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Further Characterization of Seven Bis(naphtho-γ-pyrone) Congeners of Ustilaginoidins, Coloring Matters of *Claviceps virens* (*Ustilaginoidea virens*)

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Seven new bis(naphtho-γ-pyrone) derivatives, named ustilaginoidins D (**8**), E (**9**), F (**10**), G (**11**), H (**12**), I (**13**), and J (**14**), were isolated in addition to ustilaginoidins A (**1**), B (**2**), and C (**3**) from *Claviceps virens*. Their structures were elucidated chiefly on the basis of nuclear magnetic resonance data.

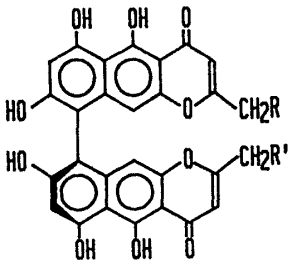
Keywords——*Claviceps virens*; *Ustilaginoidea virens*; ustilaginoidin; bis(naphtho-γ-pyrone); mycotoxin; ^{1}H-NMR; ^{13}C-NMR

Ustilaginoidins A (**1**), B (**2**), and C (**3**) are red pigments of smutted balls formed by the infection of *Claviceps virens* (COOKE) TAKAHASHI (anamorph: *Ustilaginoidea virens*) on the rice plant, and their structures (**1**—**3**) were established as hexahydroxybis(naphtho-γ-pyrone) derivatives by chemical, physical, and synthetic methods.[1,2] The absolute configurations proposed[3] were confirmed by our recent work.[4] Chaetochromin A (**4**) is a related tetrahydrohexahydroxybis(naphtho-γ-pyrone) produced by *Chaetomium* spp.[5] and exhibits toxicity to experimental animals[6] as well as antitumor activities.[7] The characterization of the congeners, chaetochromins B (**5**), C (**6**), and D (**7**), and the determination of the absolute configurations were also reported in previous papers.[4,8]

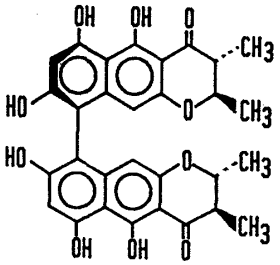
For biological and chemical studies of these naphtho-γ-pyrone derivatives, the metabolites of *Claviceps virens* were reexamined and, besides the hitherto known ustilaginoidins A, B, and C (**1**—**3**), seven new congeners, named ustilaginoidins D—J (**8**—**14**), which will be interesting materials for studies on structure–activity relationships, were isolated. This paper presents details of the isolation and structure elucidation of the metabolites.

Silica gel chromatography of the ethyl acetate extract of the infected rice afforded, besides the major metabolite ustilaginoidin A (**1**), minor metabolites named ustilaginoidins D (**8**), E (**9**), F (**10**), and G (**11**) with CH_2Cl_2 as the developing solvent and ustilaginoidins A (**1**), H (**12**), B (**2**), I (**13**), C (**3**) and J (**14**) with ethyl acetate–hexane (2 : 1) as the developing solvent. Further purification was performed by high-performance liquid chromatography (HPLC) on Nucleosil 50-5.

Ustilaginoidins D (**8**), E (**9**), and F (**10**) showed nearly the same ultraviolet (UV) and infrared (IR) spectra as ustilaginoidin A (**1**) and chaetochromin A (**4**), suggesting the presence of the same naphtho-γ-pyrone chromophore in the molecules. Molecular formulae determined by high-resolution mass spectroscopy (MS) indicated $C_{30}H_{26}O_{10}$, $C_{29}H_{24}O_{10}$, and $C_{28}H_{22}O_{10}$, respectively, for **8**, **9**, and **10**. Proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra (Tables I and II) of **8**, **9**, and **10** indicated the presence of two *trans*-2,3-dimethyl groups, one *trans*-2,3-dimethyl group and one 2-methyl group, and two 2-methyl groups, respectively, on the 2,3-dihydropyran-4-one ring. Thus they must be stereoisomers of chaetochromin A (**4**), chaetochromin C (**6**), and cephalochromin (**15**), a



1 : R=R′=H
2 : R=H, R′=OH
3 : R=R′=OH



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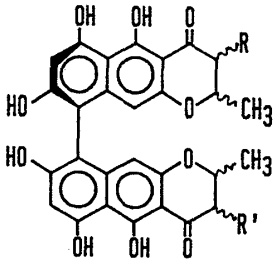
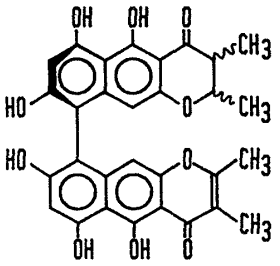
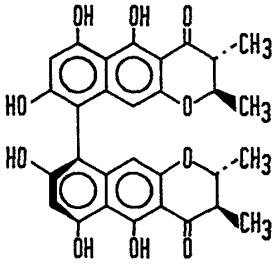


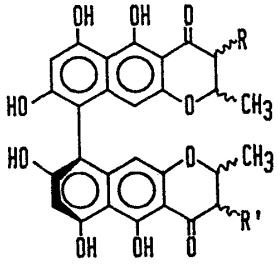
5 : R=R′=CH_3
6 : R=H, R′=CH_3
15 : R=R′=H



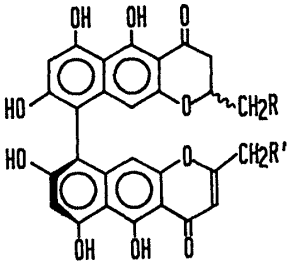
7



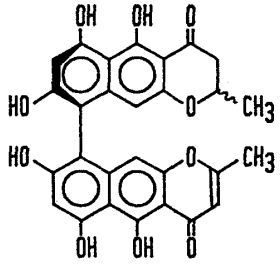
8



9 : R=H, R′=CH_3
10 : R=R′=H



11 : R=R′=H
12 : R=H, R′=OH
13 : R=OH, R′=H
14 : R=R′=OH



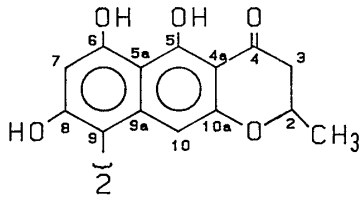
16

Chart 1

metabolite of *Cephalosporium* sp. (later assigned as *Acremonium butyri*), *Nectria viridescens* (anamorph: *Acremonium butyri*) and *N. flavoviridis* (anamorph: *Fusarium melanochlorum*), and *Verticillium* sp.,[4,9-11] respectively. The circular dichroism (CD) of **8**, **9**, and **10** showed negative Cotton effects around 295 nm and positive Cotton effects around 265 nm, as found for ustilaginoidin A (**1**), suggesting the *R* configuration at the 9–9′ positions, which is antipodal to that in **4**, **6**, and **15**.[4] Although the ^{13}C-NMR spectra of **8**, **9**, and **10** were nearly the same as those of **4**, **6**, and **15**, respectively, comparison of the melting points (**8**, mp 244—247 °C; **9**, mp 215—218 °C; **10**, mp 285—288 °C; **4**, mp 207—210 °C; **6**, mp 214—217 °C; and **15**, mp > 300 °C) and ^{1}H-NMR spectral data of 2,2′-H and 3,3′-H suggested that **8**, **9**, and **10** were diastereomers rather than enantiomers of **4**, **6**, and **15**, respectively. Thus the structures of ustilaginoidins D, E, and F were determined as **8**, **9**, and **10**.

Ustilaginoidins G (**11**), H (**12**), I (**13**), and J (**14**) also showed nearly the same UV and IR spectra as ustilaginoidin A (**1**), indicating the presence of the same naphtho-γ-pyrone chromophore in the molecules. Molecular formulae determined by high MS (by fast atom bombardment (FAB)-MS for **14**) indicated $C_{28}H_{20}O_{10}$, $C_{28}H_{20}O_{11}$, $C_{28}H_{20}O_{11}$ and $C_{28}H_{20}O_{12}$, respectively. The ^{1}H- and ^{13}C-NMR spectra of **11**—**14** (Tables I and II) showed

TABLE I. ^{1}H-NMR Data for Ustilaginoidins and Related Compounds



Proton	1	2	3	4	6	8	9
2, 2′				4.17 dq J=11.0, 6.1	4.17 dq J=11.0, 6.1 4.49 ddq J=9.8, 5.5, 6.1	4.11 dq J=10.7, 6.1	4.08 dq J=10.4, 6.1 4.39 ddq J=11.6, 3.7, 6.1
3, 3′	6.16 s[a]	6.16 s[a] 6.17 s[a]	6.17 s[a]	2.62 dq J=11.0, 6.7	2.62 dq J=11.0, 6.7 2.68 d J=9.8 2.68 d J=5.5	2.67 dq J=10.7, 7.3	2.66 dq J=10.4, 6.7 2.64 dd J=17.7, 3.7 2.72 dd J=17.7, 11.6
7, 7′	6.58 s	6.59 s 6.60 s	6.61 s	6.48 s	6.50 s 6.47 s	6.53 s	6.51 s 6.53 s
10, 10′	6.18 s[a]	6.17 s[a] 6.20 s[a]	6.21 s[a]	5.93 s	5.93 s 5.93 s	5.94 s	5.90 s 5.91 s
2, 2′-CH_3	2.23 s	2.25 s		1.42 d J=6.1	1.42 d J=6.1 1.38 d J=6.1	1.42 d J=6.1	1.39 d J=6.1 1.40 d J=6.1
2, 2′-$C\underline{H}_2OH$		4.27 d J=6.1	4.28 d J=5.9				
2, 2′-$CH_2O\underline{H}$		5.76 t J=6.1	5.76 t J=5.9				
3, 3′-CH_3				1.24 d J=6.7	1.24 d J=6.7	1.24 d J=7.3	1.22 d J=6.7
5, 5′-OH	15.81 s	15.53 br 15.53 br	[b]	15.27 s	15.25 s 15.06 s	15.36 s	15.04 s 15.29 s
6, 6′-OH	10.01 s	10.05 s 10.05 s	10.01 s	9.65 s	9.63 s 9.63 s	9.76 s	9.70 s 9.73 s
8, 8′-OH	9.76 s	9.83 s 9.81 s	9.85 s	5.70 br	5.84 br 5.84 br	5.44 s	5.71 s 5.83 s

that the two halves of these molecules are not the same. In the ^{13}C-NMR spectrum of ustilaginoidin A (**1**) fourteen signals appear, while in those of **11**—**14** twenty-eight signals, two signals corresponding to each one signal in **1**, were observed. In ustilaginoidin G (**11**), the presence of one methyl group on a secondary carbon atom and one olefinic carbon atom was shown by ^{1}H- and ^{13}C-NMR (Tables I and II). These data suggested that, in ustilaginoidin G (**11**), one half of the molecule is a naphtho-γ-pyrone as in **1**, while the other half of the molecule is a naphtho-2,3-dihydro-γ-pyrone as in cephalochromin (**15**). Since the CD of **11** showed the same Cotton effects as **1**, the absolute configuration of the 9–9′ bond must be *R*. Comparison of the melting points (**11**, mp 257 °C and **16**, mp > 300 °C) and ^{1}H-NMR data suggested that the compound (**11**) is not an enantiomer but a diastereomer of dihydroisoustilaginoidin A (**16**) from *Verticillium* sp.[10]

Comparison of the ^{1}H- and ^{13}C-NMR spectra of ustilaginoidin H (**12**) with those of G (**11**) (Tables I and II) clearly indicated that the methyl group at C-2′ of the pyran-4-one ring in

TABLE I. (continued)

Proton	10	11	12	13	14	15	16
2, 2′	4.43 ddq J=11.2, 4.1, 5.9	4.42 ddq J=11.6, 3.1, 6.1	4.44 ddq J=2.4, 11.6, 6.1	4.34 ddt J=12.2, 3.1, 10.4	4.34 ddt J=12.2, 2.4, 11.0	4.47 tq J=7.3, 6.1	4.47 ddq J=12.2, 6.7, 3.1
3, 3′	2.68 dd J=17.6, 4.1 2.75 dd J=17.6, 11.2	2.80 dd J=17.7, 11.6 2.67 dd J=17.7, 3.1	2.81 dd J=17.1, 11.6 2.68 dd J=17.1, 2.4	2.91 dd J=17.7, 3.1 2.60 dd J=17.7, 12.2	2.91 dd J=17.1, 12.2 2.60 dd J=17.1, 2.4	2.66 d J=7.3	2.80 dd J=17.4, 12.2 2.68 dd J=17.4, 3.1
		5.64 s	5.65 s	5.70 s	5.69 s		5.66 s
7, 7′	6.55 s	6.45 s 6.57 s	6.46 s 6.58 s	6.46 s 6.57 s	6.46 s 6.58 s	6.49 s	6.47 s 6.58 s
10, 10′	5.95 s	6.17 s 6.18 s	6.20 s 6.21 s	6.17 s 6.19 s	6.20 s 2H	6.16 s	6.17 s 6.18 s
2, 2′-CH_3	1.41 d J=5.9	1.27 d J=6.1 2.26 s	1.27 d J=6.1	2.27 s		1.39 d J=6.1	1.28 d J=6.7 2.27 s
2, 2′-$C\underline{H}_2OH$			4.30 d J=6.1	3.52 dd J=10.4, 4.9	3.52 dd J=11.0, 4.9 4.30 d J=5.5		
2, 2′-$CH_2O\underline{H}$			5.77 t J=6.1	4.98 t J=4.9	4.98 t J=4.9 5.78 t J=5.5		
5, 5′-OH	15.16 s	*b)*	*b)*	*b)*	*b)*	14.90 s	*b)*
6, 6′-OH	9.74 s	10.00 s 2H	9.99 s 2H	9.97 s 2H	9.98 s 2H	9.57 s	9.95 s 9.83 s
8, 8′-OH	5.55 s	9.76 s 2H	9.79 s 9.77 s	9.77 s 9.77 s	9.79 s 9.80 s	5.90 s	9.78 s 2H

Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard and coupling constants are given in Hz (s, singlet; d, doublet; t, triplet; br, broad). Measured in DMSO-d_6 to **1**—**3**, **11**—**14**, **16** and in $CDCl_3$ to **4**, **6**, **8**—**10**, **15**. *a)* Assignments may be interchanged in each column. *b)* *ca.* 15 ppm, br.

11 was replaced by a hydroxymethyl group.

Ustilaginoidin I (**13**) was proved to be an isomer of **12** by the MS, and ^{1}H- and ^{13}C-NMR data of **12** (Tables I and II) showed the methyl group at C-2 of the 2,3-dihydropyran-4-one ring in **11** was replaced by a hydroxymethyl group.

The molecular formula of ustilaginoidin J (**14**) showed the presence of one more hydroxyl group than in **12** and **13**, and the ^{1}H- and ^{13}C-NMR data (Tables I and II) indicated that both of the two methyl groups in **11** were replaced by hydroxymethyl groups.

The CD of **12**—**14** showed negative Cotton effects around 295 nm and positive Cotton effects around 265 nm as in the case of ustilaginoidin A (**1**), suggesting the *R* configuration of the 9–9′ positions.[4] Thus the structures (**12**, **13**) of ustilaginoidins H and I correspond to the dihydro derivatives of ustilaginoidin B (**2**) from the same source,[2] while the structure (**14**) of ustilaginoidin J corresponds to the dihydro derivative of ustilaginoidin C (**3**) from the same source.[2]

The results of the biological tests and the structure–activity relationship of ustilaginoidins and related compounds will be reported in a forthcoming paper.

Experimental

All melting points were determined on a Yanagimoto MP micromelting point apparatus and are uncorrected.

TABLE II. ^{13}C-NMR Data for Ustilaginoidins and Related Compounds

Carbon atom	1	2	3	4	6	8	9
2, 2′	169.7	169.8	172.5	78.4	78.4	78.4	73.4
		172.5			73.4		78.4
3, 3′	105.9	103.4	103.5	46.2	46.2 (d)	46.1	43.1 (t)
		105.9			43.3 (t)		46.0 (d)
4, 4′	183.3	183.4	183.6	200.8	200.8	200.7	198.2
		183.5			198.3		200.7
4a, 4′a	101.7	101.7	102.3	101.8	101.9	101.6	101.7
		102.2			101.6		101.9
5, 5′	162.6	162.6	162.7	164.4	164.7	164.6	164.4
		162.7			164.5		164.5
5a, 5′a	105.9[a]	105.9[a]	106.0[a]	105.6	105.6	105.6	105.5
		106.0[a]			105.6		105.5
6, 6′	158.0[b]	158.1[b]	158.1[b]	159.8	160.0	159.9	160.0
		158.1[b]			159.8		160.2
7, 7′	98.2[c]	98.2[c]	98.3[c]	99.7	99.8[a]	99.7	100.0[a]
		98.4[c]			99.7[a]		100.1[a]
8, 8′	159.2[b]	159.2[b]	159.3[b]	160.8	161.1	161.1	160.9
		159.3[b]			161.0		161.0
9, 9′	106.4[a]	106.5[a]	106.5[a]	102.0	102.6	102.0	102.2
		106.5[a]			102.0		102.4
9a, 9′a	139.8	139.9	140.0	141.9	142.0	141.7	141.8
		140.0			141.9		141.9
10, 10′	100.8[c]	100.9[c]	101.0[c]	99.3	99.5[a]	99.2	99.3[a]
		101.0[c]			99.3[a]		99.5[a]
10a, 10′a	151.9	151.7	151.8	156.3	156.4	156.2	156.0
		152.0			156.3		156.1
2-CH_3, 2′-CH_3	20.2	20.3		19.6	20.9	19.6	19.6
					19.6		20.9
2-CH_2OH, 2′-CH_2OH		59.9	59.9				
3-CH_3, 3′-CH_3				9.9	10.1	10.1	10.0

The ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 (^{1}H 400 MHz and ^{13}C 100 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are recorded in ppm (δ). MS were taken on JEOL JMS-D300 and JEOL JMS-HX100 instruments. UV and IR spectra were measured with a Shimadzu UV-240 spectrophotometer and a JASCO A-102 infrared spectrophotometer. The $[\alpha]_D$ values were measured with a JASCO DIP-140 digital polarimeter. CD spectra were recorded on a JASCO J-20 spectropolarimeter.

Kieselgel 60 F_{254} (Merck) precoated plates were used for thin-layer chromatography (TLC) and the spots were detected by UV illumination. Column chromatography was carried out on 70—230 mesh silica gel (Merck). HPLC was carried out by using a Waters M45J pump with an Oyo-Bunko Uvilog 7 UV detector.

Isolation of Metabolites from *Claviceps virens*——Infected rice (43 g), collected at Kamikita and Nishitsugaru, Aomori, in September 1985, was extracted three times with EtOAc (200 ml) and three times more with acetone (200 ml), each for 24 h at room temperature. Each extract was concentrated under reduced pressure. Each residue was dissolved in EtOAc–hexane and chromatographed over silica gel (treated with 3% oxalic acid) with CH_2Cl_2 as the developing solvent to afford fractions 1A and 2A, and with EtOAc–hexane (2 : 1) to afford fraction 3A from the EtOAc extract, and fraction 1B from the acetone extract. Each fraction was purified by HPLC (Nucleosil 50-5, treated with 3% oxalic acid) with EtOAc–hexane (1 : 4) as the developing solvent to yield ustilaginoidins D (**8**) (7.2 mg), E (**9**) (10.4 mg), F (**10**) (5.2 mg), G (**11**) (33.2 mg), and A (**1**) (322.4 mg) from fraction 2A, with EtOAc–hexane (1 : 1) to yield ustilaginoidins A (**1**) (9.3 mg), H (**12**) (7.2 mg), B (**2**) (99.7 mg), I (**13**) (15.6 mg), C (**3**) (14.5 mg), and J (**14**) (5.2 mg) from fraction 3A, and with EtOAc–hexane (1 : 1) to yield ustilaginoidins A (**1**) (37 mg), B (**2**) (28 mg), I (**13**) (3 mg), and C (**3**) (2 mg) from fraction 1B. All fractions were washed with H_2O to remove oxalic acid.

Ustilaginoidins A (1), B (2), and C (3)[2]——Ustilaginoidin A (**1**) was recrystallized from dioxane as red prisms, mp >300 °C, $[\alpha]_D^{20}$ $-329°$ ($c=0.08$, dioxane). MS m/z: 514.0912 (M^+, Calcd for $C_{28}H_{18}O_{10}$: 514.0900). UV $\lambda_{max}^{dioxane}$ nm (ε): 210 (71600), 220 (71400), 250 (40400), 270 (46800), 289 (55300), 329 (4300), 346 (2800), 410 (6400). IR ν_{max}^{KBr} cm^{-1}: 3380, 1655, 1645, 1620, 1580, 1360, 1270, 1150, 1080, 955, 835. CD (dioxane) $[\theta]^{20}$ (nm): 0 (222), +133600

TABLE II. (continued)

Carbon atom	10	11	12	13	14	15
2, 2′	73.3	72.9	72.7	77.4	77.4	73.3
		169.8	172.3	169.8	172.5	
3, 3′	43.1	42.7	42.6	37.8	37.8	43.1
		105.8	103.2	105.9	103.4	
4, 4′	198.2	183.3	183.4	183.4	183.6	198.4
		198.1	198.0	198.1	198.1	
4a, 4′a	101.9	101.7	101.6	101.7	101.9	102.2
		101.7	102.0	101.8	102.2	
5, 5′	164.6	162.6	162.4	162.7	162.6	164.4
		164.8	164.6	164.7	164.7	
5a, 5′a	105.5	104.3[a]	104.2[a]	104.4[a]	104.4[a]	105.4
		105.8[a]	105.7[a]	105.9[a]	105.9[a]	
6, 6′	160.2	157.9[b]	157.8[b]	157.9[b]	157.9[b]	160.1
		158.8[b]	158.7[b]	158.8[b]	158.9[b]	
7, 7′	100.1	98.1[c]	98.1[c]	98.1[c]	98.3[c]	100.1
		98.5[c]	98.3[c]	98.6[c]	98.6[c]	
8, 8′	161.1	159.1[b]	159.0[b]	159.1[b]	159.2[b]	160.7
		160.1[b]	160.0[b]	160.1[b]	160.1[b]	
9, 9′	102.4	106.6[a]	106.5[a]	106.7[a]	106.7[a]	102.4
		107.0[a]	106.8[a]	107.0[a]	107.0[a]	
9a, 9′a	141.9	139.8	139.7	139.8	139.9	142.1
		141.7	141.5	141.7	141.7	
10, 10′	99.4	100.1[c]	99.9[c]	100.1[c]	100.1[c]	99.6
		100.8[c]	100.7[c]	100.8[c]	100.9[c]	
10a, 10′a	156.2	151.9	151.5	152.0	151.7	156.3
		154.9	154.8	154.9	154.9	
2-CH_3, 2′-CH_3	20.9	20.2	20.3	20.2		20.9
		20.4				
2-CH_2OH, 2′-CH_2OH			59.7	62.8	59.9	
					62.8	

Measured in DMSO-d_6 for **1**—**3**, **11**—**14**, and in $CDCl_3$ for **4**, **6**, **8**—**10**, **15**. *a*—*c*) Assignments may be interchanged in each column.

(231), +308400 (261), 0 (275), −469800 (290), 0 (328), +48300 (345), +3100 (425). Its identity with an authentic sample of ustilaginoidin A was confirmed by UV, IR, TLC, and CD.

Ustilaginoidin B (**2**) was recrystallized from EtOAc as red prisms, mp 255—257 °C, $[\alpha]_D^{20}$: −297° (c=0.06, dioxane). MS m/z: 530.0845 (M^+, Calcd for $C_{28}H_{18}O_{11}$: 530.0849). UV $\lambda_{max}^{dioxane}$ nm (ε): 205 (26700), 223 (37100), 255 (32000), 272 (46600), 290 (56200), 330 (4700), 345 (4500), 410 (9000). IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1650, 1620, 1590, 1370, 1270, 1155, 1090, 960, 840. CD (dioxane) $[\theta]^{20}$ (nm): +62900 (232), +143700 (263), 0 (276), −224600 (291), 0 (323), +22900 (346), +1800 (427). Its identity with an authentic sample of ustilaginoidin B was confirmed by UV, IR, TLC, and CD.

Ustilaginoidin C (**3**) was recrystallized from EtOAc–hexane as a red powder, mp >300 °C, $[\alpha]_D^{20}$ −255° (c=0.09, dioxane). FAB-MS m/z: 547.0898 ($(M+H)^+$, Calcd for $C_{28}H_{19}O_{12}$: 547.0878). UV $\lambda_{max}^{dioxane}$ nm (ε): 225 (34900), 252 (29800), 275 (43900), 289 (52900), 329 (4300), 346 (2900), 410 (6700). IR ν_{max}^{KBr} cm^{-1}: 3350, 1652, 1645, 1620, 1615, 1581, 1360, 1260, 1150, 1096, 1080, 1066, 1010, 960, 832. CD (dioxane) $[\theta]^{20}$ (nm): +105100 (231), +73600 (241), +233600 (264), 0 (276), −373700 (292), 0 (330), +38000 (346), +2600 (430). Its identity with an authentic sample of ustilaginoidin C was confirmed by UV, IR TLC, and CD.

Ustilaginoidin D (8)——Recrystallized from CH_2Cl_2–hexane as a yellow powder, mp 244—247 °C, $[\alpha]_D^{20}$ −188° (c=0.03, dioxane). MS m/z: 546.1522 (M^+, Calcd for $C_{30}H_{26}O_{10}$: 546.1526). UV $\lambda_{max}^{dioxane}$ nm (ε): 210 (109200), 227 (66500), 270 (49100), 282 (45900), 294 (50600), 325 (13300), 338 (7900), 415 (8700). IR ν_{max}^{KBr} cm^{-1}: 3400, 1655, 1650, 1640, 1630, 1590, 1440, 1380, 1360, 1350, 1260, 1150, 1130, 1090, 1015, 840. CD (dioxane) $[\theta]^{20}$ (nm): 0 (217), −72800 (224), 0 (232), +92700 (241), +473900 (266), 0 (275), −191900 (282), −503000 (294), −32400 (325), 0 (331), +34400 (338), +1300 (430).

Ustilagionoidin E (9)——Recrystallized from CH_2Cl_2–hexane as a yellow powder, mp 215—218 °C, $[\alpha]_D^{20}$ −216° (c=0.05, dioxane). MS m/z: 532.1332 (M^+, Calcd for $C_{29}H_{24}O_{10}$: 532.1369). UV $\lambda_{max}^{dioxane}$ nm (ε): 205 (18100), 270 (39100), 293 (50300), 325 (13700), 339 (9500), 415 (9200). IR ν_{max}^{KBr} cm^{-1}: 3400, 1644, 1635, 1590, 1507, 1465, 1449, 1386, 1368, 1348, 1150, 1130, 1090, 1080, 1061, 1023, 842. CD (dioxane) $[\theta]^{20}$ (nm): −68600 (224), 0 (230), +91500 (241), +411900 (265), 0 (274), −1487300 (280), −446200 (294), −30300 (322), 0 (331), +30300 (337), +1100 (430).

Ustilaginoidin F (10)——Recrystallized from CH_2Cl_2–hexane as a yellow powder, mp 285—288 °C, $[\alpha]_D^{20}$ −252° (c=0.02, dioxane). MS m/z: 518.1191 (M^+, Calcd for $C_{28}H_{22}O_{10}$: 518.1213). UV $\lambda_{max}^{dioxane}$ nm (ε): 233 (40700), 270 (45500), 292 (56800), 326 (15600), 340 (10000), 414 (10900). IR ν_{max}^{KBr} cm^{-1}: 3340, 1630, 1620, 1572, 1492, 1435, 1373, 1355, 1335, 1325, 1136, 1112, 1070, 925, 860, 828. CD (dioxane) $[\theta]^{20}$ (nm): +57900 (213), 0 (219), −65700 (225), 0 (233), +90200 (241), +465600 (266), 0 (275), −228400 (283), −512400 (295), −30100 (325), 0 (332), +30100 (339), +2100 (426).

Ustilaginoidin G (11)——Recrystallized from EtOAc as red prisms, mp 257 °C, $[\alpha]_D^{20}$ −306° (c=0.05, dioxane). MS m/z: 516.1076 (M^+, Calcd for $C_{28}H_{20}O_{10}$: 516.1056). UV $\lambda_{max}^{dioxane}$ nm (ε): 208 (24300), 225 (35000), 253 (31200), 270 (47100), 290 (56700), 325 (7900), 345 (4900), 410 (8100). IR ν_{max}^{KBr} cm^{-1}: 3400, 1655, 1650, 1640, 1635, 1620, 1590, 1450, 1380, 1360, 1340, 1280, 1150, 1092, 960, 840. CD (dioxane) $[\theta]^{20}$ (nm): +45500 (236), +182100 (264), 0 (276), −232700 (293), 0 (332), +17700 (346), +2000 (425).

Ustilaginoidin H (12)——Recrystallized from dioxane as red prisms, mp 245 °C, $[\alpha]_D^{20}$ −279° (c=0.10, dioxane). MS m/z: 532.1023 (M^+, Calcd for $C_{28}H_{20}O_{11}$: 532.1005). UV $\lambda_{max}^{dioxane}$ nm (ε): 211 (44800), 226 (41700), 255 (27500), 272 (42800), 292 (51900), 326 (8700), 339 (5600), 413 (8100). IR ν_{max}^{KBr} cm^{-1}: 3400, 1655, 1645, 1640, 1630, 1620, 1585, 1400, 1380, 1360, 1340, 1150, 1080, 840. CD (dioxane) $[\theta]^{20}$ (nm): 0 (231), +122200 (240), +341100 (265), 0 (276), −458200 (294), 0 (332), +28500 (343), +3600 (429).

Ustilaginoidin I (13)——Recrystallized from EtOAc–hexane as a yellow powder, mp 253 °C, $[\alpha]_D^{20}$ −262° (c=0.18, dioxane). MS m/z: 532.1023 (M^+, Calcd for $C_{28}H_{20}O_{11}$: 532.1005). UV $\lambda_{max}^{dioxane}$ nm (ε): 211 (33500), 226 (38900), 255 (30500), 270 (45100), 290 (54100), 325 (9000), 338 (6300), 410 (8400). IR ν_{max}^{KBr} cm^{-1}: 3400, 1658, 1650, 1640, 1635, 1590, 1390, 1365, 1345, 1280, 1265, 1180, 1150, 1083, 1020, 958, 838. CD (dioxane) $[\theta]^{20}$ (nm): −53800 (225), 0 (230), +125500 (241), +370600 (265), 0 (275), −481200 (293), 0 (331), +30200 (343), +4800 (432).

Ustilaginoidin J (14)——Recrystallized from EtOAc as red prisms, mp 205—208 °C, $[\alpha]_D^{20}$ −215° (c=0.07, dioxane). FAB-MS m/z: 549.1041 ($(M+H)^+$, Calcd for $C_{28}H_{21}O_{12}$: 549.1035). UV $\lambda_{max}^{dioxane}$ nm (ε): 205 (22500), 230 (33000), 255 (27600), 272 (42600), 290 (50500), 325 (9200), 339 (6500), 410 (8200). IR ν_{max}^{KBr} cm^{-1}: 3380, 1655, 1645, 1640, 1625, 1588, 1360, 1340, 1275, 1270, 1150, 1082, 1018, 842, 838. CD (dioxane) $[\theta]^{20}$ (nm): −2300 (227), 0 (230), +62200 (241), +159300 (265), 0 (276), −217600 (294), 0 (331), +12800 (343), +1900 (430).

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References

1) T. Yabuta and Y. Sumiki, *Nippon Nogei-Kagaku Kaishi*, **9**, 478 (1933); *idem*, *ibid.*, **13**, 106 (1937); T. Yabuta, Y. Sumiki, and H. Igarashi, *ibid.*, **13**, 110 (1937).
2) S. Shibata, A. Ohta, and Y. Ogihara, *Chem. Pharm. Bull.*, **11**, 1174 (1963); S. Shibata, Y. Ogihara, and A. Ohta, *ibid.*, **11**, 1179 (1963); S. Shibata and Y. Ogihara, *ibid.*, **11**, 1576 (1963); E. Morishita and S. Shibata, *ibid.*, **15**, 1772 (1967).
3) S. Shibata and Y. Ogihara, *Tetrahedron Lett.*, **1963**, 1777.
4) K. Koyama, S. Natori, and Y. Iitaka, *Chem. Pharm. Bull.*, **35**, 4049 (1987).
5) S. Sekita, K. Yoshihira, and S. Natori, *Chem. Pharm. Bull.*, **28**, 2428 (1980).
6) K. Ohtsubo, *Proc. Jpn. Assoc. Mycotoxicol.*, **12**, 28 (1980); *idem*, *ibid.*, **15**, 25 (1982); Y. Ito and K. Ohtsubo, *ibid.*, **16**, 22 (1982); *idem*, *Bull. Environ. Contam. Toxicol.*, **39**, 299 (1987).
7) The screening was performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, U.S.A.
8) K. Koyama and S. Natori, *Chem. Pharm. Bull.*, **35**, 578 (1987).
9) G. Tertzakian, R. H. Haskins, G. P. Slater, and L. R. Nestbitt, *Proc. Chem. Soc., London*, **1964**, 195.
10) M. Matsumoto, H. Minato, E. Kondo, T. Mitsugi, and K. Katagiri, *J. Antibiot. Ser. A*, **28**, 602 (1975).
11) S. T. Carey and M. S. R. Nair, *Lloydia*, **38**, 448 (1975).