Isolation, Identification and Pharmacological Studies on Three Toxic Metabolites from a Mushroom, *Hebeloma spoliatum*

Haruhiro FUJIMOTO, Yasuhiro TAKANO and Mikio YAMAZAKI*

Faculty of Pharmaceutical Sciences, Chiba University, 1–33, Yayoi-cho, Chiba 260, Japan. *Received September 17, 1991*

Three metabolites, tentatively named HS-A, -B and -C, were isolated from a mushroom, *Hebeloma spoliatum*, as the fatal toxic principles to mice. HS-A was identified as 3-acetyl-2-(3′-hydroxy-3′-methyl)glutarylcrustulinol, which has been isolated from *Hebeloma crustuliniforme* and *H. sinapizans* as a cytotoxic principle. HS-B and -C were deduced to be 3,21-diacetyl-2-(3′-hydroxy-3′-methyl)glutarylcrustulinol and 3-acetyl-2-(3′-hydroxy-3′-methyl)glutarylanhydrocrustulinol, respectively, from their chemical and spectral data.

Intraperitoneal administration of HS-A, -B and -C at a dose of 100 mg/kg caused death after paralysis of the limbs in mice. The compounds caused relaxation of mouse small intestine contracted by acetylcholine chloride or barium chloride treatment *in vitro*. They appear to exhibit a paraverine-like relaxation effect.

Keywords Basidiomycetes; *Hebeloma spoliatum*; mushroom toxin; 3-acetyl-2-(3′-hydroxy-3′-methyl)glutarylcrustulinol; lanostane-type triterpene ester; smooth muscle; relaxation effect; Magnus method

In our screening program on the toxic principles of mushrooms, nine new neurotoxic glycosides have been isolated from *Hebeloma vinosophyllum*.[1] Our next target was *Hebeloma spoliatum* (Japanese name: ashinaganumeri). The methanolic extract of *H. spoliatum* caused depression in mice, and three toxic principles were isolated from the extract. This report deals with the isolation, structure elucidation and pharmacological study of the toxic principles of *H. spoliatum*.

Intraperitoneal administration of the methanolic extract of dried fruit-bodies of *H. spoliatum* (collected at Mt. Kiyosumi, Chiba, Japan) at a dose of 500 mg/kg caused depression in mice. The extract was divided into supernatant and precipitate by treatment with a mixture of 60% methanol and acetone (3 : 5, v/v). The supernatant was further divided into five fractions, I—V, by chromatography on a silica gel column. Fractions III and IV both caused death after paralysis of the limbs in mice at a dose of 500 mg/kg. Fraction IV was further purified by medium-pressure liquid chromatography (MPLC) with a silica gel column and by high-performance liquid chromatography (HPLC) with a reversed-phase octadecylsilica gel (ODS) column successively to afford a toxic principle tentatively named HS-A (**1**) (yield from the dried fruit-bodies: 1.10%). Fraction III was also purified by HPLC and MPLC successively to afford two other toxic principles tentatively named HS-B (**2**) and -C (**3**) (yield from the dried fruit-bodies: 0.71 and 0.02%, respectively). Intraperitoneal administration of **1**, **2** or **3** caused death after paralysis of the limbs in mice at a dose of 100 mg/kg.

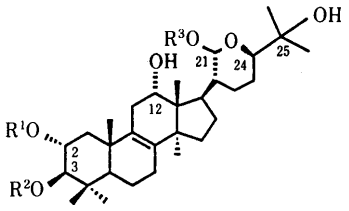
HS-A (**1**), a colorless powder, $C_{38}H_{60}O_{11}$, was positive to the Griess test and soluble in 0.5 N $NaHCO_3$, and also positive to the Liebermann-Burchard reaction. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** in deuterochloroform ($CDCl_3$) gave signals of eight tertiary methyls, one acetyl methyl, four hydrogens in a system of –CC$\underline{H}_2$CO–, two hydrogens of –C$\underline{H}$(O–)–, two vicinal hydrogens of –OC$\underline{H}$C$\underline{H}$(CH_2–)O– and one hydrogen of –CC$\underline{H}$(O–)O–. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** in pyridine-d_5 (C_5D_5N) afforded signals of nine methyls, ten methylenes, three methines in a system of –C$\underline{C}$H(C–)C–, four methines of –O$\underline{C}$H(C–)C–, one methine of –O$\underline{C}$H(C–)O–, four quaternary carbons of –C$\underline{C}$(C–)(C–)C–, two quaternary carbons of –O$\underline{C}$(C–)(C–)C–, two quaternary olefinic carbons and three ester carbonyls (see Table I).

The physico-chemical and spectral data of **1** were very similar to the corresponding data of a lanostane-type triterpene ester, 3-acetyl-2-(3′-hydroxy-3′-methyl)glutaryl-crustulinol, which was isolated from *Hebeloma crustulini-*

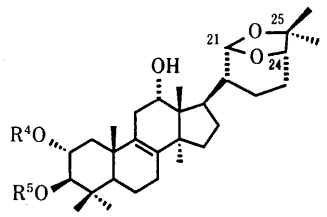
TABLE I. ^{13}C-NMR Data for HS-A (**1**), -B (**2**), -C (**3**), Crustulinol (**5**) and Anhydrocrustulinol (**6**), δ (ppm) from TMS in C_5D_5N

Position	1	1[2)]	2	3	5	5[2,a)]	6
1	40.9	40.9	41.2	41.0	44.5	44.8	44.5
2	70.3	70.2	70.3	70.2	69.0	69.8	69.0
3	80.2	80.2	80.1	80.2	83.6	84.2	83.6
4	39.5	39.5	39.5	39.6	39.9	40.3	39.7
5	50.2	50.2	50.1	50.2	51.1	51.9	51.0
6	18.3	18.3	18.3	18.3	18.7	19.4	18.7
8	135.6[b,c)]	135.8	135.6[b)]	135.6[b)]	134.6	136.1	134.6
9	132.9[d)]	132.8	132.3	133.9	134.0	134.2	134.1
10	38.2	38.2	38.1	38.3	38.4	39.2	38.4
12	72.7	72.6	71.4	72.5	72.8	73.9	72.6
13	50.5	50.4	49.8	50.3	51.0	51.2	50.4
14	50.0	50.0	49.4	50.2	50.1	50.9	50.2
17	39.9	39.8	38.4	37.2	39.9	40.5	37.2
18	17.9[e)]	17.6[e)]	17.6[e)]	17.7[e)]	17.5[e)]	17.6[e)]	17.5[e)]
19	19.9	19.8	19.9	19.9	20.3	20.4	20.3
20	44.0	43.9	42.4	42.6	44.0	44.5	42.6
21	93.3	93.2	93.0	103.2	93.3	94.2	103.2
24	75.0	74.9	77.2	80.4	75.0	75.5	80.4
25	71.2	71.2	70.9	80.1	71.2	72.6	80.1
26	26.1	26.1	25.6	20.6	26.2	25.8	20.6
27	26.8	26.6	26.9	28.8	26.8	25.8	28.8
28	24.2	24.2	25.7	24.1	24.3	24.4	24.2
29	28.3	28.3	28.3	28.3	29.1	29.1	29.1
30	17.2[e)]	17.2[e)]	17.3[e)]	16.5[e)]	17.3[e)]	17.3[e)]	16.5[e)]
1′	171.3	171.2	170.2	171.3			
2′	46.5	46.5	46.5	46.4			
3′	69.9	69.8	69.9	69.9			
4′	46.6	46.3	46.4	46.6			
5′	174.8	174.3	174.6	174.7			
6′	28.4	28.3	28.4	28.4			
C$\underline{O}$$CH_3$	170.8	170.6	170.8	170.8			
CO$\underline{C}$$H_3$	21.1	21.0	21.0	21.1			

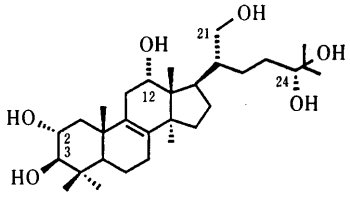
a) Measured in CD_3OD. *b*) Overlapped with one of the signals of the solvent. *c*) This signal appeared at δ 135.8 when measured in CD_3COCD_3. *d*) This signal appeared at δ 133.4 when measured in CD_3COCD_3. *e*) Assignments may be interchanged.



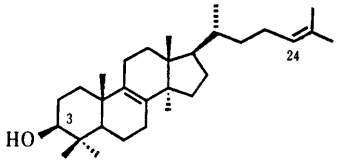
1 : $R^1=COCH_2C(CH_3)(OH)CH_2COOH$, $R^2=Ac$, $R^3=H$
2 : $R^1=COCH_2C(CH_3)(OH)CH_2COOH$, $R^2=R^3=Ac$
5 : $R^1=R^2=R^3=H$



3 : $R^4=COCH_2C(CH_3)(OH)CH_2COOH$, $R^5=Ac$
6 : $R^4=R^5=H$



4



7

Chart 1

forme and *H. sinapizans* as a cytotoxic principle by Bernardi *et al.*[2] The structure of 3-acetyl-2-(3′-hydroxy-3′-methyl)-glutarylcrustulinol was confirmed on the basis of derivation to fasciculol C (**4**) (a lanostane-type triterpene from *Naematoloma fasciculare*[3a,b] and *N. sublateritium*[3c]) *via* crustulinol (**5**) by Italian researchers.[2] Authentic **4** was synthesized from **1** *via* an intermediate compound, colorless needles, $C_{30}H_{50}O_6$, whose physico-chemical and spectral data were identical with those of **5** reported in the literature.[2] Thus, HS-A was deduced to be 3-acetyl-2-(3′-hydroxy-3′-methyl)glutarylcrustulinol (**1**), as shown in Chart 1.

HS-B (**2**): a colorless amorphous solid, $C_{40}H_{62}O_{12}$. Comparison of the ^{1}H-NMR spectrum of **2** with that of **1** indicated that the signals of two acetyl methyls are present at δ 2.02 and 2.03, the signal at δ 5.46 is shifted to δ 6.14, and other signals are similar to those of **1**. All signals in the ^{13}C-NMR spectrum of **2** were quite similar to those of **1** except that the signals of one more acetyl group are observed at δ 21.4 (CH_3) and 171.2 (CO), and the signals of C-20, 21 and 24 are shifted to δ 42.4 (−1.6), 93.0 (−0.3) and 77.2 (+2.2), respectively (see Table I). These data suggested that **2** may be an acetylated derivative of **1** at position 21. On treatment with 0.25% potassium hydroxide in methanol at room temperature, one acetyl group in **2** was removed to afford a deacetyl derivative which was identical with **1**. Thus, HS-B was deduced to be 3,21-diacetyl-2-(3′-hydroxy-3′-methyl)-glutarylcrustulinol (**2**), the 21-acetylated derivative of **1**, as shown in Chart 1.

HS-C (**3**): a colorless amorphous solid, $C_{38}H_{58}O_{10}$. All signals in the ^{13}C-NMR spectrum of **3** were quite similar to those of **1** except that the signals of C-20, -21, -23, -24, -25, -26 and -27 are shifted to δ 42.6 (−1.4), 103.2 (+10.0), 22.3 (−4.1), 80.4 (+5.4), 80.1 (+8.9), 20.6 (−5.5) and 28.8 (+2.0) (see Table I). The ^{13}C-NMR data of **3** suggested that the partial structure from position 20 to 27 in **3** may be similar to that of the corresponding part in anhydrocrustulinol (**6**), which is an anhydro derivative prepared from **5** through dehydration of the hydroxyl groups at positions 21 and 25,[2] and the remaining part of **3** may be identical with that of the corresponding part in **1** (see Chart 1). HS-C was identical with a dehydro product newly derived from **1** on treatment with 0.1 N hydrochloric acid in acetone. On hydrolysis with 0.25% potassium hydroxide in methanol, **3** afforded a product, colorless needles, $C_{30}H_{48}O_5$, which was identical with **6** prepared from **5**.[2] Accordingly, the structure of HS-C was deduced to be 3-acetyl-2-(3′-hydroxy-3′-methyl)glutarylanhydro-crustulinol (**3**), as shown in Chart 1.

Intraperitoneal administration of HS-A, B or C (**1**, **2** or **3**) caused death after paralysis of the limbs in mice at the dose of 100 mg/kg, but similar administration caused diarrhea in mice at the dose of 45 mg/kg. The toxic effect of these compounds therefore seemed to affect not only the central nervous system, but also the autonomic nervous system in mice. Compounds **1**, **2** and **3** caused relaxation of the contracted small intestine of mice treated with acetylcholine chloride (Ach) *in vitro*. The contraction of the intestines induced by Ach (1.0×10^{-6} M) was reversed to the extents of 33 and 10% by addition of **1** at the concentrations of 1.0×10^{-4} M (see Fig. 1) and 1.0×10^{-5} M, respectively. But, **1** showed no clear relaxation at the concentration of 1.0×10^{-6} M. At the concentration of 1.0×10^{-5} M, compounds **2**, **3**, **4**, **5** and **6** also reversed the contraction of the intestines induced by Ach by 10, 73, 37, 68 and 96%, respectively. But, a lanostane-type compound, lanosterol (**7**) (see Chart 1), showed no relaxation at 1.0×10^{-5} and 1.0×10^{-4} M of the contraction of the smooth muscle induced by Ach or barium chloride (1.0×10^{-3} M).

The dose–response curves between Ach and **6** (1.0×10^{-5} M) or atropine sulfate (Atr) (1.0×10^{-8} M) were obtained by the cumulative method[4] using smooth muscle preparations of the small intestine from mice, as shown in Fig. 2. Comparison of the curve between Ach and **6** with that between Ach and Atr indicated that **6**, which differs from Atr, acts non-competitively with respect to Ach on the smooth muscle and causes relaxation of the Ach-induced contraction of the muscle. It is well known that the contraction induced by barium salt is directly relaxed by papaverine hydrochloride (Pap) in a smooth muscle

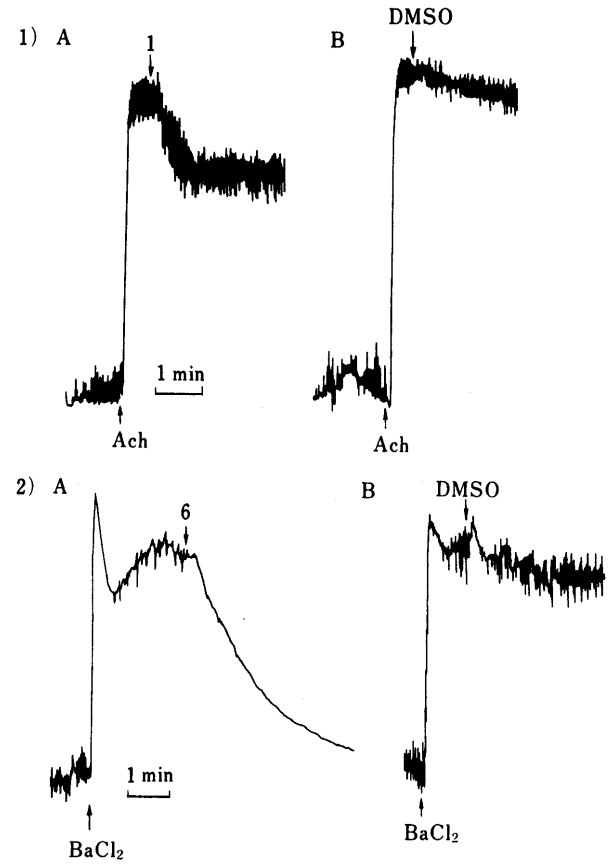


Fig. 1. Relaxating Effect of HS-A (**1**) and Anhydrocrustulinol (**6**) on the Contraction Induced by Ach or Barium Chloride ($BaCl_2$) in Mouse Small Intestine

1) Relaxating effect of **1** on the contraction by Ach (A), and control (B). 2) Relaxating effect of **6** on the contraction by $BaCl_2$ (A), and control (B). **1**, 1.0×10^{-4} M/1% (v/v) dimethylsulfoxide (DMSO)/Tyrode solution; Ach, 1.0×10^{-6} M/Tyrode solution; DMSO, 1% (v/v)/Tyrode solution; **6**, 1.0×10^{-5} M/1% (v/v) DMSO/Tyrode solution; $BaCl_2$, 1.0×10^{-3} M/Tyrode solution.

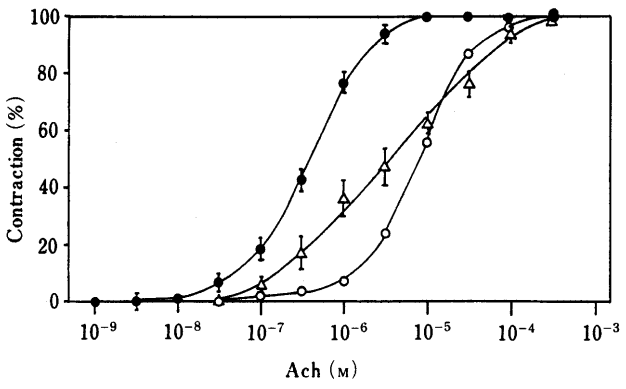


Fig. 2. Dose–Response Curves between Ach and Anhydrocrustulinol (**6**) or Atr in 1% (v/v) DMSO/Tyrode Solution

△, Ach and **6** (1.0×10^{-5} M); ○, Ach and Atr (1.0×10^{-8} M); ●, control.

preparation.[5] The contraction of the smooth muscle of mouse small intestine by barium chloride at 3.0×10^{-3} M was relaxed by treatment with **6** at 1.0×10^{-5} M (see Fig. 1) as well as by Pap at 1.0×10^{-5} M. Therefore, it seemed that **6** and the related compounds **1**—**5** may release the contraction of the smooth muscle induced by Ach or barium chloride in a similar manner to that exhibited by Pap but different from that by Atr.

Experimental

The melting points were measured on a Yanagimoto micro melting point apparatus (hot stage type) and are uncorrected. The optical rotations were measured with a JASCO DIP-140 digital polarimeter. The ultraviolet (UV) spectra were recorded with a Hitachi U-3400 spectrometer, the infrared (IR) spectra with a Hitachi EPI-G3 spectrometer, the electron impact mass spectra (EI-MS) with a Hitachi M-60 spectrometer, the high-resolution EI-MS (HREI-MS) with a Hitachi RMU-7M or JEOL JMS-HX110 spectrometer, the fast atom bombardment mass spectra (FAB-MS) with a JEOL JMS-SX102 spectrometer using *m*-nitrobenzyl alcohol as a matrix, the high-resolution FAB-MS (HRFAB-MS) with a JEOL JMS-HX110 spectrometer using *m*-nitrobenzyl alcohol as a matrix, the ^{1}H-NMR spectra with a JEOL JNM-GX270, JEOL JNM-GSX400 or JEOL JNM GSX-500 at 270, 400 or 500 MHz, and the ^{13}C-NMR spectra with a JEOL JNM GSX-270 or JEOL JNM GSX-400 at 67.8 or 100.4 MHz. Chemical shifts are expressed in δ (ppm) values from tetramethylsilane (TMS) as an internal standard. The thin layer chromatographic (TLC) analyses were carried out with a) silica gel plates (Merck Kieselgel 60G or $60F_{254}$), b) silica gel plates impregnated with 10% (w/v) oxalic acid or c) reversed-phase silica gel plates (Merck RP-18 F_{254}), the chromatographic separation with silica gel columns (Wakogel C-200), the MPLC separations with an MPLC system [a silica gel column (Fujigel CQ-3, 45 i.d. × 450 mm) and a Kusano KP-H6 micro pump], and the HPLC separations with an HPLC system [an ODS column (Senshu 5251-S, 20 i.d. × 250 mm), a Senshu Flow System 3100, a Senshu Pressure Control 3110 and an Erma RI-Detector ERC-7522]. The fruit-bodies of *H. spoliatum* were collected at Mt. Kiyosumi, Chiba, Japan in September, 1988. The *in vivo* toxicity of each sample was examined by intraperitoneal injection of 0.1 ml of a solution of each sample in dimethylsulfoxide (DMSO) into each mouse (ddY, male, 20—25 g), which was observed for 72 h thereafter (3 mice were employed for each sample). The small intestines of mice were prepared as described in the literature,[4] and mounted in an organ bath containing Tyrode solution (5.0 ml) bubbled with 95% O_2 and 5% CO_2 at 37 °C.[4] The *in vitro* effect of each sample on the preparations was examined by addition of 0.05 ml of a solution of each sample in DMSO to the organ bath of a Magnus apparatus, and longitudinal contractions or relaxations of the preparations were recorded by attaching the upper end of the preparations to a light spring connected to a Nihonkohden TD-112S isotonic transducer. The following drugs were used for the Magnus experiment: Ach (Nacalai Tesque, GR), Atr (Nacalai Tesque, GR), and Pap (Nacalai Tesque, GR).

Isolation of HS-A (1), -B (2) and -C (3) Dried fruit-bodies (100.00 g) were cut into small pieces and shaken for 3 h in MeOH (2.0 l) at room temperature 3 times to afford a methanolic extract (17.8 g). The methanolic extract was divided with 60% (v/v) aqueous MeOH–acetone (3 : 5, v/v) (0.8 l) into a supernatant part (after evaporation of the solvent, 8.2 g) and a precipitate part (after drying, 7.9 g). The supernatant part was chromatographed on a silica gel column (72 i.d. × 270 mm) with $CHCl_3$–MeOH (100 : 1), (100 : 1), (50 : 1), (15 : 1) and (5 : 1, v/v) to afford fractions I (0.29 g), II (0.31 g), III (1.00 g), IV (1.44 g) and V (2.53 g), respectively. Fraction IV was subjected to MPLC with *n*-hexane–acetone (2 : 1) and (1 : 1, v/v) at a flow rate of 12 ml/min, and HPLC with MeOH–H_2O (40 : 1, v/v) at a flow rate of 4.5 ml/min successively, to afford **1** (1.10 g), which was treated with *n*-hexane–acetone to give **1** in a pure state. Fraction III was subjected to HPLC with CH_3CN–acetone (3 : 1, v/v) at a flow rate of 2.5 ml/min and MPLC with $CHCl_3$–MeOH (70 : 1, v/v) to afford **2** (710 mg) and **3** (20 mg).

HS-A (**1**): a colorless powder, mp 201—202 °C (lit.[2] mp 204—205 °C), $[\alpha]_D^{17}$ −7.9° (*c* = 1.0, MeOH) (lit.[2] $[\alpha]_D^{20}$ −10.83° (*c* = 1, MeOH)), UV (in MeOH): end absorption. IR_{max}^{KBr} cm^{-1}: 3425 (O–H), 2930 (C–H), 1735 (C=O), 1375 (C–O). FAB-MS positive ion *m/z*: 715 [(M+Na)$^+$]; positive ion (plus KI) *m/z*: 731 [(M+K)$^+$]; negative ion *m/z*: 691 [(M−H)$^-$]. *Anal.* Calcd for $C_{38}H_{60}O_{11}$: C, 65.87; H, 8.73. Found: C, 65.45; H, 8.67. ^{1}H-NMR (in $CDCl_3$) δ: 0.61, 0.91, 0.94, 1.05, 1.11, 1.15, 1.18, 1.37 (each 3H, s, H_3-18, -30, -29, -19, -26, -27, -28, -6'), 2.06 (3H, s, CH_3CO), 2.62, 2.66 (each 1H, AB-type d, *J* = 14.8 Hz), 2.65, 2.71 (each 1H, AB-type d, *J* = 15.5 Hz) (H_2-2', -4'), 3.72 (1H, dd, J_1 = 11.6, J_2 = 2.0 Hz, H-24), 3.87 (1H, d, *J* = 8.2 Hz, H-12), 4.79 (1H, d, *J* = 10.3 Hz, H-3), 5.18 (1H, td, J_1 = 10.3, J_2 = 5.0 Hz, H-2), 5.46 (1H, br s, H-21).

HS-B (**2**): a colorless amorphous solid, $[\alpha]_D^{25}$ −21.4° (*c* = 0.70, MeOH), IR_{max}^{KBr} cm^{-1}: 3450, 2950, 1740, 1370. FAB-MS positive ion *m/z*: 757 [(M+Na)$^+$]; positive ion (plus KI) *m/z*: 773 [(M+K)$^+$]; negative ion *m/z*: 733 [(M−H)$^-$]. HRFAB-MS positive ion (plus KI) *m/z* Calcd for $C_{40}H_{62}KO_{12}$ [(M+K)$^+$]: 773.3879. Found: 773.7859; negative ion *m/z* Calcd for $C_{40}H_{61}O_{12}$ [(M−H)$^-$]: 733.4163. Found: 733.4156. ^{1}H-NMR (in CD_3COCD_3) δ: 0.70, 0.91, 0.95, 1.07, 1.07, 1.14, 1.16, 1.35 (each 3H, s, H_3-18, -30, -29, -19, -26, -27, -28, -6'), 2.02, 2.03 (each 3H, s, CH_3CO), 2.61, 2.64 (each 1H, AB-type d, *J* = 15.1 Hz), 2.65, 2.70 (each 1H, AB-type

d, $J=15.7$ Hz) (H_2-2', -4'), 3.51 (1H, dd, $J_1=10.8$, $J_2=1.9$ Hz, H-24), 3.77 (1H, d, $J=7.2$ Hz, H-12), 4.76 (1H, d, $J=10.8$ Hz, H-3), 5.16 (1H, td, $J_1=10.8$, $J_2=4.4$ Hz, H-2), 6.14 (1H, br s, H-21).

HS-C (**3**): a colorless amorphous solid, $[\alpha]_D^{25}$ −3.1° ($c=0.38$, MeOH), IR_{max}^{KBr} cm^{-1}: 3525, 2970, 1745, 1380. FAB-MS positive ion (plus KI) *m/z*: 713 $[(M+K)^+]$; negative ion *m/z*: 673 $[(M-H)^-]$. HRFAB-MS positive ion *m/z* Calcd for $C_{38}H_{59}O_{10}$ $[(M+H)^+]$: 675.4109. Found: 675.4124; positive ion (plus KI) *m/z* Calcd for $C_{38}H_{58}KO_{10}$ $[(M+K)^+]$: 713.3667. Found: 713.3657; negative ion *m/z* Calcd for $C_{38}H_{57}O_{10}$ $[(M-H)^-]$: 673.3952. Found: 673.3954. ^{1}H-NMR (in C_5D_5N) δ: 0.55, 0.96, 0.97, 1.10, 1.27, 1.37, 1.37, 1.79 (each 3H, s, H_3-18, -30, -29, -19, -26, -27, -28, -6'), 2.19 (3H, s, CH_3CO), 3.18, 3.19 (each 1H, AB-type d, $J=14.7$ Hz), 3.22 (2H, s) (H_2-2', -4'), 3.82 (1H, d, $J=2.9$ Hz, H-24), 3.95 (1H, d, $J=9.1$ Hz, H-12), 5.13 (1H, d, $J=10.3$ Hz, H-3), 5.55 (1H, td, $J_1=10.3$, $J_2=4.4$ Hz, H-2), 5.84 (1H, s, H-21).

Derivation of HS-A (1) to Fasciculol C (4) *via* Crustulinol (5) Compound **1** (95 mg) was dissolved in a solution of 0.25% (w/v) KOH in MeOH (20 ml), and refluxed for 7 h under an argon gas flow. The reaction mixture was neutralized with 0.1 N HCl under ice-cooling, diluted with ice-H_2O, and extracted with AcOEt. The AcOEt layer was treated as usual to afford a crude product (74 mg), which was crystallized from aqueous EtOH to give **5** (50 mg), colorless needles, mp 238—240 °C (lit.[2] mp 238—240 °C), $[\alpha]_D^{18}$ +16.5° ($c=0.94$, MeOH), IR_{max}^{KBr} cm^{-1}: 3375, 2950, 1370. HREI-MS *m/z* Calcd for $C_{30}H_{50}O_6$ (M^+): 506.3604. Found: 506.3588 (lit.[2] EI-MS: *m/z* 506 (M^+)). ^{1}H-NMR (in CD_3OD) δ: 0.66, 0.83, 1.02, 1.05, 1.05, 1.15, 1.15 (each 3H, s, H_3-18, -30, -29, -19, -26, -27, -28), 2.92 (1H, d, $J=9.6$ Hz, H-3), 3.63 (1H, m), 3.66 (1H, br d, $J=11.7$ Hz) (H-2, -24), 3.88 (1H, d, $J=8.2$ Hz, H-12), 5.43 (1H, s, H-21).

A solution of $NaBH_4$ (35 mg) in H_2O (3 ml) was added to a solution of **5** (100 mg) in EtOH (13 ml), and stirred for 1.5 h under an argon gas flow. The reaction mixture was neutralized by addition of AcOH under ice-cooling, diluted with H_2O, and extracted with AcOEt. The AcOEt layer was treated as usual to afford a crude product (90 mg). The crude product was crystallized from MeOH–AcOEt to give colorless prisms (50 mg); this product was identical with authentic **4**[3] on the basis of mixed melting point (186—188 °C), IR spectral comparison (KBr) and TLC behavior [1) plate b; solvent, $CHCl_3$–MeOH (5 : 1, v/v); 2) plate c; solvent, MeOH–H_2O (40 : 1, v/v); 3) plate c; solvent, acetone–H_2O (9 : 1, v/v)].

Derivation of HS-B (2) to HS-A (1) Compound **2** (100 mg) was dissolved in a solution of 0.25% (w/v) KOH in MeOH (20 ml), and the solution was stirred at room temperature for 30 min under an argon gas flow. The reaction mixture was treated in a similar way to that described for derivation of **1** to **5** to afford a crude product (90 mg). The crude product was crystallized from *n*-hexane–acetone to give a colorless powder (30 mg), which was identical with **1** on the basis of mixed melting point and IR (KBr), ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (C_5D_5N) spectral comparisons.

Preparation of Anhydrocrustulinol (6) from Crustulinol (5) Compound **5** (150 mg) was dissolved in a solution of 0.1 N HCl in acetone (30 ml), and stirred at room temperature for 3 h under an argon gas flow. The reaction mixture was diluted with H_2O, neutralized with 0.1 N NaOH under ice-cooling, and extracted with EtOAc. The EtOAc layer was treated as usual to give a mixture (164 mg), which was chromatographed on a silica gel column (18 i.d. × 70 mm) with $CHCl_3$–MeOH (70 : 1, v/v) to give a product. This product was crystallized from aqueous EtOH to afford **6** (50 mg), colorless needles, mp 269—270°C (lit.[2] mp 271—272 °C), $[\alpha]_D^{15}$ +40.1° ($c=0.13$, MeOH). IR_{max}^{KBr} cm^{-1}: 3490, 2940, 1380. EI-MS *m/z* (%): 489 ($(M+H)^+$, 10), 488 (M^+, 30), 473 (27), 456 (34), 455 (100), 437 (40) (lit.[2] EI-MS *m/z*: 488 (M^+)). ^{1}H-NMR (CD_3COCD_3) δ: 0.62, 0.84, 1.03, 1.06, 1.10, 1.22, 1.41 (each 3H, s, H_3-18, -30, -29, -19, -26, -27, -28), 2.93 (1H, d, $J=9.5$ Hz, H-3), 3.62 (1H, m, H-2), 3.86 (1H, d, $J=9.1$ Hz, H-12), 3.97 (1H, d, $J=3.3$ Hz, H-24), 5.60 (1H, s, H-21).

Derivation of HS-C (3) from HS-A (1) Compound **1** (200 mg) was dissolved in a solution of 0.1 N HCl in acetone (20 ml), and stirred at room temperature for 2 h under an argon gas flow. The reaction mixture was treated and purified in a similar way to that described for preparation of **6** from **5** to afford a product (116 mg), which was identical with **3** on the basis of IR (KBr), ^{1}H-NMR (CD_3COCD_3) and ^{13}C-NMR (C_5D_5N) spectral comparisons and TLC behavior [1) plate b; solvent, $CHCl_3$–MeOH (4 : 1, v/v); 2) plate c; solvent, MeOH–H_2O (40 : 1, v/v)].

Derivation of HS-C (3) to Anhydrocrustulinol (6) Compound **3** (10 mg) was dissolved in a solution of 0.25% (w/v) KOH in MeOH (10 ml), and refluxed for 7 h under an argon gas flow. The reaction mixture was treated in a similar way to that described for derivation of **1** to **5** to afford a crude product (9 mg). This crude product was crystallized from aqueous EtOH to give colorless needles (4 mg). This product was identical with **6** prepared from **5** in terms of mixed melting point and IR (KBr), EI-MS and ^{1}H-NMR (CD_3COCD_3) spectral comparisons.

Acknowledgement We are grateful to Dr. A. Suzuki, Chiba University, for his help in collecting fruit-bodies of *H. spoliatum* grown at Mt. Kiyosumi. We are also grateful to Dr. S. Horie of our Faculty for his advice on the pharmacological experiment. We thank Prof. S. Nozoe and Dr. G. Kusano, Tohoku University, for their gift of an authentic specimen of fasciculol C, Mr. A. Higuchi, Taisho Pharmaceutical Co., Ltd., for his measurement of FAB-MS, and Mrs. H. Seki, Miss R. Hara, Mr. T. Kuromochi and Mrs. S. Imamoto, Analysis Center, Chiba University, for their measurement of NMR, EI-MS, HREI-MS, and HRFAB-MS and elemental analyses.

References

1) H. Fujimoto, K. Maeda and M. Yamazaki, *Chem. Pharm. Bull.*, **39**, 1958 (1991); H. Fujimoto, H. Hagiwara, K. Suzuki and M. Yamazaki, *ibid.*, **35**, 2254 (1987); H. Fujimoto, K. Suzuki, H. Hagiwara and M. Yamazaki, *ibid.*, **34**, 88 (1986); H. Fujimoto, M. Yamazaki and A. Suzuki, *Trans. Mycol. Soc. Jpn.*, **23**, 405 (1982).
2) M. D. Bernardi, G. Fronza, M. P. Gianotti, G. Mellerio, G. Vidari and P. Vita-Finzi, *Tetrahedron Lett.*, **24**, 1635 (1983).
3) *a*) M. Ikeda, G. Niwa, K. Tohyama, T. Sassa and Y. Miura, *Agric. Biol. Chem.*, **41**, 1803 (1977); *b*) I. Kubo, A. Matsumoto, M. Kozuka and W. F. Wood, *Chem. Pharm. Bull.*, **33**, 3821 (1985); *c*) M. D. Bernardi, G. Mellerio, G. Vidari, P. Vita-Finzi, G. Fronza, M. Kocor and J. S. Pyrek, *J. Nat. Prod.*, **44**, 351 (1981).
4) K. Kubota (ed.), "Kiso Yakurigaku Jikken," Nankodo Inc., Tokyo, 1987, pp. 84—96; I. Takayanagi, *Ohyo Yakuri*, **2**, 131 (1968).
5) T. Shibuya (ed.), "Shin Yakurigaku Nyumon," Nanazando Inc., Tokyo, 1981, pp. 263—272, pp. 336—348; K. Takagi (ed.), "Saishin Yakurigaku Kohgi," Hirokawa Shoten Inc., Tokyo, 1987, pp. 137—146; M. Ito (ed.), "Yakurigaku Jikken Shishin," Hirokawa Shoten Inc., Tokyo, 1987, pp. 87—95.