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MASS-SPECTROMETRIC ANALYSIS OF PHYTOL DERIVATIVES FROM THE LEAVES OF *Platanus orientalis*

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Products of the oxidation, polymerization, and esterification of tocopherols and esters of phytol with aliphatic acids have been identified in fractions of an alcoholic extract of shed plane leaves by means of mass-spectrometric analysis.

The monounsaturated diterpene alcohol phytol ($C_{20}H_{39}OH$) occupies a special position in the plant world. A phytol residue esterifies the porphyrin nucleus of chlorophyll, which increases its lipophilicity [1]. It is also attached to the aromatic skeletons of the molecules of vitamins E and K, which take part in processes of electron transport in the cell [1]. In an investigation of the the leaves of *Acer platanoides* [2] and of *Fatsia japonica* [3], the waxes were found to contain free phytol and its esters with fatty acids. Phytol esters have also been found in marine and fresh-water flagellate algae. In marine algae, the esterifying acids are the polyunsaturated 16:3, 18:5, 20:5, and 22:6 species [4], and in fresh-water algae the 16:0 and 18:0 species [5]. There are reports of phytyl phytonate [6] and of a macrocyclic biphytanyl products localized in the membranes of bacteria of the Caldariella group [7]. Synthetic esters of phytol with saturated fatty acids have been patented as antiulcer and antiinflammatory agents [8].

On analyzing the total extractive substances from shed leaves of the plane *Platanus orientalis* L., fam. Platanaceae, we detected fractions enriched with esters and other derivativs of phytol. Only phenolic carboxylic acids and some flavonoids and anthocyamidins have been isolated from this source previously [9].

We have established that a considerable accumulation of phytol derivatives in plane leaves takes place in the autumn, i.e., in the leaf-shedding phase. In order to make a detailed study of the essential set of extractive substances of the leaves, the experiment was performed with a large amount of raw material. After five extractions of 20 kg of leaves with ethanol the extract was concentrated to 18 liters. The concentrate obtained was separated into fractions for investigation. Fraction A (0.2 g) was obtained by treating the alcoholic concentrate with hexane, followed by column chromatography with mixtures of hexane and chloroform having different polarities. To obtain fractions B (1.6 g) and C (4.9 g), the alcohol was distilled off from another part of the concentrate, the dry residue was treated with chloroform, the solution was separated from the insoluble part, and the chloroform was distilled off to give a new dry residue. In the case of fraction B, the dry residue was dissolved in hexane and was fractionated with mixtures of hexane and chloroform having different polarities, while in the case of fraction C a hexane–ether system was used for chromatography.

Phytol derivatives were represented most widely in fraction A. They formed two groups of different natures: 1) tocopherol metabolites, including products of their oxidation, dimerization, and esterification; and 2) esters of phytol with fatty acids. Such an inhomogeneous composition of the fractions demanded the use for the identification of the components of a complex group of mass-spectrometric methods, including the spectra of metastable ions, high-resolution mass spectrometry, and multipeak monitoring [10], permitting an analysis of the change in the composition of the fraction over a wide range of temperatures. A typical spectrum of fraction A is given in the Experimental part. Below, we give the results of an investigation of mass spectra.

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* Deceased.

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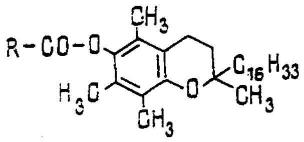
TABLE 1. α-Tocopherol Esters of Fractions A and B



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Compound	$M^{+\cdot}$	R	Esterifying acid
4a	612	$C_{11}H_{23}$	12:0 (B)
4b	640*	$C_{13}H_{27}$	14:0 (B)
4c	668	$C_{15}H_{31}$	16:0 (B)
4d	638	$C_{13}H_{25}$	14:1 (A)
4e	666	$C_{15}H_{29}$	16:1 (A)

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*Some of the ions with m/z 640 relate to the M^{*} ion of compound (5).

TABLE 2. Elemental Compositions of the Ions in the Mass Spectrum of Fraction A

m/z of the ion	Composition	m/z of the ion	Composition
444	$C_{29}H_{48}O_3$	645	$C_{43}H_{65}O_4$ (1)
485	$C_{33}H_{57}O_2$		$C_{44}H_{85}O_2$ (10)
533	$C_{35}H_{49}O_4$ (1)*	646	$C_{43}H_{66}O_4$ (1)
	$C_{36}H_{69}O_2$ (15)		$C_{44}H_{86}O_2$ (3)
554	$C_{38}H_{66}O_2$	662	$C_{46}H_{78}O_2$
561	$C_{38}H_{73}O_2$	666	$C_{45}H_{78}O_3$
580	$C_{38}H_{60}O_4$	746	$C_{49}H_{78}O_5$ (5)
589	$C_{39}H_{57}O_4$ (1)		$C_{51}H_{86}O_3$ (1)
	$C_{40}H_{77}O_2$ (5)	748	$C_{49}H_{80}O_5$ (3)
592	$C_{39}H_{60}O_4$		$C_{51}H_{88}O_3$ (1)
617	$C_{41}H_{61}O_4$ (1)	762	$C_{52}H_{90}O_3$
	$C_{42}H_{81}O_2$ (5)	830	$C_{56}H_{94}O_4$
631	$C_{42}H_{63}O_4$ (1)	856	$C_{58}H_{96}O_4$
	$C_{43}H_{83}O_2$ (1)	858	$C_{58}H_{98}O_4$
632	$C_{42}H_{64}O_4$ (1)	872	$C_{58}H_{96}O_5$
	$C_{43}H_{84}O_2$ (1)	874	$C_{58}H_{98}O_5$

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*Ratio of the intensities of the peaks of the isobaric ions in the doublets in the high-resolution regime.

The products of the oxidation of tocopherol appeared in the form of the peaks of molecular ions with m/z 444 (1), 446 (2), and 462 (3). In the analysis of such materials, special measures must be used to confirm the native nature of the oxidation products, since it is known that when solutions of pure tocopherol are stored it undergoes oxidation. This property forms the basis of the antioxidant action of the tocopherols, which are traps for peroxide radicals and protect the lipids of cell membranes from oxidation [11].

The elemental composition of the ion with m/z 444 was $C_{29}H_{48}O_3$. In the literature [11], two stereoisomeric forms of 1,2,8-trimethyl-4-methylene-8-phytyl-7-oxaspiro(4.5)dec-1-ene-3,6-dione, with a spiro center, were proposed for the corresponding molecule (1), and its mass-spectral fragments were described. The m/z 446 ion was the $M^{+\cdot}$ ion of α-tocopherylquinone (2) [11, 12]. The molecular ion of α-tocopherylquinone 2,3-oxide (3) with m/z 462 [11, 12] readily breaks down with the formation of characteristic fragments having m/z 419 and 402, through which this substance can be recognized.

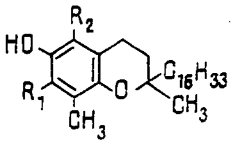
TABLE 3. Mass Numbers of the Daughter Ions in the *B/E* Linked-Scanning Spectra of Key Ions (fraction A)

Parental ions, *m/z*	Daughter ions, *m/z*
890	874, 872, 858, 663, 566, 480, 430
874	858, 840, 830, 746, 648, 646, 632, 631, 618, 605, 592, 589, 580, 566, 480, 444, 430, 428, 416
872	840, 830, 746, 648, 646, 632, 631, 618, 605, 592, 589, 580, 566, 480, 444, 430, 416
858 and 856	840, 824, 632, 631, 618, 617, 592, 589, 580, 480, 444, 430, 428, 416, 367
666	430, 428
632	617, 589, 561, 533, 430, 428, 367, 296, 278
592 and 589	561, 547, 533, 430, 428, 416, 367, 296, 278
554	511, 430, 428

In the region of higher mass numbers of the spectrum of fraction A, the peaks of ions with *m/z* 638, 662, 666, 746, 748, and 750 were observed. The ions with *m/z* 638 and 666 were the molecular ions of esters of α-tocopherol with unsaturated acids (**4d, e**) (Table 1). This was shown by the elemental composition of the ions with *m/z* 666 (Table 2) and by well-defined metastable transitions to an ion with *m/z* 430 in the *B/E* spectra [13] of the ions with *m/z* 638 and 666. The metastable defocusing (MD) spectrum of the ion with *m/z* 430 also showed transitions of the ions with *m/z* 638 and 666 (Table 4). The ions with *m/z* 746 and 748 were doublets the components of which contained either three or five oxygen atoms (see Table 2). This also complicated the pattern of the *B/E* spectrum, preventing the unambiguous determination of their structures.

In contrast to the spectrum of fraction ~~A, in the~~ spectrum of fraction B we observed the peaks of ions with *m/z* 612, 640, and 668, which, judging from their elemental compositions and the existence of transitions to ions with *m/z* 430 in the *B/E* spectra, corresponded to esters of tocopherol with saturated acids (see Table 1). We may note that for the ion with m/z 640 an additional transition to an ion with *m/z* 416 was detected, i.e., it corresponded in part to to an ester of β- or γ-tocopherol (**5**). The ratio of the peaks of the metastable ions with *m/z* 416 and 430 was 1:8, respectively.

In the spectrum of fraction A, peaks of an ion with *m/z* 554, composition $C_{38}H_{66}O_2$ and of a fragment of it with *m/z* 485 ($C_{33}H_{57}O_2$) stood out. In combination with the presence in the spectrum of doublets of ions with *m/z* 296, 295, and 278, 277, this would mean the presence in this fraction of the phytyl ester of an 18:4 acid; however, no genetic link between these ions was detected. The *B/E* spectrum of the ion with *m/z* 554 contained transitions to ions with *m/z* 511, 485, and 430-428. On the other hand, the MD spectra of the ions with *m/z* 428, 430, and 485 unambiguously showed the existence of a precursor with *m/z* 554. On the basis of these directions of fragmentation and in the light of the isoprene rule, it is possible to propose for the compound with $M^{+\cdot}$ 554 the structure of a C-dihydrogeraniol derivative of β- or γ-tocopherol (**4**), formed as the result of a Claisen rearrangement of an O-alkenyl ether:



6a. $R_1 = C_{10}H_{19}$; $R_2 = CH_3$
6b $R_1 = CH_3$; $R_2 = C_{10}H_{19}$

In the region of high mass numbers in the spectrum of fraction A, the peaks of ions with *m/z* 858 and 830 stood out, these corresponding, according to their elemental compositions, to dimeric forms of α- and β-tocopherols, respectively. We shall discuss this section of the spectrum in detail below. The central part of the spectrum of fraction A was the most complex for interpretation. Here, at a moderate temperature were observed a series of peaks of ions with *m/z* 533, 561, 589, terminated by homologs with *m/z* 603, 617, 631, 645. All these peaks were accompanied by peaks of ions with mass numbers 1 a.m.u. or greater. The majority of ions had a doublet nature and contained two-oxygen and four-oxygen components (see Table 2). With a rise in the temperature, the intensities of the peaks of the ions with *m/z* 533, 561 and 589 in this region of the spectrum increased greatly. The intensities of the peaks of the phytyl fragments with *m/z* 278 and 296 increased simulta-

TABLE 4. Mass Numbers of the Parental Ions in the Metastable Defocusing (MD) Spectra, of Key Ions (fraction A)

Daughter ions, *m/z*	Parental ions, *m/z*	Daughter ions, *m/z*	Parental ions, *m/z*
762	890	430	446, 458, 505, 533, 554
632	700, 748, 856		566, 589, 617, 632, 638, 645,
631	856, 890		659, 666, 700
618	830, 856, 874	430	446, 458, 485, 700, 748,
617	748, 830	(2 kV)	856
592	830, 856, 874	428	446, 458, 533, 554, 566,
589	816, 830, 856	(2 kV)	592, 856, 874
533	748, 816		
485	554		

TABLE 5. Phytol Esters in Fractions A and B, $R-CO-O-C_{20}H_{39}$

Compound	$M^{+\bullet}$	R	Esterifying acid
7a	534	$C_{15}H_{31}$	16:0 (B)
7b	562	$C_{17}H_{35}$	18:0 (B)
7c	590	$C_{19}H_{39}$	20:0 (B)
7d	604	$C_{20}H_{41}$	21:0 (A)
7e	618	$C_{21}H_{43}$	22:0 (A)
7f	632	$C_{22}H_{45}$	23:0 (A)
7g	646	$C_{23}H_{47}$	24:0 (A)

neously. In the light of the fact that in the compositions of the ions with *m/z* 533 and 589 the two-oxygen components predominated under these conditions, while the ion with *m/z* 561 was singlet of analogous nature, it may be concluded that these ions were fragments of the spectra of esters of phytol with saturated fatty acids.

In the spectra of fraction B we recorded the peaks of the $M^{+\cdot}$ ions of esters of phytol with the 16:0, 18:0, and 20:0 acids (*m/z* 534, 562, 590). But indications of the presence of homologs with higher mass numbers were insignificant. On the other hand, in the spectrum of fraction A such peaks had medium intensity. This means that the series of even- and odd-electron fragments in the 533-646 a.m.u. interval can be regarded as a combination of molecular and fragmentary ions of esters of phytol with the acids from 16:0 to 24:0 (7). In order to confirm this conclusion, let us turn to the *B/E* spectrum of the ion with *m/z* 632. In it we observed weak peaks of transitions to ions with *m/z* 617, 603, 589, 561, and 533. It is most important that in this spectrum there were peaks of transitions to phytyl fragments with *m/z* 278 and 296. These facts can be interpreted unambiguously as showing the presence of phytol esters (Table 5).

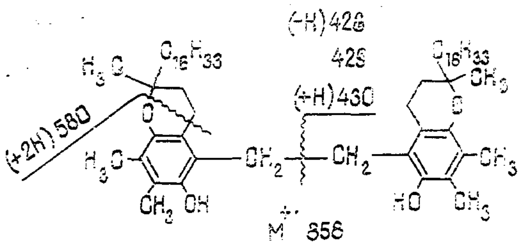
In order to elucidate the nature of the four-oxygen fragments in the central part of the spectrum of fraction A we made a further separation of this fraction by TLC. Using the benzene–ethyl acetate (24:1) system, we studied the mas spectra of eluates of three zones, with R_f 0.9 (1), 0.77 (2), and 0.52 (3).

In the spectrum of the eluate of zone 3 we established the presence of a heptadecaprene with $M^{+\cdot}$ 1152 and the peaks of wax molecules, while in the eluate of zone 1 peaks of the characteristic ions of sitosterol esters [14] predominated.

The spectrum of zone 2 obtained in the temperature interval of 170-190°C proved to be the most suitable for determining the origin of the four-oxygen fragments in the central part of the spectrum of fraction A. Under these conditions the intensities of peaks with *m/z* 618 ($C_{41}H_{62}O_4$) and 632 ($C_{42}H_{64}O_4$) increased. Peaks of the two-oxygen components of these ions and also the peaks of phytol residues were absent. The intensities of the peaks of ions of dimeric tocopherols with *m/z* 830, 856, 858, 872, 874, and 890 rose simultaneously. The *B/E* spectra of these ions showed transitions to ions with *m/z* 533, 589, 618, 631, and 632 (see Table 3). In its turn, in the MD spectrum of the ion with *m/z* 618, transitions of ions with *m/z* 830, 856, and 874 were found, and, in the analogous spectra of the ion with *m/z* 632, transitions of an ion with *m/z* 856. So far as concerns the *B/E* spectra of the ions with *m/z* 618 and 632, they revealed transitions to daughter ions with *m/z* 430 and 428. According to its *B/E* spectrum, another four-oxygen fragment with *m/z* 592 ($C_{39}H_{60}O_4$) broke down with the formation of ions having *m/z* 430 and 416. Thus, it was established that the fragments under consideration in the central part of the spectrum were intermediate products of the breakdown of dimeric forms of tocopherol under electron impact. Analysis of the spectra of the metastable ions permitted the structural characteristics of the dimers to be deduced.

In [15], using a model derivative of α-tocopherol – 6-hydroxy-2,2,5,7,8-pentamethylchromane – as an example, it was established that the dimerization of the molecule in the presence of an oxidizing agent may proceed as far as the formation of a spirodimer and a trimeric form. The peaks with *m/z* 828, 856, 872 in the spectrum of the eluate of zone 2 may be assigned to spirodimeric varieties of tocopherol. Another group of molecular ions with *m/z* 830, 858, and 874 permits the suggestion of the presence of so-called dimers of the diphenylethane type [16]. Ions with *m/z* 872 and 874 included an additional oxygen atom. The spectrum also contained the fairly stable peak of an ion with *m/z* 890 hving two additional oxygen atoms. The strongest peak was that of the ion with *m/z* 858. The intensities of the other peaks in relation to this one were: 830 – 10%; 856 – 10%; 872 – 10%; 828 – 5%; 874 – 30%; and 890 – 10%. However, with a rise in the temperature, the ratio of the intensities of these peaks changed sharply in favor of the peaks of the ions with *m/z* 856 and 858.

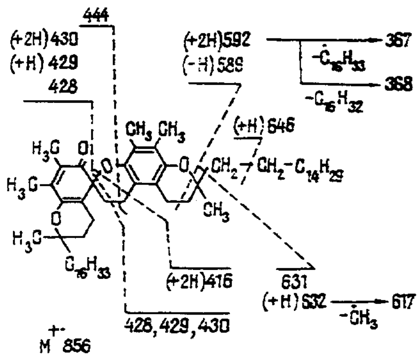
One of the most reliable methods of proving that the parental ions with *m/z* 858 and 856 corresponded to dimers of different types was an investigation of features of the spectra of the daughter ions of each of these two $M^{+\cdot}$ ions separately. This problem was solved by narrowing the β-slit of the mass-spectrometer analyzer in order to prevent the simultaneous passage of the two parental ions. Under these conditions, the *B/E* spectrum of the *m/z* 858 ion showed relatively weak peaks of transitions to ions with *m/z* 632, 631, 589, 580, and 428-430. On the other hand, in an analysis of th *B/E* spectrum of the ion with *m/z* 856 it was found that the peaks corresponding to transitions to the same daughter ions were an order of magnitude higher (see Table 3). These facts confirmed the idea of the presence of dimers with a diphenylethane bridge ($M^{+\cdot}$ 858) breaking down through the cleavage of the ethane C–C bond (Scheme 1), which leads to weak metastable peaks. The breakdown of the dihydropyran ring in the molecular ion of the spirodimer ($M^{+\cdot}$ 856) has the nature of a rearrangement process and the heights of the peaks in the corresponding *B/E* spectrum are therefore increased.



Scheme 1. Method of formation of the ions with *m/z* 580 and the main fragmentary ions from the $M^{+\cdot}$ ion of the diphenylethane-type dimer.

Transitions to ions with *m/z* 589, 617, 618, 631, and 632 arose on the splitting out of the isoprenoid chains from the dihydropyran rings. Moreover, in the *B/E* spectrum of the ion with *m/z* 856, there were transitions to an ion with *m/z* 592 formed by a retrodiene breakdown of a dihydropyran ring. These processes are analogous to those in the fragmentation of tocopherol monomers [17]. One of the strongest metastable peaks in the *B/E* spectrum of the *m/z* 858 ion corresponded to a transition to an ion with *m/z* 580. In the general spectrum of fraction A this daughter ion was weak. A probable analog of this fragment in the spectrum of α-tocopherol – an ion with *m/z* 152 – was also weak. However, in a spectrum of an eluate of zone 2 obtained at a high temperature, the intensity of the peak of the *m/z* ion had increased. On the basis of the composition of this ion ($C_{38}H_{60}O_4$) it could be considered that it was formed by the ejection of a dehydrated phytol residue, $C_{20}H_{38}$, from the ion with *m/z* 858 (see Scheme 1).

In the *B/E* spectrum of the *m/z* 856 ion a transition to an ion with *m/z* 368 was observed. An ion with the same mass number arose on the breakdown of the *m/z* 592 fragment, i.e., this daughter ion was formed as the result of the sum of two retrodiene decompositions of a dihydropyran unit of the spirodimer taking place successively or synchronously (Scheme 2).



Scheme 2. Main directions of the fragmentation of the M^+ ion of the spirodimer of α-tocopherol.

Now let us dwell on the possible structures of the products of the oxidation of the dimers. The *B/E* spectra of the ions with *m/z* 874 and 872 show the breakdown of these $M^{+\cdot}$ ions in two alternative directions the relative contributions of which depend on the temperature regime: 1) with the loss of an oxygen atom followed by the fragmentation that is characteristic of the molecular ions of the corresponding unoxidized dimers, and 2) with the formation of fragmentary ions retaining the additional oxygen atom. What we have in view is the existence of metastable transitions to analogs of the ions with *m/z* 589 and 631 – ions with *m/z* 605, 646, and 648. It is unlikely that the compounds under consideration were tocopherylquinones in a dimeric variant, since quinone structures do not break down with the loss of an oxygen atom. It is most likely that they were products of the epoxidation of the π-bonds of the aromatic rings, for which the loss of an oxygen atom under electron impact is characteristic [18].

The *B/E* spectrum of the *m/z* 890 ion showed transitions to ions with *m/z* 874 and 858 (loss of one and two oxygen atoms, respectively) and transitions to an analog of the ion with *m/z* 631 – an ion with *m/z* 663 – and to the ion of the monomer with *m/z* 430. These facts are in harmony with the probable presence in the dimer molecule of the diphenylethane type of peroxide chain. No such grouping is realized in the molecule of the spirodimer. In actual fact, the $M^{+\cdot}$ ion with *m/z* 888 (856 + O_2) was insignificant in the spectra of all the fractions.

In the general spectrum of fraction A at a high temperature, peaks were recorded in the 900-1350 a.m.u. region among which the peaks of ions with *m/z* 1286 and 1284, corresponding to tocopherol trimers stood out. There are reports in the literature on the existence of such substances, synthesized from compounds modeling α- and β-tocopherols, in the molecules of which the phytyl residue $C_{16}H_{33}$ has been replaced by a methyl group [15]. In order to confirm the nature of these peaks we obtained the spectrum of an eluate of zone 2 under the same temperature conditions. Here the contribution of these ions had increased and the peak of an ion with *m/z* 1059, corresponding to the elimination of a $C_{16}H_{33}$ radical from a $M^{+\cdot}$ ion with *m/z* 1284 had appeared.

EXPERIMENTAL

General spectra were obtained on a MKh 1310 instrument with a SVP-5 system for direct injection, at temperatures of 120-200°C. Measurements of elemental compositions and the recording of the linked-scanning (*B/E* = const) spectra of the metastable ions and of the metastable defocusing spectra were carried out as described in [13, 14].

Production of the Alcoholic Extract. Leaves shed from one plane tree were collected to an amount of 20 kg, cleaned, dried, comminuted, and extracted by steeping with alcohol for seven days, five times, and the alcoholic extract was concentrated. This gave 18 liters of concentrate, which was subsequently investigated in portions.

Fraction A. In a rotary evaporator, 200 ml of the alcoholic concentrate was evaporated to 80 ml. The resulting viscous concentrate was diluted with water and was then extractd several times with hexane. The hexane extract yielded 18.4 g of total material, which was separated on a column (4.2 × 120 cm) with KSK silica gel as adsorbent at a ratio of adsorbent to substance of 20:1. The eluents were hexane–chloroform (10:1), (5:1), (2:1), and (1:1). Fraction A (200 mg) was isolated. Mass spectrum, 170°C, *m/z* (%): 1288(0.03), 1287(0.14), 1286(0.14), 1284(0.03), 1282(0.03), 1175(0.07), 1174(0.07), 1059(0.07), 1000(0.03), 986(0.03), 918(0.14), 908(0.14), 906(0.3), 892(0.4), 891(0.5), 890(2.8), 888(0.3), 875(8), 874(14), 872(2), 860(14), 859(21), 858(66), 856(5), 854(1.4), 844(0.4), 842(0.5), 831(1), 830(2.5), 828(0.3), 778(0.5), 765(1), 764(2.5), 762(1), 751(1), 750(3.5), 749(1.7), 748(4.2), 746(1.4), 744(0.5), 734(0.4), 733(0.5), 708(0.5), 694(0.7), 690(0.5), 688(0.2), 680(0.5), 667(1), 666(2.8), 664(0.3), 662(1), 660(0.5), 659(0.7), 647(0.5), 646(1), 645(3.5), 639(0.5), 638(1.4), 632(3.5), 631(7.1), 618(3.5), 617(3), 604(1.4), 603(3.5), 593(0.7), 592(0.5), 591(2.1), 590(8), 589(21.3), 580(0.7), 576(0.5), 575(1.7), 562(4), 561(11), 555(2.8), 554(4.2), 547(1.7), 534(7), 533(21.3), 525(1.4), 517(0.7), 505(1), 486(2.5), 485(6.4), 472(1), 467(0.7), 446(1.4), 445(0.7), 444(0.7), 443(1.4), 442(0.7), 431(42), 430(100), 429(6), 428(17), 424(4), 416(4), 410(7), 368(2), 367(4), 366(0.4), 295(10), 294(5), 278(9), 277(31), 276(16), 275(14).

Fraction B. In a rotary evaporator, 2000 ml of the alcoholic concentrate was evaporated to a dry residue weighing 170 g, and this was treated with chloroform. The 100 g of total substances so obtained was separated on an 8 × 120 cm column at a ratio of adsorbent to substance of 15:1. Elution was conducted with hexane and mixtures of hexane and chloroform (50:1), (25:1), (10:1), (5:1), and (2:1). This gave 1.6 g of fraction B.

Fraction C was obtained in a similar way to fraction B, but with the use of hexane–ether as eluent. The amount of fraction C obtained from 85 g of total substances was 4.9 g.

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