ceric sulphate as spray reagent. The EtOH extractive of the dry plant material (2 kg) was macerated with EtOAc and the soluble fraction (92 g) was chromatographed over Si gel (1 kg). Fifty fractions (500 ml each) were collected using hexane containing increasing amounts of Et_2O. The hexane–Et_2O (1:1) eluates (fraction 22–24, 3.686 g) yielded substance E (1.068 g) after purification by PLC. Subsequent fractions 35–47 of the same eluant (2.786 g) on crystallization with MeOH yielded substance F (500 mg).

Substance E (*5,7-dihydroxy-3-8,4'-trimethoxyflavone*). Yellow needles from dil. MeOH mp 172–73°, R_f 0.35 (hexane–Et_2O 1:1). It produced magenta colour with Mg/HCl and an intense yellow with ammonia. UV (MeOH): 277, 312, 358 nm (log ε 4.37, 3.83, 3.7); MeOH/$AlCl_3$: 285, 311, 352, 412 nm; MeOH/NaOAc: 285, 307, 390 nm: MeOH/NaOMe: 285, 305, 415 nm. PMR (acetone-d_6) ppm: 3.9 (9H, *s*, 3OMe), 6.3 (1H, *s*, C6), 7.1 (2H, *d*, $J = 9$ Hz, C3'5'), 8.1 (2H, *d*, $J = 9$ Hz, C2'6'). MS: *m/e* 344 (M^+). Found: C, 62.2; H, 4.2 $C_{18}H_{16}O_7$ requires C, 62.0; H, 4.2%. The diacetate ($C_{22}H_{20}O_9$) crystallized from $CHCl_3$–MeOH, mp 160–62°. UV (MeOH): 265 and 340 nm. PMR ppm: 2.35, 3.45 (3H each, *s*, OAc), 3.84 (3H, *s*, OMe), 3.9 (6H, *s*, 2OMe), 6.8 (1H, *s*, C6), 7.12 (2H, *d*, $J = 9$ Hz, C3',5'), 8.12 (2H, *d*, 9 Hz, C2',6'). The monomethylether ($C_{19}H_{18}O_7$) crystallized from dil. EtOH, mp 161°. PMR ppm: 3.9 (12H, *s*, 4OMe), 6.4 (1H, *s*, C6), 7.0 (2H, *s*, $J = 9$ Hz, C3',5'), 8.05 (2H, *d*, $J = 9$ Hz, C2',6'). The dimethyl ether ($C_{20}H_{20}O_7$) crystallized from dil. MeOH, mp 156°. UV (MeOH): 272, 310, 350 nm. PMR ppm: 3.9 (15H, *s*, 5OMe), 6.4 (1H, *s*, C6), 7.0 (2H, *d*, $J = 9$ Hz, C3',5'), 8.05 (2H, *d*, $J = 9$ Hz, C2',6').

Substance F (conyzatin). Yellow needles from MeOH, mp 209°. With Mg/HCl it gave a magenta colour and an intense yellow spot with NH_3. R_f 0.4 (hexane–Et_2O 1:3), UV (MeOH): 276, 314, 362, 402 nm (log ε 4.42, 4.25, 3.90, 3.98); MeOH/$AlCl_3$: 286, 317, 355, 400, 421 nm: MeOH/NaOAc: 287, 305, 414, MeOH/NaOMe: 288, 305, 416 nm. PMR (DMSO-d_6) ppm: 3.83 (3H, *s*, OMe), 3.9 (12H, *s*, 4OMe), 6.25 (1H, *s*, C6). 7.4 (2H, *s*, C2',6'), MS: *m/e* (M^+) 404. Found: C, 58.35: H, 6.1 $C_{20}H_{20}O_9$ requires C, 58.30; H, 6.05%. The diacetate was obtained as amorphous powder from hexane–C_6H_6, mp 152°. PMR (CCl_4) ppm: 2.23, 2.29, (3H each, *s*, OAc), 3.8 (12H, *s*, 4OMe), 3.9 (3H, *s*, OMe), 6.45 (1H, *s*, C6), 7.3 (2H, *s*, C2',6'). PMR (DMSO-d_6) ppm: 2.3 (6H, *br s*, 2OAc), 3.75–3.85 (12H, *br s*, 4OMe), 4.0 (3H, *s*, OMe), 7.05 (1H, *s*, C6), 7.38 (1H, *s*, C2'), 7.6 (1H, *s*, C6'). Found: C, 58.1; H, 4.8 $C_{24}H_{24}O_{11}$ requires C, 59.12, H, 4.9%. The monomethyl ether crystallised from MeOH, mp 170°. UV (MeOH): 275, 308, 340, 362 nm; MeOH/$AlCl_3$: 285, 320, 356, 400 nm. PMR (acetone-d_6) ppm: 3.8 (3H, *s*, OMe), 3.85 (3H, *s*, OMe), 3.86 (9H, *s*, 3OMe), 3.91 (3H, *s*, OMe), 6.4 (1H, *s*, C6), 7.46 (2H, *s*, C2',6'). MS: *m/e* 418 (M^+). The dimethyl ether crystallized from MeOH, mp 192°. UV (MeOH): 277, 308, 356 nm; UV ($AlCl_3$, NaOAc): no change PMR (DMSO-d_6) ppm: 3.78 (3H, *s*, OMe), 3.8 (3H, *s*, OMe), 3.85 (3H, *s*, OMe), 3.88 (9H, *s*, 3OMe), 3.98 (3H, *s*, OMe), 6.3 (1H, *s*, C6), 7.42 (2H, *s*, C2',6'). MS: *m/e* 432 (M^+).

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REFERENCES

1. Domiainguer, X. A., Quintero, G. and Butruille, D. (1972) *Phytochemistry* **11**, 1855.
2. Saleh, M. R. I. (1957) *Proc. Pharm. Soc. Egypt Sci. Ed.* **39**, 107.
3. Bohlmann, F., Bornowski, H. and Arndt, C. (1965) *Chem. Ber.* **98**, 1736.
4. Bohlmann, F. and Grenz, M. (1972) *Chem. Ber.* **105**, 3123.
5. Bhakuni, D. S., Dhar, M. L., Dhawan, B. N. and Mehrotra, B. N. (1969) *Indian J. Exp. Biol.* 7, 250.
6. Brown, R. F. C., Gilham, P. T., Hughes, G. K. and Ritche, E. (1954) *Australian J. Chem.* 7, 181.
7. Dawson, R. M., Henrick, C. A., Jefferies, P. R. and Middleton, E. J. (1965) *Australian J. Chem.* **18**, 1871.
8. Henrick, C. A. and Jefferies, P. R. (1964) *Australian J. Chem.* **17**, 934.
9. Joshi, B. S. and Kamat, V. N. (1970) *Phytochemistry* **9**, 889.

Phytochemistry, 1977, Vol. 16, pp. 1456–1457. Pergamon Press Printed in England.

BIFLAVONES AND FLAVONOL-*O*-GLYCOSIDES FROM *JUNIPERUS MACROPODA*

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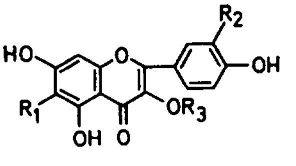
Key Word Index—*Juniperus macropoda*; Cupressaceae; amentoflavone; hinokiflavone; isocryptomerin; quercetagetin-3-*O*-rhamnoside; quercetin-3-*O*-rhamnoside; kaempferol-3-*O*-glucoside.

Powdered and dried leaves of *Juniperus macropoda* Boiss. were extracted with acetone in a Soxhlet. The extract was dried under reduced pressure and treated with hot water. The insoluble portion was purified by column chromatography on silica gel to give three biflavones by preparative TLC. They were characterized as amentoflavone, hinokiflavone and isocryptomerin, by mp and comparison of NMR spectra of their methyl and acetyl derivatives with those of authentic samples.

The water soluble portion was extracted with butanol and separated by column chromatography followed by TLC on silica gel into three fractions A, B, and C. On hydrolysis with 10% HCl fractions A and B gave quercetagetin, mp 320–324° and quercetin, mp 315° respectively and a common sugar rhamnose (TLC), while fraction C gave kaempferol, mp 284° and glucose. The aglycones were characterized by co-chromatography on TLC and by comparing the mp's of their acetates with authentic samples. The glycosides were characterized as quercetagetin-3-O-α-L-rhamnoside (**1**), mp 195°, quercetin-3-O-α-L-rhamnoside (**2**), mp 322° and kaempferol-3-O-β-D-glucoside (**3**), mp 180°, by UV (using diagnostic reagents) and NMR.



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

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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REFERENCES

1. Swada, T. (1958) *J. Pharm. Soc.* (*Japan*) **78**, 1023.
2. Kariyone, T. (1962) *J. Pharmacog. Soc.* (*Japan*) **16**, 1.
3. Mashima, T., Okigawa, M. and Kawano, N. (1970) *J. Pharm. Soc.* (*Japan*) **90**, 512.
4. Pelter, A., Warren, R., Hameed, N., Ilyas, M. and Rahman, W. (1971) *J. Indian Chem. Soc.* **48**, 204.
5. Mashima, T., Okigawa, M. and Kawano, N. (1970) *Yakugaku Zasshi* **90**, 512.
6. Hameed, N., Ilyas, M., Rahman, W., Okigawa, M. and Kawano, N. (1973) *Phytochemistry* **12**, 1494.

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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

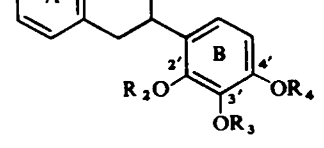
INTRODUCTION

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: $R_1 = R_4 = H$; $R_2 = R_3 = Me$