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C-Prenylflavonoids from *Derris heyneana*

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2',4'-Dihydroxy-3'-(3-methylbut-2-enyl)-4-methoxychalcone (**1**) and three known *C*-prenylflavonoids, namely, lupinifolin (flemichin B), hiravanone, and derrubone were isolated from the leaves of *Derris heyneana* (Febaceae). The structures were established by 2D NMR analysis.

Keywords: *Derris heyneana*, Febaceae, *C*-prenylflavonoids, chalcone, flavanones, isoflavone, antimicrobial.

Derris heyneana "Benth," (Fabaceae) is a woody climber with large red flowers distributed throughout the forests and hills of Southeast Asia. In India, it is commonly found growing in the Western Ghats and the hills of Travancore up to an altitude of 5000 ft [1]. Over the years, *Derris* sp. have been reported to produce numerous chalcones, flavones, isoflavones and flavanones possessing oxygenation at C-5,7,3' and 4', with the notable presence of a methylenedioxy group in the B-ring and prenyl units linked at C-6 and 8 of the A-ring [2-8]. The present study describes the isolation and structure determination of four *C*-prenylflavonoids.

A new 4,2',4'-trioxygenated 3'-prenychalcone, which was named heyneanachalcone (**1**), and three known *C*-prenylflavonoids (**2-4**) were isolated from the chloroform extract of the leaves of *D. heyneana* by sequential chromatographic techniques. The known compounds were identified by comparison of physical and spectral data (m.p., ESI MS, ^{1}H and ^{13}C NMR) with those reported in the literature.

Metabolite **1** appeared as a yellow spot when observed on TLC under UV light. On fuming with ammonia, the color of the spot changed to an intense red, suggesting a chalcone [9]. The molecular formula of **1** was determined to be $C_{21}H_{22}O_4$ by ESI-HRMS in accordance with eleven degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) suggested a prenylated chalcone with signals typical of a 3-methylbut-2-enyl side chain, along with the presence of two *trans*-olefinic protons at δ 7.47 and 7.86 (J = 15.4 Hz), assigned to the α and β positions of the chalcone bridge, respectively. In addition, the spectrum also displayed signals for a chelated hydroxyl, an aromatic hydroxyl, a methoxyl, two 2H doublets at δ 7.61 and δ 6.94 (J = 8.7 Hz), corresponding to four aromatic protons showing an A_2B_2 pattern distinctive of an oxygen substituent at C-4 in the B-ring. Analysis of the COSY spectrum indicated that signals attributed to the methylene protons (δ 3.40) were coupled to resonances at δ 5.30 and 1.77, assignable to H-1'', H-2'' and H-5'' of the prenyl unit, while the downfield aromatic spin system was made up of six aromatic protons at δ 6.42, 7.73 (J = 8.9 Hz) and δ 6.94, 7.61 (J = 8.7 Hz), respectively, that could be assigned to H-5'/6' and H-3,5/2,6 of the A and B-rings. The ^{13}C NMR spectrum showed 21 signals (Table 1) that included two intense ones at δ 130.3 and 114.4 that were assigned to two equivalent aromatic carbons of the symmetrical B-ring and five signals that were due to the prenyl unit in the A-ring, while the remaining signal at δ 55.4, from its chemical shift and ^{13}C NMR spectrum, was that of a methoxyl group. The most downfield signals

Table 1: ^{1}H , ^{13}C , and HMBC spectral data of heyneanachalcone (**1**).

C/H Position	δ_H	δ_C	^{2}J(HMBC)	^{3}J(HMBC)
1		127.5		
2,6	7.61 (d, *J* = 8.7 Hz)	130.3		C- β
3,5	6.94 (d, *J* = 8.7 Hz)	114.4	C-4	C-1
4		161.5		
4-OCH_3	3.86 s	55.4		C-4
α	7.47 (d, *J* = 15.4 Hz)	117.9	CO	
β	7.86 (*d*, *J* = 15.4 Hz)	144.1	C-1	CO, C-2, C-6
CO		192.1		
1’		114.0		
2’		161.7		
2’-OH	13.09 s			CO
3’		114.1		
4’		163.8		
4’-OH	6.17 s			
5’	6.42 (d, *J* = 8.9 Hz)	107.7		C-1’, C-3’
6’	7.73 (d, *J* = 8.9 Hz)	129.2		C-2’, C-4’, CO
1’’	3.40 (d, *J* = 7.1 Hz)	21.8	C-2’’, C-3’	C-2’, C-4’, C-3’’
2’’	5.30 (t, *J* = 5.8 Hz)	121.1		
3’’		135.8		
4’’	1.85 s	18.3		
5’’	1.77 (d, *J* = 1.0 Hz)	25.8		C-2’’

of the carbon spectrum at δ 163.8, 161.7 and δ 192.1 (CO) were characteristic of quaternary carbons bearing oxygen functions.

The arrangement of these substituents and the placement of the 3-methylbut-2-enyl side chain in the A ring was achieved through the HMBC experiment (Figure 1, Table 1). The methoxyl group at δ 3.86 exhibited a ^{3}J correlation between its protons and the quaternary carbon at C-4 (δ 161.5). Furthermore, the H-3/ H-5 protons showed consistent ^{2}J correlations with the quaternary carbon suggesting that the methoxyl was bonded at C-4 of the B-ring. The Ar-methines at δ 6.42 and 7.73 revealed the expected ^{3}J correlations with the quaternary carbons C-1’, C-3’ and C-2’, C-4’ and the carbonyl group (δ 192.1), clearly suggesting that the methines remained unsubstituted and therefore the aromatic hydroxyl was located at C-4’ of the A-ring. The placement of the side chain at C-3’ of a trisubstituted A-ring was supported by a clear ^{2}J correlation between the H-1’’ of the prenyl unit and C-3’ (δ 114.1) and ^{3}J correlations with the oxygen bearing carbons C-2’ (δ 161.7), C-4’ (δ 163.8), the quaternary carbon C-3’’ (δ 135.8) and a ^{4}J correlation to C-1’ (δ 114.0), respectively. Therefore (**1**) was considered as a 2’,4’-dihydroxychalcone having a prenyl unit at C-3’ and a lone methoxyl at C-4. Further confirmation came from comparing the observed ^{1}H and ^{13}C chemical shifts with those of its related isomer, 4-hydroxyderricin **1a** reported from the roots of *Angelica keiskei* [10]. These data allowed the identification of **1** as 2’.4’-dihydroxy-4-methoxy-3’-(3-methylbut-2-enyl)-chalcone, a new prenylchalcone.

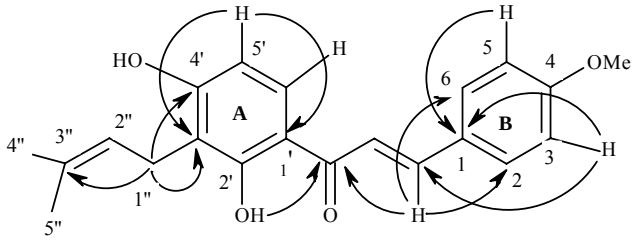
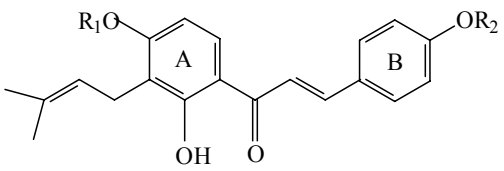


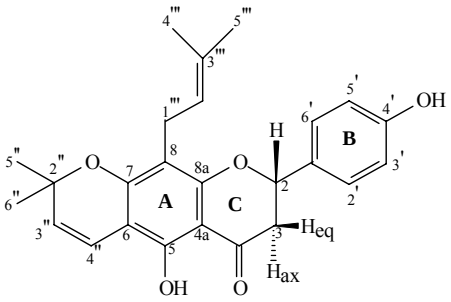
Figure 1: Correlations observed with heteronuclear long-range coupling ^{13}C-^{1}H-COSY spectra (HMBC) of heyneanachalcone (**1**).

Two C-6/8 prenylated flavanones having oxygenation at C-5, and 4’ identified as lupinifolin (**2**) and hiravanone (**3**), and a 5,7-dihydroxy-3’,4’-methylenedioxy isoflavone, named, derrubone (**4**) were characterized by analysis of their 1D and 2D-NMR and comparison with reported literature data [5, 11, 12]. The ^{1}H and ^{13}C NMR assignments were confirmed by HSQC (^{1}J C-H), COSY and HMBC (^{2}J, ^{3}J) heteronuclear coupling experiments.

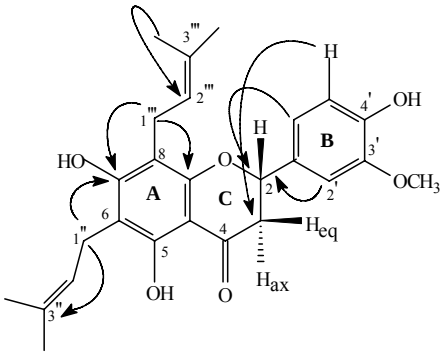


R_1=H, R_2=CH_3., Hyneanachalcone **1**

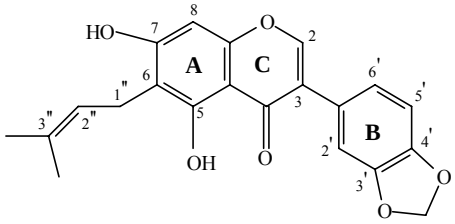
R_1=CH_3, R_2=H., Hydroxyderricin **1a**



Lupinifolin **2**



Important HMBC Correlations of Hiravanone **3**



Derrubone **4**

Flavonoids have been widely reported to possess several biological properties that include anticancer, antioxidant, antimicrobial, and anti-inflammatory properties [13]. Metabolite **1** proved to be moderately active, when tested against a series of microorganisms [14] that included *Bacillus pumilis* (NCIM 2327), *Staphylococcus aureus* (NCIM 2492), *Escherichia coli* (NCIM 2345) and *Proteus vulgaris* (NCIM 2027) with minimal inhibitory concentrations (MICs) of 30, 7.5, 15 and 30 μg/ml, respectively. The above results were compared with the standard, Chloramphenicol.

From a chemotaxonomic perspective, the isolation of polyoxygenated *C*-prenylflavonoids **1-4** from *D. heyneana* is quite consistent with reports prior to this study, regarding the chemical profile of *Derris*. Flavonoids associated with *Derris* have shown oxygenation at C-5/7/3'/4' and prenylation restricted to C-6/8 of the A-ring, which in certain cases have undergone cyclization, as in **2** (linear). Compounds **2** and **4** have been previously reported from *Derris reticulata* [5] and *D. robusta* [12], while this is the first record of **3** from a *Derris* species.

Experimental

General experimental techniques: Melting points (uncorrected) were measured on a Cipla I-28 digital melting point apparatus. Silica gel (Acme, 60-120 mesh) was used for column chromatography and silica gel (Acme) for preparative thin layer chromatography. Spots on plates were detected under UV light (λ 254 and 366 nm) and by spraying with 5% H_2SO_4 in methanol followed by gentle heating. ESI mass spectra were recorded on a Quattro Triple Quadrupole Mass Spectrometer, Finnigan TSQ 7000 with nano-ESI API ion source. EI spectra were recorded on a Finnigan MAT 95 spectrometer at 70 eV. The NMR experiments (both 1D and 2D) were obtained on a Varian Unity 300 spectrometer operating at 300.145 MHz for ^{1}H and 75.77 MHz for ^{13}C, respectively.

Plant material: The leaves (1 kg) of *Derris heyneana* were collected from Amboli Ghat, Konkan coast of Maharastra, India, in July, 2004. The sample was authenticated by Dr P.S.N. Rao, Joint Director, Botanical Survey of India, Western Circle-7, Pune, India. A voucher specimen (SG/DHL/04/333) has been deposited at the Herbarium, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

Extraction and isolation: Extraction of the leaves (1 kg) with chloroform (at room temp. and the solvent removed *in vacuo*) yielded 12 g of crude extract. This extract was applied to a silica gel (275 g) column, using *n*-hexane, *n*-hexane-chloroform and chloroform as eluents, resulting in 10 major fractions (A-J). Fractions A-C were combined after they were found to be similar on TLC and yielded stigmasterol

(12 mg; 0.12 %). Fraction D-E on crystallization using Petroleum ether-benzene yielded **1** as a yellow crystalline powder (16 mg; 0.16 %). Fraction F, on purification using PTLC followed by crystallization using chloroform afforded **2** as yellow needles (7 mg; 0.07 %) while G, on repeated purification using PTLC (petroleum ether-$CHCl_3$; 4:6) gave **3** as a pale yellow powder (9 mg; 0.09 %). Fractions H-I were mixed, followed by TLC as a final purification step to give **4** as a pale yellow powder (5 mg; 0.05 %). Flavonoids **1-4** fluoresced yellow on TLC and intensified on exposure to ammonia vapor.

Heyneanachalcone [**2',4'-Dihydroxy-3'-(3-methylbut-2-enyl)-4-methoxychalcone** (**1**)]

Deep yellow powder.
MP: 112-114 ºC.
^{1}H NMR($CDCl_3$): Table 1.
^{13}C NMR ($CDCl_3$): Table 1.
HMBC ($CDCl_3$): Table 1 and Figure 1.
(+)-ESI HRMS *m/z*: 339.15926 $[M+H]^+$), calcd for $C_{21}H_{23}O_4S$: 339.411; found: 339.159.
Anal. Calcd for $C_{21}H_{23}O_4$: C, 74. 31; H, 6.83; O, 18.85. Found C, 74.23; H, 6.92, O =18.78.

Lupinifolin [**5,4'-dihydroxy-8-(3-methylbut-2-enyl)- 5'',6''-dimethyl-6,7-pyranochalcone** (**2**)]

Pale yellow needles.
MP: 114-116 ºC (Lit. MP: 110 ºC [5]).
^{1}H NMR and ^{13}C NMR data were in agreement with the published data [5].
(+)-ESI MS *m/z*: 407.1 ($[M+H]^+$) according to $C_{25}H_{27}O_5$.
Anal. Calcd for $C_{25}H_{27}O_5$: C, 74.53; H, 6.55; O, 20.41. Found C, 74.42; H, 6.58; O = 20.14.

Hiravanone [**4',5,7-trihydroxy-3'-methoxy-6,8-diprenylchalcone** (**3**)]

Pale yellow powder.
MP:186 ºC.
^{1}H NMR and ^{13}C NMR, COSY and HMBC data were in accord with the published data [11].
(+)-ESI HRMS *m/z*: 439.21145 ($[M+H]^+$) calcd for $C_{26}H_{31}O_6$ S: 439.528; found: 439.212.
Anal. Calcd for $C_{26}H_{31}O_6$: C, 71.21; H, 6.89; O, 21.89. Found C, 71.15; H, 6. 96; O, 21.83.

Derrubone [**5,7-dihydroxy-3',4'-methylenedioxy-6-prenylisoflavone** (**4**)]

Pale yellow powder.
MP: 202-204 ºC (Lit. MP: 210-211 ºC [12]).
^{1}H NMR and ^{13}C NMR data were in agreement with that published in the literature [12].
(+)-ESI HRMS *m/z*: 367.11762 ($[M+H]^+$) calcd for $C_{21}H_{18}O_6S$: 366.370; found: 366.110.

Antimicrobial testing: Minimum inhibitory concentrations (MICs) of heyneanachalcone were determined by two-fold serial dilution technique as described by the Indian Pharmacopeia (1985). The bacterial inoculum was prepared in 100 ml nutrient agar broth and incubated at 37°C, over night. The study involved a series of ten test tubes against the microorganisms, *Bacillus pumilis* (NCIM 2327), *Staphylococcus aureus* (NCIM 2492), *Escherichia coli* (NCIM 2345) and *Proteus vulgaris* (NCIM 2027). Heyneanachalcone was dissolved in 30% dimethylsulfoxide (DMSO). To the first test tube 1.8ml of seeded broth was transferred and then 0.2ml of the test compound (1mg/ml) was added to it and mixed thoroughly. To the remaining nine assay tubes, 1ml of seeded broth was then transferred. From the first test tube, 1ml of the content was pipetted out into a second tube and this was mixed thoroughly. The two fold serial dilution was prepared up to the tenth test tube. The addition of the compound and the serial dilution was done under aseptic conditions. Solvent control and the standard, chloramphenicol were maintained through out the entire experiment. The test tubes were placed in the incubator at (37 ºC) and left for about 24 hours. The antimicrobial testing was conducted in duplicate. MICs were determined as the lowest concentration of the test compound that completely inhibited the growth of micro organisms.

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