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Note

Capillary gas chromatography–mass spectrometry of very-long-chain α,ω-dicarboxylic acid dimethyl esters from *Equisetum* (horsetail)

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The occurrence of α,ω-dicarboxylic acids in nature is much smaller than that of fatty acids. Moreover, among these dicarboxylic acids are those having hydrocarbon chain lengths greater than C_{22} as a minor component[1]. There are several types of plant material in which α,ω-dicarboxylic acids represent an important component, *e.g.*, in the bark of needle-leaved trees[2,3] or leafy trees[4] and fossil sediments like peat[5] or shales[6]. Only the last two kinds of materials contain α,ω-dicarboxylic acids with chain lengths up to C_{28} and C_{32}, respectively.

It was concluded that the only available material containing very small amounts of long-chain α,ω-dicarboxylic acids is horsetail, a precursor of fossil sediments[7,8].

In our previous work[9] it was mentioned that only Ryhage and Stenhagen[10,11] had dealt systematically with the mass spectrometry of dimethyl esters of α,ω-dicarboxylic acids (DEDAs), however, only up to the C_{22} chain. In another paper[8], solely the DEDA-C_{30} mass spectrum was shown. For this reason we have characterized other very-long-chain DEDAs up to C_{34}.

EXPERIMENTAL

Preparation of DEDAs

The cones from *Equisetum arvense* L. were collected about 30 km south of Prague in the first half of April 1988. Further isolation from spores was carried out according to the method of Adams and Bonnett[8]. The total gain was 47 mg of waxy substance, from which the methyl esters were prepared by treatment with diazomethane. DEDAs were separated by thin-layer chromatography (TLC) on silica gel G using the solvent system hexane–diethyl ether (7:3, v/v); the R_F was 0.63 and the total gain was 31.3 mg.

Gas chromatography–mass spectometry (GC–MS) of DEDAs

The mixture of DEDAs was chromatographed on a non-polar capillary column

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60 mm × 0.32 mm I.D.) SPB-1 (Supelco, Bellefonte, PA, U.S.A.) using splitless injection and helium as a carrier gas. The oven temperature was programmed from 100 to 300°C at 4° min^{-1}. Mass detection was carried out by a coupled mass spectrometer, QP 1000 (Shimadzu, Kyoto, Japan) which uses a glass separator as a connection between the chromatograph and mass spectrometer. The ionization energy was 70 eV and the electron multiplier voltage was 2.5 kV.

RESULTS AND DISCUSSION

Capillary GC

Fig. 1. shows the baseline separation of the homologous saturated DEDAs on a non-polar capillary column. Previously we have demonstrated[9] a certain discrimination of higher homologues, *i.e.* of DEDAs with chain lengths higher than C_{20}. To avoid such losses, splitless injection was used, which helps decrease the discrimination of higher boiling components. By this method, even DEDA-C_{34} could be detected.

The effective chain length (ECL) of DEDAs between C_{20} and C_{34} is about 3.8 units higher than that of the methyl esters of the respective fatty acids (FAMEs), see Fig. 2. The data for the lower-chain-length DEDAs have been extracted from our previous measurements[9]. Fig. 2 shows an increasing Δ(ECL) for higher mass molecules which causes a gradual deterioration in separation quality between DEDAs and FAMEs.

The distribution of DEDA homologous is shown in Fig. 1; in horsetail, α,ω-dicarboxylic acids were found ranging between C_{10} and C_{34}. Adams *et al.*[7,8], however, reported that more than 90% of the total dicarboxylic acids was DEDA-C_{30}. Dicarboxylic acids with hydrocarbon chains longer than C_{30} were not found; among the

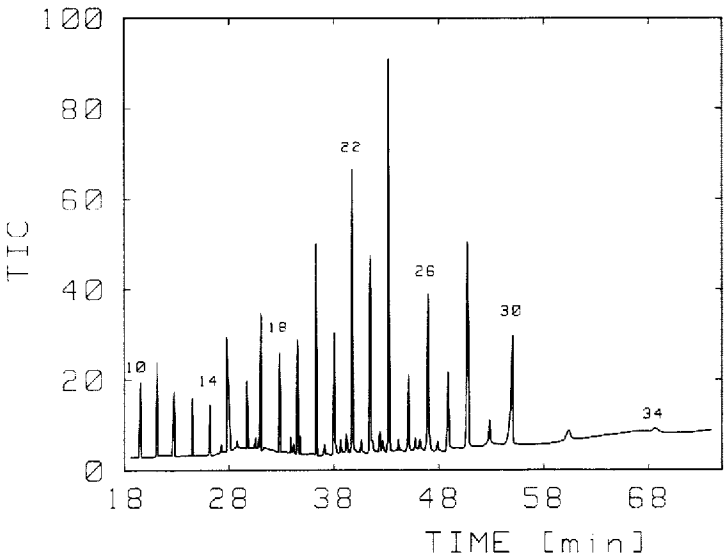

Fig. 1. Capillary GC–MS of DEDAs (the numbers at the peaks indicate the carbon chain lengths) from *Equisetum arvense* L. on a SPB-1 column (Supelco), 60 mm × 0.32 mm I.D. splitless injection, oven programmed from 100 to 300°C at 4° min^{-1}. TIC = total ion current.

TABLE I

ELECTRON IMPACT MASS SPECTRA OF DEDAs OBTAINED BY MEANS OF A QUADRUPOLE MASS SPECTROMETER

Acid	*Mol.wt.*	*M−31*	*M−32*	*M−63*	*M−64*	*M−73*	*M−91*	*M−92*	*M−105*	*M−106*	*M−123*	*112*	*111*	*98*	*97*	*87*	*84*	*83*	*74*	*73*	*69*	*59*	*57*	*56*	*55*
C_{21}	384	14.3	3.7	2.8	5.2	6.8	0.9	1.0	9.5	7.1	3.8	31.5	10.9	100	20.8	14.7	45.7	10.7	29.4	5.8	10.5	14.7	20.1	7.7	54.6
C_{22}	398	10.9	4.3	3.7	4.1	7.0	1.8	2.5	7.6	4.9	3.6	32.3	14.3	100	27.6	12.1	42.6	27.2	37.0	5.4	35.7	18.3	23.9	8.7	63.9
C_{23}	412	10.1	3.9	6.7	11.7	5.2	1.6	3.8	6.5	5.3	4.1	29.1	18.6	100	36.6	13.8	50.7	39.8	29.3	6.5	44.9	18.5	17.2	13.8	64.9
C_{24}	426	7.3	3.7	2.5	2.9	4.8	0.7	1.2	5.5	4.7	2.4	28.3	14.3	100	27.3	5.5	41.8	26.3	26.3	5.5	36.0	16.7	21.3	8.4	63.3
C_{25}	440	1.7	0.9	6.2	12.9	0.5	0.8	1.5	9.5	7.9	1.5	29.1	5.6	100	12.1	8.4	32.3	25.8	25.1	4.8	29.9	12.7	17.4	7.3	51.6
C_{26}	454	9.7	4.0	3.6	5.2	5.2	3.5	3.1	6.9	4.2	3.4	25.7	11.1	100	21.6	4.5	38.6	21.4	27.3	4.2	27.3	11.6	7.5	5.5	45.8
C_{27}	468	7.9	4.4	6.4	13.4	3.9	1.6	1.7	5.5	4.7	2.5	21.3	24.2	100	44.6	4.9	29.7	49.1	22.7	4.8	60.9	16.7	7.6	11.4	19.2
C_{28}	482	10.4	8.9	4.8	8.1	4.1	1.1	2.9	8.2	6.0	2.4	26.6	7.3	100	10.6	7.0	37.3	23.8	36.4	6.5	17.6	11.4	8.1	3.5	12.2
C_{29}	496	8.1	9.1	2.2	4.3	1.1	0.8	3.7	9.8	6.1	1.1	29.3	8.4	100	24.2	3.8	42.1	14.3	29.8	3.2	35.2	10.3	7.8	9.8	10.9
C_{30}	510	13.4	9.5	2.4	3.8	0.8	2.1	3.0	9.7	11.5	0.5	28.0	8.9	100	14.0	6.7	35.3	15.8	31.3	5.0	9.0	7.2	6.9	7.2	14.1

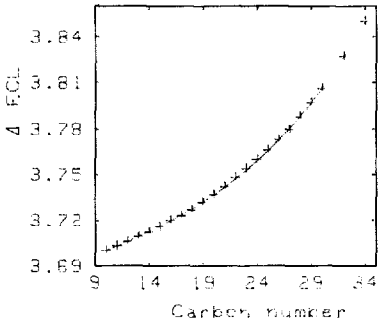

Fig. 2. The relationship between the carbon number of DEDAs and the difference in ECL between DEDA and FAME.

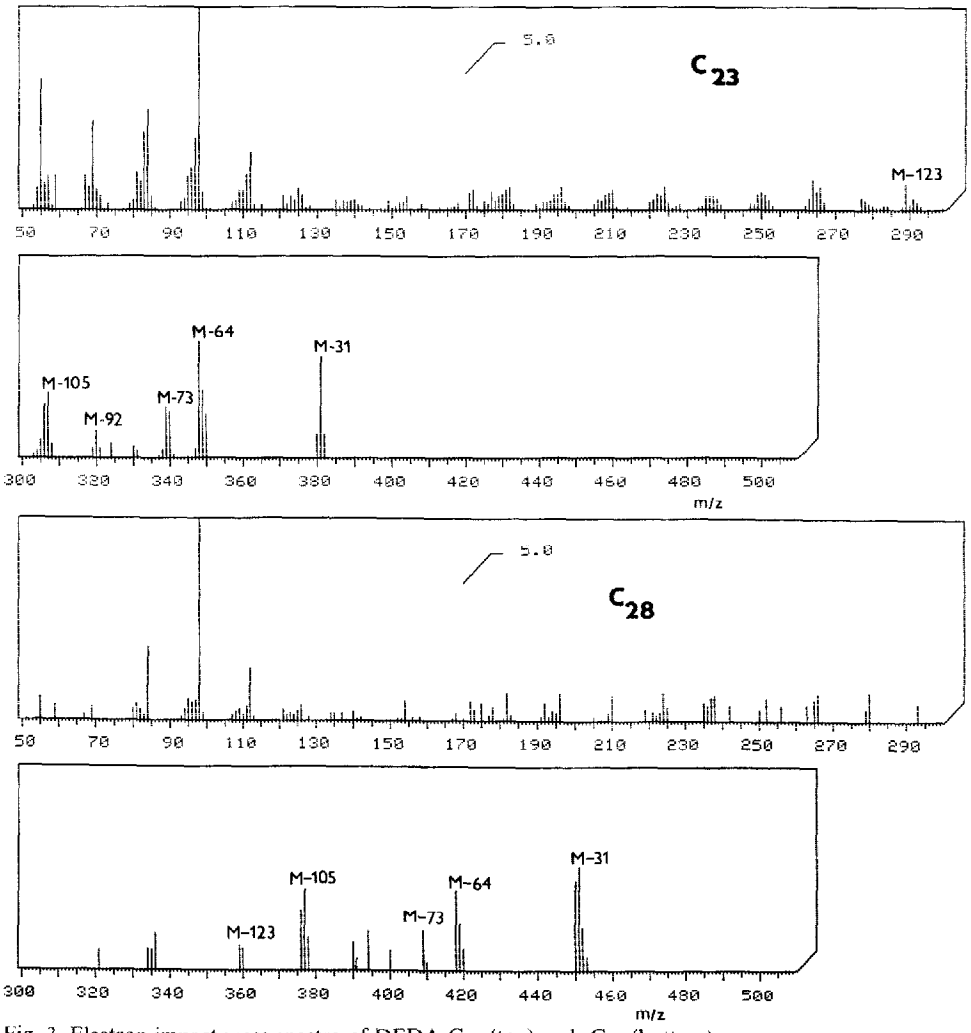

Fig. 3. Electron impact mass spectra of DEDA-C_{23} (top) and -C_{28} (bottom).

others the content of DEDA-C_{28} lay between 1.7 and 5%, and the contents of C_{22} and C_{24} were at most 2%. Our results substantially differ from this distribution, which can be attributed to different varieties of horsetail.

Mass spectra of long-chain DEDAs

The most characteristic feature of the mass spectra of long chain DEDAs (distinguishing them unambiguously from short chain DEDAs up to C_{10} as well as from the FAME analogues) are the homologues ions at *m/z* 84, 98 and 112 found in almost constant abundance ratio, approximately 4:10:3, the ion at *m/z* 98 being the base peak of the mass spectra for all DEDAs starting with C_{11}; the structure of these ions was discussed elsewhere[9,11,12].

In the absence of a molecular ion (ions of abundance lower than 0.5% of the base peak were not recorded) there are two important ions in the high mass region: $[M-31]^+$ corresponding to the loss of one methoxy group similarly to FAMEs and a rearrangement ion $[M-64]^+$ formed by the loss of two molecules of methanol.

Mass spectra of rarely occurring (C_{23} and C_{28}) long chain DEDAs are displayed as an example in Fig.3 and the most significant ions of all C_{21}–C_{30} DEDAs are summarized in Table I. Some differences in ion abundance especially in the high mass region of C_{21}–C_{24} DEDAs, if compared to our previous report[9], are largely due to the different instrumentation (HP5995 and Shimadzu QP 1000) and experimental conditions employed.

CONCLUSIONS

Very-long-chain α,ω-dicarboxylic acids in the form of dimethyl esters can be succesfully analyzed by capillary GC on a non-polar column (because high temperatures are necessary) in combination with mass spectrometric detection, which enables besides qualitative analysis also quantitative estimates of the long-chain DEDAs, based upon the characteristic ions (*m/z* 84, 98, 112, M − 123, M − 146) for this group of compounds. If the analyzed material contains FAMEs along with DEDAs, there is hardly another analytical method available because of the similar chemical character of these two groups of compounds, especially for the C_{20}–C_{34} DEDA region, where the effective chain length difference from the interfering FAME decreases substantially.

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