Thonningianins A and B, New Antioxidants from the African Medicinal Herb *Thonningia sanguinea*

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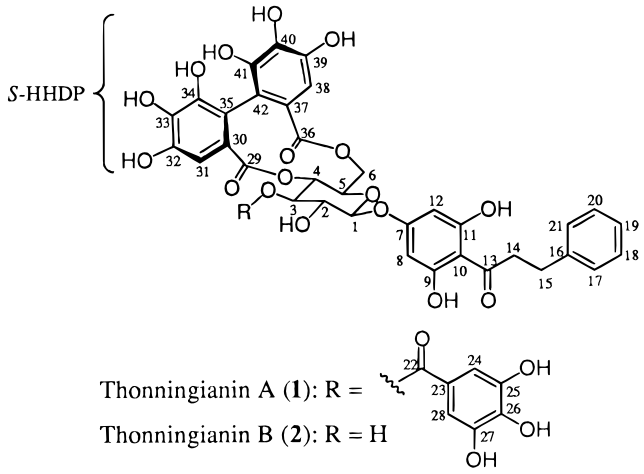
Two new ellagitannins, thonningianins A (**1**) and B (**2**), have been isolated from the African medicinal herb *Thonningia sanguinea* and their structures elucidated by interpretation of spectroscopic data. Both **1** and **2** showed strong free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) as shown by ESR analysis.

The medicinal herb *Thonningia sanguinea* vahl (Balanophoraceae) is used prophylactically against bronchial asthma in Ghana.[1] An extract of this herb showed scavenging action of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), inhibition of H_2O_2-induced lipid peroxidation in liver microsomes, and hepatoprotective effect against murine acute hepatitis induced by carbon tetrachloride, galactosamine, and aflatoxin B_1.[2] We have now succeeded in the identification of two new ellagitannins, thonningianins A (**1**) and B (**2**), as antioxidant constituents. In this report we describe the isolation, structure elucidation, and free radical scavenging action of **1** and **2**.



The roots of *T. sanguinea*, collected in a forest of eastern Ghana, were dried in an oven, crushed into pieces, and extracted with dichloromethane followed by methanol. Fractionation of the extract was guided by monitoring radical scavenging action with DPPH. Separation of the concentrated methanol extract (405 mg) on Sephadex LH-20 (MeOH) followed by HPLC on ODS (MeOH−H_2O) afforded thonningianins A (**1**, 14 mg) and B (**2**, 9 mg) as pale yellow solids.

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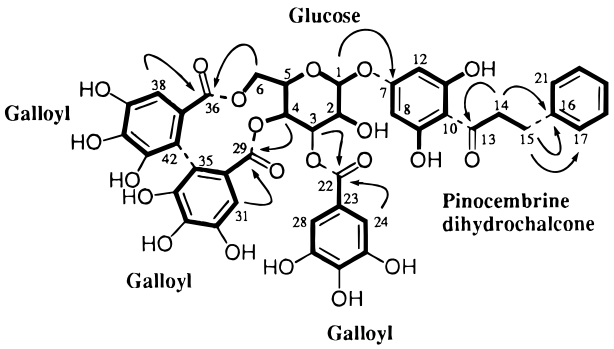

Figure 1. Partial structures (bold line) and HMBC correlations (arrows) for thonningianin A (**1**).

The molecular formula of thonningianin A (**1**) was determined as $C_{42}H_{34}O_{21}$ (26 deg of unsaturation) by high-resolution FABMS ([M − H]$^-$ *m/z* 873.1473 Δ −4.2 mmu). Interpretation of the NMR spectra (Table 1) suggested the presence of a glucose (C-1 to C-6), a 1,3,5-trihydroxyphenyl (C-7 to C-12), a propanoyl (C-13 to C-15), a phenyl (C-16 to C-21), and three galloyl (C-22 to C-28; C-29 to C-35; C-36 to C-42) groups. The presence of a 1,3,4,6-tetra-*O*-substituted-*β*-glucose was deduced from the ^3J coupling constants [7.7 Hz (H-1, H-2), 9.1 Hz (H-2, H-3), 10.0 Hz (H-3, H-4; H-4, H-5)] and chemical shift values [*δ* 5.17 (H-1), 3.83 (H-2), 5.43 (H-3), 5.09 (H-4), 5.37 (H-6), 3.88 (H-6′)]. Connectivity of the partial structures was shown by HMBC cross-peaks [H-1, H_2-8,12 (*δ* 6.12)/C-7 (*δ* 164.7); H-3, H_2-24,28 (*δ* 7.01)/C-22 (*δ* 168.0); H-4, H-31 (*δ* 6.46)/C-29 (*δ* 169.3); H-6′, H-38 (*δ* 6.58)/C-36 (*δ* 169.6); H_2-14 (*δ* 3.37), H_2-15 (*δ* 2.96), H_2-17,21, H_2-18,20 (*δ* 7.24)/C-16 (*δ* 143.0)], as shown in Figure 1. The NMR data for C-7 to C-21 were superimposable with those reported for the pinocembrine dihydrochalcone.[3] This together with the glucose and three galloyl groups accounts for 25 of the 26 deg of unsaturation. The remaining unsaturation must be due to the presence of an additional ring containing a hexahydroxydiphenoyl (HHDP) group formed by fusing the two galloyloxyl groups at the C-4 and C-6 of the glucose. The presence of HHDP (C-29 to C-42) and another galloyl moiety (C-22 to C-28) is supported by similar NMR data with those reported for related compounds.[4] The CD spectrum of **1** shows a negative Cotton effect at 268.5 nm (Δϵ −6.3) and a positive effect at 238.5 nm (Δϵ +23.0), indicating an *S*-configuration of the HHDP group.[5] The absolute configuration of glucose was proposed to be D by comparison of its optical rotation, $[\alpha]_D$ −44° (*c* 0.08, MeOH), with those reported for related

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Table 1. ^{1}H and ^{13}C NMR Data for Thonningianin A (**1**) in CD_3OD

no.	^{13}C NMR[a]		^{1}H NMR		[mult. *J* (Hz)][b]	HMBC (10 Hz)	HMBC (5 Hz)
1	101.5	d	5.17	d	7.7	H-2	
2	73.4	d	3.83	dd	7.7, 9.1	H-3	H-3
3	76.0	d	5.43	dd	9.1, 10.0	H-2, H-4	H-4
4	71.3	d	5.09	t	10.0	H-3	H-3
5	72.9	d	4.28	dd	6.4, 10.0	H-3, H-4, H-6′	H-4, H-6′
6	63.9	t	5.37	dd	13.1, 6.4		
6′			3.88	d	13.1		
7	164.7	s				H_2-8,12	H-1, H_2-8,12
8	96.4	d	6.12	s		H-12	
9	165.4	s					
10	107.1	s				H_2-8,12	H_2-8,12
11	165.4	s					
12	96.4	d	6.12	s		H-8	
13	206.8	s				H_2-14	
14	47.1	t	3.37	t	7.7		H_2-15
15	31.9	t	2.96	t	7.7		H_2-14, H_2-17,21
16	143.0	s				H_2-14, H_2-15, H_2-17,21, H_2-18,20	H_2-14, H_2-15, H_2-17,21, H_2-18,20
17	129.4	d	7.24	m		H-18	H_2-15, H-19
18	129.4	d	7.24	m		H-17	H-19
19	126.9	d	7.15	m			H_2-17,21, H_2-18,20
20	129.4	d	7.24	m		H-21	H-19
21	129.4	d	7.24	m		H-20	H_2-15, H-19
22	168.0	s				H-3, H_2-24,28	H-3, H_2-24,28
23	121.1	s				H_2-24,28	H_2-24,28
24	110.5	d	7.01	s			
25	146.3	s				H-24	
26	139.9	s				H_2-24,28	H_2-24,28
27	146.3	s				H-28	
28	110.5	d	7.01	s			
29	169.3	s				H-4, H-31	H-4, H-31
30	126.2[c]	s					
31	108.2	d	6.46	s			
32	145.8	s					H-31
33	137.6	s				H-31	H-31
34	145.0	s					
35	116.5	s				H-31	H-31
36	169.6	s				H-38	H-6′, H-38
37	125.9[c]	s					
38	108.5	d	6.58	s			
39	145.8	s					H-38
40	137.6	s				H-38	H-38
41	145.0	s					
42	116.8	s				H-38	H-38

[a] CD_3OD (49.0 ppm) signal was used as internal standard for ^{13}C (125 MHz) NMR. [b] CD_2HOD (3.30 ppm) signal was used as internal standard for ^{1}H NMR (500 MHz). [c] Interchangeable signals.

compounds.[4] Thus, the structure of thonningianin A (**1**) was determined as shown.

Thonningianin B (**2**) had a molecular formula of $C_{35}H_{30}O_{17}$ as determined by high-resolution FABMS ($[M - H]^-$ *m*/*z* 721.1367 Δ −3.8 mmu). The ^{1}H and ^{13}C NMR spectra of **2** (Table 2) are similar to those of **1**, except for the absence of the signals corresponding to one galloyl group. Compared with **1**, the H-3 signal is shifted upfield (δ 3.70 in **2**, δ 5.43 in **1**), indicating that **2** is a desgalloyl thonningianin A. Detail NMR analysis supported this conclusion. The absolute configurations of HHDP and the sugar moiety were determined in the same manner as **1**.

Both thonningianins A (**1**) and B (**2**) showed strong scavenging action against the DPPH radical, as revealed by an ESR study (Figure 2). The DPPH radical was scavenged completely by a 25 μM solution of **1** and 90% by a 34.5 μM solution of **2**. In a separate experiment, IC_{50} values of **1** and **2** were determined to be 8 and 21 μM, respectively. The more potent activity of thonningianin A (**1**) as compared to B (**2**) is presumably due to the presence of an additional galloyl group in A.

Experimental Section

General Experimental Procedures. The IR spectra were measured using a JASCO FT/IR-300. The UV spectra were obtained in methanol using a JASCO UVDEC 610 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL α-500 spectrometer and ESR spectra on a JEOL JES-FR30 instrument using manganese oxide (MnO) as an internal standard.

Plant Materials. The roots of *T. sanguinea* were collected in a forest of the Eastern Region of Ghana by the staffs of the Center for Scientific Research into Plant Medicine (CSRPM) on April 12, 1994. Taxonomic examination of the plant was carried out at CSRPM. A voucher specimen (No. CSRPM 406) has been deposited at CSRPM Herbarium, Akwapim-Mampong, Ghana.

Guide for Separation. To a solution of 0.1 mM DPPH in EtOH (ca. 0.2 mL) was added a small portion of sample solution (ca. 0.02 mL). When the color of DPPH changed from purple to pale yellow in 30 s, the sample was judged active.

Scavenging Action to DPPH Radical (ESR). A test solution was prepared by dissolving 12−25 μM thonningianin A or B in methanol and diluting with 100-fold volume of distilled water. The test solution (100 μL) was mixed with 100 μL of 100 μM DPPH ethanolic solution in a test tube by shaking for 10 s. The mixture was transferred to a flat cell for analysis of the amount of DPPH radical. ESR spectra were recorded after 40 s of mixing the solutions. The signal intensity was evaluated by dividing the peak height of the third of the five line signals of the DPPH radical with the height of the MnO signal to give relative peak height. The conditions of the

Table 2. ^{1}H and ^{13}C NMR Data for Thonningianin B (**2**) in CD_3OD

no.	^{13}C NMR[a]		^{1}H NMR		[mult. *J* (Hz)][b]	HMBC (10 Hz)
1	101.5	d	5.00	d	7.7	H-2
2	75.3	d	3.54	dd	7.7, 9.1	H-3
3	75.8	d	3.70	dd	9.1, 9.6	H-2
4	73.2	d	obscured by CD_2HOD signal			H-3, H-6′, H-5
5	73.1	d	4.07	dd	6.4, 9.8	H-3, H-6′
6	64.3	t	5.26	dd	6.4, 13.1	
6′			3.86	d	13.1	
7	164.8	s				H-1, H_2-8,12
8	96.4	d	6.10	s		H-12
9	165.4	s				
10	107.0	s				H_2-8,12
11	165.4	s				
12	96.4	d	6.10	s		H-8
13	206.8	s				H_2-14, H_2-15
14	47.1	t	3.37	t	7.7	H_2-15
15	31.9	t	2.96	t	7.7	H_2-14,
16	143.1	s				H_2-14, H_2-15, H_2-17,21, H_2-18,20
17	129.4	d	7.24	m		H_2-15, H-18, H-19, H-21
18	129.4	d	7.24	m		H-17, H-19, H-20
19	126.9	d	7.15	m		H_2-17,21, H_2-18,20
20	129.4	d	7.24	m		H-18, H-19, H-21
21	129.4	d	7.24	m		H_2-15, H-17, H-19, H-20
29	169.6	s				H-31
30	126.4[c]	s				
31	108.6	d	6.68	s		
32	145.8[d]	s				H-31
33	137.6	s				H-31
34	144.8[e]	s				
35	116.9	s				H-31
36	169.9	s				H-6, H-38
37	126.5[c]	s				
38	108.4	d	6.55	s		
39	145.9[d]	s				H-38
40	137.4	s				H-38
41	144.9[e]	s				
42	116.6	s				H-38

[a] *C*D_3OD (49.0 ppm) signal was used as internal standard for ^{13}C (125 MHz) NMR. [b] CD_2*H*OD (3.30 ppm) signal was used as internal standard for ^{1}H NMR (500 MHz). [c–e] Interchangeable signals.

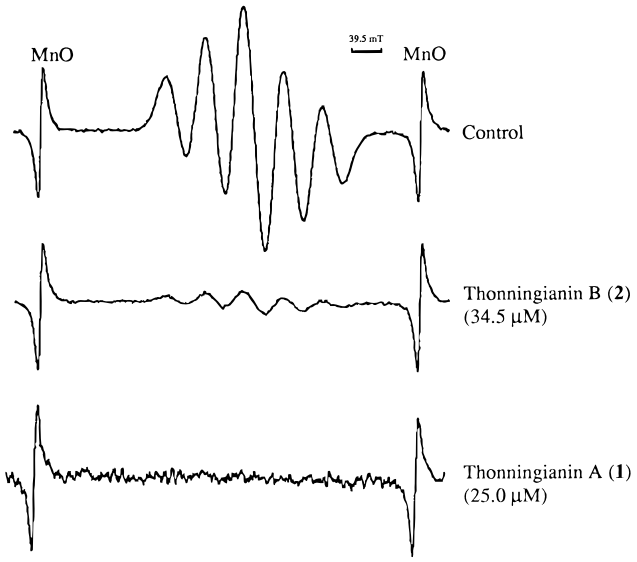

Figure 2. Effect of thonningianins A (**1**) and B (**2**) on the ESR signals of DPPH.

ESR spectrometer were set at temperature 15 ± 1 °C, power 4 mW, magnetic field 335.350 ± 5 mT, field modulation width 0.1 mT, sweep time 1 min, and time constant 0.1 s.

Extraction and Isolation. The oven-dried roots (5.3 g) were crushed into pieces and successively extracted with CH_2Cl_2 and MeOH. The MeOH extract was concentrated to dryness, and the residue (405 mg) was separated on Sephadex LH-20 (MeOH). The active fractions were combined and further separated by HPLC on ODS (MeOH−H_2O, 3:1 and 1:1) to afford thonningianins A (**1**, 14 mg) and B (**2**, 9 mg).

Thonningianin A (1): pale yellow solid; $[\alpha]_D$ −44° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 279.6 (4.45), 216.8 nm (4.64); IR (film) ν_{max} 3850, 1715, 1630, 1445, 1350, 1240, 1040 cm^{-1}; CD (MeOH) 268.5 nm ($\Delta\epsilon$ −6.3), 238.5 nm ($\Delta\epsilon$ +23.0); ^{1}H and ^{13}C NMR data are shown in Table 1; HRFABMS *m*/*z* 873.1473 ([M − H]$^-$, calcd for $C_{42}H_{33}O_{21}$ 873.1515, Δ −4.2 mmu).

Thonningianin B (2): pale yellow solid; $[\alpha]_D$ −141° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 280.0 (4.41), 224.8 (4.60), 208.4 nm (4.57); IR (film) ν_{max} 3850, 1730, 1630, 1600, 1435, 1360, 1235, 1200, 1180, 1080, 1050, 1025 cm^{-1}; CD (MeOH) 267.5 nm ($\Delta\epsilon$ −6.1), 239.0 nm ($\Delta\epsilon$ +17.5); ^{1}H and ^{13}C NMR data are shown in Table 2; HRFABMS *m*/*z* 721.1367 ([M − H]$^-$, calcd for $C_{35}H_{29}O_{17}$ 721.1405, Δ −3.8 mmu).

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