

STRUCTURE OF A NEW ISOFLAVONE FROM FUNGI AND STREPTOMYCES  
INHIBITING DOPA DECARBOXYLASE

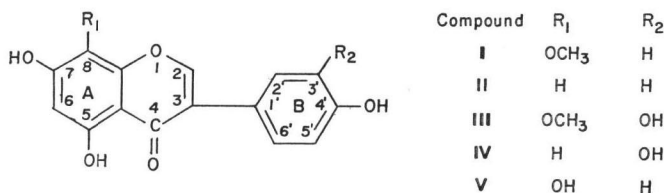
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A new dopa decarboxylase inhibiting isoflavone has been isolated from culture filtrates of fungi and streptomyces and shown to be 3',4',5,7-tetrahydroxy-8-methoxyisoflavone.

As reported in a previous paper,<sup>1)</sup> five isoflavone compounds inhibiting dopa decarboxylase were isolated from culture filtrates of *Aspergillus niger* NRRL-3122 and *Streptomyces neyagawaensis* var. *orobolere* and identified to be psi-tectorigenin (I),<sup>2,3)</sup> genistein (II),<sup>4)</sup> orobol (IV),<sup>5)</sup> 8-hydroxy genistein (V)<sup>2)</sup> and a new compound. In this paper, we report on the structure of this compound, which was elucidated to be 3',4',5,7-tetrahydroxy-8-methoxyisoflavone (III).



III has the molecular formula C<sub>16</sub>H<sub>12</sub>O<sub>7</sub> (MW 316.26), m.p. 252°C (dec.). Anal. Found: C, 60.43; H, 3.86; O, 35.39. Calcd.: C, 60.76; H, 3.82; O, 35.41. M<sup>+</sup>, *m/e* 316. IR  $\nu_{\text{KBr}}$ : 3400, 1660, 1530, 1450, 1380, 1270, 1240, 1180, 1115, 1065, 1035, 995, 910, 865, 830, 780, 730 and 680 cm<sup>-1</sup>. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  (log  $\epsilon$ ): 269 nm (4.48) and 295 nm (shoulder). The isoflavone structure was suggested by the UV spectrum and the presence of a singlet proton signal at  $\delta$  8.08 which is characteristic of the C<sub>2</sub>-proton of an isoflavone compound. III gave positive ferric chloride (dark blue) and GIBB's (brown) tests for a phenolic hydroxyl group. The NMR spectrum in octadeuteriodioxane showed the presence of one methoxyl group ( $\delta$  3.83), a hydrogen-bonded hydroxyl group ( $\delta$  12.65), three deuterium-exchangeable hydroxyl groups ( $\delta$  8.73,  $\delta$  7.68 and  $\delta$  7.55), the C<sub>2</sub>-proton ( $\delta$  8.08), one proton on ring A ( $\delta$  6.30, singlet) and three aromatic protons on ring B ( $\delta$  7.15,  $\delta$  6.87 and  $\delta$  6.85). Acetylation of III with acetic anhydride and sulfuric acid afforded the tetraacetyl derivative, m.p. 158°C. Treatment of III with dimethyl sulfate afforded the tetramethyl ether derivative, m.p. 164°C.

Addition of either aluminum chloride or anhydrous sodium acetate to a solution of III caused red shifts of the main UV absorption band by 14 or 11 nm respectively. These properties are characteristics of hydroxyl groups located at the 5 and 7 positions.<sup>9)</sup> A bathochromic shift was not observed by addition of NaOAc/H<sub>3</sub>BO<sub>3</sub> to a methanolic solution of III. This suggested that III did not contain orthodihydroxyl groups at C-6,7 or C-7,8.<sup>10)</sup> These data indicated that two of the four hydroxyl groups were present at the 5 and 7 positions in the ring A of the isoflavone. The presence of the 5-hydroxyl group was also supported by its NMR spectrum ( $\delta$  12.65 described above) indicating the formation of a hydrogen bond with the carbonyl group. By oxidative degradation with alkaline hydrogen peroxide,

the tetramethyl ether of **III** gave 3,4-dimethoxy benzoic acid which was identified by direct comparison of its IR spectrum with that of an authentic sample. Therefore, the other two hydroxyl groups are located at the 3' and 4' positions in ring B of the isoflavone.

The five methoxyl peaks in the NMR spectrum of the tetramethyl ether of **III** in deuteriochloroform appeared at  $\delta$  4.00, 3.95, 3.91, 3.90 and 3.88, and shifted to  $\delta$  3.78, 3.50, 3.47, 3.41 and 3.26 when hexadeuterobenzene was used as the solvent. This solvent shift is consistent with a 5,7,8-oxygenation pattern in the ring A as reported by WILSON *et al.*<sup>7)</sup> and SIDWELL *et al.*<sup>9)</sup> Nuclear OVERHAUSER effects were observed between the aromatic proton ( $\delta$  6.00, benzene- $d_6$ ) of ring A and the 7 position<sup>7)</sup> methoxyl signal ( $\delta$  3.26) (+11.4%  $\text{CH}\{\text{OCH}_3\}$ ), and, the 5 position<sup>7)</sup> methoxyl signal ( $\delta$  3.47) (+11.8%  $\text{CH}\{\text{OCH}_3\}$ ). These results indicated the presence of an aromatic proton at the 6 position. The presence of a methoxyl group at the 8 position of ring A was deduced from the UV shifts caused by addition of aluminum chloride and anhydrous sodium acetate. It was further confirmed by the absence of a nuclear OVERHAUSER effect between the singlet proton ( $\delta$  6.30) at the 6 position and the methoxyl group in the NMR spectrum of **III**.

Thus, the structure of **III** was deduced to be 3',4',5,7-tetrahydroxy-8-methoxyisoflavone.

### Experimental

UV spectra were measured by a Hitachi EPS-3T UV spectrometer, IR spectra by a Hitachi EPI-S2 Infrared spectrometer, NMR spectra by a Varian HA-100D spectrometer, mass spectra by a Hitachi RMU-6M spectrometer. The ferric chloride color reaction was achieved with 1% ferric chloride solution in water (w/v). The color by GIBB's reagent [1% solution of 2,6-dichloroquinonechlorimide in ethanol (w/v)] was developed with ammonia vapor after spraying.

The UV spectrum with  $\text{AlCl}_3$  was measured immediately after the addition of three drops of 10%  $\text{AlCl}_3$  in ethanol to an ethanolic solution of **III**. The UV spectrum with NaOAc was determined immediately after the addition of excess anhydrous NaOAc (powder) to an ethanolic solution of **III**. The NaOAc/ $\text{H}_3\text{BO}_3$  spectrum was determined immediately after the addition of excess NaOAc and  $\text{H}_3\text{BO}_3$  to a methanolic solution of **III**. **III** was dissolved to give a 4.5  $\mu\text{g}/\text{ml}$  concentration in all cuvettes.

#### Tetraacetate of **III**.

To 0.5 ml of an acetic anhydride solution of **III** (20.5 mg) was added one drop of conc. sulfuric acid. The reaction mixture was allowed to stand for 1 hour at room temperature. To the mixture was added 3 ml of water and the precipitate was collected and washed with water on a glass filter. The precipitate was crystallized from methanol, 10.8 mg, (yield 38%), m.p. 158°C, UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (log  $\epsilon$ ): 250 nm (4.72) and 310 nm (shoulder), NMR  $\delta_{\text{TMS}}$  ( $\text{CDCl}_3$ ): 2.29 (6H, singlet), 2.39 (3H, singlet), 2.40 (3H, singlet), 3.98 (3H, singlet), 6.83 (1H, singlet), 7.2~7.45 (3H, ABX system) and 7.98 (1H, singlet), IR  $\nu_{\text{KBr}}$ : 3450, 1770, 1650, 1510, 1490, 1415, 1370, 1295, 1270, 1200, 1180, 1110, 1060, 1030, 1010, 905, 895, 840 and 810  $\text{cm}^{-1}$ .

#### Tetramethyl ether of **III**.

To 8 ml of a refluxing acetone solution of **III** (15.0 mg) and potassium carbonate (180 mg) was added dropwise dimethyl sulfate in five portions (total 0.2 ml) at 10-minute intervals. The reaction mixture was allowed to stand overnight and then evaporated under reduced pressure at 40°C. The residue was suspended in water and filtered on a glass filter. After being washed with water, the tetramethyl ether of **III** was obtained as a white powder (12.2 mg, yield 69%), m.p. 164°C, UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (log  $\epsilon$ ): 264 nm (4.56) and 290 nm (shoulder), NMR  $\delta_{\text{TMS}}$  (benzene- $d_6$ ): 3.26 (3H, singlet), 3.42 (3H, singlet), 3.47 (3H, singlet), 3.50 (3H, singlet), 3.78 (3H, singlet), 6.00 (1H, singlet), 6.66 (1H, doublet  $J=8$  Hz), 7.08 (1H, double doublet), 7.29 (1H, doublet  $J=2$  Hz) and 7.38 (1H, singlet), IR  $\nu_{\text{KBr}}$ : 3450, 2950, 1660, 1605, 1575, 1520, 1470, 1410, 1360, 1315, 1270, 1210, 1140, 1090, 1070, 1020, 980, 880 and 820  $\text{cm}^{-1}$ .

Oxidative degradation of the tetramethyl ether of III.

Hydrogen peroxide (30%, w/v) was added dropwise with stirring in five portions (total 0.3 ml) at 10-minute intervals to a solution of the tetramethyl ether (12.5 mg) in 5% ethanolic (w/v) potassium hydroxide at 50°C. After one hour at 50°C, the reaction mixture was evaporated under reduced pressure and the residue was dissolved in 20 ml of water. The aqueous layer was washed with three 10-ml portions of chloroform, acidified with 1 N HCl, and then extracted with chloroform and ethyl acetate (10-ml portions each). The combined organic extracts were evaporated under reduced pressure to give an acidic material (2.8 mg, yield 46%) which was identified as 3,4-dimethoxy benzoic acid by direct comparison of its IR spectrum with that of an authentic sample.

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