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6-DEMETHYLVIGNAFURAN AS A PHYTOALEXIN OF *TETRAGONOLOBUS MARITIMUS*

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Key Word Index—*Tetragonolobus maritimus*; Leguminosae; Loteae; *Helminthosporium carbonum*; 2-arylbenzofuran; pterocarpene; phytoalexin; antifungal activity; isoflavonoid; benzofuran synthesis.

Abstract—A phytoalexin isolated from the fungus-inoculated leaves of *Tetragonolobus maritimus* has been identified as 6-demethylvignafuran (2-[4-hydroxy-2-methoxyphenyl]-6-hydroxybenzofuran). The synthesis of 6-demethylvignafuran and two isomeric benzofurans is described. The presence of 3,9-dihydroxypterocarp-6a-ene in *T. maritimus* is also reported.

INTRODUCTION

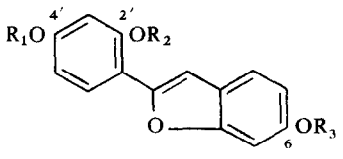
Isoflavonoid phytoalexins have been isolated from the fungus-inoculated leaves of many species belonging to the Leguminosae (subfamily Papilionoideae). Although leguminous species may also produce non-isoflavonoid phytoalexins either exclusively or in association with isoflavonoid derivatives (e.g. the furanoacetylenes of *Vicia faba* [1–3] and the stilbenes of *Arachis hypogaea* [4, 5]), such compounds appear to be comparatively rare. During a recent survey of the tribe Loteae a new isoflavan phytoalexin (4',7-dihydroxy-2'-methoxyisoflavan or isovestitol) was isolated from the fungus-infected leaves of *Tetragonolobus maritimus* [6]. In addition, small quantities of a non-isoflavonoid compound (provisionally identified as a dihydroxy-monomethoxy 2-arylbenzofuran) were also detected. The identification of this compound as 6-demethylvignafuran (**1**) and its total synthesis by a route based on its possible biogenetic origin are described in the present paper.

RESULTS AND DISCUSSION

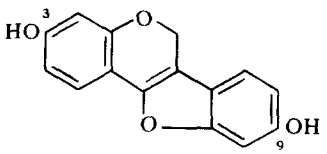
Detached leaves of *Tetragonolobus maritimus* (L.) Roth were inoculated with a conidial suspension of *Helminthosporium carbonum* Ullstrup and incubated for 48 hr. Diffusate extracts (EtOAc) were chromatographed (Si gel TLC; $CHCl_3$–MeOH, 50:1) to afford isovestitol (*ca* R_f 0.47) admixed with traces of a substance (**1**) having UV (EtOH) maxima (see Experimental) characteristic of a 2-arylbenzofuran. Such a compound (vignafuran, (**5**)) has already been isolated as a phytoalexin from the *Colletotrichum lindemuthianum*-inoculated leaves of cowpea (*Vigna unguiculata*) [7]. Because diffusates contained only small amounts of the *Tetragonolobus* benzofuran, further quantities were isolated from leaf tissues underlying the inoculum droplets; chlorophyll and other pigments present in the resulting leaf extracts (EtOH) were removed by base–acid partition (see Experimental) prior to Si gel TLC. On average. this procedure afforded 27 μg/g fr. tissue (based on $\log \varepsilon = 4.59$ at 320 nm for vignafuran [7]) of a phenolic compound which gave an intense purple–pink colouration when sprayed with diazotised *p*-nitroaniline; it did not react to Gibbs reagent. MS analysis gave the M^+ at *m/e* 256 (corresponding to $C_{16}H_{14}O_3$) whilst methylation (CH_2N_2) afforded a diMe ether (M^+ 284) identical (UV, MS, TLC) with 4'-*O*-methylvignafuran (**6**). Leaf extracts also contained extremely small quantities of 3,9-dihydroxypterocarp-6a-ene (**2**); the identity of (**2**) was established by comparison with a synthetic sample prepared from 2',4',7-trihydroxyisoflavone.

A number of 2-arylbenzofurans, namely pterofuran from *Pterocarpus indicus* [8], neoraufurane from *Neorautanenia edulis* [9] and vignafuran (**5**) from *Vigna unguiculata* [7] co-occur in these leguminous plants with isoflavonoids having similar substitution patterns; it is likely therefore that such compounds share similar biosynthetic origins with the isoflavonoids [8, 10, 11]. Plausible biosynthetic sequences involving loss of one carbon atom from an isoflavone, pterocarpan, pterocarpene or coumestan may readily be devised. Indeed, such a sequence has been employed during the degradation and structure determination of coumestans [12]. In view of its co-occurrence with isovestitol and the pterocarpene (**2**), compound (**1**) was tentatively assigned a 2',4',6-substitution pattern and the three possible dihydroxy-monomethoxy 2-arylbenzofurans (**1**), (**3**) and (**4**) were synthesised.

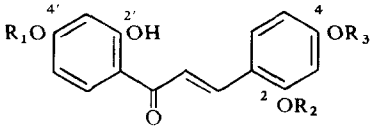
A number of synthetic routes to 2-arylbenzofurans have been described and there are published syntheses of the naturally-occurring compounds pterofuran [13, 14] and vignafuran [7, 14]. However, all these methods suffer from low yields and/or lack of specificity in ring closure; they also employ relatively inaccessible starting materials. The present approach to benzofuran synthesis exploits the similarity between these compounds and the isoflavonoids. It involves as a key step the formation of deoxybenzoins via base hydrolysis of the corresponding 2'-benzyloxyisoflavones [15]; removal of the benzyl protecting group coupled with acid-catalysed ring closure then affords the desired benzofuran derivative. Isoflavones are readily obtained from suitable chalcones by means of the thallium nitrate rearrangement [16],



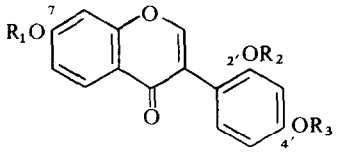
(1) $R_1 = R_3 = H$, $R_2 = Me$
(3) $R_1 = R_2 = H$, $R_3 = Me$
(4) $R_1 = Me$, $R_2 = R_3 = H$
(5) $R_1 = H$, $R_2 = R_3 = Me$
(6) $R_1 = R_2 = R_3 = Me$



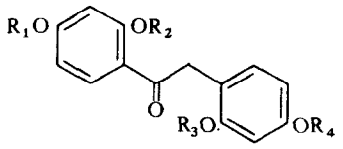
(2)



(7) $R_1 = R_2 = R_3 = CH_2Ph$
(8) $R_1 = R_2 = CH_2Ph$, $R_3 = Me$
(9) $R_1 = Me$, $R_2 = R_3 = CH_2Ph$



(10) $R_1 = R_2 = R_3 = CH_2Ph$
(11) $R_1 = R_2 = CH_2Ph$, $R_3 = Me$
(12) $R_1 = Me$, $R_2 = R_3 = CH_2Ph$



(13) $R_1 = R_3 = R_4 = CH_2Ph$, $R_2 = H$
(14) $R_1 = R_3 = R_4 = CH_2Ph$, $R_2 = Me$
(15) $R_1 = R_3 = CH_2Ph$, $R_2 = H$, $R_4 = Me$
(16) $R_1 = Me$, $R_2 = H$, $R_3 = R_4 = CH_2Ph$

the chalcones themselves being easily synthesised by condensation of the accessible acetophenone and aromatic aldehyde starting materials.

Base condensation of 4′-benzyloxy-2′-hydroxyacetophenone with 2,4-dibenzyloxybenzaldehyde yielded 2,4,-4′-tribenzyloxy-2′-hydroxychalcone (**7**) which was converted via thallium nitrate oxidation and treatment with acid into 2′,4′,7-tribenzyloxyisoflavone (**10**). Base hydrolysis of this isoflavone produced the deoxybenzoin (**13**) which was methylated to give (**14**). Hydrogenolysis of (**14**) in EtOH–HCl and subsequent purification yielded the benzofuran (**1**) as the major product. The benzofurans (**3**) and (**4**) were obtained by similar treatment of the deoxybenzoins (**15**) and (**16**) derived from 2′,7-dibenzyloxy-4′-methoxyisoflavone (**11**) and 2′,4′-dibenzyloxy-7-methoxyisoflavone (**12**) respectively. The three benzofurans (**1**), (**3**) and (**4**) could be resolved by Si gel TLC, and by a comparison of TLC, UV and MS data for the compounds, their acetates and methyl ethers, it was established that (**1**) was identical to 2-(4-hydroxy-2-methoxyphenyl)-6-hydroxybenzofuran. The common name 6-demethylvignafuran is suggested for this previously undescribed natural product. The above procedure has also been employed to synthesis vignafuran (**5**) in 20% overall yield from 2′,7-dibenzyloxy-4′-methoxyisoflavone (**11**) [17].

In a TLC bioassay [18] against the spore germination of *Cladosporium herbarum* Fr., 6-demethylvignafuran (10 and 20 μg based on log ε at 320 nm for (**5**) [7]) gave inhibition zones of 50 and 72 mm^2 respectively; antifungal activity was apparent even at an applied level of 1 μg. In contrast, inhibition zones given by the common isoflavonoid phytoalexin medicarpin (10 and 20 μg) were 28 and 41 mm^2. Although 6-demethylvignafuran has not been tested against the mycelial growth of *H. carbonum*, synthetic vignafuran is highly active (ED_{50} 15–20 μg/ml [19]). Vignafuran totally inhibits spore germination of *Colletotrichum lindemuthianum* at a concentration of 8 μg/ml [7]. Because of the small quantities of material available, pterocarpene (**2**) has not been tested for antifungal activity.

The extraction of 6-demethylvignafuran involved base–acid partition, a procedure which might dehydrate (or alter in some other way) certain labile isoflavonoids (e.g. 6a-hydroxypterocarpans). It seems unlikely, however, that (**1**) is an artifact since trace quantities of this compound have been isolated from *T. maritimus* diffusates by a process not involving exposure to either acid or base. 6-Demethylvignafuran has been provisionally identified as a minor leaf phytoalexin of kidney-vetch (*Anthyllis vulneraria*) [19], a species closely related to *T. maritimus;* it is also produced by the *H. carbonum*-infected leaves of *Coronilla emerus* (tribe Coronilleae) together with a second unidentified benzofuran (M^+ 270) separable (by Si gel TLC) from but isomeric with vignafuran [19]. The co-occurrence in *T. maritimus* of 6-demethylvignafuran with the isoflavonoids isovestitol, demethylvestitol [6] and 3,9-dihydroxypterocarp-6a-ene strengthens the hypothesis that these compounds are all biosynthetically related, and that 2-arylbenzofurans may be derived from isoflavonoid precursors by a process involving loss of the C-2 carbon atom. However, direct experimental evidence for such conversions is at present unavailable.

EXPERIMENTAL

Mass and UV spectra were determined as previously described [20]. TLC was carried out using either 0.25 or 0.5 mm layers of Si gel (Merck Kiesel gel GF_{254}).

Induction, isolation and purification of (**1**) *and* (**2**). Leaflets of *Tetragonolobus maritimus* (L.) Roth (collected from established and authenticated plants growing at the University of Reading Botanic Garden) were inoculated with a condial suspension of *Helminthosporium carbonum* Ullstrup [21] and incubated for 48 hr [21, 22]. Inoculated tissues were than excised and extracted (× 5) with EtOH [6]. The extracts were bulked, concd (*in vacuo*; 40°) to *ca* 20 ml and de-ionized H_2O (200 ml) added; the vol. was again reduced (to 20–30 ml) before final dilution (H_2O; 100 ml) and extraction with EtOAc (4 × 100 ml). Bulked EtOAc fractions were reduced to dryness and the residue dissolved in CCl_4 (100 ml). This soln was extracted (× 4) with equal vol. cold (4°) aq. NaOH (0.2 N). The NaOH fraction

was acidified (pH 3; 2N HCl) and immediately extracted (×3) with equal vol. EtOAc. After removal of EtOAc, the residue was chromatographed ($CHCl_3$–MeOH, 25:1) to afford (R_f 0.28) a mixture of (**1**) and (**2**). Elution (EtOH) and additional TLC (*n*-pentane–EtO_2–HOAc, 75:25:3) gave pure (**1**) (R_f 0.38) and (**2**) (R_f 0.47). Compounds (**1**) and (**2**) were not obtained from leaflets treated with de-ionized H_2O.

3,9-Dihydroxypterocarp-6a-ene (**2**). Diazotized *p*-nitroaniline, brown; λ_{max} EtOH (nm) 215, 250*sh*, 287*sh*, 318*sh*, 335, 353; λ_{max} EtOH + NaOH (nm) 218, 250*sh*, 300*sh*, 334*sh*, 353, 372; MS *m/e* (rel. int.) 255(30), 254 (M^+; 100), 253(52).

Synthesis of (**2**) [23] $NaBH_4$ (30 mg) was added to a soln of 2′,4′,7-trihydroxyisoflavone [24] (10 mg) in MeOH (3 ml) and the mixture stirred at room temp. for 20 min; conc HCl (0.2 ml) was added dropwise and stirring continued for a further 10–15 mins. The soln was allowed to stand *ca* 18 hr at room temp., diluted with de-ionized H_2O (80 ml) and extracted (×2) with equal vol. EtOAc. Removal of EtOAc (*in vacuo*) and TLC (*n*-hexane–EtOAc–MeOH, 60:40:1) of the residue afforded (**2**) at R_f 0.55. MS *m/e* (rel. int.) 255(10), 254 (M^+; 61), 253 (100); UV maxima (EtOH; EtOH + NaOH) as given for the natural product. Synthetic and natural (**2**) were inseparable by TLC in (a) C_6H_6–MeOH, 9:1, R_f 0.27, (b) $CHCl_3$–MeOH, 25:1, R_f 0.31 and (c) *n*-pentane–Et_2O–HOAc, 75:25:3, R_f 0.46.

2-(4-Hydroxy-2-methoxyphenyl)-6-hydroxybenzofuran (**1**). Diazotized *p*-nitroaniline, purple/pink; λ_{max} EtOH (nm) 212, 228 *sh*, 249*sh*, 282, 307*sh*, 321, 337; λ_{max} EtOH + NaOH (nm) 217, 299, 326*sh*, 343, 359; MS *m/e* (rel. int.) 257(19), 256 (M^+; 100), 255(26), 242(7), 241(31), 213(48). *DiMe ether* (**6**) (CH_2N_2) (R_f 0.90, $CHCl_3$–CCl_4, 3.1). λ_{max} EtOH (nm) 212, 229*sh*, 250*sh*, 272*sh*, 284, 307*sh*, 321, 337; MS *m/e* (rel. int.) 285(12), 284 (M^+; 100), 270(15), 269(39).

2′,4′,7-Tribenzyloxyisoflavone (**10**). 4′-Benzyloxy-2′-hydroxyacetophenone (2.4 g) and 2,4-dibenzyloxybenzaldehyde (3.2 g) in warm EtOH (100 ml) were treated with KOH (20 g) in H_2O (20 ml), and stirred *ca* 18 hr at room temp. The ppt. was filtered, washed with H_2O, then recrystallized from $CHCl_3$–MeOH to give *2,4,4′-tribenzyloxy-2′-hydroxychalcone* (**7**) (1.55 g), mp 141–2°. (Found: C, 79.1; H_1 5.50. $C_{36}H_{30}O_5$ requires: C, 79.7; H, 5.54%). This chalcone (1.55 g) was acetylated (dry Py–Ac_2O, *ca* 18 hr, room temp.), and the reaction mixture poured into H_2O, and extracted with EtOAc (2×). The extract was washed with dil. HCl (2×), then H_2O, and evapd and dried. The acetate, without further purification, was dissolved in warm MeOH (400 ml), treated with $Tl(NO_3)_3 . 3H_2O$ (1.4 g), then stirred for *ca* 18 hr at room temp. Solid KOH (2 g) was added, and the mixture stirred for a further 1 hr. After neutralization with conc HCl, dil. HCl (10%, 40 ml) was added, and the mixture heated under reflux for 1 hr, then filtered hot. The filtrate was concd under red. press., diluted with H_2O and extracted with EtOAc (2×). The EtOAc extract, on evapn, yielded *2′,4′,7-tribenzyloxyisoflavone* (**10**) which was recrystallized from MeOH. Yield 0.60 g mp 154–7°. (Found: C, 79.7; H, 5.50. $C_{36}H_{28}O_5$ requires: C, 80.0; H, 5.19%).

2′,7-Dibenzyloxy-4′-methoxyisoflavone (**11**). Base condensation as above of 4′-benzyloxy-2′-hydroxyacetophenone (3 g) and 2-benzyloxy-4-methoxybenzaldehyde (3 g) gave *2,4′-dibenzyloxy-2′-hydroxy-4-methoxychalcone* (**8**) (3.3 g), mp 142–4° (from $CHCl_3$–MeOH) (lit. [16] 142–4°). $Tl(NO_3)_3$ oxidation of the chalcone (1 g) as its acetate, gave *2′,7-dibenzyloxy-4′-methoxyisoflavone* (**11**) (0.62 g), mp 131–2° (from MeOH) (lit. [16] 132–4°).

2′,4′-Dibenzyloxy-7-methoxyisoflavone (**12**). Base condensation of 2′-hydroxy-4′-methoxyacetophenone (1.3 g) and 2,4-dibenzyloxybenzaldehyde (2.5 g) gave *2,4-dibenzyloxy-2′-hydroxy-4′-methoxychalcone* (**9**) (1.2 g) mp 157–164° (from $CHCl_3$–MeOH). (Found: C, 76.7; H, 5.72. $C_{30}H_{26}O_5$ requires: C, 77.3; H, 5.58%). $Tl(NO_3)_3$ oxidation of this chalcone (1 g) as its acetate, gave *2′,4′-dibenzyloxy-7-methoxyisoflavone* (**12**) (0.9 g) mp 140–1° (from MeOH). (Found: C, 77.1; H, 5.57. $C_{30}H_{24}O_5$ requires: C, 77.6; H, 5.17%).

Base hydrolysis of isoflavones → deoxybenzoins. 2′,4′,7-Tribenzyloxyisoflavone (**10**) (0.60 g) in EtOH (40 ml) was heated under reflux for 1.5 hr with KOH (2 g) in H_2O (20 ml). EtOH was removed under red. press. and the residue was acidified with dil. HCl and extracted with EtOAc (2×). The EtOAc extract on evapn yielded *2,4-dibenzyloxybenzyl-4-benzyloxy-2-hydroxyphenylketone* (**13**) (0.30 g) mp 137–8° (from $CHCl_3$–MeOH) (Found: C, 78.9; H, 6.04. $C_{35}H_{30}O_5$ requires: C, 79.2; H, 5.66%). NMR (60 MHz, $CDCl_3$, TMS); δ 4.07 (2H, *s*, CO—C<u>H</u>$_2$—Ar), 4.93 (4H, *s*, O—C<u>H</u>$_2$—Ph), 4.98 (2H, *s*, O—C<u>H</u>$_2$—Ph), 6.19–6.60 (4H, *m*, H-3,5,3′,5′), *ca* 7.05 (1H, partially overlapped *d*, H-6′), 7.18 (5H, *s*, O—CH_2—<u>Ph</u>), 7.30 (10H, *s*, O—CH_2—<u>Ph</u>), 7.67 (1H, *d*, J = 9 Hz, H-6), 12.75 (1H, *s*, OH). Similarly, 2′,7-dibenzyloxy-4′methoxyisoflavone (**11**) (0.50 g) yielded *2-benzyloxy-4-methoxybenzyl-4-benzyloxy-2-hydroxyphenylketone* (**15**) (0.43 g) mp 113–5° (from MeOH) (Found: C, 76.2; H, 5.55. $C_{29}H_{26}O_5$ requires: C, 76.6; H, 5.73%). NMR (60 MHz, $CDCl_3$, TMS): δ 3.72 (3H, *s*, OMe), 4.12 (2H, *s*, CO—C<u>H</u>$_2$—Ar), 4.97 (2H, *s*, O—C<u>H</u>$_2$—Ph), 5.02 (2H, *s*, O—C<u>H</u>$_2$—Ph), 6.17–6.52 (4H, *m*, H-3,5,3′,5′), *ca* 7.09 (1H, partially overlapped *d*, H-6′), 7.25 (5H, *s*, O—CH_2—<u>Ph</u>), 7.35 (5H, *s*, O—CH_2—<u>Ph</u>), 7.71 (1H, *d*, J = 9 Hz, H-6), 12.73 (1H, *s*, OH), and 2′,4′-dibenzyloxy-7-methoxyisoflavone (**12**) (0.80 g) gave *2,4-dibenzyloxybenzyl-2-hydroxy-4-methoxyphenylketone* (**16**) (0.46 g) mp 143–5° (from $CHCl_3$–MeOH). (Found: C, 76.1; H, 5.88. $C_{29}H_{26}O_5$ requires: C, 76.6; H, 5.73%). NMR (60 MHz, $CDCl_3$, TMS): δ 3.78 (3H, *s*, OMe), 4 14 (2H, *s*, CO—C<u>H</u>$_2$—Ar), 5.01 (4H, *s*, O—C<u>H</u>$_2$—Ph), 6.18–6.65 (4H, *m*, H-3,5,3′,5′), *ca* 7.12 (1H, partially overlapped *d*, H-6′), 7.25 (5H, *s*, O—CH_2—<u>Ph</u>), 7.36 (5H, *s*, O—CH_2—<u>Ph</u>), 7.74 (1H, *d*, J = 9 Hz, H-6), 12.80 (1H, *s*, OH). Deoxybenzoin (**13**) (0.20 g) in DMF (20 ml) was stirred at 60° with anhyd. K_2CO_3 (2 g) and Me_2SO_4 (0.057 g) for 1.5 hr. The mixture was poured into H_2O, extracted with EtOAc (2×). The extract was washed with H_2O, evapd and purified by TLC (C_6H_6–EtOAc–*iso*-PrOH, 90:10:1) to give *2,4-dibenzyloxybenzyl-4-benzyloxy-2-methoxyphenylketone* (**14**) as a gum (0.14 g) (Found: C, 78.8; H, 6.03. $C_{36}H_{32}O_5$ requires: C, 79.4; H, 5.88%). NMR (60 MHz, $CDCl_3$, TMS): δ 3.62 (3H, *s*, OMe), 4.13 (2H, *s*, CO—C<u>H</u>$_2$—Ar), 4.87 (2H, *s*, O—C<u>H</u>$_2$—Ph), 4.89 (2H, *s*, O—C<u>H</u>$_2$—Ph), 4.95 (2H, *s*, O—C<u>H</u>$_2$—Ph), 6.10–6.60 (4H, *m*, H-3,5,3′,5′), *ca* 6.95 (1H, partially overlapped *d*, H-6′), 7.12 (5H, *s*, O—CH_2—<u>Ph</u>), 7.26 (10H, *s*, O—CH_2—<u>Ph</u>), 7,61 (1H, *d*, J = 9 Hz, H-6).

Hydrogenolysis of deoxybenzoins → 2-arylbenzofurans. Deoxybenzoin (**14**) (10 mg) in EtOH (10 ml) containing conc HCl (0.1 ml) was hydrogenated over Pd/C catalyst for 1 hr. The reaction mixture was filtered, evapd to dryness and the product purified by TLC (C_6H_6–EtOAc–MeOH–petrol (60–80°), 6:4:1:6) to yield *2-(4-hydroxy-2-methoxyphenyl)-6-hydroxybenzofuran* (**1**). Diazotized *p*-nitroaniline, purple/pink; UV and MS as given for natural compound. *DiMe ether*. TLC, UV and MS as given above. *Diacetate* (Py–Ac_2O) (R_f 0.90, $CHCl_3$). λ_{max} EtOH (nm) 210, 236, 244*sh*, 268*sh*, 279, 291, 315, 330; MS *m/e* (rel. int.) 341(4), 340(M^+; 19), 299(5), 298(31), 257(18), 256(100), 255(16), 241(7), 213(10), 212(7). Natural and synthetic (**1**) were indistinguishable by Si gel TLC in the following solvents: (a) $CHCl_3$–MeOH (100:3), R_f 0.42 (b) C_6H_6–MeOH (9:1) R_f 0.38 (c) $CHCl_3$–HOAc (5:2) R_f 0.83 and (d) Et_2O–*n*-hexane (3:1) R_f 0.66. Similarly, deoxybenzoin (**15**) gave *2-(2,4-dihydroxyphenyl)-6-methoxybenzofuran* (**3**). Diazotized *p*-nitroaniline, yellow/brown; Gibbs reagent, blue/gray; λ_{max} EtOH (nm) 213, 230*sh*, 275*sh*, 284, 306*sh*, 322, 337; λ_{max} EtOH + NaOH (nm) 216, 250*sh*, 292, 334*sh*, 350; MS *m/e* (rel. int.) 257(16), 256(M^+; 100), 255(4), 242(7), 241(68), 152(17), 149(31), 137(8), 135(15), 128(19), 111(12). *DiMe ether*. TLC, UV and MS as given for diMe ether of (**1**). *Diacetate* (R_f 0.82, $CHCl_3$). λ_{max} EtOH (nm) 208, 230*sh*, 282*sh*, 316, 329; MS *m/e* (rel. int.) 341(3), 340 (M^+; 15), 299(4), 298(27), 257(14), 256(100), 255(10), 242(8), 241(53), 227(10). Compound (**3**) was readily separated from natural (**1**) by Si gel TLC in $CHCl_3$–MeOH (100:3) ((**1**): R_f 0.42; (**3**): R_f 0.37). Deoxybenzoin (**16**) yielded *2-(2-hydroxy-4-methoxyphenyl)-6-hydroxybenzofuran* (**4**). Diazotized *p*-nitroaniline, yellow/brown; Gibbs reagent, blue/grey; λ_{max} EtOH (nm) 212, 248*sh*, 274*sh*, 282, 307*sh*, 322, 337; λ_{max} EtOH + NaOH (nm) 213, 272, 286, 348, 364; MS *m/e* (rel. int.) 257(13), 256(M^+; 100), 255(5), 242(9), 241(56), 157(6), 128(19), 111(14). *DiMe ether*. TLC, UV and MS as given

for diMe ether of (**1**). *Diacetate* (R_f 0.61, $CHCl_3$), λ_{max} EtOH (mn) 208, 225*sh*, 247*sh*, 277*sh*, 288*sh*, 309, 322; MS *m/e* (rel. int.) 341(3), 340 (M^+; 22), 299(4), 298(24), 257(15), 256(100), 255(17), 241(32). Compound (**4**) readily separated from natural (**1**) upon Si gel TLC in $CHCl_3$–MeOH (100:3) ((**1**): R_f 0.42; (**4**): R_f 0.61).

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