Communications to the Editor

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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_2O : 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 + Pentagalloylglucose	7
8	70 : 30 : 0 (100)			
9	70 : 30 : 0 (100)			
10	60 : 40 : 0 (100)	0.29	Pentagalloylglucose	14
11	60 : 40 : 0 (100)	0.32	Pentagalloylglucose + Hexagalloylglucose	20
12	54 : 36 : 10 (100)			
13	54 : 36 : 10 (100)	0.55	Hexagalloylglucose	
14	48 : 32 : 20 (100)			
15	48 : 32 : 20 (100)	0.94	Heptagalloylglucose + Octagalloylglucose	18
16	42 : 28 : 30 (100)			17
17	42 : 28 : 30 (100)	0.46	Octagalloylglucose	
18	36 : 24 : 40 (100)	0.62	Nonagalloylglucose + Decagalloylglucose	11
19	36 : 24 : 40 (100)			10
20	36 : 24 : 40 (100)	0.08	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.

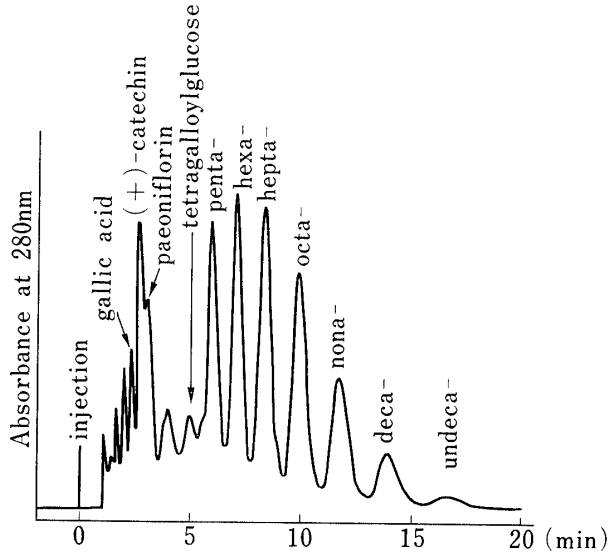

Fig. 1. High Performance Liquid Chromatogram of the Acetone Extract from Paeoniae Radix

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

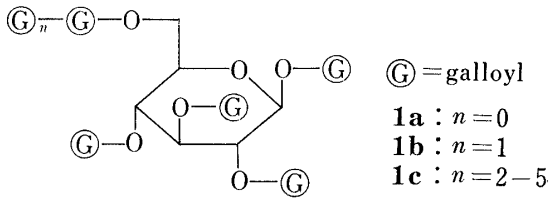

Fig. 2. Structure of the Gallotannins in Paeoniae Radix

Pentagalloylglucose (**1a**), $[\alpha]_D^{20}$ +33.4° (c=3.1, acetone), UV: λ_{max}^{EtOH} 280 nm (log ε=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-pentakistri-O-methylgalloyl-β-D-glucose by comparison of its spectral data with those of authentic sample.[1] The ^{13}C-NMR spectrum[2] 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-glucose[3] and 1,2,3,4,6-pentabenzoyl-β-D-glucose.[4]

Hexagalloylglucose (**1b**), UV λ_{max}^{EtOH} nm (log ε): 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*)-Calcidiol Lactone (25-Hydroxyvitamin D_3 26,23-Lactone)[1]

(23*R*)-Calcidiol lactone(25-hydroxyvitamin D_3 26,23-lactone) was synthesized from bisnorcholenic acid. The configuration at C-23 was determined by transformation to 22- and 23-hydroxycholesterols.

Keywords——calcidiol lactone; 25-hydroxyvitamin D_3 26,23-lactone; metabolite of vitamin D_3; 22-hydroxycholesterol; 23-hydroxycholesterol

Calcidiol lactone[2] is a new metabolite of Vitamen D_3. 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 calcidiol lactone for biological investigation. We describe herein a synthesis of (23*R*)-calcidiol 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-H_3), 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. Iodolactonization[6] 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-H_3), 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-H_3), 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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