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Equivalent chain lengths of all *C*4–*C*23 saturated monomethyl branched fatty acid methyl esters on methylsilicone OV-1 stationary phase

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ABSTRACT

Isomer mixtures of monomethyl branched saturated *C*7–*C*23 fatty acid methyl esters (FAME) were prepared by performing a methylene insertion reaction to the straight chain FAME and this study model was completed by using commercially available standards of *C*4–*C*7 FAME. The equivalent chain lengths (*ECL*) of all 220 *C*4–*C*23 monomethyl branched FAME on OV-1 stationary phase were measured, achieving an average repeatability of ±0.0004 *ECL* units. The monomethyl branched FAME was identified by GC on the basis of regularity of the fractional chain lengths (*FCL*) dependence on the number of carbon atoms (C_z) of individual homologous series of methyl 2-, 3-, . . ., 21-FAME. The prediction of retention of the first homologues, having the new position of methyl group beginning at higher carbon atoms number, and analogously for the second, third, fourth, and other members of the homologous series, allowed the dependence $FCL = f(C_z)$ for the first and subsequent members of beginning homologous of monomethyl derivatives of FAME. The identification was confirmed by mass spectrometry. All of the methyl isomers of FAME, which could not be completely separated by gas chromatography due to having a methyl group in surroundings of the middle of the carbon chain, were resolved by mass spectrometry using deconvolution in a SIM-mode. Measured gas chromatographic and mass spectrometric data were applied for identification of the monomethyl branched saturated FAME in tongue coating.

1. Introduction

Fatty acids with methyl branches are commonly present in many organisms from bacteria to mammals, and their traces can be found in most plants as well [1]. In humans, the monomethyl fatty acids have been found in the skin, brain, blood, and cancer cells [2]. Frequently, only a single methyl branch is present, while multi-branched fatty acids are found in ruminant tissues and some other tissues. The *iso*- and *anteiso*-isomers, i.e. with the methyl branch on the penultimate and antepenultimate carbon atoms respectively, are those most often found in nature. Typically, the acyl chain of the fatty acid molecule is saturated, but in some bacteria there may also be a single double bond. It was found that the methyl branched fatty acids *C*15–*C*17 have anticancer activity, with their cytotoxicity being comparable to that of the conjugated linoleic acid [3]. Kaneda [4] reviewed the biosynthesis process, as well as the function and taxonomic significance of the *iso* and *anteiso* fatty acids in bacteria. The monomethyl saturated fatty acid methyl esters (FAME) may be considered as an endogenous marker in exhaled breath [5]. The compositional analysis of the exhaled breath can provide insights into different biochemical processes in both healthy and diseased human bodies.

GC–MS is proved to be a reliable tool for identification and analysis of monomethyl saturated FAME [6]. The analysis of individual monomethyl branched FAME in a wide range of carbon atoms (up to *C*23) is complicated by their multicomponent nature, resulting in isomers with methyl branches in surroundings of the middle of the carbon chain showing very similar retention behavior. Another problem is the lack of standard reference materials and the absence and/or relative poorer reproducibility of published retention data. Finally, the interpretation of mass spectra from incompletely separated positional isomers using GC–MS hyphenated techniques is still imperfect. The use of GC retention data as identification complementary information to the mass spectra data can provide an interesting additional information, as some isomers have identical or nearly identical fragmentation patterns.

Apon and Nicolaides [6] studied the problem of determination of the methyl branch position in the *C*11–*C*18 FAME by capillary GC–MS. They used a complete series of standard methyl monomethyloctadecanoates with the methyl branch in location

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Table 1
Measured equivalent chain lengths (*ECL*) of *C*4–*C*23 monomethyl branched *C*4–*C*23 fatty acid methyl esters on OV-1 and their standard deviations *s*, fractional chain lengths (*FCL*) and specific MS ions *m*/*z*.

Methyl x-Methyl-y-oate			*ECL*	*s*	*FCL*	MS ions *m*/*z*
	x	y				
MeC3	2-	propan	3.5343	0.00078	0.5343	57, 88, 101
MeC4	3-	butan	4.4658	0.00056	0.4658	74, 75, 101
	2-	butan	4.4828	0.00075	0.4828	57, 88, 101
MeC5	2-	pentan	5.4143	0.00052	0.4143	57, 88, 101
	3-	pentan	5.5492	0.00048	0.5492	74, 75, 101
	4-	pentan	5.6182	0.00062	0.6182	74, 87, 115
MeC6	2-	hexan	6.4128	0.00041	0.4128	57, 88, 101
	3-	hexan	6.4861	0.00041	0.4861	74, 75, 101
	5-	hexan	6.6285	0.00041	0.6285	74, 101, 129
	4-	hexan	6.7166	0.00081	0.7166	55, 74, 87
MeC7	2-	heptan	7.4032	0.00030	0.4032	57, 88, 101
	3-	heptan	7.4653	0.00066	0.4653	74, 75, 101
	4-	heptan	7.6231	0.00041	0.6231	74, 87, 115
	6-	heptan	7.6342	0.00047	0.6342	74, 115, 143
	5-	heptan	7.6987	0.00057	0.6987	74, 101, 129
MeC8	2-	octan	8.3918	0.00066	0.3918	57, 88, 101
	3-	octan	8.4428	0.00048	0.4428	74, 75, 101
	4-	octan	8.5823	0.00037	0.5823	74, 87, 115
	5-	octan	8.5930	0.00059	0.5930	74, 101, 129
	7-	octan	8.6475	0.00060	0.6475	74, 129, 157
	6-	octan	8.6917	0.00043	0.6917	74, 115, 143
MeC9	2-	nonan	9.3806	0.00018	0.3806	57, 88, 101
	3-	nonan	9.4277	0.00032	0.4277	74, 75, 101
	5-	nonan	9.5480	0.00055	0.5480	74, 101, 129
	4-	nonan	9.5574	0.00037	0.5574	74, 87, 115
	6-	nonan	9.5868	0.00049	0.5868	74, 115, 143
	8-	nonan	9.6460	0.00067	0.6460	74, 143, 171
	7-	nonan	9.7134	0.00032	0.7134	74, 129, 157
MeC10	2-	decan	10.3841	0.00038	0.3841	57, 88, 101
	3-	decan	10.4257	0.00033	0.4257	74, 75, 101
	5-	decan	10.5242	0.00050	0.5242	74, 101, 129
	6-	decan	10.5455	0.00038	0.5455	74, 115, 143
	4-	decan	10.5471	0.00033	0.5471	74, 87, 115
	7-	decan	10.6071	0.00068	0.6071	74, 129, 157
	9-	decan	10.6528	0.00050	0.6528	74, 157, 185
	8-	decan	10.7145	0.00087	0.7145	74, 143, 171
MeC11	2-	undecan	11.3763	0.00035	0.3763	57, 88, 101
	3-	undecan	11.4177	0.00035	0.4177	74, 75, 101
	5-	undecan	11.5006	0.00075	0.5006	74, 101, 129
	6-	undecan	11.5090	0.00094	0.5090	74, 115, 143
	4-	undecan	11.5337	0.00094	0.5337	74, 87, 115
	7-	undecan	11.5530	0.00055	0.5530	74, 129, 157
	8-	undecan	11.5969	0.00075	0.5969	74, 143, 171
	10-	undecan	11.6509	0.00043	0.6509	74, 171, 199
	9-	undecan	11.7170	0.00096	0.7170	74, 157, 185
MeC12	2-	dodecan	12.3714	0.00029	0.3714	57, 88, 101
	3-	dodecan	12.4101	0.00018	0.4101	74, 75, 101
	5-	dodecan	12.4826	0.00047	0.4826	74, 101, 129
	6-	dodecan	12.4840	0.00028	0.4840	74, 115, 143
	7-	dodecan	12.5147	0.00047	0.5147	74, 129, 157
	4-	dodecan	12.5240	0.00028	0.5240	74, 87, 115
	8-	dodecan	12.5398	0.00058	0.5398	74, 143, 171
	9-	dodecan	12.5965	0.00045	0.5965	74, 157, 185
	11-	dodecan	12.6488	0.00038	0.6488	74, 185, 213
	10-	dodecan	12.7174	0.00045	0.7174	74, 171, 199
MeC13	2-	tridecan	13.3666	0.00055	0.3666	57, 88, 101
	3-	tridecan	13.4050	0.00033	0.4050	74, 75, 101
	6-	tridecan	13.4656	0.00033	0.4656	74, 115, 143
	5-	tridecan	13.4693	0.00033	0.4693	74, 101, 129
	7-	tridecan	13.4859	0.00033	0.4859	74, 129, 157
	8-	tridecan	13.4984	0.00055	0.4984	74, 143, 171
	4-	tridecan	13.5155	0.00076	0.5155	74, 87, 115
	9-	tridecan	13.5377	0.00076	0.5377	74, 157, 185
	10-	tridecan	13.5932	0.00076	0.5932	74, 171, 199
	12-	tridecan	13.6486	0.00033	0.6486	74, 199, 227
	11-	tridecan	13.7175	0.00077	0.7175	74, 185, 213
MeC14	2-	tetradecan	14.3621	0.00033	0.3621	57, 88, 101
	3-	tetradecan	14.4010	0.00032	0.4010	74, 75, 101
	6-	tetradecan	14.4524	0.00052	0.4524	74, 115, 143
	5-	tetradecan	14.4592	0.00031	0.4592	74, 101, 129
	7-	tetradecan	14.4612	0.00031	0.4612	74, 129, 157
	8-	tetradecan	14.4689	0.00053	0.4689	74, 143, 171
	9-	tetradecan	14.4961	0.00030	0.4961	74, 157, 185

Table 1 (*Continued*)

Methyl x-Methyl-y-oate			*ECL*	*s*	*FCL*	MS ions *m*/*z*
	x	y				
	4-	tetradecan	14.5102	0.00052	0.5102	74, 87, 115
	10-	tetradecan	14.5320	0.00020	0.5320	74, 171, 199
	11-	tetradecan	14.5913	0.00053	0.5913	74, 185, 213
	13-	tetradecan	14.6471	0.00028	0.6471	74, 213, 241
	12-	tetradecan	14.7194	0.00043	0.7194	74, 199, 227
MeC15	2-	pentadecan	15.3595	0.00021	0.3595	57, 88, 101
	3-	pentadecan	15.3977	0.00053	0.3977	74, 75, 101
	6-	pentadecan	15.4409	0.00031	0.4409	74, 115, 143
	8-	pentadecan	15.4460	0.00044	0.4460	74, 143, 171
	7-	pentadecan	15.4481	0.00054	0.4481	74, 129, 157
	5-	pentadecan	15.4496	0.00031	0.4496	74, 101, 129
	9-	pentadecan	15.4649	0.00021	0.4649	74, 157, 185
	10-	pentadecan	15.4893	0.00032	0.4893	74, 171, 199
	4-	pentadecan	15.5061	0.00031	0.5061	74, 87, 115
	11-	pentadecan	15.5300	0.00021	0.5300	74, 185, 213
	12-	pentadecan	15.5922	0.00014	0.5922	74, 199, 227
	14-	pentadecan	15.6477	0.00016	0.6477	74, 227, 255
	13-	pentadecan	15.7215	0.00059	0.7215	74, 213, 241
MeC16	2-	hexadecan	16.3561	0.00022	0.3561	57, 88, 101
	3-	hexadecan	16.3950	0.00035	0.3950	74, 75, 101
	8-	hexadecan	16.4296	0.00059	0.4296	74, 143, 171
	6-	hexadecan	16.4318	0.00058	0.4318	74, 115, 143
	7-	hexadecan	16.4360	0.00059	0.4360	74, 129, 157
	9-	hexadecan	16.4398	0.00034	0.4398	74, 157, 185
	5-	hexadecan	16.4435	0.00022	0.4435	74, 101, 129
	10-	hexadecan	16.4542	0.00035	0.4542	74, 171, 199
	11-	hexadecan	16.4845	0.00022	0.4845	74, 185, 213
	4-	hexadecan	16.5027	0.00013	0.5027	74, 87, 115
	12-	hexadecan	16.5283	0.00033	0.5283	74, 199, 227
	13-	hexadecan	16.5906	0.00032	0.5906	74, 213, 241
	15-	hexadecan	16.6471	0.00031	0.6471	74, 241, 269
	14-	hexadecan	16.7239	0.00029	0.7239	74, 227, 255
MeC17	2-	heptadecan	17.3534	0.00032	0.3534	57, 88, 101
	3-	heptadecan	17.3930	0.00058	0.3930	74, 75, 101
	8-	heptadecan	17.4136	0.00048	0.4136	74, 143, 171
	9-	heptadecan	17.4142	0.00023	0.4142	74, 157, 185
	7-	heptadecan	17.4231	0.00059	0.4231	74, 129, 157
	6-	heptadecan	17.4236	0.00033	0.4236	74, 115, 143
	10-	heptadecan	17.4242	0.00023	0.4242	74, 171, 199
	5-	heptadecan	17.4376	0.00048	0.4376	74, 101, 129
	11-	heptadecan	17.4493	0.00033	0.4493	74, 185, 213
	12-	heptadecan	17.4822	0.00060	0.4822	74, 199, 227
	4-	heptadecan	17.5011	0.00035	0.5011	74, 87, 115
	13-	heptadecan	17.5256	0.00061	0.5256	74, 213, 241
	14-	heptadecan	17.5914	0.00023	0.5914	74, 227, 255
	16-	heptadecan	17.6472	0.00063	0.6472	74, 255, 283
	15-	heptadecan	17.7263	0.00039	0.7263	74, 241, 269
MeC18	2-	octadecan	18.3510	0.00039	0.3510	57, 88, 101
	3-	octadecan	18.3917	0.00038	0.3917	74, 75, 101
	8-	octadecan	18.4034	0.00038	0.4034	74, 143, 171
	9-	octadecan	18.4040	0.00024	0.4040	74, 157, 185
	10-	octadecan	18.4057	0.00065	0.4057	74, 171, 199
	7-	octadecan	18.4133	0.00024	0.4133	74, 129, 157
	6-	octadecan	18.4168	0.00038	0.4168	74, 115, 143
	11-	octadecan	18.4237	0.00065	0.4237	74, 185, 213
	5-	octadecan	18.4354	0.00024	0.4354	74, 101, 129
	12-	octadecan	18.4464	0.00037	0.4464	74, 199, 227
	13-	octadecan	18.4790	0.00062	0.4790	74, 213, 241
	4-	octadecan	18.4988	0.00065	0.4988	74, 87, 115
	14-	octadecan	18.5262	0.00049	0.5262	74, 227, 255
	15-	octadecan	18.5925	0.00034	0.5925	74, 241, 269
	17-	octadecan	18.6467	0.00033	0.6467	74, 269, 297
	16-	octadecan	18.7288	0.00032	0.7288	74, 255, 283
MeC19	2-	nonadecan	19.3483	0.00041	0.3483	57, 88, 101
	3-	nonadecan	19.3908	0.00028	0.3908	74, 75, 101
	8-	nonadecan	19.3920	0.00035	0.3920	74, 143, 171
	9-	nonadecan	19.3932	0.00059	0.3932	74, 157, 185
	11-	nonadecan	19.3938	0.00037	0.3938	74, 185, 213
	10-	nonadecan	19.4029	0.00030	0.4029	74, 171, 199
	7-	nonadecan	19.4053	0.00029	0.4053	74, 129, 157
	6-	nonadecan	19.4132	0.00036	0.4132	74, 115, 143
	12-	nonadecan	19.4199	0.00030	0.4199	74, 199, 227
	5-	nonadecan	19.4314	0.00031	0.4314	74, 101, 129
	13-	nonadecan	19.4430	0.00059	0.4430	74, 213, 241
	14-	nonadecan	19.4775	0.00034	0.4775	74, 227, 255

Table 1 (*Continued*)

Methyl x-Methyl-y-oate			*ECL*	*s*	*FCL*	MS ions *m*/*z*
	x	y				
	4-	nonadecan	19.4970	0.00037	0.4970	74, 87, 115
	15-	nonadecan	19.5243	0.00060	0.5243	74, 241, 269
	16-	nonadecan	19.5922	0.00042	0.5922	74, 255, 283
	18-	nonadecan	19.6462	0.00046	0.6462	74, 283, 311
	17-	nonadecan	19.7306	0.00051	0.7306	74, 269, 297
MeC20	2-	eicosan	20.3462	0.00043	0.3462	57, 88, 101
	10-	eicosan	20.3797	0.00042	0.3797	74, 171, 199
	8-	eicosan	20.3828	0.00042	0.3828	74, 143, 171
	9-	eicosan	20.3847	0.00070	0.3847	74, 157, 185
	11-	eicosan	20.3860	0.00041	0.3860	74, 185, 213
	3-	eicosan	20.3879	0.00070	0.3879	74, 75, 101
	12-	eicosan	20.3986	0.00042	0.3986	74, 199, 227
	7-	eicosan	20.3992	0.00070	0.3992	74, 129, 157
	6-	eicosan	20.4087	0.00017	0.4087	74, 115, 143
	13-	eicosan	20.4138	0.00042	0.4138	74, 213, 241
	5-	eicosan	20.4289	0.00041	0.4289	74, 101, 129
	14-	eicosan	20.4409	0.00042	0.4409	74, 227, 255
	15-	eicosan	20.4757	0.00040	0.4757	74, 241, 269
	4-	eicosan	20.4965	0.00039	0.4965	74, 87, 115
	16-	eicosan	20.5243	0.00039	0.5243	74, 255, 283
	17-	eicosan	20.5925	0.00066	0.5925	74, 269, 297
	19-	eicosan	20.6469	0.00036	0.6469	74, 297, 325
	18-	eicosan	20.7328	0.00034	0.7328	74, 283, 311
MeC21	2-	heneicosan	21.3441	0.00037	0.3441	57, 88, 101
	10-	heneicosan	21.3703	0.00038	0.3703	74, 171, 199
	9-	heneicosan	21.3716	0.00057	0.3716	74, 157, 185
	11-	heneicosan	21.3716	0.00039	0.3716	74, 185, 213
	8-	heneicosan	21.3736	0.00039	0.3736	74, 143, 171
	12-	heneicosan	21.3775	0.00038	0.3775	74, 199, 227
	3-	heneicosan	21.3854	0.00057	0.3854	74, 75, 101
	13-	heneicosan	21.3920	0.00057	0.3920	74, 213, 241
	7-	heneicosan	21.3933	0.00027	0.3933	74, 129, 157
	6-	heneicosan	21.4064	0.00038	0.4064	74, 115, 143
	14-	heneicosan	21.4123	0.00039	0.4123	74, 227, 255
	5-	heneicosan	21.4248	0.00038	0.4248	74, 101, 129
	15-	heneicosan	21.4373	0.00027	0.4373	74, 241, 269
	16-	heneicosan	21.4747	0.00040	0.4747	74, 255, 283
	4-	heneicosan	21.4951	0.00041	0.4951	74, 87, 115
	17-	heneicosan	21.5233	0.00041	0.5233	74, 269, 297
	18-	heneicosan	21.5923	0.00043	0.5923	74, 283, 311
	20-	heneicosan	21.6448	0.00055	0.6448	74, 311, 339
	19-	heneicosan	21.7347	0.00047	0.7347	74, 297, 325
MeC22	2-	docosan	22.3413	0.00028	0.3413	57, 88, 101
	10-	docosan	22.3590	0.00045	0.3590	74, 171, 199
	11-	docosan	22.3604	0.00046	0.3604	74, 185, 213
	9-	docosan	22.3631	0.00057	0.3631	74, 157, 185
	12-	docosan	22.3644	0.00057	0.3644	74, 199, 227
	8-	docosan	22.3665	0.00028	0.3665	74, 143, 171
	13-	docosan	22.3699	0.00057	0.3699	74, 213, 241
	3-	docosan	22.3856	0.00028	0.3856	74, 75, 101
	14-	docosan	22.3883	0.00088	0.3883	74, 227, 255
	7-	docosan	22.3890	0.00028	0.3890	74, 129, 157
	6-	docosan	22.4033	0.00057	0.4033	74, 115, 143
	15-	docosan	22.4080	0.00046	0.4080	74, 241, 269
	5-	docosan	22.4237	0.00057	0.4237	74, 101, 129
	16-	docosan	22.4292	0.00018	0.4292	74, 255, 283
	17-	docosan	22.4721	0.00058	0.4721	74, 269, 297
	4-	docosan	22.4918	0.00028	0.4918	74, 87, 115
	18-	docosan	22.5218	0.00058	0.5218	74, 283, 311
	19-	docosan	22.5920	0.00028	0.5920	74, 297, 325
	21-	docosan	22.6431	0.00028	0.6431	74, 325, 353
	20-	docosan	22.7357	0.00060	0.7357	74, 311, 339

from the 2-position to the 17-position on the carbon chain. Their model was elaborated using standards of the *iso* acids with an even number of *C*-atoms ranging from *C*12 to *C*20 and the *anteiso* acids with an odd number of *C*-atoms *C*13–*C*21. The published mass spectra of some monomethyl branched FAME up to *C*18 [6] were used to establish the retention order of closely eluting peaks. These analytical procedures were applied in a study of the branched fatty acids of human sebaceous type excretions [7]. Only the methyl branches on even numbered *C*-atoms, except for the *iso* compounds, were identified. In some other works, derivatives of methyl-isomers of FAME were used for better resolution of mass spectra. For example, Simon et al. [8] localized the branch in monomethyl branched fatty acids by converting the FAME to the corresponding branched alkanes. Blomquist et al. [9] identified 18 methyl-branched *C*15–*C*19 fatty acids from the housefly (*Musca domestica* L.) by GC–MS after reduction to the corresponding hydrocarbons. Yu et al. [10] localized methyl branching in fatty acids by GC–MS of 4,4-dimethyloxazoline derivatives.

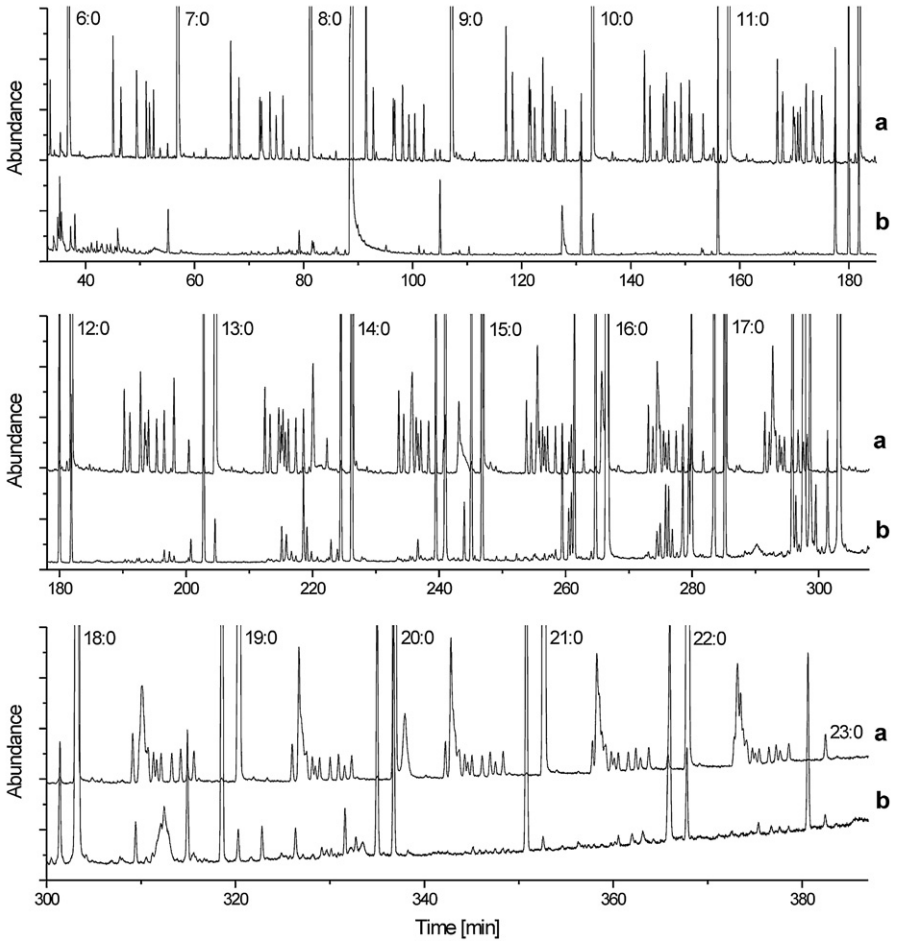

Fig. 1. Chromatogram of the GC separation of the *C*6–*C*23 monomethyl branched fatty acid methyl esters on column coated with OV-1 stationary phase; (a) product of methylene insertion reaction, (b) tongue coating sample.

The aim of this study was the investigation of GC retention behavior of all 220 monomethyl branched saturated *C*4–*C*23 FAME on methylsilicone OV-1 stationary phase. Since standard materials were unavailable, the monomethyl FAME were prepared from a mixture of the *C*6–*C*22 straight chain FAME by methylene insertion reaction [11], and completed by commercial standards of the *C*4–*C*6 monomethyl branched FAME. For the purpose of GC identification of the monomethyl branched FAME, the structure–retention correlations previously published for hydrocarbon homologues series [12–14] were used and identification was confirmed by GC–MS [6]. The retention data of the GC unseparated isomers were obtained by the MS deconvolution [14].

2. Experimental

The model mixtures of monomethyl branched FAME *C*7–*C*23 were prepared from the commercially available mixture of *C*6–*C*22 straight chain FAME (Supelco, Bellefonte, PA, USA) by methylene insertion reaction [11], by using gaseous diazomethane and UV radiation in an apparatus by Glastrup [15]. The recovery of this reaction was about 4%. This mixture was completed by the individual *C*3–*C*5 straight chain FAME and the *C*4–*C*7 monomethyl branched FAME (Sigma–Aldrich, Steinheim, Germany).

The biological sample used in this study was prepared using the white coating collected from root of the tongue of a patient suffering under immunodeficiency. The sample amount taken was approximately 50 μl. In order to avoid contamination of the sample, the patient took only a fat free food within 24 h before sample collection. The sample was driven to dryness at 50 °C. The lipids were extracted with 1 ml of chloroform. After removal of the solvent from the extract under stream of nitrogen at 50 °C, 200 μl of hexane and 40 μl of methyl acetate were added and mixed. Methyl esters of fatty acids were prepared using 100 μl of 0.5 M sodium methoxide in dry methanol and for 15 min allowed to react at room temperature with occasional mixing. Then vial was cooled at −20 °C for 10 min, added 60 μl of oxalic acid (0.5 g in 15 ml diethyl ether), and mixed. Vial was centrifuged to settle sodium oxalate precipitate. The upper phase with FAME solution was used for analysis by GC–MS.

GC–MS measurements were performed on an Agilent Technologies 6890N gas chromatograph with a 5973 Network mass-selective detector (Agilent, Waldbronn, Germany). The 1 μl of sample injection operated at the temperature 320 °C in the split injection mode with a split ratio 100:1 and in the splitless mode for the standard mixture and biological sample, respectively. The monomethyl branched FAME mixture was separated using capillary column 100 m × 0.25 mm i.d. coated with a film thickness of 0.25 μm of methylsilicone OV-1 as stationary phase (Supelco, Bellefonte, PA, USA). The column temperature was 30 °C initially, then the temperature was increased to 310 °C at ramp rate of 1 °C min^{-1}, temperature was held at the final temperature 310 °C for 5 min. Helium carrier gas with constant flow of 1.6 ml min^{-1} was used. The transfer line temperature was set at 330 °C. The quadrupole conditions were as follows: electron energy 70 eV, and ion source temperature 230 °C. The scan range of the MS-SIM was from m/z 57 to 311.

The retention data at temperature programmed gas chromatography were expressed as equivalent chain lengths (*ECL*) with the saturated straight chain FAME as reference compounds [16]. The *ECL* values of the monomethyl branched FAME were calculated from three parallel measurements with an average repeatability of ±0.0004 *ECL* units. The specific monomethyl branched FAME of the model mixture were identified on the basis of structure–retention relationships of fractional chain lengths (*FCL*) of individual monomethyl branched FAME homologous series on the number of carbon atoms [17] and confirmed by the GC–MS [6].

3. Results and discussion

The GC chromatogram obtained for the *C*6–C23 monomethyl branched FAME, prepared by methylene insertion reaction to linear chain FAME, has the peaks of straight chain FAME assigned as shown in Fig. 1a. The characteristic mixtures of all isomeric monomethyl branched FAME were obtained. The measured *ECL* values and their standard deviations *s*, as well as *FCL* values for monomethyl branched FAME on methylsilicone stationary phase, are given in Table 1. *FCL* is the fraction of a carbon number attributed to the methyl branched at a specific position [17]. The *FCL* values were obtained as the difference of *ECL* value of the given monomethyl branched FAME homologue and those of straight chain FAME with the same carbon atom number in main acid chain. Thus, the *FCL* value characterizes the contribution of a certain position of methyl group to the *ECL* value of monomethyl branched FAME.

Despite using a 100 m long high-resolution capillary column with calculated separation number SN = 50 for pair of the straight chain *C*16–*C*17 FAME at given gas chromatographic condition, the gas chromatographic separation of several monomethyl branched FAME were not obtained. The most difficult to separate isomers are those with methyl substitution in the surroundings of the middle of the molecular carbon chain. The separation was becoming even more difficult with increasing number of *C*-atoms in the FAME molecules.

The *ECL* values of gas chromatographically unseparated monomethyl branched FAME isomers were calculated by the MS deconvolution. The GC–MS deconvolution was performed by using of the characteristic fragment ions formed by cleavage of the carbon–carbon bond adjacent to the tertiary carbon atoms [6]. The

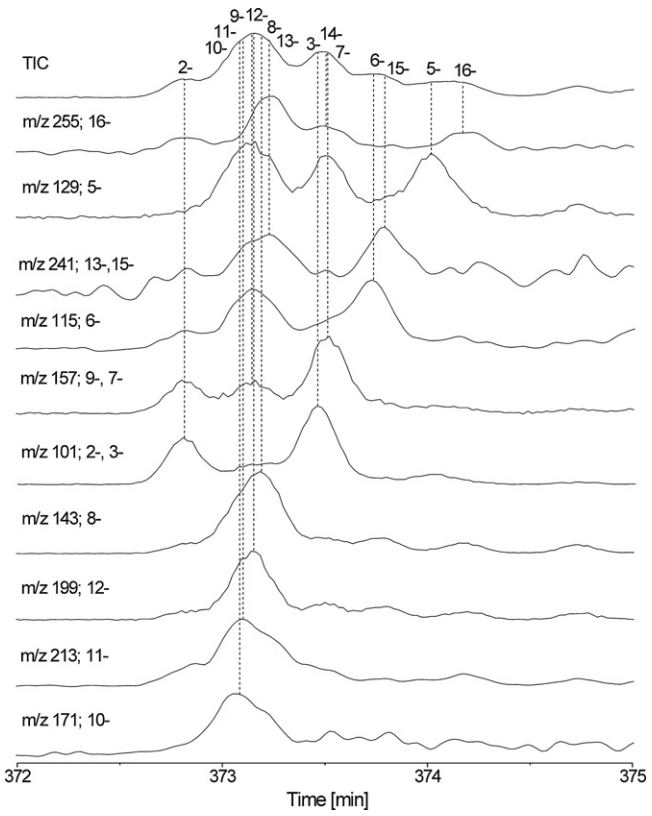

Fig. 2. Mass spectrometric deconvolution of the GC unseparated isomers of the methyl monomethyldocosanoates.

first characteristic mass ion *m*/*z* 74 is the base peak for most methyl esters except of those with methyl position 2- and for methyl-4-methyl-hexanoate. It consists of the methoxycarbonyl group of the ester, the next methylene group and an *H*-atom attached from the third *C*-atom of the chain. The second selected fragmentation mass ion corresponds to the methoxycarbonyl group and the part of *C*-chain up to (but excluding) the tertiary *C*-atom. The third characteristic fragmentation mass ion corresponds to the methoxycarbonyl group and the part of *C*-chain including the tertiary *C*-atom. The difference between third and second charac-

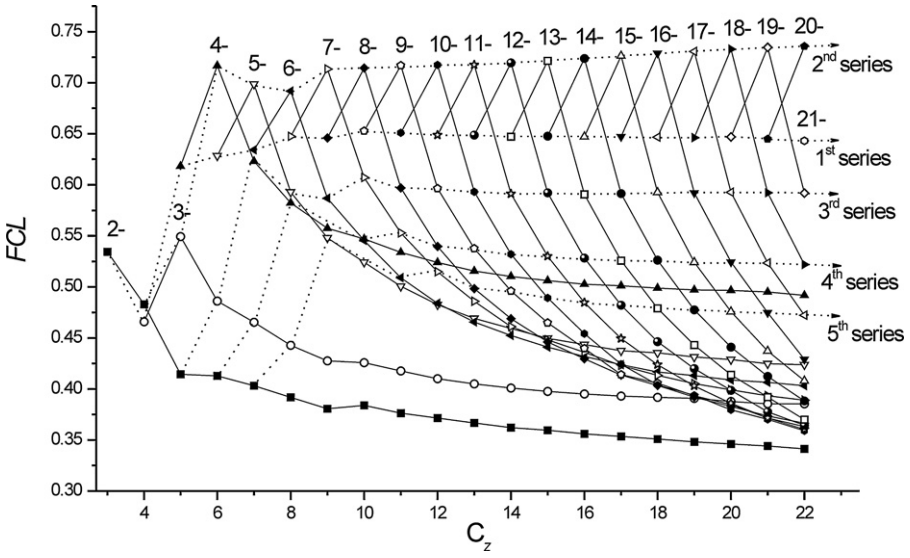

Fig. 3. Dependence of fractional chain lengths (*FCL*) values on the number of carbon atoms (C_z) of the monomethyl fatty acid methyl esters homologues series. 1st series, the first members of homologues series; 2nd series, the second members of homologues series; 3rd series, the third members of homologues series; etc.

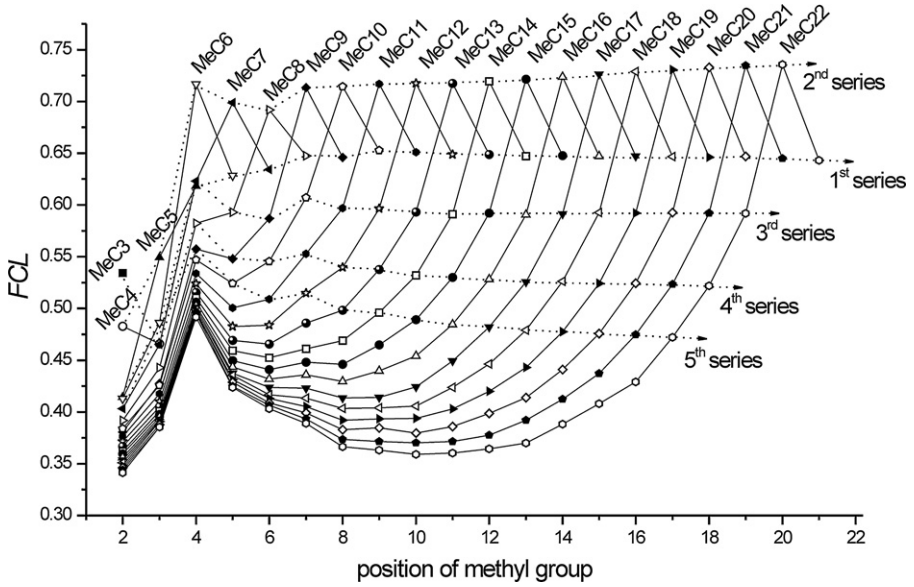

Fig. 4. Dependence of the fractional chain lengths (*FCL*) values on the position of methyl group of the monomethyl fatty acid methyl esters homologues series. 1st series, the first members of homologues series; 2nd series, the second members of homologues series; 3rd series, the third members of homologues series; etc.

teristic fragmentation mass ions is 28 amu. The MS ions specific for identification of the monomethyl branched FAME are summarized in Table 1. Fig. 2 shows the mass spectrometric deconvolution of gas chromatographic unseparated isomers 2-, 3-, 5- to 16-monomethyl branched *C*22 FAME. All these isomers were mass spectrometrically deconvoluted by detection of specific fragment mass ions, which allowed to determine their retention times and *ECL* values. For the deconvoluted 7- and 14-monomethyl *C*22 FAME isomeric pair, the retention time difference is only 0.01 min corresponding to an *ECL* value difference of 0.0007 *ECL* units.

The GC identification of monomethyl branched FAME reaction products was obtained from measured temperature programmed *ECL* values, with the structure–retention relationships based on regularity of the dependence of the fractional chain lengths on the number of carbon atoms for individual homologous series, i.e. for the 2-, 3-, 4-, . . ., 22-monomethyl branched FAME. The GC identification of the monomethyl branched FAME from the model mixture up to *C*7 was done by using of commercial reference materials. The *ECL* values of monomethyl branched FAME >*C*7 were calculated by progressive carbon by carbon extrapolation of *FCL* values in monomethyl branched FAME homologues series. Because of non-linearity of the dependence of retention data on the number of carbon atoms for the first 5–6 homologues, the extrapolated *FCL* values were little different than the measured *ECL* values. Nevertheless, the precision was sufficient for identification of these analytes in model mixture. For the following retention extrapolation of higher homologues, the measured *FCL* values were used. However, the dependence $FCL = f(C_z)$ (Fig. 3) still did not allow the retention prediction of the first homologues of homologues series beginning at a higher number of *C*-atoms, and the prediction of retention of second, third, fourth, etc. members of homologues series was less precise because of non-linearity of these dependencies. These issues were resolved by a net extrapolation of the *FCL* values for the first and subsequent members of different homologues series, with almost linear dependencies (signed by dotted lines in Fig. 3). Similar precision of prediction of the *FCL* values (better as 0.01 *FCL* units) was obtained by the dependencies of the *FCL* values on the position of methyl group of monomethyl branched FAME, as presented in Fig. 4.

Relationships found between the *FCL* on the number of carbon atoms in the fatty acid chain excluding the methyl branch allows to achieve a precise retention prediction of the saturated monomethyl branched FAME with a number of the *C*-atoms >23. The preliminary identification of the monomethyl branched FAME as model

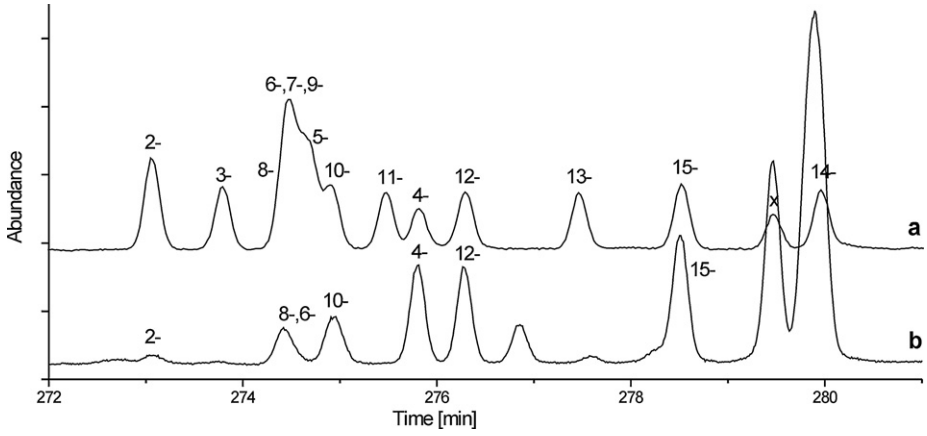

Fig. 5. Chromatograms of the GC separation of the isomeric monomethylhexadecanoates; (a) a product of the methylene insertion reaction, (b) a tongue coating sample; (x) ethyl hexadecanoate.

analytes obtained by methylene insertion reaction to the straight chain FAME was confirmed by GC–MS. All 220 of the *C*4–*C*23 saturated monomethyl branched FAME have been characterized by the gas chromatographic and mass spectrometric data.

Formerly, Apon and Nicolaides [6] published retention data as equivalent chain lengths of monomethyl branched FAME with chain length *C*11 for 5 isomers, *C*12 6 isomers, *C*13 6 isomers, *C*14 7 isomers, *C*15 7 isomers, *C*16 8 isomers, *C*17 8 isomers, and for 16 isomers *C*18. Together, the *ECL* data were published [6] for 63 *C*12–*C*19 monomethyl branched FAME, measured on laboratory prepared capillary columns of either 1000 ft or 500 ft with i.d. of 0.030 in. coated with Pentasil stationary phase and Igepal-*C*o-880 addition, and column temperature programmed from 170 °C to 210 °C by 0.5 °C min^{-1}. *ECL* data for the isomeric *C*18 monomethyl branched FAME were measured in 500 ft column at 200 °C. In the complete mixture of 16 methyl monomethyl octadecanoates, the clusters of methyl isomers 8-, 9-, 10-, and 6-, 11-, and 5-, 12- were not separated and some other isomers were only partially separated. Also some isomers of uncomplete series of *C*11–*C*17 monomethyl branched FAME were not resolved, i.e. at *C*17 isomers 5- and 10-, at *C*14 isomers 6- and 8-, at *C*12 isomers 4- and 8-.

In the study of Bal and *C*zarnowski [18], the coated tongue was found to be one of the 4 specific clinical markers for typhoid fever, in the addition to high fever, loose bowel movements and bradycardia, and may provide an important diagnostic clue. The GC–MS chromatogram of the coated tongue sample is presented in Fig. 1b. The *C*17 monomethyl-branched FAME as the most characteristic methyl fatty acid methyl esters of the tongue coating, in methyl positions 2-, 4-, 6-, 8-, 10-, 12-, 14-, and 15-, thus methyl isomers with even position except of *iso*, were identified by GC/MS-SIM. From Fig. 5 it can be seen, that the content of *anteiso* 17:0 to be the most abundant and next most abundant is *iso* 17:0 among isomers of monomethyl branched 17:0 FAME. It is notable that *anteiso* 17:0 is a major lipid constituent of many bacterial membranes [4].

4. Conclusions

The structure–retention correlation in the homologues series, based on fraction chain lengths in hyphenation with mass spectra and mass spectrometric deconvolution of gas chromatographic unseparated positional isomers, allowed identification of all the 210 *C*7–*C*23 monomethyl branched FAME in the product of methyl insertion reaction of the straight chain *C*6–*C*22 FAME. *ECL* data of monomethyl branched FAME were substantially completed and are more precise compared with those published previously. In the tongue coating, which is one of the specific clinical markers for the typhoid fever, the methyl isomers with even position of methylhexadecanoates were ascertained as the most characteristic compounds. The obtained *ECL* values and MS data will be applied for identification of the monomethyl FAME in the breath analysis [14,19].

Acknowledgements

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