Chemistry of Natural Compounds, Vol. 31, No. 5, 1995

USE OF SPHERON FOR SEPARATING FLAVONOIDS

V. S. Nikitina, O. É. Orazov, and G. V. Shendel'

UDC 615.322.547.814.5

With the aim of the structural-group separation of flavonoids and their glycosides, we have tested the sorption capacity of Spheron 40 (LC) — a copolymer of 2-hydroxyethyl methacrylate with ethylene dimethacrylate produced by Lachema, Brno, Czechoslovakia. We have studied concentrates of the polyphenolic compounds obtained from the leaves of two species of willow: *Salix triandra* L., and *S. acutifolia* L. (fam. Salicaceae). The objects of investigation were chosen in view of the promising nature of their practical use as a raw material for obtaining a number of biologically active substances: rutin, quercetin, kaempferol, luteolin, luteolin 7-O-glucoside, isorhamnetin, and tannins [1-4]. As standard substances we used quercetin and its 3-O-rhamnoglucoside (rutin). A chromatogram of a mixture of them is given in Fig. 1.

The plant mixtures of flavonoids were separated into hydrophilic fractions of glycosides and hydrophobic fractions of aglycons with purification both from low-molecular-mass and condensed phenolic compounds (Fig. 2). The first peak on the chromatogram corresponds to water-soluble low-molecular-mass impurities and amounts to 5% of the weight of sample added to the column. Then the polyphenols were eluted, the first fraction of them consisting of biocides with two sugar residues in the molecule and dimeric biflavonoids. These were represented by the second peak, including 30-35% by weight of the sample. The third peak corresponded to a fraction of flavonoid monosaccharides and amounted to about 40% by weight of the sample being analyzed. Aglycons were eluted with 70-90% ethanol and accounted for about 15% by weight — the fourth peak. The condensed polyphenols were eluted in the form of diffuse peaks. By a 20-25% increase in the rate of feed of solvent and a change in the ethanol gradient it was possible to achieve a greater compression of the peaks with no deterioration in the chromatographic separation.

The use of precolumn purification of the fractions by selective extraction successively with ether, ethyl acetate, and butanol enabled us to obtain separate chromatographic profiles of the aglycons, monoxides, and biocides (Fig. 3). As can be seen from the chromatograms, diethyl ether and ethyl, acetate as selective extractants did not enable sufficiently pure aglycon and monoxide fractions to be obtained, but their selectivity could be enhanced by optimizing the extraction conditions. The ether and ethyl acetate extracts contained no low-molecular-mass impurities, which is of interest in a more detailed study of the structural composition of the samples.

Air-dry leaves from branches of the same age gathered simultaneously at the end of the vegetation period were investigated. The isolation and purification of the flavonoid concentrates were carried out by known extraction methods [5]. The flavonoids from the fractions were subjected to additional purification successively with diethyl ether, ethyl acetate, and

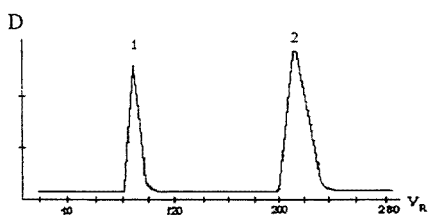

Fig. 1. Separation of an artificial mixture of rutin (*1*) and quercetin (*2*).

Institute of Biology, Ufa Scientific Center, Russian Academy of Sciences, Ufa, fax (3472) 35 61 03. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 755-756, September-October, 1995. Original article submitted September 6, 1994.

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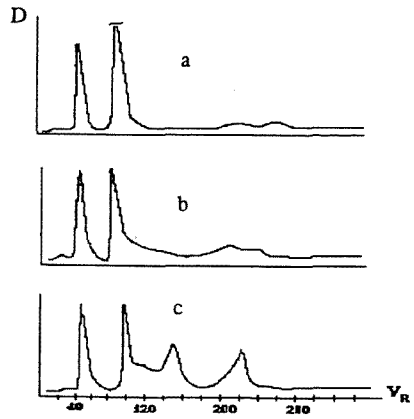

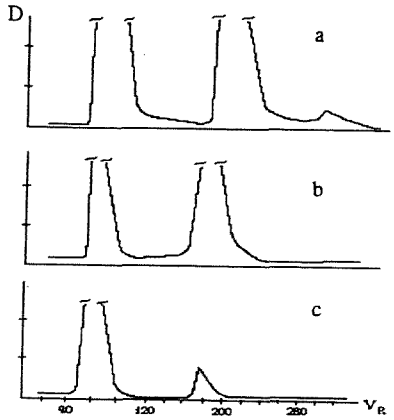

Fig. 2

Fig. 3

Fig. 2. Chromatic profiles of flavonoid preparations from the leaves of *Salix triandra* L., male plants (*a*) and female plants (*b*); and of *Salix acutifolia* L., female plants (*c*).

Fig. 3. Chromatograms of fractions of aglycons (*a*), monoxides (*b*), and biocides (*c*) after extractive purification by solvents from the leaves of *Salix triandra* L., form *concolor*.

n-butanol. The CC of the mixtures of flavonoids was carried out on a laboratory apparatus in the semipreparative variant. The apparatus consisted of a glass column (8 × 1270 mm) with valves, peristaltic pump for feeding the solvent, a system of linking tubes with cocks, a manometer, and an FCC 61 automatic fraction collector (Laboratorni Pristroje, Prague). Chromatography was conducted in a gradient elution system consisting of aqueous alcoholic mixtures with an increasing concentration of ethanol throughout chromatography. Detection was effected on an SF-26 (LOMO) spectrophotometer at λ = 254 nm.

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