Antidepressant Principles of the Roots of *Polygala tenuifolia*

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Received May 7, 2006

[^{125}I]RTI-55-membrane binding assay-guided fractionation and separation of a water-soluble extract of the roots of *Polygala tenuifolia* gave five new oligosaccharide derivatives, polygalatenosides A−E (**1**−**5**). The structures of these new oligosaccharides were established on the basis of spectroscopic evidence. Polygalatenosides A and B (**1** and **2**) showed significant inhibitory activity, with IC_{50} values of 30.0 and 6.04 μM, respectively, in this membrane binding assay and acted as norepinephrine reuptake inhibitors through blocking norepinephrine transport.

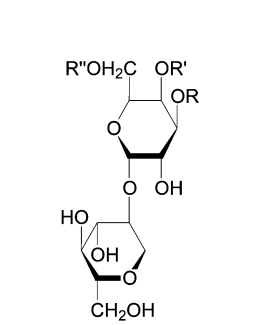
Psychotic diseases such as anxiety and neurosis have increased in recent years, and various antidepressant medications are now available. A number of studies have demonstrated that the newer antidepressant agents, such as venlafaxine (norepinephrine reuptake inhibitor) and bupropion (norepinephrine/dopamine reuptake inhibitor), exert their actions through an interaction with multiple receptors within the central nervous system.[1,2]

Extensive studies to search for new active extracts or components derived from various plants that can be used in the treatment of psychotic diseases have been carried out, in attempts to discover additional therapeutic drugs that possess both high efficacy and safety.[2−5] Although the mechanisms of action of plant extracts that have been used medicinally and traditionally should be investigated further, it is thought that they might contain various active components responsible for mediating atypical properties of the central nervous system.

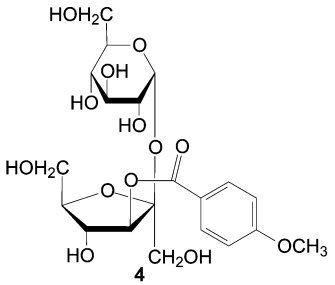
"Yuan Zhi" (the roots of *Polygala tenuifolia* Willd., Polygalaceae) is an important herb prescribed in traditional Chinese medicine to mediate sedative, antipsychotic, cognitive improving, neuron protective, and anti-inflammatory therapeutic effects on the central nervous system. It has also been used for insomnia, neurasthenia, amnesia, palpitations with anxiety, restlessness, and disorientation, and to prevent dementia and memory failure.[2,6−9] Various xanthones, saponins, and oligosaccharide esters have been reported from this plant.[10−17] To extend our understanding of the pharmacological actions of the constituents of *P. tenuifolia* on the central nervous system, in the present study the inhibitory action of the methanol extract of roots of *P. tenuifolia* was investigated against [^{125}I]RTI-55 [3β-(4-iodophenyl)tropan-2β-carboxylic acid methyl ester]-membrane protein binding. We report herein on the bioassay-guided separation of five new oligosaccharide derivatives (**1**−**5**) of an extract from *P. tenuifolia* found to inhibit the [^{125}I]RTI-55 binding to norepinephrine transporter protein.

Results and Discussion

The air-dried roots (1.25 kg) of *P. tenuifolia* were powdered and extracted with water under reflux. The water extract was subjected to Diaion HP-20 column chromatography and eluted, in turn, with H_2O, 50% aqueous MeOH, and 100% MeOH. The 50% aqueous MeOH eluate was chromatographed on a silica gel column using $CHCl_3$−MeOH to afford six fractions. Among them, fraction 2 inhibited [^{125}I]RTI-55 binding to norepinephrine transporter in MDCK cells in a concentration-dependent manner. The IC_{50} value was calculated to be 4.6 μg/mL. The selected fraction 2 was then subjected to preparative HPLC, using reversed-phase (ODS), which led to the isolation of five new oligosaccharide derivatives, polygalatenosides A−E (**1**−**5**).



1 R = H, R' = H, R" = benzyl
2 R = benzyl, R' = H, R" = H
3 R = H, R' = benzyl, R" = H



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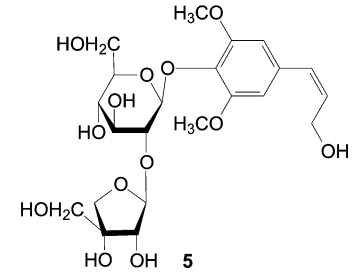

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Polygalatenoside A (**1**) was isolated as a colorless syrup. The HRFABMS of **1** showed a protonated molecular ion peak at *m*/*z* 431.1557 $[M + H]^+$, consistent with a molecular formula of $C_{19}H_{26}O_{11}$. The UV spectrum showed absorption maxima suggesting the presence of a benzoyl residue. In the IR spectrum, bands at 3411 and 1713 cm^{-1} revealed the presence of hydroxyl and conjugated ester carbonyl groups. The ^{1}H NMR spectrum displayed signals for a benzoyl group (δ 7.99, 2H d; 7.61, 1H, t; 7.47, 2H, d) in addition to signals due to galactosyl and polygolitosyl residues (Table 1). In addition, the ^{13}C NMR signals due to benzoyl, galactosyl, and polygolitosyl moieties (Table 2) also indicated **1** to be a polygolitosylgalactoside of benzoic acid. All proton and carbon NMR signals were assigned by ^{1}H− ^{1}H COSY, HMQC, and HMBC NMR experiments. The downfield shift of C-2 of the polygolitosyl unit by 4 ppm compared to that of polygolitol,[18] and the downfield shift of C-1 of the galactosyl unit to δ_C 95.7, suggested the interglycosidic linkage in **1** as polygolitosyl-(2→1)-α-galactoside. This was supported by a ^3J correlation between H-1 of the galactosyl unit (δ_H 5.00) and C-2 of the polygolitol moiety (δ_C 73.3) in a HMBC experiment (Figure 1). In the NMR spectra, downfield shifts of the methylene proton signals of the galactosyl residue to δ_H 4.49 and 4.39 and its C-6 carbon to δ_C 64.3 were used to establish the

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10.1021/np060207r CCC: $33.50
Published on Web 09/09/2006

Table 1. ^{1}H NMR Spectroscopic Data of **1**−**5** in CD_3OD

proton(s)	**1**[a]	**2**	**3**	**4**	**5**
sugar moiety					
galactose-1	5.00 d (4.2)	5.06 d (4)	5.09 d (3.5)		
2	3.78 dd (10.8, 4.2)	4.21 dd (10.5, 4)	3.94 dd (9.5, 3.5)		
3	3.84 dd (10.8, 3.6)	5.29 dd (10.5, 2.5)	4.10 dd (9.5, 3.5)		
4	4.02 m	4.25 br.	5.59 br s		
5	4.42	4.34 t (6)	4.47 t (5.5)		
6	4.49 m	3.78 dd (8.5, 6)	3.57 d (5.5)		
	4.39 m	3.72 dd (8.5, 6)	3.57 d (5.5)		
polygolitol-1	4.03 m	4.13 dd (11, 5)	4.14 dd (11, 5)		
	3.18 dd (11.4, 9.6)	3.30 dd (11, 9)	3.30 t (11)		
2	3.50 td (9.6, 5.4)	3.68 td (9, 5)	3.68 td (11, 6)		
3	3.43 (t (9.6)	3.53 t (9)	3.51 dd (11, 8.5)		
4	3.19 t (9.6)	3.33 t (9)	3.32 dd (8.5, 2)		
5	2.91 td (9.6, 1.8)	3.22 dd (9, 6)	3.23 dd (5.5, 2)		
6	3.45 dd (12.6, 9.6)	3.86 d (12)	3.86 d (12.5)		
	3.72 dd (12.6, 1.8)	3.64 dd (12, 6)	3.60 dd (12.5, 5.5)		
glucose-1				5.41 d (3.6)	5.10 d (7.6)
2				3.42 dd (9.2, 3.6)	3.69 dd (9.2, 7.6)
3				3.61 t (9.2)	3.54 t (9.2)
4				3.38 t (9.2)	3.45 t (9.2)
5				3.88 m	3.16 ddd (9.2, 4.8, 2.4)
6				3.76 dd (12, 4)	3.62 dd (11.6, 4.8)
				3.73 dd (12, 4.4)	3.73 dd (11.6, 2.4)
fructose-1				3.67 d (12)	
				3.58 d (12)	
2					
3				5.56 d (7.6)	
4				4.42 t (7.6)	
5				3.96 ddd (7.6, 6, 3.2)	
6				3.83 br d (9.2)	
				3.81 dd (9.2, 6)	
apiose-1					5.47 br s
2					4.00 br
3					
4					3.68 d (9.6)
					4.03 d (9.6)
5					3.59 d (11.6)
					3.72 d (11.6)
aglycon moiety					
2	7.99 d (8.4)	8.12 d (7.5)	8.06 d (7.5)	8.06 dt (9.6, 2)	6.55 s
3	7.47 m	7.50 t (7.5)	7.50 t (7.5)	7.01 dd (9.6, 2)	
4	7.61 m	7.62 t (7.5)	7.63 t (7.5)		
5	7.47 m	7.50 t (7.5)	7.50 t (7.5)	7.01 dd (9.6, 2)	
6	7.99 d (8.4)	8.12 d (7.5)	8.06 d (7.5)	8.06 dt (9.6, 2)	6.55 s
7					6.48 br d (11.6)
8					5.80 dt (11.6, 6.4)
9					4.34 dd (6.4, 1.6)
OMe				3.86 s	3.83 s

[a] Recorded in D_2O.

position of the benzoyl group linkage at C-6. This was confirmed by the ^3J correlation between H-6 (δ_H 4.49 and 4.39) and the ester carbonyl carbon of the benzoyl residue (δ_C 168.2). Analysis of all the available data led us to conclude that polygalatenoside A (**1**) is 6-*O*-benzoyl polygolitosyl-(2→1)-α-galactose.

Polygalatenosides B (**2**) and C (**3**) were isolated as colorless syrups. The HRFABMS of **2** and **3** gave protonated molecular ion peaks at *m*/*z* 431.1552 and 431.1554 $[M + H]^+$, respectively, and their ^{13}C NMR data were consistent with a molecular formula of $C_{19}H_{26}O_{11}$, the same as that of **1**. The UV spectra each showed absorption maxima corresponding to a benzoyl residue. The IR bands at 3415 and 1713 cm^{-1} indicated the presence of hydroxyl and conjugated ester carbonyl groups, respectively. In turn, the NMR spectra of polygalatenosides B and C were similar to those of **1**, showing a benzoyl residue as an ester moiety and galactosyl and polygolitosyl residues as sugar moieties. The substitution sites of these residues were determined with the aid of NOE (Figure 2) and HMBC (Figure 1) NMR observations after assignment of all proton signals from the $^1H-^1H$ COSY and HMQC spectra. The appearance of downfield shifted signals for C-2 of the polygolitosyl and C-1 of the galactosyl moieties in the ^{13}C NMR spectra and a ^3J correlation between H-1 (galactosyl) and C-2 (polygolitosyl) in the HMBC spectra of both compounds **2** and **3** confirmed the sugar residues as polygolitosyl-(2→1)-α-galactoside, the same as in **1**. However, these compounds were found to differ in the site of the ester linkages. In **2**, the downfield shifts of the H-3 and C-3 signals of the galactosyl unit to δ_H 5.29 and δ_C 75.3, respectively, suggested that the benzoyl group is located at C-3 of the galactosyl residue. In **3**, the signals of H-4 and C-4 of the galactosyl unit were shifted downfield to δ_H 5.59 and δ_C 73.5, respectively, and suggested that the benzoyl group is linked at the galactosyl C-4. This was supported by the ^3J correlation between H-4 (δ_H 5.59) of the galactosyl unit and the carbonyl carbon of the benzoyl group C-7 (δ_C 167.9) in the HMBC spectrum. Accordingly, **2** was defined structurally as 3-*O*-benzoylpolygolitosyl-(2→1)-α-galactose and **3** as 4-*O*-benzoylpolygolitosyl-(2→1)-α-galactose.

Polygalatenoside D (**4**) was obtained as a colorless syrup. The HRFABMS displayed a protonated molecular ion at *m*/*z* 477.1606, corresponding to the molecular formula $C_{20}H_{29}O_{13}$. The UV spectrum of **4** showed absorptions at 216 and 258 nm. The IR bands at 3414 and 1708 cm^{-1} were consistent with the presence of hydroxyl and conjugated ester carbonyl groups. The ^1H NMR

Table 2. ^{13}C NMR Spectroscopic Data of **1**−**5** in CD_3OD

carbon	**1**[a]	**2**	**3**	**4**	**5**
sugar moiety					
galactose-1	95.7	97.8	98.9		
2	67.9	67.6	70.5		
3	69.1	75.3	69.7		
4	69.2	68.8	73.5		
5	68.7	72.0	71.4		
6	64.3	62.4	62.1		
polygolitol-1	65.8	68.0	68.1		
2	73.3	76.7	76.7		
3	75.2	78.0	78.1		
4	80.3	72.0	71.9		
5	69.7	82.4	82.5		
6	61.0	63.1	63.1		
glucose-1				93.9	103.2
2				73.6	79.2
3				75.4	79.2
4				71.6	71.8
5				75.2	78.5
6				62.7	63.1
fructose-1				65.6	
2				105.3	
3				80.6	
4				74.4	
5				84.7	
6				63.6	
apiose-1					110.9
2					78.6
3					81.4
4					76.1
5					66.9
aglycon moiety					
1	129.1	131.2	131.4	122.8	134.9
2	129.6	130.9	130.8	133.6	108.5
3	128.7	129.5	129.6	115.4	154.7
4	133.9	134.3	134.4	165.8	135.5
5	128.7	129.5	129.6	115.4	154.7
6	129.6	130.9	130.8	133.6	108.5
7	168.2	168.7	167.9	167.7	132.0
8					132.8
9					60.3
OMe				56.5	57.5

[a] Recorded in D_2O.

spectrum displayed signals for A_2B_2-type aromatic protons (δ 8.06 and 7.01, each 2H) and a methoxy group (δ 3.86, 3H, s), in addition to signals due to a sucrose moiety. The ^{13}C NMR spectrum of **4** also showed signals due to a *p*-methoxybenzoyl group and a sucrose residue (Table 2). Full assignments of the ^1H and ^{13}C NMR signals were secured by COSY, HMQC, and HMBC experiments. The presence of the downfield-shifted oxymethine proton and carbon (H-3 and C-3) of the fructosyl moiety at δ_H 5.56 and δ_C 80.6, respectively, suggested that the *p*-methoxybenzoyl moiety is located at C-3′ in **4**. This was supported by the HMBC spectrum, since H-3 of the fructosyl residue (δ_H 5.56) was correlated to an ester carbonyl carbon of a *p*-methoxybenzoyl moiety at δ_C 167.7. Thus, the structure of **4** was deduced as 3′-*O*-*p*-methoxybenzoylsucrose.

Polygalatenoside E (**5**) was isolated as colorless syrup and was deduced as having an elemental composition of $C_{22}H_{32}O_{13}$ from its HRFABMS ($[M + H]^+$ *m*/*z* 505.1920). The IR absorption bands at 3400, 1585, 1505, and 1464 cm^{-1} indicated the presence of hydroxyl and aromatic moieties. NMR data of **5** showed a *cis*-sinapyl alcohol moiety signal including two equivalent aromatic protons at δ_H 6.55 (2H, s), two methoxy groups at δ_H 3.83 (6H, s), two *cis*-olefinic protons at δ_H 6.48 (1H, d, J = 11.6 Hz) and 5.80 (1H, dt, J = 11.6, 6.4 Hz), and oxymethylene protons at δ_H 4.34 (2H, dd, J = 6.4, 1.6 Hz), in addition to signals of a glucosyl at δ_C 103.2, 79.2, 79.2, 71.8, 78.5, and 63.1 and an apiosyl at δ_C 110.9, 78.6, 81.4, 76.1, and 66.9 in the ^{13}C NMR spectrum of **5**. By comparison of these data with those of kalopanaxin D,[19] the sugar portion was deduced as a β-apiosyl-(1→2)-β-glucoside moiety. This was supported by the downfield shift of C-2 of glucosyl to δ_C 79.2 and a ^3J correlation between the H-2 proton (δ_H 3.69) of the glucosyl unit and the C-1 of the apiosyl (δ_C 110.9) unit in the HMBC experiment (Figure 1). In addition, sinapyl alcohol was located at glucosyl C-1 on the basis of the observed HMBC correlations between the glucosyl H-1 (δ_H 5.10) and the *cis*-sinapyl C-4 (δ_C 135.5). From these data, the structure of **5** was elucidated as sinapyl alcohol 4-*O*-β-apiosyl-(1→2)-β-glucose.

Compounds **1**−**5** were tested in vitro for their ability to inhibit isotope-labeled RTI-55 binding to norepinephrine transporter protein.[20] In this membrane-binding assay, polygalatenosides A (**1**) and B (**2**) showed significant inhibitory activities, with IC_{50} values of 30.0 and 6.04 μM, respectively. Desipramine, a tricyclic antidepressant, also inhibited [^{125}I]RTI-55 binding to NET with an IC_{50} value of 0.93 nM. These results indicated that **1** and **2** may act as norepinephrine reuptake inhibitors by specific blockage of NETs.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-370 polarimeter. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer. The IR spectra were measured on a JASCO IR Report-100 spectrophotometer as KBr disks. HPLC was performed on a Shimadzu LC-10AT_{VP} (Japan) system using a Cosmosil 5C-18-MS-II column (20 × 250 mm and 4.6 × 250 mm, 5 μm). ^1H, ^{13}C, HMQC, HMBC, and NOESY NMR spectra were recorded on Bruker AMX-400 and Varian-400 Unity Plus NMR spectrometers, using tetramethylsilane (TMS) as internal standard; all chemical shifts are reported in parts per million (ppm, δ). Mass spectra (EI or FAB) were performed on a VG 70-250 S spectrometer.

Plant Material. The roots of *P. tenuifolia* were purchased from a market in Taipei, Taiwan, in May 2003 and authenticated by Prof. C. S. Kuoh (Department of Life Science, National Cheng Kung University). A voucher specimen of the plant (No. 920021) has been deposited at the herbarium of Medical and Pharmaceutical Industry Technology and Development Center, Taipei County, Taiwan.

Extraction and Isolation. The air-dried roots of *P. tenuifolia* (1.25 kg) were powdered and extracted twice with H_2O (5 L) for 2 h under reflux. The H_2O extract was subjected to Diaion HP-20 column chromatography and eluted with H_2O (45 L), 50% MeOH (30 L), and MeOH (25 L), successively. The 50% MeOH eluate was concentrated under reduced pressure to give a pale yellow syrup (44 g) and was chromatographed on a silica gel column using mixtures of $CHCl_3$−MeOH to afford six fractions. Fraction 2 (1.0 g) was subjected to preparative HPLC [ODS-5 (20 × 250 mm) using H_2O−CH_3CN (70:30) as mobile phase] to give three subfractions, 2-1 (125.3 mg), 2-2 (121.3 mg), and 2-3 (27.1 mg). Subfraction 2-2 was separated by HPLC [column: ODS-5 (4.6 × 250 mm) with the mobile phase H_2O−MeOH (80:20); flow rate 1.0 mL/min; UV 230 nm] to afford **2** (retention time: 13.3 min) (3.1 mg), **5** (retention time: 17.7 min) (1.6 mg), **4** (retention time: 19.4 min) (3.8 mg), **3** (retention time: 20.9 min) (4.6 mg), and **1** (retention time: 22.2 min) (33.8 mg), successively.

Polygalatenoside A (1): colorless syrup, $[\alpha]_D$ +171 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (4.12), 273 (3.41), 279 (sh) (3.37) nm; IR (KBr) ν_{max} 3411, 1713, 1634, 1603, 1585, 1285, 1080 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 431 ($[M + H]^+$, 3), 307 (40), 291 (24), 289 (18), 267 (8), 154 (100), 139 (11), 138 (28), 137 (56), 136 (58), 107 (15); HRFABMS *m*/*z* 431.1557 $[M + 1]^+$ (calcd for $C_{19}H_{27}O_{11}$, 431.1553).

Polygalatenoside B (2): colorless syrup, $[\alpha]_D$ +343.1 (*c* 0.003, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (3.94), 272 (3.69) nm; IR (KBr) ν_{max} 3415, 2927, 1713, 1602, 1452, 1280 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 431 $[M + H]^+$, 307, 291, 289, 154, 137, 136, 107; HRFABMS *m*/*z* 431.1552 $[M + 1]^+$ (calcd for $C_{19}H_{27}O_{11}$, 431.1553).

Polygalatenoside C (3): colorless syrup, $[\alpha]_D$ +256.6 (*c* 0.005, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (3.87), 273 (3.72), 301 (3.55) nm; IR (KBr) ν_{max} 3402, 1713, 1631, 1602, 1452, 1280 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 431 ($[M + H]^+$, 3.4), 307 (33), 291 (21), 289 (15), 267 (8), 155 (27), 154 (100), 139 (11), 138 (29), 137 (57), 136 (61), 107 (16); HRFABMS *m*/*z* 431.1554 $[M + 1]^+$ (calcd for $C_{19}H_{27}O_{11}$, 431.1553).

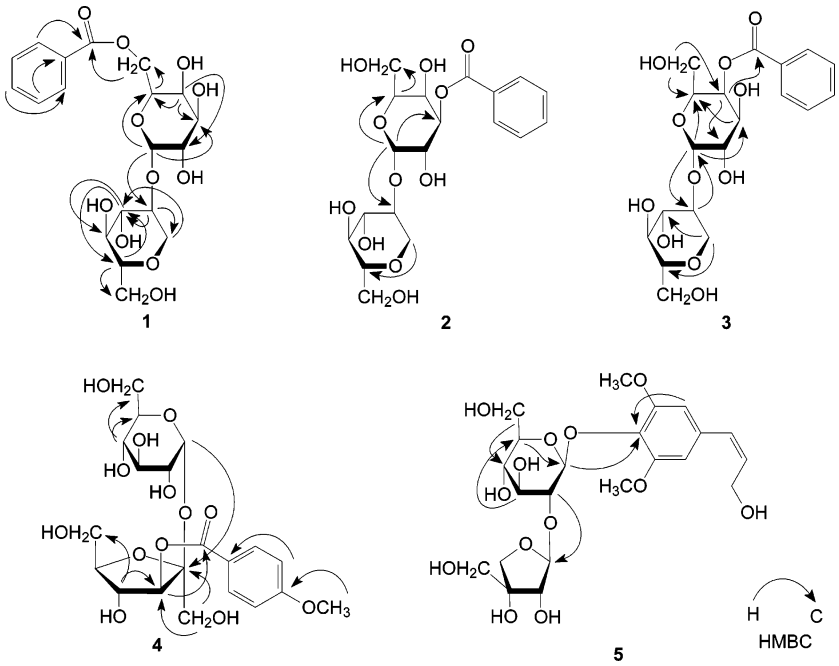

Figure 1. HMBC correlations of compounds **1**−**5**.

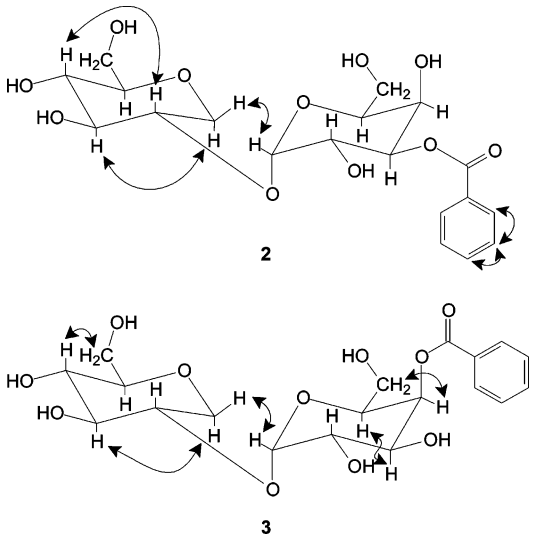

Figure 2. NOESY correlations of compounds **1**−**5**.

Polygalatenoside D (4): colorless syrup, $[\alpha]_D$ +103.7 (*c* 0.004, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (3.98), 258 (4.02) nm; IR (KBr) ν_{max} 3414, 1708, 1606, 1512, 1464 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m*/*z* 477.1606 $[M + 1]^+$ (calcd for $C_{20}H_{29}O_{13}$, 477.1611).

Polygalatenoside E (5): colorless syrup, $[\alpha]_D$ +616.8 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ϵ) 258 (4.15) nm; IR (KBr) ν_{max} 3400, 1585, 1505, 1464, 1405 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 505 ($[M + H]^+$, 0.5), 503 (2), 459 (3), 371 (3), 369 (3), 297 (4), 277 (11), 241 (16), 185 (100), 149 (28), 117 (10), 93 (98), 75 (40); HRFABMS *m*/*z* 505.1920 $[M + 1]^+$ (calcd for $C_{22}H_{33}O_{13}$: 505.1923).

Membrane Binding Assay. Membranes from dog kidney MDCK cells, which were stably transfected with the human norepinephrine transporter, were used. Total cell membranes were prepared from transfected cells grown to confluence in 500 cm^2 tissue culture dishes. Cells were scraped into a centrifuge tube and pelleted at 900*g* and 4 °C for 10 min. The pellets were resuspended in modified Tris-HCl buffer (50 mM Tris-HCl, 100 mM NaCl, 1 μM leupetin, 10 μM PMSF; pH 7.4) and centrifuged at 17000*g* and 4 °C for 30 min. Then, the pellets were resuspended, homogenized with a glass homogenizer with a Teflon pestle, and centrifuged at 17000*g* and 4 °C for 90 min. Pellets were collected and resuspended in modified Tris-HCl buffer. Protein concentrations were determined using BCA protein assay reagent (Pierce, Rockford). For the binding assay, a 40 μg aliquot of membrane protein was incubated with 0.2 nM [^{125}I]RTI-55 [3β-(4-iodophenyl)tropan-2β-carboxylic acid methyl ester] at 4 °C for 3 h. The binding was terminated by rapid vacuum filtration over Whatman GF/B filters soaked in 0.3% polyethylineimine followed by three rapid 1 mL washes in ice-cold buffer. Bound radioactivity was measured by γ emission spectrometry. Nonspecific binding was determined in the presence of 10 μM desipramine and was subtracted from the data in the absence of desipramine to yield specific binding.

Acknowledgment. We thank the National Science Council and the Ministry of Economic Affairs, Republic of China, for financial support of this research.

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NP060207R