Isolation of New Tremorgenic Metabolites from an Ascomycete, *Corynascus setosus*

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A new meroterpenoid named setosusin and two new tryptoquivalines were isolated as tremorgenic principles from an Ascomycete, *Corynascus setosus*, together with four known metabolites, fiscalin B, helvolic acid, helvolinic acid, and 2-(1-oxo-2-hydroxyethyl)furan. The structure of setosusin was determined from the chemical and spectral data and by X-ray crystallographic analysis of its deacetyl-dehydrated derivative.The two new tryptoquivalines were deduced to be 27-*epi*-isomers of tryptoquivaline and nortryptoquivaline from their spectral data supported by X-ray analysis.

Key words fungal metabolite; Ascomycete; *Corynascus setosus*; tremorgen; meroterpenoid; tryptoquivaline

During our search for biologically active new fungal metabolites, we have already isolated neurotoxic,[1] immunosuppressive,[2] and monoamine oxidase-inhibitory metabolites[3] from Ascomycetous and Basidiomycetous fungi. We have now found that the ethyl acetate extract of an Ascomycete, *Corynascus setosus* (DADE) VON ARX IFM4648,[4] causes tremor in mice. By means of solvent partition followed by repeated chromatography of the extract, a new meroterpenoid named setosusin and two new 27-*epi*-isomers of tryptoquivalines were isolated as tremorgenic principles of this fungus, together with four known metabolites. This report deals with the isolation, structure elucidation, and examination of tremorgenic activity of these fungal metabolites.

Results and Discussion

The AcOEt extract of *C. setosus* cultivated on sterilized rice caused weak tremor in mice on intraperitoneal (i.p.) injection at a dose of 500 mg/kg. The extract was partitioned into fatty and defatted layers, and the defatted layer was further partitioned into AcOEt and aqueous layers. Among these layers, only the AcOEt layer caused weak tremor in mice on i.p. injection at 250 mg/kg. Thus, the AcOEt layer was subjected to chromatography on silica gel columns to give four fractions I—IV, among which III and I caused weak tremor in mice at 50 mg/kg. Fraction III was further chromatographed repeatedly to afford a tremorgenic principle tentatively named CS-A (**1**). Fraction I was also chromatographed to give two other tremorgenic components, tentatively named CS-B (**2**) and -C (**3**). Besides **1**—**3**, four non-tremorgenic components tentatively named CS-D (**4**)—G (**7**) were obtained on repeated chromatography of the AcOEt layer.

CS-A (**1**) was obtained as colorless needles, $C_{29}H_{38}O_8$. The IR and UV spectra suggested the presence of ester and conjugated ketone groups in **1**. The ^{1}H- and ^{13}C-NMR spectral data, including spin-decoupling ^{1}H-NMR and two-dimensional ^{1}H–^{1}H (^{1}H–^{1}H COSY) and ^{13}C–^{1}H shift correlation (^{13}C–^{1}H COSY) NMR spectra suggested that **1** consisted of eight tertiary methyls (including one acetoxyl and three olefinic), five methylenes, four methines (including one oxygen-bearing and one olefinic), and twelve quaternary carbons (including three oxygen-bearing, two olefinic, three ester carbonyls, one olefinic oxygen-bearing, and one ketone carbonyl) (see Table 1). The ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) NMR spectrum experiment showed that **1** might be composed of three partial structures, *a*, *b* (acetoxyl), and *c* (carboxyl), as shown in Chart 1.

On treatment with 0.1% KOH/MeOH, **1** gave colorless prisms (**8**), $C_{27}H_{34}O_6$. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **8** with those of **1** showed that all signals in the ^{1}H- and ^{13}C-NMR spectra of **8** were similar to those of **1** except that the signals due to an H–C=C–H group [δ 5.88, 6.47 (each d, 12.0 Hz), 121.4, 157.6 (each d)] newly appeared, and those of an acetoxyl group [δ 2.10 (3H, s), 21.0 (q), 170.4 (s)] disappeared, indicating that **8** might be a deacetyl-dehydrated derivative of **1** (see Table 1). The relative stereostructure of **8** was solved directly by X-ray crystallographic analysis of **8** (see Experimental), as shown in Chart 2. It was considered that an acetoxyl group at position 1 in **1** was removed, followed by dehydration to give **8** during the treatment with alkali. In a nuclear Overhauser effect (NOE) experiment, significant NOEs were observed between the signal of CH_3-10 (δ 1.21) and that of H-1 attached to C-1 bearing the acetoxyl group (δ 4.88), and between the signal of CH_3-10 and that of one of the two methyls at position 4 (δ 1.52). Thus, the relative configurations of H-1 and one of the two CH_3-4 at δ 1.52 were shown to be the same (β) as that of CH_3-10 (the relative configuration of CH_3COO-1 was shown to be α). Accordingly, the relative stereostructure of CS-A was deduced to be **1**, which was constructed from the partial structures, *a*, *b*, and *c*, as shown in Chart 2. We propose to name CS-A setosusin. The structure of setosusin (**1**) seems to be similar to those of fungal meroterpenoids, andilesin A (**9**)[5] and anditomin (**10**),[6] from *Aspergillus variecolor* (Chart 2).

CS-C (**3**) was obtained as colorless prisms, $C_{29}H_{30}N_4O_7$. The IR spectrum suggested the presence of hydroxyl, ester and amide groups, and the UV spectrum suggested the presence of quinazolone- and indole-related moieties in **3**. The ^{1}H- and ^{13}C-NMR spectra showed that **3** was composed of five methyls (including one acetoxyl,

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Table 1. ^{1}H-NMR and ^{13}C-NMR Data for Setosusin (**1**) and Deacetyl-Dehydrated Setosusin (**8**), δ (ppm) from Tetramethylsilane (TMS) as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	**1**			**8**		
	^{1}H-NMR	^{13}C-NMR	HMBC (^{1}H-NMR/^{13}C-NMR)	^{1}H-NMR	^{13}C-NMR	HMBC (^{1}H-NMR/^{13}C-NMR)
1	4.88 (t, 4.1)	70.3 (d)	4.88/15.1, 35.3, 39.2, 43.9, 50.3, 170.4	6.47 (d, 12.0)	157.6 (d)	6.47/44.7, 55.9, 167.8
2	3.10 (2H, d, 4.1)	35.3 (t)	3.10/43.9, 70.3, 170.4	5.88 (d, 12.0)	121.4 (d)	5.88/42.9, 167.8
3	—	170.4 (s)	—	—	167.8 (s)	—
4	—	85.6 (s)	—	—	85.5 (s)	—
5	2.05 (br d, 10.8)	50.3 (d)	2.05/15.1, 21.2, 24.0, 31.7, 43.9, 85.6	2.07 (dd, 10.7, 4.7)	55.9 (d)	2.07/14.8, 22.5, 42.9
6	1.60, 1.85 (each m)	21.2 (t)	1.60/50.3, 85.6 1.85/41.0, 43.9, 85.6	1.67 (2H, m)	22.5 (t)	1.67/
7	1.52, 1.65 (each m)	31.7 (t)	1.52/21.2, 50.3 1.65/21.2, 39.2, 41.0, 50.3	1.46, 1.67 (each m)	31.4 (t)	1.46/22.5, 55.9 1.67/
8	—	41.0 (s)	—	—	41.4 (s)	—
9	2.30 (dd, 8.5, 7.6)	39.2 (d)	2.30/15.1, 16.8, 23.0, 41.0, 43.9, 90.3	1.99 (br d, 8.5)	44.7 (d)	1.99/14.8, 16.5, 24.2, 41.4, 42.9, 90.3
10	—	43.9 (s)	—	—	42.9 (s)	—
11	1.96 (2H, m)	23.0 (t)	1.96/39.2, 129.9, 130.5	2.17 (2H, m)	24.2 (t)	2.17/
12	5.76 (t-like)	129.9 (d)	5.76/19.6, 23.0, 39.2	5.88 (t-like)	130.2 (d)	5.88/
13	—	130.5 (s)	—	—	131.0 (s)	—
14	—	90.3 (s)	—	—	90.3 (s)	—
15	2.45, 2.89 (each d, 15.9)	32.7 (t)	2.45/41.0, 87.0, 90.3, 130.5, 169.0, 199.7 2.89/41.0, 87.0, 90.3, 130.5, 169.0, 199.7	2.46, 2.89 (each d, 15.9)	32.3 (t)	2.46/41.4, 86.9, 90.3, 131.0, 169.2 2.89/41.4, 86.9, 90.3, 131.0, 169.2, 199.6
16	1.55 (3H, s)	34.6 (q)	1.55/24.0, 50.3, 85.6	1.46 (3H, s)	32.5 (q)	1.46/27.0, 55.9, 85.5
17	1.52 (3H, s)	24.0 (q)	1.52/34.6, 50.3, 85.6	1.47 (3H, s)	27.0 (q)	1.47/32.5, 55.9, 85.5
18	0.94 (3H, s)	16.8 (q)	0.94/31.7, 39.2, 41.0, 90.3	1.02 (3H, s)	16.5 (q)	1.02/31.4, 41.4, 44.7, 90.3
19	1.21 (3H, s)	15.1 (q)	1.21/39.2, 43.9, 50.3, 70.3	1.34 (3H, s)	14.8 (q)	1.34/42.9, 44.7, 55.9, 157.6
20	1.93 (3H, s)	19.6 (q)	1.93/90.3, 129.9, 130.5	1.96 (3H, d, 1.5)	19.4 (q)	1.96/90.3, 130.2, 131.0
1′	—	169.0 (s)	—	—	169.2 (s)	—
2′	—	87.0 (s)	—	—	86.9 (s)	—
3′	—	199.7 (s)	—	—	199.6 (s)	—
4′	—	110.6 (s)	—	—	110.7 (s)	—
5′	—	185.7 (s)	—	—	185.8 (s)	—
6′	2.27 (3H, s)	15.0 (q)	2.27/110.6, 185.7	2.27 (3H, s)	15.0 (q)	2.27/110.7, 185.8
7′	1.71 (3H, s)	5.8 (q)	1.71/110.6, 185.7, 199.7	1.71 (3H, s)	5.8 (q)	1.71/110.7, 185.8, 199.6
1″	—	170.4 (s)	—	—		—
2″	2.10 (3H, s)	21.0 (q)	2.10/170.4	—		—

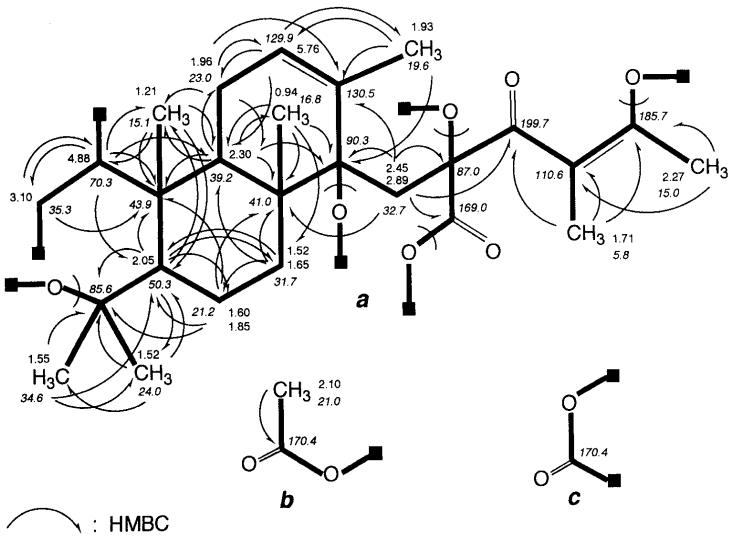


Chart 1. Partial Structures *a*—*c* for Setosusin (**1**)

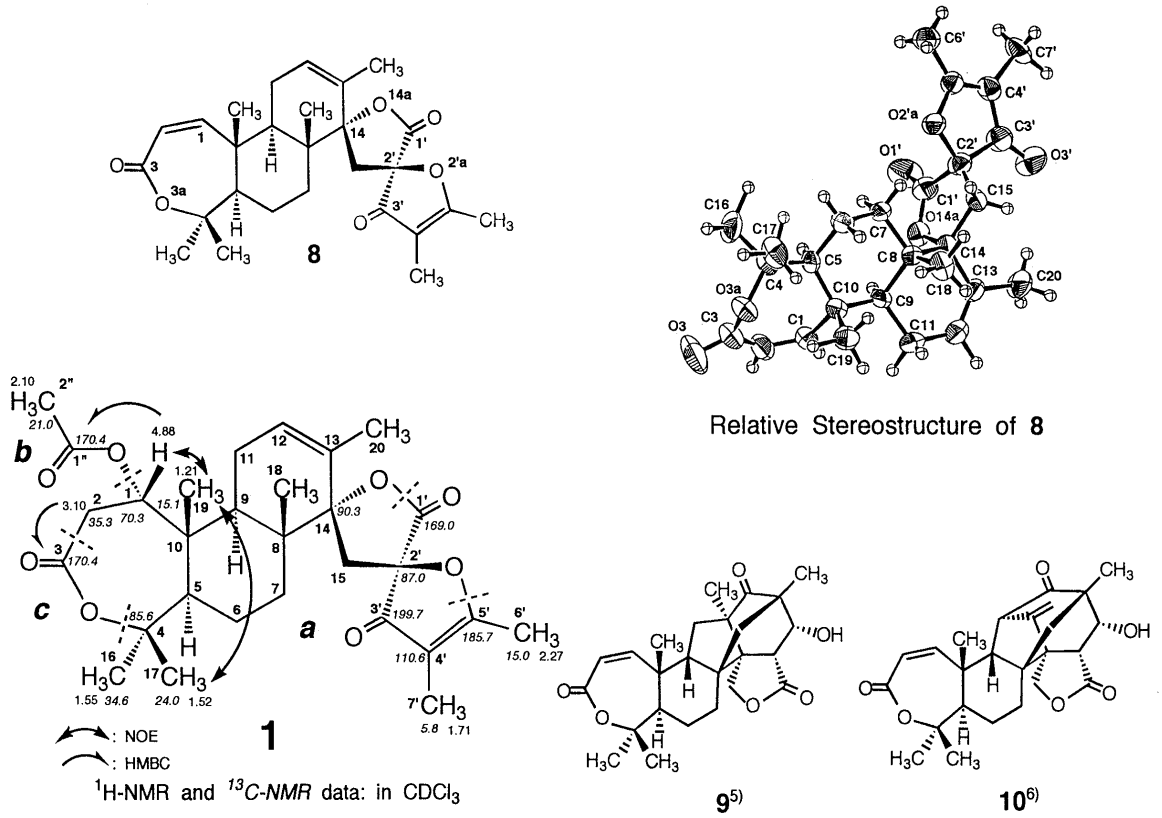


Chart 2. Structures of Setosusin (**1**), Deacetyl-Dehydrated Setosusin (**8**), Andilesin A (**9**), and Anditomin (**10**)

two secondary and two tertiary), one methylene, twelve methines (including three oxygen- or nitrogen-bearing and eight aromatic), eleven quaternary carbons (including two oxygen- or nitrogen-bearing, five aromatic and four ester or amide carbonyls), and one hydroxyl group. These data were similar to those of tryptoquivaline (FTC), from *Aspergillus clavatus*[7] and *A. fumigatus*[8] (Chart 3). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of authentic FTC showed that all of the signals of **3** resembled the corresponding signals of FTC, except that the signals of C-26 and -27 were shifted to δ 151.5 (-1.0) and 78.9 ($+1.7$) (see Table 2). X-Ray crystallographic analysis of **3** was executed in the same way as for **8** to give a stereostructure of **3**, which corresponded to the diastereomer of FTC at position 27[9] (Chart 3). FTC, which has (2*S*,3*S*,12*R*,27*S*)-configuration[10] gave positive (the first) and negative (the second) Cotton effects at 307 and 253 nm in the CD spectrum. The CD spectrum of **3** was similar to that of FTC except that an additional positive Cotton effect newly appeared at 281 nm (see Experimental). These data suggested that **3** might be a diastereomer of FTC, but not an enantiomer. Accordingly, CS-C was deduced to be 27-*epi*-tryptoquivaline (**3**) (27*R*-configuration), as shown in Chart 3.

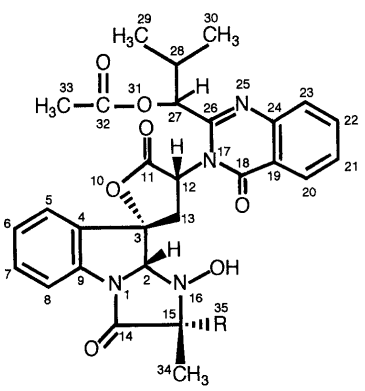
CS-B (**2**) was obtained as colorless needles, $C_{28}H_{28}N_4O_7$. The IR and UV spectra of **2** were quite similar to those of **3**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **3** showed that all of the signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were similar to the corresponding signals of **3** except that the $>C(CH_3)_2$ group at position 15 was replaced with a $>CHCH_3$ group [4.35 (q, $J=7.2$ Hz), 1.59 (3H, d, $J=7.2$ Hz), 67.9 (d), 11.0 (q)] (see Table 2), suggesting that **2** might also be a diastereomer of nortryptoquivaline (FTD) [FTD, isolated together with FTC from *A. clavatus*[7] and *A. fumigatus*[8] has (2*S*,3*S*,12*R*,15*S*,27*S*)-configuration[10] (Chart 3)]. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of authentic FTD showed that all of the signals of **2** resembled the corresponding signals of FTD except that the signals of C-26 and -27 were shifted to δ 151.5 (-1.1) and 79.0 ($+1.8$) (Table 2). The CD spectrum of **2** resembled that of **3**, and was also similar to that of FTD, except for the appearance of an additional positive Cotton effect at 278 nm in the spectrum of **2** (see Experimental), indicating that CS-B is the diastereomer of FTD at position 27 [27-*epi*-nortryptoquivaline (**2**)] (27*R*-configuration) (see Chart 3).

CS-D (**4**), obtained as colorless powder, $C_{23}H_{22}N_4O_2$, was deduced to be identical with fiscalin B, a substance P-inhibitor, from *Neosartorya fischeri*,[11] based on a comparison of the physicochemical and spectral data of **4** (Table 2 and Experimental) with those of fiscalin B in the literature.[11] Fiscalin B is biogenetically related to tryptoquivalines, as shown in Chart 3.

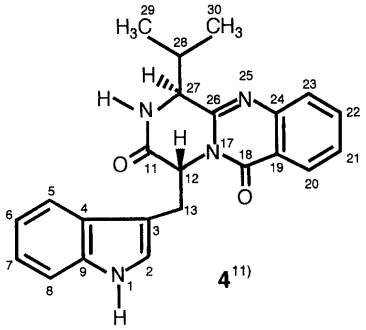
CS-E (**5**), obtained as colorless needles, $C_{32}H_{44}O_8$, was identical with helvolic acid from *Aspergillus fumigatus* m. *helvola*[12a] and *Cephalosporium caerulens*,[12b] based on direct comparison with an authentic sample. CS-F (**6**), obtained as colorless needles, was deduced to be helvolinic acid (6-deacetylhelvolic acid), from *C. caerulens*[12a,c] (Chart 4), from the similarity of the physicochemical data to those of helvolinic acid in the literature.[12c] All of the signals in the ^{1}H-NMR spectrum of **6** were similar to the corresponding signals of **5**, except that the signal of

Table 2. ^{1}H-NMR and ^{13}C-NMR Data for CS-B (**2**), CS-C (**3**), CS-D (**4**), Nortryptoquivaline (FTD), and Tryptoquivaline (FTC) δ (ppm) form TMS in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	**2**		FTD		**3**		FTC		**4**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	—	—	—	—	—	—	—	—	8.21 (br s)	—
2	5.23 (s)	89.9 (d)	5.21 (s)	90.2 (d)	5.05 (s)	87.6 (d)	5.03 (s)	87.9 (d)	6.53 (d, 2.1)	122.6 (d)
3	—	83.9 (s)	—	84.3 (s)	—	83.9 (s)	—	84.2 (s)	—	108.2 (s)
4	—	133.7 (s)	—	133.8 (s)	—	133.7 (s)	—	133.8 (s)	—	126.2 (s)
5	7.41 (br d, 7.6)	123.7 (d)	7.41 (br d, 7.6)	124.0 (d)	7.41 (br d, 7.6)	123.7 (d)	7.40 (br d, 7.5)	123.9 (d)	7.35 (br d, 7.8)	117.6 (d)
6	7.28 (td, 7.6, 1.1)	125.7 (d)	7.26 (td, 7.6, 1.2)	125.6 (d)	7.28 (td, 7.6, 1.1)	125.6 (d)	7.26 (td, 7.5, 1.1)	125.5 (d)	6.84 (td, 7.8, 1.0)	118.9 (d)
7	7.49 (td, 7.6, 1.1)	131.9 (d)	7.48 (td, 7.6, 1.2)	132.0 (d)	7.48 (td, 7.6, 1.1)	131.9 (d)	7.48 (td, 7.5, 1.0)	131.9 (d)	7.04 (td, 7.8, 1.0)	121.5 (d)
8	7.63 (br d, 7.6)	116.1 (d)	7.63 (br d, 7.6)	116.1 (d)	7.64 (br d, 7.6)	116.1 (d)	7.63 (br d, 7.8)	116.1 (d)	7.21 (br d, 7.8)	110.0 (d)
9	—	137.8 (s)	—	137.8 (s)	—	138.1 (s)	—	138.0 (s)	—	135.0 (s)
10	—	—	—	—	—	—	—	—	6.10 (br s)	—
11	—	169.3 (s)	—	169.4 (s)	—	169.4 (s)	—	169.6 (s)	—	168.5 (s)
12	5.87 (t-like, 10.1)	54.6 (d)	5.69 (t-like, 9.9)	54.9 (d)	5.88 (t-like, 10.0)	54.7 (d)	5.67 (t-like, 9.9)	54.9 (d)	5.59 (dd, 5.5, 2.8)	55.7 (d)
13	3.09	34.1 (t)	3.04	33.6 (t)	3.12	34.6 (t)	3.08	33.9 (t)	3.56	26.3 (t)
	(dd, 13.3, 9.5)		(dd, 13.9, 9.3)		(dd, 13.5, 9.7)		(dd, 13.7, 9.5)		(dd, 14.8, 5.5)	
	3.15		3.21		3.14		3.18		3.65	
	(dd, 13.3, 10.7)		(dd, 13.9, 10.5)		(dd, 13.5, 10.1)		(dd, 13.7, 10.5)		(dd, 14.8, 2.8)	
14	—	169.2 (s)	—	169.3 (s)	—	169.4 (s)	—	169.6 (s)	—	—
15	4.35 (q, 7.2)	67.9 (d)	4.37 (q, 7.1)	68.1 (d)	—	71.4 (s)	—	71.6 (s)	—	—
16-OH	7.16 (s)	—	7.29 (s)	—	6.99 (s)	—	7.12 (s)	—	—	—
18	—	161.8 (s)	—	161.6 (s)	—	161.7 (s)	—	161.5 (s)	—	159.9 (s)
19	—	120.3 (s)	—	120.2 (s)	—	120.3 (s)	—	120.3 (s)	—	119.1 (s)
20	8.22 (dd, 8.4, 1.5)	126.7 (d)	8.23 (dd, 7.7, 1.3)	126.8 (d)	8.25 (dd, 7.2, 1.3)	126.8 (d)	8.25 (dd, 7.9, 1.3)	126.9 (d)	8.29 (dd, 7.9, 1.5)	125.9 (d)
21	7.55 (td, 8.4, 1.3)	128.3 (d)	7.55 (td, 7.7, 1.3)	128.2 (d)	7.55 (td, 7.2, 1.2)	128.2 (d)	7.55 (td, 7.9, 1.3)	128.1 (d)	7.45 (td, 7.9, 1.1)	126.0 (d)
22	7.84 (td, 8.4, 1.5)	135.5 (d)	7.84 (td, 7.7, 1.3)	135.5 (d)	7.84 (td, 7.2, 1.3)	135.5 (d)	7.83 (td, 7.9, 1.3)	135.5 (d)	7.69 (td, 7.9, 1.5)	133.6 (d)
23	7.79 (br d, 8.4)	128.0 (d)	7.79 (br d, 7.7)	127.9 (d)	7.79 (br d, 7.2)	128.0 (d)	7.79 (br d, 7.9)	127.9 (d)	7.48 (br d, 7.9)	126.1 (d)
24	—	146.5 (s)	—	146.6 (s)	—	146.5 (s)	—	146.6 (s)	—	146.0 (s)
26	—	151.5 (s)	—	152.6 (s)	—	151.5 (s)	—	152.5 (s)	—	149.2 (s)
27	5.66 (d, 9.8)	79.0 (d)	5.57 (d, 9.1)	77.2 (d)	5.65 (d, 10.1)	78.9 (d)	5.59 (d, 9.0)	77.2 (d)	2.67 (d, 2.4)	57.1 (d)
28	2.68 (m)	31.8 (d)	2.66 (m)	31.9 (d)	2.69 (m)	31.8 (d)	2.66 (m)	31.9 (d)	2.57 (m)	28.5 (d)
29	0.98 (3H, d, 6.6)	19.0 (q)	1.05 (3H, d, 6.9)	18.8 (q)	1.06 (3H, d, 6.8)	18.9 (q)	1.06 (3H, d, 6.6)	18.9 (q)	0.57 (3H, d, 7.0)	13.7 (q)
30	1.16 (3H, d, 6.6)	18.9 (q)	1.20 (3H, d, 6.9)	18.7 (q)	1.17 (3H, d, 6.8)	18.9 (q)	1.20 (3H, d, 6.6)	18.7 (q)	0.59 (3H, d, 7.0)	17.7 (q)
32	—	170.5 (s)		171.2 (s)	—	170.6 (s)	—	171.1 (s)	—	—
33	2.21 (3H, s)	20.8 (q)	2.19 (3H, s)	20.7 (q)	2.22 (3H, s)	20.8 (q)	2.19 (3H, s)	20.8 (q)	—	—
34	1.59 (3H, d, 7.2)	11.0 (q)	1.60 (3H, d, 7.1)	11.2 (q)	1.49 (3H, s)	16.9 (q)	1.49 (3H, s)	17.1 (q)	—	—
35	—	—		—	1.50 (3H, s)	22.8 (q)	1.51 (3H, s)	22.9 (q)	—	—



2 [R=H, (*R*)-configuration at position 27]
3 [R=CH_3, (*R*)-configuration at position 27]
FTD [R=H, (*S*)-configuration at position 27][10)]
FTC [R=CH_3, (*S*)-configuration at position 27][10)]



4[11)]

Chart 3. Structures of CS-B (**2**), CS-C (**3**), CS-D (**4**), Nortryptoquivaline (FTD), and Tryptoquivaline (FTC)

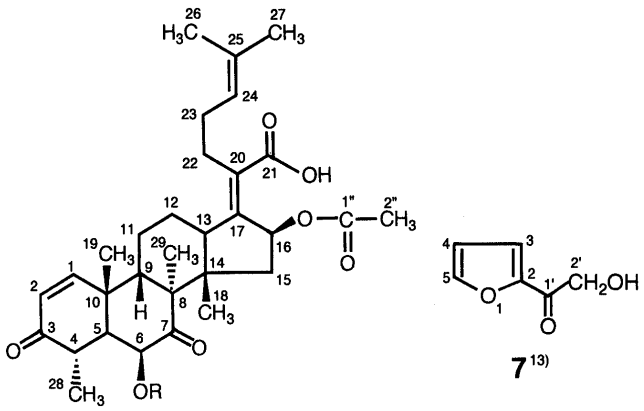
CH_3COO at position 6 [δ 2.12 (3H, s)] disappeared and that of H-6 was shifted to δ 4.02 (-1.21) (Table 3). CS-G (**7**), obtained as colorless needles, was identical with a fungal furan, 2-(1-oxo-2-hydroxyethyl)furan, from *Rhizoctonia solani*[13)] (Chart 4), based on a comparative examination of the physicochemical and spectral data (see Experimental).

On i.p. injection at 30 mg/kg, setosusin (**1**) caused tremor

lasting for *ca.* 30 min in mice. To our knowledge, setosusin is the first fungal meroterpenoid having tremorgenic activity. Tryptoquivaline (FTC) has been described as tremorgenic,[7] but the tremorgenic activities of FTC and nortryptoquivaline (FTD) were subsequently reported to be so weak as to be unrecognizable.[8] In mice, 27-*epi*-tryptoquivaline (**3**) and 27-*epi*-nortryptoquivaline (**2**), caused weak tremor with paralysis, which gradually appeared at *ca.* 30 and 120 min after injection and continued for *ca.* 120 and 30 min, respectively, on i.p. injection at 50 mg/kg. Meanwhile, on i.p. injection at 50 mg/kg in mice, FTC caused weak tremor with paralysis, which also gradually appeared at *ca.* 60 min after injection and continued for *ca.* 90 min. FTD did not cause tremor.

Table 3. ^{1}H-NMR and ^{13}C-NMR Data for CS-E (**5**) and -F (**6**), δ (ppm) from TMS in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	5		6
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	7.32 (d, 10.2)	157.3 (d)	7.31 (d, 10.1)
2	5.87 (d, 10.2)	127.8 (d)	5.85 (d, 10.1)
3		201.4 (s)	
4	2.78 (dq, 12.5, 6.8)	40.4 (d)	3.02 (dq, 11.6, 6.8)
5	2.28 (d, 12.5)	47.2 (d)	2.20 (d, 11.6)
6	5.23 (br s)	73.8 (d)	4.02 (br s)
6-O$\underline{C}$OCH$_3$		168.9 (s)	
6-OCO$\underline{CH_3}$	2.12 (3H, s)	20.7 (q)	
7		208.8 (s)	
8		52.7 (s)	
9	2.62 (dd, 13.0, 2.6)	41.7 (d)	2.63 (dd, 10.3, 2.9)
10		38.2 (s)	
11	1.58, 1.98 (each m)	23.9 (t)	1.56, 1.99 (each m)
12	1.81 (dd, 12.7, 3.4)	25.9 (t)	1.81 (br d, 12.5)
	2.42 (m)		2.39 (m)
13	2.57 (br d, 11.0)	49.4 (d)	2.56 (br d, 11.6)
14		46.6 (s)	
15	1.90 (d, 14.7)	40.6 (t)	1.97 (d, 15.2)
	2.21 (dd, 14.7, 8.3)		2.22 (dd, 15.2, 8.7)
16	5.91 (d, 8.3)	73.5 (d)	5.90 (d, 8.7)
16-O$\underline{C}$OCH$_3$		170.3 (s)	
16-OCO$\underline{CH_3}$	1.96 (3H, s)	20.5 (q)	1.99 (3H, s)
17		147.6 (s)	
18	0.93 (3H, s)	17.9 (q)	0.98 (3H, s)
19	1.45 (3H, s)	27.5 (q)	1.52 (3H, s)
20		130.5 (s)	
21		174.2 (s)	
22	2.47 (2H, m)	28.6 (t)	2.46, 2.53 (each m)
23	2.10, 2.14 (each m)	28.3 (t)	2.12 (2H, m)
24	5.11 (t, 7.2)	122.8 (d)	5.11 (t, 7.3)
25		132.9 (s)	
26	1.61 (3H, s)	17.8 (q)	1.61 (3H, s)
27	1.69 (3H, s)	25.7 (q)	1.69 (3H, s)
28	1.28 (3H, d, 6.8)	13.1 (q)	1.23 (3H, d, 6.8)
29	1.18 (3H, s)	18.3 (q)	1.13 (3H, s)



5 (R=COCH$_3$)[12a, b]

6 (R=H)[12a, c]

7[13]

Chart 4. Structures of CS-E (**5**), CS-F (**6**), and CS-G (**7**)

Experimental

The general procedures for the chemical experiments were the same as described in our preceding report.[2c]

Isolation of CS-A (1)—G (7) *Corynascus setosus* IFM4648[4] was cultivated on sterilized rice (200 g/flask × 300) at 25 °C for 17 d. The moldy rice was extracted with AcOEt (72 l) with shaking at room temperature for 2 h two times to give a crude extract (331 g), which was partitioned with *n*-hexane–MeOH (1 : 1, v/v) (9.3 l) into the *n*-hexane (fatty) layer (156 g) and the MeOH (defatted) layer (154 g). The defatted layer was then partitioned with AcOEt–H_2O (1 : 1) (4.15 l) into the AcOEt layer (129.2 g) and the aqueous layer (23.9 g). The AcOEt layer was chromatographed on a silica gel column with *n*-hexane–AcOEt (2 : 1), (1 : 1), and (1 : 2), AcOEt, and MeOH two times to give fractions I (10.2), II (18.6), III (5.6), and IV (10.5 g). Fraction III was further chromatographed on silica gel columns with *n*-hexane–AcOEt and $CHCl_3$–MeOH repeatedly to afford **5**, **6**, **1**, and **7** (yields after trituration with MeOH, MeOH, MeOH, and *n*-hexane–AcOEt: 150, 10, 37, and 220 mg, respectively). Fraction I was chromatographed on silica gel columns with *n*-hexane–AcOEt repeatedly and on an octadecyl silica gel (ODS) column with CH_3CN–H_2O to furnish **3** and **2** (yields after trituration with MeOH and MeOH: 196 and 133 mg, respectively). Fraction II was also chromatographed in a similar manner to afford **4** (yield after trituration with $CHCl_3$–MeOH: 29 mg).

CS-A (**1**): Colorless prisms, mp 174.5—175.5 °C (from *n*-hexane–AcOEt). $[\alpha]_D^{24}$ +67° (*c*=0.77, MeOH). HRFAB-MS *m/z*: 515.2634 ($C_{29}H_{39}O_8$ requires 515.2645 [(M+H)$^+$]). UV λ_{max}^{MeOH} nm (log ε): 274 (3.95). IR ν_{max}^{KBr} cm^{-1}: 1770, 1730, 1640, 1440, 1240, 1160.

CS-B (**2**): Colorless needles, mp 236—238 °C (from MeOH). $[\alpha]_D^{18}$ +196° (*c*=0.292, $CHCl_3$). HRFAB-MS *m/z*: 533.2023 ($C_{28}H_{29}N_4O_7$ requires 533.2036 [(M+H)$^+$]). UV λ_{max}^{MeOH} nm (log ε): 209 (4.64), 290 (sh, 3.80), 307 (sh, 3.53), 318 (sh, 3.43). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3000, 1780, 1720, 1660, 1600. CD $\Delta\varepsilon$ (nm): 0 (325), +1.9 (305), +4.1 (278), 0 (259), −2.6 (252) (*c*=0.011, EtOH). [FTD[8]: mp 255-257 °C, $[\alpha]_D^{25}$ +115°, CD $\Delta\varepsilon$ (nm): 0 (325), +2.3 (305), 0 (285), −6.3 (252) (*c*=0.010, EtOH)].

CS-C (**3**): Colorless needles, mp 225—227 °C (from MeOH). $[\alpha]_D^{25}$ +138° (*c*=0.020, $CHCl_3$). HRFAB-MS *m/z*: 547.2183 ($C_{29}H_{31}N_4O_7$ requires 547.2193 [(M+H)$^+$]). UV λ_{max}^{MeOH} nm (log ε): 209 (sh, 4.67), 289 (sh, 3.83), 306 (sh, 3.54), 318 (sh, 3.44). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3000, 1790, 1735, 1675, 1610. CD $\Delta\varepsilon$ (nm): 0 (325), +1.8 (305), +3.6 (281), 0 (260), −1.9 (253) (*c*=0.011, EtOH). [FTC[8]: mp 245—247 °C, $[\alpha]_D^{25}$ +90.5°, CD $\Delta\varepsilon$ (nm): 0 (330), +2.3 (307), 0 (285), −5.3 (253) (*c*=0.010, EtOH)].

CS-D (**4**): Colorless powder, mp 164.5—170.5 °C (from $CHCl_3$–MeOH). $[\alpha]_D^{21.5}$ −124° (*c*=0.021, MeOH). HRFAB-MS *m/z*: 425.1389 ($C_{23}H_{22}N_4O_2K$ requires 425.1380 [(M+K)$^+$]). UV λ_{max}^{MeOH} nm (log ε): 220 (4.65), 273 (4.06), 290 (3.90), 305 (3.60), 318 (3.47). IR ν_{max}^{KBr} cm^{-1}: 3258, 1680, 1600, 1460.

CS-E (**5**): Colorless needles, mp 217—218.5 °C (from MeOH). $[\alpha]_D^{22}$ −129° (*c*=1.00, MeOH). This compound was identical with authentic helvolic acid in terms of mixed mp, ^{1}H- and ^{13}C-NMR spectra.

CS-F (**6**): Colorless needles, mp 201—204 °C (from MeOH) (lit.[12c] 202.5—203 °C).

CS-G (**7**): Colorless needles, mp 87—88 °C (from $CHCl_3$–Et_2O) (lit.[13] 83—84 °C). UV ν_{max}^{MeOH} nm (log ε): 269 (4.15) [lit.[13] 269 (4.20)]. IR ν_{max}^{KBr} cm^{-1}: 3400, 1680, 1470, 1420, 1280, 1120. ^{1}H-NMR ($CDCl_3$): δ 3.27 (t, *J*=4.9 Hz, OH-2'), 4.75 (2H, d, *J*=4.9 Hz, H_2-2'), 6.61 (dd, *J*=3.6, 1.7 Hz, H-4), 7.31 (dd, *J*=3.6, 0.7 Hz, H-3), 7.64 (dd, *J*=1.7, 0.7 Hz, H-5). ^{13}C-NMR ($CDCl_3$): δ 65.1 (t, C-2'), 112.6 (d, C-4), 117.9 (d, C-3), 147.1 (d, C-5), 187.7 (s, C-1').

Alkaline Treatment of CS-A (1) A solution of **1** (20 mg) in 0.1% KOH–MeOH (1.5 ml) was stirred at room temperature for 45 min, and then neutralized with Amberlite MB-3. The reaction mixture was chromatographed on a silica gel column with $CHCl_3$ and $CHCl_3$–MeOH

Table 4. Fractional Coordinates and Isotropic Thermal Parameters for Non-hydrogen Atoms of Deacetyl-Dehydrated Setosusin (**8**) with Estimated Standard Deviations in Parentheses

Atom	x	y	z	B_{eq}
C- 1	0.6388 (6)	0.8499 (4)	0.2212 (7)	4.1 (2)
C- 2	0.7372 (6)	0.8448 (5)	0.2677 (8)	4.5 (2)
C- 3	0.8388 (6)	0.8436 (5)	0.2024 (7)	4.8 (2)
C- 4	0.7938 (5)	0.9242 (4)	0.0187 (7)	3.5 (2)
C- 5	0.6720 (5)	0.9363 (4)	0.0407 (6)	3.0 (1)
C- 6	0.6217 (6)	0.9753 (4)	−0.0694 (7)	3.6 (2)
C- 7	0.5104 (5)	1.0057 (4)	−0.0405 (8)	3.3 (2)
C- 8	0.4346 (5)	0.9389 (4)	−0.0078 (6)	3.0 (1)
C- 9	0.4846 (5)	0.8911 (4)	0.0992 (6)	3.0 (1)
C-10	0.6052 (5)	0.8668 (3)	0.0884 (6)	3.1 (1)
C-11	0.4130 (7)	0.8209 (5)	0.1282 (10)	4.4 (2)
C-12	0.2962 (6)	0.8434 (4)	0.1322 (7)	4.1 (2)
C-13	0.2537 (5)	0.9085 (4)	0.0899 (7)	3.6 (2)
C-14	0.3256 (5)	0.9717 (4)	0.0385 (6)	3.1 (1)
C-15	0.2724 (6)	1.0276 (4)	−0.0570 (7)	3.5 (2)
C-16	0.8540 (7)	0.9986 (6)	0.061 (1)	5.5 (3)
C-17	0.8235 (7)	0.9058 (7)	−0.1120 (8)	5.1 (2)
C-18	0.4110 (7)	0.8884 (5)	−0.1239 (8)	4.1 (2)
C-19	0.6230 (8)	0.7917 (5)	0.0119 (9)	4.4 (2)
C-20	0.1343 (7)	0.9243 (7)	0.098 (1)	5.5 (3)
C- 1′	0.3211 (6)	1.0979 (4)	0.1263 (7)	4.1 (2)
C- 2′	0.2767 (5)	1.1080 (4)	−0.0008 (7)	3.7 (2)
C- 3′	0.1694 (6)	1.1544 (4)	−0.0036 (8)	4.5 (2)
C- 4′	0.1974 (5)	1.2289 (4)	−0.0550 (7)	3.7 (2)
C- 5′	0.2993 (5)	1.2278 (4)	−0.0846 (7)	3.4 (2)
C- 6′	0.3694 (8)	1.2870 (5)	−0.141 (1)	4.6 (2)
C- 7′	0.1155 (8)	1.2948 (5)	−0.0670 (10)	4.7 (2)
O- 3	0.9193 (5)	0.8221 (4)	0.2516 (6)	8.4 (2)
O- 3a	0.8435 (4)	0.8578 (3)	0.0827 (5)	4.5 (1)
O-14a	0.3472 (3)	1.0226 (2)	0.1462 (4)	3.5 (1)
O- 1′	0.3344 (5)	1.1467 (3)	0.2015 (5)	6.4 (2)
O- 2′a	0.3513 (3)	1.1591 (2)	−0.0624 (4)	4.0 (1)
O- 3′	0.0854 (4)	1.1269 (3)	0.0299 (7)	6.7 (2)

(1 : 1) to give **8** (12 mg) and **1** (recovered, 5 mg). Compound **8**: Colorless prisms, mp 244—246 °C [from *n*-hexane–CH_3CN–acetone (1 drop)], $[\alpha]_D^{24}$ +170° (c=0.17, MeOH). HRFAB-MS m/z: 455.2428 ($C_{27}H_{35}O_6$ requires 455.2433 $[(M+H)]^+$. UV λ_{max}^{MeOH} nm (log ε): 274 (3.97). IR ν_{max}^{KBr} cm^{-1}: 1770, 1695, 1620, 1430, 1220, 1150.

X-Ray Crystallographic Analysis of Deacetyl-Dehydrated Setosusin (8) For X-ray crystallographic analysis of **8** [orthorhombic, space group $P2_12_12_1$, lattice constants a=12.514(1), b=17.110(2), c=10.952(1) Å, V=2344.9(4) Å^3, Z=4, D_{calcd}=1.287 g/cm^3], the data on 2057 observed reflections [$I>3.00\sigma(I)$] within the range of $0°<2\theta<135.1°$, measured with CuK_α radiation, were solved directly by the SHELXS86 program,[14] and the solution was refined by the full-matrix least-squares method with anisotropic and isotropic temperature factors for all non-hydrogen atoms and all hydrogen atoms, respectively, to give a final R value of 0.067, including the contributions of all hydrogen atoms in **8**.[15] The final fractional coordinates of all non-hydrogen atoms with estimated standard deviations are listed in Table 4.

Evaluation of Tremorgenic Activity of Sample Compounds Polyoxyethylene sorbitan monooleate (Tween 80, Nacalai) (0.04 ml) was added to a powdered sample compound (6.0 mg) under stirring to make a gruel. Saline (Otsuka) was added to the gruel under stirring to prepare a suspension (1.2 ml). This suspension (0.27—0.30 ml) was injected intraperitoneally into a mouse (ddY, male, 27—30 g). Three mice were employed for evaluation of the tremorgenic activity of each sample compound.

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