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ANTIPLASMODIAL AND LARVICIDAL FLAVONOIDS FROM *DERRIS TRIFOLIATA*

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ABSTRACT. From the dichloromethane-methanol (1:1) extract of the seed pods of *Derris trifoliata,* a new flavanone derivative (*S*)-lupinifolin 4´-methyl ether was isolated. In addition, the known flavonoids lupinifolin and rotenone were identified. The structures were determined on the basis of spectroscopic evidence. Lupinfolin showed moderate *in vitro* antiplasmodial activity against the D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) strains of *Plasmodium falciparum.* The different parts of this plant showed larvicidal activities against *Aedes aegypti* and rotenoids were identified as the active principles.

KEY WORDS: *Derris trifoliata*, Leguminosae, Flavanone, (*S*)-Lupinifolin 4´-methyl ether, Lupinifolin, Antiplasmodial, Rotenoid, Larvicide, *Aedes aegypti*

INTRODUCTION

The genus *Derris* (Leguminosae) is a rich source of isoflavonoids, especially rotenoids [1]. Some *Derris* species are in fact cultivated and commercialized as a source of the insecticide rotenone [2]. In Kenya this genus is represented by *Derris trifoliata.* From the roots [3] and seeds [4] of this plant we have isolated unique isoflavonoid derivatives, rotenoloids and a spirohomo-oxarotenoid. Here we report the isolation and characterization of a new flavanone derivative (**1**) named (*S*)-lupinifolin 4´-methyl ether. The anti-plasmodial and larvicidal activities of some of the flavonoids of this plant are also presented.

RESULTS AND DISCUSSION

HRMS analysis of compound **1** showed a $[M+1]^+$ peak at *m/z* 421.2002 corresponding to the molecular formula of $C_{26}H_{28}O_5$. The ^1H (δ 5.35 *dd*, J = 3.0, 13.0 for H-2_{ax}; 2.80 *dd*, J = 3.0, -17.0 for H-3_{eq}; and 3.05 *dd*, J = 13.0, -17.0 for H-3_{ax}) and ^{13}C (δ 78.5 for C-2, 43.1 for C-3, 196.4 for C-4) NMR spectra showed this compound to be a flavanone derivative. Furthermore, the presence of a chelated hydroxyl at C-5, a methoxyl, a 2,2-dimethylpyran, and a 3,3-dimethylallyl substituents was established from the NMR spectra (Table 1). In the ^1H NMR spectrum, the presence of an AA´XX´ spin system suggested that B-ring is substituted at C-4´. This substituent was established to be a methoxy (δ_H 3.84, δ_C 55.3) from NOESY (interaction of the methoxy protons with C-3´/5´) and HMBC (correlation of H-2´/6´ with C-4´, H-3´/5´; and OCH_3 with C-4´) spectra. It follows then that ring-A is fully substituted with hydroxyl (at C-5), the 2,2-dimethylpyran and the 3,3-dimethylallyl groups. This was supported by the presence of an EI-MS fragment ion at *m/z* 271 (**1a**) which resulted from a *retro*-Diels Alder fragmentation

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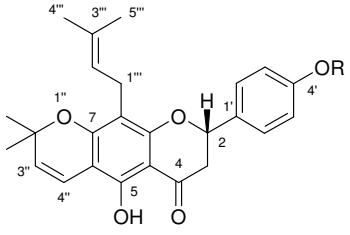
of ring-C with loss of a methyl group from ring-A. The placement of the 3,3-dimethylallyl group at C-8 and the 2,2-dimethylpyran group at C-6/7 was established from the HMBC spectrum (correlation of the methylene protons of the 3,3-dimethylallyl group with C-7 and C-8a; H-4´´ with C-5 and C-7; and correlation of the chelated OH with C-4a and C-6). The placement of the 2,2-dimethylpyran group at C-6/7 was confirmed by NOE interaction of H-4´´ with the chelated OH. Therefore this compound was assigned structure **1** for which the trivial name lupinifolin 4´-methyl ether is suggested by relating it to lupinifolin (**2**) a compound, which co-occurs in this plant. The CD spectrum and the negative optical rotation of compound **1** are consistent with an *S* configuration at C-2 [5]. Methylation of compound **2** gave the methoxy derivative **1**.

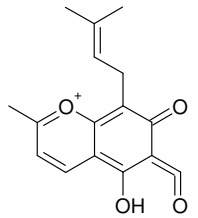
Table 1. NMR data for compounds **1** and **2** ($CDCl_3$), ^1H (500 MHz) and ^{13}C (150 MHz).

Position	**1**			**2**	
	δ_C	δ_H (*J* in Hz)	HMBC	δ_C	δ_H (*J* in Hz)
2	78.5	5.35 *dd* (3.0, 13.0)	C-4	78.5	5.35 *dd* (3.3, 12.6)
3_{eq}	43.1	2.80 *dd* (3.0, -17.0)	C-2, 4, 1´	44.1	2.79 *dd* (3.3, -17.0)
3_{ax}		3.05 *dd* (13.0, -17.0)	C-2, 4, 1´		3.04 *dd* (12.6, -17.0)
4	196.4			196.6	
4a	102.7*			102.5*	
5	156.6			156.5	
6	102.8*			102.8*	
7	159.8			160.0	
8	108.6			108.7	
8a	159.4			159.4	
1´	130.9			130.8	
2´/6´	127.5	7.37 *d* (8.4)	C-3´/5´, 4´	127.7	7.31 *d* (8.4)
3´/5´	114.1	6.94 *d* (8.4)	C-1´, 4´	115.5	6.87 *d* (8.4)
4´	159.8			156.0	
2´´	78.0			78.1	
3´´	125.9	5.49 *d* (10.0)	C-6	126.0	5.50 *d* (10.2)
4´´	115.7	6.63 *d* (10.0)	C-5, 7	115.6	6.63 *d* (10.2)
1´´´	21.5	3.21 *d* (7.2)	C-7, 8, 8a, 2´´, 3´´´	21.5	3.20 *d* (7.2)
2´´´	122.6	5.16 *t* (7.2)		122.6	5.16 *t* (7.2)
3´´´	131.6			131.1	
4´´´	25.8	1.65 *s*	C-3´´´	25.8	1.65 *s*
5´´´	17.8	1.65 *s*	C-3´´´	17.8	1.65 *s*
OCH_3	55.3	3.84 *s*	C-4´		
5-OH		12.26 *s*	C-4a, 5, 6		12.23 *s*

*Assignments may be interchangeable.



1 R = CH_3
2 R = H



m/z 271

1a

The seed pods of *Derris trifoliata* showed weak activity against the D6 and W2 strains of *Plasmodium falciparum* (Table 2). The major compounds isolated from this plant were also tested (Table 2), with the flavanone lupinfolin (**2**) being the most active with IC_{50} values of 2.6 ± 0.8 and 3.7 ± 1.4 µg/mL against the D6 and W2 strains of *Plasmodim falciparum*, respectively. Lupinifolin 4´-methyl ether (**1**) is less active (IC_{50} values of 12.9 ± 1.6 and 15.0 ± 2.5 µg/mL against the D6 and W2 strains, respectively) than lupinifolin showing the importance of free hydroxyl group at C-4´ in compound **2** for anti-plasmodial activity. We have observed a similar trend for flavanones isolated from *Erythrina* species [6]. The *in vivo* antiplasmodial activity of these compounds is questionable. Compound **2** has recently been reported to exhibit a marked inhibitory effect on mouse skin tumor promotion in an *in vivo* two-stage carcinogenesis test [7]. To the best of our knowledge, the cancer chemoprevention is one of the major activities of these compounds.

Table 2. *In vitro* IC_{50} values of the crude seed pods extract and flavonoids from *D. trifoliata* against W2 and D6 strains of *Plasmodium falciparum*.

Tested samples	IC_{50} (µg/mL)	
	D6	W2
Seed pods extract	12.2 ± 2.4	13.4 ± 2.6
Lupinifolin 4'-methyl ether (**1**)	12.9 ± 1.6	15.0 ± 2.5
Lupinifolin (**2**)	2.6 ± 0.8	3.7 ± 1.4
Rotenone	3.8 ± 1.6	6.6 ± 2.2
Deguelin [3]	6.3 ± 1.8	8.9 ± 2.0
6.7-Dimethyl-4-chromanone [4]	13.2 ± 1.2	15.7 ± 2.3
Chloroquine	0.008 ± 0.004	0.051 ± 0.010
Mefloquine	0.042 0 ± 0.008	0.015 ± 0.002

The various parts of *Derris trifoliata* were also tested for larvicidal activities against 2nd instar larvae of *Aedes aegypti* and the results are summarized in Table 3. The seeds were the most active with LC_{50} value of 0.7 ± 0.2 µg/mL at 24 h. The seed extract is also potent against 3rd (LC_{50} = 3.7 ± 1.5 µg/mL) and 4th (LC_{50} = 8.5 ± 2.3 µg/mL) instar larvae of *Aedes aegypti*. The major compounds isolated from this plant [3, 4] have also been tested and we have identified rotenoids, especially rotenone, to be the most active ingredient of this plant (Table 3).

Table 3. Activities of crude CH_2Cl_2/MeOH (1:1) extracts and pure compounds from *Derris trifoliata* against 2nd instar larvae of *Aedes aegypti*.

Tested samples	LC_{50} (µg/mL) at 24 h
Crude extracts (plant part)	
Seeds	0.7 ± 0.2
Roots	1.4 ± 0.5
Stem	8.6 ± 1.3
Leaves	7.5 ± 1.7
Seed pods	> 20
Pure compounds	
Lupinifolin 4'-methyl ether (**1**)	> 20
Lupinifolin (**2**)	> 20
Rotenone	0.5 ± 0.2
Deguelin [3]	1.6 ± 0.9
Dehydrodeguelin [4]	> 20
Tephrosin [4]	1.5 ± 0.8
6,7-Dimethoxy-4-chromone [4]	13.4 ± 2.7

EXPERIMENTAL

General

Analytical TLC: Merck pre-coated silica gel $60F_{254}$ plates. CC on oxalic acid impregnated silica gel 60 (70-230 mesh). UV spectra were measured on UV-1700 PharmaSpec, Shimadzu (Japan). EIMS: direct inlet, 70 eV, on a SSQ 710, Finnigan MAT mass spectrometer (USA). HRMS were recorded with electrospray ionisation on a ESI-QTOFmicro mass spectrometer from Waters Inc. (USA). ^{1}H-NMR (500 or 200 MHz) and ^{13}C-NMR (125 or 50 MHz) on Bruker (Germany) or Varian-Mercury spectrometers (USA) using TMS as internal standard. HMQC and HMBC spectra were acquired using the standard Bruker software. CD spectra were recorded on Jasco J-715 spectropolarimeter (Japan) and Optical rotations on a Jasco P-1020 polarimeter (Japan).

Plant material

For collection and authentication of *Derris trifoliata*, refer to reference [4].

Extraction and isolation

The dried and ground seed pods (280 g) of *D. trifoliata* were extracted with dichloromethane-methanol (1:1) by cold percolation (3 × 500 mL). Removal of the solvent under reduced pressure afforded 27 g of crude extract. The extract was subjected to CC on silica gel (300 g) and eluted with hexane containing increasing amounts of dichloromethane. A total of 20 fractions each of ca. 750 mL were collected. The fraction eluted with 20 % CH_2Cl_2 in hexane was further purified by CC on Sephadex LH-20 (eluting with CH_2Cl_2-MeOH; 1:1) and then on prep TLC (hexane-CH_2Cl_2-EtOAc; 6:3:1) to give compound **1** (35 mg). The fractions eluted with 30 % CH_2Cl_2 in hexane showed two spots on TLC and these were separated by prep TLC (hexane-CH_2Cl_2-EtOAc; 6:3:1) to give rotenone (36 mg) and compound **2** (67 mg). The fractions eluted with 40 % and 50 % CH_2Cl_2 in hexane were combined and upon crystallization afforded more amounts of compound **2** (136 mg).

*Lupinifolin 4'-methyl ether (**1**)*

Pale yellow oil. UV λ_{max}^{MeOH} (log ε) nm: 273 (4.3), 312 (3.8), 367 (3.2). $[\alpha]_D^{26}$ = -65.7° (MeOH, c 0.01). CD (MeOH, 0.01): $[\Theta]_{329}$ + 9472, $[\Theta]_{288}$ -3278. ^{1}H and ^{13}C NMR (Table 1). EIMS *m/z* (rel int): 420 (57, $[M]^+$), 405 (69, $[M\text{-}Me]^+$, 271 (52), 215 (100, $C_{16}H_{15}O_4$). HR-EIMS [M+1] found *m/z* 421.2002 $C_{26}H_{29}O_5$, calculated for 421.2015.

*Methylation of lupinifolin (**2**)*

Lupinifolin (10 mg) in dry acetone (10 mL), anhydrous K_2CO_3 (1 g) and dry dimethyl sulfate (20 µL) was refluxed for 2 h. It was then filtered, the solvent removed and was purified by CC (over silica gel eluting with CH_2Cl_2) to give compound **1** (7.5 mg). The identity was confirmed by direct co-TLC and ^{1}H NMR comparison with the sample isolated from the seed pods of *D. trifoliata*.

In-vitro antiplasmodial activity assay

The crude extract and pure compounds were assayed using a non-radioactive assay technique [8] with modifications to determine 50 % growth inhibition of cultured parasites. This is an accepted method for assaying *in vitro* drug susceptibility using the fluorochrome called "Sybr

Green", a non-radioactive intercalating DNA marker that accurately depicts *in vitro* parasite replication. This test replaces the older, ^{3}H-hypoxanthine uptake assay, is fully endorsed by the WHO. Briefly, two different strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), of *P. falciparum* were grown as described in the literature [9]. The culture-adapted *P. falciparum* were added on to the plate containing dose range of drugs and incubated in a gas mixture (5 % CO_2, 5 % O_2, and 90 % N_2) at 37 °C for 72 h and frozen at –80 °C.

After thawing, lysis buffer containing SYBR green I (1 x final concentration) was added directly to the plates and gently mixed by using the Beckman Coulter Biomek 2000 automated laboratory workstation (Beckman Coulter, Inc., Fullerton, USA). The plates were incubated for 5-15 minutes at room temperature in the dark. Parasite growth inhibition was quantified by measuring the per-well relative fluorescence units (RFU) of SYBR green 1 dye using the Tecan Genios Plus (Tecan US, Inc., Durham, USA) with excitation and emission wavelengths of 485 nm and 535 nm, respectively, and with the gain set at 60. Differential counts of relative fluorescence units (RFUs) were used in calculating IC_{50}s for each drug using Prism 4.0 software for Windows (Graphpad Software, San Diego, USA). A minimum of three separate determinations was carried out for each sample. Replicates had narrow data ranges hence presented as mean ± SD.

Preparation of extracts for larvicidal assay

The dry and ground plant materials (10 g each) were extracted with dichloromethane/methanol (1:1) by cold percolation. The solvents were removed under vacuum and each extract stored for larvicidal test.

Larvicidal activity assay

The larvicidal activity assay against 2nd instar larvae of *Aedes aegypti* was done as described in reference [10]. LC_{50} values were calculated (from the average of three observations for each concentration) using Finney's probit analysis for quantal data [11].

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