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Triterpenes and 4α-Methylsterols in Birch Wood

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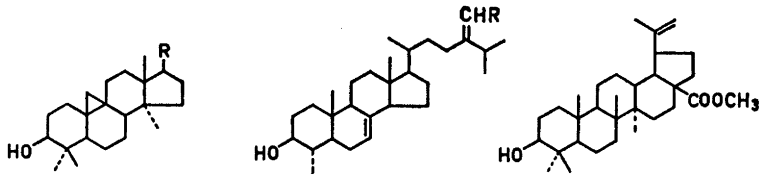
Cycloartenol (I), 24-methylene-cycloartanol (II), 4α-methylstigmasta-7,24(28)-dien-3β-ol ('α-sitosterol') (III) and the acetate of methyl betulinate (V) have been isolated from birch wood. The composition of the wood extractives is given in Table 2. The mass spectrum of 4α-methylstigmasta-7,24(28)-dien-3β-ol is discussed.

Fatty acid esters of steroid and terpenoid alcohols make up a large part of the wood extractives from birch (*Betula verrucosa* Erh.).[1,2] Chromatography on a silicic acid column divides the alcoholic moiety of these esters into four fractions, two of which have been described earlier. The first fraction was shown to consist of aliphatic C_{30}, C_{35}, C_{40}, and C_{45} terpinols.[1] The fourth fraction is composed mainly of β-sitosterol.[1,2]

The second fraction has now been found to contain two components which are indistinguishable by TLC (= thin layer chromatography) and GLC (= gas liquid chromatography) from cycloartenol (I) and 24-methylene-cycloartanol (II), respectively, two substances previously isolated from plants.[3] The components were separated from each other by preparative TLC and their identities with the above-mentioned triterpenes were confirmed by comparison with authentic samples.

Recrystallisation of the third fraction yielded a preparation whose analysis agrees with the formula $C_{30}H_{50}O$. Its properties and reactions were similar to those reported for α-sitosterol and citrostadienol (see experimental part). Both these substances have the structure (III), 4α-methylstigmasta-7,24(28)-dien-3β-ol.[4,5] It has been claimed that they are the $\Delta^{24,(28)}$ *cis-trans* isomers of this structure.[6] A recent study has shown that, in an α-sitosterol preparation from potato leaves, the methylstigmastadienol (III) is mixed with one of its lower homologues, 4α-methylergosta-7,24(28)-dien-3β-ol (IV).[7]

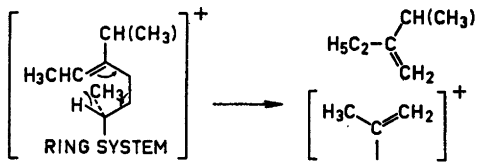
The mass spectra of the acetylated samples of α-sitosterol, citrostadienol and the birch preparation are almost identical, except in the molecular peaks



I R = -CHMe-$(CH_2)_2$-CH=CMe_2 III R = CH_3 V

II R = -CHMe-$(CH_2)_2$-C($CHMe_2$)=CH_2 IV R = H

for their components (see below). Two strong fragmentation peaks are characteristic for the spectra. One of these is ascribed to an elimination of C_7H_{14} from the molecular ion and the other of the side-chain at C-17 (below called R) plus two hydrogen atoms. The existence of a connecting metastable ion shows that the $[M^+-(R + 2H)]$ fragment is formed from the $[M^+-C_7H_{14}]$ fragment. Electron impact on the methylergostadienol (IV) forms, in a similar manner, a $[M^+-(R + 2H)]$ and a $[M^+-C_6H_{12}]$ fragment.



Scheme 1. Degradation by electron impact of the side-chain of $\Delta^{24(28)}$-steroids.

The cyclic rearrangement in Scheme 1 is assumed to be the first step in this two step fragmentation. The second step involves an elimination of the isopropylidene side-chain plus two hydrogen atoms. Analogues of the second step are found in the $[M^+-C_3H_7]$ ions produced by electron impact on $\Delta^{20(29)}$-lupene derivatives.[8] It is not known which of the hydrogen atoms are split off together with the side-chain.

Fucosterol [stigmasta-5,24(28)-dien-3β-ol], which has the same side-chain as α-sitosterol, is strongly fragmented to $[M^+-C_7H_{14}]$ but the $[M^+-(R + 2H)]$ ion is only formed in small amounts (Fig. 1). The second step is perhaps facilitated by the Δ^7-bond, which is not present in fucosterol.

The proton magnetic frequencies for the angular 18- and 19-methyl groups agree with those calculated for the structure (III) (Table 1) assuming that the 24-ethylidene and the equatorial 4α-methyl groups do not affect these frequencies. Also in other respects the structure (III) is supported by the NMR spectrum (see Table 1).

From the above it is concluded that the main constituent of the birch preparation is one of the isomers of the methylstigmastadienol (III). GLC shows it to be mixed with other substances which, according to the mass spectrum, may have the formula $C_{29}H_{48}O$, $C_{29}H_{50}O$ (perhaps β-sitosterol), $C_{30}H_{52}O$ and $C_{31}H_{52}O$. The mass spectrum of a citrostadienol sample also shows the presence of these compounds but in other proportions.

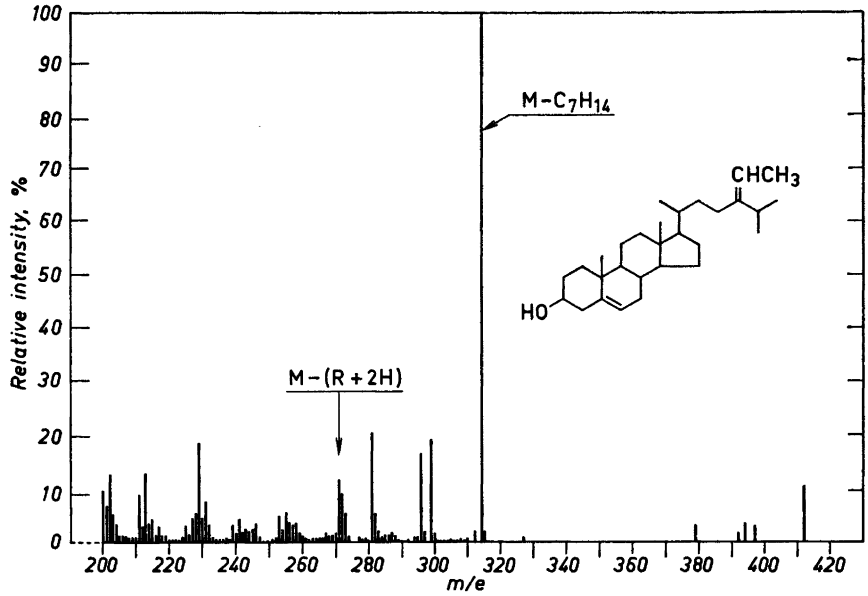


Fig. 1. Mass spectrum of fucosterol.

A substance identified as the acetate of methyl betulinate (V) was isolated from the extractives of a sulphite birch pulp and its presence in extractives of stored wood was indicated by TLC. On storing, the triglycerides are hydrolysed and oxidised by air. That facilitates the identification of the triterpene which moves in TCL with the same rate as the triglycerides.

The methyl betulinate [9] present in the waste liquor after alkaline pulping of birch wood is probably formed by saponification of the acetate. The methyl

Table 1. Proton magnetic signals for the α-sitosterol acetate preparation isolated from birch wood.

Shift [a] ppm	Peak type	Assignment Protons on carbon atom
0.53[b]	singlet	18
0.82[c]	singlet	19
0.97	doublet $J = 7$[d]	26, 27
1.56	doublet $J = 7$	29
2.02	singlet	CH_3COO
2.82	multiplet $J = 7$	25
4.3–4.5	multiplet [e]	3
5.0–5.3	multiplet [e]	7, 28

[a] Given in δ-values. Carbon tetrachloride solution. 60 Mc. [b] Calc. 0.53 ppm for 3β-acetoxy-cholest-7-ene. [14] [c] Calc. 0.82 ppm for 3β-acetoxy-cholest-7-ene.[14] [d] Given in cps. [e] Unresolved.

ester group in the sterically strongly hindered C-28 position survives the alkaline sulphate cooking.

To the authors' knowledge, the acetate of methyl betulinate is the only ester of a 28-triterpenoic acid that has been reported to exist in nature. Recently Erdtman *et al.*[10] have isolated the methyl esters of common resin acids from the oleoresins of various *Pinales* species.

The analysis of the light petroleum soluble portion of the extractives from a newly cut tree is summed up in Table 2.

Table 2. The composition of the light petroleum soluble portion of the extractives from a newly cut birch tree.[a]

Component	Percentage of the extractives	
Squalene		3
Fatty acid esters of		
betulaprenols	12	
cycloartenol (I)	2	
methylene-cycloartanol (II)	4	29
methylstigmastadienol (III) [b]	5	
β-sitosterol	6	
Triglycerides		48
Acetate of methyl betulinate (V)		present [c]
β-Sitosterol		4

[a] This portion was 1.0 % of the wood weight.
[b] Includes some other substances, see the text.
[c] The amount isolated from the sulphite pulp was about 0.2 % of the pulp extractives.

EXPERIMENTAL

General. Trimethyl silyl ethers were prepared according to Sweeley *et al.*[11] The acetylations were performed with acetic anhydride and pyridine at room temperature, the reaction time being about 18 h.

For the GLC analyses a stainless steel column of 1 % XE-60 on silanized Gas Chrom-P was used. The column temperature was increased during a period of 23.5 min (1.7°/min) from 205 to 245°C, and then kept constant. Under these conditions the retention times for the trimethyl silyl ethers of the following compounds were: cholesterol 15.0 min, β-sitosterol 19.7 min, cycloartenol 20.9 min, 24-methylene-cycloartanol 22.7 min, and 4α-methylstigmasta-7,24(28)-dien-3β-ol (the main constituent of the third, D, fraction) 24.1 min.

The isolation of fractions C and D is described in the previous paper.[1]

TLC analyses were run on plates of silicic acid (Merck G) and of silicic acid containing silver nitrate. The solvents used for the terpene alcohols were hexane-isopropyl ether (1/1 v/v), for their acetates, hexane-benzene (2/3 v/v) and for their trimethyl silyl ethers, hexane-benzene (5/1 v/v).

The optical rotations were determined in chloroform solutions, the concentration being about 0.5 %.

Cycloartenol (I) and 24-methylene-cycloartanol (II). (These triterpenes and their acetates were recrystallised from methanol-ethanol mixtures before the m.ps. and $[\alpha]_D$-values were determined). Fraction C consisted of two components, whose acetates and

trimethyl silyl ethers by TLC and GLC were indistinguishable from the same derivatives of cycloartenol and 24-methylene-cycloartanol. The area of the GLC peak of methylene-cycloartanol was twice that of cycloartenol.

Acetylation of the crude material gave crystals which melted at 107–110°C, $[\alpha]_{578}$ + 63°. The mass spectrum of the acetylated material gave a molecular peak for $C_{31}H_{51}(OCOCH_3)$. NMR showed the characteristic pair of doublet signals at δ = 0.32 ppm and 0.58 ppm for 4,4-dimethyl-9,19-cyclosterols.[12]

The acetylated mixture (120 mg) was separated by preparative TLC, on silicic acid containing silver nitrate. The faster moving acetate (30 mg) melted at 116–122°C. Saponification with potassium hydroxide in methanol-ethanol solution at 100°C (1 h) yielded cycloartenol, m.p. 92–97 and 106–109°C (two m.ps.), undepressed on admixture with an authentic sample.

The slower moving triterpene acetate (80 mg) melted at 104.5–105°C and 114.5–115°C (two m.ps.). Saponification as above yielded 24-methylene-cycloartanol, m.p. 116–119°C undepressed on admixture with an authentic sample, but depressed to 110°C on admixture with cycloartenol.

4α-Methylstigmasta-7,24(28)-dien-3β-ol (III). Recrystallisations from ethanol of the fraction D yielded a product with m.p. 166–167°C, $[\alpha]_{578}$ + 7° and $[\alpha]_{364}$ −3°. (Found: C 84.39; H 11.63; O 3.73. Calc. for $C_{30}H_{50}O$ (426.7): C 84.44; H 11.81; O 3.75).

Citrostadienol has m.p. 168°C, $[\alpha]_D$ + 24°, α-sitosterol, m.p. 164°C, $[\alpha]_D$ + 1°.[4,5]

The acetylated material was purified by preparative TLC on silicic acid containing silver nitrate. This removed a small amount of a faster moving component. Recrystallisations from ethanol yielded a product with m.p. 149–152°C, $[\alpha]_{578}$ + 34° and $[\alpha]_{364}$ + 83°. Like citrostadienol [4] and α-sitosterol [5] the preparation gave a positive Fieser selenium dioxide reaction, and, on ozonolysis, it yielded acetaldehyde.

Citrostadienol acetate has m.p. 146°C and $[\alpha]_D$ + 43°.[4] The acetate of an α-sitosterol sample, isolated from *Solanum tuberosum* by Schreiber and Osske,[5] has m.p. 144°C and $[\alpha]_D$ + 33°. The m.p. of this sample was undepressed on admixture with the acetylated birch preparation, m.p. 149–152°C.

The mass spectrum of the unacetylated birch preparation showed distinct peaks for *m/e* 440 (0.2 % of the base peak), 428 (2), 426 (7), 424 (2), 414 (5), 412 (4), 411 (7), 328 (60), 313 (9), 300 (3), 285 (100), 269 (10), 260 (9), 245 (8), and 227 (12). A peak for a metastable ion was observed at *m/e* 248–249.

The proton magnetic signals for the acetylated preparation are shown in Table 1.

GLC of the acetylated material gave a large, irregular peak (retention time was 24.1 min) plus a few small peaks.

The acetate of methyl betulinate (V). Sulphite pulp (2500 g), prepared in the laboratory from stored wood, was extracted with dichloromethane. A part (20 g) of the light petroleum soluble fraction (27.7 g) of the extractives was chromatographed on a silicic acid column (Mallinckrodt, length 70 cm, diameter 7.5 cm) using, as solvent, light petroleum containing stepwise increasing amounts of isopropyl ether. A fraction (1.2 g) eluted with light petroleum-isopropyl ether (85/15 v/v) gave, after recrystallisations from ethanol, crystals (60 mg) with m.p. 203–204°C and $[\alpha]_D$ + 14°. Mixed m.p. with an authentic sample of the acetate of methyl betulinate was undepressed. The mass spectrum and the frequencies of the proton magnetic signals were as expected for the acetate of methyl betulinate.[8,13]

Stored wood (320 g) was extracted with acetone. The light petroleum soluble extractives (1.6 g) were analysed by TLC. A weak spot of a substance moving with the same rate as the acetate of methyl betulinate was observed.

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