temperature. The waxes were saponified with 2 N ethanolic caustic soda on the boiling water bath for 10-12 h.

The lipids were separated into classes of compounds and analyzed as described in [5].

SUMMARY

1. The surface lipids of *Ferula* seeds are characterized by high amounts of saturated hydrocarbons with numbers of carbons atoms from 28 to 32.

2. The triacylglycerols make up 50% of the total lipid extract of *Ferula* seeds.

3. In the triacylglycerols of the seed kernels, as in the triacylglycerols of the coat lipids, the amount of petroselinic acid is greater than that of oleic acid.

4. A fairly high percentage of linoleic acid is characteristic for the fatty oils of *Ferula*.

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FIELD DESORPTION MASS SPECTRA OF FLAVONOID ACYLGLYCOSIDES.

I. NATURAL ACETYL DERIVATIVES

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UDC 547.972:543.51

The field desorption mass spectra (FD spectra) of 17 natural flavonoid acetylglycosides have been studied. In the spectra of each of the O-monoglycosides the molecular ion (M or M + H) appears as the main peak and it is accompanied by the ions of the aglycone (A or A + H) and of the acylated anhydrosugar (S). The intensity of the latter peak is largely connected with the structure of the substance. In the FD spectra of flavone O-biosides, fragment S is absent but its mass can be calculated from the difference (M − A). Useful information for establishing the position of the acetyl group is given by the fragments S_1 and ($M - S_1$) corresponding to the detachment of the terminal sugar residue. The FD spectra of flavone C-glycosides differ greatly from the spectra of the O-glycosides: In them the main peak is that of the ion (M), but peaks (A) and (S) are absent and the ions present resemble the fragmentation of the C-glycosides under the action of electron impact.

Electron impact mass spectrometry (EI-MS) has been well developed for flavonoid aglycones and serves as a reliable tool in their structural analysis. These results have been given in detail in a review [1]. Because of their low volatility and thermal instability, flavonoid O-glycosides decompose in the mass spectrometer under the action of electron impact and give mass spectra which are practically those of their aglycones. Workers therefore have recourse

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to obtaining acetylated derivatives and trimethylsilyl ethers, and also methyl or deuteromethyl ethers [1-9]. An although the EI spectra measured for these derivatives give a considerable amount of information on the structure of the compounds, a number of factors make this information problematical: difficulty of obtaining full methyl and deuteromethyl ethers, the possible elimination of acyl groups in this process, and the excessively high molecular masses of the silyl ethers of the glycosides, which sometimes lie beyond the limits of the scale of measurement.

Many years' interest in flavonoids and the isolation in recent years of a large group of new O-acylated glycoside derivatives and the determination of their structure in our laboratory [10] have impelled us to study the possibility of field desorption mass spectrometry (FM-MS) for the structural analysis of these compounds.

In the ideal case, a FD spectrum contains the radical ion $M^{\cdot +}$ and/or the ion arising on protonation $(M + H)^+$, and also the ions $(M + Li)^+$, $(M + Na)^+$, and $(M + K)^+$ which are formed either on the inclusion of these metals from a contaminated sample or from a contaminated emitter. In actual fact, in the FD spectra, in addition to the molecular ion, several fragments are observed the formation of which depends on the temperature of the emitter or (since this temperature cannot be measured) on the current through the emitter.

At the time when our work was begun [10, 11] there were no reports in the literature on the application of FD-MS to acylated glycosides of flavonoids and in only two papers were the results given of a study of the FD spectra of flavonoid biosides: rutin, naringin, and hesperidin [12], and chrysoeriol apioglucoside [13]. In recent years, reports have appeared in which FD spectra have been used to confirm the composition of two flavonoid acylglucosides [14, 15], and also a paper [16] in which a systematic analysis of the FD-MS fragmentation of a series of flavonoid glycosides and of three acylated derivatives is given. It was shown that the fragmentation of the glycosides largely resembles the pattern of breakdown of glycosides on thermolysis and mainly affects the glycosidic bond. In the TLC of the products of thermolysis the same substances were found as arise on FD-MS: diglycosides from triglycosides, monoglycosides from biosides, and aglycones from monoglycosides.

We have analyzed the FD spectra of the 17 acylglycosides below:

	Compound	Literature
1.	Quercetin 3-(6"-O-acetylgalactoside)	17
2.	Quercetin 3-(3"-O-acetylgalactoside)	17
3.	Quercetin 3-(6"-O-acetylglucoside)	18
4.	Isorhamnetin 3-(6"-O-acetylglucoside)	18
5.	Isorhamnetin 3-(6"-O-acetylgalactoside)	18
6.	Herbacetin 8-(3"-O-acetylarabinoside) = acetylrhodalgin	19, 20
7.	Herbacetin 8-(3"-O-acetylxyloside) = acetylrhodalin	21
8.	Herbacetin 8-(2",3"-di-O-acetylxyloside) = diacetylrhodalin	19, 20
9.	Herbacetin 8-(2",3",4"-tri-O-acetylxyloside) = triacetylrhodalin	19, 20
10.	Luteolin 7-(6"-O-acetylglucoside) = acetylcynaroside	22
11.	Acacetin 7-(6"-O-acetylglucoside) = agastachoside	23
12.	Acetate of ethyl (apigenin 7-glucosid)uronate	24
13.	Luteolin 8-C-(2"-O-acetylglucoside) = acetylorientin	25
14.	Luteolin 7-[2"-(3"'-O-acetylapio)xyloside] = campanoside	26
15.	Pectolinarigenin 7-(3"'-O-acetylrutinoside) = acetylpectolinarin	27, 28
16.	Isoscutellarein 7-(acetylglucoglucoside) = acetylpalustaside	29
17.	4'-Methoxyisoscutellarein 7-(acetylglucoglucoside) = acetylpalustasidin	29

It must be mentioned that in the EI spectra of the acylated compounds investigated only the ions of the aglycone and of its characteristic fragments were recorded in all cases. The only exceptions are some herbacetin 8-glycosides containing acetylated arabinose or xylose residues (compounds 6-9), for which at 70 eV and a temperature of 200-220°C it was possible to obtain the molecular ions with a relative intensity of 0.5% by creating a high concentration of the substance in the ionization chamber [20].

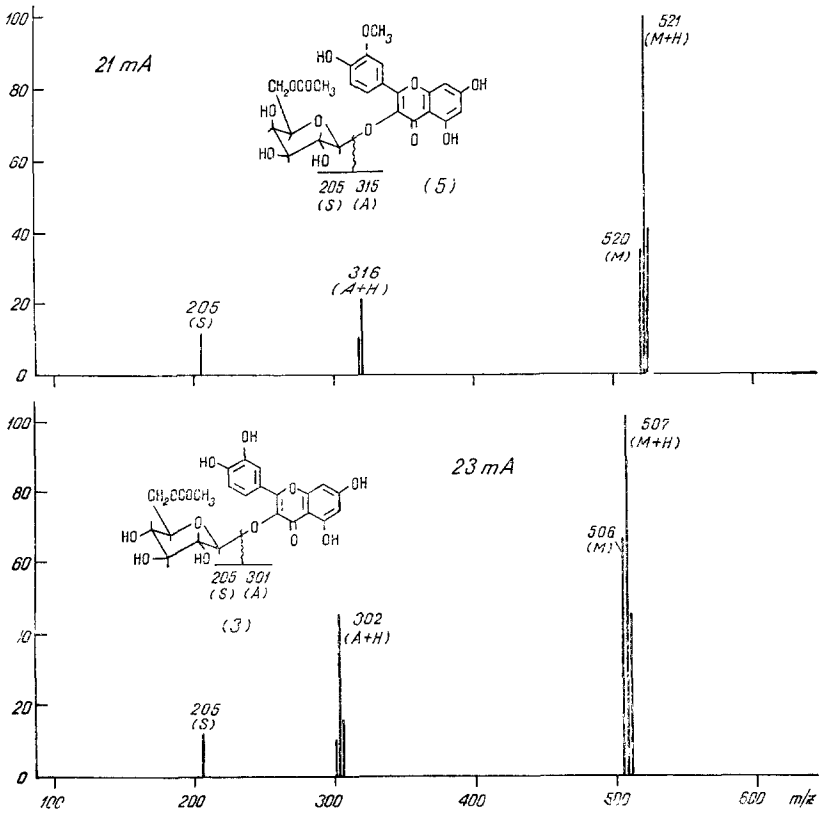


Fig. 1. FD spectra of compounds (3) and (5).

The aim of the present work was to find the laws of the fragmentation of flavonoid acylglycosides under the conditions of FD-MS and to determine the diagnostic ions for the analysis of structural analogues of such compounds. Since the basis of the acylglycosides that we isolated consisted of monoglycosides and biosides of flavonols and flavones, we also studied the FD spectra of some deacylated analogues.

Flavone and Flavonol Glycosides (Deacylglycosides)

We measured the FD spectra of a large group of flavone and flavonol O-monoglycosides containing glucopyranose, galactopyranose, rhamnopyranose, xylopyranose, arabinopyranose, and arabinofuranose residues. The field desorption mass spectra of the O-monoglycosides are easy to interpret. They always contain the molecular ion (M), as the main peak and the fragmentary ion of the flavonoid aglycone (A), sometimes accompanied by a peak with the mass of the anhydro sugar at m/z 133 in the pentosides, m/z 147 in the rhamnosides, and m/z 163 in the hexosides. The sugar fragment was most stable in the arabinofuranosides (juglanin, avicularin), but in the majority of other glycosides it was absent although its mass was determined unambiguously from the difference (M − A).

We studied a number of O-biosides containing residues of rhamnosylglucose (rutinose), glucosylglucose, and apiosylxylose. In the FD spectra of the biosides, likewise, in addition to the molecular ion, one of the main peaks was that of the aglycone and it was therefore possible to determine the mass of the carbohydrate substituent, the ion of which was not formed, from the difference (M − A). The structure of the aglycone had practically no influence on the nature of the fragmentation but this was appreciably affected by the position of attachment of the biose to the flavonoid. For example, the spectra of the 3-rutinosides of (of datiscetin, kaempferol, and quercetin) contained the ions of the terminal sugar residue and the monoglycoside corresponding to this elimination, while the spectra of the 7-rutinosides (pectolinarin, etc.) did not contain these ions.

Flavonol O-Acetyl-O-monoglycosides

In this group, we considered the FD spectra of compounds (1-9) taken for each compound repeatedly with a variation in the heating current of the emitter. The spectra of the quer-

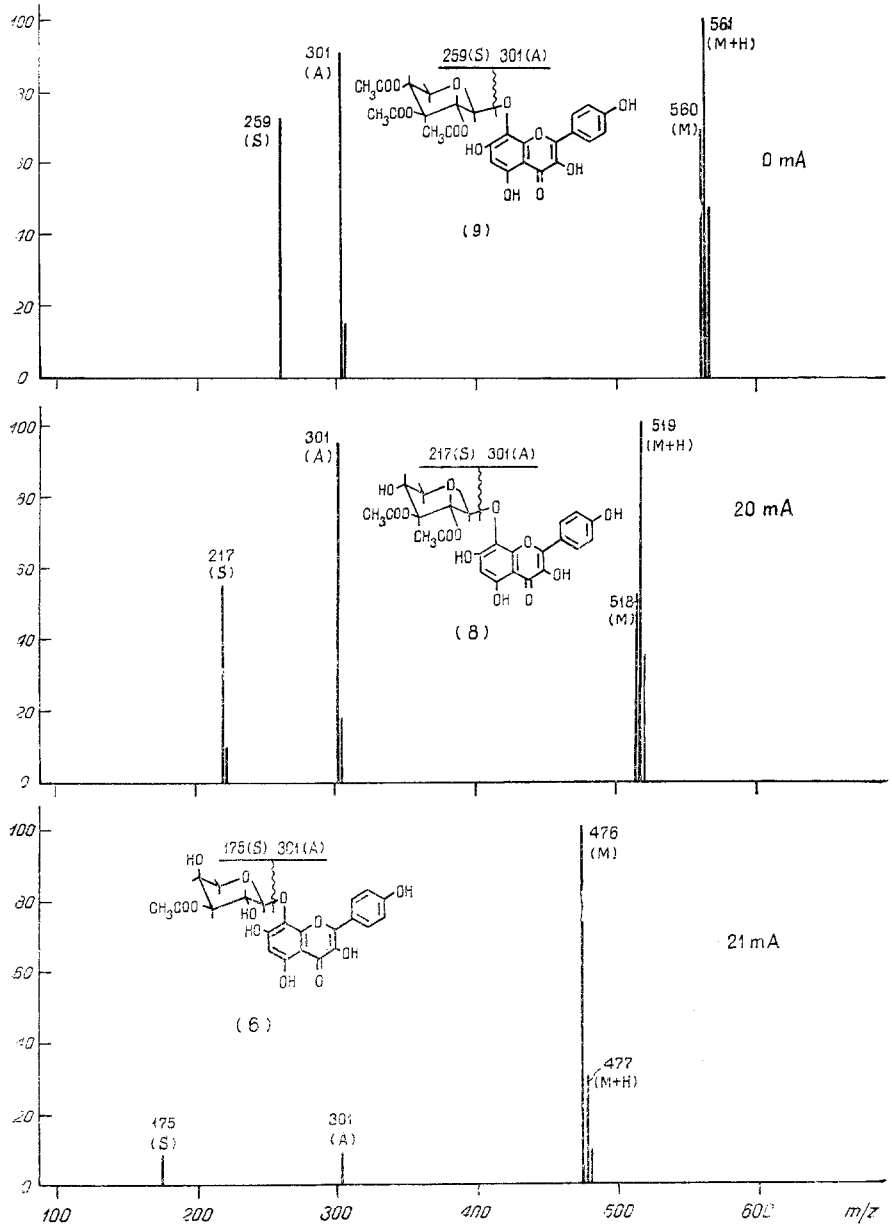


Fig. 2. FD spectra of compounds (6), (8), and (9).

cetin glycosides (1-3) were similar to one another and also to the spectra of the isorhamnetin analogues (4, 5), but the isorhamnetin glycosides were desorbed at a lower emitter current (21 mA) than the quercetin glycosides (23 mA) (see Fig. 1). In the spectra of compounds (1-5), the main peak was that of the (M + H) ion, and it was accompanied by the M and (M + 2H) ions. The group of aglycone ions (A + H) also included the peaks of the ions A and (A + 2H). The spectra each included the fragment of the acylated anhydro sugar S.

In a study of the FD spectra of two monoacetylated 8-glycosides of herbacetin — acetylrhodalgin (6) (Fig. 2), and acetylrhodalin (7) — no fundamental differences were observed in the nature of the fragmentation as compared with the preceeding compounds (1-5).

Interesting features were revealed in the FD spectra of two derivatives of polyacetylated xylosides — diacetylrhodalin (8) and triacetylrhodalin (9) (Fig. 2). Characteristic for them was the presence of strong fragmentary peaks of the di- and triacetylated anhydro sugars S (m/z 217 and 259, respectively).

For compound (8), the peak of the S ion (m/z 217) was the main one at 19 mA and the fragmentary ion of the aglycone A had an intensity of 93%, the spectrum also including the molecular ion (m/z, 518, 58%) and the (M + H) ion (98%). Further heating led to the appearance of the ions (M + 2H), (M — H), (A + H), and (S + H).

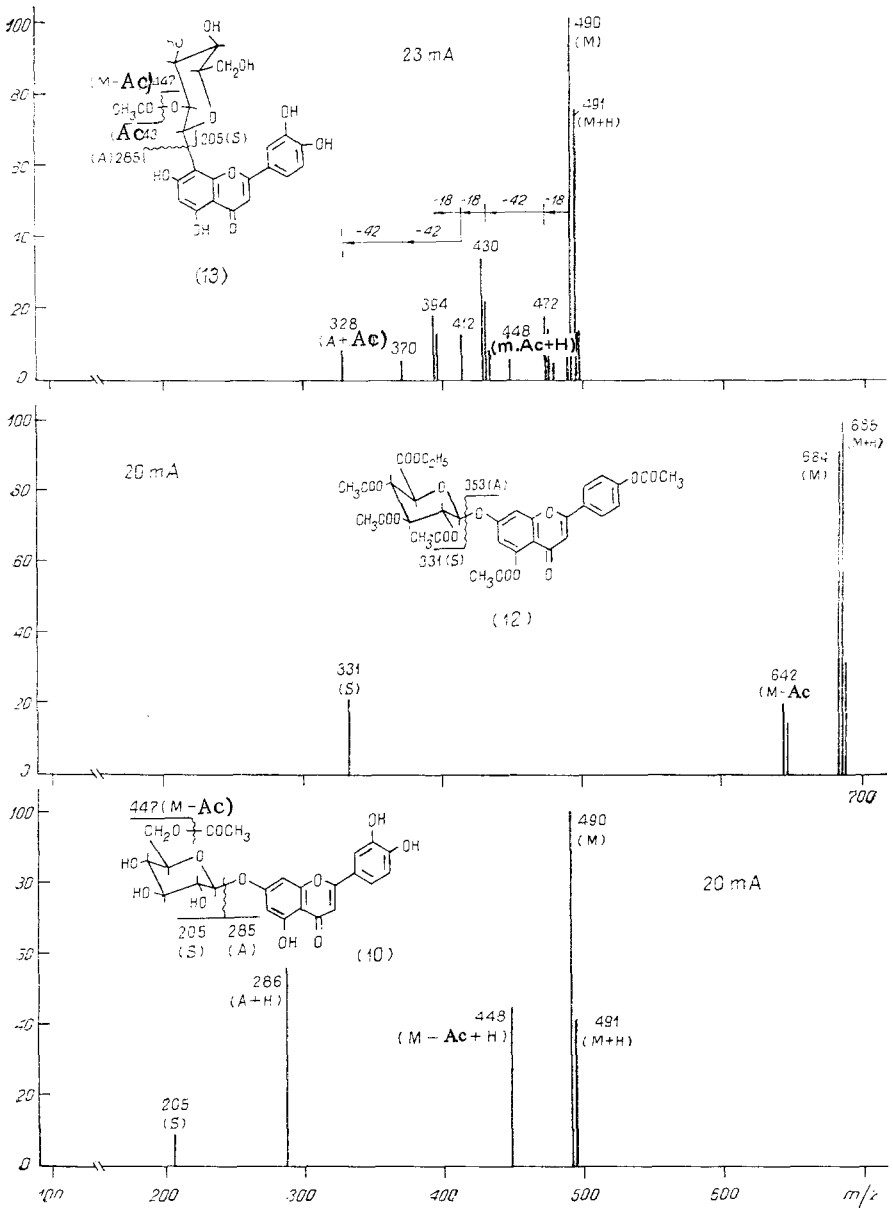


Fig. 3. FD spectra of compounds (10), (12), and (13).

In the case of triacetylrhodalin (9), the residue S (anhydroglucose with three acetyl groups) was readily split off even at I = 0 mA when the spectrum (Fig. 2) contained strong peaks of ions of the aglycone A, of the molecular ion M, and of the (M + H) ion. Heating the emitter led to a decrease in the intensity of the fragmentary ions S and A and to the appearance of rearranged (A + H), (M — H), and (M + 2H) ions.

O-Acetyl-O-monoglycosides of Flavones

The field desorption mass spectra of three flavone O-monoglycosides (10-12) were investigated. For acetylcynaroside (10), 13 spectra were taken at different anode-heating currents. The majority of them contained only the peaks of the M and (M + H) ions. At 20 mA (Fig. 3), in addition to the peaks mentioned, ions with m/z 448 (M—Ac+H) and with m/z 286 (A + H) and the ion of the acylated anhydrosugar S with m/z 205 appeared.

For acacetin 7-O-(6"-O-acetyl-β-D-glucopyranoside) (11) (the sample was kindly provided by A. M. Zakharov, VILR [All-Union Institute of Medicinal Plants], a variation in the heating current of the emitter did not lead to the appearance of any ions whatever apart from the molecular ion.

The behavior of the full acetates of flavone glycosides appeared of interest, and we measured the FD spectra of the pentaacetate of apigenin 7-(ethyl glucosiduronate) (12). We

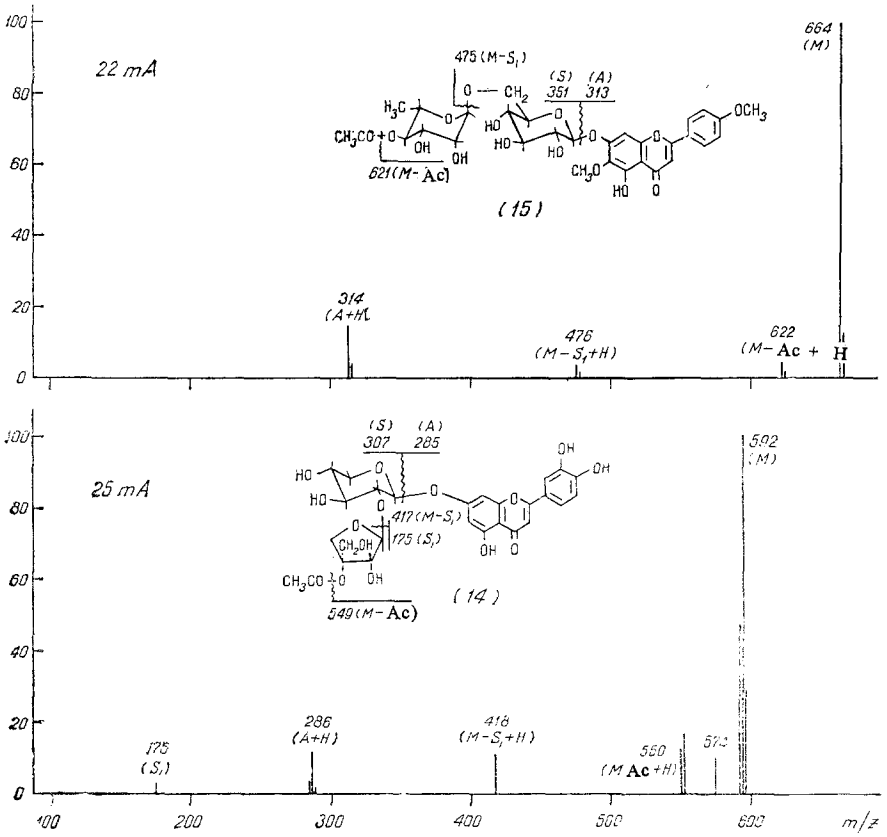


Fig. 4. FD spectra of compounds (14) and (15).

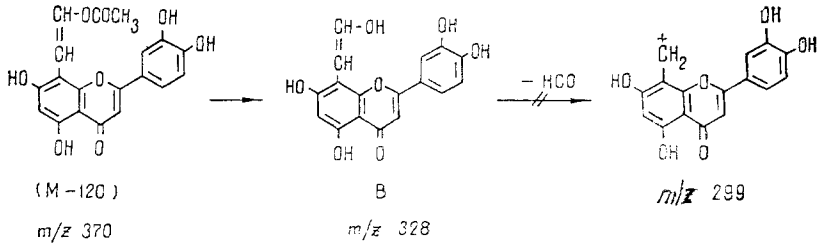
took 15 spectra with a variation in the current through the emitter of from 10 to 21 mA. The expected group of ions of the aglycone A (m/z 353), (A − 1 Ac) (m/z 311), and (A − 2 Ac) (m/z 269) or their protonated analogues were not detected in the spectra, although these ions are always present in electron-impact spectra. At 20 mA (Fig. 3), the spectrum contained the fragmentary ion of the acylated carbohydrate fragment S (m/z 331), and a group of peaks of the molecular ion. With the exception of the formation of the (M − 1 Ac + H) ion with m/z 642, no deacylation of the molecule, which readily occurs on bombardment with ionizing electrons, took place, either.

Thus, in comparison with the flavonol 3- and 8-glycosides the fragmentation of the flavone 7-glycosides was very difficult (as also is their hydrolytic cleavage by acids), and the fragmentary ions formed had a low intensity. The formation of the deacylated monoglycoside (M − Ac) observed for the 7-glycosides was not recorded for any of the other nine flavonol O-(acylglycosides).

O-Acetyl-C-monoglycosides of Flavones

In the FD spectra of an acylated C-glycoside (2"-O-acetylorientin, 13), as in the spectra of the O-glycosides, the main peak was that of the molecular ion (m/z 490) (see Fig. 3). The ion of the deacylated glycoside (M − Ac + H) (m/z 448) was formed with a low intensity (5%). Neither the aglycone A nor the carbohydrate fragment S appeared in the spectra. They did contain the (A + 43) ion with m/z 328 and other fragmentary ions formed in the successive splitting out of water and ketene (Fig. 3). This breakdown resembles the fragmentation of the 8-C-glycosides under the action of electron impact [2]. The molecular ion is not always observed in the EI spectra of the C-glucosylflavones and the (M − H_2O), (M − $2H_2O$), and (M − $3H_2O$) ions are the characteristic ones. The strongest here is the (M − 149) ion − the benzyl cation of the aglycone, the formation of which in the 8-glucosides (as metastable ions show) takes place in two stages: first $C_4H_8O_4$ is eliminated from the molecular ion, leading to (M − 120) ions, and the subsequent loss of CHO leads to (M − 149) ions. It has been shown that the (M − 120) ion is characteristic only for the 8-C-glucosides and is absent from the spectra of the 6-C-glucosides [2].

Under the conditions of the field desorption of acetylorientin (13), the ion with m/z 328 obviously corresponds to ion B in the EI spectra, but it does not breakdown further into the benzyl cation:



O-Acetyl-O-Biosides of Flavones

In this group we considered four compounds: campanoside (14), acetylpectolinarin (15), acetylpalustaside (16), and acetylpalustasidin (17), samples of which were kindly provided by T. V. Zinchenko (Kiev, Institute of Advanced Medical Studies).

All the compounds mentioned are 7-biosides and their fragmentation is very difficult, as in the case of the 7-monoglycosides. Thus, in the spectra of compounds (16) and (17) there were strong molecular ions, while the intensities of the ions of the deacylated glycosides (M − Ac + H) and of the aglycone (A + H) did not exceed 5-15%. In the spectrum of acetylpectolinarin (15, Fig. 4), an additional ion (M − S_1 + H), corresponding to the detachment of the terminal sugar residue arose. The bioside campanoside (14, Fig. 4) includes the furanose residue of apiose, which is readily split off, forming the ion of the terminal sugar residue S_1 (m/z 175). In addition to the ions mentioned for (15), the spectrum of (14) also contains the (M − H_2O) ion with m/z 574.

Thus, the field-desorption mass spectra of flavonoid acetylglycosides are easy to interpret since, apart from the molecular ion, they contain only a few characteristic fragmentary ions.

For none of the three compounds investigated did we observe the transacylation (formation of diacylglycosides from monoacylglycosides) reported in [16].

In the taking of the FD spectra of several samples (compounds 3, 11, 16, and 17) made available by other workers we obtained weak (6-16%) peaks of the cations $(M + {}^{23}Na)^+$, which, after the additional purification of the substances (by chromatography on polyamide or by recrystallization) disappeared from the spectra. We assume that the presence of these cations is due to the contamination of the samples caused by the high capacity of the flavonoids for chelate-formation.

EXPERIMENTAL

The field-desorption mass spectra were measured on a Varian MAT-731 mass spectrometer with a combined EI/FD/FI ion source. The recording and processing of the field-desorption mass spectra were carried out with the aid of a SS-100 MS data-processing system. The spectra were scanned at the rate of 4 sec/deg at a resolution R = 1000 (at the 10% level).

Field emitters (anodes) of tungsten wire with a diameter of 10 μm activated in an atmosphere of benzonitrile in a Varian activation apparatus using the method described by Beckey [30] were used. The samples, dissolved in acetone or methanol (10-20 mg/ml), were deposited on the surface of the activated emitter by immersing the emitter in the solution. Then the excess of solvent was evaporated off and the anode with the sample was introduced through the vacuum lock into the source, the temperature of which was about 90°C.

For optimizing the current by adjusting the focussing system acetone was passed into the source through the inlet system. The emitter heating current was set in such a way as to produce a sufficiently large current of the main (the molecular) ion. Depending on the structure of the compound, it ranged from 0 to 25 mA.

Before each measurement the emitter was heated with a current of about 100 mA (approximately 1200°C) in order to eliminate the preceeding compounds effectively.

The mass spectrometers were calibrated in the electron-impact regime using perfluorokerosine.

CONCLUSIONS

The field-desorption mass spectra of 17 natural flavonoid acetylglycosides have been studied. It has been shown that the spectra of the monoglycosides contain as the main peak in each case the molecular (M) ion or the (M + H) ion, which is accompanied by the ions of the aglycone (A) and of the acylated anhydrosugar (S). The intensity of the latter peak is largely connected with the structure of the substance and depends on the magnitude of the emitter heating current.

Fragment S is absent from the FD spectra of flavone biosides, but its mass can be calculated from the difference M − A. Useful information for determining the position of an acetyl group is given by the fragments S_1 and $(M - S_1)$ corresponding to the detachment of the terminal sugar residue.

The FD spectra of acylated C-glycosides differ sharply from the spectra of O-glycosides and resemble the EI spectra of these compounds.

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