Characterization of bile acid methyl ester acetate derivatives using gas–liquid chromatography, electron impact, and chemical ionization mass spectrometry

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Abstract The gas–liquid chromatographic retention times on 0.5% SP-525 for 48 bile acids and related compounds as their methyl ester acetate derivatives are given. Ion tables for electron impact spectra have been compiled that permit direct access to ion structures for any given ion mass. Chemical ionization yields highly simplified mass spectra with two or three ions predominating for each compound. When the relative retention times of bile acids as their methyl ester acetates are combined with selective ion monitoring techniques in chemical ionization mass spectrometry, the retention time and ion mass number form a coordinate system which can be a powerful tool in the characterization of bile acid mixtures.

Supplementary key words Selective ion monitoring

The characterization of individual bile acids by mass spectrometry or of mixtures of bile acids by combined gas–liquid chromatography–mass spectrometry has been extensively described by Bergström (1), Bergström, Ryhage, and Stenhagen (2), Ryhage and Stenhagen (3), Eneroth et al. (4, 5), Sjövall (6, 7), Sjövall, Eneroth, and Ryhage (8), Egger (9), and Elliott (10). These workers have provided detailed explanations for the fragmentation of bile acids under electron impact ionization, and this has permitted the identification of these compounds when available in isolated form or when cleanly resolved by gas–liquid chromatography. Most studies involving gas–liquid chromatographic separations prior to mass spectrometry have been constrained by the availability of stationary phases that have both good separation characteristics and low column bleed. Since column bleed is dependent upon column temperature, this in turn required that the derivatives have high volatility, and most workers have used either the methyl ester trifluoroacetates or trimethylsilyl ethers; as a consequence, a majority of the spectra reported have been obtained on these bile acid derivatives.

Our laboratory has been faced with the requirements for a bile acid derivative that has (*a*) high stability (to permit shipment or storage of samples), (*b*) a low molecular weight, and (*c*) does not introduce further isotopic complexity (such as is present in trimethylsilyl ethers). These requirements are best fulfilled by the methyl ester acetates, and the commercial availability of new GLC stationary phases with good temperature stability, low bleed, and good separation characteristics for these derivatives has expedited their use in identification and measurement of stable isotopic ratios.

In the course of our studies, we have compiled the GLC retention times of almost fifty bile acids and related compounds on columns of 0.5% SP-525 and have determined their electron impact spectra. Since the ultimate basis of bile acid identification is electron impact mass spectrometry, the latter data have been interpreted on the basis of previously reported fragmentation pathways to provide the analyst with an ion table for the bile acid methyl ester acetates. In this "reverse catalogue," ions are listed in order of increasing molecular weight, and the structures of all ions with that mass arising from bile acid structures are listed. This permits the analyst to look up each major ion and tabulate the limited alternatives, rather than requiring the use of a computer for spectra matching. Complete spectra of all compounds listed are available, and copies can be obtained from the authors upon request.

In addition to the electron impact spectra, the chemical ionization spectra of this collection have been obtained. Because of their great simplicity, the ion

Abbreviations: GLC, gas–liquid chromatography; EI, electron impact; CI, chemical ionization; MS, mass spectrometry; RRT, relative retention time; SIRMID, stable isotope ratiometer multiple ion detector.

intensities can be tabulated in a compact form. By combining these characteristics with the relative retention time data, it is possible to specify a pair of coordinates made up of an ion mass and a relative retention time for each of the bile acids, thus implementing the use of selective ion monitoring techniques. The absence of extensive fragmentation in chemical ionization mass spectrometry means that these coordinates provide a system of bile acid identification of exceptional selectivity and sensitivity.

MATERIALS AND METHODS

Chemicals

Most of the bile acids used in this study were the gifts of collaborators, notably Dr. Alan Hofmann of Mayo Clinic. Additional bile acids were purchased either as the free acids, methyl esters, or methyl ester acetates. A few bile acids were prepared in our laboratory by Dr. David Hachey and Mr. William Samuels. All compounds proved to be of satisfactory purity by GLC. A complete list of the compounds studied and their suppliers is given in **Table 1**. The bile acids are listed in order of increasing complexity, beginning with the mono-, di- and trihydroxy bile acids, then the keto-bile acids, followed by the allo-bile acids, and finally those compounds with C_{22}, C_{23}, or C_{27} structures. This listing is intended to provide the reader with an entry key to subsequent retention time and mass spectral data, and the number assigned in Table 1 is therefore the identification number for the compound throughout the paper.

Underivatized or partially derivatized bile acids were methylated using an excess of diazomethane, acetylated (11), and dissolved in acetone. Mass spectra were obtained on an excess of sample

TABLE 1. Bile acids and related compounds studied and their sources

No.	Structure	Parent Compound (IUPAC)	Source
1	C_{24} 5β 3α OAc	5β-Cholan-24-oic acid	*a*
2	C_{24} 5β 3α OAc Δ11	5β-Cholan-24-oic acid	*b*
3	C_{24} 5β 12α OAc Δ3	5β-Cholan-24-oic acid	*b*
4	C_{24} 5β 3α OAc 6α OAc	5β-Cholan-24-oic acid	*c*
5	C_{24} 5β 3α OAc 6β OAc	5β-Cholan-24-oic acid	*b*
6	C_{24} 5β 3α OAc 7α OAc	5β-Cholan-24-oic acid	*d*
7	C_{24} 5β 3α OAc 7β OAc	5β-Cholan-24-oic acid	*c*
8	C_{24} 5β 3α OAc 12α OAc	5β-Cholan-24-oic acid	*e*
9	C_{24} 5β 3α OAc 12β OAc	5β-Cholan-24-oic acid	*c*
10	C_{24} 5β 3α OAc 7α OAc Δ11	5β-Cholan-24-oic acid	*f*
11	C_{24} 5β 3α OAc 12α OAc Δ8(14)	5β-Cholan-24-oic acid	*b*
12	C_{24} 5β 3α OAc 6α OAc 7α OAc	5β-Cholan-24-oic acid	*g*
13	C_{24} 5β 3α OAc 6β OAc 7α OAc	5β-Cholan-24-oic acid	*c*
14	C_{24} 5β 3α OAc 6β OAc 7β OAc	5β-Cholan-24-oic acid	*c*
15	C_{24} 5β 3α OAc 7α OAc 12α OAc	5β-Cholan-24-oic acid	*e*
16	C_{24} 5β 3β OAc 7α OAc 12α OAc	5β-Cholan-24-oic acid	*c*
17	C_{24} 5β 3α OAc 7β OAc 12α OAc	5β-Cholan-24-oic acid	*c*
18	C_{24} 5β 3α OAc 7α OAc 12α OH	5β-Cholan-24-oic acid	*c*
19	C_{24} 5β 3α OMe 7α OAc 12α OAc	5β-Cholan-24-oic acid	*f*
20	C_{24} 5β 3α OEt 7α OAc 12α OAc	5β-Cholan-24-oic acid	*f*
21	C_{24} 5β 3 = 0	5β-Cholan-24-oic acid	*b*
22	C_{24} 5β 3 = 0 7α OAc	5β-Cholan-24-oic acid	*c*
23	C_{24} 5β 3 = 0 12α OAc	5β-Cholan-24-oic acid	*c*
24	C_{24} 5β 3α OAc 7 = 0	5β-Cholan-24-oic acid	*c*
25	C_{24} 5β 3α OAc 12 = 0	5β-Cholan-24-oic acid	*c*
26	C_{24} 5β 3 = 0 7α OAc 12α OAc	5β-Cholan-24-oic acid	*c*
27	C_{24} 5β 3α OAc 7 = 0 12α OAc	5β-Cholan-24-oic acid	*c*
28	C_{24} 5β 3α OAc 7α OAc 12 = 0	5β-Cholan-24-oic acid	*c*
29	C_{24} 5β 3α OAc 12 = 0 Δ9(11)	5β-Cholan-24-oic acid	*b*
30	C_{24} 5β 3 = 0 6 = 0	5β-Cholan-24-oic acid	*b*
31	C_{24} 5β 3 = 0 7α OAc 12 = 0	5β-Cholan-24-oic acid	*b*
32	C_{24} 5β 3α OAc 7 = 0 12 = 0	5β-Cholan-24-oic acid	*b*
33	C_{24} 5α 3α OAc 12α OAc	5α-Cholan-24-oic acid	*c*
34	C_{24} 5α 3β OAc 12α OAc	5α-Cholan-24-oic acid	*c*
35	C_{24} 5α 3α OAc 7α OAc 12α OAc	5α-Cholan-24-oic acid	*c*
36	C_{24} 5α 3 = 0	5α-Cholan-24-oic acid	*c*
37	C_{24} 5α 3 = 0 12α OAc	5α-Cholan-24-oic acid	*c*
38	C_{24} 5α 3 = 0 12 = 0	5α-Cholan-24-oic acid	*c*
39	C_{22} 3β OAc Δ5	24,23-Dinorchol-5-en-22-oic acid	*b*
40	C_{22} 5β 3α OAc 12α OAc	24,23-Dinor-5β-cholan-22-oic acid	*f*

TABLE 1. (*Continued*)

No.	Structure	Parent Compound (IUPAC)	Source
41	C_{22} 5β 3α OAc 11α OAc	24,23-Dinor-5β-cholan-22-oic acid	*c*
42	C_{22} 5β 3α OAc 7α OAc 12α OAc	24,23-Dinor-5β-cholan-22-oic acid	*h*
43	C_{23} 5β 3α OAc 12α OAc	24-Nor-5β-cholan-23-oic acid	*f*
44	C_{23} 5β 3α OAc 11α OAc	24-Nor-5β-cholan-23-oic acid	*c*
45	C_{23} 5β 3α OAc 7α OAc	24-Nor-5β-chol-22-ene	*f*
46	C_{23} 5β 3α OAc 12α OAc	24-Nor-5β-chol-22-ene	*f*
47	C_{23} 5β 3α OAc 7α OAc 12α OAc	24-Nor-5β-chol-22-ene	*f*
48	C_{27} 5β 3α OAc	Cholest-5-ene	*f*
49	C_{27} 5β 3α OAc 7α OAc 12α OAc 25 OAc	5β-Cholestane	*i*
50	C_{27} 5β 3α OAc 7α OAc 12α OAc 24 OAc	5β-Cholestan-26-oic acid	*j*
51	C_{27} 5β 3α OAc 7α OAc	5β-Cholestan-26-oic acid	*j*
52	C_{27} 5β 3α OAc 7α OAc 12α OAc	5β-Cholestan-26-oic acid	*j*

[a] Nutritional Biochemicals Corp., Cleveland, Ohio.
[b] Steraloids, Inc., Pawling, N.Y.
[c] Dr. Alan Hofmann, Mayo Clinic, Rochester, Minn.
[d] Weddel Pharmaceutical Co., Ltd., London, England.
[e] Aldrich Chemical Company, Milwaukee, Wis.
[f] Dr. David Hachey, Argonne National Laboratory, Argonne, Ill.
[g] Dr. Robert Palmer, Rockefeller University, New York, N.Y.
[h] Mr. William Samuels, Argonne National Laboratory, Argonne, Ill.
[i] Dr. Gerald Salen, V.A. Hospital, Newark, N.J.
[j] Dr. Russell Hanson, University of Minnesota, Minneapolis, Minn.

(between 5 and 10 μg) in order to minimize background contributions.

GLC and GLC–MS

Bile acid retention times were determined on a Varian 1400 series gas chromatograph (Varian Associates, Palo Alto, Cal.) using a glass column (1 mm × 183 cm) packed with 0.5% SP-525 (Supelco Inc., Bellefonte, Pa.) on 100/200 mesh Gas-Chrom Q. Column temperature was 240°C; the injector and detector were maintained at 280°C. Nitrogen flow was 15 ml/min. Two to four analyses were made on each sample using a Varian Aerograph Model 475 digital integrator to establish retention times.

For electron impact mass spectrometry, samples were analyzed on a Perkin-Elmer 270 mass spectrometer (Perkin-Elmer Corp., Norwalk, Conn.), with sample introduction through the gas chromatographic inlet. A glass column (1 mm × 183 cm) packed with 0.25% SP-525 on 100/200 mesh Gas-Chrom Q was used. The column and inlet system were silanized to minimize decomposition. Column temperature was varied between 250°C and 285°C. The injector and manifold were maintained at 300°C; the ion source was 150°C. Electron energy was 70 eV. Spectral data were acquired on a PDP-12 computer (Digital Equipment Corporation, Maynard, Mass.) calibrated against a perfluorokerosene standard using the standard software provided by the manufacturer. Mass spectral analysis was performed on normalized spectra and mass ion tables generated by the computer system.

Chemical ionization mass spectra were obtained on a Biospect quadrupole mass spectrometer (Scientific Research Instruments, Baltimore, Maryland). The ion source temperature was between 160°C and 180°C, and the source pressure was maintained at 0.6 mm isobutane. Bile acid samples were introduced into the ion source in silanized quartz probe tubes using the direct insertion probe. The probe was heated to a constant temperature (typically between 150°C and 190°C) to produce a constant ion current of sufficient intensity to permit measurement of all mass peaks greater than 0.1% relative abundance. Mass spectra were recorded on light-sensitive paper; the data were then normalized and expressed as a percent of the total ionization. All ions greater than 0.1% relative abundance are reported.

Selective ion monitoring was accomplished using the stable isotope ratiometer–multiple ion detector (SIRMID) previously described (12). This instrument is used to carry out quantitation of isotope ratios or ion intensities in organic molecules as well as to monitor up to six ions in a cyclic fashion. The output from the ion monitoring process is a parallel series of analog voltages which are recorded on a multipen recorder.

RESULTS AND DISCUSSION

Gas–liquid chromatography

Our laboratory is currently involved in a number of collaborative research programs utilizing a gas chromatograph–mass spectrometer for the analysis

TABLE 2 Relative retention times of bile acids as methyl ester acetates on 0.5% SP525[a]

No.	Functional Groups		Relative Retention Time[b]
3	C_{24} 5β 12α OAc Δ3		0.31
2	C_{24} 5β 3α OAc Δ11		0.70
1	C_{24} 5β 3α OAc	(Lithocholate)	0.71
21	C_{24} 5β 3 = 0		0.75
11	C_{24} 5β 3α OAc 12α OAc Δ8(14)		0.86
20	C_{24} 5β 3α OEt 7α OAc 12α OAc		0.92
19	C_{24} 5β 3α OMe 7α OAc 12α OAc		0.92
9	C_{24} 5β 3α OAc 12α OAc		0.93
36	C_{24} 5α 3 = 0		0.93
8	C_{24} 5β 3α OAc 12α OAc	(Deoxycholate)	1.00
33	C_{24} 5α 3α OAc 12α OAc	(Allodeoxycholate)	1.14
23	C_{24} 5β 3 = 0 12α OAc		1.14
10	C_{24} 5β 3α OAc 7α OAc Δ11		1.26
6	C_{24} 5β 3α OAc 7α OAc	(Chenodeoxycholate)	1.30
34	C_{24} 5α 3β OAc 12α OAc		1.45
15	C_{24} 5β 3α OAc 7α OAc 12α OAc	(Cholate)	1.62
22	C_{24} 5β 3 = 0 7α OAc		1.62
37	C_{24} 5α 3 = 0 12α OAc		1.62
16	C_{24} 5β 3β OAc 7α OAc 12α OAc		1.65
5	C_{24} 5β 3α OAc 6β OAc		1.69
4	C_{24} 5β 3α OAc 6α OAc	(Hyodeoxycholate)	1.82
26	C_{24} 5β 3 = 0 7α OAc 12α OAc		1.82
35	C_{24} 5α 3α OAc 7α OAc 12α OAc	(Allocholate)	1.83
7	C_{24} 5β 3α OAc 7β OAc	(Ursodeoxycholate)	1.93
24	C_{24} 5β 3α OAc 7 = 0		1.95
25	C_{24} 5β 3α OAc 12 = 0		2.02
29	C_{24} 5β 3α OAc 12 = 0 Δ9(11)		2.11
13	C_{24} 5β 3α OAc 6β OAc 7α OAc	(α-Muricholate)	2.26
12	C_{24} 5β 3α OAc 6α OAc 7α OAc	(Hyocholate)	2.36
27	C_{24} 5β 3α OAc 7 = 0 12α OAc		2.40
38	C_{24} 5α 3 = 0 12 = 0		2.42
30	C_{24} 5β 3 = 0 6 = 0		2.80
14	C_{24} 5β 3α OAc 6β OAc 7β OAc	(β-Muricholate)	3.10
28	C_{24} 5β 3α OAc 7α OAc 12 = 0		3.12
18	C_{24} 5β 3α OAc 7α OAc 12α OH		(3.59)
31	C_{24} 5β 3 = 0 7α OAc 12 = 0		3.62
32	C_{24} 5β 3α OAc 7 = 0 12 = 0		4.20
39	C_{22} 3β OAc Δ5		0.38
40	C_{22} 5β 3α OAc 12α OAc	(Bisnordeoxycholate)	0.46
41	C_{22} 5β 3α OAc 11α OAc		0.64
42	C_{22} 5β 3α OAc 7α OAc 12α OAc	(Bisnorcholate)	0.75
43	C_{23} 5β 3α OAc 12α OAc	(Nordeoxycholate)	0.73
44	C_{23} 5β 3α OAc 11α OAc		0.98
48	C_{27} 3α OAc Δ5	(Cholesterol)	0.37
49	C_{27} 5β 3α OAc 7α OAc 12α OAc 25 OAc		0.76
50	C_{27} 5β 3α OAc 7α OAc 12α OAc 24 OAc	(Varanic acid)	0.98
51	C_{27} 5β 3α OAc 7α OAc		2.03
52	C_{27} 5β 3α OAc 7α OAc 12α OAc		2.50

[a] Run on a Varian 1400 gas chromatograph using a glass column (183 cm × 1 mm) packed with 0.5% SP-525 on 100/200 mesh Gas-Chrom Q; column at 240°C; injector and detector at 280°C; N_2 flow rate 15 ml/min.
[b] Retention time relative to deoxycholate at 665 seconds.

of bile acids (13–18). The measurement of stable isotope ratios requires that the samples analyzed either be pure or that they be separable into their individual components prior to analysis. Derivatization of the sample to enhance its volatility for gas chromatographic separation is always necessary. It is especially critical that the derivative chosen yield mass ions that have a low molecular weight and do not contain complex clusters of isotopic species which could interfere with the analysis. Therefore, the methyl ester acetate was the logical choice of derivative for stable isotope measurements. Additionally, the procedures used provide virtually complete derivatization of the samples with few, if any, side products (19) and are simple enough to be performed in clinical laboratories with good reproducibility. The high stability of the methyl ester acetate derivatives permits shipment and storage for long periods of time.

TABLE 3. Effect of epimer configuration on relative retention time (RRT) on 0.5% SP-525

Epimer Position	RRT	RRTα/RRTβ
3 C_{24} 5β 3α 7α 12α	1.62	1.02
C_{24} 5β 3β 7α 12α	1.65	
6 C_{24} 5β 3α 6α	1.82	0.93
C_{24} 5β 3α 6β	1.69	
C_{24} 5β 3α 6α 7α	2.36	0.96
C_{24} 5β 3α 6β 7α	2.26	
7 C_{24} 5β 3α 6β 7α	2.26	1.37
C_{24} 5β 3α 6β 7β	3.10	
C_{24} 5β 3α 7α	1.30	1.48
C_{24} 5β 3α 7β	1.93	
C_{24} 5β 3α 7α 12α[a]	1.76	1.46
C_{24} 5β 3α 7β 12α[a]	2.57	
12 C_{24} 5β 3α 12α	1.00	0.93
C_{24} 5β 3α 12β	0.93	

[a] Calculated from 0.25% SP-525 data.

It is equally important that the methods chosen offer good separation of similar bile acids, such as the dihydroxy compounds, in a reasonable amount of time. The aromatic hydrocarbon SP-525 is currently being used in our laboratory for the GLC separation of bile acid methyl ester acetates. Our preliminary work with this phase was done using a 0.25% coating. However, the sensitivity of this phase to oxidation makes the use of higher loadings more desirable; 0.5–1.0% coatings are currently being used. **Table 2** lists the retention times relative to deoxycholate of 48 bile acids and related compounds on a 0.5% coating of SP-525. This combination of derivative and column has proved useful for determining stable isotope ratios and, more recently, in the identification of samples containing mixtures of bile acids.

Values for the relative retention times on SP-525 reflect the structural differences—i.e., type, position, number, and configuration of the functional groups present and length of the side chain—in the bile acid molecule. By comparing the relative retention times for two compounds that differ by a single variable, it is possible to assign secondary separation factors for each of these changes in the molecule. A comparison of the relative retention times for the epimer pairs of importance in the bile acid structure and the separation factors (RRTα/RRTβ) resulting from inversion at the 3, 6, 7, and 12 positions are listed in **Table 3**. The separation factors for the allo-compounds can also be calculated if one bears in mind that the change from the 5β to the 5α position also results in a change in the orientation of the 3α OAc from the equatorial to the axial position, and thus necessitates a corresponding change in this epimeric separation factor. These separation factors can be combined in a variety of ways to predict the relative retention times and elution sequences of a series of epimeric compounds. The application of separation factors for the prediction of bile acid sequences and relative retention times has been described elsewhere in detail (20, 21). While such predictions are not always completely accurate (being influenced by the effect of vicinal groups and other group interactions (6)), their use will be more apparent in the discussion on the use of selective ion monitoring techniques in the identification of bile acid mixtures presented later.

Electron impact mass spectrometry

Table 4 (page 319) is a compilation of the mass spectral data obtained from the electron impact ionization of the bile acid methyl ester acetates and related compounds examined in this study. It contains the ions of significant diagnostic value which comprise these spectra, tabulated by increasing molecular weight, and includes representative ion structures for each mass ion listed. Some information about the stereochemistry of the compounds that form the mass ion—i.e., number and types of functional groups present and length of the side chain if it is not a C_{24}-bile acid—is given for most ion species.

It should be realized that the structures of the fragments given are tentative, as detailed mechanistic studies of these bile acid derivatives have not been made. However, the general fragmentation patterns of bile acids are well-known, and the suggested structures of the fragments are as proposed by references, which are also listed in the table. Those structures for which no references are given are generally derivative or side chain variants which have been observed in our laboratory, and whose steroid fragmentation patterns follow the fragmentation rules. Fragments reported for the 3,6,7-triacetoxy bile acids have not been reported elsewhere, and their structure is hypothetical.

It should be further emphasized that Table 4 contains only those mass ions that constitute the mass spectra we examined, that is, the ions from the 48 bile acid methyl ester acetates and related compounds obtained in a low resolution mass spectrometer under the conditions given earlier. Therefore, many of the high molecular weight ions reported in the mass spectra obtained in other labo-

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry

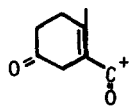
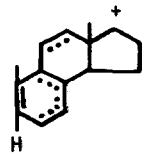
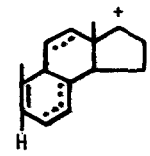
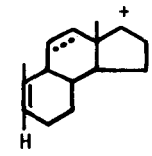
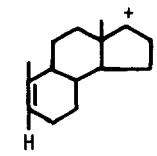
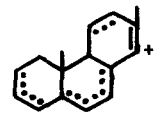
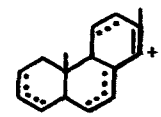
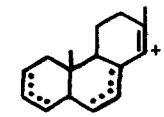
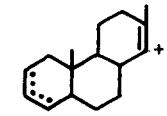
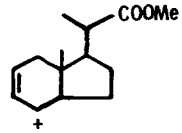
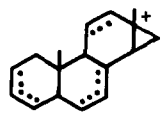
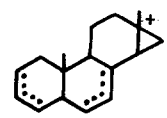
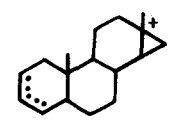
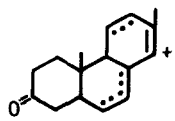
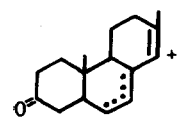
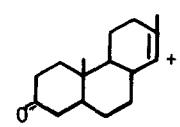
m/e	Representative ion structure	References
121	Structure unknown 12-O specific	k
137		
197 199 201 203	 1 C-O + 1 C-C  2 C-C  1 C-C 	c , d , e , f , i , k
209 211 213 215	 1 C-O + 2 C-C  1 C-O + 1 C-C or 3 C-C  1 C-O or 2 C-C  1 C-C	b , c , d , e , f , i , j , k , l
221	 Bisnor structure with either 5-ene, 6-ene, 6-OH or 7-OH	
226 228 230	 3 C-C  2 C-C  1 C-C	c , e , i , k , l
227 229 231	 1 C-O + 2 C-C  1 C-O + 1 C-C  1 C-O	f , h , i , k , l

TABLE 4. Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

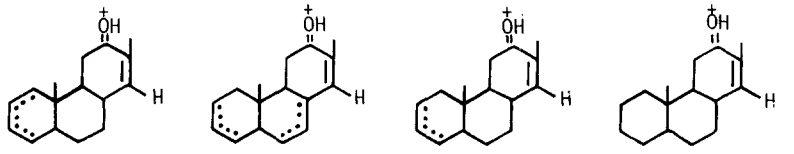
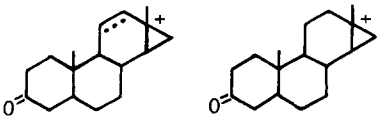
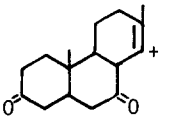
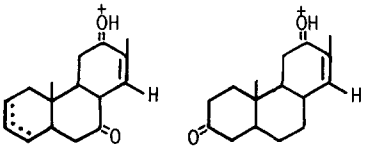
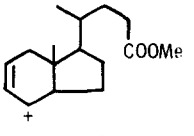
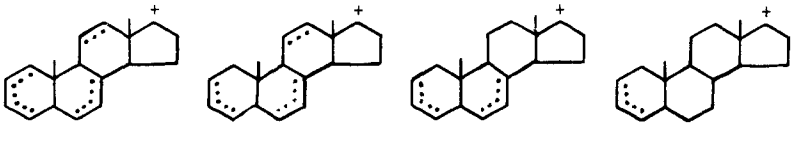
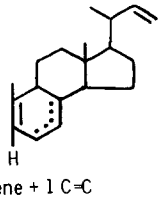
m/e	Representative ion structure	References
229 231 233	 12 =O + 1 C=O 12 =O specific 12 =O + 2 C=C 12 =O + 1 C=C 12 =O	h, i, j, k, l
244 246	 1 C=O + 1 C=C 1 C=O	f, k, l
245	 2 C=O	e, k, l
245 247	 12 =O + 1 C=O + 1 C=C 12 =O + 1 C=O	e, f, i, j, k, l
249	 C_{24} side chain with either 5-ene, 6-ene, 6-OH, or 7-OH	h, j, k, l
251 253 255 257	 1 C=O + 2 C=C 1 C=O + 1 C=C or 3 C=C 1 C=O or 2 C=C 1 C=C	a, b, c, d, e, f, h, i, j, k, l
256	 22-ene + 1 C=C	

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

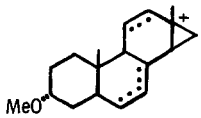
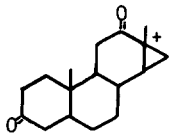
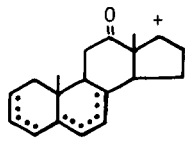
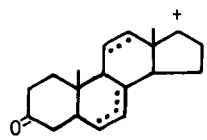
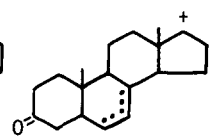
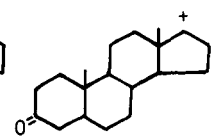
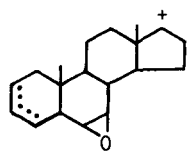
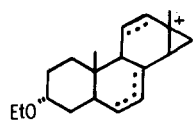
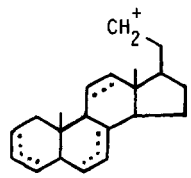
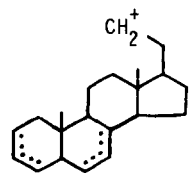
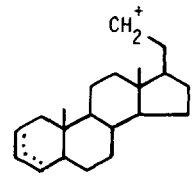
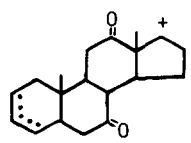
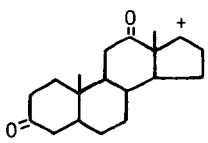
m/e	Representative ion structure	References
258	 Methoxy + 2 C=C	n
260	 2 C=O	e , k , l
267 269 271 273	 2 C=O + 1 C=C  1 C=O + 2 C=C or 2 C=O  1 C=O + 1 C=C  1 C=O	c , f , h , i , k , l
271	 Specific for 3, 6, 7 triacetate	
272	 Ethoxy + 2 C=C	n
281 283 285	 1 C=O + 1 C=C or 3 C=C  1 C=O or 2 C=C  1 C=C	c , k
285 287	 2 C=O + 1 C=C  2 C=O	e , f , i , k , l

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

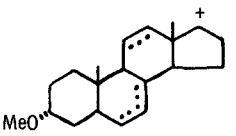
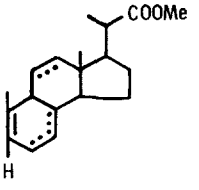
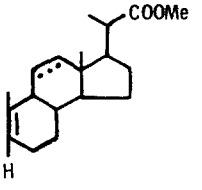
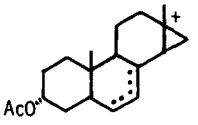
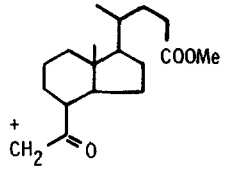
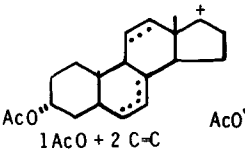
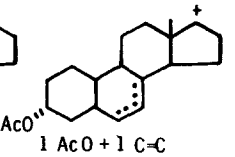
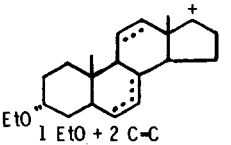
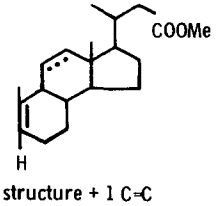
m/e	Representative ion structure	References
285	 Methoxy + 2 C=C Specific for methoxy group	n
286 288	 Bisnor structure + 2 C=C  Bisnor structure + 1 C=C	c
288	 1 AcO + 1 C=C	l
292	 Specific for 7 =O	e , h , k , l
293 295	 1 AcO + 2 C=C Loss of $-CH_3$  1 AcO + 1 C=C	
299	 1 EtO + 2 C=C Specific for ethoxy compound	g (noted but not identified) n
302	 Nor structure + 1 C=C	

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

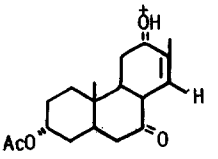
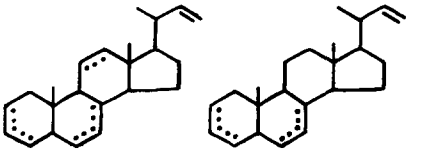
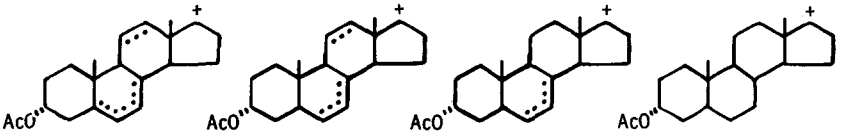
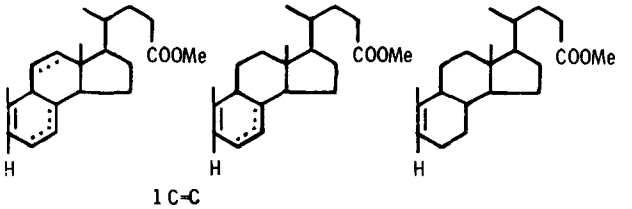
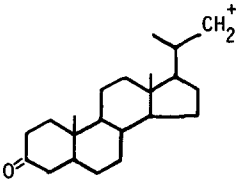
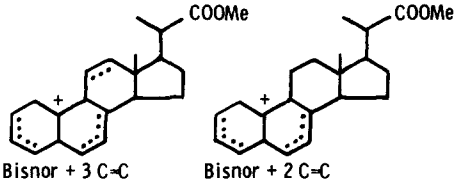
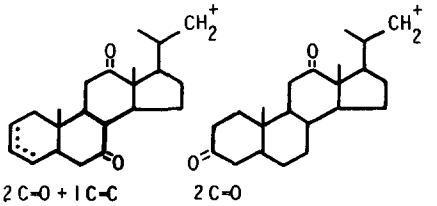
m/e	Representative ion structure	References
305	 1 AcO + 12 -O + 1 C=O	i , k ,
308 310	 22-ene + 3 C=C 22-ene + 2 C=C	
311 313 315 317	 1 AcO + 1 C=O + 1 C=C 1 AcO + 2 C=C 1 AcO + 1 C=C 1 AcO	c , d , i , j , l , m
314 316 318	 2 C=C 1 C=C	c , d , e , f , h , i , k , l ,
315		f , k , l ,
325 327	 Bisnor + 3 C=C Loss of $-CH_3$ Bisnor + 2 C=C	c
327 329	 2 C=O + 1 C=C 2 C=O	i , k

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

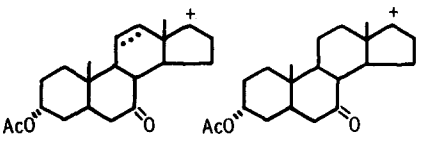
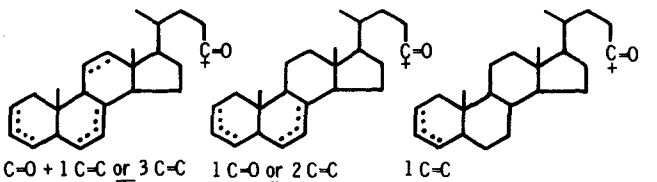
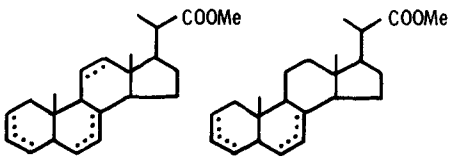
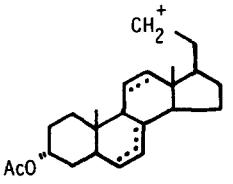
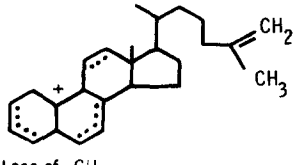
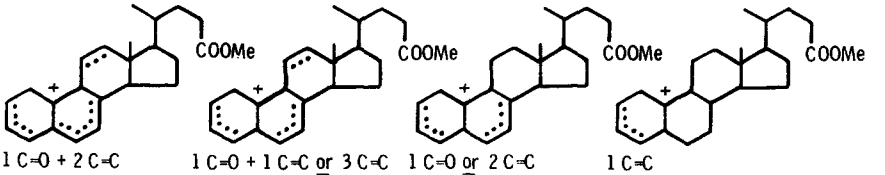
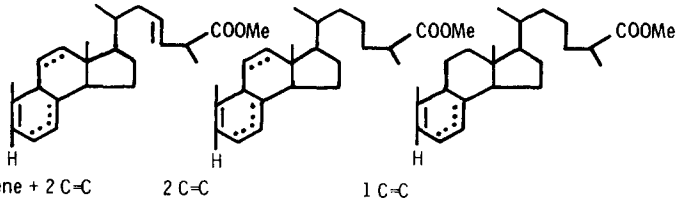
m/e	Representative ion structure	References
329 331	 1 AcO + 1 C=O + 1 C=C 1 AcO + 1 C=O	c , i
337 339 341	 1 C=O + 1 C=C or 3 C=C 1 C=O or 2 C=C 1 C=C	c , h , k
340 342	 Bisnor + 3 C=C Bisnor + 2 C=C	c
341	 1 AcO + 2 C=C	
349	 Loss of $-CH_3$	
351 353 355 357	 1 C=O + 2 C=C Loss of $-CH_3$ 1 C=O + 1 C=C or 3 C=C 1 C=O or 2 C=C 1 C=C	c , d , e , i , k , l
354 356 358	 23-ene + 2 C=C 2 C=C 1 C=C	c

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

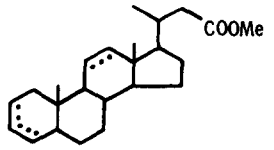
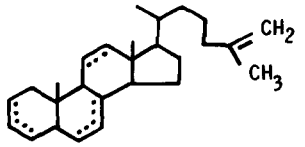
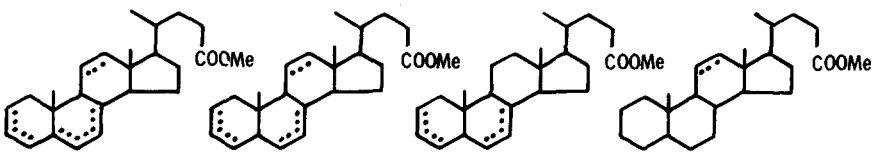
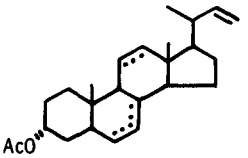
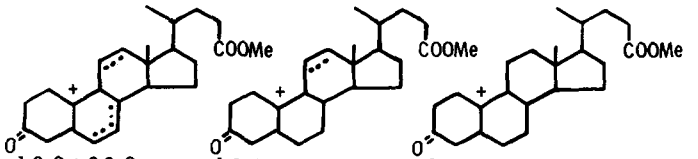
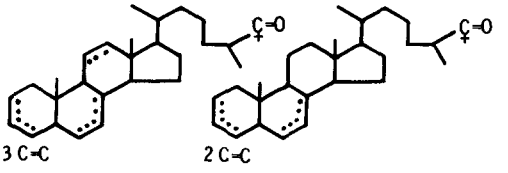
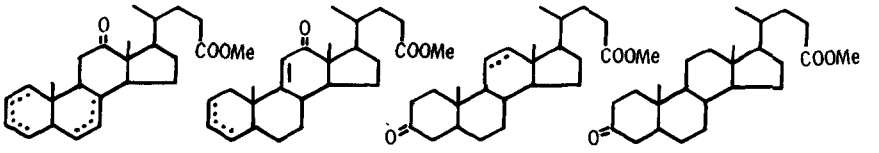
m/e	Representative ion structure	References
356	 Nor + 2 C=C	
364		
366 368 370 372	 1 C=O + 2 C=C 1 C=O + 1 C=C or 3 C=C 1 C=O or 2 C=C 1 C=C	b , c , d , e , f , h , i , j , k, l
368	 22-ene + 1 AcO + 2 C=C	
369 371 373	 1 C=O + 2 C=C Loss of $-CH_3$ 1 C=O + 1 C=C 1 C=O	f , k , l
379 381	 3 C=C 2 C=C	c
382 384 386 388	 2 C=O + 1 C=C 1 C=O + 2 C=C 1 C=O + 1 C=C 1 C=O M^+	c , i , k , l

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

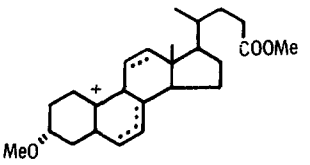
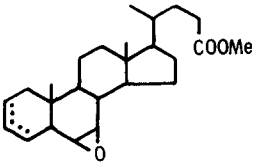
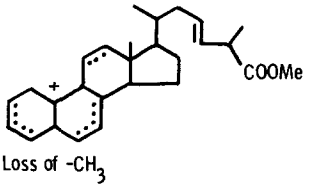
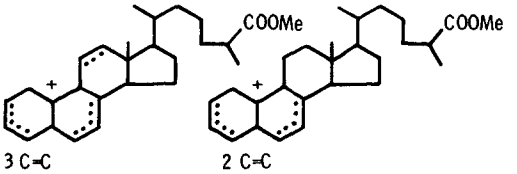
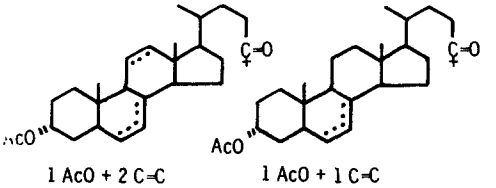
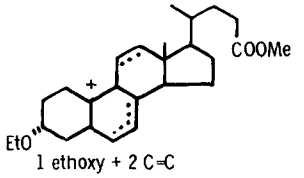
m/e	Representative ion structure	References
385	 1 methoxy + 2 C=C Loss of methyl group Specific for methoxy group	n
386	 Specific for 3, 6, 7, -triacetate	
393	 Loss of $-CH_3$	
395 397	 3 C=C Loss of $-CH_3$ 2 C=C	c , e
397 399	 1 AcO + 2 C=C 1 AcO + 1 C=C	l
399	 1 ethoxy + 2 C=C Loss of $-CH_3$	n

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

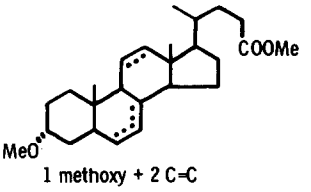
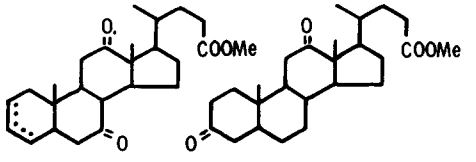
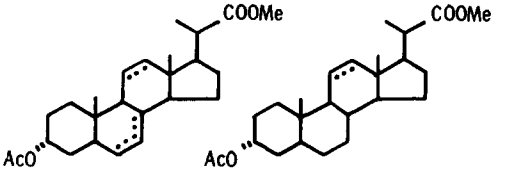
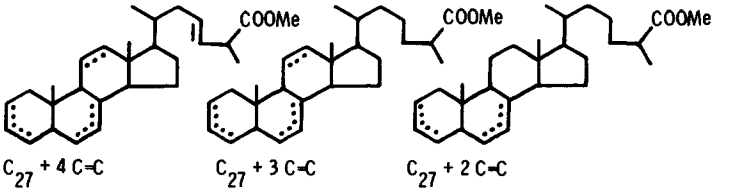
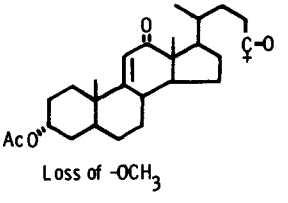
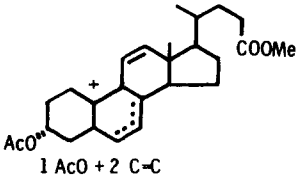
m/e	Representative ion structure	References
400	 1 methoxy + 2 C=C	n
400 402	 2 C=O + 1 C=C 2 C=O M^+	i , k , l
400 402	 Bisnor + 1 AcO + 2 C=C Bisnor + 1 AcO + 1 C=C	c
408 410 412	 C_{27} + 4 C=C C_{27} + 3 C=C C_{27} + 2 C=C	c , e , m
413	 Loss of $-OCH_3$	
413	 1 AcO + 2 C=C Loss of $-CH_3$	d

TABLE 4 . Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

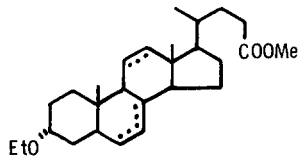
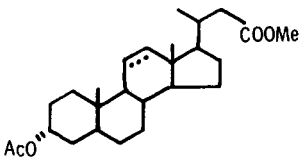
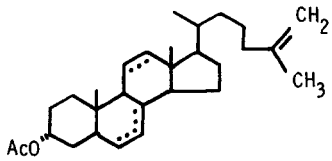
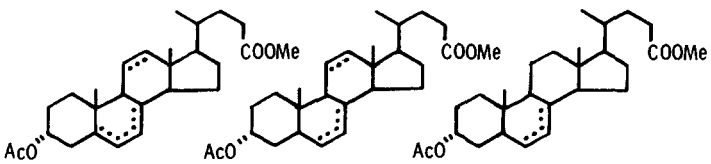
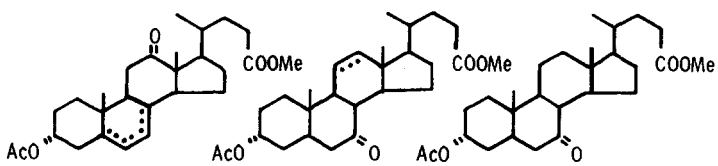
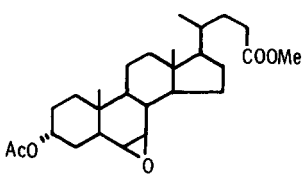
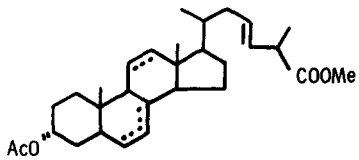
m/e	Representative ion structure	References
414	 1 EtO + 2 C=C Specific for ethoxy compound	n
416	 Nor structures with 1 AcO + 1 C=C	
424	 1 AcO + 1 side chain C=C + 2 C=C Found in tetraacetates	
426 428 430	 1 AcO + 1 C=O + 1 C=C 1 AcO + 2 C=C 1 AcO + 1 C=C	a , b , c , d , i , k , l
442 444 446	 1 AcO + 2 C=O 1 AcO + 1 C=O + 1 C=C 1 AcO + 1 C=O Loss of H_2O from diketone M^+	c , i
446	 Specific for 3, 6, 7 triacetate	

TABLE 4. Ion table of bile acid methyl ester acetates by electron-impact mass spectrometry (con't)

m/e	Representative ion structure	References
468	 1 AcO + 1 side chain C=C + 2 C=C Found in tetraacetates	c

a Bergström, S. 1958. Mass spectrometric studies on bile acids and other steroid derivatives. Acta Chem. Scand. 12: 1349-1358.

b Ryhage, R. and E. J. Stenhagen. 1960. Mass spectrometry in lipid research. J. Lipid Res. 1: 361-390.

c Bergström, S. R. Ryhage, and E. Stenhagen. 1961. Mass spectrometric studies on sterols and bile acids. Sven. Kem. Tids. 73: 566-581

d Budzikiewicz, H., C. Djerassi, and D.H. Williams. 1964. in Structure Elucidation of Natural Products By Mass Spectrometry. Vol.II Holden-Day, Inc. San Francisco, Calif. Chapters 18-21.

e Dean, P.D.G., and R.T. Aplin. 1966. Mass spectrometric studies on bile acids: the differentiation between chenodeoxycholic acid and deoxycholic acid and the identification of 3α, 7α-dihydroxy-5β-cholestanoic acid in alligator bile. Steroids 8: 565-579.

f Eneroth, P., B. Gordon, R. Ryhage, and J. Sjövall. 1966. Identification of mono- and dihydroxy bile acids in human feces by gas-liquid chromatography and mass spectrometry. J. Lipid Res. 7: 511-523

g Eneroth, P., B. Gordon, and J. Sjövall. 1966. Characterization of trisubstituted cholanoic acids in human feces. J. Lipid Res. 7: 524-530.

h Sjövall, J. 1967. Applications of gas chromatography-mass spectrometry to the analysis of bile acids in biological materials. in The Gas Liquid Chromatography of Steroids. Mem. Soc. Endocrinol. No. 16. J.K. Grant, Editor. Cambridge University Press, London. pp 243-257.

i Egger, H. 1968. Zur massenspektrometrie von Gallensaurenderivativen. Monatsch. Chem. 99: 1163-1174.

j Sjövall, J. 1969. Use of gas chromatography-mass spectrometry in the study of bile acid metabolism. in Bile Salt Metabolism. L. Schiff, J. Carey Jr. and J. Dietschy, Eds. Charles C. Thomas, Springfield, Ill. pp 205-222.

k Sjövall, J., P. Eneroth, and R. Ryhage. 1971. Mass spectra of bile acids. in The Bile Acids, Vol.I: Chemistry. P.P. Nair and D. Kritschevsky, Eds. Plenum Press, New York, N.Y. pp 209-248.

l Elliott, W.H. 1972. Bile Acids. in Biochemical Application of Mass Spectrometry. G. R. Waller, Editor. Wiley-Interscience, New York, N.Y. pp 291-312.

m Eyssen, H., G. Parmentier, F. Compernolle, J. Boon, and E. Eggermont. 1972. Trihydroxycoprostanic acid in the duodenal fluid of two children with intrahepatic bile duct anomalies. Biochim. Biophys. Acta 273: 212-221.

n Hachey, D., P. Szczepanik, J. Watkins, A. Tercyak and P. Klein. 1974. Bile salt alkyl ethers: evidence for a new class of biliary lipids in human bile. Gastroenterology 67: 795

ratories, especially those employing other instruments using lower ionization potentials (4–8), will not be found in this table. Moreover, monoacetoxy- and monoketo-bile acids that were not in our collection are not represented.

The identification of bile acid methyl ester acetates without using library search techniques or computer-matching routines can be simplified by the use of Table 4 and is perhaps quicker than the use of the above methods alone. One need only look up the mass ions in the unknown spectrum and compile a set of ion structures that are con-

TABLE 5. Chemical ionization characteristics of bile acid methyl ester acetates using isobutane reagent gas

No.	Functional Groups	Mol. Wt.	MH^+	$MH^+ - 60$	$MH^+ - (2 \times 60)$	$MH^+ - (3 \times 60)$
				Percent of total ionization		
1	C_{24} 5β 3α OAc	432	0.0	71.8		
2	C_{24} 5β 3α OAc Δ11	430	11.8	63.4		
3	C_{24} 5β 12α OAc Δ3	430	0.7	72.0		
4	C_{24} 5β 3α OAc 6α OAc	490	0.0	11.9	63.0	
5	C_{24} 5β 3α OAc 6β OAc	490	0.0	10.0	62.8	
6	C_{24} 5β 3α OAc 7α OAc	490	0.0	6.5	66.8	
7	C_{24} 5β 3α OAc 7β OAc	490	0.0	10.4	65.4	
8	C_{24} 5β 3α OAc 12α OAc	490	0.0	25.7	43.8	
9	C_{24} 5β 3α OAc 12β OAc	490	0.0	1.6	61.5	
10	C_{24} 5β 3α OAc 7α OAc Δ11	488	1.2	21.5	45.4	
11	C_{24} 5β 3α OAc 12α OAc Δ8(14)	488	0.5	18.5	5.0	
12	C_{24} 5β 3α OAc 6α OAc 7α OAc	548	0.8	7.8	25.4	24.0
13	C_{24} 5β 3α OAc 6β OAc 7α OAc	548	7.0	7.9	24.1	22.3
14	C_{24} 5β 3α OAc 6β OAc 7β OAc	548	0.5	9.0	15.9	16.8
15	C_{24} 5β 3α OAc 7α OAc 12α OAc	548	0.0	0.0	58.5	12.3
16	C_{24} 5β 3β OAc 7α OAc 12α OAc	548	0.0	1.4	45.1	20.2
17	C_{24} 5β 3α OAc 7β OAc 12α OAc	548			Sample unavailable	
18	C_{24} 5β 3α OAc 7α OAc 12α OH	507	0.0	52.8	21.7	
19[a]	C_{24} 5β 3α OMe 7α OAc 12α OAc	520	0.0	0.7	68.0	
20[b]	C_{24} 5β 3α OEt 7α OAc 12α OAc	534	0.0	0.1	54.4	
21	C_{24} 5β 3 = 0	388	72.8			
22	C_{24} 5β 3 = 0 7α OAc	446	0.0	66.2		
23	C_{24} 5β 3 = 0 12α OAc	446	0.0	66.2		
24	C_{24} 5β 3α OAc 7 = 0	446	0.0	69.1		
25	C_{24} 5β 3α OAc 12 = 0	446	57.3	10.3		
26	C_{24} 5β 3 = 0 7α OAc 12α OAc	504	0.0	0.0	70.8	
27	C_{24} 5β 3α OAc 7 = 0 12α OAc	504	0.0	52.6	13.8	
28	C_{24} 5β 3α OAc 7α OAc 12 = 0	504	40.9	26.0		
29	C_{24} 5β 3α OAc 12 = 0 Δ9(11)	444	53.5	17.1		
30	C_{24} 5β 3 = 0 6 = 0	402	68.2			
31	C_{24} 5β 3 = 0 7α OAc 12 = 0	460	50.3	22.4		
32	C_{24} 5β 3α OAc 7 = 0 12 = 0	460	55.4	9.3		
33	C_{24} 5α 3α OAc 12α OAc	490	0.0	13.7	59.0	
34	C_{24} 5α 3β OAc 12α OAc	490	0.0	31.6	38.9	
35	C_{24} 5α 3α OAc 7α OAc 12α OAc	548	0.0	1.6	61.9	9.5
36	C_{24} 5α 3 = 0	388	72.5			
37	C_{24} 5α 3 = 0 12α OAc	446	0.5	73.1		
38	C_{24} 5α 3 = 0 12 = 0	402	74.3			
39	C_{22} 5β 3β OAc Δ5	402	10.6	65.5		
40	C_{22} 5β 3α OAc 12α OAc	462	0.7	54.8	13.1	
41	C_{22} 5β 3α OAc 11α OAc	462	0.8	28.7	43.9	
42	C_{22} 5β 3α OAc 7α OAc 12α OAc	520	0.0	6.5	56.1	12.2
43	C_{23} 5β 3α OAc 12α OAc	476	0.0	33.1	39.2	
44	C_{23} 5β 3α OAc 11α OAc	476	1.3	20.5	50.0	
45	C_{23} 5β 3α OAc 7α OAc Δ22	430	0.0	1.7	66.7	
46	C_{23} 5β 3α OAc 12α OAc Δ22	430	0.0	2.3	70.2	
47[c]	C_{23} 5β 3α OH 7α OH 12α OH Δ22	362	1.0[c]	11.7[c]	20.8[c]	
48	C_{27} 5β 3β OAc Δ5 (cholesterol)				Not applicable	
49[d]	C_{27} 5β 3α OAc 7α OAc 12α OAc 25 OAc	604	0.0	0.0	1.0	50.0
50[e]	C_{27} 5β 3α OAc 7α OAc 12α OAc 24 OAc	648	0.0	0.2	0.2	31.4
51	C_{27} 5β 3α OAc 7α OAc	532	0.0	4.2	65.3	
52	C_{27} 5β 3α OAc 7α OAc 12α OAc	590	0.0	0.7	46.3	24.2

[a] Additional ions at $MH^+ - 32 - 60$, 1.5%; $MH^+ - 32 - (2 \times 60)$, 4.0%.
[b] Additional ions at $MH^+ - 46 - 60$, 3.6%; $MH^+ - 46 - (2 \times 60)$, 12.8%.
[c] Run as free alcohol only; ions represent MH^+, $MH^+ - 18$, $MH^+ - (2 \times 18)$.
[d] $MH^+ - (4 \times 60)$, 10.5%.
[e] Run with methane reagent gas.

sistent in their stereochemistry to reconstruct a probable structure for the unknown compound. The epimeric configuration or position of the functional groups may not always be apparent from the intensity of the mass ions and their representative structures. However, at this point, the identification of the unknown compound can usually be narrowed to two or three compounds whose mass spectra and rela-

TABLE 6. Comparison of ion yield for selected bile acid methyl ester acetates by chemical ionization (CI) vs electron impact (EI) modes

Bile Acid	*m/e*	CI % Σ^a	EI % Σ^b	CI/EI
Lithocholate	373[c]	71.8	2.21	32.5
Deoxycholate	371[c]	43.8	1.09	40.2
Chenodeoxycholate	371[c]	65.4	5.35	12.2
Cholate	369[c]	12.3	1.52	8.1

[a] Percent of total ionization for ions above *m/e* 100; values obtained on Biospect chemical ionization quadrupole mass spectrometer.

[b] Percent of total ionization for ions above *m/e* 100; values obtained on Perkin-Elmer DF-MS 270 by electron impact magnetic sector mass spectrometry. Total ionization yields on this instrument cannot be compared directly to those on the Biospect. See text for estimations made in this comparison.

[c] EI ion is not protonated; this mass is 1 unit lower.

tive retention times can easily be compared with that of the unknown compound to establish the identification.

Chemical ionization mass spectrometry

Chemical ionization mass spectrometry provides a unique analytical tool which may be used to complement and enhance the structural information provided by electron impact mass spectrometry. Chemical ionization (CI) is a very mild ionization process in which a proton is transferred from an ionized reagent gas to the compound of interest. This gentle ionization process results in a simple fragmentation pattern with only two or three ions predominating.[1] For steroid molecules, the ions obtained by CI generally result from elimination of oxygen-containing functional groups, e.g., trimethylsilanol, water, or acetic acid are eliminated from the corresponding TMS, alcohol, or acetate derivatives (22–25). Fragmentation of the carbon skeleton seldom occurs with methane or isobutane CI. When carbon–carbon fragmentation does occur in CI, it is rarely more than a few percent of the total ionization, and the fragments produced can be identified as resulting from fission of the side chain. The fragmentation pattern observed for bile acid derivatives under CI conditions is temperature dependent. With increasing temperature, more extensive loss of hetero atom functional groups is observed. At very high source temperatures (>225°C), fragments resulting from side chain fission become apparent in compounds possessing a functional group in the C ring. Occasionally, a small amount of an ion resulting from hydride abstraction (as opposed to proton transfer) is present preceding a major ion by two amu (23). The ions resulting from carbon–carbon fission and hydride abstraction generally occur in trace amounts; for this reason they are not included in **Table 5**.

For simple acetate derivatives, elimination proceeds in a sequential manner to produce ions at MH^+, $MH^+ - 60$, . . . , $MH^+ - (n \times 60)$, where n = 1–4. The molecular ion is usually too weak to record, but may be apparent in compounds that have a functional group in the C ring, or that possess a double bond in the steroid nucleus (compounds 2, 3, 10, 11, 39, 40, 41, 44); it is also present in compounds that possess a 6,7-glycol structure (compounds 12, 13, 14). These compounds cannot readily eliminate all their acetate groups, which appear to be stabilized by intramolecular bonding of the 6,7-glycol structure.

Keto bile acids are very stable under CI conditions and exhibit an intense molecular ion (compounds 21, 30, 36, 38). When acetate groups are also present, elimination proceeds in the manner described above. Bile acids that possess both a 12-keto group and acetate groups usually do not undergo elimination. In all such cases that we examined (compounds 25, 28, 29, 31, 32), the base peak in the spectrum was the molecular ion (MH^+).

The highly simplified spectra resulting from chemical ionization processes as shown in Table 5 have two further advantages in the analysis of bile acid mixtures. The first advantage is that the yield of ions at a mass characteristic of that bile acid is significantly increased over that obtained with electron impact ionization. We have compared the yield of ions at masses representative of the intact carbon skeleton for four bile acids (lithocholic, deoxycholic, chenodeoxycholic, and cholic acid) by chemical ionization with the yield by electron impact ionization as shown in **Table 6**. All of the chemical ionization spectra display single characteristic ions which comprise 12–70% of the total ions. (In the case of cholic, an ion at *m/e* 429, reflecting retention of one acetate group, is present at 58.5% of the total ionization.) By contrast, the extensive fragmentation of the molecules by electron impact ionization diminishes the yield to 1–5% for the same ions. Chemical ionization thus results in an ion yield for a given mass fragment that is between one and two orders of magnitude larger, solely because of the uniformity and low energy with which ion formation takes place. There is an additional factor of one to two orders of magnitude in sensitivity, which results from the overall rates at which ions are formed and

[1] In a C_{24} bile acid methyl ester acetate, the ^{13}C satellite peak is usually 27–28% of the normal ion. This means that in any spectrum in which the normal ion is 70% of the total ionization or greater, there are no ions other than isotopic satellites present.

TABLE 7. Selective ion monitoring–relative retention time coordinates for bile acid methyl ester acetates by gas–liquid chromatography–chemical ionization mass spectrometry

m/e	Relative Retention Time on 0.5% SP-525									
369[a]	0.86 (11)	1.26 (10)	1.62 (15)	1.65 (16)	1.83 (35)	2.26 (13)	2.36 (12)	3.10 (14)		
371	0.31 (3)	0.70 (2)	0.93 (9)	1.00 (8)	1.14 (33)	1.30 (6)	1.45 (34)	1.69 (5)	1.82 (4)	1.93 (7)
373		0.71 (1)								
385	1.82 (26)	2.11 (29)	2.40 (27)							
387	1.14 (23)	1.62 (22)	1.62 (37)	1.95 (24)						
389	0.75 (21)	0.93 (36)								
401	0.925 (19)	3.62 (31)	4.20 (32)							
403	2.42 (38)	2.80 (30)								
415	0.915 (20)									
429[a]	0.86 (11)	1.26 (10)	1.62 (15)	1.65 (16)	1.83 (35)	2.26 (13)	2.36 (12)	3.10 (14)		
431	1.00 (8)	1.14 (33)	1.45 (34)	1.69 (5)	1.82 (4)	1.93 (7)				
445	2.11 (29)	2.40 (27)	3.12 (28)							
447	2.01 (25)									
461	3.62 (31)	4.20 (32)								
489[a]	2.26 (13)	2.36 (12)	3.10 (14)							
505	3.12 (28)									
549	2.26 (13)									

[a] In addition to the compounds listed, cholate epimers may occur at the relative retention times predicted in Table 3.

extracted in the chemical ionization source as opposed to the electron impact source, but unless these measurements are made in the same instrument, it is difficult to assign exact values to this enhancement. Miyazaki and Hashimoto (26) have shown evidence that this value may be as large as 10 in the same instrument.

The second advantage of the simplified chemical ionization spectra is that there are virtually no ions at any masses other than those listed in Table 5 and their isotopic satellites. This is particularly important in using the mass spectrometer as a selective ion monitoring device to detect the presence of a particular bile acid in a mixture where a major constituent may have a similar retention time. In electron impact mass spectrometry, the generation of ions at each and every mass number increases with the concentration of bile acid molecules entering the ion source. Hence the background against which the minor constituent must be detected is increased by the presence of high concentrations of a neighboring bile acid and sensitivity is diminished. The result is that the dynamic range of detection in electron impact ionization is a function of, and

is limited by the composition of, the sample being analysed. No such limitation is present when chemical ionization is used for the analysis of bile acids.

Selective ion monitoring by chemical ionization and mass spectrometry

The GLC data and chemical ionization spectra presented above can be applied to the analysis of complex mixtures of bile acids. In this instance, it is necessary to enumerate the components as well as to estimate their quantity when they are present in concentrations from 0.9 down to 5×10^{-4} mole fraction. Fragmentation of the bile acid molecule in the chemical ionization process is generally limited to the removal of acetate groups. Under these circumstances, all bile acids of the same category—i.e., with the same number of hydroxyl groups and the same carbon skeleton—will give rise to an ion at the same mass, regardless of hydroxyl position or epimer configuration. Usually formation of this ion will be limited to these compounds and the corresponding unsaturated bile acids. Accordingly, if one uses the gas chromatograph–mass spectrometer in a selective ion monitoring mode, all members of the same class can be monitored at one mass ion but, unlike selected ion monitoring with electron impact ionization, no other classes will be detected (27). The specificity of the intersection between ion mass and retention time offers a great enhancement over conventional GC or GC–EI–MS identification, since the CI mass ion and the relative retention time combine to form a unique index for each bile acid. **Table 7** lists the selective mass ion and relative retention time coordinates for the bile acids and related compounds in our collection. The numerical reference for each compound is given in parentheses below its relative retention time coordinate.

The SIRMID system, described earlier (12), is used to drive a four pen recorder. On the basis of the information given in Table 7, one can select four mass ions to be monitored that will detect up to 25 bile acids plus all their epimers and any unsaturated counterparts in a single GLC run. Therefore, in two or three replicate injections it is possible to screen any bile acid mixture to determine the presence or absence of any of the bile acids listed in Table 1, using the mass ion–relative retention time coordinate system. At the same time, an approximation of the quantity present can be made on the basis of peak height. Since each mass ion has a separate gain control, minor constituents can be amplified to make them more visible, and by this means we have detected constituents that comprise exceedingly small mole fractions of the total mixture. Although selective ion monitoring requires the establishment of response curves for each constituent to be truly quantitative, approximations to ±10% of the individual values are feasible by internal comparison to a major constituent whose concentration has been determined by gas chromatography. A detailed report on the use of this technique in the analysis of bile samples from patients treated with chenodeoxycholic acid for gallstone therapy will be presented at a later date.

We believe that the combination of GLC retention data on SP-525, the electron impact fragmentation characteristics, and the chemical ionization spectra of the bile acids listed in this catalogue form a balanced and integrated system for the identification of individual bile acid methyl ester acetates. The specific combination of gas–liquid chromatography–chemical ionization mass spectrometry with selective ion monitoring offers a unique and powerful tool in the characterization of bile acid mixtures.

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