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Neo-lignans in the Seed Crusts of *Trewia nudiflora*

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Abstract: Four new neo-lignans were isolated from the chloroform portion of 70% ethanol extract of the seed crusts of *Trewia nudiflora* L., and were determined to be 9'-methoxy-7'-en-3',8:4',7-di-epoxyneolignan-3,4,9-triol **1**, 9'-methoxy-7'-en-3',7:4',8-diepoxyneolignan-3,4,9-triol **2**, 9'- ethoxy-7'-en-3',8:4',7-diepoxyneolignan-3,4,9-triol **3**, 9'-butoxy-7'-en-3',8:4',7-diepoxy-neolignan -3,4,9-triol **4**, respectively, on the basis of spectral data. The antimicrobial bioassays showed that **4** had evident inhibitory activity against *Mycobacterium tuberculosis*.

Keywords: *Trewia nudiflora*, neo-lignans, antibacterial activity.

The genus *Trewia* (Euphorbiaceae) only has one species, which is tall arbor and distributes in the tropical districts of India, Malaysia and China. Some maytansinoids isolated from the *T. nudiflora* seeds are tumor inhibitors[1,2], and may be responsible for the resistance of the seeds to fungal degradation. However, there were no reports about the isolation of antibacterial components from the seeds of *T. nudiflora* from the seed crusts. We report here the isolation, structure identification and the antibacterial activity against *Mycobacterium tuberculosis* of four neo-lignans.

Compound **1**, obtained as white powders, $[\alpha]_D^{23}$ -6.67 (*c* 0.30, MeOH). The HRESIMS determined the molecular formula to be $C_{19}H_{20}O_6$ (*m/z* 367.1167 $[M + Na]^+$, calcd. 367.1157). The IR (KBr) spectrum of **1** revealed the presence of the hydroxyl (3430 cm^{-1}), ether (1274 cm^{-1}) groups, double bond (1615 cm^{-1}) and aromatic (1586, 1507 cm^{-1}) ring and its UV (MeOH) spectrum has absorption at λ_{max} (log ε) 204.2 (4.70), 268.8 (3.32), 340.8 (2.73) and 375.6 (2.48). The ^{1}H-NMR spectrum indicated the presence of a *trans*-double bond [δ 6.49 (d, 1H, J = 15.9 Hz), δ 6.13 (dd, 1H, J = 6.2, 15.8 Hz)], two 1,3,4-trisustituted benzene rings [δ 6.85 (s, 1H), δ 6.81 (d, 1H, J = 8.3 Hz), δ 6.75 (d, 1H, J = 7.5 Hz)] and [δ 6.94 (s, 1H), δ 6.91 (d, 1H, J = 8.5 Hz), δ 6.89 (d, 1H, J = 6.1 Hz)], and the structure unit involving 1,4-dioxane ring [δ 4.02 (m, 1H), δ 4.78 (d, 1H, J = 8.0 Hz)], indicating that **1** was a neolignan. The HMBC experiments showed the ^{1}H-^{13}C long-range correlations between δ_H 3.34 (H-10') and δ_C 74.1 (C-9'), δ_H 3.92 (H-9') and δ_C 58.0 (OMe-9'), 124.7 (C-8') and 133.6 (C-7'), and the structure of the cafestol moiety was determined. The structure of nine-carbon (C-1 – C-9) moiety was determined based on the HMBC correlations between δ_H 3.65 (H-9) and δ_C 77.5

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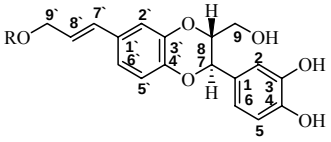

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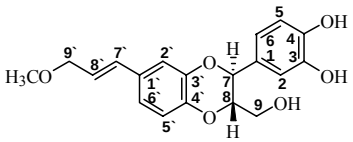

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Table 1 The NMR data of compounds **1** and **2** in CD_3OD[a](δppm)

Position	Compound **1**			Compound **2**		
	δ_C	δ_H	HMBC	δ_C	δ_H	
1	129.4			129.4		
2	115.7	6.85, s	C-7, C-6, C-4	115.6	6.85, s	C-7, C-6, C-4
3	146.4			146.7		
4	146.9			147.2		
5	116.3	6.81,d, 8.3	C-1, C-3	116.4	6.81,d, 8.3	C-1, C-3
6	120.4	6.75, d, 8.5	C-7, C-2, C-1, C-4	120.4	6.77, d, 8.5	C-7, C-2, C-4
7	77.5	4.78, d, 8.0	C-8, C-2, C-1, C-4'	77.7	4.82, d, 8.1	C-8, C-2, C-1
8	79.8	3.96, m		80.0	4.02, m	C-7
9	62.0	3.65, d, 11.4	C-7	62.1	3.71, d, 11.4	C-7, C-8
		3.46,dd,3.7,12.2			3.49,dd,3.7,12.2	C-8, C-3' (w)
1'	131.6			131.8		
2'	115.8	6.94,s	C-6', C-7', C-4'	115.8	7.05,s	C-6', C-7', C-3'
3'	144.7			144.9		
4'	145.1			145.5		
5'	117.9	6.91,d, 8.5	C-1', C-7', C-4'	117.3	6.99, d, 8.5	C-1', C-4'
6'	121.3	6.89,d, 8.4	C-2', C-1', C-7' C-4'	121.0	6.90,d, 8.4	C-2',
7'	133.6	6.49, d, 15.9	C-9', C-2', C-1' (w)	133.6	6.53, d, 15.9	C-9', C-2', C-6'
8'	124.7	6.13,dd,6.2,15.8	C-9', C-1', C-7'	125.0	6.16,dd,6.2,15.7	C-9', C-1'
9'	74.1	4.01, d, 6.1	OCH_3, C-8', C-7'	74.2	4.04, m	OCH_3,C-8',C-7'
	58.0	3.34, s, 3H	74.1	58.0	3.34, s, 3H	74.1

(C-7), δ_H 4.78 (H-7) and δ_C 62.0 (C-9), 79.8 (C-8), 129.4 (C-1) and 115.7 (C-2). The linkage of the two moieties was determined by the weak three-bond ^1H-^{13}C long- range correlations between δ_H 4.78 (H-7) and δ_C 145.1 (C-4'). The ROESY experiment showed ^1H-^1H correlations between δ_H 4.78 (H-7) and δ_H 3.46 (H-9b) and 3.65 (H-9a), indicating the *trans*-form of C-7/C-8 in the dioxane ring. Therefore, compound **1** was determined to be 9'-methoxy-7'-en-3',8:4',7-diepoxy-neolignan-3,4,9-triol, namely 9'-methyl americanol A [3].

Compound **2**, the molecular formula was determined by HRESIMS to be $C_{19}H_{20}O_6$ (*m/z* 367.1152 $[M + Na]^+$, calcd. 367.1157). The ^1H- and ^{13}C-NMR spectra of **2** showed great similarities to those of compound **1**. The ROESY experiment showed ^1H-^1H correlations between δ_H 4.82 (H-7) and δ_H 3.49 (H-9b), indicating the *trans*-form of C-7/C-8 in the dioxane ring. Therefore, compound **2** was determined to be 9'-methoxy-7'-en-3',7:4',8-diepoxyneolignan-3,4,9-triol, namely 9'-methyl isoamericanol A [3].

Table 2 The NMR data of compounds **3** and **4** in CD_3OD[a](δppm)

Position	Compound **3**			Compound **4**		
	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
1	128.0			129.6		
2	114.2	6.80, s	C-6, C-1(w), C-4	115.5	6.85, s	C-6, C-4
3	146.7			146.6		
4	147.2			147.1		
5	115.3	6.72, d, 8.5	C-2, C-1, C-3	116.4	6.80,d, 8.3	C-1, C-3
6	119.6	6.74, d, 8.5	C-4, C-2	120.4	6.76, d, 8.5	C-2, C-4
7	77.7	4.77, d, 8.2	C-8, C-1, C-5	77.7	4.79, d, 8.1	C-9, C-2, C-4' (w)
8	80.0	3.92, br, s	C-2 (w)	80.1	3.99, m	C-2 (w)
9	62.1	3.71, d, 11.4	C-7, C-8	62.1	3.67, d, 11.4	
		3.49,dd,3.7,12.2	C-8, C-3' (w)		3.48,dd,3.7,12.2	C-7, C-3' (w)
1'	131.8			131.9		
2'	114.8	6.93, s	C-6', C-7', C-3'	115.8	6.94,s	C-7', C-6', C-3'
3'	144.5			144.9		
4'	144.9			145.3		
5'	116.8	6.83,d, 8.3	C-1'	118.0	6.91,d, 8.5	C-2', C-4'
6'	120.0	6.84,d, 8.4	C-2', C-7', C-3'	120.9	6.89,d, 8.4	C-2', C-1', C-7'
7'	132.3	6.46, d, 16.0	C-9', C-2', C-1'	133.3	6.50, d, 15.9	C-9', C-2', C-1'
8'	123.9	6.10,dd,7.8,14.0	C-9', C-1'	125.3	6.16,dd, 6.2, 15.8	C-9', C-8'
9'	73.1	4.07, d, 6.2	O<u>C</u>H$_2$CH$_3$, C-8', C-7'	72.5	4.08, ddd, 6.1, 6.2, 6.2	O<u>C</u>H$_2$(CH$_2$)$_2$CH$_3$, C-8', C-7'
		4.02, d, 6.2				
	65.6	3.48, q, 6.4, 2H	15.0	71.5	3.48, m, 2H	20.3, 32.9, 72.5,
	15.0	1.23, t, 6.3, 3H	65.6	32.9	1.57, m, 2H	14.2, 20.3, 71.5
				20.3	1.41, q, 7.7, 2H	14.2, 32.9, 71.5
				14.2	0.94, t, 7.3, 3H	20.3, 32.9

[a] The 1D-NMR data for all compounds were recorded on Bruker AM-400, and 2D-NMR data for all compounds were recorded on Bruker AM-500, respectively.

Compound **3**, the molecular formula was determined by HRESIMS to be $C_{20}H_{22}O_6$ (*m/z* 381.1312 $[M + Na]^+$, calcd. 381.1314). The data of ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of compound **1** except for the replacement of methoxyl group by ethoxyl at C-9'. The HMBC experiments showed weak four-bond ^{1}H-^{13}C long-range correlations between δ_H 3.49 (H-9b) and δ_C 144.5 (C-3'), and the ROESY experiment showed ^{1}H-^{1}H correlations between δ_H 4.77 (H-7) and δ_H 3.71 (H-9a), indicating the *trans*-form of C-7/C-8 in the dioxane ring. Therefore, compound **3** was determined to be 9'-ethoxy-7'-en-3',8:4',7-diepoxyneolignan-3,4,9-triol, namely 9'-ethyl americanol A[3].

Compound **4**, the HRESIMS determined the molecular formula to be $C_{22}H_{26}O_6$ (*m/z* 409.1614 $[M + Na]^+$, calcd. 409.1627). The ^{1}H- and ^{13}C-NMR spectra of **4** showed great similarities to those of compound **1** except for the replacement of methoxyl group by butoxyl at C-9'. The HMBC experiments showed ^{1}H-^{13}C long-range correlations between δ_H4.79 (H-7) and δ_C 145.3 (C-4'), and weak four-bond ^{1}H-^{13}C long-range correlations between δ_H 3.48 (H-9b) and δ_C 144.9 (C-3'). Therefore, compound **4** was determined to be 9'-butoxy-7'-en-3',8:4',7-diepoxyneolignan-3,4,9-triol, namely 9'-butyl americanol A [3].

The antimicrobial bioassays showed that **4** had evident inhibitory activity against *Mycobacterium tuberculosis* but the activity was a little weaker than that of rifampicin in

an agar diffusion assay, other compounds did not exhibit antibacterial activity at 100 μg/disc.

Acknowledgments

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