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ANALYSIS OF 1,2-DIOLS OF LINOLEIC, α-LINOLENIC AND ARACHIDONIC ACID BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY USING CYCLIC ALKYL BORONIC ESTERS

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SUMMARY

Arachidonic acid is metabolized by hepatic and renal cortical microsomes in the presence of NADPH to vicinal dihydroxyeicosatrienoic acids as some of the major metabolites. Other polyunsaturated, long-chain fatty acids might be metabolized to vicinal dihydroxy acids (1,2-diols) in the same way. To facilitate identification of 1,2-diols in biological samples, a series of unsaturated 1,2-diols were synthesized from linoleic, α-linolenic and arachidonic acid and the electron-ionization mass spectra of cyclic methane- and n-butaneboronic ester derivatives and of trimethylsilyl (TMS) ether derivatives were compared. The TMS ether derivatives gave rise to weak molecular ions but prominent informative fragmentation ions were formed by α-cleavage as well as cleavage between the carbons with the TMS ethers. The TMS ether derivative of methyl 15,16-dihydroxy-9,12-octadecadienoate had a considerably larger carbon value than the other C_{18} diols, while the cyclic boronates were poorly separated on gas chromatography. The methane- and n-butaneboronic acid derivatives showed strong molecular ions and a characteristic but not very informative fragmentation, although the position of the hydroxyls could be deduced from one or two fragments formed by α-cleavage. Linoleic and α-linolenic acid are metabolized in the rabbit to many polar products by hepatic and renal cortical microsomes and NADPH. 12,13-Dihydroxy-9-octadecenoic acid and other metabolites of linoleic acid were identified by gas chromatography—mass spectrometry.

INTRODUCTION

Cyclic boronic ester derivatives were introduced by Brooks and Watson [1] for the analysis of bifunctional compounds by gas chromatography—mass spectrometry (GC—MS). Cyclic alkyl boronic esters are formed under mild conditions and have suitable GC—MS properties [2]. They have been used successfully for analysis of various 1,2- and 1,3-diols, steroids, prostaglandins, catecholamines and cannabinoids (see ref. 3 for review). Stable GC—MS deriva-

tives are only formed with bifunctional compounds, which allow formation of the cyclic boronic esters [3]. This selectivity is advantageous and may reduce the need for extensive purification of biological samples.

Arachidonic acid is metabolized by hepatic and renal microsomes to vicinal diols as major metabolites [4—6]. These diols are formed by epoxidation catalyzed by cytochrome P-450 (LM$_2$ or PB-B$_2$) and the epoxides are converted to 1,2-diols by epoxide hydrolase [7]. As shown in the present report, linoleic and α-linolenic acid are also metabolized to polar metabolites by hepatic and renal cortical microsomes and NADPH. It seemed likely that cyclic boronic esters might be a useful complement to TMS ether derivatives for identification of some of these unknown metabolites and also for studying the metabolism of arachidonic acid to 1,2-diols. This prompted a systematic study of the mass spectra of cyclic boronic esters of unsaturated 1,2-diols, chemically prepared from linoleic, α-linolenic and arachidonic acid. The results show that these cyclic boronic esters have a rather non-specific fragmentation pattern. They can nevertheless be used for identification of metabolites in biological samples by comparison with authentic material as demonstrated for a 1,2-diol of linoleic acid, which is formed by renal cortical microsomes and NADPH.

MATERIALS AND METHODS

[1-^{14}C] Arachidonic, [1-^{14}C] linolenic and [1-^{14}C] linoleic acids (all 56—58 mCi/mmol), were from the Radiochemical Centre, Amersham, Great Britain. Arachidonic acid and NADPH were from Sigma, St. Louis, MO, U.S.A. Linoleic acid, α-linolenic acid and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were from Supelco, Bellefonte, PA, U.S.A. m-Chloroperoxybenzoic acid (85%, technical) was from Aldrich, Beerse, Belgium. Methane- and n-butaneboronic acid were from Applied Science Labs., State College, PA, U.S.A. Thin-layer chromatography (TLC) was performed with glass plates, precoated with a 0.25-mm thick layer of silica gel 60 (10 × 20 cm plates), which, together with most other chemicals, were from Merck, Darmstadt, G.F.R. Equipment for high-performance liquid chromatography (HPLC) was from Laboratory Data Control, Riviera Beach, FL, U.S.A. (cf. ref. 9), and the following columns were used (Waters Assoc., Milford, MA, U.S.A.): octadecasilane bonded to 10-μm silica gel (μBondapak C$_{18}$, 300 × 7.8 mm) and 10-μm silica gel (μPorasil, 300 × 3.9 mm). Radioactivity was measured by liquid scintillation counting (PLD PRIAS, Packard, Downers Grove, IL, U.S.A.) using Ready Solv HP (Beckman, Fife, Scotland) as scintillator.

Synthesis of 1,2-diols

14,15-Dihydroxy-, 11,12-dihydroxy-, 8,9-dihydroxy- and 5,6-dihydroxy-C$_{20:3}$ were synthesized as described previously [7—9]. The other diols were obtained as follows: 30—70 mg of α-linolenic and linolenic acid were methylated with diazomethane. After evaporation, the fatty acid methyl esters were reacted with 1.1 equiv. of m-chloroperoxybenzoic acid in 1—2 ml of dichloromethane at room temperature overnight. The reaction was ended by washing with 10% Na$_2$SO$_3$ and with 5% NaHCO$_3$ in water (cf. ref. 10). After evaporation, the residue was purified by preparative TLC (silica gel 60,

15% ethyl acetate in hexane). The epoxides were converted to 1,2-diols as described [7, 9, 11] and purified by TLC (50% ethyl acetate in hexane; R_F values are given in Table I). [5,6,8,9,11,12,14,15-$^2H_8$]-14,15-Dihydroxy-5,8,11-eicosatrienoic acid was synthesized as described [9].

Experimental

Male white New Zealand rabbits (2—3 kg) were killed by a blow to the head. Microsomes were prepared from a 20% tissue homogenate as described [9] and resuspended so that 1 ml of microsomal suspension corresponded to microsomes from 0.4 g of hepatic tissue or 0.8 g of renal cortical tissue. The microsomes were used immediately after preparation. The microsomal suspensions (typically 10 ml) were incubated with 1 mM NADPH and the fatty acid (0.18 mM, added in 50 μl of ethanol) for 15 min at 37°C under a normal atmosphere. An aliquot of the microsomal suspension (0.5 ml) and NADPH were incubated with 1-^{14}C-labelled fatty acid (1—2 μCi) in the same way and the two incubations combined at the end. Proteins were precipitated with methanol (4 volumes) and spun down. The pellet was washed once with methanol. After evaporation, the residue was dissolved in water and extracted twice with dichloromethane at pH 3. The organic layers were combined, washed with water and dried over Na_2SO_4. Following evaporation, the residue was dissolved in water and extracted twice with ethyl acetate at pH 3. After evaporation, the residue was dissolved in methanol—water, centrifuged, and the clear supernatant was purified by reversed-phase HPLC (μBondapak C_{18}; methanol—water—acetic acid, 73:27:0.2, v/v; flow-rate 2 ml/min per fraction). The renal metabolites of $C_{18:2}$, which were analyzed by GC—MS, were further purified by normal-phase HPLC (μPorasil; gradients of chloroform in hexane as described below).

GC—MS derivatives

An ethereal solution of diazomethane was used for methylation. Methane- and n-butaneboronic acid were dissolved in dimethoxypropane (Fluka; 2.5 mg/ml) and 30 μl were used for derivatization. Initially the reaction mixture was heated to 60—70°C for a few minutes but this step was subsequently omitted since 10 min at room temperature seemed to be sufficient. A fraction of the dimethoxypropane solution was injected directly into the GC—MS system. Silylation was performed with 10 μl of BSTFA in pyridine (1:1) for about 10 min at 70°C and then evaporated to dryness under nitrogen; the residue was dissolved in hexane. Methoximes were prepared as described [7].

GC—MS analysis

Analysis were usually performed on a Finnigan 4000 quadrupole mass spectrometer equipped with a Finnigan Incos data system. The GC column was 1% SP-2100 (90 cm), operated isothermally at 210—230°C. The electron energy was 70 eV and the temperature of the ion source was set to 300°C. Occasionally, samples were run on an LKB 2091 mass spectrometer using a GC column with 1% SE-30. C values were extrapolated from the retention times of the following saturated fatty acid methyl esters: C_{18}, C_{20}, C_{22} and C_{24}.

TABLE I

SALIENT FEATURES OF THE TMS ETHER METHYL ESTER DERIVATIVES OF UNSATURATED 1,2-DIOLS FROM LINOLEIC α-LINOLENIC AND ARACHIDONIC ACID

Compound	R_F*	C values			Salient features of the TMS derivatives § m/z (relative intensity)							
		MeB	BuB	TMS	M+ §§	a-90	a	b	c	d	d-90	[R_m]+ TMS §§§
Methyl 9,10-(HO)₂-C₁₈:₁	0.57	19.9	22.1	21.1	472	225(1.3)	315(0)	259(39)	213(33)	361(4.0)	271(19)	230(0)
Methyl 12,13-(HO)₂-C₁₈:₁	0.62	19.9	22.0	21.3	472	185(9.3)	275(25)	299(23)	173(32)	40(0)	311(1.2)	270(9.3)
Methyl 9,10-(HO)₂-C₁₈:₂	0.55	19.9	22.1	21.4	470	223(0)	313(0)	259(43)	211(9.3)	361(4.4)	271(16)	230(0)
Methyl 12,13-(HO)₂-C₁₈:₂	0.63	19.8	22.0	21.4	470	183(17)	273(15)	299(21)	171(49)	401(0.2)	311(27)	270(9.3)
Methyl 15,16-(HO)₂-C₁₈:₂	0.53	19.9	22.1	22.2	470	143(30)	233(21)	389(5.3)	131(97)	441(0)	351(0.9)	310(8.2)
Methyl 5,6-(HO)₂-C₂₀:₃	0.50**	21.0	23.7	22.7	496***	305(64)	395(0)	203(100)	298(5)	305(64)	215(29)	174(0)
Methyl 8,9-(HO)₂-C₂₀:₃	0.66	21.0	23.7	22.6	496***	265(26)	355(26)	243(59)	253(11)	345(22)	255(100)	214(26)
Methyl 11,12-(HO)₂-C₂₀:₃	0.69	21.0	23.6	22.7	496***	225(21)	315(31)	283(16)	213(41)	385(3)	295(64)	254(11)
Methyl 14,15-(HO)₂-C₂₀:₃	0.70	21.0	23.6	22.8	496***	185(17)	275(59)	323(7)	173(100)	425(0)	335(0.9)	294(10)

*TLC on glass plates, precoated with silica gel 60 (system: 50% ethyl acetate in hexane).

**R_F for the δ 5-lactone was 0.41.

***Complete mass spectra have been published [4—7] and mass spectra of the octadeuterated analogues [9].

§The fragmentation ions m/z a, b, c and d refer to: R_m—CH(OTMS)—CH(OTMS)—R_w, where R_m and R_w are the side-chains con-taining the methyl ester and the omega end, respectively. Relative intensities within parentheses. TMS stands for Si(CH₃)₃.

§§The intensity of the molecular ions is usually low.

§§§See text for explanation and refs. 12—14.

RESULTS

Synthesis of 1,2-diols

Methyl linoleate was oxidized by *m*-chloroperoxybenzoic acid to methyl 9(10)oxido-12-octadecaenoate and methyl 12(13)oxido-9-octadecaenoate, which were separated by TLC. The mass spectra of both epoxides showed signals at m/z 310 (M$^+$), 292 (M$^+$ — 18), 279 (M$^+$ — 31) and 261 [M$^+$ — (18 + 31)]. The C value was 19.3. The least polar epoxide (R_F 0.62) also showed a strong signal at m/z 239 (M$^+$ — 71, loss of carbons 14—18) and it was hydrolyzed to methyl 12,13-dihydroxy-9-octadecaenoate (Table I). The other product (R_F 0.57) showed a relatively strong signal at m/z 199 (M$^+$ — 111, loss of carbons 11—18) and it was hydrolyzed to methyl 9,10-dihydroxy-12-octadecaenoate (Table I).

Methyl linolenoate was oxidized by *m*-chloroperoxybenzoic acid to several products as judged from TLC, but only two spots contained the monooxygenated products. The least polar one was methyl 12(13)oxido-9,15-octadecadienoate (R_F 0.56). The mass spectrum of this compound showed strong signals at m/z 308 (M$^+$), 290 (M$^+$ — 18), 277 (M$^+$ — 31) and 259 [M$^+$ — (18 + 31)], 239 (M$^+$ — 69, loss of carbons 14—18) and 221 [M$^+$ — (69 + 18)]. After hydrolysis, it was converted to methyl 12,13-dihydroxy-9,15-octadecadienoate (Table I). The other product (R_F 0.50) was a mixture of methyl 9(10)oxido- and 15(16)oxido-octadecadienoate, as indicated by a mass spectrum with strong signals at m/z 308 (M$^+$), 290 (M$^+$ — 18), 279 [M$^+$ — 29, loss of CH$_2$CH$_3$ from the 15(16)oxide], 277 (M$^+$ — 31), 261 [M$^+$ — (29 + 18)] and at m/z 199 [M$^+$ — 109, presumably cleavage between carbons 10 and 11 of the 9(10)-oxide]. After hydrolysis, this material was converted to methyl 9,10-dihydroxy-12,15-octadecadienoate and methyl 15,16-dihydroxy-9,12-octadecadienoate, which were separated by TLC (Table I).

TMS ether methyl ester derivatives

Important features of the TMS ether methyl ester derivatives of the unsaturated vicinal dihydroxy acids, which were derived from linoleic, α-linolenic and arachidonic acid are summarized in Table I and will only be discussed briefly. Complete mass spectra of the 1,2-diols derived from arachidonic acid have been published previously [4—7] but data are included in Table I for comparison.

The TMS ether methyl ester derivatives of these unsaturated 1,2-diols showed a characteristic fragmentation. The molecular ion (M$^+$) was usually relatively weak and weak signals were also observed at m/z M$^+$ — 15, M$^+$ — 31 and M$^+$ — 90. The position of the vicinal TMS ethers could be deduced from either one or two fragments formed by α-cleavage (i.e. a and d in Table I) and by cleavage between the carbons carrying the TMS ethers (fragments b and c in Table I). Many of these fragments can also lose trimethylsilanol, and the fragments a—90 and d—90 were observed in almost all scans and were often relatively intense (Table I). Occasionally, signals at m/z b—90 (cf. Fig. 2D) or c—90 were also observed.

The mass spectra of several of the 1,2-diols showed prominent fragmentation ions, which were presumably formed by a common rearrangement reac-

tion. The TMS ether methyl ester derivative of 14,15-dihydroxy-$C_{20:3}$ showed a strong signal at m/z 294 ($M^+ - 202$) and racemic methyl [14-^{18}O]- and [15-^{18}O]-14,15-dihydroxy-$C_{20:3}$ showed a signal at m/z 294 but not at m/z 296 [5]. The mass spectrum of methyl [5,6,8,9,11,12,14,15-$^2H_8$]-14,15-dihydroxy-$C_{20:3}$ showed that m/z 294 had shifted to m/z 300 (cf. ref. 9). It is therefore likely that the signal at m/z 294 originates from $[R_m]^+Si(CH_3)_3$, where R_m denotes the side-chain formed by α-cleavage and containing the methyl ester. $Si(CH_3)_3$ is presumably transferred to the carbonyl group [12–14]. Other examples of this rearrangement are given in Table I (cf. m/z 310 in Fig. 2D).

The C values of the TMS ether methyl ester derivatives of 1,2-diols with the same number of carbon atoms were similar with one exception. 15,16-Dihydroxy-9,12-octadecadienoic acid had a C value that was 0.8 longer than that of the other C_{18} diols (Table I). This is presumably due to the TMS ethers being located rather close to the ω end (cf. ref. 15).

Cyclic alkyl boronic ester derivatives of 1,2-diols

General features. The *n*-butaneboronic acid methyl ester derivatives (BuB) showed strong molecular ions, and signals were noted at $M^+ - 31$ (loss of OCH_3), $M^+ - 57$ (loss of C_4H_9), $M^+ - 84$ (loss of C_4H_9BO), $M^+ - 102$ (loss of $C_4H_9B(OH)_2$) and $M^+ - 115$ (i.e. loss of $84 + 31$). These signals were observed in all the mass spectra but with variable intensity. The methaneboronic acid derivatives (MeB) also showed strong molecular ions, and signals were noted at $M^+ - 31$ (or $M^+ - 32$ in a few cases), $M^+ - 42$ (loss of CH_3BO), $M^+ - 60$ (loss of $CH_3B(OH)_2$) and $M^+ - 73$ (i.e. loss of $42 + 31$). The signal at $M^+ - 15$ of the MeB derivatives were much weaker than $M^+ - 57$ in the mass spectra of the BuB derivatives. Signals at $M^+ - 88$ (loss of $57 + 31$) or $M^+ - 46$ (loss of $15 + 31$) in the mass spectra of the BuB and MeB derivatives, respectively, were inconspicuous and could usually not be observed.

The mass spectra of the MeB and BuB derivatives showed fragments that presumably were formed by α-cleavage (e.g. a and b in Table II). These important fragments often gave strong signals and in some cases strong signals were also found at b—32 (presumably loss of methanol). Due to the natural abundance of ^{10}B and ^{11}B, which is 1:4.2, a boron-containing fragment (Y) gave signals both at m/z Y—1 and Y, which gave them a characteristic appearance [3].

The C values of the MeB and BuB derivatives are given in Table I and show little variation between diols with the same number of carbons, even if the cyclic alkyl boronic esters are situated near the ω end.

Mass spectra of individual 1,2-diols. For brevity, many of the general features discussed above will not be repeated in the description below.

1,2-Diols derived from linoleic acid

Methyl 9,10-dihydroxy-$C_{18:1}$. A mass spectrum of the MeB derivative is shown in Fig. 1A. Important signals are noted at m/z 241 ($M^+ - 111$) and at m/z 209 (241 — 32). The BuB derivative showed corresponding strong signals at m/z 283 ($M^+ - 111$) and 251 (283 — 32). The MeB derivative showed a
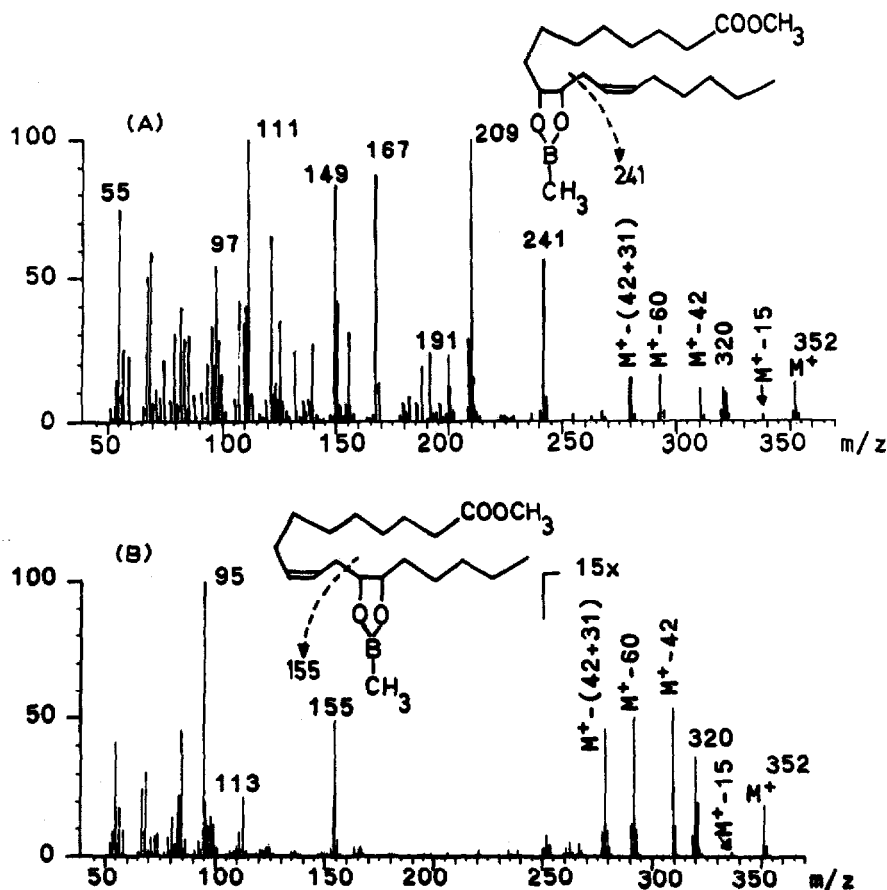
Fig. 1. Electron-ionization mass spectra of the cyclic methaneboronic ester methyl ester of 9,10-dihydroxy-12-octadecaenoic acid (A) and 12,13-dihydroxy-9-octadecaenoic acid (B).

rather strong signal at m/z 320 (M$^+$ − 32) but the corresponding fragment of the BuB derivative was inconspicuous.

Methyl 12,13-dihydroxy-$C_{18:1}$. A mass spectrum of the MeB derivative is shown in Fig. 1B. A fragment formed by α-cleavage could be m/z 155 (cleavage between carbons 11 and 12). This fragment could be expected to shift to 197 in the BuB derivative. A mass spectrum of the BuB derivative is shown in Fig. 5, although the diol in this case was isolated from a biological source (see below). This mass spectrum was, however, identical with that of the authentic material and a strong signal is noted at m/z 197.

1,2-Diols derived from α-linolenic acid

Methyl 9,10-dihydroxy-$C_{18:2}$. A mass spectrum of the BuB derivative is shown in Fig. 2A. The signals at m/z 235 and 283 might be due to α-cleavage (insert in Fig. 2A). A strong signal is found at m/z 251 (283 − 32). These three signals presumably correspond to the signals at m/z 193 (235 − 42), 241 (283 − 42) and 209 (241 − 32) of the MeB derivative.

Methyl 12,13-dihydroxy-$C_{18:2}$. A mass spectrum of the BuB derivative is shown in Fig. 2B. The signals at m/z 195 and 323 might be due to α-cleavage

(insert in Fig. 2B). Strong signals are also noted at m/z 291 (323 − 32) and 290 (M^+ − 102). The MeB derivative showed strong signals at m/z 281 (323 − 42), 153 (possibly 195 − 42) and 249 (281 − 32).

Methyl 15,16-dihydroxy-$C_{18:2}$. A mass spectrum of the BuB derivative is shown in Fig. 2C. The fragments formed by α-cleavage presumably give rise to the signals at m/z 363 (M^+ − 29, loss of C_2H_5), 332 (M^+ − (29 + 31)) and 155 (insert in Fig. 2C). The MeB derivative showed a very strong signal at m/z 113 (155 − 42), but a signal at m/z 321 (M^+ − 29) was not observed.
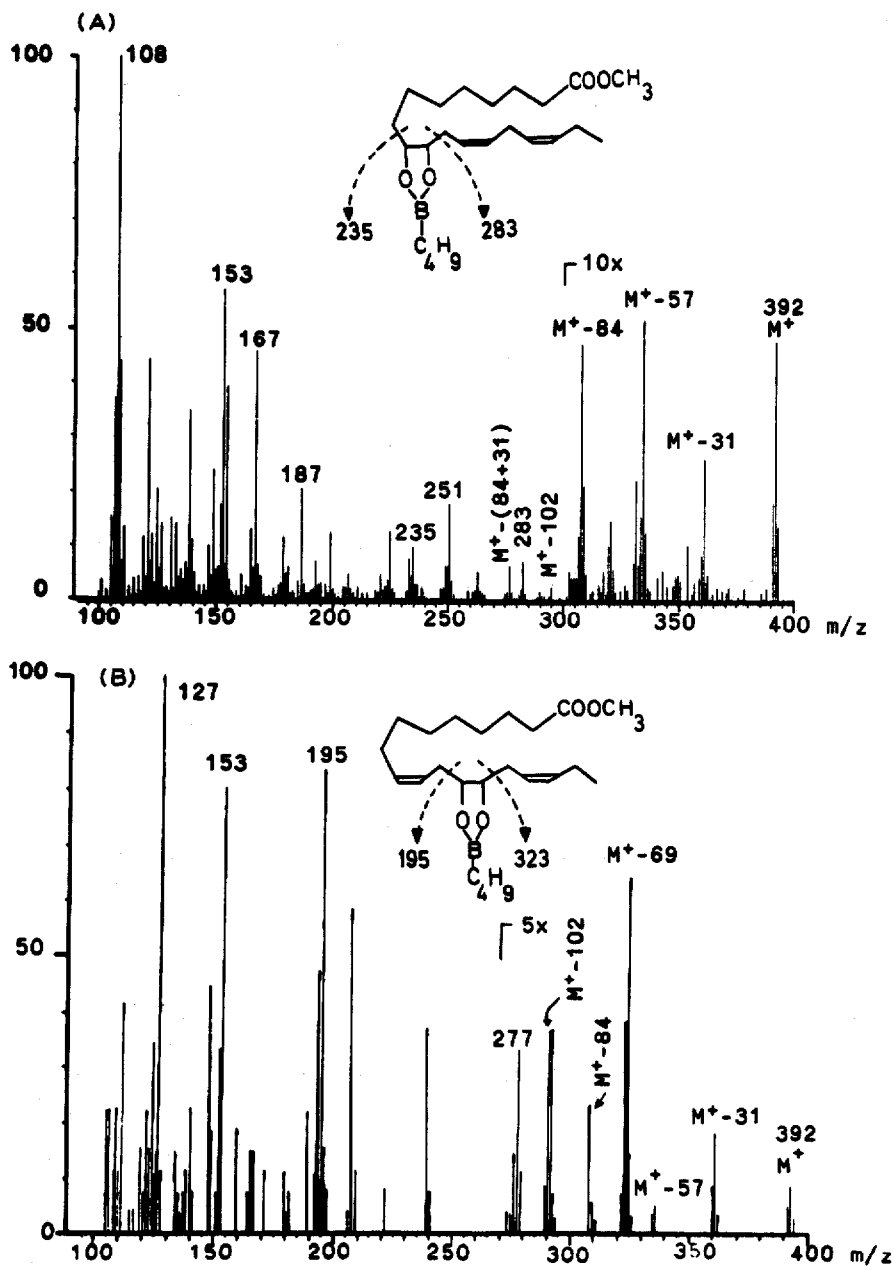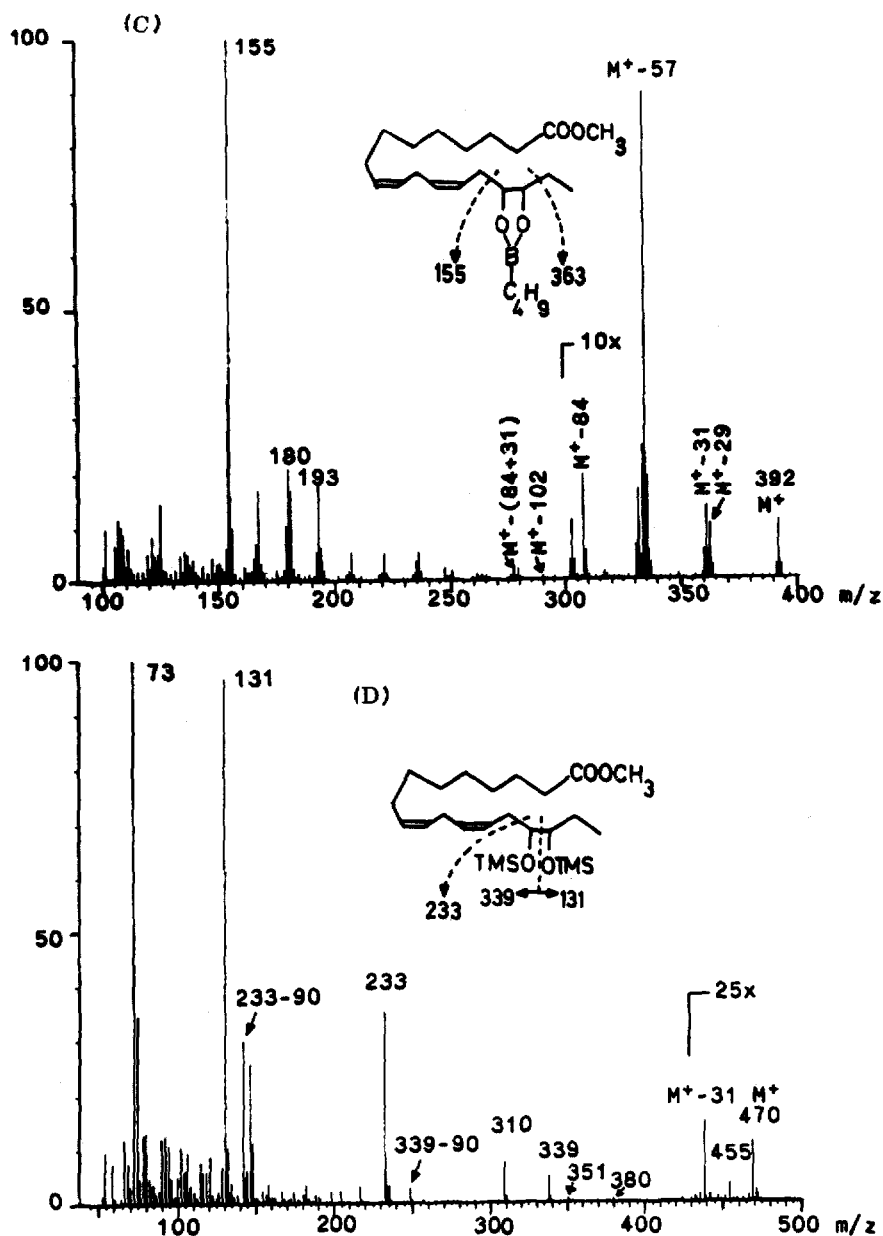

Fig. 2.

Fig. 2. Electron-ionization mass spectra of three vicinal dihydroxy acids, which were derived from α-linolenic acid. A, B and C show mass spectra of n-butaneboronic acid methyl ester derivatives of 9,10-dihydroxy-, 12,13-dihydroxy- and 15,16-dihydroxy-$C_{18:2}$, respectively. D shows a mass spectrum of the TMS ether methyl ester derivative of 15,16-dihydroxy-9,12-octadecadienoic acid. TMS stands for $Si(CH_3)_3$.

1,2-Diols derived from arachidonic acid

Important features of the MeB and BuB derivatives are summarized in Table II, which also shows the fragments that are presumably formed by α-cleavage (a and b in Table II). Signals at b—32 were noted in several of

TABLE II

SALIENT FEATURES OF THE CYCLIC n-BUTANEBORONIC ACID AND METHANEBORONIC ACID DERIVATIVES OF 1,2-DIOLS DERIVED FROM ARACHIDONIC ACID

Relative intensities of n-butaneboronic acid derivatives

Compound	M+ 418	M+-31 387	M+-57 361	M+-84 334	M+-102 316	M+-115 303	a* (%)	b* (%)	Base peak (100%)	Fragments in the upper mass range and their relative intensities (%)
Methyl 5,6-$(HO)_2$-$C_{20:3}$	0.5	1.1	0.3	1.6	2.3	0.6	317(4.0)	227(7.2)	55	336(1.9) 306(1.6) 279(1.4) 252(5.3) 195(34)**
Methyl 8,9-$(HO)_2$-$C_{20:3}$	2.3	0.2	0.7	0.6	1.4	4.3	277(5.9)	267(2.4)	67	317(1.4) 289(2.4) 277(5.7) 263(2.3)
Methyl 11,12-$(HO)_2$-$C_{20:3}$	1.2	0.2	0.5	0.6	1.2	1.7	237(4.0)	307(3.8)	69	281(1.4) 275(1.4)** 263(1.2)
Methyl 14,15-$(HO)_2$-$C_{20:3}$	0.8	0.1	2.7	1.5	2.7	3.2	197(33)	347(2.9)	55	336(6.8) 289(3.6) 276(3) 263(3.6)

Relative intensities of methaneboronic acid derivatives

Compound	M+ 376	M+-31 345	M+-42 334	M+-60 316	M+-73 303	a* (%)	b* (%)	Base peak (100%)	Fragments in the upper mass range and their relative intensities (%)
Methyl 5,6-$(HO)_2$-$C_{20:3}$	0.5	1.9	1.8	1.4	0.6	275(3.9)	185(12)	111	302(0.6) 294(2.6) 275(3.9) 236(4.8) 153(66)**
Methyl 8,9-$(HO)_2$-$C_{20:3}$	1.5	0.1	0.5	1.1	0.8	235(7.9)	225(0)	67	291(0.8) 275(1.4) 261(3.6)
Methyl 11,12-$(HO)_2$-$C_{20:3}$	0.6	0.1	0.4	0.8	0.8	195(2.6)	265(2.8)	69	291(0.4) 275(0.6) 265(2.9) 233(1.9)**
Methyl 14,15-$(HO)_2$-$C_{20:3}$	0.7	0.1	1.2	2.0	1.5	155(30)	305(1.7)	95	294(3.1) 273(0.4)** 263(1.1) 261(1.2)

*The fragmentation ions a and b refer to R_m —CH OB(X)OCH— R_w, where R_m and R_w denote the side-chains carrying the methyl ester and the omega end, respectively, and X = CH_3 or C_4H_9.

**These signals might, at least partly, be formed from b with loss of 32 (see text).

the mass spectra but they were not very intense in most of them. Mass spectra of some of the diols showed strong ions, which presumably are formed by rearrangement reactions.

Methyl 5,6-dihydroxy-$C_{20:3}$. Mass spectra of the MeB and BuB derivatives are shown in Fig. 3 and the fragmentation is summarized in Table II. The relatively strong signals at m/z 336 ($M^+ - 82$) of the BuB derivative and m/z 294 ($M^+ - 82$) of the MeB derivative were also noted in the mass spectra of 14,15-dihydroxy-$C_{20:3}$ but not in any of the other diols.
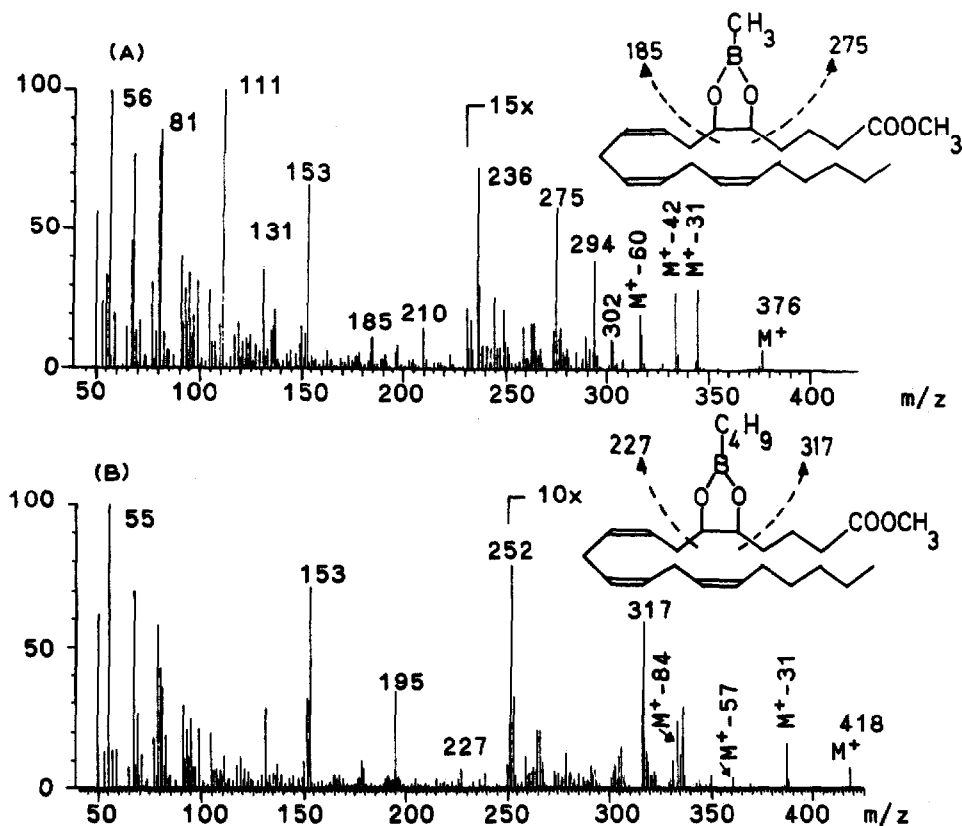

Fig. 3. Electron-ionization mass spectrum of the cyclic methaneboronic (A) and *n*-butane-boronic ester methyl ester (B) of 5,6-dihydroxy-8,11,14-eicosatrienoic acid.

Methyl 8,9-dihydroxy-$C_{20:3}$. The MeB and BuB derivatives are summarized in Table II and the mass spectra revealed few other interesting features.

Methyl 11,12-dihydroxy-$C_{20:3}$. A mass spectrum of the BuB derivative has been published previously [4]. The mass spectrum of the MeB derivative was also without conspicuous rearrangement ions.

Methyl 14,15-dihydroxy-$C_{20:3}$. A mass spectrum of the BuB derivative of the diol from a biological source has been published previously [4]. As shown by Table II, both the BuB and the MeB derivatives showed strong signals at $M^+ - 82$, i.e. at m/z 336 and 294, respectively. These fragments were the strongest in the upper mass range of these mass spectra (cf. Table II). The

BuB derivative of methyl $[5,6,8,9,11,12,14,15-^2H_8]$-14,15-dihydroxy-$C_{20:3}$ showed signals at m/z 426 (M^+), 369 ($M^+ - 57$), 355 ($M^+ - 71$), 342 ($M^+ - 84$) and 338 ($M^+ - 88$, possibly 57 + 31). The latter was the strongest signal but it can hardly correspond to m/z 336 of the protium form of the diol. The deuterated compound therefore seems to form partly other fragmentation ions than the protium form, when the BuB derivative is used.

Metabolism of linoleic and α-linolenic acid by hepatic and renal cortical microsomes and NADPH

$[1-^{14}C]$ Linoleic and $[1-^{14}C]$ linolenic acid were metabolized to many polar metabolites by rabbit renal cortical or hepatic microsomes and NADPH. Virtually no metabolism was noted in the absence of this cofactor. Fig. 4 shows the elution of radiolabelled metabolites from a reversed-phase HPLC column. Three peaks of radioactivity eluted (I—III in Fig. 4). Hepatic and renal cortical microsomes gave a very similar elution pattern. It is notable that peak II from the incubation of α-linolenic acid had the same elution volume as authentic $[^{14}C]$-9,10-dihydroxy- and -15,16-dihydroxy-$C_{18:2}$, but the various metabolites of α-linolenic acid were not further analyzed in this study.

Some metabolites of linoleic acid, which were formed by renal microsomes and NADPH, were purified and analyzed by GC—MS (GC column with 3% SP-2100). The material of peak II was subjected to normal-phase HPLC (a linear gradient of 30% chloroform in hexane to 90% chloroform in hexane in 60 min with 0.4% acetic acid, flow-rate 1 ml/min) and a major peak eluted after 58—60 min. The mass spectrum of the BuB derivative of the methyl ester is shown in Fig. 5. Signals were noted, inter alia, at m/z 394 (M^+), 363
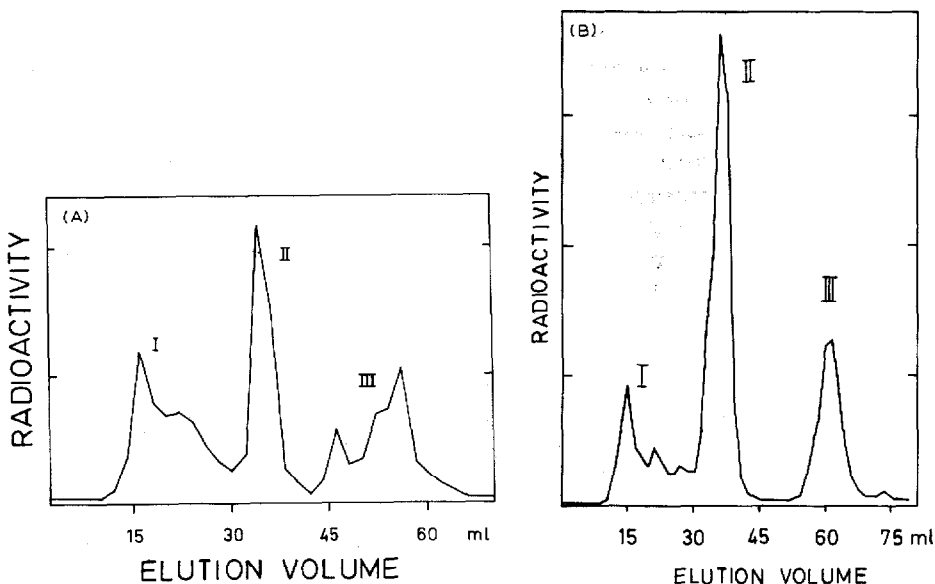

Fig. 4. Reversed-phase HPLC of metabolites formed by hepatic microsomes and NADPH from $[^{14}C]$linoleic (A) and α-$[^{14}C]$linolenic acid (B) (μBondapak C_{18}). Virtually no metabolites were formed if NADPH was omitted from the incubations.
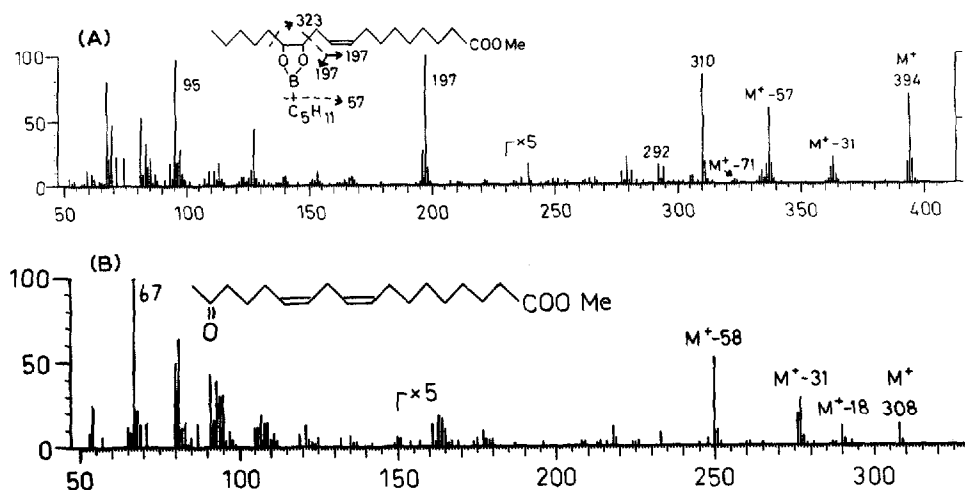
Fig. 5. Mass spectra of two metabolites of linoleic acid formed by renal cortical microsomes of the rabbit in the presence of NADPH. (A) n-Butaneboronic acid methyl ester derivative of 12,13-dihydroxy-9-octadecenoic acid. (B) Methyl ester derivative of 17-oxo-linoleic acid.

$(M^+ - 31)$, 337 $(M^+ - 57)$, 323 $(M^+ - 71)$, 310 $(M^+ - 84)$, 279 $[M^+ - (84 + 31)]$, 239, and a very strong signal at m/z 197. The two fragments formed by α-cleavage are indicated by the insert in Fig. 5. The C value was 22.2. This mass spectrum was identical with that of the authentic 12,13-dihydroxy-9-octadecenoic acid derivative.

The material of peak III was found to contain several metabolites. One was presumably 17-oxo-9,12-octadecadienoic acid (17-oxo-linoleic acid). It eluted after about 13 min on normal-phase HPLC (gradient as above). The mass spectrum of the methyl ester of this metabolite is shown in Fig. 5B. Signals were noted at m/z 308 (M^+), 290 ($M^+ - 18$), 277 and 278 ($M^+ - 31$ and $M^+ - 32$), 250 ($M^+ - 58$, presumably loss of CH_3COCH_3) and 67 (base peak). The C value was 19.3. The methoxime methyl ester derivative showed signals, inter alia, at m/z 337 (M^+), 306 ($M^+ - 31$) and 87 [base peak, presumably $CH_3C(NOCH_3)CH_3$]. The C value was 20.1. Assuming that the double bonds have not changed, as indicated by UV analysis, this compound was tentatively identified as 17-oxo-linoleic acid. Another metabolite peak III was 13-hydroxy-9,11-octadecadienoic acid, since the TMS ether methyl ester derivative gave a mass spectrum that was almost identical with that previously reported [16].

DISCUSSION

The present work compares the use of TMS ether derivatives and cyclic alkyl boronic ester derivatives for GC—MS analysis of 1,2-diols, which were chemically derived from three long-chain polyunsaturated fatty acids. The biological interest in these diols is based on the metabolism of arachidonic acid to vicinal diols by cytochrome P-450 in a reconstituted system [4, 6, 7] and by isolated renal cells and hepatocytes [9]. The present work also demon-

strates that linoleic and α-linolenic acid are metabolized to many polar products by cytochrome-P-450-containing microsomes and NADPH. It may be of interest to determine these metabolites, and the results of the present study might be helpful.

There are several features which make the cyclic boronates useful for analysis of 1,2-diols in biological samples. First, the cyclic boronates are only formed with a restricted number of bifunctional compounds, mainly 1,2-diols, 1,3-diols and α-hydroxy carboxylic acids [3]. The very formation of these derivatives provides important structural information. Furthermore, their electron-ionization mass spectra are easily recognized by the occurrence of abundant molecular ions with the characteristic isotope distribution and the fragmentation of the alkyl boronic esters. The mass spectra also indicate the position of the boronic esters by one or two fragments formed by α-cleavage.

The cyclic boronic ester derivatives also have some limitations. They are of little value for analysis of fatty acids with two or more vicinal diols, since diagnostically reliable fragmentation ions are not formed [17]. The mass spectra of the boronate derivatives of some of the polyunsaturated diols were difficult to interpret due to strong fragmentation ions, which presumably are formed by a rearrangement mechanism (e.g. 5,6-dihydroxy- and 14,15-dihydroxy-$C_{20:3}$ as discussed above). The present report also indicates that the n-butaneboronic ester derivative of octadeuterated 14,15-dihydroxy-$C_{20:3}$ can form partly other fragmentation ions than the protium form. This may limit the use of this derivative for mass fragmentography employing the deuterated internal standard.

The TMS ether methyl ester derivatives of the 1,2-diols gave rise to rather weak molecular ions, but still had many advantages. Strong fragmentation ions were formed by α-cleavage and by cleavage between the vicinal diols, giving up to four diagnostically very useful fragmentation ions. In this respect the TMS ether derivatives were clearly superior to the boronic esters. The TMS derivatives of vicinal diols also give important rearrangement ions, which were originally observed by Capella [12] and Capella and Zorzut [13]. These fragments, denoted $[R_m]^+Si(CH_3)_3$ in Table I, are of considerable practical value since they are intense, and often occur in the mass spectra of some metabolites of the 1,2-diols, e.g. metabolites formed by ω and $\omega-1$ oxidation [7].

Long-chain fatty acids can be metabolized by hepatic and renal cortical microsomes, fortified by NADPH, to many products. ω and $\omega-1$ oxidation have been studied in detail and both saturated and unsaturated fatty acids are oxygenated in this way by cytochrome P-450 [4, 18, 19]. Unsaturated fatty acids are also oxygenated by cytochrome-P-450-catalyzed epoxidation and by subsequent hydrolysis to vicinal diols [4—7, 20]. Finally, unsaturated fatty acids may undergo lipid peroxidation and autooxidation.

12,13-Dihydroxy-9-octadecenoic acid was identified as a metabolite of linoleic acid in rabbit renal cortical microsomes fortified with NADPH. This metabolite is presumably formed by $\omega6$ epoxidation and by hydrolysis. Another metabolite, 17-oxo-linoleic is likely formed by $\omega-1$ oxidation. In many respects, the metabolism of linoleic seems to be analogous to the cytochrome-

P-450-linked oxygenation of arachidonic acid. It will be of interest to determine whether α-linolenic acid and other polyunsaturated fatty acids of the ω3 series are metabolized in a similar fashion.

ACKNOWLEDGEMENTS

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