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Note

Application of gas chromatography to the analysis of sesquiterpene lactones from *Lactarius* (*Russulaceae*) mushrooms*

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Sesquiterpene lactones have received considerable attention during recent decades. This is mainly due to the biological activity that many of them exhibit¹⁻⁶, such as cytotoxic and antitumor effects, antibiotic effects on bacteria and fungi, allergic effects (contact dermatitis) in man, toxic effects as the toxic principles of some poisonous plants, and allelopathic effects e.g. on seed germination and seedling growth.

Sesquiterpene lactones have been isolated mainly from the order *Asterales* (families *Asteraceae* and *Cichoriaceae*) but also from umbelliferous plants (fam. *Apiaceae*). Isolated occurrences have been reported as well in other dicotyledonous families: *Acanthaceae*, *Aristolochiaceae*, *Cortiariaceae*, *Illiciaceae*, *Lauraceae*, *Magnoliaceae*, *Menispermaceae* and *Winteraceae*^{4,7}. Among cryptogams sesquiterpene lactones are evidently rare, but some are known to occur in the liverwort genera *Diplophyllum*, *Frullania* and *Conocephalum*⁸⁻¹⁰. They have further been isolated from fungi belonging to some Basidiomycete (*Clitocybe*^{11,12}, *Fomes*¹³, *Lactarius* (e.g. refs. 14-17), *Marasmius*^{18,19}) and Ascomycete (*Aspergillus*²⁰) genera.

The preparative separation of individual sesquiterpene lactones from crude extracts has mainly been carried out by column chromatography (adsorbent SiO₂ or Al₂O₃) or preparative thin-layer chromatography (TLC) (e.g. ref. 5). Recently preparative high-performance liquid chromatography (HPLC) has been used as well^{14,21,22}.

The qualitative composition of sesquiterpene lactone fractions has been analysed mainly by TLC (e.g. refs. 5 and 23). In the resolution of complex mixtures TLC does not always give a satisfactory separation, however, and in this respect open tubular column gas-liquid chromatography (GLC) would seem more promising. A couple of reports^{16,24} have appeared describing the GLC analysis of unsubstituted sesquiterpene lactones. Owing to thermal decomposition, however, many heteroatom-substituted sesquiterpene lactones cannot be analysed by GLC without derivatization (cf. p. 468). Accordingly we converted hydroxy-substituted sesquiterpene lactones into

* Part III in the series "Sesquiterpenoids in Finnish *Lactarius* mushrooms".

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their trimethylsilyl ethers and analysed them on glass capillary open tubular columns. GLC analysis of trimethylsilyl ethers of hydroxy-substituted sesquiterpene lactones is mentioned also in two very recent reports^{25,26}. In these, however, packed columns were used, which give a poorer resolution than open tubular columns.

EXPERIMENTAL

Compounds studied

A fraction containing sesquiterpenoid compounds was isolated from frozen Finnish *Lactarius torminosus* mushrooms, mainly according to Daniewski *et al.*²⁷. From this fraction six individual compounds (Fig. 1) were separated^{15,28}. Both the fraction and the isolated compounds were used for the GLC experiments.

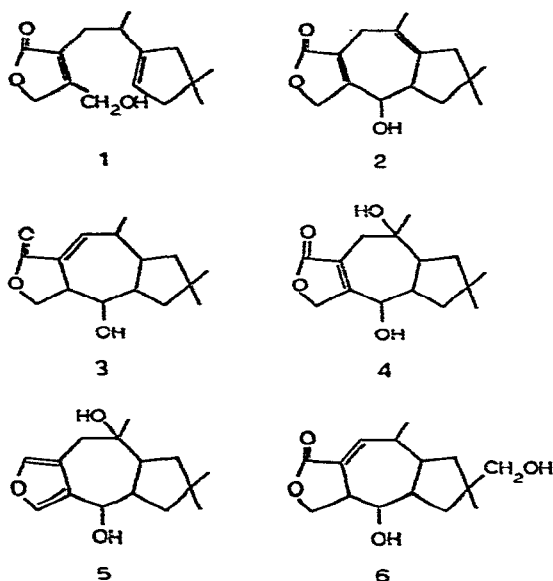


Fig. 1. Sesquiterpenoids of *Lactarius torminosus*: 1 = blennin C; 2 = anhydrolactarorufin A; 3 = blennin A; 4 = lactarorufin A; 5 = furanalcohol-2; 6 = 15-hydroxyblennin A.

Preparation of trimethylsilyl ethers

A 10-mg amount of the compound or sesquiterpenoid mixture was dissolved in 200 μ l of a mixture of 0.5 ml BSA (N,O-bis(trimethylsilyl)acetamide) (Supelco, Bellefonte, Pa., U.S.A.) and 9.5 ml dry benzene and allowed to stand at 60° for 15 min. The BSA and benzene were evaporated in a nitrogen flow and the solid residue dissolved in 1 ml of dry benzene.

Gas-liquid chromatography experiments

The GLC experiments were carried out immediately after the silylations. Several types of glass capillary GLC columns varying in acidity, length and polarity were tested. The most suitable for the detection of silylated derivatives were OV-101

liquid phase coated columns, constructed by a method described in detail by Grob *et al.*^{29,30}. In the GLC experiments a splitless injection technique was used, and the temperature of the glass-coated injection oven was 200°. A 1- μ l volume of the solution was injected and the GLC oven was temperature programmed from 50° to 240° at 8°/min. Hydrogen (2 ml/min) was used as carrier gas and the instrument (Carlo-Erba 2300) was equipped with a flame ionization detector.

RESULTS AND DISCUSSION

When the compounds were analysed on the gas chromatograph before derivatization, only furan alcohol 2 eluted without decomposition, as was expected. Accordingly, trimethylsilyl ethers of the compounds to be studied were prepared. OV-101 proved the most suitable stationary phase for the analysis of the silyl ethers. Fig. 2 shows a gas chromatogram obtained for the sesquiterpenoid fraction of *Lactarius torminosus* mushrooms; for comparison, Fig. 3 shows the best resolutions obtained for the same fraction using TLC, when more than 20 different solvent systems had been tested.

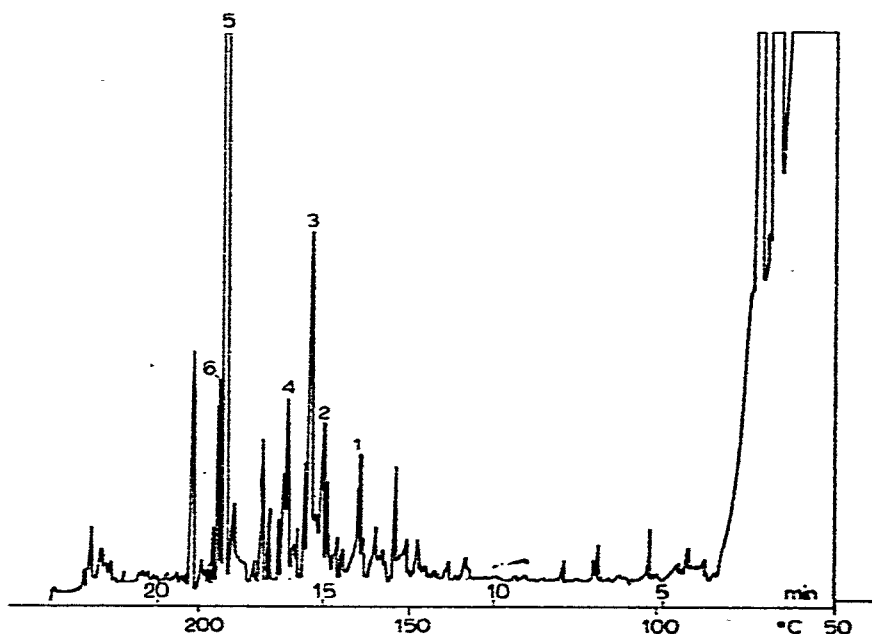


Fig. 2. Gas chromatographic separation of trimethylsilyl ethers of the sesquiterpenoid mixture from *Lactarius torminosus*.

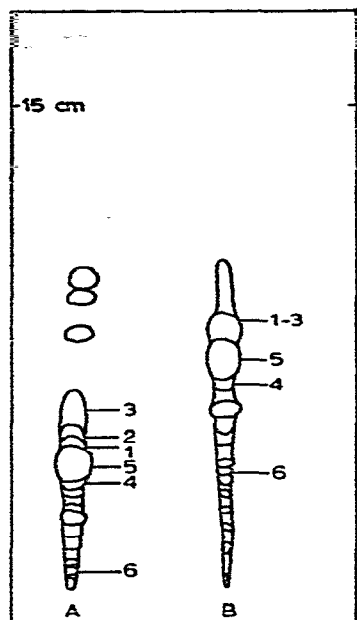


Fig. 3. TLC separation of the same mixture as in Fig. 2. Compound numbering as in Fig. 1. Adsorbent: SiO_2 . Solvent: CH_2Cl_2 - $\text{C}_2\text{H}_5\text{OH}$ (98:2) A, (90:10) B. Spray reagent: vanillin-sulphuric acid.

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