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BRANCHED AND VERY LONG-CHAIN DICARBOXYLIC ACIDS FROM *EQUISETUM* SPECIES

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Key Word Index—*Equisetum*; horsetail; dicarboxylic acids; methyl-branched dicarboxylic acids; GC-MS; NMR.

Abstract—Ten species of *Equisetum* growing in the Czech Republic were analysed for the lipidic content of their spores. GC-MS was used after extraction of spores to determine the qualitative and quantitative abundance of dicarboxylic acids. Together with known acids with an even number of carbon atoms (C_{22}–C_{30}), two new acids, 14-methylnonacosanedioic acid and 14,15-dimethyltriacontanedioic acid were identified from *E. litorale*. Their structures were confirmed by means of mass, as well as NMR spectra. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Very long-chain monocarboxylic acids are often found in natural materials [1]. Dicarboxylic acids with chain-lengths more than C_{10} are less common. In the plant kingdom, they usually exist in the form of esters as a part of cutins and suberins [2]. Except for this source, they were also identified in fossil sediments like peat [3] or shales [4]. Even longer homologues occur very rarely, [5]. For instance, even carboxylic acids C_{32}–C_{36} were found to predominate in the bacterium, *Sarcina ventriculi*, and one of them, a long-chain dioic acid had an unusual structure with two vicinal methyl residues in the centre of the chain [6].

As early as 1949, Sosa [7] described the presence of equisetolic acid (C_{37} dioic acid) in the spores of horsetails. More than 20 years later, the structure of equisetolic acid was defined with more precision and minor homologues were identified [8].

In our previous work on the long-chain dioic acids in the horsetail, *Equisetum arvense*, the mixture of homologues was determined where the single homologues were represented almost equally [9]. Horsetails are very ancient plants, so that their spores are found in peat and lignite, as well as in coal. Dicarboxylic acids present in fossil fuel [10] can also have their origin in plant spores. To elucidate this hypothesis we investigated the structure of the dicarboxylic acids in ten species of horsetails.

RESULTS AND DISCUSSION

Nine out of the ten species of horsetails examined showed a similar distribution of dioic acids (Table 1). However, *E. litorale* had a different composition. This species is a hybrid *E. arvense* and *E. limosum*, and forms predominantly sterile stalks with very small spore spikes and underdeveloped spores [11].

The structure of all homologues of dicarboxylic acids was established using GC-mass spectrometry, using the corresponding dimethyl and dipicolinyl esters. In the mass spectra of dimethyl esters, the base peak ion is m/z 98 with significant ions at $[M-31]^+$, $[M-32]^+$ and $[M-64]^+$ [9]. The m/z values of separate ions including their relative abundance are listed in Table 2; they are in good agreement with published data [12]. For picolinyl esters, only Harvey [13] has published a spectrum of the dipicolinyl ester of 1,12-dodecanedicarboxylic acid. The major ion in the high mass region of these derivatives was the $[M]^+$ and $[M-107]^+$ (in [13] described as ion *l*.) Relative abundances for these ions are listed in Table 2.

Two new acids were identified in *E. litorale*, GC-mass spectrometry showing resolution of peaks in the C_{30} and C_{32} methyl ester region. The mass spectra of these compounds showed ions which suggested branching in the chain. Preparative RP-HPLC was used to isolate these acids as their dimethyl esters and, based on ^1H NMR, ^{13}C NMR and mass spectra, their structures were determined.

In the first branched acid, 14-methylnonacosanedioic, a signal in the ^1H NMR spectra at δ 0.77 ($J = 7.1$ Hz) was observed, indicating substitution of the aliphatic chain by a methyl in the

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Table 1. Content of dicarboxylic acids in ten horse-tail species

	E. arvense	*E. fluviatile*	*E. hiemale*	*E. litorale*	*E. palustre*	*E. pratense*	*E. ramosissimum*	*E. silvaticum*	*E. telmateia*	*E. variegatum*
Spores (mg)	70	70	110	540	60	80	12	21	23	17
Total lipids (μg)	510	630	910	6250	570	850	137	218	192	145
Dioic acid (No. C)*	% of total acids									
10	2.85	2.60	0.74	0.23	0.78	1.61	2.30	2.15	2.67	2.32
11	3.70	1.82	0.47	0.02	0.91	1.38	1.56	1.91	1.36	1.89
12	2.28	2.38	1.17	0.28	1.17	2.76	1.75	2.58	2.86	2.97
13	1.99	1.53	0.70	0.00	1.15	2.53	2.77	2.31	2.29	2.11
14	1.71	2.86	1.89	0.59	1.36	2.57	2.14	3.29	3.04	3.16
15	4.48	2.62	1.41	0.22	0.97	3.22	4.09	2.79	2.61	2.56
16	2.56	3.65	2.82	0.79	3.69	2.30	5.69	3.43	3.43	3.55
17	5.13	1.55	1.64	0.89	3.30	2.69	3.70	1.93	2.48	2.53
18	3.49	4.94	5.40	2.05	4.46	3.80	4.47	4.51	4.10	4.86
19	3.99	2.34	4.46	0.56	2.67	3.45	3.31	3.22	3.65	3.58
20	7.12	5.99	5.87	1.32	5.63	6.21	5.60	5.36	5.52	5.68
21	4.27	3.13	6.34	1.21	2.72	5.06	5.45	2.90	4.19	4.42
22	8.83	9.11	9.19	1.84	6.80	8.05	7.59	7.30	6.67	6.95
23	7.69	3.63	4.93	6.53	3.63	5.29	6.42	5.15	4.76	4.81
24	13.31	14.51	13.43	7.63	9.13	11.21	9.90	8.37	9.90	9.89
25	3.42	2.64	9.86	3.56	4.49	7.36	4.45	4.58	5.71	5.64
26	4.84	10.67	9.15	7.21	9.90	9.43	8.75	10.70	8.95	7.58
27	3.13	3.39	3.76	0.71	6.88	3.91	3.50	3.86	3.05	4.41
28	7.40	7.55	5.31	6.49	9.56	6.44	5.06	7.52	7.81	8.15
29	1.14	1.60	1.88	1.05	5.44	2.25	2.33	4.56	3.62	2.95
30	4.56	5.21	4.40	22.37†	6.99	4.14	3.76	4.94	6.77	5.62
31	0.29	0.78	0.94	0.26	1.39	2.07	1.50	2.36	1.33	1.05
32	0.85	3.68	2.52	35.53‡	3.88	1.15	2.72	3.00	2.10	1.85
33	0.28	0.52	0.49	0.53	0.58	0.46	0.39	0.21	0.38	0.63
34	0.57	1.04	0.99	0.75	2.14	0.69	0.78	1.07	0.57	0.84
35	0.00	0.26	0.23	0.35	0.39	0.00	0.00	0.00	0.19	0.00

* No. of total carbon atoms in dioic acid.

† Predominantly 14-methylnonacosanedioic acid, (broad peak, included as major compound branched acid, as minor compound unbranched acid).

‡ Predominantly 14,15-dimethyltriacontanedioic acid, (broad peak, included as major compound branched acid, as minor compound unbranched acid).

Table 2. Structure and abundance of major ions from esters of dioic acids

	Dimethyl esters*											Dipicolinyl esters†			
Dioic acid‡	M_r§	$[M-31]^+$	%‖	$[M-32]^+$	%‖	$[M-64]^+$	%‖	$[M-92]^+$	%‖	$[M-105]^+$	%‖	$[M]^+$	%‖	$[M-107]^+$	%‖
10	230	199	14	198	10	166	14	135	6	125	36	384	57	277	94
11	244	213	13	212	6	180	27	149	5	139	25	398	43	291	86
12	258	227	19	226	7	194	31	163	6	153	23	412	31	305	78
13	272	241	23	240	8	208	35	177	6	167	21	426	35	319	63
14	286	255	25	254	8	222	24	191	5	181	22	440	30	333	57
15	300	269	24	268	4	236	30	205	4	195	14	454	32	347	72
16	314	283	20	282	6	250	41	219	3	209	11	468	27	361	66
17	328	297	18	296	7	264	31	233	2	223	10	482	25	375	58
18	342	311	21	310	3	278	35	247	3	237	12	496	31	389	62
19	356	325	17	324	5	292	27	261	3	251	11	510	23	403	56
20	370	339	15	338	4	306	29	275	2	265	9	524	17	417	61
21	384	353	13	352	5	320	23	289	4	279	7	538	19	431	50
22	398	367	10	366	3	334	24	303	2	293	5	552	14	445	48
23	412	381	7	380	4	348	16	317	3	307	3	566	15	459	57
24	426	395	6	394	3	362	14	331	2	321	2	580	13	473	46
25	440	409	4	408	2	376	9	345	1	335	1	594	11	487	53
26	454	423	4	422	2	390	7	359	2	349	2	608	10	501	41
27	468	437	3	436	2	404	6	373	1	363	1	622	8	515	48
28	482	451	4	450	1	418	7	387	1	377	1	636	9	529	36
29	496	465	2	464	1	432	3	401	1	391	1	650	5	543	49
30	510	479	3	478	1	446	5	415	1	405	1	664	7	557	31
31	524	493	1	492	1	460	2	429	1	419	1	678	6	571	27
32	538	507	2	506	1	474	1	443	1	433	1	692	5	585	34
33	552	521	1	520	1	488	1	457	1	447	1	706	4	599	45
34	566	535	2	534	1	502	1	471	1	461	1	720	5	613	21
35	580	549	1	548	1	516	1	485	1	475	1	734	4	627	33

* Base peak for all dimethyl esters of dioic acids was ion at *m/z* 98.

† Base peak for all dipicolinyl esters of dioic acids was ion at *m/z* 108.

‡ Number, i.e. no. of total carbon atoms in dioic acid.

§ M_r for dimethyl ester of appropriate dioic acid.

‖ % of base peak.

vicinity of the centre of the chain. This was confirmed by a signal at δ 19.8 in the ^{13}C-NMR spectrum. The formula $C_{32}H_{62}O_4$ was deduced from high resolution measurements and fragmentation ions at *m/z* 227, 255 and 283 confirmed the structure of the dimethyl ester. This was supported by the corresponding ions *m/z* 304, 332 and 360 in the mass spectrum of the dipicolinyl derivative.

The structure of the compound with the longer retention time in GC-MS analysis, 14,15-dimethyltriacontanedioic acid, was established from the following evidence. The ^{1}H NMR spectrum confirmed the presence of branching with methyl groups (doublet at δ 0.76, $J = 7.3$ Hz); values for other signals and their assignment are given in Experimental. The ^{13}C NMR spectrum shows signals at δ 14.8 and 15.1, confirming the proposed structure. These values are in good agreement with published data for diabolic and similar acids [6, 15]. An aliphatic chain substituted by two vicinal methyls near the centre of the chain was thus predicted. The two ^{13}C NMR signals also indicate that the molecule is asymmetrical. Mass spectrometry enabled full elucidation of the suggested structure. The formula $C_{36}H_{66}O_4$ was deduced from high resolution measurements of the dimethyl ester and fragment ions with *m/z* values 227, 255, 283 and 311 indicated the presence of vicinal methyl residues in positions 14 and 15, confirming the asymmetry of the molecule. In accordance with these data were the values measured for the corresponding dipicolinyl ester. Fragments with *m/z* values of 304, 332, 346, 360 and 388, and especially their abundance suggest branching of the chain with two methyl groups in positions 14 and 15. Because of fragmentation of the molecule from each ester group the ions with *m/z* 388 and 304 exhibit higher intensity. Ions with *m/z* 332 and 360 correspond to cleavage of the bond between carbon atoms 15 and 16, and 14 and 15, respectively. Alternatively, ions with *m/z* 304 and 332 have their origin from cleavage of bonds between carbon atoms 13 and 14, and 14 and 15, respectively, and ion *m/z* 388 corresponds to the cleavage of the bond between carbon atoms 13 and 14. The chain-length 31.3 published by Adams and Bonnett [8] 25 years ago also suggests the presence of branched homologues.

The finding of novel branched-chain dioic acids in a horsetail is of interest. These acids probably arose from tail-to-tail coupling of appropriate fatty acids (i.e. pentadecanoic and heptadecanoic or two pentadecanoics). Such a "mixed" biogenesis has not been recognized before, since previous studies have only reported symmetrical dimers. Only one exception has been described by Carballeira *et al.* [16] who referred to "mix" biosynthesis, but only with even carbon number fatty acids. This is the first mention presumably about condensation of odd carbon number fatty acids.

The function of dicarboxylic acids from spores is well known. They act predominantly, like the other lipids, waxes and hydrocarbons, as a hydrophobic layer on the spore surface, which decreases their wettability. In contrast to bacteria, where they are a part of phospho- and glycolipids [6], in horsetails they exist in the free form. We have identified in all ten horsetail species studied the whole homologous series. It is difficult to say whether differences from earlier work is caused by different geographical origin of our horsetails (central Europe in comparison with Pacific coast of Canada [8]), or if our modern instruments made the difference. On the basis of our previous analyses of fossil organic rocks [10] we can say that at least part of dicarboxylic acids can have their origin in horsetails. This is in good accordance with the abundance of separate homologues in fossil fuels where the maximum abundance related to the chain length was documented in even carbon numbered C_{22}–C_{28} acids. It also corresponds well with the data listed in Table 1.

EXPERIMENTAL

Cones from the different *Equisetum* species were collected in the area of central Bohemia in the period from May–August 1996 and April–May 1997. Cones were air-dried and the Me esters of the dioic acids prepared according to Ref. [9] and analyzed by GC-MS. DiMe esters and/or picolinyl esters (prepared according to Ref. [13]) were analyzed using a SPB-1 column (30 m × 0.25 mm I.D.). The column temp. was programmed from 100° (maintained for 1 min) at 4° min^{-1} to 280° for diMe esters and from 130° at 4° min^{-1} to 310° for dipicolinyl esters. Direct probe MS of mono- and dibranched esters was also carried out. Dioic acid diMe esters from cones of *E. litorale* were separated on a RP-HPLC column and two branched homologues were isolated. A semi-prep. Supelcosil LC-18 column (25 cm × 10 mm I.D.) was used with a linear gradient of MeOH–THF from 9:1 to 1:9 during 30 min.

Dimethyl ester of 14-*methylnonacosanedioic acid.* ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.77 *d* ($J = 7.1$ Hz; CH_3; i.e. C-30 → Me on C-14), 1.31 *m* ($22 \times CH_2$), 1.35 *m* ($1 \times H$; —$C\underline{H}CH_3$—), 1.61 *m* ($4 \times H$; OC—CH_2—$C\underline{H}_2$), 2.28 *t* ($4 \times H$; OC—$C\underline{H}_2$), 3.66 *s* ($6 \times H$; CH_3–O). ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.8 ($C_{30} \rightarrow$ Me on C_{14}), 25.0 (C-3 and C-27), 27.2 and 27.2 (C-12 and C-16), 29.2–29.8 (C-4—C-10. and C18–C26), 30.1 (C-11 and C-17), 33.0 (C-14), 34.1 (C-2 and C-28), 37.3 and 37.2 (C-13 and C-15), 52.2 ($2 \times CH_3$—O), 175.9 and 175.8 (C-1 and C-29). HR-MS found 510.84838, calcd 510.84904 for $C_{32}H_{62}O_4$. GC-MS *m/z* (rel. int.): 510 ($[M]^+$, 35), 495 ($[M-Me]^+$, 3). 479 ($[M-MeO]^+$. 17), 478 ($[M-MeOH]^+$. 54), 446 ($[M-2 \times MeOH]^+$, 37). 297 (14), 283 (41), 255 (69), 227 (73), 213 (28). 143 (55), 129 (74), 115 (41). 101 (65), 98 (100), 87 (84), 74 (90). Dipicolinyl ester *m/z* (rel. int.): 664 ($[M]^+$, 43), 557 ($[M-l]^+$, 71), 528 (3), 514 (2), 500 (4), 486 (5), 472 (4), 458 (6), 444 (8), 430 (9), 416 (7), 402 (11), 388 (9), 374 (12), 360 (19), 346 (3). 332 (32), 318 (5), 304 (26), 290 (18), 276 (13), 262 (19). 248 (15), 234 (12).

220 (20), 206 (16), 192 (23), 178 (25), 164 (44), 151 (67), 108 (100), 92 (75).

Dimethyl ester of 14,15-*dimethyltriacontanedioic acid.* ^{1}H NMR (500 MHz, CDCl$_3$): δ 0.76 *d* ($J = 7.3$ Hz; $2 \times$ CH$_3$; i.e. C-30 and C-31 → Me on C-14 and C-15), 1.30 *m* ($22 \times$ CH$_2$), 1.34 *m* ($1 \times$ H; —C$\underline{\mathrm{H}}$CH$_3$—), 1.61 *m* ($4 \times$ H; OC—CH$_2$—C$\underline{\mathrm{H}}_2$), 2.27 *t* ($4 \times$ H; OC—C$\underline{\mathrm{H}}_2$), 3.66 *s* ($6 \times$ H; CH$_3$—O). ^{13}C NMR (125 MHz, CDCl$_3$) 14.8 and 15.1 (C-31 or C-32; i.e. Me's on C-14 or C-15), 25.1 (C-3 and C-28), 27.4 and 27.4 (C-12 and C-17), 29.26–29.93 (C-4—C-10 and C-19—C-27), 30.1 and 30.1 (C-11 and C-18), 34.27 (C-2 and C-29), 34.6 and 34.6 (C-13 and C-16), 36.7 and 37.1 (C-14 and C-15), 52.4 ($2 \times$ CH$_3$—O), 175.48 and 175.70 (C-1 and C-30). HR-MS found 538.90261, calcd 538.90272 for $C_{34}H_{66}O_4$. GC-MS *m/z* (rel. int.): 538 ($[M]^+$, 38), 523 ($[M-Me]^+$, 14), 507 ($[M-MeO]^+$, 23), 506 ($[M-MeOH]^+$, 37), 474 ($[M-2 \times MeOH]^+$, 18), 325 (13), 311 (42), 283 (69), 255 (77), 2500 (3), 486 (5), 472 (4), 458 (4), 444 (5), 430 (7), 416 (12), 402 (21), 388 (18), 374 (7), 360 (5), 346 (6), 332 (6), 318 (17), 304 (23), 290 (11), 276 (9), 262 (10), 248 (12), 234 (16), 220 (15), 206 (17), 192 (14), 178 (19), 164 (41), 151 (57), 108 (100), 92 (68).

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