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Homologous Aliphatic C_{30}—C_{45} Terpenols in Birch Wood

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The wood extractives of birch (*Betula verrucosa* Erh.) have been analysed. A large part of them are fatty acid esters of terpenoid and steroid alcohols. About half of the alcohols are compounds with the general formula

$$H-[CH_2-C(CH_3)=CH-CH_2]_n-OH$$

n being 6, 7, 8, and 9. About 60 % of their double bonds have the *cis* configuration. The name betulaprenols is proposed for the alcohols.

Birch wood extractives have been studied by Kahila and Rinne,[1] by Perilä and Toivonen,[2] and by Selleby,[3] all of whom have mainly analysed the fatty acids and the sterols. Paasonen [6] and Lindgren [6] have found that a great part of the extractives are fatty acid esters of steroid and terpenoid alcohols. This paper gives some notes about the composition of the wood extractives and reports a structural determination of the aliphatic terpene alcohols, which amount to about half of the total alcohols.

Analysis of the extractives. The analysis was carried out on the light petroleum soluble part of the wood extractives from birch (*Betula verrucosa* Erh.). This part, which amounted to 1.0 % of the wood weight, was fractionated by column chromatography into:

I. Unsaturated hydrocarbons (4 % of the light petroleum soluble extractives). They consisted mainly of squalene whose presence in the birch wood has been shown by Paasonen.[4]
II. Fatty acid esters of terpenoid and steroid alcohols (29 %).
III. Triglycerides (48 %).
IV. A mixture of unidentified substances (4 %).
V. Unesterified sterols (4 %).

The fatty acid components of fraction II (the sterol and terpene esters) and fraction III (the triglycerides) had about the same composition. Linoleic acid predominated and palmitic, oleic, and linolenic acids were present in considerable amounts. The composition agrees with that reported in earlier studies.[1-3]

A second portion of the extractives was saponified. The neutral part obtained was divided by column chromatography into five fractions (A)—(E). (A) was composed mainly of squalene and, as judged from TLC, the others consisted of four alcohol moieties which are formed by saponification of fraction II.

The first alcohol fraction (B), containing almost half of the (A)—(E) material, was an oil. A similar fraction has been isolated by Kahila and Rinne [1] and by Paasonen.[4] The latter found that its IR spectrum resembles that of an isoprenoid alcohol. Our studies (see below) have shown that the oil consists of alcohols with the formula (I, R = H, n = 6—9).

The second fraction (C) contained cycloartenol and 24-methylene-cycloartanol and the third fraction (D), α-sitosterol.[6] GLC of fraction (E) showed the presense of β-sitosterol (reported also in Refs. 3, 4) and an unidentified compound, in the weight ratio 100:15 as estimated from the peak areas.

The presence of stigmastanol in birch wood has been reported.[3] The chromatogram of fraction (E) contained a peak which suggested the presence of this stanol. Its content was low, the peak area being only one % of that of β-sitosterol.

Birch wood extractives are thus characterised by their high content of non-cyclic terpenoids (squalene and betulaprenols). The extractives of pine, spruce, and aspen contain no observable amounts of betulaprenols and, compared with birch, much smaller amounts of squalene.

Aliphatic terpene alcohols. The oily alcohol fraction (B) had no optical activity. According to GLC this fraction consisted of several components for which we have proposed the name betulaprenols. The following structural study was performed on the betulaprenol mixture without attempts at its fractionation.

The IR spectrum of the betulaprenol mixture contains peaks for methyl groups (C—H symmetrical bending frequency at 1375 cm^{-1}), trisubstituted double bonds (C—C stretching frequency at 1662 and C—H 'out-of-plane' deformation frequency at 828 cm^{-1}) and allylic hydroxyl groups (C—O stretching frequency at 995 cm^{-1}). The spectrum is similar to that [7] of solanesol (I, R = H, n = 9, all-*trans*),[7-9] and this suggested the formula (I, R = H) for the betulaprenols.

$$\underset{\alpha}{(H_3C)_2C}=\underset{\varepsilon}{CH}-\underset{\gamma}{CH_2}-[\underset{\gamma}{CH_2}-\underset{\alpha}{C(CH_3)}=\underset{\varepsilon}{CH}-\underset{\gamma}{CH_2}]_{n-2}-\underset{\gamma}{CH_2}-\underset{\beta}{C(CH_3)}=\underset{\varepsilon}{CH}-\underset{\delta}{CH_2OR}$$

I, R = H or $OCCH_3$

The suggestion was confirmed by NMR. Table 1 shows the shifts, peak areas and assignments for the proton magnetic signals of the acetylated betulaprenol mixture. For comparison, the shifts of the solanesol signals taken from the paper by Burgos *et al.*[10] are included. As is evident from the table, the spectrum contains all the signals expected from a mixture of substances with the formula (I, R = $OCCH_3$) and no other. The peak areas agree with the mean number of isoprene units in the betulaprenols being 7.5.

As seen in Table 1 all signals observed are for allylic or vinylic protons. This shows that all double bonds must be located between the two middle carbon atoms of the isoprene units.

The betulaprenol acetate mixture was perhydrogenated to an oil which gave NMR signals for methyl groups, methylene groups, acetyl groups and protons geminal to acetyl groups. The last two signals were weak, their peak areas corresponding to only one acetyl group for 60—70 isoprene units. As expected for an allylic ester, the greater proportion of the acetyl groups had been hydrogenolysed and this has given rise to a mixture of saturated hydrocarbons and small amounts of saturated acetates. Solanesol acetate reacts in the same way.[7]

The betulaprenol mixture was ozonolysed to laevulic acid, laevulic aldehyde, acetone and glycolic acid in agreement with the proposed formula. When the ozonides were degraded by hydrolysis no succinic acid was found thus indicating that no isoprene units are linked 'tail-tail' as are the two middle units of squalene.

The NMR spectrum of the betulaprenol acetate mixture contains one small and two large signals for allylic methyl groups. They are assigned by applying the results of Bates and Gale [11] (see notes *e-h* in Table 1) as has been

Table 1. The proton magnetic resonance signals from the betulaprenol acetate mixture.[a]

Assignment[b]	Solanesol (I, R = H, n = 9) Shift[c] ppm	Betulaprenol acetates (I, R = CH_3CO, n = 7.5) Shift ppm	Peak areas Found	Peak areas Calc.
trans α[d,e]	1.58	1.57	9[i]	
cis α[f], *trans* β[g]	1.66	1.65	14[i]	26
cis β[h]	—	1.72	3[i]	26
CH_3CO	—	1.93		
γ	1.98	2.01[j]	30	29
	2.02	2.06[j]		
δ R = H	4.00[k]	(3.95)[l]		
δ R = Ac	—	4.54[m]	2	2
ε	5.05	5.18[n]	7.4	7.5

[a] The 60 Mc spectrum of a carbon tetrachloride solution. The peak positions are given as δ values.

[b] The Greek letters refer to the symbols below formula (I).

[c] From the paper by Burgos *et al.*[10]

[d] *Trans* and *cis* refer to the relationship between the methyl group and the vicinal olefinic hydrogen atom.

[e-h] According to Bates and Gale [11] the shift for these types of methyl are *e*) 1.59 ppm *f*) 1.66 ppm *g*) 1.65 ppm (R = H), and *h*) 1.73 ppm (R = H).

[i] The values are approximate because the peaks partly overlapped.

[j] Complicated multiplet, in which the two strongest signals are the ones tabulated.

[k] Doublet, coupling constant was 6 cps.

[l] This signal was present in the spectrum of the betulaprenols but not in the one of their acetates. The signal was a doublet with the coupling constant 7 cps.

[m] Doublet, coupling constant was 8 cps.

[n] Unresolved multiplet.

done recently for the methyl signals of solanesol and dolichol (II, mainly *cis*, *n* about 20).[10]

The two large signals are ascribed to the methyl groups of the non-alcoholic isoprene units; the downfield one to the methyl groups *cis*-located to their vicinal olefinic hydrogen atoms, and the other to methyl groups in the *trans*-position. The area ratio between the two signals implies that about 60 % of the non-end isoprene units have the *cis*-configuration.

$$H-[CH_2-C(CH_3)=CH-CH_2]_{n-1}-CH_2-CH(CH_3)-CH_2-CH_2OH$$

II

Of the three methyl signals, the smallest one occurs at the lowest field. It is attributed to *cis*-methyl groups in the alcoholic end units. Its tabulated peak area corresponds to one group for every betulaprenol molecule. Owing to partial overlapping the value is, however, not accurate, and a significant amount of alcoholic end units with a *trans* bond may be present. A signal from the methyl protons of such units cannot be observed as it is overlapped by the large *cis*-methyl peak of the non-alcoholic units.

The GLC retention times for the betulaprenol acetates are shown in Table 2. The ratios between retention times for consecutive acetates are 2.6, 2.9, and

Table 2. Gas liquid chromatographic retention times for derivatives of betulaprenols, solanesol and squalene.[a]

Derivatives of	*n*[b]	Retention times, min.		
		Acetate	Perhydro-acetate	Saturated hydrocarb.
Betulahexaprenol	6	4.2	3.4	2.2
» hepta »	7	14	11	8
» octa »	8	40	24	21
» nona »	9	103[c]	*d*	63
Squalene[e]	6			2.0
Solanesol	9	120[c]	*d*	64

[a] Pye fractometer. Column: 1% Se-30 on Celite. Temperature 228—230°C. The argon pressure at the inlet was 1.0 atm.
[b] The number of isoprene units in the molecule.
[c] Approximate value as the peak was broad and unsharp.
[d] No peak was observed.
[e] The retention time for squalene was 3.0 min.

3.3. As the values are roughly the same the betulaprenols may be members of a homologous series.[12]

GLC on the hydrogenated betulaprenol acetates gave peaks which could be divided into two groups. One may correspond to the saturated hydrocarbons and the other to the saturated acetates (Table 2). Every acetate peak had a slightly larger retention time and was much smaller than the cor-

responding hydrocarbon peak. Within both groups the ratio of the retention times between consecutive members was, as in the series of unsaturated acetates, about 3.

The GLC peaks of the betulaprenol and solanesol acetates were much broader than the peaks of other substances with similar retention times, for example the perhydrogenated samples. The broadening is probably due to rearrangements of the oligoprenols during the GLC analysis.

The slowest component in the hydrogenated betulaprenol acetate mixture was retained to the same extent as perhydrogenated solanesol acetate. It has then the same number of isoprene units as solanesol, *i.e.* nine. Perhydrosqualene moved with about the same rate as the fastest hydrocarbon, which thus has six units. The betulaprenols therefore consist of alcohols with 6, 7, 8, and 9 isoprene units.

According to the GLC peak areas, the betulaprenol mixture consists mainly of the heptamer and octamer. As the degree of degradation during the analysis is unknown, the content of the nonamer might, however, be higher than suggested by the peak areas.

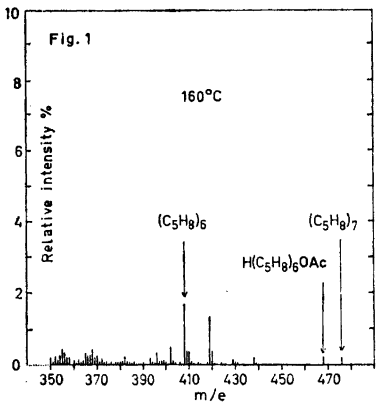
The mass spectrum of the betulaprenol acetate mixture was determined at varying inlet temperatures. A temperature raise increases the amount of ions from the higher betulaprenols more than from the lower ones due to the difference in volatility. At 160°C the peak corresponding to the $(C_5H_8)_6^+$ ion from the hexamer was considerably higher than the $(C_5H_8)_7^+$ peak of the heptamer (Fig. 1). At 200°C the later was somewhat larger than the former (Fig. 2). A weak peak for the $(C_5H_8)_8^+$ fragment but none for $(C_5H_8)_9^+$ was observed.

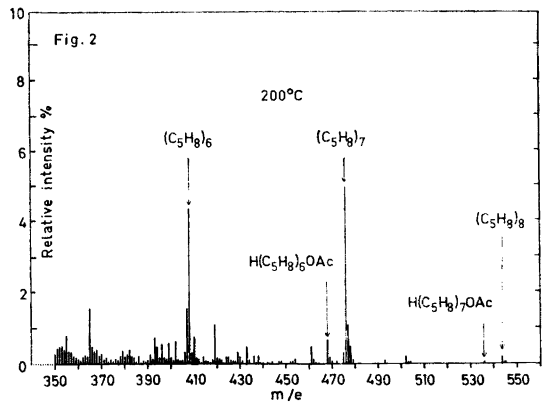
The molecular ions of the hexa-and heptamer acetates were also noticed in these spectra and their relative intensities followed the same pattern as those for the $(C_5H_8)_n^+$ peaks.

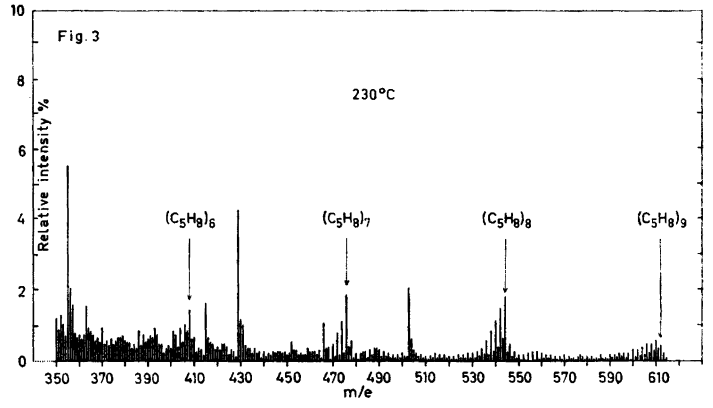
The hydrogenated betulaprenol acetate mixture gave distinct peaks for the $(C_5H_{10})_n^+$ fragments of the perhydrogenated acetates of the hepta-, octa-, and nonamers as well as smaller peaks for the molecular ions of the corresponding saturated hydrocarbons (Fig. 4).

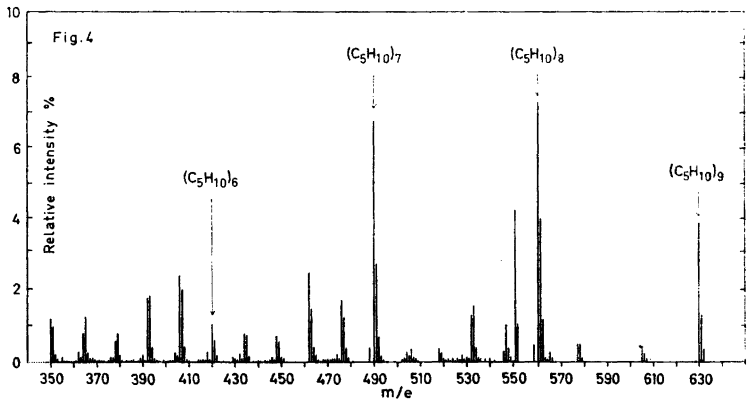
The mass spectra contained intervals with practically no peaks which hampered the mass number determination for the highest molecular ions. No difficulties were found in the evaluation for the peaks attributed to $(C_5H_8)_n^+$, $n = 6$ and 7 in Figs. 1–2 and $(C_5H_{10})_n^+$, $n = 6$, 7, and 8 in Fig. 4, thus confirming the empirical formula for the hexa- and heptamers and also partly for the octamer. For the octamer, nonamer and the hydrogenated nonamer the mass number determination was impaired by an error of at most $\pm$ 5 units. The values obtained corresponded, within the limits of the experimental error, to the proposed formula.

The mass spectrum of solanesol,[9] determined at an inlet temperature of 250°C, showed that considerable thermal destruction had occurred during the run. Instead of one distinct mass peak for $(C_5H_8)_9^+$, the spectrum contained a group of peaks with *even* mass numbers around the one arising from $(C_5H_8)_9^+$. The betulaprenol acetates were degraded in the same way when the spectrum was recorded at 230°C. Groups of peaks were found for the even mass numbers around those due to $(C_6H_8)_n^+$, n being 7, 8, and 9 (Fig. 3).









Figs. 1—4. Mass spectra of the betulaprenol acetate mixture (Figs. 1—3) and of the hydrogenated betulaprenol acetate mixture (Fig. 4). The spectra are recorded at 70 eV with an inlet temperature of 160°C (Fig. 1), 200°C (Fig. 2), and 230°C (Fig. 3).

This thermal reaction may involve an exchange of hydrogen atoms between the betulaprenol molecules, thus transforming the substances into a mixture of more or less unsaturated species. The driving force may be a tendency of forming conjugated double bond systems.

An attempt was made to isolate an all-*trans* fraction from the betulaprenol mixture by forming a thiourea inclusion compound, which can be obtained from all-*trans* substances such as squalene [13] and solanesol.[10] Since no such fraction was obtained, most, if not all, of the betulaprenol molecules contain *cis* double bonds. Whether these are distributed at random in the molecule or according to a special pattern cannot be ascertained at present.

This type of oligoterpenes may be rather common in nature. Solanesol was isolated by Rowland *et al.*[7] from tobacco leaves where it was found partly as fatty acid esters.[14] Recently Morton *et al.* have discovered several substances of this type. Dolichol [10] (II, mainly *cis*, *n* about 20) has been isolated by them from animal sources. A C_{110} substance with mainly *cis* bonds has been identified as a metabolic product of *Aspergillus fumigatus*,[15] while the spadix of *Arum maculatum* contains dolichol and an all-*trans* C_{50} alcohol.[16] Chestnut leaves contain a substance which may be closely related to the birch oligoprenols since it is probably a C_{60} alcohol with mainly *cis* double bonds and no saturated end isoprene unit.[17]

EXPERIMENTAL

Analysis of the extractives

Extraction. Shavings (1.45 kg dry weight) of freshly cut birch wood were extracted with acetone for 24 h. Evaporation of the acetone yielded a residue which was extracted with ether. The dried ether solution was evaporated to a syrup (18.9 g), part of which (14.4 g) was soluble in light petroleum (200 ml).

Fractionation of the extractives. The light petroleum soluble part (10.5 g) was placed on the top of a silicic acid (Mallinckrodt) column (diameter 4.4 cm, length 38 cm) which

Table 3. The fractions obtained by chromatography of the light petroleum soluble extractives from birch wood.

No.	Solvent		Fraction	
	IPE[a]	Volume, l	Weight, g	Composition
I	0	2.1–6.6	0.46	Squalene
II	5	6.6–8.4	3.24	Esters of sterols and terpene alcohols
III	5	8.4–10.5	3.74	Triglycerides
	10	10.5–12.6	1.37	
IV	15	12.6–15.1	0.47	Unidentified substances
V	25	15.1–18.0	0.45	Sterols

[a] The percentage of isopropyl ether in the solvent.

was then eluted with light petroleum containing stepwise increasing proportions of isopropyl ether. The elution was followed by TLC on the eluate. The result of the fractionation is given in Table 3.

Fraction I. TLC and GLC showed that the fraction was composed mainly of squalene. The hydrogen chloride addition product [18] prepared from the fraction melted at 111 – 129°C and had the same IR spectrum as that of an authentic sample.

Fraction II. The fraction contained several compounds moving closely together on TLC.

The material (2.42 g) in propanol (25 ml) was added to N methanolic potassium hydroxide solution (20 ml), and the mixture was refluxed for 1 h. The product was divided into an acidic and a neutral fraction.

The acidic material was methylated and analysed by GLC on the methyl esters of fatty acids.[19] The relative peak areas were for palmitic 10, oleic plus a small amount of stearic 23, linoleic 100, and linolenic acid 15.

On TLC, the neutral fraction gave the same four spots as given by fractions *B* – *E* described below.

Fraction III. The material was saponified as described for fraction II. The neutral, ether soluble part was only 4 % of fraction III.

The acidic fraction was methylated and analysed by GLC on the methyl esters of fatty acids.[19] The relative peak areas were for palmitic 18, oleic plus a small amount of stearic 27, linoleic 100, and linolenic acid 2.

Fraction IV. TLC showed spots of several compounds.

Fraction V. The fraction was recrystallised from ethanol-chloroform. M.p. 120 – 128°C, $[\alpha]_D$ – 24° ($CHCl_3$). TLC revealed only one spot indistinguishable from that given by β-sitosterol and similar sterols.

Chromatography of the neutral part of the saponified extractives. Light petroleum soluble extractives (9.34 g) were saponified as described for fraction II above. The neutral fraction (2.51 g) was put on the top of a silicic acid (Mallinckrodt) column which was then eluted with light petroleum-isopropyl ether (1:1 v/v). The elution was followed by TLC. Table 4 gives the fractions collected with their weights and compositions.

Table 4. Fractions obtained by chromatography of the 'unsaponifiable' material from the extractives.

No.	Solvent, ml	Weight, g	Composition
A	270 – 420	0.40	Squalene
B	421 – 570	0.80	Betulaprenols
C	571 – 780	0.20	Cycloartanol derivative
	781 – 810	0.01	
D	811 – 1140	0.30	α-Sitosterol
E	1141 – 1590	0.47	Sterols

Fraction A. TLC and GLC indicated that it consisted mainly of squalene.

Fraction B. The study of this material is described below under the heading 'Betulaprenols'.

Fractions C and D. These fractions are being examined at present.[6]

Fraction E. After recrystallisation from ethanol, the material had m.p. 137 – 139°C, $[\alpha]_D$ – 26° ($CHCl_3$). On TLC it was indistinguishable from β-sitosterol and similar substances. The fraction was acetylated with pyridine and acetic anhydride at room temperature. The acetylated material was analysed by GLC on a XF 1105 (1 %) column (lenght 180 cm) at 205°C. Peaks for the acetate of β-sitosterol (retention time was 77 min; relative peak area 100), of stigmastanol (59 min; area 1), and of an unidentified compound (140 min; areas 15) were observed.

Betulaprenols

The material from fraction B above was an oil which had $[\alpha]_D$ 0.0 ($CHCl_3$; *c*, 1.02). It gave only one spot on TLC.

Acetylation and further purification. The material was dissolved in acetic anhydride-pyridine (1:1 v/v) and left over night at room temperature. Water was added and the acetylated material was extracted with hexane. The hexane solution was washed free from acetic acid and pyridine with water, dried and evaporated. The oily residue was analysed. (Found: C 82.3; H 11.7. $H(C_5H_8)_{7.5}OCOCH_3$ requires: C 83.1; H 11.3).

The residue was purified by chromatography on a silicic acid column (Mallinckrodt, deactivated by addition of 5% of water) using light petroleum-isopropyl ether (98:2 v/v) as solvent.

The proton magnetic signals of the purified acetylated material are given in Table 1. GLC of the material is described in Table 2. Mass spectra are shown in Figs. 1–3.

Hydrogenation. The purified acetylated material was hydrogenated in ethanol solution at room temperature and 1 atm hydrogen pressure. Adam's platinum catalyst was used. GLC of the hydrogenated material is described in Table 2, and its mass spectrum is shown in Fig. 4. Its NMR spectrum (in carbon tetrachloride solution) contained the following signals: peaks at 0.77, 0.84, and 0.87 ppm (attributed to methyl groups), an unresolved multiplet at 1.20 ppm (methylene groups), a singlet at 1.88 ppm (acetyl groups), and a triplet at 4.02 ppm (protons geminal to acetyl groups.)

Ozonolysis. The oligoprenol mixture was dissolved in ethyl acetate and ozone was passed through the solution at about −15°C until a bromine test showed that all double bonds had reacted. The solution was evaporated to dryness at 20°C. Water was added to the residue. The greater part of the water was distilled off. Acetaldehyde was isolated from the distillate in the form of the dinitrophenylhydrazone. It was found that ethyl acetate gave acetaldehyde by the above-mentioned ozone treatment.

The aqueous residue which did not distil was diluted with water and filtered. To a part of the filtrate dinitrophenylhydrazone solution was added. The precipitate obtained melted at 234–238°C after recrystallisations from nitrobenzene. Mixed m.p. and IR-spectrum showed it to be leavulic aldehyde bis-dinitrophenylhydrazone.

Another part of the filtrate was evaporated to dryness. Paper chromatography [20] of the residue showed spots for glycolic or succinic acid (they have the same R_F values) and for laevulic acid. The residue was refluxed with methanolic sulphuric acid (3 % by volume) for 5 h, and the solution was neutralized with sodium hydrogen carbonate, filtered and evaporated. The residue was analysed by GLC on (*a*) a poly-diethylene glycol succinate and (*b*) a poly-propylene glycol column, both at 125°C. The residue was also treated with ethanolic sulphuric acid and the product was analysed on (*c*) the poly-propylene glycol column at 125°C. In all three analyses a dominant peak for laevulic acid (as methyl and ethyl esters, respectively) was observed. Analysis (*c*) showed the absence of succinic acid. In analysis (*a*) the methyl succinate peak coincided with that of methyl laevulic acid. In analysis (*b*) a peak with a slightly lower R_F value than that of methyl succinate was observed.

When the ozonides were split by hydrogen peroxide [21] the presence of glycolic acid was shown by the GLC method *a* above.

The betulaprenol mixture (0.11 g) was ozonolysed in a carbon tetrachloride solution (50 ml) at −15°C. Zinc powder (0.5 g) and a drop of acetic acid were added to the solution, which was then stirred for 1 h. The solution was filtered, a dinitrophenylhydrazine solution (50 ml, saturated solution in 2 N hydrochloric acid) was added and the mixture was stirred for 3 h. The precipitate and the carbon tetrachloride solution were collected separately. The precipitate was found to consist of laevulic aldehyde bis-dinitrophenyl-hydrazone (mixed m.p. and IR-spectrum). Preparative paper chromatography [10] of the material in the carbon tetrachloride solution yielded a fraction which, after a recrystallisation from methanol, melted at 120–122°C. It was found to be aectone dinitrophenylhydrazone (mixed m.p. and paper chromatography [10]).

Inclusion compound with thiourea. Acetylated betulaprenol mixture (0.5 g) was dissolved in benzene (5 ml). The solution was added to a saturated solution of thiourea in methanol (40 ml). Part of the mixture precipitated as an oil. Benzene was then added slowly with stirring until the oil dissolved. Upon the addition of benzene crystals pre-

cipitated. They were collected, dried and dissolved in water. Undissolved oil in the aqueous solution weighed 18 mg.

NMR spectrum of the oil was recorded in a carbon tetrachloride solution. The area ratio between the proton resonance signals at 1.66 (*cis* methyl) and 1.58 ppm (*trans* methyl) was about 1.

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