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Short Communication

The Isolation of a New Isochromanone from Injured Fruit Bodies of *Tricholoma scalpturatum*

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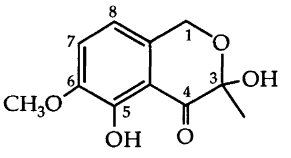
In the course of an on-going investigation of the secondary metabolites of fungi, the fruit bodies of *Tricholoma scalpturatum*, which are considered edible,[1] were examined. In view of the findings that similarly edible mushrooms produce new metabolites as a response to physical injury,[2,3] the contents of intact as well as injured specimens were examined. A TLC comparison of ethyl acetate extracts of mushrooms that had been ground in a meat grinder together with the solvent (in order to extract the metabolites present originally in the intact mushrooms), with extracts made of specimens that had been injured by grinding them in the meat grinder 15 min prior to extraction, showed that several compounds are formed as a response to injury of the fruit bodies of this species as well. Some of the compounds formed are 2,2′-biindoline-3,3′-diones, similar to the metabolites formed in injured fruit bodies of *Collybia peronata*, and the isolation and structure elucidation of those will be reported elsewhere. An additional compound that could be detected only in the extracts of injured fruit bodies was isolated by chromatography on silica gel and its structure (**1**) was determined by spectroscopic methods.



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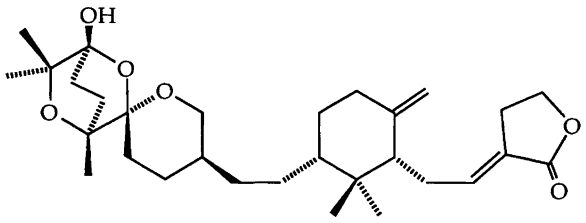

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Fig. 1. Significant NOESY (left) and long-range $^1H-^{13}C$ (right) correlations observed for compound **1**.

Results and discussion

The compound, which is new, was shown to be 3,5-dihydroxy-6-methoxy-3-methylisochroman-4-one (**1**) (see Fig. 1). The elucidation of its structure is based on NMR spectroscopy and the suggestion from high resolution mass spectroscopy data that its elemental composition is $C_{11}H_{12}O_5$. The ^1H and ^{13}C NMR data are given in the Experimental section, while the result of NOESY and long-range $^1H-^{13}C$ correlation experiments are summarized in Fig. 2. The aromatic carbons were partly assigned by the long-range correlations between the hydroxy proton, which from its chemical shift (11.63 ppm) is evidently hydrogen bonded, to C-4a, C-5 and C-6, as well as between the methoxy protons and C-6. The methoxy protons show a NOESY correlation with one of the two aromatic protons, while the other correlates to the protons of the benzylic CH_2 group. Long-range $^1H-^{13}C$ correlations between the two aromatic protons, which couple to each other with $J = 8.2$ Hz, and the carbons indicated in Fig. 2 identify the remaining aromatic carbons, and determine the position of the benzylic methylene group. Further long-range correlations between 1-H_2 and C-3, as well as between 3-CH_3 and C-3 and C-4 unambiguously determine the structure as an isochromanone, and a keto function at C-4 is in accordance with the observed hydrogen bonding of the C-5 hydroxy proton. While chroman-4-ones are very common in nature, only few isochroman-4-ones have been reported,[4] and this is, to our knowledge, the first 3-methylisochroman-4-one isolated. Compound **1** is optically inactive, and believed to be a racemate.

Another compound that neither forms nor disappears in response to injury to the fruit bodies was isolated and found to be identical with saponaceolide B (**2**), previously isolated from *Tricholoma saponaceum*,[5] together with hydroxylated derivatives,[5,6] and from *T. terreum*.[7] The structure of saponaceolide B (**2**) in Fig. 1 shows the absolute configuration.[8] The NMR data were found to be identical with those reported in Ref. 7, although in our opinion the reported assignments of 3′-H (2.16 ppm) and 4′-H (1.86 ppm) should be reversed. The saponaceolides possess an unusual skeleton and have so far been isolated only from *Tricholoma* species. They do not show any antimicrobial activity, but are highly cytotoxic. Most active is saponaceolide B (**2**), which has an ID_{50} of 0.16 μg per ml on human colon adenocarcinoma cells,[5] and it is questionable whether mushrooms containing such metabolites should be classified as edible even if the amounts of saponaceolide B (**2**) isolated in this case are limited. The biological activity of scalpturan (**1**) has so far not been investigated.

Experimental

Fruit bodies of *Tricholoma scalpturatum* (Fr.) Quél. were collected in Lund in the Autumn of 1992, and extracted the same day. Some specimens were ground in a meat grinder together with ethyl acetate (2 l per kg fruit bodies), in order to investigate the original contents, while others were ground without solvent and left as a mush for 15 min before they were extracted with ethyl acetate. The extracts were compared by TLC analyses, and the compounds were isolated by chromatography on SiO_2 columns eluted with different mixtures of ethyl acetate and heptane. Five hundred grams injured fruit bodies of *T. scalpturatum* yielded 2 mg of isochromanone (**1**) and 4 mg of saponaceolide B (**2**). The NMR data, from 1D ^1H and ^{13}C spectra and extensive 2D correlation spectroscopy, were obtained with a Bruker ARX500 spectrometer, and the assignment of the carbons was made from HMQC and HMBC spectra. Coupling constants are given in Hz. The UV spectra were recorded with a Cary 219, the IR spectra with a Perkin Elmer 257, and the mass spectra with a Jeol SX102 spectrometer.

3,5-Dihydroxy-6-methoxy-3-methylisochroman-4-one (**1**) was obtained as a yellow oil. UV (ethanol) λ_{max} (ε): 216 (18300), 259 (11700), 263 (11799), 268 (10500) and 354 nm (5100). IR (KBr): 3400, 1660, 1450, 1270, 1070, 1010, 930, 810 and 800 cm^{-1}. ^1H NMR data ($CDCl_3$, $CHCl_3$ at 7.255 ppm): δ 11.63 (s, 5-OH), 7.07 (d, $J_{7,8} = 8.2$, 7-H), 6.59 (d, $J_{7,8} = 8.2$, 8-H), 5.23 (d, $J_{1a,1b} = 15.1$, 1-Ha), 4.66 (d, $J_{1a,1b} = 15.1$, 1-Hb), 3.89 (s, 6-OCH_3), 1.68 (s, 3-CH_3). ^{13}C NMR ($CDCl_3$ at 77.2 ppm): δ 196.8 (C-4), 153.3 (C-5), 147.3 (C-6), 133.0 (C-8a), 118.9 (C-7), 113.9 (C-8), 112.6 (C-4a), 95.9 (C-3), 61.0 (C-1), 56.6 (6-OCH_3), 23.9 (3-CH_3). MS (EI, 70 eV), *m/z*: 224.0692 (M^+, 12%, $C_{11}H_{12}O_5$ requires 224.0685), 164 (100%), 149 (38), 136 (82), 93 (51).

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