Characterization of the Hexahydropolyrenols of Aspergillus fumigatus Fresenius

By K. J. Stone, P. H. W. Butterworth and F. W. Hemming

Department of Biochemistry, University of Liverpool

(Received 7 July 1966)

The isolation and properties of a group of alcohols from the mycelium of Aspergillus fumigatus Fresenius are described. Mass-, nuclear-magnetic-resonance- and infrared-spectrometric studies coupled with evidence from ozonolytic degradation and chromatography show the mixture to contain hexahydroprenols-18, -19, -20, -21, -22, -23 and -24. Each contains a saturated ‘hydroxy-terminal’ isoprene residue, a saturated ω-terminal isoprene residue and a saturated β-isoprene residue (adjacent to the ω-residue). The presence of only two trans-isoprene residues is also a feature of the series of alcohols, but the precise position of these in each molecule is not known.

The presence and nature of the mixtures of polyrenols in leaves of higher plants have been described recently (Wellburn & Hemming, 1966; Wellburn, Stevenson, Hemming & Morton, 1967; Stone, Wellburn, Hemming & Pennock, 1967). These alcohols are allylic and differ from the polyrenols (dolichols) of pig liver (Burgos, Hemming, Pennock & Morton, 1963) and of Saccharomyces cerevisiae (baker's yeast) (J. Burgos, J. F. Pennock & F. W. Hemming, unpublished work) in which the 2,3-double bond is saturated. In all these polyrenols there is a predominance of cis- over trans-isoprene residues. Another shorter member of the dolichol series of compounds, bactoprenol, has been isolated from Lactobacillus casei (Thorne & Kodicek, 1966). The configuration of the isoprene units in this alcohol is not known.

An alcohol preparation similar in properties to pig-liver dolichol was isolated from Aspergillus fumigatus Fresenius (Packter, 1962; Packter & Glover, 1964) during studies on the biosynthesis of ubiquinone. The incorporation of [2,14C]-mevalonate into the preparation has also been studied (Butterworth, Burgos & Hemming, 1966). It has now become clear that the alcohol preparation is in fact a mixture of hexahydropolyrenols. The isolation and characterization of these alcohols is reported in the present paper.

METHODS

Isolation and purification of the hexahydropolyrenols. Aspergillus fumigatus Fresenius (L.S.H.T.M. A.48; C.M.I. 89353) was grown in batches of 100 penicillin jars by using the large-scale facilities of the Department of Organic Chemistry, University of Liverpool. The authors are grateful to Dr Holker who made these facilities available. The mould was first cultured on slopes of potato–dextrose–agar (Oxoid) in Roux bottles for 9 days at 30°. A suspension of spores from one Roux bottle in 400 ml of sterile water was prepared and 4 ml of this was injected into each penicillin jar containing 1 l of Raulin–Thom medium (see Anelov & Raistrick, 1938) sterilized by autoclaving (15lb./in.2 for 15 min.). After 9 days' growth at 30° the surface of the medium was covered with a thick pad of mycelium and the medium had become purple. The cultures were autoclaved again and the mycelium (grey-green) was removed. It was spun free of medium and washed with water in a domestic spin-dryer. Each batch of 100 jars yielded 1–2 kg of mycelium harvested in this way.

Portions (300 g.) of mycelium were each blended with methanol (900 ml) containing pyrogallol (1% w/v) in an Ultra-Turrax homogenizer. Aq. 60% (w/v) KOH (450 ml) was then added and the mixture was boiled under reflux for 1 hr. This mixture was then filtered, while still hot, through fluted filter paper and cold water (1 l.) was added to the filtrate. When cool, the insoluble material in the filter paper was washed with diethyl ether (4 x 500 ml). Each time the ether washings were shaken with the original diluted filtrate paper and, on separating, the upper (etheral) layer was retained. The combined ethereal extracts were washed free of alkali and, after drying over anhydrous Na2SO4, the solvent was removed by evaporation, finally under nitrogen.

The unsaponifiable lipid (approx. 1·5 g.) from each 600 g. of mycelium was chromatographed on an alumina (Woelm; acid-washed, Brockmann grade 3) column (100 g.; 14 cm. x 3·8 cm., fitted with a centre rod; 2% (v/v) diethyl ether in light petrol (b.p. 40–60°) (11.) eluted non-polar materials such as hydrocarbons, and 13% (v/v) diethyl ether in light petrol (b.p. 40–60°) (11.) then eluted an alcohol fraction (150 mg.) containing, as well as other materials, ubiquinone-10. The alcohols were acetylated by mixing a solution of this fraction in benzene (5 ml.) with A.R. acetic anhydride (5 ml.) and 2 drops of A.R. pyridine.
and leaving at room temperature for 16 hr. The ethereal extract (see e.g. Wellburn et al. 1967) was evaporated to dryness and was chromatographed on an alumina (Woelm; acid-washed, Brockmann grade 3) column (15g.; 16 cm. x 1-6 cm.): 2% (v/v) diethyl ether in light petroleum (b.p. 40-60°) (100 ml.) eluted hexahydropolypropenyl acetates (45 mg.), which were hydrolysed by boiling under reflux a solution in benzene (10 ml.) with 15% (w/v) KOH in ethanol-water (17:3, v/v) (10 ml.) for 15 min. The ethereal extract of this mixture yielded 40 mg. of an impure hexahydropolypropenyl acetates. Preparation. Preparative chromatography of this material on silica gel G (two layers; 30 cm. x 20 cm., 600 μ thick) with methanol-benzene (1:99, v/v) as developing solvent gave a good sample of hexahydropolypropenyl acetates (35 mg.) (Rf 0-4). Infrared spectroscopy confirmed that the preparation was free of contaminating material.

Material (20 μg./spot) purified in this way was subjected to reversed-phase partition thin-layer chromatography with paraffin-impregnated kieselguhr (200 μ thick, impregnated with 31% (v/v) liquid paraffin in light petroleum (b.p. 40-60°)) as stationary phase and dry acetone, saturated with paraffin, as mobile phase. When the developed plate was sprayed with a solution of anisaldehyde in a mixture of ethanol and conc. H2SO4 and then heated at 110° for 10 min., seven components ranging in Rf from 0-14 to 0-35 were seen. Six of the components (those with highest Rf values) of the mixture (70 mg.) were separated and recovered by preparative reversed-phase partition thin-layer chromatography with 70 layers (20 cm. x 20 cm.) and the same system above in a manner similar to that described by Wellburn et al. (1967). In some cases, because of overlapping of bands of material, the chromatography had to be repeated. The individual components were labelled bands 1 to 6 in order of decreasing Rf value. The paraffin was removed from each band by chromatography on columns of alumina (see Wellburn et al. 1967) and the band was then further purified by preparative chromatography on thin layers of silica gel G (200 μ thick) as described above. The final yields of material from each band were: band 1, 0-9 mg.; band 2, 2-9 mg.; band 3, 1-5 mg.; band 4, 19-1 mg.; band 5, 9-0 mg.; band 6, 3-0 mg. These weights probably are a reliable guide to the composition of the mixture, for they confirm the relative intensities of stains taken up by the material on component when the mixture was subjected to reversed-phase partition thin-layer chromatography as described above. On such a chromatogram, material with an Rf lower than that of band 6 could only just be detected. Detection of this material and of band 1 material was made easier by two-dimensional chromatography with the same system in both dimensions. In this way more material (up to 40 μg. of mixture) could be chromatographed. Rf values in the second dimension could be measured accurately.

Spectroscopy. Infrared spectra were determined on a Perkin-Elmer Infracon model 237 spectrometer as solvent-free films between rock-salt disks. Nuclear-magnetic-resonance spectra of the single alcohols were recorded by Dr J. Feeney of Varian Associates Ltd., Walton-on-Thames, Surrey, using a Varian HA 100 M cy/sec. spectrometer. A spectrum of the mixture of alcohols was recorded at 60 M cy/sec. by Dr R. J. Abraham and Miss R. Adlard of the Department of Organic Chemistry, using a Varian A-60 instrument.

Mass spectra were determined by Dr W. Vetter and Dr P. Meyer of the Physicochemical laboratories of F. Hoffmann-La Roche and Co. Ltd., Basle, Switzerland, in an MS9 instrument.

Micro-hydrogenation. Three samples (each 3-4 mg.) of the mixture of acetates were weighed accurately on a micro-balance and were then hydrogenated quantitatively at atmospheric pressure in a Towers micro-hydrogenation apparatus. The solvent was cyclohexane-ethanol-acetic acid (1:1:1, by vol.) and platinum oxide was used as catalyst. This equipment has been shown to give values 5-10% higher (mean for eight different compounds) than the theoretical values. The results have been corrected for this error.

Ozonolysis degradation. Ozonolyses were carried out in essentially the same manner as described by Donninger & Popjak (1966) for squalene. The mixture of Aspergillus alcohols (14-4 mg.) was dissolved in ethyl chloride (25 ml.) and was subjected to ozonolysis at -73°. Ozone was passed through the solution and a deep-blue colour developed.

After a further 30 min. the excess of ozone was displaced by a stream of oxygen and the temperature was allowed to rise to 0°. During this stage the solvent evaporated. To a solution of the ozonide in diethyl ether (5 ml.) was added, dropwise and slowly, a saturated filtered etheral solution of LiAlH4 until there was no further visible reaction. Excess (1 ml.) of the LiAlH4 solution was then added and the mixture was shaken at room temperature for 0-5 hr. A solution of 10% (v/v) acetic anhydride in diethyl ether was added, dropwise and slowly, until there was no further visible reaction. A further 5 ml. of this solution was then added. After carefully removing the diethyl ether by evaporation on a water bath at 40°, acetic anhydride (3 ml.) and pyridine (3 drops) were added. The mixture was heated under reflux for 2 hr. on an oil bath at 130° to ensure complete acetylation. The excess of acetic anhydride was hydrolysed by the addition of water (40 ml.) and heating under reflux on a boiling-water bath for 15 min. Solid NaHCO3 was then added until there was no further effervescence and the resulting slightly alkaline solution was extracted with diethyl ether (5 x 25 ml.). The ethereal extract was washed well with water, N HCl and water, in that order, and dried over anhydrous Na2SO4. After removing the solvent by evaporation at 40°, 27 mg. of mixed acetates remained (75% of the theoretical).

Samples of pig-liver dolichol (32 mg.) and squalene (50 mg.) were also degraded in the same manner. The degradation products (acetates) were studied by gas-liquid chromatography. Retention times were compared with those of reference compounds.

Preparation of reference acetates for gas-liquid chromatography. 3-Methyl-α-hexene-1,6-diyi dicetate was prepared by ozonolytic degradation of citronellol (53 mg.) in the same manner as described above. The reactions involved are shown in Scheme 1. Some of the isopropyl acetate was probably lost by evaporation, but this was not important as it was shown that in the gas-liquid-chromatographic system used subsequently isopropyl acetate travels with the solvent peak. The material appeared pure as judged by infrared spectroscopy but on gas-liquid chromatography it gave a minor second peak.

n-Pentane-1,4-diyi dicetate was prepared by reducing laevulinic acid with LiAlH4 (Scheme 2) and acetylatyng the resulting n-pentane-1,4-diol. The conditions used were the
HEXAHYDROPOLYPRENOLS OF *ASPERGILLUS*

\[
\begin{align*}
\text{Citronellol} & \quad \xrightarrow{O_2} \\
\text{Isopropyl acetate} & \quad + \quad 3\text{-Methyl-}n\text{-hexane-1,6-diyl diacetate}
\end{align*}
\]

\[\text{Scheme 1.}\]

same as those used for the reductive acetylation of the ozonides. Preparative thin-layer chromatography of the products (236 mg.) on silica gel G (five layers; 20 cm. x 20 cm., 600 \( \mu \) thick) with methanol–benzene (1:25, v/v) as developing solvent provided a sample that ran as a single compound (\( R_f 0.55 \)) when rechromatographed in the same system (stain: phosphomolybdic acid) and which was identified on the basis of infrared spectroscopy.

6,10-Dimethyl-\( n \)-undecan-2-yl acetate was prepared from geranylacetone (Scheme 3), the latter compound being a gift from Professor G. Popjak. The material (151 mg.) was dissolved in cyclohexane–ethanol (1:1, v/v) and was hydrogenated for 1 hr. at atmospheric pressure with platinum oxide as catalyst in a Towers micro-hydrogenation apparatus. The product, 6,10-dimethyl-\( n \)-undecan-2-one, was reduced with LiAlH\(_4\) to form 6,10-dimethyl-\( n \)-undecan-2-ol, which was then acetylated to form the acetate (174 mg.) as in the above preparations. Infrared spectroscopy and thin-layer chromatography [silica gel G; developing solvent methanol–benzene (1:40, v/v); \( R_f 0.65 \)] showed that the product was a good sample of 6,10-dimethyl-\( n \)-undecan-2-yl acetate.

Bioch. 1967, 102
n-Butane-1,4-diyl diacetate was prepared by acetylation of n-butane-1,4-diol (British Drug Houses Ltd., Poole, Dorset) in the same manner as for the other alcohols, great care being taken not to evaporate the product when distilling off the diethyl ether. An infrared spectrum indicated that the preparation was of high purity. This compound was used in a preliminary study of the ozonolysis products of squalene. On gas-liquid chromatography it had a retention time of 5.4 min. (cf. Fig. 6).

Gas-liquid chromatography of products of ozonolytic degradation. All the acetates were studied by gas-liquid chromatography in a manner similar to that used by Donninger & Popjak (1966) for studying the ozonolytic degradation products of squalene. An Aerograph Autoprep 700 gas chromatograph (Wilkens Instrument and Research Inc.) fitted with a flame ionization detector was used. The silane-treated stainless-steel column (9 ft. x 1/8 in.) was packed with silane-treated Celite (Gas-Chrom CLH; 100-120 mesh) coated with ethylene glycol succinate polyester (15%, w/w). Chromatography was isothermal at 150° and the carrier gas was nitrogen at a flow rate of 90 ml/min. The splitting ratio was 4:7:1 in analytical work and 23:1 when the instrument was used preparatively. Benzene solutions of the samples were injected directly on to the column.

RESULTS

Mass spectrometry. The mass spectra of bands 6, 5, 4 and 3 gave prominent molecular ions at m/e 1588, 1520, 1452 and 1384 respectively. These correspond to alcohols containing 23, 22, 21 and 20 isoprene units respectively, each alcohol having three of its isoprene units saturated. Most of the spectra showed that each band also contained small proportions of alcohols one isoprene unit smaller and one isoprene unit larger than the main one. Thus in the spectrum of band 3 there were peaks of low intensity at m/e 1316 and 1452 and in the spectrum of band 6 peaks of low intensity at m/e 1452, 1520 and 1656 (± 2) were observed. Of particular note are the peaks at m/e 1316 and 1656, for these correspond to alcohols of the same series containing 19 and 24 isoprene units respectively. Thus the size of the molecular ions offers good evidence for the presence of hexahydropropenols-19, -20, -21, -22, -23 and -24.

One noteworthy aspect of the spectra is that the (M + 1)⁺ peak is of greater intensity than the M⁺ peak. This tendency became less marked as the size of the molecules decreased. The greater intensity of the (M + 1)⁺ peak can be forecast in molecules of this size on the basis of the natural abundance of ¹³C and ²H (see e.g. Beynon & Williams, 1963). In all of these spectra M⁺ has been identified in the cluster of peaks in this area of the spectrum by its much greater intensity than (M - 1)⁺, a difference in intensity that is consistently large in all prenols so far studied (e.g. solanesol, castaprenols and ficeaprenols).

Another noteworthy feature of the spectra is the virtual absence of a peak corresponding to loss of water, i.e. (M - 18)⁺. In the allylic prenols so far studied (Wellburn et al. 1967), (M - 18)⁺ is much more intense than M⁺. In polypropenols that have the ‘hydroxy-terminal’ isoprene residue saturated (i.e. they are not allylic), e.g. the yeast and pig-liver dolichols, the reverse situation holds and M⁺ can reach an intensity five times that of (M - 18)⁺ (J. F. Fennock & F. W. Hemming, unpublished work). However, even in these spectra (M - 18)⁺ is clearly prominent, whereas in the Aspergillus alcohols, which are known to have a saturated ‘hydroxy-terminal’ isoprene residue (see below), (M - 18)⁺ is almost absent. This fact does not necessarily mean that the Aspergillus alcohols do not lose water during mass spectrometry. It may simply be that (M - 18)⁺ is not a stable ion. The reasons for this are not clear but it is relevant that to explain the presence of some prominent ions at lower values of m/e it is necessary to postulate that loss of a molecule of water is involved (see below).

The spectrum of band 6 (mainly hexahydropropenol-23; m/e 1588) is illustrated in Fig. 1. Scrutiny of this spectrum shows the presence of small amounts of hexahydropropenols-21 (m/e 1452) and -22 (m/e 1520). A cluster of peaks corresponding in position to hexahydropropenol-24 of intensity 0.18% of that at m/e 271 was also apparent in the spectrum (not shown in Fig. 1). Because the intensity of the peaks between m/e 1600 and 1650 was very low, the exact value of M⁺ in this cluster could not be determined. It was measured to be between m/e 1654 and 1658. The expected value for hexahydropropenol-24 is m/e 1656.

The ‘cracking’ pattern in each spectrum was essentially the same as that given in Fig. 1. The first prominent ion below M⁺ is (M - 155)⁺. From then on the major peaks in the spectrum, apart from minor fluctuations, correspond to ions differing by 68 mass units (i.e. the mass of an isoprene unit). This continues down to m/e 481. At values of m/e above 750 this main series of peaks is accompanied by a second, slightly less prominent, series of peaks 14 mass units above the main series. Below m/e 1000 it is possible to discern a third series, 6 mass units below the main series. This series gradually increases in intensity until between m/e 407 and 271 it provides the most prominent peaks in the spectrum. Below m/e 400 a fourth series of peaks 20 mass units lower than the main series becomes increasingly prominent until below m/e 200 it becomes the most prominent series.

The most likely explanation of these four series of peaks is given in Table 1. At high values of m/e the A₅ + B₆ series and A₆ + B₅ series of peaks dominate the spectrum. At lower values of m/e series Cₓ provides the most prominent peaks, whereas at the lowest values of m/e series Cₓ takes
over the major role. The fact that each series contributes peaks 68 mass units apart, and that these series explain the origin of the more prominent peaks in the spectrum, provides strong evidence for the polyisoprenoid nature of the *Aspergillus* alcohols. It is probably overlapping of

---

**Fig. 1.** Histogram of the more prominent ions in the mass spectrum of *Aspergillus* hexahydroprenols. (a) Mixture of *Aspergillus* hexahydropolyprenols. (b), (c), (d) and (e) *Aspergillus* hexahydroprenol-23. Only peaks with intensities greater than the following limits [relative to 100 for m/e 81 in (a) and to 100 for m/e 271 in (b), (c), (d) and (e)] are shown: 10 in the range 40–170; 1 in range 170–280 (a); 10 in the range 270–400 (b); 4 in the range 400–550; 2 in the range 550–650; 1 in the range 650–900; 0.5 in the range 900–1000; 0.2 in the range 1000–1100; 0.13 in the range 1250–1610. Intensities of the peaks in the ranges 180–240, 240–280 (a), 500–605, 605–940, 940–1275 and 1275–1610 have been increased by the factors 5, 10, 5, 10, 20 and 40 respectively. The positions (m/e) of the most prominent peaks are indicated on the histogram. The position of each of the most prominent peaks expected on the basis of Table 1 is shown in parenthesis where this does not coincide with the actual most prominent peak in this region of the spectrum.
Table 1. Origin of the prominent peaks in the mass spectrum of Aspergillus hexahydroprenol-23

\[
M^+ (1588) = \left[ \begin{array}{c}
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{H} \cdot [\text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2]_2 \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{C} \cdot \text{C} \cdot \text{C} \cdot \text{CH}_2 \cdot \text{OH}
\end{array} \right]^{+}
\]

(A) 1433 = 
\[
\left[ \begin{array}{c}
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3
\end{array} \right]^{+} + \text{C} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}
\]

followed by fracture at points X or Y in the chain to give series \( A_X \) and \( A_Y \).

(B) 1433 = 
\[
\left[ \begin{array}{c}
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3
\end{array} \right]^{+} + \text{H} \cdot [\text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2]_2 \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{I}^{+}
\]

followed by fracture at points X or Y in the chain to give series \( B_X \) and \( B_Y \).

(C) or carrying an isomeric form of the dehydro-\( \alpha \)-isoprene unit, followed by fracture at points X or Y in the chain to give series \( C_X \) and \( C_Y \).

<table>
<thead>
<tr>
<th>Series ( A_X + B_Y ) (series 2)</th>
<th>Series ( A_Y + B_X ) (main series)</th>
<th>Series ( C_X ) (series 3)</th>
<th>Series ( C_Y ) (series 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1433 + + + + 767 + + + + )</td>
<td>( 1433 + + + + 753 (+ + + +) )</td>
<td>( 1413 + + 747 + + )</td>
<td>( 1413 + + 733 + )</td>
</tr>
<tr>
<td>( 1379 + + + + 699 (+ +) )</td>
<td>( 1365 + + + + 685 (+ + + +) )</td>
<td>( 1259 + + 679 + + + )</td>
<td>( 1345 + + 665 + + )</td>
</tr>
<tr>
<td>( 1311 + + + + 631 + + )</td>
<td>( 1297 + + + + 617 ++ + + + )</td>
<td>( 1291 + + 611 + + + )</td>
<td>( 1277 + + 597 + + )</td>
</tr>
<tr>
<td>( 1243 + + + + 563 + + )</td>
<td>( 1229 + + + + 549 ++ + + + )</td>
<td>( 1223 + + 543 + + + )</td>
<td>( 1209 + + 529 + + )</td>
</tr>
<tr>
<td>( 1175 + + + + 495 + )</td>
<td>( 1161 + + + + 481 + + + + )</td>
<td>( 1155 + + 475 + + + + )</td>
<td>( 1141 + + 461 + + + )</td>
</tr>
<tr>
<td>( 1107 + + + + 427 )</td>
<td>( 1093 + + + + 413 + + )</td>
<td>( 1087 + + 407 + + + + )</td>
<td>( 1073 + + 393 + + + + )</td>
</tr>
<tr>
<td>( 1039 + + + + 359 )</td>
<td>( 1025 + + + + 345 + )</td>
<td>( 1019 + + 339 + + + + )</td>
<td>( 1005 + + 325 + + + + + )</td>
</tr>
<tr>
<td>( 971 + + + + 291 )</td>
<td>( 957 (+ + + + +) 277 + )</td>
<td>( 951 (+ +) 271 + + + + )</td>
<td>( 937 + + 257 + + + + )</td>
</tr>
<tr>
<td>( 903 (+ + + +) 223 )</td>
<td>( 889 (+ + + + +) 209 )</td>
<td>( 883 + + 203 + + + + )</td>
<td>( 869 + + 189 + + + + + )</td>
</tr>
<tr>
<td>( 835 + + + + 155 )</td>
<td>( 821 (+ + + + +) 141 )</td>
<td>( 815 + + 135 + + + + )</td>
<td>( 801 + + 121 + + + + + )</td>
</tr>
</tbody>
</table>

* The number of plus signs indicates the intensities of these ions in the actual spectrum relative to the corresponding ions in the other series. Plus signs in parentheses indicate that intensity of a peak close to but not in exactly the same position as that listed. Absence of plus signs indicates that the ion was very weak in the spectrum.

The mass spectra of castaprenols, solanesol and ficaprenols show the most prominent ion to be at m/e 69. This corresponds to the unsaturated \( \omega \)-terminal isoprene residue retaining the charge on fracture of the molecule (see e.g. Wellburn et al. 1967). In the spectra of the Aspergillus alcohols this peak does not dominate, presumably because the \( \omega \)-terminal isoprene residues are saturated. Instead, the ion with m/e 81 is the most abundant and is consistent with being derived by fracture of the final member of series \( C_X \) or \( C_Y \) to give:

\[
\text{CH}_3
\]

or an isomer thereof.

It is clear then that all the major peaks in the
Fig. 2. Curve A, tracing of the nuclear-magnetic-resonance spectrum at 60Mcyc./sec. of a solution of the Aspergillus alcohols mixture in benzene. Curve B, the smaller peaks of curve A amplified. Curve C, as for curve A, but after shaking with D$_2$O.

mass spectra of the Aspergillus alcohols can be explained on the basis of the general structure (I). Other evidence (see below) supports this structure. This means that the Aspergillus alcohols are tetrahydrodolichols, or more generally and precisely $\omega$\psi-hexahydropolyprenols where $\omega$ and $\psi$ describe the ultimate and the penultimate isoprene residues and $\alpha$ describes the 'hydroxy-terminal' isoprene residue in each molecule. Throughout the rest of this paper the alcohols are referred to as hexahydropolyprenols-24, -23, -22, -21 etc., in which $n = 21$, 20, 19, 18 etc. respectively.

**Nuclear magnetic resonance.** The nuclear-magnetic-resonance spectrum at 60Mcyc./sec. of a solution of the mixture of Aspergillus hexahydropolyprenols in benzene is shown in Fig. 2. The assignments of the peaks together with the measured relative areas and the expected relative areas based on structure (I) and the known composition of the mixture are listed in Table 2. The agreement is good. The spectra of the separated hexahydropolyprenols-20, -21 and -22 were determined in benzene at 100Mcyc./sec. These gave satisfactory spectra in agreement with that of the mixture except for the presence of a rather sharp peak at 8-61$\tau$ due to the presence of a contaminant. This made it impossible to measure accurately the areas of the peaks in the 8-5–8-8$\tau$ region of the spectrum. It is probable that the new peak at 8-61$\tau$ was the result of the presence of a small proportion of a decomposition product formed during manipulation and storage before the nuclear-magnetic-resonance studies at 100Mcyc./sec. Decomposition, possibly involving cyclization, has proved a serious problem generally when handling polyprenols, especially with those related to the dolichols.

A feature of these spectra, in common with that of pig-liver dolichol (Burgos et al. 1963), is the fact that the resonance of the protons on the methylene group next to the hydroxyl group appears as a triplet (6-45, 6-55 and 6-65$\tau$). This is consistent with the carbon atom $\beta$ to the hydroxyl group carrying two hydrogen atoms and supports the idea that these alcohols are not allylic.

The pattern of peaks in the 9-0–9-2$\tau$ region has the appearance of two overlapping and distorted doublets: one at 9-17 and 9-08$\tau$ and the other at 9-13 and 9-04$\tau$. A coupling constant of 0-09$\tau$ (5-4cyc./sec.) is reasonable for spin–spin interaction in the group -CH-CH$_3$. It is likely that the former doublet corresponds to the resonance of the methyl protons in the saturated isoprene residue nearest to the hydroxyl group and the latter doublet to the methyl protons in the other saturated isoprene residues. This is supported by the position of a single doublet in the spectrum of phytol and by the position of a single doublet in the spectrum of citronellol (J. Feeney & F. W. Hemming, unpublished work). Also, assuming the total area of this region to be equivalent to four methyl groups, the ratio of the areas of the 9-17 and 9-08$\tau$ to the 9-13 and 9-04$\tau$ doublet is clearly close to 1:3, in agreement with this assignment.

The pattern in the region 8-5–8-8$\tau$ is complex, as one would expect from overlapping peaks for methylene and methane protons in slightly different chemical environments and subject to multiple splitting by protons on adjacent carbon atoms. Similar patterns have been observed in the spectra of pig-liver dolichol (Burgos et al. 1963) and phytol and citronellol (J. Feeney & F. W. Hemming, unpublished work). The area of this region relative to that in the region 9-0–9-2$\tau$ is informative, for if one of the saturated units had not been $\omega$-terminal but internal instead, these regions would have been in the ratio 28:12. It is clear from the observed relative areas (16:11) that one of the saturated isoprene residues has to be $\omega$-terminal.

Thus nuclear-magnetic-resonance studies, also, show that there is a 'hydroxy-terminal' saturated
isoprene residue and an $\omega$-terminal saturated isoprene residue. The exact position of the third saturated isoprene residue cannot be predicted on the basis of nuclear magnetic resonance.

The peak at 84.27 can be assigned to protons of a methyl group cis and that at 8.377 to protons of a methyl group trans to the olefinic proton in internal isoprene residues. The measurement of the relative areas of these two peaks in the 60 MHz spectrum was difficult, but by transferring the spectrum to millimetre graph paper and counting the squares under each peak it was deduced that about 8% of the methyl groups were in the trans configuration. This corresponds to between one and two trans-methyl groups. The 100 MHz spectra gave better resolution of these two peaks and the integration indicated the situation summarized in Table 3. The values in this Table favour two trans-methyl groups. The presence of three trans-methyl groups is most unlikely, but, bearing in mind the 60 MHz spectrum, the possibility of slight overlap of the resonance for the protons on the methylene group $\beta$ to the hydroxyl group with the 8.377 peak and the possibility of error in measuring the areas of peaks the presence of only one trans-methyl group cannot be entirely ruled

### Table 2. Positions, assignments and relative areas of the peaks in the nuclear-magnetic-resonance spectrum at 60 MHz/sec. of a solution in benzene of the mixture of Aspergillus hexahydropolyproprenols

<table>
<thead>
<tr>
<th>Peak position ($\tau$)</th>
<th>Assignment*</th>
<th>Relative areas</th>
<th>Measured</th>
<th>Calculated†</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.30, bilateral</td>
<td>O-H</td>
<td>1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>9.17, bilateral</td>
<td>$\text{CH}_2$</td>
<td>11</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>9.13, bilateral</td>
<td>$\text{CH}_3$</td>
<td>16</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>9.08, bilateral</td>
<td>$\text{CH}$</td>
<td>56</td>
<td>53.4</td>
<td></td>
</tr>
<tr>
<td>9.04, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{OH}$</td>
<td>20</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>8.72, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>8.62, bilateral</td>
<td>$\text{CH}_3$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>8.58, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{OH}$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>8.37, bilateral</td>
<td>$\text{CH}_3$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>8.24, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>7.95, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>7.83, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>7.78, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>6.65, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>6.55, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>6.45, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>4.73, bilateral</td>
<td>$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$</td>
<td>18</td>
<td>17.8</td>
<td></td>
</tr>
</tbody>
</table>

* The resonating protons are in italics.

† Calculated assuming structure (1) to be correct and the mixture to contain 1.7, 5.7, 29.6, 37.6, 19.5 and 5.9% of hexahydroproprenols-18, -19, -20, -21, -22 and -23 respectively.

‡ Disappeared on shaking with D$_2$O.

### Table 3. Relative areas of resonance peaks for protons of methyl groups cis and trans to olefinic protons in the nuclear-magnetic-resonance spectra at 100 MHz/sec. of benzene solutions of Aspergillus hexahydroproprenols-20, -21 and -22

<table>
<thead>
<tr>
<th>Hexahydropropenol-20 (band 3)</th>
<th>Hexahydropropenol-21 (band 4)</th>
<th>Hexahydropropenol-22 (band 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis (8.28, $\tau$)</td>
<td>44–46</td>
<td>49–52</td>
</tr>
<tr>
<td>trans (8.37, $\tau$)</td>
<td>5–7</td>
<td>5–7</td>
</tr>
</tbody>
</table>

* Calculated assuming the total areas of this region of the spectra of hexahydroproprenols-20, -21 and -22 to be 51, 54 and 57 respectively; these values are in fact the number of protons/mol. expected to resonate in this region.
out. Nevertheless, the areas as measured consistently are in favour of two internal trans-isoprene residues in each alcohol and this number is clearly the most likely.

Another noteworthy feature of the 8:2-8:8% region of the spectra is the absence of peaks either side of the internal trans-methyl peak. These peaks (8:32 and 8:43%) are characteristic of most spectra of benzene solutions of prenols and correspond to the cis- and trans-methyl protons (relative to the olefinic hydrogen) in the ω-terminal isoprene residue respectively (see e.g. Wellburn et al. 1967). This observation also confirms that in these alcohols the ω-terminal isoprene residue is saturated.

Infrared spectroscopy. The infrared absorption spectra of Aspergillus bands 3, 4 and 5 (predominantly hexahydroprenols-20, -21 and -22 respectively) are reproduced in Fig. 3. No absorption peaks occurred in the region 1700-2500cm.⁻¹ except for weak absorption at 1750cm.⁻¹ in the spectra of hexahydroprenols-20 and -21, almost certainly caused by the presence of trace amounts of carbonyl-containing impurity. The spectra are almost identical, as would be expected for a series of isoprenologues of this type. Spectra of bands 1, 2 and 6 (predominantly hexahydroprenols-18, -19 and -23 respectively) were qualitatively essentially the same as those in Fig. 3. The spectra are very similar to that of pig-liver dolichol (Burgos et al. 1963).

The C-O stretching (O-H deformation) band occurs at 1060cm.⁻¹, confirming that the compounds are primary alcohols and that the carbon atom β to the hydroxyl group is fully substituted. In allylic alcohols the C-O stretching band occurs at 900cm.⁻¹. The bands at 835cm.⁻¹ (C-H deformation of a trisubstituted olefin), at 1450cm.⁻¹ (C=C stretching) and at 3028cm.⁻¹ (C-H stretching of =CH), together with the relative intensities of the bands at 1450cm.⁻¹ (C-H deformation of -CH₃ and -CH₂) and at 1365cm.⁻¹ (C-H deformation of -CH₃), are in keeping with a polyisoprenoid structure (Bellamy, 1958). Small peaks at 886, 1090, 1130, 1240 and 1308cm.⁻¹ are consistent with the predominantly cis configuration of the isoprene residues, as is the absence of a shoulder at 795cm.⁻¹ on the side of the strong peak at 835cm.⁻¹ (Burgos et al. 1963).

---

**Fig. 3.** Tracings of infrared spectra of solvent-free films of (a) hexahydroprenol-20 (band 3), (b) hexahydroprenol-21 (band 4) and (c) hexahydroprenol-22 (band 5). The films differed in thickness.

**Fig. 4.** Photographs of thin-layer chromatograms of the individual hexahydropolyprenols (stain: anisaldehyde). (a) Reversed-phase partition on paraffin-impregnated kieselguhr with acetone saturated with paraffin as mobile phase. (b) Adsorption on silica gel G with methanol-benzene (1:99, v/v) as developing solvent. Spots 1, 2, 3, 4, 5 and 6 correspond to hexahydroprenols-18, -19, -20, -21, -22 and -23 respectively. A ring has been drawn round the area of stain.
Thin-layer chromatography. On adsorption thin-layer chromatography [silica gel G with methanol–benzene (1:99, v/v) as developing solvent] the alcohols travelled as single compounds with essentially the same \( R_p \) (ranging from 0.37 to 0.39 for hexahydroprenols-18 to -23). A typical chromatogram is shown in Fig. 4(a). Each sample is predominantly one compound. A plot of the \( R_m \) values of these positions against the value of \( n \) (structure I) in each of hexahydroprenols-19 to -23 gave a straight line (Fig. 5). The \( R_m \) value of the material in band 1 also fell on the same straight line when it was assumed that the main component was hexahydro-prenol-18 (\( n=15 \)). The amount of hexahydro-prenol-24 present was too small to be seen clearly by single-dimension chromatography either of band 6 or of the mixture. However, two-dimensional chromatography of larger quantities of the mixture (see the Methods section) showed the presence of seven compounds with \( R_m \) values corresponding to hexahydroprenols-18 to -24.

Thus there is chromatographic evidence in favour of the presence of hexahydroprenols-18 and -24 as well as those of intermediate chain length.

Micro-hydrogenation. Three samples of the mixture of \textit{Aspergillus} alcohols gave values for moles of hydrogen taken up per 100g. mixture of 1.284, 1.243 and 1.273. The mean value of 1.267 is within 3% of the expected value (1.233) based on structure (I) and the known composition of the mixture (see Table 2).

\textit{Ozonolysis.} Reductive acetylation of the ozonide of structure (I) would be expected to yield 1mol.prop. each of 6,10-dimethyl-n-undecan-2-yl acetate, 3-methyl-n-hexane-1,6-diyl diacetate and \( n \) mol.prop. of n-pentane-1,4-diyi diacetate (Scheme 4).

The ozonolytic degradation products of the mixture of \textit{Aspergillus} alcohols were compared with those of pig-liver dolichol and with reference compounds by gas-liquid chromatography. The results are shown in Fig. 6. As expected, both pig-liver dolichol and the \textit{Aspergillus} hexahydroprenols yielded mainly a compound with a retention time (3.85min.) corresponding to that of n-pentane-1,4-diyi diacetate (peak 4). Both preparations also gave a peak (peak 6) with a retention time (10.18min.) corresponding to that of 3-methyl-n-hexane-1,6-diyl diacetate. The chromatogram of

![Diagram](image)

**Fig. 5.** Plot of the number of unsaturated isoprene residues in each molecule (\( n \) in structure I) against the \( R_m \) value of these molecules during reversed-phase partition thin-layer chromatography (see the text), showing these molecules to be members of a series of isoprenologues.

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
\text{H} \cdot [\text{CH}_2 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2]_2 \cdot [\text{CH}_2 \cdot \text{C} \cdot \text{CH}_2 \cdot \text{CH}_3]_n & \quad \text{CH}_3 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH} \\
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
\text{H} \cdot [\text{CH}_2 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2]_2 \cdot \text{CH}_3 \cdot \text{CH} \cdot \text{O} \cdot \text{CO} \cdot \text{CH}_3 & \quad (n-1) \text{CH}_3 \cdot \text{CO} \cdot \text{O} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{O} \cdot \text{CO} \cdot \text{CH}_3 \\
6,10\text{-Dimethyl-}n\text{-undecan-2-yl acetate} & \quad n\text{-Pentane-1,4-diyl diacetate} \\
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
+ \text{CH}_3 \cdot \text{CO} \cdot \text{O} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{O} \cdot \text{CO} \cdot \text{CH}_3 & \quad 3\text{-Methyl-}n\text{-hexane-1,6-diyl diacetate}
\end{align*}
\]

Scheme 4.
the *Aspergillus* alcohol degradation products differed from the pig-liver dolichol products in the expected manner inasmuch as the former showed a peak (peak 2) with retention time (1.47 min.) identical with that of 6,10-dimethyl-n-undecan-2-yl acetate. These peaks were not present in the chromatogram of the "blank" run. The relative intensities of these peaks were much as expected. An accurate assessment of the peak areas was not attempted since the sensitivity of the detector to the different compounds had not been determined.

Peaks 1 and 3 (retention times 0.85 and 2.43 min. respectively) in the *Aspergillus* alcohols and pig-liver dolichol chromatograms also appeared in the chromatograms of the squalene ozonolysis products and in the "blank" chromatogram. In the "blank" the degradative procedure was carried out entirely as before but with no solute in the ethyl chloride. Clearly peaks 1 and 3 originate in some way from the reagents. The presence of small peaks with similar relative retention times (with n-pentane-1,4-diyli diacetate as a standard) can be observed in the chromatograms of the ozonolysis products of squalene obtained by Donninger & Popjak (1966) with the same degradative and gas–liquid chromatographic procedure.

Peak 5 remains unaccounted for. It is noteworthy that the ozonolytic degradation of citronellol, though giving rise mainly to peak 6 as expected (Scheme 1), also gave a small proportion of peak 5. The relative proportions of peaks 5 and 6 are very similar to this in the chromatograms of pig-liver dolichol and of the *Aspergillus* hexahydroprenols. Peaks 5 and 6 were both absent from the chromatograms of the squalene ozonolysis products and of the blank. It appears that peak 5 is present only in chromatograms of compounds containing a saturated 'hydroxy-terminal' isoprene residue. Its

![Fig. 6. Tracings of the gas–liquid chromatograms of the ozonolytic degradation products of: curve A, pig-liver dolichol (curve A', larger sample injected on to the column); curve B, *Aspergillus* hexahydroprenols (curve B', larger sample injected on to the column). Curve C, blank run with peaks obtained with reference compounds superimposed in broken lines. See the text for explanation of the numbers. S, Solvent.](image)

---

Scheme 5.
retention time (7.02 min.) is consistent with a compound intermediate in chain length between that of n-pentane-1,4-diyldiacetate (1-methyl-n-butane-1,4-diyldiacetate) (peak 4) and 3-methyl-n-hexane-1,5-diyldiacetate (peak 6). Possible compounds are 2-methyl-n-pentane-1,5-diyldiacetate and 3-methyl-n-pentane-1,5-diyldiacetate. If in a small proportion of the prenol molecules the double bond nearest to the hydroxyl group migrated one carbon atom closer to the hydroxyl group one would expect a small proportion of the latter compound from citronellol and a small proportion of both compounds from pig-liver dolichol and from the Aspergillus hexahydropropenols (Scheme 5). It is doubtful whether one could distinguish between these two isomers with the gas-liquid chromatographic system used.

Thus peak 5 could be accounted for by migration of the 6,7-double bond to the 5,6-position in a small proportion of the molecules. 2-Methyl-n-pentane-1,5-diyldiacetate could also be formed by migration of other double bonds in the chain in the same direction. However, this would inevitably involve the production of other diacetates on degradation and there is no evidence for this in the gas-liquid chromatograms. Similarly there is no evidence for migration of double bonds away from the hydroxyl group.

In fact some of the mixture of Aspergillus alcohols that was ozonized had been biosynthesized from [2-14C]malonic acid. This offered a convenient method of checking some of the above conclusions. By using the gas-liquid chromatogram preparatively, samples of peaks 1–6 were collected and assayed for 14C. Peak 4 (357 counts/min.) contained 80% of the radioactivity recovered, the rest being distributed mainly between peaks 2 and 6, with peak 5 containing 13% of the total recovered. Less than 3 counts/min. above background (counting efficiency 56-1%) was associated with peaks 1 and 3. This distribution of radioactivity is essentially consistent with the identification of the peaks made above.

DISCUSSION

The results presented above provide strong evidence for the presence of hexahydropropenols-19, -20, -21, -22 and -23 in the unsaponifiable lipid of Aspergillus fumigatus Fresenius. Chromatographic evidence also supports the presence of hexahydropropenols-18 and -24, with the latter compound also indicated by mass spectrometry. Of the mixture, hexahydropropenol-21 (38%) is the major component and hexahydropropenols-20 (30%) and -22 (20%) are the next two most abundant. Most, and probably all seven, of the alcohols have the 'hydroxy-terminal' (α) isoprene residue, the ω-isoprene residue and the isoprene residue adjacent to the ω-residue (the ψ-isoprene residue) saturated. The three major components of the mixture each contain two of the remaining internal isoprene residues in the trans configuration, and it is likely that all seven alcohols are alike in this respect also. The alcohols appear to differ only in the number of internal cis-isoprene residues.

The precise position of the trans-isoprene residues in each molecule is not known but it is tempting to assume that these are immediately adjacent to the saturated ψ-isoprene residue. If the saturated ω- and ψ-isoprene residues are biogenetically trans it would then follow that the Aspergillus hexahydropropenols could have been formed by addition of cis-isoprene residues to all-trans-geranylerganylporphosphate or a dihydro or tetrahydro derivative thereof. If the rather remote possibility that these alcohols contain only one trans-isoprene residue proves to be correct then trans-trans-farnesyl pyrophosphate, or a dihydro or tetrahydro derivative thereof, could be postulated as a likely precursor.

The adoption of a trivial name of these compounds presents some difficulties. The full chemical name is clearly impossibly long for general use and a trivial name is essential. The poly-cis nature of these polypropenols and the saturated nature of the 'hydroxy-terminal' isoprene residue makes them members of the dolichol series of alcohols. In fact, when first isolated, the term 'Aspergillus dolichol' was used to describe the preparation. The authors consider the continued use of the trivial name dolichol or of a derived name such as tetrahydrol-dolichol to describe the Aspergillus alcohols to be undesirable. It is likely to lead to less confusion if the trivial name 'dolichol' is retained only for the 2,3-dihydrolpolyprenols. The term 'hexahydropropenylenols' for the Aspergillus alcohols is immediately more informative and less confusing than the term 'dolichols' or 'tetrahydrodolichols'. A more precise generic name for this series of alcohols would be 'αψω-hexahydropropenylenols'. To avoid any confusion this could be prefixed by the word 'Aspergillus'. It may also be necessary at times to indicate the number of trans-isoprene residues. The same arguments hold for the individual alcohols except that here one can be more precise about the position of the saturated isoprene residues. Thus a more precise name for Aspergillus hexahydropropenol-20 would be 2,3,7,4,7,8,7,97-hexahydroisoprenosaprolactone. Alternatively, by analogy with the dihydrobiquinones and dihydromenaquinones (I.U.P.A.C.—I.U.B. Commission on Biochemical Nomenclature, 1965), this compound could be given the name I,XIX,XX-hexahydropropenol-20, where the roman numerals indicate the isoprene residues carrying the extra hydrogen atoms, the 'hydroxy-terminal' isoprene residue being I.
Unfortunately, both of these alternatives are cumbersome, and until other related alcohols are isolated it seems preferable to use terms such as ‘Aspergillus hexahydropolyprenols’ and ‘Aspergillus hexahydroprenol-20’. In most contexts the prefix ‘Aspergillus’ could be dropped. As yet, further precision in the trivial names is not essential.

These hexahydropolyprenols provide another group of the increasingly large family of predominantly cis polyrenols. Alcohols of this type have now been isolated from mammalian, avian and higher-plant tissues and from bacteria, fungi and probably algae (Wellburn & Hemming, 1966). The hexahydropolyprenols provide the largest polyrenol yet isolated in the form of hexahydroprenol-24.

The biochemical function of these widespread polyrenols is not known. The concentration of the hexahydropolyprenols in Aspergillus follows closely the same changes with growth of the mould, as do the concentrations of other constituents such as ubiquinone and ergosterol (K. J. Stone & F. W. Hemming, unpublished work). For example, there is no evidence of more rapid biosynthesis at the onset of sporulation or at any other change in the morphology of the mould. It has been shown (Butterworth et al. 1966) that the biosynthesis of the compound continues at a rate intermediate between that of ubiquinone and ergosterol. Possibly, this points to a need for these alcohols by the mould.

The authors acknowledge in particular the skill and efforts of Dr W. Vetter and Dr P. Meyer in obtaining the mass spectra and of Dr J. Feeney and Dr R. J. Abraham in determining the nuclear-magnetic resonance spectra. Without these spectra the authors could not have deduced the structures. The work was helped and encouraged by the constant interest of Professor R. A. Morton. Both K. J. S. and P. H. W. B. are indebted to the Agricultural Research Council for the award of Research Studentships. The work was aided, in part, by Grant AM05282-04 from the U.S. Public Health Service.

REFERENCES


