Microbiological Oxidation of Long-chain Aliphatic Compounds. Part II.[1] Branched-chain Alkanes

By **D. F. Jones**, Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

In a medium containing glucose, the yeast *Torulopsis gropengiesseri* converts long-chain methyl-branched alkanes into glycolipids by one or more of three metabolic pathways, namely, (i) alkane ⟶ alkan-1-ol ⟶ alkanoic acid ⟶ ω- and/or ω-1-hydroxyalkanoic acid ⟶ glycolipid, (ii) alkane ⟶ alkan-1-ol ⟶ glycolipid, and (iii) alkane ⟶ alkan-1-ol ⟶ ω- and/or ω-1-hydroxyalkan-1-ol ⟶ glycolipid. Pathways (ii) and (iii) are important for the metabolism of alkan-1-ols whose dehydrogenation to alkanoic acids is inhibited by one or more methyl substituents close to the primary alcohol group. Initial oxidation of 2,2-dimethylhexadecane occurs exclusively at the less hindered terminal position which is the predominant site of the initial oxidation of 2-methylhexadecane. Alkanoic acids and alkan-1-ols which have methyl substituents close to the functional group give ω- and ω-1-hydroxy-derivatives; alkanoic acids and alkan-1-ols which have methyl substituents at the ω-1-position give ω-hydroxy-derivatives.

MICROBIAL growth on branched-chain compounds has been the subject of many reports (reviewed by van der Linden and Thijsse [2]) but relatively little is known of the details of the microbiological oxidation of branched-chain alkanes. Terminal oxidation has been reported by Thijsse and van der Linden,[3] who isolated both 2-methylhexanoic acid and 5-methylhexanoic acid from cultures *Pseudomonas* sp. growing on 2-methylhexane, and by Takahashi and Foster [4] who isolated isovaleric acid from cultures of a bacteria growing on 2-methylbutane. Recently, McKenna and Kallio [5] have reported that a *Corynebacterium* sp. degrades 2,6,10,14-tetramethylpentadecane to give α-methylglutaric acid.

In a previous paper [1] it has been shown that the yeast *Torulopsis gropengiesseri* oxidises both ends of a long-chain alkane to give ω- and ω-1-hydroxy-acids and an αω-dicarboxylic acid. To determine the effect of chain-branching on alkane oxidation by *T. gropengiesseri*, fermentations of methyl-substituted alkanes, alkan-1-ols, and alkanoates have been carried out, and metabolic pathways defined.

Fermentations were performed in a medium containing glucose to which the substrates were added as aqueous emulsions. Methyl esters were used in preference to carboxylic acids because the esters gave the more stable emulsions. The fermentation products were presumed to be glycolipids because they were insoluble in light petroleum and co-chromatographed with glycolipids of defined structure.[6,7] The glycolipid mixtures were extracted with ethyl acetate and subjected to acid-catalysed methanolysis to release the lipid constituents. The lipid mixtures were separated by column chromatography and identified by methods which included gas–liquid chromatography, mass spectrometry, and n.m.r. spectroscopy. The lipids obtained from a variety of methyl-branched compounds are listed in Tables 1—3. In addition to the compounds tabulated, the lipid mixtures contained saturated and unsaturated, oxygenated C_{16} and C_{18} esters, which were derived from the endogenous glycolipids of *T. gropengiesseri*.[1]

TABLE 1

Lipid constituents obtained by methanolysis of glycolipids derived by fermentation of 2-methylhexadecane, 2-methylhexadecan-1-ol, methyl 2-methylhexadecanoate, and methyl 15-methylhexadecanoate

Compound fermented	% Utilised	Lipid constituents of glycolipid methanolysis products; yields * (%) shown in parentheses			
$(CH_3)_2CH\cdot[CH_2]_{13}\cdot CH_3$	92	$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_3$	(6)	$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_3$	(trace)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(1)	$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(3)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(0·7)	$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(trace)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(27)	$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(3)
$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_3$	95	$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_3$	(20)		
		$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(2·5)	$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(2)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(0·5)	$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(0·7)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(10)	$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(trace)
$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_3$	98			$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(7)
				$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(12)
				$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(15)
$(CH_3)_2CH\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	96	$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(40)		

* Corrected for recovered starting material.

All the oxidation products of branched-chain alkanes, listed in Tables 1—3, have a terminal oxygen function, and the second oxygen function, which is present in alkanediols and hydroxy-esters, occupies either the ω- or the ω-1-position. This pattern of oxygenation is similar to that found for n-alkane oxidation by *T. gropengiesseri*,[1] which suggests that the branched-chain alkanes

[1] Part I, D. F. Jones and R. Howe, preceding paper.
[2] A. C. van der Linden and G. J. E. Thijsse, *Adv. Enzymol.*, 1965, **27**, 469.
[3] G. J. E. Thijsse and A. C. van der Linden, *Antonie Van Leewenhoek*, 1961, **27**, 171.
[4] J. Takahashi and J. W. Foster, *Bact. Proc.*, 1966, p. 86.
[5] E. J. McKenna and R. E. Kallio, *Bact. Proc.*, 1964, p. 104.
[6] D. F. Jones, *J. Chem. Soc.* (*C*), 1967, 479.
[7] A. P. Tulloch, A. Hill, and J. F. T. Spencer, *Chem. Comm.*, 1967, 584.

TABLE 2

Lipid constituents obtained by methanolysis of glycolipids derived by fermentation of 2,2-dimethylhexadecane, 2,2-dimethylhexadecan-1-ol, methyl 2,2-dimethylhexadecanoate, 3,3-dimethylheptadecan-1-ol, 3,3-dimethylheptadecanoic acid, and 2,15-dimethylhexadecane

Compound fermented	% Utilised	Lipid constituents of glycolipid methanolysis products; yields * (%) shown in parentheses	
$(CH_3)_2C\cdot[CH_2]_{13}\cdot CH_3$	40	$HO\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(35)
$HO\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_3$	75	$HO\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_3$	(11)
		$HO\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(10)
		$HO\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(1)
		$HO\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(12)
$CH_3\cdot O_2C\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_3$	50	$CH_3\cdot O_2C\cdot C(CH_3)_2\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(20)
		$CH_3\cdot O_2C\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(trace)
		$CH_3\cdot O_2C\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(8)
$HO\cdot[CH_2]_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_3$	40	$HO\cdot CH_2\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_3$	(25)
		$HO\cdot CH_2\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(6)
		$HO\cdot CH_2\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(0·5)
		$HO\cdot CH_2\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(12)
$HO_2C\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_3$	30	$CH_3\cdot O_2C\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{12}\cdot CH(OH)\cdot CH_3$	(30)
		$CH_3\cdot O_2C\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CH_2\cdot OH$	(trace)
		$CH_3\cdot O_2C\cdot CH_2\cdot C(CH_3)_2\cdot[CH_2]_{13}\cdot CO_2\cdot CH_3$	(6)
$(CH_3)_2CH\cdot[CH_2]_{12}\cdot CH(CH_3)_2$	93	$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(CH_3)_2$	(12)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(CH_3)\cdot CH_2\cdot OH$	(20)
		$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(CH_3)_2$	(3)
		$CH_3\cdot O_2C\cdot CH(CH_3)\cdot[CH_2]_{12}\cdot CH(CH_3)\cdot CH_2\cdot OH$	(20)

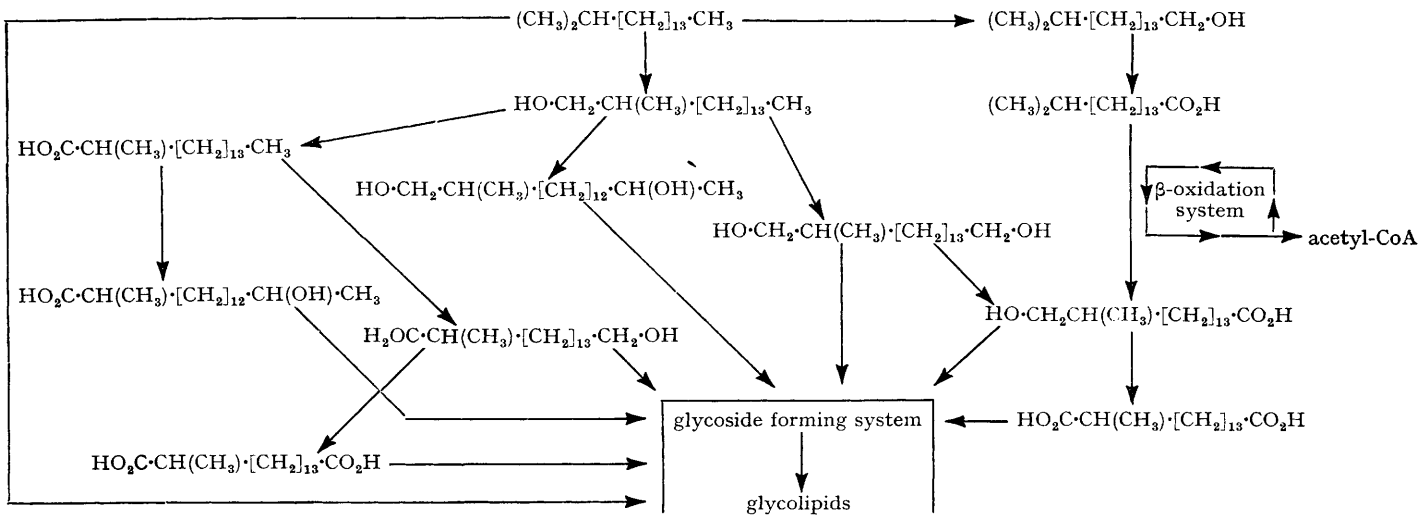
* Corrected for recovered startng material.

TABLE 3

Lipid constituents obtained by methanolysis of glycolipids derived by fermentation of 2,6,10,14-tetramethylpentadecane, 2,6,10,14-tetramethylhexadecane, 3,7,11,15-tetramethylhexadecan-1-ol, and methyl 3,7,11,15-tetramethylhexadecanoate

Compound fermented	% Utilised	Lipid constituents of glycolipid methanolysis products; yields (%) * shown in parentheses	
$(CH_3)_2CH\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_3$	5	$HO\cdot CH_2\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_3$	(30)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot OH$	(16)
		$CH_3\cdot O_2C\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot OH$	(trace)
$(CH_3)_2CH\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CH_3$	14	$HO\cdot CH_2\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CH_3$ and $(CH_3)_2CH\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CH_2\cdot OH$	(20)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CH_2\cdot OH$	(16)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CO_2\cdot CH_3$	(1)
$(CH_3)_2CH\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CH_2\cdot OH$	25	$(CH_3)_2CH\cdot\{[CH_2]_3\cdot CH(CH_2)\}_3\cdot CH_2\cdot CH_2\cdot OH$	(60)
		$HO\cdot CH_2\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_2)\}_3\cdot CH_2\cdot CH_2\cdot OH$	(16)
$(CH_3)_2CH\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CO_2\cdot CH_3$	10	$HO\cdot CH_2\cdot CH(CH_3)\cdot\{[CH_2]_3\cdot CH(CH_3)\}_3\cdot CH_2\cdot CO_2\cdot CH_3$	(35)

* Corrected for recovered starting material.



Proposed pathways of metabolism of 2-methylhexadecane in *T. gropengiesseri*

and n-alkanes are metabolised by closely similar pathways.

Metabolic pathways, which will account for the results of fermentation of 2-methylhexadecane (Table 1), are shown in the Figure. It is proposed that initial oxidation of the alkane takes place at each terminal position to give both 2-methylhexadecan-1-ol and 15-methylhexadecan-1-ol. Dehydrogenation of the latter alkanol would yield 15-methylhexadecanoic acid, which on ω-oxidation would yield 16-hydroxy-15-methylhexadecanoic acid. Subsequent incorporation of this ω-hydroxy-acid into a glycolipid would protect it from further degradation. This pathway, which accounts for the major product of 2-methylhexadecane fermentation, is supported by the results of fermentation of methyl 15-methylhexadecanoate (Table 1). Hydrolysis of the ester linkage in the latter compound is presumed to occur during fermentation, by analogy with the results obtained on fermentation of methyl n-alkanoates.[1]

Metabolism of 2-methylhexadecane by way of 2-methylhexadecan-1-ol is indicated by the isolation of this alkanol from the fermentation product. Dehydrogenation of 2-methylhexadecan-1-ol would give 2-methylhexadecanoic acid which could undergo ω- and ω-1-oxidation to give 15-hydroxy- and 16-hydroxy-2-methylhexadecanoic acids. Dehydrogenation of some of the ω-hydroxy-acids would give 2-methylhexadecane-1,16-dioic acid. Incorporation of this di-acid, and the hydroxy-acids, into glycolipids would protect these compounds from further degradation. This pathway of 2-methylhexadecane metabolism is supported by the results of fermentation of methyl 2-methylhexadecanoate (Table 1).

Compared with n-hexadecane fermentation,[1] which yielded only a trace amount of a glycolipid incorporating n-hexadecan-1-ol, 2-methylhexadecane fermentation gave a high yield of glycolipid incorporating 2-methylhexadecan-1-ol. This suggests that the dehydrogenation of 2-methylhexadecan-1-ol is hindered by the presence of the methyl substituent, and that, consequently, an unusually high proportion of the alkanol is able to reach the site of glycosidation, where it is protected from further oxidation by incorporation into a glycolipid. The diminished susceptibility to dehydrogenation of 2-methylhexadecan-1-ol could also allow some of this alkanol to undergo ω- and ω-1-oxidation followed by glycosidation to give glycolipids incorporating 2-methylhexadecane-1,16-diol and 2-methylhexadecane-1,15-diol (Table 1).

The results of 2-methylhexadecan-1-ol fermentation (Table 1) support the metabolic pathways proposed above, and indicate an additional route to 16-hydroxy-15-methylhexadecanoic acid. This acid presumably arises from 2-methylhexadecane-1,16-diol, which is dehydrogenated at the less sterically hindered primary alcohol group. This pathway could account for some of the 16-hydroxy-15-methylhexadecanoic acid formed from 2-methylhexadecane, but the major route to this acid must involve 15-methylhexadecan-1-ol, as described above, because fermentation of 2-methylhexadecane gave a higher yield of the ω-hydroxy-acid than did the fermentation of 2-methylhexadecan-1-ol (Table 1).

Methyl 16-hydroxy-15-methylhexadecanoate is the major product derived from 2-methylhexadecane fermentation. This indicates that initial oxidation of the alkane occurs preferentially at the less hindered terminal position. A similar finding has been reported by Thijsse and van der Linden [3] for 2-methylhexane oxidation by a *Pseudomonas sp.* However, the results in Table 1 show that terminal oxygenation of both alkanes and alkanoic acids is not prevented by the presence of a methyl substituent adjacent to the reaction site. This is confirmed by the formation of glycolipids incorporating 2,15-dimethylhexadecan-1-ol, 2,15-dimethylhexadecane-1,16-diol, and 16-hydroxy-2,15-dimethylhexadecanoic acid on fermentation of 2,15-dimethylhexadecane (Table 2).

The results of 2,2-dimethylhexadecane fermentation (Table 2) show that *gem*-dimethyl substitution of an alkane at a penultimate carbon atom reduces the efficiency of assimilation but does not prevent oxidative metabolism. 16-Hydroxy-15,15-dimethylhexadecanoic acid, the lipid constituent of the fermentation product, is probably derived from 2,2-dimethylhexadecane by the sequence: (i) terminal oxidation to give 15,15-dimethylhexadecan-1-ol, (ii) dehydrogenation of this alkanol to give 15,15-dimethylhexadecanoic acid, and (iii) terminal oxidation of this acid to give the ω-hydroxy-acid.

Metabolism of 2,2-dimethylhexadecane by way of initial oxidation at the hindered terminal position can be ruled out by the absence in the fermentation product of glycolipids incorporating 2,2-dimethylhexadecan-1-ol, 2,2-dimethylhexadecane-1,16-diol, and 2,2-dimethylhexadecane-1,15-diol. These glycolipids, in addition to the glycolipid incorporating 16-hydroxy-15,15-dimethylhexadecanoic acid, were formed on fermentation of 2,2-dimethylhexadecan-1-ol (Table 2).

Comparison of the results of fermentation of 2,2-dimethylhexadecane with those of the fermentation of the methyl ester of 2,2-dimethylhexadecanoic acid (Table 2) confirms that the latter acid is not an intermediate in the metabolism of 2,2-dimethylhexadecane. Thus, initial oxygenation of 2,2-dimethylhexadecane must occur exclusively at the less hindered terminal position.

The possibility of ω-oxidation of a fatty acid having a highly hindered terminal methyl group has been investigated previously by Tryding and Westöö.[8] These authors fed rats with 2,2,17,17-tetramethyl[1-^{14}C]octadecanoic acid, and isolated as urinary metabolites 2,2-dimethyladipic acid, 2,2-dimethylglutaric acid, and 2,2-dimethylpimelic acid. This result, it was suggested, indicated that the tetramethyloctadecanoic acid was degraded by a mechanism different from that of the ordinary β- and ω-oxidations. However, only a small proportion of the fed activity was recovered in the

[8] N. Tryding and G. Westöö, *Arkiv Kemi*, 1957, **11**, 291.

urine, and approximately 85% of the activity was located in an undefined form in the faeces.

The results of fermenting 2,2-dimethylhexadecan-1-ol, methyl 2,2-dimethylhexadecanoate, 3,3-dimethylheptadecan-1-ol, and 3,3-dimethylheptadecanoic acid (Table 2) indicate that ω- and ω-1-hydroxylation of long-chain alkanols and alkanoic acids is not prevented by the presence of *gem*-dimethyl substitution near the terminal polar group, although the efficiency of hydroxylation is reduced. The origin of the lipid constituents of the fermentation products can be accounted for by metabolic pathways analogous to those proposed above for the metabolism of 2-methylhexadecan-1-ol and methyl 2-methylhexadecanoate. The absence of 15- and 16-hydroxy-2,2-dimethylhexadecanoates in the products derived from 2,2-dimethylhexadecan-1-ol, and of 16- and 17-hydroxy-3,3-dimethylheptadecanoates in the products derived from 3,3-dimethylheptadecan-1-ol, indicates that *gem*-dimethyl substitution near to a primary alcohol group inhibits its dehydrogenation.

The results obtained on fermentation of 2,6,10,14-tetramethylpentadecane and 2,6,10,14-tetramethylhexadecane (Table 3) show that four methyl substituents on a long-chain alkane markedly reduce the ease of its utilisation, although oxidative metabolism still occurs. The origin of the lipid constituents of the glycolipids formed from these highly branched alkanes, and from methyl 3,7,11,15-tetramethylhexadecanoate and 3,7,11,15-tetramethylhexadecan-1-ol (Table 3), can be accounted for by metabolic pathways analogous to those described for mono- and di-methyl substituted alkanes. The formation of both 2,6,10,14-tetramethylhexadecan-1-ol and 3,7,11,15-tetramethylhexadecan-1-ol during the fermentation of 2,6,10,14-tetramethylhexadecane confirms that initial oxygenation of a branched-chain alkane can take place at both terminal positions.

It is likely that some of branched-chain alkanoic acids produced during the fermentation of branched-chain alkanes undergo β-oxidation as an alternative to ω- and ω-1-hydroxylation. However, this metabolic pathway is probably unimportant for acids having methyl substituents at the α- or β-positions, because the low efficiency of utilisation of methyl 3,7,11,15-tetramethylhexadecanoate suggests that *T. gropengiesseri* lacks an efficient mechanism for the degradation of such branched-chain fatty acids.

The close similarities in the metabolic pathways proposed for branched-chain alkanes and those defined for n-alkanes [1] suggest that the oxygenations of branched-chain alkanes and alkanoic acids and those of n-alkanes and alkanoic acids are catalysed by the same enzyme (or enzymes). The efficiencies of utilisation given in Tables 1—3 suggests that the action of this enzyme (or enzymes) is inhibited more by methyl substitution at intermediate positions along the alkyl chain than by methyl substitution near the sites of hydroxylation. This suggests that a long unsubstituted chain of methylene groups may play an important part in the binding of the substrates to the enzyme. The similarity in the oxygenation patterns between branched-chain alkanediols and branched-chain hydroxy-acids reinforces a previous suggestion [1] that ω- and ω-1-hydroxylations of alkanols are catalysed by the enzyme (or enzymes) responsible for the ω- and ω-1-hydroxylations of alkanoic acids.

Several of the alkanoic acid hydroxylations, performed by *T. gropengiesseri*, have close analogies in mammalian systems. The ω- and ω-1-hydroxylations of n-alkanoic acids, reported previously,[1] closely parallel the ω- and ω-1-hydroxylations of n-alkanoic acids in a rat liver homogenate.[9] The ω- and ω-1-hydroxylations of 2-methylhexadecanoate and 2,2-dimethylhexadecanoate, reported in the prepsent paper, closely parallel the ω- and ω-1-hydroxylations of 2,2-dimethyloctanoic acid in rate liver slices.[10] ω-Oxidation in rats of 2-methyloctadecanoic acid,[11] 2,2-dimethyloctadecanoic acid,[12] and 2,2-dimethylnonadecanoic acid [13] has also been reported. These similarities between *T. gropengiesseri* and rat liver systems suggest a similarity in enzyme content. Assuming that all the alkanoic acid oxidations performed by *T. gropengiesseri* are catalysed by the same enzyme, it seems likely that many, if not all, of the alkanoic acid hydroxylations reported in this paper may also take place in mammalian systems.

EXPERIMENTAL

Materials.—Alkanoates. Methyl 2-methylhexadecanoate was prepared by methylation (diazomethane) of 2-methyl hexadecanoic acid, m.p. 44—45° (lit.,[14] 45·5—46·5°), which was prepared from 1-bromotetradecane and diethyl methylmalonate by the method of Schneider and Speilmann.[15] Methyl 15-methylhexadecanoate, b.p. 122—124°/0·18 mm., was prepared by electrolytic coupling of 8-methylnonanoic acid (prepared by the method of Milburn and Truter [16]) and methyl hydrogen azelate, and purified by column chromatography on aluminium oxide. Saponification gave 15-methylhexadecanoic acid, m.p. 60° (lit.,[17] 60—62°). Methyl 2,2-dimethylhexadecanoate, b.p. 140—150°/6 mm., was prepared by electrolytic coupling of tetradecanoic acid and 3-methoxycarbonyl-3-methylbutyric acid, and purified by column chromatography on aluminium oxide. Methyl 3,7,11,15-tetramethylhexadecanoate (methyl phytanoate), b.p. 152—155°/0·45 mm., was prepared by methylation (diazomethane) of 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid), b.p. 159°/0·15 mm. (lit.,[18] 161—162°/0·2

[9] B. Preiss and K. Bloch, *J. Biol. Chem.*, 1964, **239**, 85.
[10] H. Den, *Biochim. Biophys. Acta*, 1965, **98**, 462.
[11] N. Tryding and G. Westöö, *Acta Chem. Scand.*, 1957, **11**, 427.
[12] S. Bergström, B. Bergström, N. Tryding, and G. Westöö, *Biochem. J.*, 1954, **58**, 604.
[13] N. Tryding and G. Westöö, *Acta Chem. Scand.*, 1956, **10**, 1234.
[14] K. E. Schulte, W. Weiszkopf, and J. Kirschner, *Z. physiol. Chem.*, 1951, **288**, 69.
[15] A. R. Schneider and M. A. Speilmann, *J. Biol. Chem.*, 1942, **142**, 351.
[16] A. H. Milburn and E. V. Truter, *J. Chem. Soc.*, 1954, 3344.
[17] A. T. Crossley and B. H. Craig, *Canad. J. Chem.*, 1955, **33**, 426.
[18] V. Kvita and J. Weichet, *Coll. Czech. Chem. Comm.*, 1960, **25**, 254.

mm.). This was prepared from crude phytol (B.D.H.) by hydrogenation over Adams catalyst, followed by oxidation with chromium trioxide in aqueous sulphuric acid and chromatography of the products on a column of Celite–silica gel (1 : 1). 3,3-Dimethylheptadecanoic acid, m.p. 37—38°, was kindly supplied by Dr. R. Howe of these laboratories.

Alkanols. 2-Methylhexadecan-1-ol, m.p. 34—37°, 2,2-dimethylhexadecan-1-ol, m.p. 29—30°, b.p. 115°/0·02 mm., 3,3-dimethylheptadecan-1-ol, b.p. 155°/0·1 mm., 3,7,11,15-tetramethylhexadecan-1-ol (phytanol), b.p. 148—150°/0·4 mm. (lit.,[18] 137°/0·2 mm.) were prepared from the corresponding alkanoates by reduction with lithium aluminium hydride in ether.

Alkanes. 2-Methylhexadecane, b.p. 105—107°/0·6 mm. (lit.,[19] 155°/10 mm.), 2,2-dimethylhexadecane, b.p. 90°/0·06 mm., and 2,6,10,14-tetramethylhexadecane (phytane), b.p. 110°/0·16 mm. [lit.,[20] 69—71°(bath)/0·001 mm.] were prepared from the corresponding alkan-1-ols by reduction of the toluene-*p*-sulphonyl derivatives (prepared with toluene-*p*-sulphonyl chloride and pyridine, 5°, 18 hr.) with lithium aluminium hydride in ether. 2,15-Dimethylhexadecane, b.p. 90°/0·7 mm., was prepared by electrolytic coupling of 8-methylnonanoic acid. 2,6,10,14-Tetramethylpentadecane was purchased from Koch–Light, Colnbrook, Bucks.

All the above compounds were of high purity, as judged by thin-layer and gas chromatography, and gave satisfactory elementary analyses and n.m.r. spectra.

Methods.—Equipment and procedures described previously [1] were employed in the fermentation of branched-chain compounds, and in the determination of the yields and structures of the lipid constituents of the glycolipids produced in these fermentations. Mass spectra were determined on an AEI MS 9 double-focussing mass spectrometer. Acidic and neutral products, obtained by oxidation of mixtures of hydroxylated branched-chain compounds, were separated on columns of silica gel. Relationships between structure and chromatographic properties, similar to those established for straight-chain compounds,[1] were found to apply to the branched-chain analogues. Typical g.l.c. retention times are in Table 4. Where chromatographic properties provided the only evidence of identity, these properties were compared directly with those of authentic compounds.

Compounds isolated from Fermentation Products.—In addition to characterisation by the chromatographic techniques described above, the following compounds were isolated.

(a) *From 2-methylhexadecane fermentation.* (i) 2-Methylhexadecan-1-ol, m.p. 33—36° (Found: C, 79·9; H, 14·4. Calc. for $C_{17}H_{36}O$: C, 79·6; H, 14·2%), τ ($CDCl_3$) multiplet centred at 6·55 [$CH(CH_3)CH_2OH$], broad singlet at 8·5 (O*H*), multiplet centred at 8·74 [$(CH_2)_n$], and multiplets [$CH(CH_3)$, CH_2CH_3]. (ii) *Methyl 16-hydroxy-15-methylhexadecanoate*, m.p. 40—42° (Found: C, 71·8; H, 12·0. $C_{18}H_{36}O_3$ requires C, 71·95; H, 12·1%), (τ CCl_4) singlet at 6·48 (CH_3O), multiplet centred at 6·72 [$CH(CH_2OH)$], triplet centred at 7·83 (CH_2CH_2CO), singlet at 8·22 (O*H*), multiplet centred at 8·78 [$(CH_2)_n$], and doublet centred at 9·16 [$CH(CH_3)$]; saponification gave *16-hydroxy-15-methylhexadecanoic acid*, m.p. 71—74° (Found: C, 71·3; H, 12·0. $C_{17}H_{34}O_3$ requires C, 71·3; H, 12·0%); oxidation, followed by treatment with ethereal diazomethane, gave liquid dimethyl 2-methylhexadecane-1,16-dioate, which was identified by comparison of its g.l.c. retention time and its n.m.r. spectrum with those of an authentic specimen. (iii) Methyl 15-hydroxy-2-methylhexadecanoate; this was not obtained pure, but oxidation, followed by chromatography gave a liquid product which contained 95% (g.l.c.) of methyl 2-methyl-15-oxohexadecanoate, which was identified by comparison (g.l.c., t.l.c., n.m.r.) with an authentic specimen.

(b) *From methyl 15-methylhexadecanoate fermentation.* Methyl 16-hydroxy-15-methylhexadecanoate, m.p. 42—44° (Found: C, 71·7; H, 11·9%); the n.m.r. spectrum was identical with that given above for this compound.

(c) *From methyl 2-methylhexadecanoate fermentation.* (i) *15-Hydroxy-2-methylhexadecanoic acid*, m.p. 57—60° (Found: C, 70·9; H, 11·7. $C_{17}H_{34}O_3$ requires C, 71·3; H, 12·0%); treatment with ethereal diazomethane gave a methyl ester showing τ (CCl_4) singlet at 6·36 (CH_3O), multiplet centred near 6·4 [$CH(OH)CH_3$], broad singlet at 7·32 (O*H*), multiplet centred at 7·74 [$CH_2CH(CH_3)CO$], multiplet 8·74 [$(CH_2)_n$], and doublets centred at 8·88 [$CH_3CH(OH)$ and $CH(CH_3)CO_2H$]; oxidation gave *2-methyl-15-oxohexadecanoic acid*, m.p. 60—63° (Found: C, 71·7; H, 11·5. $C_{17}H_{32}O_3$ requires C, 71·8; H, 11·3%), τ ($CDCl_3$) multiplet centred near 7·6 [CH_2CH_2CO and $CH_2CH(CH_3)CO$], singlet at 7·9 (CH_3CO), multiplet centred at 8·76 [$(CH_2)_n$], and doublet centred at 8·84 [$CH(CH_3)CO_2H$]. (ii) 2-Methylhexadecane-1,16-dioic acid, m.p. 88—90° (lit.,[21] 89—90°) (Found: C, 67·7; H, 10·6. Calc. for $C_{17}H_{32}O_4$: C, 68·0;

TABLE 4

G.l.c.* retention times of some oxidised derivatives of branched-chain alkanes on a silicone column

Compound	Retention time (sec.) †
$CH_3{\cdot}[CH_2]_{13}{\cdot}CH(CH_3){\cdot}CO_2{\cdot}CH_3$	59
$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{12}{\cdot}CH(CH_3){\cdot}CO_2{\cdot}CH_3$	116
$HO{\cdot}CH_2{\cdot}[CH_2]_{13}{\cdot}CH(CH_3){\cdot}CO_2{\cdot}CH_3$	149
$CH_3{\cdot}O_2C{\cdot}[CH_2]_{13}{\cdot}CH(CH_3){\cdot}CO_2{\cdot}CH_3$	159
$CH_3{\cdot}[CH_2]_{13}{\cdot}C(CH_3)_2{\cdot}CH_2{\cdot}CO_2{\cdot}CH_3$	100
$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{12}{\cdot}C(CH_3)_2{\cdot}CH_2{\cdot}CO_2{\cdot}CH_3$	190
$(CH_3)_2CH{\cdot}\{[CH_2]_3{\cdot}CH(CH_3)\}_3{\cdot}CH_2{\cdot}CO_2{\cdot}CH_3$	91
$HO{\cdot}CH_2{\cdot}CH(CH_3){\cdot}\{[CH_2]_3{\cdot}CH(CH_3)\}_3{\cdot}CH_2{\cdot}CO_2{\cdot}CH_3$	216
$(CH_3)_2CH{\cdot}\{[CH_2]_3{\cdot}CH(CH_3)\}_3{\cdot}CH_2{\cdot}CH_2{\cdot}OH$	89
$HO{\cdot}CH_2{\cdot}CH(CH_3){\cdot}\{[CH_2]_3{\cdot}CH(CH_3)\}_3{\cdot}CH_2{\cdot}CH_2{\cdot}OH$	212
$CH_3{\cdot}O_2C{\cdot}CH(CH_3){\cdot}\{[CH_2]_3{\cdot}CH(CH_3)\}_3{\cdot}CH_2{\cdot}CO_2{\cdot}CH_3$	221
$CH_3{\cdot}O_2C{\cdot}[CH_2]_{13}{\cdot}C(CH_3)_2{\cdot}CO_2{\cdot}CH_3$	182
$CH_3{\cdot}[CH_2]_{13}{\cdot}C(CH_3)_2{\cdot}CO_2{\cdot}CH_3$	62
$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{12}{\cdot}C(CH_3)_2{\cdot}CO_2{\cdot}CH_3$	120
$CH_3{\cdot}[CH_2]_{13}{\cdot}C(CH_3)_2{\cdot}CH_2{\cdot}OH$	63
$CH_3{\cdot}CH(OH){\cdot}[CH_2]_{12}{\cdot}C(CH_3)_2{\cdot}CH_2{\cdot}OH$	126
$CH_3{\cdot}[CH_2]_{13}{\cdot}C(CH_3)_2{\cdot}CH_2{\cdot}CH_2{\cdot}OH$	102
$HO{\cdot}CH_2{\cdot}[CH_2]_{13}{\cdot}C(CH_3)_2{\cdot}CH_2{\cdot}CH_2{\cdot}OH$	245
$(CH_3)_2CH{\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	67
$HO{\cdot}CH_2{\cdot}CH(CH_3){\cdot}[CH_2]_{13}{\cdot}CO_2{\cdot}CH_3$	163
$(CH_3)_2CH{\cdot}[CH_2]_{12}{\cdot}CH(CH_3){\cdot}CO_2{\cdot}CH_3$	70
$HO{\cdot}CH_2{\cdot}CH(CH_3){\cdot}[CH_2]_{12}{\cdot}CH(CH_3){\cdot}CO_2{\cdot}CH_3$	168
$(CH_3)_2CH{\cdot}[CH_2]_{12}{\cdot}CH(CH_3){\cdot}CH_2{\cdot}OH$	68
$HO{\cdot}CH_2{\cdot}CH(CH_3){\cdot}[CH_2]_{12}{\cdot}CH(CH_3){\cdot}CH_2{\cdot}OH$	163

* Glass column (5 ft. × 5 mm. i.d.) packed with Chromosorb G (80—100 mesh) with a coating (2·5%) of silicone rubber gum (S.E. 30), operated at 220° with a nitrogen flow rate of 40 ml./min. Under these conditions methyl hexadecanoate and methyl 15-hydroxyhexadecanoate had retention times of 48 and 96 sec. respectively. † Measured from solvent ($CHCl_3$) peak.

[19] E. Terres, F. Gerbert, D. Fischer, and G. Modak, *Brenstoff-Chem.*, 1959, **40**, 279.

[20] J. S. Sorensen and N. A. Sorensen, *Acta Chem. Scand.*, 1949, **3**, 939.

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

H, 10·7%); treatment with ethereal diazomethane gave the liquid dimethyl ester which showed τ (CCl_4) singlet at 6·42 (CH_3O), multiplet centred at 7·75 [CH_2CH_2CO and $CH_2CH(CH_3)CO$], multiplet centred at 8·73 [$(CH_2)_n$], and doublet at 8·88 [$\cdot CH(CH_3)\cdot$]. (iii) 16-*Hydroxy*-2-*methylhexadecanoic acid*, m.p. 66—70° (Found: C, 70·7; H, 11·5. $C_{17}H_{34}O_3$ requires C, 71·3; H, 12·0%); treatment with ethereal diazomethane gave the methyl ester which showed τ ($CDCl_3$) singlet at 6·32 (CH_3O), multiplet centred at 6·35 (CH_2CH_2OH), multiplet centred at 7·7 [$CH_2CH(CH_3)CO$], broad singlet at 8·3 (OH), multiplet centred at 8·74 [$(CH_2)_n$], and doublet centred at 8·85 [$CH(CH_3)CO$]; oxidation gave 2-methylhexadecane-1,16-dioic acid, m.p. 86—88°.

(d) *From* 2-*methylhexadecan*-1-*ol fermentation*. (i) 2-Methylhexadecan-1-ol, identified by g.l.c., t.l.c., m.p., mixed m.p., and n.m.r. spectrum. (ii) 2-*Methylhexadecane*-1,15-*diol*, m.p. 65—68° (Found: C, 74·6; H, 13·0. $C_{17}H_{36}O_2$ requires C, 74·9; H, 13·3%), τ ($CDCl_3$) multiplet centred at 6·25 [$CH_2CH(OH)CH_3$], multiplet centred at 6·6 [$CH(CH_3)CH_2OH$], broad singlet at 8·44 (OH), multiplet centred at 8·74 [$(CH_2)_n$], doublet centred at 8·84 [$CH_3CH(OH)$], and doublet centred at 9·12 [$CH(CH_3)$].

(e) *From* 2,15-*dimethylhexadecane fermentation*. (i) 2,15-*Dimethylhexadecan*-1-*ol*, m.p. 42° (Found: C, 80·0; H, 13·9. $C_{18}H_{38}O$ requires C, 79·9; H, 14·2%), τ (CCl_4) multiplet centred at 6·72 [$CH(CH_3)CH_2OH$], multiplet centred at 8·78 [$(CH_2)_n$], and multiplets 9·12—9·21 [$CH(CH_3)$] and [$C(CH_3)_2$]. (ii) 2,15-*Dimethylhexadecane*-1,16-*diol*, m.p. 75—77° (Found: C, 75·5; H, 13·6. $C_{18}H_{38}O_2$ requires C, 75·5; H, 13·4%), τ (CCl_4) multiplets centred at 6·72 [$HOCH_2CH(CH_3)$], multiplet centred at 8·87 [$(CH_2)_n$], and doublets centred at 9·16 [$CH(CH_3)$]; oxidation gave 2,15-*dimethylhexadecane*-1,16-*dioic acid*, m.p. 96—98° (Found: C, 68·9; H, 10·75. $C_{18}H_{34}O_4$ requires C, 68·8; H, 10·9%), τ ($CDCl_3$) multiplet centred at 7·6 [$CH_2CH(CH_3)CO$], multiplet centred at 8·76 [$(CH_2)_n$], and doublets centred at 8·86 [$CH(CH_3)CO$]. (iii) 16-*Hydroxy*-2,15-*dimethylhexadecanoic acid*, m.p. 76—77° (Found: C, 71·8; H, 11·8. $C_{18}H_{36}O_3$ requires C, 71·95; H, 12·1%); treatment with ethereal diazomethane gave a liquid methyl ester which showed τ ($CDCl_3$) singlet at 6·33 (CH_3O), multiplet centred at 6·5 [$HOCH_2CH(CH_3)$], multiplet centred near 7·7 [$CH_2CH(CH_3)CO$], broad singlet at 8·07 (OH), multiplet centred at 8·72 [$(CH_2)_n$], doublet centred at 8·86 [$CH(CH_3)CO$], and doublet centred at 9·09 [$CH(CH_3)CH_2OH$]; oxidation gave 2,15-dimethylhexadecane-1,16-dioic acid, m.p. 95°.

(f) *From* 2,2-*dimethylhexadecane fermentation*. A mixture (10 mg.) containing 96% (g.l.c.) of 16-hydroxy-15,15-dimethylhexadecanoate, 3·5% of methyl 17-hydroxyoctadecanoate, and 0·5% of methyl 17-hydroxyoctadec-9-enoate; the major component of this mixture had the same retention time as methyl 17-hydroxyoctadecanoate but the retention time of its propionyl derivative was slightly shorter than that of methyl 17-propionyloxyoctadecanoate; the n.m.r. spectrum of the mixture showed τ (CCl_4) singlet at 6·42 (CH_3O), singlet at 6·81 [$HOCH_2C(CH_3)_2$], triplet centred at 7·8 (CH_2CH_2CO), broad singlet at 8·51 (OH), multiplet centred at 8·74 [$(CH_2)_n$], and singlet at 9·18 [$C(CH_3)_2$]; the mass spectrum of the mixture included ions at *m/e* 314 (M^+) and 284 $(M - CH_2O)^+$, and a series of ions corresponding to $[(CH_2)_n\cdot CO_2CH_3]^+$, where $n = 2$ to 13, and an ion at *m/e* 74 $[CH_2{:}C(OH)OCH_3]^+$; the latter ions are typical of a long-chain fatty ester;[22] oxidation of the mixture, followed by methylation with diazomethane, gave dimethyl 2,2-dimethylhexadecane-1,16-dioate, a liquid which showed τ (CCl_4) singlet at 6·49 (CH_3O), triplet centred at 7·85 (CH_2CH_2CO), multiplet centred at 8·79 [$(CH_2)_n$], and singlet at 8·93 [$C(CH_3)_2CO$]; the mass spectrum included an ion at *m/e* 342 (M^+), a series of ions corresponding to $[(CH_2)_n\cdot CO_2CH_3]^+$ where $n = 2$ to 12, and ions at *m/e* 74 $[CH_2{:}C(OH)OCH_3]^+$ and 102 $[C(CH_3)_2{:}C(OH)OCH_3]^+$.

(g) *From methyl* 2,2-*dimethylhexadecanoate fermentation*. (i) Dimethyl 2,2-dimethylhexadecane-1,16-dioate; identified by g.l.c. and n.m.r. and mass spectra (see above). (ii) A mixture (30 mg.) containing methyl 15-hydroxy-2,2-dimethylhexadecanoate (92% by g.l.c.), methyl 17-hydroxyoctadec-9-enoate (5%), and methyl 17-hydroxyoctadecanoate (3%); the n.m.r. spectrum of this liquid mixture showed τ (CCl_4) singlet at 6·4 (CH_3O), broad singlet at 8·3 (OH), multiplet centred at 8·73 [$(CH_2)_n$], singlet at 8·87 [$C(CH_3)_2CO$], and a doublet centred at 8·89 [$CH_3CH(OH)$]; the mass spectrum included ions at *m/e* 314 (M^+), 296 $(M - H_2O)^+$, 102 $[C(CH_3)_2{:}C(OH)CH_3]^+$, and 45 $(CH_3CH{:}OH)^+$; oxidation gave a neutral ketonic product (24 mg.), which contained 90% of methyl 2,2-dimethyl-15-oxohexadecanoate which showed τ (CCl_4) singlet at 6·42 (CH_3O), triplet centred at 7·7 (CH_2CH_2CO), singlet at 7·96 (CH_3CO), multiplet centred at 8·74 [$(CH_2)_n$], and singlet 8·87 [$C(CH_3)_2CO$]; the mass spectrum included ions at *m/e* 312 (M^+), 269 $(M - CH_3O)^+$, 255 $(M - CH_3COCH_2)^+$, and 102 $[C(CH_3)_2{:}C(OH)OCH_3]^+$.

(h) *From* 2,2-*dimethylhexadecan*-1-*ol fermentation*. (i) 2,2-*Dimethylhexadecan*-1-*ol*, m.p. 28—30° (Found: C, 80·2; H, 14·0. $C_{18}H_{38}O$ requires C, 79·9; H, 14·2%); identified by mixed m.p., g.l.c., t.l.c., and n.m.r. spectrum. (ii) A liquid mixture (30 mg.) containing methyl 16-hydroxy-15,15-dimethylhexadecanoate (97% by g.l.c.) and methyl 17-hydroxyoctadecanoate (3%); the major constituent was identical (g.l.c., t.l.c., n.m.r., and mass spectroscopy) with the methyl 16-hydroxy-15,15-dimethylhexadecanoate obtained above. (iii) A mixture (19 mg.) containing 2,2-dimethylhexadecane-1,15-diol (92%) and an unidentified impurity; the n.m.r. spectrum showed τ (CCl_4) multiplet centred near 6·4 [$CH_3CH(CH_3)CH_2$], singlet at 6·8 [$HOCH_2C(CH_3)_2$], broad singlet at 8·3 (OH), multiplet centred at 8·74 [$(CH_2)_n$], doublet centred at 8·8 [$CH_3CH(OH)$], and singlet at 9·16 [$C(CH_3)_2$]; oxidation, followed by methylation with diazomethane, gave methyl 2,2-dimethyl-15-oxohexadecanoate identified by g.l.c., t.l.c., n.m.r., and mass spectrum (see above).

(i) *From* 3,3-*dimethylheptadecan*-1-*ol fermentation*. (i) 3,3-*Dimethylheptadecan*-1-*ol*, b.p. 155°/0·1 mm. (Found: C, 79·9; H, 13·9. $C_{19}H_{40}O$ requires C, 80·2; H, 14·2%); the n.m.r. spectrum showed τ (CCl_4) triplet centred at 6·47 ($HOCH_2CH_2$), broad singlet at 7·0 (OH), singlet and triplet centred at 9·12 [$C(CH_3)_2$ and CH_3CH_2]. (ii) A liquid mixture (23 mg.) containing methyl 17-hydroxy-15,15-dimethylheptadecanoate (90% by g.l.c.) and methyl 17-hydroxyoctadecanoate (10%); the n.m.r. spectrum showed τ (CCl_4) singlet at 6·41 (CH_3O), triplet centred at 6·45 ($HOCH_2CH_2$), triplet centred at 7·85 (CH_2CH_2CO), multiplet centred at 8·74 [$(CH_2)_n$], and singlet at 9·16 [$C(CH_3)_2$]; oxidation, followed by chromatography on silica gel and methylation with diazomethane, gave methyl 3,3-dimethylheptadecane-1,17-dioate (10 mg., 99% pure by g.l.c.); the mass spectrum included ions at *m/e* 356 (M^+), 115

[22] S. Abrahamsson, S. Stalberg-Stenhagen, and E. Stenhagen, *Progr. Chem. Fats and Lipids*, 1963, **7**, Part 1, p. 41.

$[C(CH_3)_2CH_2CO_2CH_3]^+$, and 74 $[CH_2{:}C(OH)OCH_3]^+$, and the n.m.r. spectrum showed τ ($CDCl_3$) singlets at 6·42 and 6·44 (CH_3O), triplet centred at 7·75 (CH_2CH_2CO), singlet at 7·86 $[(CH_3)_2CH_2CO]$, multiplet centred at 8·78 $[(CH_2)_n]$, and singlet at 9·06 $[C(CH_3)_2]$. (iii) A mixture (10 mg.) containing 3,3-dimethylheptadecane-1,16-diol (98% by g.l.c.); the n.m.r. spectrum ($CDCl_3$) showed multiplet centred near 6·4 $[CH_3CH(OH)]$, triplet centred at 6·4 (CH_2CH_2OH), multiplet centred at 8·79 $[(CH_2)_n]$, doublet centred at 8·86 $[CH_3CH(OH)]$, and singlet at 9·16 $[C(CH_3)_2]$; oxidation, followed by methylation with diazomethane, gave methyl 3,3-dimethyl-16-oxoheptadecanoate, a glass which showed τ ($CDCl_3$) singlet at 6·44 (CH_3O), triplet centred at 7·62 and singlet at 7·93 ($CH_3COCH_2CH_2$), singlet at 7·86 $[C(CH_3)_2CH_2CO]$, and singlet at 9·06 $[C(CH_3)_2]$; the mass spectrum included ions at *m/e* 326 (M^+), 115 $[C(CH_3)_2CH_2CO_2CH_3]^+$, 74 $[CH_2{:}C(OH){\cdot}OCH_3]^+$, and 43 $(CH_3CO)^+$.

(j) *From* 3,3-*dimethylheptadecanoic acid fermentation.* Methyl 16-hydroxy-3,3-dimethylheptadecanoate, a liquid 98% pure by g.l.c.; the n.m.r. spectrum showed τ (CCl_4) singlet at 6·42 (CH_3O), multiplet centred near 6·4 $[CH_3CH(OH)]$, singlet at 7·87 $[(CH_3)_2CH_2CO]$, singlet at 8·3 (OH), multiplet centred at 8·75 $[(CH_2)_n]$, doublet centred at 8·87 $[CH_3CH(OH)]$, and singlet at 9·06 $[C(CH_3)_2]$; the mass spectrum included ions at *m/e* 328 (M^+), 115 $[C(CH_3)_2CH_2CO_2CH_3]^+$, 74 $[CH_2{:}C(OH)CH_3]^+$, and 45 $(CH_3CH{:}OH)^+$; oxidation gave methyl 3,3-dimethyl-16-oxoheptadecanoate identified by t.l.c., g.l.c., and n.m.r. spectrum (see above).

(k) *From methyl* 3,7,11,15-*tetramethylhexadecanoate fermentation.* A liquid mixture (20 mg.) containing methyl 16-hydroxy-3,7,11,15-tetramethylhexadecanoate (97% by g.l.c.); the n.m.r. spectrum showed τ (CCl_4) singlet at 6·4 (CH_3O), multiplet centred at 6·65 $[HOCH_2CH(CH_3)]$, triplet centred at 7·8 $[CH(CH_3)CH_2CO]$, broad singlet at 8·3 (OH), multiplet centred at 8·74 $[(CH_2)_n]$, and multiplets 9·04—9·17 $[CH(CH_3)]$; the mass spectrum showed ions at *m/e* 342 (M^+), 327 $(M - CH_3)^+$, and 324 $(M - H_2O)^+$, and included ions at *m/e* 74, 101, 143, 171, 213, 241, and 283 (the latter ions, which were formed by fissions of the alkyl chain at each side of the points of methyl branching, are present in the mass spectrum of methyl 3,7,11,15-tetramethylhexadecanoate [22]); oxidation of the liquid mixture, followed by methylation with diazomethane, gave a product containing 95% (g.l.c.) of methyl 2,6,10,14-tetramethylhexadecane-1,16-dioate which showed τ (CCl_4) singlets 6·48 (CH_3O), multiplets 7·5—8·0 (CH_2CH_2CO) and $[CH_2CH(CH_3)CO]$, multiplet centred at 8·77 $[(CH_2)_n]$, doublet centred at 8·94 $[CH(CH_3)CO]$, and multiplets 9·08—9·22 $[CH(CH_3)]$; spin-decoupling, by double irradiation of protons having τ 7·6—7·8, caused the doublet at 8·94 to collapse into a singlet, confirming the presence of the group $CH(CH_3)CO$; the mass spectrum showed ions at *m/e* 370 (M^+), 283 $(M - CH_3CHCOOCH_3)^+$, 74 and 101 [indicative of the group $CH_2CH(CH_3)CH_2CO_2CH_3$ [22]], and *m/e* 88 [indicative of the group $CH_2CH_2CH(CH_3)CO_2CH_3$ [22]].

(l) *From* 2,6,10,14-*tetramethylhexadecane fermentation.* (i) 2,6,10,14-Tetramethylhexadecan-1-ol and 3,7,11,15-tetramethylhexadecan-1-ol (phytanol); these compounds were obtained as a liquid mixture (2 mg.) which behaved as a single compound on t.l.c. (R_F = phytanol) and on g.l.c. (retention time = phytanol, retention time of propionates = phytanyl propionate); the mass spectrum lacked molecular ions but was consistent with that of the proposed mixture of alkanols because it showed an ion at *m/e* 280, $(M - H_2O)^+$ and ions at 252 and 238 which could be attributed to elimination of ethylene and propene from the ions of *m/e* 280; such eliminations are typical of the behaviour of alkyl derivatives having the terminal groups CH_2CH_3 and $CH(CH_3)_2$; [23] oxidation of the mixture of alkanols, followed by methylation with diazomethane, gave a mixture of esters which behaved as a single compound on t.l.c. (R_F = methyl phytanoate) and g.l.c. (retention time = methyl phytanoate); the n.m.r. spectrum (CCl_4) of this mixture of closely resembled that of methyl phytanoate but showed an additional weak doublet near τ 8·9 $[CH(CH_3)CO]$; the mass spectrum was consistent with that of a mixture of methyl 2,6,10,14-tetramethylhexadecanoate and methyl 3,7,11,15-tetramethylhexadecanoate, and showed ions at *m/e* 326 (M^+), 74 and 101 [indicative of $CH_2CH(CH_3)_2CO_2CH_3$ [22]], and 88 [indicative of $CH_2CH_2CH(CH_3)CO_2CH_3$ [22]]. (ii) A mixture (3 mg.) containing 2,6,10,14-tetramethylhexadecane-1,16-diol (90% by g.l.c.); the n.m.r. spectrum ($CDCl_3$) showed multiplets in the region τ 6·3—6·7 $[HOCH_2CH(CH_3)]$ and $(HOCH_2CH_2)$, multiplet centred near 8·8 $[(CH_2)_n]$, and multiplets 9·1—9·2 $[CH(CH_3)]$; oxidation, followed by methylation with diazomethane, gave dimethyl 2,6,10,14-tetramethylhexadecane-1,16-dioate identified by g.l.c., t.l.c., n.m.r., and mass spectroscopy (see above).

(m) *From* 2,6,10,14-*tetramethylpentadecane fermentation.* A liquid mixture (20 mg.) containing 2,6,10,14-tetramethylpentadecan-1-ol (97% by g.l.c.); the n.m.r. spectrum showed τ (CCl_4) multiplet centred at 6·7 $[HOCH_2CH(CH_2)]$, broad singlet at 8·06 (OH), multiplet centred near 8·83 $[(CH_2)_n]$, and multiplets 9·14—9·21 $[CH(CH_3)]$; the mass spectrum included ions at *m/e* 284 (M^+) and 266 $(M - H_2O)^+$; oxidation, followed by treatment with diazomethane, gave methyl 2,6,10,14-tetramethylpentadecanoate, τ (CCl_4) singlet at 6·46 (CH_3O), multiplet centred near 7·75 $[CH_2CH(CH_3)CO]$, and multiplets 9·12—9·21 $[CH(CH_3)]$. (ii) A liquid mixture (20 mg.) containing 2,6,10,14-tetramethylpentadecane-1,15-diol (90% by g.l.c.); the n.m.r. spectrum showed τ (CCl_4) multiplet centred at 6·7 $(HOCH_2CH(CH_3))$, broad singlet 8·1 (OH), multiplet centred near 8·8 $[(CH_2)_n]$, and multiplets 9·14—9·21 $[CH(CH_3)]$; the mass spectrum showed ions at *m/e* 300 (M^+), 282 $(M - H_2O)^+$, and 252 $(282 - CH_2O)^+$; oxidation followed by treatment with diazomethane gave dimethyl 2,6,10,14-tetramethylpentadecane-1,15-dioate, which showed τ (CCl_4) singlet at 6·46 (CH_3O), multiplets centred near 7·75 $[CH_2CH(CH_3)CO]$, multiplet centred near 8·8 $[(CH_2)]$, doublet centred at 8·93 $[CH(CH_3)CO]$, and multiplets 9·12—9·21 $[CH(CH_3)]$.

I thank Mr. G. W. Elson and Mr. B. F. Holmes for carrying out the fermentations, and Mr. R. Wilkins for technical assistance.

[8/430 *Received, March 26th*, 1968]

[23] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Interpretation of Mass Spectra of Organic Compounds,' Holden-Day, San Francisco, 1964, p. 35.