THE CHARACTERISTIC ODOR OF *COPRINUS PICACEUS*: A RAPID ENRICHMENT PROCEDURE FOR APOLAR, VOLATILE INDOLES

HARTMUT LAATSCH AND LUTZ MATTHIES

_Institut für Organische Chemie, Universität Göttingen, Tammannstraße 2, D-3400 Göttingen, Federal Republic of Germany_

Skatole is detected and recognized by the human olfactory sense at very low concentrations. As early as 100 years ago, the odor of indole and skatole led to their discovery in different biological sources (Dunstan, 1889; Hesse, 1899). In line with its natural occurrence as a component of jasmine oil and orange-blossom oil, indole has been used to fix fragrances and is, therefore, found in many perfumes (Walker, 1974). Skatole is a minor component (0.1%) of civet, a glandular secretion from the civet cat (_Viverra civetta_), and is also used in many perfume compositions (Neumüller, 1988). Furthermore, skatole has been identified in several plants, e.g., _Celtis reticulosa_ L. (Greshoff, 1898) and _Tecoma stans_ Miquel (Kunapuli and Vaidyanathan, 1984). In addition both indoles are distributed ubiquitously in feces.

Certain odors of mushrooms can be used as an aid in distinguishing similar species, which has resulted in many attempts being made in the past to identify the substances responsible for mushroom odors (e.g., Gmelin et al., 1976; Watson et al., 1986; Murahashi, 1938).

Hilber (1968) identified indole and/or skatole as major components of odor in various _Tricholoma_ species using thin layer chromatography. Later investigators (Watson et al., 1986) performed analysis via combined gas chromatography/mass spectrometry (GC/MS) and reported that a mixture of aromatic aldehydes and 1-octen-3-ol are responsible for the “coal tar odor” or “smelling of illuminating gas” of at least _Tricholoma inamoenum_ (Fr.) Quel. In this species, indole was found only in trace amounts, and skatole could not be detected. Indeed, it appears that skatole has not yet been conclusively identified as a component of mushrooms.

We felt that the characteristic, disagreeable odor of the stipes of _Coprinus picaceus_ (Bull.) Fr. (Phillips, 1981) was very similar to the well-known skatole odor. Hence, we checked the mushroom for volatile indoles and found skatole and traces of indole to be present not only in the stipes but also in the less odorous caps.

A major problem in handling small quantities of indoles in the laboratory is their high vapor pressure. They are steam volatile and also distill with organic solvent vapors. For this reason enrichment of indoles must be performed very carefully. In experiments to enrich apolar indoles from _Coprinus picaceus_ the conventional solvent/solvent extraction procedure often leads to substantial losses of indole and skatole.

Fruit bodies of _Coprinus picaceus_ were collected on several occasions from Pleesse citadel near Göttingen (German topographical map 25: 4425/2 Göttingen) during Sept. and Oct., 1990. Mushrooms that had not reached the umbellate stage were collected as these exhibited the skatole odor. Reference material is deposited in the herbarium at the University of Leipzig (LZ 4154). On the same day the specimens were collected, the stipes and caps were separated, washed in water and placed in 250 ml Erlenmeyer flasks. The flasks were corked, sealed with parafilm and stored at −20 °C in a freezer.

All chemicals were HPLC or analytical grade and were obtained from E. Merck (Darmstadt).

One hundred fifty g of clean, frozen stipes (or caps) of _Coprinus picaceus_ were minced with an ultra turrax (Janke and Kunkel, Staufen im Breisgau, Germany) and the homogenate was subsequently placed into centrifuge tubes. During the centrifugation (15 min, 3200 rpm, room temperature) the homogenate was allowed to thaw slowly. Using a syringe, 25 ml of the supernatant was subsequently passed through a C-18 silica gel cartridge (“Sep-Pak”; Waters, Milford, Massachusetts), which had been pre-equilibrated with dichloromethane. Adsorbed apolar substances were subsequently eluted with 3 ml dichloromethane/1% methanol. The water was initially reduced by freezing the eluate at −20 °C and decanting. Further drying was accomplished by ad-
Fig. 1. Temperature programmed capillary GC separation (CP Sil 5 CB) of an indole/skatole mixture from Coprinus picaceus after Sep-Pak enrichment.

dition of ca 20 pears of molecular sieve 0.4 nm/2 mm. For further purification, the solution was filtered over a 0.5 × 6 cm column of aluminum oxide, and skatole/indole fractions were collected.

Indole/skatole fractions were obtained as described above and subjected to capillary GC (Fig. 1) using a Carlo Erba Mega 5300 gas chromatograph (column CP Sil 5 CB, 25 m × 0.32 mm,
injector temperature 280 °C, detector temperature 300 °C). The temperature program consisted in running the GC at 100 °C for 6 min followed by an increase in temperature (5 °C/min) to 260 °C. Skatole retention time (t<sub>r</sub>) was 7.13 min (96% of total amount); indole t<sub>r</sub> was 9.61 min (4% of total amount) (Fig. 1). The authentic compounds revealed identical behavior.

Combined gas chromatography/mass spectrometry (GC/MS) was carried out using a Varian 3700 gas chromatograph (column: CP Sil 5, 20 m × 0.32 mm; Chrompack) fitted with a Varian MAT 311 A mass spectrometer. The GC was run with increasing temperature (5 °C/min) up to 280 °C. The main fragments are as follows: skatole, t<sub>r</sub> = 6.0 min: m/z = 132 (8%), 131 (60; M+), 130 (100), 103 (10), 102 (8), 77 (18), 65 (16), 51 (17). These data were identical to those given in the literature (Beynon and Williams, 1959).

After the enrichment procedure, skatole and traces of indole were identified by TLC using silica gel plates (DC Micro Cards SI F 5 × 10 cm, layer thickness 0.2 mm; Riedel-de Haen, Seelze, Germany). After development with di-
chloromethane, the plates were subsequently sprayed with van Urk reagent (Stahl et al., 1961): Indole ($R_F = 0.6$) gave a weak pink; skatole ($R_F = 0.65$), a blue color (Fig. 2). All features were identical to the commercial reference compounds.

This study demonstrates that skatole is the major component of the odor in *Coprinus picaceus* stipes. In conclusion, as skatole smells much stronger than indole, our GC/MS and TLC analysis revealed that the disagreeable odor of *Coprinus picaceus* is due to skatole as the main component. A very dilute aqueous solution of skatole has a similar odor to fresh mushrooms. This is the first description of skatole being a constituent of a mushroom verified by GC/MS analysis.

ACKNOWLEDGMENTS

We are indebted to Dr. G. Remberg for GC/MS-measurements and for helpful discussions. We thank F. Hettich, Deutsche Gesellschaft für Mykologie, for supplying a part of the mushroom material. This study was supported by the Niedersächsische Kultusministerium, grant No. 213/7112-5 to L.M., and the Fonds der Chemischen Industrie.

Key Words: analysis, *Coprinus picaceus*, indole, mushroom, odor, skatole

LITERATURE CITED


