(8*E*)-10-Hydroxydec-8-enoic Acid: its Isolation from Injured Fruit Bodies of *Cantharellus tubaeformis* and Synthetic Preparation

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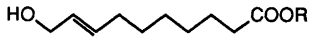
The chemical contents of both intact and injured fruit bodies of the edible mushroom *Cantharellus tubaeformis* have been investigated and compared with those of *C. cibarius* (chanterelle). Ethyl acetate extracts of intact fruit bodies of the two species were found to contain the same major components, but extracts of injured fruit bodies differ with respect to two metabolites. No traces of cibaric acid, formed enzymatically in injured fruit bodies of *C. cibarius*, could be detected in the fruit bodies of *C. tubaeformis*, which instead forms (8*E*)-10-hydroxy-8-decenoic acid in response to injury. The structure of (8*E*)-10-hydroxy-8-decenoic acid was elucidated by spectroscopic methods, and confirmed by synthesis.

Enzymatic conversions of secondary metabolites in mushrooms in response to physical injury have been observed in several species,[1-3] and appear in some cases to be part of chemical defence systems that protect the fruit bodies from parasites. For example, the rapid formation of pungent unsaturated dialdehydes possessing antibiotic[4] and antifeedant[5] activities in injured fruit bodies of species belonging to Russulaceae,[1] would help to protect an injury from infection by microorganisms and to discourage parasites such as insects and mammals from consuming the mushroom. The possibility of finding ecologically significant new compounds with interesting and potentially useful biological activity, is the motivation for a chemical comparison of intact and injured fruit bodies and an investigation of the biological activities of any metabolites formed in response to injury. In addition, consumers of wild mushrooms may be warned against chemical health hazards not previously considered. The fruit bodies of the two species *Cantharellus cibarius* (chanterelle) and *C. tubaeformis* are among the most popular edible mushrooms, and because of their apparent resistance to the insects and snails which normally attack and devour mushrooms, they have been suspected to contain some protective agent. We have previously shown that fruit bodies of *C. cibarius* produce large amounts of a new polyunsaturated fatty acid (cibaric acid, **2**) in response to injury,[6] and in this paper we report the results from a similar investigation with fruit bodies of *C. tubaeformis*.

Results and discussion

Fresh fruit bodies of *Cantharellus tubaeformis* were extracted with ethyl acetate on the day of the collection. In order to extract the original contents of the fruit bodies, they were ground together with the solvent, to produce extract A. To obtain compounds formed as a response to injury, the mushrooms were first ground and then left as a mush at room temperature for 15–30 min before extraction (extract B). No differences between extract A and extracts of *C. cibarius* obtained in the same way[4] were indicated by a TLC comparison, and the original contents of the fruit bodies of the two species (fats, fatty acids, steroids, etc.) appear to be identical. This was also confirmed by isolation and spectral characterization of the major metabolites. However, extracts of specimens of the two species that had been injured prior to extraction showed some differences. There were no traces of cibaric acid **2**, which is formed in *C. cibarius* (approximately 100 mg kg^{-1} fresh mushroom), in extract B of *C. tubaeformis*. Instead, a compound which not could be detected in injured specimens of *C. cibarius*, was shown to be present in the extract B of *C. tubaeformis*. The compound was isolated (approximately 50 mg kg^{-1} fresh mushroom) and its spectral data suggested it to be (8*E*)-10-hydroxydec-8-enoic acid **1a**.



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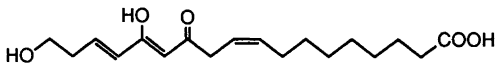

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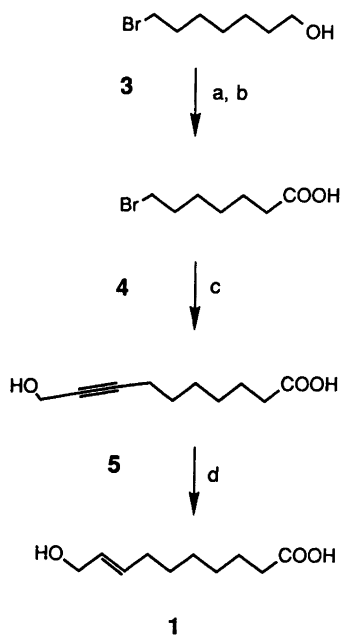
Fig. 1. **a**, R=H; **b**, $R=CH_3$.

In order to confirm this structure, and to obtain larger amounts for biological testing, we decided to develop a synthesis for compound **1a**. This is based on the same route developed previously for the *Z* isomer,[7] and is depicted in Scheme 1. Commercially available 7-bromoheptanol **3** was oxidized in two steps[8] to 7-bromoheptanoic acid **4**, which thereafter was coupled with the dianion of propargyl alcohol to 10-Hydroxydec-8-ynoic acid **5**.[7] (8*E*)-10-hydroxydec-8-enoic acid **1a** was obtained by the reduction of compound **5** with sodium in liquid ammonia. The product thus obtained was identical (as compared by TLC, NMR and mass spectroscopy) with the natural product isolated from *C. tubaeformis*.



Scheme 1. a, b, PCC and Ag_2O oxidation; c, coupling with the dianion of propargyl alcohol; d, reduction with sodium in liquid ammonia.

(8*E*)-10-hydroxydec-8-enoic acid **1a** has previously been mentioned in a study of Claisen rearrangements of macrocyclic lactones,[9] although nothing is stated about its preparation or spectral data. 10-Hydroxydec-8-enoic acid (double bond stereochemistry not assigned, structure determination based on GC–MS data of the methyl ester and the trimethylsilyl ether–methyl ester[10]) has been reported to be formed, presumably enzymatically, in fruit bodies of *Agaricus campestris* to which linoleic acid had been added (2.3 mg g^{-1} linoleic acid). Linoleic acid is one of the major fatty acids of *C. tubaeformis*, and is a likely precursor of the (8*E*)-10-hydroxydec-8-enoic acid **1a** isolated in this investigation. It is possible that compound **1a** is formed by chemical transformations (i.e. fatty acid peroxidation) initiated when the fruit bodies are injured, but the fact that this compound does not appear to be formed in injured specimens of *C. cibarius*, although the two species initially contain the same fatty acids, suggests that some specific system (i.e. enzymes) is involved in its formation. Studies of the biological activities of both (8*E*)-10-hydroxydec-8-enoic acid **1a** and cibaric acid **2** are presently in progress.

Experimental

Fruit bodies of *Cantharellus tubaeformis* Fr. [syn. *C. infundibuliformis* (Scop.) Fr.] were collected in the vicinity of Kaiserslautern (FRG) and Nice (France), and ethyl acetate extracts were prepared both of the intact mushrooms and of specimens that had been injured for 15–30 min. Injury to the fruit bodies was simulated by grinding them at room temperature in an ordinary meat grinder, and the mush was extracted after 15–30 min. No differences were noted by TLC analysis of extracts prepared from mushrooms collected at different sites. TLC analysis of the extracts were made on Merck Kieselgel 60 F_{254} SiO_2 plates developed with ethyl acetate–heptane mixtures and visualized by spraying with anisaldehyde–sulfuric acid and warming to 120 °C. The high resolution mass spectrum was recorded with a Jeol SX102 mass spectrometer. NMR spectra were recorded with a Varian XL300 spectrometer, the chemical shifts are reported in ppm with tetramethylsilane as an internal standard and the coupling constants (*J*) are given in Hz. The IR spectrum was recorded with a Perkin-Elmer 257 spectrometer and the UV spectrum with a Cary 219 spectrometer.

(8E)-10-Hydroxydec-8-enoic acid **1a** (50 mg kg^{-1} ground *Cantharellus tubaeformis*) was obtained as a colourless oil by chromatography of ethyl acetate extracts on SiO_2 eluted with different mixtures of ethyl acetate and heptane. R_F 0.18 (ethyl acetate–heptane 1:1). Anal. $C_{10}H_{18}O_3$. MS[EI 70 eV *m/z* (% rel. int.)]: 168 (M^+, $-H_2O$) (32), 108 (48), 98 (59), 81 (60), 73 (72), 55 (100). Mol. wt. ($-H_2O$): obs. 168.1142. Calc. for $C_{10}H_{16}O_2$ 168.1150. ^1H NMR (300 MHz, $CDCl_3$): δ 5.68 [dd, C(8)H, $J_{7,8}$ = 5.5, $J_{8,9}$ = 15.3], 5.62 [dd, C(9)H, $J_{8,9}$ = 15.3, $J_{9,10}$ = 4.8], 4.09 [d, C(10)H_2, $J_{9,10}$ = 4.8] 2.35 [t, C(2)H_2, $J_{2,3}$ = 7.5], 2.04 [m, C(7)H_2], 1.63 [m, C(3)H_2], 1.35 [m, C(4)H_2, C(5)H_2, and C(6)H_2]. ^{13}C NMR (76 MHz, $CDCl_3$): δ 179.2 C(1), 133.3 and 128.6 C(8) and C(9), 63.5 C(10), 34.0, 32.0, 28.8, 28.8, 28.6 and 24.6 C(2), C(3), C(4), C(5), C(6) and C(7). IR (KBr): 3290, 2900, 2600, 1700, 1460, 970 cm^{-1}. UV [ethanol]: no maximum above 210 nm.

(8E)-10-Hydroxydec-8-enoic acid methyl ester **1b** was obtained in quantitative yield by the treatment of compound **1a** with diazomethane in diethyl ether. MS [CI NH_3 *m/z* (% rel. int.)]: 183 (M^+, $-H_2O$)+H (17), 150 (41), 108 (38), 98 (25), 87 (100), 74 (77), 40 (90). ^1H NMR (300 MHz, $CDCl_3$): δ 5.68 [dd, C(8)H, $J_{7,8}$ = 5.5, $J_{8,9}$ = 15.3], 5.62 [dd, C(9)H, $J_{8,9}$ = 15.3, $J_{9,10}$ = 4.8], 4.08 [d, C(10)H_2, $J_{9,10}$ = 4.9], 3.67 [s, (MeO)CH_3], 2.31 [t, C(2)H_2, $J_{2,3}$ = 7.5], 2.02 [m, C(7)H_2], 1.63 [m, C(3)H_2], 1.35 [m, C(4)H_2, C(5)H_2, and C(6)H_2].

*Synthetic preparation of (8*E*)-10-hydroxydec-8-enoic acid* **1a**. 1.84 g (10 mmol) of 10-hydroxydec-8-ynoic acid **5**, prepared as described previously,[7,8] were dissolved in 4 ml THF and added to 50 ml liquid ammonia to which approximately 0.5 g of finely divided sodium metal had been added. The reaction mixture was kept at −33 °C for 2 h whereafter the remaining sodium was destroyed by adding ammonium nitrate. The ammonia was evaporated, 50 ml 2 M hydrochloric acid were added and the mixture was extracted three times with 50 ml ethyl acetate. After drying with sodium sulphate, evaporation of the solvent and column chromatography on silica gel, 1.73 g (93 %) of (8*E*)-10-hydroxydec-8-enoic acid **1a** were obtained. No trace of the *Z* isomer was indicated in the ^{1}H NMR spectrum.

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