GAS-CHROMATOGRAPHIC DETERMINATION OF THE RATIO OF VISNADIN AND DIHYDROSAMIDIN IN MIXTURES OF THEM

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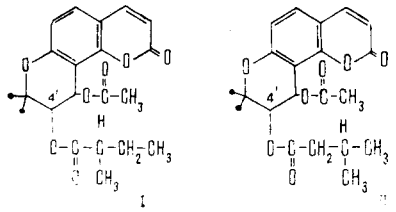
Visnadin and dihydrosamidin – diacyldihydropyranocoumarins from the khellactone group – are the active substances of some spasmolytic preparations: vibeline (France) [1], carduben (GFR) [2], and visnadin and dimidin (USSR) [3, 4]. These substances are obtained from plant raw material which simultaneously contains visnadin and dihydrosamidin (Ammi visnaga and Phlojodicarpus sibiricus [5, 6]). The only difference of visnadin (I) from dihydrosamidin (II) is in the structure of the 4'-acyl group, namely: the first is acylated by α-methylbutyric and the second by β-methylbutyric (isovaleric) acids. Such closeness in the structure makes the chemical and physicochemical properties of these substances so similar that if they are present simultaneously in the initial raw material it is practically impossible to separate them completely either by repeated recrystallization or by chromatography. In the manufacture of the preparations, it is very important to determine the ratio of visnadin and dihydrosamidin in their mixtures and medicinal forms.



I

The method of determination using a PMR spectrometer [7] requires complex apparatus and cannot be used under industrial conditions. There has been no other, more convenient method for their separate determination. However, if the ratio between the 4'-acyl groups is determined this will correspond to the ratio of the substance themselves.

We have developed a method for determining the ratio of visnadin and dihydrosamidin in mixtures of them which consists in first hydrolyzing the mixture of substances with alcoholic alkali, then acidifying the hydrolyzate to convert the salts formed from the acyl groups and the free acids, extracting the latter with ether, and injecting part of the ethereal extract into a gas chromatograph. The calculation of the ratio of visnadin and dihydrosamidin in the mixture can be performed in the usual way from the areas of the corresponding peaks, taking the sum of the areas as 100%. To separate the α- and β-methylbutyric acids, which have close boiling points (177.0 and 176.7°C [8]) we used behenic acid [9] deposited on an inert support. The partition coefficient of these acids depends on the temperature (K at 120, 115, 110, and 102°C = 0.92, 1.06, 1.11, and 1.27, respectively). Figure 1 shows the results of the separation of an artificial mixture of the isomeric acids from C_2 to C_5, inclusive at 110°C and K = 1.11 ("Ch" ["pure"] isovaleric acid gives two peaks). However, in this method of separation the peaks of the α- and β-methylbutyric acids partially overlap and it is difficult to measure the half-width of the peaks accurately; consequently, to calculate the ratio we used the method of determining the product of the height of the peak by the retention time of the component, which is equivalent to the calculation of areas [10]. Figure 2 gives typical chromatograms: a) a mixture enriched with visnadin; b) the sum of the visnadin and dihydrosamidin obtained from Phlojodicarpus sibiricus; and c) "Ch" isovaleric acid (which corresponds to a mixture enriched with dihydrosamidin); the method of calculation from chromatogram b is also given.

The first component that issued from the chromatogram is β-methylbutyric acid (peak D – dihydrosamidin) and the second is α-methylbutyric acid (peak V – visnadin). The height of the peak corresponding

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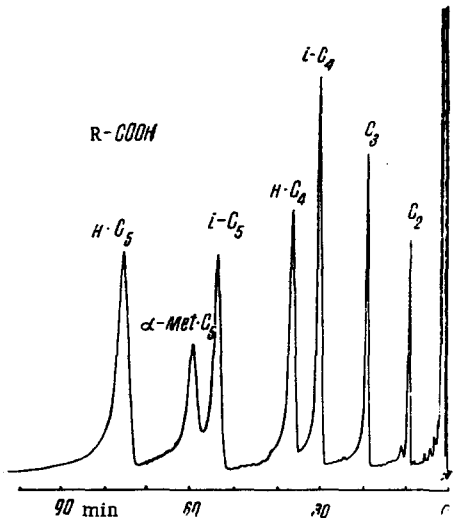

Fig. 1. Chromatogram of a synthetic mixture of acetic, propionic, isobutyric, butyric, "Ch" isovaleric, and valeric acids (in molar ratio).

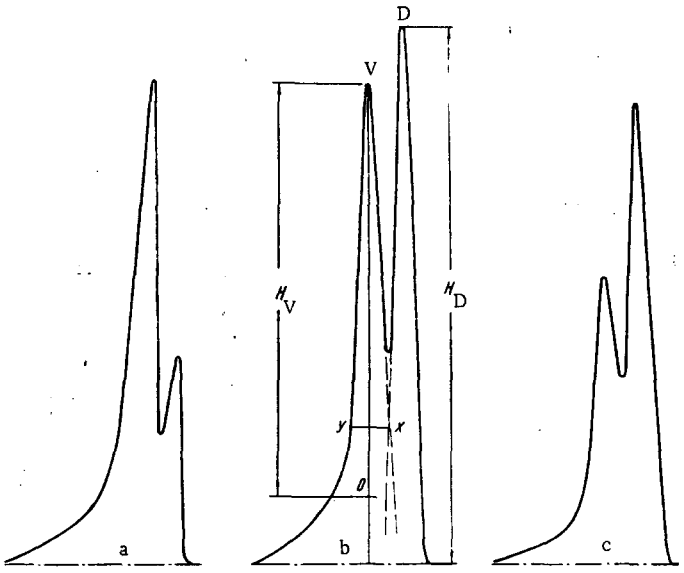

Fig. 2. Typical chromatograms of various mixtures of visnadin and dihydrosamidin.

to dihydrosamidin (peak D) is equal to the difference between the maximum of peak D and the zero line H_D. To determine the height corresponding to visnadin (H_V) the "tail" of the preceding peak D must be excluded from peak V. For this purpose, the point of intersection (X) between the tangents drawn through the points of inflection of the rear line of peak D and the line of the front of peak V is found (Fig. 2b). From point X a straight line is drawn parallel to the zero line to intersect the rear line of peak V (point Y). Then the segment XY obtained is moved parallel to itself along the rear line of peak V until the end X coincides with the perpendicular drawn from the point of the maximum of peak V (point O). The value of the intercept OV is taken as the height of peak V (H_V).

The calculation is performed from the formula

$$\%_V = \frac{H_V \times T_V \times 100}{H_V \times T_V + H_D \times T_D}, \qquad \%_D = \frac{H_D \times T_D \times 100}{H_V \times T_V + H_D \times T_D},$$

where $\%_V$ and $\%_D$ are the amounts of visnadin and dihydrosamidin; H_V and H_D are the heights of the peaks; and T_V and T_D are the retention times of the acids corresponding to visnadin and dihydrosamidin.

The error of the determination does not exceed 10% relative.

Determinations of the ratio of visnadin and dihydrosamidin in one and the sample of natural mixture enriched with visnadin by this method (69 and 31%, see Fig. 2a) and by the PMR method (67 and 33%) agree satisfactorily. The composition of the mixture of visnadin and dihydrosamidin from P. sibiricus (Fig. 2b) is 46 and 54%, respectively.

The "Ch" isovaleric acid contained 36.5% of α-methylbutyric acid (Fig. 2c). According to our results, this method can also be used successfully to evaluate the quality of the raw material used for the production of the preparation and for the search and selection of plants having a high content of visnadin or dihydrosamidin.

EXPERIMENTAL

Method of Analysis. A 10-ml flask with a ground-in stopper was charged with 15-20 mg of the mixture, and then 0.5 ml of a 5% solution of KOH in methanol was added and the flask was heated on the water bath with a reflux condenser for 30 min and cooled, after which 1 ml of water, 1 ml of diethyl ether, and, in drops, 0.2 ml of concentrated HCl were added. The flask was closed, shaken vigorously, and left for the mixture to separate. About 20 μl of the ethereal layer was injected by a syringe into a chromatograph (Khrom-2). The stainless-steel column, 3 m × 4 mm (internal diameter), was filled with 20% of behenic acid and 0.4% of H_3PO_4 on Chromaton NAW HMDS, 0.16-0.20 mm. The rate of flow of the carrier gas, nitrogen was 31 ml/min, that of hydrogen 55 ml/min, and that of air 600 ml/min, the temperature of the evaporator and of the column was 110°C, and the rate of movement of the paper strip of the recorder 2 mm/min; the time of analysis was about 75 min.

SUMMARY

A gas-chromatographic method for determining the ratio of visnadin and dihydrosamidin in mixtures of them has been developed.

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