Rotenoid derivatives from Kenyan *Millettia* and *Derris* species as larvicidal agents

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INTRODUCTION

Rotenone (**1**), one of the most extensively used natural insecticides, was reported to be highly toxic to the 4th instar larvae of *Aedes aegypti* (Abe et al., 1985). The insecticidal activities of rotenone and some other rotenoids, including deguelin (**2**) and tephrosin (**3**) against a variety of insect species are well known (Fukami and Nakajima, 1971). Commercially, rotenone is mainly extracted from the roots of *Derris* species in Asia and *Lonchocarpus* species in South America (Fukami and Nakajima, 1971). Rotenone and other flavonoids are also known to occur in several plants, including *Millettia,* belonging to the family Leguminosae (Dewick, 1994).

We have studied the larvicidal activities of several rotenoids isolated from *Millettia* and *Derris* species. The structures and larvicidal activities of these rotenoids are reviewed in this paper.

RESULTS AND DISCUSSION

The genus *Millettia*

In Kenya the genus *Millettia* is represented by six species, namely *M. dura, M. lasiantha, M. leucantha, M. oblata, M. tanaensis* and *M. usaramensis*. We have analysed so far two species for their flavonoid and isoflavonoid contents.

Milletia dura

The crude chloroform extract of seeds of *Millettia dura* (Leguminosae) showed high larvicidal activity (LD_{50} = 3.5 μg/ml at 24 hours) against second-instar larvae of the mosquito, *Aedes aegypti* (Diptera: Culicidae). Extracts obtained from the stem bark and root bark and leaves were inactive even at 20 μg/ml. Previous phytochemical investigations have showed that isoflavones are found in different parts of this plant, while rotenoids are restricted to the seeds (Derese et al., 2003; Yenesew et al., 1996; Ollis et al., 1967; Dagne et al., 1991). Indeed the rotenoids deguelin (2) and tephrosin (3) isolated from the seeds of this plant also showed potent activities with LD_{50} vales of 1.6 and 1.4 μg/ml at 24 h, respectively. Interestingly, the related rotenoids millettone (4) and millettosine (5) were completely inactive even at 20 μg/ml. The presence of methoxyl groups at C-2 and/or C-3 in deguelin and tephrosin appear to be important for the observed larvicidal activity. The wide insecticidal activities of deguelin and tephrosin are well known (Fukami and Nakajima, 1971). Dehydrodeguelin was also inactive indicating the importance of saturation at the B/C-ring junction for larvicidal activity of rotenoids.

The powdered seeds were also tested for larvicidal activity and interestingly a one mg portion in twenty milliliters of brine solution of the powdered seeds caused 100% mortality to the 2nd instar larvae of *Aedes aegypti*. This observation indicates the potency of the seeds and the ease with which local communities can use it to control mosquitoes.

Millettia usaramensis* subspecies *usaramensis

The seeds and the stem bark of *Millettia usaramensis* subspecies *usaramensis* showed very good activity against the second instar larvae of *Aedes aegypti.* The stem bark elaborates rotenoids chalcones and isoflavones (Yenesew et al., 1998; 2003b). These compounds have been isolated and tested for larvicidal activities in this study. The results are summarized in table 1. Degulin and tephrosin again are mainly responsible for the larvicidal activity of the seeds. The stem bark mainly elaborate rotenoids with trans B/C-ring junction which are not effective as insecticides (Fukami and Nakajima, 1971). These rotenoids also lack the two methoxy groups at C-2 and C-3, which are required for larvicidal activity (Yenesew et al., 2003). The compounds responsible for the larvicidal activity of the stem bark of this plant are not identified as yet.

Although the rotenoids, usararotenoid-A (**6**), -B (**7**) and –C (**8**) (Yenesew et al., 1998; 2003), isolated from the stem bark did not show larvicidal activities, thesecompounds represent the only natural rotenoids with 6a*S*,12a*R* stereochemistry at the B/C ring junction. The stereochemistry was established from negative Cotton effect at ca. 340 in the CD spectra of these rotenoids. X-ray crystallography of compound **8** confirmed the 6a*S*,12a*R* stereochemistry in this compound and by extension in the other rotenoids of the stem bark of this plant (Yenesew et al., 2003).

Derris trifoliata

There is one *Derris* species, *Derris trifoliata* in Kenya and we have studied the roots and seeds of this plant. The acetone extract of the roots *Derris trifoliata* showed potent larvicidal activity against the second instar larvae of *Culex quinquefasciatus* and *Aedes agypti.* From this extract a new isoflavonoid derivative, named 7a-*O*-methyldeguelol (**9**), a modified rotenoid with an open ring-C, representing a new sub-class of isoflavonoids (the sub-class named as rotenoloid), was isolated and characterised. In addition, the known rotenoids rotenone, deguelin and α-toxicarol (**10**) were identified. Biogenetically, compound **9** appear to have been derived from deguelin through opening of Ring-C to form an intermediate, **11**, which upon subsequent methylation gives compound **9**. 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* (Table 1 and 2). The charachterization and larvidial activity of these compounds have been published recently (Yenesew et al., 2005).

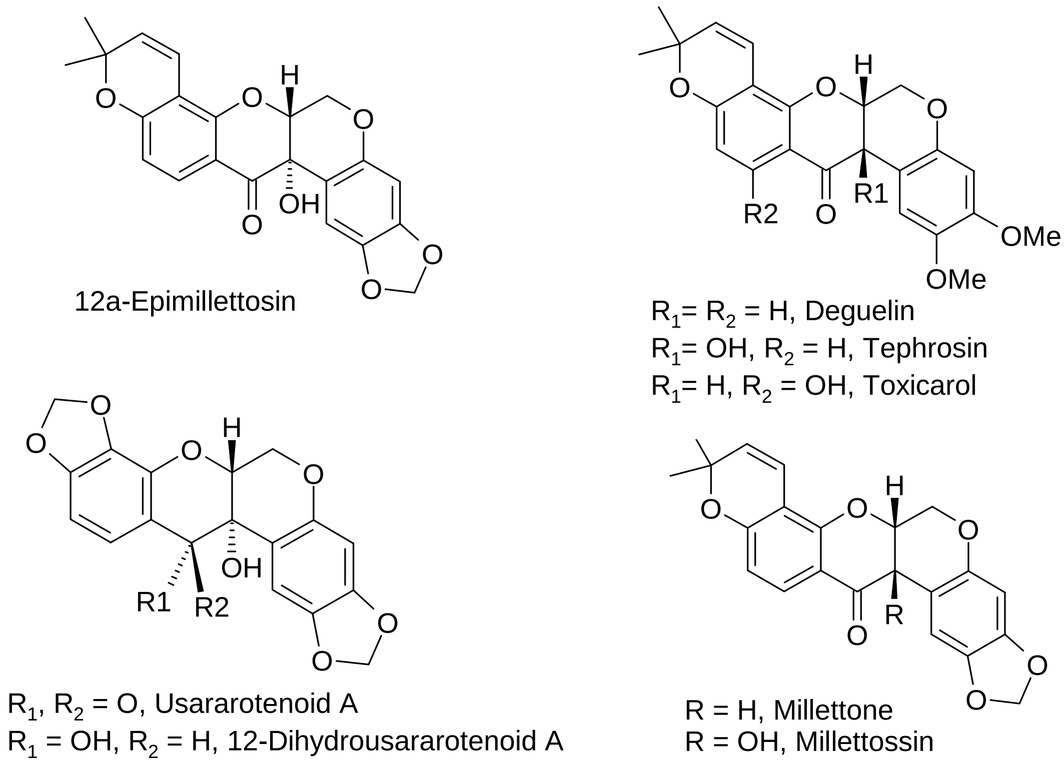
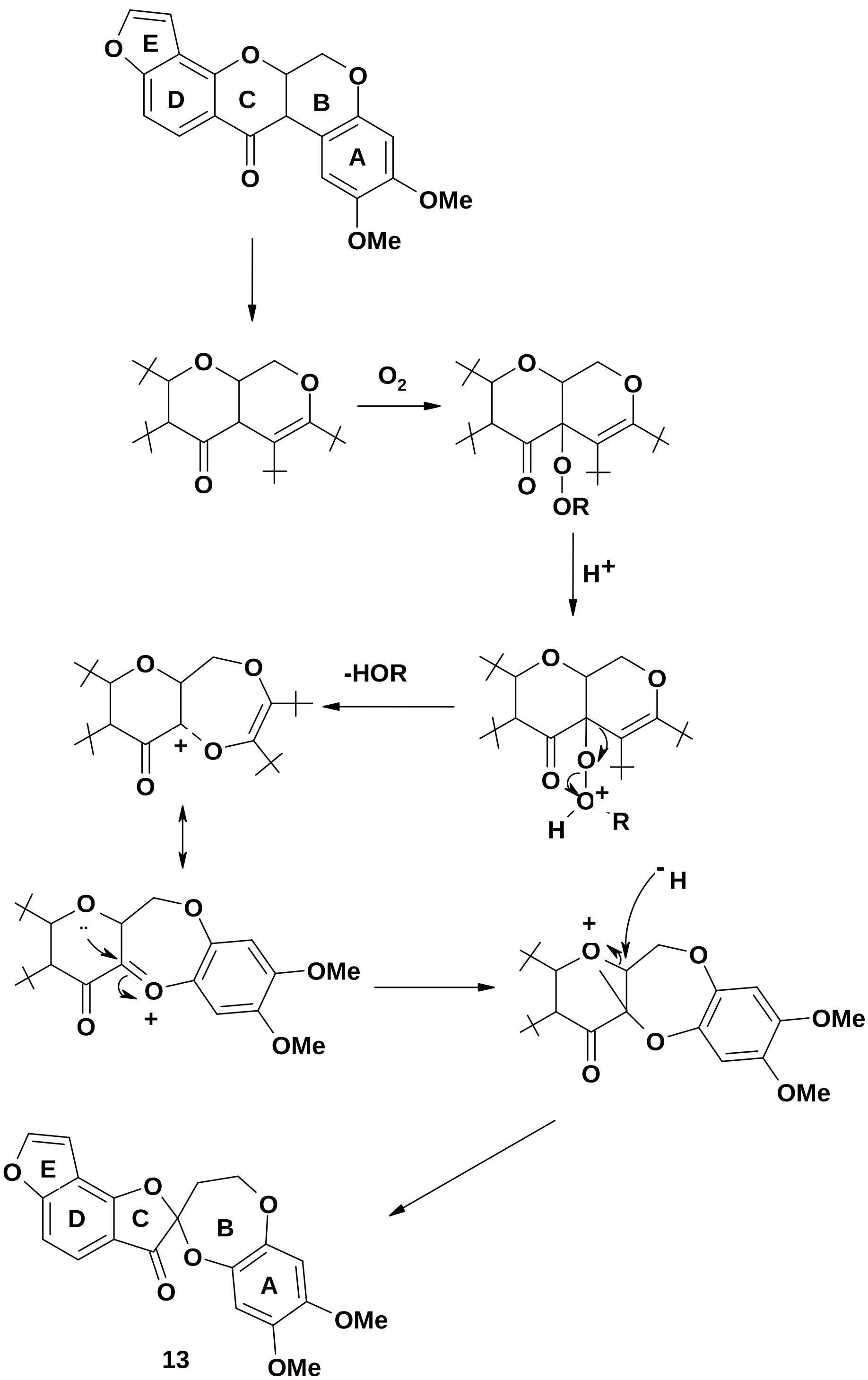


Fig. 1. Chemical structures of rotenoids and other flavonoids isolated from *Millettia* species

Dried and ground seeds of *Derris trifoliata* were extracted with methanol by cold percolation for 24 Hrs at room temperature. The MeOH extracts showed larvicidal activities against the 2nd instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* mosquito with LD_{50} of 0.56 µg/ml and 0.63 µg/ml respectively. Rotenone is the major compound in this extract and it precipitated out upon removal of the solvents. The larvicidal activity of the mother liquor decreased with the LD_{50} of 2.32 µg/ml and 3.05 µg/ml respectively.

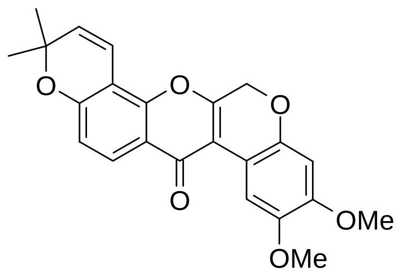
The crude methanol extract of the seeds of *Derris trifoliata* showed potent and dose dependent larvicidal activity against the 2nd instar larvae of *Aedes aegypti*. From this extract a further two modified rotenoid derivatives, a rotenoloid, 7a-*O*-methyl-12a-hydroxydeguelol (**12**) and a spirohomooxarotenoid, named spiro-13-homo-13-oxaelliptone (**13**), were isolated and characterized. In addition a rare natural chromanone 6,7-dimethoxy-4-chromanone (**14**) and the known rotenoids rotenone, tephrosin and dehydrodeguelin (**15**) were identified. spectroscopic evidence. The characterization of this compounds have been published (Yenesew et al., 2006). 7a-*O*-methyl-12a-hydroxydeguelol (**12**) could have been derived through oxidation at C-12a of 7a-*O*-methyldeguelol (**12**) or cleavage of the C-6a O-7 bond in tephrosine. The biogenesis of spiro-13-homo-13-oxaelliptone (**13**) could have followed a radical mechanism as shown in scheme 1.



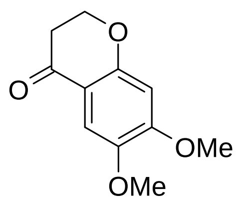
Scheme1: Proposed biogenesis of compound **13** from elliptone

Rotenone is the major compound in this extract and the larvicidal activity of the crude extract is mainly due to this compound with LD_{50} of 0.45 µg/ml. Compound 14 and tephrosin were also tested against 2nd instar mosquito larvae of *A. aegypti* with an LC_{50} of 13.4 µg/ml and 1.5 µg/ml respectively..

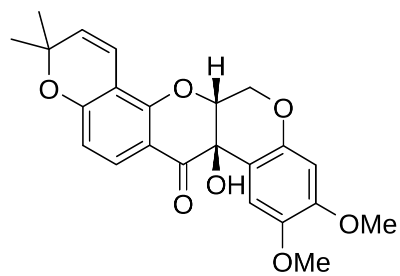
The crude acetone extract of the stem of *Derris trifoliata* was also tested and gave a LD_{50} of 7.53 µg/ml. The presence of the rotenoids rotenone, (6aα,12aα)-12a-hydroxyelliptone, elliptone, deguelin, α-toxicarol and tephrosin has been reported (Ito et al., 2004). These rotenoids appear to be responsible for the larvicidal activity of the stems of this plant.



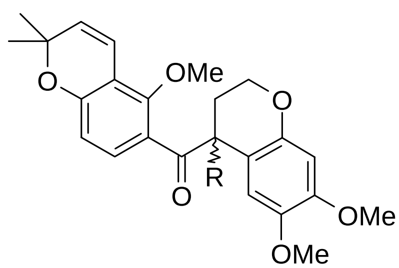
Dehydrodeguelin (**3**)



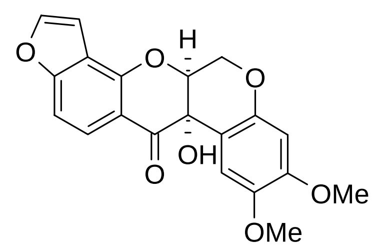
6,7-Dimethoxy-4-chromone (**4**)



Tephrosin (**5**)



R = H, 7-O.Metrhyldeguelol (**6**)
R = OH, 12a-Hydroxy-
7-O-Metrhyldeguelol (6)



(6α ,12α) -12a-hydroxyelliptone (**7**)

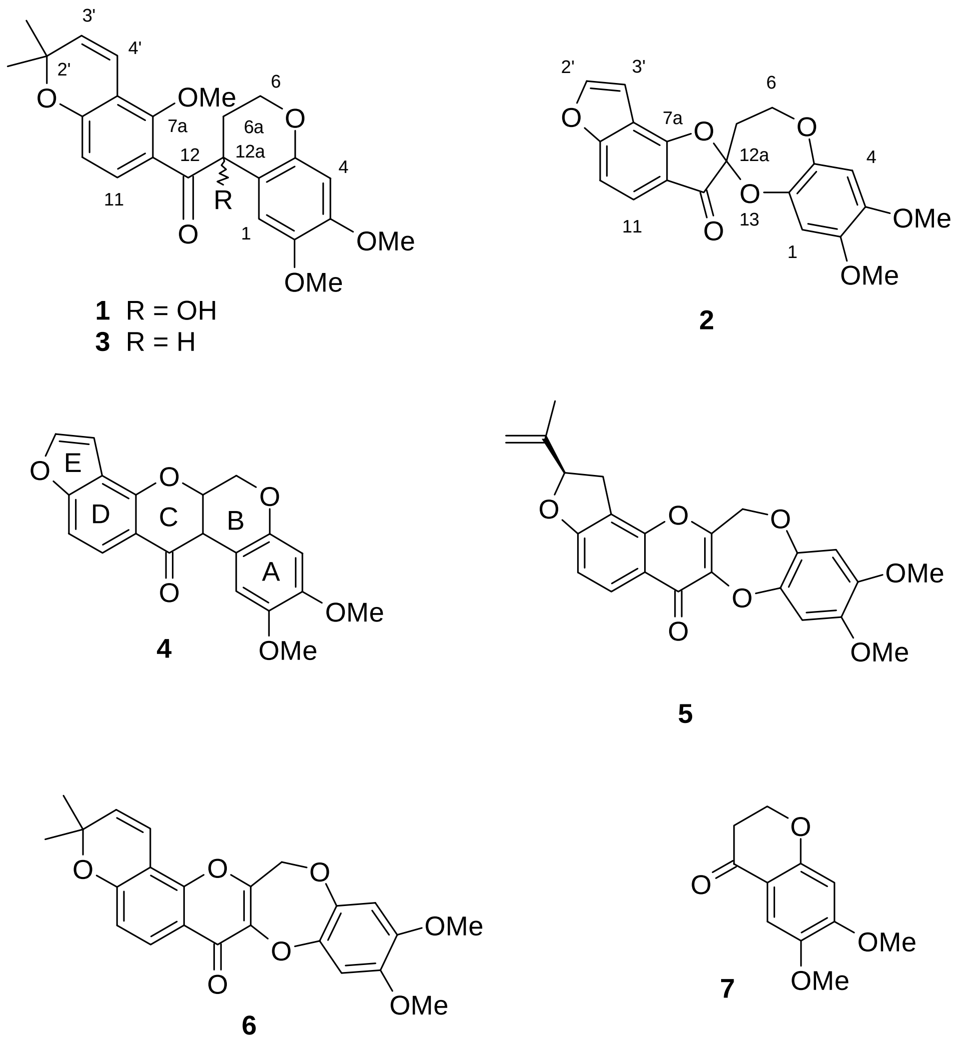


Figure 2: Chemical structures of compounds isolated from *Derris trifoliata*

Table 1. Activities (LC$_{50}$ (μg/ml) of crude extracts obtained from different plants against Brine Shrimps, *Aedes agypti* and *Culex quinquefasciatus*

Plant sample	Brine Shrimps	*Aedes agypti*	*Culex quinquefasciatus*
Derris trifoliata			
Seeds	<0.5	0.56	0.63
Roots	0.5-1.0	0.74	0.45
Stem	1.0-3.0	7.53	8.23
Pods and leaves	1.0-2.0	NT	NT
Millettiia dura			
Seeds	3.4	2.7	3.1
Stem bark	27.2	>20	>20
Millettiia usaramensis			
Seeds	5.6	3.5	3.2
Stem bark	12.5	9.4	8.3

NT = Not Tested

Table 2. List of rotenoids identified from different plants and their LC_{50} values (μg/ml) against Brine shrimps (BS), *Aedes agypti* (AA) and *Culex quinquefasciatus* (CQ)

Rotenoid	BS	AA	CQ.	Plant source (plant part)
Deguelin	0.8	1.6	1.8	*Millettia dura* (seeds) *Millettia usaramensis* (seeds) *Derris trifoliata* (roots, seeds, stem)
12-Dihydrousararotenoid A	>20	>20	NT	*Millettia usaramensis* (stem, bark)
12a-Epimillettosin	2.0	>20	NT	*Millettia usaramensis* (seeds, stem bark)
7a-*O*-Methyldeguelol	NT	NT	NT	*Derris trifoliata* (roots)
Millettone	2.3	>20	NT	*Millettia dura* (seeds)
Millettosin	2.72	>20	NT	*Millettia dura* (seeds)
Rotenone	<0.75	0.52	0.45	*Derris trifoliata* (roots, seeds, stem)
Tephrosin	0.6	1.5	NT	*Millettia dura* (seeds) *Millettia usaramensis* (seeds) *Derris trifoliata* (seeds, stem)
Toxicarol	NT	NT	NT	*Derris trifoliata* (roots, stem)
12a-Hydroxyelliptonol	NT	NT	NT	*Derris trifoliata* (seeds)
7a-*O*-Methyl-12a-hydroxyelliptonol	NT	NT	NT	*Derris trifoliata* (seeds)
6a,12a-Deydroellipton	NT	NT	NT	*Derris trifoliata* (seeds)
Usararotenoid A	6.5	9.3	NT	*Millettia usaramensis* (stem bark, seeds)
Usararotenoid B	9.3	>20	NT	*Millettia usaramensis* (stem bark, seeds)
6,7-Dimethoxy-4-chromone	NT	13.4	NT	*Derris trifoliata* (seeds)
(6aα,12aα)-12a-Hydroxyelliptone	NT	NT	NT	*Derris trifoliata* (stem)
Jamaicin	>20	>20	>20	*Millettia usaramensis* (stem bark, seeds)
4'-*O*-Geranylisoliquiritigenin			NT	*Millettia usaramensis* (stem bark, seeds)

NT = Not Tested

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