ISOLATION AND STRUCTURES OF THREE SECO-LIMONOIDS, INSECT ANTIFEEDANTS FROM *TRICHILIA ROKA* (MELIACEAE)

Munehiro Nakatani*, Miyako Okamoto, Takashi Iwashita†, Kosei Mizukawa†, Hideo Naoki†, and Tsunao Hase
Department of Chemistry, Faculty of Science, Kagoshima University, Kagoshima 890, Japan
†Suntory Institute for Bioorganic Research, Mishima-gun, Osaka 618, Japan

Abstract - The stem bark of African medicinal plant *Trichilia roka* has been found to contain a number of limonoids. Three of these has been isolated as insect antifeedant; They are all of the ring B-cleaved meliacan group and are closely related to known prieurianin.

Trichilia roka is a large tree found in drier part of East Africa. It has been used medicinally and a decoction of the bark is used as a purgative[1]. We have already described the isolation of limonoids, trichilin A-F and 7-acetyltrichilin A , as insect antifeedant from the root bark[2]. We now describe the isolation and structures of three prieurianin type limonoids from the stem bark. The group of limonoid related to prieurianin[3], mainly found from *Trichilia* species, is rapidly growing and more than 20 are now known, in which hispidins from *Trichilia hispida* have been reported to have cytotoxic properties[4]. The compounds from the stem bark of *T. roka* also exhibited insect antifeedant activity against the larvae of the Japanese pest insect, "nekiri-mushi", *Ajrotis sejetum Denis* (200 ppm) with the leaf disk choice test.

The stem bark (1.3 Kg)[5] was deffated with petroleum ether and extracted with ether to yield 5.2 g of an extract. An insoluble resin (1.1 g) of the extract in ether contained various congeners of limonoids. The isolation was a tedius process required very careful use of HPLC and pure compounds also showed 5-7 peaks on the C_{18} reversed-phase column. Because of these chromatographic properties, it was expected that these compounds would belong to the ring B-cleaved meliacan group of limonoids. As is common with this type of extract, separation was a major problem

, owing to the presence of multiple conformational isomers[3] and decomposition during chromatography[6]. A combination of column chromatography on silica and HPLC on normal- and reversed-phase columns gave three pure substances; Tr-A, 3.0 mg: Tr-B, 3.1 mg: Tr-C, 3.4 mg.

These compounds remained amorphous and the structures of Tr-A (**1**), B (**2**) and C (**3**) were elucidated mainly by ^{1}H NMR decoupling studies and comparison of their spectra with those published for other related limonoids, in particular, prieurianin[3], rohituka substances[6,7,8] and hispidins[4]. The ^{1}H NMR spectra of **1-3** showed very broad signals due to restricted rotation of the molecule about C-9, C-10 bond at lower temperatures and so all spectra were measured at 45° C to obviate the difficulties of analysis.

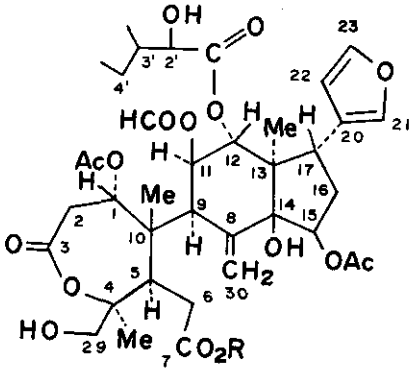
Tr-A (**1**), $C_{39}H_{54}O_{16}$ (FD-MS: m/z 778, M^+), and Tr-C (**3**), $C_{38}H_{52}O_{16}$ (FD-MS: 764, M^+), exhibit the following spectral data. **1**: IR($CHCl_3$) 3500, 1730 cm^{-1}; UV(MeOH) 207 nm(ε 3300); CD(MeOH) $\Delta_{\varepsilon_{208}}$ +7.3. **3**: IR($CHCl_3$) 3550, 1730 cm^{-1}; UV(MeOH) 206

Table 1. ^{1}H NMR data for Tr-A (**1**), B (**2**) and C acetate (**3a**), in ppm (J value, Hz)

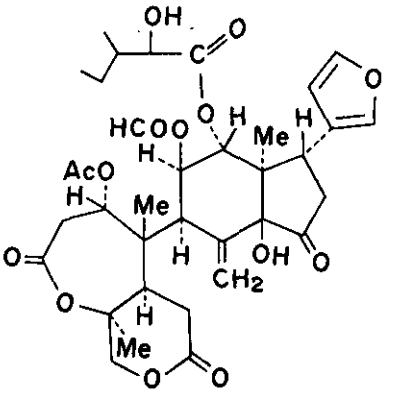
proton	**1**[a]	**2**[b]	**3a**[b]	proton	**1**[a]	**2**[b]	**3a**[b]
1	6.18 br d (11)	5.26 m	5.56 m	29a	3.86 s	4.21 q (12)	4.49 d (13.5)
2α	3.75 br d (16)	3.16 m	2.98 m	29b	3.87 s		4.03 d (13.5)
2β	3.34 dd (16,11)	3.28 m	2.75 m	30a	5.35 br s	5.92 br s	5.27 br s
				30b	5.16 br s	5.50 br s	5.17 br s
9	3.68 d (9)	3.78 d (7)	3.20 d (8)	CMe(18)	1.05 s	0.98 s	0.97 s
11	5.80 dd (11,9)	5.47 dd (11,7)	5.55 m	(19)	1.78 s	1.82 s	1.68 s
				(28)	1.98 s	1.79 s	1.55 s
12	6.69 d (11)	6.16 d (11)	5.97 d (11.5)	(3'-)	1.04 d (7)	0.84 d (7)	0.83 d (7)
15	5.93 dd (10,5)	----	6.67 m	(4'-)	0.88 t (8)	0.78 t (8)	0.78 t (8)
16α	2.28 ddd (15,9,5)	2.33 dd (20,9)	2.10 m	OCHO	8.64 s	7.76 s	8.02 s
				2'	3.33 m	3.12 dd (6,4)	4.64 d (4.5)
16β	2.60 ddd (15,10,9)	2.84 dd (20,9)	2.40 ddd (15,10,9)				
				OAc	2.10 s	2.09 s	2.06 s
17	4.39 br t (9)	3.95 br t (9)	3.92 br t (10)		2.18 s		2.10 s
							2.12 s
21	7.56	7.39	7.36				2.21 s
22	6.53	6.23	6.31				
23	7.53	7.22	7.33	CO_2R	1.10 t(7)		3.64 s
					4.12 q(7)		

[a] In C_5D_5N at 45° C, at 360 MHz. [b] In $CDCl_3$ at 45° C, at 360 MHz.

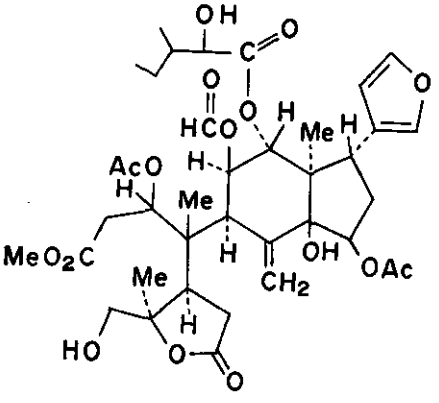
nm(ε 3700); CD(MeOH) $\Delta\varepsilon_{207}$ +7.6. The ^{1}H NMR spectrum of **3** shows all the peaks of **1** except the peak due to the alkoxyl grouping[9], in which there are resonances which can be attributed to two acetates, a formate, an exo-methylene group and 2-hydroxy-3-methylvaleric acid (Table 1). The acetate **1a** from **1** shows two additional acetyl groups, one of these being associated with the appearance of a doublet at δ 4.65(J= 4.5 Hz) which is typical of 2'-H in the acetylated hydroxy acid[6]. The spectra of **1** and **1a** are very similar to those of rohituka-2 (**4**) from *Aphanamixis polystacha* and the acetate **4a**, 29,2'-diacetate[6], but their IR bands due to the lactone carbonyl are different; in **4** it is 1775 cm^{-1}. The ε-lactone structure of the A-ring is deduced for **1** from the presence of the $-CO-CH_2-CHOAc-\overset{|}{C}-$ grouping in the ^{1}H NMR spectrum (see the 1-H, 2α-H, 2β-H and OAc peaks in Table 1) and on biogenetic grounds, in that some five Meliaceae tetranortriterpenoids have been found with the same substitution pattern[3,6,10]. The α orientation of the 1-OAc group is revealed



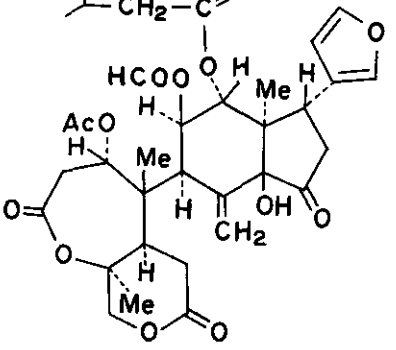
1 R = Et
3 R = Me



2



4



5

by the chemical shifts of the 2α-H at δ 3.75 and 2β-H at δ 3.34, and the coupling of 11 Hz between the 1β- and 2β-H and a small coupling between the 1β- and 2α-H. The characteristic broad 17β-H triplet at δ 4.39, showing the presence of long range coupling with a furan proton[4], is coupled to the 16α- and 16β-H at δ 2.28 and 2.60 with 9 Hz. Since these signals are coupled to the 15-H at δ 5.91 with 5 and 10 Hz, respectively, the configulation of the 15-OAc group is assigned β same with that of rohituka substances[8]. Irradiation of the 13-Me peak at δ 1.05 induced 13 % NOE on the 22-H signal[2b]. There are also 1H doublets at δ 3.68(9-H) and δ 6.69(12-H), which are coupled with 11 and 9 Hz to a doublet of doublets at δ 5.80(11-H), showing a small coupling with the -CHO. This is consistent with the location of the formate at C-11 as in **4** and, by analogy with **4** and other some substances[3,4,6], we place the hydroxy ester at C-12 and an acetate at C-1. While the hydroxymethyl group at δ 3.87 and 3.86(each 1H, s), replaced by an AB quartet(δ 4.49 and 4.03, J= 13.5 Hz) in the spectrum of the acetate **1a**, was located at C-29. We therefore assign Tr-A the structure **1** shown and so Tr-C is 15-dihydro-29-deacetylprieurianin 15β-acetate (**3**).

Tr-B (**2**), $C_{35}H_{44}O_{14}$ (FD-MS: m/z 688, M^+), shows the IR absorption at 3500 and 1725 cm^{-1} and n-π* transition of ketone at 307 nm(Δ_ε -1.6) and n-π* one of lactone at 235 nm(Δ_ε -1.8)[11] in the CD spectrum which suggest the presence of the 5-membered ketone and 6-membered lactone rings as in some seco-limonoids[7]. The ^1H NMR spectrum revealed Tr-B (**2**) different from A (**1**) and C (**3**) in the lack of carboalkoxyl group and the presence of only one acetate, though there was a quartet at δ 4.21(2H, J= 12 Hz) suggesting an acyloxylated C-29. We therefore consider **2** to belong to the 7-29 lactone group, like rohitukin (**5**)[6], rather than the ring opened group, like **1** and **3**. The presence of the 15-keto group is supported by the couplings of the 16α-H at δ 2.33(dd, J= 20 and 9 Hz) and the 16β-H at δ 2.84(dd, J= 20 and 9 Hz), and the observed downfield shift of the 30-methylene protons in the same plane of the carbonyl group; δ 5.92 and 5.50 in **2**, while δ 5.31 and 5.16 in **1** and δ 5.29 and 5.16 in **3** (both in $CDCl_3$). The other substituents including the hydroxy methylvaleric acid are same with those of **1** and **3**. The 2'-H signal shows a doublet of doublets at δ 3.12. We therefore consider that it is the hydroxy methylvalerate ester corresponding to rohitukin (**5**)[6] and has the structure **2** shown.

ACKNOWLEDGEMENTS

The Authors are grateful to Professor K. Nakanishi, Columbia University, for giving

the chance to investigate the present work. Our thanks are also due to Professor I. Kubo, University of California, for his help for the collection of *T. roka* and to Dr. S. F. Dossaji, University of Nairobi, for the identification of the plant.

REFERENCES AND NOTES

1. J. O. Kokwaro, 'Medicinal Plants of East Africa', East African Literature Bureau, Nairobi, Kenya, 1976, p. 157.
2. a. M. Nakatani, J. C. James, and K. Nakanishi, *J. Am. Chem. Soc.*, 1981, 103, 1228. b. M. Nakatani, I. Iwashita, H. Naoki, and T. Hase, *Phytochemistry*, accepted.
3. V. P. Gullo, I. Miura, K. Nakanishi, A. F. Cameron, J. D. Connolly, F. D. Dancanson, A. F. Harding, R. McCrindle, and D. A. H. Taylor, *J. Chem. Soc., Chem. Commun.*, 1975, 345.
4. S. D. Jolad, J. J. Hoffmann, K. H. Schram, J. R. Cole, M. S. Tempesta, and R. B. Bates, *J. Org. Chem.*, 1981, 46, 641.
5. Collected by the author in June 1979 at Shimba Hill near Monbasa, Kenya, and extracted at Nakanishi's laboratory of Columbia University.
6. D. A. Brown and D. A. H. Taylor, *Phytochemistry*, 1978, 17, 1995; L. K. Mclachlan and D. A. H. Taylor, *ibid.*, 1982, 21, 1701.
7. L. K. Mclachlan and D. A. H. Taylor, *ibid.*, 1982, 21, 2426 and references cited therein.
8. T. J. King and D. A. H. Taylor, *ibid.*, 1983, 22, 307.
9. **1**($CDCl_3$, at 45° C, at 360 MHz): δ 0.80(3H, t, J= 7.5Hz), 0.88(3H, d, J= 6.5Hz), 0.97(3H, s), 1.26(3H, t, J= 7.0Hz), 1.52(3H, s), 1.59(3H, s), 2.04(3H, s, OAc), 2.12(1H, m, C_{16}-H), 2.22(3H, s, OAc), 2.37(1H, m, C_{16}-H), 3.22(1H, d, J= 8.0Hz, C_9-H), 3.84(2H, br d, J= 6.5Hz, C_{29}-H), 3.96(1H, dd, J= 11.0 and 9.0Hz, C_{17}-H), 4.15(2H, q, J= 7.0Hz, $OC\underline{H}_2CH_3$), 5.16(1H, s, C_{30}-H), 5.31(1H, s, C_{30}-H), 5.40(1H, dd, J= 10.5 and 8.0Hz, C_{11}-H), 5.66(1H, m, C_1-H), 5.66(1H, dd, J= 9.5 and 5.0 Hz, C_{15}-H), 6.05(1H, d, J= 10.5Hz, C_{12}-H), 6.25(1H, C_{22}-H), 7.17(1H, C_{23}-H), 7.33(1H, C_{21}-H), 8.01(1H, s, C$\underline{H}$O).
3($CDCl_3$, at 45° C, at 360 MHz): δ 0.79(3H, t, J= 7.5Hz), 0.88(3H, d, J= 7.0Hz), 0.96(3H, s), 1.52(3H, s), 1.59(3H, s), 2.04(3H, s, OAc), 2.12(1H, m, C_{16}-H), 2.22(3H, s, OAc), 2.37(1H, m, C_{16}-H), 3.22(1H, d, J= 8.0Hz, C_9-H), 3.68(3H, s, OMe), 3.84(2H, br d, J= 6.5Hz, C_{29}-H), 3.94(1H, dd, J= 11.0 and 8.5Hz, C_{17}-H),

5.16(1H, s, C_{30}-H), 5.29(1H, s, C_{30}-H), 5.40(1H, dd, J= 10.5 and 8.0Hz, C_{11}-H), 5.66(1H, m, C_1-H), 5.66(1H, dd, J= 9.5 and 5.0Hz, C_{15}-H), 6.05(1H, d, J= 10.5, C_{12}-H), 6.25(1H, C_{22}-H), 7.16(1H, C_{23}-H), 7.34(1H, C_{21}-H), 7.99(1H, s, CHO).

10. J. D. Connolly, D. A. Okorie, L. D. De Wit, and D. A. H. Taylor, *J. Chem. Soc., Chem. Commun.*, 1976, 909.

11. J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 1965, 7211.

Received, 8th June, 1984