Tannins of Tamaricaceous Plants. VI.[1] Four New Trimeric Hydrolyzable Tannins from *Reaumuria hirtella* and *Tamarix pakistanica*

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Two novel ellagitannin trimers, hirtellins T_2 (1) and T_3 (25), besides previously reported hirtellin T_1 (28), have been isolated from the leaves of *Reaumuria hirtella* JAUB. *et* SP. (Tamaricaceae). Hirtellin T_2 has a 36-membered macro-ring structure (1) with three dehydrodigalloyl (*m*-GOG) units connecting monomers, while hirtellin T_3 (25) possesses one *m*-GOG and one hellinoyl (*m*-GO-*m*-GOG) unit forming a rigid macro-ring, as found in hirtellin B. Two new tannins, tamarixinins T_1 (26) and T_2 ($T_{2a}+T_{2b}$) (27), having a *m*-GOG and/or isodehydrodigalloyl (*p*-GOG) unit were also isolated from the flowers of *Tamarix pakistanica* QUAISER. The structures of these new ellagitannin trimers were elucidated based on chemical methods and spectroscopic analyses, including two-dimensional nuclear magnetic resonance measurements.

Keywords ellagitannin; *Reaumuria hirtella*; *Tamarix pakistanica*; hirtellin T_1; hirtellin T_2; tamarixinin T_1

In our series of studies on the tannin constituents of medicinal plants, Tamaricaceous plants have been shown to produce unique ellagitannin oligomers possessing a *m*-GOG (dehydrodigalloyl) or *p*-GOG (isodehydrodigalloyl), and/or a *m*-GO-*m*-GOG (hellinoyl) group as the connecting units, at O-1 of the *C1* glucopyranose core of one monomer and O-2 of another monomer.[1,3] Tamaricaceous ellagitannin oligomers are regarded as metabolites biogenetically produced by two characteristic oligomerization modes yielding linear type and macrocyclic type oligomers.[1,3] This paper deals with the isolation and structure elucidation of four new trimers of these types isolated from *Reaumuria hirtella* and *Tamarix pakistanica*.

The new compounds, named hirtellins T_2, T_3 (from *R. hirtella*), and tamarixinins T_1 and T_2 (from *T. pakistanica*), were shown to be trimeric ellagitannins by their positive coloration with the $FeCl_3$ and $NaNO_2$–AcOH reagents[4] and retention time in normal-phase HPLC, which are similar to those of a previously isolated trimer (hirtellin T_1).[1]

Structure of Hirtellin T_2 (1) Hirtellin T_2 (**1**) was obtained as an off-white amorphous powder from the *n*-BuOH extract of *R. hirtella* leaves. Methylation of **1** with dimethyl sulfate and anhydrous potassium carbonate in dry acetone gave the permethylated derivative **1a**, which upon methanolysis furnished methyl tri-*O*-methylgallate (**5**), dimethyl hexamethoxydiphenate (**6**) and dimethyl

TABLE I. ^{1}H-NMR Spectral Data for Glucose Moieties of **12**, **13**, **14**, **25**, **28** and **1** (500 MHz, Acetone-d_6+D_2O, *J* in Hz)

H	12	13	14	25	28	1
Glucose-I						
1	6.20 d (*J*=8)	6.07 d (*J*=8)	6.16 d (*J*=8)	5.47 d (*J*=8)	6.12 d (*J*=8.5)	5.87 br
2	5.58 dd (*J*=8, 9.5)	5.58 dd (*J*=8, 10)	5.66 dd (*J*=8, 10)	5.53 dd (*J*=8, 10)	5.53 dd (*J*=8.5, 10)	5.45 br
3	5.75 t (*J*=9.5)	5.77 t (*J*=10)	5.74 t (*J*=10)	5.69 t (*J*=10)	5.78 t (*J*=10)	5.70 t (*J*=10)
4	5.14 t (*J*=9.5)	5.22 t (*J*=10)	5.14 t (*J*=10)	5.15 t (*J*=10)	5.18 t (*J*=10)	5.13 t (*J*=10)
5	4.41 dd (*J*=5.5, 9.5)	4.53 dd (*J*=6, 10)	4.51 dd (*J*=6, 10)	4.27 dd (*J*=6.5, 10)	4.48 dd (*J*=6.5, 10)	4.30 br
6	5.33 dd (*J*=5.5, 13.5)	5.33 dd (*J*=6, 13)	5.35 dd (*J*=6, 13)	5.31 dd (*J*=6.5, 13)	5.30 dd (*J*=6.5, 13.5)	5.25 dd (*J*=6, 13.5)
	3.80 d (*J*=13.5)	3.98 d (*J*=13)	3.86 d (*J*=13)	3.84 d (*J*=13)	3.94 d (*J*=13.5)	3.78 d (*J*=13.5)
Glucose-II						
1′		5.35 d (*J*=8)	5.60 d (*J*=8.5)	6.02 d (*J*=8.5)	5.77 d (*J*=8.5)	5.87 br
2′		5.55 dd (*J*=8, 10)	5.36 dd (*J*=8.5, 10)	5.72 dd (*J*=8.5, 10)	5.51 dd (*J*=8.5, 10)	5.45 br
3′		5.64 t (*J*=10)	5.69 t (*J*=10)	5.56 t (*J*=10)	5.63 t (*J*=10)	5.70 t (*J*=10)
4′		5.13 t (*J*=10)	5.19 t (*J*=10)	5.11 t (*J*=10)	5.13 t (*J*=10)	5.13 t (*J*=10)
5′		4.22 dd (*J*=6.5, 10)	4.35 ddd (*J*=1.5, 7, 10)	4.40 dd (*J*=6.5, 10)	4.32 ddd (*J*=7.5, 10)	4.30 br
6′		5.29 dd (*J*=6.5, 13)	5.31 dd (*J*=7, 13)	5.31 dd (*J*=6.5, 13.5)	5.29 dd (*J*=7.5, 13.5)	5.25 dd (*J*=6, 13.5)
		3.82 d (*J*=13)	4.13 dd (*J*=1.5, 13.5)	3.94 d (*J*=13)	3.83 dd (*J*=13.5)	3.78 d (*J*=13.5)
Glucose-III						
1″				5.63 d (*J*=8)	5.50 d (*J*=8)	5.87 br
2″				5.36 dd (*J*=8, 10)	5.47 dd (*J*=8, 10)	5.45 br
3″				5.68 t (*J*=10)	5.52 t (*J*=10)	5.70 t (*J*=10)
4″				5.14 t (*J*=10)	5.14 t (*J*=10)	5.13 t (*J*=10)
5″				4.34 ddd (*J*=1.5, 6.5, 10)	4.25 dd (*J*=6.5, 10)	4.30 br
6″				5.26 dd (*J*=6.5, 13.5)	5.30 dd (*J*=6.5, 13)	5.25 dd (*J*=6, 13.5)
				4.05 d (*J*=1.5, 13.5)	3.89 dd (*J*=13)	3.78 d (*J*=13.5)

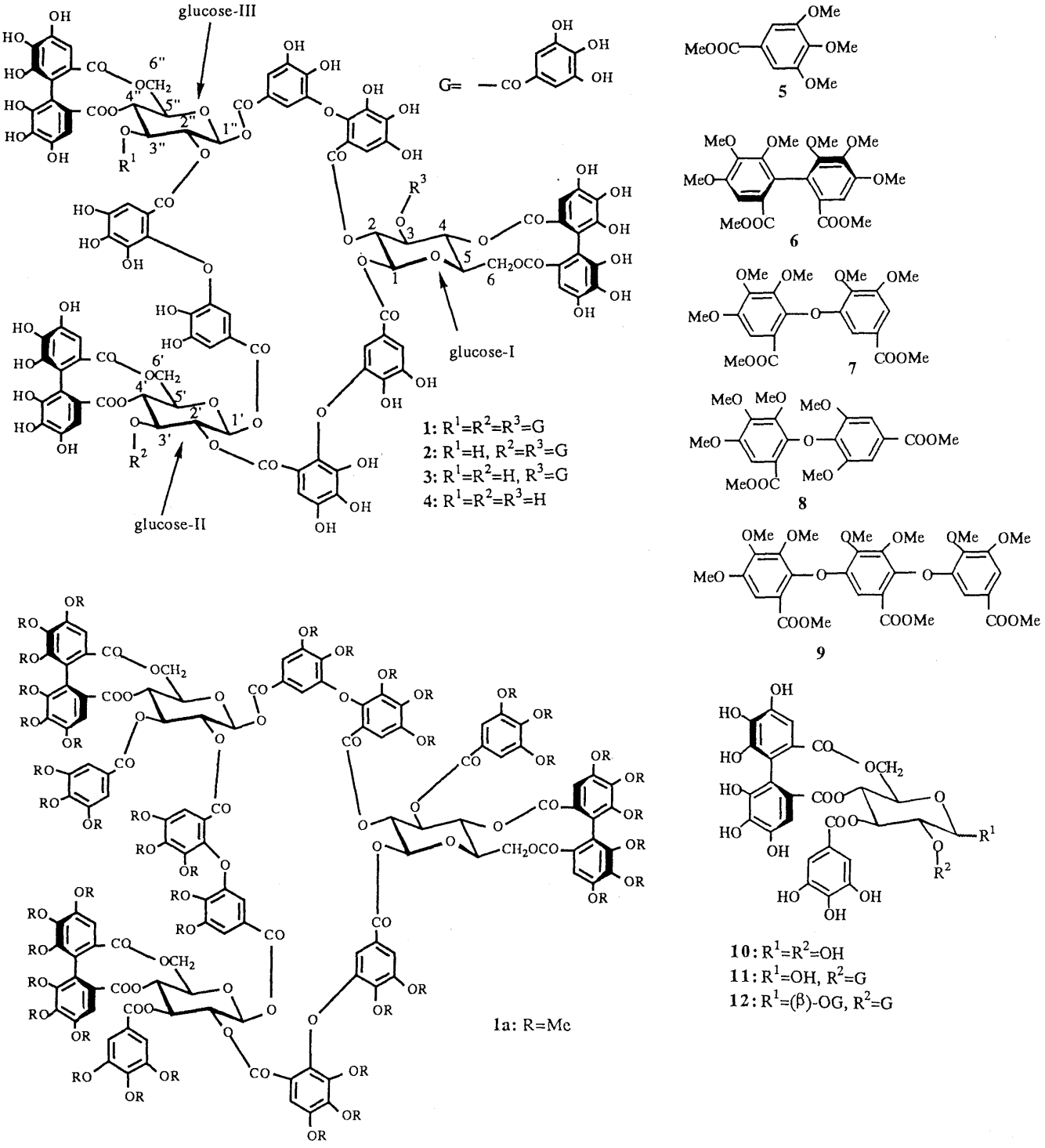


Chart 1-1

penta-*O*-methyldehydrodigallate (**7**). The sugar component was shown to be glucose by acid hydrolysis. In spite of the trimeric nature exhibited by **1** upon HPLC,[5] the ^{1}H-NMR spectrum of hirtellin T_2 (**1**) looks like that of a monomeric ellagitannin *i.e.*, it shows a sequentially coupled seven-spin system due to a fully acylated glucose core, and the signals due to a galloyl, a hexahydroxydiphenoyl (HHDP) and a *m*-GOG[6] residue. A symmetrical structure, as found in tamarixinin B[3d] and isohirtellin C,[3c] is plausible for hirtellin T_2 (**1**). The glucose proton signals (Table I) were assigned with the aid of the ^{1}H–^{1}H shift correlation spectrum (COSY) and by comparison with the glucose-II proton signals of hirtellin T_1 (**28**).[1] The ^{1}H-NMR spectrum of hirtellin T_2 (**1**) showed broadening and/or multiplication of the same proton signals, particularly for H-1, H-2 and H-5 of the glucose moiety and the aromatic proton signals of the *m*-GOG unit (see Experimental). This spectral feature, which is similar to that of the macrocyclic oligomers, hirtellins C and F,[3c] was also observed in the spectrum

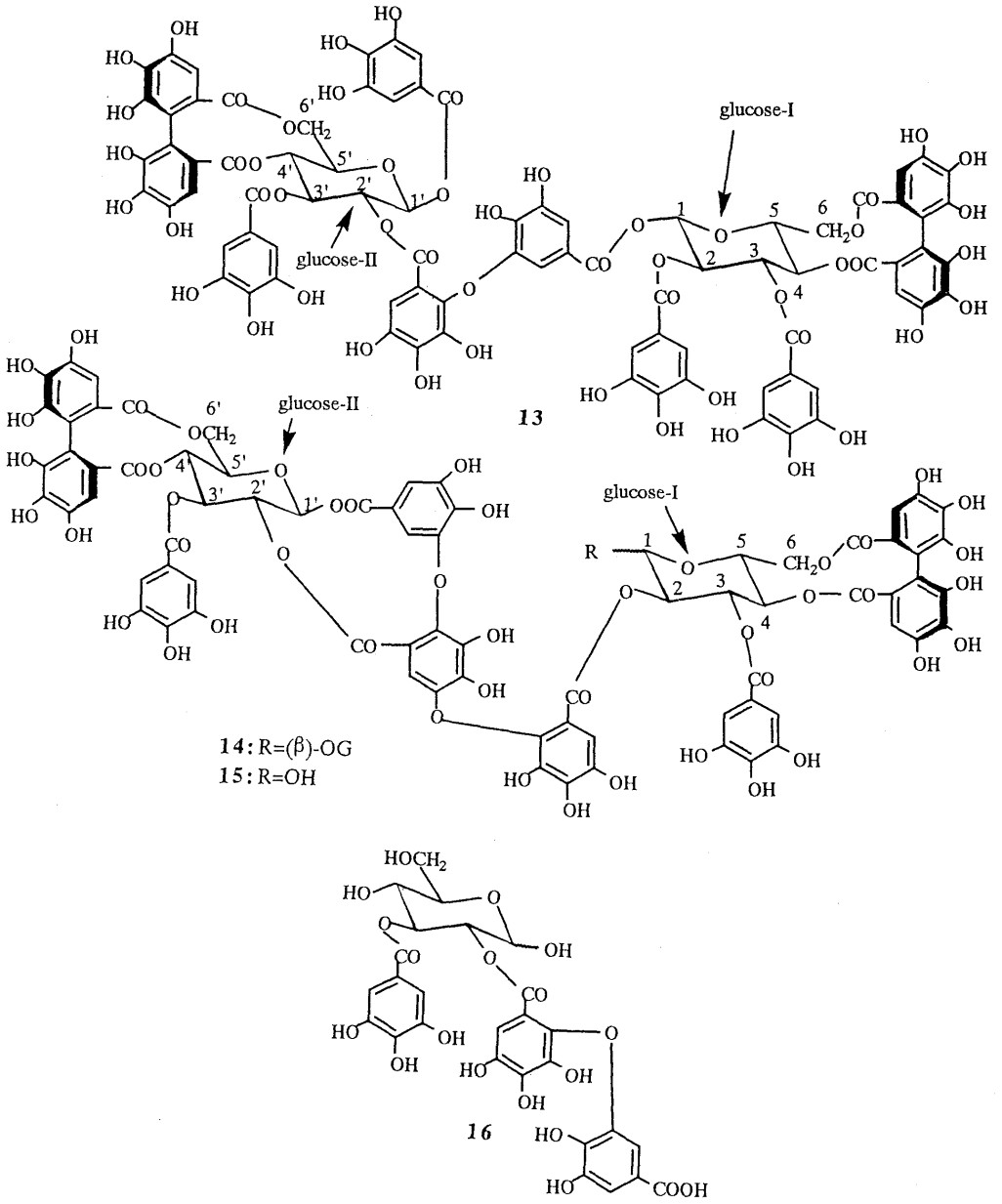


Chart 1-2

of the methylated derivative (**1a**), in which all sugar proton signals were notably broadened, suggesting the presence of conformational isomers of the macro-ring. The position of the HHDP group on the glucose residue was indicated to be at O-4 and O-6 by the large difference ($\Delta\delta$ *ca.* 1.5) between the chemical shifts of the C-6 *geminal* protons.[7] Although the anomeric proton signal of the glucose moiety in **1** is considerably broadened, its significant upfield shift relative to that of tellimagrandin II (**12**) implies the location of the *m*-GOG unit at O-2.[3] The location of the galloyl group was determined to be at O-3 of the glucose residue as follows. Treatment of hirtellin T_2 (**1**) with tannase[8] gave the partial hydrolyzates **2**, **3** and **4** which were produced by sequential removal of galloyl groups from O-3 of the glucose cores, as demonstrated by the decrease of the galloyl signals accompanied by a remarkable upfield shift ($\Delta\delta$ *ca.* 1.7) of H-3 in the ^{1}H-NMR spectrum. The trimeric nature of **1** was clearly shown by the ^{1}H-NMR spectra of the degalloylated products **2** and **3**, in which the structural symmetry observed in **1** was lost owing to the removal of one or two galloyl groups, and twenty-one aliphatic proton signals attributable to three glucopyranose residues were apparent. The structural symmetry was again observed for **4**, which has no galloyl group in the molecule, and shows a monomer-like ^{1}H-NMR spectrum. From the above observations, hirtellin T_2 (**1**) was concluded to be an ellagitannin trimer possessing three units each of galloyl group, HHDP group, *m*-GOG unit, and glucose core, and was presumed to be formulated as **1** in which the *m*-GOG units link the three glucose cores in the following sequence; O-2→O-1″, O-2′→O-1 and O-2″→O-1′ forming a 36-membered ring.

The proposed structure **1** was chemically substantiated by partial acid hydrolysis, yielding several monomeric (**10**,

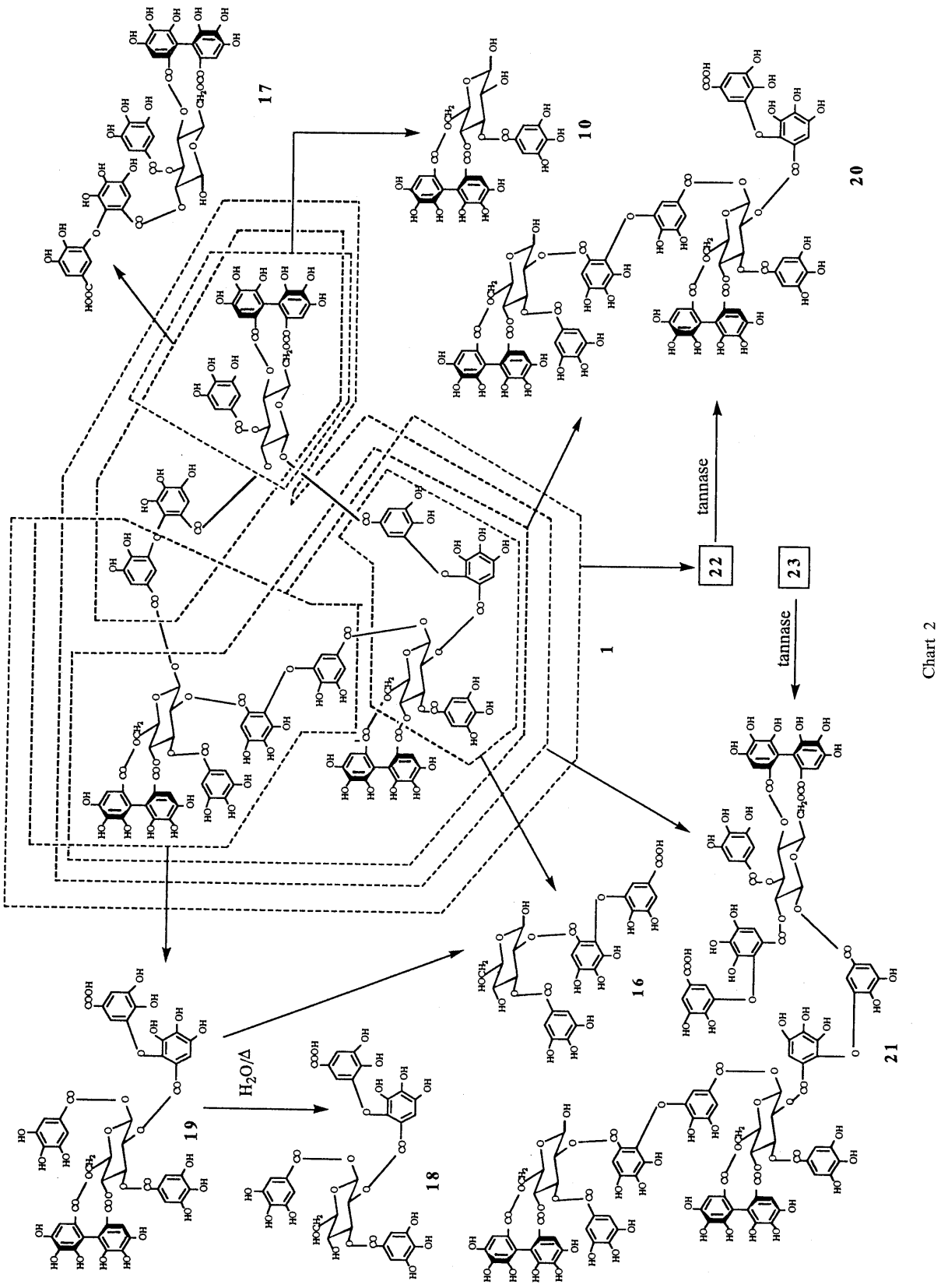


Chart 2

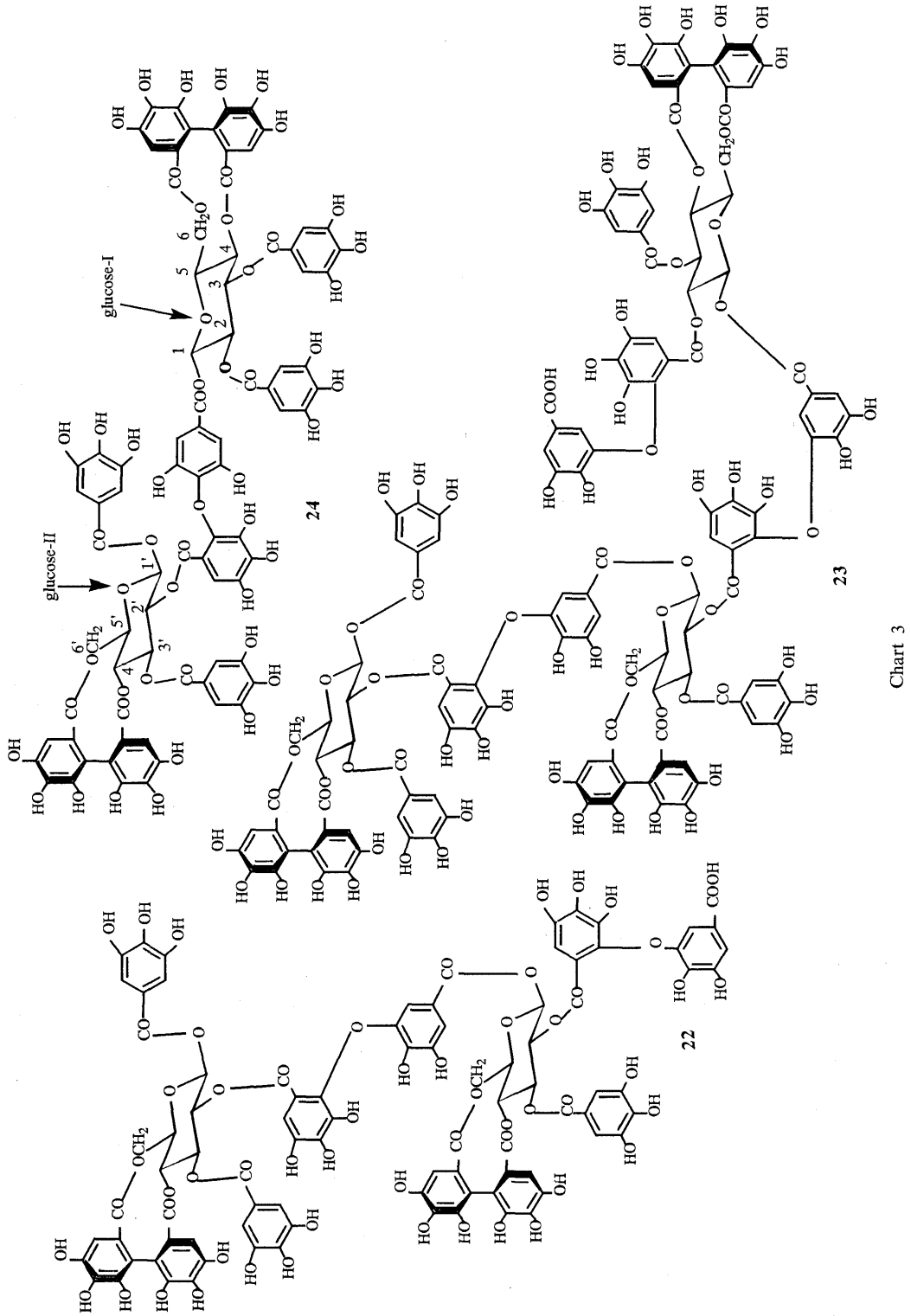
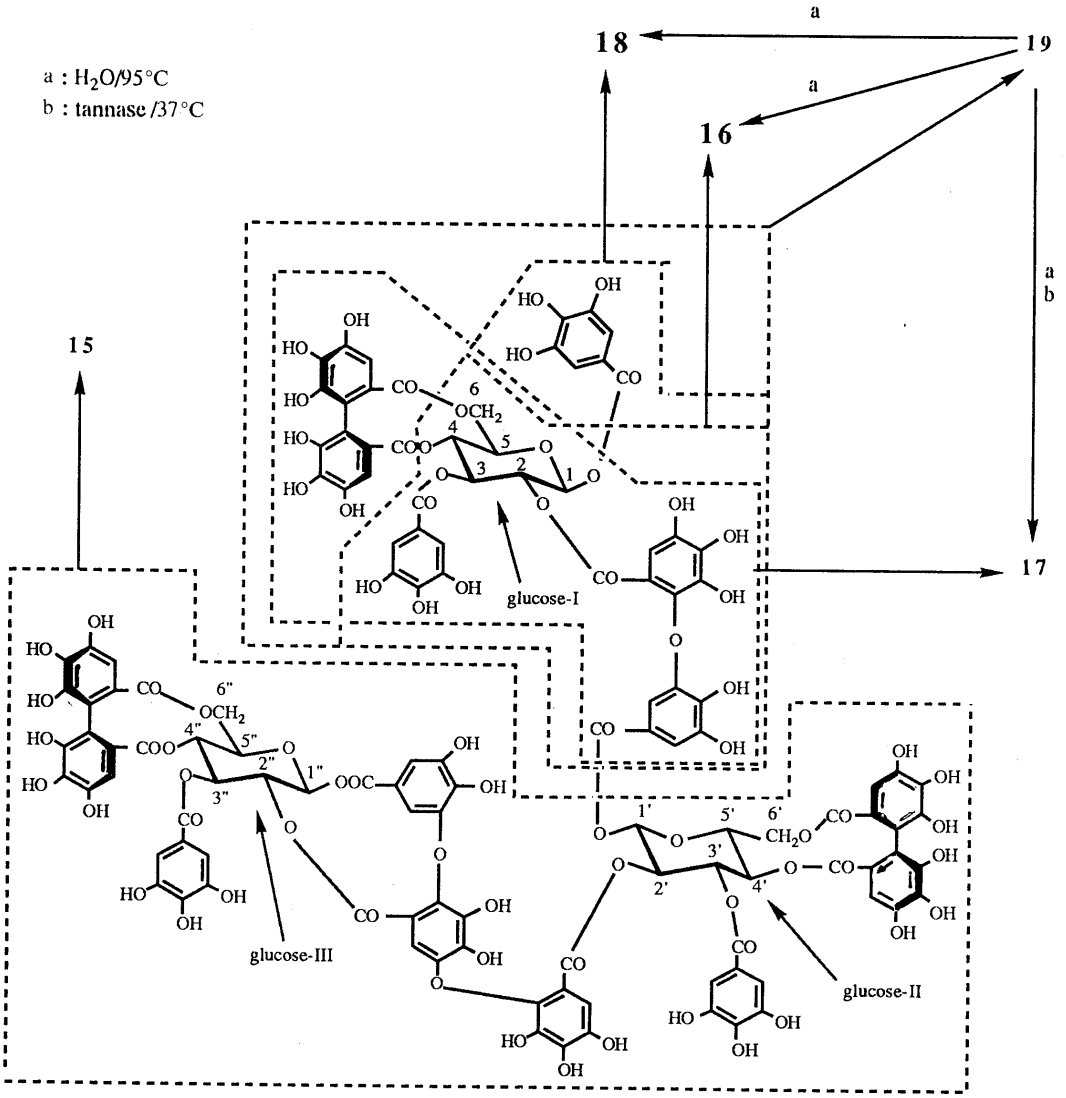


Chart 3

16—**19**), dimeric (**10**, **22**) and trimeric (**21**) hydrolyzates (Chart 1). The hydrolysis of **1** at the anomeric centers resulted in the formation of **10**, **16**, **17**, **20** and **21**, each existing as an equilibrium mixture of anomers. The monomeric hydrolyzates (**10**, **16** and **17**) were identified by co-chromatography with authentic samples.[1,3] The structures of **20** and **21** were confirmed by their reduction with $NaBH_4$, which yielded the products identical with those prepared by selective degalloylation of the known oligomers **22** and **23**,[1] respectively, with tannase, followed by $NaBH_4$ reduction (see Experimental). The hydrolyzates **19** and **22** are regarded as formed through a linear congener (**28**) (observed as a very minor peak in the HPLC of the hydrolyzate mixture) produced by cleavage of the ether bond of a *m*-GOG unit in **1**.[9]

From the above findings, hirtellin T_2 (**1**) was assigned the structure **1**. Hirtellin T_2 (**1**) is biogenetically regarded as a metabolite produced by intramolecular oxidative coupling between the galloyl groups at O-1″ and O-2 of hirtellin T_1 (**28**). It is noteworthy that hirtellin T_2 (**1**) is the first natural trimeric tannin having a symmetrical structure containing three *m*-GOG units.

Structure of Hirtellin T_3 (25) Hirtellin T_3 (**25**) was concurrently eluted with the other trimers, **1** and **28**, upon the column chromatography over Toyopearl HW-40 and/or MCI-gel CHP-20P of the *n*-BuOH extract from *R. hirtella*. The retention time in normal phase-HPLC was similar to those of **1** and **28**, indicating its trimeric nature.[5] The methylation of **25** with diazomethane followed by methanolysis with sodium methoxide afforded **5**, **6**, **7** and trimethyl hepta-*O*-methylhellinate (**9**).[3a] The ^{1}H-NMR spectrum of **25** exhibited two *meta*-coupled doublets (each 1H, $J=2$ Hz) at δ 6.88 and 5.75, two 1H singlets at δ 7.57 and 7.71 which are characteristic of a *m*-GO-*m*-GOG[6] unit, in addition to the other two 1H doublets ($J=2$ Hz) at δ 7.30 and 6.50, and 1H singlet at δ 7.07 attributable to a *m*-GOG unit. The presence of four 2H- and six 1H-singlets in the aromatic region at δ 7.04—6.46, due to four galloyl and three HHDP units, was also observed. The sugar liberated after complete acid hydrolysis of **25**



25

Chart 4

was identified as glucose. The chemical shifts and the coupling pattern of the sugar proton signals assigned by ^1H–^1H COSY (Table I) indicated that the three glucoses in **25** adopt the *C1* conformaton and are fully acylated. The chemical shift difference ($\Delta\delta$ 1.2—1.5) between the C-6 *geminal* protons of each glucose core indicated that the three HHDP groups are located at O-4/O-6 of each glucose residue.[7] The CD spectrum of **25** exhibited a strong positive Cotton effect at 233 nm $[\theta]$ $+51.1\times10^4$), indicating that the three HHDP groups in **25** have the *S*-configuration,[10] which is identical with those established for the ellagitannins, hirtellin B (**14**)[3a] and tamarixinin A (**15**). The comparison of the sugar proton signals in the ^1H-NMR spectrum of **25** with those of hirtellins A (**13**) and B (**14**) (Table I) revealed that the chemical shifts for two glucose cores (glucose-II and III) in **25** were almost the same as those of **14**, whereas those for the remaining glucose residue (glucose-I) were almost identical with those of glucose-II in **13**.[3b] These findings and the remarkable upfield shifts of H-1 (δ 5.47) and H-1″ (δ 5.63) are in agreement with the presence of a *m*-GOG unit at O-2 and of a *m*-GO-*m*-GOG unit at O-1″ and O-2″ as found in **13** and **14**. Therefore, hirtellin T_3 (**25**) is an ellagitannin trimer biogenetically producible by intermolecular oxidative coupling of the galloyl group at O-2 of tellimagrandin II (**12**) with the galloyl group at O-1′ of hirtellin B (**14**), accompanied with the formation of an additional *m*-GOG linking unit. This structure (**25**) for hirtellin T_3 was substantiated by partial hydrolysis in hot water, yielding two major hydrolyzates of known structures, **19** (monomer) and **15** (dimer), and also **16**, **17** and **18** (Chart 4).

Structure of Tamarixinin T_1 (26) Tamarixinin T_1 (**26**), isolated from the EtOAc extract of *T. pakistanica*, showed the $[M+Na]^+$ ion peak at *m/z* 2833 in the FAB-MS, corresponding to the molecular formula $C_{123}H_{86}O_{78}$. Methylation of **26** with diazomethane followed by methanolysis with sodium methoxide afforded **5**, **6** and **8**, which were identical with those obtained by similar treatment of tamarixinins B and C (**24**).[3d] The ^1H-NMR signals in the aromatic region of **26** indicated the presence of five galloyl groups, two *p*-GOG[6] units and three HHDP groups, by three 2H singlets (δ 7.10, 7.00 and 6.98), two 4H singlets (δ 7.02 and 6.97), two 1H singlets (δ 6.86 and 6.84), and four 1H and one 2H singlets at (δ 6.66—6.94). The spectrum in the aliphatic region showed the presence of three fully acylated *C1* glucopyranose cores which were distinguished from each other in the ^1H–^1H COSY spectrum as shown in Table II. The remarkable difference ($\Delta\delta$ *ca.* 1.5) in the chemical shifts between the C-6 *geminal* protons of each glucose moiety indicated the location of HHDP groups to be at O-4 and O-6 of each glucose core.[7] The (*S*)-configuration[10] of the HHDP groups in **26** was determined by the positive Cotton effect ($[\theta]$ $+26.4\times10^4$) at 235 nm in the CD spectrum. As shown in Table II, the chemical shifts of H-1—H-6 of glucose-I of **26** are similar to those of glucose-I of tamarixinin C (**24**), whereas those of protons of the other two glucose cores (II and III) were almost identical with those of glucose-II of **24**. These findings and the upfield shifts (δ 6.06 and 6.04) of the anomeric proton signals of glucose-II and III in **26** relative to those of tellimagrandin II (**12**), as found in tamarixinin B,[3d] suggested that tamarixinin T_1 (**26**) is a trimer composed of tamarixinin C (**24**) and **12**, which are linked

TABLE II. ^1H-NMR Spectral Data for Glucose Moieties[a] of **24**, **26** and **27** (500 MHz, Acetone-d_6+D_2O, *J* in Hz)

H	24	26	27	
			T_{2a}	T_{2b}
Glucose-I				
1	6.24 d (*J*=8)	6.23 d (*J*=8)	6.24 d (*J*=8.5)	6.15 d (*J*=8.5)
2	5.63 dd (*J*=8, 10)	5.64 dd (*J*=8, 10)	5.61 dd (*J*=8.5, 10)	5.53 dd (*J*=8.5, 10)
3	5.85 t (*J*=10)	5.82 t (*J*=10)	5.83 t (*J*=10)	5.741 t (*J*=10)
4	5.20 t (*J*=10)	5.20 t (*J*=10)[b]	5.16 t (*J*=10)	5.20 t (*J*=10)
5	4.49 dd (*J*=6.5, 10)	4.46 dd (*J*=6.5, 10)[c]	4.46 dd (*J*=6.5, 10)	4.49 dd (*J*=6.5, 10)
6	5.35 dd (*J*=6.5, 13.5)	5.343 dd (*J*=6.5, 13)	5.30 dd (*J*=6.5, 13)	5.32 dd (*J*=6.5, 13)
	3.85 d (*J*=13)	3.86 d (*J*=13)[d]	3.83 d (*J*=13)	3.86 d (*J*=13)
Glucose-II				
1′	6.07 d (*J*=8.5)	6.06 d (*J*=8)	6.09 d (*J*=8)	5.38 d (*J*=8)
2′	5.65 dd (*J*=8.5, 10)	5.68 t (*J*=10)	5.65 dd (*J*=8, 10)	5.57 d (*J*=8, 10)
3′	5.81 t (*J*=10)	5.81 t (*J*=10)	5.79 t (*J*=10)	5.65 t (*J*=10)
4′	5.23 t (*J*=10)	5.22 t (*J*=10)[b]	5.21 t (*J*=10)	5.21 t (*J*=10)
5′	4.57 dd (*J*=6.5, 10)	4.48 dd (*J*=6.5, 10)[c]	4.46 dd (*J*=6.5, 10)	4.21 dd (*J*=6.5, 10)
6′	5.37 dd (*J*=6.5, 13.5)	5.34 dd (*J*=6.5, 13)	5.32 dd (*J*=6.5, 13)	5.33 dd (*J*=6.5, 13.5)
	3.92 d (*J*=13.5)	3.88 d (*J*=13)[d]	3.86 br d (*J*=13)	4.00 br d (*J*=13.5)
Glucose-III				
1″		6.04 d (*J*=8)	5.42 d (*J*=8)	6.05 d (*J*=8)
2″		5.65 dd (*J*=8, 10)	5.55 dd (*J*=8, 10)	5.63 dd (*J*=8, 10)
3″		5.81 t (*J*=10)	5.67 t (*J*=10)	5.81 t (*J*=10)
4″		5.20 t (*J*=10)[b]	5.14 t (*J*=10)	5.22 t (*J*=10)
5″		4.56 dd (*J*=6.5, 10)[c]	4.25 dd (*J*=6.5, 10)	4.56 dd (*J*=6.5, 10)
6″		5.34 dd (*J*=6.5, 13)	5.30 dd (*J*=6.5, 13)	5.33 dd (*J*=6.5, 13)
		3.91 d (*J*=13)[d]	3.95 d (*J*=13)	3.89 d (*J*=13)

a) Assignments of **26** and **27** were based on the COSY, and correspondences with known compounds **12**, **13**, **24**, and **28**. Assignments marked *b*), *c*), or *d*) may be interchanged.

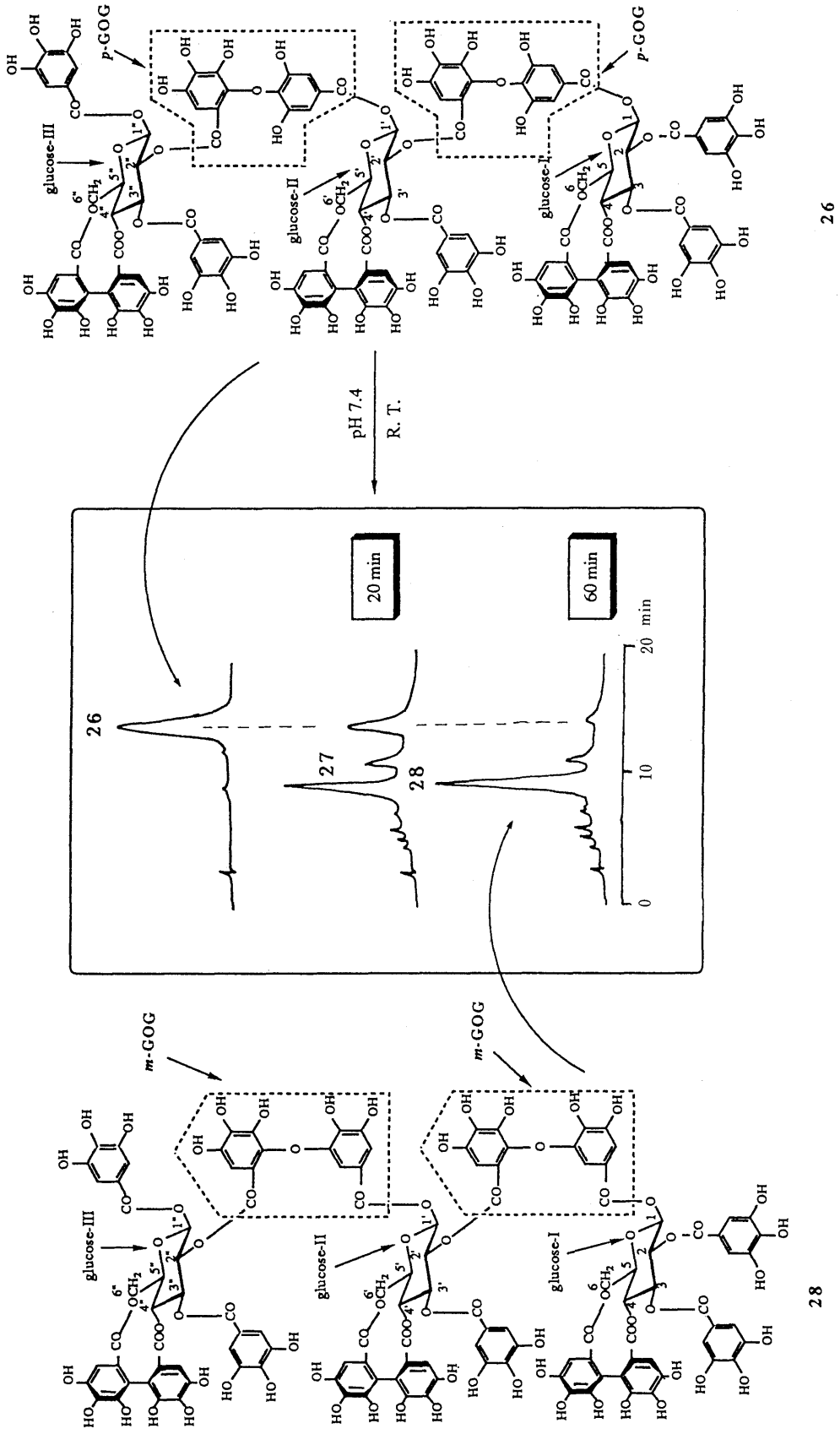
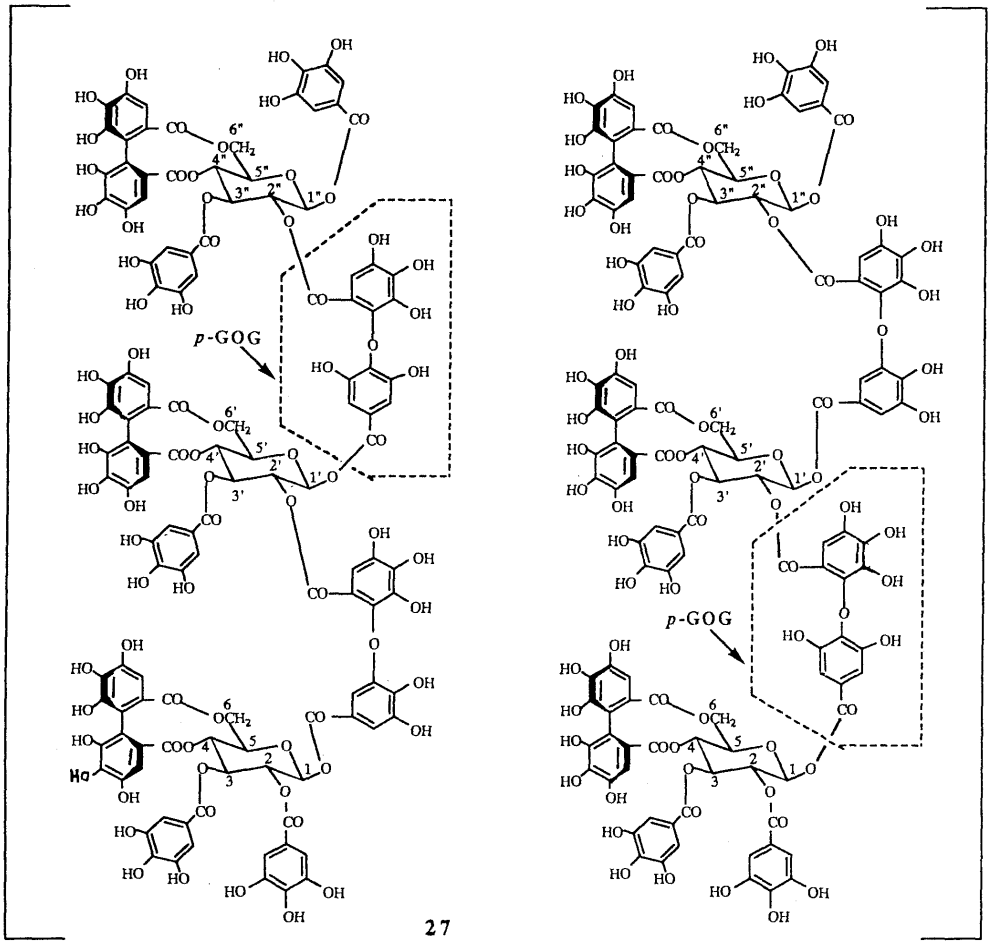


Chart 5. Conversion of Tamarixinin T_1 (**26**) into Hirtellin T_1 (**28**) at pH 7.4

Reversed-phase HPLC conditions: column, LiChrospher RP-18 (Merck); temperature, 40°C; mobile phase, 0.01 M KH_2PO_4-0.01 M H_3PO_4-EtOH-EtOAc (41.5:41.5:12:5); flow rate, 1.0 ml min^{-1}; UV detection at 280 nm.



27

Chart 6

to each other through an additional *p*-GOG unit. The binding modes of the *p*-GOG units in the structure **26** were confirmed by Smiles rearrangement,[3c,d] by which the two *p*-GOG units in **26** were isomerized into *m*-GOG units, giving hirtellin T_1 (**28**)[1] (Chart 5). In the reversed-phase HPLC monitoring of this reaction, the peak of **28** at an early stage was accompanied with another peak having a longer retention time, which became a minor peak as time passed. Separation of this minor compound (**27**) followed by isomerization through Smiles rearrangement afforded **28**, suggesting that **26** isomerized in a stepwise manner (**26**→**27**→**28**). The intermediary product (**27**) was an inseparable mixture of two isomers concerning the positions of the *m*-GOG and *p*-GOG units in the molecule, as revealed by the ^{1}H-NMR spectrum, which showed duplicated signals ascribable to five galloyl, a *m*-GOG, a *p*-GOG and three glucose residues (see Experimental). Tamarixinin T_2 (a mixture of isomers), obtained along with tamarixinin T_1 (**26**) from the ethyl acetate extract of *Tamarix pakistanica*, was identical with **27**. The possibility that the minor constituent, **27**, is an artefact produced from **26** during the extraction procedure can not be ruled out.

Experimental

General The materials (*R. hirtella* and *T. pakistanica*), instruments and chromatographic methods employed in this work were the same as those described in the preceding paper.[3c]

Isolation of Tannins from *R. hirtella* A part (35 g) of the *n*-BuOH extract obtained from the aqueous acetone homogenate of *R. hirtella* was subjected to column chromatography over Toyopearl HW-40 (Coarse)[3c] using aqueous MeOH and MeOH–H_2O–acetone. A part (600 mg) of the trimer mixture (4.3 g) obtained from the MeOH–H_2O–acetone (5:2:3) eluate was subjected to repeated chromatography over MCI-gel CHP-20P and/or preparative HPLC[1] to yield hirtellins T_2 (**1**) (50 mg) and T_3 (**25**) (56.8 mg) in addition to the previously isolated major trimer, hirtellin T_1.[1]

Isolation of Tannins from *T. pakistanica* The earlier fractions of the MeOH–H_2O–acetone (5:2:3) eluate from the column chromatography over Toypearl HW-40 (coarse) of a part (10.5 g) of the EtOAc-soluble portion of the crude extract[3d] gave a crude trimer, which, upon purification over MCI-gel CHP-20P and preparative HPLC,[1] yield tamarixinin T_2 (**27**) (9.5 mg) as a mixture of two regioisomers (T_{2a} and T_{2b}). The later fractions gave another crude trimer which was similarly purified to yield tamarixinin T_1 (**26**) (39.4 mg).

Hirtellin T_2 (1) An off-white amorphous powder, $[\alpha]_D$ +26° (c=1.0, MeOH). *Anal.* Calcd for $C_{123}H_{84}O_{78}\cdot 22H_2O$: C, 46.01; H, 4.02. Found: C, 45.73; H, 3.71. UV λ_{max}^{MeOH} (log ε): 216 nm (log 5.35), 274 (5.03). CD (MeOH) [θ] (nm): $+28.1\times10^4$ (237), -12.5×10^4 (263), $+7.5\times10^4$ (287) and -3.1×10^4 (320). ^{1}H-NMR (acetone-d_6+D_2O): δ 7.02 [6H, s, galloyl (Gal)×3], 6.61, 6.49 (each 3H, s, HHDP×3), 6.96—6.90 (6H in total), 6.46 (3H in total) (*m*-GOG×3), glucose protons, see Table I.

Methylation of Hirtellin T_2 (1) Followed by Methanolysis A mixture of hirtellin T_1 (**1**) (20 mg), anhydrous K_2CO_3 (200 mg) and Me_2SO_4 (0.4 ml) in dry acetone (5 ml) was stirred overnight at room temperature, and then refluxed for 7 h. After removal of the inorganic materials by filtration, the filtrate was concentrated *in vacuo*, and subjected to preparative TLC (Kieselgel PF_{254} benzene–acetone, 5:1) to give the permethylated derivative (**1a**) (10.5 mg) of hirtellin T_2 as a white amorphous powder. ^{1}H-NMR (acetone-d_6): δ 7.26 (6H, s, Gal×3), 7.19—7.16 (3H, br), 7.11, 6.71 (each 3H, br) (*m*-GOG×3), 6.88, 6.81

(each 3H, s, HHDP), 6.10—6.20 [br s, glucose (Glc) H-1, 1′, 1″], 5.58 (3H, br t, Glc H-2, 2′, 2″), 5.75 (3H, br, Glc H-3, 3′, 3″), 5.31 (3H, br, Glc H-4, 4′, 4″), 4.39 (3H, br, Glc H-5, 5′, 5″), 5.31 (3H, br, Glc H-6, 6′, 6″), other Glc H-6, 6′ and 6″ are overlapped by the OMe signals; 3.88, 3.84, 3.83, 3.74, 3.68 (br), 3.64, 3.62, 3.61 (br) (each 9H, s, OMe × 24) 3.86 (36H, s, OMe × 12), 3.78 (18H, s, OMe × 6), 3.83 (12H, s, OMe × 4).

Methanolysis of **1a** (1 mg) with 1% NaOMe (50 μl) in MeOH (0.2 ml) followed by usual work up[3c] gave methyl tri-*O*-methyl gallate (**5**), dimethyl hexamethoxydiphenate (**6**) and dimethyl penta-*O*-methyldehydrodigallate (**7**) (EI-MS: M^+, *m/z* 436) which were identical with authentic samples upon TLC co-chromatography.[3b]

Enzymatic Hydrolysis of 1 with Tannase A solution of **1** (10 mg) in H_2O (2 ml) was incubated with tannase (20 drops) at 37 °C for 60 h. The reaction mixture was acidified with 2 N HCl (3 d) and the precipitate was removed by centrifugation. The filtrate was placed on a BondElut C18 cartridge and eluted with H_2O and then with MeOH. The H_2O eluate gave gallic acid (1 mg), and the MeOH eluate was further purified by preparative HPLC[1] to give degalloylated compounds **2** (1.5 mg), **3** (1.5 mg) and **4** (1 mg), in addition to unreacted hirtellin T_2 (1.5 mg).

2: An off-white amorphous powder. ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.13 (2H, br s, *m*-GOG), 7.01 (5H, s, Gal × 2, *m*-GOG), 6.91, 6.92 (2H each, br, *m*-GOG), 6.72 (2H, br, *m*-GOG), 6.62, 6.57, 6.49 (2H each, s, HHDP × 3), 5.80—6.00 (3H, br, Glc H-1, 1′, 1″), 5.44 (2H, br Glc H-2, 2′), 5.70 (2H, br, Glc H-3, 3′), 5.13, 5.14 (1H each, t, *J* = 10 Hz, Glc H-4, 4′), 4.30 (2H, br, Glc H-5, 5′), 5.25, 5.26 (1H each, dd, *J* = 6.5, 13.5 Hz, Glc H-6, 6′), 3.80, 3.78 (1H each, d, *J* = 10 Hz, Glc H-6, 6′), 5.22—5.28 (1H, overlapped with H-6, 6′, Glc H-2″), 4.05 (2H, br, Glc H-3″, H-5″), 4.97 (1H each, t, *J* = 10 Hz, Glc H-4″), 5.16—5.11 (1H, overlapped by H-4, 4′, Glc H-6″), 3.73 (1H, d, *J* = 13.5 Hz, Glc H-6″).

3: An off-white amorphous powder. ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.12, 7.9 (br s each, 3H in total, *m*-GOG), 7.01 (2H, s, Gal × 1), 6.91—6.96 (3H, br, *m*-GOG), 6.74, 6.71 (br s each, 3H in total, *m*-GOG), 6.61, 6.57, 6.49 (2H each, s, HHDP × 3), 5.50—5.80 (3H, br Glc H-1, 1′, 1″), 5.44 (1H, br m, Glc H-2), 5.71 (1H, t, *J* = 10 Hz, Glc H-3), 5.14 (1H overlapped by H-6′, 6″, Glc H-4), 4.30 (1H br, Glc H-5), 3.77 (1H, d, *J* = 13.5 Hz, Glc H-6), 5.25 (3H, m, Glc H-6, 2′, 2″), 4.04 (4H, m, Glc H-3′, 3″, 5′, 5″), 4.97 (2H, t, *J* = 10 Hz, Glc H-4′, 4″), 5.13 (2H, overlapped by H-4, Glc H-6′, 6″), 3.73, 3.74 (1H each, d, *J* = 13 Hz, Glc H-6′, 6″).

4: An off-white amorphous powder. ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.08, 7.00 (3H each, br s), 6.71 (3H, br s), 6.58 (6H, s) (*m*-GOG × 3 and HHDP × 3), 5.40—5.75 (3H, br, Glc H-1, 1′, 1″), 5.18 (3H, m, Glc H-2, 2′, 2″), 4.02 (6H, m, Glc H-3, 3′, 3″, 5, 5′, 5″), 4.96 (3H, t, *J* = 10 Hz, Glc H-4, 4′, 4″), 5.15 (3H, dd, *J* = 6.5, 13 Hz, Glc H-6, 6′, 6″), 3.71 (3H, d, *J* = 13 Hz, Glc H-6, 6′, 6″).

Acid Hydrolysis of 1 A solution of **1** (1 mg) in 2 N HCl (1 ml) in a sealed ampule was heated in a water bath at 95 °C for 10 h. After cooling and filtration, the filtrate was extracted with EtOAc, and HCl in the aqueous layer was removed by adding Ag_2O and filtration. The TLC co-chromatography of the filtrate with an authentic sample [Kieselgel PF_{254}, *n*-BuOH–AcOH–water (4 : 1 : 2); detection: 2-aminobiphenylhydrogen oxalate reagent] showed the presence of glucose.

Partial Acid Hydrolysis of 1 A solution of **1** (1 mg) in 5% H_2SO_4 (1 ml) was heated in a water bath at 80 °C, with monitoring by HPLC, which showed the formation of compound **16** (t_R; 2.65 min), gemin D (**10**) (t_R; 2.76 and 3.05 min), compound **18** (t_R; 3.66 min), remurin B (**17**) (t_R; 3.36 and 4.23 min), remurin A (**19**) (t_R; 7.70 min), and compounds **20** (t_R; 4.52 and 7.90 min), **21** (t_R; 5.64 and 9.95 min) and **22** (t_R; 12.18 min). The identities of the first five hydrolyzates and compound **22** were confirmed by co-chromatography with authentic samples. Compounds **20** and **21** were identical with the products prepared by the degalloylation of compounds **22** and **23**, respectively, with tannase (37 °C, 20 min). The identities of remurin B (**17**), compounds **20** and **21** were further confirmed by the reduction ($NaBH_4$, 2 h) of a portion of the hydrolyzate mixture from hirtellin T_2 (**1**), followed by co-chromatography (HPLC) with the reduction products (t_R; 3.95, 6.78 and 8.43 min) from **17**, **20** and **21**. HPLC conditions: LiChrospher RP-18 cartridge (4 mm i.d. × 250 mm; Merck); solvent system, 0.01 M KH_2PO_4–0.01 M H_3PO_4–acetonitrile (45 : 45 : 10), at 40 °C.

Hirtellin T_3 (25) An off-white amorphous powder, $[\alpha]_D$ +90° (*c* = 1.0, MeOH). *Anal.* Calcd for $C_{123}H_{84}O_{78} \cdot 22H_2O$: C, 46.01; H, 4.02. Found: C, 45.77; H, 3.77. UV λ_{max}^{MeOH} nm (log ε): 217 (5.34), 274 (4.97). CD (MeOH) [θ] (nm): $+51.1 \times 10^4$ (233), -8.2×10^4 (260), $+6.1 \times 10^4$ (283), -2.0×10^4 (325). Normal-phase HPLC, t_R 17.07 (N2). ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.04, 6.97, 6.91, 6.78 (2H each, s, Gal × 4), 6.70, 6.64, 6.62, 6.55, 6.50, 6.46 (1H each, s, HHDP × 3), 7.30, 6.88, 6.50, 5.75 (1H each, d, *J* = 2 Hz), 7.57, 7.07, 6.71 (1H each, s) (*m*-GOG × 1 and *m*-GO-*m*-GOG × 1), and glucose protons, see Table I.

Methylation of Hirtellin T_3 (25) Followed by Methanolysis A solution of **25** (1.2 mg) in MeOH (0.5 ml) was methylated with ethereal CH_2N_2 at room temperature overnight. After removal of the solvent, the residue was treated with 1% NaOMe (50 μl) in MeOH (1 ml) at room temperature for 10 h. The reaction mixture was acidified with a drop of HOAc and the solvent was evaporated off. The residue was submitted to preparative TLC (Kieselgel PF_{254}, benzene–acetone, 4 : 1) to give **5** (0.2 mg) (EI-MS: M^+, *m/z* 226), **6** (0.3 mg) (EI-MS: M^+, *m/z* 450), **7** (0.1 mg) (EI-MS: M^+, *m/z* 436) and **9** (0.3 mg) (EI-MS: M^+, *m/z* 646), which were identical with authentic samples in TLC co-chromatography.

Acid Hydrolysis of Hirtellin T_3 (25) A solution of **25** (1 mg) in 2 N HCl (1 ml) in a sealed ampule was heated in a water bath at 95 °C for 10 h. After cooling and filtration, the filtrate was worked up in a similar way to that described for hirtellin T_2 (**1**) to show the presence of glucose as the sugar component.

Partial Hydrolysis of (25) A solution of **25** (8 mg) in H_2O (5 ml) was heated at 95 °C for 3 h, then allowed to cool. The precipitate was collected by centrifugation and was identified as ellagic acid (0.8 mg). The concentrated supernatant was subjected to preparative HPLC[1] using 0.01 M phosphate buffer–acetonitrile (88 : 12) as a mobile phase to afford gallic acid (0.5 mg) and compounds **16** (0.3 mg), **18** (0.2 mg), remurin A (**19**) (0.7 mg), remurin B (**17**) (0.2 mg) and tamarixinin A (**15**) (1.2 mg). These hydrolyzates were identified by HPLC co-chromatography with authentic samples. The identity of remurin A (**19**) was further confirmed by examination of the ^{1}H-NMR spectrum.

Tamariximin T_1 (26) An off-white amorphous powder, $[\alpha]_D$ +38° (*c* = 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 216 (5.39), 272 (5.05). CD (MeOH) [θ] (nm): $+26.4 \times 10^4$ (235), -8.4×10^4 (260), $+7.3 \times 10^4$ (285). FAB-MS: *m/z* 2833 $(M+Na)^+$. ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.10, 7.00, 6.98 (2H each, s), 7.02, 6.97 (4H each, s), 6.86, 6.84 (1H each, s) (Gal × 5 and *p*-GOG × 2), 6.66, 6.64, 6.63, 6.48 (1H each, s), 6.49 (2H, s) (HHDP × 3), and glucose protons, see Table II.

Methylation of Tamarixinin T_1 (26) Followed by Methanolysis A solution of **26** (2 mg) in MeOH (0.5 ml) was methylated with ethereal CH_2N_2 at room temperature overnight. The residue obtained after removal of the solvent was methanolyzed with 1% NaOMe (50 μl) in MeOH (0.5 ml) at room temperature for 8 h to give **5**, **6** and **8**, which were shown to be identical with those produced from tamarixinin C (**11**)[3d] by TLC co-chromatography.

Conversion of Tamarixinin T_1 (26) into Hirtellin T_1 (28) A solution of **26** (10 mg) in 0.1 M phosphate buffer (pH 7.4) (1 ml) was left standing at room temperature for 60 min, under monitoring by HPLC. The reaction mixture was acidified with 2 N HCl and evaporated. The residue was subjected to preparative HPLC[1] using 0.01 M phosphate buffer–EtOAc–EtOH (80 : 15 : 5) as a mobile phase to give hirtellin T_1 (**28**) (4 mg) and tamarixinin T_2 (**27**) (0.5 mg). The identities of both trimers were proved by co-chromatography on HPLC with authentic samples. The identity of hirtellin T_1 (**28**) was further confirmed by ^{1}H-NMR spectral comparison with an authentic specimen, and tamarixinin T_2 was converted into hirtellin T_1 (**27**) by Smiles rearrangement as described below.

Tamarixinin T_2 (27) An off-white amorphous powder. ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.11, 7.02, 6.99, 6.98, 6.96, 6.94 (2H each, s), 7.03, 6.97 6.97 (4H each, s), 6.82, 6.81 (1H each, s) (Gal × 5 × 2 and *p*-GOG × 1 × 2), 6.67, 6.67, 6.66, 6.63, 6.62, 6.61, 6.48, 6.47 (1H each, s), 6.49, 6.48 (2H, s) (HHDP × 3 × 2), 7.34, 7.28, 6.51, 6.49 (1H each, d, *J* = 2 Hz), 7.10, 7.07 (1H each, s) (*m*-GOG × 1 × 2) and glucose protons, see Table II.

Conversion of Tamarixinin T_2 (27) into Hirtellin T_1 (28) A solution of **27** (0.5 mg) in 0.1 M phosphate buffer (pH 7.4) (0.5 ml) was left standing for 45 min at room temperature, under monitoring by HPLC. The reaction mixture was acidified with 2 N HCl and evaporated. The main product was identified as hirtellin T_1 (**28**) by co-chromatography with an authentic sample.

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