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STRUCTURE OF A LIMONOID ANTIFEEDANT FROM *TRICHILIA ROKA*

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Key Word Index—*Trichilia roka*; Meliaceae; antifeedant; limonoid; 7-acetyltrichilin A.

Abstract—A new limonoid, 7-acetyltrichilin A, has been isolated from the root bark of *Trichilia roka* and identified as an antifeedant against North American and Japanese pest insects.

INTRODUCTION

Trichilia roka P. Br. is a large tree found in East Africa. It has been used medicinally for the treatment of a variety of human disorders. In Kenya, an infusion of the pounded bark is used as a remedy for pneumonia. It acts as an emetic and causes profuse perspiration. A decoction of the roots is taken as a remedy for colds, as a diuretic or to induce labour in pregnant women [1].

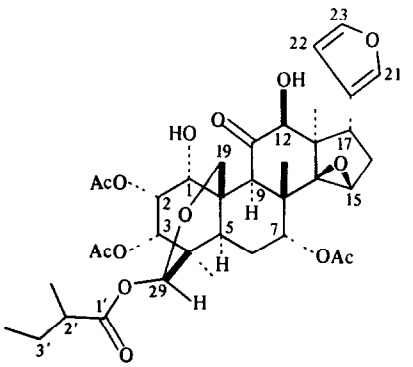
In our investigation of insect antifeedants from the African medicinal plant, we have isolated a number of limonoids, the trichilins A–F [2]. We now describe a new limonoid isolated from the ether extract of the root bark, which showed antifeedant activity against the pest insects of North American plants, i.e. the Southern army worm (*Spodoptera eridonia*) and the Mexican bean beetle (*Epilachna varivestis*), and Japanese plants, i.e. Awayotou (*Spodoptera littoralis* Boisd.). The isolation was monitored by antifeeding assay and the structure was established by chemical and spectroscopic means.

RESULTS AND DISCUSSION

The antifeeding compounds in the ether extract from *T. roka* were very sensitive to traces of acid and gradually decomposed on a silica column [2, 3]. It was therefore necessary to use flash chromatography [4] and HPLC separation techniques to obtain pure compounds. From the less polar fraction of the ether extract, compound **1** was isolated in an amorphous form by repeated HPLC on a normal-phase column.

The mass spectrum of **1** revealed a molecular ion at m/z 716 $[M]^+$ corresponding to $C_{37}H_{48}O_{14}$, and fragmentation ions at m/z 614 $[M - C_4H_9CO_2H]^+$ and 554 $[M - C_4H_9CO_2H - HOAc]^+$. This fragmentation pattern and the IR, UV and circular dichroism (CD) spectra (see Experimental) showed the presence of similar groups in **1** to those in the trichilins [2]. The ^1H NMR spectrum of **1** showed the presence of three acetyl groups (δ2.03, 2.12 and 2.16) and suggested that it resembled trichilin A (**2**) and trichilin B (**3**) except for the presence of an additional acetyl group. A ^1H NMR study at 360 MHz allowed us to assign all the peaks in the complex spectrum as well as to derive structure **1** (Table 1). Two *W*-type long-range couplings were observed between the 1β-H at δ3.99 (*t*, (*br*), J = 4 Hz), coupling with OH at 2.46 and 2β-H at 5.91, and the 3β-H at 5.52 (*d*, (*br*), J = 4.5 Hz), and one proton of the 19-methylene at 4.50 (AB type) and the 5α-H at 2.60 (*dd* (*br*), J = 15 and 3.5 Hz). These observations revealed the substitution pattern around the A-ring. Another long-range coupling was observed between the 17β-H at δ3.99 (*dd* (*br*), J = 11 and 6.5 Hz) and the 22-H at 6.34, and the chemical shift of the 17β-H was indicative of the presence of a β-hydroxyl group at C-12. Irradiation of the 13-Me peak at δ1.28 induced a 7% nuclear Overhauser effect (NOE) on the 22-H signal and a 32% NOE on the 9-H signal, which was indicated by the characteristic resonance at δ4.71 attributed to the effect of the 1α-hydroxyl group in a 1,3-diaxial relation.

The configuration of the 12-hydroxyl group was assigned β from the chemical shift of the 1β-H (δ3.99) and a CD study of the 12-benzoate **4**. The 1β-H resonance frequencies of the 12α-epimers in trichilins are subjected to greater paramagnetic anisotropy by the 11-keto group and appear at lower field ($\Delta\delta$0.4–0.5) than those of the 12β-epimers: 3.98 in **2** and 4.58 in **3**. Recently, we showed exciton-split CD curves arising from interactions between different chromophores and its application in structure studies [5]. The CD curve of **4** showed a positive



1		**4**	12 – OBz
2	7 – OH	**5**	7 – OH, 12 – OAc
3	7 – OH, 12α – OH	**6**	12 – OAc

Table 1. ^{1}H NMR spectral data of **1** (360 MHz, $CDCl_3$, TMS as internal standard)

H		H	
1	3.99 *t* (*br*) (4)	21	7.19
2	5.91 *t* (4.5)	22	6.34
3	5.52 *d* (*br*) (4.5)	23	7.37
5	2.60 *dd* (*br*) (15, 3.5)	28 (4-Me)	0.78 *s*
6α	1.83 *dt* (15, 3)	29	5.73 *s*
6β	2.07 *td* (15, 3)	30 (8-Me)	1.08 *s*
7	4.86 *t* (*br*) (3)	2′	2.49 *tq* (7,.7)
9	4.71 *s*	2′-Me	1.19 *d* (7.5)
12	3.74 *d* (3)	3′a	1.52 *ddq* (14, 7, 7)
15	3.43 *s*	3′b	1.72 *ddq* (14, 7, 7)
16α	2.26 *dd* (13, 6)	3′-Me	0.92 *t* (7.5)
16β	1.73 *dd* (*br*) (12)	Ac(2-)	2.12 *s*
17	3.39 *dd* (11, 6.5)	(3-)	2.03 *s*
18(13-Me)	1.28 *s*	(7-)	2.16 *s*
19a	4.50 AB (14.5)	OH(1)	2.46 *d* (3)
19b	4.52 AB (14.5)	(12-)	3.14 *d* (3)

Coupling constants (Hz) are in parentheses.

interaction of one the bands of the benzoate group with a furan ring at 237 nm ($\Delta\varepsilon$ +3.6) similar to the one observed for the 12-benzoate of **2**. The 12-benzoate of **3** had a negative band at the same position ($\Delta\varepsilon$ −7.0). Although we did not succeed in preparing **1** from **2**, **1** and **2** gave the same derivative, 7,12-diacetyltrichilin A (**6**), on acetylation. We therefore assigned structure 7-acetyltrichilin A to **1**.

EXPERIMENTAL

^{1}H NMR: 360 MHz, TMS as internal standard. Bioassay of the antifeedant was done by the leaf-disk method against the larvae of the insects.

Plant material. The root bark was collected in June 1979 at Simba Hill near Mombasa, Kenya and identified by Dr. S. F. Dossaji (University of Nairobi, Kenya).

Extraction and isolation. The root bark (365 g) was defatted with petrol and extracted with Et_2O to yield 2.9 g of an extract. The extract was flash chromatographed with Et_2O–hexane, and the active fraction was rechromatographed on a flash column with 0.6% MeOH–CH_2Cl_2. Repeated passage through a HPLC, Whatman Partisil M9 semiprep. column with 0.6–1.0% MeOH–CH_2Cl_2 as the solvent finally gave 74 mg **1**, $C_{37}H_{48}O_{14}$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1740 (COO), 1720 (sh) (>CO); UV λ_{max}^{MeOH} nm (log ε): 206 (3.65); CD (MeOH) nm: $\Delta\varepsilon_{213}$ +3.3 ($\pi\pi^*$ of furan), $\Delta\varepsilon_{305}$ ($n\pi^*$ of ketone); EIMS *m/z* (rel. int.): 716 $[M]^+$ (14), 656 $[M-HOAc]^+$ (6), 614 $[M-MeCH_2CH(Me)CO_2H]^+$ (19), 596 $[614-H_2O]^+$ (8), 554 $[614-HOAc]^+$ (35).

Benzoate of **1**. A pyridine (1 ml) soln of **1** (10 mg) was added to benzoyl chloride (0.2 ml) and left to stand overnight. Work-up as usual gave **4** (2 mg), $C_{44}H_{52}O_{15}$; FDMS *m/z*: 820 $[M]^+$; UV λ_{max}^{MeOH} nm (log ε): 203 (3.59), 215 (3.79); CD (MeOH) nm: $\Delta\varepsilon_{216}$ +0.4, $\Delta\varepsilon_{237}$ +3.6 (interaction band of $\pi\pi^*$), $\Delta\varepsilon_{306}$ −5.4 ($n\pi^*$ of ketone).

Benzoate of trichilin A (**2**). Benzoylation of **2** (benzoyl chloride–pyridine at 30° for 20 hr) gave the 12-benzoate (14%), $C_{42}H_{50}O_{14}$; CIMS *m/z*: 779 $[M+1]^+$; UV λ_{max}^{MeOH} nm (log ε): 204 (3.81), 229 (3.76); CD (MeOH) nm. $\Delta\varepsilon_{215}$ +0.6, $\Delta\varepsilon_{237}$ +3.4 ($\pi\pi^*$ interaction band), $\Delta\varepsilon_{306}$ −5.1 ($n\pi^*$ of ketone).

Benzoates of trichilin B (**3**). Benzoylation of **3** (benzoyl chloride–pyridine at 56° for 48 hr) gave the 12-benzoate (43%) and the 7,12-dibenzoate (7%). 12-Benzoyltrichilin B, $C_{42}H_{50}O_{14}$; CIMS *m/z*: 779 $[M+1]^+$; UV λ_{max}^{MeOH} nm (log ε). 208 (4.00), 229 (4.06): CD (MeOH) nm: $\Delta\varepsilon_{215}$ +4.4, $\Delta\varepsilon_{238}$ −7.0 ($\pi\pi^*$ interaction band), $\Delta\varepsilon_{273}$ +1.0, $\Delta\varepsilon_{280}$ +0.8, $\Delta\varepsilon_{312}$ −1.2. 7,12-Dibenzoyltrichilin B, $C_{49}H_{54}O_{15}$; CIMS *m/z*: 883 $[M+1]^+$; UV λ_{max}^{MeOH} nm (log ε): 205 (4.15), 229 (4.36): CD (MeOH) nm: $\Delta\varepsilon_{218}$ +9.4 ($\pi\pi^*$ interaction band), $\Delta\varepsilon_{245}$ −7.3 ($\pi\pi^*$ interaction band), $\Delta\varepsilon_{275}$ +1.3, $\Delta\varepsilon_{280}$ +1.4, $\Delta\varepsilon_{315}$ −0.6.

Acetylation of **1**. A pyridine (2 ml) soln of **1** (20 mg) was added to Ac_2O (0.5 ml) and left to stand for 3 days. Work-up as usual gave **6** (15 mg), $C_{39}H_{50}O_{15}$; CIMS *m/z*: 759 $[M+1]^+$; ^{1}H NMR ($CDCl_3$): δ0.80 (3H, *s*), 0.92 (3H, *t*, *J* = 6.6 Hz), 1.16 (3H, *s*), 1.25 (3H, *d*, *J* = 7.4 Hz), 1.38 (3H, *s*), 2.04, 2.11, 2.18, 2.23 (each 3H, *s*), 3.5 (1H, *s*), 4.06 (1H, *m*), 4.46 (2H, *s*), 4.70 (1H, *s*), 4.83 (1H, *m*), 5.25 (1H, *s*), 5.52 (1H, *d*, *J* = 4.9 Hz), 5.77 (1H, *s*), 5.90 (1H, *t*, *J* = 4.9 Hz), 6.06, 7.09, 7.37 (each 1H)

Acetylation of **2** (i) Acetylation of **2** (Ac_2O–pyridine at 25° for 24 hr) gave the 12-acetate **5** (70%), $C_{37}H_{48}O_{14}$; CIMS *m/z*. 717 $[M+1]^+$; ^{1}H NMR ($CDCl_3$): δ0.84 (3H, *s*), 0.92 (3H, *t*, *J* = 6.6 Hz), 1.09 (3H, *s*), 1.19 (3H, *d*, *J* = 7.2 Hz), 1.41 (3H, *s*), 2.05, 2.15, 2.24 (each 3H, *s*), 3.59 (1H, *s*), 3.70 (1H, *m*), 4.06 (1H, *d* (*br*), *J* = 4.6 Hz), 4.46 (2H, *s*), 4.69 (2H, *s*), 4.69 (1H, *s*), 5.22 (1H, *s*), 5.53 (1H, *d*, *J* = 4.6 Hz), 5.76 (1H, *t*, *J* = 4.6 Hz), 6.06, 7.09, 7.35 (each 1H). (ii) Acetylation of **2** at 45° gave **5** (52%) and **6** (21%), identical to the acetate of **1** (^{1}H NMR).

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