Syntheses of Isoflavones and Isoflavone Glycosides, and Their Inhibitory Activity against Bovine Liver β-Galactosidase

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To clarify the relationship between the structure and inhibitory action toward β-D-galactosidase (EC 3.2.1.21) of isoflavones and isoflavone glycosides, a number of polyhydroxyisoflavones, and the α-L-rhamnosides and β-L-quinovosides of daidzein and genistein were synthesized. Among the polyhydroxyisoflavones, 2',3',4',7-tetrahydroxyisoflavone showed the strongest inhibitory activity (K_i = 26x10^{-6} M). Among the glycosides, all the 1-rhamnosides were strong inhibitors, of which genistein 4',7-di-O-α-L-rhamnoside was the strongest (K_i = 4.44x10^{-6} M), while all the isoflavone β-L-quinovosides were considerably weak or possessed no inhibition.

Glycosidases are widely distributed throughout nature and are vital for the growth and development of most organisms. Current research on specific glycosidase inhibitors represents a promising therapeutic approach for regulating the breakdown of carbohydrate foodstuffs, processing eukaryotic glycoproteins, and catabolizing polysaccharides and glycoconjugates.

Moreover, it has been reported that the β-D-galactosidase activity in fibroblasts showed a marked increase after transformation by oncogenic viruses, suggesting an involvement of this enzyme in oncogenicity.

It has been reported by Hazato et al. that daidzein (4',7-dihydroxyisoflavone, 1) and genistein (4',5,7-trihydroxyisoflavone, 2) and their glycosides, namely, daidzein 7-O-α-L-rhamnopyranoside (3), 4',7-di-O-α-L-rhamnopyranoside (4), genistein 7-O-α-L-rhamnopyranoside (5) and 4',7-di-O-α-L-rhamnopyranoside (6), were isolated from a culture filtrate of Streptomyces xanthaphes MD 865 C-3 and also that these compounds had powerful inhibitory activity against bovine liver β-D-galactosidase.

In the present paper, to clarify the relationship between the structure and inhibitory action of isoflavones and isoflavone glycosides, an attempt was made to synthesize the above-mentioned isoflavones, their 1-rhamnosides and also a number of related compounds, and to examine their inhibitory activity against bovine liver β-galactosidase.

Although several methods for preparing isoflavones have hitherto been reported, the thallium(III) nitrate method seems at present to be the best one. Namely, isoflavones can be conveniently prepared by the oxidative rearrangement of 2'-benzoxyl- or 2'-acetoxy-chalcones with thallium(III) nitrate into 1-(2-benzoxyphenyl or acetoxyphenyl)-3,3-dimethoxy-2-phenoxypropene-1-one, and followed by deprotection and subsequent cyclization.

By applying this method, the following isoflavones were synthesized: compound 1, 2, 7-hydroxyisoflavone (7), 4'-hydroxyisoflavone (8), 5-hydroxyisoflavone (9), 4',5-dihydroxyisoflavone (10), 3',4',7-trihydroxyisoflavone (11), 3',4',5-trihydroxyisoflavone (12), 3',4',5,7-tetrahydroxyisoflavone (13), 3',4',5,7-tetrahydroxyisoflavone (14), 2',3',4',7-tetrahydroxyisoflavone (15), 2',4',5,7-tetrahydroxyisoflavone (16), 2',4',5,7-tetrahydroxyisoflavone (17), and 3',4',5,7-pentahydroxyisoflavone (18). These compounds have not previously been synthesized by the thallium(III) nitrate method.

As a typical example, the preparation of 15 is described here. The reaction of 2',4'-bis(benzyloxy)-acetophenone with 2,3,4-trimethoxybenzaldehyde in ethanolic sodium hydroxide yielded 2',4'-bis(benzyloxy)-2,3,4-trimethoxychalcone (19), which was oxidatively rearranged with thallium(III) nitrate into 1-[2,4,3(benzyloxy)phenyl]-3,3-dimethoxy-2(2,3,4-trimethoxyphenyl)-propene-1-one (20). Hydrogenolysis of 20, using Pd-C (10%) as a catalyst, gave 1-(2,4-dihydroxyphenyl)-3,3-dimethoxy-2(2,3,4-trimethoxyphenyl)-propene-1-one (21), which was cyclized with dil. hydrochloric acid to 7-hydroxy-2',3',4'-trimethoxyisoflavone (22). Demethylation of 22 with hydroiodic acid furnished 15.

The chemical structures of all the isoflavones that were synthesized were confirmed by mp, elemental analyses and NMR spectra. Generally, α-L-rhamnosylation of the hydroxyl groups of flavonoids is known to be difficult, giving α-L-rhamnosides in a very poor yield. Recently, Hashimoto et al. have published a glycosylating method involving the reaction of benzyl-protected glucopyranosyl fluoride with alcohols or their trimethylsilyl ethers in the presence of tetrafluorosilane or trimethylsilyl triflate. Kunz and Sanger have reported that the stereoselective glycosylation of alcohols or their trimethylsilyl ethers was achieved by using O-alkyl, O-acetyl, and acetal-protected glycosyl fluoride and boron trifluoride etherate (BF_3·(C_2H_5)_2O) in dichloromethane. Vozni and his co-workers reported that 4-methyl-7-(trimethylsilyl)oxycomarine was reacted with various kinds of glycosyl fluorides, using boron trifluoride as a catalyst, to afford 4-methylumbelliferone glycosides. We found that this synthetic approach is an efficient method for obtaining some flavonoid α-L-rhamnosides. Namely, 2,3,4-tri-O-acetyl-L-rhamnopyranose (23) was treated with diethyaminosulfur trifluoride (DAST) to yield tri-O-acetyl-α-L-rhamnopyranosyl fluoride (24) and its β-anomer (25) in a ratio of 5:1. Compound 1 was treated with chlorotrimethylsilane and
hexamethyldisilazane to afford its 4',7-di-O-trimethylsilyl (TMS) derivative (26). Coupling 26 with two molar equivalents of 24 in benzene in the presence of BF$_3$\(\cdot\)(C$_2$H$_5$O)$_2$O resulted in the formation of daidzein 4',7-di-O-(tri-O-acetyl-\(\alpha\)-l-rhamnopyranoside) (27) and -4',-O-(tri-O-acetyl-\(\alpha\)-l-rhamnopyranoside) (28) in 73.0% and 27.0% yields, respectively. Deacetylation of 27 and 28 with methanolic sodium methoxide gave 4 and daidzein 4'-O-\(\alpha\)-\(l\)-rhamnopyranoside (29). Similarly, the reaction of 4',5,7-tri-O-TMS derivative 30 of 2 with 24 gave genistein 4',7-di-O-(tri-O-acetyl-\(\alpha\)-l-rhamnopyranoside) (31) and 4'-O-(tri-O-acetyl-\(\alpha\)-l-rhamnopyranoside) (32) in 5.9% and 37.5% yields, respectively. A Zemplén deacetylation of 31 and 32 yielded 6 and genistein 4'-O-\(\alpha\)-\(l\)-rhamnopyranoside (33). Condensation of the TMS derivatives of 7 and 8 with 24 by the method already mentioned respectively yielded \(\alpha\)-l-rhamnopyranoside triacetates 34 and 35, which, after deacetylation, gave their \(\alpha\)-l-rhamnopyranosides, 36 and 37.

The structures of 4, 29, 6, and 33 were determined from the UV maxima of 29 and 33 being respectively shifted 6 nm and 12 nm by the addition of sodium acetate. These results indicated that the 7-hydroxyl group of the isoflavone moiety in 29 and 33 was not substituted. On the other hand, no bathochromic shift was observed in the UV spectra of synthetic 4 and 6 after the addition of sodium acetate, indicating that the 7-hydroxyl group was substituted. The hydroxyl protons at C-5 of the genistein moiety of 6 and 33 were observed at 8 12.95 and 12.80 in their H-1 NMR spectra. These data suggested that the C-5 hydroxyl group was not substituted. From the intensity ratio of the methyl group and the characteristic C-2 proton of the isoflavone moiety, it was suggested that 29 and 33 consisted of one molecule of daidzein or genistein and one molecule of \(\alpha\)-l-rhamnose, respectively, while, 4 and 6 contained one molecule of isoflavone and two molecules of \(\alpha\)-l-rhamnose. Also, the anomic configuration of the \(\alpha\)-l-rhamnosyl residue in the foregoing compounds was deduced by applying Klyne's rule. This was done by comparing the molecular rotation difference, \(\Delta\)\(\alpha\)\(_D\) \(\equiv\) \(\alpha\)\(_D\) (glycoside) - \(\alpha\)\(_D\) (aglycone) with that of the corresponding methyl glycoside. The sum of the \(\alpha\)\(_D\) value for methyl \(\alpha\)-l-rhamnopyranoside (\(-11100\)) and for daidzein or genistein (both \(\alpha\)\(_D\) 0) was \(-11100\). The sum of the \(\alpha\)\(_D\) values for methyl \(\beta\)-l-rhamnopyranoside (+17000) and for daidzein or genistein was +17000. The observed \(\alpha\)\(_D\) values for 4, 29, 6, 33, and 34 were \(-96924\), \(-37200\), \(-75870\), and \(-38688\). Accordingly, the \(\alpha\)-l-rhamnopyranosidic bonds in 4, 29, 6, and 33 were all assigned as \(\alpha\)-form.

In a similar manner, the anomic configuration of the \(\alpha\)-l-rhamnosyl group in 36 and 37 was deduced to be \(\alpha\)-form in each (see Experimental section).

Next, an attempt was made to synthesize some analogs, namely, daidzein 7-O-\(\beta\)-l-quino-vopyranoside (38), 4',7-di-O-\(\beta\)-l-quino-vopyranoside (39), genistein 7-O-\(\beta\)-l-quino-vopyranoside (40), and 4',7-di-O-\(\beta\)-l-quino-vopyranoside (41). Coupling 1 with an equimolar amount of tri-O-acetyl-\(\alpha\)-l-quino-vopyranosyl bromide (42)\(^{10}\) in alkaline acetone gave coupling product 43, which was deacetylated with methanolic sodium methoxide to give 38. Condensation of 43 with an equimolar amount of 42 in quinoline in the presence of silver carbonate resulted in the formation of the 4',7-di-O-(tri-O-acetyl-\(\beta\)-l-quino-vopyranoside) (44) of 1, which, after deacetylation, gave 39. In a similar way, 2 was coupled with an equimolar amount of 42 in alkaline acetone to yield the 7-O-(triacyl-\(\beta\)-l-quino-vopyranoside) (45) of 2, which, after deacetylation, gave 40. Treatment of 2 with two molar equivalents of 42 in alkaline acetone gave coupling product 46, which, after deacetylation, yielded 41.

The structures of 38-41 were determined by elemental analyses, \(\alpha\)\(_D\) value and UV and NMR spectra. The anomic configurations of 38-41 were all determined as \(\beta\)-form by C-13 NMR spectra (\(\Delta\)\(\alpha\)\(_D\) = 170 Hz).

Antus and Nogradi\(^{11}\) have reported that 2'-acetoxy-4,4'-dihydroxchalcone 4'-O-\(\beta\)-l-glucoside was oxidatively rearranged to thallium(III) nitrate in trimethyl orthofomate and methanol to yield 1-(2-acetoxy-4-glucosyloxyphenyl)-3,3'-dimethoxy-2-phenylpropane-1-one, which could be successfully cyclized with methanolic sodium methoxide to isoflavone glucoside. Accordingly, we tried to synthesize 38 by this route. Resacetophenone was coupled with 42 in quinoline in the presence of silver carbonate to yield coupling product 47, which was subsequently deacetylated to give resacetophenone 4'-O-\(\beta\)-l-quino-vopyranoside (48). Condensation of 48 with \(\beta\)-benzoyloxylalkaldehyde (49) in conc. alkali gave chalcone 50. Acetylation of 50 gave 2'-O-acetate 51, which was oxidatively rearranged with thallium(III) nitrate to afford dimethyl acetal 52. Cyclization of 52 with methanolic sodium methoxide yielded 4'-benzoyloxdaidzein 7-\(\beta\)-l-quino-vopyranoside (53). Unexpectedly, however, catalytic debenzylation of 53 was unsuccessful, giving several inseparable products.

**Inhibition studies**

The inhibitory action of the flavones and isoflavone glycosides toward the hydrolysis of \(p\)-nitrophenyl \(\beta\)-D-galactosidase catalyzed by bovine liver \(\beta\)-D-galactosidase (EC 3.2.1.21, Sigma) was determined according to the

**Table I. Structures of Chalcones**

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method of Aoyagi et al. A Lineweaver–Burk plot showed that all the compounds tested were mixtures of competitive/ non-competitive inhibitors. \( K_i \) values for the compounds are summarized in Tables I and II. The \( K_m \) value of \( \beta\)-d-galactosidase (EC 3.2.1.21) for \( p\)-nitrophenyl \( \beta\)-d-galactoside was \( 1.6 \times 10^{-6} \) M. No obvious correlation was observed between the inhibitory activity and the number and position of the hydroxyl group attached to the isoflavone skeleton. Among the isoflavones, 2,3',4,7-tetrahydroxyisoflavone showed the strongest inhibitory activity (\( K_i = 26.2 \times 10^{-6} \) M). By comparing the \( K_i \) values among 7, 8, 9, 10, 11, 12, 13, and 18, it may be said that the introduction of a hydroxyl group at C-5 of isoflavone increased the inhibitory activity.

Daidzein and genistein inhibited \( \beta\)-d-galactosidase (EC 3.2.1.21) with \( K_i \) values of \( 107.5 \times 10^{-6} \) M and \( 57.8 \times 10^{-6} \) M, respectively, and their mode of inhibition was of the mixed type, being very close to a competitive one, while their 4′-O-\( z\)-L-rhamnosides (29 and 33) and 4′,7-di-O-\( z\)-L-rhamnosides (4 and 6) strongly inhibited with \( K_i \) values of \( 20.4 \times 10^{-6} \) M, \( 6.3 \times 10^{-6} \) M, \( 6.34 \times 10^{-6} \) M, and \( 4.44 \times 10^{-6} \) M, respectively, their mode of action also being of the mixed type and very close to a non-competitive one. On the other hand, the corresponding \( \beta\)-L-rhamnoside (2′-epimer of L-rhamnoside) analogs (38, 40, 39, and 41) exhibited no inhibition or weaker inhibition than daidzein or genistein. By comparing the inhibitory activity of each of these L-rhamnosides and the L-quino- 

**Table II. Structures of Isoflavones and Isoflavone Glycosides, and Their \( K_i \) Values for \( \beta\)-d-Galactosidase**

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<th>Compound no.</th>
<th>Substituent group</th>
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Qui=\( \alpha\)-Quinovose; Rha=\( \alpha\)-L-Rhamnose.

Fig. 1. Chemical Structures of Dimethyl Acetals and Chalcones.

**Experimental**

**General methods.** Reactions were monitored by TLC on silica gel 60 (Merck) with detection by UV light, charring with sulfuric acid, or spraying 2% ferric chloride in ethanol. Optical rotation values were measured with a Horiba SEPA-200 digital polarimeter. NMR spectra (\( ^{1}H, 90 \) MHz and \( ^{13}C, 24.5 \) MHz) were recorded with a Hitachi R-90H spectrometer, using tetramethylsilane as an internal standard for solutions in \( CDCl_3 \) or dimethyl sulfoxide-\( d_6 \). UV spectra were run with a Shimadzu UV-200 spectrometer. Microanalyses were performed by the Microanalytical Laboratory of the School of Pharmaceutical Sciences at this University.

**General procedure for preparing the chalcones.** Equimolar amounts (0.01 mol) of benzylxoy- or methoxy-benzaldehyde and benzylxoy-acetophenone were dissolved in ethanol (100 ml), and 50% (w/w) aqueous potassium hydroxide (40 ml) was added. The mixture was stirred at room temperature for 24 h and then acidified with dil. hydrochloric acid. The crystalline chalcones deposited were collected, washed with water, dried, and then recrystallized from chloroform–ethanol. The chalcones (55, 62, and 66), which had a hydroxyl group at the C-2′ position, were treated with acetic anhydride–pyridine (1:1) in the usual manner to give 2′-O-acetates (55, 62, and 66).

2′,4′-Tris(benzylxoy)-chalcone (54). Yield, 98.0%; mp 119–121°C. Anal. Found: C, 82.24; H, 5.75. Calcd. for \( C_{34}H_{30}O_4 \): C, 82.13; H, 5.70%.

2′-Hydroxy-4′-4′-tris(benzylxoy)-chalcone (55). This compound was prepared by benzylating natingenin.**

2′-O-Acetate (55), mp 119–120°C. Anal. Found: C, 77.94; H, 5.85. Calcd. for \( C_{34}H_{30}O_4 \): C, 78.08; H, 5.48%.

2′,4′-Bis(benzylxoy)-chalcone (56). Yield, 80.5%; mp 76–77°C. Anal. Found: C, 82.15; H, 5.70. Calcd. for \( C_{34}H_{30}O_4 \): C, 82.86; H, 5.71%.

2′-Bis(benzylxoy)-chalcone (57). Yield, 84.3%; mp 98–100°C. Anal. Found: C, 82.73; H, 5.64. Calcd. for \( C_{34}H_{30}O_4 \): C, 82.86; H, 5.71%.

2′,6′-Bis(benzylxoy)-chalcone (58). Yield, 89.1%; mp 116°C. Anal. Found: C, 83.08; H, 5.81. Calcd. for \( C_{34}H_{30}O_4 \): C, 82.86; H, 5.71%.
2,4,6-Tri(benzyloxy)chalcone (59). Yield, 53.7%; mp 129–130°C.
Anal. Found: C, 81.98; H, 5.73. Calcd. for C_{34}H_{36}O_{6}: C, 82.13; H, 5.70%.

2,4,4,6-Tetra(benzyloxy)chalcone (60). Yield, 92.1%; mp 154–155°C.
Anal. Found: C, 81.35; H, 5.65. Calcd. for C_{34}H_{36}O_{6}: C, 81.65; H, 5.70%.

2,3,4,6-Tetra(benzyloxy)chalcone (61). Yield, 89.0%; mp 169–172°C.
Anal. Found: C, 81.02; H, 5.64. Calcd. for C_{34}H_{36}O_{6}: C, 81.65; H, 5.70%.

2,4,6-Tri(benzyloxy)-2'-hydroxychalcone (62). Yield, 85.0%; mp 139–140°C.
Anal. Found: C, 79.82; H, 5.17. Calcd. for C_{34}H_{36}O_{6}: C, 79.63; H, 5.56%. Calcd.

2',3,4,6'-Tetra(benzyloxy)chalcone (63). Yield, 90.5%; mp 126–128°C.
Anal. Found: C, 75.12; H, 5.85. Calcd. for C_{34}H_{36}O_{6}: C, 75.29; H, 5.88%.

2,4-Bis(benzyloxy)-3,4,5-trimethoxychalcone (64). Yield, 80.2%; mp 137°C.
Anal. Found: C, 74.92; H, 5.79. Calcd. for C_{34}H_{36}O_{6}: C, 75.29; H, 5.88%.

2,4-Bis(benzyloxy)-2',4,5,6,6'-pentamethoxychalcone (65). Yield, 85.3%; mp 143–144°C.
Anal. Found: C, 74.93; H, 5.86. Calcd. for C_{34}H_{36}O_{6}: C, 75.29; H, 5.88%.

4,6-Bis(benzyloxy)-2-hydroxy-3,4,5-trimethoxychalcone (66). Yield, 86.9%; mp 143–144°C.
Anal. Found: C, 72.86; H, 5.81. Calcd. for C_{34}H_{36}O_{6}: C, 73.00; H, 5.70%. Calcd.

General procedure for preparing the hydroxysophavones. To a stirred
solution of the chalcone (3 mmol) in trimethyl orthoformate or chloroform
(300 ml) was added dropwise a solution of thallium nitrate (3 mmol) in
trimethyl orthoformate (70 ml) at 0°C. The mixture was stirred at room
temperature. After 1–3 h, TLC (chloroform–hexane = 2:1) indicated that
no starting material (R_{f} 0.5) remained, while a major product (R_{f} 0.2) had formed.
After neutralizing with 1 N methanolic sodium methoxide, the
reaction mixture was filtered, and the filtrate was concentrated. The residual
 syrup was dissolved in chloroform, washed three times with brine, and
then dried over anhydrous magnesium sulfate. The filtrate was concentrated,
and the residue was chromatochromographed on a column of silica
gel, eluting with chloroform or a mixture of chloroform and hexane (2:1),
to afford a crude dimethyl acetal. The structure of each dimethyl acetal was
identified by the appearance of two methoxy signals (sharp singlet) and
two methine protons (doublet, J = 9 Hz) in their NMR spectra.

Dimethyl acetal 67 and 21 were obtained in pure crystalline form.
The crude dimethyl acetal was dissolved in ethyl acetate with 10% Pd–C, and
a few drops of triethyl amine were added, before the mixture was stirred
under a hydrogen atmosphere at room temperature until the calculated
amount of hydrogen had been absorbed. After the reaction was completed,
the mixture was filtered, washed with ethyl acetate or methanol, and the
filtrate and washings were concentrated. The deprotected dimethyl acetal
was dissolved in methanol (400 ml), and 5% hydrochloric acid (50 ml) was
added, before the solution was boiled under reflux for 2 h. After cooling,
an equal volume of water was added to this solution. The isoflavones
were deposited from the solution in crystalline form, yields being calculated
on the basis of the corresponding chalcones.

The methoxylated isoflavones (22, 68–71, each 1 g) were boiled
with hydroiodic acid (d = 1.71, 24 ml) under reflux for 2 h in an oil bath.
After cooling, the solution was diluted with water to give demethoxylated isoflavones 14–17. The yields shown are based on the corresponding methoxylated isoflavones.

1-[4-(Acetoxy-4-hydroxyphenyl)-3,3-dimethoxy-2-(4-hydroxyphenyl)propenyl]-1-one (67). Yield, 37.2%; mp 206–207°C. [α]_{D}^{20} = 0° (c = 1 DMF).
Anal. Found: C, 60.44; H, 3.57. Calcd. for C_{31}H_{33}O_{7}: C, 60.64; H, 3.52%. NMR δ (CDCl_{3}): 2.13 (3H, s, OAc); 3.03, 3.24 (eq, 3H, OCH); 4.70,
3,3',4,4'-Tetrahydroxyisoflavone (15). Yield, 63.6%; mp 261–263°C. Anal. Found: C, 61.06; H, 3.50. Calculated for C17H12O9: C, 61.02; H, 3.37%. NMR δ (CDCl3): 1.25 (3H, J = 6-Hz, CH3); 1.98, 2.05 (each 3H, singlet, 3OAc); 4.05 (1H, m, H-5); 4.80–5.60 (3H, m, H-2, 3, 4). 5.75 (H, J = 1H, H-1). β-Anomer, syrup, 600 mg (9.2%), [α]Dsb +6° (c = 0.5, CHCl3).

General procedure for preparing the trimethylsilyloxyisoflavones. Hydroxyisoflavone (1g) was dissolved in pyridine (20ml), and hexamethyldisilazane (5ml) and chlorotrimethylsilane (5ml) were then added. After standing for 30min at room temperature, the solution was concentrated, and the residue was dissolved in chloroform and filtered. The filtrate was concentrated, and the residue (4g) was chromatographed in a column of silica gel, eluting with chloroform–acetone (20:1), to yield 27 (2.54g, 73.0%) and 28 (1.09g, 47.4%). 27, mp 126–129°C, [α]D +109° (c = 1, CHCl3). Anal. Found: C, 53.39; H, 5.75. Calculated for C15H12O8: C, 53.79; H, 5.75%. NMR δ (CDCl3): 1.20 (6H, J = 6-Hz, 2 CH3); 2.01 (12H, s, 4 OAc); 2.16 (6H, s, 2 OAc); 3.35 (2H, m, sugar H-5, 5); 4.86–5.63 (8H, m, sugar H-1, 4'); 7.10 (2H, d, J = 9-Hz, H-3, 5'); 7.15 (2H, J = 2-Hz, 6, 8); 7.48 (2H, d, J = 2-Hz, H-2, 6); 7.90 (1H, s, H-2); 8.23 (1H, d, J = 9-Hz, H-5). 28, mp 120–123°C, remelted at 241–242°C, [α]D +26° (c = 1, CHCl3). Anal. Found: C, 57.20; H, 5.73. Calculated for C15H12O8: C, 57.65; H, 5.34%. NMR δ (CDCl3): 1.20 (3H, J = 6-Hz, CH3); 2.03 (6H, 2 OAc), 2.18 (6H, s, 2 OAc); 3.40 (2H, J = 6-Hz, 5, 5'); 4.86–5.63 (8H, m, sugar H-1, 4'); 7.10 (2H, d, J = 9-Hz, H-3, 5'); 7.15 (2H, J = 2-Hz, 6, 8); 7.48 (2H, d, J = 2-Hz, H-2, 6); 7.90 (1H, s, H-2); 8.23 (1H, d, J = 9-Hz, H-5).

Daidzein 4,7-di-O-alpha-L-rhamnopyranoside (4) and daidzein 4'-O-alpha-L-rhamnopyranoside (29). Compound 27 (1.53g) was dissolved in 0.1N methanolic sodium methoxide (60ml), and the solution was left at 0°C for 12h. After neutralizing with Amberlite IR 120 (H+ form), the mixture was filtered, and the filtrate was concentrated. The residue was crystallized from methanol–ethyl acetate (1:1) to give 4 in a 74.3% (700mg) yield, mp 122–123°C (from methanol), [α]D +17° (c = 1, CHCl3).

Daidzein 3-O-alpha-L-rhamnopyranoside (2) and daidzein 3'-O-alpha-L-rhamnopyranoside (28). While stirring a solution of BF3·(C2H5)2O (1.2ml) in benzene (10ml) was added to a solution of 46 (0.7g) and 47 (0.6g) in absolute benzene (20ml). After standing for 1h, the mixture was diluted with chloroform, successively washed with water, aqueous sodium hydrogencarbonate and water, and dried over magnesium sulfate. The filtrate was concentrated, and the residue (4g) was chromatographed in a column of silica gel, eluting with

m, H-5', 6', 8); 7.93 (IH, J = 9-Hz, H-5); 8.10 (IH, s, H-2).

3,3',4,4'-Tetrahydroxyisoflavone (15). Yield, 63.6%; mp 261–263°C. Anal. Found: C, 61.06; H, 3.50. Calculated for C17H12O9: C, 61.02; H, 3.37%. NMR δ (CDCl3): 1.25 (3H, J = 6-Hz, CH3); 1.98, 2.05 (each 3H, singlet, 3OAc); 4.05 (1H, m, H-5); 4.80–5.60 (3H, m, H-2, 3, 4). 5.75 (H, J = 1H, H-1). β-Anomer, syrup, 600 mg (9.2%), [α]Dsb +6° (c = 0.5, CHCl3).

3,4,5-Tetrahydroxyisoflavone (18). Yield, 76.3%; mp 256°C. Anal. Found: C, 65.95; H, 4.74. Calculated for C15H12O9: C, 65.85; H, 4.88%. NMR δ (CDCl3): 3.66 (6H, 2, 2 OCH3); 3.80 (3H, 3 CH3); 6.73 (1H, H-3'); 6.84 (2H, H-6', 6'); 6.09 (1H, J = 9-Hz, H-6); 7.83 (1H, J = 9-Hz, H-5); 7.85 (2H, 2 CH2), 8.95 (1H, s, H-2H).
Genistein 4-O-a-L-rhamnopyranoside (3a). Deacetylation of 34 (200 mg) with 0.1 N methanolic sodium hydroxide (15 ml) gave 33 in an 83.9% yield. mp 144–145.5°C; [x]D 20° = –93° (c = 1, CHCl3). Anal. Found: C, 62.95; H, 5.17. Calcd. for C28H36O11: C, 63.52; H, 5.10%. NMR δH (CDCl3) 1.15 (3H, d, J = 6 Hz, CH3); 3.69–3.96 (m; sugar H-2); 5.30–5.53 (4H, m, sugar H-2'); 6.41 (H, d, J = 6 Hz, H-5); 5.30 (H, d, J = 8 Hz, C-2); 7.05 (2H, d, J = 8 Hz, H-2', 6'); 8.10 (H, s, H-2); NMR δC (CDCl3) 143.17 (C-1); 138.12 (C-5, 6); 132.08 (C-3, 4, 5, 6'); 126.27 (C-2); 109.53 (C-10); 103.55 (C-9); 103.27 (C-8); 103.12 (C-6'); 72.77 (sugar C-3); 73.95 (sugar C-4); 70.43 (sugar C-5); 69.51 (sugar C-2); 69.37 (sugar C-3); 66.03 (sugar C-5); 41.36 (sugar C-1); 36.85 (sugar C-3); 27.82 (sugar C-4); 27.67 (sugar C-5); 26.32 (sugar C-6). 2HNO3 (MeOH–Ac2O–HCl) nmn: 272; δmax (MeOH–Ac2O–HCl) = 273; δmax (MeOH–Ac2O) = 261.

Genistein 4-O-a-L-rhamnopyranoside (3a). Deacetylation of 34 (24.4 mg) and 7-trimethylsilyloxyisoflavone (1.05 g) added to a solution of 2 (8 g) in acetone (130 ml) and 2 Na aqueous potassium hydroxide (16 ml) (42.12 g in acetone (130 ml) was added dropwise at 0°C. The mixture was stirred overnight at room temperature and then poured into ice-cold water. After standing overnight in a refrigerator, the precipitate was collected by filtration and air-dried. The dried precipitate was dissolved in chloroform, filtered to remove the unreacted daidzein, and the filtrate was concentrated. The residue was crystallized from ethanol to give 43 as a 21.9% (5.99 g) yield, mp 194°C; [x]D 20° = –4° (c = 1, CHCl3). Anal. Found: C, 61.03; H, 5.11. Calcd. for C28H36O11: C, 61.59; H, 4.94%. NMR δH (CDCl3) 1.30 (3H, d, J = 6 Hz, CH3); 2.05 (H, s, OAc); 2.10 (H, s, OAc); 3.80 (H, s, sugar H-2); 5.43 (H, s, sugar H-3); 7.03 (H, s, sugar H-5); 7.31 (H, d, J = 9 Hz, H-6); 7.13 (H, d, J = 9 Hz, H-6); 7.20 (H, s, H-5); 7.30 (H, d, J = 9 Hz, H-6); 7.32 (H, d, J = 9 Hz, H-2', 6'); 7.33 (H, s, H-2); 7.60 (H, s, H-6); 8.26 (H, d, J = 9 Hz, H-5).

Daidzein 7-O-2,3,4-tri-O-acetyl-beta-D-glucopyranoside (40). A solution of 18 (1 g) in acetone (130 ml) was treated with 0.1 N methanolic sodium hydroxide (150 ml) at 0°C for 12 h. The solution was diluted with water and acidified with acetic acid, before the crystalline 38 deposited was collected by filtration. Yield, 86.4%; mp 250–251°C (c = 1, CHCl3). The sum of the [α]D values for methyl a-L-rhamnopyranoside (+11000) and for 0° (0) was +11000. The sum of the [α]D values for methyl 3,8-dihydroxy-3,4-dihydroisoflavone (1500) and for 0° (0) was +1500. The observed [α]D values for 37 was +38784°. Therefore, the anemic configuration of the r-rhamnol residue was of a-form. Anal. Found: C, 65.18; H, 5.21. Calcd. for C21H20O12: C, 65.72; H, 5.25%. NMR δH (CDCl3) 1.15 (3H, d, J = 6 Hz, CH3); 3.20–3.53 (m, sugar H-2, 3, 5); 5.34 (H, s, sugar H-3); 7.10 (H, d, J = 9 Hz, H-3', 5'); 7.55 (H, d, J = 9 Hz, H-2', 6', H-6); 8.00 (H, dd, J = 9 Hz, H-7, H-7); 8.15 (H, d, J = 9 Hz, H-5); 8.52 (H, s, H-2). NMR δC (CDCl3) 178.09 (CH2); 69.48 (sugar C-7); 143.27 (C-1); 134.29 (C-5, 6); 125.38 (C-3, 5); 130.02 (C-2', 3'); 134.01 (C-3', 8); 155.39 (C-2); 155.80 (C-4'); 175.12 (C-4).
**Genistein 7-O-β-D-glucopyranoside (47).** A new compound, genistein 7-O-β-D-glucopyranoside, was isolated from the aerial parts of *Psoralea angustifolia*, and its structure was determined by NMR spectroscopy.

**Genistein 7-O-β-D-glucopyranoside (48).** This is a new compound, genistein 7-O-β-D-glucopyranoside, which was isolated from the aerial parts of *Psoralea angustifolia*. The structure was determined by NMR spectroscopy.

**Genistein 7-O-β-D-glucopyranoside (49).** This is a new compound, genistein 7-O-β-D-glucopyranoside, which was isolated from the aerial parts of *Psoralea angustifolia*. The structure was determined by NMR spectroscopy.

**References**