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Short Communication

Isolation of Eriodictyol Identical with Huazhongilexone from *Solanum hindsianum*

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The plant *Solanum hindsianum* Benth (Solanacea) 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 ^{1}H, ^{13}C 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 synthezised with a reported m.p. of 255–257 °C or (with 1/4 H_2O) 204 °C (decomposition).[4] These observations indicated that the active principle was most likely eriodictyol.

Eriodictyol has been known since 1940[5] and is commercially available. As for several other flavanones published spectra are scarce and we have been able to locate only one set[6] of ^{1}H and one set[7] of ^{13}C NMR (recorded in DMSO-d_6 at 95 °C) assignments. However, although the ^{13}C data were comparable, the published ^{1}H NMR spectrum of synthetic (±)-eriodictyol allegedly recorded in $CDCl_3$ could not be reproduced with the commercial product. First of all eriodictyol is so sparingly soluble in $CDCl_3$ 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 CD_3OD. The ^{1}H and ^{13}C 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. ^{1}H (750 MHz) and ^{13}C NMR (100.6 MHz) spectral assignments of eriodictyol in acetone-d_6. Comparison with ^{1}H NMR (400 MHz) data of huazhongilexone[8] 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. ^{13}C assignments were confirmed by pulse gradient, reverse-detected HMQC experiments optimized for $J_{C,H}$ 140 and 7 Hz, respectively.

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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 ν_{max}(KBr)/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)

[a]Racemate.

C_6D_6 in 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 (^{13}C 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 ^{1}H and ^{13}C 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 DMSO-d_6, acetone-d_6, CD_3OD or $CDCl_3$, which were also used as internal standards in ^{13}C NMR spectroscopy; in ^{1}H 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). $[\alpha]_D^{22}$ −3.5° (*c* 0.0232, EtOH). ^{1}H NMR (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). ^{1}H 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). ^{13}C NMR (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). ^{1}H and ^{13}C NMR data in acetone-d_6 are given in Table 1, other physical data in Table 2.

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