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Note

Separation of isomeric glycoflavones by high-pressure liquid chromatography

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A large variety of flavonoid glycosides have been encountered in nature[1] but only a few papers dealing with their separation by high-pressure liquid chromatography have as yet been published. Wulf and Nagel[2] separated some aglycones, monosides and biosides on chemically bonded silica gel (μBondapak/C_{18}) using water–methanol–acetic acid (65:30:5) as eluent. A micro-C_{18} column coupled with water–acetonitrile (80:20) as eluent has been used for the resolution and quantitation of naringenin rutinoside and naringin in grapefruit juice[3].

We were mainly interested in separating the O-glycosides of glycoflavones which differ only in the position of the hydrolysable sugar on the flavone skeleton and which are only poorly separated by other chromatographic methods.

EXPERIMENTAL

The naturally occurring glycoside samples were available from our previous work from extracts of *Gentiana*[4,5]. Separations were carried out on saturated methanol solutions employing a Hewlett-Packard Model 1080 liquid chromatograph with gradient capability. The columns used were of stainless steel, 25 cm × 4.0 mm I.D., and were slurry packed with microporous chemically bonded silica gel (LiChrosorb RP8 of particle size 5 μm and LiChrosorb NH_2 of particle size 10 μm, from Merck, Darmstadt, G.F.R.). Detection was made by UV absorption at 254 nm.

RESULTS AND DISCUSSION

Whereas various flavone O- and C-glycosides were separated on LiChrosorb C_8 using different mixtures of acetonitrile–water[6], the more polar isoorientin derivatives, isoorientin-3′-O-glucoside (I) and isoorientin-4′-O-glucoside (II) could not be resolved.

Since LiChrosorb NH_2 had been used for the separation of monosaccharides[7], we tried this packing material for the separation of the isomers I and II. By using a gradient starting with acetonitrile–water (1:9) and switching to acetonitrile–water

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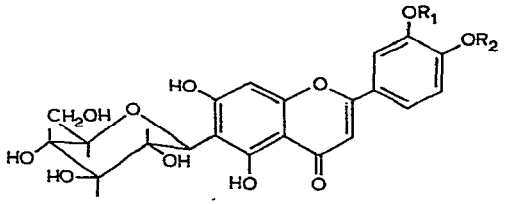

I: R_1 = β-D-glucosyl
R_2 = H
II: R_1 = H
R_2 = β-D-glucosyl

(9:1) in 15 min, a satisfactory separation of the isomers was achieved as shown in Fig. 1. The NH_2-chemically bonded silica gel seems to be an ideal phase for separation of both polyphenolic aglycones and glycosides. Recently, we reported its use for the separation of polar aglycones, whereas less polar aglycones are better resolved on a more polar phase such as CN-chemically bonded silica gel[8].

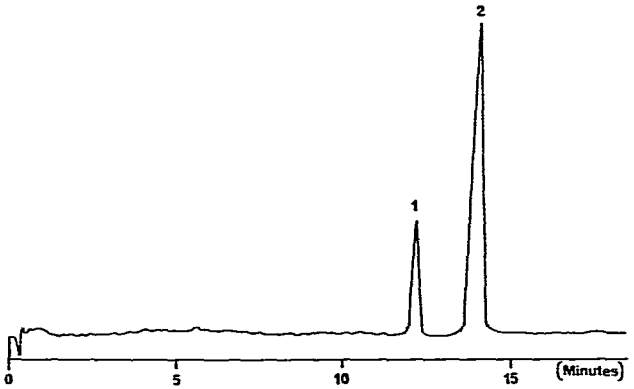

Fig. 1. Separation of isoorientin-3'-O-glucoside (1) and isoorientin-4'-O-glucoside (2). Column, 25 cm × 4 mm I.D. LiChrosorb NH_2 (particle size 10 μm); gradient starting with acetonitrile–water (1:9) and switching to acetonitrile–water (9:1) in 15 min; flow-rate, 2 ml/min; sample volume, 2.5 μl; detection, UV absorbance at 254 nm.

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