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ISOLATION OF OXYGEN-CONTAINING MONOTERPENOIDS OF ESSENTIAL OILS BY PREPARATIVE ADSORPTION CHROMATOGRAPHY WITH GRADIENT ELUTION

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UDC 665.52:543.544

A simple and effective procedure for the comparative chromatography of 1-2 ml of essential oils on a 40 × 250 mm column of silica gel with gradient elution by hexane–diethyl ether has been developed. A simple system of preparing the mobile phase permits the creation of a continuous gradient during chromatography. The efficacy of the procedure has been shown taking as an example the isolation of fenchone from fennel essential oil.

Oxygen-containing monoterpenoids possess a wide spectrum of action and a higher biological activity and lower toxicity than monoterpene hydrocarbons and sesquiterpenoids [1]. The isolation of these substances from essential oils (EOs) in fairly large amounts (more than 0.1 g) is frequently necessary for their identification (by the methods of UV, IR, NMR, and mass spectrometry), the study of their biological activity, and their use as comparative substances for the identification and the quantitative determination of the components of EOs by chromatographic methods.

Our aim was the development of an effective procedure for the preparative chromatographic separation of oxygen-containing monoterpenoids of EOs that would permit the isolation of a substance with a purity of not less than 95% in amounts (more than 0.1 g) sufficient for use as comparison substances for GLC analysis.

The simplest and most effective method of fractionating terpenoids is their adsorption chromatography on silica gel [2]. In the separation on a silica gel column of a mixture of substances with close polarities, such as monoterpene hydrocarbons [3], isocratic elution with pentane is used. The collection of a number of small fractions leads to the separation or enrichment of certain substances in different fractions. To separate mixtures of substances differing appreciably in polarity, the method of gradient elution is used [4], this consisting in the fact that during chromatography the eluting power of the mobile phase is gradually increased. This is achieved by the successive use of a number of solvents ("eluotropic series") or mixtures of two solvents with gradual increase in the proportion of the more polar component. A continuous gradient changes the values of the partition coefficients of substances between the mobile and stationary phases. The increasing eluting power of the mobile phase compresses the band of the sample, as the result of which the peaks become narrower and the "tail" of a peak decreases even with large loads on the column.

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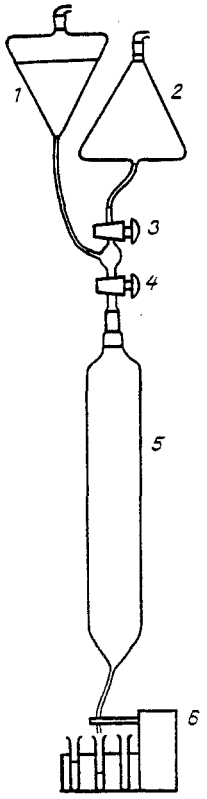

Fig. 1

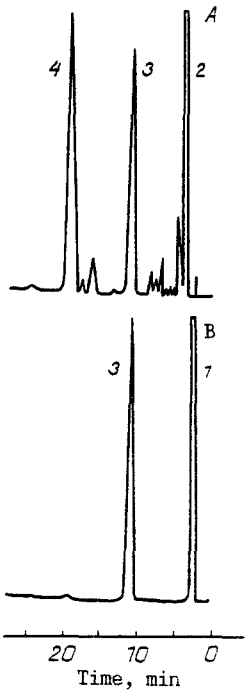

Fig. 2

Fig. 1. Apparatus for the preparative chromatography of essential oils: 1) flask containing hexane; 2) flask containing a 50% solution of diethyl ether in hexane; 3, 4) mixer cocks; 5) glass column containing silica gel; 6) fraction collector.

Fig. 2. Chromatograms of fennel essential oils (A) and of a fenchone-containing fraction isolated from it (B); 1, 2) solvents; 3) fenchone; 4) anethole.

Gradient elution can be used for shortening the time of chromatography and raising the efficiency of separation. But on passing from analytical to preparative chromatography problems arise in the creation of devices that will reproduce complex gradients on working with large volumes of mobile phase [4]. In order to eliminate this complication, so-called "stepwise" gradient elution is frequently used, with some loss of efficiency. Thus, the separation of a model mixture of oxygen-containing monoterpenoids [5] was carried out with the successive use as the mobile phase of 2.5, 5, 10, 25, and 50% solutions of diethyl ether in pentane. This procedure permitted the mixture to be separated into individual groups of substances. Esters were eluted first, and then aldehydes, ketones, and, lastly, alcohols.

We have developed a simple system for preparing a mobile phase (Fig. 1) which permits a continuous gradient to be obtained with good reproducibility during chromatography. The system consists of two 500-ml conical flasks connected by Teflon tubes (diameter 2.5 mm) with a mixer. The flask with the narrow end downward contains hexane during chromatography, and the flask with the narrow end upward a mixture (1:1) of hexane and diethyl ether. During working, the same level of liquid is established and automatically maintained in the two flasks. A gradually decreasing amount of hexane and a gradually increasing amount of the mixture of hexane and ether pass into the mixer. In this way a constant smooth increase in the polarity of the mobile phase is created.

The working diameter of the column (40 mm) was calculated so that the height of a layer of EO with a volume of 2 ml did not exceed 1 mm. In order to bring the "dead" volume to a minimum, the top and bottom of the column were made conical (the top with a diameter of 15 mm and the bottom with a diameter of 1.5 mm). The collection of small fractions of eluate (with a volume of 15 ml) enabled the secondary mixing of the separated components to be avoided.

Because of the factor mentioned, it was possible in a single chromatographic run to isolate with high purity a number of oxygen-containing monoterpenoids from various EOs. For example, Fig. 2 shows gas–liquid chromatograms of the EO of fennel Foeniculum vulgare Mill. remaining after the freezing out of anethole and of a fraction of eluate with the isolated fenchone. If it is considered that anethole and fenchone have close retention times on a silica gel column [5] and the peak of fenchone appears on the "tail" of the anethole peak, this example well illustrates the efficiency of the procedure developed.

The four fractions containing fenchone were combined. After the solvent had been separated off completely in a current of air, the isolated fenchone had a purity of 98.2% (GLC, normalization method).

The sequence of elution of monoterpenoids when the procedure developed was used agreed with that given by Scheffer et al. [5].

The proposed procedure can be used: 1) for the isolation of individual oxygen-containing monoterpenoids from EOs on single or multiple chromatography; 2) for the fractionation of EOs with the aim of their subsequent GLC analysis; and 3) to obtain valuable information on the nature of the functional groups of the components of an EO from the sequence of their elution from the column.

EXPERIMENTAL

Preparation of the Sorbent and Filling the Column. Preparation was carried out on silica gel L 40/100 (for chromatography, not more than 10% of moisture, Lachema/Chemapol, Czechoslovakia). It is generally accepted [4] that the "active sites" upon which adsorption takes place are the silanol (–SiOH) groups of the silica gel surface. Some closely located silanol groups form hydrogen bonds with one another. The addition of small amounts of water to the silica gel makes its surface more homogeneous through the formation of hydrogen bonds between the water molecules and the free silanol groups. This permits the conditions of equilibration of the column to be simplified, reproducibility to be improved, irreversible sorption to be increased, the capacity for the sample to be increased, and regeneration to be improved.

Silica gel was treated in a beaker with a double volume of hexane (H), and, with good stirring, the suspension was poured into the column set vertically in the end of which a small piece of glass cloth had been placed while the eluate discharged tube was closed by means of a pinchcock. After the particles of silica gel had settled, the pinchcock was opened and hexane was run out from the column until its level was 1 cm above the surface of the silica gel. Then the procedure was repeated until the column was filled with silica gel to a level of 1-2 cm below the ground joint.

Chromatographic Procedure. With cocks 3 and 4 (Fig. 1) closed, 300 ml of hexane was introduced into flask 1 and 500 ml of a 50% solution of medicinal diethyl ether in hexane into flask 2. The level of hexane in flask 1 was made 1-1.5 cm higher than that of the solution in flask 2. On the surface of the silica gel was carefully placed 1-2 ml of EO, and then the pinchcock was opened so that the EO passed completely into the layer of silica gel, and the eluate outlet was again closed. The column was filled completely with hexane, the mixer was attached to the column, and cocks 3 and 4 were opened successively. The same level of solvents was then established in the two flasks. The pinchcock on the bottom tube was opened and it was attached to a LCC-60 fraction collector (Laboratorni pristroje, Czechoslovakia). The rate of elution was set at 2 ml/min, and the collector was set for the conditions of collecting 15-ml fractions. Chromatography was continued for ~7 h.

Each of the fractions collected was evaporated in a current of air to a volume of 1 ml. The compositions of the fractions were checked with the aid of GLC on a LKhM-80 instrument (6-th model) with a flame-ionization detector. GLC was conducted on a 3 mm × 3 m column containing 5% Superox 20M on Chromosorb W-HW (100-120 mesh) (Alltech Associates, USA) with programming of the temperature from 100 to 190°C at the rate of 3°C/min. The detector and evaporator were thermostated at 220 and 190°C [sic], respectively. The rates of flow of the carrier gas (helium), hydrogen, and air were 25, 25, and 250 ml/min, respectively.

Regeneration of the Column. Regeneration after the end of chromatography was carried out with hexane, 200 ml of which was sufficient to eliminate the diethyl ether from the column. On storage, bubbles of gas may form in the filled column through the evaporation of

the hexane, which lowers the efficiency of separation. After a long interruption, it may therefore be recommended to fill the column anew as described above.

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TERPENOIDS OF Crepis tectorum. MOLECULAR AND CRYSTAL STRUCTURE OF THE SESQUITERPENE LACTONE 8-EPIDEACYLCYNAROPICRIN

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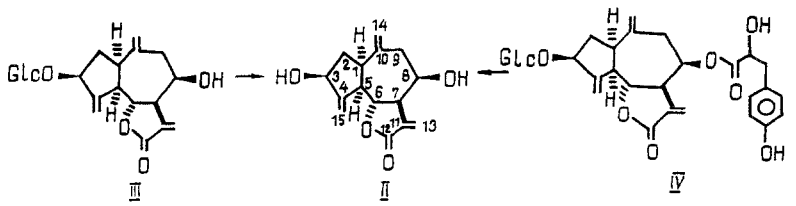
UDC 547.314:548.737

Sesquiterpene lactones of the guaianolide type – isolipidiol (I) and 8-epideacylcynaropicrin (II) – have been isolated from narrow-leaf hawk's-beard, Crepis tectorum L. On the basis of the results of an x-ray experiment the structure of (1R,3S,5R,6R,7R,8R)-3,8-dihydroxy-cis,trans-guaia-4(15),10(14),-11(13)-trien-6,12-olide is proposed for (II).

By extraction with chloroform followed by chromatography of the total material obtained on a column of KSK silica gel, we have isolated two crystalline substances (I) and (II) from the epigeal part of Crepis tectorum (narrow-leaf hawk's-beard) gathered in the flowering phase in the environs of the village of Egindybulak, Karaganda province, Kazakh SSR. From their IR and PMR spectra a comparison with literature information they have been identified as the guaianolides isolipidiol [1] and 8-epideacylcynaropicrin [2], respectively. This is the first time that isolipidiol and 8-epideacylcynaropicrin have been isolated from narrow-leaf hawk's-beard.

The isolation from the roots of narrow-leaf hawk's-beard of the 3β-glucoside of 8β-hydroxy-1α,5α,7α(H)-guaia-4(15),10(14),11(13)-trien-6,12-olides (III) and of tectoroside – the 3-(4-hydroxyphenyl)lactate of the glucoside of 8-epideacylcynaropicrin (IV) has been described previously [3, 4]. One must assume the possibility of the formation of (II) in the plant organism on the enzymatic hydrolysis of (III) or (IV) by the following scheme:



With the aim of elucidating the spatial structure and the configurations of the asymmetric centers of compound (II), we have made an x-ray structural investigation of its mole-

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Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 638-642, September-October, 1991. Original article submitted May 15, 1990; revision submitted March 12, 1991.

0009-3130/91/2705-0562$12.50