

IDENTIFICATION AND BIOLOGICAL ACTIVITY OF THE VOLATILE ORGANIC SUBSTANCES EMITTED BY PLANTS AND INSECTS
II. SESQUITERPENE COMPOSITION OF THE NATIVE SCENT OF LEAVES OF THE POTATO *Solanum tuberosum*

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*By dynamic gas extraction of the volatile components of the native scent of the leaves of the potato *Solanum tuberosum* L. growing under natural conditions, followed by the gas-chromatographic separation and mass-spectrometric analysis of the substances obtained, the following sesquiterpene compounds have been identified: trans- and cis-caryophyllenes, α -ylangene, α -copaene, β -bourbonene, β -elemene, Z- β -farnesene, aromadendrene, α -humulene, α -elemene, β -bisabolene, γ -cadinene, and δ -cadinene.*

According to the literature [2, 3], C₆ and C₇ aliphatic alcohols and aldehydes and geraniol, linalool, caryophyllene, and terpineol have been isolated and identified from aqueous extracts of leaves of the potato *Solanum tuberosum* L. At the same time, it is known that the composition and structure of extractive substances differ substantially from the hydrocarbon components constituting the native scent of living plants [4].

We have previously reported the identification of *trans*-caryophyllene as the main component of the native scent of potato leaves and of the volatiles emitted by the Colorado beetle *Leptinotarsa decemlineata* Say [1, 5]. The aim of the present work was to establish the composition and structure of the native scent of potato plants, which will lead to a deeper understanding of the mechanisms of chemocommunication in the biological pair of potato—Colorado beetle and also to the creation of effective methods of combating this pest.

To identify the volatile substances emitted into the atmosphere by potato leaves we used a procedure [6—11] based on the dynamic gas extraction of these compounds with the aid of an effective adsorbent under the conditions of the natural growth of the plant, followed by the desorption and analysis of the terpenes obtained with the aid of capillary gas chromatography and mass spectrometry, and also by comparing the hydrocarbons obtained with authentically known specimens.

A chromatogram of the mixture of substances constituting the native scent of a potato of the variety Adretta, which is shown in Fig. 1, contains more than a score of peaks in the region characteristic for the retention times of sesquiterpenes ($\tau = 12$ —20 min). We analyzed peaks 1—21 the intensities of which in the mixture exceeded a level of 1%. For the majority of the peaks (excluding 15, 16, and 19) the mass spectrum (MS) contained the molecular ion (m/z 204) witnessing that they belonged to the sesquiterpene series (empirical formula C₁₅H₂₄). Each of the chromatographic peaks was identified from its retention index (*I*), which was calculated as described in the literature [6, 9] and was compared with the published values, I_{lit} , for the sesquiterpene series [9]. Their agreement (within the limits of standard deviations) was a necessary condition for the identification of the compounds. A second criterion was the agreement of the parameters of the MS of the compound undergoing analysis with its structural characteristics and with the information given in the Hewlett-Packard MS Data Base [12].

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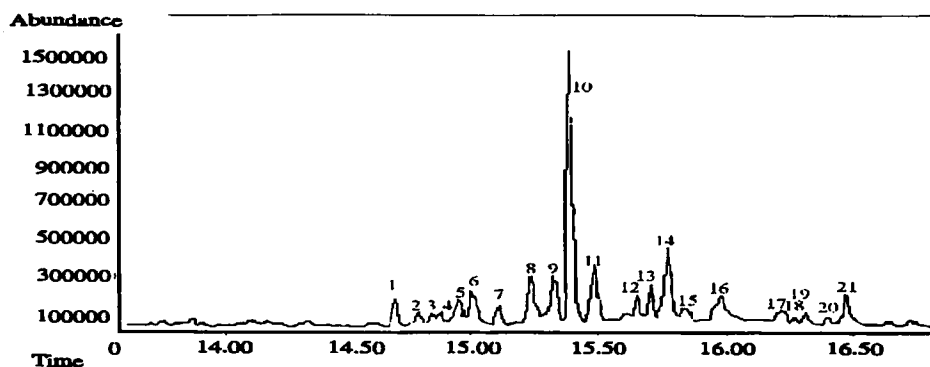


Fig. 1. Chromatogram of the native scent of leaves of a potato of the variety Adretta.

The results of the identification are given in Table 1. The chromatographic characteristics of the main component (peak 10, 26.6%, $I = 1419$) are identical with those of an authentic specimen and correspond to those given in the literature for *trans*-caryophyllene (1) ($I_{lit} = 1417 \pm 6$). Chromatographic peak 11 ($I = 1427$) belongs to *cis*-caryophyllene (2). In its MS we observed ions with m/z 189 and 161, corresponding to the splitting out from $[M^+]$ of methyl and isopropyl groups, respectively. The breakdown of the cyclobutane ring took place with the formation of an ion having m/z 148, and the cleavage of the cyclononene fragment of the molecule was accompanied by the appearance of tropylium ions (m/z 133 and 119) and a dimethylcyclopentadienyl cation (m/z 93).

Chromatographic peak 1 ($I = 1357$) coincided (within the limits of standard deviations) with the values of I for α -longipinene ($I_{lit} = 1358 \pm 2$). The MS of peak 1 contained $[M^+]$ 204 and also intense fragmentary ions with m/z 189 and 161, corresponding to the splitting out of methyl and isopropyl groups, the ion with m/z 161 being the maximum ion. At the same time, according to the literature [12], in the MS of α -longipinene the ion with m/z 119 has the maximum intensity, while in the MS that we observed the relative intensity of the ion with m/z 119, which corresponds to the formation of a dimethyltropylium cation, amounted to 20%. This introduces uncertainty into the identification of peak 1 as α -longipinene.

The MS of peak 2 ($I = 1366$), coinciding in retention index with α -ylangene (3) ($I_{lit} = 1364 \pm 5$), contained a characteristic fragmentary ion with m/z 161, corresponding to the splitting out of an isopropyl group from $[M^+]$, and also intense ions corresponding to dimethyltropylium and dimethylcyclopentadienyl cations with m/z 119 and 93, respectively.

Both from its retention index ($I_{lit} = 1372 \pm 8$) and from the characteristics of its MS, component (3) ($I = 1371$) corresponded to the tricyclic sesquiterpene lactone α -copaene (4). Peak 4 ($I = 1375$) corresponded to the structure of β -bourbonene (5), the MS of which is characterized by the cleavage of the cyclobutane ring with the ejection of ions having m/z 81 and 123. According to the literature [12], the mass spectral characteristics of peak 5 coincided completely with those of γ -gurjunene (6). However, the absence from the data base [9] of a retention index for γ -gurjunene does not permit a positive identification of this peak as compound (6).

The MS of peak 6 corresponded completely to that given in the literature for β -elemene (7), and their retention indices were also close. Chromatographic peak 7 ($I = 1394$) had an MS corresponding to the norsesquiterpene ketone (8) for which, however, no figures for I are given in the literature. The MS characteristics of peak 8 ($I = 1406$) were close to those of isocaryophyllene (No. 128696 in the Hewlett-Packard MS Data Base [12]). The identity of isocaryophyllene with *cis*-caryophyllene has been reported in the literature [14]. Consequently, the eighth peak apparently belonged to a sesquiterpene having a structure close to that of *cis*-caryophyllene.

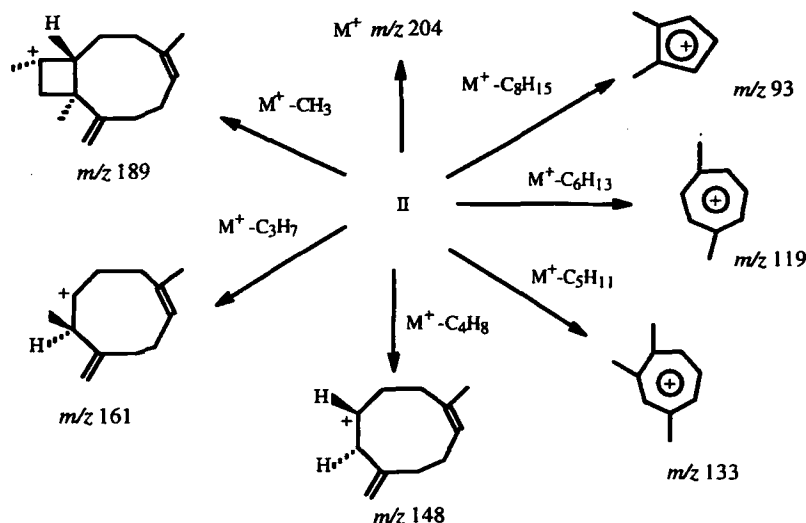
The MS of peak 9 contained the intense $[M^+]$ and fragmentary ions characteristic for longibornene [12]; however, the absence of I values for longibornene from the literature does not permit the identification to be considered definitive.

The only alicyclic sesquiterpene in the series considered — Z- β -farnesene (9, peak 12) — had a maximum yield of ions with m/z 69, corresponding to cleavage of the C—C bond in the allyl position to the double bonds.

The structure of component 13 corresponded completely to aromadendrene (10) (according to I [9] and to MS [12]). In the MS of peak 14, identified from I as the monocyclic sesquiterpene α -humulene (11), we observed a characteristic ion with the maximum intensity (m/z 93) corresponding to the complete cleavage of the ring at the two saturated C—C bonds with the formation of a metastable dimethylcyclopentadienyl cation [14]. The MS of chromatographic peak 15 ($I = 1457$) contained $[M^+]$ (m/z 222) and also the fragmentary ions m/z 207 $[M - CH_3]^+$, 193 $[M - CH_2CH_3]^+$, 177 $[M - 3CH_3]^+$, and 161 $[M - 3CH_3, H_2O]^+$ characterizing the structure of 2,5-di-*tert*-butylhydroquinone (12).

TABLE 1. Chromatographic Parameters and Mass-spectrometric Characteristics of Components of the Native Scent of Leaves of the Potato *Solanum tuberosum*

Peak No	Amount in the mixture, %	Empirical formula (mol. mass, M)	Mass spectrum, m/z (I_{rel} , %)	Compounds identified	Retention index, I	Lit. [9], I_{lit}
1	2.7	C ₁₅ H ₂₄ (204)	204(14), 189(56), 161(100), 119(20), 105(21)	-	1357	-
2	1.9	C ₁₅ H ₂₄ (204)	204(10), 189(12), 161(100), 133(32), 119(44), 105(57), 93(100)	α -Ylangene (3)	1366	1364 \pm 5
3	1.2	C ₁₅ H ₂₄ (204)	204(24), 189(9), 161(100), 119(94), 105(89), 93(44)	α -Copaene (4)	1371	1372 \pm 8
4	1.3	C ₁₅ H ₂₄ (204)	204(12), 161(33), 123(49), 81(82), 57(100)	β -Bourbonene (5)	1375	1385 \pm 11
5	2.6	C ₁₅ H ₂₄ (204)	204(23), 189(16), 161(47), 81(93), 57(100)	γ -Gurjunene (6)	1381	-
6	3.9	C ₁₅ H ₂₄ (204)	204(8), 189(38), 161(51), 147(59), 107(72), 93(100), 81(93)	β -Elemene (7)	1386	1394 \pm 12
7	2.4	C ₁₄ H ₂₀ O (204)	204(7), 189(11), 161(10), 148(97), 133(100), 106(94)	4-(2',7',7'-Trimethylbicyclo[3.2.0]hept-2'-en-1'-yl)but-3-en-2-one (8)	1394	-
8	7.4	C ₁₅ H ₂₄ (204)	204(31), 189(31), 175(14), 161(54), 133(95), 93(100)	-	1406	-
9	5.5	C ₁₅ H ₂₄ (204)	204(82), 189(51), 161(67), 148(54), 133(59), 119(57), 93(100)	-	1413	-
10	26.6	C ₁₅ H ₂₄ (204)	204(19), 189(26), 161(52), 148(37), 147(38), 133(98), 93(100), 69(76), 41(46)	<i>trans</i> -Caryophyllene (1)	1419	1417 \pm 6
11	7.3	C ₁₅ H ₂₄ (204)	204(11), 189(21), 161(31), 148(14), 133(26), 119(84), 93(100), 69(46)	<i>cis</i> -Caryophyllene (2)	1427	1422
12	3.4	C ₁₅ H ₂₄ (204)	204(16), 161(36), 133(37), 93(80), 69(100), 41(65)	Z- β -Farnesene (9)	1441	1442 \pm 10
13	3.1	C ₁₅ H ₂₄ (204)	204(20), 189(34), 161(53), 133(79), 119(56), 107(66), 93(100), 41(52)	Aromadendrene (10)	1446	1447 \pm 16
14	10.2	C ₁₅ H ₂₄ (204)	204(18), 161(12), 147(31), 121(36), 93(100), 80(35)	α -Humulene (11)	1451	1447 \pm 6
15	3.2	C ₁₄ H ₂₂ O ₂ (222)	222(52), 207(100), 193(18), 177(22), 61(37)	2,5-Di- <i>tert</i> -butylhydroquinone (12)	1457	-
16	4.3	C ₁₅ H ₂₂ (202)	202(44), 161(27), 145(29), 132(100), 119(3), 105(57), 55(39), 41(37)	<i>ar</i> -Curcumene (13)	1466	-
17	2.4	C ₁₅ H ₂₄ (204)	204(58), 189(39), 161(98), 133(32), 119(44), 105(100)	α -Elemene (14)	1488	1486 \pm 3
18	1.0	C ₁₅ H ₂₄ (204)	204(28), 189(19), 161(33), 109(39), 93(96), 69(100)	β -Bisabolene (15)	1492	1496 \pm 7
19	1.4	C ₁₅ H ₂₄ O (220)	220(28), 205(100), 177(12), 145(11), 57(32)	2,6-Di- <i>tert</i> -butyl- <i>para</i> -methylphenol (16)	1496	-
20	1.4	C ₁₅ H ₂₄ (204)	204(32), 161(100), 133(28), 119(42), 105(48)	γ -Cadinene (17)	1505	1510 \pm 13
21	4.3	C ₁₅ H ₂₄ (204)	204(58), 189(19), 161(100), 134(42), 119(48), 105(43)	δ -Cadinene (18)	1512	1515 \pm 7

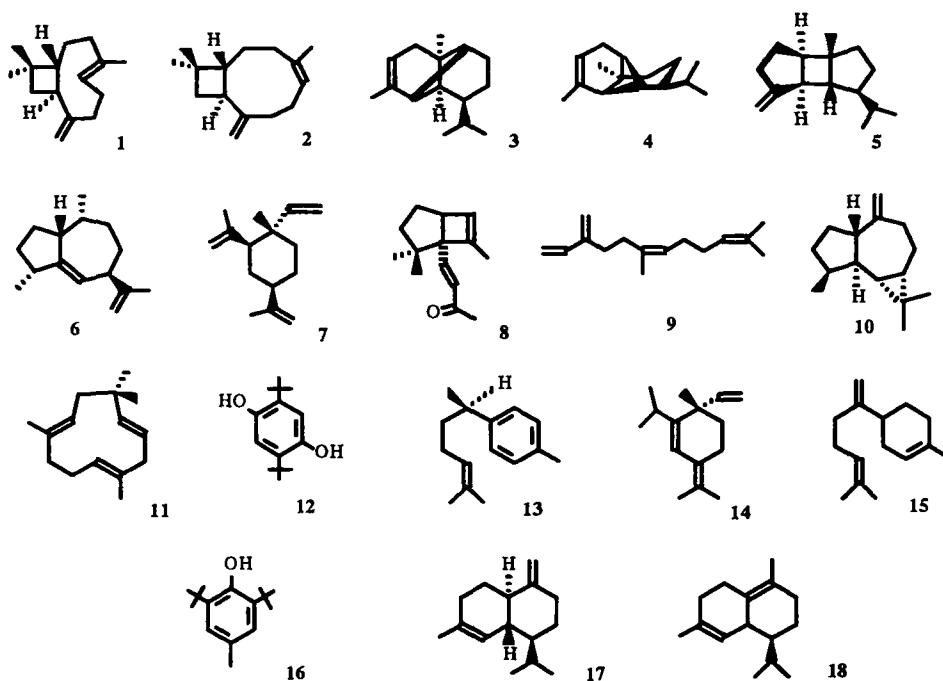


From its MS and *I*, chromatographic peak 16 was identified as α -curcumene (13). Characteristic for the MS of peak 17, as also for α -elemene, with a close *I* value, was a high intensity of M^+ 204 and of the fragmentary ions 189 and 161 resulting from the splitting out of methyl and isopropyl groups, respectively. The structure of component 18 (*I* = 1492) as β -bisabolene (15) was confirmed by the closeness of their MSs [12]. The intense M^+ (m/z 220) and the fragmentary ion with m/z 205 ($M - CH_3$)⁺ for chromatographic peak 19 (*I* = 1496) corresponded to the structure of 2,6-di-*tert*-butyl-4-methylphenol (16)*.

The compounds corresponding to peaks 20 and 21 on the chromatograms had close MSs, and a comparison of their *I* values with the literature [9] permitted their assignment to γ -cadinene (17) and δ -cadinene (18), respectively.

Thus we have identified 13 sesquiterpenes (1—5, 7, 9—11, 14, 15, 17, and 18) as components of the native scent of leaves of the potato *Solanum tuberosum*.

The structures of compounds (6), (8) and (13) have been proposed solely on the basis of mass-spectroscopic characteristics. Three chromatographic peaks (1, 8, and 9) have remained unidentified and represent an object of further investigation.



*Compounds (12) and (16) were apparently of anthropogenic origin.

EXPERIMENTAL

Analysis of the volatile organic compounds in the atmosphere of the plants was carried out by dynamic gas extraction followed by GC-MS detection. In order to take samples of the native potato scent, the plant under investigation was covered with a filter-paper cap having a diameter of 500 mm with an opening in the center for a glass sorption tube with dimensions of 4 mm × 11.5 cm filled with the adsorbent Tenax GC or arbotrap (20/40 mesh). The volatile products were trapped in the tube during the aspiration of air at the rate of 100 ml/min for 15 min. Then the sorption tube was placed in a Supelco thermodesorber (USA) and the adsorbed compounds were blown out with helium at a rate of flow of 5 ml/min for 10 min into a glass tube with dimensions of 2 mm × 11.5 cm filled with the same sorbent.

Prefocusing was carried out to eliminate water vapor and to accelerate the transfer of the sample from the sorption tube into the capillary column. GC-MS analysis was conducted on a Hewlett-Packard MS-Engine (HP Ultra-2 quartz capillary column (0.32 mm × 50 m), linear programming of the temperature: 32°C for 5 min, then heating at a rate of 4°C/min to 250°C]. MS parameters: ionizing energy 70 eV, working temperature 200°C.

As the object of investigation we chose the potato variety Adretta as the most representative among a series of varieties studied in terms of the amount and diversity of the sesquiterpene content. The experimental plot was located 50 km to the south of Ufa. The experiments were conducted in the summer of 1998.

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