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FLAVONOIDS OF *BAPTISIA AUSTRALIS* (LEGUMINOSAE)

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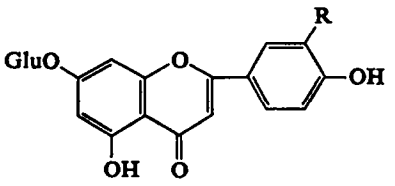
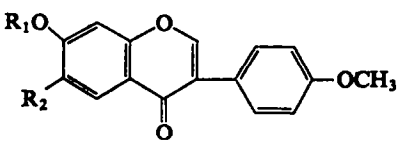
Abstract—Five flavonoids, apigenin 7-*O*-β-D-glucoside, luteolin 7-*O*-β-D-glucoside, formononetin 7-*O*-β-D-glucoside, formononetin, and afrormosin have been isolated from *B. australis* together with the chromanocoumarin trifolirhizin. Afrormosin 7-*O*-β-D-glucoside was identified but not isolated. Formononetin 7-*O*-β-D-glucoside, afrormosin 7-*O*-β-D-glucoside, afrormosin and trifolirhizin are new to the genus *Baptisia*.

INTRODUCTION

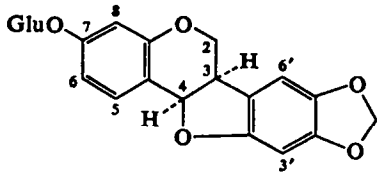
THE GENUS *Baptisia* (Leguminosae) is at present under intensive chemical investigation in connection with a broad biochemical systematic program,[1–4] and the present communication



(I) R = H
(II) R = OH



(III) R_1 = H, R_2 = H
(IV) R_1 = glucose, R_2 = H
(V) R_1 = H, R_2 = —OCH_3
(VI) R_1 = glucose, R_2 = —OCH_3



(VII)

[1] T. J. MABRY, J. KAGAN and H. RÖSLER, *Phytochem.* **4**, 487 (1965).
[2] H. RÖSLER, T. J. MABRY and J. KAGAN, *Chem. Ber.* **98**, 2193 (1965).
[3] R. E. ALSTON, H. RÖSLER, K. NAIFEH and T. J. MABRY, *Proc. Natl Acad. Sci. U.S.* **54**, 1458 (1965).
[4] M. F. CRANMER and T. J. MABRY, *Phytochem.* **5**, 1113 (1966).

describes some of the flavonoid chemistry of *B. australis*.[5] Although detailed information is now available on the lupine alkaloid constituents of *B. australis*,[4,6] this is the first description of the flavonoid constituents of the species. We now report the isolation and characterization of apigenin 7-*O*-β-D-glucoside (I), luteolin 7-*O*-β-D-glucoside (II), formononetin (III), formononetin 7-*O*-β-D-glucoside (IV), afrormosin (V), afrormosin 7-*O*-β-D-glucoside (VI) and trifolirhizin (VII) from leaf, stem, root and flower material of *B. australis*.

RESULTS AND DISCUSSION

Apigenin and luteolin monoglucosides. The 7-*O*-β-D-glucosides of both apigenin and luteolin are commonly occurring natural products,[7] and all physical and spectral properties observed for the *Baptisia australis* samples were identical with those already published for these compounds. The aglycones, isolated from acid hydrolysis of I and II, were also identical with authentic apigenin and luteolin. Both glucosides could be hydrolyzed with β-glucosidase.

Paper chromatographic evidence indicates that apigenin, luteolin and luteolin 7-*O*-glucoside occur widely in the genus *Baptisia*.[8] Apigenin 7-*O*-glucoside, however, has so far been found only in two species of the genus, *B. leucophaea* and *B. megacarpa*, and was identified only by R_f values and u.v. spectroscopy.[8] Thus, the present report of the isolation of crystalline apigenin and luteolin 7-*O*-glucosides constitutes the first full characterization of these compounds from any *Baptisia* species.

Formononetin, afrormosin and their 7-O-β-D-glucoside derivatives. The identification of formononetin 7-*O*-β-D-glucoside (IV) was based on the identity of its m.p., optical rotation and u.v. spectrum with those reported in the literature[9]. Additional characterization was by means of quantitative acid hydrolysis, hydrolysis with β-glucosidase, the recording of the NMR (Table 1) and i.r. spectra and the preparation of a tetra-acetate. The aglycone isolated from flower material, was identified by co-chromatography with formononetin, by lack of m.p. depression of its acetate when mixed with formononetin monoacetate and from the NMR spectrum of the acetate (Table 1).

Afrormosin (V) was recognized by the identity of its u.v. and i.r. spectra with those previously published for this compound.[10] It was characterized by the preparation of the acetate, the m.p. of which agreed with that for afrormosin acetate,[10] and by the determination of an NMR spectrum (Table 1), which was in accord with structure (V). The 7-*O*-β-D-glucoside was not isolated but was detected as a spot on 2D paper chromatograms. It was identified as an afrormosin glycoside by β-glucosidase hydrolysis which produced afrormosin, and as a monoglucoside by its R_f values which are almost the same as those of formononetin 7-glucoside.

The isolation and characterization of formononetin 7-*O*-β-D-glucoside (ononin) and afrormosin from, and the identification of afrormosin 7-*O*-β-D-glucoside in *B. australis*, constitutes their first recognition in any species of *Baptisia*. (Formononetin has been previously isolated from *B. sphaerocarpa*.[11]) The presence of these isoflavones in *B. australis* is

[5] *B. minor* is now recognized as a variety of *B. australis* (B. L. TURNER, private communication).
[6] L. MARION and J. OUELETT, *J. Am. Chem. Soc.* **70**, 691 (1948).
[7] W. KARRER, *Konstitution und Vorkommen der Organischen Pflanzenstoffe*. Birkhauser, Basel (1958).
[8] Preliminary survey carried out on *Baptisia* species at the University of Texas.
[9] G. ZEMPLEN, L. FARKAS and A. BIEN, *Chem. Ber.*, **77**, 452 (1944); L. FARKAS and J. VARADAY, *Chem. Ber.* **92**, 819 (1959).
[10] T. B. H. MCMURRY and C. Y. THENG, *J. Chem. Soc.* 1491 (1960); J. B. HARBORNE, O. R. GOTTLIEB and M. T. MAGALHAES, *J. Org. Chem.* **28**, 881 (1963).
[11] H. RÖSLER, unpublished results.

TABLE 1. NMR DATA FOR *Baptisia australis* ISOFLAVONOIDS*

Compound	Protons												
	2	3	4	5	6	8	2′	3′	5′	6′	—OCH_3	Sugar H-1	Other
Trifolirhizin†	3·3–3·8 (hidden)	4·15 (m)	5·37 (d, J=6)	7·31 (d, J=9)	6·67 (q, J=9) J=2·5)	6·55 (broad)		6·33		6·60		4·80	5·82 (O—CH_2—O)
Formononetin 7-*O*-glucoside†	7·85			8·16 (d, J=10)	7·02 (q, J=10 J=2·5)	6·93	7·46 (d, J=9)	6·86 (d, J=9)	6·86 (d, J=9)	7·46 (d, J=9)	3·77	4·94	
Formononetin monoacetate	7·98			8·33 (d, J=9)	*ca.* 7·30	*ca.* 7·30	7·52 (d, J=9)	7·02 (d, J=9)	7·02 (d, J=9)	7·52 (d, J=9)	3·85		2·35 (Acetate)
Afrormosin	8·03			7·83		*ca.* 7·30	7·56 (d, J=9)	7·03 (d, J=9)	7·03 (d, J=9)	7·56 (d, J=9)	3·88 3·98		

* Spectra were determined in $CDCl_3$ on a Varian A-60 spectrometer unless otherwise noted and values are given in ppm relative to tetramethylsilane as internal standard; d = doublet, q = quartet and m = multiplet. J = coupling constant in c/s.

† Spectra measured for the trimethylsilyl ether derivatives using CCl_4 as solvent.

not surprising since a wide variety of isoflavones including biochanin A, orobol, oroboside, tectoridin, genistein, genistin and genistein 7-rutinoside have recently been detected in this genus.[12] Afrormosin, afrormosin 7-glucoside and formononetin 7-glucoside have all previously been isolated from other genera of the Leguminosae. Afrormosin was first isolated in 1960 from *Afrormosia elata*. Harms[10] and since then has been reported to occur in *Myrocarpus*, *Myroxylon*, *Wistaria*, *Amphimas*,[13a] and also in *Castanospermum australe*,[13b] where it co-occurs with formononetin. The only known glycoside, afrormosin 7-*O*-glucoside, has to date been reported in only one plant, the common garden *Wistaria floribunda*.[14] Formononetin 7-glucoside has been isolated only from the roots of *Ononis spinosa* L.,[15] whereas the aglycone, formononetin, has been detected in *Trifolium*.[15] *Cicer*.[15] *Baptisia*.[11] *Ononis*[15] and *Castanospermum*.[13b]

Trifolirhizin. Compound VII was initially identified as a chromanocoumarin by its highly characteristic u.v. spectrum (see ref. 16). The i.r. spectrum was virtually superimposable with that published[16] for the chromanocoumarin trifolirhizin, and an analysis of the NMR spectrum (Table 1) confirmed not only the oxidation pattern but also the presence of a methylenedioxy group and one glucose residue. The oxidation pattern in structure VII, involving a 4',5'-methylenedioxy bridge, is based on the presence of *singlet* H-3' and H-6' proton signals in the NMR spectrum. This is in agreement with recent structure proposals for trifolirhizin and its aglycone (maackiain).[17] The melting point, elemental analysis and optical rotation were determined for compound VII and its tetra-acetate and all are in good agreement with those published[16] for trifolirhizin and its tetra-acetate. The optical rotation of −174 distinguishes this compound from its enantiomer sophojaponicin[18] and establishes the absolute stereochemistry as 3R, 4R.[17]

Trifolirhizin was first isolated in 1961 from the roots of red clover (*Trifolium pratense* L.)[16] and its aglycone maackiain[19] (inermin[20]) has been found in *Andira*, *Maackia* and *Sophora*, all members of the Leguminosae. The present isolation of trifolirhizin from *B. australis* is the first report of chromanocoumarins in the genus *Baptisia*. Trifolirhizin was isolated only from root material in *B. australis* however, and, since root material has not usually been investigated in this genus, it is possible that other species also contain chromanocoumarins. Its occurrence in the genus *Baptisia* together with a wide variety of isoflavones is not surprising in view of the possible close biosynthetic relationship between chromanocoumarins, isoflavanones and isoflavones.[21]

The distribution of the compounds I–VII within the plant was determined by paper chromatography of unpurified extracts: leaf, stem and flower material contained compounds I–VI but no trifolirhizin (VII) and root material contained trifolirhizin together with formononetin, formononetin 7-glucoside and possibly afrormosin, but no luteolin or apigenin glycosides.

[12] Data from Alston, Rösler, Kagan and Mabry as cited by J. B. HARBORNE, In *Comparative Phytochemistry* (Edited by T. SWAIN), p. 271. Academic Press, New York (1966).

[13a] See J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, pp. 93, 169. Academic Press, New York (1967); [b] R. A. EADE, H. HINTERBERGER and J. J. H. SIMES, *Australian J. Chem.* **16**, 188 (1963).

[14] S. SHIBATA, T. MURATA and M. FUJITA, *Chem. Pharm. Bull. Tokyo* **11**, 372 (1963).

[15] See F. M. DEAN, *Naturally Occurring Oxygen Ring Compounds*, p. 368. Butterworth, London (1963).

[16] J. B-SON. BRENDENBERG and P. K. HIETALA, *Acta Chem. Scand.* **15**, 696, 936 (1961).

[17] H. SUGINOME, *Bull. Chem. Soc. Japan* **39**, 1544 (1966).

[18] S. SHIBATA and Y. NISHIKAWA, *Chem. Pharm. Bull.* **11**, 167 (1963).

[19] H. SUGINOME, *Bull. Chem. Soc. Japan* **39**, 1529 (1966).

[20] W. LOCKER, T. DAHL, C. DEMPSEY and T. B. H. MCMURRAY, *Chem. Ind.* 216 (1962).

[21] W. D. OLLIS, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 355. Pergamon Press, Oxford (1962).

Additional flavonoid constituents of *B. australis* are presently under investigation in connection with a continuing chemotaxonomic analysis of all the species in this genus.

EXPERIMENTAL

Extraction and Purification Procedures

Pulverized leaf and stem material,* prewashed with $CHCl_3$, was soxhlet extracted with methanol. The dry extract was dissolved in hot water and left to stand. The first solid to separate consisted largely of the flavone glycosides and the second of formononetin 7-glucoside. Further purification was achieved through polyamide column chromatography using water–methanol solvent mixtures as eluent, and by preparative paper chromatography.

Fresh root material was treated in the same manner. The aqueous solution on standing first yielded crude formononetin 7-glucoside and then, trifolirhizin. Both were purified by recrystallization.

Pulverized flower material† was shaken with $CHCl_3$ for two days. The extract yielded a gum on evaporation which contained afrormosin and formononetin. These were purified by preparative paper chromatography using the upper layer of benzene:HOAc:H_2O, 6:7:3. (R_f afrormosin 0·9, formononetin 0·6).

Apigenin 7-O-glucoside (*I*), a minor constituent, was isolated crystalline by preparative paper chromatography (R_f 0·60 in BuOH:HOAc:H_2O, 4:1:5), λ_{max} (EtOH) 269, 340 nm; ($AlCl_3$) 279, 301, 343, 385 nm; (NaOAc) 268, 350, 402 nm; (NaOAc/H_3BO_3) 268, 342 nm; (NaOEt) 270, 275, 388 nm. The i.r. spectrum was superimposable with that of synthetic apigenin 7-*O*-β-D-glucoside.[22] The aglycone (from both acid and β-glucosidase hydrolyses) was chromatographically indistinguishable from apigenin.

Luteolin 7-O-β-D-glucoside (*II*) was isolated in 0·8% yield from dry leaf material and crystallized from aq. ethanol, m.p., 240–280° (lit.[23] 238–281°), $\alpha_D = -81°$ (c, 0·78; EtOH), λ_{max} (EtOH) 257, 268, 353 nm; ($AlCl_3$/HCl) 275, 360, 393 nm; (NaOAc) 257, 410 nm; (NaOAc/H_3BO_3) 264, 384 nm; (NaOEt) 270, 401, nm. (Found: C, 52·98; H, 5·24. Calc. for $C_{21}H_{20}O_{11} \cdot 1{\cdot}5\ H_2O$: C, 53·05; H, 4·87%). The i.r. spectrum of this material was identical with that published for hydrated luteolin 7-*O*-β-D-glucoside.[22]

Acid hydrolysis of the glucoside (5 mg) with 2N HCl gave the aglycone (2·9 mg). λ_{max} (EtOH) 256, 269, 353 nm; ($AlCl_3$/HCl) 263, 276, 360, 392 nm; (NaOAc) 270, 390 nm; (NaOAc/H_3BO_3) 261, 372 nm; (NaOEt) 271, 405 nm. The sugar moiety was paper chromatographically inseparable from glucose (R_f 0·20 in BuOH:HOAc:H_2O, 4:1:5 and 0·35 in 80% aq. phenol). Enzymatic hydrolysis of the glucoside (10 mg) with β-glucosidase (20 mg) in a pH 5 citrate-phosphate buffer (50 ml) for 15 hr at 35° also gave the aglycone.

Formononetin 7-O-β-D-glucoside (*IV*) was isolated in 0·2% yield from dried leaf material and in 0·8% yield from fresh root material. It crystallized from aq. ethanol, m.p. 211–216° (lit.[9] 214°, hydrated), $\alpha_D = -44°$ (c, 0·886, pyridine), (Found: C, 61·24; H, 5·24. Calc. for $C_{22}H_{22}O_9$: C, 61·39; H, 5·15%). The u.v. spectrum, λ_{max} (EtOH) 230 (infl), 250 (infl), 261, 300 (infl) nm, was unchanged by the addition of either NaOEt or $AlCl_3$. The i.r. spectrum was identical with that determined for authentic formononetin 7-*O*-β-D-glucoside ν_{max} (KBr) 3·05, 3·45w, 6·15s, 6·6m, 6·93m, 7·28w, 7·4w, 7·6w, 7·7w, 7·82, 8·0s, 8·38w, 8·7w, 9·05s, 9·3s, 9·55w, 9·92m, 11·1m, 11·25m, 11·85m, 12·2m, 12·42m, 12·8m, 12·9m, 13·5w, 14·5w μ. NMR data is presented in Table 1.

Acetylation produced a tetra-acetate, m.p., 184° (lit.[9] 183–184°), (Found: C, 60·85; H, 5·0; OCH_3, 4·90. Calc. for $C_{30}H_{30}O_{13}$: C, 60·20; H, 5·05; OCH_3, 5·18%), $\alpha_D = -28{\cdot}5°$ (c, 0·531 g/100 ml $CHCl_3$).

Acid hydrolysis of the glucoside (267 mg) produced glucose together with the aglycone (160 mg), m.p., 255–260° (lit.[9] 255–265°). The u.v. spectrum, λ_{max} (EtOH) 251, 305 (infl), nm, and the i.r. spectrum were identical with those of authentic formononetin. Treatment of IV with β-glucosidase in H_2O also produced formononetin.

Formononetin, isolated with afrormosin by paper chromatography of flower material, was characterized by preparation of its mono-acetate, m.p., 171–172°; mixed with authentic formononetin mono-acetate, m.p., 171–172°. The NMR spectrum is reported in Table 1.

Afrormosin (*V*) was isolated by paper chromatography in 0·01% yield from dry flower material as a white solid, m.p., 212–220° (lit.[10] 228–229°), λ_{max} (MeOH) 226, 254, 326 nm; (NaOMe) 258, 349 nm; (NaOAc) 258, 333 nm. The i.r. spectrum (in $CHCl_3$) was almost identical with that previously published[10] for this compound. NMR data is presented in Table 1. The mono-acetate was prepared and recrystallized from methanol, m.p. 167° (lit.[10] 165–167°).

Afrormosin 7-O-β-D-glucoside (*VI*). β-Glucosidase hydrolysis of the formononetin 7-*O*-glucoside spot, cut from a 2D (TBA/HOAc) paper chromatogram of a crude methanol extract of *B. australis* stem, leaf and flower

* Origin: Botanical Garden of the Medicine Faculty of Lyon. Voucher specimen, University of Texas Herbarium (256154).

† Voucher specimen, University of Texas Herbarium (253910).

[22] R. TEOULE, J. CHOPIN and C. MENTZER. *Bull. Soc. Chim. France* 2116 (1960).

[23] S. HATTORI, In *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 324. Pergamon Press, Oxford (1962).

material, yielded formononetin plus one other isoflavone aglycone. This additional aglycone co-chromatographed with afrormosin in three solvents: *t*-BuOH:HOAc:H_2O, 3:1:1 (TBA), 15% aq. HOAc, and the benzene layer of a 6:7:3 mixture of Benzene:HOAc:H_2O. Approximate R_f values for afrormosin 7-*O*-β-D-glucoside are 0·62 in TBA and 0·75 in 15% aq. HOAc, cf. formononetin 7-*O*-glucoside 0 65 and 0·77, respectively.

Trifolirhizin (*VII*) was isolated in 0·5% yield from fresh root material and crystallized from methanol, m.p. 140° (lit.[16] 142–144°), $\alpha_D = -174°$ (c, 0·526, EtOH) (lit.[16] −183°), (Found: C, 56·87; H, 5·41. Calc. for $C_{22}H_{22}O_{10}$. H_2O: C, 56·89; H, 5·21%). The u.v. spectrum, λ_{max} (EtOH), 280 (infl), 286, 312 nm, is identical with that published for trifolirhizin[16] and is unaffected by the addition of NaOEt. The i.r. spectrum is superimposable with that of trifolirhizin. NMR data is presented in Table 1. Glucose, identified by paper chromatography, was produced by acid hydrolysis of VII but the aglycone was not isolated.

Pyridine/acetic anhydride treatment of trifolirhizin gave the tetra-acetate, m.p. 187–189° (lit.[16] 188–189°), $\alpha_D = -125°$ (c, 0·51, EtOH) (lit.[16] −126°), (Found: C, 58·44; H, 4·82. Calc. for $C_{30}H_{30}O_{14}$: C, 58·63; H, 4·91%).

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