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STRUCTURES OF INSECT ANTIFEEDING LIMONOIDS, TRICHILINS F AND G, FROM *TRICHILIA ROKA*

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Abstract - Two new limonoids, trichilins F and G, have been isolated from the root bark of *Trichilia roka* and identified as antifeedant against some pest insects. Their structures were determined from spectral data and chemical means, in which the treatment of trichilin A with zinc borohydride induced an acyl migration to yield trichilin F.

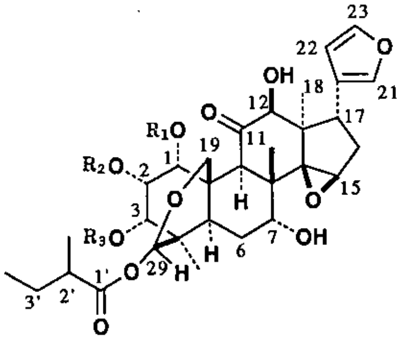
Trichilia roka (Meliaceae) is an East African medicinal plant and, in kenya, a decoction of the root is taken as a remedy for colds, as a diuretic or to induce labour in pregnant women.[1] It is a rich source of limonoids, "trichilins"[2,3] and seco-limonoids,[4] which are antifeedants against North American pest insects, the Southern army worm (*Spodoptera eridania*) and the Mexican bean beetle (*Epilachna varivestis*), and a Japanese pest insect, *Spodoptera littoralis* Boisd. Trichilins A-E are one of the few antifeedants active against the voracious *S. eridania* catapillar.[5]

In continuous isolation study of antifeedant, we got two new trichilins, F (**1**) and G (**2**), from the root bark of *T. roka*. Their structures were established by chemical and spectroscopic means. In particular, treatment of trichilin A (**3**) with zinc borohydride induced acetyl migration to give trichilin F (**1**) and this unexpected conversion established its structure.

RESULTS AND DISCUSSION

The antifeeding limonoids from *T. roka* were very sensitive to a trace of acid and gradually decomposed on a sillica column.[2,6] Therefore, the use of flash chromatographic and hplc separation techniques is essential for the isolation, which was monitored by antifeeding assay against a North American pest insect, *S. eridania*. From the ether extract of the dried root bark (365 g), two new limonoids of trichilins F (**1**), 1.3 mg, and G (**2**), 2.0 mg, were isolated as insect antifeedant.

^{1}H Nmr study of trichilin F (**1**), $C_{35}H_{46}O_{13}$, CI-ms m/z 675 $(M+1)^+$, taking account the cd data ($\Delta\varepsilon_{300}$ -3.7; n-π^* absorption of 11-keto group),[2] allowed us to expect it to be an isomer of trichilin A (**3**). The complex spectrum was very similar to that of **3**, including the signals due to a β-furan ring, two acetyl and one 2-methylbutanoyl groups, except for some changes of chemical shifts and splittings (Table 1). Although there are two series of trichilin having 12β-OH and its epimer, the fact that the 12-OH in **1** is β same as in **3** was deduced from the chemical shift of 17-H. The low shift of δ 3.42 in **1** (δ 3.39 in **3**) could be attributed to the effect of the 12β-OH in a 1,3-diaxial relation, whereas its signal was observed at δ 3.15 in 12-acetyltrichilin A and in trichilin B (12α-OH) it was shifted upfield to δ 3.02.[7] On the other hand, the substitution pat-



	R₁	R₂	R₃
trichilin F (**1**):	OAc	OAc	OH
trichilin G (**2**):	OH	OH	OAc
trichilin A (**3**):	OH	OAc	OAc
(**4**):	OAc	OH	OAc

tern around the A-ring, namely, that **1** has a free 3-OH and 1,2-diacetyl groups different from **3**, was shown by the fact that the 9-H signal in **1** was at δ 4.13, whereas in **3** it was shifted down-field to δ 4.70 by the effect of the 1-OH in a 1,3-diaxial relation.

The structure (**1**) for trichilin F including its stereochemistry was finally determined by an unexpected acetyl migration in ring A with zinc borohydride.[8] Treatment of **3** with zinc borohydride in dry 2-propanol in

Table 1. ^{1}H Nmr data for trichilins F (**1**), G (**2**), A (**3**) and compound **4**.

H	**1**		**2**		**3**		**4**	
	δ	Mult(J/Hz)	δ	Mult(J/Hz)	δ	Mult(J/Hz)	δ	Mult(J/Hz)
1	5.40	dd(5.0, 1.0)	3.90	m	3.98	br t(4.5)	5.32	d(4.2)
2	5.91	t(5.0)	4.73	t(4.2)	5.90	t(4.5)	4.80	m
3	4.05	m	5.41	d(4.2)	5.54	br d(4.5)	5.46	d(4.2)
5	2.66	dd(14, 4.5)	2.72	dd(14, 4.3)	2.72	dd(14, 4.0)	2.68	dd(14, 4.5)
7	3.69	m	3.72	m	3.72	m	3.71	m
9	4.13	s	4.70	s	4.70	s	4.17	s
12	3.50	d(1.7)	3.74	d(2.0)	3.74	d(2.0)	3.55	d(1.0)
15	3.48	s	3.50	s	3.50	s	3.47	s
17	3.42	dd(11, 6.0)	3.40	dd(11, 6.0)	3.40	dd(11, 7.0)	3.45	dd(11, 6.0)
18(Me)	1.16	s	1.30	s	1.30	s	1.18	s
19a	4.53	br s	4.48	br s	4.48	br s*	4.55	br s
19b								
21	7.17		7.19		7.19		7.16	
22	6.32		6.35		6.35		6.32	
23	7.35		7.35		7.37		7.35	
28(Me)	0.99	s	0.79	s	0.88	s	0.82	s
29	5.77	s	5.71	s	5.76	s	5.75	s
30(Me)	1.01	s	0.97	s	1.02	s	1.00	s
2'-Me	1.15	d(7.1)	1.14	d(6.5)	1.19	d(7.0)	1.15	d(6.9)
3'-Me	0.89	t(7.3)	0.88	t(7.0)	0.92	t(7.5)	0.89	t(7.3)
Ac	2.02		2.12		2.03		2.04	
	2.06				2.14		2.10	

Measured in $CDCl_3$ at 250 MHz.

* Observed as two signals at δ 4.47 (d, J= 13) and 4.53 (d, J= 13) at 400 MHz.

attempt to reduce the 11-keto led to an acyl migration in ring A and gave a mixture of **3** and its 1,2-diacetyl (**1**) and 1,3-diacetyl isomers (**4**), trichilin E, which have been separated.[2]

Second antifeedant, trichilin G (**2**), $C_{33}H_{44}O_{12}$, CI-ms m/z 633 $(M+1)^+$, showed the presence of only one acetyl group along with a β-furan ring and a 2-methylbutanoyl group in the ^1H nmr spectrum (Table 1). In addition, by D_2O addition four signals due to OH group at δ 1.63, 2.28, 2.80 and 3.11 disappeared and three methine signals at δ 3.90 (m), 3.72 (m) and 3.74 (d, J= 2.0) were collapsed to doublet (J= 4.2), sharp multiplet and singlet, respectively. The ^1H nmr spectrum resembled with that of trichilin A (**3**) very well except for the lack of one acetyl group and one additional OH group. Its cd absorption at 302 nm (Δ_ε -3.6, n-π* of 11-keto) and the chemical shift of 17-H at δ 3.40 suggested the presence of an 11-keto, 12β-OH group identical with **1** or **3**. The β configuration of the 12-OH was also supported by the chemical shift of the 1β-H at δ 3.90 which, in **3**, was observed at δ 3.98. In trichilin B (12α-epimer of **3**) and 12α-hydroxyamoorastatin[9] (2-deoxy compound), the 1β-H signal was observed at δ 4.58 and 4.48, which shifted to δ 4.42 and 4.27 in their 12-acetates, and observed at δ 4.23[7] and 4.19 in trichilin D (12-deoxytrichilin A) and amoorastatin.[10] A similar relation on the chemical shift of the 1β-H was also observed in **1** (δ 5.40) and its 12α-epimer[11] (δ 5.93, 12-acetate: δ 5.75). These observation greatly suggested that the 1β-H in the 11-keto, 12α-OH compounds was subjected to greater paramagnetic anisotropy by the 11-carbonyl group, resulted in the conformation change of the ring C due to a five-membered hydrogen bonding between the 12-OH and 11-keto groups. The chemical shift of δ 3.72 and the half height width ($\Delta H\frac{1}{2}$= 6.5 Hz) of the 7-H signal revealed the same α, axial orientation of the 7-OH group as in other trichilins. As the 9-H signal was observed at δ 4.70 suggesting the presence of the 1α-OH group in 1,3-diaxial relation, compound (**2**) was presumed to be different from **3** only at C_2 or C_3 in ring A. The substitution pattern around the ring A in **2** was readily deduced from a doublet (J= 4.2) at δ 5.41 due to 3β-H under acetoxyl group, which coupled to the 2β-H signal (t, J= 4.2) at δ 4.73 coupling with the 1β-H signal at δ 3.90 (m). The 1β-H signal was W-coupled with the 3β-H signal and another W-type long range coupling was observed between one proton of the 19-methylene at δ 4.48 and the 5α-H (dd, J= 14 and 4.3) at δ 2.72.

The antifeeding activities of trichilins F (**1**) and G (**2**) were tested by the conventional leaf disk method[12] against the Southern army worm. Independent of the substitution pattern in ring A, both **1** and **2** were active at 300 ppm concentration similarly to trichilin A (**3**).[13]

EXPERIMENTAL

^{1}H Nmr spectra were measured in $CDCl_3$ at 250 MHz with TMS as internal standard. Uv and cd spectra were measured in MeOH.

Extraction and isolation of trichilins F (1) and G (2). The dried root bark (365 g) of *T. roka* was defatted with petrol (3 l) for 2 weeks at 22°C and extracted with ether (2 l) to yield 2.9 g of an extract. The extract was flash chromatographed on SiO_2 with ether-hexane, and an active fraction was rechromatographed on a flash column with 0.6% MeOH-CH_2Cl_2. Final purification was done by hplc on a Whatman Partisil M9 semiprep. column using 0.6 - 1.0% MeOH-CH_2Cl_2 solvent system to give **1** (1.3 mg) and **2** (2.0 mg).

1: powder; CI-ms m/z 675 $(M+1)^+$, 615 (675 - 60), 573 (675 - 102); uv 210 nm (ε 5100); cd 211 ($\Delta\varepsilon$ +2.2), 303 nm ($\Delta\varepsilon$ -4.3). **2**: mp 222-224°C from CH_2Cl_2-petroleum ether; CI-ms m/z 633 $(M+1)^+$, 615 (633 - 18), 573 (615 - 42), 555 (573 - 18), 531 (633 - 102); uv 212 nm (ε 4200); cd 209 ($\Delta\varepsilon$ +3.0), 302 nm ($\Delta\varepsilon$ -3.6).

Treatment of trichilin A (3) with zinc borohydride. A solution of trichilin A (**3**; 10 mg, 0.015 mmol) in dry 2-propanol (0.5 ml) was stirred with 1.3 M ether solution (0.1 ml, 0.13 mmol) of zinc borohydride for 40 h at room temperature and then acetone (1 ml) was added. After an additional stirring of 2 h, the reaction products were purified by column chromatography on SiO_2 and hplc with the Partisil column to give **3** (7.9 mg), **1** (0.6 mg) and **4** (1.1 mg). **4**: CI-ms m/z 675 $(M+1)^+$, 657 (675 - 18), 615 (657 - 42), 573 (675 - 102); uv 215 nm (ε 4100); cd 211 (Δ_ε +2.7), 300 nm (Δ_ε -3.7).

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