164.5	C-13	13.8
C-4	129.8	C-14	70.0
C-5	40.5	C-15	134.8
C-6	77.0	C-1′	162.9
C-7	53.8	C-2′	138.4
C-8	68.9	C-3′	60.8
C-9	38.0	C-4′	125.8
C-10	40.0		

by the 5-H (*dddd*, J = 0.8, 1.0, 1.8, 12 Hz) and 7-H (*ddd*, J = 11, 11, 12 Hz) multiplicities. The ^{13}C NMR data are tabulated in Table 2. The signals due to C-5 and C-10 overlapped to give a relatively intense peak. The assignment of each peak was based on the observed multiplicity in a single-frequency off-resonance decoupled spectrum.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker FT-400 instrument using TMS as an internal standard in $CDCl_3$. ^{13}C NMR spectra were determined on JEOLCO PH-100 instrument. MS were obtained with a Hitachi Perkin–Elmer, model RMU-6D, single-focusing spectrometer. UV spectra were recorded on a Hitachi UV-100-80 spectrophotometer with 10 mm cell. IR spectra were taken on a Jasco IRA-1 Grating Infrared Spectrophotometer.

Sample preparation. The air-dried leaves (450 g) of *V. amygdalina*, collected near Nairobi, Kenya, were ground and extracted with Et_2O and the extracts were concentrated under red. pres. to a thick oil. The crude material (4 g) was chromatographed on 500 g silica gel. After eluting with 5 l. of $CHCl_3$ further elution with 1.5 l. $CHCl_3$–MeOH (99:1) resulted in the separation of an antifeedant active fraction. This fraction (0.5 g) was dissolved in MeOH and after filtration subjected to further purification by reversed-phase semi-preparative HPLC. Mobile phase, MeOH–H_2O (1:1) at 3 ml/min; Whatman C_{18} semi-preparative stainless-steel column (50 cm × 9.4 mm i.d.) equipped with Whatman stainless-steel guard column (7 cm × 2.1 mm i.d.) packed with pellicular Co:Pell ODS; typical column head pressures under these conditions were 157 bar. Solvents were thoroughly degassed by applying a helium degassing system. The compounds were monitored by UV absorption at a wavelength of 220 nm.

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