SUMMARY

The roots of *Ferula akitschkensis* have yielded two new esters — akiferidin and akiferidinin — for which the structures of 6-O-(3',4'-dihydroxybenzoyl)ferutinol and 2-O-angeloyl-6-O-(3',4'-dihydroxybenzoyl)akichenol, respectively, have been proposed.

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FEATURES OF THE MASS-SPECTROMETRIC FRAGMENTATION OF THE TEUCRINS H

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UDC 543.51+547.913

At the present time, about 16 diterpene lactones that are derivatives of clerodane and 18-norclerodane are known, these having been isolated mainly from species of *Teucrin* (family Labiatae) and being characterized by the presence of a C_{20}—C_{12}-γ-lactone group; a C_{17}-methyl group, and a furan ring at C_{12} formed by the C_{13}, C_{14}, C_{15}, and C_{16} atoms of the clerodane system [1-5]. These lactones also include the teucrins H1-H4 (I, II, III, and IV) which we have isolated from *Teucrium hyrcanicum* L. (Hyrcanian germander) [6, 7].

The lactones of this group have been little studied mass-spectrometrically. They all differ considerably in their degree of oxidation, which is responsible for the different variants of the localization of the positive charge in the molecular ions and, consequently, for the diversity of the pathways of mass-spectrometric fragmentation and for the complexity of the interpretation of the spectra.

However, a consideration of the few mass-spectrometric characteristics of the known lactones of this series given in the literature and, in particular, the analysis of the spectra of teucrins H1-H4 and the products of their transformation (V-VII) have enabled us to isolate for analytical purposes a number of common fragments and fragmentation pathways of the molecules under the action of electron impact (Schemes 1-3).

Certain authors have already reported the presence in the spectra of lactones of this group of strong peaks at m/e 95, 94, and 81 corresponding to the ions **q**, **r**, and **s** and due to the presence in the compounds studied of a β-substituted furan fragment [1-3]. The ions **q**, **r**, and **s** were also observed in the mass spectra of the teucrins and their derivatives that we studied, with relative intensities of from 12.5 to 100% and the elementary compositions C_6H_7O, C_6H_6O, and C_5H_5O (from the results of high-resolution spectroscopy).

The presence in teucrins H of a C_{20}—C_{12}-γ-lactone is obviously responsible for the ejection of β-vinylfuran from the molecular ions and the formation of the ion radical **a** $(M - 94)^+$. The formation of ion **a** is not characteristic for the diterpene furolactones marrubiin, columbin, and isocolumbin, which do not contain such a lactone system [8, 9].

The ejection of β-vinylfuran in the case of compounds (I), (IV), (V), and (VI) is also observed from the ions $M^+ - ROH$, which leads to the ions **b** $(M - ROH - 94)^+$, while for teucrin H3 (III) the elimination of β-vinylfuran takes place after the splitting out of formaldehyde and ketene, giving the ion **c** $(M - CH_2O - CH_2CO - 94)^+$.

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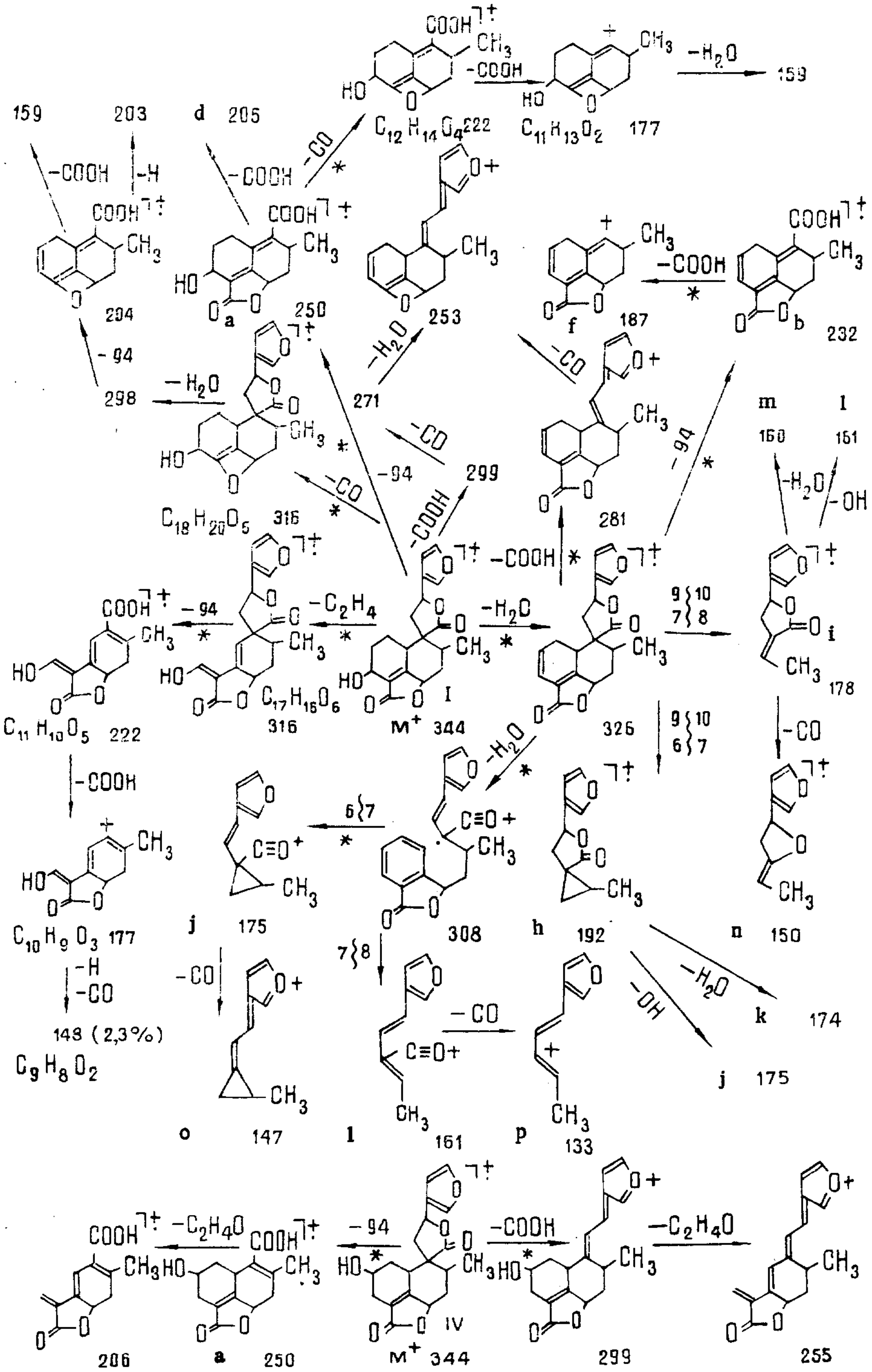
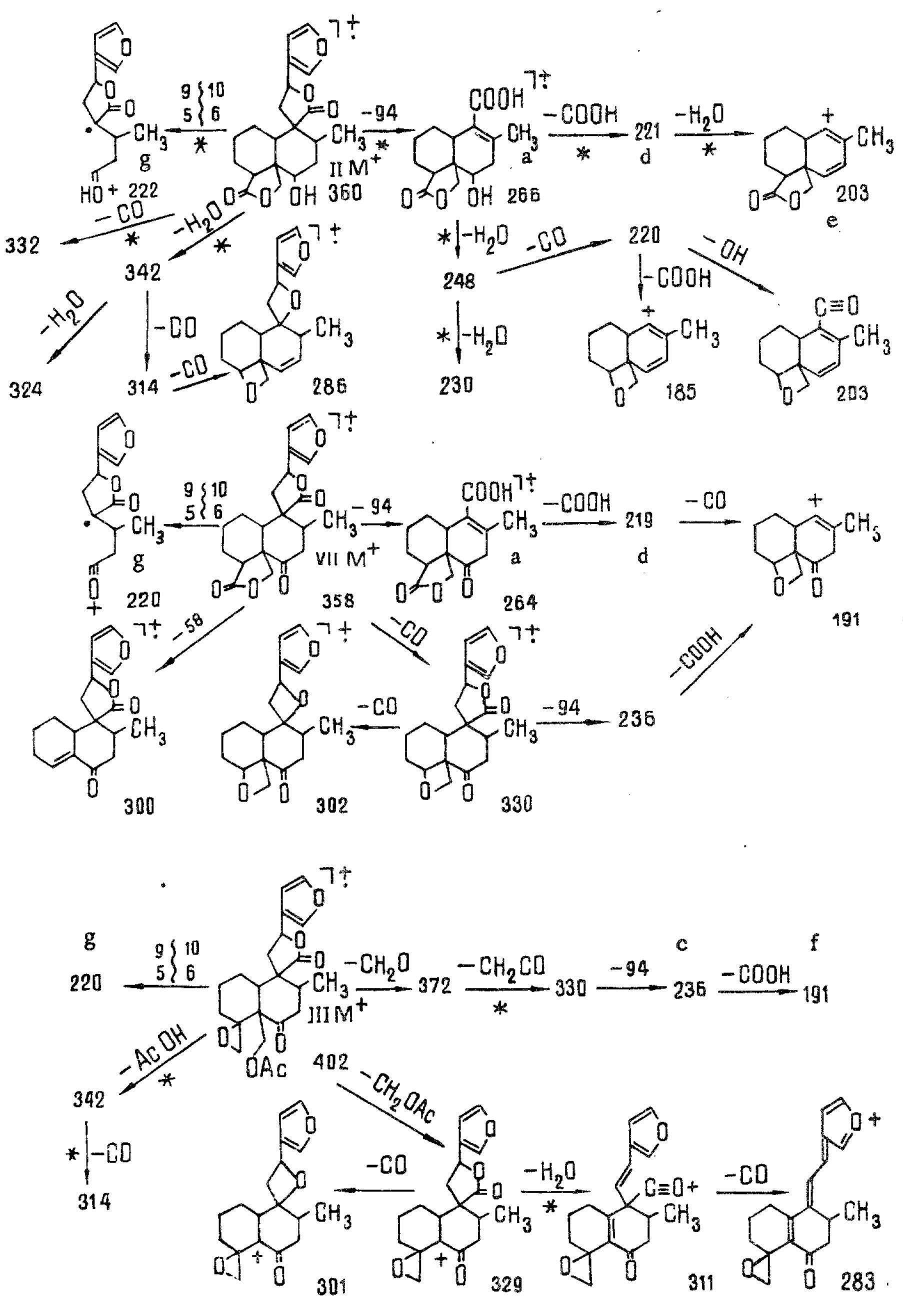
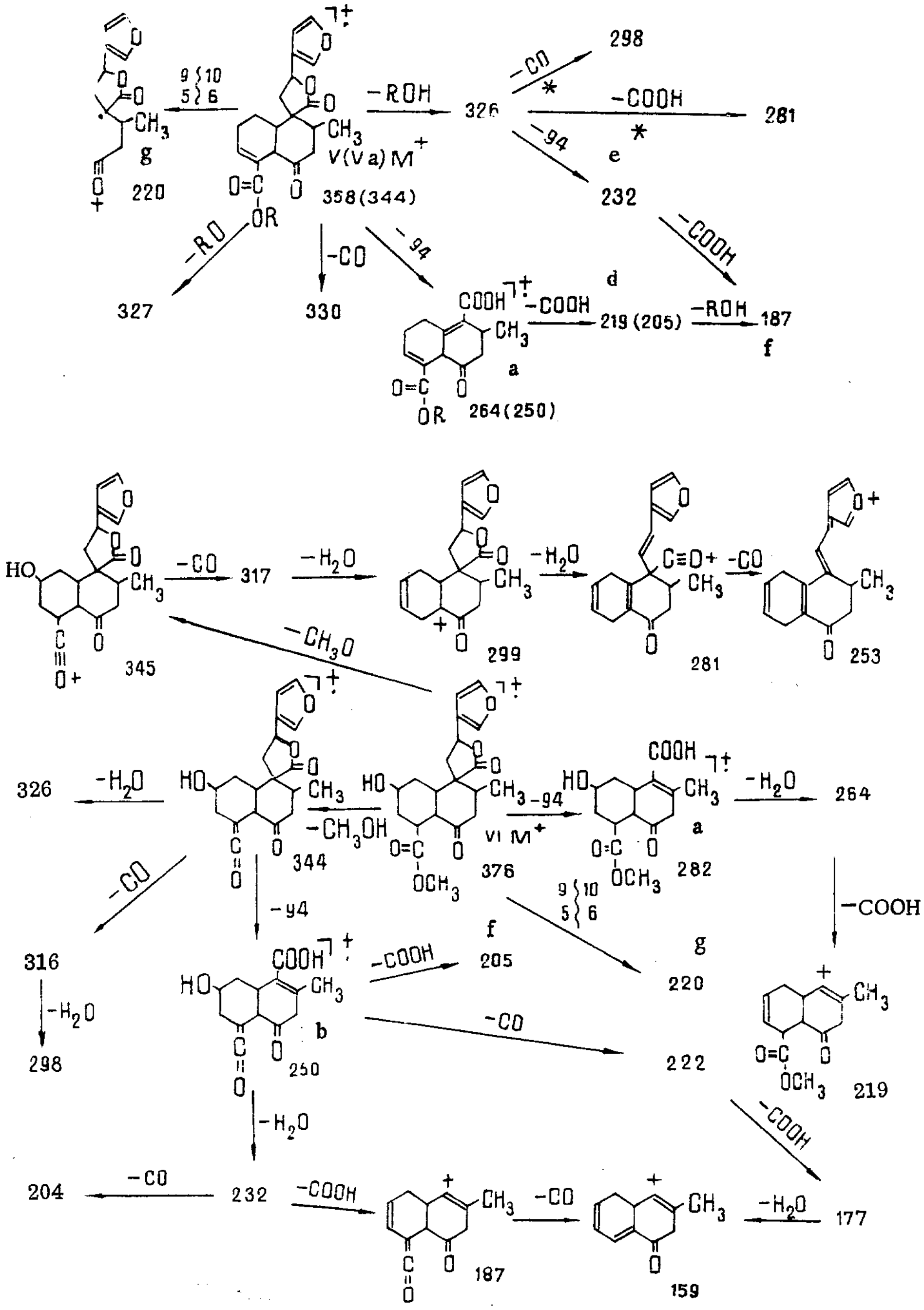


COOH
CH3
-COOH
HO
O
C12H14O4 222
C11H13O2
177
-H2O
159
159
203
d
205
-COOH
-H
-COOH
-CO
*
O+
COOH
CH3
294
HO
a
250
253
+
CH3
-COOH
*
f
187
b
232
-94
298
-H2O
-H2O
271
-CO
316
C18H20O5
-CO
-94
-COOH
299
281
-94
m
l
160
161
-H2O
-OH
COOH
-94
HO
C11H10O5 222
-C2H4
C17H16O6
316
I
M+ 344
-H2O
326
9{10
7{8
i
178
9{10
6{7
-H2O
-CO
-COOH
6{7
C≡O+
j
175
308
h
192
n
150
C10H9O3 177
-H
-CO
148 (2,3%)
C9H8O2
-CO
7{8
-CO
-OH
-H2O
k
174
o
147
l
161
p
133
j
175
-C2H4O
-94
IV
M+ 344
-COOH
-C2H4O
206
a
250
299
255

SCHEME 1

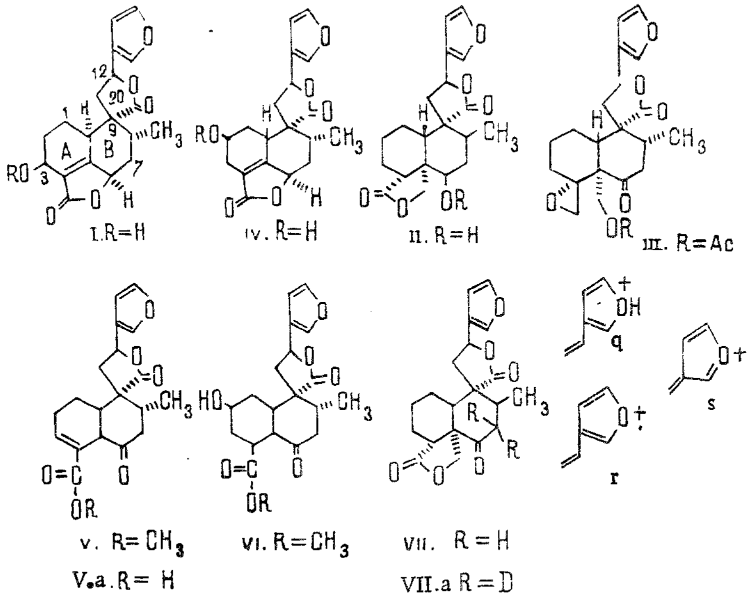

9 { 10
5 { 6
g
HO+ 222
II M+
360
−94
COOH
a
266
−COOH
221
d
−H2O
+
CH3
203
e
−CO
332
−H2O
342
−H2O
324
−CO
314
−CO
286
−H2O
248
−CO
220
−OH
−COOH
185
C≡O
203
−H2O
230
g
220
VII M+
358
−94
264
−COOH
219
d
−CO
191
−58
300
302
−CO
330
−94
236
−COOH
g
220
III M+
402
OAc
−CH2O
372
−CH2CO
330
−94
c
236
−COOH
f
191
−AcOH
342
−CO
314
−CH2OAc
−CO
301
329
−H2O
C≡O+
311
−CO
283

SCHEME 2



9 ⌠10
5 ⌡ 6
g
220
V (V a) M⁺
358 (344)
−ROH
326
−CO
298
−COOH
281
−94
e
232
−COOH
−RO
327
−CO
330
−94
d
COOH
−COOH
219 (205)
−ROH
187
f
a
264 (250)
OR
CH₃
HO
345
−CO
317
−H₂O
299
−H₂O
281
−CO
253
−CH₃O
326
−H₂O
344
−CH₃OH
VI M⁺
376
3 −94
282
a
−H₂O
264
−COOH
219
−CO
316
−H₂O
298
−94
b
250
−COOH
205
9 ⌠10
5 ⌡ 6
g
220
−CO
222
−COOH
177
−H₂O
232
−CO
204
−COOH
187
−CO
159
OCH₃

SCHEME 3



I. R=H IV. R=H II. R=H III. R=Ac

V. R=CH_3 VI. R=CH_3 VII. R=H

V.a. R=H VII.a R=D

The elimination of β-vinylfuran from the molecular and intermediate ions is obviously accompanied by the appearance of a carboxy group at C_9 through the cleaveage of the lactone ring and the migration of hydrogen from C_{10} or C_8. The appearance of a carboxy group is shown by the ejection by part of the ions **a**, **b**, and **c** of a carboxy group and their conversion into the ions **d** $(M - 94 - COOH)^+$, **e** $(M - 94 - COOH - H_2O)^+$, and **f** $(M - ROH - 94 - COOH$ and $M - CH_2O - CH_2CO - 94 - COOH)^+$, which have a relatively low intensity.

The reality of the transitions described and of the further fragmentations of these ions is confirmed by the corresponding metastable peaks and by the accurate determination of the masses of the ions.

The subsequent qualitative similarity of the fragmentation of the teucrins under the action of electron impact must be due to cleavage at the C_9—C_{10} bond accompanied by cleavages at C_7—C_8, C_6—C_7, and C_5—C_6, depending on the substituents in ring B. These directions of fragmentation, extremely different quantitatively for (I-VII), lead to the appearance of several types of furan-containing fragmentary ions which may be considered as characteristic. The first type of ions (the ions **g** with even mass numbers) is formed in the case of compounds (II), (III), (V), (VI), and (VII) as the result of double C_9—C_{10}, and C_5—C_6 cleavage. The ions of the second type, the **h** ions (with m/e 192; $C_{11}H_{12}O_3$) and **i** ions (with m/e 178; $C_{10}H_{10}O_3$) are formed by double C_9—C_{10}, C_6—C_7, and C_9—C_{10}, C_7—C_8 cleavages, respectively. The ions of the third type, ions with odd mass numbers — **j** (with m/e 175; $C_{11}H_{11}O_2$), **l** (with m/e 161; $C_{10}H_9O_2$), **o** (with m/e 147; $C_{10}H_{11}O$) and **p** (with m/e 133; C_9H_9O) — are obviously genetically dependent on the ions **h** and **i**, as is shown by the presence in the spectrum of teucrin H2 (II) of metastable peaks at m/e 141.5 and 117.5 corresponding to the transitions m/e 178 → m/e 161, and m/e 192 → m/e 147. On the other hand, the metastable peak at m/e 99.5 in the case of teucrin H4 (IV) (m/e 308 → m/e 175) shows the possibility of a second pathway for the formation of these ions, as well. Finally, the fourth type of ions in this group is obtained, judging from their compositions, from the ions **h** and **i** by the splitting out of water — **k** ($h - H_2O$; m/e 174), **m** ($i - H_2O$; m/e 160) — and by the ejection of a carbonyl group from ion **i** — **n** ($i - CO$; m/e 150).

The facts given and the detection of ions **h**-**p** in the mass spectra of all the compounds studied has permitted structures shown in the Schemes to be assigned to them.

The formation of a carboxy group at C_9 as the result of the migration of hydrogen from C_{11} to the oxygen of the upper lactone group, which also leads to the appearance of a C_{11}—C_{12} double bond, must also be regarded as a characteristic feature of the mass-spectrometric fragmentation of the teucrins.

This process is, in all probability, connected with the appearance of ions with m/e 308, 299, 281, and 253 in the spectra of (I) and (IV), 324 [for (II)], 311 [for (III)], and 326 [for (VI)], and also with the transformation of the ions **h** and **i** into the ions **j**-**p**. The ion with m/e 308 is formed as the result of the splitting out of water through the

TABLE 1. Main and Characteristic Ions in the Mass Spectra of Teucrins H1-H4 and Their Derivatives

Ion	I	II	III	IV	V [Va]†	VI	VII [VIIa]†
M^+	$C_{19}H_{20}O_6$ 344 (5,6)	360 (82)	$C_{22}H_{26}O_7$ 402 (0,5)	$C_{19}H_{20}O_6$ 344 (5,8)	358 (22) [344 (64)]	$C_{20}H_{24}O_7$ 376 (17.2)	358 (48) [360 (100)]
$(M-ROH)^+$	326 (10,3) $C_{19}H_{18}O_5$	342 (3)	342 (0,8) $C_{20}H_{22}O_5$	326 (3,3) $C_{19}H_{18}O_5$	326 (49) [(74)]	344 (30,3) $C_{20}H_{22}O_6$	—
(M—CO)	316 (0,7) $C_{18}H_{20}O_5$	332 (3)	—	316 (0,2) $C_{18}H_{20}O_5$	330 (2) [316 (5)]	—	330 (2,5) [332 (7)]
$(M-ROH-H_2O)$	380 (3,0) $C_{19}H_{16}O_4$	324 (2)	—	308 (0,4) $C_{19}H_{16}O_4$	—	326 (4,2) $C_{19}H_{18}O_5$	—
a (M—94)	250 (1,9) $C_{13}H_{14}O_5$	266 (52)	—	250 (41,5) $C_{13}H_{14}O_5$	264 (1) [250 (6)]	282 (35,2) $C_{11}H_{18}O_6$	264 (12) [266 (16)]
b (M—ROH—94)	232 (32,5) $C_{19}H_{12}O_4$	—	—	232 (26.7) $C_{13}H_{12}O_4$	232 (15) [(40)]	250 (75,8) $C_{13}H_{14}O_5$	—
c (M—30—42—94)	—	—	236 (30) $C_{13}H_{16}O_4$	—	—	—	—
d (a—COOH)	205 (1) $C_{12}H_{13}O_3$	221 (6)	—	205 (2,6) $C_{12}H_{13}O_3$	219 (2) [205 (11)]	—	219 (15) [221 (17)]
e $(a-COOH-H_2O)$	—	203 (11)	—	—	—	—	—
f (b—COOH) (c—COOH)	187 (3,9) $C_{12}H_{11}O_2$		191 (3,8) $C_{12}H_{15}O_2$	187 (5.5) $C_{12}H_{11}O_2$	187 (26) [(25)]	205 (4,7) $C_{12}H_{13}O_3$	—
g	—	222 (21)	220 (2,8) $C_{12}H_{12}O_4$	—	220 (98) [(100)]	220 (35,3) $C_{12}H_{12}O_4$	220 (18) [222 (21)]
h *m/e* 192 $C_{11}H_{12}O_3$	(3,8)	(12)	(2)	(1)	(8) [(6)]	(3,2)	(5) [194 (5)]
i *m/e* 178 $C_{10}H_{10}O_3$	(10,5)	(16)	(13,9)	(8,4)	(100) [(68)]	(53,4)	(18) [178 (31)]
j *m/e* 175 $C_{11}H_{11}O_2$	(2,5)	(11)	(4,2)	(3,9)	(8) [(7)]	(3,2)	(6) [177 (4)]
k *m/e* 174 $C_{11}H_{10}O_2$	(1,7)	(11,	(1,3)	(1,1)	(8) [(7)]	(1,8)	(5) [176 (20)]
l *m/e* 161 $C_{10}H_9O_2$	(2,4)	(11)	(11)	(7,3)	(25) [(26)]	(20,8)	(6) [(13)]
m *m/e* 160 $C_{10}H_8O_2$	(1,4)	(2)	(2;2)	(1,8)	(17) [(12)]	(8,6)	(3) [(4)]
n *m/e* 150 $C_9H_{10}O_2$	(1)	(4)	(2,6)	(0,5)	(5) [(5)]	(2,7)	(9) [(10)]
o *m/e* 147 $C_{10}H_{11}O$	(5,3)	(17)	(6,5)	(3,0)	(16) [(8)]	(8,9)	(6) [149 (13)]
p *m/e* 133C_9H_9O	(5,3)	(14)	(12,9)	(7,6)	(13) [(6)]	(12)	(14) [(11)]
q *m/e* 95 C_6H_7O	(27,6)	(100)	(75,5)	(58)	(30) [(33)]	(49,5)	(68) [(87)]
r *m/e* 94 C_6H_6O	(100)	(73)	(26,7)	(100)	(22) [(20)]	(100)	(46) [(62)]
s *m/e* 81 C_5H_5O	(12,5)	(38)	(57)	(29)	(20) [(24)]	(30,2)	(36) [(37)]
Other ions	316 (0,3) $C_{17}H_{16}O_6$	314 (3)	372 (0,6) $C_{21}H_{24}O_6$	299 (1,3) $C_{18}H_{19}O_4$	327 (29) [(17)]	358 (3,3) $C_{20}H_{22}O_6$	313 (2) [315 (4)]
	299 (0,5) $C_{18}N_{19}O_4$	310 (10)	330 (68,7) $C_{19}H_{22}O_5$	298 (1,4) $C_{18}H_{18}O_4$	298 (10)](12)]	345 (18,2) $C_{19}H_{21}O_6$	312 (2) [314 (5)]
	298 (1,5) $C_{18}H_{18}O_4$	286 (7)	329 (47,9) $C_{19}H_{21}O_5$	281 (4,1) $C_{18}H_{17}O_3$	281 (3) [(4)]	317 (4,5) $C_{18}H_{21}O_5$	302 (2) [304 (2)]

TABLE 1 (Continued)

Ion	I	II	III	IV	V [Va]	VI	VII [VIIa]
	281 (2,0) $C_{18}H_{17}O_3$	248 (36)	314 (2,0) $C_{19}H_{22}O_4$	271 (1,1) $C_{17}H_{19}O_3$	280 (3) [(4)]	316 (3,6) $C_{18}H_{20}O_5$	300 (2) [302 (4)]
	271 (1,1) $C_{17}H_{19}O_3$	273 (13)	311 (100) $C_{19}H_{19}O_4$	255 (0,4) $C_{16}H_{15}O_3$	270 (3) [(7)]	299 (5,1) $C_{18}H_{19}O_4$	285 (3) [287 (8.5)]
	245 (6) $C_{14}H_{13}O_4$	230 (7)	301 (9) $C_{18}H_{21}O_4$	253 (1,2) $C_{17}H_{17}O_2$	255 (9) [(11)]	298 (3,8) $C_{18}H_{18}O_4$	236 (6) [(238 (6)]
	222 (13,8) $C_{11}H_{10}O_5$	220 (20)	285 (7,7) $C_{18}H_{21}O_3$	245 (1,7) $C_{14}H_{13}O_4$	204 (4)[(21)]	281 (2,6) $C_{18}H_{17}O_3$	234 (6) [236 (5)]
	222 (3,3) $C_{12}H_{14}O_4$	194 (12)	283 (6,2) $C_{18}H_{19}O_3$	222 (11,2) $C_{12}H_{14}O_4$	202 (25) [(26)]	264 (3,7) $C_{14}H_{16}O_5$	202 (10) [204 (11)]
	221 (10,8) $C_{12}H_{13}O_4$	185 (4)	267 (3,3) $C_{18}H_{19}O_2$	221 (6,4) $C_{12}H_{13}O_4$	186 (17) [(18)]	253 (2,7) $C_{17}H_{17}O_2$	191 (6) [193 (5)]
	204 (1) $C_{12}H_{12}O_3$		235 (10,7) $C_{13}H_{15}O_4$	206 (0,9) $C_{11}H_{11}O_4$	134 (19) [(10)]	232 (16,7) $C_{13}H_{12}O_4$	149 (22) [151 (6)] [149 (13)]
	203 (2) $C_{12}H_{11}O_3$		218 (10,5) $C_{13}H_{14}O_3$	204 (10,2) $C_{12}H_{13}O_3$	121 (51) [(22)]	222 (12,6) $C_{12}H_{14}O_4$	121 (16) [(12)]
	201 (2,6) $C_{13}H_{13}O_2$		207 (8,5) $C_{12}H_{15}O_3$	203 (2,4) $C_{12}H_{11}O_3$		219 (2,2) $C_{13}H_{15}O_3$	
	199 (7.2) $C_{13}H_{11}O_2$		189 (6,5) $C_{12}H_{13}O_2$	201 (4,5) $C_{13}H_{13}O_2$		204 (12,1) $C_{12}H_{12}O_3$	
	177 (0,6) $C_{11}H_{13}O_2$		185 (16.5) $C_{13}H_{13}O$	199 (11,8) $C_{13}H_{11}O_2$		202 (11,3) $C_{12}H_{10}O_3$	
	177 (2,4) $C_{10}H_9O_3$		173 (15,6) $C_{12}H_{13}O$	177 (4,5) $C_{11}H_{13}O_2$		187 (8,8) $C_{11}H_7O_3$	
	176 (7,4) $C_{10}H_8O_3$		121 (6,5) $C_7H_5O_2$	176 (2,5) $C_{11}H_{12}O_2$		177 (4,2) $C_{11}H_{13}O_2$	
	159 (2,0) $C_{11}H_{11}O$			159 (4,8) $C_{11}H_{11}O$		159(7,4) $C_{11}H_{11}O$	
	135 (11,0) $C_8H_7O_2$			133 (0,8) $C_8H_5O_2$		155 (12,3) $C_8H_{11}O_3$	
	133 (1,7) $C_8H_5O_2$					137 (5,1) $C_8H_9O_2$	
						121 (27,2) $C_7H_5O_2$	

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*The relative intensities of the ions in percentages are given in parentheses.

†The mass numbers of the corresponding ions of (VIa) and (VIIa) and their intensities in percentages are given in brackets.

hydroxy group of the carboxyl and the hydrogen at C_1 with the simultaneous cleaveage of the C_9—C_{10} bond. In fact, it is the further decomposition of ions of the m/e 308 type that leads to the ions **j**, **l**, **o**, and **p**.

Compounds (I-VII) are lactones, and therefore the ejection of 28 m.u. from their molecular or fragmentary ions, which is extremely characteristic for their spectra, is due almost exclusively to the splitting out of CO. Only in the case of (I), thanks to the presence of a Δ^4 bond and the possibility of the occurrence of decomposition in the manner of a retrodiene reaction does the elimination of the M^+ 28 m.u. ion in the form of C_2H_4 take place with the formation of an ion having m/e 316 ($C_{17}H_{16}O_6$). In the case of teucrin H4, indeed, decomposition of the retrodiene type with the elimination now of 44 m.u. (C_2H_4O) takes place in the case of the ions with m/e 299 and 250, giving ions with m/e 255($C_{16}H_{15}O_3$) and 206 ($C_{11}H_{10}O_4$).

Teucrin H4 differs from teucrin H1 by the position of the hydroxy group and the stereochemistry at C_{10} and C_{12}. Their structural closeness has also found its reflection in the mass spectra which, in the main, differ only qualitatively [for example, for (IV) the main direction of fragmentation of the M^+ ion is the elimination of β-vinylfuran, and for (I) it is the elimination of water]. The small qualitative differences in their spectra are connected with different ions from the retrodiene type of fragmentation.

Characteristic directions of the fragmentation of the M^+ ion of teucrin H3 (III) are, on the one hand, the ejection of a CH_2—OAc group and, on the other hand, the ejection of formaldehyde, obviously as the result of transannular cleavage [10]. The ions so formed, with m/e 329 and 372, lead to a series of ions with m/e 311, 283, 301, 207, 285, and 191, and m/e 330, 236, and 173, respectively.

A characteristic direction of the decomposition of the M^+ ion of the keto derivatives (V) and (VI) is the elimination of methanol, and for the M^+ ion of (Va) the elimination of water at the expense of the acid hydroxyl.

All the most intensive ions and also those characteristic for compounds (I-VII) are given in Table 1.

The at least 15 fragmentary ions characteristic for teucrins H and their derivatives that have been detected on the basis of the analysis performed may play an auxiliary analytical role in the structural investigations of new diterpene lactones of the clerodane series.

EXPERIMENTAL

The mass spectra of the compounds were obtained on an MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source; the temperature of the inlet system was 90-120°C, and the ionizing voltage was 40 V; mass spectra were taken on an LKB-9000 instrument at the same temperature with an ionizing voltage of 70 V. The high-resolution mass spectra were obtained on an MS-902 instrument with a DS-30 data-processing system (Institute of Bioorganic Chemistry of the Academy of Sciences of the USSR).*

SUMMARY

Characteristic directions of the mass-spectrometric fragmentation of teucrins H are the elimination of β-vinylfuran and a carboxy group, and also the formation of furan-containing ions with the compositions $C_{11}H_{12}O_3$ (m/e 192), $C_{10}H_{10}O_3$ (m/e 178), $C_{11}H_{11}O_2$ (m/e 175), $C_{11}H_{10}O_2$ (m/e 174), $C_{10}H_9O_2$ (m/e 161), $C_{10}H_8O_2$ (m/e 160), $C_9H_{10}O_2$ (m/e 150), $C_{10}H_{11}O$ (m/e 147), C_9H_9O (m/e 133), C_6H_7O (m/e 95), C_6H_6O (m/e 94) C_5H_5O (m/e 81).

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CHROMATO-SPECTROPHOTOMETRIC METHOD OF DETERMINING ECDYSTERONE IN PLANT RAW MATERIAL

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Ecdysones are natural polyhydroxysteroid compounds possessing the activity of hormones for the moulting and metamorphosis of arthropods. Ecdysterone (β-ecdysone) is found most frequently in the vegetable kingdom [1, 2]; this possesses an anabolic effect [3].

In connection with the search for the most profitable source of ecdysterone the necessity has arisen for the development of a method for its quantitative determination in plant raw material. The qualitative identification of ecdysterone is carried out with the aid of TLC, GLC, mass spectrometry, and other physicochemical methods [4]. For quantitative purposes, at the present time fluorimetry in sulfuric acid [5], thin-layer chromatography with subsequent analysis by the photochemical method [6, 7], and gas–liquid chromatography of the trimethylsilyl derivatives of ecdysterone [8, 9] are used.

We have developed a chromato-spectrophotometric method of determining ecdysterone in the epigeal part and in the roots of *Rhaponticum orientale* Soscov and *Rhaponticum integrifolium* C. Winkl (family Compositae), which were known to contain cedysones from previous investigations [10, 11].

The UV spectrum of ecdysterone [12] has the absorption maximum (π → π* transition) λ_{max}^{MeOH} 242 nm (log ε 4.07; $E_{1\,cm}^{1\%}$ = 255 ± 4.5) because of the presence of a Δ^7-6-keto grouping in the molecule [13]. The intensity of this extremum permits it to be used as an ana-

TABLE 1. Statistical Treatment of the Results of the Chromato-spectrophotometric Determination of Ecdysterone in the Roots of *Rhaponticum orientale* (P = 0.95; n = 6)

Found, % (x)	$(x_{av} - x)$ %	$(x_{av}-x)^2 \cdot 10^{-6}$ %	Metrological characteristics
0,141	0,008	64	$S=\sqrt{\frac{\Sigma (x_{av}-x)^2}{n-1}}$
0,157	−0,008	64	
0,151	−0,002	4	$S=\sqrt{\frac{226 \cdot 10^{-6}}{5}}=\pm 0,0067\%$
0,146	0,003	9	
0,156	−0,007	49	$\varepsilon_\alpha = \pm \frac{S \cdot t_{\alpha n}}{\sqrt{n}}$
0,143	0,006	36	
			$\varepsilon_\alpha = \frac{0,0067 \cdot 2,271}{\sqrt{6}} = \pm 0,0069\%$
$x_{av}=0,149$		$(x_{av}-x)^2=226$	$x=0,149 \pm 0,0069\%$

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