Chemistry of Natural Compounds, Vol. 31, No. 6, 1995

POLYMERIC ADSORBENTS OF THE AFFINITY TYPE IN THE INVESTIGATION OF PHYSIOLOGICALLY ACTIVE SUBSTANCES. VIII. INVESTIGATION OF THE CHROMATOGRAPHIC SEPARATION OF THE TOTAL PHENOLIC COMPOUNDS OF *Campanula rapunculoides*

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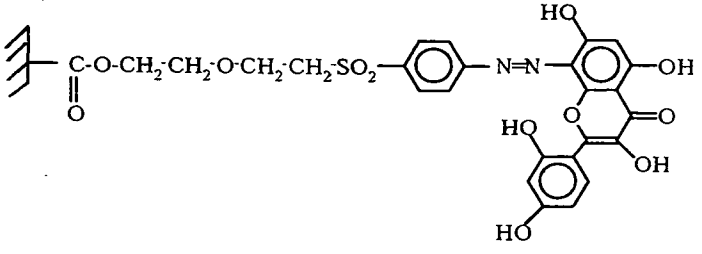
UDC 577.15.07+543.544+547.814

The possibility of separating the components of the polyphenolic compounds of creeping bellflower Campanula rapunculoides *L. on the adsorbent morin-azophenylsulfonylethoxyethyl-Spheron by the method of nonclassical low-pressure affinity chromatography has been studied. It has been shown to be feasible to separate phenolcarboxylic acids and flavone glycosides and aglycons.*

At the present time investigations in the field of nonclassical affinity chromatography (NAFC) on adsorbents of the affinity type (AAFTs) for developing new methods of separating, purifying, and analyzing plant biochemically active substances (pBASs) are recognized as one of the urgent tasks of pharmacy at the modern stage [1] that is being successfully solved by domestic scientists [2-5].

We have previously shown the effective application of AAFTs for the separation of flavonoid glycosides (FLs) — glycosides of herbacetin, quercetin, and luteolin, and their analogs — from the aglycons of these FLs [6, 7], the possibility of isolating trace amounts of impurity FLs in combined preparations [2, 8, 9], and also the separation of structural isomers of the drug troksevazin [2].

Azo adsorbents, obtained by the epoxy activation method, with a number of immobilized ligands [resorcinol (RC), catechin (CC), morin (MRN)] on supports of the agarose (AG) or Toyopearl (TP) type [2, 6] have recommended themselves quite well for these purposes. Nevertheless, according to the results of a more detailed screening of these AAFTs in a study of the separation of FL glycosides [7] the best separating capacity is possessed by MRN-azophenylsulfonylethoxyethyl-Spheron (MRN-SP), the structural formula of which is given below:



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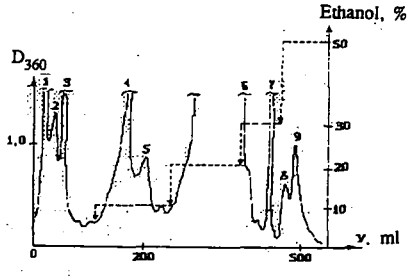

Fig. 1. Chromatograms of the separation of the polyphenolic compounds of creeping bellflower (for the conditions of separation, see text). Peaks: 1-3 and 7) phenolcarboxylic acids [1 and 7) unidentified; 2) CGA; 3) NCGA]; 4) quercetin 3-dirhamnoglucoside; 5) three FL biosides (one of them being RUT); 6) HPS contaminated with an unidentified bioside; 8 and 9) unidentified (FL aglycons).

In our opinion, the immobilization of the ligand (MRN) takes place at position 8 of the molecule, which is the most active in reactions with electrophiles. As a rule, the formation of polymeric bis-azo derivatives, either by cross-linking or by a process of the "crownization" type [3, 10] under the conditions for performing the reaction given in [9] (large excess of the ligand) is excluded. It is true that according to [11] a small percentage of such structures may apparently be formed as a "polymeric" impurity, but its influence on the chromatographic process has scarcely been investigated.

In the present paper we give the results of the chromatographic separation on MRN-SP of the total polyphenols of creeping bellflower isolated by a known method [12]. The choice of the object of investigation is connected with the chemotaxonomic aspects of the study of the phenolic compounds of the Campanulaceae family that is being conducted in the Department of Phamacognosy of the St. Petersburg Institute of Pharmaceutical Chemistry (head of the department Prof. G. P. Yakovlev).

The experimental results obtained (Fig. 1) permit the conclusion that we have convincingly shown the basic possibility of the effective use of polymeric AAFTs — specifically MRN-SP — under the conditions of low-pressure NAFC for the micropreparative separation and isolation of individual pBASs from a complex native natural mixture. Attention is attracted by the confirmation of a phenomenon that we have observed previously — the separation of FL tri(bi)osides from monosides and the aglycons. The latter are capable of being concentrated and are readily eluted with 50% aqueous alcohol.

The successful separation of a number of model mixtures of phenolcarboxylic acids on polymeric AAFTs described in [11] agrees well with the results of our work on the separation of chlorogenic and neochlorogenic acids (CGA and NCGA) from the total polyphenolic compounds. As was assumed, the most polar phenolcarboxylic acids issued from the column in the first three elution peaks (see Fig. 1), well separated from FL glycosides. It has not yet been possible to establish the nature of the acid of peak 7, but it must apparently possess a high hydrophobicity.

It is interesting that for the analysis of the total free phenolic acids and aldehydes under the conditions of high-performance liquid chromatography on the adsorbent Viospher C_6 (5 μm) Galletti et al. [13] used among their eluents the toxic methanol, and detection was performed electrochemically.

Thus, the use of polymeric AAFTs for polyphenol-ligand chromatography in the field of particularly pure substances [2, 3] will permit a substantial simplification of the technique of phytochemical analysis, opening up broad prospects for introducing new, alternative to classical, methods for the separation, isolation, and purification of natural compounds.

EXPERIMENTAL

The sample under study was separated on a glass column (0.8 × 30 cm) filled with 5 ml (packed volume) of the gel MRN-SP. The following mobile phases were used successively: distilled water, and a stepwise gradient (5, 10, 20, 30, and 50%) of aqueous ethanol. The volume of the eluted fractions was 4 ml and the rate of chromatography about 20-40 ml/h. De-

tection was carried out at a wavelength of 360 nm on a SF-26 instrument (CIS). The chromatographic column was charged with 1 ml of a solution in 2 ml of water of the evaporated residue of a combined extract of the polyphenolic compounds of creeping bellflower.

The course of the chromatographic separation was followed with the aid of paper chromatography in the systems: 15% acetic acid, and *n*-butanol–acetic acid–water (4:1:2; BAW), with the use of UV spectrophotometry where necessary. The peak fractions were taken for chromatographic analysis. As markers (15% acetic acid system) we used chlorogenic acid (CGA) (R_f 0.61), neochlorogenic acid (NCGA) (R_f 0.58), rutin (RUT) (R_f 0.66), and hyperoside (HPS) (R_f 0.37). The paper chromatograms were revealed by the usual methods.

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