

## ISOLATION AND STRUCTURAL ELUCIDATION OF POTENTIALLY INSECTICIDAL AND ACARICIDAL ISOFLAVONE-TYPE COMPOUNDS FROM *NEORAUTANENIA MITIS*

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**ABSTRACT.**—Investigation of the roots of *Neorautanenia mitis*, collected in Rwanda (Central Africa), revealed the presence of three compounds, neotenone [1], dolineone [3], and pachyrrhizine [2], which were previously isolated from *Neorautanenia pseudopachyrrhiza*, the former name of the title plant. However, neotenone [1], which was previously reported as an optically inactive substance, was isolated now in optically active form for the first time. In addition, four isoflavanone-type compounds were isolated for the first time from *N. mitis*, including dehydroneotenone [5], neoduline [4], 12a-hydroxydolineone [6], and 12a-hydroxyrotenone [7]. Detailed 360 MHz nmr spectra of all compounds are reported for the first time, providing correction to previously incorrectly reported low resolution data. Also, the <sup>13</sup>C-nmr and mass spectral data of all these compounds are reported for the first time. The petroleum ether total extract of *N. mitis* roots showed significant acaricidal properties that were attributed to the presence of 12a-hydroxyrotenone [7].

*Neorautanenia* species are a widespread group of Fabaceae (Papilionaceae) plants of Central and South Africa and are known for their poisonous properties (1). These plants are used by the native population as fish poison (2-5) and insecticide (6,7), while in the traditional medicine of Central Africa they are used against syphilis (8) and the treatment of female frigidity.<sup>4</sup> The possible use of some of these plants for killing bilharzia-carrying fresh water snails of the genera *Bulinus* and *Biophalaria* has also been reported (9).

Several chemical studies have been performed already on *Neorautanenia* species, among others *N. ficifolia* (3,14,15), *N. edulis* (2,4,12,16,18), *N. pseudopachyrrhiza* (10,11,13), *N. amboensis* (5,12,17,19), and the related *Pachyrrhizus erosus* (20-22). In the course of our investigation on medicinal plants from Rwanda (Central Africa) we investigated *Neorautanenia mitis* (A. Rich.) Verdcourt (local name "Igitembatenibe" or "Amakubwe") on its insecticidal and acaricidal properties because of some promising preliminary results. This plant was previously named *Dolichos pseudopachyrrhizus*, *Dolichos mitis*, *Neorautanenia orbicularis*, or *Neorautanenia pseudopachyrrhiza* but is now generally accepted as *N. mitis* (9). This plant has been investigated already two decades ago (under the name of *N. pseudopachyrrhiza*), which resulted in the isolation of various isoflavanone-type compounds, including neotenone [1], pachyrrhizine [2], dolineone [3], and nepseudin (10,11). The first three compounds (1, 2, and 3) were also isolated by us from roots of *N. mitis*, collected in Rwanda, but nepseudin was not found. However, we isolated and fully identified four compounds from this plant source, namely neoduline [4], dehydroneotenone [5], 12a-hydroxydolineone [6], and 12a-hydroxyrotenone [7]. Compounds 4-7 are now described for the first time from *N. mitis* but

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<sup>4</sup>According to our personal enquiries directed towards traditional healers in Rwanda (Central Africa).

TABLE 1. <sup>1</sup>H-nmr Spectrometric Data (δ) of Compounds 1-7

Protons	Neoteneone [1] (360 MHz; CDCl <sub>3</sub> )	Pachyrrhizine [2] (360 MHz; CDCl <sub>3</sub> )	Dolineone [3] (360 MHz; CDCl <sub>3</sub> )
1	—	—	6.72 (s) (overlap)
2	4.50 (dxd, J=5.4 Hz, 10.8 Hz)	—	—
	4.58 (dxd, J=10.8 Hz, 11.4 Hz)	—	—
3	4.31 (dxd, J=5.4 Hz, J=11.4 Hz)	—	—
4	—	7.80 (s)	6.44 (s)
5	8.25 (s)	7.69 (s)	—
6	—	—	4.19 (d, broadened, J=12.0 Hz);
	—	—	4.63 (dxd, J=12.0 Hz, J=3.2 Hz)
7	—	—	—
8	7.08 (br s)	7.46 (br s)	7.05 (br s)
10	—	—	—
11	—	—	8.21 (s)
3'	6.57 (s)	6.64 (s)	—
4'	—	—	—
5'	—	—	—
6'	6.62 (s)	6.91 (s)	—
7'	—	—	—
8'	—	—	—
2''	7.57 (d, J=2.3 Hz)	7.70 (d, J=2.2 Hz)	7.54 (d, J=2.3 Hz)
3''	6.76 (dxd, J=2.3 Hz, J=1 Hz)	6.84 (dxd, J=2.2 Hz, 1 Hz)	6.73 (dxd, J=2.3 Hz, J=1 Hz)
2'''	5.90 (s)	5.98 (s)	5.80 and 5.86 (each 1H, each d, J=1.3 Hz)
6a	—	—	4.96 (dxdxd, J=3.2 Hz, J=1.0 Hz, J=3.9 Hz)
11a	—	—	—
12a	—	—	3.89 (d, J=3.9 Hz)
OCH <sub>3</sub>	3.72 (s)	3.79 (s)	—
OH	—	—	—

have all four been described from different plant sources, including neoduline [4] (4,5) and dehydroneoteneone [5] (13) from *N. edulis*, dehydroneoteneone [5] (14,20) and 12a-hydroxydolineone [6] (22) from *P. erosus*, and dehydroneoteneone [5] (12), 12a-hydroxydolineone [6] (19), and 12a-hydroxyrotenone [7] (19) from *N. amboensis*.

All compounds (1-7) were characterized by spectrometric methods (<sup>1</sup>H nmr, <sup>13</sup>C nmr, ir, and ms) and by comparison with the data in the literature. Not all spectral data have been reported hitherto and, therefore, a detailed spectrometric study was undertaken (including 360 MHz <sup>1</sup>H nmr and 20 MHz <sup>13</sup>C nmr). A comparison was made between some reported data from the literature and those found in this communication. The <sup>1</sup>H-nmr spectrometric data of compounds (1-7) are compiled in Table 1, while Table 2 describes the <sup>13</sup>C-nmr data of these compounds. Table 3 entails some physical data, ir spectral data, and mass spectral fragmentations.

Neoteneone [1], i.e., 2'-methoxy-4',5'-methylenedioxy-2,3-dihydrofuro [3'', 2''-6, 7] isoflavone, was isolated as light green-yellow crystals, mp 152° (CHCl<sub>3</sub>/MeOH). Literature reports claimed melting points of neoteneone of 180.5° (11) (MeOH or CHCl<sub>3</sub>/MeOH) and 180° (Me<sub>2</sub>CO) (12). The compound with mp 180.5° corresponded to the racemate because of the absence of optical rotation [ $\alpha$ ]<sub>D</sub><sup>20</sup> 0° (11). However, it was reported (12) that a second crystalline modification, mp 149° (MeOH), reverted to mp 180° on recrystallization from C<sub>6</sub>H<sub>6</sub>. It is assumed that 1, isolated by us from *N.*

TABLE I. Continued

Protons	Neoduline [4] (360 MHz; CDCl <sub>3</sub> )	Dehydroneoteneone [5] (60 MHz; CDCl <sub>3</sub> -DMSO, 1:4)	12a-Hydroxydoloneone [6] (360 MHz; CDCl <sub>3</sub> )	12a-Hydroxyrotenone [7] (60 MHz; CDCl <sub>3</sub> )
1	7.72 (s)	—	6.52 (s) <sup>a</sup>	6.53 (s)
2	—	7.98 (s)	—	—
3	—	—	—	—
4	7.09 (br s)	—	6.48 (s) <sup>a</sup>	6.44 (s)
5	—	8.28 (s)	—	—
6	4.28 (dxd, <i>J</i> =4.8 Hz, <i>J</i> =10.8 Hz, H <sub>eq</sub> ); 3.71 (dxd, <i>J</i> =10.8 Hz, <i>J</i> =10.4 Hz, H <sub>ax</sub> )	—	4.50 (dxd, <i>J</i> =12.9 Hz, <i>J</i> =2.0 Hz) 4.63 (dxd, <i>J</i> =2.4 Hz, <i>J</i> =12.9 Hz)	4.5 (m)
7	6.75 (s)	—	—	—
8	—	7.63 (br s)	7.02 (s, br)	—
10	6.44 (s)	—	—	6.80 (d, <i>J</i> =8.5 Hz)
11	—	—	8.19 (s)	7.7 (d, <i>J</i> =8.5 Hz)
3'	—	6.64 (s)	—	—
4'	—	—	—	~3 (m)
5'	—	—	—	5.2 (m)
6'	—	6.70 (s)	—	—
7'	—	—	—	4.8-5.1 (m)
8'	—	—	—	1.73 (s, br)
2"	7.56 (d, <i>J</i> =2.2 Hz)	7.88 (d, <i>J</i> =2 Hz)	7.55 (d, <i>J</i> =2.2 Hz)	—
3"	6.72 (dxd, <i>J</i> =2.2 Hz, <i>J</i> =1 Hz)	6.93 (dxd, <i>J</i> =2 Hz, <i>J</i> =1 Hz)	6.74 (dxd, <i>J</i> =2.2 Hz, <i>J</i> =1 Hz)	—
2"	5.90 and 5.93 (each 1 H, each d, <i>J</i> =1.3 Hz)	5.86 (s)	5.80 and 5.85 (each 1 H, each d, <i>J</i> =1.2 Hz)	—
6a	3.59 (dxdxd, <i>J</i> =7 Hz, <i>J</i> =4.8 Hz, <i>J</i> =10.4 Hz)	—	4.62 (1H, br)	4.5 (m)
11a	5.67 (d, <i>J</i> =7.0 Hz)	—	—	—
12a	—	—	—	—
OCH <sub>3</sub>	—	3.78 (s)	—	3.70 and 3.78 (s)
OH	—	—	4.46	4.5 (br)

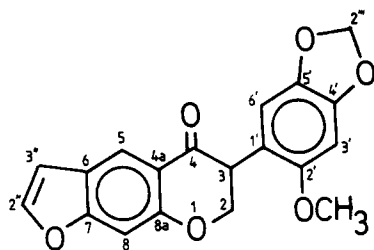
<sup>a</sup>Attribution may be inverted.

*mitis*, corresponded to the latter "crystalline modification," although we believe that we did not isolate the racemate ( $\pm$ )-neoteneone, but instead an optically active substance due to its optical rotation,  $[\alpha]^{20}_D$  (*c* 18.1 mg/10 ml)  $-5.5^\circ$  (589 nm),  $-8.8^\circ$  (578 nm),  $-14.9^\circ$  (546 nm),  $-134.3^\circ$  (436 nm). The <sup>1</sup>H-nmr spectral data agreed with those found in the literature (11), but some more accurate (and corrected) <sup>1</sup>H-nmr data (360 MHz) are reported now together with the 20 MHz <sup>13</sup>C-nmr data and the mass spectral data, both the latter being reported for the first time. The structure of **1** was unambiguously proved by X-ray crystallographic analysis (to be published separately).

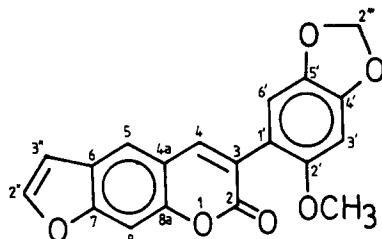
Pachyrrhizine [**2**], previously named neorautone (4), revealed identical <sup>1</sup>H-nmr data as those reported previously (13). The compound was easily identified by its ir spectrum, which pointed to a lactone structure ( $\nu_{C=O}$  1728 cm<sup>-1</sup>) instead of the usual isoflavanone type structure. The <sup>13</sup>C-nmr and mass spectral data are reported here for the first time.

Doloneone [**3**], previously named dolichone (10), was identical with the reported compound (11). However, a much more detailed 360 MHz <sup>1</sup>H-nmr spectrum is presented here while also complete <sup>13</sup>C-nmr data and mass spectral fragmentations are reported.

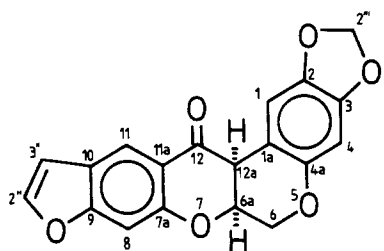
Neoduline [**4**], previously named edulin (4), was now isolated for the first time



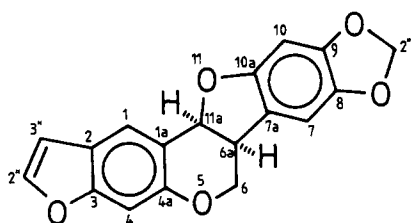
1 neoteneone



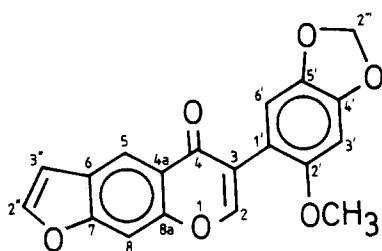
2 pachyrrhizine



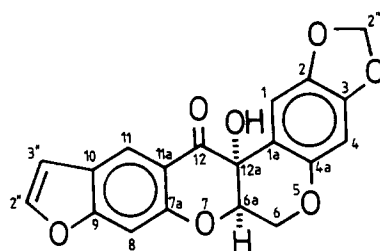
3 dolineone



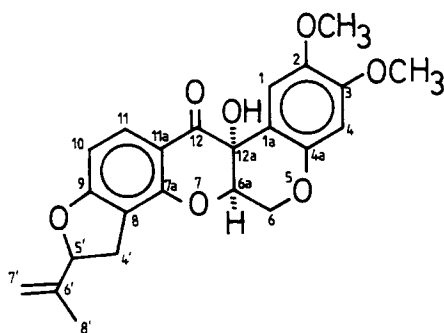
4 neoduline



5 dehydroneoteneone



6 12a-hydroxydolineone



7 12a-hydroxyrotenone

from *N. mitis* and agreed with the reported  $^1\text{H}$ -nmr data (4, 14). Again we present for the first time high resolution  $^1\text{H}$ -nmr data (360 MHz). The  $^{13}\text{C}$ -nmr data are nearly identical (shift difference of about 1 ppm) to those reported (23).

Dehydroneoteneone [5] was easily identified due to its analogy with neoteneone [1]. Its isolation from *N. mitis* is reported for the first time in this article, while full spectral data, including  $^1\text{H}$ -nmr, ir, and ms data, are reported for the first time. The mp  $239\text{--}240^\circ$  agreed with the value reported in the literature [mp  $240\text{--}241^\circ$  (12)].

12a-Hydroxydolineone [6] is reported now for the first time from *N. mitis*, while complete and detailed  $^1\text{H}$ -nmr (360 MHz) and  $^{13}\text{C}$ -nmr data are given. Our more detailed  $^1\text{H}$ -nmr values corresponded with those reported earlier (19, 22).

TABLE 2.  $^{13}\text{C}$ -nmr Spectrometric Data ( $\delta$ ; 20 MHz) of Compounds 1-7

Carbons	Neotenone [1] (CDCl <sub>3</sub> )	Pachyrrhizine [2] (CDCl <sub>3</sub> -DMSO, 1:5)	Dolineone [3] (CDCl <sub>3</sub> )	Neoduline [4] (CDCl <sub>3</sub> )	12a-Hydroxy-dolineone [6] (CDCl <sub>3</sub> )	12a-Hydroxy-rotenone [7] (CDCl <sub>3</sub> )
1	—	—	106.9 (d)	122.9 (d)	106.8 (d) <sup>a</sup>	108.8 (d)
2	71.3 (t)	173.2 (s)	143.2 (s)	122.4 (s)	142.3 (s)	142.9 (s)
3	48.3 (d)	124.0 (s) <sup>a</sup>	147.9 (s) <sup>a</sup>	155.7 (s)	149.5 (s) <sup>b</sup>	151.2 (s)
4	192.6 (s)	142.4 (d)	98.9 (d) <sup>b</sup>	99.9 (d)	99.9 (d) <sup>c</sup>	101.1 (d)
5	120.8 (s)	119.6 (d)	—	—	—	—
6	115.6 (s)	124.8 (s) <sup>a</sup>	66.4 (t)	67.0 (t)	63.9 (t)	63.9 (t)
7	159.2 (s) <sup>a</sup>	156.2 (s)	—	104.7 (d)	—	—
8	99.6 (d)	99.5 (d)	99.8 (d) <sup>b</sup>	141.8 (s)	100.0 (d) <sup>f</sup>	113.2 (s)
9	—	—	158.6 (s) <sup>c</sup>	148.2 (s)	158.3 (s)	168.0 (s)
10	—	—	123.1 (s)	93.8 (d)	123.3 (s)	105.3 (d)
11	—	—	121.0 (d)	—	121.0 (d)	130.2 (d)
12	—	—	190.6 (s)	—	192.9 (s)	191.1 (s)
1'	122.6 (s)	—	—	—	—	—
2'	152.8 (s)	151.7	—	—	—	—
3'	95.4 (d)	95.5 (d)	—	—	—	—
4'	147.8 (s)	148.8 (s)	—	—	—	31.1 (t)
5'	141.4 (s)	141.3 (s)	—	—	—	88.0 (d)
6'	109.8 (d)	110.3 (d)	—	—	—	142.9 (s)
7'	—	—	—	—	—	112.6 (t)
8'	—	—	—	—	—	17.1 (q)
2 <sup>u</sup>	146.0 (d)	146.7 (d)	146.2 (d)	145.1 (d)	146.4 (d)	—
3 <sup>u</sup>	107.0 (d)	106.4 (d)	106.9 (d)	106.3 (d)	105.7 (d) <sup>a</sup>	—
2 <sup>w</sup>	101.3 (t)	101.5 (t)	101.2 (t)	101.3 (t)	101.3 (t)	—
1a	—	—	105.3 (s)	116.5 (s)	109.2 (s)	109.5 (s)
4a	118.8 (s)	116.2 (s)	148.5 (s) <sup>a</sup>	153.5 (s)	149.6 (s) <sup>b</sup>	148.4 (s)
6a	—	—	72.1 (d)	40.6 (d)	75.9 (d)	76.1 (d)
7a	—	—	159.8 (s) <sup>c</sup>	117.9 (s)	160.3 (s)	157.7 (s)
8a	159.9 (s) <sup>a</sup>	156.2	—	—	—	—
10a	—	—	—	154.2 (s)	—	—
11a	—	—	116.1 (s)	79.2 (d)	114.6 (s)	111.8 (s)
12a	—	—	45.3 (d)	—	68.3 (s)	67.6 (s)
OCH <sub>3</sub>	56.5 (q)	56.9 (q)	—	—	—	55.9 and 56.4 (q)

<sup>a,b,c</sup>Attribution may be inverted.

12a-Hydroxyrotenone [7] is also isolated for the first time from *N. mitis*. The 360 MHz  $^1\text{H}$ -nmr data are more complete and more detailed than those reported (19). In addition, the  $^{13}\text{C}$ -nmr spectral data and the mass spectral fragmentations are reported.

The configuration of compounds 6 and 7 at the B/C fusion is *cis* (thermodynamically most stable form), which is easily established by the  $\delta$  value of the shielded proton at the 1-position ( $\delta$  6.5 ppm) (13,29).

The petroleum ether extract of the roots of *N. mitis* inhibits completely the oviposition of the female tick *Rhipicephalus appendiculatus* at a concentration of 2.5 mg/ml (25). The only product responsible for this activity was shown to be 12a-hydroxyrotenone (7), which has a MIC (minimum inhibitory concentration) of 50  $\mu\text{g}/\text{ml}$  in the test described earlier (25). The reference used in this text was ethyl quinolin-8-yl phenylphosphonothionate (Backdip; Quinthiophos; Bayer 9037) (an organo-phosphorus compound used as an acaricide in dipping-tanks in Rwanda), which has a MIC value of 0.2  $\mu\text{g}/\text{ml}$ .

A MeOH extract of the roots of *N. mitis* showed also an insecticidal activity against the defoliant caterpillar, *Acraea accrata*, which attacked the leaves of the sweet potato (*Ipomoea batatas*) (results to be published). These results agree with the known insecticidal properties of rotenoids (26) and neobanone (27), the latter being isolated from *N. amboensis*.

TABLE 3. Physical, ir, and ms Data of Compounds 1-7

	mp	lit. mp	ir (KBr) $\text{cm}^{-1}$	ms (70 eV) $m/z$ (%)
Neoteneone [1] . . . . .	152 <sup>a</sup>	149° (12)	1680 (C=O)	338 ( $\text{M}^+$ ; 27); 178(100); 165(9); 163(15); 149(5); 133 (33%); 105(9); 77(14); 57(6); 55(5); 53(8); 51(6)
Pachyrrhizine [2] . . . . .	204-205°	180.5° (11) 206.5-207.5° (18) 207-209° (11)	1620 (C=C) 1728 (C=O)	336 ( $\text{M}^+$ ; 100); 321(10); 293(35); 265(16); 199(19); 168(13); 154(16)
Dolineone [3] . . . . .	235°	233-235° (11)	1679 (C=O) 1622 (C=C)	336 ( $\text{M}^+$ ; 27); 176(100); 175(60); 105(16)
Neoduline [4] . . . . .	225°	225° (4)	1610-25 (C=C)	308 ( $\text{M}^+$ ; 100); 291(11); 162(35); 158(23)
Dehydroteneone [5] . . . . .	239-240°	240-241° (11)	1624-45 (C=O and C=C)	336 ( $\text{M}^+$ ; 100); 305(77); 290(14); 263(14); 176(14); 175(28); 161(35); 153(14); 152(14)
12a-Hydroxydolineone [6] . . . . .	189°	194-195° (19) 180-181° (22)	1682 (C=O) 1625 (C=C)	352 ( $\text{M}^+$ ; 24); 334(13); 192(100); 191(70); 161(30); 149(36); 106(28); 97(40); 95(25); 85(46); 83(34); 81(37); 78(20); 73(23); 71(35); 69(52); 57(70); 55(44); 43(50)
12a-Hydroxyroteneone [7] . . . . .	87-90°	oil (19) 88° (24)	3443 (OH) 1670 (C=O) 3420 (OH)	410 ( $\text{M}^+$ ; 14); 395(4); 208(100); 207(36)

<sup>a</sup>See comments in the text regarding the melting point.

## EXPERIMENTAL

<sup>1</sup>H-nmr spectra were recorded with Varian T-60 (60 MHz) and Bruker WH-360 (360 MHz) nmr spectrometers. Ir spectra were measured with a Perkin-Elmer model 1310 spectrometer while mass spectra were obtained from a Varian-MAT 112 mass spectrometer (70 eV). Optical rotations were measured in MeOH at ambient temperature using a Perkin-Elmer 141 polarimeter. Melting points were determined on a Kofler hotstage.

**PLANT MATERIAL.**—Roots of *N. mitis* were collected in the prefecture of Kibungo (South-East of Rwanda) in April 1981, for extraction procedure A and in our Botanical Garden (prefecture of Butare, South-West of Rwanda) in July 1983, for extraction procedure B. The plant was identified by our botanist F.X. Ayobangira, and voucher herbarium specimens are deposited in our Department of Botany and in the Herbaria of the INRS (Institut National de Recherche Scientifique) at Butare. The roots were air-dried and powdered mechanically.

**EXTRACTION AND ISOLATION.**—*Procedure A.*—Powdered roots (1.5 kg) were extracted in a percolator with petroleum ether (40–60°) (9 liters). After evaporation in vacuo, a brown syrup (24.2 g, yield 1.6%) was obtained. An amount of 12 g of the petroleum ether extract was chromatographed on a Si gel column (600 g) (Merck, 230–400 mesh) in petroleum ether and eluted with a petroleum ether/C<sub>6</sub>H<sub>6</sub>/CHCl<sub>3</sub>/MeOH gradient.

Neoduline [4] was isolated from the petroleum ether–C<sub>6</sub>H<sub>6</sub> (2:8) fraction (186 mg, yield 0.025%) and was recrystallized from CHCl<sub>3</sub>/MeOH to afford small, white crystals, mp Table 3; λ max CHCl<sub>3</sub> 305 nm.

$$[\alpha]_{20D} = \frac{580 \quad 578 \quad 546 \quad 436}{-42.9 \quad -50.7 \quad -57.1 \quad -94.4} \quad (\text{MeOH}; c = 14 \text{ mg}/10 \text{ ml}).$$

Dolineone [3] was isolated from the C<sub>6</sub>H<sub>6</sub>–CHCl<sub>3</sub> (9:1) fraction (122 mg, yield 0.016%) and recrystallized from CHCl<sub>3</sub>/MeOH to afford light brown crystals, mp Table 3.

$$[\alpha]_{20D} = \frac{589 \quad 578 \quad 546 \quad 436}{+36.9 \quad +39.3 \quad +46.7 \quad +105.7} \quad (\text{MeOH}; c = 12.2 \text{ mg}/10 \text{ ml}).$$

Neotenenone [1] was isolated from the C<sub>6</sub>H<sub>6</sub>–CHCl<sub>3</sub> (7:3) fraction (769 mg, yield 0.1%) and recrystallized from CHCl<sub>3</sub>/MeOH to afford light yellow-green crystals, mp Table 3.

$$[\alpha]_{20D} = \frac{589 \quad 578 \quad 546 \quad 436}{-5.5 \quad -8.8 \quad -14.9 \quad -134.3} \quad (\text{MeOH}; c = 18.1 \text{ mg}/10 \text{ ml}).$$

12a-Hydroxydolineone [6] was isolated from the CHCl<sub>3</sub>–MeOH (99:1) fraction after preparative tlc (Merck, Si gel 60, 2 mm) using CHCl<sub>3</sub>–MeOH (96:4) as the eluent (54 mg, yield 0.007%) and recrystallized from C<sub>6</sub>H<sub>6</sub>/*n*-hexane to afford white crystals, mp Table 3.

12a-Hydroxyrotenone [7] was isolated from the CHCl<sub>3</sub>–MeOH (99:1) fraction after preparative tlc (Merck, Si gel 60, 2 mm) using CHCl<sub>3</sub>–MeOH (96:4) as the eluent (72 mg, yield 0.009%) and recrystallized from *n*-hexane to afford small colorless crystals, mp Table 3.

**Procedure B.**—Powdered roots (5.0 kg) were extracted in a percolator with petroleum ether (40–60°) (25 liters). After concentration in vacuo, the petroleum ether phase was extracted with MeOH–H<sub>2</sub>O (9:1). The petroleum ether phase was then evaporated in vacuo to give a brown syrup (18.1 g, yield 0.36%). Afterwards, the MeOH/H<sub>2</sub>O phase was concentrated in vacuo in order to evaporate the MeOH. The H<sub>2</sub>O phase was extracted with CHCl<sub>3</sub> which gave, after evaporation in vacuo, a brown residue (24.8 g, yield 0.49%). 20 g of the CHCl<sub>3</sub> extract was absorbed on Si gel (75 g) and slurried onto the top of a column containing 525 g Si gel (Merck, 230–400 mesh) in petroleum ether and eluted with a petroleum ether/toluene/CHCl<sub>3</sub>/Me<sub>2</sub>CO/MeOH gradient.

Neoduline [4] was isolated from the toluene–CHCl<sub>3</sub> (9:1) fraction (567 mg, yield 0.014%).

Dolineone [3] was isolated from the CHCl<sub>3</sub>–Me<sub>2</sub>CO (99:1; 99:3) fraction (328 mg, yield 0.008%). Afterwards, three complex fractions were isolated: fraction I eluted with CHCl<sub>3</sub>–Me<sub>2</sub>CO (97:3) (3990 mg), fraction II eluted with CHCl<sub>3</sub>–Me<sub>2</sub>CO (97:3) (3990 mg), fraction II eluted with CHCl<sub>3</sub>–Me<sub>2</sub>CO (95:5) (4979 mg), and fraction III also eluted with CHCl<sub>3</sub>–Me<sub>2</sub>CO (95:5) (5900 mg). Fraction I was absorbed on 20 g of Si gel and chromatographed on a Si gel column (170 g) (Merck, 230–400 mesh) in *n*-hexane and eluted with a *n*-hexane/toluene/CHCl<sub>3</sub>/EtOAc/MeOH gradient.

Neotenenone [1] was isolated from the toluene–CHCl<sub>3</sub> (7:3; 6:4; 5:5; 4:6) fraction (1940 mg, yield 0.048%).

Dolinenone [3] was isolated from the toluene–CHCl<sub>3</sub> (9:1, 9:2) fraction (98 mg, yield 0.002%).

Fraction II was absorbed on 20 g of Si gel and chromatographed on a Si gel column (190 g) (Merck, 230–400 mesh) in *n*-hexane and eluted with a *n*-hexane/toluene/CHCl<sub>3</sub>/EtOAc/MeOH gradient.

Pachyrrhizine [2] was isolated from the toluene–CHCl<sub>3</sub> (2:8) fraction (709 mg, yield 0.017%).

Fraction III was absorbed on 20 g of Si gel and chromatographed on a Si gel column (190 g) (Merck, 230–400 mesh) in *n*-hexane and eluted with a *n*-hexane/toluene/CHCl<sub>3</sub>/EtOAc/MeOH gradient.

Dehydronootenone [5] was isolated from the toluene- $\text{CHCl}_3$  (2:8) fraction (1198 mg, yield 0.029%).

**BIOLOGICAL ACTIVITY.**—The acaricidal activity of the extracts and purified products was determined following the method of complete immersion of female ticks *Rhipicephalus appendiculatus* during 10 min in test tubes containing the plant extracts and measurement of the oviposition after 10 and 20 days as described earlier (25).

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