

Communications to the Editor

[Chem. Pharm. Bull.]
[28(9)2850—2852(1980)]Structure of Gallotannins in *Paeoniae Radix*

The gallotannins (polygalloylglucoses) were isolated homogeneously from *Paeoniae Radix* and their structures were elucidated by ^{13}C -nuclear magnetic resonance spectroscopy.

Keywords—gallotannin; *Paeoniae Radix*; *Paeonia albiflora* var. *trichocarpa*; high performance liquid chromatography; ^{13}C -NMR; Sephadex LH-20 chromatography

Six gallotannins (penta—decagalloylglucoses) were obtained homogeneously from *Paeoniae Radix* (*Paeonia albiflora* PALLAS var. *trichocarpa* BUNGE) which has been used as an important Chinese medicine. The mixture of gallotannins was fractionated by column chromatography over Sephadex LH-20 (Table), and the homogeneity of each gallotannin, after rechromatography over Sephadex LH-20, was confirmed by high performance liquid chromatography (HPLC).

TABLE I. Column Chromatography of Acetone Extract from *Paeoniae Radix* on Sephadex LH-20^{a)}

Fraction NO.	Elution solvent (ml) EtOH:H ₂ O:acetone	Weight (g)	Substances	Composition ratio ^{b)} (%)
1	100 : 0 : 0 (200)	6.82	Glucosides	
2	100 : 0 : 0 (200)	1.46	Gallic acid, digallic acid	
3	100 : 0 : 0 (100)	0.45		
4	90 : 10 : 0 (100)			
5	90 : 10 : 0 (100)	0.31		
6	80 : 20 : 0 (100)			
7	80 : 20 : 0 (100)	0.66	Tetragalloylglucose	7
8	70 : 30 : 0 (100)		+	
9	70 : 30 : 0 (100)		Pentagalloylglucose	
10	60 : 40 : 0 (100)	0.29	Pentagalloylglucose	14
11	60 : 40 : 0 (100)	0.32	Pentagalloylglucose	
12	54 : 36 : 10 (100)		+	20
13	54 : 36 : 10 (100)	0.55	Hexagalloylglucose	
14	48 : 32 : 20 (100)		Hexagalloylglucose	
15	48 : 32 : 20 (100)	0.94	Heptagalloylglucose	18
16	42 : 28 : 30 (100)		+	
17	42 : 28 : 30 (100)	0.46	Octagalloylglucose	17
18	36 : 24 : 40 (100)	0.62	Nonagalloylglucose	
19	36 : 24 : 40 (100)		+	10
20	36 : 24 : 40 (100)	0.08	Decagalloylglucose	
			Undecagalloylglucose	3

a) To the 3.5 ID × 20 cm column was applied 13.3 g of the extract.

b) [amount of each gallotannin/total amount of the gallotannins (2.92 g, yield 0.41%)] × 100.

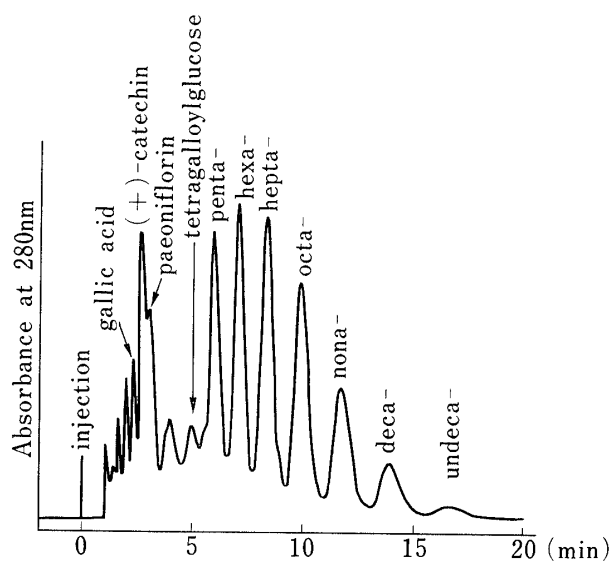


Fig. 1. High Performance Liquid Chromatogram of the Acetone Extract from *Paeoniae Radix*

Column: Nucleosil 50-10 (3ID×300mm) glass column.
Solvent: *n*-hexane-MeOH-THF-HCOOH (52:33:11:1)
(oxalic acid 40mg/100ml).

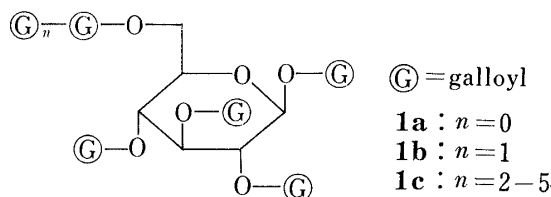


Fig. 2. Structure of the Gallotannins in *Paeoniae Radix*

Pentagalloylglucose (**1a**), $[\alpha]_D^{20} +33.4^\circ$ ($c=3.1$, acetone), UV: $\lambda_{\text{max}}^{\text{EtOH}}$ 280 nm ($\log \epsilon=4.59$), gave on methylation with diazomethane a permethyl derivative, MS m/e : 1150 (M^+), IR: no hydroxyl absorption, which was identified as 1,2,3,4,6-penta-kistri-O-methylgalloyl- β -D-glucose by comparison of its spectral data with those of authentic sample.¹⁾ The ^{13}C -NMR spectrum²⁾ of **1a** revealed the signals due to the glucose carbons at 93.3 (C-1), 74.0 (C-5), 73.3 (C-3), 71.7 (C-2), 69.3 (C-4) and

62.7 ppm (C-6). The assignment of these signals was performed by comparison with those of 1,2,3,4,6-pentaacetyl- β -D-glucose³⁾ and 1,2,3,4,6-pentabenzoyl- β -D-glucose.⁴⁾

Hexagalloylglucose (**1b**), UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm ($\log \epsilon$): 280 (4.65), 300 sh. (4.47), gave equimolar amounts of methyl gallate and **1a** on methanolysis with aqueous methanol (acetate buffer, pH 5.5).⁵⁾ The ^{13}C -NMR spectrum of **1b** showed the signals of the glucose carbons at 93.3 (C-1), 73.8 (C-5), 73.3 (C-3), 71.7 (C-2), 69.4 (C-4) and 63.1 ppm (C-6). In comparison with the resonances of **1a**, those of C-6 and C-4 were shifted to the downfield by 0.4 and 0.1 ppm, respectively, whereas that of C-5 was shifted to the upfield by 0.2 ppm. The downfield shift of C-6 was similar to that of the ester methyl carbon of methyl gallate when the *m*-hydroxyl group was galloylated.⁶⁾ Thus, the structure of **1b** was elucidated to be 6-*m*-digalloyl-1,2,3,4-tetragalloyl- β -D-glucose.

Hepta—decagalloylglucose (**1c**) also gave **1a** and methyl gallate on methanolysis, indicating that these gallotannins are consisted of pentagalloylglucose core. The position of the polygalloyl side chain was determined to be C-6 in the glucose moiety by the presence of similar C-4, -5, -6 carbon signals as those of **1b** on the ^{13}C -NMR spectra.

The heterogeneity of gallotannins in Chinese and Turkish galls was recognized by many investigators.⁷⁾ However, homogenous gallotannins having the polygalloyl side chain have not yet been isolated from these galls. Gallotannins have been isolated homogeneously for the first time by combination of Sephadex LH-20 chromatography and HPLC, and ^{13}C -NMR spectroscopy proved to be a useful method for the structure determination of gallotannins.

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Synthesis of (23*R*)-Caldiol Lactone (25-Hydroxyvitamin D₃ 26,23-Lactone)¹⁾

(23*R*)-Caldiol lactone(25-hydroxyvitamin D₃ 26,23-lactone) was synthesized from bisnorcholenic acid. The configuration at C-23 was determined by transformation to 22- and 23-hydroxycholesterols.

Keywords—caldiol lactone; 25-hydroxyvitamin D₃ 26,23-lactone; metabolite of vitamin D₃; 22-hydroxycholesterol; 23-hydroxycholesterol

Caldiol lactone²⁾ is a new metabolite of Vitamin D₃. Although the preliminary tests of this metabolite showed interesting biological activity,^{2,3)} the configuration at C-23 and C-25 positions as well as the biological role of this metabolite are unknown yet. In order to confirm the reported structure and to elucidate the configuration at C-23 and C-25, it is necessary to synthesize four possible isomers. It would be also urged to prepare the natural caldiol lactone for biological investigation. We describe herein a synthesis of (23*R*)-caldiol lactone.

The 22-aldehyde 3-THP ether **1**⁴⁾ derived from 22,23-bisnorcholenic acid was coupled with vinylmagnesium bromide to give a mixture of the 22-alcohols (**2**) in a 6:1 ratio. The less polar major alcohol, mp 155–156°, possesses the 22*R*-configuration according to the precedents for this mode of reaction.⁵⁾ The 22-alcohol **2** was reacted with ethyl orthopropionate and propionic acid as catalyst in refluxing xylene to give 22,23-*trans* 26-ethyl ester **3a** [mp 105–107.5°; NMR δ 1.11 ppm (3H, d, $J=6.6$ Hz, 27-H₃), 5.20–5.50 (3H, m, 6-H, 22-H, 23-H); IR 1720 cm⁻¹] in 96% yield. The 26-ester **3a** was hydrolysed to the 3-hydroxy-26-acid **3b**, mp 175–178°, by HCl-methanol and then KOH-methanol treatment. Iodolactonization⁶⁾ of the acid with iodine in acetonitrile at –0° gave regio- and stereoselectively a single product **4a** [mp 220–224°; 94% yield; NMR δ 1.28 (3H, d, $J=6$ Hz, 27-H₃), 2.50–3.10 (1H, m, 25-H), 4.10 (1H, dd, $J=5.1, 5.7$ Hz, 22-H), 4.60 (1H, m, 1/2w, 30 Hz, 23-H); IR 1768 cm⁻¹]. The iodolactone **4a** was then reduced by freshly distilled tributyltinhydride in dry THF at room temperature to the lactone **5a** [mp 223–224°; 85% yield; NMR δ 1.27 (3H, d, $J=7.5$ Hz, 27-H₃), 4.52 (1H, m, 1/2w 26 Hz, 23-H); IR 1760 cm⁻¹; CD (dioxane) $[\theta]^{20}$ (nm): –19 (249) (negative maximum).

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