

Separation of Trimethylsilyl Ethers of Triterpenes by Thin Layer Chromatography: Triterpenes in Wood from *Populus tremula* L.

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The following alcohols have been isolated from *Populus tremula* wood: α - and β -amyrine, cycloartenol, 24-methylenecycloartanol, lupeol, butyrospermol, 4 α -methyl- $\Delta^{7,24(28)}$ -stigmastadienol-(3 β) (" α -sitosterol"), and β -sitosterol. The separation of the various compounds was achieved by argentative thin layer chromatography of the respective trimethylsilyl ethers. The mass spectra of the 4,4-dimethyl-9,10-cyclosterols are described.

Recently, Croon¹ has shown that wood from the European aspen (*Populus tremula* L.) contains about 0.25 % of higher alcohols and about 0.3 % of fatty acid esters of such alcohols ("waxes"). This paper describes the analysis of the triterpenes and the sterols of these alcohols. The alcohols present in the wood from a closely related species, trembling aspen (*P. tremuloides* Michx.), have recently been carefully studied by Abramovitch and Micetich.²⁻⁴

Our analysis was performed as follows. The light petroleum soluble part of the extractives from a newly cut tree was isolated and saponified. The neutral ("unsaponifiable") material obtained was chromatographed on a silica gel column. β -Sitosterol, 4 α -methyl- $\Delta^{7,24(28)}$ -stigmastadienol-(3 β) (" α -sitosterol")⁵ and a triterpene alcohol mixture were obtained.

Triterpene mixtures have previously been analysed by argentative thin layer chromatography (=TLC), either on the free alcohols⁶ or on their acetates.⁷⁻⁹ However, since a trimethylsilyl ether is less polar than its corresponding acetate, we expected to obtain a better separation with the ethers. The trimethylsilyl ethers of some terpenols and sterols were therefore prepared and characterised (Table 1). Their IR spectra exhibit a distinct peak at 1250 cm^{-1} (KBr) for methyl groups linked to silicon. The proton magnetic signal of the trimethylsilyl group in α -amyrine trimethylsilyl ether appears at δ 0.09 ppm and that of the 3 α -H, at 3.13 ppm.

Table 1. Some properties of the triterpene alcohol present in aspen wood. (Cholesterol is included for comparison.)

| Alcohol | Trimethylsilyl ethers | | | Acetates | |
|--------------------------------|-----------------------|---|------------------------------------|------------------------------------|--|
| | m.p.°C | $[\alpha]_{578}^c$ c, 0.5: CHCl ₃ | GLC ^a R _t | GLC ^a R _t | |
| Cholesterol | 128.5–129 | –33° | 1.00 ^b | 1.00 ^c | |
| α -Amyrine | 107–111 | +84° | 1.50 | 1.40 | |
| β -Amyrine | 156.5–159.5 | +84° | 1.41 | 1.31 | |
| Butyrospermol oil | | –6° | 1.41 | 1.30 | |
| Cycloartenol | 103–106.5 | +45° | 1.50 | 1.36 | |
| Lupeol | 176.5–178.5 | +33° | 1.56 | 1.43 | |
| 24-Methylene- -cycloartanol | 140–143 | +45° | 1.62 | 1.46 | |

^a Stationary phase, 1% XE-60 on silanized Gas-ChromP; flow rate, 30 ml/min; the temperature was raised from 205°C to 245°C with a rate of 1.7°C/min and then kept constant.

^b Retention time was 11 min. ^c Retention time was 19 min.

These ethers were found to be stable enough to be used in preparative TLC. A comparison of Figs. 1 and 2 shows that a better separation was achieved with the trimethylsilyl ethers than with the corresponding acetates.

The triterpene fraction was silylated and the resulting trimethylsilyl ether mixture was fractionated by preparative argentative TLC. The fractions consisted of 1) α - and β -amyrine, 2) saturated fatty alcohols, 3) cycloartenol and butyrospermol, and 4) lupeol and 24-methylene-cycloartanol. The constituents of fraction 3 were obtained in pure form by argentative TLC of their acetates. The components of fraction 4 were separated by recrystallisation. The amyrynes were recognised by GLC and by GLC combined with mass spectrometry.

The relative amounts of aspen terpene alcohols were estimated to be approximately: α -amyrine 5, β -amyrine 5, cycloartenol 5, 24-methylene-cycloartanol 9, lupeol 3, butyrospermol 15, and the fatty alcohols 1.

The mass spectra of cycloartenol and 24-methylene-cycloartanol showed a characteristic peak corresponding to the elimination of a C₉H₁₆O unit (see Fig. 3).¹⁶ The peak for the elimination of the side-chain and the D ring (M⁺ – R – C₃H₆) was very small. The first fragmentation may involve the loss of all the atoms on the left side of the line α (see the formula in Fig. 3) together with a hydrogen atom, or all the atoms on the left side of the line β and a hydrogen atom. Probably the cyclopropane ring contributes to this characteristic cleavage by formation of relatively stable cyclopropyl carbonium ions.^{10,11}

If the 9,10-cyclo-compounds were analysed as their acetates or their trimethylsilyl ethers at a high inlet temperature, 250°C, (the conditions for analysing directly after GLC) the (M⁺ – C₉H₁₆O) fragmentation was not as equally distinct as for the corresponding free alcohols.

All the triterpenes which we have found in *P. tremula*, with the exception of cycloartenol, have been isolated by Abramovitch and Micetich⁴ from the heartwood of *P. tremuloides*. However, a crude sample of 24-methylene-

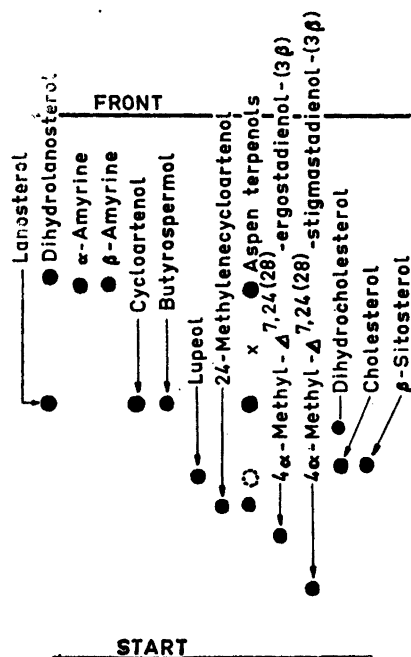


Fig. 1. Argentative thin layer chromatography of the trimethylsilyl ethers of some terpenoid alcohols.

Adsorbent: Silicagel G containing 20 % of silver nitrate. Solvent: hexane-benzene (5:1 v/v). Detection: spraying with aqueous phosphoric acid (1:1 v/v) followed by carbonisation. Amounts of alcohols: 5 μ g (the aspen terpenol mixture, 25 μ g). x) The position of the fatty alcohol mixture (not observable when only 25 μ g of aspen terpenols are put on the plate).

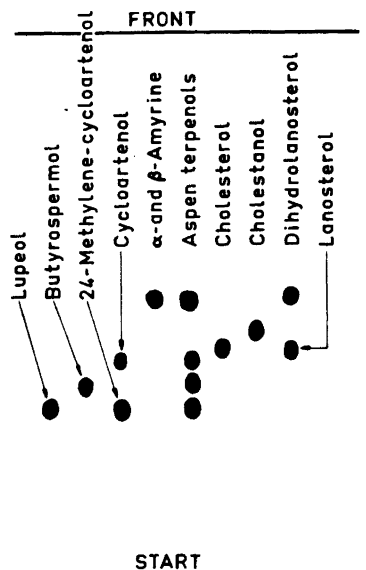


Fig. 2. Argentative thin layer chromatography of the acetates of some terpenoid alcohols.

Adsorbent: Silicagel G containing 20 % of silver nitrate. Solvent: benzene-hexane (3:2 v/v). Detection: spraying with aqueous phosphoric acid (1:1 v/v) followed by carbonisation. Amounts of alcohols: 5 μ g (the aspen terpenol mixture, 25 μ g).

cycloartenol from *P. tremuloides*, kindly sent by Dr. Abramovitch, contained cycloartenol as was shown by GLC and argentative TLC.

$\Delta^{3,5}$ -Stigmastadienone-(7) ("tremulone") has been isolated from *P. tremuloides*.³ This substance might, however, be an artefact formed by autoxidation of β -sitosterol³ (cf. also Rowe¹²). We did not observe the ketone in the newly cut wood of *P. tremula*.

EXPERIMENTAL

General

Unless otherwise stated, the optical rotations were determined in chloroform solutions, c being about 0.4; the recrystallisations were carried out in methanol-ethanol mixtures; the acetylations were performed with acetic anhydride in pyridine at room temperature; the saponifications of the acetates were carried out using 0.5 N methanolic KOH solution; the GLC analyses were carried out as described in Table 1.

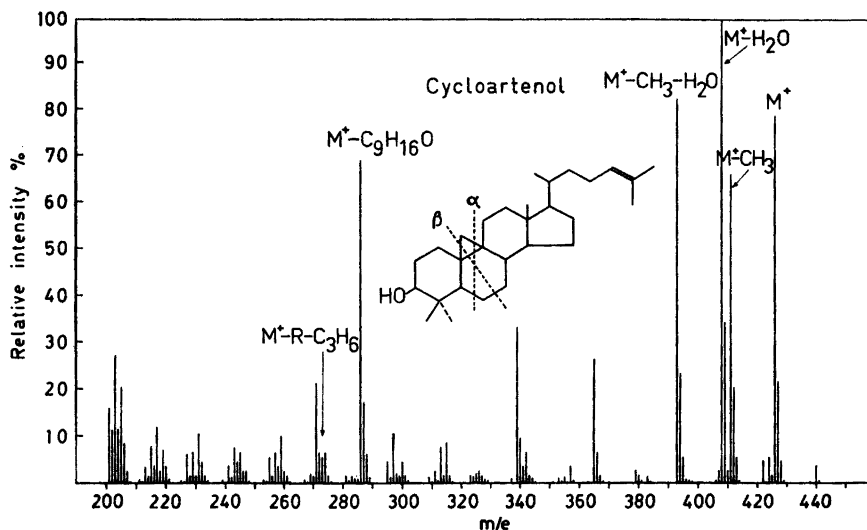


Fig. 3. The mass spectrum of cycloartenol.

TLC of terpenoid trimethylsilyl ethers. A plate (20 by 20 cm) was coated with a layer (1 mm thick) of a suspension of silica gel HF₂₅₄ (15 g) in an aqueous silver nitrate solution (10 %, 37 ml). The plate was dried at 110°C. About 80 mg of the triterpene alcohol mixture could be separated on one plate. The chromatogram was developed with hexane-benzene (5:1 v/v). The positions of the fractions were determined with Rhodamine 6G and they were extracted as described by Avigan *et al.*⁷ For complete removal of Rhodamine 6G, the chloroform solutions were filtered through a layer (thickness 0.5–1 cm) of silica gel. If the extraction was carefully done the fractions obtained contained only a small amount of hydrolysed material.

TLC of terpenoid acetates. The separation was carried out as described above for the silyl ethers except that the ratio between hexane-benzene was altered to 2:3 v/v. About 45 mg of an acetate mixture was used for one plate.

Preparation and hydrolysis of terpenoid trimethylsilyl ethers. The silyl ethers of the terpenoid alcohols were prepared and hydrolysed as described below for cholesterol. Their characteristics are given in Table 1.

Preparation. A mixture of trimethylchlorosilane and hexamethyldisilazane (1:2 v/v)¹³ (5 ml) was added to a solution of cholesterol (1 g) in pyridine (5 ml), followed, after about 5 min, by hexane (50 ml). The mixture was shaken twice with water (50 ml), the hexane layer was separated off, dried over anhydrous calcium sulphate overnight, and evaporated. The residue was recrystallised from methanol-acetone and the product (yield 91 %) melted at 128.5–129.0°C, $[\alpha]_{D}^{25} -33.0^\circ$. (Found: Si 6.10. Calc. for C₃₀H₅₄OSi: Si 6.12).

Hydrolysis. Cholesterol trimethylsilyl ether (150 mg) was shaken with a solution (30 ml) of 0.01 N hydrogen chloride in isopropyl alcohol-water (9:1 w/w) until it was dissolved (generally about half an hour). The solvent was then evaporated, the residue (125 mg, yield 99 %, m.p. 147–148°C) was cholesterol.

Fractionation of extractives

The neutral material (39 g) obtained from the saponified extractives was isolated and chromatographed as described for the corresponding material from birch.¹⁴ The following fractions (A–D) were obtained:

A. (18 g). Methyl esters of fatty acids (obtained by trans-esterification during the saponification in methanolic KOH) and unsaponified esters of terpenoid alcohols.

B. (5.7 g). A part of the material (0.680 g) was silylated and the product was fractionated by argentative TLC as described in the general part. Four fractions were obtained:

1. (120 mg). GLC of the fraction showed the presence of two components indistinguishable from α -amyryne and β -amyryne (Table 1). Using a gas chromatograph combined with a mass spectrometer, the fraction was divided into the two components, the mass spectra of which were determined. The spectra were identical with those of α -amyryne and β -amyryne. As expected from Ref. 15, the only distinct difference between the spectra of these two triterpenes was in the peak heights for m/e 203 and m/e 218.

2. (12 mg). The hydrolysed fraction melted, after recrystallisations, at 72–75°C. The IR spectrum (KBr plate) was characteristic for fatty alcohols.

3. (237 mg). GLC and TLC of the fraction showed the presence of two components indistinguishable from cycloartenol and butyrospermol (Table 1). The acetylated fraction (264 mg) was divided into two fractions by preparative TLC (see general part).

Recrystallisations of the first fraction (68 mg) yielded a product (38 mg) with m.p. 118–122°C and $[\alpha]_{578} + 50.0^\circ$, and identical with cycloartenol acetate (mixed m.p., NMR, mass spectrum).

Recrystallisations of the second fraction (155 mg) yielded a product with m.p. 140–144°C and $[\alpha]_{578} + 13.1^\circ$. Its proton magnetic signals agree with those published⁴ for butyrospermol acetate. Saponification of the acetate yielded a product, which after recrystallisations from acetonitrile, melted at 105–108°C (undepressed by admixture with authentic butyrospermol).

4. (152 mg). GLC of the fraction showed the presence of three components. Two of them were indistinguishable from 24-methylene-cycloartanol and lupeol. The GLC peak areas were for methylene-cycloartanol 20, lupeol 6 and the unidentified substance 3. The GLC retention time for the silyl ether of the unidentified substance was 1.44 (relative to cholesterol silyl ether, see Table 1). Using a gas chromatograph combined with a mass spectrometer, the presence of lupeol and 24-methylene-cycloartanol was further confirmed.

Recrystallisations of the silylated fraction from methanol-acetone yielded a product with m.p. 140–143°C (undepressed by admixture of the trimethylsilyl ether from authentic 24-methylene-cycloartanol) and $[\alpha]_{578} + 45.0^\circ$, $[\alpha]_{313} + 190^\circ$. It exhibited proton magnetic signals which can be attributed to a 9,10-cyclo-structure, to a trimethylsilyl group, and to two vinyl protons. GLC showed that the product still contained the above-mentioned unidentified substance.

The silylated product in the methanol-acetone mother liquor was hydrolysed and acetylated. Argentative TLC followed by recrystallisations yielded a product with m.p. 217.5–218.5°C and $[\alpha]_{578} + 41^\circ$ (c, 0.15; CHCl_3), which was identical with lupeol acetate (mixed m.p.).

C. (2.5 g). The fraction was divided by preparative TLC on silica gel using light petroleum-isopropyl ether (1:1 v/v) as eluant. Besides material belonging to fractions B and D a small amount of a product with m.p. 158°C (not sharp) and $[\alpha]_{578} - 2^\circ$ was obtained. After silylation or acetylation it gave two peaks with about the same area on GLC. The retention times for the slower component was the same as that of 4 α -methyl- $\Delta^{7,24(28)}$ -stigmastadienol-(3 β). The mass spectrum of the material was identical with the characteristic spectrum⁹ of this compound.

D. (4.0 g). GLC of the silylated fraction gave only one large peak, namely that of the silyl ether of β -sitosterol. Recrystallisations gave a β -sitosterol preparation (2.2 g) (m.p. 136–138°C, $[\alpha]_{578} - 28^\circ$) which after further recrystallisations from light petroleum had $[\alpha]_{578} - 42^\circ$.

Acknowledgement. We thank Docent Sture Forsén and his group, Royal Institute of Technology, Stockholm, for determining the NMR spectra, and Docent Ragnar Ryhage and his group, Royal Caroline Medico-Chirurgical Institute, Stockholm for running the mass spectra. Reference samples have kindly been supplied by Professor R. A. Abramovitch, Saskatoon, and Professor T. G. Halsall, Oxford.

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Received April 1, 1966.