LOCATION OF ETHYLENIC BONDS IN FATTY/ALKANES COMPOUNDS

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Abstract. The oxidative degradation of unsaturated long-chain compounds liberate short-chain fatty acids which are difficult to analyse due to their volatility and solubility in water. However, this difficulty is overcome by the development of an analytical technique which recommends the direct analysis of short-chain fatty acids in aqueous media onto a short (18 × ⅛ in) Porapak Q column. This physicochemical method is very useful for the structure elucidation and positional isomers of alcohols, acids and hydrocarbons of animal and vegetable origin.

Generally there are two phases of problems in organic chemistry. One relates to the synthetic field and the other to the structure elucidation of a compound. The isolation and identification of a compound can be accomplished by various means and methods i. e. distillation, crystallisation, m.p. chromatography, spectroscopy, NMR, mass spectrometry and chemical methods etc.

There are many techniques for the determination of ethylenic bonds in unsaturated molecules,[1-3] but most depend upon sophisticated instrumentation which is not, as yet, widely available e.g. combined chromatography—mass spectrometry[4-7] (GC-MS). Alternatively, Gunstone[8] has shown that the retention data obtained by analysis of unsaturated compounds (either methyl esters or acetates) on capillary columns can indicate the position of a double bond. These methods require either expensive instrumentation GC-MS or a supply of all isomers for the respective monoene fatty alcohols and acids for capillary column gas liquid chromatography. Many workers do not have access to either of these facilities and are forced to determine the position of the double bond by more classical chemical means i.e. oxidation.

The oxidation cleaves the double bond position and the identification of the products reflect upon the position of double bond. There are various oxidizing agents e.g. per acids,[9] osmium tetraoxide,[10] hydrogen peroxide in t-butanol[11-12] sodium chlorate with traces of osmium tetraoxide,[13] ozonolysis,[14-16] potassium permanganate,[17] sodium periodate and potassium permanganate [18-21] (Von Rudloff's oxidation). The choice of an oxidizing agent depends upon the simple and easy handling, availability, cost and to minimise the danger of over oxidation.

In most of these studies, the major problem has been the analysis of the water soluble short-chain acids which are produced due to the oxidation of unsaturated long-chain compounds. On reviewing for the analysis of short-chain fatty acids, it is found that previous workers consumed too much time even 24 hours for the extraction[22-23] of these acids. After extraction, long-chain derivative [24-26] as butylphenyl and decyl esters are prepared for their analysis on GLC. The analysis of free fatty acids in organic solvent[27] is also possible on GLC. Later on, it is found that free fatty acids upto valeric acid[28] in aqueous media could be analysed on a column known as Porapak Q.

In the recent development of an analytical technique, the Von Rudloff's oxidation is modified by using double molar solution of potassium permanganate and sodium metaperiodate, by carrying out oxidation for 1 hr, no destruction of the oxidizing agent by sulphur dioxide or sodium bisulphite, no extraction by organic solvent, no separation and methylation of short-chain fatty acids. These short-chain acids, in the aqueous media, are analysed directly onto a column of Porapak Q alongwith the difunctional groups and oxidants without interference. There is no such easy technique to solve the complexity of the problem by determining the location of double bonds and positional isomers which reflect upon the structure elucidation and biosynthetic relationship respectively. Keeping in view the utility of the technique, it is applied to hydrocarbons of *Ligia oceanica*, alcohols of spermwhale, Jojoba wax esters and natural oil.

Ligia Oceanica. The crustacean *Ligia oceanica* is a member of the order Isopoda subclass Malacostraca in the Phylum Arthropoda. It is related to the terrestrial wood louse (Slater) *Porcellio scaber* and is sometimes called the shore slater. *Ligia oceanica* is restricted to the seashore around the British Isles, inhabiting rocky beaches above high tide marks. Occupying the middle ground between the terrestrial and the marine environments the shore slater represents a type of crustacean whose chemical composition has not been examined to any great extent. Like terrestrial arthropods, *Ligia oceanica* has a layer of wax over the cuticle as its primary adaptation towards water proofing. It has been suggested that the cuticle is poorly developed because the creature inhabits a damp environment.[29]

Since the wood louse is reported[30] to contain a series of unusual alcohols e.g. slaterol, it was decided to investigate the surface constituents of *Ligia oceanica*, in order to compare the composition and character of the wax with those of other arthropods and other marine species. Hydrocarbons are separated as a first fraction from the surface lipids of *Ligia oceanica.* The mono and diunsaturated hydrocarbon in alkane fraction [31] is heptadec-9-ene and heptadec-9, 13-diene respectively.

Spermwhale Wax. In the animal kingdom, the monster of sea i.e. spermwhale is well known and valuable for its oil and for spermaceti, the waxy

like fatty substances obtained from a cavity in its head. Beneath the skin lies the rich coating of fat or blubber which is commercially important. The composition of the spermwhale wax was determined by previous workers [32-33] Rider [34] made the acetate derivatives of spermwhale wax alcohols and separated unsaturated alcohol acetates of different chain length by the method of Hutchins and Martin[35] for the oxidation of purified oleyl acetates. She concluded from her oxidation of spermwhale wax alcohols that the position of double bond in each even number unsaturated higher alcohols $\Delta^{9:10}$ (The rotation indicates a double bond position by counting carbon atom from the carboxylic or alcoholic end) and that there are also other unidentifiable products. It is believed that the other products were not detected because the short-chain acid could not be analysed. Therefore, further work is required to investigate the position of the double bonds in these monounsaturated molecules from spermwhale alcohols.[36]

Jojoba Wax. Among the vegetable kingdom, the seeds of *Simmondsia californica* Nutt, the ever green desert shrubs indigenous to southern California, Arizona and northern Mexico, is a source for the liquid wax ester called Jojoba wax. Earlier worker on unsaturated acids and alcohols of Jojoba wax claimed that the position of unsaturation was W 9 (The notation W indicates a double bond position by counting carbon atom from the methyl end) viz. eicos-11-enol and eicos-11-enoic acid. It was realized that due to the difficulty for the analysis of short-chain acids, the positional isomers could not be determined and, therefore, the work was undertaken.[37]

Natural Oil. The most abundant unsaturated fatty acids in nature have ethylenic bonds at either the 9, 12 or 15 positions, i.e. olive oil is very rich in oleic acid ($\Delta^{9:10}$), cottonseed oil in linoleic acid ($\Delta^{9:10}$ $\Delta^{12:13}$), and linseed oil, in linoleic acid ($\Delta^{9:10}$, $\Delta^{12:13}$, $\Delta^{15:16}$), oxidation of these oil, will result in cleavage of the fatty acid at the double bond with consequent liberation of short chain fatty acid, C9, C6 or C3 respectively. In the experiment, the readily available oils (olive, linseed and cottonseed) are used.[38]

The short-chain fatty acids liberated after the oxidation of long-chain molecule can be analysed happily even if the quantity of the material is less than 1 mg. So the ideal degradation of pure unsaturated compound will consist of two parts, one is of short-chain fatty acids and the other is difunctional part. After the aliquot has been removed for analysis of monocarboxylic acids, the residual solution is extracted for acetylation of difunctional group or methylation of dicarboxylic acid and monocarboxylic acids above C10. They are further separated by TLC. These products are identified by using 1% Apiezon column in a pye 104 analytical GLC.

Results and Discussion

The analysis of short-chain fatty acids C3-C9 on Porapak Q (18 × ⅛ in) was accomplished under different conditions, firstly to know the retention time as a standard for the oxidized unknown sample and secondly to check up the reproducibility of results. It can be seen (Fig 1) that the most satisfactory peak separations were obtained under the following conditions :

The standard sample of free fatty acids C3-C9 in t-butanol : water (1 : 2) was injected onto the column Porapak Q, in a Pye 104 analytical GLC with a flame ionization detector which does not respond to water molecules at 130° which temperature was maintained until the C3 acid was eluted. The temperature was increased from 130° to 200° at 6°/min and using this temperature programme, C4 and C5 acids were eluted. The temperature was kept constant at 200° so that C6, C7 and C8 acids were eluted and the temperature was increased from 200° to 230° for the elution of C9 acid.

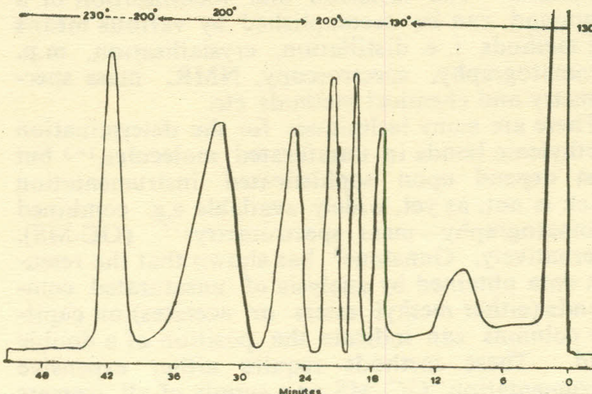
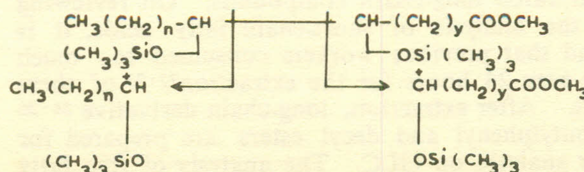


Fig. 1. Analysis of C3—C9 by combination of isothermal and temperature programming 130°—230°C at 6°/minute.

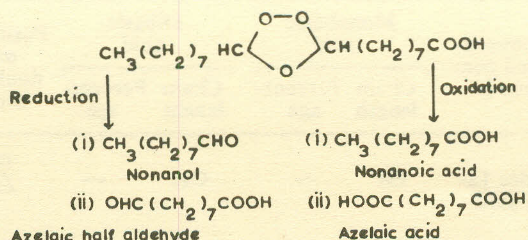
In order to describe the experimental errors and the reproducibility of these results one has to calculate the standard deviation and the coefficient of variation. By series of experiments the peak areas measured by triangulation were compared statistically with the known percentage by weight. The analysis of the acids was accomplished with a coefficient of variation[39] of no greater than ± 7.5%.

In combined gas chromatography—mass spectrometry usually the unsaturated centre is hydroxylated and the resultant diol converted into a trimethylsilyl ether derivatives which has better gas chromatographic properties and breaks down in the mass spectrometer to readily assignable fragment ions:



In addition to GC—MS the oxidative degradation of unsaturated compounds is also prac-

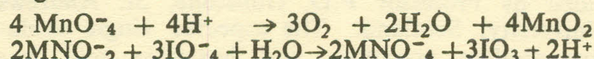
ticised. One of them is ozonolysis which is a popular way of cleaving the double bond with the formation of an ozanide, being an explosive in nature, is further hydrolysed[48] either in the presence of an oxidizing or reducing agent to get the respective products for identification.

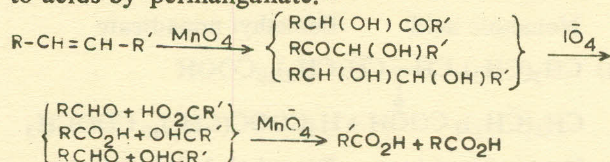


No doubt, ozonolysis employed on an industrial scale due to cheapness of ozone. But due to the explosive nature of ozonide, it may be replaced by other oxidizing agents as mentioned before. The oxidation with per acids is a tedious method followed periodate or lead tetracetate cleavage and subsequent oxidation of aldehyde. Osmium tetraoxide is not used due to its higher cost and toxicity, so the best oxidising agent which minimizes the chances for over-oxidation and which is easily available is sodium metaperiodate containing catalytic amount of potassium permanganate investigated by Von Rudloff. In the Von Rudloff's oxidation a low concentration of potassium permanganate is maintained throughout the reaction because periodate regenerates permanganate from manganate.

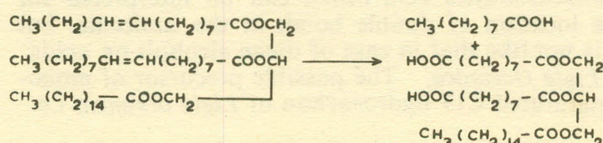
$$4\,MnO^{-}_{4} + 4H^{+} \rightarrow 3O_2 + 2H_2O + 4MnO_2$$
$$2MNO^{-}_{2} + 3IO^{-}_{4} + H_2O \rightarrow 2MNO^{-}_{4} + 3IO_3 + 2H^{+}$$

The Von Rudloff's oxidant solution of potassium permanganate and sodium periodate at the pH of 7-8, convert the olefin into ketal and some diol both of which are cleaved by periodate to acidic and aldehydic compounds, the latter then oxidised to acids by permanganate.[40]





so the analysis of short-chain fatty acids resulted from the monounsaturated hydrocarbon can reflect upon the position of double bond. Similarly the direct oxidation of natural oil e.g. olive oil can liberate short-chain fatty acids for analysis just to detect the presence of unsaturated long-chain fatty acids in triglyceride molecule without the interference of azeleoglyceride.[38]



In the Von Rudloff's oxidation, dilute oxidant solution is used, oxidation is carried out for 2 hr, after oxidation the oxidants are destroyed by sulphur dioxide or sodium sulphite then neutralized by sodium carbonate to a pH 7-8. The mono-basic and dibasic fatty materials are extracted by the solvent very carefully, methylated and separated for further identification. The losses of short-chain fatty acids could not be avoided during the process of recovery of acids from the aqueous media and secondly during the separation and purification of mono and dibasic acids. However, the losses could be minimised by the formation of long-chain derivatives.[24-26] In the modified Von Rudloff's technique oxidation was carried out for 1 hr and all the steps, except the double molar concentration of the stock oxidant solution were avoided altogether. After oxidation an aliquot was taken immediately out of the mixture containing short-chain fatty acids, dibasic acids or the difunctional group, potassium permanganate and sodium periodate, water, t-butanol etc. and was injected directly onto the Porapak column Q to analyse short-chain fatty acids C_2–C_{10}.

In lipid studies the investigation of various constituents of lipids particularly alcohols and acids among the animal and vegetable kingdom is not completed unless the position of double bond has not been found. If the ethylenic bonds are at different positions in one homologue, it is called double bond positional isomers e.g. the oxidation of the oleyl alcohol from some natural source shows the double bond position at $\Delta^{9:10}$ $\Delta^{10:11}$ and $\Delta^{11:12}$. So the presence of three double bonds at different position in one homologue show three positional isomers of oleyl alcohols as given below:

(i) $CH_3\,(CH_2)_7\,CH{=}CH\,(CH_2)_7\,CH_2OH$
(ii) $CH_3\,(CH_2)_6\,CH{=}CH\,(CH_2)_8\,CH_2OH$
(iii) $CH_3\,(CH_2)_5\,CH{=}CH\,(CH_2)_9\,CH_2OH$

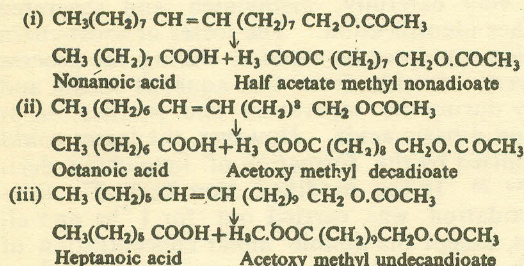
The acids and alcohols are methylated and acetylated respectively for better resolution on GLC. After the analysis of short-chain fatty acids in aqueous media, it was extracted, methylated and separated for monocarboxylic acid higher than decanoic acid, dicarboxylic acid and the half acetylated methylated alcohol.

The difunctional material resulting from oxidation was compared with the standard ω-acetoxy methyl decanoate on TLC and GLC. The different forms of ω-acetoxy decanoic acid (ω-acetoxy methyl decanoate, ω-hydroxy decanoic acid, ω-hydroxy methyl decanoate), octadecanol, stearic acid, azelaic acid, methyl azelate and methyl laurate were checked on TLC to evaluate their *Rf* value with respect to the polarity and consequently for the accurate analysis of the difunctional material (Fig. 2, Table 1). The lower acids analysed were in proportion as expected from the corresponding half acetate methyl ester or methylated dicarboxylic acid.

The oxidation of alcohol, acid or hydrocarbon (4 mg) was carried out by dissolving it in purified t-butanol (1 ml) and shaking it for 1 hr in a stock oxidant solution (2 ml) prepared by mixing sodium

periodate (0.2M) and potassium permanganate (0.005M).

The oxidative degradation of oleyl alcohol acetate at $\Delta^{9:10}$, $\Delta^{10:11}$ and $\Delta^{11:12}$ shows the following products respectively :



1. W—Acetoxy methyl decanoate
2. W—Acetoxy decanoic acid
3. W—Hydroxy decanoic acid
4. W—Hydroxy methyl decanoate
5. Dimethyl nonanedioate
6. Octadecanol (stearyl alcohol)
7. Methyl dodecanoate (lauric acid)
8. Octadecanoic acid (stearic acid)
9. Nonanedioic acid (azelaic acid)

Fig. 2. Thin layer chromatography of mono and difunctional compounds on silica gel using the petroleum ether: diethyl ether (v/v).

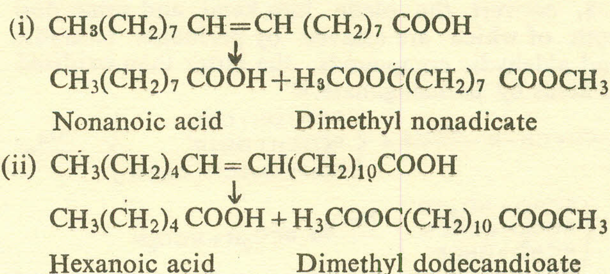
TABLE 1. THE R_f VALUE OF DIFFERENT COMPOUNDS ON SILICA GEL.

Compounds	R_f
Methyl dodecanoate (lauric acid)	0.68
ω-Acetoxyl methyl decanoate	0.45
Dimethyl nonanedioate	0.44
Octadecanoic acid (stearic acid)	0.34
Octadecanol (stearyl alcohol)	0.32
ω-Acetoxy decanoic acid	0.29
ω-Hydroxy methyl decanoate	0.25
ω-Hydroxy decanoic acid	0.15
Nonanedioic acid (azelaic acid)	0.04

TABLE 2. MONOCARBOXYLIC ACID AND DIFUNCTIONAL PORTION FROM THE OXIDATION OF MONOUNSATURATED LONG CHAIN COMPOUNDS.

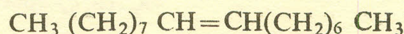
Monounsaturated compounds	Monobasic acid		Dibasic acid		Position of double bond
	Chain length	Percentage	Chain lenght	Percentage	
Octadec 12-enoic acid	C6	—	C12	—	Δ^{12}
Octadec-9-enoic acid	C9	—	C9	—	Δ^{9}
Oleyl alcohol	C7	17.6	C11	17.3	Δ^{11}
	C8	6.8	C10	8.4	Δ^{10}
	C9	75.6	C9	74.3	Δ^{9}

The positional isomers of oleyl alcohol, as mentioned above may be questioned due to the overoxidation of it. To check up the said possibility it was , however, applied to synthetic pure octadec-*cis*-9-enoic acid and octadec-*cis*-12-enoic acid provided by Professor F.D. Gunstone, St. Andrews University, Scotland. The expected single oxidative product nonanoic and hexanoic acid was obtained respectively (Table 2) from octadec-9-enoic acid and octadec-12-enoic acid.

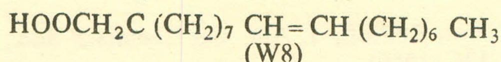


The modified Von Rudloff's oxidation was applied to the mono and diunsaturated C17 hydrocarbon of *Ligia oceanica* and also to the monoene alcohols of spermwhale and Jojoba wax. The technique can be applied even to the diunsaturated hydrocarbon due to the presence of two methyl groups at both ends of the molecule liberate two monocarboxylic acid which can be interpreted for the location of double bonds in the molecule, but it is not like that in case of diene alcohols or acids.

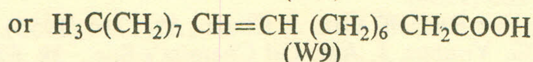
Ligia Oceanica. The possible precursor of monounsaturated C17 hydrocarbon of *Ligia oceanica* i.e.



could be either



$HOOCH_2C(CH_2)_7CH=CH(CH_2)_6CH_3$ (W8)



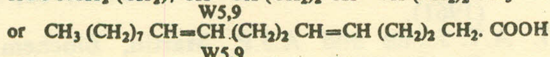
or $H_3C(CH_2)_7CH=CH(CH_2)_6CH_2COOH$ (W9)

It is believed that W9 could be precursor for the monounsaturated C17 due to the usual position of carboxylic acid in such a system. Similarly the oxidation products of diunsaturated C17 are C4 and C9 monobasic acids which reflect the position of double bonds at position W4 and W8.

$H_3C_2(CH_2)_7CH=CH(CH_2)_2CH=CH(CH_2)_2CH_3$

The possible precursor for diunsaturated C17could be either

$HOOC.CH_2(CH_2)_7CH=CH(CH_2)_2CH=CH(CH_2)_2CH_3$ W5,9



or $CH_3(CH_2)_7CH=CH(CH_2)_2CH=CH(CH_2)_2CH_2.COOH$ W5,9

so W5, 9 could be most likely precursor of diunsaturated C17. In the *Ligia oceanica* C18 mono and diunsaturated acids have been found out in the esterified form and also in free form. Presumably the unsaturated centres in monounsaturated and diunsaturated C17 hydrocarbons are formed on the dicarboxylation of C18 acid.

Spermwhale Alcohols.[36] The spermwhale wax was hydrolysed and free alcohols, separated from the nonsaponifiable, were acetylated. The preparative GLC (20% Apiezon column) was used for the separation of different molecular weight alcohols, i.e. C12 – C20. Each homologue was oxidised to get the positional isomers (Table 3).

TABLE 3. SPERMWHALE MONOUNSATURATED ALCOHOLS.

Chain length	Double bond position (Percentage isomer)							Published isomers distribution (Ref 36
	11	10	9	8	7	6	5	
14:1	—	—	48	—	40	—	12	9 ω
16:1	—	—	71	—	24	—	5	7 ω
18:1	4	Traces	84	Traces	9	Traces	3	9 ω
20:1	3	—	73	—	24	—	—	

These results show the higher proportion of nonanoic acid and thus indicating the unsaturated centres at $\Delta^{5:6}$ $\Delta^{7:8}$ $\Delta^{9:10}$ and $\Delta^{11:12}$ in each homologue C14:1, C16:1, C18:1 and C20:1 respectively. This suggests that there is one biosynthetic family and C14:1 alcohol may be the precursor of C20:1 alcohol.

C14:1 (Δ^5) —— C16:1 (Δ^7) —— C18:1 (Δ^9) C20:1 (Δ^{11}). This pathway would be similar to the one suggested for the production of triacontenoic acid (C30:1) from octadecenoic acid[40] (C18:1).

18:1 (Δ^9) — 20:1(Δ^{11}) — 22:1(Δ^{13}) — 24:1(Δ^{15}) — 26:1 (Δ^{17})—— 28:1 (Δ^{19}) —— 30:1 (Δ^{21})

TABLE 4. COMPOSITION AND ISOMER DISTRIBUTION OF THE MONOUNSATURATED ALKYL MOIETIES IN WAX ESTERS FROM JOJOBA OIL.

Chain lengths of alkyl moiety	Composition %	Position of double bond (% composition)					
		ω9	ω8	ω7	ω6	ω5	ω4
18:1	1.0	69	—	3	16	12	—
20:1	48.7	83	11	4	2	—	—
22:1	4.9	89	4	5	2	—	—
24:1	6.4	63	—	2	3	5	27

so the various positional isomers of monoene alcohols of spermwhale wax are interpreted on the basis of biosynthetic and dietary origin.

Jojoba Wax.[37] As usual the Jojoba wax was hydrolysed to get alcohols for acetylation. Each alcohol acetate C18:1, C20:1, C22:1 and C24:1 was obtained by preparative GLC as in case of spermwhale alcohols. Further purification of monoene alcohol acetate was obtained by 16.7% $AgNO_3$[41] impregnated silica gel TLC using petroleum ether – diethylether (9:1) as an eluting solvent. Later on oxidation is carried out to determine the positional isomers (Table 4). Earlier worker on unsaturated acids and alcohols of Jojoba wax claimed that the position of unsaturation was W9 i.e. eicos-11-enol and eicos–11-enoic. Many comparisons have been made between the acids and alcohols of Jojoba and those of Lunaria seed oil. Fatty alcohols and acids of Jojoba wax are claimed to be similar to Lunaria wax composition. Miwa[42] analysed the monobasic acids obtained after these oxidation of the Lunaria monoenoic acid and showed their composition to be in the ratio of 2:1:3:94 (hexanoic – heptanoic – octanoic – nonanoic acid.) The modified Von Rudloff's oxidation is first time applied to find out the positional isomers of monoene alcohols. It is found that tetracosenol has 27% of the W4 isomer and octadecenol has significant proportion of W5(12%) and W6(16%) in addition to major W9 double bonds positional isomers.

The absence of monobasic acids of C4, C5 and C6 in the oxidation of the acids of Jojoba wax alcohols in earlier work makes it seem unlikely that Schlenk's[43] concept of rapid equilibrium between acids and alcohols which hold in fish applies in Jojoba weeds wax alcohols.

Conclusively it is stated that the location of unsaturated centres by the application of a newly developed analytical technique for short-chain fatty acids analysis can eventually lead to the structural elucidation and determination of positional isomers to show the biosynthetic relationship of unsaturated hydrocarbons, fatty alcohols and acids of animal and vegetable origin. However, the following points can be summed up for the said technique.

(1) The smallest quantity of the material required

for oxidation is 50 – 100 mg for the analysis of lower fatty acids liberated after oxidation.

(2) The yield of oxidation is 87%.

(3) The method is very encouraging for the analysis of short-chain fatty acids in aqueous media, avoiding overoxidation and it is without much undue experimental difficulties.

(4) The time required for oxidation is short i.e. one hour as compared to the previous quoted figures for 5 – 24 hr.

(5) Large quantity of the oxidised sample (100 - 150 ml) is injected onto the Porapak Q column as compared to the recommended quantity of 0.24ml/component.

(6) It is an easy, quick method without any destruction of the oxidising agent, no removal of sodium periodate and potassium permanganate, no extraction with ether is required because the analysis of lower acids in aqueous solution is directly accomplished onto the Porapak Q column.

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