

Notes

Munchiwarin, a Prenylated Chalcone from *Crotalaria trifoliastrum*

Shu-Wei Yang,[†] Geoffrey A. Cordell,^{*†} Hermann Lotter,[‡] Hildebert Wagner,[‡] Basuvoju C. Mouly,[‡] A. V. N. Appa Rao,[§] and Poruri S. Rao^{||}

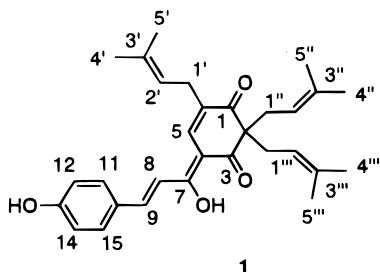
Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, Institut für Pharmazeutische Biologie, Universität München, Karlstrasse 29, D-80333, Munich, Germany, S.R.A.S. College, Kothagudem, 507 101, India, Institute of Pharmaceutical Sciences, Sri Padmavathi Mahila Viswavidyalayam, Tirupathi, 517 502, India, and Department of Chemistry, Kakatiya University, Warangal 506 009, India

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Munchiwarin, a chalcone with the first 2,2,6-tri-isoprenyl-cyclohex-5-ene-1,3-dione skeleton, was isolated from *Crotalaria trifoliastrum* and structurally identified by various NMR techniques in combination with X-ray crystallography.

It was observed that the roots of several *Crotalaria* species (Leguminosae) are pigmented with yellow to orange substances. Some of these pigments, including medicagenin from *Crotalaria medicagenia*¹ and crotramomin and ramosimin from *Crotalaria ramosissima*,^{2,3} have been isolated and identified.

In a continuation of these studies, two colored substances, a novel chalcone, munchiwarin (**1**) (orange color),



and medicagenin (yellow color) were isolated and identified from the roots of *Crotalaria trifoliastrum* Willd. (Leguminosae). Munchiwarin, an isomer of sophoradin, is being reported for the first time. Pyrrolizidine alkaloids were isolated and identified previously from *Crotalaria trifoliastrum*.^{4,5} Chalcones with a tri-isoprene substitution on the 1,3-diphenyl-propene skeleton, such as sophoradin,⁶ have been isolated previously from *Sophora subprostrata* and possess anti-ulcer activity.^{6–10}

We describe here the structure elucidation of **1** and the unambiguous assignment by various NMR techniques, including 2D ¹H–¹H COSY, HETCOR, and HMBC, as well as X-ray crystallography. Munchiwarin (**1**) is the first natural product possessing a 2,2,6-tri-isoprenyl-cyclohex-5-ene-1,3-dione ring system.

Munchiwarin (**1**) was isolated, by Si gel column chromatography, as orange needlelike crystals. The molecular

weight (*m/z* 460) of **1** was deduced from the negative FAB at *m/z* 459 (*M*⁺ – 1). UV spectrometry indicated a highly conjugated system (λ 422 nm; log ϵ 4.48), and the IR spectrum showed two highly conjugated ketone groups (ν 1595 and 1581 cm^{-1}), numerous unsaturated carbons, and hydroxy (ν 3345 cm^{-1}) functionalities. In the ¹H NMR and ¹H–¹H-COSY spectra, a pair of doublets at δ 7.50 (H-11 and H-15, *J* = 8.6 Hz) and 6.91 (H-12 and H-14, *J* = 8.6 Hz) indicated the presence of a *p*-hydroxy substituted phenyl group, and another pair of doublets at δ 7.76 (H-9, *J* = 15.4 Hz) and 6.93 (H-8, *J* = 15.4 Hz) indicated a *trans*-configured double bond. In the upfield region, six methyl groups at δ 1.75 (H₃-4', *s*), 1.66 (H₃-5', *s*), and 1.52 (H₃-4'', H₃-4''', H₃-4''', and H₃-5''', *s*) combined with three =CH–CH₂– groups in a typical isoprene pattern suggested that three isoprene units were present. One of the isoprene units was observed at δ 3.01 (H₂-1', *d*, *J* = 7.0 Hz), 5.14 (H-2', *t*, *J* = 7.0 Hz), and at δ 1.75 (H₃-4', *s*) and 1.66 (H₃-5', *s*). The other two sets of isoprene signals overlapped and appeared as a doublet at δ 2.63 (H₂-1'' and H₂-1''', *J* = 7.5 Hz) and a triplet at δ 4.77 (H-2'' and H-2''', *J* = 7.5 Hz), indicating that these two isoprene units are magnetically equivalent. These suggestions were confirmed by ¹³C NMR and HMBC (H₃-4' and H₃-5' to C-2' and C-3'; H₃-4'' and H₃-5'' to C-2'' and C-3''), which also showed that the two identical isoprene units are proximally located (described later).

In the ¹³C NMR spectrum (in addition to the *p*-hydroxyphenyl, one *trans*-olefin, and three isoprene units), two conjugated ketone functionalities, one =CH– (singlet proton in an isolated environment), and one saturated and three unsaturated quaternary carbons were noted. From the HMBC spectrum, the *p*-hydroxyphenyl group was connected with a CH=CH group (H-11 to C-9; H-9 to C-11; H-8 to C-10), and one of the olefinic carbons (C-8, δ 113.9) was three bonds away from a hydroxy proton (7-OH δ 16.71, *s*) which might form a hydrogen bond with an oxygen atom (3-C=O). The other two long-range hetero-correlations from this hydroxy proton through two and three bonds are to δ 174.1 (C-7) and 107.7 (C-4), suggesting the presence of =C–OH (C-7, δ 174.1) and =C< (C-4, 107.7) carbons, which are connected to –CH=CH– (C-8 and C-9),

* To whom correspondence should be addressed. Tel.: (312) 996-7245. Fax: (312) 996-7107. E-mail: cordell@uic.edu.

[†] University of Illinois at Chicago.

[‡] Universität München.

[§] S.R.A.S. College.

^{||} Sri Padmavathi Mahila Viswavidyalayam.

^{||} Kakatiya University.

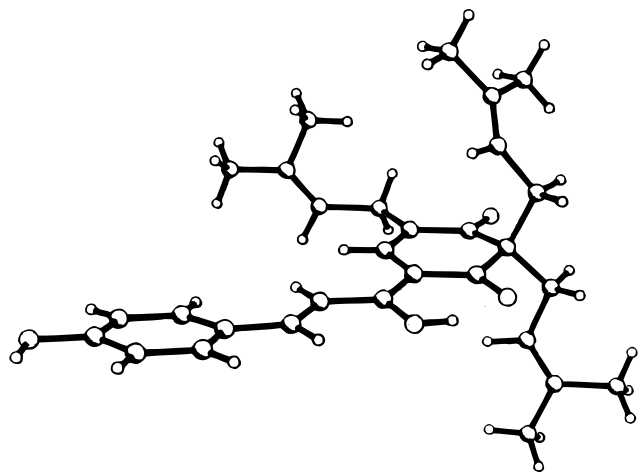


Figure 1. Crystal structure of munchiwarin (**1**).

based on the observation of correlations of C-7 to H-8 and H-9. The tri-isoprenyl-substituted cyclohex-5-ene-1,3-dione ring system could be established by the HMBC spectrum according to the following correlations: H-1'' and H-1''' to one aliphatic quaternary carbon C-2 (δ 63.4) and two conjugated ketone groups, C-1 (δ 200.2) and C-3 (δ 206.0). Because these two isoprene units are symmetrically and magnetically equivalent, the most probable arrangement is di-isoprenyl substitution on the same carbon (C-2) and adjacent to two keto groups (C-1 and C-3). The correlations of H-1' to C-1 and C-5 (a =CH-) revealed the position of the third isoprene substitution, that is, neighboring to one of the carbonyl groups (C-1) and to an olefinic CH (C-5). The correlations of H-5 to C-1, C-3, C-6, and C-7 successfully connected both structural units (phenyl-propenyl and tri-isoprene-diketo units). The location of the unsaturated quaternary C-6 was also confirmed by the correlation of H-2' to C-6. The suggested structure shows the proximity of the 7-OH and the oxygen on the 3-keto group, in which a hydrogen bond could be formed that could explain the very downfield chemical shift of the 7-OH (δ 16.71). The unambiguous assignments were achieved by the interpretation of ^1H - ^1H COSY, HETCOR, and HMBC spectra.

Due to the unusual downfield chemical shifts of C-2 and C-7 and their functionalities, the lack of certain long-range correlations such as H-8 and H-5 to C-4 and of H-2'' and H-2''' to C-2 in the HMBC spectrum, the existence of several unsaturated and quaternary carbons, and the symmetrical disubstitution (2,2-diisoprene), as well as the unusual skeleton, the proposed structure of munchiwarin (**1**) was established by X-ray crystallography.

The crystal structure of munchiwarin (**1**) is shown in Figure 1. The basic skeleton is a long conjugated system from the phenol to a keto-stabilized resorcinol group with three isopentenyl units attached. The conjugated unit is rather planar with a mean deviation from the best plane of only 0.09 Å; hence the orange color of the substance. The planarity of the structure is additionally supported by a hydrogen bond between the hydroxy group at the C-7 position and the carbonyl at position 3. All three isopentenyl side chains show extremely high-temperature vibrations.

In conclusion, munchiwarin (**1**) is the first prenylated chalcone containing a 2,2,6-tri-isoprenylated cyclohexene moiety. The isolate is therefore an isomer of sophoradin.⁶ The highly conjugated system makes the two-ring system coplanar, and the two isoprene units on C-2 are oriented each perpendicular to the plane. The 7-hydroxy proton is

Table 1. Unambiguous Assignments of the ^1H and ^{13}C Resonances and HMBC Correlations of Compound **1**

1	^1H ppm (Hz) ^a	^{13}C ppm ^c	HMBC
1		200.2 (s)	H-5, H ₂ -1', H ₂ -1'', H ₂ -1'''
2		63.4 (s)	H ₂ -1'', H ₂ -1'''
3		206.0 (s)	H-5, H ₂ -1'', H ₂ -1'''
4		107.7 (s)	7-OH
5	7.32 (1H, s)	136.1 (d)	H ₂ -1'
6		129.9 (s)	H-5, H ₂ -1'
7		174.1 (s)	H-5, H-8, H-9, 7-OH
8	6.93 (1H, d, 15.4)	113.9 (d)	7-OH
9	7.76 (1H, d, 15.4)	142.5 (d)	H-11, H-15
10		127.6 (s)	H-8, H-12, H-14
11	7.50 (2H, d, 8.6)	130.3 (d)	H-9, H-15 (H-9, H-11)
12 (14)	6.91 (2H, d, 8.6)	116.1 (d)	
13		158.3 (s)	H-11, H-12, H-14, H-15
1'	3.01 (2H, d, 7.0)	28.0 (t)	H-5
2'	5.14 (1H, t, 7.0)	121.3 (d)	H ₂ -1', H ₃ -4', H ₃ -5'
3'		133.7 (s)	H ₂ -1', H ₃ -4', H ₃ -5'
4'	1.75 (3H, s)	25.8 (q)	H-2', H ₃ -5'
5'	1.66 (3H, s)	17.9 (q)	H-2', H ₃ -4'
1'' (1''')	2.63 (4H, d, 7.5)	38.4 (t)	
2''	4.77 (2H, t, 7.5)	117.9 (d)	H ₂ -1'', H ₃ -4'', H ₃ -5'' (H ₂ -1''', H ₃ -4''', H ₃ -5''')
3''		135.0 (s)	H ₂ -1'', H ₃ -4'', H ₃ -5'' (H ₂ -1''', H ₃ -4''', H ₃ -5''')
4''	1.52 (6H, s) ^b	25.8 (q)	H-2'', H ₃ -5'' (H-2''', H ₃ -5''')
5''	1.52 (6H, s) ^b	17.9 (q)	H-2'', H ₃ -4'' (H-2''', H ₃ -4''')
7-OH	16.71 (1H, s)		
13-OH	6.11 (1H, s)		

^a Data were obtained at 300 MHz in CDCl_3 and the coupling constants were recorded in Hz. ^b The ^1H signals of H₃-4'' (H₃-4''') and H₃-5'' (H₃-5''') overlapped and were observed as a singlet. ^c Multiplicity was determined by an APT experiment. HETCOR was used to correlate the corresponding ^1H resonances.

proximal to the 3-keto oxygen atom and forms a stable hydrogen bond. Munchiwarin (**1**) was evaluated for cytotoxic activity against a panel of human cancer cell lines and for inhibition of HIV-1 reverse transcriptase. The results show that compound **1** is inactive against various cell lines,¹¹ including KB (IC₅₀ 11 $\mu\text{g}/\text{mL}$), KB-V(+VLB) (13 $\mu\text{g}/\text{mL}$), KB-V(-VLB) (14 $\mu\text{g}/\text{mL}$), Col2 (9 $\mu\text{g}/\text{mL}$), BCL (9 $\mu\text{g}/\text{mL}$). It did not show inhibition of HIV-1 reverse transcriptase.¹²

Experimental Section

General Experimental Procedures. ^1H , ^{13}C , APT, HETCOR, and ^1H - ^1H COSY spectra were recorded on a Varian XL-300 instrument operating at 300 MHz. HMBC spectrum was performed on a GE Omega 500 MHz instrument (499.9 MHz for ^1H NMR) with the standard programs. CDCl_3 was used as an internal standard ($\delta_{\text{CHCl}_3} = 7.24$ ppm), and chemical shifts are reported in parts per million on the δ scale; coupling constants (J) are in Hertz.

Plant Material. Plants of *Crotalaria trifoliolatum* were collected from the garden of the Chemistry Department, Kakatiya University, Warangal, in October 1993. The plant material was identified by Dr. V. S. Raju in the Plant Systematics Laboratory, Department of Botany, Kakatiya University, Warangal. A voucher specimen of the plant material (P. S. R.-C. R. T.) is being maintained in the Chemistry Department of Kakatiya University. Roots were separated from the freshly collected plants and used for extraction.

Isolation and Purification of 1. Roots (1 kg) separated from the whole plants of *C. trifoliolatum* were extracted with Me_2CO for a week at room temperature. The Me_2CO extract of the roots was decanted and concentrated under reduced pressure. The Me_2CO extract (8.5 g) was triturated with

n-hexane (3 × 100 mL), and the decantate was evaporated to yield a yellow residue (1.4 g).

TLC of the *n*-hexane extract in CHCl₃ revealed the presence of two spots, one orange (*R_f* 0.5) and the other yellow (*R_f* 0.2). The *n*-hexane extract (1.4 g) was dissolved in CHCl₃ and adsorbed on to Si gel (10 g). This material was chromatographed on a column packed with Si gel H (200 to 400 mesh, 50 g), and elution was carried out with C₆H₆, C₆H₆-CHCl₃ mixtures, and finally with CHCl₃. Elution with C₆H₆-CHCl₃ (1:3) gave orange-red crystals of munchiwarin (**1**) (25 mg, yield 0.0025%).

Elution with CHCl₃ yielded a yellow crystalline compound (20 mg, yield 0.002%), identified as medicagenin by melting point, MS, and ¹H NMR.¹

Munchiwarin (1): C₃₀H₃₆O₄, obtained as orange needles; mp 130 °C; UV (MeOH) λ_{max} (log ε) 347 (sh, 4.25), 422 (4.48), and 445 nm (sh, 4.41); IR ν_{max}, 3345 (OH), 2982, 2932, 1595 (C=O), 1581 (C=O), 1539, 1447, 1269, 1169, and 829 cm⁻¹; for the unambiguous assignments of ¹H NMR and ¹³C NMR, see Table 1. Negative FAB MS *m/z* 459 [M - 1]⁺; EIMS 386, 371, 253, and 119; in HRFABMS, the accurate MS could not be obtained due to the low intensity of the molecular ion peak.

X-ray Crystallographic Analysis of 1. Munchiwarin (**1**) crystallized from CHCl₃ in the form of transparent, very tiny, orange platelets. A crystal of size 0.7 × 0.1 × 0.01 mm was selected for the measurements on a Siemens R3m diffractometer. The unit cell dimensions were determined from 25 centered reflections and least-squares calculations. Crystal data: C₃₀H₃₆O₄, *M* = 460.6, triclinic space group *P*₁, *a* = 10.263 (3), *b* = 11.257 (4), *c* = 12.947 (4) Å, α = 106.70 (2), β = 103.68 (2), γ = 92.93 (2)°, *Z* = 2, *D* = 1.108 g/cm³.

A total of 3714 reflections were measured with Ni-filtered Cu Kα radiation in Ω scan mode and scan speed 1/min up to an 2Θ range of 114°. Because of the small crystal size, only 2083 could be treated as observed with *F* > 4 δ (*F*), *R*_{int} = 0.0%. Due to the extreme platelet shape of the crystal an empirical absorption correction was applied to the measurements (μ = 0.57 mm⁻¹). The crystal deteriorated during the measuring period, as observed by repeated measurement of two control reflections, whose intensities had dropped by 12% by the end of the data collection.

The structure was solved by direct methods using SHELX-TL.¹³ The decision for the centered space group could clearly

be made by the statistic of the E value. Hydrogen atoms were calculated from the positions of the heavier atoms to which they are bound. The refinement initially with isotropic and finally anisotropic temperature factors converged at wR = 6.8% for the observed data (w = unit weight). Surprisingly, application of the absorption correction did not improve the final R value. Full crystallographic data are deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

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