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5,2'-DIHYDROXY-6,7-METHYLENEDIOXYISOFLAVONE FROM SEED BALLS OF SUGAR BEET

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Key Word Index—*Beta vulgaris*; Chenopodiaceae; sugar beet; 5,2'-dihydroxy-6,7-methylenedioxyisoflavone; 6-oxo-2-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo-[3.3.0]-octane; indole-3-carboxylic acid.

Abstract—5,2'-Dihydroxy-6,7-methylenedioxyisoflavone was identified from the seed balls of sugar beet. A neolignan, 6-oxo-2-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo-[3.3.0]-octane, and indole-3-carboxylic acid were also isolated.

INTRODUCTION

In the previous paper, we reported the isolation and structure elucidation of eight phenolic compounds [1, 2] as germination inhibitors and two phenolic amides [3] from the seed balls of sugar beet (*Beta vulgaris* L. var. *saccharifera* Alefeld). Two phytoalexins have also been isolated from the leaves of sugar beet infected with *Cercospora beticola* [4]. We have studied the constitutive antifungal constituents in these plants. This paper is concerned with the structure elucidation of a new isoflavone (1) biogenetically related to one of the phytoalexins, betavulgarin [4], together with a neolignan, 6-oxo-2-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo-[3.3.0]-octane (2), and indole-3-carboxylic acid (3) from the seed balls of sugar beet.

RESULTS AND DISCUSSION

The molecular formula of 1 was estimated to be $C_{16}H_{10}O_6$ by elementary analysis and mass spectrometry. The IR (1675 cm^{-1}, C=O), UV (218, 243, 269, 340 nm), and ^1H NMR (δ8.3, 1H, *s*, C-2 proton) spectra indicated that 1 was an isoflavone. The presence of two phenolic hydroxyl groups was indicated by colour reactions and by the ^1H NMR signals at δ12.8 (1H, *s*) and 9.42 (1H, *s*), which disappeared on addition of D_2O. In addition, acetylation and methylation of 1 gave a diacetate and a dimethyl ether respectively. Also, the ^1H NMR signal at δ6.16 (2H, *s*), IR absorption band at 920 cm^{-1}, and Hansen test [5] revealed the presence of a methylenedioxy group in 1. A bathochromic shift was observed in the UV of 1 on addition of $AlCl_3$–HCl, locating a hydroxyl group at C-5. The MS-fragmentation ion at *m/z* 180 (base peak), arising from the retro-Diels–Alder cleavage, disclosed that the one hydroxyl group and the methylenedioxy group were in ring A, and the other hydroxyl group was in ring B. The presence of an $[M-31]^+$ peak in the MS of the dimethyl ether of 1 suggested that the hydroxyl group in ring B of 1 was at C-2' [6]. The position of the methylenedioxy group on ring A was suggested to be between C-6 and C-7, because a singlet at δ6.64 (1H) in the dimethyl ether was assigned to the C-8 proton by comparison with betavulgarin [4]. Thus 1 must be 5,2'-dihydroxy-6,7-methylenedioxyisoflavone. The structure was confirmed by synthesis of its dimethyl ether by Hoesch condensation of 3-methoxy-4,5-methylenedioxyphenol with 2-methoxyphenylacetonitrile, formylation and cyclization. The synthetic isoflavone was identical (spectra, mp) with the natural dimethyl ether. Although the antifungal activity of 1 and its diacetate against *C. beticola* was lower than that of the dimethyl ether, 1 may be a precursor of the phytoalexin, betavulgarin.

Compound 2 was obtained as colourless needles, mp 197–198°, $[M]^+$ 280 and was shown to be the neolignan, 6-oxo-2-(4-hydroxy-3,5-dimethoxyphenyl-3,7-dioxabicyclo-[3.3.0]-octane by means of UV, ^1H NMR and IR, and those of the acetate. Identity was confirmed by comparison of these spectral data with those of an authentic sample obtained from hydrolysis products of *Quercus mongolica* [7]. Also, compound 3 was identified as indole-3-carboxylic acid by means of mp and spectral comparison with published data (see Experimental) [8].

EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in EtOH. ^1H NMR spectra were measured at 100 MHz using TMS as an int. standard. Mass spectra were recorded by direct inlet at 70 eV. Silica gel (Wakogel C-200) was used for CC and silica gel (Kieselgel G Typ 60) for TLC.

Isolation. Seed balls of sugar beet (*Beta vulgaris* L. var. *saccharifera* Alefeld) (26.5 kg) were ground finely and extracted with MeOH (13.5 l.). The methanolic extract was concd to 1.5 l. *in vacuo* at 40° and Me_2CO (4 l.) was added to the concn. The filtrate was evaporated *in vacuo* to give a reddish brown syrup (132 g). The syrup was chromatographed on a silica gel column (4.4 × 72 cm) using C_6H_6 in EtOAc (F1–F6, 20% stepwise). The fraction F_3 (1.54 g) was rechromatographed on a silica gel column with $CHCl_3$ to give 1 (9.1 mg) as pale yellow crystals. The fraction F_4 (3.96 g) was subjected to a silica gel column with

$CHCl_3$–EtOH (99:1) to yield **2** (8 mg) and **3** (17 mg) as colourless needles, respectively.

5,2'-Dihydroxy-6,7-methylenedioxyisoflavone (**1**). Mp 238–239° (EtOAc); (Found: C, 64.20; H, 3.28. $C_{16}H_{10}O_6$ requires: C, 64.43; H, 3.38%); UV λ_{max}^{EtOH} nm (log ε): 217 (4.51), 243 (4.24), 269 (4.27), 290 sh (4.17), 340 sh (3.59); + $AlCl_3$; 220, 242 sh, 282, 317. IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1675 (C=O), 1620, 1550, 920 (O–CH_2–O). EIMS *m/z* (rel. int.): 298 $[M]^+$ (37.8), 281 (1.2), 280 (1.3), 181 (3.0), 180 (100). ^1H NMR (DMSO-d_6): δ12.8 (1H, *s*, OH at C-5, disappeared by D_2O addition), 9.42 (1H, *s*, OH at C-2, disappeared by D_2O addition), 8.31 (1H, *s*, H-2), 6.8–7.3 (5H, *m*, H-3', 4', 5', 6', 8), 6.16 (2H, *s*, O–CH_2 O). Colour test: diazotized sulphanic acid: orange, Folin–Ciocalteu; greyish blue, Hansen test; violet.

Methylation of **1**. Compound **1** (10 mg) was refluxed with Me_2SO_4 (0.02 ml), K_2CO_3 (100 mg) and Me_2CO (2 ml) for 42 hr. The reaction mixture was poured in ammoniacal water and extracted with $CHCl_3$. After evapn of the solvent, the syrup was chromatographed on a silica gel column with $CHCl_3$ EtOH (19:1) to give the dimethyl ether (4 mg) as colourless needles, mp 148–150°. UV λ_{max}^{EtOH} nm: 216, 244, 280 sh, 318. IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O), 1615, 920 (O–CH_2–O). EIMS *m/z* (rel. int.): 326 $[M]^+$ (100), 325 (15.3), 308 (17.0), 298 (4.3), 297 (8.7), 296 (9.3), 295 $[M - 31]^+$ (69.1), 280 (25.4), 267 (35.1), 265 (13.9), 195 (29.9), 194 (14.6), 166 (33.9), 131 (34.4). High resolution MS *m/z*: 326.0781. $C_{16}H_{14}O_6$ requires: 326.0772. ^1H NMR ($CDCl_3$): δ7.77 (1H, *s*, H-2), 7.35 (2H, *dt*, *J* = 1.5, 8 Hz, H-3', 5'), 6.99 (2H, *dt*, *J* = 1.5, 8 Hz, H-4', 6'), 6.64 (1H, *s*, H-8), 6.06 (2H, *s*, O–CH_2–O), 4.06 (3H, *s*, MeO), 3.79 (3H, *s*, MeO).

Acetylation of **1**. Cold Ac_2O (0.5 ml) was added to **1** (5 mg) in pyridine (0.5 ml) at 0° and the mixture left at room temp. for 24 hr. The product was extracted with $CHCl_3$ and subjected to silica gel CC. Elution with $CHCl_3$–EtOH (19:1) gave the diacetate (5 mg) as colourless needles, mp 165–167°. UV λ_{max}^{EtOH} nm: 242, 260 sh, 316. IR ν_{max}^{KBr} cm^{-1}: 1760 (Ac C=O), 1750 (Ac C=O), 1650 (C=O), 1620, 1590, 910 (O–CH_2 O). EIMS *m/z* (rel. int.): 382 $[M]^+$ (2.1), 340 (60.0), 298 (71.8), 280 (8.0), 180 (100). High resolution MS *m/z*: 382.0638. $C_{20}H_{14}O_8$ requires: 382.0689. ^1H NMR ($CDCl_3$): δ7.75 (1H, *s*, H-2), 7.15–7.4 (4H, *m*, H-3', 4', 5', 6'), 6.82 (1H, *s*, H-8), 6.14 (2H, *s*, O CH_2–O), 2.40 (3H, *s*, Ac), 2.16 (3H, *s*, Ac).

Synthesis of 5,2'-dimethoxy-6,7-methylenedioxyisoflavone. (1) 2-Hydroxy-6-methoxy-4,5-methylenedioxyphenyl-2-methoxybenzylketone. After 3-methoxy-4,5-methylenedioxyphenol (1.4 g), *o*-methoxyphenylacetonitrile (1.5 g) and fused $ZnCl_2$ (1 g) were successively dissolved in dry Et_2O (30 ml), the soln was satd with dry HCl gas at 0° and allowed to keep overnight. The Et_2O soln was decanted off and the brownish oily ketimine hydrochloride was washed twice with dry Et_2O. The oil was then refluxed for 1 hr with H_2O (30 ml). After cooling, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on a silica gel column and eluted with C_6H_6 monitoring with UV lamp (365 nm). The yellow fluorescent fraction was concd to give the ketone (168 mg) as yellow needles: mp 118 119° (lit. [9–11] 115–116°). Found: C, 64.62; H, 4.95. $C_{17}H_{16}O_6$ requires: C, 64.55; H, 5.10%. UV λ_{max}^{EtOH} nm (log ε): 242 (4.68), 282 (4.74), 348 (4.30). IR ν_{max}^{KBr} cm^{-1}: 1625 (C=O), 928 (O–CH_2–O). EIMS *m/z* (rel. int.): 316 $[M]^+$ (4.6), 195 (100), 180 (87.1), 121 (1.3). ^1H NMR (Me_2CO-d_6): δ13.51 (1H, *s*), 6.77–7.35 (4H, *m*), 6.13 (1H, *s*), 5.97 (2H, *s*), 4.25 (2H, *s*), 4.01 (3H, *s*).

(2) 5,2'-Dimethoxy-6,7-methylenedioxyisoflavone. To a soln of the diketone (111 mg) in dry pyridine (3 ml) were added ethyl orthoformate (3 ml) and piperidine (5–6 drops). The soln was refluxed for 4 hr and set aside at 0° overnight. The reaction mixture was added to H_2O (20 ml) and extracted with $CHCl_3$. After removal of the solvent, the $CHCl_3$ extract was chromatographed on a silica gel column and eluted with $CHCl_3$ monitoring with UV lamp (365 nm). The blue fluorescent fraction was concd to give tlatlancuayin (63 mg) as colourless needles. Mp 150–151° (147–148° [10, 12], 145–147° [13]). Found: C, 66.20; H, 4.39. Calc. for $C_{18}H_{14}O_6$: C, 66.26; H, 4.32%. Its spectral data were identical with those of **1** dimethyl ether.

6-Oxo-2-4-hydroxy-3,5-dimethoxyphenyl-3,7-dioxabicyclo-[3.3.0]-octan (**2**). Mp 197–198°. UV λ_{max}^{EtOH} nm: 237, 270, 280 sh. IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O), 1610, 1515. EIMS *m/z* (rel. int.): 280 $[M]^+$ (100), 249 (2.6), 196 (2.4), 195 (20.0), 182 (32.3), 181 (18.4), 167 (62.1). FDMS *m/z* (rel. int.): 281 $[M + H]^+$ (16.9), 280 $[M]^+$ (100). High resolution MS *m/z*: 280.0939 $[M]^+$. $C_{14}H_{16}O_6$ requires: 280.0945. ^1H NMR ($CDCl_3$): δ6.57 (2H, *s*), 5.55 (1H, *s*, disappeared with D_2O), 4.59 (1H, *d*, *J* = 7 Hz), 4.51 (1H, *dd*, *J* = 7, 10 Hz), 4.39 (1H, *t*, *J* = 9 Hz), 4.34 (1H, *dd*, *J* = 2, 10 Hz), 4.20 (1H, *dd*, *J* = 4, 9 Hz), 3.91 (6H, *s*), 3.40–3.54 (1H, *m*), 3.05–3.18 (1H, *m*).

Acetylation of **2**. Acetylation of **2** (2 mg) in dry pyridine (1.5 ml) and Ac_2O (0.5 ml) gave a monoacetate (2 mg) as colourless needles. Mp 146–147°. UV λ_{max}^{EtOH} nm: 206, 225 sh, 266, 277 sh. IR ν_{max}^{KBr} cm^{-1}: 1770, 1760 (C=O). FDMS *m/z* (rel. int.): 323 $[M + H]^+$ (18.7), 322 $[M]^+$ (100), 280 (6.9), 279 (17.6). ^1H NMR ($CDCl_3$): δ6.58 (2H, *s*), 4.64 (1H, *d*, *J* = 7 Hz), 4.56 (1H, *dd*, *J* = 7, 10 Hz), 4.39 (1H, *t*, *J* = 9 Hz), 4.36 (1H, *dd*, *J* = 2, 10 Hz), 4.21 (1H, *dd*, *J* = 4, 9 Hz), 3.83 (6H, *s*), 3.5 (1H, *m*), 3.14 (1H, *m*), 2.34 (3H, *s*).

Indole-3-carboxylic acid (**3**). Mp 220° (222° [8]). UV λ_{max}^{EtOH} nm (log ε): 212 (4.11), 225 sh (3.82), 246 sh (3.55), 280 (3.62), 287 sh (3.59). IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O). EIMS *m/z* (rel. int.): 161 $[M]^+$ (100), 144 (85.4), 116 (7.5), 89 (6.5). High resolution MS *m/z*: 161.0469 $[M]^+$. $C_9H_7NO_2$ requires: 161.0474. ^1H NMR (Me_2CO-d_6): δ10.6–11.4 (2H, *m*), 8.16 (1H, *m*), 8.06 (1H, *d*), 7.5 (1H, *m*), 7.2 (2H, *m*). Colour test: Ehrlich reagent, violet; 4-dimethylaminocinnamaldehyde, pink.

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