

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

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**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<sup>1</sup>. We have already described the isolation of limonoids, trichilin A-F and 7-acetyltrichilin A, as insect antifeedant from the root bark<sup>2</sup>. We now describe the isolation and structures of three prieurianin type limonoids from the stem bark. The group of limonoid related to prieurianin<sup>3</sup>, 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<sup>4</sup>. 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 segetum* Denis (200 ppm) with the leaf disk choice test.

The stem bark (1.3 Kg)<sup>5</sup> was defatted 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 tedious process required very careful use of HPLC and pure compounds also showed 5-7 peaks on the C<sub>18</sub> 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<sup>3</sup> and decomposition during chromatography<sup>6</sup>. 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 <sup>1</sup>H NMR decoupling studies and comparison of their spectra with those published for other related limonoids, in particular, prieurianin<sup>3</sup>, rohituka substances<sup>6,7,8</sup> and hispidins<sup>4</sup>. The <sup>1</sup>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.

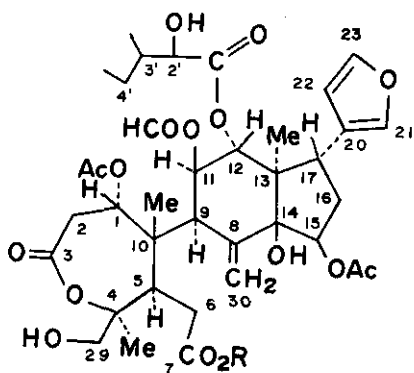
Tr-A (1), C<sub>39</sub>H<sub>54</sub>O<sub>16</sub> (FD-MS: m/z 778, M<sup>+</sup>), and Tr-C (3), C<sub>38</sub>H<sub>52</sub>O<sub>16</sub> (FD-MS: 764, M<sup>+</sup>), exhibit the following spectral data. 1: IR(CHCl<sub>3</sub>) 3500, 1730 cm<sup>-1</sup>; UV(MeOH) 207 nm(ε 3300); CD(MeOH) Δ<sub>ε208</sub> +7.3. 3: IR(CHCl<sub>3</sub>) 3550, 1730 cm<sup>-1</sup>; UV(MeOH) 206

Table 1. <sup>1</sup>H NMR data for Tr-A (1), B (2) and C acetate (3a), in ppm (J value, Hz)

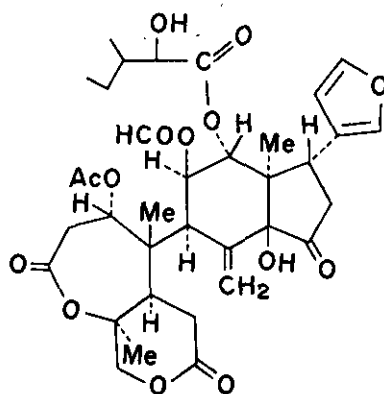
| proton | 1 <sup>a</sup>        | 2 <sup>b</sup>    | 3a <sup>b</sup>       | proton            | 1 <sup>a</sup> | 2 <sup>b</sup>   | 3a <sup>b</sup>  |
|--------|-----------------------|-------------------|-----------------------|-------------------|----------------|------------------|------------------|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

<sup>a</sup> In C<sub>5</sub>D<sub>5</sub>N at 45° C, at 360 MHz. <sup>b</sup> In CDCl<sub>3</sub> at 45° C, at 360 MHz.

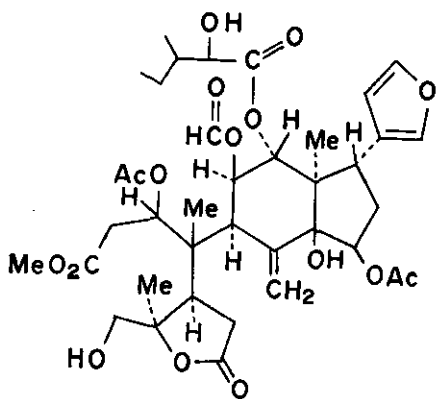
nm( $\epsilon$  3700); CD(MeOH)  $\Delta\epsilon_{207}$  +7.6. The  $^1\text{H}$  NMR spectrum of **3** shows all the peaks of **1** except the peak due to the alkoxy grouping<sup>9</sup>, in which there are resonances which can be attributed to two acetates, a formate, an exo-methylene group and 2-hydroxy-3-methylvaleric 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  $\delta$  4.65 ( $J$  = 4.5 Hz) which is typical of 2'-H in the acetylated hydroxy acid<sup>6</sup>. 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<sup>6</sup>, but their IR bands due to the lactone carbonyl are different; in **4** it is  $1775\text{ cm}^{-1}$ . The  $\epsilon$ -lactone structure of the A-ring is deduced for **1** from the presence of the  $-\text{CO}-\text{CH}_2-\text{CHOAc}-\text{C}-$  grouping in the  $^1\text{H}$  NMR spectrum (see the 1-H,  $2\alpha$ -H,  $2\beta$ -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<sup>3,6,10</sup>. The  $\alpha$  orientation of the 1-OAc group is revealed



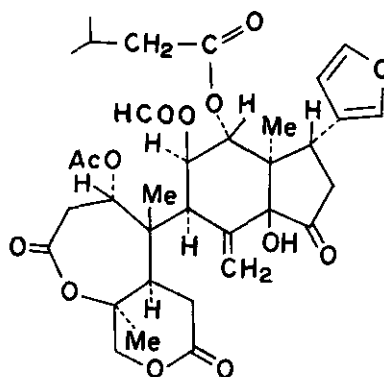
1R = Et  
3R = Me



2



4



5

by the chemical shifts of the 2 $\alpha$ -H at  $\delta$  3.75 and 2 $\beta$ -H at  $\delta$  3.34, and the coupling of 11 Hz between the 1 $\beta$ - and 2 $\beta$ -H and a small coupling between the 1 $\beta$ - and 2 $\alpha$ -H. The characteristic broad 17 $\beta$ -H triplet at  $\delta$  4.39, showing the presence of long range coupling with a furan proton<sup>4</sup>, is coupled to the 16 $\alpha$ - and 16 $\beta$ -H at  $\delta$  2.28 and 2.60 with 9 Hz. Since these signals are coupled to the 15-H at  $\delta$  5.91 with 5 and 10 Hz, respectively, the configuration of the 15-OAc group is assigned  $\beta$  same with that of rohituka substances<sup>8</sup>. Irradiation of the 13-Me peak at  $\delta$  1.05 induced 13 % NOE on the 22-H signal<sup>2b</sup>. There are also 1H doublets at  $\delta$  3.68(9-H) and  $\delta$  6.69(12-H), which are coupled with 11 and 9 Hz to a doublet of doublets at  $\delta$  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<sup>3,4,6</sup>, we place the hydroxy ester at C-12 and an acetate at C-1. While the hydroxymethyl group at  $\delta$  3.87 and 3.86 (each 1H, s), replaced by an AB quartet ( $\delta$  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 15 $\beta$ -acetate (3).

Tr-B (2), C<sub>35</sub>H<sub>44</sub>O<sub>14</sub> (FD-MS: m/z 688, M<sup>+</sup>), shows the IR absorption at 3500 and 1725 cm<sup>-1</sup> and n- $\pi$ \* transition of ketone at 307 nm ( $\Delta\epsilon$  -1.6) and n- $\pi$ \* one of lactone at 235 nm ( $\Delta\epsilon$  -1.8)<sup>11</sup> in the CD spectrum which suggest the presence of the 5-membered ketone and 6-membered lactone rings as in some seco-limonoids<sup>7</sup>. The <sup>1</sup>H 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  $\delta$  4.21(2H, J= 12 Hz) suggesting an acyloxyated C-29. We therefore consider 2 to belong to the 7-29 lactone group, like rohitukin (5)<sup>6</sup>, rather than the ring opened group, like 1 and 3. The presence of the 15-keto group is supported by the couplings of the 16 $\alpha$ -H at  $\delta$  2.33(dd, J= 20 and 9 Hz) and the 16 $\beta$ -H at  $\delta$  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;  $\delta$  5.92 and 5.50 in 2, while  $\delta$  5.31 and 5.16 in 1 and  $\delta$  5.29 and 5.16 in 3 (both in CDCl<sub>3</sub>). 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  $\delta$  3.12. We therefore consider that it is the hydroxy methylvalerate ester corresponding to rohitukin (5)<sup>6</sup> and has the structure 2 shown.

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9.  $^1\text{H}$  (CDCl<sub>3</sub>, at 45° C, at 360 MHz):  $\delta$  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<sub>16</sub>-H), 2.22(3H, s, OAc), 2.37(1H, m, C<sub>16</sub>-H), 3.22(1H, d, J= 8.0Hz, C<sub>9</sub>-H), 3.84(2H, br d, J= 6.5Hz, C<sub>29</sub>-H), 3.96(1H, dd, J= 11.0 and 9.0Hz, C<sub>17</sub>-H), 4.15(2H, q, J= 7.0Hz, OCH<sub>2</sub>CH<sub>3</sub>), 5.16(1H, s, C<sub>30</sub>-H), 5.31(1H, s, C<sub>30</sub>-H), 5.40(1H, dd, J= 10.5 and 8.0Hz, C<sub>11</sub>-H), 5.66(1H, m, C<sub>1</sub>-H), 5.66(1H, dd, J= 9.5 and 5.0 Hz, C<sub>15</sub>-H), 6.05(1H, d, J= 10.5Hz, C<sub>12</sub>-H), 6.25(1H, C<sub>22</sub>-H), 7.17(1H, C<sub>23</sub>-H), 7.33(1H, C<sub>21</sub>-H), 8.01(1H, s, CHO).  
 $^3\text{H}$  (CDCl<sub>3</sub>, at 45° C, at 360 MHz):  $\delta$  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<sub>16</sub>-H), 2.22(3H, s, OAc), 2.37(1H, m, C<sub>16</sub>-H), 3.22(1H, d, J= 8.0Hz, C<sub>9</sub>-H), 3.68(3H, s, OMe), 3.84(2H, br d, J= 6.5Hz, C<sub>29</sub>-H), 3.94(1H, dd, J= 11.0 and 8.5Hz, C<sub>17</sub>-H),

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