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CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF ALIPHATIC SATURATED α,ω-DICARBOXYLIC ACID DIMETHYL ESTERS AND DIRECT INLET MASS SPECTROMETRY OF THE CORRESPONDING FREE ACIDS

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SUMMARY

Fused-silica capillary gas chromatography on a 40-m FS-DC 510 column has been studied for dimethyl esters of C_3–C_{24} aliphatic saturated α,ω-dicarboxylic acids. The electron-impact mass spectra of free α,ω-dicarboxylic acids (from malonic, C_3, to thapsic, C_{16}) and of the dimethyl esters of such acids ranging from C_3 to C_{24} is discussed. Despite the absence of molecular ions, sufficient ions characteristic for the hydrocarbon chain length of α,ω-dicarboxylic acids (and their dimethyl esters) are formed. Fragmentation paths for both the free acids (direct inlet mass spectrometry) and the corresponding esters (gas–liquid chromatography–mass spectrometry) are suggested. Acids with long hydrocarbon chains (up to C_{24}) have been found in the fungus *Fomes igniarius*.

INTRODUCTION

Although α,ω-dicarboxylic acids are less abundant in nature than fatty acids, they have been determined in various kinds of biological and other materials. In plants they have been observed as a wax component[1], and in apples as one of the components of cutine[2,3] or suberine[4,5]. Other unconventional sources of α,ω-dicarboxylic acids are shales[6] or the alga *Botryococcus braunii*[7], where dimethyl esters of C_{14}–C_{19} acids were identified by gas–liquid chromatography–mass spectrometry (GLC–MS).

As for the animal kingdom, attention has been paid especially to α,ω-dicarboxylic acids as anomalous metabolites. The relationships between anomalous metabolisms and the corresponding diseases were reviewed by Jellum[8] and Liebich[9]. C_6–C_{10} α,ω-Dicarboxylic acids were found in the urine from patients suffering from

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"type I and type III glycogen storage disease"[10], C_3–C_{10} acids in urine were identified by GLC–MS[11] and dicarboxylic acids up to C_{18} were identified by capillary GLC–MS in patients with Reye's syndrome[12].

The products of oxidation of unsaturated monocarboxylic acids are another possible source of α,ω-dicarboxylic acids. Oxidative cleavage is used not only for analytical determination of double bonds[13] but also for the preparation of α,ω-dicarboxylic acids[14]. Polymer chain splitting of condensation-type polymers, *e.g.* alkyds, unsaturated polyesters, polyamides, yields polycarboxylic acids. Herrmann *et al.*[15] investigated the resulting dimethyl esters of C_3–C_{12} dicarboxylic acids by using a 25-m Ultra 1 (Hewlett-Packard) non-polar capillary column and a quadrupole-type (Varian MAT 44S) mass analyzer.

Only few papers have dealt systematically with the mass spectrometry of α,ω-dicarboxylic acids. Ryhage and Stenhagen[16,17] have studied the behaviour of dicarboxylic acid esters under electron impact. The set of standards analyzed included dimethyl esters of acids with 6, 8, 9, 13, 18 and 22 carbon atoms. Other published results[1,3,7,12] are restricted to graphical mass spectra without any commentary on the formation of ions, etc.

As dicarboxylic acids have recently received attention in many fields (above all as metabolites), we decided to carry out a thorough GLC–MS investigation of these compounds.

EXPERIMENTAL

GLC–MS of dimethyl esters of α,ω-dicarboxylic acids (d.e.d.a.s)

The mixture of d.e.d.a. standards, prepared from free acids by the method of Metcalfe and Schmitz[18] (C_3–C_{16} acids) to which d.e.d.a.s from the fungus *Fomes igniarius* were added, was chromatographed on a FS-DC 510 capillary column (Macherey-Nagel; fused silica, 40 m × 0.32 mm I.D.); the oven temperature was increased from 60 to 250°C at 6°/min. The compounds were identified by a quadrupole mass spectrometer HP 5995 (Hewlett-Packard).

The same mixture was analyzed on a sector-type GLC–MS instrument LKB 9000 (LKB Produkter AB, Sweden) using a glass column (5 m × 3 mm I.D.) packed with 5% OV-101 on Chromaton N-AW-DMCS (Lachema, Brno, Czechoslovakia) to enable comparison between the quadrupole and sector-type mass spectra of α,ω-dicarboxylic acids. The GLC–MS conditions were as follows: injection port temperature, 160°C; oven temperature increased from 60 to 250°C at 10°/min; separator temperature, 270°C; ion-source temperature, 290°C; electron-impact energy, 70 eV; accelerating voltage, 3.5 kV.

MS of free α,ω-dicarboxylic acids (f.d.a.s)

The f.d.a. standards, C_3, C_4, C_6–C_{10}, C_{14} (Foxboro/Analabs, CT, U.S.A.), C_5, C_{11}, C_{12}, C_{16} (Fluka, Buch, Switzerland), C_{13} (Tokyo Chemical Industry, Tokyo, Japan) and C_{15} (Dr. Tax, Institute of Microbiology), were analyzed on a MAT 311 instrument (Varian, Bremen, F.R.G.). The samples were placed in a gold sample holder (a crucible) and passed through the direct inlet into the ion source, to be heated gradually from 70 to 200°C. The 70-eV mass spectra were recorded at the point of maximum total ion gain.

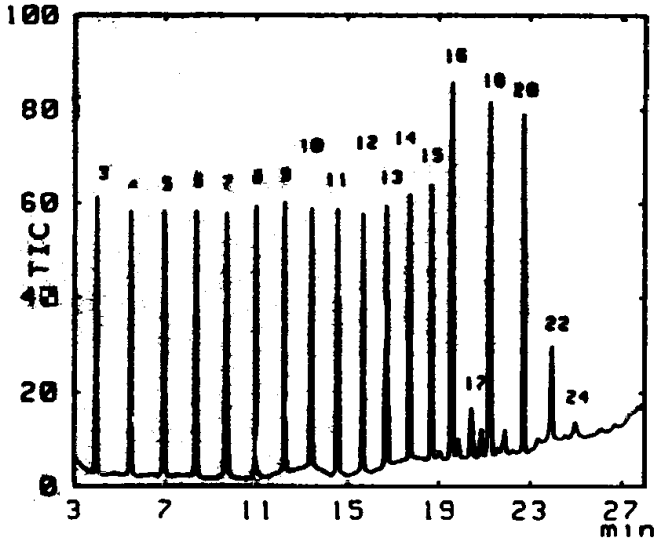

Fig. 1. GLC–MS analysis of a mixture of C_3–C_{24} d.e.d.a. standards on a 40-m capillary column FS-DC 510 (0.32 mm I.D.). Oven temperature gradient: from 60 to 250°C at 6°/min.

RESULTS

Capillary gas chromatography

The homologous saturated dimethyl esters of α,ω-dicarboxylic acids were baseline separated on a non-polar capillary column as shown in Fig. 1. However, the d.e.d.a.s higher than C_{18} are (quantitatively) discriminated.

The effective chain length (ECL) of d.e.d.a.s higher than C_{10} was found to be about 3.7–3.8 greater than those of the corresponding FAMEs (monocarboxylic fatty acid methyl esters), *e.g.*, the ECL of C_{16} d.e.d.a. is 19.72 and that of C_{24} d.e.d.a. is 27.76.

Larsson *et al.*[19] and Schomburg *et al.*[20] have studied the effect of the injector temperature and of various injection techniques, split, splitless and on-column, on the quantitative response of FAMEs up to C_{26} and of hydrocarbons up to C_{36}. Both groups reported discrimination of the highest-boiling components.

It can, therefore, be concluded that split injection without discrimination may be used for d.e.d.a.s with a maximum of 20 carbon atoms, which is equivalent to FAMEs containing 24 carbon atoms; for higher α,ω-dicarboxylic acid dimethyl esters, which are very rare in biological material, a different injection method must be used.

Mass spectra of d.e.d.a.s

The most important ions of dimethyl esters of C_3–C_{24} acids as measured by the quadrupole analyzer (HP 5995) are summarized in Table I. The base peaks for different carbon chain lengths of d.e.d.a.s are shown in Table II.

A comparison of the mass spectra of the C_{13} acid dimethyl ester obtained the quadrupole and sector-type mass analyzers is shown in Fig. 2. Some differences are due to the calibration of the quadrupole analyzer, but mainly arise from the combined effects of all the instrument constants. However, the characteristic ions, including

their relative intensities are identical in the two cases. Two examples of the mass spectra of the C_7 and C_{22} d.e.d.a.s are shown in Fig. 3.

TABLE I

ELECTRON IMPACT MASS SPECTRA OF d.e.d.a.s

Acid	*MW*	*M−31*	*M−32*	*M−59*	*M−60*	*M−63*	*M−64*	*M−73*	*M−91*	*M−92*	*M−105*	*M−106*
C_3	132	100	–	–	1	8	–	82	5	–	–	–
C_4	146	100	24	13	2	1	–	–	42	–	3	–
C_5	160	83	62	47	78	8	1	19	5	2	41	–
C_6	174	70	25	21	96	67	4	62	24	12	7	3
C_7	188	41	12	6	41	48	28	100	24	9	37	3
C_8	202	44	5	2	12	27	95	100	27	23	60	4
C_9	216	39	5	1	8	15	91	55	29	38	65	10
C_{10}	230	36	3	1	5	5	31	38	22	47	74	15
C_{11}	244	31	4	–	1	2	8	34	12	32	72	20
C_{12}	258	18	–	–	1	2	6	26	5	10	44	16
C_{13}	272	12	–	–	–	1	5	17	2	3	22	7
C_{14}	286	7	–	–	–	–	3	12	1	2	13	5
C_{15}	300	4	–	–	–	–	2	7	1	1	8	4
C_{16}	314	4	–	–	–	–	–	6	–	1	8	4
C_{17}	328	4	–	–	–	–	–	5	–	–	6	4
C_{18}	342	4	–	–	–	–	–	3	–	–	3	2
C_{20}	370	4	–	–	–	–	–	2	–	–	3	3
C_{22}	398	3	–	–	–	–	–	2	–	–	2	2
C_{24}*	426	3	–	–	–	–	–	1	–	–	2	1

M−123	*112*	*111*	*98*	*97*	*84*	*83*	*87*	*74*	*73*	*69*	*59*	*57*	*56*	*55*
–	–	–	–	–	–	–	–	43	–	–	82	24	–	–
–	–	–	–	–	1	–	–	–	–	–	31	4	–	–
–	–	–	1	8	–	–	–	9	–	5	100	1	–	–
1	5	67	–	4	3	28	12	36	24	7	100	5	12	78
1	1	18	2	24	2	37	19	56	24	39	50	5	5	59
2	2	27	5	60	8	31	31	61	6	53	46	6	13	73
3	6	65	20	29	26	53	30	67	7	31	52	8	9	100
6	7	10	49	39	35	30	31	76	8	29	46	8	11	100
20	27	20	100	37	42	26	35	86	9	46	48	13	9	94
31	43	23	100	27	49	33	34	71	7	40	42	14	9	76
24	36	13	100	27	38	32	31	69	6	30	35	12	8	73
14	33	15	100	28	37	28	29	57	6	31	30	12	7	60
8	38	16	100	26	41	25	30	63	5	32	30	14	8	68
9	41	19	100	28	43	29	36	63	5	35	31	17	8	62
5	41	13	100	24	34	27	29	65	3	24	25	14	6	62
3	34	14	100	25	41	26	37	76	6	35	31	24	10	84
1	37	17	100	22	45	22	38	74	6	44	27	20	12	97
1	37	17	100	24	40	29	40	76	6	36	23	36	13	97
1	14	17	86	19	14	16	33	58	42	61	19	37	14	100

* Very low spectral intensity (small chromatographic peak).

TABLE II

BASE PEAK FORMATION IN d.e.d.a.s UNDER ELECTRON IMPACT

Acid	*Base peak (m/z)*	*Fragment or substance formed*
C_3	101	M−31
C_4	115	M−31
C_5, C_6	59	$-COOCH_3$
C_7	115	M−73
C_8	129	M−73
C_9, C_{10}	55	Hydrocarbon fragment
C_{11}–C_{22}	98	Cyclohexanone
C_{24}*	55	Hydrocarbon fragment

* Very low spectral intensity (small chromatographic peak).

Mass spectra of f.d.a.s

The mass spectra of C_3–C_{16} f.d.a.s are summarized in Table III. Up to C_{10}, the base peaks are found in the low mass region with the exception of C_6 for which *m/z* 100 (M−46) is the most abundant ion. When the effect of the hydrocarbon chain becomes dominant, the base peak is found at *m/z* 98.

DISCUSSION

Molecular ion

Ryhage and Stenhagen[16,17] in their extensive work on dicarboxylic acid esters

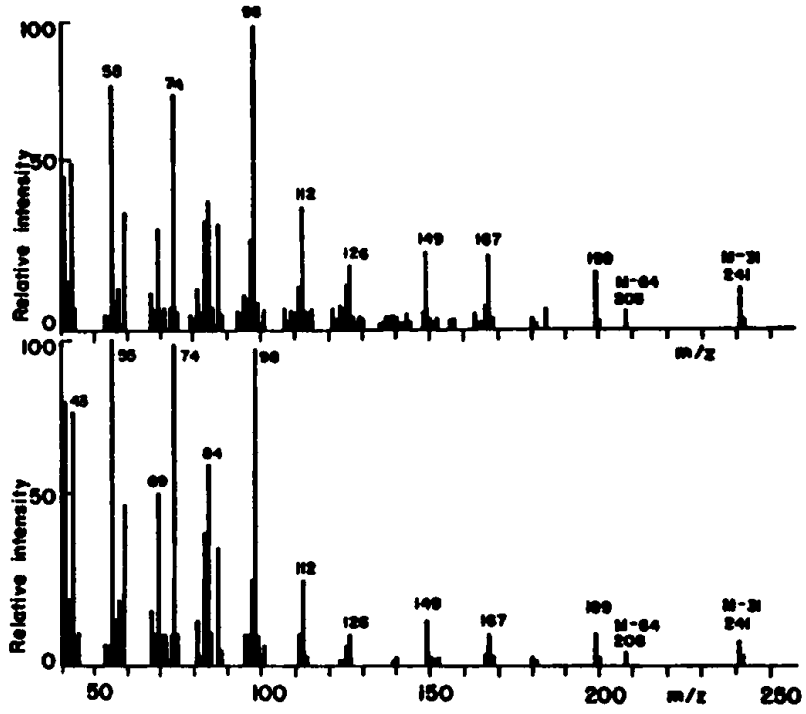

Fig. 2. Mass spectra of C_{13} d.e.d.a. measured by a quadrupole analyzer (upper) and by a sector-type mass analyzer (lower).

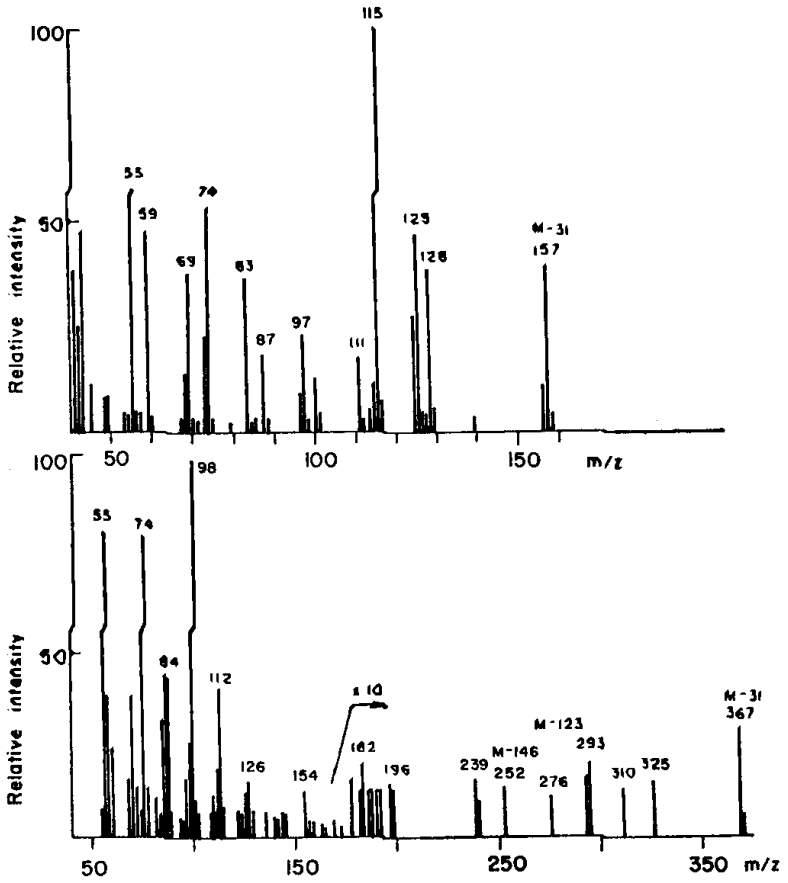

Fig. 3. Mass spectra of the C_7 (upper) and C_{22} d.e.d.a.s (lower).

found both M^+ or $(M+1)^+$ for different esters. We have observed M^+ only for dimethyl malonate; C_4–C_{11} d.e.d.a.s exhibited traces of $(M+1)^+$ plus similarly abundant $(M+15)^+$, while higher d.e.d.a.s did not exhibit either of these quasimolecular ions.

The spectra of f.d.a.s also contain $(M+1)^+$ but none M^+ which, however, almost merge with the background noise.

Fragmentation ions

Because of the very low intensity of (quasi)molecular ions it is necessary to consider characteristic fragmentation ions of both f.d.a.s and d.e.d.a.s. These can be divided into three principal groups: identical ions (A), analogous ions (B) and ions specific for f.d.a.s (C_1) as well as for d.e.d.a.s (C_2).

(A) Two sets of ions arising from the hydrocarbon moiety are characteristic for both f.d.a.s and d.e.d.a.s, the series of *m/z* 27, 41, 55, 69, etc., being more abundant than that of *m/z* 29, 43, 57, 71, etc. More specific for both of these types of compounds are the ions at *m/z* 84, 98, 112, the most abundant of which is that at *m/z* 98. As for its structure, Field[21] suggested a cyclohexanone, while Ryhage and Stenhagen[16] suggested a cyclohexenol form. In our work the nature of this ion was determined by high-resolution measurement as C_5H_8O. In our opinion a cycloalkane structure is appropriate (Fig. 4).

TABLE III

ELECTRON-IMPACT MASS SPECTRA OF f.d.a.s

Acid	*MW*	*M+1*	*M−17*	*M−18*	*M−36*	*M−44*	*M−46*	*M−59*	*M−64*	*M−77*	*M−95*
C_3	104	–	9	–	–	53	–	64	–	–	–
C_4	118	1	27	49	–	100	–	–	–	–	–
C_5	132	0.5	9	15	–	–	77	22	–	65	–
C_6	146	0.7	6	10	4	–	100	36	17	44	–
C_7	160	0.5	4	–	17	–	50	43	16	76	–
C_8	174	0.5	5	–	50	–	6	15	23	60	–
C_9	188	0.4	3	–	33	–	2	5	25	32	1
C_{10}	202	0.4	3	–	13	–	–	2	24	27	12
C_{11}	216	0.5	8	–	7	1	–	–	16	17	16
C_{12}	230	0.5	2	–	5	5	–	–	6	10	15
C_{13}	244	0.3	2	–	7	4	–	–	4	6	10
C_{14}	258	0.1	2	–	5	3	–	–	3	4	6
C_{15}	272	0.2	1	–	2	2	–		1	3	3
C_{16}	286	0.1	2	1	2	3	–	–	2	3	3

112	*98*	*84*	*83*	*73*	*69*	*60*	*55*	*44*	*41*	*Other ions [m/z (%)]*
–	–	–	–	–	–	–	–	100	–	42(85), 45(64), 69(45)
–	–	–	–	84	–	–	92	7	–	45(88)
–	–	–	–	22	–	49	65	19	44	42(100), 58(32)
–	–	–	15	31	44	57	59	20	63	43(73), 68(14), 111(5)
–	–	4	76	40	19	79	100	29	56	68(28)
–	–	11	14	31	63	100	52	30	50	68(23)
–	23	50	56	38	50	75	100	50	78	
13	100	55	33	31	38	54	91	50	62	
26	100	63	29	40	59	53	87	25	80	129(9), 185(3)
21	100	73	20	40	56	48	91	59	79	129(10)
25	100	76	30	37	39	42	77	68	64	126(8), 129(10), 157(10) 171(2)
28	100	75	35	33	45	34	85	17	74	126(7), 129(10), 171(2)
30	100	87	30	27	40	30	75	27	57	126(18), 129(6), 185(6), 199(1)
27	100	65	33	27	46	31	79	45	63	126(11), 129(8), 171(3), 185(3), 199(2)

Less intense ions at *m/z* 84 (a cyclopentanone) and at *m/z* 112 (a cycloheptanone or a methylcyclohexanone) are also formed.

(B) The analogous ions are summarized in Table IV. From f.d.a.s or d.e.d.a.s the fragments –OH or $-OCH_3$, –COOH or $-COOCH_3$, $-CH_2COOH$ or $-CH_2COOCH_3$, respectively, and molecules water or methanol, formic acid or methyl formate, acetic acid or methyl acetate, respectively, are lost. Figs. 5 and 6 show the analogy between f.d.a. and d.e.d.a. mass spectra for C_8 and C_{12} acids. The compo-

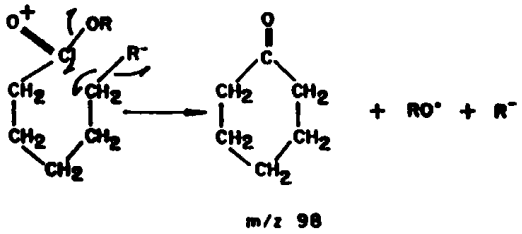

Fig. 4. Suggested structure for the cycloalkane ion, *m/z* 98.

sition of the ion corresponding to $(M-95)^+$ was determined by high-resolution measurement for the C_{12} acid (MW = 230) as $C_{10}H_{15}$; difference between theoretical and measured masses −1.8 mg/kg, unsaturation 3.5. These data imply the loss of all four oxygen atoms, which leaves no other real possibility than the elimination of a $-CH_2COOH$ fragment followed by the elimination of two molecules of water (in the case of d.e.d.a.s, elimination of $-CH_2COOCH_3$ followed by elimination of water and methanol):

TABLE IV

FRAGMENTATION UNDER ELECTRON IMPACT, ANALOGOUS TO BOTH d.e.d.a.s AND f.d.a.s

Dimethyl ester		*Free acid*		*Fragment from* $ROCO(CH_2)_nCOOR$
Fragment	*Occurrence*	*Fragment*	*Occurrence*	
M − 31	All; C_3 and C_4, base peak; higher than C_{13}, 10%	M − 17	All (low intensity)	−OR
M − 32	All lower except C_3; higher than C_7, 10%; higher than C_{11}, 0	M − 18	Only C_4–C_6	ROH
M − 59	All lower except C_3; higher than C_7, 10%; higher than C_{11}, 0	–		$-COOCH_3$
M − 60	C_3 and C_4, about 2%; all others similar to M − 32 fragmentation	M − 46	Only C_5–C_9	HCOOR
M − 63	Lower d.e.d.a.s; higher than C_9, 10%; higher than C_{13}, 0	–		−OR + ROH
M − 64	All lower except C_3 and C_4; higher than C_{10}, 10%; higher than C_{15}, 0	M − 36	All higher than C_5	2 ROH
M − 73	All except C_4; higher than C_{14}, 10%	M − 59	C_3, 64%; all lower acids except C_4; higher than C_{10}, 0	$-CH_2COOR$
M − 74	Higher than C_6	–		CH_3COOCH_3
M − 91	All except C_{16}–C_{24}	M − 63	Traces	−COOR + ROH
M − 92	All except C_3,C_4 and C_{17},C_{24}	M − 64	Higher than C_5	HCOOR + ROH
M − 105	All	M − 77	Higher than C_4	$-CH_2COOR$ + ROH
M − 106	Higher than C_5	–		CH_3COOCH_3 + CH_3OH
M − 123	Higher than C_9	M − 95	Higher than C_8	ROH + H_2O + CH_2COOR

$$HOOC(CH_2)_9\text{-}CH_2COOH \xrightarrow{-CH_2COOH} HOOC(CH_2)_8CH_2^{\cdot} \xrightarrow{-2H_2O} C_{10}H_{15}^{+}$$

The ion $C_{10}H_{15}^{+}$ is probably stabilized by conjugation of three double bonds, which means that this type of fragmentation occurs for α,ω-dicarboxylic acids with hydrocarbon chains containing more than eight carbon atoms. Analogous ions $(M-123)^{+}$ for d.e.d.a.s are important for the identification of the esters investigated (Table V), especially when quasimolecular ions are absent.

(C_1) The ions specific for f.d.a.s include: *m/z* 44, found for all f.d.a.s analyzed, may be related to $(M-44)^{+}$ occurring with malonic acid (McLafferty's rearrangement), succinic acid (base peak) and C_{11}–C_{16} f.d.a.s in ≤5% abundance; *m/z* 60, formed by McLafferty's rearrangement similarly to monocarboxylic acids, and found for > C_4 f.d.a.s; *m/z* 73, formed by elimination of a $-CH_2CH_2COOH$ fragment similarly to monocarboxylic acids, and found for > C_3 f.d.a.s.

(C_2) The ions specific for d.e.d.a.s include: *m/z* 59, formed by elimination of a $-COOCH_3$ fragment; similarly formed but less abundant are homologous ions at *m/z* 73, 87; *m/z* 74, this ion is analogous to *m/z* 60 formed for f.d.a.s (McLafferty); a series of even-mass ions (Table VI), corresponding to (M – 146) which are entirely specific to the d.e.d.a.s examined and are absent from the spectra of f.d.a.s; they are denoted in Table VI.

CONCLUSIONS

Three principal groups of ions were selected: identical ions (A), analogous ions (B) and ions specific for f.d.a.s (C_1) and d.e.d.a.s (C_2). The ions having a cycloal-

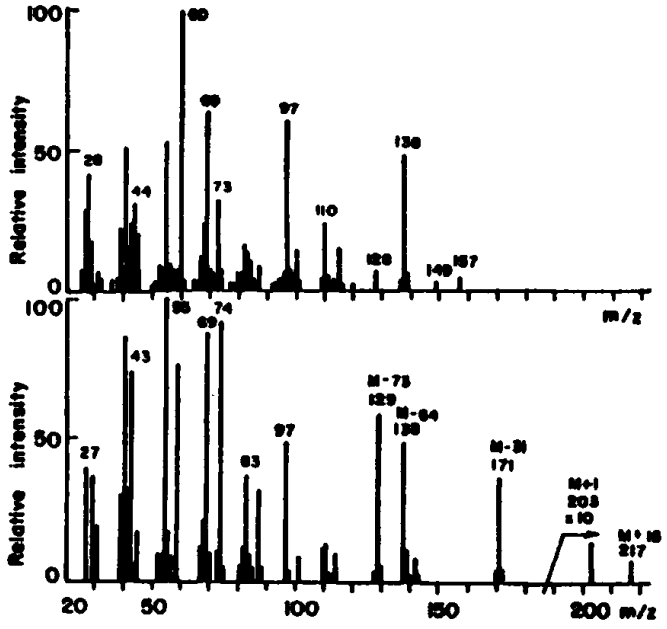

Fig. 5. Mass spectra of the C_8 f.d.a. (upper) and d.e.d.a. (lower). The molecular weight of the free α,ω-dicarboxylic acid is 174.

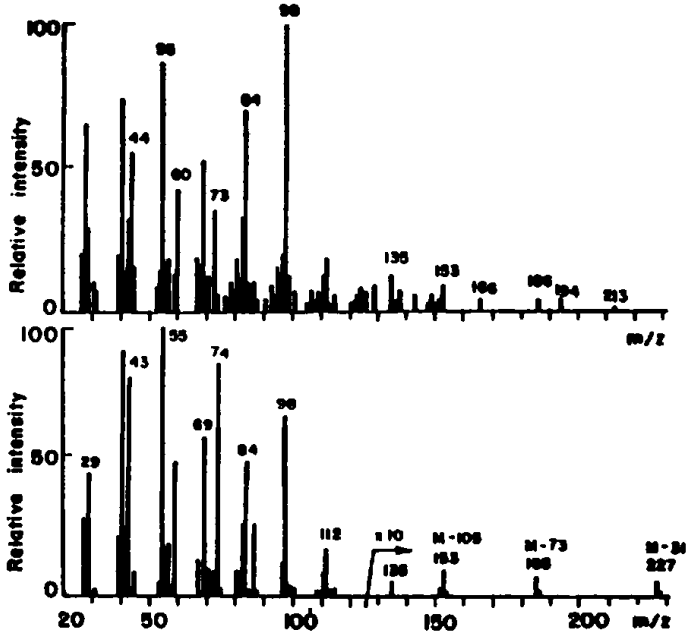

Fig. 6. Mass spectra of the C_{12} f.d.a. (upper) and d.e.d.a. (lower). The molecular weight of the free α,ω-dicarboxylic acid is 230.

kanone structure (*m/z* 98 being the base peak for dicarboxylic acids higher than C_{10}) are characteristic for group A. Group B comprises ions analogous for both f.d.a.s and d.e.d.a.s,which are formed by loss of analogous fragments or neutral molecules (Table IV); important ions $(M-123)^+$, Table V, and $(M-146)^+$, Table VI, for the identification of esters were determined.

TABLE V

ABUNDANCE (rel. %) OF $(M-123)^+$ FOR d.e.d.a.s

Acid	*m/z*										
	107	*121*	*135*	*149*	*163*	*177*	*191*	*205*	*219*	*247*	*275*
C_{10}	6	14	1	–	–						
C_{11}	3	20	14	–	2						
C_{12}	7	3	31	8	–						
C_{13}	6	6	2	24	4						
C_{14}	5	9	4	2	14						
C_{15}	5	9	9	2	1	8					
C_{16}	5	9	9	5	1	–	9				
C_{17}	5	1	8	4	6	–	–	5			
C_{18}	3	7	6	3	2	1	–	–	3		
C_{20}	3	6	7	5	1	1	–	–	1	1	
C_{22}	2	7	6	2	2	2	1	–	–	–	1

TABLE VI

ABUNDANCE (rel. %) OF $(M-146)^+$ FOR d.e.d.a.s

Acid	*m/z*							
	126	*140*	*154*	*168*	*182*	*196*	*224*	*252*
C_{13}	19	4	1	3	–	–	–	–
C_{14}	11	12	4	–	2	–	–	–
C_{15}	10	7	12	2	–	2	–	–
C_{16}	14	7	10	7	1	–	–	–
C_{17}	15	6	8	7	3	–	–	–
C_{18}	11	8	9	5	2	4	–	–
C_{20}	5	9	10	5	2	1	1	–
C_{22}	14	5	10	4	2	2	1	2

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