Note

6-Hydroxyflavonoids as α-Glucosidase Inhibitors from Marjoram (Origanum majorana) Leaves

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A methanol extract of marjoram leaves strongly inhibited rat intestinal α-glucosidase. Five 6-hydroxyflavonoids, 6-hydroxyapigenin (scutellarein; IC$_{50}$ for sucrose hydrolysis by rat intestinal α-glucosidase, 12 μM), 6-hydroxyapigenin-7-O-β-D-glucopyranoside (>500 μM), 6-hydroxyluteolin-7-O-β-D-glucopyranoside (300 μM), 6-hydroxyapigenin-7-O-(6-O-feruloyl)-β-D-glucopyranoside (>500 μM), and 6-hydroxyluteolin-7-O-(6-O-feruloyl)-β-D-glucopyranoside (>500 μM), were isolated as active principles and related compounds. The two feruloylglucosides are novel compounds.

Key words: 6-hydroxyflavone; Origanum majorana; marjoram; α-glucosidase inhibitor

Intestinal α-glucosidases have a physiologically important role in the digestion of dietary carbohydrates. Hence, suppression of the activity of the enzymes decreases glucose absorption and thus reduces the postprandial blood glucose concentration. 1) While searching for food-derived α-glucosidase inhibitors, we isolated and identified quercetin, sulfolipids, N-p-coumaroyltyramine, baicalein (1), and ellagitannins from tochu-cha, 2) hijiki, 3) welsh onion, 4) ougon, 5) and clove, 6) respectively. Strong inhibitory activity was found in marjoram extracts in screening for such inhibitors from herbs. In this paper, we report the isolation of two new flavonoid acylglucosides and of three structurally related flavones as rat intestinal α-glucosidase inhibitors from leaves of marjoram (Origanum majorana, Labiatae).

Solvent partitions of aqueous methanol extracts of dried leaves (100 g) of marjoram purchased from a local market gave a 1-butanol-soluble fraction that inhibited rat intestinal α-glucosidase. 5,6) The fraction was separated by reverse-phase ODS column chromatography (water-methanol (6:4 and 4:6)) followed by preparative HPLC (Inertsil PREP-ODS, GL Science, 20.0 × 250 mm; mobile phase, 23–30% MeCN containing 0.1% trifluoroacetic acid; flow rate, 5 mL/min; UV 254 nm), which yielded compounds 2 (25 mg), 4 (42 mg), 5 (334 mg), 6 (33 mg), and 7 (12 mg), all of which have a 5,6,7-trihydroxyflavone skeleton. Compounds 2, 4, and 5 were identified as 6-hydroxyapigenin (scutellarein), 7) its 7-O-β-D-
and H-3, respectively. Compounds C31H28O4 (found, 624.1465; calcd., 624.1479). The desorption (FD) mass spectrum and high-resolution 6.70 (1H, d, 15.8 Hz, H-8) and a pair of doublets of H-2, similar to those found with glucopyranoside,9) and 6-hydroxyluteolin-7-

To the 1H NMR spectrum (Table 1) had signal patterns similar to those found with 4, which included two singlets of H-3 and H-8 and a pair of doublets of H-2(6') and H-3(5') for the 6-hydroxyapigenin-7-glucoside. A series of coupled β-glucose protons, except for an additional characteristic set of 1,2,4-trisubstituted benzene protons at δH 4.68 (1H, d, J = 8.1 Hz, H-5'), 6.55 (1H, dd, J = 8.1 and 1.7 Hz, H-6'), and 6.70 (1H, d, J = 1.7 Hz, H-2') together with a couple of doublets of trans-olefinic protons at δH 6.20 (1H, d, J = 15.8 Hz, H-8') and 7.44 (1H, d, J = 15.8 Hz, H-7') and a methoxyl singlet at δH 3.63 (3H, s, H-3'OMe). The difference in the molecular formulae of 6 and 4 was C10H10O5, which suggests the presence of a feruloyl group in 6 when the NMR signals just mentioned were taken into account. The feruloyl residue was confirmed by strong three-bond correlations of H-2'' (δH 6.70) / C-4'' (δC 150.4), H-6'' (δH 6.55) / C-4'' (δC 149.0), methoxyl (δH 3.63) / C-3'' (δC 127.3), H-8'' (δH 6.20) / C-1'', and H-7'' (δH 7.44) / C-9'' (δC 168.7) in the heteronuclear multiple bond correlation (HMBC) spectrum. The connectivity of the aglycon, glucose, and ferulic acid was also determined by HMBC correlation peaks between an anomic H-1'' (δH 5.10) and C-7 (δC 152.5) of the aglycon, which was further coupled weakly with H-8 (δH 6.84) by two-bond connectivity, and between nonequivalent methylene protons of H-6'' (δH 4.23 and 4.78), shifted downfield, and a carbonyl carbon (C-9'') of the feruloyl residue. The structure of 6 was thus concluded to be 6-hydroxyapigenin-7,7'-disubstituted B-ring protons at δH 7.14 (1H, d, J = 7.3 Hz, H-5'), 7.19 (1H, br s, H-2'), and 7.23 (1H, dd, J = 7.3 and 2.0 Hz, H-6') appeared in 7 in place of p-disubstituted B-ring protons at δH 6.81 (2H, d, J = 8.8 Hz, H-2'(6')) in 6. In consequence, the structure of 7 was found to be 6-hydroxyluteolin-7-O-(6-O-feruloyl)-β-D-glucoside.

The five isolates and 6-hydroxyluteolin (3),12) an aglycon of 5 and 7, which was prepared by enzymatic hydrolysis of 5, were tested for inhibition of rat intestinal α-glucosidase with sucrose as the substrate. The activity of each flavone at 500 μM was as follows: 2 (81%), 3 (92%), 4 (44%), 5 (55%), 6 (25%), and 7 (26%). The IC50 values were 2 (12 μM), 3 (10 μM), 4 (>500 μM), 5 (300 μM), 6 (>500 μM), and 7 (>500 μM). All of the isolates were flavone derivatives with a characteristic 6-hydroxyl substituent. As previously reported, baicalin (5,6,7-trihydroxyflavone),1 isolated from ougon (Scutellaria baicalensis) potently inhibits rat intestinal α-glucosidase (IC50, 32 μM).5) These results suggest that the 5,6,7-trihydroxyflavone structure was crucial for high activity regardless of B-ring hydroxylation, considering that common flavones such as apigenin (4',5,7-trihydroxyflavone, 8) and luteolin (3',4',5,7-tetrahydroxyflavone, 9), both of which lack the 6-hydroxyl substituent found in the active principles in marjoram, had negligible activity (12% and 22%, respectively), at 500 μM in our enzyme inhibitory assay. In addition, glycosylation of the 7-hydroxyl group on the A ring reduces the inhibition, and acylation of the sugar further reduces inhibition.

| Table 1. NMR Data for 6 and 7 (methanol-d4)6 |
|---|---|---|
|  | 13C | 1H | 1H |
| 2 | 166.5 | — | — |
| 3 | 103.2 | 6.42 s | 6.36 s |
| 4 | 184.3 | — | — |
| 5 | 148.0 | — | — |
| 6 | 131.8 | — | — |
| 7 | 152.5 | — | — |
| 8 | 95.4 | 6.84 s | 6.80 s |
| 9 | 151.1 | — | — |
| 10 | 107.4 | — | — |
| 1 | 123.0 | — | — |
| 2 | 129.2 | 7.67 d (8.8)b | 7.19 br s |
| 3 | 116.8 | 6.81 d (8.8) | — |
| 4 | 162.7 | — | — |
| 5 | 116.8 | 6.81 d (8.8) | 7.14 d (7.3) |
| 6 | 129.2 | 7.67 d (8.8) | 7.23 dd (7.3, 2.0) |
| 1 | 101.7 | 5.10 d (7.5) | 5.09 d (7.6) |
| 2 | 74.5 | 3.63 dd (9.0, 7.5) | 3.63 dd (9.0, 7.6) |
| 3 | 77.5 | 3.56 t (9.0) | 3.56 t (9.0) |
| 4 | 72.5 | 3.41 t (9.0) | 3.41 t (9.0) |
| 5 | 75.6 | 3.88 m | 3.87 m |
| 6 | 64.7 | 4.23 dd (11.8, 8.4) | 4.26 dd (11.6, 8.9) |
| 7 | 47.8 dd (11.8, 2.5) | 4.75 dd (11.6, 2.7) |
| 1 | 127.3 | — | — |
| 2 | 111.2 | 6.70 d (1.7) | 6.71 d (1.8) |
| 3 | 149.0 | — | — |
| 4 | 150.4 | — | — |
| 5 | 116.3 | 6.48 d (8.1) | 6.48 d (8.1) |
| 6 | 123.7 | 6.55 dd (8.1, 1.7) | 6.55 dd (8.1, 1.8) |
| 7 | 147.4 | 7.44 d (15.8) | 7.43 d (15.8) |
| 8 | 114.9 | 6.20 d (15.8) | 6.21 d (15.8) |
| 9 | 168.7 | — | — |
| OMe | 56.0 | 3.63 s | 3.63 s |

5) In Hz.
6) J. K. AWABATA, J. K. et al.
7) R. K. AWABATA, R. et al.
8) T. K. AWABATA, T. et al.
9) U. K. AWABATA, U. et al.
10) S. K. AWABATA, S. et al.
12) A. K. AWABATA, A. et al.
13) N. K. AWABATA, N. et al.
6-Hydroxyflavonoids are found in few plants and the compounds are interesting phylogenic markers.\textsuperscript{11)} This is the first report of the importance of the 6-hydroxyl substituent in the flavone skeleton for inhibition of a mammalian intestinal \(\alpha\)-glucosidase.

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**References**


