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A NEW ISOFLAVONE FROM SOYA BEANS

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Abstract—A new isoflavone was isolated from soya beans and shown to be 7,4′-dihydroxy, 6-methoxyisoflavone, for which the name glycitein is proposed.

INTRODUCTION

SOYA BEANS contain glycosides of the isoflavones genistein and daidzein.[1,2] György *et al.*[3] reported also the presence of 6,7,4′-trihydroxyisoflavone in fermented soya beans, which, however, was shown to be a product of the fermentation process. In this communication evidence is presented for the occurrence of an additional isoflavone—glycitein—in soya beans, the structure of which has been determined.

RESULTS AND DISCUSSION

TLC showed that the acid hydrolysate of a soya extract contained genistein and daidzein and an additional flavonoid aglycone which was isolated by preparative TLC and crystallized from 90% MeOH giving a UV and NMR spectra typical of an isoflavone.

The addition of $AlCl_3$, $AlCl_3$–HCl or NaOAc–H_3BO_3 gave no shift in the UV spectrum showing ortho dihydroxyl groups were not present at 6,7 or 7,8 nor was there a free 5-hydroxyl group. The MS of the new isoflavone gave a molecular ion at *m/e* 284 ($C_{16}H_{12}O_5$), and after acetylation peaks at *m/e* 368, *m/e* 326 (M-42) and at *m/e* 284 (M-84) indicating the presence of two hydroxyl groups. Cleavage of the γ pyrone ring gave also peaks at *m/e* 166 and 118 indicating the presence of an OH and an OMe group in ring A and another OH group in ring B.[4] The presence of one methoxyl and two hydroxyl groups was shown also by the NMR spectra. The parent compound showed a signal at δ 4·00 for OCH_3, and the spectrum of the diacetate gave three proton singlets, one at δ 3·95 for the OCH_3 group and two at δ 2·37 and δ 2·32 for the two acetates. In view of the MS and the UV spectra it was assumed that the OCH_3 substituent is located at C6, one OH group at C4′ and by analogy to a great number of isoflavones, the second OH group is at C7, providing the glycosidic linkage to the sugar moiety.

This structure was confirmed by methylation and hydrolysis, when afrormosin was obtained, and its identity established by direct comparison in GLC and with a variety of solvents in TLC. Thus the new isoflavone, for which the name glycitein is proposed, is 7,4′-

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dihydroxy, 6-methoxyisoflavone. Since acid and enzymic hydrolysis gave only glucose, the glycoside of glycitein is a 7-*O*-β-glucoside. It should be pointed out that the 6,7,4′-oxygenation pattern was also assigned to texasin,[5] which, however, differs from glycitein by having the OMe group at C4′ rather than C6.

EXPERIMENTAL

M.ps are uncorrected. UV spectra (in MeOH) were measured on a Beckman DB-G spectrophotometer. IR spectra (in KBr) were recorded on a Perkin–Elmer Infracord Model 137 spectrophotometer. NMR spectra were determined in $CDCl_3$ with $SiMe_4$ as internal standard on a Varian A60 spectrometer. MS were recorded on an Atlas CH4 mass spectrometer. GLC was performed on a Packard 7200–7400 gas chromatograph equipped with a fid, using columns of 0·75% SE-30 on GCQ at an operating temperature of 220°.[6] R_fs are quoted relative to formononetin (R_{For}). Analytical and preparative TLC were carried out on Kieselgel G or HR (Merck) Plates, respectively, with the solvents (I)-$CHCl_3$–MeOH (9:1);[7] (II)-C_6H_6–EtOAc–light petrol. (60–80°)–MeOH (6:4:3:1);[8] (III)-ether–light petrol. (40–60°) (7:3).[8] Spots were visualized either in UV light (360 nm) or by spraying with the Folin–Ciocalteu reagent.[7] PC of sugars was carried out with the upper phase of C_6H_6–BuOH–pyridine–H_2O (1:5:3:3)[9] and spots were visualized with the $AgNO_3$–NaOH reagent.[10]

Extraction and isolation procedure. Finely ground soya bean flour (2 kg) (Wayne variety, 1970 crop) was exhaustively extracted with light petrol, and then with 60% EtOH. The EtOH extract was concentrated to a syrup, stirred with 2 vol. Me_2CO for 2 hr at room temp., filtered and the filtrate concentrated to a syrup, which was adsorbed on ~ 200 g Kieselgel G. The cake was extracted with Et_2O for 3 days with Me_2CO for another 3 days. The solvents were removed, the Me_2CO residue crystallized from 80% EtOH and washed with $CHCl_3$ to give a mixture of glycosides of genistein, daidzein and glycitein. Genistein which comprised the major part of the mixture was removed by chelating with Al^{3+}. The mixture of glycosides was adsorbed on aluminium oxide G (Merck), from which the glycosides of daidzein and glycitein were extracted with 50% MeOH. After evaporation of the solvent the glycosides were subjected to acid hydrolysis in 2N H_2SO_4 in 50% EtOH for 3 hr. PC showed the presence of glucose as the sole sugar component. The Et_2O extract of the hydrolysate gave a mixture of daidzein and glycitein, from which the latter was isolated by preparative TLC (multiple development, two runs) with solvent III. The following $R_{Genistein}$ values were found: solvent I: 0·90, II: 0·53, III: 0·32. Glycitein twice crystallized from 90% MeOH had m.p. 311–313°. Acetylation with pyridine–acetic anhydride gave diacetate, m.p. 212–214° (from EtOH), GLC of glycitein gave a peak at R_{For} 2·24.

Spectral analyses. UV: λ_{max} (MeOH) 256, 319 nm (log ε 4·35, 3·98); λ_{max} (NaOMe): 259, 344; λ_{max} ($AlCl_3$): 257, 317; λ_{max} ($AlCl_3$–HCl): 257, 317; λ_{max} (NaOAc): 255, 347; λ_{max} (NaOAc–H_3BO_3): 255, 320. IR (in KBr): ν_{max} 3395 (broad) –OH; 1608 (C=O pyrone) 1568, 1552, 1508 (C=C); 1270 (aromatic C–O). NMR in deuterated pyridine of glycitein showed δ 4·00 (OCH_3). NMR of acetylated glycitein (in $CDCl_3$) showed δ 8·02 singlet (2H), δ 7·78 singlet (5H), δ 7·62 doublet (2′H, 6′H) J = 8 Hz, δ 7·28 overlap due to the proton of the solvent, δ 7·18 doublet (3′H, 5′H) J = 8 Hz, δ 3·95 singlet (OCH_3), δ 2·37 and δ 2·32 singlets (two methylacetates). MS of parent compound: M^+ 284. After acetylation: M^+ 368, 326 (M-42) 296, 284 (M-84), 254, 166, 138, 118. The relative intensities of the peaks were 7·2, 8·3, 4·8, 29·0, 6·5, 2·2, 1·8 and 2·2% respectively.

Conversion of glycitein to afrormosin. After removal of genistin by chelation as described above, the mixture of glycosides was treated with CH_2N_2 in MeOH for 6 days. Acid hydrolysis of the products gave the methylated isoflavones formononetin (from daidzein) and afrormosin (from glycitein) identified by direct comparison by GLC and TLC with solvents I, II and III.

Enzymic hydrolysis. 5 mg of a mixture of isoflavone glycosides were digested with 1 mg almond β-glucosidase (Sigma Chemicals Co.) at pH 5·0, 37° for 4 hr. The reaction products were identified chromatographically.

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