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Prenylated chalcones isolated from *Crotalaria genus* inhibits in vitro growth of the human malaria parasite *Plasmodium falciparum*☆

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Abstract—A prenylated chalcone **2** named crotaorixin, has been isolated from the aerial parts of the *Crotalaria orixensis*. Its structure has been established by extensive 1D and 2D NMR measurements. In vitro antimalarial activity of crotaorixin as well as few prenylated chalcones isolated from *C. medicagenia* and *C. ramosissima* were evaluated at three concentrations (50, 10 and 2 μg/ml) against *Plasmodium falciparum* (Strain NF-54). Compound **3** has exhibited 100% inhibition of schizont maturation at 2 μg/ml concentration.

1. Introduction

The genus *Crotalaria* belongs to the family of Leguminosae, sub-family papilionaceae, tribe genistae and it is essentially restricted to the tropical and subtropical areas of the world. About 15 species of *Crotalaria* have been reported to occur in India. The phytochemistry of this genus has been quite well investigated in view of its importance in Indian traditional medicine, and our own studies of this genus have provided several new polyphenolic compounds.[1–4] In a continuing search for chalcones, we report here the structure of a chalcone **2** from the aerial parts of the *Crotalaria orixensis*[16] and evaluation of in vitro antimalarial activity of compound **2** and few previously isolated compounds, that is, chalcone **3** from *C. medicagenia*[5] and chromenodihydrochalcones **4**–**6** from *C. ramosissima* (Fig. 1).[6]

2. Results and discussion

Compound **2**, $C_{21}H_{22}O_5$, was isolated from the ethyl acetate extract of the aerial parts of *C. orixensis* as yellow crystals.

The UV spectrum showed absorptions at 254, 288 and 375 nm, indicating the likely presence of substituted aromatic rings and an α–β unsaturated ketone in the molecule.[7] Its IR spectrum showed typical absorptions for a hydroxyl group (3360 cm^{-1}) and a carbonyl group (1650 cm^{-1}). The phenolic nature of the compound was indicated by characteristic colour reaction ($FeCl_3$, purple). The ^1H and ^{13}C NMR spectra of compound **2** (Table 1) showed the presence of a 3-methyl-2-butenyl (3,3-dimethylallyl) fragment at δ 1.65, 1.77 for two methyl groups as singlets corresponding to six protons, 3.36 (2H, d, J = 3.0 Hz), and 5.22 (1H, m) were assigned to the olefinic and methylene protons.

The singlet at δ 3.90 corresponds to a methoxyl group. A pair of doublets at 7.40 and 7.79 (d, J = 14.9 Hz) for the chalcone double bond, and a singlet for chelated hydroxyl at 13.8 (interchangeable with D_2O) provide further evidence for the 2′-OH chalcone system. The phenyl ring attached to C-β carries a *para*-hydroxyl group and an additional methoxy group at C-3, as shown by the correlation between the methoxy protons and C-3. The

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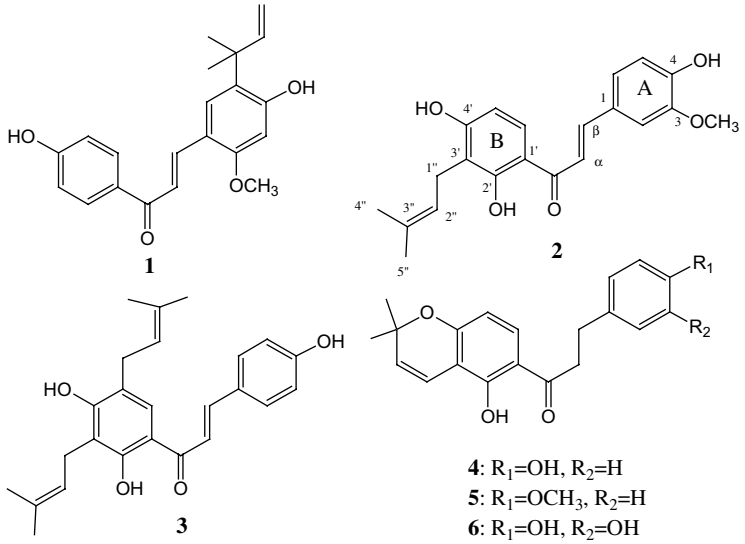

Figure 1. Licochalcone A (**1**) isolated from the licorice roots and crotaorixin (**2**) from *Crotalaria orixensis*; medicagenin (**3**) from *C. medicagenia* and crotaramosmin (**4**), crotaramin (**5**) and crotin (**6**) from *C. ramosissima*.

Table 1. ^{1}H and ^{13}C chemical shifts for compound **2** (300 MHz, $CDCl_3$ + DMSO-d_6) including ^{13}C, ^{1}H long-range correlations (HMBC, optimized to 7 Hz)

Position	δ ^{1}H (J_{HH}, Hz)	δ ^{13}C	HMBC ^{13}C-partners
1	—	126.7	—
2	7.10 (2.1)	110.8	3,4,6,β
3	—	149.4	—
4	—	147.7	—
5	6.89 (8.1)	115.4	1,3,4
6	7.19 (8.1) (2.1)	123.4	2,4,β
α	7.40 (14.9)	117.1	1,C=O
β	7.79 (14.9)	143.9	1,2,6,α,C=O
C=O	—	191.2	—
1′	—	112.7	—
2′-OH	13.8	163.6	—
3′	—	114.7	—
4′-OH	9.6	162.0	—
5′	6.52 (7.9)	107.1	1′,3′,4′
6′	7.60 (7.9)	128.7	2′,4′,C=O
1″	3.36	21.2	2″,2′,4′
2″	5.22	122.2	1″,4″,5″
3″	—	130.3	—
4″	1.65	25.3	5″
5″	1.77	17.4	4″
OCH_3	3.90	55.6	3

corresponding three-proton spin system fits to that structure in its typical ^{1}H NMR chemical shifts and coupling constants.[8] The substituent at the carbonyl group is a tetra-substituted benzene ring with two *ortho*-positioned hydrogens coupling with each other. *ortho*-Coupled doublets are seen at δ 6.52 (1H, d, J = 7.9 Hz) and δ 7.60 (1H, d, J = 7.9 Hz) due to the 5′ and 6′ protons in B-ring. The long-range ^{13}C, ^{1}H couplings (HMBC, Table 1) provided further support for the proposed structure.[9]

The presence of a prenyl group in B-ring is indicated by mass fragmentation *m*/*z* 205. A complementary pair of ions at *m*/*z* 205 and 149 accounts for the mass of the molecular ion together. The pairs of signals at 205/149, and 204/150 evidenced the chalcone–flavanone isomerisation. These fragments result only if B-ring contains the prenyl group along with the two hydroxyls.

While preparation of this manuscript in progress, a paper describing the enzymatic synthesis of compound **2** by prenylating 2′,4′,4-trihydroxy-3-methoxy chalcone with prenyltransferase enzyme derived from microsomal fractions of cell cultures of *Morus nigra* was published.[10] To our knowledge compound **2** is not known as a plant secondary metabolite in the literature.

3. In vitro antimalarial activity

Licochalcone A **1** isolated from Chinese licorice[11] roots has been reported for its antimalarial activity and prompted us to screen our compound's activity as a part of our drug discovery programme. In vitro antimalarial activity[12–15] of all the compounds was studied at three concentrations (50, 10 and 2 μg/ml) against malaria parasite *P. falciparum* (Strain NF-54). Compound **2** has exhibited 100% inhibition of maturation of parasites from ring stage to schizont stage both at 50 and 10 μg/ml concentrations. The diprenylated compound **3**, which was isolated from the roots of *C. medicagenia* has inhibited the parasites 100% at 2 μg/ml concentration while the chromenodihydrochalcones **4**–**6** isolated from the aerial parts of *C. ramosissima* showed lower order of activity. Compound **4** in which the 4′hydroxyl group is involved in the chromene formation and α–β unsaturated double bond was absent has 100% inhibition at the concentration of 10 μg/ml and its 4-*O*-methyl derivative **5** has shown same inhibition at 50 μg/ml concentration, whereas compound **6** was inactive at 50 μg/ml also. Our in vitro antimalarial activity results disclose that the substitution at the 4′hydroxyl group in ring-B as in compound **4**–**6** and 4-hydroxyl as in compound

5, respectively decrease the activity. The substitution pattern in ring-A play an important role in the activity. The compound which has 3,4-disubstituted benzene system (ring-A) as in compound **2** has exhibited lower order of activity (10 μg/ml) and compound **6**, which has similar disubstituted benzene system (ring-A) and whose 4′hydroxyl group is involved in the chromenylation was inactive even at 50 μg/ml. The diprenylated compound **3** which has shown more significant activity than the other chalcones indicates that the prenylation with free 4,4′-dihydroxy system lead to improved activity The reference drug chloroquine has exhibited 100% inhibition at 0.25 μg/ml concentration in the same test system.

4. Conclusion

In summary we have isolated and characterized a prenylated chalcone, hitherto not reported as a plant secondary metabolite from *C. orixensis* and screened for its in vitro antimalarial activity along with a few previously isolated prenylated chalcones and dihydrochromenochalcones isolated from two different species of *Crotalaria* genus to provide a scientific rational for the antiprotozoal potency of plants used in ethnomedicine in the search of new antiprotozoal drugs. Prenylated chalcones are new class of lead compounds, which have shown antiparasitic activity in our studies, and synthesis of appropriate substituted derivatives may pave the way to develop a potent synthetic antimalarial drug.

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15. In vitro antimalarial activity: the in vitro antimalarial activity of compound was assessed by evaluating the minimum inhibitory concentration (MIC) against *Plasmodium falciparum* by following Rieckman's microassay technique with minor modification. Asynchronous parasites obtained from cultures of *P. falciparum* (Strain NF-54) were synchronized after 5% sorbital treatment so as to obtain only ring stage parasites. Parasite suspension medium RPMI-1640 at 1–2% parasitimia and 3% hematocrit was dispensed into sterile 96 well plates; and test compounds were serially diluted in duplicate wells so as to obtain final concentrations of 50, 10 and 2 μg/ml. The culture plates were incubated in a candle jar at 37 °C for 34–36 h. Thin blood smears from each well were microscopically examined and the concentration, which fully inhibited the maturation of the ring stage parasites in to schizont stage, was recorded as MIC.
16. Experimental: plant material was collected from Regional Engineering College campus, Warangal, India and identified by Dr. V. S. Raju and Dr. A. Ragan, Department of Botany, Kakatiya University. Voucher specimen (No. CO-12) is deposited in the Department of Botany, Kakatiya University, Warangal, India. The fresh aerial plant parts (5 kg) of *C. orixensis* were macerated with ethanol in a blender and kept under the solvent for a period of two weeks. The solvent was removed by rotary evaporator. The concentrate was extracted with toluene to remove chlorophyll. Subsequent extraction with ethyl acetate and column chromatography of ethyl acetate fraction (silica gel 100–200 mesh and hexane/ethyl acetate, 85:15) gave compound **2** after evaporation as yellow crystals (50 mg), mp 154–156 °C, homogenous by TLC in hexane/ethyl acetate (65:35 $R_f = 0.55$) solvent system. Silica gel for column chromatography (Merck, 100–200 mesh) was used as received. Thin layer chromatography was carried out using Merck pre-coated silica gel sheets (0.25 mm, 60 F_{254}). UV spectra were recorded on a Hitachi UV 3200 spectrophotometer, IR spectra on a JASCO 302-A spectrophotometer. Melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. Mass data was obtained on a Varian MAT 311A (EI) mass spectrometer, ^{1}H and ^{13}C NMR spectra on Bruker DRX-300 spectrometer.