# THE GAS-LIQUID CHROMATOGRAPHY OF SOME NATURAL COUMARINS AND COUMARIN FRACTIONS FROM PLANTS

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In recent years, several papers have been devoted to the gas-liquid chromatography (GLC) of the coumarins [1-4]. Based on a study of a series of substances, some characteristic features have been noted for the behavior of coumarin derivatives during their separation which are a function of the molecular structure, and of the number and position of the substituents.

This paper gives the results of the GLC of 32 representatives of natural coumarins, and also coumarin fractions from the roots of Prangos pabularia Lindl, and from the roots and fruit of P. hissarica Korov. [5]. For separating the coumarins, of the various conditions for performing GLC (temperature, programming, etc.) we decided on a packed column with the stationary phase SE-30 at a column temperature of 200° C.

The majority of the coumarins used for the investigation of their behavior on GLC were well separated under the conditions mentioned. We calculated the relative retention times (RRT) of pure samples using osthole as a standard, this being the most widely distributed compound in the plants of the family Umbelliferae that we studied (Table 1). The table gives the RRT values that we obtained for known coumarins, for only nine of which was there previous information on their GLC behavior. These compounds were osthole, scoparone, angelicin, psoralen, bergapten, xanthotoxin, pimpinellin, and isopimpinellin, but with RRT values differing from ours, since GLC was carried out under different conditions [1, 2]. Our results are in good agreement with literature data [1, 2, 4] and permit several additional conclusions to be drawn concerning the behavior of the coumarins on GLC separation. In GLC separation, a clear interrelationship is observed between the retention times and the structures of the coumarins: the RRT depend on the number of ether and other side chains and their position in the coumarin molecule, and change according to the length or angular structure of the furocoumarin or pyranocoumarin skeleton. A coumarin having no ether groups or side chains has the shortest retention time.

An increase in the number of methoxy groups leads to an increase in the retention time. For example, the RRT of the monomethoxycoumarins xanthotoxin and bergapten are less than 1.00, and those of the dimethoxycoumarins isopimpinellin and pimpinellin are 1.00 and above.

The appearance of an isoprenoid chain in the molecule of a coumarin or furocoumarin leads to a greater increase in the retention times. Furocoumarins containing an isopentenyl group attached to the aromatic nucleus through an ethereal oxygen, for example, imperatorin or isoimperatorin, are well separated under our experimental conditions, although this had not been achieved previously [1, 2, 4].

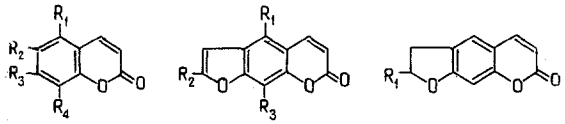
The introduction of hydroxyl groups into the side chain also lengthens the retention time, this increase being proportional to the number of hydroxyl groups. Thus, pranferol, with one hydroxyl group, has RRT 4.31, and hydroxypeucedanin hydrate, with two hydroxyl groups, has RRT 6.21.

We have found that coumarins containing epoxidized isoprenoid chains isomerize under the given conditions. Meranzin, oxypeucedanin, and prangenin give two or three peaks. One of the peaks clearly corresponds to the RRT value of the corresponding isomeric compound (isomeranzin, isooxypeucedanin).

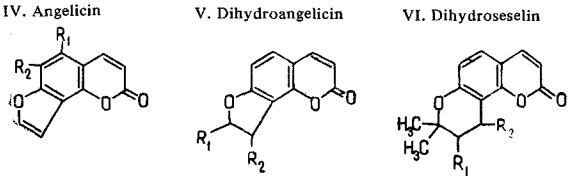
The results that we have obtained have confirmed information in the literature [2] according to which angular furocoumarins have shorter retention times than linear ones. For example, for isopimpinellin the RRT is 1.33 and for pimpinellin it is 1.00. The retention times of the angular dihydropyranocoumarins are shorter than those of linear and angluar dihydrofurocoumarins with substituents of the same nature and number. For the angular dihydropyranocoumarin pteryxin, RRT is 6.68, for the angular dihydrofurocoumarin libanotin it is 6.80, and for the linear dihydrofurocoumarin pranchimgin it is 6.79. An increase in the number of ester groups, like an increase in the size of the hydrocarbon chain of the acid residue, leads to an increase in the retention time: xanthogalin with one

Table 1. Relative Retention Times of Coumarins

Groups: I. Coumarin II. Psoralen III. Dihydropsoralen



IV. Angelicin V. Dihydroangelicin VI. Dihydroseselin



| Group | Substance | Substituents | RRT |
|---|---|---|---|
| I | Coumarin | $R_1=R_2=R_3=R_4=H$ | 0,10 |
| | Scoparone | $R_1=R_4=H$, $R_2=R_3=-OCH_3$ | 0,54 |
| | Osthole | $R_1=R_2=H$, $R_3=-OCH_3$; $R_4=-CH_2-CH=C(CH_3)_2$ | 1,00 |
| | Meranzin | $R_1=R_2=H$, $R_3=-OCH_3$; $R_4=-CH_2-CH(O)-C(CH_3)_2$ | 1,39<br>2,03 |
| | Isomeranzin | $R_1=R_2=H$, $R_3=-OCH_3$; $R_4=-CH_2-CO-CH(CH_3)_2$ | 1,35 |
| | Meranzin hydrate | $R_1=R_2=H$, $R_3=-OCH_3$; $R_4=-CH_2-CH(OH)-C(OH)(CH_3)_2$ | 2,54 |
| | Meranzin acetate | $R_1=R_2=H$, $R_3=-OCH_3$; $R_4=-C_5H_9(OH)(OAc)$ | 2,27 |
| II | Psoralen | $R_1=R_2=R_3=H$ | 0,39 |
| | Xanthotoxin | $R_1=R_2=H$, $R_3=-OCH_3$ | 0,75 |
| | Bergapten | $R_2=R_3=H$, $R_1=-OCH_3$ | 0,87 |
| | Isopimpinellin | $R_2=H$, $R_1=R_3=-OCH_3$ | 1,23 |
| | Imperatorin | $R_1=R_2=H$; $R_3=-CH_2-CH=C(CH_3)_2$ | 2,00 |
| | Prangenin | $R_1=R_2=H$; $R_3=-CH_2-CH(O)-C(CH_3)_2$ | 2,53<br>2,75<br>3,21 |
| | Prangenin hydrate | $R_1=R_2=H$; $R_3=-CH_2-CH(OH)-C(OH)(CH_3)_2$ | 5,07 |
| | Isoimperatorin | $R_2=R_3=H$; $R_1=-CH_2-CH=C(CH_3)_2$ | 2,25 |
| | Oxypeucedanin | $R_2=R_3=H$ | 3,21 |
| | | $R_1=-CH_2-CH(O)-C(CH_3)_2$ | 4,00 |

| Group | Substance | Substituents | RRT |
|---|---|---|---|
| | Isooxypeucedanin | $R_2=R_3=H$; $R_1=-CH_2-CO-CH(CH_3)_2$ | 3,18 |
| | Pranferol | $R_2=R_3=H$; $R_1=-CH_2-CH(OH)-CH(CH_3)_2$ | 4,31 |
| | Oxypeucedanin hydrate | $R_2=R_3=H$; $R_1=-CH_2-CH(OH)-C(OH)(CH_3)_2$ | 5,94 |
| III | Marmezin | $R_1=-C(CH_3)_2$ (OH) | 1,78 |
| | Pranchimgin | $R_1=-C(CH_3)_2$ ($OCO-CH=C(CH_3)_2$) | 6,79 |
| IV | Angelicin | $R_1=R_2=H$ | 0,33 |
| | Pimpinellin | $R_1=R_2=-OCH_3$ | 1,00 |
| V | Peucenidin | $R_1=-C(CH_3)_2$ ($OCOCH_3$) $R_2=-OCOCH=C(CH_3)_2$ | 8,06 |
| | Libanotin | $R_1=-C(CH_3)_2$ ($OR_3$) $R_2=-OR_4$; $R_3$ and $R_4$ = –AC и $-CO-C(CH_3)=CH_2$ | 6,80 |
| | Athamantin | $R_1=-C(CH_3)_2$ ($OCO-CH_2-CH(CH_3)_2$) $R_2=-OCO-CH_2-CH(CH_3)_2$ | 11,59 |
| VI | Xanthogalol | $R_1=OH$ $R_2=H$ | 1,59 |
| | Xanthogalin | $R_1=-OCO-C(CH_3)=CH(CH_3)$; $R_2=H$ | 3,93 |
| | Visnadin | $R_1=-OCO-CH(CH_3)-CH_2-CH_3$; $R_2=-OCOCH_3$ | 5,86 |
| | Dihydrosamidin | $R_1=-OCO-CH_2-CH(CH_3)_2$; $R_2=-OCOCH_3$ | 5,91 |
| | Pteryxin | $R_1=-OCOCH_3$; $R_2=-OCO-C(CH_3)=CH(CH_3)$ | 6,68 |
| | trans-Anomalin | $R_1=R_2=-OCO-C(CH_3)=CH(CH_2)$ | 12,50 |

ester group has RRT 3.93, pteryxin with two such groups 6.68, and trans-anomalin a still higher value, 12.5.

Under the conditions of GLC, furocoumarins substituted in positions 5 and 8 of the benzene ring behave differently. We have previously [6] reported that these compounds differ in the nature of their absorption in the UV and IR regions. It can be seen from Table 1 that the retention times of 8-substituted furocoumarins are shorter than those of 5-substituted furocoumarins with the same substituents. Thus, the 8-substituted furocoumarins xanthotoxin and imperatorin issue more rapidly than the corresponding 5-substituted furocoumarins bergapten and isoimperatorin. We confirmed these results by the GLC separation of a mixture prepared artificially from nine pure coumarin derivatives.

The substances were readily separated and they issued in the sequence shown in Fig. 1 (peaks nos. 1–9). We also studied the coumarin fractions from the roots of P. pabularia (Fig. 2), and also those from the fruit and roots of P. hissarica (Figs. 3 and 4, respectively) [5].

The chromatogram of the coumarin fraction from the fruit of P. hissarica had 13 peaks (see Fig. 3). Table 2 gives the comparative RRT values of standard samples and of the substances from the coumarin fraction.

Table 2. Comparison of the RRT of Components of the Fruit of P. hissarica with Standard Samples

| Peak No. | Compound | Substance from the fraction (RRT) | RRT of standard substances |
|---|---|---|---|
| 1 | Psoralen | 0,43 | 0,39 |
| 2 | Bergapten | 0,83 | 0,87 |
| 3 | Osthole | 1,00 | 1,00 |
| 4 | Unidentified | 1,18 | — |
| 5 | Unidentified | 1,54 | — |
| 6 | Marmezin | 1,68 | 1,78 |
| 7 | Imperatorin | 1,89 | 2,00 |
| 8 | Isoimperatorin | 2,21 | 2,25 |
| 9 | Unidentified | 3,29 | — |
| 10 | Unidentified | 4,03 | — |
| 11 | Prangenin hydrate | 5,03 | 5,07 |
| 12 | Oxypeucedanin hydrate | 5,96 | 5,94 |
| 13 | Pranchimgin | 6,80 | 6,79 |

The GLC results agree well with those of paper and column chromatography. We isolated bergapten, osthole, isoimperatorin, and oxypeucedanin hydrate and identified them by their melting points and IR spectra.

The chromatogram of the coumarin fraction from the roots of P. hissarica showed nine peaks (see Fig. 4). Psoralen, bergapten, osthole, marmezin and isoimperatorin (one peak), oxypeucedanin hydrate, and deltoin were identified from their RRT. Three peaks have not yet been identified. Some of the compounds mentioned (osthole, isoimperatorin, marmezin, oxypeucedanin hydrate) were isolated and identified. It may be considered provisionally that the roots of P. hissarica contain meranzin hydrate and imperatorin.

## EXPERIMENTAL

The work was carried out on a Pye instrument with a flame ionization detector. The chromatography of the coumarins was carried out with a packed column (1.5 m long) containing SE 30 (3%) on Gas Chrom Q (100/100 mesh) as stationary phase.

The rate of flow of argon as the carrier gas was 150 ml/min. In two cases, hydrogen was used as the carrier gas in GLC (see Figs. 2 and 4). The working temperature of the column was 200° C. The coumarin fractions, obtained by Svendsen's method [7], and the samples of the pure compounds were dissolved in acetone (0.1–1.0% solution), and 0.1–10 ml was introduced into the column. Osthole was used as the standard for measuring the RRT.

The sample of psoralen was kindly given to us by N. K. Abubakirov, scoparone by S. Serkerov, isopimpinellin, pimpinellin, angelicin, peucenidin, libanotin, athamantin, and visnadin by N. F. Komissarenko and A. P. Prokopenko, and xanthogalin, dihydrosamidin, pteryxin, and trans-anomalin by G. K. Nikonov.

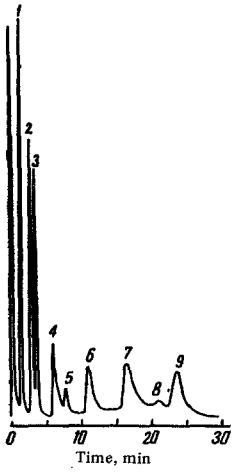



Fig. 1. Chromatogram of a synthetic mixture of nine coumarin derivatives: 1) psoralen, 2) bergapten, 3) osthole, 4) marmezin, 5) isoimperatorin, 6) isooxypeucedanin, 7) prangenin hydrate, 8) oxypeucedanin hydrate, 9) pranchimgin (carrier gas Ar, 150 ml/min).

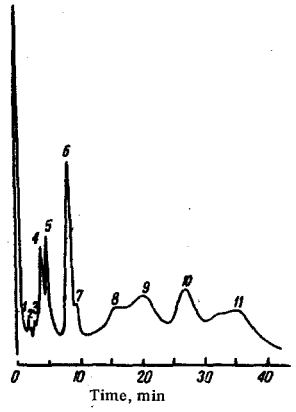



Fig. 2. Chromatogram of the coumarin fraction from the roots of Prangos pabularia: 1) psoralen, 2) unidentified compound, 3) xanthotoxin, 4) bergapten, 5) osthole, 6) marmezin, 7) imperatorin, 8) prangenin, 9) pranferol, 10) hydroxypeucedanin hydrate, 11) deltoin (carrier gas $H_2$, 49 ml/min).

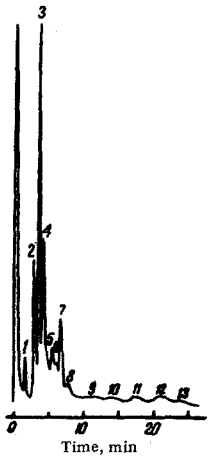



Fig. 3. Chromatogram of the coumarin fraction from the fruit of Prangos hissarica: 1) psoralen, 2) bergapten, 3) osthole, 4, 5) unidentified compounds, 6) marmezin, 7) imperatorin, 8) isoimperatorin, 9, 10) unidentified compounds, 11) prangenin hydrate; 12) oxypeucedanin hydrate, 13) pranchimgin (carrier gas Ar, 150 ml/min).

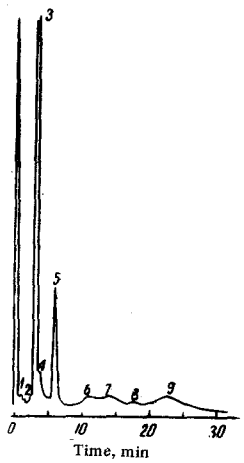



Fig. 4. Chromatogram of the coumarin fraction from the roots of Prangos hissarica: 1) psoralen, 2) bergapten, 3) osthole, 4) unidentified compound, 5) marmezin and isoimperatorin, 6, 7) unidentified compounds, 8) oxypeucedanin hydrate, 9) deltoin (carrier gas $H_2$, 74 ml/min).

## CONCLUSIONS

The possibility of using gas-liquid chromatography for the analysis of coumarin fractions from plants and for the identification of the substances has been shown. The relative retention times for 32 coumarin derivatives have been calculated. The coumarin composition of the roots and fruit of Prangos hissarica have been studied for the first time. Osthole, imperatorin, isoimperatorin, marmezin, oxypeucedanin hydrate, deltoin, and pranchimgin were found in them by gas chromatography.

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