Sparticarpin: A Pterocarpan Phytoalexin from *Spartium junceum*

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A new phytoalexin isolated from the fungus-inoculated leaflets of *Spartium junceum* (Spanish broom) has been identified as (−)-6a*R*; 11a*R*-2,3-dimethoxy-9-hydroxypterocarpan (sparticarpin). The total synthesis of sparticarpin is described.

Introduction

Although pterocarpan phytoalexins commonly accumulate in the fungus-inoculated tissues of papilionate legumes, only three compounds of this type possess oxygenation at C-2 [1, 2]. They are 2,3,9-trimethoxypterocarpan (**1**), 2,9-dimethoxy-3-hydroxypterocarpan (2-methoxymedicarpin, **2**) and 2,3,9-trimethoxy-4-hydroxypterocarpan (**3**) all of which co-occur with pisatin in the *Fusarium solani* f. sp. *pisi*-infected epicotyls of garden pea, *Pisum sativum* (Leguminosae, tribe Vicieae) [3, 4]. Compounds **1**–**3** have not been isolated from any other legume. We have recently discovered that detached leaflets of Spanish broom (*Spartium junceum* L.; tribe Genisteae) produce a phenolic pterocarpan phytoalexin (designated sparticarpin) following short wavelength UV irradiation or treatment with conidial suspensions of the fungus *Helminthosporium carbonum* Ullstrup. In this paper we present evidence to show that sparticarpin is identical with 2,3-dimethoxy-9-hydroxypterocarpan (**4**).

Results and Discussion

Si gel TLC ($CHCl_3$:MeOH, 25:1) of the fungus-induced diffusate extracts (EtOAc) gave two phenolic compounds (R_F 0.55 and 0.10) both of which significantly inhibited the growth of *Cladosporium herbarum* Fr. (TLC bioassay [5, 6]). The lower compound was subsequently identified (UV, TLC) as the previously reported isoflavone, 2′-hydroxygenistein **5** (5,7,2′,4′-tetrahydroxyisoflavone) [7] whilst the phytoalexin at R_F 0.55 (sparticarpin) was provisionally assigned a pterocarpan structure on the basis of its UV (EtOH) spectrum which resembled that of 2-methoxymedicarpin (**2**) [3]. Methylation (CH_2N_2) afforded a monomethyl ether indistinguishable (UV, MS, TLC) from authentic **1** [3]. The MS of sparticarpin (M^+ 300) exhibited a prominent fragment at *m/e* 285 ($M^+ - Me$) together with ions of lower intensity at *m/e* 191/178 (pterocarpan with two OMe groups on same aromatic ring) and 147/134 (pterocarpan with single OH group on aromatic ring). Because sparticarpin is known to possess a 2,3,9-oxygenation pattern, the two OMe groups must be assigned to ring A and the single OH group to ring D as only this arrangement is consistent with the observed MS ions (*cf.* **2**, M^+ 300, *m/e* 299, 177/164 (A ring) and 161/148 (D ring)) [3, 8, 9]. As sparticarpin has a negative optical rotation (about −170 ° (0.1 mg in 1 ml MeOH)) it can thus be fully represented as (−)-6a*R*; 11a*R*-2,3-dimethoxy-9-hydroxypterocarpan (**4**) [10]. Racaemic **4** was readily synthesized by $NaBH_4$ reduction of 6,7-dimethoxy-2′,4′-dihydroxyisoflavone (see Experimental) obtained *via* the now standard $Tl(NO_3)_3$ oxidation of a suitably benzylated chalcone [11]. The synthetic and *Spartium*-derived, pterocarpans were essentially indistinguishable by UV, MS and co-TLC.

Levels of **4** and **5** in *Spartium* diffusates and/or leaf tissues after treatment with *H. carbonum*, H_2O or short wavelength (254 nm) UV light are given in Table I. Attempts to isolate sparticarpin from petals of *S. junceum* were unsuccessful [12].

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Table I. Typical concentrations of sparticarpin (**4**) and 2′-hydrogenistein (**5**) in 48 h diffusates and leaf tissues of *S. junceum* following treatment with fungal spores, H_2O or UV light [a].

Sample	Treatment	Compound	
		4	**5**
Diffusate	*H. carbonum*	15	11
	H_2O	–	TR
Tissue	*H. carbonum*	31	ND
	H_2O	–	ND
	$UV_{(254\,nm)}$ light	9	ND

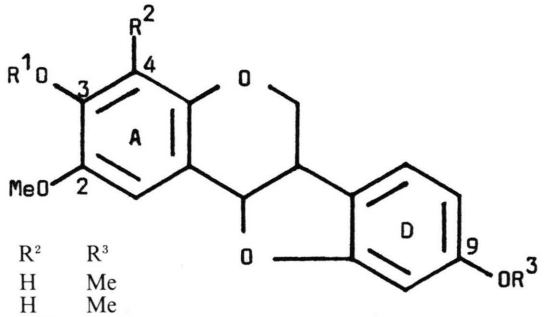
TR, trace; ND, not determined; –, not detected.
[a] Isoflavonoid concentrations (μg/ml diffusate or μg/g fr.wt. tissue) were determined spectrophotometrically using the following extinction coefficients: **4**, log ε = 4.01 at 292 nm for **2** [4]; **5**, log ε = 4.63 at 262 nm for 5,7,4′-trihydroxyisoflavone (genistein) [7].

The production of a pterocarpan phytoalexin by *S. junceum* is interesting because surveys of other Genisteae [9] suggest that isoflavone phytoalexins predominate in this legume tribe. For example, diffusates from leaflets of *Laburnum anagyroides* contain four related isoflavones, namely genistein (3 μg/ml) and **5** (19 μg/ml) as well as their 6-prenylated analogues, wighteone (4 μg/ml) and luteone (9 μg/ml) [9]. Genistein (trace), **5** (69 μg/g fr. tissue) and luteone (57 μg/g) similarly accumulate in the etiolated hypocotyls of *Lupinus albus* (cultivar Kievskij Mutant) following inoculation with *H. carbonum* [9]. In a previous study both luteone and wighteone were found to occur pre-infectionally in the leaves of various *Lupinus* species [9, 13, 14].

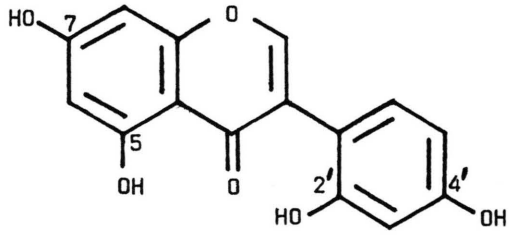
Experimental

Induction and extraction of **4** *and* **5**. Leaflets of *Spartium junceum* L. (collected from bushes growing in the University of Reading Botanic Garden) were treated with droplets of de-ionised H_2O or spore suspensions of *Helminthosporium carbonum* as reported elsewhere [15, 16]. UV irradiation (3 h) was undertaken as previously described [17]; the leaflets, without further treatment, were then incubated for 2 days [17] prior to extraction. TLC (Merck Si gel, F 254, layer thickness 0.25 mm) of diffusate extracts (see Results and Discussion) gave **4** and **5** together with a third, very minor, component (R_F 0.17) provisionally identified (TLC) as genistein (5,7,4′-trihydroxyisoflavone). Traces of the latter isoflavonoid were also present in control diffusates. Compounds **4** and **5** were eluted (EtOH) and further purified by TLC in *n*-pentane:Et_2O:HOAc (PEA) 75:25:3 (**4**, R_F 0.28) or 75:25:6, ×3 (**5**). Sparticarpin was isolated from tissues underlying the applied droplets by extraction (EtOH) and TLC (Et_2O:*n*-hexane, 3:1) [18]. The band at R_F 0.50 was eluted and the pterocarpan purified by successive TLC in PEA, 75:25:3, ×3 and C_6H_6:MeOH, 9:1 (R_F 0.61). UV irradiated leaflets were extracted using the base/acid procedure previously reported [19]. PLC (Merck, Si gel, F 254, layer thickness 0.5 mm) using $CHCl_3$:MeOH (25:1) gave **4** (R_F 0.50) which was eluted and rechromatographed (PEA followed by C_6H_6:MeOH) as outlined above.

2,3-Dimethoxy-9-hydroxypterocarpan (**4**) (*sparticarpin*). Diazotised *p*-nitroaniline, orange. λ_{max} (nm) [7]: EtOH 211 (100%), 232 sh (29%), 290 sh (22%), 294 (23%), 302 sh (13%); EtOH + NaOH 215, 248 sh, 299. MS [7, 17] (rel. int.) 301 (16), 300 (M^+; 100), 299 (15), 285 (19), 270 (12), 269 (8), 191 (5), 178 (5), 167 (14), 149 (41), 147 (12), 137 (6), 135 (11), 134 (10), 123 (12). *Monomethyl ether* (**1**) (CH_2N_2; R_F 0.22, $CHCl_3$:CCl_4, 3:1). UV as lit. [3]. MS (rel. int.) 315 (24), 314 (M^+; 100), 313 (21), 300 (7), 299 (39),



	R^1	R^2	R^3
1	Me	H	Me
2	H	H	Me
3	Me	OH	Me
4	Me	H	H



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271 (9), 211 (10), 191 (6), 185 (8), 178 (6), 163 (10), 162 (8), 161 (17), 157 (9), 152 (7), 149 (11), 148 (49), 139 (8), 137 (7), 133 (17), 128 (14), 127 (12). *Monoacetate* (Py-Ac_2O; R_F 0.48, $CHCl_3$) λ_{max} (nm): EtOH 211 (100%), 232 sh (40%), 283 sh (25%), 290 (28%), 302 sh (20%). MS (rel. int.) 343 (11), 342 (M^+; 40), 301 (11), 300 (100), 299 (18), 285 (25), 178 (7), 167 (21), 147 (8), 134 (10).

Synthesis of **4**. *2'-Hydroxy-4',5'-dimethoxyacetophenone.* 2',4',5'-Trihydroxyacetophenone (1 g) [20], Me_2SO_4 (1.6 g), dry Me_2CO (50 ml) and dry K_2CO_3 (5 g) were stirred under reflux for 4 h. The mixture was filtered, and the filtrate evapd. The residue was treated with MeOH (2–3 ml) and allowed to crystallise; the crystals were collected by filtration, washed with a little MeOH and then recrystd. from MeOH, mp. 112–113 ° (lit. 111–112 ° [21]). Yield 0.78 g. Further product (0.12 g) was obtained by TLC (C_6H_6:EtOAc:MeOH:petrol (60–80 °), 6:4:1:6) of the mother liquors.

2'-Hydroxy-4',5'-dimethoxy-2,4-dibenzyloxychalcone. KOH (5 g) in H_2O (5 ml) was added to a soln of 2'-hydroxy-4',5'-dimethoxyacetophenone (0.5 g) and 2,4-dibenzyloxybenzaldehyde (0.8 g) in warm EtOH (30 ml). The mixture was stirred at room temp. (16 h), poured onto ice, acidified with conc. HCl and then extracted with EtOAc (×3). The extracts were washed with H_2O, evapd. and the residue recrystd. from $CHCl_3$-MeOH, mp. 139–140 °. Yield 0.5 g. MS (rel. int.) 497 (3), 496 (M^+; 9), 405 (3), 389 (3), 388 (6), 373 (3), 244 (3), 181 (12), 92 (8), 91 (100).

6,7-Dimethoxy-2',4'-dibenzyloxyisoflavone. The above chalcone (0.45 g) was acetylated (Py (10 ml)–Ac_2O (1 ml), room temp. 16 h) and the reaction mixture poured into H_2O and extracted with EtOAc (×2). The extracts were washed successively with dil. HCl (×2) and H_2O, and then evapd. to dryness. The acetate, without further purification, was dissolved in MeOH (100 ml) and stirred with $Tl(NO_3)_3 \cdot 3H_2O$ (0.45 g) for 16 h (room temp.). Solid KOH (1 g) was then added and the mixture stirred for a further 1 h. After neutralisation with conc. HCl, the reaction mixture was acidified with 10% HCl (20 ml) and heated under reflux for 2 h. After filtration, the mixture was concd. *in vacuo*, diluted with H_2O and extracted with EtOAc (×2). The extracts were evapd. to dryness and the product isolated by TLC (C_6H_6:EtOAc:MeOH:petrol (60–80 °), 6:4:1:6) to give a gum which slowly crystallised. Recrystallisation from $CHCl_3$-MeOH gave the desired isoflavone, mp. 180–181 °. Yield 0.28 g. MS (rel. int.) 495 (3), 494 (10), 404 (3), 403 (11), 181 (3), 92 (8), 91 (100).

6,7-Dimethoxy-2',4'-dihydroxyisoflavone. The above isoflavone (150 mg) was heated at 70 ° for 2 h with conc. HCl (10 ml) in glacial HOAc (20 ml). The reaction mixture was poured into H_2O, extracted with EtOAc (×2) and the combined extracts washed with aqueous $NaHCO_3$ (×2) followed by H_2O. Removal of EtOAc gave a residue which slowly crystallised on addition of MeOH. The crystals were filtered off, washed with MeOH and then recrystd. from MeOH, mp. 238–240 °. Yield 74 mg. MS (rel. int.) 315 (20), 314 (M^+; 88), 313 (5), 297 (18), 182 (10), 181 (100), 180 (81), 165 (52), 161 (12), 157 (6), 152 (7), 137 (31), 134 (31), 109 (15), 105 (18).

2,3-Dimethoxy-9-hydroxypterocarpan. Solid $NaBH_4$ (200 mg) was added in three portions over 2 h to a stirred soln of 6,7-dimethoxy-2',4'-dihydroxyisoflavone (50 mg) in THF (5 ml) and absolute EtOH (5 ml). After stirring for a further 16 h, Me_2CO (3 ml) was added and the mixture concd. *in vacuo*. The residue was then treated with dil. HCl, extracted with EtOAc (×2) and the extracts washed (H_2O) and evapd. The pterocarpan was isolated by TLC (C_6H_6:EtOAc:MeOH:petrol (60–80 °), 6:4:1:6) and crystallised from MeOH, mp. 223–225 °. Yield 25 mg. UV and MS as given for natural sparticarpin (**4**).

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