

Short Communication

Isolation of Eriodictyol Identical with Huazhongilexone from *Solanum hindsianum*

D. Rosalba Encarnación,^a Clara L. Nogueiras,^a Victor H. A. Salinas,^a Uffe Anthoni,^b Per H. Nielsen^b and Carsten Christophersen^{b,*}

^aDepartamento de Agronomía, Universidad Autónoma de Baja California Sur, A.P. 19-B, La Paz, B. C. S. 23080, México and ^bMarine Chemistry Section, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark

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The plant *Solanum hindsianum* Benth (Solanaceae) is commonly used in the traditional medicine of Baja California Sur (México) and referred to as 'mariola'. Medicinally it is employed against fever, to normalize menstruation, to facilitate placenta expulsion, and for the relief of earache, gonorrhoea, cough and diarrhoea.¹ In addition to these uses extracts were found to be active against the bacteria *Staphylococcus aureus* and *Bacillus subtilis* and the yeast *Candida albicans*. Bioassay-guided fractionation (activity against *B. subtilis* in the filter paper disk assay) of an ethanolic extract of the branches of *S. hindsianum* eventually resulted in a compound active against *B. subtilis* and *S. aureus*. From the ¹H, ¹³C NMR and mass spectrum it was concluded that the structure was that of 2',4',5,7-, 3',4',5,7-(eriodictyol), 3',5',5,7- or 2',5',5,7-tetrahydroxyflavanone. The last two structures are excluded since the NMR values do not fit the published spectra.^{2,3} 2',4',5,7-Tetrahydroxyflavanone has never been described from Nature but has been synthesized with a reported m.p. of 255–257 °C or (with 1/4 H₂O) 204 °C (decomposition).⁴ These observations indicated that the active principle was most likely eriodictyol.

Eriodictyol has been known since 1940⁵ and is commercially available. As for several other flavanones published spectra are scarce and we have been able to locate only one set⁶ of ¹H and one set⁷ of ¹³C NMR (recorded in DMSO-*d*₆ at 95 °C) assignments. However, although the ¹³C data were comparable, the published ¹H NMR spectrum of synthetic (±)-eriodictyol allegedly recorded in CDCl₃ could not be reproduced with the commercial

product. First of all eriodictyol is so sparingly soluble in CDCl₃ that 80 scans did not show a single line originating from the flavanone. Moreover, the published spectra were different from those obtained from commercial eriodictyol in DMSO-*d*₆, acetone-*d*₆ or CD₃OD. The ¹H and ¹³C NMR spectra of commercial eriodictyol are given in Table 1. A reliable diagnostic feature of eriodictyol is that H-6 and H-8 become isochronous in 10%

Table 1. ¹H (750 MHz) and ¹³C NMR (100.6 MHz) spectral assignments of eriodictyol in acetone-*d*₆. Comparison with ¹H NMR (400 MHz) data of huazhongilexone⁸ in acetone-*d*₆.

Position	δ _C	δ _H (J _{H/H} /Hz)	δ _H (J _{H/H} /Hz) ⁸
2	79.3	5.40 (12.6, 3.1)	5.40 (12.7, 2.8)
3a	42.9	3.14 (17.1, 12.6)	3.25 (17.2, 12.7)
3b		2.73 (17.1, 3.1)	2.73 (17.2, 2.8)
4	196.5		
4a	102.0		
5	164.6 ^b		
6	95.2 ^a	5.94 (2.2) ^a	5.97 (m)
7	166.6		
8	96.1 ^a	5.95 (2.2) ^a	5.97 (m)
8a	163.7 ^b		
1'	129.7		
2'	115.4 ^c	7.04 (1.7)	7.05 (s)
3'	145.7 ^d		
4'	145.4 ^d		
5'	114.1 ^c	6.87 (8)	6.88 (s)
6'	118.6	6.88 (8, 1.7)	6.88 (s)
5-OH		12.17	Not given
7,3',4'-OH		8.5 and 2.98 br	Not given

^{a-d}Signals may be interchanged. ¹³C assignments were confirmed by pulse gradient, reverse-detected HMQC experiments optimized for J_{C,H} 140 and 7 Hz, respectively.

* To whom correspondence should be addressed.

Table 2. Comparison of physical properties from eriodictyol and huazhongilexone.⁸

Physical property	Eriodictyol	Huazhongilexone
M.p./°C	259–261, ⁹ 265, ⁵ 267, ^{a,10} 271, ^{a,10}	286–287
UV λ_{\max}/nm , (log ϵ)	EtOH: 230 (sh, 4.28), 288 (4.26), 328 (sh, 3.64)	MeOH: 230 (sh), 289 (4.83), 325 (sh)
MS m/z	288 (M^+), 179, 153, 136	288 (M^+), 179, 153, 136
IR $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$	3367, 1636, 1602, 1519, 1447, 1160, 1086	3360, 1640, 1602, 1535, 1455, 1160, 1085
CD θ/nm	330, 290 (positive Cotton effect)	330, 290 (positive Cotton effect)

^aRacemate.

C_6D_6 in $\text{DMSO}-d_6$, even at 750 MHz, appearing at δ_{H} 6.01 (s). Another characteristic change in the latter solvent is that the pattern of the low field dd (at δ_{H} 6.88) originating from H-6' partially overlaps one branch of the high field signal δ_{H} 6.87 d originating from H-5' interchange so that the former high field signal now becomes the low field signal and *vice versa*. The data obtained from the flavanone from *S. hindsianum* were identical thus identifying this natural product as eriodictyol.

Huazhongilexone has been isolated from leaves of *Ilex centrochinensis* S. Y. Hu and assigned the structure (2*S*)-3',5',5,7-tetrahydroxyflavanone.⁸ However, this assignment was proved wrong by comparison of the data published with those of an authentic synthetic sample.³ Table 1 compares reassigned proton NMR data (¹³C NMR not given in Ref. 8), and Table 2 other physical data for huazhongilexone and eriodictyol. Since these are virtually identical, we submit that huazhongilexone is actually eriodictyol.

Materials and methods

The ¹H and ¹³C NMR spectra were recorded on a Varian UNITY 400 spectrometer, operating at 400 MHz for protons and at 100.6 MHz for carbons, respectively. Spectra were recorded for samples in $\text{DMSO}-d_6$, acetone- d_6 , CD_3OD or CDCl_3 , which were also used as internal standards in ¹³C NMR spectroscopy; in ¹H NMR spectroscopy TMS was used as an internal standard. Mass spectra were obtained on a JEOL JMS-HX/HX110A spectrometer using the direct inlet system. The ethanol used was of commercial quality and distilled. Methanol (99.9%) was obtained from Baker as was acetone (99.5%). Chloroform (99.8%) was purchased from VWR Brand. Eriodictyol was purchased from Roth Biochemicals.

Isolation and purification of 3',4',5,7-tetrahydroxyflavanone. The plant material was collected near the road 14 km towards Todos Santos on September 5, 1986. The sample was identified by Ing. Jorge Agúndez, Agronomy Department, Universidad Autónoma de Baja California Sur (UABCS) by comparison with an authentic specimen retained at the Pharmacognosy Laboratory of the Agronomy Department at UABCS (México). The aerial part of the dried plant (340 g) was subjected to exhaustive

Soxhlet extraction with petroleum ether, CHCl_3 and EtOH (95%). Concentration under reduced pressure gave the crude extracts (1.7, 7.7 and 38 g, respectively). The ethanolic crude extract (8.5 g) was absorbed on silica gel (45 g) and Soxhlet extracted with acetone, EtOAc, EtOAc–EtOH (8:2), EtOH and MeOH, respectively. The acetone extraction gave a residue (1.5 g) active against *B. subtilis* and *S. aureus* measured by the agar diffusion method at 2 mg/disk. Further purification was affected by two column chromatographic separations (silica gel) using CHCl_3 –EtOAc and CHCl_3 –acetone with a polarity gradient. The resulting fraction (15 mg) showed activity against the same bacteria. A third column (silica gel) fractionation with CHCl_3 –acetone as the eluent provided an almost pure compound (3 mg). Autobiography (about 600 μg applied) showed activity against *B. subtilis* and *S. aureus*.

Eriodictyol: light yellow solid. EIMS m/z (% rel. int.) 288 (100, M^+), 287 (38), 179 (24), 166 (40), 153 (75), 136 (34), 123 (16). $[\alpha]_{\text{D}}^{22} -3.5^\circ$ (c 0.0232, EtOH). ¹H NMR ($\text{DMSO}-d_6$): δ 2.66 (H-3a, dd, 17.0, 3.1), 3.15 (H-3b, dd, 17.0, 12.5), 5.36 (H-2, dd, 12.5, 3.1), 5.85 (H-6, H-8, two s with a spacing of 1.4 Hz), 6.74 (H-5', H-6', two singlets with a spacing of 1.1 Hz), 6.87 (H-2', s), 9.05 (br, 3 H, OH-3', 4' and OH-7, s), 12.15 (br, OH-5). ¹H NMR (CD_3OD): δ 2.74 (H-3a, dd, 17, 3), 3.11 (H-3b, dd, 17, 13), 5.31 (H-2, dd, 13, 3), 5.94, 5.92 (H-6, H-8, two doublets, 2), 6.83 (H-4', H-5', two apparent singlets with a spacing of 0.9 Hz), 6.96 (H-2', dd, 0.7, 1.3). ¹³C NMR ($\text{DMSO}-d_6$): δ 42.1 (C-3), 78.5 (C-2), 95.0 (C-8), 95.8 (C-6), 101.8 (C-4a), 114.4 (C-2'), 115.4 (C-5'), 117.9 (C-6'), 129.5 (C-1'), 145.2 (C-3'), 145.7 (C-4'), 163.6 (C-5), 163.5 (C-8a), 166.6 (C-7), 196.3 (C-4). ¹H and ¹³C NMR data in acetone- d_6 are given in Table 1, other physical data in Table 2.

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