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ISOPTEROFURAN, A NEW 2-ARYLBENZOFURAN PHYTOALEXIN FROM *CORONILLA EMERUS*

PAUL M. DEWICK* and JOHN L. INGHAM†

*Department of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, U. K.; †Phytochemical Unit, Department of Botany, University of Reading, Reading, RG6 2AS, U.K.

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Key Word Index—*Coronilla emerus*; Leguminosae; Coronilleae; 2-arylbenzofurans; phytoalexins; isopterofuran; 6-demethylvignafuran; antifungal activity.

Abstract—Two 2-arylbenzofuran phytoalexins isolated from the fungus-inoculated leaflets of *Coronilla emerus* (scorpion senna) have been identified as 6-demethylvignafuran and the previously unreported 2-(4-hydroxy-2,3-dimethoxyphenyl)-6-hydroxybenzofuran (isopterofuran). The synthesis of isopterofuran is described.

INTRODUCTION

Whilst many plant species belonging to the Leguminosae (subfamily Papilionoideae) synthesize isoflavonoid phytoalexins when their tissues are challenged by fungi, a few characteristically produce non-flavonoid compounds either exclusively or in association with isoflavonoid derivatives [1]. These non-flavonoid phytoalexins include furanoacetylenes, stilbenes and 2-arylbenzofurans [1]. As part of a chemosystematic survey of the Leguminosae, we have investigated the phytoalexins which accumulate when leaflets of scorpion senna, *Coronilla emerus* (tribe Coronilleae) are subjected to fungal inoculation. The isolation of two 2-arylbenzofuran phytoalexins from the leaf diffusates and underlying leaf tissues of this leguminous shrub, and their identification as 6-demethylvignafuran (**1**) and the previously unreported 2-(4-hydroxy-2,3-dimethoxphenyl)-6-hydroxybenzofuran (isopterofuran, **2**) are described in the present paper.

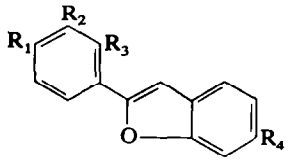
RESULTS AND DISCUSSION

Detached leaflets of *Coronilla emerus* were inoculated with a conidial suspension of *Helminthosporium carbonum* and incubated for 48 hr. Diffusate extracts (EtOAc) were then chromatographed (Si gel TLC; $CHCl_3$–MeOH, 50:1) to give two phenolic compounds (R_f 0.48 and 0.21) both of which exhibited a blue fluorescence when developed TLC plates were viewed under long wavelength UV light. The UV maxima (EtOH) of these compounds were characteristic of 2-arylbenzofurans, the lower band (λ_{max} 321 and 337 nm) subsequently being identified as 6-demethylvignafuran (**1**) after UV, MS and TLC comparison with an authentic specimen isolated from *Tetragonolobus maritimus* (tribe Loteae) [2].

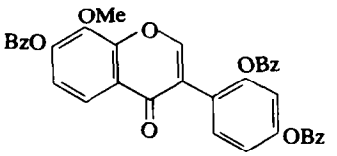
The second band had UV maxima at 318 and 332 nm shifting to 342 and 358 nm on addition of aqueous NaOH. The mass spectrum for this compound exhibited a molecular ion at *m/e* 286 (base peak) although little structural information could be obtained from the observed major fragments at *m/e* 271 (M-15), 243 (M-15-28) and 228 (M-15-28-15). Methylation (CH_2N_2) gave a dimethyl ether (M^+ 314) whilst acetylation (Py—Ac_2O) afforded a diacetate (M^+ 370). These data are consistent with the formulation of **2** as a 2-arylbenzofuran having two hydroxyl and two methoxyl substituents. Such a compound, pterofuran 2-(3-hydroxy-2,4-dimethoxyphenyl)-6-hydroxybenzofuran) (**3**), is already known to occur in nature as a heartwood constituent of the leguminous tree, *Pterocarpus indicus* (tribe Pterocarpeae) [3]. The neutral UV spectrum of **2** was almost identical to that of pterofuran (λ_{max} 317 and 332 nm) indicative perhaps of the same oxygenation pattern. However, pterofuran and **2** could readily be separated by Si gel TLC ($CHCl_3$–MeOH, 50:1, ×3) (**2**, lower zone; **3**, upper zone) although their dimethyl ethers (**4**) proved to be identical (UV, MS, TLC). Because of its co-occurrence with 6-demethylvignafuran (**1**) and its failure to react with Gibbs reagent, compound **2** was tentatively assigned the structure 2-(4-hydroxy-2,3-dimethoxyphenyl)-6-hydroxybenzofuran. This provisional structure was later confirmed by unambiguous synthesis.

A number of natural 2-arylbenzofuran derivatives appear to be related biogenetically to the isoflavonoids [4], and chemical modification of suitable isoflavones provides a convenient synthetic approach to these compounds. This route has been used successfully to synthesize vignafuran (**5**) [4] and 6-demethylvignafuran as well as two structural isomers of the latter compound [2]. 2′,4′,7-Tribenzyloxy-8-methoxyisoflavone (**6**) was synthesized via Tl $(NO_3)_3$ oxidation [5] of the corresponding chalcone. Upon base hydrolysis, this isoflavone yielded the deoxybenzoin (**7**) which was methylated to give **8**. Catalytic hydrogenation of **8** in acid solution afforded **2** which

proved to be indistinguishable (UV, MS and TLC in $CHCl_3$–MeOH, 50:1, R_f 0.48; *n*-pentane–Et_2O–HOAc, 75:25:3, R_f 0.41; and C_6H_6–MeOH, 9:1, R_f 0.31) from the natural product. The common name isopterofuran is proposed for this previously undescribed benzofuran.



	R_1	R_2	R_3	R_4
1	OH	H	OMe	OH
2	OH	OMe	OMe	OH
3	OMe	OH	OMe	OH
4	OMe	OMe	OMe	OMe
5	OH	H	OMe	OMe



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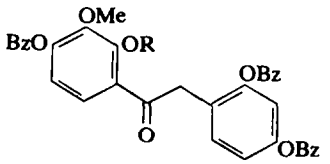

7 R = H
8 R = Me

There was no evidence to suggest that *C. emerus* produced either medicarpin (a common legume phytoalexin) or the simple isoflavan phytoalexins (e.g. vestitol and sativan) commonly associated with species belonging to the closely related tribes Carmichaelieae (Ingham, J. L., unpublished data), Loteae [6, 7] and Trifolieae [8, 9]. Vignafuran (**5**), a phytoalexin of *Lablab niger* and *Vigna unguiculata* (tribe Phaseoleae) [10, 11] was also absent from diffusates of *C. emerus*.

Isopterofuran is highly fungitoxic. When tested (TLC bioassay) [12, 13] against spore germination of *Cladosporium herbarum*, the compound (10 μg based on log $\varepsilon = 4.58$ at 317 nm for pterofuran) [3] gave an inhibition zone of *ca* 230 mm^2; fungal growth was totally inhibited over an area of *ca* 80 mm^2. Corresponding values for a 20 μg sample of **2** were *ca* 530 and 150 mm^2, respectively.

In leaf diffusates the average concentrations of **2** and **1** were 14 μg/ml (based on log ε for **3**) and 8 μg/ml (based on log ε 4.59 at 320 nm for vignafuran) [10], respectively. Extracts of inoculated leaf tissues gave reasonable quantities of **2** (34 μg/g fr. wt) but only small amounts (<5 μg/g fr. wt) of **1**. Neither phytoalexin was isolated in measurable quantities from control leaflets treated with deionized H_2O. Isopterofuran and 6-demethylvignafuran have not been detected in diffusates from the *H. carbonum*-inoculated leaflets of *C. cretica*, *C. valentina* and *C. varia* all of which contain various bright-blue fluorescent compounds of undetermined constitution (Ingham, J. L., unpublished data).

EXPERIMENTAL

Mass and UV spectra were determined as previously described [6, 14]. TLC separations were undertaken using precoated, glass-backed plates (Merck Si gel F/GF 254, layer thickness, 0.25 or 0.5 mm).

Isolation of compounds **1** *and* **2** . Leaflets of *Coronilla emerus* L. (collected from an established plant growing at the University of Reading Botanic Garden) were inoculated with droplets of fungal spore suspension [12, 15] and the resulting diffusates collected after 48 hr incubation [15]. Control leaflets received droplets of deionised H_2O. Si gel TLC ($CHCl_3$–MeOH, 50:1) of diffusate extracts (EtOAc) gave isopterofuran (**2**) and 6-demethylvignafuran (**1**) at R_f 0.48 and 0.21, respectively (cf. vignafuran **5**, R_f 0.68). These zones were eluted (EtOH) and their components subjected to additional TLC in *n*-pentane–Et_2O–HOAc, 75:25:1 (**2**, R_f 0.28; **1**, R_f 0.14) prior to UV and MS analysis. Tissues underlying the inoculum droplets were excised and extracted as described elsewhere [2]. Si gel TLC ($CHCl_3$–MeOH, 50:1) of the extract gave **2** together with very small quantities of **1**; both compounds were further purified as outlined above.

Isopterofuran (**2**). Diazotized *p*-nitroaniline, purple/brown; Gibbs reagent, no reaction. When TLC plates were examined under long wavelength UV light, **2** was visible as a dark-blue fluorescent band unaffected by NH_3 vapour. λ_{max} EtOH nm :213 (90%), 245 sh (33%), 285 (47%), 249 sh (51%), 304 sh (62%), 318 (100%), 332 (94%); λ_{max} EtOH + NaOH nm :218 (100%), 300 sh (12%), 326 sh (23%), 342 (38%), 358 (35%). MS *m/e* (rel. int.): 286 (M^+;100), 285 (5), 272 (2), 271 (31), 253 (13), 243 (33), 228 (41), 227 (6), 225 (20), 215 (5), 213 (6), 200 (9), 197 (15), 172 (31), 171 (39), 168 (7), 165 (6), 158 (6), 147 (6), 146 (8), 144 (8), 143 (9), 139 (7), 115 (31). *DiMe ether* (**4**) (CH_2N_2) (R_f 0.77, $CHCl_3$—CCl_4, 1:1) λ_{max} EtOH nm: 212 (88%), 245 sh (40%), 284 sh (50%), 292 sh (53%), 303 sh (67%), 316 (100%), 331(95%). MS *m/e* (rel. int.): 314 (M^+; 100), 313 (4), 300 (6), 299 (38), 284 (12), 271 (11), 269 (10), 256 (9), 241 (11), 239 (3), 213 (9), 211 (4), 186 (4), 185 (33), 170 (13), 157 (8), 142 (10), 139 (3). *Diacetate* (Py—Ac_2O) (R_f 0.76, $CHCl_3$) λ_{max} EtOH nm: 214 (74%), 240 sh (29%), 278 sh (43%), 286 sh (53%), 294 sh (64%), 307 (100%), 321 (86%). MS *m/e* (rel. int.): 370 (M^+; 3), 329 (2), 328 (13), 286 (100), 271 (17). Comparative data recorded for authentic pterofuran **3** were as follows. Diazotized *p*-nitroaniline, orange/brown; Gibbs reagent, purple/black. **3** exhibited a dark-blue fluorescence (unaffected by NH_3) under long wavelength UV light. λ_{max} EtOH as lit. [3]; $\lambda_{max}^{EtOH+NaOH}$ nm: 213 (100%), 238 (26%), 300 sh (14%), 322 sh (22%), 337 (33%), 352 (33%). MS *m/e*(rel. int.): 286 (M^+; 100), 285 (14), 272 (7), 271 (62), 243 (21), 228 (26), 227 (4), 225 (30), 224 (6), 215 (14), 200 (16), 197 (9), 172 (24), 171 (33), 158 (5), 155 (4), 146 (5), 144 (7), 143 (8), 115 (24).

Synthesis of 2-(4-hydroxy-2,3-dimethoxyphenyl)-6-hydroxybenzofuran (isopterofuran). A solution of 4'-benzyloxy-2'-hydroxy-3'-methoxyacetophenone [16] (1 g) and 2,4-dibenzyloxybenzaldehyde (1.3 g) in warm EtOH (75 ml) was treated with KOH (10 g) in H_2O (10 ml), and then stirred at room temp. for two days. The ppt. was removed by filtration,

washed with H_2O, and recrystallized from $CHCl_3$–MeOH to give 2,4,4'-tribenzyloxy-2'-hydroxy-3'-methoxychalcone (1.05 g), mp 150–51°. (Found: C, 77.36; H, 5.59. $C_{37}H_{32}O_6$ requires: C, 77.62; H, 5.59%). This chalcone (1 g) was acetylated (Py–Ac_2O, room temp., 18 hr), the reaction mixture poured into H_2O, and the precipitated acetate filtered off. The acetate was then dissolved in EtOAc, and the resulting soln washed successively with 5% HCl (2×) and H_2O (2×) before being evapd to dryness. Without further purification, the acetate was dissolved in $CHCl_3$–MeOH (1:1, 100 ml) and stirred (room temp.) with $Tl(NO_3)_3 \cdot 3H_2O$ (0.85 g) for 3 hr. The reaction mixture was then neutralized with NaOH in MeOH, filtered, and the filtrate concd and poured into H_2O. The mixture was extracted with $CHCl_3$ (2×), the $CHCl_3$ extracts evapd, and the residues stirred with KOH (1 g) in MeOH (100 ml) at room temp. for 1 hr. After neutralization with conc HCl, 10% HCl (20 ml) was added, and the mixture heated under reflux for 2 hr, and then cooled. The precipitated 2',4',7-tribenzyloxy-8-methoxyisoflavone (**6**) was filtered off and recrystallized from $CHCl_3$–MeOH. Yield 0.83 g, mp 155–56°. (Found: C, 77.65; H, 5.32. $C_{37}H_{30}O_6$ requires: C, 77.89; H, 5.26%). This isoflavone (0.6 g) in EtOH (40 ml) was heated under reflux with KOH (2 g) in H_2O (20 ml) for 1.5 hr. After removal of the EtOH, the mixture was acidified, extracted with EtOAc (2×), and the extracts washed with H_2O and evapd. The product, 2,4-dibenzyloxybenzyl-4-benzyloxy-2-hydroxy-3-methoxyphenylketone (**7**) was recrystallized from $CHCl_3$–MeOH. Yield 0.43 g, mp 139–40°. (Found: C, 77.16; H, 5.90. $C_{36}H_{32}O_6$ requires: C, 77.14; H, 5.71%). The above deoxybenzoin (0.20 g) was methylated by stirring under reflux with Me_2SO_4 (40 μl) and dry K_2CO_3 (2 g) in dry Me_2CO (20 ml) for 7 hr. The mixture was filtered, the filtrate evapd, and the residue recrystallized from $CHCl_3$–MeOH to yield 2,4-dibenzyloxybenzyl-4-benzyloxy-2,3-dimethoxyphenylketone (**8**) (0.17 g) mp 92–3°. (Found: C, 77.32; H, 6.06. $C_{37}H_{34}O_6$ requires: C, 77.35; H, 5.92%). Hydrogenation of this compound (50 mg) over Pd–C catalyst (10%, 20 mg) in EtOH (20 ml) containing conc HCl (0.2 ml) yielded isopterofuran (**2**), which was obtained as an oil after TLC purification (C_6H_6–EtOAc–MeOH–petrol (60–80°), 6:4:1:6); synthetic isopterofuran was identical (UV, MS, TLC) to the natural product. NMR (60 MHz, $CDCl_3$, TMS): δ 3.90 (3H, *s*, OMe), 3.97 (3H, *s*, OMe), 6.75 (1H, *dd*, $J = 8$, 2 Hz, H-5), 6.80 (1H, *d*, $J = 8.5$ Hz, H-5'), 6.98 (1H, *d*, $J = 2$ Hz, H-7), 7.08 (1H, *s*, H-3), 7.38 (1H, *d*, $J = 8$ Hz, H-4), 7.58 (1H, *d*, $J = 8.5$ Hz, H-6'). Acetylation (Py–Ac_2O) of isopterofuran yielded a diacetate (12 mg) which was recrystallized from aq. MeOH, mp 113–4°. (Found: C, 64.69; H, 4.98. $C_{20}H_{18}O_7$ requires: C, 64.86; H, 4.86%).

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