The Structure of Novel C_{35} Pentacyclic Terpenes from Acetobacter xylinum

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A novel C_{35} terpene and its monounsaturated analogue were isolated from cultures of Acetobacter xylinum, together with traces of their C_{36} homologues. These substances were found to be hopane derivatives substituted by a five-carbon chain bearing four vicinal hydroxyl groups. For the parent hydrocarbon the term bacteriohopane is proposed. The elucidation of the structures utilized high-resolution mass spectrometry of the terpenes, degradation to C_{32} hydrocarbons and detailed mass-spectrometric comparison of these with C_{32} hydrocarbons synthesized from known pentacyclic triterpenes. High-resolution mass-spectral data of the terpenes are presented. N.m.r. data are in agreement with the proposed structures, which are further supported by the isolation of the same organism from 22-hydroxyhopane and derivative hopene(s).

Acetobacter xylinum synthesizes, and excretes into the medium, a complex lipid that is involved in the alignment of extracellular cellulose microfibrils. Two related lipids have been isolated that also have some degree of activity in microfibril alignment. The isolation and bioassay of these compounds and the possible relevance of their activity to cell-wall synthesis in higher plants are discussed in the accompanying paper (Haigh et al., 1973). The present paper describes the structural characterization of the non-saponifiable portion of the most-active compound (VI) and related compounds. The non-saponifiable portion is active in itself but was isolated and purified as the inactive acetate ester to avoid problems of instability.

Methods and Results
Source of compounds
The isolation, purification and bioassay of the non-saponifiable compound with the greatest activity and of related compounds are described in the accompanying paper (Haigh et al., 1973).

Instrumentation
Low-resolution mass spectra were recorded by means of a Hitachi RMU-6D or an Atlas model CH4

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mass spectrometer. The g.l.c.-m.s. (gas-liquid chromatography–mass spectrometry) analysis employed a Perkin–Elmer 990 gas chromatograph–Hitachi RMU-6L mass spectrometer combination, which is operated on line with an IBM 1800 computer (Hites & Beimann, 1968). High-resolution mass spectra were obtained from a CEC-21-110B mass spectrometer with photoplate recording. The line positions and intensities were measured with a D.W. Mann (Burlington, Mass., U.S.A.) microdensitometer operated on line with an IBM 1800 computer which is programmed to calculate accurate masses and elemental compositions (Biemann, 1968). I.r. spectra were recorded with a Perkin–Elmer Infracord, model 237. The 60 MHz proton n.m.r. spectra were observed by means of a Varian A60A spectrometer and a Hitachi R20B spectrometer with A-1600A signal-averaging analyser. The 100 MHz proton n.m.r. spectra were recorded with a Varian HA100 spectrometer. Molecular-weight determination by osmometry was by means of a Mechrolab model 301A osmometer.

Saponification of fraction 2
Fraction 2, the acetylated material (Haigh et al., 1973), had a molecular weight of 732±3%. For saponification, it was heated under reflux with methanolic 2M-KOH for 30 min, the mixture was diluted with water and the alcohol extracted with diethyl ether.
High-resolution mass spectrometry of fraction 2 and the alcohol from fraction 2

The high-resolution mass spectra of fraction 2 and the alcohol from fraction 2 were measured. Their characteristic ions are listed in Table 1.

I.r. spectra of fraction 2 and the alcohol from fraction 2

The i.r. spectrum of fraction 2 in carbon disulphide exhibited strong bands characteristic of an acetate ester (1760 and 1220 cm\(^{-1}\)), gem-dimethyl groups (1375 cm\(^{-1}\)), methyl and methylene groups (2940, 2925 and 2860 cm\(^{-1}\)), and a weak band (1650 cm\(^{-1}\)) indicating a double bond. The i.r. spectrum of the alcohol from fraction 2 in chloroform exhibited strong bands for an alcohol (3605, 3450, 1110 and 1020 cm\(^{-1}\)), methyl and methylene groups (2930 and 2865 cm\(^{-1}\)), but no carbonyl absorption.

N.m.r. spectra of fraction 2

The 100 MHz proton n.m.r. spectrum of fraction 2 in deuterochloroform showed methyl signals at 0.77, 0.68 and 0.80 ppm, together with a broad envelope for aliphatic hydrogens between 1.20 and 1.85 ppm (\(\sim 30\)H), signals for acetate-methyl protons at 2.12 and 2.16 ppm (12H), two complex signals centered at 4.25 ppm (2H) and 5.25 ppm (3H) indicative of \(\sim\)CH\(_2\)-OAc and \(\sim\)CH-OAc groups and an olefinic proton signal at 5.50 ppm (1H).

Degradation of the alcohol from fraction 2 to \(C_{32}\) hydrocarbons (Scheme 1)

Because of scarcity of material, the degradation sequence was begun with 2.5 mg of alcohol from fraction 2 and carried through all consecutive steps without isolation of the product, which was characterized at each step by low- and high-resolution mass spectra and i.r. spectra where indicated. The same approach was used for preparation of samples for comparison as outlined in later paragraphs. The oxidation of the alcohol from fraction 2 with an excess of \(H_2IO_6\) in tetrahydrofuran-water (95:5, v/v) yielded two carbonyl compounds of elemental composition \(C_{32}H_{54}O\) and \(C_{32}H_{52}O\), which exhibited a strong absorption at 1725 cm\(^{-1}\) (in chloroform). The further oxidation of the carbonyl compounds with Jones reagent (Augustine, 1969) yielded two \(C_{32}\) carboxylic acids of elemental composition \(C_{32}H_{54}O_2\) and \(C_{32}H_{52}O_2\). The \(C_{32}\) carbonyl compounds were reduced to the corresponding alcohols (\(C_{32}H_{56}O\) and \(C_{32}H_{54}O\)) with LiAlH\(_4\) in dimethoxyethane. Reaction of the \(C_{32}\) alcohols with phosphorus tribromide in toluene gave

0.68, 0.77, 0.80, 0.86, 0.94 and 1.10 (\(\delta\) in p.p.m. from tetramethylsilane as internal standard), totalling eight methyl groups, a broad envelope for aliphatic hydrogens between 1.20 and 1.85 ppm, signals for acetate-methyl protons at 2.12 and 2.16 ppm (12H), two complex signals centered at 4.25 ppm (2H) and 5.25 ppm (3H) indicative of \(\sim\)CH\(_2\)-OAc and \(\sim\)CH-OAc groups and an olefinic proton signal at 5.50 ppm.

### Table 1. Elemental compositions of characteristic ions in the high-resolution mass spectra of fraction 2 and the alcohol from fraction 2

<table>
<thead>
<tr>
<th>m/e</th>
<th>Elemental composition</th>
</tr>
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<tbody>
<tr>
<td>73.0297</td>
<td>(C_6H_5O_2)</td>
</tr>
<tr>
<td>145.0506</td>
<td>(C_6H_6O_4)</td>
</tr>
<tr>
<td>189.1653</td>
<td>(C_7H_{13}O_6)</td>
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<td>191.1810</td>
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<td>217.0731</td>
<td>(C_8H_{13}O_6)</td>
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<td>289.0947</td>
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<tr>
<td>367.3378</td>
<td>(C_{27}H_{33})</td>
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<tr>
<td>369.3547</td>
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<td>369.3516</td>
<td>(C_{27}H_{31})</td>
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<tr>
<td>544.4495</td>
<td>(C_{35}H_{45}O_4)</td>
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<tr>
<td>546.4610</td>
<td>(C_{35}H_{45}O_4)</td>
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</table>

#### Fig. 1. Total ionization plot of the gas chromatogram of the \(C_{32}\) hydrocarbon mixture obtained by degradation of the alcohol from fraction 2

The relative intensity is given on the ordinate from 0 to 100.
the C32 bromides (C32H55Br and C32H53Br), which were further reduced with LiAlH4 in dimethoxyethane to C32 hydrocarbons (C32H56 and C32H54). Low- and high-resolution mass spectra of all intermediates were determined. The major ions of their mass spectra are listed in Table 2. The molecular ions in the mass spectra of the degradation products were always accompanied by a minor set of ions that differed in elemental composition by an additional CH2 group, reflecting the parallel degradation of two higher homologues.

G.l.c.–m.s. of C32 hydrocarbons

The mixture of hydrocarbons obtained in the degradation of the saponified fraction 2 was analysed by a gas–liquid chromatograph–mass spectrometer–computer system (Hites & Biemann, 1968). The mixture was chromatographed isothermally at 280°C on 1.5m (5 ft) columns (3% OV-17 on Gas-Chrom Q).

Table 2. Prominent ions in the mass spectra of the alcohol from fraction 2 and its degradation products

<table>
<thead>
<tr>
<th></th>
<th>m/e of fragment ions</th>
<th>m/e of molecular ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C35 tetrols*</td>
<td>189 191 325 367 369</td>
<td>544 546</td>
</tr>
<tr>
<td>C32 aldehydes</td>
<td>189 191 233 367 369</td>
<td>452 454</td>
</tr>
<tr>
<td>C32 alcohols</td>
<td>189 191 235 367 369</td>
<td>454 456</td>
</tr>
<tr>
<td>C32 bromides</td>
<td>189 191 297,299 367 369</td>
<td>516, 518, 520</td>
</tr>
<tr>
<td>C33 hydrocarbons</td>
<td>189 191 219 367 369</td>
<td>438 440</td>
</tr>
</tbody>
</table>

* Alcohol from fraction 2.

![Fig. 2. Mass spectrum of C32 hydrocarbon (mol.wt. 438) obtained by degradation of the alcohol from fraction 2 (scan no. 97 in Fig. 1)](image)

The relative intensity is given on the ordinate from 0 to 100.

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reagent (Fieser & Fieser, 1968) prepared from ethyl-triphenylphosphonium chloride and phenyl-lithium in tetrahydrofuran. The resulting diene was separated from triphenylphosphine oxide by Al₂O₃ column chromatography (cyclohexane–benzene, 95:5, v/v). Hydrogenation with H₂–Pd–C in dioxan yielded mixture of two saturated C₃₂ hydrocarbons (III), which was analysed by g.l.c.–m.s. under the conditions described above. The hydrocarbons, being stereoisomers, exhibited identical mass spectra (Fig. 4). They eluted from the gas chromatograph with retention times of 3.2 and 3.5 min respectively.

**Synthesis of C₃₂ hopane hydrocarbons (Scheme 4)**

Adiantone (Berti et al., 1963) (5 mg) was reacted with an excess of propylmagnesium bromide in tetrahydrofuran. The resulting tertiary alcohol was dehydrated to a mixture of olefins with POCl₃–pyridine (Berti et al., 1963). Hydrogenation of the olefins with

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![Fig. 3. Mass spectrum of C₃₂ hydrocarbon (mol. wt. 440) obtained by degradation of the alcohol from fraction 2 (scan no. 120 in Fig. 1)](image)

The relative intensity is given on the ordinate from 0 to 100.

![Fig. 4. Mass spectrum of synthetic C₃₂ lupane-type hydrocarbon (III)](image)

The relative intensity is given on the ordinate from 0 to 100.
fraction from NOVEL C₃₅

Three saturated C₃₂ hydrocarbons under the conditions previously described. Three saturated C₃₂ hydrocarbon fractions were observed eluting after 5.1, 5.5 and 7.2 min. The mass spectra of these C₃₂ hydrocarbons are nearly identical. Fig. 5 shows the spectrum of the C₃₂ hydrocarbon which elutes from the column last.

**Periodate oxidation of the total non-saponifiable lipid fraction from Acetobacter xylinum**

The lipid residue (Haigh et al., 1973) obtained after washing by the method of Folch et al. (1957) was saponified with methanolic 3 M-KOH for 1 h and extracted with ether. The solvent was removed and the residue was dissolved in dioxan. Periodate oxidation of this extract (fourfold excess of periodate in dioxane at room temperature overnight) yielded a mixture that separated on preparative thin-layer plates (silica gel G; E. Merck, Darmstadt, Germany) into two main bands when developed with ethyl acetate–light petroleum (b.p. 30–60°C) (1:3 v/v). The band with Rₚ 0.9 consisted mainly of the C₃₂ aldehydes as confirmed by mass spectrometry. The 60 MHz proton n.m.r. spectrum (in deuterochloroform) of the material in this band was obtained by using a signal-averaging analyser (64 scans). The spectrum exhibits signals (δ in p.p.m. from tetramethylsilane as internal standard) at 0.71, 0.82–0.86, 0.98, 1.10 and 1.27 indicative of methyl groups, a broad envelope for aliphatic protons between 1.2 and 2.0, an unresolved signal at 2.20, a signal for olefinic protons at 5.49 and a signal for aldehydeic protons at 9.70.

The band with Rₚ 0.6 yielded crystalline material which, after recrystallization from chloroform–methanol, melted at 254°C. High-resolution mass spectrometry established its molecular weight to be 428 and the elemental composition C₃₂H₅₅O (determined: 428.40172; calculated: 428.40178). The i.r. spectrum exhibited a strong absorption at 3605 cm⁻¹ indicating an alcohol. The compound was identical in all spectroscopic data (i.r., n.m.r., m.s.) with 22-hydroxyhopane (m.p. 225°C) which had been prepared from adiantone (Berti et al., 1963) and methylmagnesium bromide.

**Interpretation of structural data on fraction 2 and its derivatives**

The high-resolution mass spectrum of fraction 2 reveals the major features of the structure of its components. Most of the major ions occur in pairs differing in elemental composition by two hydrogen atoms, which indicates the presence of a mixture consisting chiefly of two closely related compounds differing by a double bond or ring. The ions with elemental compositions C₃₂H₄₀O₄ and C₃₃H₄₂O₄ are most likely to be the molecular ions of the two major components of the alcohol from fraction 2. They are shifted by 4 × 42 atomic-weight units (4 × C₂H₂O) in the mass spectrum of fraction 2. A similar shift by 4 × 72 atomic-weight units (4 × C₃H₅Si) was observed when the alcohol from fraction 2 was trimethylsilylated with NO-bis(trimethylsilyl)trifluoroacetamide. In the mass spectra of fraction 2, the alcohol from fraction 2 and its trimethylsilyl derivative there was always a set of minor ions present, which differed from the molecular ions by an additional CH₂.

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![Fig. 5. Mass spectrum of synthetic C₃₂ hopane-type hydrocarbon (IV)](image_url)

The relative intensity is given on the ordinate from 0 to 100.
group. These are probably due to two higher homologues.

The characteristic O₂-free ions of m/e 369 (C₂₇H₄₅) and 367 (C₂₇H₄₃) still have the same number of double bond or ring equivalents (five and six respectively) as the molecular ions. The compounds in the alcohol from fraction 2 must thus have a large hydrocarbon portion of 27 carbon atoms to which an eight-carbon side chain with four hydroxyl groups is attached. An ion of m/e 175 (C₈H₁₃O₄) in the mass spectrum of the alcohol from fraction 2, which corresponds to this side chain, gives additional support to this general structure. The abundant hydrocarbon ions at m/e 189 (C₁₄H₂₁) and 191 (C₁₄H₂₃) are present in the spectra of fraction 2 as well as in the spectrum of the alcohol from fraction 2. They must originate from a facile cleavage through the hydrocarbon portion of the molecules by loss of a part that carries the side chain. Further, the part containing the side chain is present in the ion of m/e 493 (C₂₂H₄₁O₂) in the spectrum of fraction 2, which is shifted by 4×42 atomic-weight units to m/e 325 (C₁₄H₂₁O₂) in the spectrum of the alcohol from fraction 2. These ions must also originate from a cleavage through the hydrocarbon part of the molecules. The presence of ions with m/e 205 (C₁₂H₂₁O₂Si₂) and 307 (C₁₃H₂₁O₂Si₃) in the mass spectrum of the trimethylsilylated alcohol from fraction 2 indicates that at least three of the four hydroxyl groups are on adjacent carbon atoms of the side chain. The series of ions of m/e 73 (C₅H₉O₂), 145 (C₆H₁₃O₄), 217 (C₉H₁₃O₆) and 289 (C₁₂H₁₇O₆) in the mass spectrum of fraction 2 further confirms that the hydroxyl groups are on adjacent carbon atoms of the side chain which is thus a vicinal tetrol.

As a consequence of these results, degradation experiments were performed that had two principal goals: (a) the identification of the hydrocarbon portion and (b) the identification of the side chain, including the mode and location of its attachment to the hydrocarbon portion. The accumulation of all the hydroxyl groups in one small part of the molecules makes periodate oxidation an obvious choice for these degradation experiments. The degradation sequence is summarized in Scheme 1. Inspection of Table 2 shows that the abundant ions of m/e 189 and 191, as well as the ions of m/e 367 and 369, are present in the mass spectra of the starting material, the intermediates and the final products. However, the abundant fragment ions that include the side chain (m/e 325 in the saponified fraction 2), as well as the molecular ion, do change in mass/charge ratio and the observed shifts are an excellent indication of the chemical changes the compounds have undergone during the degradation sequence. As the hydrocarbon portion

\[
\begin{align*}
C_{27}H_{45} - C_4H_8 - [CH(OH)]_4 - H & \xrightarrow{H_2O_4} C_{27}H_{45} - C_4H_8 - C\equiv H \quad \text{(1) LiAlH}_4 \\
C_{27}H_{45} - C_4H_8 - [CH(OH)]_4 - H & \xrightarrow{H_2O_4} C_{27}H_{45} - C_4H_8 - C\equiv H \quad \text{(2) PBr}_3 \\
C_{27}H_{45} - C_4H_8 - [CH(OH)]_4 - H & \xrightarrow{H_2O_4} C_{27}H_{45} - C_4H_8 - C\equiv H \quad \text{(3) LiAlH}_4
\end{align*}
\]

Scheme 1. Degradation of the alcohol from fraction 2 to C₃₂ hydrocarbons
For details see the text.

![Scheme 2](image-url)

Scheme 2. Main fragmentation pathways of compounds (I) and (II)
See Scheme 5 for ring labelling.
(m/e 367 and 369) remains unaltered by the stepwise degradation it seems warranted to discuss the structure of the resulting C₃₂ hydrocarbons first, particularly as their gas-chromatographic separation resulted in clean spectra for both components and will permit the discussion of their structural differences.

The C₃₂ hydrocarbon of molecular weight 440 (C₃₂H₅₆) produces characteristic abundant fragment ions of m/e 191 and 369 (Fig. 3). These ions are very common in the mass spectra of saturated penta-cyclic triterpene hydrocarbons like lupane (I, R₁ = H₂, R₂ = C₃H₇) or hopane (II, R₁ = H2, R₂ = C₃H₁₁). Scheme 2 summarizes the main fragmentation pathways of these two compound types (Corbett & Young, 1966). The ion of m/e 369 arises by loss of the side chain R₂ from the molecular ion. The ion of m/e 191 (189 + 2H) in the mass spectra of lupane and hopane can be formed in two different ways by cleavage through ring C. That the A,B-ring portion and the D,E-ring portion of the molecules both contribute to the abundant ion of m/e 191 in lupane and hopane had been concluded from the mass spectrum of lupan-3-one (I, R₁ = O, R₂ = C₃H₇) which exhibits an ion of m/e 205 (189 + O) originating from rings A and B and one of m/e 191 originating from rings D and E (Corbett & Young, 1966). This can explain the fragment ion of m/e 219 (C₁₄H₂₇) as being derived from the D,E-ring portion of a penta-cyclic triterpene like compound I (R₁ = H₂, R₂ = C₃H₁₁) or II (R₁ = H₂, R₂ = C₃H₁₁). Fig. 3 shows that the ion of m/e 219 is the most-intense (base) peak in the spectrum of the C₃₂ hydrocarbon of molecular weight 440 derived by degradation of the alcohol from fraction 2. This indicates preferential charge retention in rings D and E, which is of particular interest since the cited lupan-3-one investigation had demonstrated preferential charge retention in the A,B-ring portion for lupane and its derivatives. However, the evidence (Corbett & Young, 1966) for the preferential charge retention in the A and B rings of lupane had been obtained from oxygenated lupane derivatives and could also reflect the influence of the substitution with heteroatoms on the retention of charge. Therefore authentic C₃₂ triterpene hydrocarbon models of the naturally derived C₃₂ hydrocarbon were synthesized and their mass spectra compared. The synthesis (Scheme 3) of the C₃₂ lupane-type hydrocarbon (III) employing a Wittig synthesis (Fieser & Fieser, 1968) is unambiguous and yields only the stereoisomers at C-20 of lupane.

A comparison of Fig. 3 and Fig. 4 shows that the mass spectra of the naturally derived C₃₂ hydrocarbon and of compound III are markedly different. In the mass spectrum of the synthetic C₃₂ lupane-type hydrocarbon the ion of m/e 191 is the base peak, whereas in the spectrum of the naturally derived C₃₂ hydrocarbon the abundance of the m/e 191 ions reaches only 75% of the abundance of the base peak (m/e 219). That the two compounds are not identical is further indicated by their vastly different gas-chromatographic retention times (3.5 versus 7.5 min).

It was therefore suspected that the natural compound may contain a stereochemically different ring system and the analogous C₃₂ hydrocarbon of the hopane type (IV) was synthesized (Scheme 4) as a further model compound. The synthesis of the C₃₂ hopane-type hydrocarbon gives, by analogy with the dehydration of 22-hydroxyhopane (Budzikiewicz et al., 1963) under the same conditions a mixture of olefins with a 21–22, 22–29 or 22–30 double bond. The hydrogenation of this mixture can give four stereoisomeric C₃₂ hydrocarbons (at C-21 and C-22), which nevertheless all have the characteristic D–E.
chair–chair ring function of hopane. Comparison of the mass spectra of the naturally derived C\textsubscript{32} hydrocarbon (Fig. 3) with the synthetic C\textsubscript{32} hydrocarbon having the hopane stereochemistry of the ring system (Fig. 5) shows their virtual identity. The observed difference in retention time (7.4 versus 7.2 min) is most likely caused by a different branching of the side chain. Nevertheless, the result of this comparison, particularly of the mass spectra, is a clear indication for the hopane skeleton of the saturated component in fraction 2.

A comparison of the relative intensities of the peaks at m/e 191 and 219 in Fig. 4 and Fig. 5 illustrates the striking influence that the stereochemistry of the D,E-ring junction has on the charge retention in saturated triterpene hydrocarbons with 6,6,6,5,6-ring skeleton. The demonstrated preferential charge retention in rings D and E of hopanes for cleavages involving ring C (m/e 219 in Fig. 5) seems to be a general phenomenon and is also observed (at m/e 177) in the mass spectrum of adiantone (Henderson et al., 1968).

These observations might be of general interest to the mass spectrometry of triterpene hydrocarbons, particularly in view of recent findings of these compound types in geological sources (Anders & Robinson, 1971).

The C\textsubscript{32} hydrocarbon with molecular weight 438 contains one double bond. A comparison of the mass spectra of the saturated and the unsaturated C\textsubscript{32} hydrocarbon (Fig. 2 and Fig. 3) shows that the ion derived from rings A and B is shifted by two mass units to m/e 189 whereas the D,E-ring-derived ion of m/e 219 remains unaltered. If one assumes an unarranged hopane skeleton of this compound the double bond must be located in either ring A or B. Its presence in ring B would explain the high abundance of the ion of m/e 189, as cleavage through ring C would result in a carbonium ion which is conjugated to a diene system. For a bond in position 5–6 of a pentacyclic triterpene hydrocarbon one would expect (Audier et al., 1964) an ion of m/e 150 owing to a retro Diels–Alder cleavage (cleavage of two bonds of a cyclic system with formation of two stable, unsaturated fragments). However, the formation of such an ion is frequently influenced by rather subtle changes in stereochemistry (Corbett & Young, 1966) and its absence in Fig. 2 is thus no indication of the absence of a 5,6-double bond. The well-documented skeletal rearrangements (Corbett et al., 1968) of hopane cations, which could readily be formed by protonation of the double bond under the acidic conditions employed during the original isolation of the lipid, would make a structural assignment for the unsaturated hydrocarbon, on the basis of its mass spectrum alone, even more tenuous.
The assignment of hopane-type hydrocarbon portions for the main components in fraction 2 is further supported by the simultaneous presence of 22-hydroxyhopane in the non-saponifiable lipid fraction. The observed chemical shifts of the methyl signals in the proton n.m.r. spectrum of fraction 2 are all in close agreement with the chemical shifts of hopane which are reported in the literature (Berti et al., 1968).

The aldehydic fraction obtained by the periodate oxidation of the total non-saponifiable lipid fraction has an unresolved olefinic multiplet at 5.50 p.p.m. This chemical shift is within the range of chemical shifts of an olefinic proton of a 5-6 double bond which is found at 5.6±0.1 p.p.m. in a wide variety of steroids (Bhaca & Williams, 1964).

Having established a hopane-type skeleton for the hydrocarbon portion of the two components in fraction 2 the question of the structure of the remaining eight-carbon side chain redefines itself to the structure of a five-carbon unit and the point of its attachment to the hopane system (i.e. C-22 or C-29). As the periodate oxidation of the components of the alcohol from fraction 2 results in the loss of not only three carbon atoms but also of three of the four oxygen atoms, this five-carbon unit must contain four vicinal hydroxyl groups, a result which is in agreement with the high-resolution mass-spectrometric data (Table 1) discussed above. The facile further oxidation of the C23 carbonyl compounds to C32 carboxylic acids excludes the possibility of branching at the remaining oxygen-substituted carbon atom, which would result in the formation of a ketone rather than of an aldehyde. The n.m.r. spectrum of fraction 2 indicates that the three-carbon entity eliminated in the periodate oxidation is probably not branched. This spectrum is compatible with a straight-chain five-carbon unit: it exhibits a complex two-proton signal centered around 4.25 p.p.m. and a three-proton signal centered around 5.25 p.p.m., the expected chemical shifts for −CH3−OAc and −CH−OAc groups in linear systems (Wolfrom et al., 1962).

The question whether the five-carbon unit is attached to the hopane system at C-22 or at C-29 cannot be definitely answered but some of the data favours the former. The n.m.r. spectrum of fraction 2 clearly indicated eight rather than seven methyl groups. The n.m.r. spectrum of the aldehydic fraction obtained by the periodate oxidation of the total non-saponifiable lipid fraction has a large sharp methyl signal at 1.28 p.p.m., the expected chemical shift for methyl groups that are removed from a carbonyl group by an additional carbon atom.

A low-resolution spectrum from mass spectrometry of the hydrocarbon, which was isolated as a white, waxy mass from the acetylated non-saponifiable fraction (Haigh et al., 1973), showed a molecular ion of m/e 410 (Fig. 6) which, taken together with the composition, indicated a molecular formula of C39H50. In addition, the spectrum showed evidence for abundant fragment ions of m/e 395 (C29H47), 367 (C27H44), 231 (C19H29), 191 (C18H23) and 189

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![Fig. 6. Mass spectrum of the hydrocarbon from the acetylated non-saponifiable fraction](image)

The relative intensity is given on the ordinate from 0 to 100.
(C_{14}H_{21}). It is indicative of a pentacyclic triterpene hydrocarbon (Budzikiewicz et al., 1964). In particular, the ion of m/e 367, which is formed from the molecular ion by loss of a C_3 moiety, suggests a monounsaturated triterpene hydrocarbon with a 6,6,6,6,5-ring skeleton of either the lupane or hopane type (Budzikiewicz et al., 1963). This hydrocarbon may be the product of acid-catalysed dehydration of 22-hydroxyhopane (Haigh et al., 1973).

Discussion

The biosynthesis of tetrahydroxybacteriohopane (VI) and its analogues could in principle proceed in two different ways: (a) the cyclization of an acyclic C_{35} precursor followed by hydroxylations or (b) the attachment of a C_5 building block to a preformed pentacyclic system. We prefer the second possibility, in particular as 22-hydroxyhopane is simultaneously present in the total unsaponifiable lipid fraction. There are again two general possibilities for the attachment of a C_5 species to the pentacyclic system. In one case the hopane cation, the primary product of the all-chair cyclization of squalene, is trapped by an anionic C_5 species. Alternatively the C_{35} terpene may be formed by an electrophilic attack of a C_5 unit on the 22-29 double bond of hopene. However, in either case C-22 is the preferred centre of attack. These considerations represent an additional argument for the attachment of the C_5 unit at C-22 of hopane. Thus, the combination of the mass-spectrometric data and the results of the degradation experiments lead to structure (V) for the saturated terpene from A. xylinum. On the basis of additional arguments, namely n.m.r. data of the mixture, and biosynthetic considerations, structure (VI) is proposed for the

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**Scheme 6. Attachment of a C_5 unit to C-22 of hopane**

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**(V)**

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**(VI)**

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saturated component of the alcohol from fraction 2, which is termed tetrahydroxybacteriophahope. The unsaturated component in the alcohol from fraction 2 is an analogue of tetrahydroxybacteriophahope with a double bond in ring A or B.

It will be of interest to isolate the components present in the aldehyde fraction obtained by periodate oxidation of the total non-saponifiable lipid fraction to clarify the location of the double bond in the unsaturated analogue of tetrahydroxybacteriophahope. This may also provide information about the nature of the observed higher homologues.

The occurrence of a pentacyclic terpene system as part of a biologically active lipid represents one of the rare cases where a function may be assigned to pentacyclic terpenoids (Templeton, 1969). The presence of pentacyclic triterpenes in bacterial lipids has been reported only recently (De Rosa et al., 1971; Bird et al., 1971), but so far it has been considered more an exception and in one case was attributed to the extreme living conditions of a thermophilic bacterium (De Rosa et al., 1971). The presence of the hopane-type C_{35} terpenoids and 22-hydroxyohopane in A. xylinum suggests that these pentacyclic systems are more widely distributed than previously thought.

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