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GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC CHARACTERIZATION OF NATURALLY-OCCURRING ACYCLIC ISOPRENOID CARBOXYLIC ACIDS

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Abstract—Gas chromatographic retention data for the methyl esters of acyclic isoprenoid acids are discussed and shown to be useful in their recognition. Analytical methods included the use of a combined gas chromatograph–mass spectrometer; fragmentation patterns for the methyl esters of acyclic isoprenoid acids are briefly noted. Two shales of different age and different depositional history have been examined for their isoprenoid acid content. Serpiano Oil Shale, (Triassic, *ca* 210 × 10^6 yr; marine) from Switzerland contains all of the isoprenoid acids from C_{11}–C_{20} (and possibly C_{21}), the n-acids from C_8–C_{23} and a series of 'pseudo' isoprenoid acids. Green River Shale (Eocene, *ca* 60 × 10^6 yr; lacustrine) from Colorado, U.S.A. contains the C_{14}–C_{17} and C_{19}–C_{22} isoprenoid acids and the n-acids from C_{12}–C_{34}. The range of α,ω-dicarboxylic acids is considerably extended over previous findings and a series of 2-alkylsuccinic acids is provisionally reported.

INTRODUCTION

THE occurrence of some isoprenoid§ acids in a crude oil,[1a–c] in Recent sediments[2] and in Ancient sediments[3–7] has been reported and a general review of the occurrence of fatty acids in sediments has been published.[8] The isoprenoid acids are of some interest geochemically, as they are almost certainly related to the abundant and varied isoprenoid compounds found in present-day living organisms.[9,10] Indeed, stereochemical correlations between isoprenoid fatty acids from the Green River Shale and the same acids from zooplankton, fishes, marine and terrestrial mammals have recently been established using capillary gas chromatography.[11,12] The diastereoisomeric compositions are consistent with a phytol origin for phytanic acid and lower isoprenoid acids isolated from the Green River Shale.

The present paper describes in detail the isolation and characterisation of a wide range of isoprenoid acids from two shales of different ages and depositional environments. Mention is also made of α,ω-dicarboxylic and n-alkylsuccinic acids in one shale, and a number of "pseudo" isoprenoid acids in the other.

Green River Shale (Eocene, about 60 × 10^6 years) occurs as a vast, organic-rich, lacustrine deposit in Colorado, U.S.A., and contains remains of alga, fish, etc[13] and is reported as not having been subjected to high temperatures.[14,15] It is supposedly the largest, single, known deposit of hydrocarbons in the world and it has been studied both chemically and geologically. Recent studies of shale from the Mahogany

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§ The term "isoprenoid" strictly speaking describes only those compounds having carbon skeletons bases on isoprenoid, C_5 units. However, it is convenient to use the same term for those compounds believed to be derived from isoprenoid precursors, on account of the portions of their skeletons which are of isoprenoid pattern.

Zone have shown the presence of normal C_{10}–C_{34} alkanes,[16,17] with a marked predominance of those with odd carbon numbers, especially C_{27}, C_{29} and C_{31}, a characteristic of most plant waxes.[18,19] The presence of acyclic isoprenoid hydrocarbons,[16,20] steranes and triterpanes[21,22] has also been noted.

Serpiano "oil shale" (Triassic, about 210×10^6 years) is actually a bituminous dolomite found at Serpiano, Switzerland and was laid down in a shallow coastal sea near the upper boundary of the Anisian horizon of the middle Triassic. It is noted for its faunas, being rich in fishes and marine and amphibious reptiles and also for its unusually high content of tetrapyrrole pigments.[23a–c]

METHODS AND RESULTS

Isolation of isoprenoid acids

Different methods were used to isolate the fatty acids from the two shales.

Green River Shale from the 1900 ft level at Sulphur Creek was cleaned, powdered and demineralised with a mixture of hydrochloric and hydrofluoric acids. This demineralised shale was treated with methanolic potassium hydroxide to free the kerogen-bound fatty acids.[4,24] The free fatty acids were isolated from the crude lipid extract by the procedure of McCarthy and Duthie,[25] and converted to their methyl esters which were purified by TLC against standard methyl esters.

The sample of Serpiano Oil Shale, from which the total fatty acids were extracted, was from the Grenzbitumen horizon at the boundary of Anisian and Ladinian. The shale was cleaned and powdered and the lipids which were extracted with benzene/methanol were esterified. Furthermore, the extracted shale was treated with methanol/hydrochloric acid and the esters formed were removed; acids not esterified at this stage were subsequently treated with methanol/BF_3. The combined crude esters were extracted with pentane and further purified by chromatography over silica gel. The straight chain (and some Me branched) acids were removed by repeated urea clathration and the recovered, non-adducted isoprenoid acids were then treated in the same way as the acids from the Green River Shale.

Although different extraction procedures were used for the two shales, the results are in some measure comparable since demineralisation, saponification and esterification of the powdered Green River Shale is paralleled by the methanolysis of the powdered Serpiano Shale after the unbound lipids had been extracted and esterified. Thus, the acids investigated represent those which may be present as the free acids, esters, acid salts and kerogen-bound esters.

Characterisation by gas chromatography

Both purified ester fractions were examined by analytical GLC. The Green River Shale esters were partly separated by preparative GLC and small fractions trapped for mass spectrometric examination. Both ester fractions were also examined by direct combined gas chromatography-mass spectrometry (GC–MS).

James and Martin[26] first noted that a plot of the carbon number against the logarithm of the gas chromatographic retention time gave a straight line for members of a homologous series. Correspondingly, within a homologous series, the Kovats retention indices[27] or the equivalent chain lengths (ECL)[28a,b] of adjacent members differ by equal increments.

Isoprenoid compounds, although forming a related series, are not homologous

By definition, the equivalent chain length (but not the retention index) of a straight chain ester is the same on polar and nonpolar substrates. Because of their Me branching, the isoprenoid esters appear less polar than the straight chain esters, and their equivalent chain lengths on polar substrates (e.g. FFAP*, Table 4) are lower

TABLE 4. COMPARISON OF EQUIVALENT CHAIN LENGTHS OF METHYL ESTERS OF BRANCHED ACIDS (ISOPRENOID ACIDS) FROM SERPIANO SHALE ON POLAR AND NON-POLAR COLUMNS (CF. TABLE 3)

Carbon number	ECL Polar phase (FFAP)	ECL Non-polar phase (Apiezon L)	ECL Difference (non-polar − polar)	Terminal acid group
11	9·88	10·03	0·15	propanoic
12	10·87	11·11	0·24	butanoic
13	11·80	12·00	0·20	pentanoic
14	11·67	12·19	0·52	formic
15	12·81	13·26	0·45	ethanoic
16	14·03	14·35	0·32	propanoic
17	14·95	15·28	0·33	butanoic
18	15·95	16·24	0·29	pentanoic
19	15·76	16·41	0·65	formic
20	16·95	17·50	0·55	ethanoic
21	18·06	18·60	0·54	propanoic
22	19·19	19·67	0·48	butanoic

than they are on nonpolar substrates. The difference is not constant but decreases with increasing length of the terminal acid group, $-(CH_2)_nCO_2H$, one anomaly (for FFAP) being the C_{11} acid. The C_{18} isoprenoid ester, which terminates in an n-pentanoic group, resembles in this behaviour the ester of an n-acid more than the C_{19} ester with a Me substituent adjacent to the carboxyl group. The combined effect of lowering of the b.p. and of the decrease in apparent polarity with increased branching in the C_{14} and C_{19} isoprenoid esters brings about on polar columns an unusual reversal of the order of elution and of the equivalent chain lengths; the C_{14} ester elutes before the C_{13} ester and the C_{19} ahead of the C_{18} ester.

The accuracy of these retention data, measured in one laboratory and applied in another, is exemplified on the linear temperature programmed capillary chromatogram of a sample of Green River Shale esters shown in Fig 1. The equivalent chain length values of the isoprenoid esters measured directly from the original chart of Fig 1 are shown in Table 5, together with the values for samples of the same esters derived synthetically and from the Serpiano shale. The sample of the Green River Shale was from the Mahogany Zone of the Green River Formation, in contrast to the sample used for the rest of this work, which was from Sulphur Creek. The gas chromatogram, Fig 1, shows a small peak with the correct ECL value (16·24) for methyl 6,10,14-trimethylpentadecanoate (methyl norpristanate) but the mass spectrum of the equivalent region was unsatisfactory, when recorded from a GC–MS analysis in which a packed column was used. The mass spectra of the labelled peaks confirmed their structure.

* FFAP (Varian Aerograph, Walnut Creek, Calif.) Free Fatty Acid Phase.

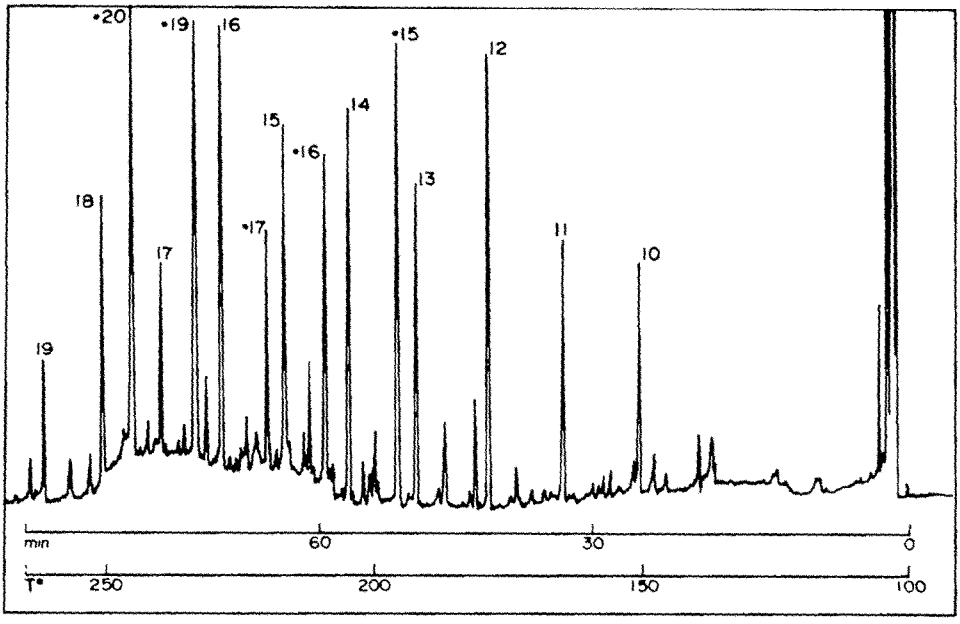


FIG 1. Gas chromatogram of the methyl ester fraction (*ca.* C_{10}–C_{20}) derived from the Green River Shale (Mahogany zone). Conditions: column 200 ft × 0·01 in. stainless steel capillary coated with Apiezon L containing 0·2% Gas Quat L; temperature programmed from 100–250° at 2°/min; nitrogen 50 psi; split ratio 10:1 for 1·5 μl injected. The normal and isoprenoid (marked with an asterisk) esters confirmed in this sample by GC–MS are numbered according to carbon number

TABLE 5. COMPARISON OF E.C.L. VALUES OF METHYL ESTERS OF BRANCHED ACIDS (ISOPRENOID ACIDS) ON APIEZON L, FROM GREEN RIVER AND SERPIANO OIL SHALES

Isoprenoid ester Carbon number	ECL (Serpiano and synthetic) (esters – *cf.* Table 3)	ECL (GRS esters (from Fig. 1)
11	10·03	
12	11·11	
13	12·00	
14	12·19	12·18
15	13·26	13·28
16	14·35	14·38
17	15·28	15·28
18	16·24	16·24
19	16·41	16·42
20	17·50	17·50
21	18·60	18·55
22	19·67	

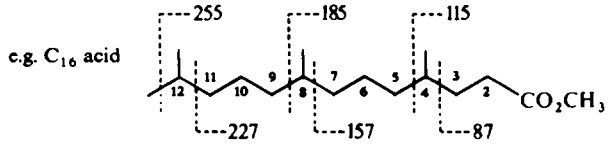
The GLC behaviour of isoprenoid and other branched acids has also been discussed by Ackman, particularly with respect to the characterisation of these acids in biological lipids.[11, 31a–e]

Characterisation by mass spectrometry

Mass spectral fragmentation patterns of some synthetic and naturally occurring branched chain[32a,b–35] and isoprenoid fatty acids[36–38] have been discussed. These patterns have already been used to characterise isoprenoid acids in petroleum[1a–c] and shales.[3,4,5,7,24] In the present paper the combined GC–MS technique, which permits sequential separation and identification, has revealed a wide range of these acids in the two sediments examined. The important mass spectral fragmentation patterns of these acids may be summarised briefly as follows.

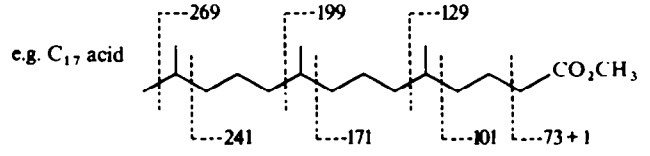
In his discussions on the fragmentation patterns of long chain, Me branched fatty acids, Ryhage noted,[32a–b,38] *inter alia*, the following features:—In unbranched methyl esters the base peak is at *m/e* 74 due to the rearranged ion $[CH_3—O—C(OH)=CH_2]^+$, a rearrangement which has been discussed by McLafferty.[39] In 2-Me substituted esters however, the rearranged ion carries the Me branch and the base peak appears at *m/e* 88. Ryhage has recorded the mass spectra of many long chain Me[32a] and poly Me[32b] substituted fatty acid esters and has shown that preferential bond breaking will occur at the tertiary C atoms. This gives rise to two peaks 28 mass units apart, which are more intense than the peak (different by 14 mass units) which lies between them. These carbomethoxy ions are of the type $[CH_3—O—CO—(CH_2)_n]^+$ and $[CH_3—O—CO—(CH_2)_nCH—CH_3]^+$, the latter of which may lose 32 mass units (MeOH) or 50 mass units (MeOH + H_2O) to give significant fragments in the spectrum. Using these criteria, the expected mass spectra of the methyl esters of isoprenoid acids between C_{11} and C_{22} are discussed briefly, those with similar substitution patterns being grouped together.

Methyl groups attached to carbon atoms 4,8 *and* 12 *etc as in methyl esters of* C_{11}, C_{16} *and* C_{21} *isoprenoid acids*



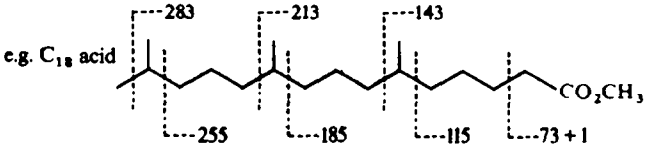
Base peak at *m/e* 87 due to 3,4 bond cleavage; peak at *m/e* 74 usually second most abundant fragment. Peaks at *m/e*: 115 > 101, 157 and 185 > 171, 227 and 255 > 241. Presence of a M-57 fragment due to loss of four-carbon fragment carrying C atoms 2,3,4, and the Me group attached to C_4. Peaks due to loss of 32 and 50 mass units from fragments with *m/e* values of 185 and 255.

Methyl groups attached to carbon atoms 5,9 *and* 13 *etc as in methyl esters of* C_{12}, C_{17} *and* C_{22} *isoprenoid acids.*



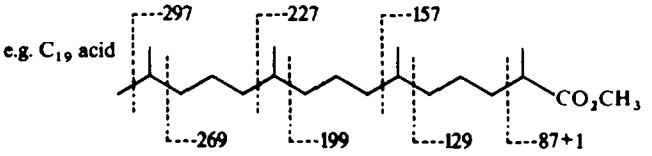
Base peak at *m/e* 74; peak at *m/e* 87 usually second most abundant fragment. Peak at *m/e*: 101 and 129 > 115, 171 and 199 > 185, 241 and 269 > 255: M-43 and M-71 present. Small peaks at M-76 and M-50. Peaks due to loss of 32 and 50 mass units from fragments with *m/e* values of 199 and 269.

Methyl groups attached to carbon atoms 6, 10 *and* 14 *etc as in the methyl esters of* C_{13} *and* C_{18} *isoprenoid acids.*



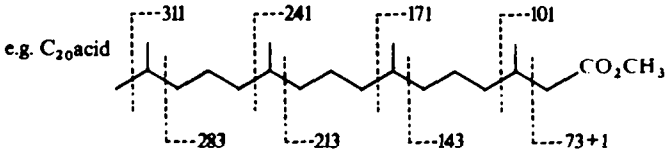
Base peak at *m/e* 74, and that at *m/e* 87 usually second most abundant fragment. Peaks at *m/e*: 115 and 143 > 129, 185 and 213 > 199, 255 and 283 > 269. M-43 and M-57 present and a substantial peak at M-50. The peak due to M-76 is very large in 6-methyl esters. Peaks due to loss of 32 and 50 mass units from fragments with *m/e* values of 213 and 283.

Methyl groups attached to carbon atoms 2, 6 *and* 10 *etc as in the methyl esters of* C_{14} *and* C_{19} *isoprenoid acids.*



Base peak at *m/e* 88, and a large peak at *m/e* 101 corresponding to the peak at *m/e* 87 in the above examples. Peaks at *m/e* 129 and 157 > 143, 199 and 227 > 213, 269 and 297 > 283. The fragmentation giving the M-76 fragment in the 6-methyl esters now gives a fragment M-90 because of the Me group on C_2. Peaks due to loss of 32 and 50 mass units from fragments with *m/e* values of 157, 227 and 297.

Methyl groups attached to carbon atoms 3, 7 *and* 11 *etc as in the methyl esters of* C_{15} *and* C_{20} *isoprenoid acids.*



Base peak at *m/e* 101 and a high abundance of the *m/e* 74 ion. Although Ryhage[32a] has established that methyl 3-methyleicosanoate has a base peak at *m/e* 74, with a high abundance of the ion at *m/e* 101, he has also shown[32b] that in the methyl esters of 3,5-dimethyltricosanoic and 3,6-dimethyltetracosanoic acids the base peak is at *m/e* 101. This has also been established by us in previous work[3] and by others[24] for the spectrum of methyl phytanate. Ions at M-43 and M-57 are present and peaks at *m/e*: 143 and 171 > 157, 213 and 241 > 227, 283 and 311 > 299. Peaks due to loss of 32 and 50 mass units from fragments with *m/e* 171, 241 and 311.

Caution should be exercised in the general application of these fragmentation rules. For example, in the C_{16} isoprenoid ester, loss of fragments due to expulsion of [C-2 + C-3] and [C-2 + C-3 and C-4, (with attached methyl)] would result in ions of relatively high abundance at M-29 and M-57, respectively, whereas the ion at M-43 would be absent, since loss of 43 mass units requires rupture of an extra C—C bond due to the presence of the 4-Me group. However, the abundant ion anticipated

at *m/e* 241 (M-29) is one that is not expected from cleavage at the C_{12} branch, where the ions with *m/e* values of 227 and 255, bracketing 241, should be more abundant; consequently, the abundance of *m/e* 241 is greater than *m/e* 255.

Isoprenoid acids in Serpiano Oil Shale

A gas chromatogram of the isoprenoid esters, obtained by urea clathration as described above, is shown in Fig 2. It shows isoprenoid esters ranging from C_{11}–C_{21}.

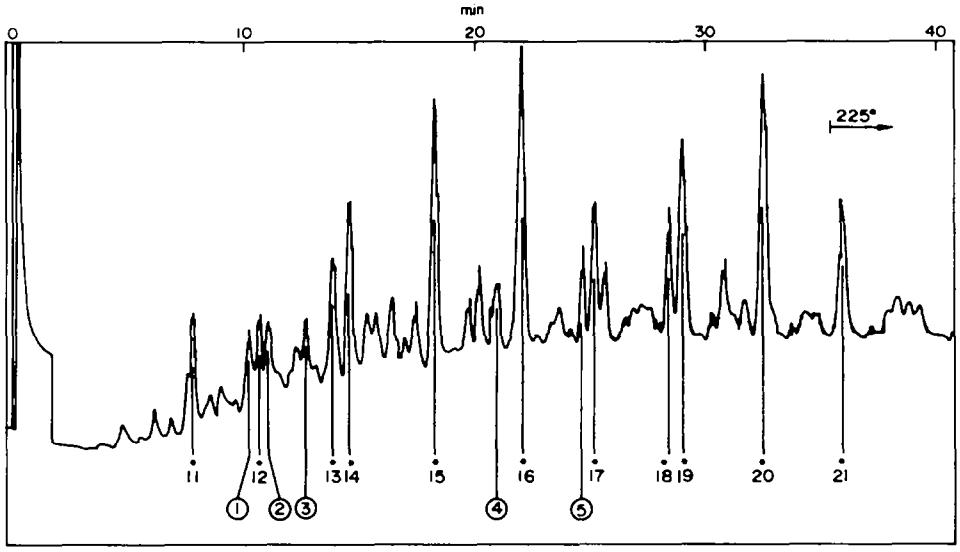


FIG 2. Gas chromatogram of the methyl ester fraction of the isoprenoid and "pseudo" isoprenoid acids isolated from Serpiano Oil Shale, shown as a GC–MS trace (total ion monitor). Conditions: column 10 ft × ⅛ in (i.d.) containing 1% APL on 100–120 mesh Gas Chrom P; temperature programmed from 100–225° at 3°/min; helium 34 ml/min. Isoprenoid esters are starred and numbered 11*–21* (according to carbon number) and the "pseudo"-isoprenoid esters, listed in Table 8, are numbered 1–5

The gas chromatographic retention values of these esters, (recorded as their equivalent chain lengths) on a polar phase and on a non-polar phase, are given in Table 4. It has already been shown above, that the ECL values for the esters of the fossil acids C_{15}, C_{16}, C_{17}, C_{19} and C_{20} are identical to those obtained for the esters of the corresponding synthetic acids and that the lowering of the ECL values (with respect to n-esters) are approximately the same for compounds with the same number of Me branches.

A separate isolation in which losses were minimised was used for the quantitative determination of the Serpiano Oil Shale esters. The isoprenoid esters were determined quantitatively from the gas chromatogram of the esters remaining after urea clathration. Methyl pristanate served as calibration standard. Quantitative determination of isoprenoid esters at the parts per billion level in a mixture of the complexity encountered here is subject to many errors; losses during isolation, volatilization of the lower boiling esters, and, in the gas chromatography, the need for a rather high background correction and the presence of unresolved minor components contribute to the uncertainties. The figures of Table 6 are therefore not

TABLE 6. COMPOSITION OF NORMAL AND BRANCHED (ISOPRENOID) ACIDS ISOLATED FROM GREEN RIVER AND SERPIANO OIL SHALES (AS METHYL ESTERS)

Carbon Number	Normal acids (ppm) Serpiano	GRS	Branched acids (ppm) Serpiano	GRS
8	0·09			
9	0·23			
10	0·37			
11	0·38		0·04	
12	0·45	0·71	0·02	
13	0·40	1·35	0·03	
14	0·42	1·27	0·03	0·95
15	0·34	1·11	0·04	2·30
16	0·57	2·86	0·08	3·17
17	0·26	1·61	0·03	2·98
18	0·30	3·57	0·03	1·38
19	0·25	2·42	0·04	9·35
20	0·21	3·47	0·05	7·41
21	0·19	2·82	0·04	2·18
22	0·11	6·93	0·02	1·21
23	0·05	3·44		
24		5·61		
25		2·43		
26		7·48		
27		2·06		
28		9·65		
29		2·27		
30		11·63		
31		1·42		
32		5·56		
33		1·73		
34		1·40		

to be taken as very precise; however, they are internally consistent and should be comparable to the data for Green River Shale.

The mass spectra of the same isolated and synthetic esters have been recorded on a combined gas chromatograph-mass spectrometer and the relevant *m/e vs* abundance values are collected in Table 7. Values in heavy type are for the base peak, the molecular ion and those ions expected for fragmentation on either side of the Me branches. Every spectrum exhibited the expected molecular ion and base peak. Further, in most of the spectra, the ions spaced 28 mass units apart due to fragmentation at the Me branches, are more abundant than the ions, different by 14 mass units, which lie between them. In some of the spectra, however, this does not hold and we note, on careful examination of published spectra, that other workers have obtained similar results.[24] We believe that this is due, in part, to dead volumes in the gas chromatograph-mass spectrometer interface allowing contamination of one solute by another. A more important cause however, is likely to be due to non-resolved components in the gas chromatograph; inspection of the total ion monitor trace Fig 2 shows a hump which is not entirely due to baseline drift, but rather to unresolved components in the mixture. The spectrum of the C_{21} isoprenoid ester is

unsatisfactory since the ion at *m/e* 101 is strong, whereas it should be very weak; however, it is probable from both the gas chromatographic and other mass spectrometric data that this compound is indeed present. The spectrum of the C_{18} isoprenoid ester agrees well with that expected from the fragmentation patterns discussed above and also with a published spectrum.[7] Mass spectra were recorded of other peaks shown in the gas chromatogram of the isoprenoid ester fraction. A number of these spectra indicated that some gas chromatographic peaks represented more than one compound and that it was not possible to assign structures to the component compounds. However, some of the peaks gave reasonably good mass spectra for which structures, or part structures, have been proposed (Table 8)—they appear to have a "pseudo" isoprenoid structure in that they have Me branches at the correct isoprenoid spacing, but have longer end groups (Et, Pr, etc, unlike the isoprenoid esters which have i-Pr end groups). Thus the peak numbered 5 in Fig 2, which emerged just before the C_{17} isoprenoid ester, gave the spectrum shown in Fig 3a. This is a C_{17} ester (m. wt. 284) with a Me group substituted at C-3 since it has a base peak at *m/e* 101, a large peak (82%) at *m/e* 74 and a smaller peak (20%) at *m/e* 87. Branching at C_7 (and possibly C_{11}) is suggested by the abundance of the ions produced by fragmentation at these branches, giving as tentative structures for this compound either methyl 3,7-dimethylpentadecanoate or methyl 3,7,11-trimethyltetradecanoate. The spectrum

TABLE 7. SIGNIFICANT IONS IN THE MASS SPECTRA OF THE METHYL ESTERS OF ISOPRENOID ACIDS. RESULTS FOR SYNTHETIC ESTERS AND THOSE ISOLATED FROM GREEN RIVER SHALE (GRS) AND SERPIANO OIL SHALE (SERP)

C_{11} Isoprenoid ester			C_{16} Isoprenoid Ester					C_{21} Isoprenoid ester			
m/e	fragment	abundance	*m/e*	fragment	abundance			*m/e*	fragment	abundance	
		SERP			GRS	SYNTH	SERP			GRS	SERP
87	base pk.	**1000**	**87**	base pk.	**1000**	**1000**	**1000**	**87**	base pk.	**1000**	**1000**
101		60	101		34	20	75	101		86	750
115		**80**	**115**		**66**	**50**	**55**	**115**		**108**	**110**
157		**45**	**157**		**79**	**120**	**90**	**157**		**122**	**190**
171		26	171		10	10	8	171		22	110
185		7	**185**		**9**	**15**	**11**	**185**		**14**	**45**
			227		**6**	**10**	**55**	**227**		**15**	**50**
			241		12	10	8	241		6	30
			255		**3**	**7**	**3**	**255**		**7**	**15**
								297		**4**	**10**
								311		9	20
								325		**4**	**20**
135		20	135		22	25	30	135		23	80
153		30	153		39	30	35	153		42	90
			205		5	11	8	205		9	50
			223		6	10	6	223		9	40
								275		3	10
								293		3	20
143	M-57	75	213	M-57	54	72	42	283	M-57	55	95
200	M^+	**8**	**270**	M^+	**12**	**24**	**7**	**340**	M^+	**25**	**80**

Figures in heavy type are for the molecular ion, base peak and fragment ions at methyl branches.

TABLE 7—*continued*

C_{12} Isoprenoid ester			C_{17} Isoprenoid ester					C_{22} Isoprenoid ester		
m/e	fragment	abundance	*m/e*	fragment	abundance			*m/e*	fragment	abundance
		SERP			GRS	SYNTH	SERP			GRS
74	base pk.	**1000**	**74**	base pk.	**1000**	**1000**	**1000**	**74**	base pk.	**1000**
87		410	87		393	310	445	87		400
101		**160**	**101**		**149**	**130**	**230**	**101**		**189**
115		40	115		36	20	30	119		50
129		**105**	**129**		**184**	**175**	**225**	**129**		**229**
171		**85**	**171**		**25**	**15**	**35**	**171**		**29**
185		6	185		16	2	15	185		20
199		7	**199**		**18**	**11**	**25**	**199**		**37**
			241		**128**	**81**	**110**	**241**		**20**
			255		7	–	5	255		6
			269		**7**	**5**	**10**	**269**		**16**
								311		**94**
								325		1
								339		**7**
149		20	149		32	21	55	149		43
167		15	167		48	26	50	167		47
			219		8	4	15	219		11
			237		10	3	10	237		11
								289		4
								307		6
138	M-76	40	208	M-76	18	7	20	278	M-76	16
143	M-71	35	213	M-71	14	6	5	283	M-71	17
164	M-50	20	234	M-50	16	8	15	204	M-50	11
171	M-43	85	241	M-43	128	81	110	311	M-43	94
214	M^+	**19**	**284**	M^+	**39**	**28**	**23**	**354**	M^+	**54**

TABLE 7—*continued*

C_{13} Isoprenoid ester			C_{18} Isoprenoid ester		
m/e	fragment	abundance	*m/e*	fragment	abundance
		SERP			SERP
74	base pk.	**1000**	**74**	base pk.	**1000**
87		770	87		810
115		**110**	**115**		**205**
129		50	129		55
143		**75**	**143**		**115**
185		**20**	**185**		**25**
199		3	199		20
213		**8**	**213**		**30**
			255		**20**
			269		10
			283		**15**
163		90	163		80
181		13	181		55
			233		15
			251		20
152	M-76	140	222	M-76	285
288	M^+	**15**	**298**	M^+	**35**

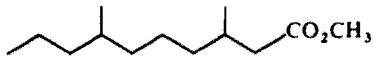
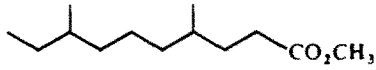
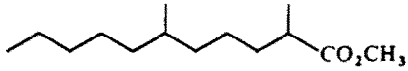
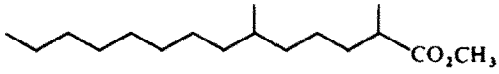
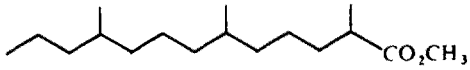
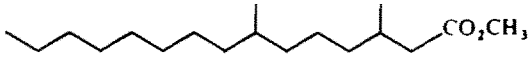
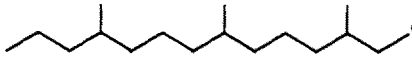
TABLE 7—*continued*

m/e	C_{14} Isoprenoid ester fragment	abundance		m/e	C_{19} Isoprenoid ester fragment	abundance		
		GRS	SERP			GRS	SYNTH	SERP
88	base pk.	**1000**	**1000**	**88**	base pk.	**1000**	**1000**	**1000**
101		426	400	101		333	430	360
129		**59**	**60**	**129**		**48**	**80**	**75**
143		11	30	143		11	20	30
157		**61**	**80**	**157**		**78**	**120**	**120**
199		**11**	**8**	**199**		**7**	**20**	**100**
213		–	5	213		4	10	90
227		**5**	**6**	**227**		**5**	**8**	**100**
				269		**3**	**11**	**65**
				283		1	–	20
				297		**3**	**10**	**45**
107		16	20	107		5	50	20
125		31	50	125		27	110	75
177		16	10	177		7	31	140
195		10	10	195		11	35	200
				247		1	10	70
				265		3	10	70
152	M-90	66	20	222	M-90	22	53	380
242	M^+	**38**	**19**	**312**	M^+	**40**	**84**	**295**

TABLE 7—*continued*

m/e	C_{15} Isoprenoid ester fragment	abundance		m/e	C_{20} Isoprenoid ester fragment	abundance		
		GRS	SERP			GRS	SYNTH	SERP
74		**816**	**870**	**74**		**728**	**610**	**680**
101	base pk.	**1000**	**1000**	**101**	base pk.	**1000**	**1000**	**1000**
143		**74**	**60**	**143**		**70**	**60**	**60**
157		8	25	157		9	10	20
171		**68**	**65**	**171**		**82**	**105**	**90**
213		**16**	**13**	**213**		**20**	**17**	**20**
227		3	3	227		1	3	–
241		**18**	**12**	**241**		**12**	**12**	**15**
				238		**13**	**11**	**15**
				297		5	5	10
				311		**14**	**14**	**12**
121		18	15	121		19	20	30
139		47	55	139		44	50	70
191		8	10	191		13	13	20
209		5	10	209		13	13	20
				261		3	5	–
				279		4	6	10
213	M-43	16	13	283	M-43	13	4	5
199	M-57	8	7	269	M-57	7	20	10
256	M^+	**42**	**12**	**326**	M^+	**38**	**65**	**15**

TABLE 8. METHYL ESTERS OF THE PROPOSED "PSEUDO" ISOPRENOID ACIDS FROM SERPIANO OIL SHALE

Peak number in Fig 2	Proposed methyl ester	Structure
1	3,7-dimethyldecanoate	
2	4,8-dimethyldecanoate	
3	2,6-dimethylundecanoate	
4	2,6-dimethyltetradecanoate	
	or	
	2,6,10-trimethyltridecanoate	
5	3,7-dimethylpentadecanoate	
	or	
	3,7,11-trimethyltetradecanoate	

(Fig 3b) of peak number 3 in the same gas chromatogram compares very favourably with that of methyl 2,6-dimethylundecanoate reported by Odham.[34, 35]

Isoprenoid acids in Green River Shale

The esters, obtained as described above, were analysed by gas liquid chromatography without further separation by urea clathration; their distribution is shown in Fig 4. Cuts taken from an equivalent preparative (3% SE-30) gas chromatographic separation for reanalysis by combined gas chromatography-mass spectrometry are also indicated in Fig 4. Each of the collected fractions 1–11 was separately analysed on a 1% SE-30 column in the combined instrument at temperatures (variously isothermal and programmed) which allowed better resolution than was obtained in the preparative chromatogram. The mass spectra of the esters from the Green River Shale are collected in Table 7, together with the spectra of the esters from the Serpiano Shale and those of authentic isoprenoid esters. Each ester from the Green River Shale shows the expected molecular ion and base peak, and again, the rules of fragmentation at the Me branches are followed; they also show good agreement with the spectra of the available synthetic esters.

Dicarboxylic acids in Green River Shale

A fraction of the Green River Shale acid esters, easily separable from the normal and isoprenoid esters by TLC, was analysed by combined gas chromatography-mass spectrometry. The methyl esters of two types of dicarboxylic acid appeared to be present in this fraction:—

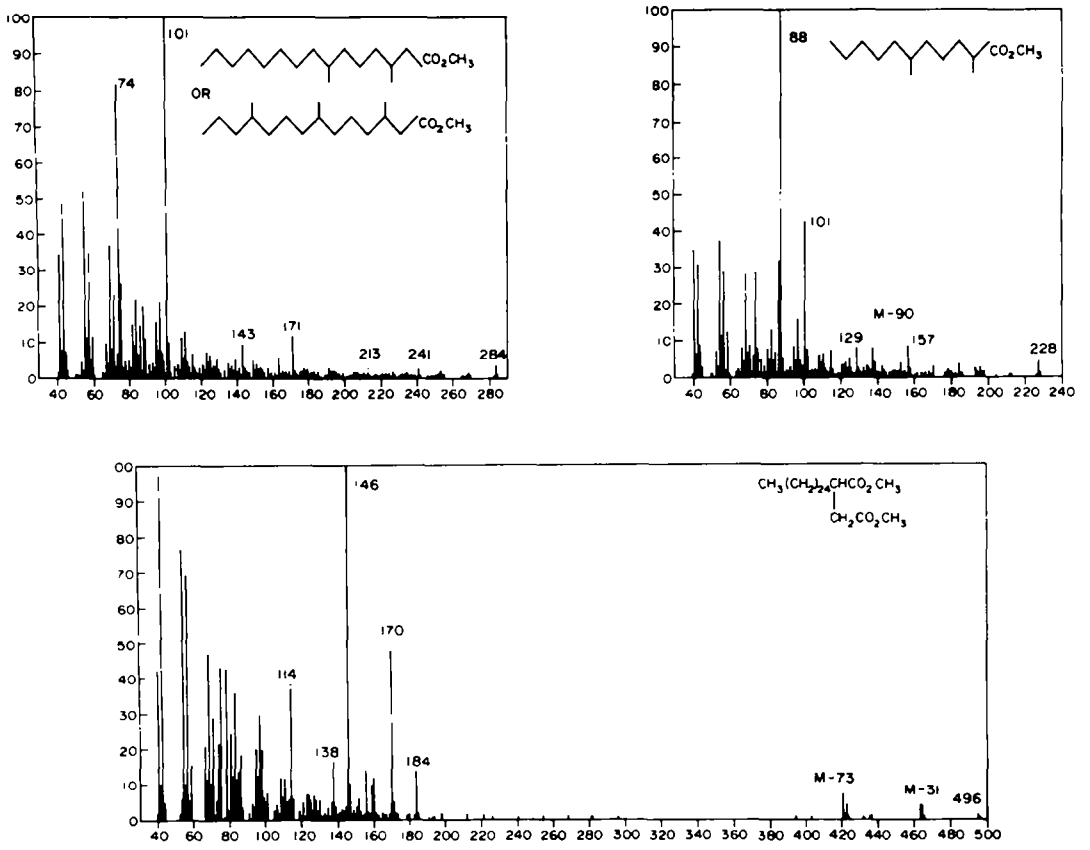


FIG 3. (a) Mass spectrum of the methyl ester of the "pseudo" isoprenoid acid from Serpiano Oil Shale (peak 5 in Fig 2) provisionally assigned the structure methyl-3,7-dimethylpentadecanoate or methyl 3,7,11-trimethyltetradecanoate.

(b) Mass spectrum of peak 3 in Fig 2, which we propose is methyl 2,6-dimethylundecanoate.

(c) Mass spectrum of the methyl ester of the acid provisionally assigned as 3-methoxycarbonyloctacosanoic acid, from Green River Shale

(i) A series of α,ω-diacids $CH_3O_2C(CH_2)_nCO_2CH_3$ in the range C_{11}–C_{32}. The spectra, in general, agreed with those previously published[5,40a,b] and showed the characteristic peaks tabulated by Ryhage and Stenhagen;[40a] the spectra of the C_{17}, C_{28} and C_{31} diacids were less satisfactory since they contained fragments due to contaminating acids.

(ii) Another series of dicarboxylic acids $CH_3(CH_2)_nCH(CO_2CH_3)CH_2CO_2CH_3$, ranging from C_{17} to C_{33}. The mass spectrum shown in figure 3c is that of the dimethyl ester of the C_{29} acid, which we believe to be 3-carboxy octacosanoic acid, since the fragmentation pattern shown is in accordance with that discussed and presented by Ryhage[40b] for the esters of such acids. Similar fragmentation patterns were found for the spectra recorded during scans of the various GC peaks and correspond to the C_{17}–C_{21}, C_{23}–C_{31} and C_{33} members of the series although the spectra for the C_{17}, C_{18}, C_{19}, C_{20} and C_{23} homologues contained additional fragments.

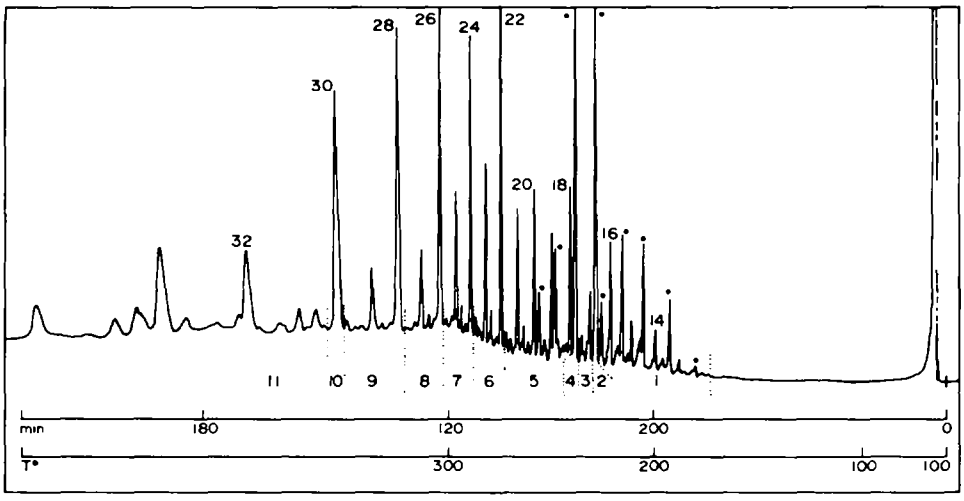


FIG 4. Gas chromatogram of the methyl ester fraction of the total acids of the Green River Shale from Sulphur Creek. Conditions: column 20 ft × 0·04 in (i.d.) containing 3% OV-1 on 100–120 mesh Gas Chrom Q; temperature programmed from 100–300° at 2°/min after 20 min isothermal at 100°; nitrogen 48 psi. Fractions marked 1–11 show where cuts were taken, for GC–MS analyses, from an equivalent preparative gas chromatographic separation. Normal acids are numbered according to carbon number. The starred peaks correspond to the methyl esters of the C_{14}–C_{22} isoprenoid acids

Normal fatty acids in Green River and Serpiano Oil Shales

In both of these shales n-acids were present; they were determined by the mass spectrometric and gas chromatographic behaviour (retention data and coinjection of standards) of the methyl esters.

A separate isolation, in which losses were minimized, was used for the quantitative determination of the Serpiano Oil Shale esters. Straight chain esters were determined from the gas chromatograms of the esters before they were subjected to urea clathration. Methyl laurate served as a calibration standard. For Green River Shale relative abundances of n- and isoprenoid acids were obtained by measuring peak heights and comparing them with the peak heights of standard n-C_{16}, C_{18}, C_{20}, C_{22} and C_{30} esters. A 5 ft × $\frac{1}{8}$ in column containing 3% OV-1 (which gave 2,400 theoretical plates for n-C_{22}) was used, from which all of the esters to n-C_{32} were eluted during the linear programme. The range and amount of acids present is given in Table 6.

DISCUSSION

There have been no previous reports of fatty acids in Serpiano Oil Shale; on the other hand, Green River Shale has been examined by a number of workers. Normal,[3,4,42,43,44] *iso*-,[43] *anteiso*-[43] and isoprenoid[3–7] acids have been variously reported. Burlingame and Simoneit noted that the C_{19} and C_{20} isoprenoid acids were the main constituents of a branched acid fraction covering the C_8–C_{22} range in a sample of the shale from Parachute Creek[6] and subsequently,[24,44] using low and high resolution mass spectrometry, they reported the presence of isoprenoid moieties

in an oxidised fraction of the kerogen (i.e. the complex organic macrostructure which is insoluble in common organic solvents) from the same shale sample. Although this last oxidative procedure may give equivocal results with respect to the occurrence in the kerogen of these isoprenoid acids *per se*, it undoubtedly confirms the presence of isoprenoid skeletons within the kerogen structures. We had previously found 2,6,10,14-tetramethylpentadecanoic (pristanic) acid and 3,7,11,15-tetramethylhexadecanoic (phytanic) acid in a demineralised sample of the shale from Sulphur Creek[3] and subsequently announced[4] the isolation of a further number of these acids that occurred both free and bound to the kerogen. Recently, Murphy *et al*[7] have used a GC–MS computer system to analyse Green River Shale acids. Mass spectra were recorded, at four second intervals, for every point on the chromatogram and selected spectra were compared with those of available authentic substances stored on magnetic discs. Isoprenoid acids, which were identified by their 2-, 3-, 4-, or 5-methyl substituents, ranged from C_{14}–C_{26}, though the C_{23} compound was absent.

The mass spectrum that we have obtained for the ester of the C_{18} isoprenoid acid isolated from the Serpiano Oil Shale is satisfactory but the spectra for the corresponding GC peaks for both samples of Green River Shale contained many spurious fragments. Even so, we believe that this acid is present in our samples and that the better results obtained with the Serpiano esters indicate that it is preferable to bring about a separation of normal and branched esters by urea clathration prior to GC–MS study.

Murphy *et al.*,[7] in reporting the occurrence of the C_{18} acid in a sample of the Green River Shale, suggest that it is formed by an essentially biochemical process, prior to deposition in the sediment. Two pathways were put forward, starting either with a squalane derivative or with pristanic acid; β-oxidation of the latter giving either 4,8,12-trimethyltridecanoic acid or the C_{18} acid (6,10,14-trimethylpentadecanoic acid), depending on whether β-oxidation takes place at the methyl or methylene group. It is of interest to note that Stokke[45] has found no evidence of β-methyl oxidation but only of β-methylene oxidation, when *in vitro* oxidation of 2,5-dimethylheptanoic acid with guinea-pig kidney slices gave 3-methylvaleric acid and not 5-methylheptanoic acid. Also, Maxwell and Cox[46] have shown that oxidation of pristane with *Mycobacterium fortuitum* gives 2,6,10,14-tetramethylpentadecanoic and, 4,8,12-trimethyltridecanoic acid and lower acids, but not, apparently, the C_{18} (6,10,14-trimethylpentadecanoic) acid. Furthermore, if a biochemical oxidation of pristanic acid can take place, one might ask why the C_{18} acid has not been found in biological systems but only in sediments which appear to have undergone considerable diagenetic change.

If the isoprenoid acids are formed by post-depositional oxidation of suitable precursors one can argue for a greater diagenetic change in the constituents of the older Triassic shale, where the ratios of the C_{20}/C_{18} or C_{20}/C_{17} isoprenoid acids are considerably less than they are in the younger Green River Shale. More extensive geochemical alteration is also evident in a comparison of the even/odd relationships of the normal fatty acids (Table 6). In our samples of the Green River Shale the ratio is 2·6:1 whereas in the Serpiano sample the ratio is 1·2:1. Furthermore, the distributions of the normal acids might be taken as evidence for marine and non-marine environments of deposition, respectively; essentially low molecular weight acids in Serpiano Shale and high molecular weight acids in Green River Shale. The relative

contributions of diagenesis, maturation and the originating source material cannot be assigned at this stage.

Blumer and Cooper[2] have established the presence in Recent sediments of the C_{16}, C_{19} and C_{20} isoprenoid acids. Other isoprenoid acids were not detected and if present at all they were at least a factor of ten less abundant. In spite of the obvious diagenetic changes which have affected the acid distribution in the Serpiano Oil Shale, these three most abundant acids of Recent sediments still predominate. This and the continued though slight predominance of the even carbon numbered straight chain acids suggests a mild diagenetic history for the organic matter of the Serpiano Oil Shale. The considerable abundance of the C_{19} and C_{20} isoprenoid acids in Green River Shale, together with the strong even/odd predominance in the series of n-acids, also suggests a mild thermal history, in agreement with evidence previously deduced from different criteria.[14,15]

The occurrence in Serpiano Shale of what we have termed "pseudo" isoprenoid acids may be of considerable geochemical significance. As far as we are aware such acids are rare in nature, although some have been found in the feather waxes of birds;[34,35] indeed, 2,6-dimethylundecanoic acid has been found in the preen gland of the oyster-catcher[35] and eider duck.[34] Whether the acids in Serpiano Shale are true biological remnants, or whether they are formed during sedimentary maturation processes is, we believe, still an open question. Rationalisation might be possible if these acids were to be found in a number of very old, as against younger, sediments.

We have previously reported α,ω-dicarboxylic acids in the algal deposit torbanite[4] (Carboniferous), and Haug *et al* have found unbranched C_{12}–C_{18} α,ω-dicarboxylic acids and three 2-methyl α,ω-dicarboxylic acids in Green River Shale.[5] In our samples, the α,ω-acids are present as a homologous series extending from C_{11} to C_{32}. Since our previous discussion[4] on the possible sources of these acids, we have found a similar series in the alga *Botryococcus Braunii*,[47] the supposed precursor of torbanite. The C_{28} and C_{30} acids have recently been reported to occur in spores of *Equisetum*, the C_{30} acid constituting 1% of the dried spores.[48]

Substituted succinic acids are not common in nature and their probable occurrence in the range C_{17} to C_{33}, in Green River Shale is of considerable interest. We do not, for the moment, intend to speculate on their origin, but would note in passing that if they are formed by the post-depositional oxidation of some suitable precursor (e.g. a n-alkylcyclic compound susceptible to oxidative attack on the ring) then further oxidation would give a corresponding alkylmalonic acid, the facile decarboxylation of which would provide a normal fatty acid. The relationship of normal and alkyl dicarboxylic acids might therefore be useful as a parameter in studies on oxidative diagenesis.

No attempt has been made in the present work to define the stereochemistry of the branched acids examined. Such studies will be an essential part of attempts to define the origin of the acids. Preliminary data obtained from a gas chromatographic analysis of the (−)-menthyl esters of the major isoprenoid acids in the Green River Shale indicates that these acids have absolute stereochemistries compatible with a chlorophyll origin.[12] Degradative and synthetic studies, designed to prepare pure, stereochemically defined individual Me branched acids, will be necessary to provide conclusive stereochemical identification of these acids herein provisionally or firmly identified. The next phase of the organic geochemical study of the carboxylic acids

present in, and obtainable from, Recent and ancient sediments will require careful examination of the components of present-day environments and the reactions proceeding during diagenesis and maturation. Only then will it be possible to unravel the various pathways by which the great variety of acids not known to be present in the sediments have been derived. Extended speculation cannot be justified at the present stage.

EXPERIMENTAL

All solvents were of Analar grade and were distilled prior to use, through a 18 in. Vigreux column packed with glass helices. Woelm alumina (grade I, neutral) and Davison silica gel (grade 922, through 200 mesh) were used for column chromatography and Kieselgel G for TLC. Analytical plates were 0·25 mm thick; they were visualized by spraying with 50% H_2SO_4 and baking at 230° for about 20 min. Preparative plates were 1·0 mm thick and were visualized by spraying with a 0·001% ethanolic soln of Rhodamine 6 G. The LKB 9000 gas chromatograph-mass spectrometer were operated as follows:—injector 260°, molecular separator 280°, electron energy 70 eV. Conditions for gas chromatographic analyses are noted in the Fig legends or in the following experimental section. IR spectra were measured in solution in carbon tetrachloride.

Green River Shale

Demineralisation, hydrolysis and extraction. Green River Shale (320 g, from the 1900 ft level at Sulphur Creek, Colorado), was broken into small pieces (1–1½ in) and cleaned ultrasonically in benzene/MeOH (1:1, 5 min). The shale was washed twice more with clean solvent, dried at room temp and pulverised in a disc mill (TEMA) to pass 200 mesh. The powdered shale (300 g) was demineralised by vigorously stirring portions (25 g) with 5N HCl (45 ml) for 30 min and then setting the mixture aside. After 3 days, 40% HF (30 ml) was added to each portion, they were allowed to stand for 10–14 days and then filtered. Each portion of shale was thoroughly washed with distilled water (300 ml) combined and boiled under reflux for 48 hr with methanolic KOH (8%, 1200 ml). The cooled alkaline mixture was acidified to pH 1 with dil H_2SO_4 acid, divided into 8 portions and centrifuged. The supernatant aqueous layers were extracted with benzene and the residual portions of shale were extracted ultrasonically with benzene/MeOH (3:1, 100 ml, ×3); the combined extracts were evaporated under reduced pressure to provide a dark gum (9·4 g).

Isolation and methylation of the carboxylic acids. The above gum in ether (60 ml) was applied to a silica gel-KOH column according to the procedure of McCarthy and Duthie.[25] Neutral lipids were eluted with ether (350 ml) and the acids were obtained by eluting with ether (200 ml) containing 2% formic acid, followed by ether alone (200 ml). The residue obtained on evaporation of the acid eluate was methylated by boiling under reflux for 12 hr with a mixture containing MeOH/benzene/H_2SO_4 (20:10:1·5, 30 ml). This mixture was diluted with water (10 ml) and extracted with n-hexane (10 ml, ×3); the hexane eluates were combined, washed with water (5 ml, ×2), evaporated and dried azeotropically with MeOH to furnish a crude ester fraction. Chromatography on an alumina column (7 × 1 in) using ether/n-hexane (2:3) as eluant gave esters in the first three 100 ml fractions, these were shown by TLC against standards to contain mono- and dicarboxylic esters and some hydrocarbon. Each fraction was separated by preparative TLC using standard long-chain hydrocarbons and esters as markers.

Recovery of the mono-esters afforded a waxy solid (74 mg, equivalent to 0·025% of esters based on 300 g of shale) with IR absorption bands at 2912, 2840 (s, νCH for CH_2 and CH_3), 1741 (s, νC=O for CO_2Me), 1458, 1429, 1372, (m, δCH for CH_2 and CH_3), 1252, 1165 and 1010 cm^{-1} (m–w, νC—O). The ratio of the optical densities νC=O(1741)/νCH(2840) was 1·04, compared with methyl stearate (1·00), regarded as standard. Recovery of the diesters from a broad band afforded a solid (27 mg equiv to 0·009% based on 300 g of shale) with IR absorption bands at 2930, 2860, 1743, 1461, 1440, 1380, 1368, 1250, 1200, 1170 and 1015 cm^{-1}.

GLC and GC–MS of esters. GLC of the above mono-carboxylic acid ester fraction indicated that it was a complex mixture of esters ranging from about n-C_{12} to above n-C_{32} (operating conditions shown in legend of Fig 4); the positions of the n-C_{14}, n-C_{16} and n-C_{18} esters were confirmed by coinjection of standards. The mixture was separated into eleven fractions by preparative GLC at the points indicated

in the analytical chromatogram, Fig 4. (Operating conditions for preparative GLC were: column 10 ft × ¼ in, containing 3% SE-30 on 100–120 mesh silanized Gas Chrom Z, helium 60 ml/min, temp programmed from 100–300° at 4°/min). The esters in each of the eleven fractions were examined and some were identified, by combined GC–MS; findings are summarised in Table 6. Operating conditions for the LKB 9000 combined gas chromatograph-mass spectrometer were as follows; column 10 ft × ⅛ in i.d. containing 1% SE-30 on 100–120 mesh Gas Chrom Q, helium 35 ml/min oven conditions for fractions 1–11 were thus: frs. 1 and 3, 80°–160° at 3°/min; frs. 2 and 4, isothermal at 135°; fr. 5 100°–200° at 3°/min; frs. 6–11 isothermal at 175°, 200°, 200°, 210°, 210° and 240° respectively.

The LKB 9000 was operated as follows, using the same chromatographic column, for analysis of the diesters. Column temp programmed from 100–280° at 3°/min, helium 30 ml/min.

Serpiano Oil Shale

Extraction and methylation of the carboxylic acids. Serpiano Oil Shale (from horizon S-XVIII of the Grenzbitumen, Anisian) was prepared and ground to pass 200 mesh, as described above. Soxhlet extraction of the powdered shale with benzene/MeOH azeotrope afforded an oil which was dissolved in the minimum quantity of $CHCl_3$ and esterified with BF_3/MeOH (50°, 15 min). The dried residual shale was suspended in anhyd MeOH/HCl (40°, 2 days), and the supernatant liquid removed and added to the benzene/MeOH extract of the shale. This extract was diluted with water, extracted with $CHCl_3$, dried (Na_2SO_4), evaporated and treated with BF_3/MeOH (to esterify acids not already esterified by MeOH/HCl). The combined ester fractions were dissolved in $CHCl_3$, washed, dried (Na_2SO_4) and extracted with cyclopentane, dried (Na_2SO_4) evaporated, re-extracted with n-pentane and chromatographed on silica gel (deactivated with 10% water). Pentane, followed by pentane containing 10%, 20% and 30% of benzene were used as eluants; the presence of esters in the eluates was established by IR spectroscopy. Because of their lower overall polarity, higher mol wt esters elute early. The resulting spread of the isoprenoid esters over several eluate fractions limits the degree to which they can be purified by column or TLC.

Separation of the isoprenoid acid esters by urea adduction. The unbranched esters were removed by forming their clathrates with urea. To the esters (~ 10 mg) in a mixture of pentane (4 ml) and acetone (2 ml), MeOH (0·5 ml) containing urea (10% w/v) was added; the solvents were removed at room temp on a rotary evaporator. The non-adducted esters were recovered by washing the crystalline adducts with n-pentane; they were then subjected to two additional adduction experiments. Normally, the third adduction removed only traces of additional straight chain or monobranched esters. The adducts were decomposed with hot water and the unbranched esters were recovered by extracting with pentane. GLC of this ester fraction indicated that it was very complex; some separation was provided by preparative TLC.

TLC and GLC of ester fractions. The ester mixture was charged to a preparative TLC plate and developed with n-hexane/ether (95:5). After visualization, four bands were removed at the following distances from the origin: band I (8·6–5·5 cm), band II (5·5–3·6 cm) band III (3·6–0·35 cm) and band IV (0·35–0 cm). They were extracted with ether and the extracts were filtered through a short column of alumina:—

Band I (3·1 mg). Predominantly hydrocarbon mixture with IR absorption bands at 2930, 2865, 1460, 1360 cm^{-1}. A weak broad band at 1710 cm^{-1} is unaccountable, and a weak band at 1740 cm^{-1} may be due to contaminating ester from band II.

Band II (1·0 mg). The IR spectrum of this fraction showed the characteristic absorption bands of long chain fatty acid methyl esters at 2960–2850, 1743, 1460, 1370, 1190 and 1170 cm^{-1}.

Bands III (1·0 mg) and IV (1·0 mg). The IR spectra of these fractions, taken together with their R_f values suggested that oxygenated molecules more polar than the fatty acid esters were present. They have not yet been investigated.

Gas chromatographic analyses of these esters were accomplished on columns from 6–12 ft. long, (i.d. 0·055 in) packed with 0·8% Apiezon L on 80–100 mesh silanized Chromosorb G or 25% FFAP on 80–100 mesh silanized Chromosorb W. Due to the extensive bleeding of this FFAP column at the temp of elution of the C_{20} and higher esters, another column was packed with a mixture (1:3) of the 25% FFAP packing and inert silanized (100–140 mesh) glass microbeads. This column was more efficient than one prepared with less liquid phase. Chromatograms were programmed at 2–4°/min and N_2 flow rates (*ca* 10 ml/min), were chosen for maximum plate efficiency.

Reference compounds

3,7,11-Trimethyldodecanoic acid was prepared from 3,7,11,15-tetramethylhexadeca-2,4-diene by ozonolysis and oxidation with alkaline H_2O_2 as previously described.[49]

In an analogous manner, 4,8,12-trimethyltridecanoic acid was prepared from 3,7,11,15-tetramethylhexadeca-1,3-diene (*cis* and *trans*) and 5,9,13-trimethyltetradecanoic acid was prepared from 7,11,15-trimethyl-3-methylenehexadeca-1-ene.

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