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Two unusual rotenoid derivatives, 7a-*O*-methyl-12a-hydroxydeguelol and spiro-13-homo-13-oxaelliptone, from the seeds of *Derris trifoliata*

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Dedicated to Professor Ermias Dagne on the occasion of his 60th Birthday.

Abstract

The crude methanol extract of the seeds of *Derris trifoliata* showed potent and dose dependent larvicidal activity against the 2nd instar larvae of *Aedes aegypti*. From this extract two unusual rotenoid derivatives, a rotenoloid (named 7a-*O*-methyl-12a-hydroxydeguelol) and a spirohomooxarotenoid (named spiro-13-homo-13-oxaelliptone), were isolated and characterised. In addition a rare natural chromanone (6,7-dimethoxy-4-chromanone) and the known rotenoids rotenone, tephrosin and dehydrodeguelin were identified. The structures were assigned on the basis of spectroscopic evidence. The larvicidal activity of the crude extract is mainly due to rotenone.

Keywords: *Derris trifoliata*; Leguminosae; Seeds; Rotenoloid; Spirohomooxarotenoid; Rotenoids; 7a-*O*-methyl-12a-hydroxydeguelol; Spiro-13-homo-13-oxaelliptone; 6,7-Dimethoxy-4-chromanone; Mosquito; Larvicides; *Aedes aegypti*

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1. Introduction

In the search for compounds with larvicidal and pesticidal activities from plants, the larvicidal activities of rotenoids isolated from the seeds of *Millettia dura* Dunn have been reported (Yenesew et al., 2003). Rotenoids are also known to occur in the genera *Derris*, *Lonchocarpus*, and *Tephrosia* of the family Leguminosae (Dewick, 1994).

In Kenya, the genus *Derris* is represented by *Derris trifoliata* Lour. From the stem of this plant, the presence of rotenoids with cancer chemopreventive properties has been reported (Ito et al., 2004). In a recent phytochemical investigation (Yenesew et al., 2005) of the roots of this plant, we have reported a modified rotenoid with an open ring-C (trivial name 7a-*O*-methyldeguelol), representing a new sub-class of isoflavonoids (the sub-class named rotenoloid). We have analyzed the seeds of this plant and report here the isolation and identification of a further two modified rotenoids (**1** and **2**) along with four known compounds.

2. Results and discussion

HR-MS of compound **1** established a molecular formula of $C_{24}H_{26}O_7$. Comparison of the ^1H and ^{13}C NMR

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Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** and **2** in CD_2Cl_2 (*J* in Hz)

	1			**2**		
	^{1}H	^{13}C	HMBC (^{2}J, ^{3}J)	^{1}H	^{13}C	HMBC (^{2}J, ^{3}J)
1	6.44 *s*	110.7	C-2, -3, -4a, -12a	6.64 *s*	105.5	C-2, -3, -4a, -1a
1a		114.2			138.1	
2		144.6			146.7	
3		151.3			145.2	
4	6.47 *s*	101.6	C-1a, -2, -3, -4a	6.48 *s*	107.8	C-1a, -2, -3, -4a
4a		149.9			143.9	
6	4.26 *ddd* (3.2, 4.3, 10.8)	63.4		4.59 *ddd* (3.5, 5.5, 12.5)	65.6	C-4a, -12a
	4.14 *ddd* (2.2, 10.8, 12.1)			4.22 *ddd* (2.0, 10.0, 12.5)		C-4a, -12a
6a	2.42 *ddd* (4.4, 12.1, 14.2)	35.6	C-6	2.65 *ddd* (3.5, 10.0, 15.5)	35.4	C-12, -12a
	1.94 *ddd* (2.2, 3.2, 14.2)		C-12a	2.23 *ddd* (2.0, 5.5, 15.5)		C-12a
7a		156.4			166.3	
8		115.9			114.3	
9		157.6			162.5	
10	6.32 *dd* (0.7, 8.7)	112.1	C-8, -11a	7.33 *dd* (1.0, 8.5)	108.2	C-8, -11a
11	6.58 *d* (8.7)	130.5	C-7, -9, -10, -12	7.64 *d* (8.5)	120.9	C-7, -9, -12
11a		122.4			113.3	
12		203.1			194.4	
12a		74.8			105.1	
2′		77.2		7.72 *d* (2.5)	146.4	C-8, -9
3′	5.70 *d* (10.1)	131.3	C-2′, -8	6.95 *dd* (1.0, 2.5)	104.0	C-2′, -8, -9
4′	6.57 *dd* (0.7, 10.1)	116.5	C-2′			
2′-Me$_2$	1.42 *s*	28.2	C-2′, -3′			
	1.39 *s*	27.9	C-2′, -3′			
OMe-2	3.67 *s*	56.7	C-2	3.68 *s*	56.4	C-2
OMe-3	3.82 *s*	56.1	C-3	3.81 *s*	56.5	C-3
OMe-7a	3.74	63.3	C-7a			
OH-12a	5.10 *br s*					

(Table 1) spectra of **1** with those of 7a-*O*-methyldeguelol (**3**) indicated that it is a rotenoloid derivative (Yenesew et al., 2005). In fact, rings A, D and E were identical to that of **3**. In ring B, however, the methylene protons at C-6 and C-6a appeared as four sets of mutually coupled doublet of doublets (Table 1) which requires that C-12a is substituted by a hydroxyl group (δ 5.10 *br s* for OH-12a in ^{1}H and 74.8 for C-12a in ^{13}C NMR spectra). In the MS, the molecular ion peak at m/z 426 ($C_{24}H_{26}O_7$) and the fragment ions at m/z 217 ($C_{13}H_{13}O_3$) and 209 ($C_{11}H_{13}O_4$) are consistent with **1** being a 12a-hydroxy derivative of **3**. Hence this new compound was identified as 7a-*O*-methyl-12a-hydroxydeguelol (**1**). Compound **1** is levorotatory ($[\alpha]_D = -9.0°$) as 7a-*O*-methyldeguelol (**3**) suggesting the same configuration at C-12a. However, the absolute configuration at C-12a in these compounds remains to be determined. Compound **1** is an addition to the new sub-class of isoflavonoids named rotenoloids (Yenesew et al., 2005).

The ^{1}H and ^{13}C NMR (Table 1) spectra of compound **2** (molecular formula $C_{20}H_{16}O_7$) showed some features similar to those of 12a-hydroxyelliptone (**4**) (Ito et al., 2004). As in **4**, ring A contains two methoxyl groups at C-2 (δ_H 3.68 and δ_C 56.4) and C-3 (δ_H 3.81 and δ_C 56.5) with the *para*-oriented protons H-1 and H-4 appearing at δ 6.64 (*s*) and δ 6.48 (*s*), respectively. The placements of the two methoxyl groups at C-2 and C-3 was confirmed through HMBC correlation (Table 1) of the methoxyl protons, δ 3.68 and 3.81, with C-2 (δ 146.7) and C-3 (δ 145.2), respectively.

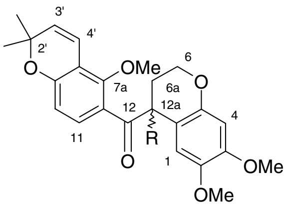
The ^{13}C NMR spectrum, however, displayed this ring to be tetra-oxygenated (instead of tri-oxygenation as in commonly found rotenoids such as **4**) viz. C-1a (δ 138.1), C-2 (146.7), C-3 (145.2) and C-4a (143.9). Such oxygenation pattern has been observed in the modified rotenoids 13-homo-13-oxa-6a,12a-dehydrorotenone (**5**) and 13-homo-13-oxa-6a,12a-dehydrodeguelin (**6**) (Fang and Casida, 1997; Wangenesteen et al., 2005). As in these compounds, ring B in **2** is also seven-membered with a second oxygen atom (O-13) bridging C-1a and C-12a.

In this ring, the ^{1}H NMR signals for the two protons at C-6 appeared as two sets of doublet of a double doublet (*ddd*) at δ 4.59 ($J = 3.5$, 5.5, 12.5 Hz) and 4.22 ($J = 2.0$, 10.0, 12.5 Hz) which suggested that the adjacent carbon atom (C-6a) also bears two protons. Indeed, a further two sets of doublet of a double doublet (*ddd*) at δ 2.65 ($J = 3.5$, 10.0, 15.5 Hz) and 2.33 ($J = 2.0$, 5.5, 15.5 Hz) could be assigned to the two protons at C-6a. The ^{1}H,^{1}H-COSY spectrum showed connectivities amongst these four protons (CH$_2$-6 and CH$_2$-6a). The ^{13}C NMR spectrum (Table 1) further showed signals for C-6 and C-6a of this ring at δ 65.6 and δ 35.4, respectively, which showed correlations with the corresponding protons (CH$_2$-6 and CH$_2$-6a, respectively) in the HMQC spectrum. Such pattern is only possible if the bond between C-6a and *O*-7 (in ring C) is broken as in the rotenoloids **1** and **3**. However, unlike in these rotenoloids where *O*-7 is methylated, this oxygen atom in **2** is attached to C-12a giving a spiro-acetal structure at the B/C ring junction; the acetal carbon (C-12a) appearing at δ 105.1 in the ^{13}C NMR spectrum. In agreement with this, the HMBC spectrum (Table 1) showed correlation of CH$_2$-6 and CH$_2$-6a with this acetal carbon (C-12a).

In ring D, H-10 and H-11 appeared at δ 7.33 (*dd*, $J = 8.5$, 1.0 Hz) and 7.64 (*d*, $J = 8.5$ Hz), respectively; with the former showing a long range (^5J) coupling with the benzofuranoic proton H-3′ (δ 6.95, *dd*, $J = 1.0$, 2.5 Hz), which in turn couples with the other benzofuranoic proton H-2′ (δ 7.72, *d*, $J = 2.5$ Hz) of ring E (Ito et al., 2004). Hence this compound was assigned the spirohomooxarotenoid structure **2**, for which the trivial name spiro-13-homo-13-oxaelliptone is assigned. Compound **2** is optically active ($[\alpha]_D = -92.9°$) and the CD spectrum (Section 3.5) showed a negative Cotton effect at 238 and 330 nm and a positive one at 275 nm. However, the absolute configuration at C-12a remains to be determined.

Compound **7** was identified as 6,7-dimethoxy-4-chromanone. This is only the second report on the natural occurrence of this compound, having recently been reported from *Sarcolobus globosus* (Asclepiadaceae) (Wangenesteen et al., 2005). It appears that **7** is formed in this plant through oxidative cleavage of the C12–C12a bond in the 12a-hydroxyrotenoloid, **1**. The known compounds rotenone, tephrosin and dehydrodeguelin were identified by comparison of their spectroscopic data with those reported in literature (Yenesew et al., 2005; Dagne et al., 1989; Ollis et al., 1967).

The MeOH extract of the seeds of this plant showed potent larvicidal activity against the 2nd instar larvae of *Aedes aegypti* with LD$_{50}$ of 0.74 ± 0.3 μg/ml. Rotenone, LD$_{50}$ of 0.47 ± 0.2 μg/ml, is the major compound in this extract and the larvicidal activity of the crude extract is mainly due to this compound. Compound **7** and tephrosin were also active with LD$_{50}$ of 14.8 ± 2.5 μg/ml and 1.6 ± 0.3 μg/ml, respectively. However, compounds **1** and **2** were isolated in small amounts and have not been tested for larvicidal activities.



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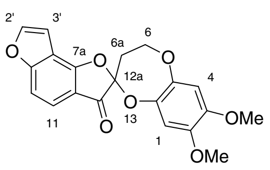

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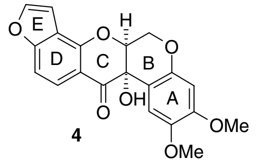

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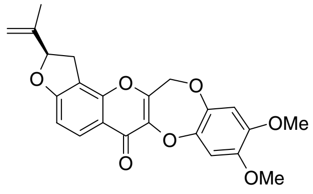

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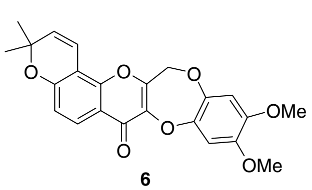

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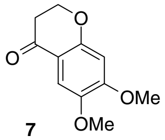

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3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F$_{254}$ plates. CC on silica gel (70–230 mesh). EIMS: direct inlet, 70 eV, on SSQ 710, Finnigan MAT mass spectrometer. ESI spectra were recorded using a Q-TOF$_{micro}$ mass spectrometer (Micromass, Manchester, UK) in positive electron spray mode. Elemental compositions were determined by accurate mass measurement with standard deviation <5 ppm. ^{1}H and ^{13}C NMR on Bruker spectrometers using TMS as internal standard. HMQC and HMBC were acquired using the standard Bruker software.

3.2. Plant material

The seeds of *D. trifoliata* Lour were collected from Coast Province, Kenya, in August 2003. The plant was identified at the University Herbarium, Botany Department, University of Nairobi, where a voucher specimen is deposited.

3.3. Extraction and isolation

The dried and ground seeds (200 g) of *D. trifoliata* were extracted with methanol by cold percolation (3 × 500 ml).

Upon concentration of the extract, rotenone (1.3 g) precipitated. Complete removal of the solvent from the mother liquor resulted in brown oily extract (19 g), which was partitioned between water and ethyl acetate. The ethyl acetate layer (15 g) was subjected to CC on silica gel (150 g) eluting with hexane containing increasing amounts (5%, 10%, 15%, 20%, 25%, and 30%) of CH_2Cl_2. Six major fractions each ca. 500 ml were collected. The fraction which was eluted with 15% CH_2Cl_2 in hexane was purified by preparative TLC (silica gel, hexane/CH_2Cl_2/EtOAc, 6:3:1) which yielded dehydrodeguelin (5 mg). Similar treatment of the fraction eluted with 20% CH_2Cl_2 in hexane yielded **1** (5 mg), **2** (20 mg) and tephrosin (20 mg). The fraction which was eluted with 30% CH_2Cl_2 in hexane yielded **7** (29 mg).

*3.4. 7a-O-methyl-12a-hydroxydeguelol (**1**)*

Colourless amorphous solid. $[\alpha]_D^{25} = -9.5°$ (c 0.2, $CHCl_3$). UV λ_{max} (MeOH, log ε) nm: 263 (4.2), 296 (3.5). ^1H and ^{13}C NMR (see Table 1). EIMS m/z (rel. int.): 426 (36, $[M]^+$), 409 (100, $[M - H_2O]^+$), 217 (13, $[C_{13}H_{13}O_3]^+$), 209 (93, $[C_{11}H_{13}O_4]^+$). HR-MS found 449.1582, $C_{24}H_{26}O_7Na$, calculated for 449.1576.

*3.5. Spiro-13-homo-13-oxaelliptone (**2**)*

Colourless amorphous solid. $[\alpha]_D^{25} = -92.9°$ (c 0.5, $CHCl_3$). UV λ_{max} (MeOH, log ε) nm: 270 (4.2), 275 (4.2), 300 (3.6), 316 (3.6). CD (c 0.02, MeOH) $[\theta]_{330} - 1421$, $[\theta]_{275} + 1662$, $[\theta]_{238} - 2234$. ^1H and ^{13}C NMR (see Table 1). EIMS m/z (rel int): 368 (100, $[M]^+$), 353 (12, $[M - Me]^+$), 200 (26), 169 (49). HR-MS found 391.0774, $C_{20}H_{16}O_7Na$, calculated for 391.0794.

3.6. Larvicidal assay

The larvicidal assay on second instar larvae of *A. aegypti* was done according to Yenesew et al. (2003). LD_{50} values were calculated (from the average of three observations for each concentration) using Finney's probit analysis for quantal data (McLaughlin et al., 1991).

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References

Dagne, E., Yenesew, A., Waterman, P.G., 1989. Flavonoids and isoflavonoids from *Tephrosia fulvinervis* and *Tephrosia pentaphylla*. Phytochemistry 28, 3207–3210.

Dewick, P.M., 1994. Isoflavonoids. In: Harborne, J.B. (Ed.), Flavonoids: Advances in Research Since 1986. Chapman and Hall, London, p. 117.

Fang, N., Casida, J.E., 1997. Novel bioactive cube insecticide constituents: isolation and preparation of 13-homo-13-oxa-6a,12a-dehydrorotenoids. Journal of Organic Chemistry 62, 350–353.

Ito, C., Itoigawa, M., Kajima, N., Tan, H.T.-W., Takayasu, J., Tokuda, H., Nishino, H., Furukawa, H., 2004. Cancer chemopreventive activity of rotenoids from *Derris trifoliata*. Planta Medica 70, 585–588.

McLaughlin, J.R., Chang, C.-J., Smith, D.L., 1991. Bench-top bioassays for discovery of bioactive natural products: an update. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, vol. 99. Elsevier Science Publishers BV, Amsterdam, p. 383.

Ollis, W.D., Rhodes, C.A., Sutherland, I.O., 1967. The extractives of *Milletta dura*. Tetrahedron 23, 4741–4760.

Wangenesteen, H., Alamgir, M., Rajia, S., Samuelsen, A.N., Malterud, K., 2005. Rotenoids and isoflavones from *Sarcolobus globosus*. Planta Medica 71, 754–758.

Yenesew, A., Derese, S., Midiwo, J.O., Hedenreich, M., Peter, M.G., 2003. Effect of rotenoids from the seeds of *Millettia dura* on larvae of *Aedes aegypti*. Pest Management Science 59, 1159–1161.

Yenesew, A., Mushibe, E.K., Induli, M., Derese, S., Midiwo, J.O., Kabaru, J.M., Heydenreich, M., Koch, A., Peter, M.G., 2005. 7a-*O*-methyldeguelol, a modified rotenoid with an open ring-C, from the roots of *Derris trifoliata*. Phytochemistry 66, 653–657.