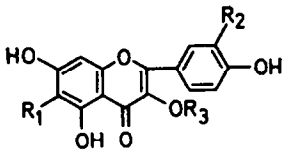
samples. The glycosides were characterized as quercetagetin-3-*O*-α-L-rhamnoside (**1**), mp 195°, quercetin-3-*O*-



1, R_1 = OH, R_2 = OH, R_3 = Rha
2, R_1 = H, R_2 = OH, R_3 = Rha
3, R_1 = H, R_2 = H, R_3 = Glc

α-L-rhamnoside (**2**), mp 322° and kaempferol-3-*O*-β-D-glucoside (**3**), mp 180°, by UV (using diagnostic reagents) and NMR.

Further confirmation of the identity of the glycosides was furnished by co-chromatography on TLC (cellulose and silica gel) using a number of solvent systems.

This is the first report of the co-occurrence of biflavones and flavonol-*O*-glycosides in a *Juniperus* species. Both classes of flavonoid have been recorded separately in other *Juniperus* species [1–6].

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AN ISOFLAVAN PHYTOALEXIN FROM LEAVES OF *GLYCYRRHIZA GLABRA*

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Key Word Index—*Glycyrrhiza*; Leguminosae; licorice; isoflavan; phytoalexin; antifungal compound.

Abstract—An isoflavonoid phytoalexin isolated from the leaves of *Glycyrrhiza glabra* has been characterised as 7,2′-dihydroxy-3′,4′-dimethoxyisoflavan

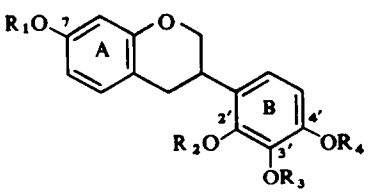
INTRODUCTION

The roots of European licorice (*Glycyrrhiza glabra* L.; Leguminosae, tribe Astragaleae) and other members of this pharmacologically important genus are rich in isoflavonoid constituents. Representatives of the coumestan [1], isoflavan [2, 3], isoflavone [4–10] and isoflavene [5] groups have all been obtained from this source. In contrast, there is apparently no evidence for the production of leaf isoflavonoids by any *Glycyrrhiza* species. However, during a recent investigation it was found that the fungus-inoculated leaves of *G. glabra* var. *glabra* accumulated large quantities of a phenolic isoflavan phytoalexin [11]. The present paper describes the isolation and identification of this compound as 7,2′-dihydroxy-3′,4′-dimethoxyisoflavan **1** (isomucronulatol).

RESULTS AND DISCUSSION

Detached leaflets were inoculated with droplets of a conidial suspension of *Helminthosporium carbonum* Ullstrup [12, 13], incubated for 48 hr (20°; *ca* 400 lx) and the resulting diffusate then collected and extracted (EtOAc) as previously described [12]. Si gel TLC ($CHCl_3$–MeOH, 50:1) afforded a single, non-fluorescent fraction (*ca* R_f 0.5) which proved to be homogeneous when chromatographed in several additional solvent systems. This compound (**1**) reacted strongly to both diazotised *p*-nitroaniline (yellow/brown) and Gibbs reagent [13] (ultramarine). Only traces of **1** were present in extracts of the control diffusate [12].

MS analysis gave the molecular ion at *m/e* 302 (corresponding to $C_{17}H_{18}O_5$) and revealed a typically



1: $R_1 = R_2 = H$; $R_3 = R_4 = Me$
2: $R_1 = R_3 = H$; $R_2 = R_4 = Me$
3: $R_1 = R_2 = R_3 = R_4 = Me$
4: $R_1 = R_4 = H$; $R_2 = R_3 = Me$

isoflavan-like fragmentation pattern [14] (see Experimental). Prominent ions at *m/e* 180 and 167 were attributed to B-ring derived fragments substituted with one OH and two OMe groups; A-ring monohydroxylation was apparent from the fragment at *m/e* 135. UV maxima recorded for **1** were virtually indistinguishable from those of 7,3′-dihydroxy-2′,4′-dimethoxyisoflavan **2** (mucronulatol) prepared by hydrogenation of authentic violanone (7,3′-dihydroxy-2′,4′-dimethoxyisoflavanone). **1** readily formed a diacetate whilst methylation of both **1** and **2** gave identical (MS, UV, TLC) diMe products (**3**). However, although clearly similar, **1** and **2** were easily resolved by Si gel TLC in a variety of solvents including C_6H_6–MeOH (9:1) (**1**, R_f 0.53; **2**, R_f 0.47) and *n*-pentane–Et_2O–HOAc (75:25:1) (**1**, R_f 0.37; **2**, R_f 0.28). From the above data, **1** was provisionally identified as 7,2′-dihydroxy-3′,4′-dimethoxyisoflavan for which the common name isomucronulatol is proposed. The B-ring hydroxyl was located at C-2′ (rather than C-4′ as in **4**) from the positive Gibbs test noted above. A sample of synthetic isomucronulatol (**15**) was indistinguishable (MS, UV, TLC) from the *Glycyrrhiza*-derived product. Like **1**, several other *Glycyrrhiza* isoflavonoids also possess a B-ring with 2′,3′,4′-substitution [2, 5]. However, **1** is apparently the first licorice isoflavonoid known to have direct 3′-oxygenation; other related compounds from *Glycyrrhiza* spp. (e.g. licoricidin [2] and glabrone [5]) are characterized either by 3′-prenylation or the possession of a 3′,4′-dimethylchromen ring.

Diffusates (48 hr) from *H. carbonum*-inoculated leaflets contained **1** at a concentration of *ca* 108 μg/ml (based on log $\varepsilon = 3.62$ at 282 nm for **2** [16]). In contrast, only traces of **1** (< 5 μg/ml) were isolated from the control diffusate. Large quantities of isomucronulatol (*ca* 750 μg/g) were also obtained from leaf tissues underlying the inoculum droplets; **1** was essentially absent from the control tissues. There was no evidence for the occurrence (in diffusate or tissue extracts) of other induced or constitutive isoflavonoids which might function as biosynthetic precursors of isomucronulatol. The concentration of **1** in diffusates collected 12, 24, 36, 48 and 72 hr after fungal inoculation was 24, 59, 125, 135 and 117 μg/ml respectively. Corresponding leaf tissue values were 128, 325, 620, 890 and 840 μg/g. In a TLC bioassay [13] isomucronulatol (*ca* 20, 30 and 40 μg/g) exhibited high antifungal activity against spore germination of *Cladosporium herbarum* Fr. thereby supporting its proposed role as a phytoalexin of *G. glabra*.

Attempts to use the drop-diffusate technique for the isolation of phytoalexins from leaves of *G. glabra* var. *glandulifera*, *G. echinata* L., *G. lepidota* Pursh and *G. uralensis* Fisch. were unsuccessful; conidial suspensions (with or without surfactant [12]) were not retained for more than a few minutes on the leaves of these species. This feature presumably reflects the production of a viscid glandular secretion by leaves of the above plants.

EXPERIMENTAL

Mass and UV spectra were determined as previously described [17]. Seeds of *Glycyrrhiza glabra* var. *glabra* (supplied by the Dept. Botany Herbarium, University of Reading) were grown in light potting compost [18] for *ca* 6 months prior to use. Diffusate extracts were chromatographed (Si gel TLC [17]) using $CHCl_3$–MeOH (50:1) as the developing solvent. Leaf tissues underlying the inoculum (or control) droplets were excised and extracted with EtOH [19]. Si gel TLC (Et_2O–hexane, 3:1) of these extracts afforded **1** at *ca* R_f 0.62; elution (EtOH) and additional TLC (*n*-pentance–Et_2O–HOAc, 75:25:1) gave pure **1** (*ca* R_f 0.35).

7,2′-Dihydroxy-3′,4′-dimethoxyisoflavan **1** (*isomucronulatol*). λ_{max} EtOH (nm) 217, 228*sh*, 276*sh*, 281, 290*sh*; λ_{max} EtOH + NaOH (nm) 222, 242*sh*, 294; *m/e* (rel. int.) 303(8), 302(M^+; 56), 181(7), 180(100), 168(28), 167(33), 165(9), 153(5), 151(9), 147(6), 135(14), 133(12), 123(13), 107(12). DiMe ether **3** (CH_2N_2) (R_f 0.87, $CHCl_3$:CCl_4, 3:1). λ_{max} EtOH (nm) 216, 227*sh*, 274*sh*, 280, 283*sh*, 289; MS *m/e* (rel. int.) 331(8), 330(M^+; 52), 195(13), 194(100), 182(27), 181(21), 180(7), 179(52), 167(8), 166(9), 165(19), 151(21), 149(41), 148(8), 137(11), 136(10), 123(11), 121(19), 109(10). Diacetate (Py–Ac_2O) (R_f 0.81, $CHCl_3$). λ_{max} EtOH (nm) 213, 227*sh*, 270*sh*, 275*sh*, 278, 284*sh*; MS *m/e* (rel. int.) 386(M^+; 23), 345(6), 344(35), 303(8), 302(51), 301(12), 181(11), 180(100), other fragments as given for **1**. Comparative data recorded for 7,3′-dihydroxy-2′,4′-dimethoxyisoflavan **2** (mucronulatol) were as follows: λ_{max} EtOH (nm) 213, 228*sh*, 276*sh*, 281, 290*sh*; λ_{max} EtOH + NaOH (nm) 223, 243, 293; MS *m/e* (rel. int.) 303(8), 302(46), 181(13), 180(100), 168(59), 167(42), 165(40), 153(10), 152(7), 151(19), 147(16), 137(11), 136(11), 135(33), 133(42), 123(20), 107(24). DiMe ether. TLC, MS and UV as given for diMe ether of **1**.

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