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MASS-SPECTROMETRIC DISTINCTION OF LINEAR AND ANGULAR 3',4'-DIHYDROXY-SUBSTITUTED DIHYDROPYRANOCOUMARINS AND THEIR DERIVATIVES

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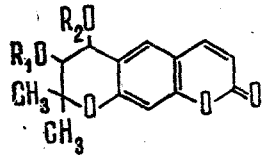
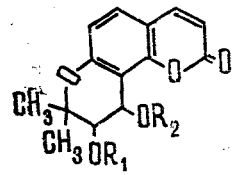
The mass-spectral behavior of angular 3',4'-dihydroxy- and 3',4'-diacyloxy-2',2'-dimethyldihydropyranocoumarins has been studied previously [1]. In the present paper we consider the dissociative ionization of a number of linear compounds of this class: isokhellactone (I), methylisokhellactone (II), and products of the partial hydrolysis and methanolysis of andelin (III, IV), and their deuterium analogs (V-VII) in order to establish the differences in the mass spectra of these compounds from the mass spectra of angular representatives of 3',4'-substituted dihydropyranocoumarins isomeric with or having a similar structure to them (VIII-X). The mass spectra of the latter were taken under conditions identical to those for the mass spectra of (I-VII). Substances (I-IV) are the products of the complete or partial hydrolysis and methanolysis of andelin [2] and their deuterium analogs were obtained synthetically.





I. $R_1=R_2=-H$; II $R_1=-H$, $R_2=-CH_3$;

III. $R_1=-COCH=C(CH_3)(CH_3)$, $R_2=-H$;

IV. $R_1=-COCH=C(CH_3)(CH_3)$, $R_2=-CH_3$;

V. $R_1=R_2=-D$; VI. $R_2=-CH_3$;

VII. $R_1=-COCH=C(CH_3)(CH_3)$, $R_2=-D$;

VIII. $R_1=R_2=-H$; IX. $R_1=-H$, $R_2=CH_3$.

X. $R_1=-COCH=C(CH_3)(CH_3)$, $R_2=-CH_3$.

The results of a comparison of the mass spectra of compounds (I and VIII) and (II and IX) (Figs. 1 and 2) show that they differ by the relative intensities of a number of fragments. As follows from the mass spectra of the deuterium analogs (V and VI), this fact is due not to differences in the mechanism of the behavior of these fragments but to the different decomposition stabilities of the linear and angular isomers of this class of substances. In the case of isokhellactone (I) and khellactone (VIII), such a difference is connected with a subsidiary direction of their dissociative ionization as a result of which the elimination of a molecule of H_2O takes place with the subsequent ejection of a CH_3 radical (Scheme 1). For the linear isomer (I), the ratio $I_{M+}/I_{(M-H_2O)+}$ is approximately 2.5 times lower than for the angular isomer (VIII) (1.6 and 4.0 units, respectively), while, conversely, the ratio $I_{(M-H_2O)+}/I_{(M-OH)+}$ is twice as great (3.5 and 1.7 units, respectively). The mass spectra of the isomers (I) and (VIII) also differ by the mutual intensities of the ions with m/e 229 and 228, 176 and 175, and 175 and 162.

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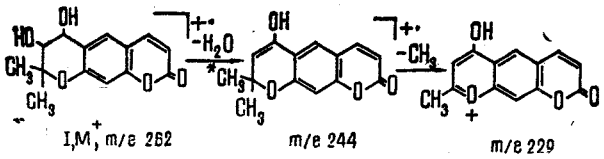
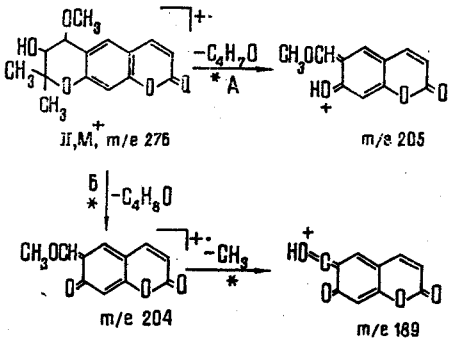
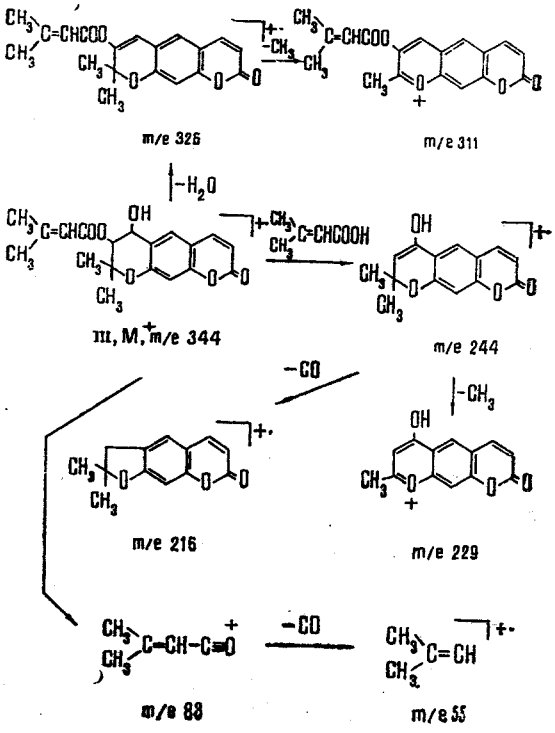


Scheme 1



Scheme 2



Scheme 3

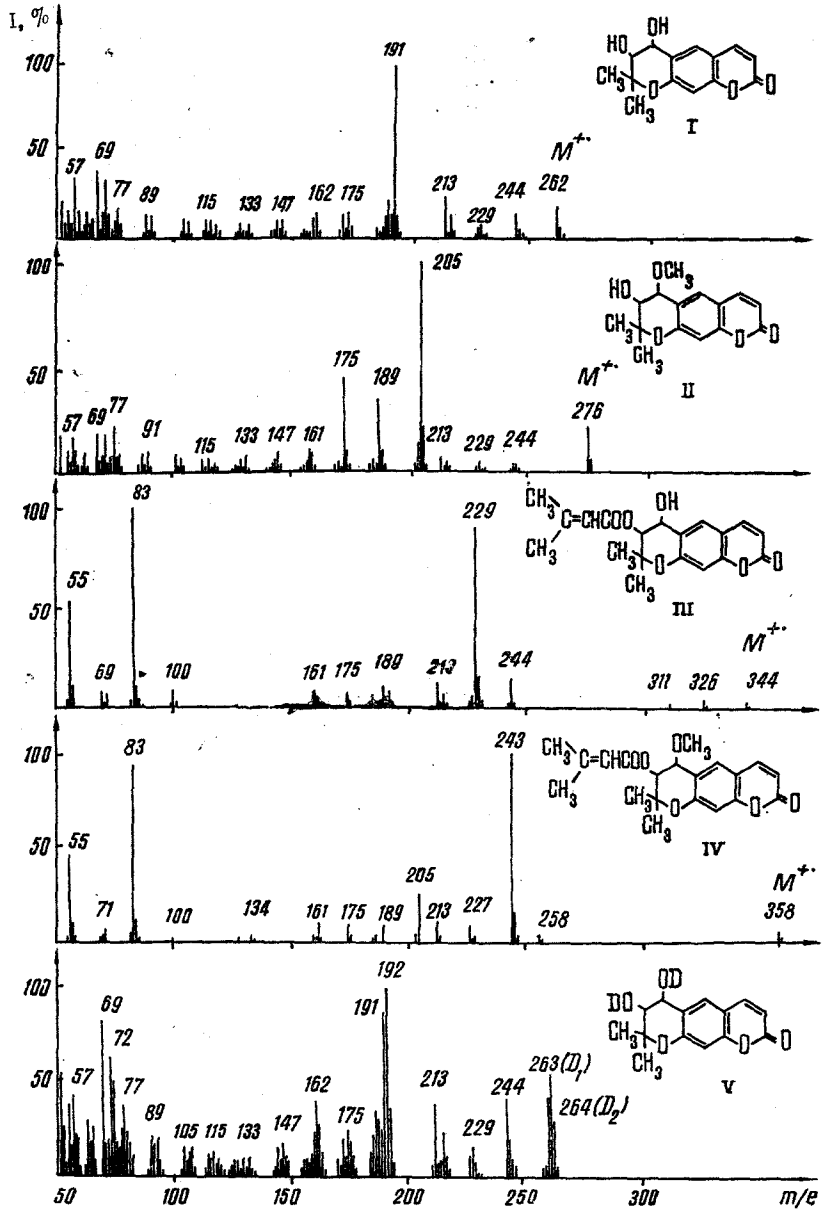


Fig. 1. Mass spectra of isokhellactone (I), methylisokhellactone (II), 4'-hydroxy-2',2'-dimethyl-3'-(senecioyloxy)-3',4'-dihydropyrano-5',6':6,7-coumarin (III), 4'-methoxy-2',2'-dimethyl-3'-(senecioyloxy)-3',4'-dihydropyrano-5',6':6,7-coumarin (IV), and [D_2]isokhellactone (V).

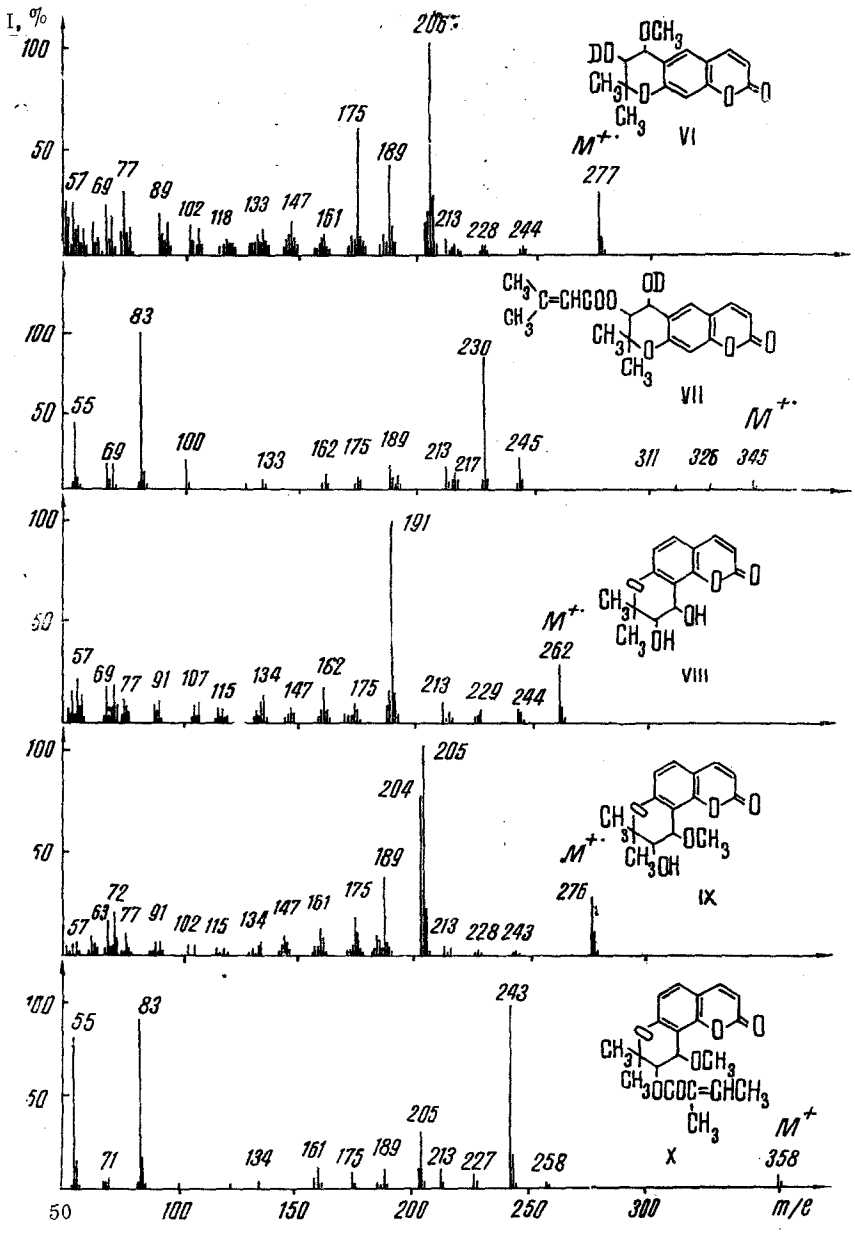


Fig. 2. Mass spectra of [D]methylisokhellactone (VI), the deutero analog of 4'-hydroxy-2',2'-dimethyl-3'-senecioyloxy-3',4'-dihydropyrano-5',6':6,7-coumarin (VII), khellactone (VIII), methylkhellactone (IX), and 3'-angeloyloxy-4'-methoxy-2',2'-dimethyl-3',4'-dihydropyrano-5',6':8,7-coumarin (X).

A comparison of the mass spectra of methylisokhellactone (II) and methylkhellactone (IX) (see Figs. 1 and 2) shows that these compounds differ by the relative intensities of the ions with m/e 205 and 204 formed in the main direction of their decomposition in a similar manner to the fragmentation of 3-hydroxy-substituted compounds [5] as a result of the cleavage of the dihydropyran ring (Scheme 2).* For the linear isomer (II), the cleavage of this ring, taking place by the mechanism of a retrodiene decomposition without the transfer of the hydrogen atom of the hydroxy group from the fragments split out to the main part of the molecule (route B, Scheme 2), occurs with a considerably lower probability than for the angular (IX). As a result of this, the ratio I_{205}/I_{204} in the mass spectrum of the linear compound (II) is approximately seven times higher than that for the angular isomer (IX) (9.0 and 1.3 units, respectively). The mass spectra of substances (II) and (IX) also differ by the ratios of the intensities of the ions with m/e 189 and 175, and 176 and 175.

The results of a study of the mass-spectral behavior of the products of the partial hydrolysis and methanolysis of andelin (III and IV) and also of the deuterium analog (VII) show that the main direction of dissociative ionization of these compounds, like that of substance (X) [1], is the aromatization of the dihydropyran ring through the elimination by the $M^{+\cdot}$ molecule of senecioic acid (m/e 244) with the subsequent ejection of a CH_3 radical (m/e 229) and also the detachment of the acyl residue with the formation of the fragment $RC\equiv\overset{+}{O}$ (m/e 83), which then splits off a CO group (m/e 55) (Scheme 3). Here, as can be seen from the mass spectrum of the deuterium analog (VII), the appearance of the rearranged ion $RCOOH^+$ (m/e 100) is accompanied by the migration to the oxygen of the carbonyl group of one of the hydrogen atoms H_3' or H_4' of the dihydropyran ring, and not the hydrogen atom of the hydroxy group. According to the McLafferty principle [3], this process probably takes place with the participation of the H_4' hydrogen atom. The ratio I_{83}/I_{55} in the mass spectra of (III) and (IV) with the presence in the latter of the ions $RC\equiv\overset{+}{O}$ corresponding to the acyl residue of senecioic acid, is approximately 1.8 times higher than in the mass spectrum of (X) [1], where the $RC\equiv\overset{+}{O}$ ion relates to the acyl residue of angelic acid. A similar rule was found previously in a study of the dissociation ionization of the acyl derivatives of monohydroxydihydropyranocoumarins and -dihydrofurocoumarins [4, 5].

A similar direction of the decomposition of the molecular ion of compound (III) is the loss by it of an H_2O molecule (m/e 326) with the subsequent splitting out of a CH_3 group (m/e 311). No analogous process is observed in the case of the fragmentation of substances (IV) and (X) (appearance of the ion $M^+ - CH_3OH$), just like the formation of an ion with m/e 216 ($M^+ - RCOOH - CO$). These factors together with the displacement of the M^+, $(M - RCOOH)^+$ and $(M - RCOOH - CH_3)^+$ ions in the mass spectra of (IV) and (X) by 14 amu in the direction of higher masses as compared with the spectrum of (III) permit the products of the partial hydrolysis and methanolysis of 3',4'-diacyloxy-2',2'-dimethyldihydropyranocoumarins to be distinguished by the mass-spectrometric method. The linear isomer (IV) differs from the angular isomer (X), as in the case of the dissociative ionization of compounds (II) and (IX), by the relative intensities of the ions with m/e 205 and 204. In the mass spectrum of (IV), the ratio I_{205}/I_{204} is twice as high as in the mass spectrum of (X) (5.9 and 2.9 units, respectively).

Thus, the mass-spectrometric method provides valuable information on the structure of 3',4'-dihydroxydihydropyranocoumarins and their derivatives.

EXPERIMENTAL

The mass spectra of compounds (I-IX) were obtained on a standard MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source at an ionizing voltage of 70 V and a recording temperature of 100°C. The deuterium analogs (IV-VI) were synthesized by the method of Shipchandler and Soine [6].

SUMMARY

The dissociative ionization of four linear dihydropyranocoumarins and three of their deuterium analogs has been studied. It has been shown that by means of the mass-spectro-

*In our previous paper [1] we suggested a different mechanism for the formation of the ions with m/e 204 and 205 which is apparently energetically less suitable than that shown in Scheme 2. This is confirmed by the results of other workers [6, 7].

metric method it is possible to determine the type of linkage of the dihydropyrane ring with the coumarin ring for each pair of linear and angular isomers in this series of substances, to determine the presence of hydroxy or methoxy groups in position 4' of their molecules, and to distinguish the presence of the acyl residue of senecioic acid from that of angelic acid in the products of the partial hydrolysis and methanolysis of 3',4'-diacyloxydihydropyranocoumarins.

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FLAVONOID INHIBITORS OF Na^+,K^+-ATPase

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Among natural compounds, cardiac glycosides are the most effective [1, 2] but not the only inhibitors of the active transport of Na^+ and K^+ and of Na^+,K^+-ATPase. A number of metabolic poisons (oligomycin, ethacrynic acid, cassaine) can also suppress these processes. In spite of the considerable difference in the structures of the cardiac glycosides and the compounds mentioned above, their inhibiting action on Na^+,K^+-ATPase is equally prevented by high concentrations of potassium.

Among more than 100 derivatives of α- and β-unsaturated ketones, Kobashi [3] found a new inhibitor of Na^+,K^+-ATPase – luteolin (3',4',5,7-tetrahydroxy flavone), which possesses the highest affinity for the transport enzyme after its specific inhibitor ouabain. The mechanism of the action of luteolin on Na^+,K^+-ATPase is different from that of the action of ouabain. Recently, the action of isoflavonoids on aerobic glycolysis on mitochondrial ATPase and on ATPase from the plasmatic membranes of tumors has been studied [4]. Of the flavonoids investigated an inhibiting effect on the ATPase of the plasmatic membranes was possessed by tetra- and penta-hydroxyflavones with the hydroxy groups in positions 3, 3', 4, 5, and 7 but the strongest of them was 2,4',5',6'-tetrahydroxychalcone.

We have studied the action on Na^+,K^+-ATPase from the microsomal cell fractions of rat and bovine cerebral cortex with a specific activity of 600-700 μmole of P_{inorg}/mg of protrin/h of four flavone aglycones and eight glycosides mainly of the same aglycone. Of the compounds investigated, the highest inhibiting activity was shown by myricetin (3,3',4',5,-5',7-hexahydroxyflavone). At a concentration of $1 \cdot 10^{-4}$ M, this aglycone completely suppresses transport ATPase. The inhibiting action can be traced fairly clearly at concentrations of $1 \cdot 10^{-5}$ and $5 \cdot 10^{-6}$ M but falls off sharply at a dilution of $1 \cdot 10^{-6}$ M (Table 1). Quercetin (3,3',4',5,7-pentahydroxyflavone) and luteolin (3',4',5,7-tetrahydroxyflavone), in spite of the differences in the number of substituting hydroxy groups, behave in approxi-

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