# SECONDARY-EMISSION MASS SPECTROMETRY OF HEDERAGENIN GLYCOSIDES AND MEDICAGENIC ACID GLYCOSIDES

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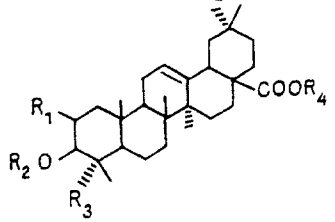
The secondary emission (SE) spectra of six glycosides of medicagenic acid (MA) and of hederagenin have been studied. The $(M + H)^+$ ions were observed in the secondary ion spectra (LSIMS) of mono- and biosides of MA and also of a tri- and a tetraoside of hederagenin in a glycerol matrix. The $(M + Na)^+$ ions were recorded in the spectra of tri- and tetraosides of MA. In the majority of cases processes involving the successive splitting out of the carbohydrate units were revealed. Positive- and negative-ion spectra have been obtained on the ionization by bombardment with fast atoms – FAB "+" and FAB "–" – of a trioside and a tetraoside of MA. The FAB "–" spectra are the most characteristic: They show intense peaks of the $(M - H)^-$ ions and of the products of the sequential elimination of the sugar residues.

The EI mass spectra of glycosides of pentacyclic triterpenoids are uninformative because of the poor volatility of these substances. Unmodified monosides do not form stable $M^{+\cdot}$ ions. The possibility of obtaining characteristic spectra of volatile derivatives of this class of compounds do not extend beyond the bi- and triosides [1, 2]. However, this problem has been solved with the introduction into practice of "mild" method of ionization [3, 4]. The group of most frequently used mass-spectral methods comprises secondary-emission (SE) methods which include the mass spectrometry of secondary-ions with the use of a liquid matrix (LSIMS) and of fast-atom-bombardment [FAB ("+" and "–")] [5, 6]. Another group includes a fairly wide set of mass-spectral methods with desorption ionization (DI), including HEI methods [7]. In order to find the optimum conditions for obtaining the necessary structural information, individual authors have investigated one and the same sample with the aid of a whole set of different methods [8]. Nevertheless, at the present time a definite specialization of particular methods in relation to compounds of certain classes have appeared. Thus, in publications devoted to the analysis of steroid and terpenoid glycosides it is mainly FAB ("+" and "–") spectra and, to a considerably smaller degree, LSIMS spectra that has been used.

In the present communication we give information on the LSIMS spectra of six triterpene oligosides of the olean-12-ene series of which the aglycons are medicagenic acid (MA) [the 3-O-β-D-glucopyranoside (I) and medicosides G (II), H (III), and J (IV)] and hederagenin [medicosides C (V) and I (VI)] isolated from Medicago sativa L. [9]. For comparison, we obtained the FAB ("+" and "–") spectra of the most polar compounds – medicosides H and J.




I. $R_1$=OH, $R_2$=β-*D*-$Glc_p$, $R_3$=COOH, $R_4$=H.
II. $R_1$=OH, $R_2$=β-*D*-$Glc_p$, $R_3$=COOH, $R_4$=β-*D*-$Glc_p$.
III. $R_1$=OH, $R_2$=β-*D*-$Glc_p$, $R_3$=COOH, $R_4$=α-*L*-$Rha_p$ (1→2)-β-*L*-$Ara_p$.
IV. $R_1$=OH, $R_2$=β-*D*-$Glc_p$, $R_3$=COOH, $R_4$=β-*D*-[illegible] (1→4)-α-*L*-$Rha_p$ (1→2)-β-*L*-$Ara_p$.
V. $R_1$=H, $R_2$=α-*L*-$Ara_p$ (1→2)-β-*D*-$Glc_p$ (1→2)-[illegible]-$Ara_p$, $R_3$=$CH_2OH$, $R_4$=H.
VI. $R_1$=H, $R_2$=α-*L*-$Ara_p$ (1→2)-β-*D*-$Glc_p$ (1→2)-α-*L*-$Ara_p$, $R_3$=$CH_2OH$, $R_4$=β-*D*-$Glc_p$.

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TABLE 1. Mass Numbers and Relative Intensities (%) of the Peaks of Diagnostic Ions in the Mass Spectra of Compounds (I-VI) Obtained by the LSIMS Method

| | |
|---|---|
| Medicagenic acid 3-O-β-D-glucopyranoside (I) | 665$(M+H)^+$(6), 619$(M-COOH)^+$(2), 503$(M-Glc+2H)^+$(5), 485$(M+H-GlcOH)^+$(9), 457(503−HCOOH)$^+$(100), 439(32) 411(18), 323(22) |
| Medicoside G (II) | 827$(M+H)^+$(3), 665$(M-Glc+2H)^+$(6), 647(665 − $H_2O$)$^+$(3), 619(665 − HCOOH)$^+$(8), 6 1(3), 503(2), 457(100), 439(58), 411(30) 3 3(19) |
| Medicoside H (III) | 987$(M+2Na-H)^+$(4), 965$(M+Na)^+$(1 ), 919(965−HCOOH)$^+$(2), 7 9(987−Rha−Ara+2H)$^+$(4), 687(965−Rha−Ara+2H)$^+$(12), 663(709−HCOOH)$^+$(4), 641(687−HCOOH)$^+$(8), 457(20), 43 (17) 421(12), 393(18), 3 1(Rha−O−Ara+Na)$^+$(100) |
| Medicoside J (IV) | a) 1119$(M+2Na-H)^+$(17), 709(23), 663(32), 433(Xyl−O−Rha−O Ara+Na)$^+$(100) b)1075$(M+H)^+$(14), 943$(M-Xyl+2H)^+$(10), 797$(M-Xyl-Rha+3H)^+$(8), 635(797− Ara+H)$^+$(18), 619(665−HCOOH)$^+$(18), 457(45), 439(27), 422(23), 393(2 ) |
| Medicoside C(V) | 899$(M+H)^+$(49), 767$(M-Ara+2H)^+$(10), 605(767−Glc)$^+$ +H)$^+$(10), 473(AglOH+H)$^+$(16), 455(95), 437(100), 427(473− HCOOH)$^+$(40), 40 (68) |
| Medicoside I (VI) | 1061$(M+H)^+$(8), 929$(M-Ara+2H)^+$(1), 899$(M-Glc+2H)^+$(3), 853(899−HCOOH)$^+$(3), 767$(M-Ara-Glc+3H)^+$(3), 455(85), 437(100), 409(75) |

TABLE 2. Mass Numbers and Relative Intensities (%) of the Peaks of Diagnostic Ions in the Spectra of Medicosides H (II) and J (IV) Obtained by the FAB ("+" and "−") Methods

| | | |
|---|---|---|
| Medicoside H (III) | FAB ·+· | 9 5 $(M+Na)^+$ (10 ), 757 (965−Glc−COOH)$^+$ (6), 687 (23), 641 (10), 457 (34), 439 (32), 421 (22), 393 (21), 301 (38) |
| | FAB ·−· | 941 $(M-H)^-$ (72), 795 (M− Rha)$^-$ (3), 779 (M−Glc)$^-$ (20), 761 (941−GlcOH)$^-$ (5), 663 (M−Rha−Ara+ H)$^-$ (46), 619 (663−$CO_2$)$^-$ (15), 501 (663 −Glc+H)$^-$ (22), 483 (663− − GlcOH) (12), 455 (5 1 − HCOOH)$^-$ (20), 439 (483− −$CO_2$)$^-$ (100) |
| Medicoside J (IV) | FAB ·−· | 457 (AglOH−COOH)$^+$ (72), 439 (48), 421 (26), 411 (36), 393 (3 ) |
| | FAB ·−· | 1073 (M−H)$^-$ (100), 941 (M−Xyl)$^-$ (9), 911 (M−Glc)$^-$ (36), 803 (1073− GlcOH)$^-$ (10), 663 (M−Xyl−Rha− Ara+2H)$^-$ (19), 617 (663− HCOOH)$^-$ (10), 501 (15), 483 (663−GlcOH)$^-$ (8), 455 (12); 439 (78), 409 (455−HCOOH)$^-$ (5). |

The main characteristic ions of the LSIMS spectra are given in Table 1, and those of the FAB ("+" and "−") spectra in Table 2.

The relatively high polarity of MA (presence of additional OH and COOH groups in ring A) led to a low stability of the $(M + H)^+$ ions in the LSIMS spectra with a glycerol matrix) of the higher glycosides (III and IV). Under the same conditions, the trioside (V) and the tetraoside (VI) of hederagenin gave more stable $(M + H)^+$ ions (Table 1). The acquisition of information spectra of medicosides H and J required the addition to the glycerol matrix of NaCl, by varying the amount of which it was possible to ensure the predominant formation of the $(M + H)^+$, $(M + Na)^+$, or $(M + 2Na - H)^+$ ions. The fragmentation pathways of these ions were different, as is demonstrated in Table 1 for the two spectra of medicoside J (IV).

The spectra of all the compounds contained the peaks of the ions formed as the result of the successive splitting out of carbohydrate units, accompanied by a series of common and individual features. Thus, the fragmentation of the oligosaccharide chains at C-28 of the $(M + H)^+$ ions took place with the successive elimination of the carbohydrate units and of the carboxy group (medicosides G and J); the fragmentation of the $(M + Na)^+$ and $(M + 2Na - H)^+$ ions took place with the one-stage loss of the oligosaccharide unit followed by the splitting out of the carboxy group (medicosides H and J). The daughter ions formed on fragmentation contained Na atoms. The fragments of the aglycon moiety did not include Na

atoms and coincided in mass numbers with the ions in the EI spectra (m/z 457, 439, 421, 411, and 393 for MA, and 455, 437, 427, 411, and 409 for hederagenin).

The fragmentation of glycosides with an oligosaccharide chain at C-3 included the successive splitting out of the corresponding carbohydrate units (medicoside C) or the alternative elimination of sugar residues from C-3 and the substituent at C-17.

We studied the nature and sequence of the splitting out of the substituents at C-3 and C-17 by the linked-scanning (B/E = const) method [10] applied to the $(M + H)^+$ ions and some fragments of MA 3-O-β-D glucoside (I) and of medicoside G (II). The B/E spectra of the $(M + H)^+$ ion with m/z 665 (I) showed that the carbohydrate unit at C-3 can be split out both in the form of GlcH and in that of GlcOH. On the breakdown of $(M + H)^+$ ion of medicoside G (m/z 827) we recorded a transition to an ion with m/z 665, while there were no transitions to ions with m/z 647 and 619. At the same time, analysis of the B/E spectra of the ion with m/z 665 showed transitions to ions with m/z 619, 503, and 485. These findings show the following facts. The first act of the breakdown of $(M + H)^+$ for (II) was the elimination of GlcH from C-28, after which followed the alternative elimination of the HCOOH molecule from the same substituent or the splitting out of GlcH or GlcOH from C-3. The main precursors of the most intense peaks of the ions with m/z 457 were ions with m/z 503 in the case of (I) and ions with m/z 619 and 503 in the case of medicoside G.

The FAB ("+") spectra of medicosides H and J (Table 2) differed greatly in their informativeness. While the first, with respect to its set of characteristic ions, was extremely close to the MS spectrum, the second contained only the peaks of ions of the aglycon moiety.

Conversely, the FAB ("−") spectra of the same compound were extremely informative. The peaks of the $(M - H)^-$ ions in them were among the most intense. The successive elimination of all the carbohydrate units, including the glucose substituent at C-3 in the form of GlcH and GlcOH was observed, which is not characteristic for the LSIMS spectra of medicosides (III) and (IV).

Thus, it can be seen from the examples given that the FAB ("−") method possesses some advantages which, however, need checking on other substances. At the same time, the LSIMS method shows a fair degree of universality.

## EXPERIMENTAL

LSIMS spectra were recorded on a M 80-A mass spectrometer (Hitachi, Japan) with a M-003 computer data-processing system. The conditions for recording them are given in [11].

The linked-scanning (B/E = const) spectra were recorded on a MKh 1310 instrument with a LSIMS ion source constructed in the Institute of Analytical Instrument Construction of the Russian Academy of Sciences (Leningrad). Ionization was achieved with a beam of accelerated $Cs^+$ ions having an energy of 7 keV; the accelerating voltage was 5 kV.

The FAB positive- and negative-ion spectra were taken on a Finnegan MAT-8430 mass spectrometer with reverse-geometry double focusing fitted with an Ion Tech. ion source and a Spectrosystem MAT-300 data-processing system. Ionization was achieved with a beam of accelerated Xe atoms having an energy of 8 keV; the accelerating voltage was 3 kV. The specimens were dispersed in glycerol and deposited on a steel target for direct introduction of the sample. The resolving power of the instrument was ~1000.

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# SYNTHESIS OF THE RACEMIC ALKALOID DIPTALINE

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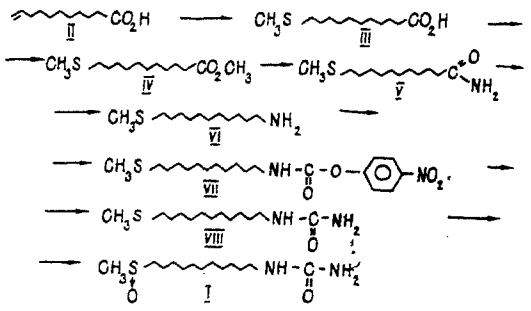
The isolation from the plant Dipthychocarpus strictus (Cruciferae) of the optically active base diptaline, identified as N-(11-methylsulfinylundecyl)urea, has been reported [1]. A synthesis of the racemic form of this alkaloid has now been achieved.

In the development of investigations on the total synthesis of racemic alkaloids of the urea type [2-4], we have devised a rational approach to the synthesis of achiral diptaline (I) based on the transformation of the readily available undec-10-enoic acid (II). Its thiylation at the $\Delta^{10}$ double bond with methyl mercaptan under UV irradiation gave a 74% yield of 12-thiatridecanoic acid (III), the methyl ester (IV) of which was converted by treatment with ammonia in methanolic solution in 74% yield into the amide (V), and this was reduced by the action of $LiAlH_4$ to the key 12-thiatridecylamine (VI) (85%). The reaction of the amine (VI) with bis(4-nitrophenyl) carbonate in $CH_2Cl_2$ solution at −10°C [5] led with a yield of 57% to the intermediate carbamate (VII), the interaction of which with ammonia under the same conditions gave an 82% yield of the sulfide precursor (VIII) of alkaloid (I). Oxidation of compound (VIII) with hydrogen peroxide completed the synthesis of racemic diptaline (I). This, unlike the native product, is a crystalline substance, which is apparently explained by the optically inactive form of the synthetic alkaloid. The overall yield of (±)-diptaline calculated on the initial acid (II) was 14%.

In the PMR spectrum of the sulfide precursor (VIII) the signals of the protons of the $CH_3$ and $CH_2$ groups adjacent to the sulfur atom were observed in the form of a singlet (δ 2.08 ppm) and a triplet (δ 2.48 ppm, J = 7.2 Hz), respectively. In the case of (±)-diptaline (I) the signals of the protons of the analogous groups appeared in a weaker field (δ 2.56 and 2.72 ppm) because of the descreening influence of the SO group.



The mass spectra of compounds (I) and (VIII) contained the peaks of molecular ions corresponding to the assumed empirical compositions ($C_{13}H_{28}N_2OS$, $C_{13}H_{28}N_2O_2S$) and the peaks of $(M + 2)^+$ ions corresponding to the natural distribution of the isotope $^{34}S$ for the pres-

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