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7a-*O*-methyldeguelol, a modified rotenoid with an open ring-C, from the roots of *Derris trifoliata*

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Abstract

From the acetone extract of the roots of *Derris trifoliata* an isoflavonoid derivative, named 7a-*O*-methyldeguelol, a modified rotenoid with an open ring-C, representing a new sub-class of isoflavonoids (the sub-class is here named as rotenoloid), was isolated and characterised. In addition, the known rotenoids, rotenone, deguelin and α-toxicarol, were identified. The structures were determined on the basis of spectroscopic evidence. Rotenone and deguelin were identified as the larvicidal principles of the acetone extract of the roots of *Derris trifoliata*.

Keywords: *Derris trifoliata*; Leguminosae; Roots; Rotenoids; Rotenoloid; 7a-*O*-methyldeguelol; Conformation; Energy calculation; Mosquito; Larvicides; *Culex quinquefasciatus*

1. Introduction

In our interest on the identification of larvicidal compounds from plants, we have reported the larvicidal activities of rotenoids isolated from the seeds of *Millettia dura* Dunn (Yenesew et al., 2003). Rotenoids also occur in the genera *Derris*, *Lonchocarpus* and *Tephrosia* of the family Leguminosae (Dewick, 1994). Some species belonging to these genera including *Derris elliptica*, are commercially cultivated as a source of insecticidal rotenoids (Fukami and Nakajima, 1971).

The genus *Derris* is represented in Kenya by *Derris trifoliata* Lour. Recent investigation of the stems of *D. trifoliata* has resulted in the identification of rotenoids with cancer chemopreventive properties (Ito et al., 2004). From the roots of this plant we report here the isolation and characterization of a novel isoflavonoid derivative (**1**) representing a new sub-class of isoflavonoids.

2. Results and discussion

The acetone extract of the roots of *Derris trifoliata* showed high toxicity against the second instar larvae of the mosquito *Culex quinquefasciatus* with LC_{50} value of 1.35 ± 0.7 μg/ml. From this extract, by the use of a combination of chromatographic methods, a novel isoflavonoid derivative (**1**) along with three known compounds was isolated. The known compounds were identified as the rotenoids, (−)-rotenone (**2**), (−)-deguelin (**3**) and (−)-α-toxicarol (**4**), by comparison of their spectroscopic data with the literature report (Dagne et al., 1989).

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Compound **1** was isolated as a colorless amorphous solid. HRMS analysis showed a molecular ion peak at *m/z* 410.1727 corresponding to the molecular formula of $C_{24}H_{26}O_6$. Comparison of the ^1H and ^{13}C NMR spectra (Table 1) of **1** with deguelin (**3**) suggested that ring-A and -D are similar in these two compounds. Thus as in deguelin, the presence of two singlets at δ 6.47 (H-1) and 6.37 (H-4) would place two of the three methoxyls in ring-A at C-2 and C-3. In ring-D an AX spin system at δ 6.39 and 7.42 (J = 7.4 Hz) were assigned to H-10 and H-11, respectively, allowing the placement of the 2,2-dimethylpyran group at C-8/C-9.

The major difference between these two compounds was on the chemical shift position and splitting pattern of the aliphatic protons at C-6, C-6a and C-12a (Table 1). The two sets of double doublets at δ 4.57 and 4.12 for the two protons on C-6 in **3** are replaced in **1** with a multiplet at δ 4.13 integrating for two protons. The one-proton signal (*ddd*) for H-6a at δ 4.87 in **3** is replaced in **1** with a two-proton multiplet at δ 2.15; while the proton at C-12a resonating as doublet at δ 3.78 in compound **3** appears as a double doublet at δ 4.67 (J = 6.4, 3.9 Hz) in **1**. This means that **1** has two protons on C-6a (instead of one proton on a rotenoid skeleton as in **3**), which could only happen if ring-C (on rotenoid skeleton) is open between C-6a and O-7. Indeed, in a $^1H,^1H$-COSY spectrum of **1**, the double doublet at δ 4.67 (H-12a) showed a correlation with the multiplet centered at δ 2.15 (CH_2-6a) which in turn correlates with the C-6 protons (δ 4.13). In agreement with this, in the ^{13}C NMR/DEPT spectra, C-6a which is oxymethine (at δ 72.4) in **3** is replaced with a methylene signal at δ 29.7 in the new compound.

It is interesting to note that the chemical shift position of H-11 is upfield shifted (Table 1) in **1** as compared to that observed in **3**. This indicates the lack of a *peri*-effect by the carbonyl at C-12 in **1**, even though its presence was evident from ^{13}C NMR (at δ 202.7) and HMBC (correlation between H-11 and C-12) spectra. Furthermore, the chemical shift position of the carbonyl in **1** is ca. 13 ppm downfield shifted compared to that of **3**. This suggested that the carbonyl in **1** is not coplanar with ring-D which also accounts why H-11 is not deshielded by the anisotropic effect of the carbonyl at C-12. These features as well as the HMBC correlation of CH_2-6a (δ 2.15) with C-12 (δ 202.7) are consistent with an open ring-C between C-6a and O-7 in **1**.

With an open ring-C, the third methoxyl (δ 3.83 in ^1H NMR and δ 64.6 in ^{13}C NMR spectra) can now be placed at C-7a. The ^{13}C NMR chemical shift value of this methoxyl (δ 64.6) is upfield shifted by ca. 10 ppm, which is typical of di-*ortho*-substituted methoxyl groups (Yenesew et al., 1998), allowing its placement at C-7a. This was supported by HMBC correlation of the methoxyl protons at C-7a (δ 3.83) with C-7a (δ 155.5). The placement of the methoxyl at C-7a was confirmed by

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) data of **1** and **3** in $CDCl_3$

Position	**1**			**3**	
	^1H (J in Hz)	^{13}C	HMBC (^2J, ^3J)	^1H (J in Hz)	^{13}C
1	6.47 *s*	112.7	C-2, -3, -4a, -12a	6.75 *s*	111.4
1a		114.9			114.8
2		149.1[a]			147.5
3		142.9			143.8
4	6.37 *s*	100.8	C-1a, -2, -3, -4a	6.38 *s*	101.0
4a		149.0[a]			149.5
6α	4.13 *m*	63.5		4.57 *dd* (12.0, 2.8)	66.2
6β	4.13 *m*			4.12 *dd* (12.0, 1.1)	
6a	2.15 *m*	29.7	C-12	4.87 *ddd* (2.8, 1.1, 3.6)	72.4
7a		155.5			156.9
8		109.9			109.7
9		157.4			160.0
10	6.39 *d* (7.4)	112.5	C-11a	6.39 *d* (8.7)	112.7
11	7.42 *d* (7.4)	130.7[b]	C-7a, -9, -12	7.68 *d* (8.7)	128.7
11a		125.2			112.7
12		202.7			189.2
12a	4.67 *dd* (6.4, 3.9)	44.6		3.78 *d* (3.6)	44.3
2′		76.9			77.6
3′	5.87 *d* (9.8)	130.9[b]	C-2′, -8	5.48 *d* (10.0)	128.5
4′	6.67 *d* (9.8)	116.4	C-2′, -9	6.59 *d* (10.0)	115.6
2′-Me_2	1.45 *s*	28.0	C-3′	1.39 *s*	28.5
	1.45 *s*	28.0		1.31 *s*	28.1
OMe-2	3.55 *s*	57.3	C-2	3.74 *s*	56.8
OMe-3	3.75 *s*	56.6	C-3	3.72 *s*	56.3
OMe-7a	3.83 *s*	64.6	C-7a		

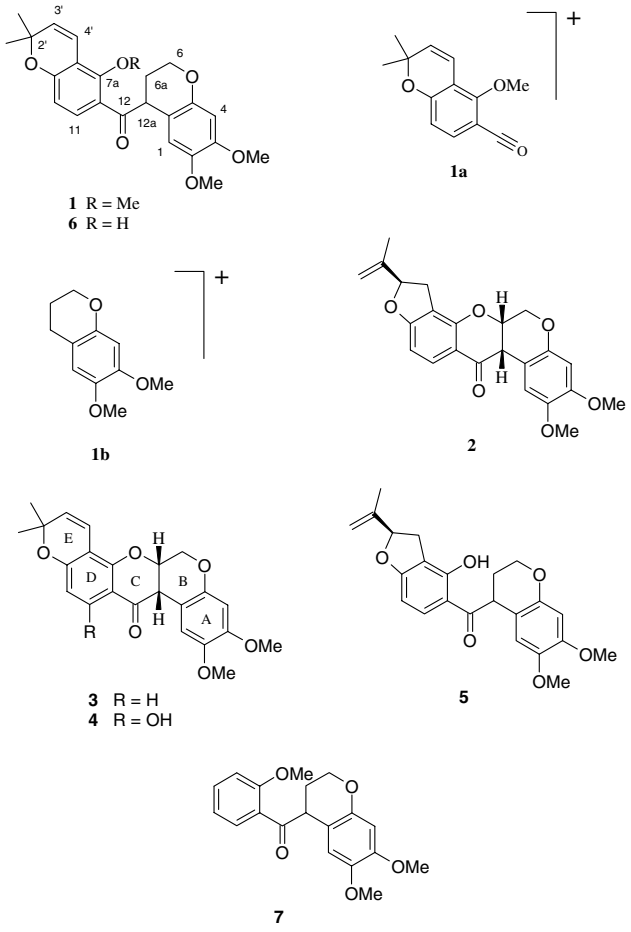
[a,b] Assignments may be interchanged.

NOE experiment where irradiation of the methoxyl at C-7a showed enhancement of H-4′ (δ 6.67). In the MS the fragment ion at *m*/*z* 217 (**1a**) is consistent with the attachment of this methoxyl and the 2,2-dimethylpyran group in ring-D, while the fragment ion at *m*/*z* 193 (**1b**) is in agreement with the two methoxyl groups being in ring-A. Hence this compound should have structure **1**.

Compound **1** is closely related to rotenol (**5**), a compound synthesized from rotenone through reductive cleavage of ring-C (Fukami and Nakajima, 1971). There is also a report on the occurrence of **5** in *Indigofera tinctoria*; however, the identification was only based on comparison of UV, IR and mp data with synthetic rotenol (Kamal and Mangla, 1993). Biogenetically, compound **1** could have been derived from deguelin through opening of ring-C to form an intermediate, **6**, which upon subsequent methylation gives compound **1**. As the name rotenol for **5** is derived from rotenone the name deguelol is assigned to **6.** Consequently, the new compound has been given the trivial name 7a-*O*-methyldeguelol. We also suggest a sub-class name rotenoloid for compounds **1**, **5** and **6**, as these are modified rotenoids. Compound **1** is optically active ($[\alpha]_D = -20.3°$), and the CD spectrum showed a positive Cotton effect at ca. 295 nm. From biogenetic considerations, the absolute configuration at C-12a in **1** is likely to be as in (−)-(6a*S*, 12a*S*)-deguelin (**3**) and other related natural rotenoids (Dewick, 1994), but opposite designation, (12a*R*)-**1**. However, this remains to be established.

To get some information about the conformation of ring-B, the energy of a model compound, (*R*)-**7**, was calculated theoretically. Two conformers (**7a** and **7b**) were found to be stable (Fig. 1), of which **7b** has the lower ground state energy by 5.6 kcal/mole. For compound **1**, although experimentally only the ^3J(H,H) coupling constants between H-12a and the two H-6a protons were available, these coupling constants were used to determine the conformation. Thus, from the theoretically calculated ground states of (*R*)-**7** the dihedral angles between these protons were extracted (Table 2). Then from these angles the theoretical coupling constants were calculated using the ALTONA-program (Cerda-Garcia-Rojas et al., 1990). The sum of the differences between calculated and experimentally determined ^3J(H,H) coupling constants is much smaller for the conformation **7b** (Table 2). Thus the actual conformation of ring-B in **1** appears to be close to that of (*R*)-**7b**.

The major compound in this extract is rotenone (**2**) and it is highly toxic towards mosquito larvae with LC_{50} value of 0.45 ± 0.1 μg/ml against the second instar larvae of *Culex quinquefasciatus*. Deguelin was less toxic with LC_{50} value of 1.8 ± 0.6 μg/ml. The new compound (**1**) and α-toxicarol (**3**) were isolated in small quantities and have not been tested for larvicidal activity at this stage.



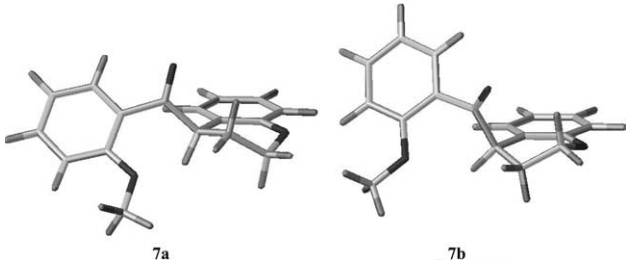


Fig. 1. The two theoretically calculated ground states of (*R*)-**7**. The right conformation (**7b**) was found to be the more stable one.

3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F_{254} plates. CC on oxalic acid impregnated silica gel 60 (70–230 mesh). EIMS: direct inlet, 70 eV, on a SSQ 710, Finnigan MAT mass spectrometer. ^{1}H and ^{13}C NMR on Bruker or Varian-Mercury spectrometers

Table 2
Experimental and calculated ^3J(H,H) coupling constants (in Hz) between H-12a and H-6a in **1** (exp.) and (*R*)-**7** (calc.), calculated dihedral angles in (*R*)-**7** (in °), and the sum of differences

Compound	Dihedral angles	Calculated ^3J(H,H)	Experimental ^3J(H,H)	$\Sigma\Delta^3J$(H,H)
(*R*)-**7a**	314.3; 195.4	5.4; 11.7	6.4; 3.9	8.8
(*R*)-**7b**	40.3; 282.1	6.2; 1.2		2.9

using TMS as int. standard. HMQC and HMBC spectra were acquired using the standard Bruker software.

3.2. Plant material

The roots of *Derris trifoliata* Lour were collected in 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 ground roots of *Derris trifoliata* (800 g) were successively extracted with hexane (3×2 l) and acetone (3×2 l) by cold percolation. The extracts were filtered and the solvents removed under vacuum to give 10.5 and 29.6 g of crude extracts, respectively. Twenty-eight grams of the acetone extract was subjected to chromatographic separation on oxalic acid impregnated silica gel (200 g) and eluted with hexane containing increasing amounts of acetone (1%, 2%, 3% and 4% acetone in hexane). A total of 15 fractions each containing ca. 250 ml were collected and labeled (A–O). Fractions L and M (eluted with 3% acetone in hexane) were active towards mosquito larvae. Thus, fraction L was further subjected to column chromatography on Sephadex LH-20 (eluted with CH_2Cl_2–MeOH; 1:1) and then preparative TLC on silica gel (solvent system: hexane-ethyl acetate, 9:1) to give **3** (57 mg), **4** (2 mg) and **1** (5 mg). Crystallization of fraction M from methanol gave **2** (255 mg).

3.4. 7a-O-methyldeguelol (*1*)

Amorphous powder. $[\alpha]_D^{25.8} = -20.3$ (*c* 0.115, MeOH). UV λ_{max} MeOH (log ε) nm: 278 (4.2). CD (*c* 6.3×10^{-4}, MeOH) $[\theta]_{295}$ +1160. ^{1}H NMR and ^{13}C NMR, see Table 1. EIMS *m/z* (rel int): 410 (5, $[M]^+$), 217 (100), 193 (35). HRMS found 410.1727, calculated for $C_{24}H_{26}O_6$, 410.1729.

3.5. Larvicidal activity assay

The larvicidal activity test on the second instar larvae of *Culex quinquefasciatus* was done according to the procedure reported earlier (Yenesew et al., 2003). LC_{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).

3.6. Theoretical calculation of conformations

The ab initio program package GAUSSIAN 98 (Frisch et al., 2002) was used for calculations. The calculations were carried out at the Hartree–Fock level by means of 6-31G** split-valence basis set (Hehre et al., 1986). The geometry optimisation of selected compounds was performed without restrictions. The quantum-chemical calculations were processed on SGI Octane and a Linux cluster at University of Potsdam. Results were visualized using molecular modelling program (SYBYL, 2002).

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References

Cerda-Garcia-Rojas, C.M., Zepeda, L.G., Joseph-Nathan, P., 1990. A PC program for calculation of dihedral angles from ^{1}H NMR data. Tetrahedron Computer Methodology 3, 113–118.

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.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Zakrzewski, V.G., Montgomery Jr., J.A., Stratmann, R.E., Burant, J.C., Dapprich, S., Millam, J.M., Daniels, A.D., Kudin, K.N., Strain, M.C., Farkas, O., Tomasi, J., Barone, V., Cossi, M., Cammi, R., Mennucci, B., Pomelli, C., Adamo, C., Clifford, S., Ochterski, J., Petersson, G.A., Ayala, P.Y., Cui, Q., Morokuma, K., Rega, N., Salvador, P., Dannenberg, J.J., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Cioslowski, J., Ortiz, J.V., Baboul, A.G., Stefanov, B.B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Gomperts, R., Martin, R.L., Fox, D.J., Keith, T., Al-Laham, M.A., Peng, C.Y., Nanayakkara, A., Challacombe, M., Gill, P.M.W., Johnson, B., Chen, W., Wong, M.W., Andres, L., Gonzalez, C., Head-Gordon, M., Replogle, E.S., Pople, J.A., 2002. Gaussian 98, Revision A.11.3. Gaussian, Inc, Pittsburgh, PA.

Fukami, H., Nakajima, M., 1971. Rotenone and rotenoids. In: Jacobson, M., Crosby, D.G. (Eds.), Naturally Occurring Insecticides. Marcel Dekker INC, New York, p. 71.

Hehre, W.J., Radom, L., Schleyer, P.v.R., Pople, J.A., 1986. Ab Initio Molecular Orbital Theory. Wiley, New York.

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.

Kamal, R., Mangla, M., 1993. *In vivo* and *in vitro* investigations on rotenoids from *Indigofera tinctoria* and their bioefficacy against larvae of *Anopheles stephensi* and adults of *Callosobruchus chinensis*. Journal of Biosciences 18, 93–101, CAN 119:24623 AN 1993:424623 CAPLUS (ACS on SciFinder (R)).

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.

SYBYL 6.9 Tripos Inc., 2002. 1699 South Hanley Road, St. Louis, MO 63144.

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., Midiwo, J.O., Miessner, M., Heydenreich, M., Peter, M.G., 1998. Two prenylated flavanones from stem bark of *Erythrina burttii*. Phytochemistry 48, 1439–1443.