Journal of Natural Products
Vol. 51, No. 1, pp. 168-171, Jan-Feb 1988

A NEW LIMONOID INSECT ANTIFEEDANT FROM THE FRUIT OF *MELIA VOLKENSII*

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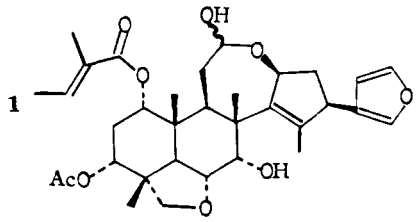
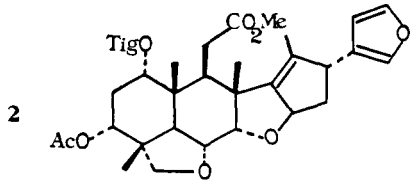
Melia volkensii Gürke (Meliaceae) is a tree found in the dry areas of East Africa. A tea prepared from the bark is used in local folk medicine to alleviate pain, and it is said to be poisonous in an overdose (1). Extracts of the seed kernels have been reported to have potent antifeedant activity against nymphs and adults of the desert locust, *Schistocerca gregaria* (2). Here we report the isolation and identification of a new limonoid, volkensin [**1**], from the whole fruits of *M. volkensii* and present evidence of its high activity as an antifeedant against larvae of the fall armyworm, *Spodoptera frugiperda*. In addition, we report the isolation of a known limonoid, salannin [**2**], and compare its antifeedant activity against the same insect.

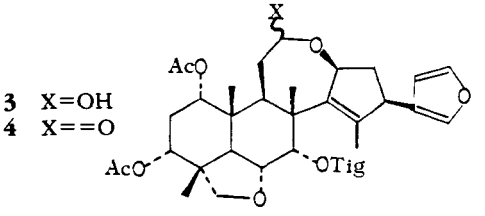
Si gel chromatography of a cold MeOH extract of crushed, fresh, whole fruit led to the isolation of a white, crystalline substance [**1**], mp 185–187°, having molecular formula $C_{33}H_{44}O_9$ (hrms). The second compound isolated was shown by identity with published values (3–5) of the ^{1}H- and ^{13}C-nmr, mass, and ir spectra, and melting point to be salannin [**2**]. Compounds **1** and **2** were isolated in approximately equal amounts and constituted the major components of the $CHCl_3$-soluble fraction of the extract.

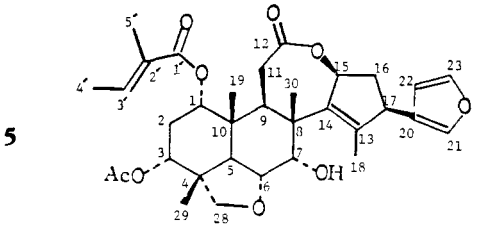
Examination of the ^{13}C- and ^{1}H-nmr spectra and the ir spectrum of **1** indicated it to be a mixture of two closely related limonoids. Indicated functionality included a carbonyl, tiglate, OH, acetate, alkene, and a β-substituted furan. A pair of singlets at 91.3 ppm and 98.4 ppm in the decoupled ^{13}C-nmr spectrum of **1**, each shown by APT techniques to bear a single attached proton, and a pair of one-proton multiplets centered at 5.20 ppm and 4.55 ppm in the ^{1}H-nmr spectrum were similar to the C-12 and H-12 absorptions reported for the C-ring lactol, nimbolinin B [**3**], isolated as a mixture of epimers from the fruit of *Melia azedarach* by Kraus and Bokel (6). As reported by those authors for **3**, we were unable to separate the epimers of **1**. Difficulties in both ^{13}C- and ^{1}H-nmr spectral analysis of **1** due to overlapping of a number of absorptions led us to convert **1** to a stable C-ring lactone for structural verification.

Careful oxidation of **1** with CrO_3, monitored by tlc, allowed selective oxidation to the hydroxylactone **5**, $C_{33}H_{42}O_9$ (hrms). In the ^{1}H-nmr spectrum of **5** the absorptions due to the 12-H epimers of **1** were no longer present; however, the 15-H multiplet at 5.2 ppm observed for **1** had shifted downfield to 5.6 ppm in **5**. These observations are consistent with those reported by Kraus and Bokel (6) for lactol **3** and by Ochi *et al.* (7) and Kraus and Bokel (6) for lactone **4**. In the ^{13}C-nmr spectrum of **5** the C-12 absorptions observed for the epimeric pair of **1** were replaced by a single lactone carbonyl absorption at 171.92 ppm. Comparison of the ^{1}H- and ^{13}C-nmr absorptions of **5** with those reported for ohchinolide-B [**4**] allowed us to place **5** in the structural class with **4**. Compound **5**, however, bears a lactone, OH, and acetate function, whereas **4** has two acetates and a tiglate. ^{1}H-^{1}H COSY experiments allowed us to establish coupling of H-2α,β to H-1 and









H-3, H-6 to H-5 and H-7, H-6α,β to H-15 and H-17, H-9 to H-11α,β, and H-22 to H-21/H-23. The magnitudes of the coupling constants of H-1 and H-3 to H-2 (J=3 Hz) established these protons as equatorial, as is generally the case in related limonoids. The very close correspondence of ^{1}H chemical shifts at positions 1 and 3 in **5** with those observed for salannin [**2**] is consistent with location of the tiglate at C-1 and the acetate at C-3. Placement of the tiglate at C-1 also accounts for the shielding of one of 12 protons in one of the epimers of volkensin [**1**] relative to that reported for H-12 in **3**. Carbon and proton chemical shifts at C-7 demonstrated this to be the location of the OH, while the H-7–H-6 coupling constant (J=3 Hz) established the 7-OH to be in the α position. An H-9–H-15 nOe experiment established the β stereochemistry of the lactone linkage at C-15 in **5**. Limonoids of this class, in which the C ring has been oxidatively cleaved, followed by rotation and closure to C-15, have β stereochemistry of the furan at C-17. Our spectral data are consistent with this D-ring structural type.

In assigning the ^{13}C absorptions (Table 1), APT techniques were used to differentiate methyls, methylenes, methines, and quaternary carbons. Heteronuclear COSY techniques were used to correlate ^{13}C- and ^{1}H-shift assignments. In addition, ^{13}C-shifts reported by Ochi *et al.* (7) and Kraus and Bokel (6) for **4** were useful in making assignments for **5**.

Volkensin [**1**] was assayed as an antifeedant against third-instar larvae of the fall armyworm, *S. frugiperda*. In choice assays using corn leaf disks, **1** exhibited high activity, limiting feeding to 50% of that observed for control, untreated disks (ED_{50}) at a concentration of 3.5 μg/cm^2 of leaf surface. In view of the lability of the 12-lactol and its possible facility for interacting with nucleophiles on the active site of a receptor protein, we were interested in comparing the activity of **1** with that of the corresponding lactone, **5**. Compound **5** exhibited an ED_{50} of 6 μg/cm^2, a level not greatly different from that observed for **1**. This reactive functional group concept is, thus, an oversimplification in this system. Salannin [**2**], a molecule in which the C ring has been cleaved and D-ring rotation and closure onto the 7-OH have occurred, exhibited an even lower ED_{50} of 13 μg/cm^2, but the extensive molecular changes in comparison with **1** and **5** do not permit useful structure-activity comparisons.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher hot-stage apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 283 spectrometer. ^{1}H- and ^{13}C-nmr spectra were obtained on

TABLE 1. ^{1}H- and ^{13}C-Nmr Spectra of Lactone [**5**].

Proton	^{1}H-nmr Assignments	^{13}C-nmr Assignments	
	δ (ppm), Multiplicity (*J*, Hz)	Carbon	δ (ppm)
1	4.96, t (3)	1	71.16
2	2.25, t (3)	2	27.47
3	4.78, t (3)	3	71.36
5	2.80, d (12.5)	4	42.55
6	4.05, dd (12.5, 3)	5	38.97
7	4.26, d (3)	6	73.63
9	3.18, dd (11,5)	7	73.49
11α,β	2.5-2.7, m	8	45.63
15	5.58, br d (7)	9	36.24
16α,β	2.5-2.7, m	10	40.45
17	3.35, br d (9)	11	32.42
18	1.82, s	12	171.92
19	1.04, s	13	139.94
21	7.32, m	14	144.85
22	6.36, m	15	86.63
23	7.29, s	16	37.22
28α,β	3.62, s	17	47.39
29	1.22, s	18	15.79
30	1.35, s	19	15.75
3′H	7.00, qq (7, 1.5)	20	126.85
4′H	1.85, dq (7, 1.2)	21	139.10
5′H	1.90, dq (1.5, 1.2)	22	110.13
3Ac	1.98, s	23	143.29
		28	78.13
		29	19.63
		30	20.87
		1′	166.20
		2′	128.73
		3′	137.94
		4′	14.53
		5′	12.06
		Ac(CO)	170.08
		Ac(Me)	20.87

a Varian XL-200 system operating at 200 MHz for ^{1}H and 50.3 MHz for ^{13}C. Low resolution mass spectra were recorded on an HP-5985 mass spectrometer operating at 70 eV. High resolution mass spectra were obtained on a VG-70E system at the Auburn University Mass Spectrometry Facility by Dr. George Goodloe.

EXTRACTION.—Ripening fruit of *M. volkensii* was collected in November 1983, in Tsavo Park, Kenya. A voucher specimen of the leaves is deposited in the University of Nairobi herbarium. Crushed whole fruit (7.9 kg) was allowed to stand 2 weeks in 8 liters of MeOH. The extract was decanted, and the residual pulp was similarly extracted a second time. The combined extracts were evaporated under vacuum, and the residue was partitioned between $CHCl_3$ and H_2O. After drying over Na_2SO_4, the $CHCl_3$ layer was evaporated under vacuum to yield 64 g of a brown oil.

ISOLATION AND IDENTIFICATION.—Column chromatography of 11.6 g of the oil on Si gel (70–30 mesh, Merck) eluted with hexane-Me_2CO (3:1) led to the isolation of **1** (1.06 g) and **2** (1.04 g), both as pale yellow solids. Crude **1** was recrystallized from Me_2CO/hexane to give 300 mg of **1** as white needles (mp 185–187°). Crude **2** was recrystallized from hexane/Et_2O to give 400 mg of pure compound as needles, mp 162–164°.

Compound **1** exhibited ir absorptions (KBr) at 3400 (br), 2950, 1740, 1710, 1650, 1270, 1160, 1080, 1050, 875 cm^{-1}. The low resolution ms displayed a weak parent ion at *m/z* 584 and a base peak at *m/z* 566 $[M-H_2O]^+$. Hrms measurements on the latter peak led to an exact mass of 566.2884, corresponding to $C_{33}H_{42}O_8$ (calcd 566.2887). The molecular formula of **1** is, thus, $C_{33}H_{44}O_9$.

OXIDATION OF VOLKENSIN.—Volkensin [**1**]

(50 mg) was dissolved in 5 ml of pyridine containing 5 mg of CrO_3, and the mixture was stirred at room temperature for 3 days. Flash chromatography over Si gel with EtOAc-$CHCl_3$ (3:1) led to isolation of 20 mg of the lactone **5**, which was recrystallized from Me_2CO/hexane to give pure **5** as white needles, mp 240–244° (dec). Hrms yielded a parent ion at *m/z* 582.2803, corresponding to $C_{33}H_{42}O_9$. The ir spectrum (KBr) displayed absorptions at 3430, 3110, 2925, 1730, 1710, 1650, 1250, 875 cm^{-1}; ^1H- and ^{13}C-nmr spectral assignments are summarized in Table 1.

Compound **2** exhibited ir, ms, ^1H- and ^{13}C-nmr spectra essentially identical with those reported in the literature for salannin [**2**] (3–5).

BIOASSAY PROCEDURES.—The compounds were evaluated for antifeedant activity in choice assays against larvae of the fall armyworm, *S. frugiperda*. Chemicals dissolved in Me_2CO were evenly distributed on the upper surface of 1-cm^2 corn-leaf disks, and the Me_2CO was allowed to evaporate. Control disks received only Me_2CO. Five treated and five untreated disks were alternately pinned in a 9-cm petri dish arena. A 1-day-old third instar was placed in the dish, and the assay was conducted at 27° for 15 h with 10 arenas per treatment. Assays were conducted at 10, 3.2, and 1 $\mu g/cm^2$. Amount of leaf material consumed was determined by weighing the oven-dried remains of disks for each assay and subtracting this from a mean initial weight obtained by drying additional disks. Percent of feeding reduction (% FR) was determined by the equation (8):

$$\% \text{ FR} = \left[1 - \left(\frac{\text{Treatment consumption}}{\text{Control consumption}}\right)\right] \times 100$$

These values were used to determine effective dosages for reduction in feeding by 50% (ED_{50}).

ACKNOWLEDGMENTS

We are indebted to Mr. L.N. Lester for assistance in obtaining nmr spectra and to the USDA for financial support through grants 85-FSTY-0102 and 86-CSRS-2-2759.

LITERATURE CITED

1. J.O. Kokwaro, "Medicinal Plants of East Africa," East African Literature Bureau, Nairobi, Kenya, 1976.
2. R.W. Mwangi, *Entomol. Exp. Appl.*, **32**, 277 (1982).
3. I. Kubo, A. Matsumoto, T. Matsumoto, and J.A. Klocke, *Tetrahedron*, **42**, 489 (1986).
4. W. Kraus and R. Cramer, *Liebigs Ann. Chem.*, 181 (1981).
5. R. Henderson, R. McCrindle, A. Melera, and K.H. Overton, *Tetrahedron*, **24**, 1525 (1968).
6. W. Kraus and M. Bokel, *Chem. Ber.*, **114**, 267 (1981).
7. M. Ochi, H. Kotsuki, M. Ido, H. Nakai, M. Shiro, and T. Tokoroyama, *Chem. Lett.*, 1137 (1979).
8. A.R. Alford, J.A. Cullen, R.H. Storch, and M.D. Bentley, *J. Econ. Entomol.*, **80**, 575 (1987).

Received 29 June 1987