Ruminal organic acid analysis by gas chromatography/mass spectrometry

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Ruminal Organic Acid Analysis by Gas Chromatography/Mass Spectrometry[1]

Sergio Daolio,* Mario Bonsembiante, Giovanni Bittante, Maurizio Ramanzin, and Piero Rinaldo

Rumen fluid was analyzed by the gas chromatography/mass spectrometry (GC/MS) technique in order to identify the organic acids contained. The gas chromatogram obtained showed more than 200 peaks, and 60 organic acids were identified from their mass spectra obtained under electron impact conditions from the relative chromatographic peaks. Keto acids, polycarboxylic acids, hydroxy acids, aromatic acids, and saturated and unsaturated fatty acids were present, which were subdivided into three main groups: (i) short- and long-chain fatty acids; (ii) polyfunctional organic acids such as intermediate metabolic products; (iii) phenolic acids mainly from lignin and tannin degradation. It was concluded that GC/MS is a very specific and sensitive technique to detect the presence of fermentation products in biological fluids and that it could allow for the simpler and cheaper GC technique to be used for routine quantitative analyses of the identified compounds.

Research on ruminal fermentation of feedstuffs is mainly based on measurements of pH, volatile fatty acids concentration, and turnover of markers. A more analytical approach is limited by difficult laboratory procedures regarding the identification and quantitation of the different biochemical pathways.

We have recently used mass spectrometry/mass spectrometry (MS/MS) techniques to analyze the gas produced during the fermentation in the rumen (Bonsembiante et al., 1987a,b), and we have also adapted the MS/MS (McLafferty, 1983) and the gas chromatography/mass spectrometry (GC/MS) (McFadden, 1973) techniques to cope with the mixture analysis in order to study the chemical changes of ensiled grass (Bonsembiante et al., 1985; Daolio et al., 1986).

The present work extends the method to analyze the nonvolatile organic acid profile of rumen fluid and to obtain the mass spectra of each component of the mixture.

This basic research is a preliminary but essential step in order to carry out a complete compound screening and to perform routine quantitative analyses with a less sophisticated technique such as gas chromatography.

MATERIALS AND METHODS

Sample Preparation. The rumen fluid (3 mL) was drawn from a wether sheep fitted with a rumen cannula and fed hay and limited amounts of concentrate, deproteinized with ethanol (18

Table I. Ionic Fragments and Their Relative Abundances Obtained from Peak 79 of the Chromatogram (Identified as 2-Hydroxyglutaric Acid)

ionic fragment, m/z	rel abund, %	ionic fragment, m/z	rel abund, %	ionic fragment, m/z	rel abund, %
32	7	75	23	157	23
43	7	85	12	203	37
44	7	129	100	294	7
45	21	130	13	231	10
55	6	131	7	247	47
69	6	133	7	248	10
73	78	147	75	249	5
74	7	148	7	349	7
				350	2

mL) and then alkalinized to pH 14 with NaOH 30% and extracted twice with an equal volume of ethyl acetate and once with diethyl ether. The aqueous phase was submitted to the following preparative steps: (i) oximation of α-keto acids with hydroxylamine hydrochloride (Adibi, 1976); (ii) silylation of carboxylic and hydroxylic functional groups with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) as a catalyst (Pierce, 1968).

The derivatized organic acids are very stable and can be run under the chromatographic conditions.

However, short-chain aliphatic acids that do not contain additional functional groups can be lost during the preparative steps or coelute with the solvent and reagents used. These low molecular weight acids are then extracted by steam distillation and analyzed by the GC technique.

Analysis. *Apparatus.* The qualitative analysis was performed by means of a HP 5792A gas chromatograph coupled with a HP 5970A mass spectrometer and a HP 9825B data acquisition system. The operative instrumental conditions were selected as follows: (i) SE 52 fused silica capillary column, 25-m length; (ii) sample volume introduced for every analysis, 1 μL (split mode 1:30); (iii) helium flow, 1.5 mL/min; (iv) injector temperature, 250 °C; (v) interface and FID detector temperatures, 275 and 300 °C, respectively (instrument is equipped with conventional FID

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Ruminal Organic Acid Analysis

J. Agric. Food Chem., Vol. 37, No. 4, 1989 **971**

Table II. Mass Spectral Data of the Identified Short- and Long-Chain Fatty Acids

GC peak no.	organic acid	GC ret time,[a] min	MW	ionic fragment[b] (relative abundance, %)											
29	1-(trimethylsilyl)heptanoic	11.26	202	187 (100)	75 (97)	117 (87)	73 (62)	131 (25)	132 (23)	129 (19)	74 (17)	188 (8)	145 (5)	143 (4)	159 (3)
89	1-(trimethylsilyl)dodecanoic (caprinic)	22.62	272	117 (100)	73 (84)	75 (83)	257 (67)	129 (46)	132 (37)	131 (29)	145 (21)	258 (13)	118 (11)	133 (7)	130 (7)
98	1-(trimethylsilyl)tridecanoic	23.82	286	73 (100)	117 (83)	43 (83)	75 (76)	45 (72)	41 (66)	129 (62)	55 (59)	58 (48)	271 (45)	69 (14)	
103	A-1-(trimethylsilyl)tridecanoic	24.52	286	117 (100)	73 (83)	75 (73)	271 (69)	129 (48)	132 (37)	131 (29)	41 (29)	145 (24)	43 (23)	272 (15)	55 (18)
112	1-(trimethylsilyl)hexadecenoic	25.28	326	311 (100)	75 (93)	254 (52)	281 (44)	73 (26)	296 (26)	312 (26)	41 (22)	43 (15)	45 (11)	55 (11)	129 (10)
114	1-(trimethylsilyl)tetradecanoic	25.66	300	117 (100)	73 (79)	75 (75)	285 (58)	129 (50)	132 (39)	145 (29)	131 (25)	55 (23)	286 (13)	118 (10)	133 (10)
116	1-(trimethylsilyl)tetradecenoic	25.96	298	75 (100)	117 (88)	73 (88)	129 (81)	55 (48)	283 (43)	41 (41)	43 (27)	145 (26)	96 (21)	67 (20)	131 (20)
118	n-1-(trimethylsilyl)tetradecanoic (miristic)	26.38	300	117 (100)	73 (86)	75 (76)	129 (48)	285 (44)	132 (39)	145 (23)	131 (20)	286 (10)	118 (10)	133 (7)	69 (7)
127	1-(trimethylsilyl)heptadecanoic	27.46	342	73 (100)	117 (86)	75 (85)	299 (78)	129 (70)	132 (54)	43 (43)	145 (39)	55 (28)	131 (24)	201 (22)	300 (21)
132	1-(trimethylsilyl)pentadecanoic	28.08	314	117 (100)	73 (88)	75 (72)	289 (56)	129 (51)	132 (46)	145 (29)	55 (24)	131 (23)	300 (14)	118 (10)	69 (8)
134	1-(trimethylsilyl)pentadecanoic	28.28	314	299 (100)	75 (96)	43 (62)	73 (60)	41 (50)	55 (39)	57 (38)	103 (32)	97 (32)	83 (31)	69 (31)	300 (26)
141	1-(trimethylsilyl)hexadecanoic	29.14	328	117 (100)	73 (73)	75 (69)	313 (59)	129 (50)	132 (48)	43 (43)	145 (24)	55 (27)	41 (25)	131 (22)	314 (16)
142	1-(trimethylsilyl)pentadecenoic	29.24	312	161 (100)	130 (98)	132 (85)	131 (76)	56 (71)	297 (67)	74 (67)	95 (62)	81 (58)	83 (58)	117 (54)	75 (50)
143	1-(trimethylsilyl)hexadecenoic	29.34	326	73 (100)	117 (95)	75 (86)	41 (50)	129 (49)	55 (48)	43 (45)	311 (27)	69 (26)	145 (23)	132 (21)	194 (13)
144	1-(trimethylsilyl)hexadecenoic	29.44	326	117 (100)	73 (89)	75 (88)	129 (70)	55 (53)	311 (50)	41 (50)	43 (29)	95 (23)	96 (23)	131 (21)	69 (20)
146	n-1-(trimethylsilyl)hexadecanoic (palmitic)	29.86	328	117 (100)	73 (83)	75 (72)	129 (52)	132 (32)	313 (28)	55 (28)	145 (27)	131 (17)	57 (15)	118 (10)	133 (7)
155	1-(trimethylsilyl)heptadecanoic	30.90	342	117 (100)	73 (88)	75 (72)	132 (56)	129 (53)	327 (50)	145 (43)	41 (32)	55 (28)	57 (23)	131 (22)	328 (15)
159	n-1-(trimethylsilyl)heptadecanoic	31.34	342	117 (100)	73 (88)	75 (75)	129 (49)	132 (44)	327 (44)	145 (32)	55 (30)	131 (17)	69 (13)	118 (12)	130 (11)
167	1-(trimethylsilyl)octadecadienoic	32.44	352	75 (100)	73 (91)	67 (67)	81 (50)	129 (44)	95 (36)	117 (34)	79 (34)	337 (22)	68 (22)	150 (18)	109 (18)
169	1-(trimethylsilyl)octadecadienoic	32.54	352	337 (100)	262 (58)	117 (56)	129 (50)	338 (27)	109 (23)	105 (23)	110 (19)	145 (18)	339 (17)	131 (16)	263 (13)
170	1-(trimethylsilyl)octadecenoic	32.64	354	117 (100)	129 (90)	145 (30)	132 (18)	131 (18)	339 (11)	110 (9)	185 (9)	222 (8)	340 (3)	264 (3)	354 (1)
173	n-1-(trimethylsilyl)octadecanoic (stearic)	32.92	356	117 (100)	73 (77)	75 (63)	129 (46)	132 (45)	145 (30)	341 (27)	131 (18)	201 (11)	69 (10)	342 (8)	118 (7)
176	1-(trimethylsilyl)octadecadienoic	33.32	352	73 (100)	75 (99)	67 (60)	81 (43)	217 (43)	337 (36)	145 (36)	150 (33)	109 (32)	54 (31)	173 (31)	129 (30)
179	1-(trimethylsilyl)nonadecenoic	33.68	368	73 (100)	143 (70)	43 (58)	57 (54)	71 (53)	117 (45)	137 (37)	103 (33)	133 (29)	83 (28)	157 (27)	353 (26)
183	1-(trimethylsilyl)nonadecenoic	34.22	368	73 (100)	117 (93)	75 (89)	129 (75)	145 (57)	41 (54)	55 (54)	132 (33)	69 (32)	43 (32)	353 (29)	96 (32)
185	n-1-(trimethylsilyl)nonadecenoic	34.46	370	131 (100)	75 (92)	73 (82)	145 (67)	55 (67)	117 (57)	132 (53)	133 (48)	56 (48)	355 (38)	69 (25)	129 (15)

[a] The 12 most significant peaks are listed in order of decreasing relative abundance (base peak first). [b] The retention times of the linear-chain fatty acids were confirmed by a standard sample solution.

detector and the same operative conditions are suitable for GC analysis of the biological fluids); (vi) oven temperature maintained at 80 °C for 4 min and then raised at 4 °C min^{-1} to 250 °C, final temperature maintained for 5 min; (vii) source electron energy, 70 eV; (viii) emission current, 250 mA; (ix) ion source temperature, 230 °C; (x) electron multiplier voltage, 1600 V; (xi) mass range, 30–600 amu.

RESULTS AND DISCUSSION

The rumen fluid was analyzed with a conventional gas chromatographic technique, and the selected conditions allowed the separation of more than 200 peaks. The mass spectrometer was used as a chromatographic detector that supplied a total ion current vs time and gave the profile shown in Figure 1.

With a very high mass scan speed (690 μ·s^{-1}), an electron impact mass spectrum for every peak was performed. Figure 2 shows the result achieved from peak 79 of the

chromatogram and Table I gives evidence for the corresponding most significant ionic fragments and their relative abundances.

The identification of the rumen fluid components was possible when the relative mass spectra gave (i) the molecular ions; (ii) trimethylsilyl group fragments that prove the presence of mono- or polyfunctional organic acids; (iii) structural specific ionic fragments due to characteristic losses.

The identification of various acids was obtained also with the aid of the mass spectra listed in a library (Chalmers and Lawson, 1982; EPA/NIH Mass Spectral Data Base, 1985) and was confirmed by the GC/MS analysis of the pure compounds (commercial or synthesized); the identified 60 organic acids are listed in Tables II–IV, and it is evident that the 2-hydroxyglutaric acid (Table III) corresponds to the compounds of Figure 2.

Table III. Mass Spectral Data of the Identified Polyfunctional Organic Acids

GC peak no.	organic acid	GC ret time, min	MW	ionic fragments[a] (relative abundance, %)											
15	2-(trimethylsilyl)lactic	8.44	234	147 (100)	73 (90)	117 (74)	191 (16)	190 (13)	75 (12)	133 (10)	66 (10)	59 (8)	118 (8)	88 (5)	219 (5)
18	2-(trimethylsilyl)glycollic	8.84	220	147 (100)	73 (70)	148 (16)	65 (17)	177 (13)	133 (10)	149 (9)	205 (9)	74 (9)	59 (8)	75 (6)	161 (5)
24	2-(trimethylsilyl)glyoxylic oxime[b]	10.34	233	73 (100)	147 (80)	218 (44)	75 (14)	74 (13)	59 (13)	148 (12)	233 (12)	190 (10)	219 (8)	149 (6)	133 (5)
25	2-(trimethylsilyl)oxalic	10.44	234	147 (100)	73 (76)	148 (19)	149 (10)	66 (9)	190 (7)	74 (6)	133 (5)	75 (4)	219 (4)	131 (4)	58 (4)
27	2-(trimethylsilyl)pyruvic oxime[b]	10.98	247	73 (100)	147 (69)	204 (30)	232 (28)	59 (24)	130 (20)	74 (14)	148 (12)	133 (10)	75 (9)	233 (7)	247 (7)
28	2-(trimethylsilyl)-3-hydroxy-n-butyric	11.18	248	147 (100)	73 (74)	117 (40)	191 (25)	75 (23)	148 (18)	233 (10)	88 (10)	74 (9)	133 (9)	130 (8)	149 (8)
30	2-(trimethylsilyl)-2-hydroxyisovaleric	11.34	262	145 (100)	73 (91)	147 (75)	75 (20)	133 (13)	148 (13)	219 (12)	74 (9)	149 (8)	59 (6)	55 (5)	131 (5)
33	2-(trimethylsilyl)-2-propenoic	11.74	232	147 (100)	73 (45)	217 (33)	75 (24)	148 (15)	74 (12)	59 (12)	149 (9)	218 (8)	232 (6)	133 (5)	131 (4)
36	2-(trimethylsilyl)malonic	12.38	248	147 (100)	73 (44)	75 (20)	148 (20)	149 (11)	66 (8)	233 (4)	133 (4)	74 (4)	99 (3)	131 (3)	234 (1)
37	2-(trimethylsilyl)-3-hydroxyisovaleric	12.50	262	147 (100)	131 (83)	73 (74)	75 (31)	247 (30)	95 (27)	115 (23)	205 (15)	148 (12)	132 (7)	147 (7)	204 (3)
44	3-(trimethylsilyl)phosphoric[c]	14.34	314	73 (100)	299 (85)	147 (59)	133 (30)	300 (21)	314 (12)	207 (12)	301 (11)	148 (8)	74 (8)	75 (8)	149 (5)
48	2-(trimethylsilyl)succinic	15.20	262	147 (100)	73 (41)	75 (20)	148 (16)	247 (8)	149 (8)	129 (7)	174 (6)	172 (5)	65 (5)	173 (4)	248 (2)
49	2-(trimethylsilyl)-4-hydroxy-2-methyl-n-valeric	15.48	276	147 (100)	73 (55)	75 (21)	261 (18)	55 (14)	148 (17)	69 (10)	217 (10)	149 (9)	74 (6)	143 (6)	70 (3)
51	3-(trimethylsilyl)glyceric	15.76	322	73 (100)	147 (73)	189 (54)	133 (23)	292 (21)	103 (20)	116 (17)	102 (16)	148 (13)	205 (12)	75 (11)	74 (8)
53	2-(trimethylsilyl)fumaric	15.98	260	245 (100)	147 (55)	73 (45)	75 (22)	246 (19)	143 (17)	247 (9)	133 (9)	148 (8)	115 (6)	149 (6)	83 (5)
61	2-(trimethylsilyl)glutaric	17.32	276	147 (100)	73 (60)	75 (39)	55 (37)	158 (19)	148 (17)	129 (17)	261 (16)	149 (14)	116 (10)	97 (8)	186 (6)
79	2-(trimethylsilyl)-2-hydroxyglutaric	21.22	364	129 (100)	73 (78)	147 (75)	247 (47)	203 (37)	157 (23)	75 (23)	130 (13)	85 (12)	248 (10)	231 (10)	349 (7)
86	3-(trimethylsilyl)-α-ketoglutaric oxime[c]	22.32	377	73 (100)	147 (65)	148 (30)	75 (21)	260 (16)	362 (12)	377 (7)	170 (7)	156 (4)	172 (3)	179 (3)	360 (3)
102	1,2,3-tricarboxy-3-(trimethylsilyl)propane	24.38	392	147 (100)	73 (79)	185 (57)	217 (26)	75 (25)	149 (23)	377 (19)	148 (16)	184 (15)	55 (14)	95 (8)	69 (8)
113	2-(trimethylsilyl)nonanedioic (azelaic)	25.50	332	73 (100)	75 (85)	129 (45)	117 (44)	149 (34)	201 (33)	147 (30)	317 (26)	152 (25)	318 (6)	76 (6)	116 (4)
124	2-(trimethylsilyl)decanedioic (sebacic)	27.26	346	73 (100)	75 (66)	129 (38)	55 (34)	117 (33)	149 (33)	215 (30)	331 (25)	147 (25)	217 (24)	204 (18)	332 (8)
128	2-(trimethylsilyl)dodecendioic	27.60	372	117 (100)	73 (90)	75 (81)	129 (50)	299 (47)	132 (39)	41 (38)	43 (30)	55 (30)	145 (29)	57 (24)	131 (22)
145	2-(trimethylsilyl)tridecandioic	29.58	388	73 (100)	147 (80)	257 (75)	373 (73)	217 (40)	204 (33)	75 (20)	374 (18)	117 (17)	148 (13)	374 (13)	83 (8)

[a]The 12 most significant peaks are listed in order of decreasing relative abundance (base peak first). [b]Oximated by hydroxylamine. [c]Inorganic acid.
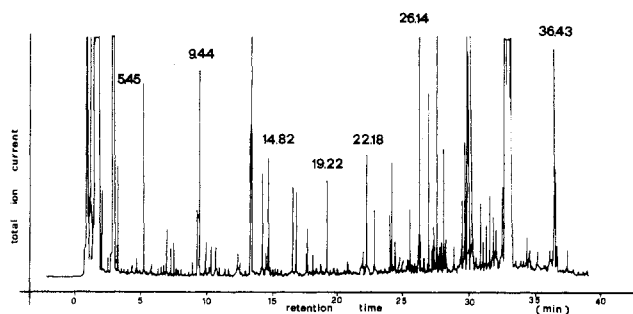

Figure 1. Chromatogram of the rumen fluid obtained by GC/MS.

Figure 2. Mass spectrum obtained under electron impact conditions from peak 79 of the chromatogram (identified as 2-hydroxyglutaric acid).

In this preliminary experiment, the MS technique coupled to the gas chromatography confirms that good results can be obtained and particularly that the structure of compounds present in complex biological mixtures can be determined: Even when the chromatographic peaks are extremely close, it is possible to obtain clear mass spectra that can be generally assumed as molecular fingerprints.

The keto acids, polycarboxylic acids, hydroxy acids, aromatic acids, and saturated and unsaturated fatty acids
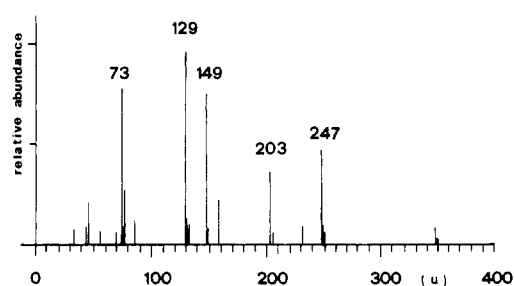
present in the rumen fluid analyzed were divided in the following three groups:

(i) The first group (Table II) was characterized by the presence of short- and long-chain fatty acids; the C_{16}–C_{18} region was especially rich in peaks (see Table II and Figure 1). Figure 3 shows the mass spectra of some typical compounds of this class, i.e. the saturated, monounsaturated, and diunsaturated C_{18} fatty acids. The TMS derivatives

Ruminal Organic Acid Analysis

J. Agric. Food Chem., Vol. 37, No. 4, 1989 **973**

Table IV. Mass Spectral Data of the Identified Aromatic (Phenolic) Acids

GC peak no.	organic acid	GC ret time, min	MW	ionic fragment[a] (relative abundance, %)											
41	1-(trimethylsilyl)benzoic	13.46	194	179 (100)	105 (79)	135 (60)	77 (49)	180 (15)	73 (15)	75 (13)	136 (10)	181 (4)	106 (4)	194 (3)	136 (3)
62	1-(trimethylsilyl)phenylpropionic	17.62	222	104 (100)	75 (79)	207 (46)	73 (38)	91 (26)	222 (16)	77 (13)	105 (12)	65 (8)	208 (7)	78 (6)	132 (5)
72	1-(trimethylsilyl)cinnamic	19.86	220	205 (100)	220 (20)	206 (14)	145 (13)	177 (12)	91 (8)	73 (7)	131 (6)	147 (6)	77 (5)	75 (5)	161 (4)
84	2-(trimethylsilyl)-4-hydroxybenzoic	22.24	282	223 (100)	267 (84)	73 (70)	193 (70)	224 (24)	268 (21)	282 (17)	126 (15)	194 (14)	75 (9)	269 (9)	195 (6)
88	2-(trimethylsilyl)-4-hydroxyphenylacetic	22.46	296	73 (100)	179 (32)	75 (21)	164 (19)	252 (15)	296 (11)	74 (10)	281 (10)	149 (7)	163 (7)	133 (6)	117 (5)
101	2-(trimethylsilyl)-3-hydroxyphenylpropionic	24.22	310	205 (100)	192 (87)	73 (34)	75 (33)	177 (28)	193 (28)	206 (17)	310 (17)	147 (12)	179 (10)	149 (9)	311 (3)
107	2-(trimethylsilyl)-4-hydroxy-3-phenylpropionic	24.86	310	179 (100)	192 (85)	73 (40)	75 (17)	177 (15)	190 (15)	193 (14)	310 (10)	149 (7)	163 (5)	181 (5)	295 (3)
126	2-(trimethylsilyl)-4-hydroxy-5-methoxyphenylpropionic	27.42	340	209 (100)	192 (57)	73 (49)	179 (30)	340 (29)	222 (25)	193 (20)	210 (19)	207 (17)	310 (16)	325 (12)	75 (12)
153	1-(trimethylsilyl)-4-hydroxy-3-methoxycinnamic	30.76	338	73 (100)	338 (99)	249 (77)	308 (68)	323 (65)	293 (48)	219 (40)	75 (34)	339 (26)	196 (23)	175 (20)	191 (20)
160	3-(trimethylsilyl)-3,4-dihydroxycinnamic	31.42	396	219 (100)	73 (54)	75 (38)	220 (32)	396 (31)	74 (25)	397 (14)	307 (13)	381 (11)	398 (8)	147 (8)	116 (5)

[a] The 12 most significant peaks are listed in order of decreasing relative abundance (base peak first.)
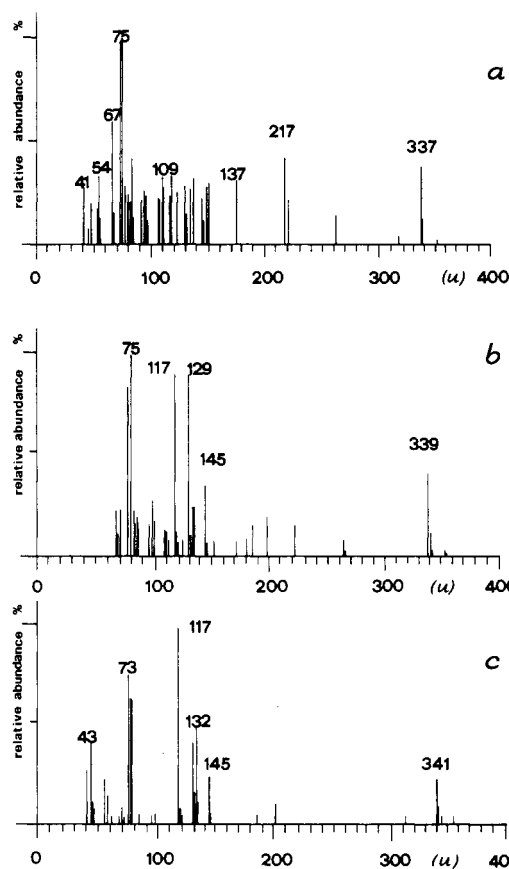

Figure 3. Mass spectra of stearic acid (a), monounsaturated C_{18} acid (b), and diunsaturated C_{18} acid (c) obtained under electron impact conditions.

Figure 4. Mass spectrum of 4-hydroxy-3-methoxycinnamic acid obtained under electron impact conditions.

give mass spectra of particular interest for quantitative analysis, showing a molecular ion accompanied by an ion representing the loss of a methyl group $[M - 15]^+$. A limit of this analytical method is its inability to locate the double bonds of the unsaturated fatty acids because of the rearrangement of the molecule under electron impact conditions, and for this goal other mass spectrometric techniques can be used (Anderson et al., 1975; Harrison and Choi, 1981; Harvey, 1982, 1984; Bambagiotti et al., 1983, 1984; Jensen et al., 1985).
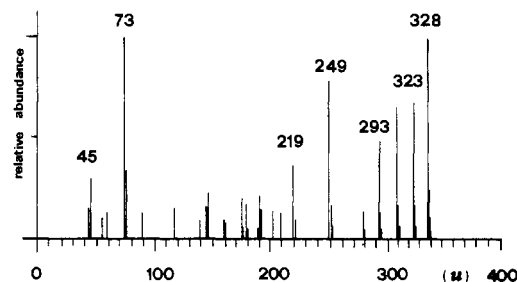
(ii) The second group (Table III) contained numerous polyfunctional organic acids produced during the ruminal fermentations. Many of them are well-known as intermediate metabolic products, and as such, it would obviously be of interest to determine their composition. A systematic study to give a name to other mass spectra of polyfunctional compounds is in progress.

(iii) Some aromatic (phenolic) acids derived in large amounts from lignin and tannin degradation were present in the third group (Table IV), and, as an example, Figure 4 shows the 4-hydroxy-3-methoxycinnamic acid spectrum. The interest in these classes of molecules has increased over the last years, and several studies on ruminal and intestinal degradation, rearrangements, and even digestion of the aromatic compounds have been published (Cymbaluk and Neudoerffer, 1970; Hartley, 1971; Cymbaluk et al., 1973; Reeves, 1985a,b; Jung et al., 1983).

Our study confirms that GC/MS techniques are powerful tools for the ruminant nutrition research: They allow a very complete, relatively simple, and very rapid analysis of numerous organic acids in the sectors of both the metabolic intermediate and the final products of the fermentation processes.

The utilization of the MS technique is not only important in the definition of the metabolic pathways of rumen fermentations but also in their quantitation at very low levels and in the analysis of the effects of ration characteristics and/or animal conditions.

Finally, it should be remembered that once the identification of the different peaks of the chromatogram is obtained, and provided that the instrumental sensitivity

is sufficiently high, the cheap, rapid, and easily available gas chromatography can be used as a routine laboratory technique for the complete organic acid profiling of rumen fluid.

Registry No. Heptanoic acid, 111-14-8; dodecanoic acid, 334-48-5; tridecanoic acid, 638-53-9; hexadecanoic acid, 57-10-3; tetradecanoic acid, 544-63-8; tetradecenoic acid, 26444-03-1; pentadecanoic acid, 1002-84-2; pentadecenoic acid, 29255-62-7; hexadecenoic acid, 28039-99-8; palmitic acid, 57-10-3; heptadecanoic acid, 506-12-7; octadecadienoic acid, 28984-77-2; octadecenoic acid, 27104-13-8; stearic acid, 57-11-4; nonadecenoic acid, 26444-05-3; lactic acid, 50-21-5; glycolic acid, 79-14-1; glyoxylic acid, 298-12-4; oxalic acid, 144-62-7; pyruvic acid, 127-17-3; 3-hydroxy-*n*-butyric acid, 300-85-6; 2-hydroxyisovaleric acid, 4026-18-0; 2-propenoic acid, 79-10-7; malonic acid, 141-82-2; 3-hydroxyisovaleric acid, 625-08-1; phosphoric acid, 7664-38-2; succinic acid, 110-15-6; 4-hydroxy-2-methyl-*n*-valeric acid, 120829-62-1; glyceric acid, 473-81-4; fumaric acid, 110-17-8; glutaric acid, 110-94-1; 2-hydroxyglutaric acid, 2889-31-8; α-ketoglutaric acid, 328-50-7; 1,2,3-tricarboxypropane, 99-14-9; azelic acid, 123-99-9; sebacic acid, 111-20-6; dodecenedioic acid, 32839-19-3; tridecanedioic acid, 505-52-2; benzoic acid, 65-85-0; phenylpropionic acid, 501-52-0; cinnamic acid, 621-82-9; 4-hydroxybenzoic acid, 99-96-7; 4-hydroxyphenylacetic acid, 156-38-7; 3-hydroxyphenylpropionic acid, 33393-93-0; 4-hydroxy-3-phenylpropionic acid, 501-97-3; 4-hydroxy-5-methoxyphenylpropionic acid, 1135-23-5; 4-hydroxy-3-methoxycinnamic acid, 1135-24-6; 3,4-dihydroxycinnamic acid, 331-39-5.

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