

STRUCTURES OF INSECT ANTIFEEDING LIMONIDS, 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* caterpillar.⁵

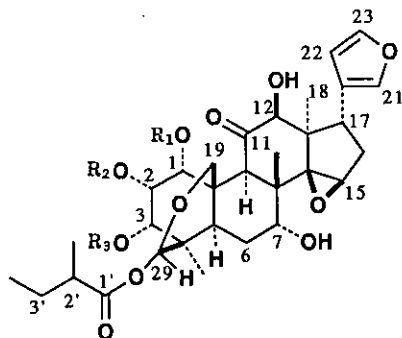
In continuous isolation study of antifeedant, we got two new trichilins, F (1) and G (2), from the root bark of *T. roka*. Their structures were established by chemical and spectroscopic means. In particular, treatment of

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 silica 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.

¹H Nmr study of trichilin F (1), C₃₅H₄₆O₁₃, CI-ms m/z 675 (M+1)⁺, taking account the cd data ($\Delta\epsilon_{300}$ -3.7; $n-\pi^*$ 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 12 β -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 12 β -OH in a 1,3-diaxial relation, whereas its signal was observed at δ 3.15 in 12-acetyltrichilin A and in trichilin B (12 α -OH) it was shifted upfield to δ 3.02.⁷ On the other hand, the substitution pat-



	R ₁	R ₂	R ₃
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. ¹H 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								
19b	4.53	br s	4.48	br s	4.48	br s*	4.55	br s
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 CDCl₃ 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 ($\Delta\epsilon$ -3.6, $n-\pi^*$ of 11-keto) and the chemical shift of 17-H at δ 3.40 suggested the presence of an 11-keto, 12 β -OH group identical with 1 or 3. The β configuration of the 12-OH was also supported by the chemical shift of the 1 β -H at δ 3.90 which, in 3, was observed at δ 3.98. In trichilin B (12 α -epimer of 3) and 12 α -hydroxyamoorastatin⁹ (2-deoxy compound), the 1 β -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 4.23⁷ and 4.19 in trichilin D (12-deoxytrichilin A) and amoorastatin.¹⁰ A similar relation on the chemical shift of the 1 β -H was also observed in 1 (δ 5.40) and its 12 α -epimer¹¹ (δ 5.93, 12-acetate: δ 5.75). These observation greatly suggested that the 1 β -H in the 11-keto, 12 α -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 1 α -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 substi-

tution 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 acetoxy 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 method¹² 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

¹H Nmr spectra were measured in CDCl₃ 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 SiO₂ with ether-hexane, and an active fraction was rechromatographed on a flash column with 0.6% MeOH-CH₂Cl₂. Final purification was done by hplc on a Whatman Partisil M9 semiprep. column using 0.6 - 1.0% MeOH-CH₂Cl₂ 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 ($\Delta\epsilon$ +2.2), 303 nm ($\Delta\epsilon$ -4.3). 2: mp 222-224°C from CH₂Cl₂-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 ($\Delta\epsilon$ +3.0), 302 nm ($\Delta\epsilon$ -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 ($\Delta\epsilon$ +2.7), 300 nm ($\Delta\epsilon$ -3.7).

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