591. *Fatty Acids. Part VIII.* The Partial Oxidation of Unsaturated Acids with Performic Acid, and a New Method of Determining the Structure of Non-conjugated Polyethenoid Acids.*

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The partial oxidation of a range of unsaturated fatty acids with performic acid has been examined. Further degradation of the reaction product provides an elegant method of determining the structure of non-conjugated polyunsaturated acids.

PARTIAL oxidation of unsaturated fatty acids has been studied by us with two main objectives: the development (i) of an improved method of determining the position of unsaturated centres in polyunsaturated acids and (ii) of a reagent for distinguishing between methylene-interrupted polyene acids such as linoleic and linolenic, and monoethenoid acids such as oleic. The attainment of the first objective is described in this paper and some results in connection with the second will be reported subsequently.

Determining the position of unsaturated centres in olefinic acids has always been an important procedure in the study of such acids and renewed interest in it has followed the development of new isolation techniques which permit the separation of highly unsaturated

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* Part VII, preceding paper.

acids present in fish glycerides and in phospholipids, and also the recognition that most lipids contain minor proportions of hitherto unrecognised acids. Although James[1] has demonstrated that chain length and the extent of unsaturation can be derived from gas-liquid chromatographic data alone there remains the task of identifying the position of the unsaturated centres in newly discovered natural acids and in acids subjected to reactions in which double-bond migration occurs. This information is almost always obtained by oxidative fission, and recent work has been directed mainly towards more selective oxidation of the unsaturated centres, freedom from secondary degradation fragments, and improved procedures for separating and identifying the oxidation products.

The reagents most widely used for this purpose include potassium permanganate in acetone and/or acetic acid solution,[2-4] potassium periodate and a trace of potassium permanganate (von Rudloff's method),[5-7] and ozone.[4,9,10]

The ideal method of structure determination must satisfy the following criteria, which, it is considered, none of the above methods does as closely as the procedure to be described below: (*a*) the experimental procedure should be as simple as possible and oxidative fission should not be accompanied by secondary degradation; (*b*) the method should be applicable to a wide range of structures; (*c*) the results should provide the maximum amount of information concerning the unsaturated acid and should lead to an unequivocal structure.

Some methods, suitable for monoethenoid acids, do not give unambiguous results with polyethenoid acids. The ambiguity can sometimes be resolved by other information, by degradation of the esters under non-hydrolytic conditions,[11] by degradation before and after chain-extension,[12] or by degradation after reduction of the acid to its alcohol by lithium aluminium hydride.[13] Difficulties of interpretation also arise with unsaturated epoxy-acids,[14] and with enynoic acids,[15] which call for modification of the usual procedures or for additional experimental results.

Compared with some of the procedures used previously, partial oxidation is a somewhat elaborate technique but it meets the remaining criteria (*b*) and (*c*) much more effectively (the approximate molecular weight is a useful but not essential datum).

Initial experiments with ozone as oxidant were discontinued when better results were obtained with performic acid. Certain difficulties in the hydroxylation of polyethenoid acids by performic acid[16] are not apparent when such acids are treated with the smaller proportion of peracid used in our experiments. The unsaturated acid is submitted to the following treatment: (*a*) hydroxylation with $\not>0{\cdot}6$ mol. of performic acid, giving a mixture of the possible dihydroxy-acids, a trace of more extensively oxidised acid, and unchanged starting material; (*b*) catalytic hydrogenation of the remaining unsaturated centres; (*c*) oxidative cleavage, by von Rudloff's reagent, of the saturated dihydroxy-acids

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[1] James, *J. Chromatog.*, 1959, **2**, 552.

[2] Armstrong and Hilditch, *J. Soc. Chem. Ind.*, 1925, **44**, 43T.

[3] (*a*) Begemann, Keppler, and Boekenoogen, *Rec. Trav. chim.*, 1950, **69**, 439; (*b*) Keppler, *ibid.*, 1957, **76**, 49.

[4] James and Webb, *Biochem. J.*, 1957, **66**, 515.

[5] Lemieux and von Rudloff, *Canad. J. Chem.*, 1955, **33**, 1701; Jones and Stolp, *J. Amer. Oil Chemists' Soc.*, 1958, **35**, 71.

[6] Tulloch and Ledingham, *Canad. J. Microbiol.*, 1960, **6**, 425; Hopkins and Chisholm, *J. Amer. Oil Chemists' Soc.*, 1960, **37**, 682.

[7] von Rudloff, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 126.

[8] Huber, *J. Amer. Chem. Soc.*, 1951, **73**, 2730.

[9] Benton, Kiess, and Harwood, *J. Amer. Oil Chemists' Soc.*, 1959, **36**, 457.

[10] Stoffel and Ahrens, (*a*) *J. Amer. Chem. Soc.*, 1958, **80**, 6604; (*b*) *J. Lipid Res.*, 1960, **1**, 139.

[11] Klenk and Bongard, *Z. physiol. Chem.*, 1952, **290**, 181, and later papers by Klenk *et al.* in that journal.

[12] Whitcutt and Sutton, *Biochem. J.*, 1956, **63**, 469.

[13] Gunstone and Morris, *J.*, 1959, 2127.

[14] Gunstone, *J.*, 1954, 1611.

[15] Gunstone and Russell, *J.*, 1955, 3782.

[16] Swern and Dickel, *J. Amer. Chem. Soc.*, 1954, **76**, 1957; McKay, Levitin, and Jones, *ibid.*, p. 2383.

to give a mixture of mono- and di-basic acids; and (*d*) the identification of these by gas-liquid chromatography.

By these steps arachidonic acid, for example, furnished five monobasic acids (C_6, C_9, C_{12}, C_{15}, and C_{20}) and four dibasic acids (C_{14}, C_{11}, C_8, and C_5). The arachidic acid (C_{20}) arises from unoxidised arachidonic acid and indicates the chain length of the acid under consideration; the remaining acids form four pairs which arise from fission at unsaturated centres in the 5,6-, 8,9-, 11,12-, and 14,15-positions. It follows that arachidonic acid is eicosa-5,8,11,14-tetraenoic acid. (In fact, the dibasic acids are accompanied by a minor amount of azelaic acid; since there is no C_{11} monobasic acid to pair with this it is concluded that this arises from bis-oxidation at the 5,6- and 14,15-centres.)

Products obtained by partial oxidation (mole %).

	Unoxidised	Monobasic oxidation products									
Acid	acid	C_{15}	C_{13}	C_{12}	C_{11}	C_{10}	C_9	C_8	C_7	C_6	C_5
Monoethenoid [a]											
Tetradec-7-enoic	44								28		
Hexadec-10-enoic	49									24	
Oleic	64						16	Tr			
Vernolic										28	
Polyethenoid [a]											
Linoleic	76						6·1			3·6	
Octadeca-7,11-dienoic	58				10·3				8·9		
γ-Linolenic	66			7·2			5·9			3·8	
Nonadeca-10,13-dienoic [b]	69 + 6						3·3			2·5	
Eicosa-7,13-dienoic	57		11·1						9·4		
Arachidonic	80	4·4		2·2			2·7			1·0	
Conjugated											
Hexadeca-8,10-dienoic [a]	55						Tr	9·6	1·7	6·2	
Octadeca-9,11-dienoic [c]	79					0·1	3·8	0·4	2·2		
Santalbic	95						0·5	0·5	0·4		
α-Elæostearic [d]	52					1·7	4·0	2·0	1·9	1·1	2·0
β-Elæostearic [d]	55					1·9	3·0	2·3	1·6	1·2	1·6
Punicic [d]	48					1·2	2·3	2·8	1·2	0·7	1·2
Octadeca-8,10,12-trienoic [e]	67				Tr	1·5	2·4	1·1	0·6	1·5	

	Dibasic oxidation products										
Acid	C_{14}	C_{13}	C_{12}	C_{11}	C_{10}	C_9	C_8	C_7	C_6	C_5	C
Monoethenoid [a]											
Tetradec-7-enoic								28			
Hexadec-10-enoic					27						
Oleic						20					
Vernolic			46			26					
Polyethenoid [a]											
Linoleic			6·2			8·3					
Octadeca-7,11-dienoic				16·6				6·6			
γ-Linolenic			5·2			6·1	Tr		5·7		Tr
Nonadeca-10,13-dienoic [b]		8·0	0·8		9·2	1·3					
Eicosa-7,13-dienoic		12·1				10·4					
Arachidonic	1·6			2·5		1·0	3·4			1·2	
Conjugated											
Hexadeca-8,10-dienoic [a]					11·6	2·0	13·9	Tr			
Octadeca-9,11-dienoic [c]				6·3	0·6	6·8	0·4				
Santalbic				2·7	0·5	0·8					
α-Elæostearic [d]		4·0	2·3	2·5	1·3	23·2	1·9				
β-Elæostearic [d]		3·0	2·5	2·1	1·5	21·8	2·9				
Punicic [d]		4·3	5·1	3·5	2·4	25·5	1·7				
Octadeca-8,10,12-trienoic [e]			1·0	2·4	1·5	2·2	18·6	Tr			

[a] These acids have the *cis*-configuration. [b] This acid contains 10% of linoleic acid. The *cis*-9,*trans*-11-isomer. [d] For configuration see text. [e] The all-*trans*-isomer. Tr = Trace.

The results which have been obtained are set out in the Table and attention is drawn to the following points:

(i) Non-conjugated ethylenic acids with up to four unsaturated centres behave very satisfactorily and there seems no reason why the method should not be successfully applied to even more highly unsaturated compounds. In all cases the expected products are formed and recognised and there is little or no evidence of secondary oxidation or of bis-oxidation of polyethenoid compounds. Any differential reactivity of the double bonds in a polyethenoid acid is too small to be detected by the experimental procedure employed.

(ii) The method is not likely to be invalidated by the presence of other functional groups or novel features in the unsaturated acids and in some cases additional valuable information is obtained. With epoxy- or *vic*-dihydroxy-acids, for example, the position of the functional group is indicated as well as the positions of unsaturation (see vernolic acid). Experiments have not been carried out with hydroxy-, keto-, or branched-chain acids but no difficulty is envisaged beyond that of recognising the rather unusual degradation fragments. Partial oxidation with peracids will also distinguish between ethylenic and acetylenic bonds since peracids react preferably with olefinic centres.[15,17] This has been confirmed in the partial oxidation of mixtures of acids [18] and in the reaction with santalbic acid (octadec-11-en-9-ynoic) even though conjugated systems are less amenable to treatment in this way. The 9,11-dienoic acid is cleaved mainly at C_9–C_{10} and C_{11}–C_{12}, but with the enynoic acid fission at C_{11}–C_{12} exceeds that at C_9–C_{10}, confirming the structures previously assigned to this acid.[15]

(iii) The results with conjugated acids do not lead to definite conclusions but are interesting nevertheless. The results for the stereoisomers, α-elæostearic (*cis*-9,*trans*-11,13), β-elæostearic (*trans*-9,11,13), and punicic (*cis*-9,*trans*-11,cis-13) acid, show no significant difference from one another and it is concluded that *cis*- and *trans*-isomers behave similarly. By comparison with the non-conjugated acids the conjugated acids afford additional products. Thus elæostearic acid gives six dibasic acids (C_8–C_{13}) and six monobasic acids (C_5–C_{10}); of these the C_9, C_{11}, and C_{13} dibasic acids and the C_5, C_7, and C_9 monobasic acids are expected, and the presence of the C_{10} and C_{12} dibasic and C_6 and C_8 monobasic acids is perhaps not surprising in view of the lability of conjugated systems, but the appearance of the C_8 dibasic acid and C_{10} monobasic acid is more difficult to explain. This could indicate contamination of the 9,11,13-trienoic acid with some 8,10,12-isomer but it is more likely that these result from side reactions, particularly as this behaviour was repeated with several different preparations of elæostearic acid, including a synthetic one, and with the natural 8,10,12-trienoic acid which gave traces of the unexpected C_7 dibasic acid and C_{11} monobasic acid.[19,20] The most unexpected feature of these results is the very high yield of azelaic acid from the 9,11,13-trienoic acids and of suberic acid from the 8,10,12-trienoic acid.

EXPERIMENTAL

Absorption spectra were determined with a Unicam quartz spectrophotometer, ethanol being the solvent. Log ε values are quoted in parentheses.

Materials.—Oleic and linoleic acid were pure samples of natural origin presented by Unilever Ltd. γ-Linolenic and arachidonic acid were synthetic products presented by Roche Products Ltd. Hexadeca-8,10-dienoic, octadeca-7,11-dienoic, and eicosa-7,13-dienoic acids were synthesised,[21] as was hexadec-10-enoic acid.[22]

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[17] Raphael, "Acetylenic Compounds in Organic Synthesis," Butterworths Scientific Publns., London, 1955, p. 33.

[18] Gunstone and Sykes, following paper.

[19] Chisholm and Hopkins, *Canad. J. Chem.*, 1960, **38**, 2500.

[20] McLean and Clark, *J.*, 1956, 777.

[21] Gunstone and Sykes, preceding paper.

[22] Baker and Gunstone, unpublished work.

Tetradec-7-enoic [23] was prepared by the procedures described in Part VII,[21] by condensation of oct-1-yne with 1-chloro-5-iodopentane followed by reaction with sodium iodide, sodium cyanide, and potassium hydroxide, and semihydrogenation in the presence of Lindlar's catalyst.

Vernolic acid (m. p. 24—28°; lit.,[24] 25—28°) was isolated from the mixed acids of *Vernonia anthelmintica* seed oil by the batch-adsorption method of Morris, Hanes, and Holman.[25]

Nonadeca-10,13-dienoic acid resulted from Arndt–Eistert chain extension of pure linoleic acid.[12] Gas chromatography of the hydrogenated product indicated that the homolinoleic acid contained about 10% of unchanged linoleic acid.

Santalbic acid, $\lambda_{\text{max.}}$ 2290 Å (4·20), $\lambda_{\text{infl.}}$ 2400 Å [lit.,[15] 2290 (4·22) and 2400 Å], was available as a specimen derived from *Santalum album* seed oil. Semihydrogenation with Lindlar's catalyst yielded octadeca-9,11-dienoic acid, $\lambda_{\text{max.}}$ 2310 Å (4·36), $\lambda_{\text{infl.}}$ 2290 and 2390 Å [lit.,[15] 2310 (4·38), 2400].

α-Elæostearic acid, m. p. 46—47°, $\lambda_{\text{max.}}$ 2610 (4·54), 2710 (4·66), and 2820 Å (4·56) [lit.,[26] 48°, 2610 (4·56), 2710 (4·67), and 2810 Å (4·58)], and β-elæostearic acid, m. p. 70·5—71°, $\lambda_{\text{max.}}$ 2580 (4·66), 2680 (4·77), and 2790 Å (4·67) [lit.,[26] 72°, 2590 (4·67), 2680 (4·79), and 2790 Å (4·69)], were prepared from tung oil by the method of Crombie and Jacklin.[26] Punicic acid was isolated from pomegranate seed oil by using the same authors instructions; it melted at 41—42° and had $\lambda_{\text{max.}}$ 2640 (4·55), 2740 (4·67), and 2850 Å (4·56) [lit.,[26] m. p. 43·5—44°, $\lambda_{\text{max.}}$ 2640 (4·54), 2740 (4·66), and 2850 Å (4·56)].

Octadeca-8,10-12-trienoic acid was obtained from Scots Marigold seed (*Calendula officinalis*) by the method of McLean and Clark.[20] It melted at 75—76° and had $\lambda_{\text{max.}}$ 2580 (4·65), 2680 (4·77), and 2790 Å (4·67) [lit.,[20] m. p. 77—78°, $\lambda_{\text{max.}}$ 2580 (4·64), 2680 (4·77), and 2800 Å (4·66)].

Partial Oxidations.—The unsaturated acid (1 mmole), 30% hydrogen peroxide (0·6 mmole), and 98% formic acid (6 ml. per g. of acid) are shaken together for 15 min. at 30° [see note (i)]. Any unchanged peracid is then destroyed with sulphur dioxide, formic acid is removed under reduced pressure at 50°, and the formyloxy-hydroxy-derivative is hydrolysed with an excess of aqueous 2N-sodium hydroxide at 100° for 1 hr. The dihydroxy-acids are liberated with dilute sulphuric acid and extracted with ether. Unsaturated compounds are then completely hydrogenated in methanol with 20% palladium–charcoal (2 hr.). After removal of catalyst and solvent the product is oxidised with potassium periodate (8 mmoles), potassium permanganate (0·134 mmole), and potassium carbonate (3 mmoles) in dilute aqueous solution (200 ml.) by shaking overnight at room temperature [see note (ii)]. The resulting solution is acidified, decolorised with sulphur dioxide, saturated with salt, and extracted six times with ether [see note (iii)]. The acids are finally treated with methanolic hydrogen chloride or diazomethane and the esters are analysed by gas–liquid chromatography [see notes (iv) and (v)].

Notes: (i) Since performic acid is unstable it is freshly prepared from 30% hydrogen peroxide and 98% formic acid for each experiment. Partial oxidations were carried out with 50—100 mg. of acid and the oxidising agent was made in 10-fold excess. For example, oleic acid (100 mg.) requires 0·62 ml. of a reagent prepared from formic acid (6·0 ml.) and peroxide (0·24 ml.) and measured from a 1-ml. burette. More formic acid (a further 10 ml. per g. of acid) was sometimes added to the reaction mixture to make it homogeneous.

(ii) The reagents for von Rudloff oxidation are weighed for each experiment. It is important not to exceed the given quantities as additional permanganate leads to secondary degradation.

(iii) Solvent ether is removed through a 15-cm. Vigreux column to minimise loss of volatile components. If difficulty is encountered in distinguishing mono- and di-basic acids by chromatography it is possible to extract the aqueous solutions first with light petroleum (b. p. 40—60°) and then with ether. The mono- and di-basic acids, respectively, will be separately concentrated in these solutions.

(iv) Gas–liquid chromatography was carried out with a Pye Argon Chromatograph fitted with a 10% Apiezon column. Each mixture was analysed at least three times and the mean result taken as the composition (% mole). Many of the degradation mixtures had to be analysed at 150° and 200°, a peak common to both analyses being used to correlate the chromatograms. Areas under the peaks are measured by planimetry or by calculation using

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[23] Taylor and Strong, *J. Amer. Chem. Soc.*, 1950, **72**, 4263.

[24] Smith, Koch, and Wolff, *J. Amer. Oil Chemists' Soc.*, 1959, **36**, 219.

[25] Morris, Hayes, and Holman, *J. Amer. Oil Chemists' Soc.*, 1961, **38**, 316.

[26] Crombie and Jacklin, *J.*, 1957, 1632.

the method of Jaulmes and Mestres.[27] A synthetic mixture (values in parentheses) was found to have the following composition (% mole) by this method: monobasic acids C_6 7·0 (7·4), C_9 3·7 (3·9), C_{14} 14·2 (14·1), C_{18} 57·4 (57·0), and dibasic acids C_9 8·8 (8·9), and C_{12} 8·9 (8·7).

(v) The recovery of material from the whole procedure (estimated chromatographically) is 70—90%. The losses are due to the volatility of short-chain monobasic acids and the water-solubility of the short-chain dibasic acids.

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[27] Jaulmes and Mestres, *Compt. rend.*, 1959, **248**, 2752.