plus que chez *Lotus corniculatus* ces flavonoïdes semblent être l'apanage du matériel floral dont l'expression métabolique peut être indépendante de l'appartenance systématique comme cela a déjà été souligné pour la corniculatusine [14].

EXPÉRIMENTAL

Le matériel végétal, *Lotus corniculatus* L., est issu de trois stations : départements de l'Ain, de la Loire et des Hautes-Alpes. L'étude des aglycones flavoniques a été conduite selon la technique du laboratoire [15].

Sexangularétine (méthoxy-8, tétrahydroxy-3,4',5,7 flavone). RMN : $(CD_3)_2CO$, 100 MHz, ppm échelle δ/TMS : 3H, *s* 3.95 (OMe); 1H, *s* 6.36 (H-6); 2H, *dd* (J = 2.5 et 8.5 Hz) 7,11 (H-3', H-5'); 2H, *dd* (J = 2,5 et 8,5 Hz) 8,30 (H-2', H-6').

Corniculatusine (méthoxy-8, pentahydroxy-3,3',4',5,7 flavone). RMN : CCl_4, 60 MHz, dérivé triméthylsilylé selon (17) : ppm échelle δ/TMS: 3H, *s* 3.90 (OMe); 1H, *s* 6.14 (H-6); 1H, *d* (J = 8.5 Hz) 6.85 (H-5'); 1H, *d* (J = 2.5 Hz) 7.69 (H-2'); 1H, *dd* (J = 8.5 et 2.5 Hz) 7.80 (H-6').

Gossypétine (hexahydroxy-3,3',4',5,7,8 flavone). $R_f \times 100$: CP AcOH–H_2O(3 : 2) : 18; BAW(4 : 1 : 5) : 35. UV λ_{max} nm : MeOH—262, 278, (310), 340, 384; NaOAc—instable; $NaOAc/H_3BO_3$—272, (284), (312), 356, 410; $AlCl_3$—289, (326), 391, 482; $AlCl_3$/HCl—272, (310), 370, 448; NaOMe—instable.

SM (les valeurs entre parenthèses représentent l'intensité relative): 319 (37%), M^+ 318 (100), 317 (24), 289 (14), 169 (25), 137 (28), 109 (11) (principaux pics en valeur *m/e*).

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5-*O*-METHYLBIOCHANIN A, A NEW ISOFLAVONE FROM *ECHINOSPARTUM HORRIDUM*

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Key Word Index—*Echinospartum horridum*; Leguminosae; Papilionoideae; Genisteae; isoflavones; phenolic compounds; chemotaxonomy; 5-methyl ether of biochanin A.

Abstract—Five known isoflavones (daidzein, formononetin, genistein, 5-*O*-methylgenistein and biochanin A) have been isolated from the leaves and stems of *Echinospartum horridum*. A sixth compound has been characterised by chemical and spectroscopic methods as the new isoflavone, 5-*O*-methylbiochanin A.

INTRODUCTION

The genus *Echinospartum* consists of 4 [1] species native to the Iberian Peninsula and southern France. The systematic position of these species is still uncertain. Originally included in *Genista*, they were later transferred to the separate genus *Echinospartum* [2, 3] and placed close to *Ulex*. Although Gibbs [4] recognized their similarity to the subgenus *Spartocarpus* of *Genista*, he maintained *Echinospartum* as a distinct genus. In contrast, Polhill [5] transferred the taxon to *Genista* regarding it as a section of the subgenus *Spartocarpus*.

A survey, using herbarium material, of flavonoids in the tribe Genisteae (Leguminosae, subfamily Papilionoideae) revealed the widespread occurrence of simple isoflavones such as daidzein (7,4'-dihydroxy- **1**), genistein (5,7,4'-trihydroxy- **2**) and 5-*O*-methylgenistein (7,4'-dihydroxy-5-methoxy- **3**). Formononetin (7-hydroxy-4'-methoxyisoflavone **4**) occurred less frequently in this group [6]. We have recently examined the fresh leaves of several Genisteae (including some of the species studied by Harborne [6]) and have found a wider spectrum of isoflavones than previously suspected. One plant, *Echinospartum horridum* (Vahl) Rothm., was particularly rich in isoflavones, containing in addition to compounds **1–4** substantial quantities of biochanin A (5,7-dihydroxy-4'-methoxyisoflavone **5**) and its 5-*O*-methyl ether (**6**), a substance not previously reported as a natural product.

RESULTS AND DISCUSSION

Six isoflavones were isolated from acid hydrolysed [7] extracts of the fresh leaves and stems of *E. horridum* (see Experimental); five of these were identified as daidzein, formononetin, genistein, 5-*O*-methylgenistein and biochanin A by UV and TLC comparison with authentic samples. Biochanin A has not previously been isolated from any member of the Genisteae.

The sixth isoflavone (M^+ 298) gave neutral (MeOH) and alkaline UV maxima similar to those of 5-*O*-methylgenistein. A reversible bathochromic shift (10 nm) was obtained with NaOAc [8] (C-7 OH) whereas the neutral spectrum was unaltered by $AlCl_3$. Acetylation gave a monoacetate whilst methylation afforded a monomethyl ether (M^+ 312) identical (MS, UV, TLC) with 5,7,4′-trimethoxyisoflavone (**7**) obtained from 5-*O*-methylgenistein. The above data suggested that the *Echinospartum* isoflavone was 5-*O*-methylbiochanin A (**6**) and this was confirmed by synthesis. Selective 7-benzylation of biochanin A ($BzCl/DMF/K_2CO_3/KI$) followed by 5-methylation ($Me_2SO_4/Me_2CO/K_2CO_3$) and debenzylation (HCl/HOAc) gave **6** indistinguishable by MS, UV and co-TLC (in six solvents) from the natural product. Although synthetic **6** has been known for many years [9] this is the first report of its occurrence in Nature. In *E. horridum*, compounds **1**–**6** presumably occur as glycosides since only traces of **4** and **5**, and no **1**, **2**, **3** or **6**, were isolated from unhydrolysed extracts (EtOH) of the leaves and stems.

The production of 4′-hydroxyisoflavones (**1**–**3**) by *E. horridum* is not unexpected since these compounds occur widely in the Genisteae; in contrast, 4′-methoxyisoflavones (**4**–**6**) are less common although formononetin has been detected in 3 of 5 species belonging to the subgenus *Spartocarpus* of *Genista* [7], the group to which *Echinospartum* is probably most closely related [4, 5]. As these species were not previously examined for biochanin A and its 5-*O*-methyl ether [7], a reinvestigation of *Spartocarpus* would appear to be justified. Although biochanin A has been obtained only from *E. horridum* in the Genisteae, it commonly occurs (together with **1**–**4**) in *Baptisia* and *Thermopsis* [10, 11] of the allied tribe Podalyrieae (Thermopsideae). *Echinospartum* might thus represent a chemical link between these two tribes.

EXPERIMENTAL

MS and UV were determined as previously described [12]. Extracts of the hydrolysed leaves and stems [7] of *Echinospartum horridum* (obtained from the University of Southampton Botanic Garden) were chromatographed (Si gel TLC [12] $CHCl_3$–MeOH, 20:1) to afford biochanin A (**5**, R_f 0.60), formononetin (**4**, R_f 0.43), 5-*O*-methylbiochanin A (**6**, R_f 0.29), genistein (**2**, R_f 0.23), daidzein (**1**, R_f 0.14) and 5-*O*-methylgenistein (**3**, R_f 0.08). Each compound was eluted (MeOH) and, apart from **1** and **4**, purified by Si gel TLC as follows: **2**, *n*-pentane–Et_2O–HOAc (75:25:6) (R_f 0.16), **3**, $CHCl_3$–MeOH (10:1) (R_f 0.17), **5**, *n*-pentane–Et_2O–HOAc (75:25:6) (R_f 0.51) and **6**, $CHCl_3$–HOAc (50:3) (× 3). In the latter system **6** (*lower zone*) gradually separated from a second substance (λ_{max} MeOH 280 nm; MS *m/e* (rel. int.) 127 (3), 126 (56), 125 (10), 109 (7), 98 (3), 97 (100)) of undetermined constitution.

5,4′-Dimethoxy-7-hydroxyisoflavone (**6**) Diazotized *p*-nitroaniline, yellow; Gibbs, no reaction; UV, fluorescent light blue (intensifying upon exposure to NH_3 vapour). λ_{max} (nm): *MeOH* 212 (88%), 256 (100%), 284 *sh* (47%), 314 *sh* (15%); *NaOH* 209 (95%), 266 (100%), 320 (33%); *NaOAc* 266, 320; *Borate* 256, 314 *sh*; *$AlCl_3$* no change; MS *m/e* (rel. int.) 313 (7), 312 (65), 311 (21), 299 (12), 298 (M^+: 65), 297 (13), 283 (M^+—Me: 15), 166 (19), 156 (9), 152 (9), 149 (10), 138 (7), 137 (10), 133 (11), 132 (53), 117 (16); the base peak occurred at *m/e* 43. *MonoMe ether* (CH_2N_2) (R_f 0.16, $CHCl_3$–CCl_4, 3:1) λ_{max} (nm): *MeOH* 210 (100%), 256 (97%), 284 *sh* (42%), 313 *sh* (15%); MS *m/e* (rel. int.) 313 (8), 312 (M^+: 46), 311 (21), 180 (5), 132 (19), base peak at *m/e* 43. *Monoacetate* (Py–Ac_2O) (R_f 0.43, $CHCl_3$) λ_{max} (nm) *MeOH* 218 (65%), 254 (100%), 318 (21%); MS *m/e* (rel. int.) 340 (M^+: 19), 298 (25), 297 (11); base peak at *m/e* 43. **6** was synthesized by the route described in [9], although the benzylating and methylating reagents used were as noted in the results section. **6** had mp 294–296° (lit. [9] 294°). NMR (60 MHz, DMSO-d_6, TMS): δ 3.75 (6H, *s*, OMe), 6.36 (2H, *s*, H-6, 8), 6.88 (2H, *d*, J = 9 Hz, H-3′,5′), 7.37 (2H, *d*, J = 9 Hz, H-2′,6′), 8.03 (1H, *s*, H-2) Other data as for the natural product. In addition to the expected molecular ion at *m/e* 298, the MS of **6** also exhibited a substantial peak at *m/e* 312. Synthetic **6** gave an identical peak even after further purification as its acetate (TLC/$CHCl_3$) and subsequent hydrolysis (aq. NH_3)/TLC ($CHCl_3$–MeOH, 20:1). The NMR of synthetic **6** is entirely in accord with its structure (see above) and any significant contamination with the methyl ether (**7**), possible *C*-methyl derivatives or homologues (e.g. ethyl ethers), all of which might account for the M + 14 peak, can be discounted. Moreover, **7** readily separates from **6** upon TLC in the solvent ($CHCl_3$–MeOH, 20:1) used to purify the *E. horridum* isoflavones (**6**, R_f 0.36, **7**, R_f 0.58) 5-*O*-methylgenistein, as well as synthetic and naturally occurring biochanin A (obtained from *Trifolium pannonicum* and *T. pratense* [13] also gave an M + 14 peak [13]. No unusual peaks were associated with the MS of prunetin (5,4′-dihydroxy-7-methoxyisoflavone), 7-*O*-methylbiochanin A, 7-acetoxybiochanin A [13] or the monoacetate of **6**. Although the origin of the M + 14 peaks has not been explained, they may be caused by the high MS temps. (e.g. source 180°; probe 310° for **6**) required for satisfactory volatilization of the isoflavone samples. Thus, the MS of biochanin A (M^+ 284) was normal at a probe temp. of 250° but exhibited the M + 14 peak at 320°.

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