Induced Isoflavonoids of *Erythrina sandwicensis*

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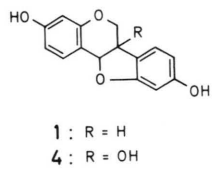
Two new phytoalexins isolated from the fungus-inoculated leaflets of *Erythrina sandwicensis* have been identified as (−)-6a*S*; 11a*S*-3,6a,9-trihydroxy-10-isopentenylpterocarpan (sandwicarpin) and (−)-6a*R*; 11a*R*-3-hydroxy-9-methoxy-10-isopentenylpterocarpan (sandwicensin). These compounds co-occur with several known pterocarpan (demethylmedicarpin, 3,6a,9-trihydroxypterocarpan, phaseollidin and cristacarpin) and isoflavan (demethylvestitol and isovestitol) derivatives. The preparation and spectral (UV, MS) characteristics of 3-methoxy-9-hydroxy-10-isopentenylpterocarpan are also described.

Introduction

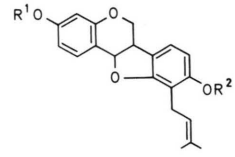
It was recently reported that demethylmedicarpin (3,9-dihydroxypterocarpan, **1**), phaseollidin (3,9-dihydroxy-10-isopentenylpterocarpan, **2**) and the previously undescribed 6a-hydroxypterocarpan, cristacarpin (3,6a-dihydroxy-9-methoxy-10-isopentenylpterocarpan, **3**) were produced as phytoalexins by fungus (*Helminthosporium carbonum*)-inoculated leaflets of the papilionate legume, *Erythrina cristagalli* (tribe Phaseoleae; subtribe Erythrininae) [1, 2]. Studies of the genus *Erythrina* have now been extended to include *E. sandwicensis*, a tree native to the Hawaiian islands. Si gel TLC examination of diffusates [3] from leaflets exposed to *H. carbonum* revealed numerous phenolic compounds which were eluted and, when necessary, further purified as outlined under Experimental. Six of these compounds were identified (UV, MS, TLC) as the known isoflavonoids **1**–**3**, 3,6a,9-trihydroxypterocarpan (**4**), demethylvestitol (7,2′,4′-trihydroxyisoflavan, **5**) and isovestitol (7,4′-dihydroxy-2′-methoxyisoflavan, **6**) [1, 4, 5]. In addition, two hitherto unreported pterocarpans were also isolated from *E. sandwicensis;* their characterisation as 3,6a,9-trihydroxy-10-isopentenylpterocarpan (sandwicarpin, **7**) and 3-hydroxy-9-methoxy-10-isopentenylpterocarpan (sandwicensin, **8**) is described in this communication.

Results and Discussion

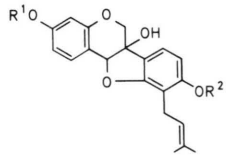
The neutral (EtOH) UV spectrum of sandwicarpin (**7**) was virtually superimposable on that of cristacarpin (**3**); upon addition of conc. HCl, **7** underwent rapid dehydration (indicative of a pterocarpan having tertiary (C-6a) hydroxylation) to yield a pterocarpene with intense UV maxima at 336 and 354 nm (*cf.* **3**, EtOH + HCl 337 and 354 nm [1]). MS analysis gave M^+ 340 together with the expected major fragments at *m/e* 322 ($M^+ - 18$ (H_2O)), 267 ($M^+ - 18 - 55$) and 266 ($M^+ - 18 - 56$); loss of 55 and/or 56 mu (isobutene) from either the parent ion or a derived fragment is characteristically observed in the MS of pterocarpans (*e. g.* **2**) and other isoflavonoids possessing an isopentenyl sidechain [6–8]. The substitution/oxygenation pattern of sandwicarpin was confirmed by PMR analysis (see Experimental) and by methylation (CH_2N_2) to afford a dimethyl ether (M^+ 368) identical (UV, MS, TLC) with 3-O-methylcristacarpin (**9**). Formation of the latter com-



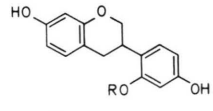
1: R = H
4: R = OH



2: $R^1 = R^2 = H$
8: $R^1 = H$; $R^2 = Me$
10: $R^1 = R^2 = Me$
11: $R^1 = Me$; $R^2 = H$



3: $R^1 = H$; $R^2 = Me$
7: $R^1 = R^2 = H$
9: $R^1 = R^2 = Me$



5: R = H
6: R = Me

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pound establishes beyond doubt that sandwicarpin is 3,6a,9-trihydroxy-10-isopentenylpterocarpan (**7**).

The second new isoflavonoid (sandwicensin, **8**) had M^+ 338 with associated fragments at *m/e* 283/282 ($M^+ - 55/56$) and could be methylated to give a monomethyl ether (M^+ 352) indistinguishable (UV, MS, TLC) from the 3,9-di-O-methyl derivative (**10**) of phaseollidin (**2**). As with cristacarpin (**3**), a distinct alkali UV maximum at approx. 250 nm (C-3 OH) and the formation of a bright yellow product with diazotised *p*-nitroaniline (C-9 OMe) allowed the OH/OMe groups of **8** to be placed at C-3 and C-9 respectively [1]. Sandwicensin is thus 3-hydroxy-9-methoxy-10-isopentenylpterocarpan. This structure was indirectly confirmed by comparison of **8** with its isomer 3-O-methylphaseollidin (**11**) prepared via selective methylation of **2**; sandwicensin and pterocarpan **11** were readily distinguished by TLC/UV (alkali maxima) and reaction to diazotised *p*-nitroaniline (see Experimental and [1]).

Fungus-induced diffusates were found to contain sandwicarpin (**7**) and sandwicensin (**8**) at concentrations (based on $\log \varepsilon = 3.78$ at 286 nm for **2** [9]) of 11–22 and < 0.3–1 μg/ml respectively. Corresponding values for the other *E. sandwicensis* isoflavonoids were: **1**, 0.5–1 μg/ml; **2**, 10–20 μg/ml; **3**, 4–10 μg/ml; **4**, 1–3 μg/ml; **5**, 9–24 μg/ml; and **6**, 2–7 μg/ml. Compounds **1–8** were not isolated from leaflets treated with de-ionised H_2O. The fungitoxic properties of compounds **1–3**, **5** and **6** have been described elsewhere [1, 5, 9, 10]. In TLC bioassays against *Cladosporium herbarum*, **7** and **8** (20 μg) had antifungal activity comparable with that of phaseollidin. 3,6a,9-Trihydroxypterocarpan (**4**) was also weakly fungitoxic; Lyne and Mulheirn [4] earlier reported that growth of an unspecified *Cladosporium* isolate was unaffected by the latter pterocarpan.

Apart from sandwicarpin and sandwicensin, all the above mentioned *Erythrina* isoflavonoids are known to occur in other legumes. Thus, phaseollidin accumulates in the *H. carbonum*-treated tissues of numerous species belonging to the pantropical Phaseoleae (subtribes Erythrininae and Phaseolinae) [1, 6, 11–13] where – as in *E. sandwicensis* – it is sometimes accompanied by traces of demethylmedicarpin. The isoflavans, demethylvestitol and isovestitol, have also been recorded in the Phaseoleae [11] although both compounds are more commonly encountered in genera comprising the largely north-temperate Loteae and Trifolieae [5, 14]. Cristacarpin is produced as a minor phytoalexin by *Psophocarpus tetragonolobus* (Phaseoleae, subtribe Phaseolinae) and by some *Erythrina* species (*e. g. E. crista-galli*) other than *E. sandwicensis* [1]. However, it is of sporadic distribution in *Erythrina*, being absent from species such as *E. corallodendron* and *E. lysistemon* where phaseollidin predominates (J. L. Ingham, unpublished data). Finally, 3,6a,9-trihydroxypterocarpan has previously been found only as a trace constituent in cotyledons of *Glycine max* (Phaseoleae, subtribe Glycininae) which are actively synthesising glyceollins I–IV [4, 15] following exposure to aqueous $CuCl_2$.

Experimental

MS/UV analyses and all chromatographic separations were undertaken as previously described [10, 16]. Seeds of *Erythrina sandwicensis* were collected from trees growing near Kekaha on the Hawaiian island of Kauai.

Induction and isolation of Erythrina isoflavonoids. E. sandwicensis was grown for approx. 6 months under conditions similar to those reported elsewhere [17]; excised leaflets were then treated with droplets of de-ionised H_2O (control) or conidial suspensions of *Helminthosporium carbonum* [3]. Extracts (EtOAc) of 48 h diffusates (between 50 and 200 ml) from *H. carbonum*-inoculated leaflets were chromatographed (Si gel TLC, $CHCl_3$:MeOH, 20:1) to afford diazotised *p*-nitroaniline-positive zones at R_F 0.74 (**8**), 0.59 (**2**), 0.42 (**3**), 0.37 (**6**), 0.32 (**1**), 0.18 (**7**) and 0.09 (**4** + **5**). These were eluted (EtOH) and with the exception of **2** and **3** further purified as follows: i) **1** and **6**, *n*-pentane:Et_2O:glacial HOAc (PEA) 75:25:3, ×3; ii) **4** + **5**, PEA 75:25:6, ×3 to give **5** (upper) and **4** (lower) as well resolved bands; iii) **7**, C_6H_6:MeOH 9:1, ×3; and iv) **8**, PEA 75:25:3 (R_F 0.63).

Compounds **1–3, 5** *and* **6**. UV and MS as lit. [1, 5, 6, 9, 10].

3,6a,9-Trihydroxypterocarpan (**4**). Diazotised *p*-nitroaniline, orange (*cf.* **1** [1, 10]. λ_{max} (nm) EtOH 214 (100%), 230 sh (71%), 283 (42%), 287 (44%), 293 sh (30%), lit. 282 and 287 nm [4]; EtOH + conc. HCl 213, 230, 240 sh, 250 sh, 283 sh, 288, 292 sh, 319 sh, 336, 353, lit. 335 and 350 nm [4]; EtOH + NaOH 218, 249, 298. An intense purple/pink colouration (λ_{max} 522 nm) rapidly developed (10–20 sec) in the presence of aqueous NaOH. MS (rel. int.) 272

(M$^+$; 2), 255 (10), 254 (71), 253 (100), 252 (3), 225 (4), 197 (14).

3,6a,9-Trihydroxy-10-isopentenylpterocarpan (**7**) (*sandwicarpin*). Diazotised *p*-nitroaniline, orange (*cf.* **2** [1, 11]). λ_{max} (nm) EtOH 212 (100%), 234 sh (37%), 281 (15%), 287 (15%); EtOH + conc. HCl 212, 244, 252 sh, 290, 320 sh, 336, 354; EtOH + NaOH 212, 250, 295. A purple/pink colour did not develop even after prolonged (30 min) exposure of **7** to aqueous NaOH (*cf.* **4**). MS (rel. int.) 340 (M$^+$; 2), 323 (6), 322 (25), 321 (8), 267 (22), 266 (100), 237 (6); PMR (360 MHz, $(CD_3)_2CO$, TMS) δ 7.34 (1 H, d, H-1), 7.03 (1 H, d, H-7), 6.55 (1 H, q, H-2), 6.45 (1 H, d, H-8), 6.30 (1 H, d, H-4), 5.26 (1 H, s, H-11a), 5.23 (1 H, br t, H-13, olefinic), 4.12/4.01 (2 H, dd, H-6,6′), 3.23 (2 H, d, H-12, methylene), 1.72 (3 H, s, methyl), 1.60 (3 H, s, methyl). The C-6a multiplet which appears at δ 4.24 in the PMR ($(CD_3)_2CO$) of phaseollidin (**2**) was absent from the spectrum of sandwicarpin. $[\alpha]_{589\,nm}$ −278 ° (approx. 0.5 mg in 1 ml MeOH); the absolute configuration of sandwicarpin is thus 6a S; 11a S [1]. *Dimethyl ether* (**9**). TLC, UV and MS data as lit. [1].

3-Hydroxy-9-methoxy-10-isopentenylpterocarpan (**8**) (*sandwicensin*). Diazotised *p*-nitroaniline, bright yellow (*cf.* **3** [1]). λ_{max} (nm) EtOH 211 (100%), 234 sh (38%), 281 (15%), 287 (16%); EtOH + NaOH 212, 252, 290, 300 sh; the MeOH spectrum was unaffected by addition of conc. HCl. MS (rel. int.) 339 (23), 338 (M$^+$; 100), 337 (7), 323 (12), 295 (23), 283 (35), 282 (84), 281 (60), 267 (24), 253 (35), 252 (10), 251 (7), 185 (7), 161 (25), 147 (27), 123 (25). $[\alpha]_{589\,nm}$ −190 ° (approx. 0.2 mg in 1 ml MeOH); the absolute configuration of sandwicensin is thus 6a R; 11a R [1]. *Monomethyl ether* (**10**) (R_F 0.68, $CHCl_3$:CCl_4, 1:1). UV as lit. [18]. MS (rel. int.) 353 (23), 352 (M$^+$; 100), 351 (8), 337 (10), 309 (15), 297 (25), 296 (54), 295 (36), 281 (13), 267 (14), 201 (8), 161 (26), 149 (15), 137 (16).

Preparation of 3-methoxy-9-hydroxy-10-isopentenylpterocarpan (**11**) (*3-O-methylphaseollidin*). CH_2N_2 was bubbled (5 min) through a solution of (−)-**2** (approx. 1 mg) in CH_2Cl_2/MeOH (1:4). Work up and Si gel TLC ($CHCl_3$:CCl_4, 1:1) gave 3-O-methylphaseollidin (approx. 0.5 mg; R_F 0.35) together with smaller quantities (approx. 0.3 mg; R_F 0.68) of the 3,9-di-O-methyl derivative (**10**). Data recorded for **11** were as follows: diazotised *p*-nitroaniline, orange (*cf.* sandwicensin, **8**). λ_{max} (nm) EtOH 211 (100%), 232 sh (57%), 281 (28%), 287 (31%); EtOH + NaOH 215, 250 sh, 282 sh, 287, 300 sh (*cf.* UV maxima of 3-hydroxy-9-methoxypterocarpan [10] and 3-methoxy-9-hydroxypterocarpan, λ_{max} (nm) EtOH 212, 230 sh, 282, 287; EtOH + NaOH 215, 250 sh, 281 sh, 287, 300 sh); MS (rel. int.) 339 (16), 338 (M$^+$; 94), 337 (6), 323 (3), 295 (11), 284 (9), 283 (46), 282 (100), 281 (53), 267 (6), 161 (21). Pterocarpan **11** could be separated from sandwicensin (**8**) by Si gel TLC in PEA 75:25:1 (**8**, R_F 0.61; **11**, R_F 0.69).

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