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Note

Separation of the isomeric isoflavones from soybeans by high-performance liquid chromatography

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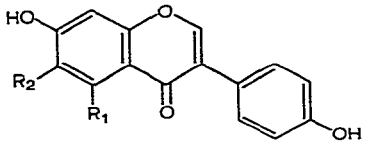
Soybeans and food products prepared from soybeans often contain isoflavone glycosides[1,2]. These isoflavones are particularly interesting due to their diverse pharmacological and antioxidant properties[1,3,4]. Genistein (I), 4′,5,7-trihydroxyisoflavone, is the principal isoflavone aglycone of soybeans and accounts for almost 90% of the total isoflavone concentration[3]. Several investigators have reported the isolation of non-glycosidically linked 4′,6,7-trihydroxyisoflavone (II) from fermented soybean preparations[5,6], but the suggestion of its natural occurrence in raw soybeans has been questioned[3].



I: R_1 = OH
R_2 = H
II: R_1 = H
R_2 = OH

The quantitation of genistein in soybeans employing gas–liquid chromatography (GLC) after the preparation of the trimethylsilyl derivative has been developed by Naim *et al.*[3], but no reports dealing with a system involving high-performance liquid chromatography (HPLC) have as yet been published. However, HPLC has been successfully employed to study natural compounds structurally related to isoflavones, such as flavonoids[7] and xanthones[8].

We were mainly interested in developing a system which would separate the isomeric trihydroxyisoflavones of soybeans and one which could be used for their quantitation in different soybean varieties and fermented soybean preparations.

EXPERIMENTAL

Authentic samples of genistein (K. & K. Labs., Plainview, N.Y., U.S.A.) and

4',6,7-trihydroxyisoflavone (Pfaltz & Bauer, Stamford, Conn., U.S.A.) were obtained and dissolved in chloroform. Soybean samples were initially defatted with petroleum ether before extracting the isoflavone glycosides with methanol. The aglycones were released after hydrolysis according to reported procedures and extracted into chloroform[9]. Samples for the detection of non-glycosidically linked isoflavones were prepared by extracting defatted soybeans with chloroform.

Separations were carried out with a mobile phase of water–acetonitrile (4:1) at a rate of 1.8 ml/min employing a Constametric II (Laboratory Data Control, Riviera Beach, Fla., U.S.A.) liquid chromatograph. The column was 25 cm × 4.6 mm I.D. stainless steel and packed with Partisil-10 ODS (Whatman, Clifton, N.J., U.S.A.). Detection was made by ultraviolet (UV) absorption employing a UV detector (Varian Vari-Chrom) at a wavelength of 260 nm.

RESULTS AND DISCUSSION

The isomeric trihydroxyisoflavones of soybeans can be separated satisfactorily with high-performance liquid chromatography as illustrated in Fig. 1. The procedure proved useful for the detection of genistein in raw soybeans and could easily be extended for use in quantitating this pharmacologically active compound in different varieties of soybeans and the many products prepared from soybeans.

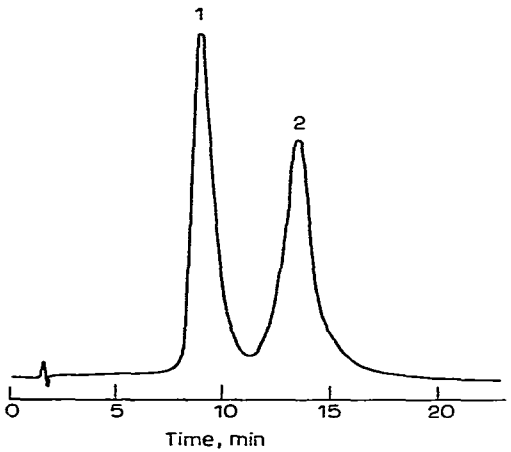


Fig. 1. Separation of 4',6,7-trihydroxyisoflavone (1) and genistein (2).

In no raw soybean extract were we able to detect the presence of 4',6,7-trihydroxyisoflavone. It is possible that this isoflavone is not a natural constituent of soybeans as has been previously suggested.

The system also substantiated the presence of only trace quantities of non-glycosidically linked genistein in the chloroform soybean extracts[3].

ACKNOWLEDGEMENT

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