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AN INSECT ANTIFEEDANT LIMONOID FROM *TURRAEA NILOTICA*

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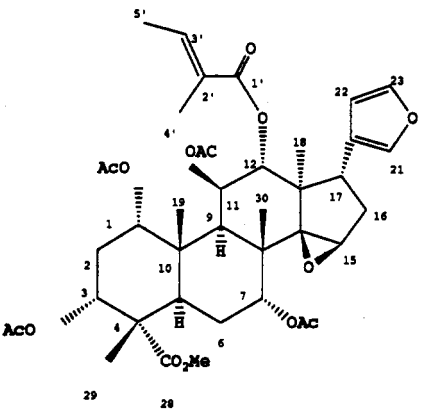
ABSTRACT.—Nilotin [**1**], a new limonoid, has been isolated from the rootbark of *Turraea nilotica* and its structure established by spectroscopic methods. It displayed significant activity as an antifeedant against larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*.

The genus *Turraea* (Meliaceae) is comprised of a group of 60–70 species of shrubs and small trees occurring in the Indian Ocean region. Several species studied have been shown to produce limonoids of the priurianin and havanensin classes (1–3). *Turraea nilotica* Kotschy et Peyr. is found in East Africa and, in traditional medicine, a decoction of the roots is taken for upset stomachs (4). From an earlier study of the stem components of this plant, protolimonoids, but not limonoids, were reported (5). In continuing our investigations on the limonoid chemistry of this genus, we have examined a MeOH extract of the root bark of *T. nilotica* and here report the isolation of a new limonoid active as an insect antifeedant.



1

Chromatography of the MeOH extract of *T. nilotica* root bark was performed on Si gel using a hexane/EtOAc gradient to obtain a fraction for which the presence of a limonoid was confirmed by ^{1}H-nmr spectroscopy. This fraction was further purified by Si gel cc (toluene/methyl ethyl ketone eluent) to obtain a new limonoid, nilotin [**1**], $C_{40}H_{52}O_{14}$. The ^{1}H-nmr spectrum of **1** (see Table 1) indicated the presence of four tertiary methyls, a carbomethoxy, four acetates, a tiglate, a β-substituted furan, and an epoxide, and was consistent with the presence of a limonoid of the havanensin class. Substitution patterns and stereochemistry were determined with the aid of ^{1}H COSY and ^{1}H NOESY nmr experiments. Important proton connectivities observed in the COSY spectrum were H-1 and H-3 with H-2; H-5 and H-7 with H-6; H-9 with H-11; and H-11 with H-12. The small coupling constants of H-1 and H-3 with H-2 were consistent with the commonly observed equatorial stereochemistry of the 1- and 3-protons. Fur-

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for **1**.

Position	^{1}H δ (ppm) *J* (Hz)	^{13}C δ (ppm)
1	4.65 t (3)	73.9
2	2.20 m	24.8[a]
3	4.97 t (3)	72.4
4	—	40.1
5	3.13 dd (12, 2)	33.4
6	1.70 m	24.2[a]
7	4.70 t (2.5)	74.0
8	—	49.6[b]
9	3.38 d (3.5)	40.3
10	—	40.1
11	5.16 t (3.5)	74.4
12	4.85 d (3.5)	79.6
13	—	48.7[b]
14	—	73.7
15	3.61 s	63.2
16	2.34 m	32.5
17	2.80 m	40.3
18	1.06 s	17.9
19	1.25 s	16.5
20	—	128.0[c]
21	7.10 m	140.6
22	6.40 m	112.3
23	7.27 m	142.3
29	1.22 s	16.5
30	1.34 s	23.6
2′	—	128.5[c]
3′	6.83 qq (7,2)	137.8
4′	1.7–1.76 m	11.6
5′	1.7–1.76 m	14.3
Me(Ac)	1.94 s	21.4
	2.05 s	21.9
	2.10 s	20.9
	2.14 s	20.9
CO_2Me	3.49 s	
C=O	—	174.0
		170.7
		169.7
		169.5
		169.2
		165.7

[a–c]Values with the same superscripts are interchangeable.

ther correlations were H-11 with H-12, H-17 with H-16, H-5′ with H-3′, and H-22 with H-21 and H-23. Using the NOESY technique, correlations between H-9 and H-11 confirmed the β stereochemistry of the ester at C-11. Correlation of the 18-methyl protons and a tiglate methyl placed the tiglate at the 12α position, while correlation of the 18-methyl with the furan H-23 confirmed the usual α-stereochemistry of the furan. Correlation of H-17 and H-12 further supported these stereochemical assignments. Correlation of H-7 with the 30-methyl corroborated the assignment of equatorial stereochemistry for H-7 indicated by its coupling constant with H-6. ^{13}C-Nmr assignments were made with the aid of APT and HETCOR techniques and are presented in Table 1. The structure of nilotin [**1**] has the same skeleton and substitution pattern as several limonoids previously isolated from *Turraea floribunda* (3), but differs in groups attached at C-3, C-11, and C-12.

Nilotin [**1**] showed significant antifeedant activity in no-choice feeding assays using 4th instar Colorado potato beetles. The dose required for 50% feeding reduction (ED_{50}) was 7 μg/ml, and was thus comparable to that determined earlier for the citrus limonoid, limonin (ED_{50}=8 μg/ml) (6). This is structurally consistent with our previous findings in structure-activity studies on citrus limonoids as Colorado potato beetle antifeedants that demonstrated that the furan and epoxide, both present in **1**, are primarily responsible for the observed activity (6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Fisher hot-stage apparatus and are uncorrected. Ir spectra were recorded on a Bio-Rad FTS-60 spectrometer. ^{1}H- and ^{13}C-nmr spectra were obtained on Varian XL-200 and XL-400 instruments, respectively, using $CDCl_3$ as solvent and TMS as internal standard.

PLANT MATERIAL.—The roots of *T. nilotica* were collected in May 1989, from Shimba Hills, Kwale District, near Mombasa, Kenya. The plant was authenticated by S.G. Mathenge of the Herbarium of the Department of Botany, University of Nairobi, and a reference specimen is on file in that department.

EXTRACTION AND ISOLATION.—The root bark of *T. nilotica* was air-dried for a week and ground into a powder. The powder (100 g) was soaked in MeOH (1 liter) for 4 weeks at room temperature, filtered, and the extract concentrated *in vacuo*. Water was added and the aqueous MeOH extract partitioned with petroleum ether. The aqueous

MeOH phase was evaporated to yield an oil (20 g), of which 18 g were chromatographed on 600 g of Si gel (Merck Kieselgel, 230–400 mesh) using a hexane/EtOAc gradient. A limonoid-containing fraction was collected. Cc of this fraction (680 mg) on Si gel eluted with toluene-methyl ethyl ketone (8:2) led to the isolation of **1** (180 mg) as a white solid: mp 138–140°; hrfabms *m/z* $[M+Li]^+$ 763.3530, $C_{40}H_{52}O_{14}Li$, calcd 763.3493; ir (KBr) ν max 2951, 1737 (br), 1365, 1232, 1126, 1032 cm^{-1}; ^1H- and ^{13}C-nmr spectral data, see Table 1.

ANTIFEEDANT BIOASSAY.—Compound **1** was subjected to no-choice antifeedant assays at three dose levels (30, 10, and 3 μg/ml) on potato leaf disks using 4th instar Colorado potato beetles according to our previously published procedure (6). Percent feeding reduction (%FR) was calculated by the equation %FR=[1-treatment consumption/control consumption]×100 and standard errors (SEM) were calculated (6). For an applied concentration of 30 μg/ml leaf disk, %FR=78 (SEM=19) (38 insects); for 10 μg/disk, %FR=61 (SEM=15) (30 insects); and for 3 μg/disk, %FR=20 (SEM=11) (8 insects).

ACKNOWLEDGMENTS

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