Tannins of *Stachyurus* Species. II.[1] Praecoxins A, B, C and D, Four New Hydrolyzable Tannins from *Stachyurus praecox* Leaves

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Four new hydrolyzable tannins, praecoxins A (1), B (2), C (3) and D (4), were isolated from leaves of *Stachyurus praecox* SIEB. *et* ZUCC. (Stachyuraceae), and their structures were elucidated.

Keywords tannin; hydrolyzable tannin; *Stachyurus praecox*; Stachyuraceae; praecoxin A; praecoxin B; praecoxin C; praecoxin D; depsidone; valoneoyl group

Leaves and twigs of *Stachyurus praecox* SIEB. *et* ZUCC. (Stachyuraceae), known to be rich in tannins,[2] have been used as diuretics in Japan.[2] Isolation and structures of several hydrolyzable tannins, including *C*-glucosidic ellagitannins, from leaves of *Stachyurus praecox*,[1] and their biogenetic relationship[3] have been reported. Our further investigation of tannins of this plant has resulted in the isolation of additional hydrolyzable tannins, including four new tannins, from the leaves. We present here the full details of the isolation and structure elucidation of these new tannins, named praecoxins A (**1**), B (**2**), C (**3**) and D (**4**).[4]

Results and Discussion

Fresh leaves of *Stachyurus praecox* SIEB. *et* ZUCC. (Stachyuraceae) were homogenized in aqueous acetone, and the concentrated filtrate from the homogenate was extracted with Et_2O and EtOAc, successively. The EtOAc extract was separated by centrifugal partition chromatography (CPC),[5] and fractions containing tannins were further purified by column chromatography over Sephadex LH-20, to give **1**—**4**, along with 1,2,6-tri-*O*-galloyl-*β*-D-glucose,[6] tellimagrandin I,[1] casuarictin (**5**),[1] rugosin C (**6**)[7] and rugosin F.[8]

Praecoxin A (**1**) was obtained as a light-brown powder. The fast-atom bombardment mass spectrum (FAB-MS) of **1** showed the $[M+Na]^+$ ion peak at *m/z* 975, which corresponds to the molecular formula $C_{41}H_{28}O_{27}$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals of a hexahydroxydiphenoyl (HHDP) group [δ 6.54, 6.49 (1H in total, each s, H-3), 6.344, 6.340 (1H in total, each s, H-3′)], a valoneoyl group [δ 7.13 (1H, s, H-6″), 6.58, 6.57 (1H in total, each s, H-3), 6.25, 6.23 (1H, in total, each s, H-3′)] and a glucopyranose core (see Experimental), and the duplication of almost all of these signals indicated that **1** forms an anomer mixture. The coupling constants of the glucose protons are characteristic of those of a glucopyranose core taking the $^4C_1$ conformation. The large difference between the chemical shifts of the two H-6 protons on the $^4C_1$ glucose of each anomer of **1** (α-anomer, $\Delta\delta$ 1.50; β-anomer, $\Delta\delta$ 1.46) indicates that the HHDP moiety of the valoneoyl group (or the HHDP group) is at O-4/O-6 of the glucose core.[9] The remaining HHDP group (or the HHDP moiety of the valoneoyl group) is hence at O-2/O-3, since the chemical shifts of the glucose protons indicate that all the hydroxyl groups on the glucopyranose core, except for the anomeric hydroxyl group, are acylated.

Among the glucose proton signals in the ^{1}H-NMR spectrum of **1**, the H-4 and two H-6 protons showed some upfield shifts, relative to those of pedunculagin (**7**)[10] [$\Delta\delta$ 0.15 (H-4), 0.15 and 0.16 (H-6) (α-anomer); $\Delta\delta$ 0.14 (H-4), 0.14 and 0.15 (H-6) (β-anomer)], while the corresponding shifts of H-2 and H-3 are within 0.09 ppm. Analogous upfield shifts were also observed for the glucose H-4 and H-6 of **6** (which has a valoneoyl group at O-4/O-6),[7] relative to those of **5** [$\Delta\delta$ 0.11 (H-4), 0.15 and 0.12 (H-6)]. Therefore, the valoneoyl group in **1** is assumed to be at O-4/O-6 of the glucose core. A broad positive peak in the short-wavelength region[7,11] ($[\theta]_{223}$ $+1.2\times10^5$, $[\theta]_{236}+1.1\times10^5$) of the circular dichroism (CD) spectrum of **1** indicated that the absolute configuration of the HHDP group and that of the valoneoyl group are both *S*. The orientation of the valoneoyl group in **1** is the same as that in **6**, since the chemical shifts of H-3 and H-3′ of the valoneoyl group in **1** are almost the same as those in **6** [δ 6.53 (H-3) and 6.23 (H-3′)].[7]

Structure **1** for praecoxin A was finally established by partial hydrolysis of **6** with tannase,[12] which afforded **1**. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** is also consistent with this structure (see Experimental).

Praecoxin B (**2**) was obtained as a light-brown powder. The ^{1}H-NMR spectrum indicated that **2** consists of two galloyl groups [δ 7.142, 7.139 (2H in total, each s), 7.13, 7.10 (2H in total, each s)], an HHDP group [δ 6.60, 6.59 (1H in total, each s, H-3), 6.39 (1H, s, H-3′)] and a $^4C_1$ glucose core (see Experimental). A positive Cotton effect at 236 nm ($[\theta]_{236}+1.0\times10^5$) in the CD spectrum of **2** indicated the (*S*)-configuration of the HHDP group.[11] Therefore, praecoxin B is an isomer of tellimagrandin I (**8**)[1,9,10] concerning the locations of the acyl groups on the glucose core.

The upfield shifts of the glucose protons H-2 and H-3 of **2**, relative to the corresponding protons of **8** having galloyl groups at O-2 and O-3 on the glucose core,[10] are ascribable to the anisotropic effects of the HHDP group, which is therefore assigned to be at O-2/O-3.[7] The ^{13}C-NMR spectrum of **2** also shows the signals of two galloyl groups, an HHDP group and a glucose core (see Experimental). The difference between the chemical shifts of the glucose C-1 signals of the two anomers of **2** ($\Delta\delta$ 3.62), which is analogous to those of the tannins having an HHDP group at O-2/O-3,[13] is also consistent with the structure **2**.

The structure **2** was further substantiated by partial hydrolysis of praecoxin B with tannase, which afforded 2,3-*O*-(*S*)-hexahydroxydiphenoyl-D-glucose (**9**).[3,11,14]

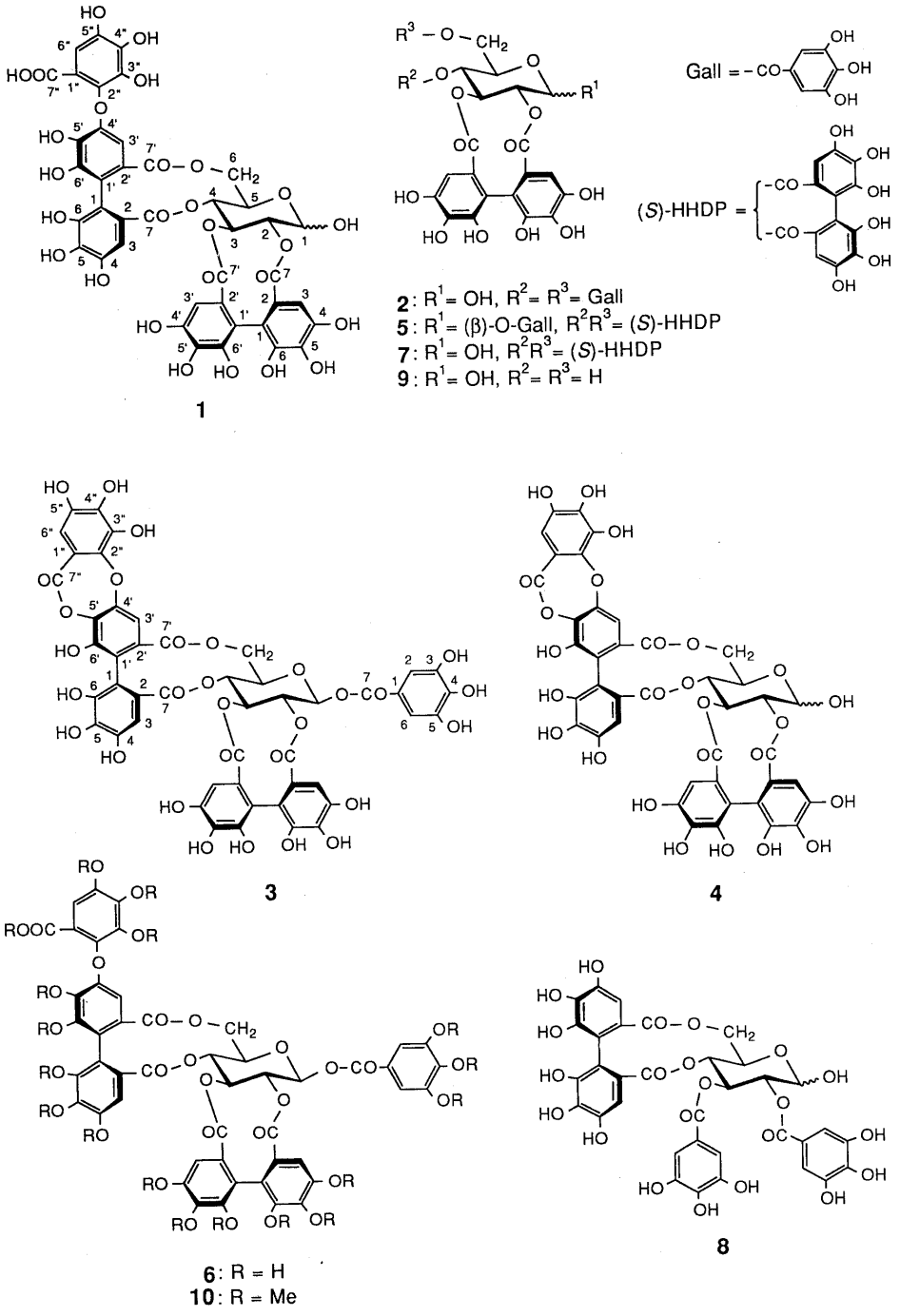

Chart 1

Praecoxin C (**3**) was obtained as a light-brown powder. The FAB-MS of **3** shows the $[M+Na]^+$ ion peak at *m/z* 1109, which corresponds to the molecular formula $C_{48}H_{30}O_{30}$. The ^{1}H-NMR spectrum shows the signals of a galloyl group [δ 7.17 (2H, s)], a valoneoyl group [δ 7.20, 6.95 and 6.58 (1H each, s)] and an HHDP group [δ 6.44 and 6.35 (1H each, s)], and also of a β-D-glucose core adopting $^4C_1$ conformation (see Experimental), suggesting that **3** has a structure closely related to **6**. The CD spectrum of **3** showed a broad positive peak at around 220—240 nm, which indicates that the absolute configuration of the HHDP group and that of the valoneoyl group are both *S*.[7,11]

Treatment of **3** with diazomethane afforded the octadecamethyl derivative (**10**) of rugosin C (**6**). Treatment of **3** in a way analogous to that employed for the selective cleavage of depside linkages in gallotannins[15] afforded **6**. From a solution containing these two compounds, **6** having a free carboxyl group was hardly extractable with EtOAc at pH 5.8, while **3** was easily extracted at this pH. These results and the molecular formula shown by the FAB-MS indicate that **3** has a depside linkage between the carboxyl group at C-1″ of the valoneoyl group and one of the phenolic hydroxyl groups in the molecule.

The ^{13}C-NMR spectrum of **3** showed the signals of a galloyl group, an HHDP group, a valoneoyl group and a glucose core as indicated by Table I. The assignments of the ^{13}C signals were based on the one-bond and long-range

TABLE I. One-Bond and Long-Range ^1H–^{13}C Correlation Data for Praecoxin C (**3**)

Carbon	δ_C	Correlated proton (δ_H)	
		Proton coupled *via* one bond	Proton coupled *via* two or three bonds[a]
Glucose (Glc)			
C-1	92.27	6.20	
C-2	75.84	5.21	
C-3	76.95	5.45	
C-4	69.37	5.15	
C-5	73.26	4.51	3.96 (Glc H-6)
C-6	63.97	5.26	
		3.96	
Galloyl (Gall)			
C-1	119.93		
C-2, C-6	110.25	7.17	
C-3, C-5	146.23		7.17 (Gall H-2, H-6)
C-4	139.88		7.17 (Gall H-2, H-6)
C-7	164.92		7.17 (Gall H-2, H-6)
Hexahydroxydiphenoyl (HHDP)			
C-1	114.92		6.44 (HHDP H-3)
C-2	126.03[b]		
C-3	107.21	6.44	
C-4	145.09		6.44 (HHDP H-3)
C-5	136.49		6.44 (HHDP H-3)
C-6	144.46[c]		
C-7	168.43		6.44 (HHDP H-3)
C-1′	114.14		6.35 (HHDP H-3′)
C-2′	126.37[b]		
C-3′	107.21	6.35	
C-4′	145.03		6.35 (HHDP H-3′)
C-5′	136.14		6.35 (HHDP H-3′)
C-6′	144.50[c]		
C-7′	169.13		6.35 (HHDP H-3′)
			5.45 (Glc H-3)
Valoneoyl (Val)			
C-1	114.58		6.58 (Val H-3)
C-2	124.98		
C-3	107.34	6.58	
C-4	145.77		6.58 (Val H-3)
C-5	136.67		6.58 (Val H-3)
C-6	145.29		
C-7	167.84		6.58 (Val H-3)
			5.15 (Glc H-4)
C-1′	122.07		7.20 (Val H-3′)
C-2′	132.82		
C-3′	111.25	7.20	
C-4′	151.67		7.20 (Val H-3′)
C-5′	135.54		7.20 (Val H-3′)
C-6′	148.49		
C-7′	167.01		
C-1″	111.38		
C-2″	141.43		6.95 (Val H-6″)
C-3″	137.10		
C-4″	143.39		6.95 (Val H-6″)
C-5″	143.80		
C-6″	109.96	6.95	
C-7″	163.21		6.95 (Val H-6″)

At 500 MHz for ^1H-NMR and 125.7 MHz for ^{13}C-NMR, in acetone-d_6. *a*) The long-range ^1H–^{13}C heteronuclear shift correlation spectrum was recorded under the average J_{CH} value of 7 Hz for two- or three-bond couplings. *b,c*) Assignments with the same superscript may be interchanged.

^1H–^{13}C shift correlation spectra.

Based on the following comparisons of the chemical shifts of the valoneoyl carbons of **3** with those of **6**,[7] the location of the depside linkage is assigned to be C-7″—O-5′ in the valoneoyl group of **3**: C-7″, δ 167.00 (**6**)→163.21 (**3**); C-4′, δ 146.97 (**6**)→151.67 (**3**); C-5′, δ 136.99 (**6**)→135.54 (**3**); C-6′, δ 144.63 (**6**)→148.49 (**3**).[16] The formula **3** in which the valoneoyl group forms a seven-membered lactone ring (a depsidone structure) was thus assigned for praecoxin C.

Transformation of rugosin C (**6**) to praecoxin C (**3**) was performed by using polyphosphoric acid as the dehydrating agent, to prove the structural correlation between **3** and **6**. The signals of the valoneoyl protons H-3′ [δ 6.23 (**6**)→7.20 (**3**)] and H-6″ [δ 7.09 (**6**)→6.95 (**3**)], which changed chemical shifts upon this transformation, were also compatible with the formation of the depside linkage. Recently, a depsidone-forming valoneoyl group has also been found in the molecule of prostratin C,[4c,17] a hydrolyzable tannin structurally related to rugosin A.[7]

Praecoxin D (**4**) was obtained as a light-brown powder. The $[M+Na]^+$ ion peak at *m/z* 957 in the FAB-MS and the microanalytical data indicated the molecular formula $C_{41}H_{28}O_{27}$ for **4**. The ^1H-NMR spectrum of **4** also shows the signals of a depsidone-forming valoneoyl group [δ 7.19, 7.17 (1H in total, each s, H-3′), 6.94 (1H, s, H-6″), 6.59, 6.568 (1H in total, each s, H-3)], along with those of an HHDP group [δ 6.571, 6.54 (1H in total, each s, HHDP H-3), 6.31 (1H, s, HHDP H-3′)] and of a glucose core (see Experimental). Duplication of these signals indicates that **4** exists as an anomeric mixture.

A broad positive peak in the short-wavelength region ($[\theta]_{220}$ $+1.0\times10^5$, $[\theta]_{235}+1.3\times10^5$) in the CD spectrum indicates that the valoneoyl group and the HHDP group in **4** have the (*S*)-configuration.[7,11]

Selective cleavage of the depside linkage in **4**, upon the incubation of its solution in a phosphate buffer (pH 5.8), afforded **1**. The structure **4** was thus assigned for praecoxin D. The ^{13}C-NMR spectrum of **4** was also consistent with this structure (see Experimental).

Experimental

Optical rotations were measured on a JASCO DIP-4 polarimeter. Ultraviolet (UV) and infrared (IR) spectra were recorded on a Hitachi 200-10 spectrophotometer and a JASCO A-102 spectrometer, respectively. CD spectra were recorded on a JASCO J-20 spectropolarimeter, or a J-500A spectropolarimeter equipped with a DP-501N data processor. Electron-impact mass spectra (EI-MS) were recorded on a Shimadzu LKB-9000 instrument, and FAB-MS spectra were recorded on a JEOL JMS-D300 or VG 70-SE mass spectrometer. ^1H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^1H-NMR and 125.7 MHz for ^{13}C-NMR) in acetone-d_6 or in acetone-d_6 containing D_2O (*ca.* 3%). Chemical shifts are given in δ values (ppm) from tetramethylsilane, based on the values of δ_H 2.04 and δ_C 29.8 of the signals of acetone-d_6. A Hitachi R22-FTS spectrometer was also used for measurements of the ^1H-NMR spectra (90 MHz). CPC was conducted on a Sanki L-90 centrifugal partition chromatograph equipped with twelve partition cell cartridges (240 ml in total). Normal-phase high-performance liquid chromatography (HPLC) was performed on a Superspher Si60 column (4×125 mm; Merck) or a Develosil 60-5 column (4×150 mm; Nomura), with *n*-hexane–MeOH–tetrahydrofuran–formic acid (55:33:11:1) containing oxalic acid (450 mg/l). Reversed-phase HPLC was performed on a LiChrospher RP-18 (5 μm) column (4×250 mm; Merck) with 0.01 M H_3PO_4–0.01 M KH_2PO_4–EtOH–EtOAc (83:83:24:10), or a LiChrosorb RP-18 (10 μm) column (4×300 mm; Merck) with 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (50:50:2:5),[3] at 40 °C. Light petroleum refers to the fraction boiling in the range of 75—120 °C. Identification of known compounds was based on comparisons of the ^1H-NMR spectral data with those of authentic samples and co-HPLC, unless otherwise mentioned.

Isolation of Tannins from Leaves of *Stachyurus praecox* Fresh leaves (3.6 kg) of *Stachyurus praecox*, collected from the trees grown at Takahashi River bank, Okayama Prefecture, in August 1982, were homogenized in a mixture of acetone and water (7:3, v/v) (15 l). The concentrated filtrate (940 ml) from the homogenate was extracted successively with Et_2O

(1 l × 10) and with EtOAc (1 l × 20), and then each solvent was evaporated off. A portion (26.6 g) of the ethyl acetate extract (48.0 g) was subjected to CPC with *n*-BuOH–*n*-PrOH–H_2O (2 : 1 : 3, v/v, normal-phase development), and the eluate was separated into fractions I—III (frs. I—III). Fraction I (3.5 g) was chromatographed over Sephadex LH-20, with EtOH→EtOH–MeOH (4 : 1), to give praecoxin C (**3**) (241 mg). Fraction II (5.1 g) was also chromatographed over Sephadex LH-20 with increasing concentrations of MeOH in EtOH, and each fraction containing tannins was further purified by column chromatography on Sephadex LH-20 or on Avicel (Funakoshi, with 5% acetic acid), to give 1,2,6-tri-*O*-galloyl-*β*-D-glucose (15 mg), praecoxin B (**2**) (198 mg), tellimagrandin I (515 mg), praecoxin A (**1**) (32 mg), praecoxin D (**4**) (188 mg), casuarictin (**5**) (248 mg), rugosin C (**6**) (337 mg) and rugosin F (43 mg). Strictinin, stachyurin, casuarinin and casuariin[1] were in fr. III from CPC.

Praecoxin A (1) A light-brown powder, $[\alpha]_D^{22}$ +45° (c=0.5, MeOH). *Anal.* Calcd for $C_{41}H_{28}O_{27}\cdot 3H_2O$: C, 48.91; H, 3.40. Found: C, 49.01; H, 3.54. FAB-MS m/z: 975 ($[M+Na]^+$). UV λ_{max}^{MeOH} nm (log ε): 217 (4.80), 260 (sh, 4.54). IR ν_{max}^{KBr} cm^{-1}: 1730, 1615. CD (MeOH): $[\theta]_{223}+1.2\times10^5$, $[\theta]_{236}+1.1\times10^5$, $[\theta]_{259}$ -5.4×10^4, $[\theta]_{282}$ $+3.4\times10^4$. ^{1}H-NMR (500 MHz, in acetone-d_6+D_2O) δ: 7.13 [α-anomer (α) and β-anomer (β); 1H, s, valoneoyl (Val) H-6″], 6.58 (α), 6.57 (β) (1H in total, each s, Val H-3), 6.54 (α), 6.49 (β) (1H in total, each s, HHDP H-3), 6.344 (α), 6.340 (β) (1H in total, each s, HHDP H-3′), 6.25 (β), 6.23 (α) (1H in total, each s, Val H-3′). Glucose protons δ: 5.36 (d, J=3.5 Hz, H-1), 5.00 (dd, J=3.5, 9.5 Hz, H-2), 5.42 (t, J=9.5 Hz, H-3), 4.95 (t, J=10 Hz, H-4), 4.52 (ddd, J=1, 6.5, 10 Hz, H-5), 5.14 (dd, J=6.5, 13 Hz, H-6), 3.64 (dd, J=1, 13 Hz, H-6) (α-anomer); 4.97 (d, J=8 Hz, H-1), 4.79 (dd, J=8, 9 Hz, H-2), 5.17 (t, J=9.5 Hz, H-3), 4.95 (t, J=9.5 Hz, H-4), 4.14 (br dd, J=6.5, 9.5 Hz, H-5), 5.17 (dd, J=6.5, 13 Hz, H-6), 3.71 (br d, J=13 Hz, H-6) (β-anomer). ^{13}C-NMR (125.7 MHz, in acetone-d_6+D_2O) δ: 63.49, 63.52 [glucose (Glc) C-6, α and β], 67.11 (Glc C-5, α), 69.56 (Glc C-4, β), 69.90 (Glc C-4, α), 72.17 (Glc C-5, β), 75.45 (Glc C-2, α), 75.65 (Glc C-3, α), 77.45 (Glc C-3, β), 78.13 (Glc C-2, β), 91.43 (Glc C-1, α), 95.12 (Glc C-1, β), 105.25, 105.34 (Val C-3′), 107.09, 107.23, 107.31, 107.57 (Val C-3, HHDP C-3 and C-3′), 109.73 (Val C-6″), 114.29, 114.38 (HHDP C-1′), 114.73, 114.80 (HHDP C-1), 115.29 (Val C-1″), 115.92 (Val C-1), 117.63, 117.66 (Val C-1′), 125.42, 125.47, 125.89, 125.93 (Val C-2 and C-2′), 126.33, 126.36, 126.53 (HHDP C-2 and C-2′), 136.01, 136.07 (HHDP C-5′), 136.27 (HHDP C-5), 136.50 (Val C-5), 136.91, 136.94 (Val C-5′), 137.04, 137.07 (Val C-2″), 139.89 (Val C-4″), 140.07 (Val C-3″), 143.18 (Val C-5″), 144.19, 144.29 (HHDP C-6 and C-6′), 144.66 (Val C-6′), 144.97, 145.06, 145.12 (Val C-6, HHDP C-4 and C-4′), 145.23 (Val C-4), 147.00, 147.03 (Val C-4′), 166.85, 166.88 (Val C-7″), 168.03, 168.08, 168.13 (Val C-7 and C-7′), 168.96, 169.07 (HHDP C-7), 169.41, 169.47 (HHDP C-7′).

Praecoxin B (2) A light-brown powder, $[\alpha]_D^{23}$ +49° (c=0.5, MeOH). *Anal.* Calcd for $C_{34}H_{26}O_{22}\cdot 3H_2O$: C, 48.57; H, 3.84. Found: C, 48.18; H, 3.82. UV λ_{max}^{MeOH} nm (log ε): 218 (4.78), 274 (4.40). IR ν_{max}^{KBr} cm^{-1}: 1710, 1615. CD (MeOH): $[\theta]_{236}$ $+1.0\times10^5$, $[\theta]_{270}-2.5\times10^4$. ^{1}H-NMR (500 MHz, in acetone-d_6+D_2O) δ: 7.142 (α), 7.139 (β) [2H in total, each s, galloyl (Gall) H-2 and H-6], 7.13 (α), 7.10 (β) (2H in total, each s, Gall H-2 and H-6), 6.60 (α), 6.59 (β) (1H in total, each s, HHDP H-3), 6.39 (α and β) (1H, s, HHDP H-3′). Glucose protons δ: 5.48 (d, J=3.5 Hz, H-1), 5.07 (dd, J=3.5, 9.5 Hz, H-2), 5.62 (t, J=9.5 Hz, H-3), 5.50 (t, J=10 Hz, H-4), 4.54 (ddd, J=2, 4, 10 Hz, H-5), 4.49 (dd, J=2, 12 Hz, H-6), 4.27 (dd, J=4, 12 Hz, H-6) (α-anomer); 5.17 (d, J=8 Hz, H-1), 4.88 (dd, J=8, 9.5 Hz, H-2), 5.35 (t, J=9.5 Hz, H-3), 5.46 (t, J=9.5 Hz, H-4), 4.22 (ddd, J=2, 5, 9.5 Hz, H-5), 4.49 (dd, J=2, 12 Hz, H-6), 4.26 (dd, J=5, 12 Hz, H-6) (β-anomer). ^{13}C-NMR (125.7 MHz, in acetone-d_6+D_2O) δ: 63.10 (Glc C-6, α), 63.16 (Glc C-6, β), 68.43 (Glc C-5, α), 68.46 (Glc C-4, β), 68.67 (Glc C-4, α), 73.08 (Glc C-5, β), 75.12 (Glc C-2, α), 75.39 (Glc C-3, α), 77.64 (Glc C-3, β), 77.73 (Glc C-2, β), 91.28 (Glc C-1, α), 94.90 (Glc C-1, β), 107.20, 107.28, 107.60 (HHDP C-3 and C-3′), 109.84, 109.99 (Gall C-2 and C-6), 114.33, 114.45 (HHDP C-1′), 114,55, 114.59 (HHDP C-1), 120.38, 120.43, 121.15, 121.21 (Gall C-1), 126.40, 126.49, 126.62 (HHDP C-2 and C-2′), 136.07, 136.16, 136.20, 136.24 (HHDP C-5 and C-5′), 138.89, 139.36 (Gall C-4), 144.23, 144.29 (HHDP C-6 and C-6′), 145.07, 145.14 (HHDP C-4 and C-4′), 145.95, 146.05 (Gall C-3 and C-5), 165.93, 166.64 (Gall C-7), 168.90, 169.06 (HHDP C-7), 169.18, 169.23 (HHDP C-7′).

Praecoxin C (3) A light-brown powder, $[\alpha]_D^{23}$ +41° (c=0.5, MeOH). *Anal.* Calcd for $C_{48}H_{30}O_{30}\cdot 5H_2O$: C, 48.98; H, 3.43. Found: C, 48.83; H, 3.78. FAB-MS m/z: 1109 ($[M+Na]^+$). UV λ_{max}^{MeOH} nm (log ε): 214 (4.92), 278 (4.49). IR ν_{max}^{KBr} cm^{-1}: 1720, 1605. CD (MeOH): $[\theta]_{223}$ $+1.1\times10^5$, $[\theta]_{233}+1.4\times10^5$, $[\theta]_{260}$ -3.2×10^4, $[\theta]_{282}+0.3\times10^4$. ^{1}H-NMR (500 MHz, in acetone-d_6) δ: 7.20 (1H, s, Val H-3′), 7.17 (2H, s, Gall H-2 and H-6), 6.95 (1H, s, Val H-6″), 6.58 (1H, s, Val H-3), 6.44 (1H, s, HHDP H-3), 6.35 (1H, s, HHDP H-3′). Glucose protons δ: 6.20 (d, J=8.5 Hz, H-1), 5.21 (t, J=9 Hz, H-2), 5.45 (dd, J=9, 10 Hz, H-3), 5.15 (t, J=10 Hz, H-4), 4.51 (br dd, J=6.5, 10 Hz, H-5), 5.26 (dd, J=6.5, 13 Hz, H-6), 3.96 (br d, J=13 Hz, H-6). ^{13}C-NMR: see Table I.

Praecoxin D (4) A light-brown powder, $[\alpha]_D^{23}$ +81° (c=0.5, MeOH). *Anal.* Calcd for $C_{41}H_{28}O_{27}\cdot 3H_2O$: C, 48.91; H, 3.40. Found: C, 49.01; H, 3.54. FAB-MS m/z: 957 ($[M+Na]^+$). UV λ_{max}^{MeOH} nm (log ε): 215 (4.87), 282 (sh, 4.44). IR ν_{max}^{KBr} cm^{-1}: 1730, 1610. CD (MeOH): $[\theta]_{220}+1.0\times10^5$, $[\theta]_{235}+1.3\times10^5$, $[\theta]_{260}-3.7\times10^4$, $[\theta]_{281}+1.8\times10^4$. ^{1}H-NMR (500 MHz, in acetone-d_6) δ: 7.19 (β), 7.17 (α) (1H in total, each s, Val H-3′), 6.94 (α and β) (1H, s, Val H-6″), 6.59 (α), 6.568 (β) (1H in total, each s, Val H-3), 6.571 (α), 6.54 (β) (1H in total, each s, HHDP H-3), 6.31 (α and β) (1H, s, HHDP H-3′). Glucose protons δ: 5.46 (d, J=3.5 Hz, H-1), 5.08 (dd, J=3.5, 9.5 Hz, H-2), 5.46 (t, J=9.5 Hz, H-3), 5.07 (t, J=10 Hz, H-4), 4.62 (ddd, J=1, 6.5, 10 Hz, H-5), 5.18 (dd, J=6.5, 13 Hz, H-6), 3.84 (dd, J=1, 13 Hz, H-6) (α-anomer); 5.06 (d, J=8 Hz, H-1), 4.84 (dd, J=8, 9 Hz, H-2), 5.24 (dd, J=9, 10 Hz, H-3), 5.06 (t, J=10 Hz, H-4), 4.24 (br dd, J=6.5, 10 Hz, H-5), 5.20 (dd, J=6.5, 13 Hz, H-6), 3.92 (br d, J=13 Hz, H-6) (β-anomer). ^{13}C-NMR (125.7 MHz, in acetone-d_6) δ: 64.41 (Glc C-6, α and β), 67.24 (Glc C-5, α), 69.78 (Glc C-4, β), 70.05 (Glc C-4, α), 72.21 (Glc C-5, β), 75.50 (Glc C-2, α), 75.56 (Glc C-3, α), 77.27 (Glc C-3, β), 78.34 (Glc C-2, β), 91.81 (Glc C-1, α), 95.51 (Glc C-1, β), 107.12, 107.21, 107.25, 107.35, 107.71 (Val C-3, HHDP C-3 and C-3′), 109.91 (Val C-6″), 111.08 (Val C-3′), 111.26 (Val C-1″), 114.02, 114.10 (HHDP C-1′), 114.57, 114.59 (Val C-1), 114.90, 114.99 (HHDP C-1), 122.12 (Val C-1′), 125.10, 125.15 (Val C-2), 126.52, 126.56, 126.58, 126.78 (HHDP C-2 and C-2′), 132.88, 132.92 (Val C-2′), 135.46, 135.49 (Val C-5′), 135.95, 136.03 (HHDP C-5′), 136.46 (HHDP C-5), 136.59 (Val C-5), 137.13 (Val C-3″), 141.50, 141.53 (Val C-2″), 143.30 (Val C-4″), 143.79, 143.81 (Val C-5″), 144.27, 144. 35, 144.39 (HHDP C-6 and C-6′), 144.95, 145.06, 145.12 (HHDP C-4 and C-4′), 145.27 (Val C-6), 145.71 (Val C-4), 148.54, 148.58 (Val C-6′), 151.60, 151.64 (Val C-4′), 163.42 (Val C-7″), 167.12, 167.22 (Val C-7′), 167.83, 167.87 (Val C-7), 168.67, 168.76 (HHDP C-7), 169.19, 169.27 (HHDP C-7′).

Partial Hydrolysis of Rugosin C with Tannase Rugosin C (**6**) (35 mg) dissolved in water (5 ml) was treated with tannase[12] at 37 °C for 24 h, and then the solvent was evaporated off. The EtOH-soluble portion of the residue was chromatographed over Sephadex LH-20 with EtOH and then with EtOH–MeOH (1 : 1), to give praecoxin A (**1**) (5 mg).

Partial Hydrolysis of Praecoxin B with Tannase Praecoxin B (**2**) (25 mg) dissolved in water (2 ml) was treated with tannase at 37 °C for 6 h. The solution was then concentrated, and was chromatographed over Sephadex LH-20 with EtOH, to afford 2,3-*O*-(*S*)-hexahydroxydiphenoyl-D-glucose (**9**) (5 mg).

Cleavage of Depside Linkage of Praecoxins C and D A solution of praecoxin C (**3**) (5 mg) in 0.03 M phosphate (KH_2PO_4–Na_2HPO_4) buffer (pH 5.8, 5 ml) was kept at 37 °C for 10 h, and then was extracted with EtOAc (to remove unchanged starting material). The aqueous layer was acidified with 10% HCl (1 ml), and was further extracted with EtOAc. Evaporation of the latter EtOAc layer gave rugosin C (**6**) (2 mg). A solution of praecoxin D (**4**) (30 mg) in phosphate buffer (15 ml) was also treated in an analogous way. After extraction of the acidified solution with EtOAc, the aqueous layer was passed through a BondElut C18 cartridge (Analytichem), and the adsorbed materials were eluted with water and then with 30% MeOH. The eluate with 30% MeOH afforded praecoxin A (**1**) (9 mg).

Treatment of Praecoxin C with Diazomethane Ethereal diazomethane was added to a solution of praecoxin C (**3**) (49 mg) in EtOH (7 ml), and the mixture was left to stand for 30 min. The solvent was evaporated off, and the residue was purified by preparative TLC on Kieselgel 60 PF_{254} with light petroleum–CH_2Cl_2–acetone (4 : 6 : 3), to give the octadecamethyl derivative (**10**) of rugosin C (**6**) (18 mg), which was identified from the ^{1}H-NMR spectrum,[18] the fragmentation pattern in the EI-MS, and $[\alpha]_D$. The identity with the derivative was further substantiated by methanolysis of this methylate to give methyl tri-*O*-methylgallate, dimethyl hexamethoxydiphenate and trimethyl octa-*O*-methylvaloneate (1 mg each),[7] which were identified by ^{1}H-NMR and EI-MS in comparison with authentic specimens.

Transformation of Rugosin C into Praecoxin C Polyphosphoric acid (250 mg) was added to a solution of rugosin C (**6**) (15 mg) in 1,4-dioxane (15 ml), and the mixture was refluxed for 1.5 h. After concentration, the reaction mixture was partitioned between water and EtOAc. The EtOAc layer was then extracted with 0.03 M phosphate buffer (pH 5.8). The organic layer was washed with 10% HCl, and the solvent was evaporated off, and

then the residue was chromatographed over Toyopearl HW-40SF with 70% EtOH, to give praecoxin C (**3**) (1 mg). The buffer solution was acidified with 10% HCl, and extracted with EtOAc, and evaporation of the EtOAc layer gave rugosin C (**6**) (3 mg).

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16) The previous assignment[4b] of the location of the depside linkage in **3** was based on a comparison of the 22.6 MHz ^{13}C-NMR spectra of **3** and **6**. The discrimination of almost all the signals of the HHDP group from those of the HHDP moiety of the valoneoyl group, in the present study, has led to the structural revision. The previously reported structure for praecoxin D[4b] was also revised as described in this paper.
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