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

S. V. Sur

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 ("elutotropic 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.
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.