Chemical Constituents of *Phyllanthus reticulatus*

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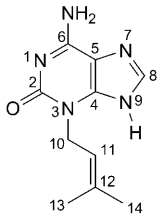
A new purine derivative, 3-(3-methylbut-2-en-1-yl)isoguanine (**1**), and a new cleistanthane-type diterpenoid glucoside, 19-hydroxyspruceanol 19-*O*-*β*-D-glucopyranoside (**2**), together with eight known compounds were isolated from the whole plant of *Phyllanthus reticulatus*. The structures were elucidated by chemical and spectroscopic methods.

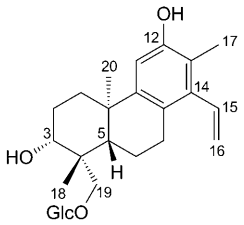
Introduction. – *Phyllanthus reticulatus* POIR. (Euphorbiaceae) is a folk medicine used for anti-inflammation and as analgetic, and for treatment of rheumatism in the Guangxi Zhuang national area of China [1]. It is a bushy shrub distributed widely in the tropics, from tropical Africa to India, China, and South-East Asia, and south to Queensland (northern Australia) [2]. Some reports have demonstrated that the extracts of this plant had antiplasmodial [3], antidiabetic [4], antimicrobial and cytotoxic [5], and hepatoprotective [6] bioactivities. Few studies on this plant revealed various chemical constituents such as triterpenoids, phytosterols, coumarin [5], flavonoids and phenols [7]. Our phytochemical investigation of the 75% EtOH extract of the title plant led to the isolation of 3-(3-methylbut-2-en-1-yl)isoguanine[1]) (**1**), a new purine derivative, and 19-hydroxyspruceanol 19-*O*-*β*-D-glucopyranoside[1]) (**2**), a new cleistanthane-type diterpene glucoside, as well as of eight known compounds, including one lignan glycoside, mananthoside I [8], one polyphenol, (−)-epigallocatechin [9], four aromatic compound glucosides, isotachioside [10], carthamoside B_5 [11], hovetrichoside A [12], and 3,4-dihydroxyphenylpropanol 3-*O*-*β*-D-glucopyranoside [13], and two megastigmane glycosides, turpenionosides A and B [14]. The above compounds are reported from this plant for the first time. In this paper, we describe the isolation and structural elucidation of **1** and **2**.

Results and Discussion. – Compound **1** showed *quasi*-molecular-ion peaks at *m*/*z* 220 ($[M+H]^+$), 242 ($[M+Na]^+$), and 218 ($[M-H]^-$) in the positive-ion- and negative-ion-mode ESI-MS, resp., in accord with the molecular formula $C_{10}H_{13}N_5O$, which was confirmed by the HR-ESI-MS. The structure of **1** was elucidated to be 3-(3-methylbut-2-en-1-yl)isoguanine[1]) on the basis of NMR analyses. The ^{1}H-NMR spectrum (*Table 1*) showed a *s* of an aromatic H-atom at δ(H) 8.11, and signals of a

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



1[1])



2[1])

3-methylbut-2-en-1-yl group at δ(H) 5.26 (t, J = 6.8 Hz, 1 H), 4.54 (d, J = 6.8 Hz, 2 H), 1.78 (s, 3 H), and 1.66 (s, 3 H). The ^{13}C-NMR and DEPT spectra (*Table 1*) displayed ten C-atom signals. Among them, five C-atoms were attributed to the (3-methylbut-2-en-1-yl) unit (δ(C) 135.7, 119.0, 40.5, 25.3, and 17.9); the remaining four sp^2 quaternary C-atoms (δ(C) 151.5, 150.6, 103.1, and 150.9), and one sp^2 CH group (δ(C) 142.0) combined with five N-atoms formed a skeleton of isoguanine [15], indicating an alkenylated isoguanine. The HMBC experiments (*Table 1*) confirmed the isoguanine unit and the 3-methylbut-2-en-1-yl group attached at N(3) by the cross-peaks H–C(8)/C(4) and C(5), as well as CH_2(10)/C(2) and C(4).

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; (D_6)DMSO) *of* **1**[1]). δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC (H → C)		δ(C)	δ(H)	HMBC (H → C)
C(2)	151.5 (s)			CH_2(10)	40.5 (t)	4.54 (d, J = 6.8)	C(2), C(4), C(12)
C(4)	150.6 (s)			H–C(11)	119.0 (d)	5.26 (t, J = 6.8)	C(13), C(14)
C(5)	103.1 (s)			C(12)	135.7 (s)		
C(6)	150.9 (s)			Me(13)	17.9 (q)	1.78 (s)	C(11), C(14)
H–C(8)	142.0 (d)	8.11 (s)	C(4), C(5)	Me(14)	25.3 (q)	1.66 (s)	C(11), C(13)

Compound **2** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{26}H_{38}O_8$ by the HR-ESI-MS. Acid hydrolysis of **2** gave a D-glucose as sugar moiety. The structure of **2** was established to be 19-hydroxyspruceanol 19-*O*-β-D-glucopyranoside[1]) by interpretation of its spectroscopic parameters and comparison with those of spruceanol (=(2*R*,4a*R*,10*S*)-8-ethenyl-1,2,3,4,4a,9,10,10a-octahydro-1,1,4a,7-tetramethylphenanthrene-2,6-diol) [16]. The ^{1}H- and ^{13}C-NMR and HMQC spectra of **2** (*Table 2*) showed that the aglycone contained 20 C-atoms and 25 C-bearing H-atoms (3 Me, 6 CH_2, 4 CH, and 7 C). Among which a pentasubstituted aromatic ring (δ(C) 154.9, 148.8, 140.4, 125.1, 121.1, and 111.2 (d); δ(H) 6.64 (s)) and attached Me group (δ(C) 13.8 (q); δ(H) 2.09 (s)), an ethenyl group (δ(C) 137.8 (d) and 119.9 (t); δ(H) 6.57, 5.46, and 5.05 (each dd, ^2J = 2.0, ^3J = 17.9, 11.4 Hz)), one isolated O-bearing CH_2 group (δ(C) 72.8; δ(H) 4.30 and 3.57 (each d, J = 10.2 Hz)), one O-bearing CH group (δ(C) 80.8; δ(H) 3.29 (dd, J = 11.3, 5.9 Hz)), and two tertiary Me groups (δ(H) 1.24 and 1.22) were assigned, indicating a spruceanol analogue [16]. Comparison of the ^{13}C-NMR data of the aglycone of **2** with those of spruceanol revealed that the most important difference was the isolated O-bearing CH_2 group of **2** instead of a Me group (δ(C) 15.4) for Me(19) of spruceanol, indicating that the

aglycone of **2** was 19-hydroxyspruceanol. The HMBC spectrum (*Table 2*) exhibited cross-peaks between $CH_2(19)$ and C(3), C(4), C(5), C(18) and C(1′), establishing that **2** is 19-hydroxyspruceanol 19-*O*-*β*-D-glucopyranoside.

Table 2. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; CD_3OD) *of* **2**[1]). *δ*(H) in ppm, *J* in Hz.

	δ(C)	*δ*(H)	HMBC (H → C)
$CH_2(1)$	39.5 (*t*)	2.27 (*dt*, *J* = 13.8, 3.3, H_α),	C(3), C(5), C(10)
		1.45 (*td*, *J* = 13.6, 3.2, H_β)	C(5), C(10), C(20)
$CH_2(2)$	29.7 (*t*)	1.79 (br. *d*, *J* = 13.4, H_α),	
		1.96 (*qd*, *J* = 13.0, 3.2, H_β)	
H–C(3)	80.8 (*d*)	3.29 (*dd*, *J* = 11.3, 5.9)	C(4), C(18), C(19)
C(4)	44.2 (*s*)		
H–C(5)	52.7 (*d*)	1.32 (*dd*, *J* = 12.3, 2.0)	C(3), C(4), C(6), C(7), C(9), C(10), C(18), C(19), C(20)
$CH_2(6)$	21.6 (*t*)	1.73–1.79 (*m*, H_α),	C(5), C(7), C(10)
		1.98 (br. *d*, *J* = 12.7, H_β)	C(4), C(5), C(7), C(8), C(10)
$CH_2(7)$	31.6 (*t*)	2.74 (*dd*, *J* = 16.9, 5.2, H_α),	C(6), C(8), C(9), C(14)
		2.45 (*ddd*, *J* = 16.9, 11.5, 7.0, H_β)	C(5), C(6), C(8), C(9), C(14)
C(8)	125.1 (*s*)		
C(9)	148.8 (*s*)		
C(10)	39.2 (*s*)		
H–C(11)	111.2 (*d*)	6.64 (*s*)	C(8), C(9), C(10), C(12), C(13), C(17)
C(12)	154.9 (*s*)		
C(13)	121.1 (*s*)		
C(14)	140.4 (*s*)		
C(15)	137.8 (*d*)	6.57 (*dd*, *J* = 17.9, 11.4)	C(8), C(13), C(14)
$CH_2(16)$	119.9 (*t*)	5.46 (*dd*, *J* = 11.4, 2.0),	C(14)
		5.05 (*dd*, *J* = 17.9, 2.0)	C(14), C(15)
Me(17)	13.8 (*q*)	2.09 (*s*)	C(8), C(9), C(11), C(12), C(13), C(14)
Me(18)	24.4 (*q*)	1.24 (*s*)	C(1), C(5), C(9), C(10), C(19)
$CH_2(19)$	72.8 (*t*)	3.57 (*d*, *J* = 10.2), 4.30 (*d*, *J* = 10.2)	C(3), C(4), C(5), C(18), C(1′)
Me(20)	26.3 (*q*)	1.22 (*s*)	C(1), C(5), C(9), C(10)
H–C(1′)	105.5 (*d*)	4.22 (*d*, *J* = 7.9)	C(19), C(2′), C(5′)
H–C(2′)	75.5 (*d*)	3.19 (*t*, *J* = 8.3)	
H–C(3′)	78.6 (*d*)	3.36 (*t*, *J* = 8.5)	
H–C(4′)	72.0 (*d*)	3.30 (*t*, *J* = 8.5)	
H–C(5′)	78.4 (*d*)	3.26 (br. *dd*, *J* = 8.5, 4.7)	
$CH_2(6')$	63.1 (*t*)	3.86 (*dd*, *J* = 12.0, 1.4),	
		3.68 (*dd*, *J* = 12.0, 4.7)	

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang*, *Co.*, *Ltd.*, P. R. China), *Sephadex LH-20* (*Pharmacia Biotech AB*, Uppsala, Sweden), *ODS-A* gel (*Mitsubishi Chemical Industries Co.*, *Ltd.*, Japan). TLC: silica gel *HSGF$_{254}$* (*Yantai Jiangyou Guijiao Kaifa Co.*, *Ltd.*,

P. R. China). Optical rotation: *Perkin-Elmer-341* polarimeter. UV Spectra: *Shimadzu-UV-2550* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Magna-750-FTIR* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker-AV-400* instrument at 400 (^1H) and 100 MHz (^{13}C); in (D_6)DMSO or CD_3OD; δ in ppm rel. to Me_4Si; J in Hz. ESI-MS and HR-ESI-MS: *Bruker-Esquire-3000-plus* and *Finnigan-LC-QDECA* mass spectrometers, resp.; in m/z (rel. int.).

Plant Material. The fresh whole plants of *P. reticulatus* were collected in the Yongning County, Guangxi Province, P. R. China. The plant was identified by Prof. *S.-J. Wei* of the Guangxi Traditional Chinese Medicine University. A voucher specimen (No. 09-108) is deposited at the Herbarium of the Shanghai Institute of Materia Medica.

Extraction and Isolation. Air-dried and powdered whole plants of *P. reticulates* (5 kg) were extracted three times with 20 l of 75% EtOH at r.t. The concentrated extract was partitioned between H_2O and petroleum ether, $CHCl_3$, AcOEt, and BuOH, resp. The BuOH fraction (108 g) was subjected to CC (SiO_2 (2 kg), column i.d. 10×90 cm, $CHCl_3$, $CHCl_3$/MeOH 100:1, 50:1, 20:1, 10:1, 6:1, and 3:1): *Frs. A – G. Fr. E* yielded a solid which was further purified by CC (*Sephadex LH-20*, MeOH): **1** (12 mg). *Fr. F* (10.9 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 10:1, 8:1, 6:1, and 3:1): *Frs. F_1 – F_4*. From *Fr. F_2* (320 mg), **2** (15 mg) was obtained after two CC (1. *ODS-A* gel, 20%, 50%, 70% and 95% MeOH/H_2O; 2. Foregoing 70% portion, *Sephadex LH-20*, MeOH). *Fr. F_3* (550 mg) was subjected to CC (SiO_2, $CHCl_3$/MeOH/H_2O 5:1:0.1): *Frs. $F_{3.1}$ – $F_{3.8}$*. *Fr. $F_{3.1}$* (45 mg) gave isotachioside (4 mg) after purification by two CC (1. *ODS-A* gel, 10 – 50% MeOH/H_2O; 2. *Sephadex LH-20*, MeOH). By the same procedure as applied to *Fr. $F_{3.1}$*, *Frs. $F_{3.4}$* (62 mg), *$F_{3.5}$* (80 mg), *$F_{3.6}$* (50 mg), *$F_{3.7}$* (45 mg), and *$F_{3.8}$* (60 mg) afforded (−)-epigallocatechin (13 mg), mananthoside I (32 mg), carthamoside B_5 (14 mg), hovetrichoside A (4 mg), and 3,4-dihydroxyphenylpropanol 3-*O*-β-D-glucopyranoside (6 mg), as well as turpenionosides A (8 mg) and B (12 mg), resp.

Acid Hydrolysis of **2**. A soln. of **2** (2 mg) in 2M HCl/dioxane 1:1 (2 ml) was refluxed for 2 h. After cooling, the soln. was neutralized with $NaHCO_3$ and then filtrated to remove the solid. The filtrate was subjected to CC (*Sephadex LH-20*, 50% MeOH/H_2O) to afford a sugar fraction. This sugar fraction and standard D-glucose (*Sigma*, USA) were each treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (1 ml) at 60° for 1 h. Then, the soln. was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.02 ml) at 60° for 1 h. Subsequently, the supernatant was subjected to GC analysis (*Supelco*, 230°, flow rate 15 ml/min): D-Glucose (t_R 24.2 min) was detected.

3-(3-Methylbut-2-en-1-yl)isoguanine (=*6-Amino-3,9-dihydro-3-(3-methylbut-2-en-1-yl)-2*H*-purin-2-one*; **1**): White amorphous powder. UV (MeOH): 203 (5.36), 291 (5.08). IR: 3325, 1767, 1660, 1604, 1576, 1493, 1448, 1410, 1302, 756, 552. ^1H- and ^{13}C-NMR and HMBC: *Table 1*. ESI-MS (pos.): 220 ($[M + H]^+$), 242 ($[M + Na]^+$), 439 ($[2\,M + H]^+$). ESI-MS (neg.): 218 ($[M - H]^-$), 437 ($[2\,M - H]^-$). HR-ESI-MS: 242.1021 ($[M + Na]^+$, $C_{10}H_{13}N_5NaO^+$; calc. 242.1018).

19-Hydroxyspruceanol 19-O-β-D-*Glucopyranoside* (=rel-*[(1*R,*2*R,*4a*R,*10a*S*)-8-Ethenyl-1,2,3,4,4a,9,10,10a-octahydro-2,6-dihydroxy-1,4a,7-trimethylphenanthren-1-yl]methyl* β-D-*Glucopyranoside*; **2**): White amorphous powder. $[\alpha]_D^{25} = -42$ ($c = 0.18$, MeOH). UV (MeOH): 211 (4.95). IR: 3421, 1632, 1425, 1398, 1269, 1078, 1040. ^1H- and ^{13}C-NMR and HMBC: *Table 2*. ESI-MS (pos.): 501 ($[M + Na]^+$). ESI-MS (neg.): 523 ($[M + HCOO^-]^-$), 955 ($[2\,M - H]^-$). HR-ESI-MS: 501.2469 ($[M + Na]^+$, $C_{26}H_{38}NaO_8^+$; calc. 501.2464).

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