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 (−)-6αS, 11αS-3,6α,9-trihydroxy-10-isopen
tenylpterocarpan (sandwicarpin) and (−)-6αR, 11αR-3-hydroxy-9-methoxy-10-isopenteny
terocarpan (sandwicensin). These compounds co-occur with several known pterocarpan (demethylmedicarpin, 3,6α,9-trihydroxyterocarpan, phaseollidin and cristacarpin) and isoflavan (demethylvestitol and isovestitol) derivatives. The preparation and spectral (UV, MS) characteristics of 3-methoxy-9-hydroxy-10-isopen
tenylpterocarpan 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 6α-hydroxypterocarpan, cristacarpin (3,6α-dihydroxy-9-methoxy-10-isopentenyl
terocarpan, 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, 3,6α,9-trihydroxypterocarpan (4), demethylvestitol (7,2',4'-trihydroxyisofla
van, 5) and isovestitol (7,4'-dihydroxy-2'-methoxyisoflavan, 6) [1, 4, 5]. In addition, two hitherto unreported pterocarps were also isolated from *E. sandwicensis*: their characterisation as 3,6α,9-trihydroxy-10-isopen
tenylpterocarpan (sandwicarpin, 7) and 3-hydroxy-9-methoxy-10-isopentenylpterocarpan (sandwicensin, 8) is described in this communica
tion.

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-6α) hydroxylation) to yield a pterocarpe 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 (H2O)), 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 substi
tution/oxygenation pattern of sandwicarpin was confirmed by PMR analysis (see Experimental) and by methylation (CH3N2) to afford a dimethyl ether (M+ 368) identical (UV, MS, TLC) with 3-O-methylcristacarpin (9). Formation of the latter com

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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 6 + 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, 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 ε = 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 H2O (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, CHCl3:MeOH, 20:1) to afford diazotised p-nitroaniline-positive zones at Rf 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:Et2O: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, C6H6:MeOH 9:1, × 3; and iv) 8, PEA 75:25:3 (Rf 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]. λmax (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 coloration (λmax 522 nm) rapidly developed (10 -20 sec) in the presence of aqueous NaOH. MS (rel. int.) 272.
3,6a,9-Trihydroxy-10-isopentenylpterocarpan (7) (sandwicarpin). Diazotised p-nitroaniline, orange (cf. 2 [1, 11]). $\lambda_{\text{max}}$ (nm) EtOH 211 (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$_3$)$_2$CO, TMS) $\delta$ 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-11 a), 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 C₆₆a multiplet which appears at $\delta$ 4.24 in the PMR ((CD$_3$)$_2$CO) of phaseollidin (2) was absent from the spectrum of sandwicarpin. [a]$\lambda_{\text{max}}$ nm $\pm$ 278° (approx. 0.5 mg in 1 ml MeOH); the absolute configuration of sandwicarpin is thus 6aS; 11aR [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]). $\lambda_{\text{max}}$ (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). [a]$\lambda_{\text{max}}$ nm $\pm$ 190° (approx. 0.2 mg in 1 ml MeOH); the absolute configuration of sandwicensin is thus 6aR; 11aR [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$_2$N$_2$ was bubbled (5 min) through a solution of (— )-2 (approx. 1 mg) in CH$_2$Cl$_2$ /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). $\lambda_{\text{max}}$ (nm) EtOH 211 (100%), 232 sh (57%), 281 (28%), 287 (31%); EtOH + NaOH 215, 250 sh, 282 sh, 300 sh (cf. UV maxima of 3-hydroxy-9-methoxypterocarpan [10] and 3-methoxy-9-hydroxypterocarpan, $\lambda_{\text{max}}$ (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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