Occurrence of a High Concentration of Spider Pheromones in the Ascomycete Fungus *Hypoxylon truncatum*

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A large amount of sex pheromones of the European spider *Linyphia triangularis*, 3*R*-hydroxybutyric acid (**1**), its dimer 3*R*-(3*R*-hydroxybutyryloxy)butyric acid (**2**), and trimer 3*R*-[3*R*-(3*R*-hydroxybutyryloxy)-butyryloxy]butyric acid (**3**) were isolated from the EtOAc extract of the Japanese inedible mushroom *Hypoxylon truncatum*.

Anderson[1] isolated 3,4-dihydro-8-hydroxy-3-methylisocoumarin from the inedible mushroom *Hypoxylon truncatum*. Later Uebler[2] and Kern[3] also reported the same compound as a trail pheromone component of the ants *Camponotus silvicola*, *C. rufipes*, and *Lasius fuliginosus*, respectively. These results opened the avenue for research on compounds occurring in both insects and mushrooms. Recently, we investigated the chemical constituents of the Japanese *H. truncatum* and isolated a novel perylenequinone, truncatone.[4] Further fractionation of the ethyl acetate extract has now resulted in the isolation of monomeric 3*R*-hydroxybutyric acid (**1**), its dimer 3*R*-(3*R*-hydroxybutyryloxy)butyric acid (**2**), and trimer 3*R*-[3*R*-(3*R*-hydroxybutyryloxy)butyryloxy]butyric acid (**3**), which are known to be sex pheromones of the spider *Linyphia triangularis* (Clerck) and related species.[5]

The EtOAc extract of *H. truncatum* was subjected to SiO_2 and Sephadex LH-20 column chromatography to give 3-hydroxybutyric acid (**1**) and a mixture of compounds **2** and **3**. On the basis of the IR spectral evidence to support the presence of a carboxylic acid group (2400−3600 cm^{-1}), samples of compound **1** and the mixture of **2** and **3** were methylated, then purified using preparative HPLC to give compounds **4**, **5**, and **6** as methylated derivatives of **1**, **2**, and **3**, respectively. On the basis of analyses of IR, ^1H and ^{13}C NMR, and MS spectra and comparisons of the spectral data with those of authentic samples and reported spectral data,[6,7] their stuctures were determined to be 3-hydroxybutyric acid methyl ester (**4**), the dimer 3-(3-hydroxybutyryloxy)butyric acid methyl ester (**5**), and the trimer 3-[3-(3-hydroxybutyryloxy)butyryloxy]butyric acid methyl ester (**6**).

To determine the absolute configurations of compounds **1**, **2**, and **3**, they were each converted to 3-acetoxybutyric acid methyl ester (**7**), which was analyzed by GC−MS on a chiral column. Compound **1** was methylated with trimethylsilyldiazomethane and acetylated to afford compound **7**. A mixture of dimer **5** and trimer **6** was saponified with potassium hydroxide in methanol, followed by methylation and acetylation to yield **7**. Compound **7** was analyzed by gas chromatography−mass spectroscopy (GC−MS) on a chiral column with authentic samples (each 3*R*- and 3*S*-acetoxybutyric acid methyl ester, which was derived from 3*R*- and 3*S*-hydroxybutyric acid, respectively) to give the chromatograms shown in Figure 2. Consequently, the configurations of all asymmetric carbons of **1**, **2**, and **3** were established to be *R*. Thus, three compounds isolated from the mushroom *H. truncatum* are monomeric 3*R*-hydroxybutyric acid, dimeric 3*R*-(3*R*-hydroxybutyryloxy)butyric acid, and trimeric 3*R*-[3*R*-(3*R*-hydroxybutyryloxy)butyryloxy]butyric acid and are identical to the sex pheromones of the spider *Linyphia triangularis* and related species.[5] Schulz and Toft[5] confirmed the presence of a very low concentration (μg order per web) of these three sex pheromones of the spider only by GC−MS and determined the absolute structures of the monomer (**1**) and dimer (**2**), but not the trimer (**3**) due to its very low concentration.

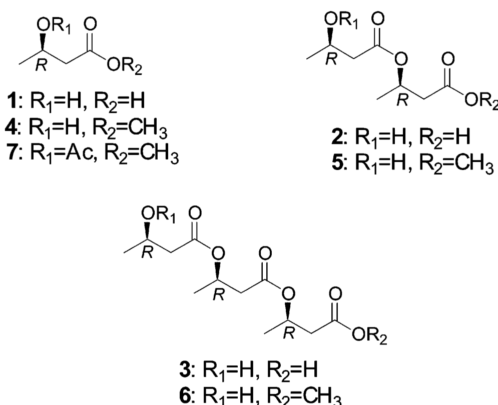

Figure 1. Structures of compounds **1**−**7**.

This is the first report of the isolation of a high concentration of spider pheromones (**1**−**3**) from the fungus and very interesting from the point of view of pheromone biogenesis in each organism.

Experimental Section

General Experimental Procedures. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C), using CD_3OD or CD_3Cl as solvent. Chemical shifts are given relative to TMS δ 0.00 as internal standard (^1H) and δ 49.0 ppm from CD_3OD or 77.03 ppm as standards (^{13}C). Mass spectra including high-resolution mass spectra were recorded on a JEOL JMS AX-500 spectrometer. GC−MS was carried out on a Hewlett-Packard mass selective detector 5971 A and a gas chromatograph 5890 Series II with chiral column β-DEX 120 (30 m × 0.25 mm, film thickness 0.25 μm). The temperature programming of GC−MS analysis was performed from a 50 °C isothermal for 3 min, then 50−230 °C at 3 °C min^{-1}, and finally an isothermal at 230 °C for 20 min. Injection temperature was 250 °C.

Fungal Material. *Hypoxylon truncatum* was collected in Tokushima, Japan, in 1992 and identified by Dr. T. Hashimoto

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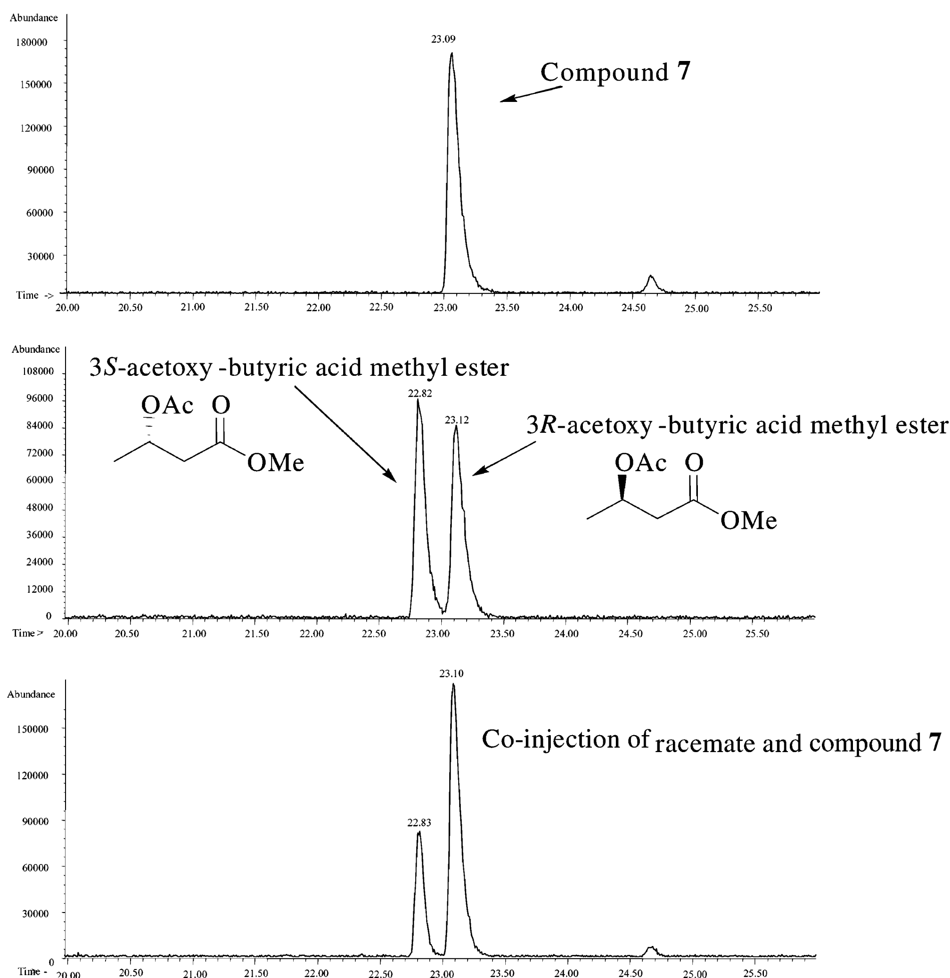

Figure 2. Determination of absolute configuration of compounds **1**, **2**, and **3** by GC–MS on a chiral column with authentic samples.

(Tokushima Bunri University, Japan). The voucher specimen (1992-1) was deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan.

Extraction and Isolation. Dried *H. truncatum* (173.2 g) was extracted with EtOAc and then evaporated to give a residue (16.6 g), which was chromatographed on SiO_2 using a $CHCl_3$/EtOAc gradient affording three fractions [fractions 1 (667 mg), 2 (1490 mg), and 3 (880.2 mg)]. Fraction 3 contained pure 3*R*-hydroxybutyric acid (**1**). A part of fraction 3 (**1**) (77.2 mg) was methylated followed by acetylation to give 3*R*-acetoxybutyric acid methyl ester (**7**) (29.4 mg). Fraction 2 was divided by Sephadex LH-20 column chromatography ($CHCl_3$/MeOH = 1:1) into three fractions: fraction 2-1 (18.8 mg), fraction 2-2 (947 mg), which contained **2** and **3** as methyl esters in the ratio 2:1 by GC, and fraction 2-3 (48.1 mg). A part of fraction 2-2 (68.8 mg) was methylated with $(CH_3)_3SiCHN_2$ (1.5 mL) in MeOH (2 mL), then purified by preparative HPLC on a Waters 5 SL-II column using hexane/EtOAc (1:3) as solvent system and Sephadex LH-20 column chromatography to give compound **5** (15.3 mg), a mixture of compounds **5** (76%) and **6** (23% in GC) (24.4 mg), and compound **6** (9.6 mg). The mixture of compounds **5** and **6** (24.4 mg) was saponified by KOH (20 mg) in MeOH (1.5 mL), neutralized by 1 N HCl, and then subjected repeatedly to column packing proton exchange resin (Amberlite IR-120B/H^+). The eluent was evaporated and methylated in the same manner as mentioned above and then acetylated with anhydride acetic (1 mL) in pyridine (1 mL) to give 3*R*-acetoxybutyric acid methyl ester (**7**) (17.2 mg).

References and Notes

(1) Anderson, J. R.; Edwards, R. L. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2185–2192.
(2) Uebler, E.; Kern, F.; Bestmann, H. J.; Boelldobler, B.; Attygalle, A. B. *Naturwissenschaften* **1995**, *82*, 523–525.
(3) Kern, F.; Klein, R. W.; Jassen, E.; Bestmann. H. J.; Attygalle, A. B.; Schaefer, D.; Maschwits, U. *J. Chem. Ecol.* **1997**, *23*, 779–792.
(4) Hashimoto, T.; Asakawa, Y. *Heterocycles* **1998**, *47*, 1067–1110.
(5) Schulz, S.; Toft, S. *Science* **1993**, *260*, 1635–1637.
(6) Li, J.; Uzawa, J.; Doi, Y. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1887–1893.
(7) Li, J.; Uzawa, J.; Doi, Y. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1683–1689.

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