TABLE 3. Activities of Catalysts 2 and 3 After Their Regeneration

Time of working of the catalyst, h	Catalyst 2		Catalyst 3	
	iodine No. of the hydrogenated oil, % I_2	activity of the catalyst $\frac{\Delta I.\ No.}{ml \cdot h}$	Iodine No. of the hydrogenated oil % I_2	activity of the catalyst $\frac{\Delta I.\ No.}{ml \cdot h}$
10	67,8	0,430	65,2	0,456
100	69,4	0,414	66,9	0,439
300	72,0	0,388	70,5	0,403
500	74,2	0,359	73,1	0,377

was calculated from the volume of hydrogen liberated during the dissolution process by means of the equation given in [4].

The iodine numbers of the hydrogenated oils were determined by the Wijs method [5].

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LIPIDS OF THE FRUIT OF Rumax paulsenianus

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UDC 633.88:581.19:665.12

The lipids of the fruit of Rumex paulsenianus have been investigated. The lipid content of the seeds was 7.8% and of the perianths 0.58%. Of the 18 classes of lipids detected, 16, including anthraquinone pigments, were identified by CC and TLC and by UV and IR spectroscopies and mass spectrometry. The low-molecular-mass triacylglycerols dimyristoylacetin, dimyristoylcaproin, myristoylcaproylacetin, and hydroxylipids have been detected in the fruit of plants of the Polygonaceae family for the first time.

Rumex paulsenianus Rech. fil. (Paulsen's dock) family Polygonaceae, grows in Central Asia, Iran, and the Hindu Kush [1]. Plants of this genus are known as sources of anthraquinones, flavonoids, and tanning substances, in view of which many species are used in the folk medicine of a number of countries as medicinal and homeopathic agents [2]. The medicinal raw material is mainly the roots, stems, and leaves of the plants, and the chemical composition of these organs has been studied to a greater degree than that of the fruit and seeds.

Information of the Remex genus is sparse. It has been reported that the amount of lipid in the seeds of individual representatives does not exceed 5%, the main fatty acids being the 18:1 and 18:2 types. The localization of the reserve lipids in the embryonal part of the seeds has been reported [3]. There is no information on the lipids of the fruit of Rumex paulsenianus.

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Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 604-611, September-October, 1990. Original article submitted December 11, 1989.

0009-3130/90/2605-0512$12.50

TABLE 1. Composition of an Extract of the Fruit of Rumex paulsenianus

	Class of lipids	Amount, % on the weight of the extract
I.	Paraffins	3,3
II.	Unidentified X_1	3,4
III.	Wax esters (WE)	0,2
IV.	Sterol esters (SE)	4,5
V.	Triacylglycerols (TAG-1)	55,3
VI.	Orange pigment	tr.
VII.	Low-molecular-mass triacylglycerols (TAG-2)	1,0
VIII.	Chrysophanol	0,8
IX.	Physcion + aloe-emodin (tr.)	0,4
X.	Free fatty acids (FFA)	11,4
XI.	Pink pigment	tr.
XII.	Fatty alcohols	2,0
XIII.	Orange pigment	tr.
XIV.	Hydroxylipid-1	3,1
XV.	Unidentified X_2	
XVI.	Hydroxylipid-2	1,7
XVII	Phytosterols	4,1
XVI	Hydroxylipids-3–5	2,9
XIX	Hydroxylipid-6	1,0
XX.	Hydroxylipid-7	4,0
XXI	Brown pigments	0,9

TABLE 2. Composition of the Fatty Acids of the Lipids of the Fruit of Rumex paulsenianus

Acid*	Fruit					Perianths	
	WES	SES	TAG-1	TAG-2	FFAs	TAGs	FFAs
10:0	Tr.	—	0,7	Tr.	1,1	—	—
12:0	Tr.	0,9	0,4	1,2	0,3	1,1	0,9
14:0	0,8	2,4	4,9	97,2	5,8	17,8	5,0
16:0	3,9	11,6	6,5	1,6	16,8	12,0	38,9
16:1 (9)	Tr.	Tr.	0,4	—	Tr.	—	—
18:0	20,8	4,5	0,5	—	2,9	3,4	8,5
18:1 (9)	12,3	16,6	48,3	—	40,2	26,1	30,2
18:2 (9,12)	—	19,5	35,2	—	27,0	12,5	4,4
18:3 (9,12,15)	—	—	0,5	—	0,7	—	—
20:0	21,8	2,5	2,3	—	4,4	18,5	3,3
22:0	2,4	23,4	0,3	—	0,8	Tr.	Tr.
24:0	38,0	11,8	—	—	—	5,9	8,8
26:0	Tr.	6,8	—	—	—	—	—
Σ sat	87,7	63,9	15,6	100,0	31,0	60,3	62,0
Σ unsat	12,3	36,1	84,4	—	69,0	39,7	38,0

*All the classes of lipids of the fruit and perianths contained in trace amounts the 11:0, 13:0, 15:0, and 17:0 acids, and the TAG-1 and FFAs of the fruit contained the 20:1 (11), 17:1 (9), and 16:2 acids.

We have investigated the lipids of the species growing in the Kazak SSR, which is promising for cultivation in Central Asia [4]. The fruit of the dock consists of a triquetrous achene included in a perianth [1]. We mainly investigated the neutral lipids of the dry fruit. In addition, to elucidate the localization of the individual components in the anatomical parts of the fruit we separated it into seeds (29% of the mass of the fruit) and perianth (71%) and made a comparative analysis of the amounts and composition of the lipids isolated.

The lipids were extracted from the comminuted samples with hexane at room temperature. The yield of extract from the fruit amounted to 2.2%, that from the seeds to 7.8%, and that from the perianths to 0.58% (on the weight of the sample). Thus, taking into account the ratio of the anatomical parts of the fruit, the proportion of extractive components of the perianths in an extract of the fruit did not exceed 15% and the latter reflected mainly the set of lipids of the seeds.

The extract of the fruit was fractionated by a combination of column chromatography (CC) and preparative thin-layer chromatography (PTLC) on silica gel, which led to a set of lipids and lipophilic substances (Table 1).

The extract consisted of compounds characteristic, as a rule, for reserve lipids and contained the lipophilic anthroquinone pigments characteristic of the Rumex genus. Components found more rarely among the reserve lipids include low-molecular-mass triacylglycerols (TAG-2) and a complex set of hydroxylipids.

Analysis of aliquot samples of three extracts by TLC in systems 1-4 showed a similarity of the compositions of the lipids of the fruit and the seeds. An extract of the perianths was simpler in terms of the set of lipid classes, with a predominance of TAGs and free fatty acids (FFAs) which are rarely found in extracts of seed coats [5]. From the extract of the perianths, the TAGs and FFAs were isolated by PTLC in system 2 for analysis of their acid compositions.

The fatty acids (FAs) were isolated from the lipids by hydrolysis with methanolic KOH, the alcoholic part of the lipids being separated, where necessary, by extraction of the saponified solution with hexane. The acids in the form of methyl esters (MEs) were investigated by UV and IR spectroscopies, GLC, Ag^+–TLC, and oxidative degradation. The results of the GLC analysis are given in Table 2 (% on the weight of the MEs).

It can be seen that the set of FAs of the fruit of R. paulsenianus is fairly broad including medium-mass (10:0-14:0) and high-mass (20:0-26:0) homologs. The specificity of the composition of the acyl radicals of the WEs, where almost 60% of the weight of the acids consisted of the 20:0 and 24:0 varieties, and the considerable degree of unsaturation of the acyl moiety of SEs must be mentioned.

The fatty acids of the lipids of the perianths were less diverse, the levels of total unsaturation of the acyl radicals of the TAGs and TFAs being almost the same, which is rarely observed in analogous classes of reserve feed lipids.

The fatty acids of the bound (TAGs) and free (FFAs) forms in the dock fruit had transparent UV spectra and, according to their IR spectra, contained no trans-olefinic bonds (absence of bands at 950-990 cm^{-1}).

To elucidate the structures of the acids, the FFAs of the fruit were converted into MEs and these were separated by preparative Ag^+–TLC in system 5. This yielded narrow fractions of saturated, monoenic, dienic, and trienic acids with the composition given below (% by weight, GLC):

Saturated		Monoenic	
10:0	1,3	16:1	0,4
11:0	0,4	17:1	1,0
12:0	0 8	18:1	96,6
13:0	0,5	20:1	2,0
14:0	23,3	Dienic	
15:0	0,5		
16:0	58,5	16:2	1,0
17:0	0,2	18:2	99,0
18:0	8,1		
20:0	4,7		
22:0	1,7		

In the trienic acid fraction, only the 18:3 species was present.

After periodate-permangate degradation of the 18:3 MEs and of the dienic acids, with subsequent analysis by GLC of the acid fragments, the only dicarboxylic acid that had been formed was shown to be nonanedioic, while in the case of the MEs of the monoenic acids, undecanedioic acid was also formed. Caproic acid (6:0) was extractable from the aqueous reaction solution of the monocarboxylic fragment of the dienic acids, and the 7:0, 8:0, and 9:0 acids from the monocarboxylic fragments of the monoenic acids. The water-soluble fragments from oxidation, being unextractable, were not investigated.

The formation of the above-mentioned fragments on the oxidation of the unsaturated fatty acids of R. paulsenianus showed that the first olefinic bond in the chain of the 16:1, 16:2, 17:1, and C_{18} acids was located at C-9 (from the COOH), and in the 20:1 chain at C-11. Among the monoenic acids, in addition to the 18:1 (9) acid, the presence of its isomer, the 18:1 (11) (vaccenic) acid was also possible. The positions of the other olefinic bonds in the polyenic acids of the dock lipids were the usual ones for the normal 18:2 acid (9, 12) and the α-18:3 acid (9, 12, 15).

Low-Molecular-Mass TAGs. In agreement with the fatty acid composition (Table 2), the bulk of the triacylglycerols of the fruit of R. paulsenianus (TAGs-1) consisted of the C_{50}-C_{56} ($C_{14}C_{18}C_{18}$–$C_{20}C_{18}C_{18}$) species, where the subscripts shown give the total numbers of C atoms of the three acyl residues in a TAG [6]. In the course of the CC of the lipids, the hexane–ether (96:4) system eluted the slower-migrating fraction of TAGs-2, which, on TLC in system 1, appeared in the form of a white spot with R_f 0.67 after the chromatogram had been kept in I_2 vapor and was scarcely revealed by treatment with H_2SO_4 at 110°C; in this system, the TAGs-1 had R_f of 0.75 and were stained by both revealing agents.

The pure TAGs-2 were isolated by rechromatography, first by CC in system 6 and then by PTLC in system 2.

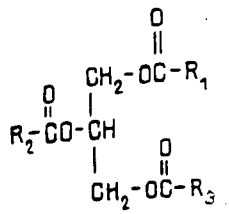
The IR spectrum of the TAGs-2 was typical for trisaturated TAGs [7] but had the following peculiarities. The band of the deformation vibrations of CH_2 groups was observed at 733 cm^{-1} and had a higher intensity, as is observed for $(CH_2)_{3-4}$ [7]; a higher intensity was also observed for the C–O bands of ester groups at 1120, 1170, and 1245 cm^{-1} (the triplet characteristic for TAGs), the intensity of the 1170 cm^{-1} band exceeding that of the 1755 cm^{-1} band (C=O in esters).

These peculiarities showed the presence of esters of glycerol with low-molecular-mass acids and, in particular, with CH_3COOH (1245 cm^{-1}) [8].

To isolate the acids from the TAGs-2 we used the barium-salt method [6], which permits the isolation of their higher and lower homologs. After the saponification of the TAGs-2 with methanolic $Ba(OH)_2$ and the decomposition of the salts, two acid fractions were obtained. The high-molecular-mass acids in the form of the MEs were analyzed by GLC (Table 2). The low-molecular-mass acids were converted into ammonium salts and, after separation by TLC on cellulose in system 7, the 2:0 (R_f 0.44, the main one), 6:0 (R_f 0.81) and 4:0 (R_f 0.68, a minor component) acids were detected.

According to these results, the TAGs-2 were myristoyl-, caproyl-, and acetyl-containing species.

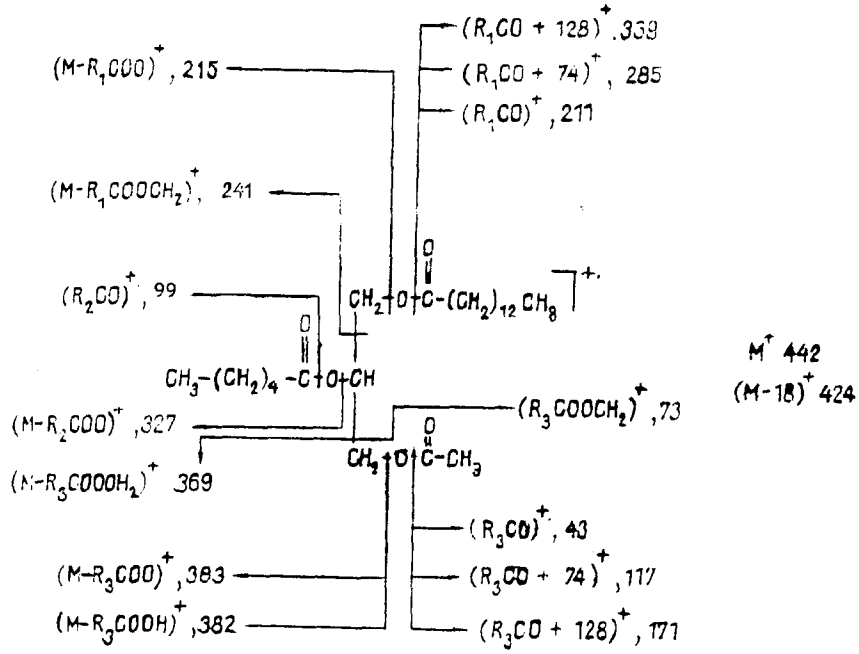
In the mass spectrum of the TAGs-2, three main M^+ peaks and those of a series of fragments characteristic for the breakdown of TAGs, consisting of residues of high- and low-molecular-mass acyl radicals [9], were observed. The molecular masses corresponded to the species $C_{14}C_6C_{14}$ (I, M^+ 610), $C_{14}C_2C_{14}$ (II, M^+ 554), and $C_{14}C_6C_2$ (III, M^+ 442). The



I. $R_1 = R_3 = -(CH_2)_{12}CH_3$; $R_2 = -(CH_2)_4CH_3$
II. $R_1 = R_3 = -(CH_2)_{12}CH_3$; $R_2 = -CH_3$
III. $R_1 = (CH_2)_{12}CH_3$; $R_2 = -(CH_2)_4CH_3$; $R_3 = -CH_2$

positions of the acid residues in I and II were substantiated by the fact that the spectrum lacked ions of the type of $(M - RCOOCH_2)^+$ where the acyloxymethylene radicals are the 6:0 and 2:0 species, respectively. It is known that the ejection of a $RCOOCH_2$ radical takes place only from the sn-1(3) positions of TAGs [9]. The analogous breakdown of III with the loss of 73 mass units in the case of the location of the 2:0 radical in the sn-1(3) position led to a fragment with m/z 369, the peak of which in the spectrum had considerable intensity. At the same time, a fragment with m/z 313 (442-129) formed on the breakdown of the isomeric species with the 6:0 radical in one of the extreme positions was absent (Fig. 1). Furthermore, the intensity of the peak of the $(M - RCOOCH_2)^+$ ion formed on the ejection of a low-molecular-mass $RCOOCH_2$ is usually higher than that of the fragment with the ejection of a high-molecular-mass radical. For the $C_{14}C_6C_2$ species, the ratio $(M - CH_3COOCH_2)^+/(M - CH_3(CH_2)_{12}COOCH_2)^+$ amounted to 5.2. The position of the acetoxy radical in the sn-3, and not in the sn-2, position of the $C_{14}C_6C_2$ species was chosen on the basis of biogenetic laws.

Thus, the 6:0 (caproic) acid esterifies the sn-2 hydroxyl of the glycerol residue in I and II, and acetic acid the sn-2(II) or the sn-3(III) hydroxyl, and the main species of the TAGs-2 of R. paulsenianus have the most probable structures of 2-caproyl-1,3-dimyristoyl glycerol (I), 2-acetyl-2,3-dimyristoylglycerol (II), and 3-acetyl-2-caproyl-1-myristoyl-sn-glycerol (II).



Mass-spectrometric characteristics of 3-acetyl-2-caproyl-1-myristoyl-sn-glycerol

In addition to the above-mentioned ions, the mass spectrum contained weak peaks of M^+ ions and of the corresponding fragments of another not less than five species of TAGs-2 with 2:0, 4:0, 6:0, 12:0, 14:0, and 16:0 acyl residues (M^+ 386, 414, 470, 526, and 582).

The monoacetyl-TAGs belong to rarely encountered species of plant acylglycerols. They have been detected in the seeds and fruit of individual representatives of the families Celastraceae, Balsaminaceae, and Polygalaceae, where their amount may reach 70-98% [10]. As a rule, the acetate group is located in the sn-3 position of such TAGs, while as the sn-1 and sn-2 acyls the aceto-TAGs contain residues of the ordinary high-molecular-mass, C_{16}–C_{18}, fatty acids. An exception is formed by the neutral lipids of Polygala virgata (family Polygalaceae), 74% of which consists of a sn-2-acetyl-TAG. Their predominating species are also symmetrical acylglycerols with high-molecular-mass fatty acids, $C_{18}C_2C_{18}$ and $C_{16}C_2C_{16}$, but among them in minor amounts of up to 4% is the $C_{14}C_2C_{14}$ species [10].

The plant species Celastrus orbiculatus (family Celastraceae) is known, the seeds of which are enriched with sn-3-acetyl-TAGs, the main species being $C_{18}C_{18}C_2$ and $C_{18}C_{16}C_2$ [6]. These types are accompanied by TAGs with other low-molecular-mass acids, including the 4:0, 6:0, and 12:0 acids.

In the lipids of the fruit of R. paulsenianus the proportion of low-molecular-mass TAGs amounts to 1% and they do not contain as structural elements the high-molecular-mass C_{18}, fatty acids while the 16:0 acid is a minor component.

Thus, the fruit of R. paulsenianus contains low-molecular-mass TAGs specific in composition and structure, of which 1,3-dimyristoyl-2-acetin is the second example of the detection of this TAG species in plants, while this is the first time that the species 1,3-dimyristoyl-2-caproin and 1-myristoyl-2-caproyl-3-acetin have been detected.

The characteristics of the hydroxylipids of R. paulsenianus will be given in a subsequent communication.

The paraffins of the fruit of R. paulsenianus had R_f 0.92 in system 3, were not revealed with I_2 vapor, and were detected feebly with 50% H_2SO_4/110°C. According to GLC results, the set of paraffins included

C_{16}	0,6	C_{22}	0,8	C_{28}	1,3
C_{17}	0,7	C_{23}	2,3	C_{29}	26,7
C_{18}	1,1	C_{24}	0,9	C_{30}	2,0
C_{19}	0,4	C_{25}	8,0	C_{31}	34,3
C_{20}	1,1	C_{26}	1,1	C_{32}	0,9
C_{21}	1,2	C_{27}	16,6		

homologs (17%). The C_{27}, C_{29}, and C_{31} hydrocarbons made up almost 78% of the mass of this fraction.

The fatty alcohols consisted of a mixture of the 23:0-32:0 alkanols with the 24:0 and 26:0 species predominating (mass spectrum, m/z 336 and 364 ($M^+ - 18$)). The alkanols of the wax esters were not investigated because of the smallness of fraction III (Table 1).

In the free sterols, campesterol (M^+ 400), stigmasterol (M^+ 412) and, as the main component, β-sitosterol (M^+ 414) were detected with the aid of mass spectrometry. The same set of sterols was obtained after the saponification of the sterol esters (fraction IV, Table 1) and the isolation, purification (TLC, system 4), and mass-spectrometric analysis of the alcoholic fraction.

Anthraquinones. Extracts of the fruit, seeds, and perianths of R. paulsenianus had a bright yellow color. The bulk of the yellow pigments was eluted from a column together with TAGs by 3-4% of diethyl ether in petroleum ether. The more polar fractions were colored pink, yellow, dark yellow, and brown.

After the end of the fractionation of the extracts, the silica gel had acquired a light lilac color which was not eliminated by washing with methanol.

On silufol in system 1 the pigments appeared in the form of two main yellow spots with R_f 0.59 and 0.48. When the plates were viewed in UV light, the yellow coloration of the spots changed to orange; qualitative reactions for anthraquinones with NH_4OH vapor and with ethanolic solutions of KOH and of $Mg(CH_3COO)_2$ [11] were positive. Two pigments were isolated by preparative TLC in systems 2 and 4 in the form of yellow and reddish-orange crystals.

The anthraquinone with R_f 0.59 had the UV spectrum λ_{max}^{MeOH} 225, 258, 278, 288, 432 nm, and in the IR spectrum vibration bands were observed at 1625, 1680, 1570, and 3400 cm^{-1}, while the mass spectrum contained the peaks of ions with m/z 255 $(M + 1)^+$ (17.6%); 254 (M^+) (100%); 226 $(M - CO)^+$ (13%); 197 $(M - CO - HCO)^+$ (12%); 152 (18%); 141 (11%); 139 (7%), on the basis of which it was identified as chrysophanol [12].

The pigment with R_f 0.48, according to its UV spectrum (λ_{max}^{MeOH} 224, 258, 267, 288, 432 nm), its IR spectrum ν_{max}^{KBr} 1620, 1670, 1570, 3400, cm^{-1}) and its mass spectrum, in which the peaks of the ions 284 (M^+) (100%), 285 $(M + 1)^+$ (18%), 256 (9.8%), and 227 (10.6%) were observed, corresponded to physcion. The same mass spectrum contained weak peaks of ions with m/z 270 (M^+), 271 $(M + 1)^+$, 242, and 213, which are characteristic for aloe-emodin [11].

The distributions of these pigments in the various parts of the fruit of R. paulsenianus were dissimilar. Analysis of aliquot samples of extracts of the seeds and perianths (TLC, systems 1-4, 8, and 9) showed that the level of the pigments in the perianths was appreciably higher and their composition was more diverse than in the seeds.

Free anthraquinones were present in the fruit and seeds of 13 out of 17 species of Rumex studied [2], but their bulk both in the free and in the bound form, accumulated in the epigeal organs of almost all the species of the genus. The roots of R. paulsenianus contained 0.4% of anthraquinones (chrysophanol, physcion). We are the first to have recorded the presence of anthraquinone pigments in the fruit of the species, including the perianths.

EXPERIMENTAL

UV and IR spectroscopies, mass spectrometry, and GLC analysis were carried out under the conditions described previously [13]. UV spectra were taken in methanol. Conditions for the mass spectrometry of the TAGs-2: temperature of the ionizing chamber 120°C and of the evaporator bulb 80°C; collector current 40μA; ionizing energy 50 eV. The R. paulsenianus fruit was collected in the environs of the village of Karkara (Kazakh SSR), by A. M. Nigmatullaev.

The lipids were isolated from the comminuted fruit, seeds, and perianths by three extractions with hexane in the course of a day.

Column chromatography was performed on a column (2.5 × 30 cm) of silica gel L 100/160 (Chemapol, Czechoslovakia) with elution of the fractions successively by hexane, hexane with the addition of 2, 4, 5, 8, 10, 20, and 50% of diethyl ether, diethyl ether, chloroform, and methanol.

Thin-layer chromatography was conducted on Silufol and silica gel of type L 5/40 (Chemapol, Czechoslovakia) with the addition of 6.5% of gypsum, and Ag^+-TLC, with 15% of $AgNO_3$, in the

following solvent systems: 1) heptane–methyl ethyl ketone–acetic acid (43:7.0.5), two runs; 2) hexane–diethyl ether–acetic acid (80:20:1); 3) hexane–diethyl ether (9:1); 4) hexane–diethyl ether (1:1); 5) benzene–chloroform–diethyl ether (50:50:25); 6) hexane–acetone (7:3); 7) butanol-1–ammonia–water (20:1:5); 8) benzene–acetone–methanol (8:10:2); and 9) hexane–ethyl acetate–acetic acid (17:4:1). The chromatograms were visualized as described in [13]. The individual lipid classes were identifided from a comparison of their chromatographic mobilities with model samples, from their chemical transformation, and from their UV, IR, and mass spectra.

The anthraquinones, fatty alcohols, and sterols were recrystallized from methanol. The saponification of the lipids and the esterification of the fatty acids obtained were performed by a known method [13]. The barium salts of the acids were obtained as described in [6].

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