New Flavonoids Isolated from Infected Sugarbeet Roots

Hajime TAKAHASHI,* Teruji SASAKI, and Masaaki ITO
Chemistry Laboratory, Department of General Education, Higashi Nippon Gakuen University, Tobetsu, Hokkaido 061-02
(Received January 8, 1987)

Synopsis. New flavonoid compounds, 3,5-dihydroxy-6,7-methylenedioxyflavanone, 2′,5-dihydroxy-6,7-methylenedioxyisoflavone, and 5-hydroxy-6,7-methylenedioxyflavone were isolated from sugarbeet roots infected with *Rhizoctonia solani*.

Results

Phytoalexin, betagarin (**1**), and betavulgarin (**2**) have been recently isolated from the infected leaves of sugarbeet with *Corcospora beticola*.[1] We now report the isolation of some flavonoids from the sugarbeet roots infected with *R. Solani*. We also report that the compounds **3** and **4** have the weak inhibition effect of hypha growth, on the other hand, the compound **5**, being only the demethylated substance of betavulgarin (**2**), haven't that effect in the least.

The sliced and infected sugarbeet roots were extracted by a dipping in methanol at room temperature. The separation of the extracts by the column and preparative plates chromatographies on silica gel allowed an isolation of the pure six flavonoids. By the IR, UV, and ^{1}H NMR spectra datas, the compounds (**1**, 6.5×10^{-4}% yield), (**2**, 2.3×10^{-2}%), and (**3**, 7×10^{-4}%) were identified with the known betagarin, betavulgarin, and dihydrooroxylin A,[2] respectively.

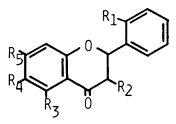
It was indicated that each of the flavonoids (**4**, 4.5×10^{-5}% yield), (**5**, 7.5×10^{-4}%), and (**6**, 1.7×10^{-2}%) had one methylenedioxy group and one or some hydroxyl groups by IR, MS, and ^{1}H NMR spectra. In the each mass spectrum of **4**, **5**, and **6**, each peak (*m/z*, 180 or 181) due to a fragment ion $C_8H_4O_5$ via retro Diels Alder pathway[3] was observed and suggested that one hydroxyl group and one methylenedioxy group attached to each A-ring of these compounds. In the ^{1}H NMR spectra of **4**, **5**, and **6**, one proton signal (singlet) respectively appeared at δ 10.92, 12.15, and 12.83, and assigned as the chelated hydroxyl group. In the UV spectra of **4**, **5**, and **6**, the respective maximum of absorption was bathochromically shifted by addition of aluminium chloride, and then, the shifted maxima were not again moved by the addition of hydrochloric acid. These results indicated that the all hydroxyl groups attached to the A-rings of **4**, **5**, and **6** oriented to the 5-positions. The Gibbes tests (all positive) of **4**, **5**, and **6** suggested that all 8-positions were free, and so all methylenedioxy groups oriented to 6- and 7-positions.

In the ^{1}H NMR of **4**, signals of two protons appeared as an AB-quartet at δ 5.09 and 4.55 (J=12 Hz) were assigned as a C-2-H and a C-3-H (trans configuration), and indicated that **4** had a flavanonol structure. Two singlets and one multiplet were also observed at δ 6.14 (1H, C-8-H), 6.04 (2H, $-OCH_2O-$), and 7.50 (5H, W_H=8 Hz, B-ring's protons), separately, in the ^{1}H NMR. By these results, it was confirmed that **4** is 3,5-dihydroxy-6,7-methylenedioxyflavanone.

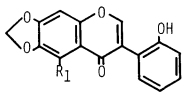
Two maxima (245 and 272 nm) in the UV of **5** and the appearance of C-2-H (δ 8.06, s) in the ^{1}H NMR indicated that **5** had an isoflavone structure. The position of B-ring's hydroxyl group of **5** was easily determined by the NMR. The splitting patterns of the aromatic four protons on the B-ring was characteristic of *ortho*-disubstituted benzene.[4] Two singlets also appeared at δ 6.61 (1H, C-8-H) and 6.15 (2H, $-OCH_2O-$) in the ^{1}H NMR. The compound **5** was ultimately derived by a demethylation of the known **2**. Thus, It was confirmed that **5** was 2′,5-dihydroxy-6,7-methylenedioxyisoflavone.

It was also estimated that the compound **6** was 5-hydroxy-6,7-methylenedioxyflavone by the followed data. In the UV spectrum of **6**, the maxima (275 and 316 nm) of the absorptions suggested that **6** had a flavone structure. In the ^{1}H NMR of **6**, the signals of the C-3-H (δ 6.61, s) and the five protons (δ 7.80, m, 2′6′-protons and δ 7.50, m, 3′4′5′-protons) also supported its structure.

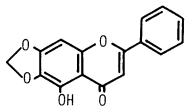
The investigation of the inhibition effect of the isolated flavonoids allowed that the compounds **3** and **4** had more weak activities than **1** and **2**, and that **5** (the demethylated compound of the active **2**) and **6** haven't the activities in the least.



1: R_1=R_3=OCH_3, R_2=H, R_4,R_5=OCH_2O
3: R_1=R_2=H, R_3=R_5=OH R_4=OCH_3
4: R_1=H, R_2=R_3=OH, R_4,R_5=OCH_2O



2: R_1=OCH_3
5: R_1=OH



6

Experimental

All melting points are uncorrected. IR, UV, and ^{1}H NMR were determined with a Shimazu IR-400 spectrometer, with a Shimazu MPS-5000 spectrometer (solvent, ethanol), and with a JEOL-JNM-FX-90 spectrometer (solvent, chloroform-*d*; internal reference tetramethylsilane). MS spectra were taken with a Shimazu KLB-5000 spectrometer. High resolution MS spectra were taken by the staff of the Faculty of Agriculture, Hokkaido University, with a JEOL-D-500 spectrometer. TLC and column chromatographies were carried out on Merck kiesel gel and Cica silica gel.

Aphanomyces euteiches and *Rhizoctonia solani* were applied for screening test of growth inhibition properties. It was surely confirmed that methanol extract of the sliced and non-

infected sugarbeet roots had never the inhibition activity.

Isolation of the Flavoniods (1)—(6). Sliced sugarbeet roots (20 kg) were infected with *R. solani,* and stayed for 3 days at 25 °C, and dried at room temperature, and dipped in methanol (10 liters). After filtration of the extracts, the methanol solution was evaporated. Ethyl acetate solution of the obtained residue was washed with saturated water by sodium chloride, and dried (sodium sulfate). The produced residue by removal of solvent was washed with hexane (this hexane solution haven't the inhibition activity). The residue (20 g) was separated on column chromatography (solvent system; benzene, chloroform, and ethyl acetate). Further, the purification of the fractions was carried out by preparative TLC plates.

The known (**1**, 13 mg), (**2**, 450 mg), and (**3**, 14 mg) were identified with the standard compound **3** and the other data.

3,5-Dihydroxy-6,7-methlenedioxyflavanone (4): Yellow crystals (9 mg), mp 179—180 °C; Gibbes test (+); MS *m/z* 300 (M^+, 48%), 180 (M−120, 100); UV, λ_{max} (EtOH) 246 nm (ε 14150), 294 (ε 16500), λ_{max} (EtOH, $AlCl_3$ or $AlCl_3$, HCl) 245 nm (ε 6430), 322 (ε 20800); IR, 3450 cm^{-1} (OH), 1670 and 1620 (C=O); ^1H NMR δ=10.92 (1H, s, C-5-OH), 7.50 (5H, m, W_H=8 Hz, B-ring protons), 6.14 (1H, s, C-8-H), 6.04 (2H, s, $-OCH_2O-$),5.09 and 4.55 (2H, AB-q, J=12 Hz, C-2-H and C-3-H); High MS. Found: 300.0635. Calcd for $C_{16}H_{12}O_6$: 300.0634.

2′,5-Dihydroxy-6,7-methylenedioxyisoflavone (5): Pale yellow crystals (15 mg), mp 230—233 °C; Gibbes Test (+); MS *m/z* 298 (M^+, 32%), 180 (M−118, 100); UV, λ_{max} (EtOH) 272 nm (ε 22600), 290 (shoulder), λ_{max} (EtOH, $AlCl_3$ or $AlCl_3$, HCl) 282 nm (ε 28000), 320 (ε 14000); IR, 3250 cm^{-1} (OH), 1670 and 1620 (C=O); ^1H NMR, δ 12.15 (1H, s, C-5-OH), 8.06 (1H, s, C-2-H), 7.30 (1H, m, C-6′-H), 7.10 (3H, m, C-3′,4′,5′-H), 6.61 (1H, s, C-8-H), 6.15 (2H, s, $-OCH_2O-$); High MS. Found: 298.0475. Calcd for $C_{16}H_{10}O_6$: 298.0477.

5 from 2: The mixture of **2** (100 mg), dry $AlCl_3$ (300 mg), and K_2CO_3 in ether (20 ml) was refluxed for 3 h. After an addition of acetic acid (10 ml) in the reaction mixture, removal of the solvent gave the residue. Chloroform solution of the residue was washed and dried (Na_2SO_4). Removal of solvent gave crystals **5**.

5-Hydroxy-6,7-methylenedioxyflavone (6): Yellow crystals (340 mg), mp 205 °C (decomp); Gibbes test (+); MS *m/z* 282 (M^+, 100%), 180 (M−102, 31), UV, λ_{max} (EtOH) 275 nm (ε 19700), 316 (ε 15200), λ_{max} (EtOH, $AlCl_3$ or $AlCl_3$, HCl) 284 nm (ε 20600), 292 (shoulder), 346 (ε 17800); IR, 3400 cm^{-1} (OH), 1670 and 1620 (C=O); ^1H NMR, δ=12.83 (1H, s, C-5-OH), 7.80 (2H, m, C-2′,6′-H), 7.50 (3H, m, C-3′,4′,5′-H), 6.68 (1H, s, C-8-H), 6.61 (1H, s, C-3-H), 6.11 (2H, s, $-OCH_2O-$); High MS, Found: 282.0539. Calcd for $C_{16}H_{10}O_5$: 282.0529.

The authors wish to express their gratitude to Dr. Ryouzoh Yokosawa, Higashi Nippon Gakuen University, for infection and screening test by him, to Mr. Juichi Sugawara, Hokkaido Togyo co. LTD., for his supply of sugarbeet, to Dr. Akiyo Sakushima, Faculty of Pharmaceutical Sciences of Higashi Nippon Gakuen University for his valuable suggestions, and to Professor Shuzo Takagi, Faculty of Pharmaceutical Sciences, Mukogawa Women's University for his kind supply of dihydrooroxylin A.

References

1) J. Geigert, F. R. Stermitz, G. Johnson, D. D. Maag, and D. K. Johnson, *Tetrahedron,* **29**, 2703 (1973).

2) H. Sauer and R. Hansel, *Planta Med.,* **15**, 443 (1967); S. Takagi, M. Yamaki, and K. Inoue, *Yakugaku Zasshi,* **100** 1220 (1980).

3) H. Budzikiewicz, C. Djerassi, and D. H. Williams. "Structure Elucidation of Natural Products by Mass Spectrometry," Holden-Day (1964); A. Pelter, P. Stainton and M. Barber, *J. Heterocycl. Chem.,* **2**, 262 (1965).

4) M. Zanger, *Org. Magn. Reson.,* **4**, 1 (1972).