Microbiological Oxidation of Long-chain Aliphatic Compounds. Part III.[1] 1-Halogenoalkanes, 1-Cyanohexadecane, and 1-Alkoxyalkanes

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In a medium containing glucose, the yeast *Torulopsis gropengiesseri* effects the following transformations: (a) long-chain 1-halogenoalkanes to αω-alkanedioic acids; (b) 1-cyanohexadecane to the cyanohydrin derivative of 16-oxohexadecanoic acid; (c) long-chain 1-methoxyalkanes and 1-ethoxyalkanes to ω-hydroxyalkanoic acids; (d) long-chain 1-propoxyalkanes to ω- and ω-1-hydroxyalkoxyalkanoic acids. The transformation products separate from the culture medium as glycolipids. Metabolic pathways which account for these transformations are proposed.

It has been shown [2] that, in a medium containing glucose, the yeast *Torulopsis gropengiesseri* oxidises long-chain alkanes to alkanoic acids. These are subsequently oxidised to ω- and ω-1-hydroxy-acids and αω-dicarboxylic acids, which separate from the culture medium as insoluble glycolipids. It has also been shown [1] that the alkane and alkanoic acid oxidations are not prevented by methyl substituents near the sites of oxidation.

To determine the effect on alkane oxidation of substitution near the sites of reaction by atoms or groups not normally found in biological systems, we have examined the fermentations by *T. gropengiesseri* of 1-halogenoalkanes, 1-cyanohexadecane, 1-methoxyalkanes, 1-ethoxyalkanes, and 1-propoxyalkanes. The fermentations were carried out in a medium containing glucose, and the glycolipids produced were subjected to acid-catalysed methanolysis to liberate the lipid constituents. The lipid mixtures were separated by column chromatography and identified by methods which included thin-layer chromatography, gas–liquid chromatography, and n.m.r. spectroscopy. In addition to the compounds described below, the lipids obtained from the fermentation products contained small amounts of saturated and unsaturated, oxygenated C_{16} and C_{18} esters which were derived from the endogenous glycolipids [2] of the yeast.

1-Halogenoalkane Fermentations.[3]—The lipids derived from the products of 1-halogenoalkane fermentations consisted of αω-dicarboxylic esters, the yields and chain-lengths of which depend upon (a) the size of the halogen atom, and (b) the chain-length of the halogenoalkane (Table 1). 1-Fluoroalkanes were not readily utilised, and approximately 75% of these compounds were recovered at the end of the incubation period. By repetition of reported [4] experiments, the glass-like glycolipid obtained from 1-bromohexadecane fermentation was shown to be identical with the macrocyclic dilactone isolated as a minor product af hexadecane fermentation.[4] The structure proposed for this dilactone now requires revision in the light of the recent report by Tulloch, Hill, and Spencer.[5]

The significant feature of the results shown in Table 1 is the exclusive formation of glycolipids incorporating αω-alkanedioic acids. Although similar glycolipids are produced as minor products from alkan-1-ols and alkanoates, the major products of these fermentations are glycolipids incorporating ω- and ω-1-hydroxy-acids.[2] Thus, metabolism of 1-halogenoalkanes by hydrolysis to alkan-1-ols, or by oxidation to fatty acids, can be excluded.

Fermentation of 2-bromohexadecane did not yield a glycolipid but gave instead a low yield of 15-bromohexadecanoic acid. This result suggested that ω-halogenoalkanoic acids might be intermediates in the conversion of halogenoalkanes into αω-dicarboxylic acids. In confirmation, it was found that fermentation of methyl

TABLE 1

Yields and chain-lengths of dimethyl αω-alkanedioates obtained by methanolysis of glycolipids produced from 1-halogenoalkanes

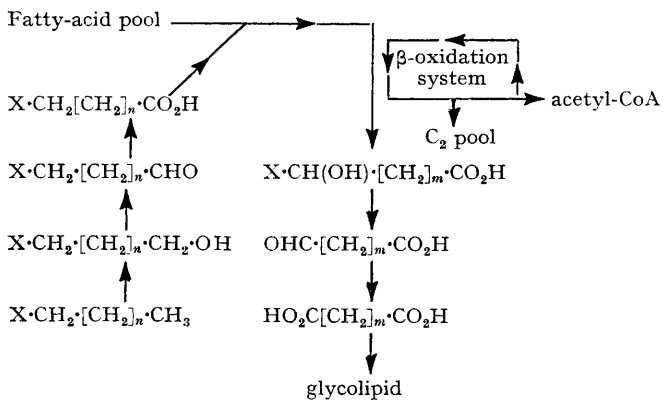
Halogenoalkane fermented	Chain-length of αω-diesters $CH_3{\cdot}O_2C{\cdot}[CH_2]_m{\cdot}CO_2{\cdot}CH_3$, with yields (%) shown in parentheses							
$CH_3{\cdot}[CH_2]_n{\cdot}X$	X = I		X = Br		X = Cl		X = F	
$n = 14$			$m = 13$	(trace)				
$n = 15$	$m = 14$	(43)	$m = 14$	(50)	$m = 14$	(33)	$m = 14$	(9)
$n = 16$			$m = 15$	(45)				
$n = 17$	$m = 16$	(15)	$m = 16$	(17)	$m = 16$	(18)	$m = 16$	(9)
	$m = 14$	(30)	$m = 14$	(29)	$m = 14$	(29)	$m = 14$	(4·5)
$n = 17(\Delta^9)$ *			$m = 16(\Delta^9)$	(26)				
			$m = 14(\Delta^7)$	(9)				
$n = 19$			$m = 14$	(34)				
$n = 21$			$m = 14$	(36)				

* Oleyl bromide.

[1] Part II, D. F. Jones, *J. Chem. Soc.* (*C*), preceding paper.
[2] D. F. Jones and R. Howe, *J. Chem. Soc.* (*C*), 1968, 2801.
[3] G. W. Elson, R. Howe, and D. F. Jones, B.P. 1,096,326 (1967).
[4] D. F. Jones, *J. Chem. Soc.* (*C*), 1967, 479.
[5] A. P. Tulloch, A. Hill, and J. F. T. Spencer, *Chem. Comm.*, 1967, 584.

16-bromohexadec-9-enoate gave a glycolipid which yielded on methanolysis dimethyl 7-hexadecene-1,16-dioate (53% yield).

The halogen atom in ω-halogenoalkanoic acids is not removed by hydrolysis because ω-hydroxyalkanoic acids are largely incorporated into glycolipids without further oxidation; dehydrogenation to αω-alkanedioic acids is a minor reaction.[2] A metabolic pathway which will account for the observed results is shown in the Figure. It is proposed that a 1-halogenoalkane is initially oxygenated at the terminal methyl group to give an ω-halogenoalkan-1-ol, which is dehydrogenated to the corresponding ω-halogenoalkanoic acid. This acid could be further metabolised, in part by β-oxidation, and in part by hydroxylation at the carbon atom bearing the halogen substituent. The latter reaction would yield an ω-halogeno-ω-hydroxyalkanoic acid, which would be expected to hydrolyse readily to an ω-oxoalkanoic acid. Dehydrogenation of this acid would yield an αω-dicarboxylic acid, which could be protected from degradation by incorporation into a glycolipid. Hydroxylation



Proposed pathway of metabolism of 1-halogenoalkanes in *T. gropengiesseri*

X = F, Cl, Br, or I; when $n = 13$, 14, and 15, $m = n$; when $n = 16$, $m = n$ and $n = 2$; when $n = 18$ or 20, $m = 14$

of the 15-bromohexadecanoic acid derived from 2-bromohexadecane is presumably inhibited by the presence of the methyl substituent at the site of oxidation, and this bromo-acid is probably metabolised by β-oxidation.

Fermentation of 1-bromo-2-methylhexadecane gave a 50% yield of methyl 2-methylhexadecane-1,16-dioate, indicating that hydroxylation of an ω-halogenoalkanoic acid is not inhibited by the presence of a methyl substituent at the carbon atom adjacent to the site of hydroxylation. A similar finding has been reported[1] for the hydroxylation of branched-chain alkanoic acids. A further correspondence in metabolism between ω-halogenoalkanoic acids and alkanoic acids is the marked dependence of the yields and chain-lengths of the oxidation products on the chain-lengths of the compounds fermented. In alkane metabolism it has been shown[2] that two processes, β-oxidation, and ω- and ω-1-hydroxylation, compete for alkane-derived alkanoic acids, and that the relative importance of these processes depends upon the alkanoic acid chain-length. The highest yields of hydroxy-acids were obtained from C_{16}—C_{18} fatty acids. Alkanoic acids shorter than C_{16} were largely metabolised by β-oxidation, and alkanoic acids longer than C_{18} were preferentially metabolised by β-oxidation to a chain-length more favourable for hydroxylation. The results of halogenoalkane fermentations can be similarly rationalised by allowing for the different sizes of the various halogen atoms. Thus, whereas octadecane gave only C_{18} products, 1-halogenooctadecanes gave mixtures of C_{18} and C_{16} dicarboxylic acids because to the hydroxylating enzyme ω-halogenoalkanoic acids are longer than alkanoic acids. In agreement with this, in 1-halogeno-octadecane fermentations, the relative proportion of the C_{16} dicarboxylic acid increases as the size of the halogen atom increases.

1-*Cyanohexadecane Fermentation*.—The lipids derived by methanolysis of the products of 1-cyanohexadecane fermentation contained methyl 16-oxohexadecanoate (8% yield). The corresponding oxo-acid seemed an unlikely end-product of fermentation because it would be expected readily to undergo dehydrogenation to give an αω-dicarboxylic acid as indicated in the Figure. Treatment of the cyanohexadecane-derived glycolipids with hot mineral acid caused the evolution of hydrogen cyanide. This suggested that the fermentation product which yielded methyl 16-oxohexadecanoate on methanolysis was a glycolipid incorporating the cyanohydrin derivative of 16-oxohexadecanoic acid. Such a glycolipid could be derived from 1-cyanohexadecane by a metabolic pathway analogous to that proposed for halogenoalkanes, *i.e.*, by way of 16-cyanohexadecan-1-ol and 16-cyanohexadecanoic acid. Hydroxylation of this acid at the carbon atom bearing the nitrile group would yield the cyanohydrin, 16-cyano-16-hydroxyhexadecanoic acid, which by reaction of its hydroxy-group, could be incorporated into a glycolipid. Presumably, under the fermentation conditions employed, a cyanohydrin group is less susceptible to hydrolysis than is the terminal halogenohydrin group which is generated on hydroxylation of an ω-halogenoalkanoic acids. In support of the proposed metabolic pathway, fermentation of ethyl 16-cyanohexadec-9-enoate gave a glycolipid which yielded hydrogen cyanide on acid hydrolysis and methyl 16-oxohexadec-9-enoate (34% yield) on methanolysis.

1-*Alkoxyalkane Fermentations*.—The lipids derived from the fermentation products of 1-methoxyalkanes, 1-ethoxyalkanes, and 1-propoxyalkanes are listed in Table 2. The results of the 1-propoxyalkane fermentations can be explained by a metabolic sequence analogous to that for defined alkane metabolism,[2] *i.e.* (1) oxidation to give a primary alcohol, (2) dehydrogenation of the alcohol to a carboxylic acid, (3) either β-oxidation of the carboxylic acid or hydroxylation to give an ω-1-hydroxy-acid and an ω-hydroxy-acid (and, by further dehydrogenation, an αω-dicarboxylic acid), and (4) protection of the oxidation products from further degradation by incorporation into water-insoluble glycolipids. The initial oxidation to a primary

alcohol can take place at either of the terminal positions of a propoxyalkane, leading to the production of oxidised derivatives of both propoxyalkanoic and alkoxypropionic acids. The only deviation from direct analogy to alkane fermentation is the production of β-(17-hydroxyoctadecyloxy)propionic acid from 1-propoxyoctadecane. The formation of this acid, which approximates in length to a C_{22} ω-1-hydroxyalkanoic acid, contrasts with the formation of a C_{18} ω-1-hydroxy-acid during docosane (C_{22}) fermentation, but was expected because β-alkoxypropionic acids are resistant to β-oxidation.[2]

In contrast with 1-propoxyalkanes, 1-methoxyalkanes yielded lipids which lacked an ethereal oxygen atom. ω-Hydroxyalkanoic acids, the major products obtained from 1-methoxyalkane fermentations (Table 2) are presumably derived from ω-methoxyalkanoic acids, because fermentation of methyl 16-methoxyhexadecanoate gave glycolipids which yielded methyl 16-hydroxyhexadecanoate (40% yield) and dimethyl hexadecane-1,16-dioate (7% yield). ω-Methoxyalkanoic acids are probably formed by dehydrogenation of ω-methoxyalkan-1-ols, formed from 1-methoxyalkanes by oxygenation of the terminal methyl group of the alkyl moiety. Demethylation of ω-methoxyalkanoic acids to give alkane at the methyl group of the methoxy-function. This would yield a hemiacetal, which on hydrolysis would give an alkan-1-ol. Further metabolism of this alkan-1-ol by the defined pathway [2] would account for the formation of an ω-1-hydroxyalkanoic acid and also some ω-hydroxyalkanoic acid.

The formation of glycolipids, incorporating ω-hydroxyalkanoic acids, by fermenting 1-ethoxyalkanes can be accounted for by a metabolic pathway similar to that of 1-methoxyalkanes. In this case an intermediate ω-ethoxyalkanoic acid should be formed. Hydroxylation of the methylene group of the ethoxy-function, and subsequent hydrolysis of the resulting hemiacetal, could yield ω-hydroxy-acid and acetaldehyde. Presumably hydroxylation of the terminal methyl groups of ω-ethoxyalkanoic acids is also possible, and compounds relating to this mode of reaction may have been present as minor products of the fermentation.

The formation of 13-hydroxytetradecyloxyacetic acid by fermenting 1-ethoxytetradecane, and of 15-hydroxyhexadecyloxyacetic acid by fermenting 1-ethoxyhexadecane, indicates that 1-ethoxyalkanes are also metabolished by a pathway involving initial oxygenation at the methyl group of the ethoxy-substituents.

TABLE 2

Major lipid constituents of the methanolysis products of glycolipids formed during fermentation of 1-alkoxyalkanes

Compound fermented	Lipid constituents of glycolipid methanolysis products; approximate yields (%) shown in parentheses					
$CH_3{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CH_3$	$HO{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(16)				
$CH_3{\cdot}O{\cdot}[CH_2]_{14}{\cdot}CH_3$	$HO{\cdot}[CH_2]_{14}{\cdot}CO_2{\cdot}CH_3$	(20)				
$CH_3{\cdot}O{\cdot}[CH_2]_{15}{\cdot}CH_3$	$HO{\cdot}[CH_2]_{15}{\cdot}CO_2{\cdot}CH_3$	(30)				
$CH_3{\cdot}O{\cdot}[CH_2]_{17}{\cdot}CH_3$	$HO{\cdot}[CH_2]_{15}{\cdot}CO_2{\cdot}CH_3$	(15)				
$CH_3{\cdot}O{\cdot}[CH_2]_6{\cdot}CH{:}CH{\cdot}[CH_2]_7{\cdot}CH_3$	$HO{\cdot}[CH_2]_6{\cdot}CH{:}CH{\cdot}[CH_2]_7{\cdot}CO_2{\cdot}CH_3$	(16)			$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{12}{\cdot}CO_2{\cdot}CH_3$	(4)
	$HO{\cdot}[CH_2]_6{\cdot}CH{:}CH{\cdot}[CH_2]_5{\cdot}CO_2{\cdot}CH_3$	(9)	$CH_3{\cdot}O_2C{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(2)	$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(7)
$CH_3{\cdot}CH_2{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CH_3$	$HO{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(8)	$CH_3{\cdot}O_2C{\cdot}[CH_2]_{14}{\cdot}CO_2{\cdot}CH_3$	(2)	$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{15}{\cdot}CO_2{\cdot}CH_3$	(14)
$CH_3{\cdot}CH_2{\cdot}O{\cdot}[CH_2]_{15}{\cdot}CH_3$	$HO{\cdot}[CH_2]_{15}{\cdot}CO_2{\cdot}CH_3$	(8)	$CH_3{\cdot}O_2C{\cdot}[CH_2]_{14}{\cdot}CO_2{\cdot}CH_3$	(1)	$CH_3{\cdot}CH(OH){\cdot}[CH_2]_6{\cdot}CH{:}CH{\cdot}[CH_2]_7{\cdot}CO_2{\cdot}CH_3$	(24)
	$HO{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(3)				
$CH_3{\cdot}CH_2{\cdot}O{\cdot}[CH_2]_{17}{\cdot}CH_3$	$HO{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(6)			$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{12}{\cdot}O{\cdot}CH_2{\cdot}CO_2{\cdot}CH_3$	(1)
$CH_3{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CH_3$	$HO{\cdot}[CH_2]_3{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(trace)			$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{14}{\cdot}O{\cdot}CH_2{\cdot}CO_2{\cdot}CH_3$	(1)
	$CH_3{\cdot}O_2C{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CH_2{\cdot}OH$	(trace)				
$CH_3{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{15}{\cdot}CH_3$	$HO{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(trace)			$CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(30)
	$CH_3{\cdot}O_2C{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CH(OH){\cdot}CH_3$	(30)				
	$CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}O{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	(17)				
	$CH_3{\cdot}O_2C{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{15}{\cdot}CH_2{\cdot}OH$	(1)	$CH_3{\cdot}O_2C{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{15}{\cdot}CO_2{\cdot}CH_3$	(1)	$CH_3{\cdot}O_2C{\cdot}[CH_2]_2{\cdot}O{\cdot}[CH_2]_{14}{\cdot}CH(OH){\cdot}CH_3$	(35)

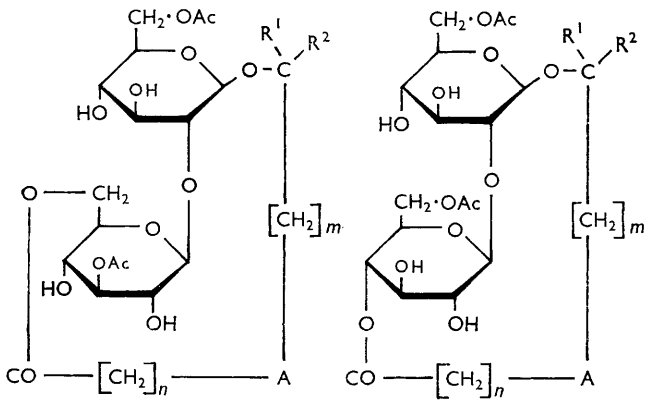
ω-hydroxyalkanoic acids probably involves hydroxylation of the methyl group of the methoxy-substituent to give a hemiacetal of the type $HO{\cdot}CH_2{\cdot}O{\cdot}[CH_2]_n{\cdot}CO_2H$. This, on hydrolysis, would yield formaldehyde and an ω-hydroxyalkanoic acid. Dehydrogenation of some of the ω-hydroxy-acid would yield αω-dicarboxylic acid and incorporation of this diacid and the remaining ω-hydroxy-acid, into glycolipids would account for the major products of 1-methoxyalkane fermentations. The chain-shortening which accompanied the conversion of 1-methoxyoctadecane into methyl 16-hydroxyhexadecanoate (Table 2) is reminiscent of the chain-length reduction which occurs during the fermentation of long-chain alkanoic and ω-halogenoalkanoic acids. It seems likely that demethylation of methoxyalkanoic acids is catalysed by the enzyme which catalyses the oxidative debromination of halogenoalkanoic acids and the hydroxylation of alkanoic acids.

The formation of glycolipids incorporating ω-1-hydroxyalkanoic acids in the fermentation of 1-methoxyalkanes indicates the existence of a metabolic pathway which probably involves initial oxygenation of a methoxy-. This oxygenation would yield β-alkoxyethanols, which by dehydrogenation of the alcohol group and hydroxylation would give hydroxyalkoxyacetic acids. The feasibility of this pathway was confirmed by fermentation of methyl pentadecyloxyacetate, which yielded glycolipids incorporating 14- and 15-hydroxypentadecyloxyacetic acids.

Properties of the Oxygenating Enzymes.—There is a close similarity between the pathways defined for alkane metabolism [2] and those proposed in the present paper for the metabolism of 1-halogenoalkanes, 1-cyanohexadecane, and 1-alkoxyalkanes. This suggests (a) that the enzyme which catalyses the oxidation of the terminal position of alkanes also catalyses the oxidation of the terminal methyl group of the alkane derivatives, and (b) that the enzyme which catalyses the ω- and ω-1-hydroxylation of alkanoic acids also catalyses the oxidative removal of the substituent from ω-halogenoalkanoic acids and ω-methoxyalkanoic acids, and the hydroxylation of 16-cyanohexadecanoic acid. It has been proposed [2] that ω- and ω-1-hydroxylations of alkanoic acids are catalysed by a single enzyme, and that

the position at which the alkanoic acid is hydroxylated is determined by the way the substrate is aligned on the enzyme surface. Assuming this, it seems likely that ω-halogenoalkanoic acids and 16-cyanohexadecanoic acid adopt the alignment on the enzyme which facilitates the ω-1-hydroxylation of an alkanoic acid; the halogen atoms and nitrile group presumably occupying the site usually occupied by the terminal methyl group of an alkanoic acid. Only one alignment of an ω-methoxyalkanoic acid on the enzyme can lead to oxygenation, since the position is equivalent to the ω-1-position of an alkanoic acid is occupied by the ethereal oxygen atom. This alignment corresponds to that which normally facilitates ω-hydroxylation of an alkanoic acid. In ω-ethoxyalkanoic acids and ω-propoxyalkanoic acids, the ether oxygen atoms does not occupy a site of oxidation, so both ω- and ω-1-hydroxylations are possible.

Glycolipid Structures.—In a previous paper,[4] the dihydro-derivative of a glycolipid obtained from methyl octadec-9-enoate fermentation was assigned structure (Ia). Largely on the basis of similarities in n.m.r. and mass spectroscopic properties, other glycolipids were assigned structures which differed from (Ia) only in the nature of the lipid fragment. However, Tulloch, Hill, and Spencer[5] have recently revised structure (Ia) to (Ib), and consequently the structures proposed for other glycolipids require a similar revision. Thus, the structure (IIa) proposed for the glycolipid incorporating hexadecane-1,16-dioic acid requires revision to (IIb),



	A	*n*	*m*	R^1	R^2
(I)	CH_2	7	7	Me	H
(II)	CH_2	6	7	=O	
(III)	CH_2	6	7	H	H
(IV)	CH_2	5	7	H	H
(V)	O	2	12	Me	H
(VI)	O	13	1	Me	H
(VII)	O	2	14	Me	H

and structure (IIIa) proposed for the glycolipid incorporating 16-hydroxyhexadecanoic acid requires revision to (IIIb).

The major glycolipid produced by 1-methoxyhexadecane fermentation was obtained as an amorphous solid which was identical (t.l.c., n.m.r., and mass spectrum) with the lactone (IIIb) previously isolated from methyl hexadecanoate fermentation.[4] The major product from 1-methoxypentadecane fermentation was an amorphous solid, m.p. 97—99°. This solid gave methyl 15-hydroxypentadecanoate on methanolysis, and its mass spectrum included ions at *m/e* 648 (M^+), 473, 427, 367, 269, 241, 233, 205, and 145. This pattern of degradation was analogous to that reported[4] for (IIIb), and indicated structure (IVb) for the 1-methoxypentadecane-derived glycolipid.

The product from 1-propoxytetradecane fermentation was repeatedly crystallised from acetone–light petroleum, and gave colourless needles, m.p. 95—98°. The mass spectrum of this solid included ions at *m/e* 692 (M^+), 674, 656, 517, 499, 471, 453, 411, 393, 375, 285 ($C_{17}H_{33}O_3$), 267, 233, 215, 205, 187, 169, 145, 127, and 109. This pattern of degradation was similar to that shown by the lactone (Ib),[4] and indicated a structure which differed from this only in the nature of the lipid fragment. Acid methanolysis of the crystalline material gave a mixture containing equal amounts of methyl β-(13-hydroxytetradecyloxy)propionate and methyl 14-(2-hydroxypropoxy)tetradecanoate. Thus, the crystalline fermentation product was a mixture of the isomeric macrocyclic lactones (Vb) and (VIb). In agreement with this, the n.m.r. spectrum ($CDCl_3$–D_2O) closely resembled that of the lactone (Ib) except that in place of a two-proton triplet centred at τ 7·68 (CH_2CH_2CO) the spectrum showed a triplet centred at 7·68 and a triplet centred at 7·45 (OCH_2CH_2CO), each signal being approximately equivalent to one proton. There was increased signal strength in the region τ 6·2—6·75, due to the group CH_2OCH_2; similarly, the n.m.r. spectrum of the crystalline peracetyl derivative, m.p. 58—61°, resembled that of the tetra-acetyl derivative of (Ib).

The major product of 1-propoxyhexadecane fermentation was obtained as a colourless amorphous solid, m.p. 101—107°. The mass spectrum of this solid showed a very weak molecular ion at *m/e* 720, and ions at 545, 527, 499, 481, 439, 421, 403, 313, 295, 233, 215, 205, 187, 169, 145, 127, and 109. This pattern of degradation was identical with that described above for compounds (Vb) and (VIb), and indicated a structure which differed from (Vb) only in the nature of lipid fragment. Acid methanolysis of the amorphous solid gave methyl β-(15-hydroxyhexadecyloxy)propionate, indicating structure (VIIb) for this glycolipid. The n.m.r. spectra of the amorphous solid and its glass-like peracetyl derivative agreed with this structure.

EXPERIMENTAL

Materials.—*1-Halogenoalkanes.* 1-Bromohexadecane and 1-bromo-octadecane were purchased from Koch-Light Laboratories, Colnbrook, Bucks. 1-Chlorohexadecane and 1-chloro-octadecane were purchased from K. and K. Laboratories, Plainview, New York. 1-Bromopentadecane and 1-bromoheptadecane were prepared by bromodecarboxylation of silver hexadecanoate and silver octadecanoate. 1-Bromoeicosane and 1-bromodocosane were prepared from the corresponding alkanols (Koch-Light) by treatment with

hydrobromic acid (48%) in conc. sulphuric acid. 1-Bromo-octadec-9-ene was prepared from oleyl alcohol by treatment with phosphorus tribromide in carbon tetrachloride. 1-Iodohexadecane and 1-iodo-octadecane were prepared from the corresponding alkanols. 1-Fluorohexadecane and 1-fluoro-octadecane were prepared from the corresponding alkyl bromides by treatment with potassium fluoride in ethylene glycol. 2-Bromohexadecane, b.p. 120/0·5 mm., 1-bromo-2-methylhexadecane, b.p. 150(bath)/0·1 mm., and 1,16-dibromohexadecane, m.p. 55—56°, were prepared from the corresponding alcohols by treatment with phosphorus tribromide and pyridine. Methyl 16-bromohexadec-9-enoate was prepared by methylation (diazomethane) of the corresponding acid (Aldrich Chemical Company, Milwaukee, Wisconsin.).

1-*Alkoxyalkanes*. 1-Methoxy-, 1-ethoxy-, and 1-propoxyalkanes were prepared from alkyl bromides and sodium alkoxides. Methyl 16-methoxyhexadecanoate was prepared by acid-catalysed methanolysis of methyl 16-methylsulphonyloxyhexadecanoate, m.p. 56°, which was prepared from methyl 16-hydroxyhexadecanoate by treatment with methylsulphonyl chloride in pyridine. Methyl pentadecyloxyacetate was prepared by methylation (diazomethane) of pentadecyloxyacetic acid, m.p. 67°, which was prepared from pentadecanol by treatment with sodium hydride and then sodium chloroacetate.

Alkenes and 1-cyanohexadecane were purchased from Koch-Light Laboratories. Ethyl 16-cyanohexadec-9-enoate was prepared from methyl 16-bromohexadec-9-enoate by the method of Mathur and Bhattacharyya.[6]

Liquids were purified by distillation under reduced pressure. Solids were purified by crystallisation and by chromatography. All compounds, except methyl 16-bromohexadec-9-enoate and ethyl 16-cyanohexadec-9-enoate (both of which contained 12% of the corresponding saturated derivatives) were of >97% purity by t.l.c. and g.l.c., and gave satisfactory elemental analyses and n.m.r. spectra.

Methods.—The equipment and procedures described previously [2] were employed for fermentation and for determination of yields and structures of the lipid constituents of the fermentation products. Where lipid constituents were identified only by the t.l.c. and g.l.c. properties of the compounds, their propionyl derivatives, and their oxidation products, these properties were compared directly with those of authentic compounds or near homologues.

Compounds isolated after Methanolysis of Fermentation Products.—In addition to characterisation by the chromatographic techniques, the following compounds were isolated.

(a) *From 1-chlorohexadecane, 1-bromohexadecane, 1-bromoeicosane, and 1-bromodocosane fermentations*. Dimethyl hexadecane-1,16-dioate, m.p. and mixed m.p. 50—51° (Found: C, 68·7; H, 11·0. Calc. for $C_{18}H_{34}O_4$: C, 68·75; H, 10·9%); the n.m.r. spectrum (CCl_4) showed two three-proton singlets at τ 6·42 and two two-proton triplets centred at 7·81 ($CH_2CH_2CO_2CH_3$), and a twenty-four-proton multiplet centred near 8·75 [$(CH_2)_{12}$]; saponification of the diester gave hexadecane-1,16-dioic acid, m.p. and mixed m.p. 122—124°.

(b) *From 1-bromoheptadecane fermentation*. Dimethyl heptadecane-1,17-dioate, m.p. 50—51° (Found: C, 69·5; H, 11·0. Calc. for $C_{19}H_{36}O_4$: C, 69·5; H, 11·0%); the n.m.r. spectrum (CCl_4) showed signals at τ 6·42 and 7·81 ($CH_2CH_2CO_2CH_3$), and 8·75 [$(CH_2)_{13}$].

(c) *From 1-bromo-2-methylhexadecane fermentation*. A dicarboxylic ester, which on saponification gave 2-methylhexadecane-1,16-dioic acid, m.p. 88—90° (lit.,[7] 89—90°) (Found: C, 67·6; H, 10·6. Calc. for $C_{17}H_{32}O_4$: C, 67·9; H, 10·7%); the n.m.r. spectrum ($CDCl_3$) showed a three-proton multiplet centred near τ 7·6 [$CH_2CH(CH_3)CO$ and CH_2CH_2CO] and a three-proton doublet centred at 8·82 [$CH(CH_3)CO$].

(d) *From methyl 16-bromohexadec-9-enoate fermentation*. Dimethyl hexadec-7-ene-1,16-dioate, a colourless oil which, like the starting material, was contaminated with approximately 12% (g.l.c.) of the corresponding saturated derivative; the n.m.r. spectrum (CCl_4) showed a multiplet centred at τ 4·7 ($CH_2CH{:}CHCH_2$), a singlet at 6·42 (CH_3O), and a multiplet between 7·6 and 8·2 (CH_2CH_2CO and $CH_2CH_2CH{:}CHCH_2CH_2$); on catalytic reduction, the mixture of diesters took up 0·8—0·9 mol. of hydrogen and gave dimethyl hexadecane-1,16-dioate, m.p. and mixed m.p. 52°.

(e) *From 2-bromohexadecane fermentation*. A mixture of glycolipids and a carboxylic acid which on methylation with diazomethane and chromatography on a column of silica gave *methyl 15-bromohexadecanoate*, m.p. 29—32° (Found: C, 59·0; H, 9·5; Br, 22·0. $C_{17}H_{33}BrO_2$ requires C, 58·5; H, 9·5; Br, 22·9%); the mass spectrum lacked a molecular ion, but included ions at *m/e* 268 $(M - HBr)^+$, 82 and 80 $(HBr)^+$, 81 and 79 (Br^+), and 74 $[CH_2{:}C(OH)OCH_3]^+$; the n.m.r. spectrum (CCl_4) showed a one-proton multiplet centred at τ 6·02, a two-proton multiplet centred near 8·3, and a three-proton doublet centred at 8·32 [$CH_3CH(Br)CH_2$], a three-proton singlet at 6·39, and a two-proton triplet centred at 7·75 ($CH_2CH_2CO_2CH_3$); methyl 15-bromohexadecanoate decomposed under the usual g.l.c. conditions; debromination, by heating in ethanol with acetone-deactivated Raney nickel, gave methyl hexadecanoate, which was identified by g.l.c. and t.l.c.

(f) *From 1-cyanohexadecane fermentation*. A crystalline material, m.p. 48—51° (26 mg., 8% yield), containing 97% (g.l.c.) of methyl 16-oxohexadecanoate and 3% of dimethyl hexadecane-1,16-dioate; the mass spectrum of the crystalline material showed ions at *m/e* 284 $(M^+ - H)$, and 74 $[CH_2{:}C(OH)OCH_3]^+$, and a series of ions corresponding to $[CH_3O_2C(CH_2)_n]^+$, where $n = 4$ to 13; the n.m.r. spectrum (CCl_4) showed a one-proton triplet centred at τ 0·3 and a two-proton triplet at 7·8 (CH_2CH_2CHO), a further two-proton multiplet at 7·8, and a three-proton singlet at 6·4 ($CH_2CH_2CO_2CH_3$); oxidation of the crystalline solid, followed by methylation with diazomethane, gave dimethyl hexadecane-1,16-dioate (identified by t.l.c., g.l.c., and mixed m.p.); reduction with sodium borohydride in methanol, followed by chromatography on aluminium oxide, gave methyl 16-hydroxyhexadecanoate, m.p. and mixed m.p. 52—54° (Found: C, 71·5; H, 11·7. Calc. for $C_{17}H_{34}O_3$: C, 71·3; H, 12·0%).

Other products of 1-cyanohexadecane fermentation will be reported in a subsequent communication.

The lipid fraction obtained from glycolipids derived from ethyl 16-cyanohexadec-9-enoate, contained 16-oxo-

[6] H. H. Mathur and S. C. Bhattacharyya, *J. Chem. Soc.*, 1963, 114.

[7] P. Chuit, F. Boelsing, J. Hausser and G. Malet, *Helv. Chim. Acta*, 1927, **10**, 167.

hexadec-9-enoate (34% yield). Oxidation of the latter followed by methylation gave dimethyl hexadec-7-ene-1,16-dioate, and reduction of the aldehyde with sodium borohydride gave methyl 16-hydroxyhexadec-9-enoate.

When the glycolipids obtained from 1-cyanohexadecane fermentation and those obtained from ethyl 16-cyanohexadec-9-enoate fermentation were heated in aqueous sulphuric acid, hydrogen cyanide was evolved. With benzidine acetate–copper acetate reagent it gave the characteristic blue colour.[8]

The following were also obtained.

(g) *From 1-methoxypentadecane fermentation.* 15-Hydroxypentadecanoic acid, m.p. and mixed m.p. 81—84°.

(h) *From 1-methoxyhexadecane fermentation.* 16-Hydroxyhexadecanoic acid, m.p. and mixed m.p. 93—95° (Found: C, 70·8; H, 11·9. Calc. for $C_{16}H_{32}O_3$: C, 70·5; H, 11·8%).

(i) *From 1-propoxytetradecane fermentation.* (i) A crystalline mixture (30 mg.) containing methyl β-(13-hydroxytetradecyloxy)propionate (88% by g.l.c.) and a mixture of C_{16} and C_{18} ω-hydroxy-esters (12%); the n.m.r. spectrum (CCl_4) showed a singlet at τ 6·35, a multiplet centred near 6·4, a triplet centred at 6·65, and a triplet centred at 7·52 ($CH_2CH_2OCH_2CH_2CO_2CH_3$), and a doublet centred at 8·85 [$CH_3CH(OH)$]; oxidation gave methyl β-(13-oxotetradecyloxy)propionate, which was identical (m.p., g.l.c., t.l.c., and n.m.r. spectrum) with an authentic sample;[2] (ii) A mixture (27 mg.) containing methyl 14-(2-hydroxypropoxy)tetradecanoate (87% by g.l.c.) and methyl 17-hydroxyoctadecanoate (13%); the n.m.r. spectrum (CCl_4) showed a multiplet centred at τ 6·28, a doublet centred at 8·85, and a broad singlet at 7·5 [$CH_2CH(OH)CH_3$], a triplet centred at 6·6 and a triplet centred at 6·75 ($CH_2CH_2OCH_2CH_2$), a singlet at 6·4 and a triplet centred at 7·75 ($CH_2CH_2CO_2CH_3$); oxidation gave *methyl 14-(2-oxopropoxy)tetradecanoate*, m.p. 45—47° (Found: C, 69·0; H, 11·1. $C_{18}H_{34}O_4$ requires C, 68·75; H, 10·9%); the n.m.r. spectrum ($CDCl_3$) showed a two-proton singlet at τ 6·06, a two-proton triplet centred at 6·57, and a three-proton singlet at 7·89 ($CH_3COCH_2OCH_2CH_2$), a three-proton singlet at 6·4 and a two-proton triplet at 7·73 ($CH_2CH_2CO_2CH_3$).

(j) *From 1-propoxyhexadecane fermentation.* (i) A crystalline mixture containing methyl 14-(2-hydroxypropoxy)-tetradecanoate (85% by g.l.c.); the n.m.r. spectrum was similar to that described above; oxidation gave methyl 14-(2-oxopropoxy)tetradecanoate, identified by m.p., g.l.c., and n.m.r. spectrum. (ii) Methyl β-(15-hydroxyhexadecyloxy)propionate; this compound and its oxidation product, methyl β-(15-oxohexadecyloxy)propionate were identified (m.p., t.l.c., g.l.c., n.m.r. spectra) by comparison with authentic samples.[2]

(k) *From methyl pentadecycloxyacetate fermentation. Methyl 14-hydroxypentadecyloxyacetate*, m.p. 44—45°, $[\alpha]_D^{22}$ +7·8° (*c* 1·0 in MeOH) (Found: C, 68·2; H, 11·7. $C_{18}H_{36}O_4$ requires C, 68·3; H, 11·5%); the n.m.r. spectrum (CCl_4) showed a two-proton singlet at τ 6·06, a three-proton singlet at 6·3, and a two-proton triplet at 6·55 ($CH_2CH_2OCH_2CH_2CO_2CH_3$), a one-proton multiplet centred near 6·3, a one-proton singlet at 8·31, and a three-proton doublet centred at 8·8 [$CH_3CH(OH)CH_2$]; oxidation gave a keto-ester which showed τ ($CDCl_3$) 5·92, 6·2, and 6·5 ($CH_2CHOCH_2CO_2CH_3$), a two-proton triplet centred at 7·6 and a three-proton singlet at 7·88 ($CH_3COCH_2CH_2$).

We thank Mr. G. W. Elson and Mr. B. F. Holmes for carrying out the fermentations, and Mr. R. Colling, Mr. R. J. Pearce, Mr. B. S. Rao, and Mr. R. Tosh for technical assistance.

[8/431 *Received, March 26th,* 1968]

[8] F. Feigl, ' Spot Tests in Organic Analysis,' 5th edn., Elsevier, Amsterdam, 1956, p. 94.