Characterization of Some Lipid Components of Asterias rubens By Mass Spectrometry

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(Received 13th February, 1983)

Summary: Fatty acid moieties of the steryl esters and triglycerides were studies by mass spectrometry and GC-MS techniques. Possible fragmentation behaviour has been discussed. GLC results were in good agreement with MS analysis. Seven fatty acids (23:0, 23:1; 24:1; 18:21:5:23:6) which have not previously been reported were identified.

Introduction

Mass spectrometry has made great contribution in the characterization of the lipids. The pattern of the electron impact induced fragmentation of fatty acid esters has been studied by Ryhage and Stenhagen [1] and Stenhagen [2]. Such and Holnan [3] have also described the mass spectrometric analysis of methylstearate, methyl 18,18-trideutero octadeconate, and triglycerides. The determination of the molecular weight and structure of mycocerosic acid has been carried out by Asselineau et al. [4].

In the present work MS and GC-MS techniques have been used to investigate the fatty acid components of the steryl esters and triglycerides of Asterias rubens.

Experimental

The steryl esters of A.rubens were separated into individual classes according to Kemp and Mercer [5]. Each lass was saponified and fatty acid methyl esters were prepared by treatment with boron trifluoride methanol complex (Morrison and Smith [6]). The mass spectra of the methyl esters were obtained at 70eV on A.E.I. MS-12 instrument.

The triglycerides were inter-esterified with methanol HCl (Gas Chrom. News-letter, 1970 (7) and the methyl esters thus obtained were subjected to the combined GC-MS system.

Results

The fatty acid moieties of the saturated esters class showed about 13 molecular ion peaks and their mass spectrometric fragmentation behaviour is given in table-1. The mono-unsaturated esters gave 14 (Table-2) while di-unsaturated and tri-unsaturated components showed 9 and 7 molecule-ion peaks respectively (Table-3 and 4).

The composition of the fatty acid methyl esters of triglyceride studied on the combined GC-MS system has been described in Fig.1 and Table-5. In Fig.1, peaks I to IV are well defined showing a single component while peaks V and VI are not so consistent and hence exhibit most probably 3 and 9 overlapping peaks respectivley.

Discussion

Methyl esters of saturated fatty acids

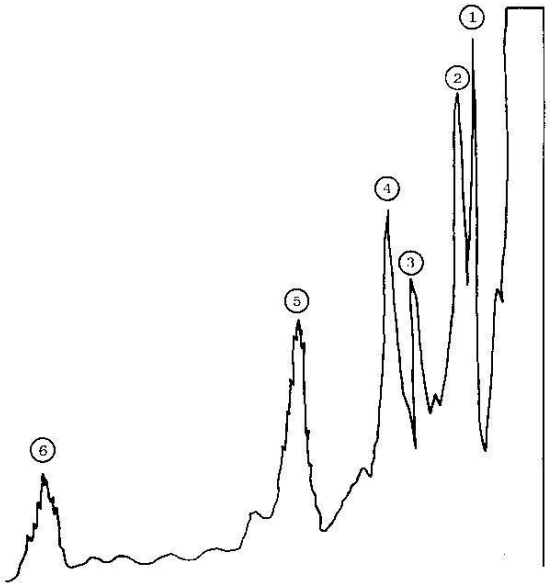

Fig.1: GLC analysis of the fatty acid methyl esters of triglycerides on 3% SILAR 10-C column.

with more than five carbon atoms fragment to give hydrocarbon ions and oxygen containing ions. The hydrocarbon ions are of little diagnostic interest. The oxygen containing ions give prominent peaks in the spectrum. These include the molecular ion peak (M^+), the acylium ion formed by loss of a methoxy group (M^+-31), the peak m/e 74 which is characteristic of most methyl esters and a series of ions corresponding to $(CH_2)_n.COOCH_3$.

Unsaturated fatty acid methyl esters follow a different fragmentation pattern. The molecular ion peak is usually quite prominent with these esters. In a mixture of components of different carbon numbers and amount of unsaturation these peaks can be seen easily. In addition to the M^+-31 peak, monounsaturated compounds also show peaks at M^+-74 and M^+-116 fragments respectively.

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Table 1: Mass spectral data for the saturated fatty acid methyl esters obtained from the steryl esters of Asterias rubens.

M^+	M^+-31	M^+-43	M^+-59	Fatty Acid	
158(5)	127(8)	115(8)	99(130)	8:0	Octanoic
172(1)	141(9)	129(12)	113(10)	9:0	Nonanoic
186(1)	155(3)	143(12)	127(10)	10:0	Decanoic
200(2)	169(4)	157(2)	141(9)	11:0	Hendecanoic
214(3)	183(4)	171(7)	155(3)	12:0	Dodecanoic
228(3)	197(4)	185(7)	169(4)	13:0	Tridecanoic
242(2)	211(4)	199(8)	183(4)	14:0	Tetradecanoic
256(2)	225(4)	213(4)	197(4)	15:0	Pentadecanoic
270(19)	239(10)	227(10)	211(4)	16:0	Hexadecanoic
284(5)	253(3)	241(4)	225(4)	17:0	Heptadecanoic
298(4)	267(3)	255(3)	239(10)	18:0	Octadecanoic
312(1)	281(1)	269(3)	253(3)	19:0	Nonadecanoic
368(8)	337(1)	325(1)	309(1)	23:0	Tricosanoic

Table 2: Mass spectral data for the monounsaturated fatty acid methyl esters obtained from the steryl esters of Asterias rubens

M^+	M^+-32	M^+-74	M^+-116	Fatty Acid	
212(2)	180(5)	138(10)	96(47)	12:1	Dodecenoic
226(1)	194(8)	152(12)	110(23)	13:1	Tridecenoic
240(2)	208(6)	166(5)	124(11)	14:1	Tetradecnoic
254(1)	222(2)	180(4)	138(9)	15:1	Pentadecenoic
268(2)	236(10)	192(3)	152(3)	16:1	Hexadecenoic
282(1)	250(6)	208(6)	166(4)	17:1	Heptadecenoic
296(1)	264(8)	222(2)	180(4)	18:1	Octadecenoic
310(1)	278(2)	236(10)	194(3)	19:1	Nonadecenoic
324(1)	292(2)	250(6)	208(6)	20:1	Eicosenoic
338(1)	306(2)	264(8)	222(2)	21:1	Heneicosenoic
352(1)	320(2)	278(3)	236(10)	22:1	Docosenoic
366(1)	334(1)	292(2)	250(6)	23:1	Tricosenoi
380(1)	348(2)	306(1)	264(8)	24:1	Tetracosenoic

Table 3: Mass spectral data for the diunsaturated fatty acid methyl esters obtained from the steryl esters of A.rubens.

M^+	M^+-31	M^+-74	Fatty Acid	
252(1)	221(4)	178(2)	15:2	Pentadecadienoic
266(1)	235(3)	192(4)	16:2	Hexadecadienoic
280(1)	249(3)	206(2)	17:2	Heptadecadienoic
294(3)	263(3)	220(3)	18:2	Octadecadienoic
308(1)	277(1)	234(2)	19:2	Nonadecadienoic
322(1)	291(2)	248(4)	20:2	Eicosadienoic
336(1)	305(2)	262(2)	21:2	Henecosadienoic
350(1)	319(1)	276(2)	22:2	Docosadoenoic
364(1)	353(1)	290(1)	23:2	Tricosadienoic

Table 4: Mass spectral data for the triunsaturated fatty acid methyl esters obtained from the steryl esters of A.rubens.

M^+	M^+-31	M^+-56	M^+-69	Fatty Acid	
278(1)	274(1)	222(4)	209(3)	17:3	Heptadecatrienoic
292(1)	261(2)	236(12)	223(2)	18:3	Octadecatrienoic
306(1)	275(1)	250(7)	237(1)	19:3	Nonadecatrienoic
320(1)	289(1)	264(3)	251(2)	20:3	Eicosatrienoic
334(1)	303(1)	278(1)	265(4)	21:3	Heneicosatrienoic
348(1)	317(1)	292(17)	279(1)	22:3	Docosatrienoic
362(1)	331(1)	306(1)	293(9)	23:3	Tricosatrienoic

Table 5: Mass Spectral data for the fatty acid methyl esters obtained from the triglycerides of Asterias rubens. m/e (Relative intensity)

M^+	M^+-31	M^+-32	M^+-43	M^+-56	M^+-59	M^+-74	M^+-116	Fatty	Acid	No*
270(50)	239(13)		227(25)	-	211(2)	-	-	16:0	Hexadecenoic	1
268(12)	-	236(35)	-	-	-	194	152	16:1	Hexadecenoic	2
298(40)	267(8)	-	255(18)	-	239(9)	-	-	18:0	Octadecanoic	3
296(12)	265(50)	264(86)	-	-	-	222(50)	180(15)	18:1	Octadecenoic	4
294(5)	263(5)	-	251(25)	-	235(10)	220(25)	-	18:2	Octadecadienoic	5
292(70)	261(10)		249(20)	236(10)	233(10)	218(10)	176(10)	18:3	Octadecatienoic	5
324(40)	-	292(70)	281(30)	-	-	250(30)	208(50)	20:1	Eicosenoic	5
316(10)	-	-	-	-	-	-	-	20:5	Eicosapentenoic	6
330(10)	-	-	-	-	-	-	-	21:5	Docosapentenoic	6
344(10)	-	-	-	-	-	-	-	22:5	Docosapentenoic	6
342(10)	-	-	-	-	-	-		22:6	Decosahexaenoic	6
356(10)	-	-	-	-	-	-	-	22:6	Decosahexaenoic	6
356(10)	-	-	-	-	-	-	-	23:6	Tricosahexenoic	6
372(10)	-	-	-	-	-	-	-	24:5	Tetracosapentenoic	6

NO* Refers to GC peak number shown in Fig.1

peaks at M^+-74 and M^+-116 fragments respectively.

In di-unsaturated fatty acid methyl esters the M^+-31 and M-74 ions are the characteristic peaks while in the triunsaturated series peaks at M^+-56 and M^+-69 are particularly high and have considerable diagnostic values.

Fatty acid methyl esters of the triglycerides also follow the same fragmentation pathway except the poly-unsaturated series. Therefore, most of the inferences in the poly unsaturated class have been made on the basis of molecular ion peak which was the only dominating factor.

Many of these fatty acids have been identified before by GLC(Allen[8] and Khan[9]). The reports on fatty acids having shorter chain lengths than C_{14} have not appeared yet. Moreover, seven fatty acids, one belonging to the category of saturated (23:0), two monounsaturated (22:1,24:1), one diunsaturated (18:2), and three polyunsaturated (21:5,24:5,23:6), which previously have not been detected were tentatively identified.

Acknowledgement

I am thankful to Mr. Garry Harriman, Department of Biochemistry, University of Liverpool, for providing the technical assistance in recording the mass spectra.

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