Journal of Chromatogrphy, 261 (1983) 311–314
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,730

Note

Chromatographic analysis of 2,2-dimethylchromene derivatives

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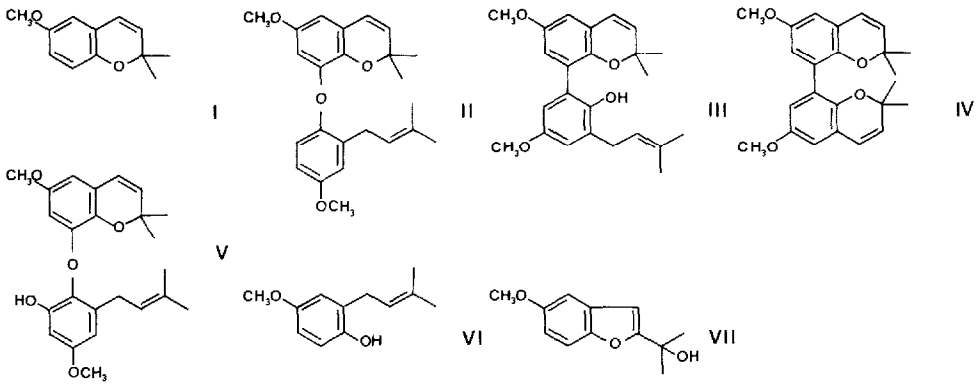
(Received January 25th, 1983)

During our studies on fungal metabolites we isolated some nw 2,2-dimethylchromenes (II–V) together with 2,2-dimethyl-6-methoxychromene (I). They occur in mushrooms with two related aromatic compounds (VI and VII)[1,2]. These metabolites were separated by thin-layer chromatography (TLC), gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).



The conditions previously devised for *Lactarius fuliginosus* were very useful for studying the composition of other *Lactarius* extracts in order to detect chromenes. So far we have found these compounds only in the following Plintogali species: *L. picinus*, *L. pterosporus* and *L. azonites*. Chemotaxonomic studies can be carried out, once the structures of the metabolites have been determined, by comparing the extracts of various species using the most suitable chromatographic methods.

A simple TLC method was used for preliminary screening of the samples. Both alumina and silica gel plates were used employing as the eluent different solvent systems (hexane–diethyl ether, diethyl ether–hexane–methanol, hexane–ethyl acetate, benzene–ethyl acetate, benzene–acetone and cyclohexane–ethyl acetate). The best results were obtained on silica gel plates using hexane–diethyl ether.

A qualitative evaluation of chromene mixture was carried out by GC using columns of different polarities (OV-1, Dexsil 300 GC, OV-17 and FFAP). The best results were obtained with a Dexsil 300 GC column and the conditions used were then employed to correlate the peaks with the compounds by GC–MS.

0021-9673/83/$03.00

EXPERIMENTAL

Standards

Reference compounds I–VII were isolated by repeated chromatography on alumina or silica gel columns from an acetone extract of *Lactarius fuliginosus* Fries[1,2].

Thin-layer chromatography

TLC analyses were performed on Kieselgel 60 F_{254} plates (0.25 mm; Merck, Darmstadt, G.F.R.) using hexane–diethyl ether (75:25) as the eluent. The spray reagent was a solution of vanillin (0.5 g) in ehtanol (20 ml) and sulphuric acid (80 ml).

Gas chromatography

A Perkin-Elmer Sigma 3B gas chromatograph with a flame-ionization detector and a 1.5% Dexsil 300 GC on Chromosorb W AW DMCS (80–100 mesh; Supelco, Bellefonte, PA, U.S.A.) packed glass silanized column, 2 m × 2 mm I.D. were used. The column temperature was increased from 80 to 280°C at 5°C/min, the carrier gas (nitrogen) flow-rate was 30 ml/min, the injector temperature was 250°C and the detector temperature was 300°C.

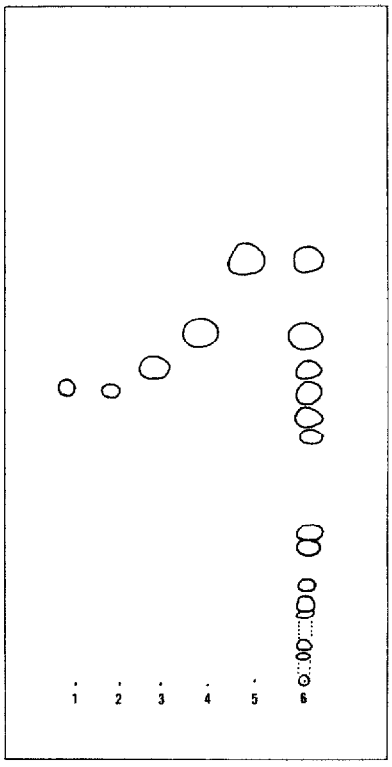


Fig. 1. TLC of *L. fuliginosus* chromenes on a Kieselgel 60 F_{254} plate with hexane–diethyl ether (75:25) as eluent. The spots were revealed with vanillin–sulphuric acid ethanolic solution spray reagent. 1 = V; 2 = III; 3 = IV; 4 = II; 5 = I; 6 = *L. fuliginosus* extract.

Gas chromatography–mass spectrometry

GC–MS was carried out on a DuPont 21-492 B mass spectrometer equipped with a DuPont data system and coupled to a Varian 2700 gas chromatograph. The column was as described under *Gas chromatography*, but of dimensions 4 m × 2 mm I.D. The column temperature was increased from 80 to 320°C at 8°C/min, the carrier gas (helium) flow-rate was 45 ml/min and the temperatures were injector 250°C, jet separator 330°C and MS source 250°C. A flame-ionization detector was used.

RESULTS AND DISCUSSION

A fatty acid-free acetone extract of *L. fuliginosus* was subjected to TLC. The spots relating to chromenes could be easily detected by the characteristic green colour after spraying the plate with vanillin–sulphuric acid ethanolic solution and heating it. An example of a TLC plate is shown in Fig. 1.

GC and GC–MS were performed on a Dexsil 300 GC column, which separated the compounds well as sharp peaks (Fig. 2), whereas on OV-1, OV-17 and FFAP columns the peaks were broad and not completely resolved.

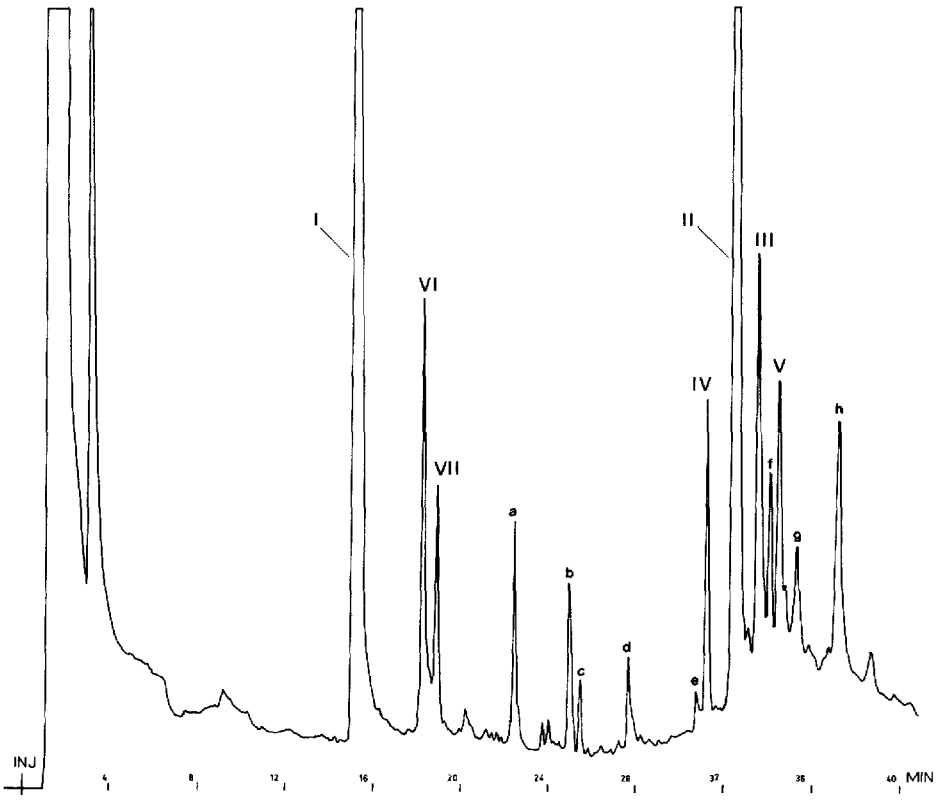


Fig. 2. GC separation of components of *L. fuliginosus* extract using a packed column coated with Dexsil 300 GC. Peaks a (MW 262), d (MW 246), f (MW 382), g (MW 380) and h (MW 398) were not identified. The MS fragmentation pattern indicated chromene structures. Peaks: b = methyl linoleate; c = methyl stearate; e = diisooctyl phthalate.

The identities of the compounds were established by their retention times and their mass spectra, using as reference compounds pure samples previously isolated[1,2]. The elution order is I, VI, VII, IV, II, III and V.

Using the same chromatographic methods and conditions we analyzed extracts of *L. picinus*, *L. azonites* and *L. pterosporus*, and in all three detected the presence of chromenes.

ACKNOWLEDGEMENT

This work was carried out with the financial support of a grant from CNR (Progetto finalizzato per la chimica fine e secondaria).

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