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2-METHOXYJUDAICIN, AN ISOFLAVENE FROM THE ROOTS OF *CICER BIJUGUM*

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Key Word Index—*Cicer bijugum*; Leguminosae; wild chickpeas; roots; isoflav-3-ene; 2-methoxyjudaicin.

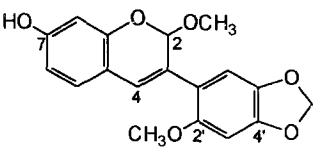
Abstract—A new isoflav-3-ene, 2,2′-dimethoxy-7-hydroxy-4′,5′-methylenedioxyisoflav-3-ene (2-methoxyjudaicin), has been isolated from the roots of *Cicer bijugum*. The structure was determined by NMR spectroscopy and FAB-mass spectrometry. Chromatographic and mass spectral data indicate that this isoflav-3-ene is also present in the plant as a glycoside. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Isoflav-3-enes are an uncommon class of isoflavonoid, with relatively few examples reported in the literature up to the end of 1993 [1–9]. More recently, a new isoflav-3-ene, 7-hydroxy-2′-methoxy-4′,5′-methylenedioxyisoflav-3-ene (judaicin), isolated from root material of *Cicer judaicum*, has been described [10]. The aglycone co-occurs with a 7-*O*-glucoside and 7-*O*-(6″-*O*-malonylglucoside), the first glycosylated isoflav-3-enes to be identified [10]. An additional example, compound **1**, has now been isolated from the roots of *C. bijugum* Rech. f., an annual herb distributed in Anatolia, Syria and Iraq [11]. A glycosidic form of **1** has also been identified, although it is present in only trace amounts. These compounds are of particular interest, as isoflavonoids substituted at the 2-position appear to be extremely rare. Biosynthetic relationships between **1** and other isoflavonoids identified in *C. bijugum* and related *Cicer* species are discussed briefly.

RESULTS AND DISCUSSION

A methanolic extract of *C. bijugum* roots, subjected to analytical HPLC with gradient elution and photodiode array detection, was found to contain five compounds with UV spectra typical of isoflav-3-enes. Three of these were identified immediately as judaicin, judaicin 7-*O*-glucoside and judaicin 7-*O*-(6″-*O*-malonylglucoside), and verified by co-chromatography



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with authentic standards [10]. The UV spectra of these isoflav-3-enes are characterized by maxima at 337 and 235 nm, and a shoulder at 300 nm [10]. Two additional compounds exhibited similar UV spectra but with band I shifted by 12 nm to 325 nm. One of these compounds, **1**, was isolated in quantities of a few milligrams when scaled-up to semi-preparative HPLC but the second could only be obtained on the microgram scale owing to its very low concentration in the root extract. The molecular structure of **1** was determined unambiguously using standard ^{1}H and ^{13}C NMR techniques and mass spectral data.

The ^{1}H NMR spectrum of **1**, although containing relatively few resonances, was complicated by overlap in the aromatic region, which was only partially resolved even at 500 MHz. Resonances could only be correctly assigned by exploiting differential ^{13}C NMR chemical shifts in an HMQC experiment, which uncovered three 1H singlets at δ 6.83 (×2) and 6.84 together with two 1H multiplets at δ 6.42 (*d*, J = 2.4 Hz) and 6.43 (*dd*, J = 8.9 and 2.4 Hz). An additional aromatic resonance was noted at δ 7.04 (*d*, J = 8.9 Hz). The remaining spectral features comprised two methoxyl 3H singlets at δ 3.33 and 3.75, a methylenedioxy 2H singlet at δ 5.99 and a 1H singlet at δ 5.83. These spectral parameters are consistent with those

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Table 1. Comparative ^{13}C NMR data for compound **1** and judaicin [10] (δ in DMSO-d_6, 67.8 MHz, 37°)

C	1	Judaicin
2	97.7	67.5
3	125.5	127.5
4	123.7	121.3
4a	113.7	115.1
5	127.7	127.4
6	109.1	108.6
7	158.8	158.2
8	102.9	102.3
8a	150.8	154.1
1′	118.4	119.5
2′	152.2	152.4
3′	95.7	95.5
4′	147.2	147.5
5′	140.9	141.0
6′	108.3	107.5
2-OMe	54.2	
2′-OMe	56.6	56.5
OCH_2O	101.1	101.1

predicted for an isoflav-3-ene derivative substituted in the C ring. Comparison of this data with corresponding ^1H NMR data for 7-hydroxy-2′-methoxy-4′,5′-methylenedioxyisoflav-3-ene (judaicin) [10] indicates that the major difference between the two compounds can be rationalized by invoking substitution at the C-2 position with an aliphatic methoxyl group. This premise was confirmed by extending the analysis to ^{13}C NMR spectra. Inspection of the data in Table 1, where comparative ^{13}C assignments for **1** and judaicin are given, shows clearly the additional methoxyl group of **1** at δ 54.2 and the dramatic increase in chemical shift at the C-2 position of +30.2 ppm caused by this substituent. The multiplicity of the ^{13}C δ 97.7 resonance of **1** was given as CH from DEPT experiments. An empirical formula for **1** of $C_{18}H_{16}O_6$ was deduced from the NMR data giving a predicted M_r of 328.31. This was in perfect agreement with the $[M]^+$ ion at m/z 328 recorded by FAB-mass spectrometry. A fragment ion at m/z 297 was also observed, corresponding to the loss of a methoxyl group. This is likely to be that at the C-2 position because no fragment ion corresponding to the loss of the C-2′ methoxyl group is found in the mass spectrum of judaicin [10]. Specific assignments for the ^1H and ^{13}C resonances of **1** were verified using DEPT, HMQC and NOE data. The close correspondence between ^{13}C chemical shift values of **1** and judaicin in the A and B rings should be noted. Compound **1** is therefore confirmed as 2,2′-dimethoxy-7-hydroxy-4′,5′-methylenedioxyisoflav-3-ene (2-methoxyjudaicin), a new isoflav-3-ene. This is the first report of an isoflav-3-ene substituted at the 2-position in the C ring (although the configuration is unknown at present). Furthermore, compounds with C-2 substituents are extremely rare within the context of the isoflavonoid class as a whole. Four relatively simple isoflavones with a 2-methyl substituent have been reported previously from roots of *Glycyrrhiza glabra*, including 2-methyl-7-hydroxy-8-acetylisoflavone (glyzarin) [12, 13]. However, it has been questioned whether these compounds, which also lack B-ring substituents, are in fact true isoflavonoids derived from chalcone-flavanone precursors [12]. The novel isoflavan, ferrugin, from *Aglaia ferruginaea* (Meliaceae), also contains a C-2 substituent as part of a bridged ring structure thought to be derived by Woodward–Hoffmann allowed cycloaddition of an ene to an ene-cation [14]. In contrast, there appear to be no examples in the literature of *O*-type substituents at C-2 as described here for compound **1**.

A second compound, **2**, noted from analytical HPLC to have an identical UV spectrum to that of **1**, was distinguished by a significantly shorter retention time. This compound was present at a much lower concentration than **1** in fresh root extracts and only microgram quantities could be obtained, despite scale-up to semi-preparative HPLC. The FAB-mass spectrum of this material exhibited two major mass ions, a $[M]^+$ at m/z 490 and a second $[M-162]^+$ ion at m/z 328, indicating loss of a glycosyl $[C_6H_{10}O_5]^+$ fragment to give the aglycone corresponding to **1**. Fragment ions at m/z 459 and 297 corresponding to loss of a methoxyl group from the glycoside and the aglycone, respectively, were also noted. It appears likely therefore, that compound **2** is a glycoside of **1**, a conclusion which finds support from the known co-occurrence of the isoflav-3-ene judaicin with its 7-*O*-glucoside and 7-*O*-(6″-*O*-malonylglucoside), both in *C. judaicum* [10] and *C. bijugum*. The most likely candidate for the glycoside moiety of **2** is clearly glucose, but it was not possible to confirm this definitively in the absence of further material.

The presence of a 2-methoxyisoflav-3-ene derivative in *C. bijugum* is also of biosynthetic interest. It is generally agreed that the biosynthesis of isoflav-3-enes follows a sequence of steps leading to the immediate precursor, 2′-hydroxyisoflavanol, which is also the key intermediate in the pathway to isoflavans and pterocarpans [15]. Isoflav-3-enes may be further elaborated to give 3-arylcoumarins and, thereafter, coumestans, both of which contain a C-2 carbonyl group. A 2-methoxyisoflav-3-ene derivative is likely to be formed by C-2 hydroxylation of an isoflav-3-ene followed by *O*-methylation. In this context, it is of interest to note that coumestans are also present in the roots of *C. bijugum* (P. C. S. and N. C. V., unpublished results). This indicates that the formation of 2-methoxyisoflav-3-enes does not block the biosynthetic route to coumestans. The putative 2-hydroxyisoflav-3-ene intermediate may therefore represent a branching point in the biosynthetic pathway from isoflav-3-enes.

EXPERIMENTAL

Plant material. Seeds of *C. bijugum* Rech. f. ICCW42 were obtained from the Genetic Resources

Unit of the International Crops Research Institute for the Semi-Arid Tropics and grown under greenhouse conditions at the Royal Botanic Gardens, Kew (accession numbers 1995-426 and 1995-427). Root material was collected when the plants were at the flowering stage (60 days after sowing).

General. NMR spectra were recorded at 500 and 67.8 MHz for ^1H and ^{13}C, respectively. Samples were dissolved in DMSO-d_6 with TMS as a primary reference. A temp. of 37° was used for all NMR expts. FAB-MS (positive mode): 3-nitrobenzyl alcohol matrix.

Extraction and isolation. Fresh root material (25 g) was ground with a pestle and mortar under a minimum vol. of MeOH before further addition of 250 ml MeOH and extraction at room temp. for 24 hr. Filtration of the resulting slurry and evapn of the filtrate under red. pres. gave a dried extract which was subsequently redissolved in MeOH to a concn of 1 g ml^{-1}, with the original plant material as reference. Filtered extract (0.45 μm Millipore filters) was injected directly in 200 μl aliquots onto a Spherisorb 5 ODS semi-prep. column 10 mm (i.d.) × 250 mm. A Waters HPLC system consisting of an LC600 pump and 996 photodiode array detector was used in gradient elution mode. A two-solvent separn system was used for the isolation of **1** and **2** with A = 90% at time $t = 0$ min, A = 45% at time $t = 20$ min and A = 0% at time $t = 25$ min, where A = 2% HOAc and B = 2% HOAc in MeCN. Compounds **1** and **2** were collected manually, with typical yields from a 200-μl aliquot being 75 μg and 2–5μg, respectively. Purified samples were dried under a stream of N_2 followed by further careful drying in a desiccator immediately before analysis.

2,2′-Dimethoxy-7-hydroxy-4′,5′-methylenedioxyisoflav-3-ene (*2-methoxyjudaicin*) (**1**). UV λ_{max}^{MeCN} nm: 235, 300sh, 325. ^1H NMR (DMSO-d_6): δ 7.04 (1H, *d*, $J = 8.9$ Hz, H-5), 6.84 (1H, *s*, H-6′), 6.83 (1H, *s*, H-3′), 6.83 (1H, *s*, H-4), 6.43 (1H, *dd*, $J = 8.9$, 2.4 Hz, H-6), 6.42 (1H, *d*, $J = 2.4$ Hz, H-8), 5.99 (2H, *s*, -OCH_2O-), 5.83 (1H, *s*, H-2), 3.75 (3H, *s*, 2′-OMe), 3.33 (3H, *s*, 2-OMe). ^{13}C NMR: Table 1. ^1H NOESY ('s', 'm', and 'w' refer to strong, medium and weak NOEs respectively): C-2H ↔ C-2OMe(s), C-2′OMe(w), C-6′H(s); C-5H ↔ C-4H(s), C-6H(m); C-2OMe ↔ C-2H(s), C-8H(w), C-6′H(w); C-2′OMe ↔ C-2H(w), C-4H(s). FAB-MS (positive) *m/z*: 328 $[M]^+$, 297 $[M-OCH_3]^+$.

2,2′-Dimethoxy-7-hydroxy-4′,5′-methylenedioxyisoflav-3-ene glycoside (*2-methoxyjudaicin glycoside*) (**2**). UV λ_{max}^{MeCN} nm: 235, 300sh, 325. FAB-MS (positive) *m/z*: 490 $[M]^+$, 459 $[M-OCH_3]^+$, 328 $[M-162]^+$ ($[A]^+$), 297 $[A-OCH_3]^+$.

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