SHORT REPORT

A C G publications

Rec. Nat. Prod. 8:3 (2014) 294-298

records of natural products

A Trimeric Proanthocyanidin from the Bark of *Acacia leucophloea* Willd.

Sarfaraz Ahmed[*1,4], Hsiao-Ching Lin[2], Iram Nizam[1], Nadeem Ahmad Khan[3], Shoei-Sheng Lee[2] and Nizam Uddin Khan[1]**

[1]*Department of Chemistry, Aligarh Muslim University, Aligarh-202 002, India*
[2]*School of Pharmacy, College of Medicine, National Taiwan University, Taipei 10051, Taiwan, Republic of China*
[3]*GVK Biosciences, 28A, IDA, Nacharam, Hyderabad-500076, India*
[4]*Department of Pharmacognosy, College of Pharmacy, King Saud University, Post box-2457, Riyadh-11451, Saudi Arabia*

(Received May 22, 2013; Revised January 29, 2014; Accepted March 26, 2014)

Abstract: (–)-Fisetinidol-(4α,8)-[(–)-fisetinidol-(4α,6)]-(+)-catechin (**1**), a proanthocyanidin, was isolated from the bark of *Acacia leucophloea*. Its structure including absolute configuration was elucidated on the basis of spectroscopic analysis and chemical correlation. The ^{1}H NMR spectrum of this compound, exhibiting exceptional complex signals attributable to rotational isomerism, and the reported data were obtained at elevated temperature in methyl ether acetate form. This work provided the ^{1}H and ^{13}C NMR assignments for **1** and its rotational isomer as the free phenolic form at ambient temperature for the first time. Compound **1** showed inhibitory activity against *α*-glucosidase type IV from *Bacillus stearothermophilus* with the IC_{50} value of 102.3 μM.

Keywords: *Acacia leucophloea*; Mimosaceae; α-Glucosidase inhibitor; proanthocyanidin.

1. Plant Source

Acacia leucophloea (Roxb.) Willd. (Mimosaceae) is a medium-sized tree wildly distributed throughout India and used as folk medicine. The bark of *Acacia leucophloea* was collected in October, 2005 from Delhi Ridge, India. A voucher specimen (No. 943) was authenticated by Dr. Athar Ali Khan (Taxonomist) and deposited in the herbarium of Department of Botany, Aligarh Muslim University, Aligarh, India.

2. Previous Studies

Previous chemical investigations of *Acacia leucophloea* had characterized kaempferol, myricetin [1], β-sitosterol [2], and three diterpenoids [3,4]. Several proanthocyanidins have also been reported from *Acacia* species [5-8].

* Corresponding author *E-mail:* ahmsarfaraz@gmail.com
** Author has equal contribution as the first author

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/01/2014 EISSN: 1307-6167

3. Present Study

In this study, a B-type proanthocyanidin was isolated from the bark of the title plant. The identification of this angular profisetinidin triflavanoid (**1**) was sophisticated due to exceptional complex NMR signals resulting from rotational isomerism and various possible configurations resulting from stereochemistry at the C-2−4 positions in each unit [9,10]. The reported data of **1** was obtained at elevated temperature as a methyl ether acetate derivative [9]. This work provides the assignment of ^{1}H and ^{13}C NMR data of compound **1** and its rotational isomer as the free phenolic form at ambient temperature for the first time. As the oligomeric procyanidin mixtures from French maritime pine bark extract (pycnogenol) exhibited anti-diabetic effects in patients [11] and showed inhibitory activity against α-glucosidase [12], a known target in the treatment of diabetes mellitus, this study also assayed the α-glucosidase inhibitory activity of compound **1.**

The EtOH extract (174.5 g) of the bark of *Acacia leucophloea* was subjected to liquid-liquid partitioning to give fractions soluble in $CHCl_3$, EtOAc, acetone and EtOH. The EtOAc fraction (50 g out of 55 g) was subjected to silica gel column to give fraction AL-1. AL-1 was fractionated and chromatographed over a Lobar RP-18 column, semi-preparative RP-18 HPLC, and Sephadex LH-20 column, leading to the isolation of (–)-fisetinidol-(4α,8)-[(–)-fisetinidol-(4α,6)]-(+)-catechin (**1**) (Figure 1), as evidenced by its CD, ^{1}H, ^{13}C and 2D NMR spectra.

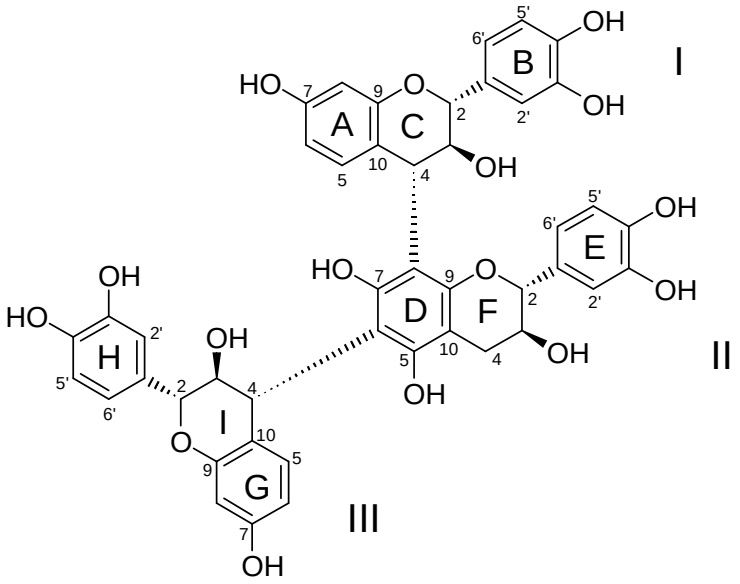

Figure 1. Structure of compound **1**

Compound **1** had the molecular formula of $C_{45}H_{38}O_{16}$, as deduced from HR-ESI-MS, showing the quasi-molecular ion $[M+Na]^+$ at m/z 857.2037, suggesting **1** to be a trimeric profisetinidin. The ^{1}H and ^{13}C NMR spectra of **1** exhibited two sets of signals arising from two diastereomers, **1a** and **1b** caused by the dynamic isomerism due to the rotational hindrance around the interflavanyl bond. The NOESY spectra of **1** showed strong exchange signals between equivalent protons of each isomeric pair, which were also verified by selective 1D NOESY data (S6) indicating **1a** and **1b** to be present in equilibrium [8]. Both ^{1}H NMR data of **1a** and **1b** revealed signals for two 3′,4′-disubstituted 5-deoxyflavan-3-ol and one 3′,4′-disubstituted flavan-3-ol moieties (Table 1) by displaying five ABX systems (δ 6.00−7.00) for the protons in B, E and H rings and resorcinol-type A and G rings, three sets of signals characteristic for the aliphatic protons in rings C, F and I (H-2−4, δ 4.30−4.75) with large coupling constants ($J_{2,3}$ and $J_{3,4}$ = 8.1−9.2 Hz), verified by selective 1D TOCSY (S7). The latter coupling constants indicated trans diaxial coupling and the configuration in rings C and I to be 2,3-*trans* and 3,4-*trans* and that in ring F to be 2,3-*trans*. The HMBC spectrum (Figure 2) showed the correlation of ring F H-2 (δ 4.43, **1a**; δ 4.46, **1b**)/ ring D C-9 (δ 153.2, **1a**; δ 153.1, **1b**), thus distinguishing the ^{13}C assignment for D C-5 (δ 154.8, **1a**; δ 155.0, **1b**) and D C-9, both being

correlated with ring F H_2-4 (δ 2.95 and δ 2.58, **1a**; δ 2.72 and δ 2.42, **1b**). Other HMBC data of **1** showed key correlation of ring C H-4 (δ 4.36, **1a**; δ 4.65, **1b**)/ ring D C-7−9, and of ring I H-4 (δ 4.68, **1a**; δ 4.67, **1b**)/ ring D C-5−7, confirming the linkage of unit I C-4 to unit II C-8 and unit III C-4 to unit II C-6 (Figure 2). These data also confirmed unit II to be the catechin residue.

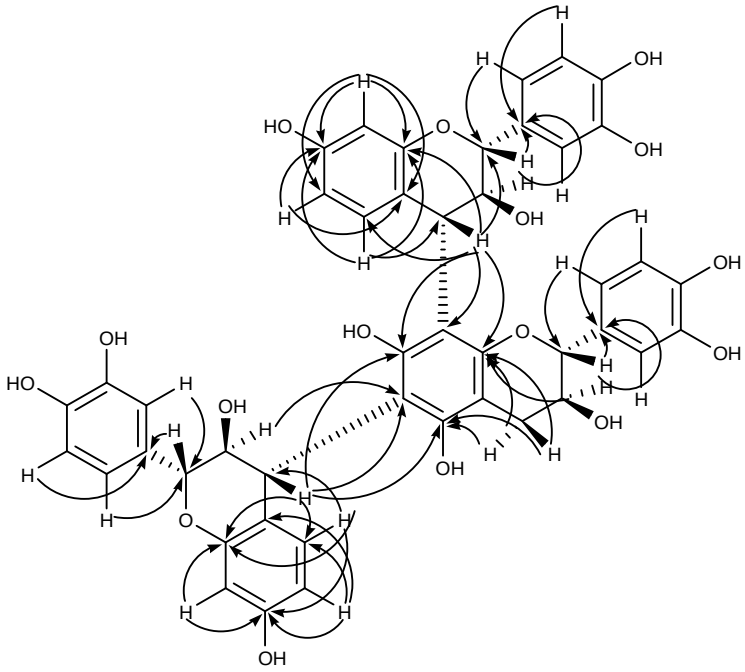

Figure 2. HMBC correlation of **1**

Compound **1** was methylated on the phenolic groups (dimethyl sulfate/K_2CO_3-Cs_2CO_3) to give a decamethylated product **1c** and then acetylated on the alcoholic groups to give a decamethyl ether triacetate **1d**. The CD data of **1d** which showed the negative Cotton Effect (*CE*) at 223 and 234 nm and together with the ^{1}H NMR data were found to be identical to the methyl ether acetate of [4,6:4,8]-all-*trans*-triflavonoid [9] thereby confirm **1** to be (–)-fisetinidol-(4α,8)-(–)-fisetinidol-(4α,6)]-(+)-catechin.

Compound **1** was reported from the heartwood of the black wattle tree (*Acacia mearnsi*) identified by chemical modification and the reported data were obtained in elevated temperature at 170 ^{0}C as methyl ether acetate form [9]. In the present study we provided the fully assigned ^{1}H and ^{13}C NMR data of compound **1** and its rotational isomer in free phenolic form at ambient temperature for the first time based on the selective TOCSY, NOESY and 2D NMR spectroscopic analyses. The inhibitory effects of compounds **1** against α-glucosidase type IV from *Bacillus stearothermophilus* was evaluated and showed the IC_{50} value of 102.3 μM.

Table 1. ^{1}H and ^{13}C NMR Data of **1a** and **1b** (methanol-d_4, AV-600)

		1a		**1b**	
Ring	No.	δ_C m	δ_H m (*J*/Hz)	δ_C m	δ_H m (*J*/Hz)
Unit I					
C	2	84.2 CH	4.45 d (8.7)	84.4 CH	4.51 d (9.0)
	3	71.3 CH	4.39 t (8.7)	71.4 CH	4.46 t (9.0)
	4	42.5 CH	4.36 d (8.7)	42.9 CH	4.65 d (9.0)
A	5	129.8 CH	6.51 d (8.3)	129.9 CH	6.65 d (8.3)
	6	109.3 CH	6.31 dd (8.3, 2.4)	109.3 CH	6.26 dd (8.3, 2.4)
	7	156.9 C		156.9 C	
	8	103.7 CH	6.17 d (2.4)	103.7 CH	6.19 d (2.4)
	9	156.5 C		156.6 C	
	10	119.2 C		119.5 C	
B	1'	132.6 C		132.5 C	
	2'	115.7—116.4 CH	6.70 d (2.0)	115.7—116.4 CH	6.70 d (2.0)
	3'	145.0—147.0 C		145.3—146.6 C	
	4'	145.0—147.0 C		145.0—147.0 C	
	5'	115.7—116.4 CH	6.66 d (8.2)	115.7—116.4 CH	6.69 d (8.4)
	6'	120.8 CH	6.49 dd (8.2, 2.0)	120.7 CH	6.55 dd (8.4, 2.0)
Unit II					
F	2	82.3 CH	4.43 d (8.3)	82.2 CH	4.46 d (8.1)
	3	69.2 CH	3.70 m	69.1 CH	3.59 m
	4a	30.1 CH_2	2.95 dd (16.0, 5.5)	29.4 CH_2	2.72 dd (16.0, 5.1)
	4b		2.58 dd (16.0, 7.9)		2.42 dd (16.0, 7.8)
D	5	154.8 C		155.0 C	
	6	111.1 C		111.7 C	
	7	153.3 C		153.3 C	
	8	110.8 C		111.0 C	
	9	153.2 C		153.1 C	
	10	103.8 C		104.4 C	
E	1'	131.8 C		131.8 C	
	2'	115.7—116.4 CH	6.54 d (2.2)	115.7—116.4 CH	6.51 d (2.0)
	3'	145.0—147.0 C		145.0—147.0 C	
	4'	145.0—147.0 C		145.0—147.0 C	
	5'	115.7—116.4 CH	6.63 d (8.0)	115.7—116.4 CH	6.60 d (8.4)
	6'	119.6 CH	6.05, dd (8.0, 2.2)	119.6 CH	6.00 dd (8.4, 2.2)
Unit III					
I	2	84.7 CH	4.62 d (9.0)	84.6 CH	4.62 d (9.2)
	3	71.8 CH	4.47 t (9.0)	72.4 CH	4.46 t (9.2)
	4	43.1 CH	4.68 d (9.0)	43.1 CH	4.67 d (9.2)
G	5	130.2 CH	6.80 d (8.4)	130.1 CH	6.70 d (8.4)
	6	110.2 CH	6.39 dd (8.4, 2.5)	109.9 CH	6.25 dd (8.4, 2.3)
	7	157.9 C		157.6 C	
	8	104.0 CH	6.31 d (2.4)	103.7 CH	6.28 d (2.3)
	9	157.0 C		156.6 C	
	10	117.5 C		118.0 C	
H	1'	132.1 C		132.2 C	
	2'	115.7—116.4 CH	6.70 d (2.0)	115.7—116.4 CH	6.94 d (2.1)
	3'	145.0—147.0 C		145.0—147.0 C	
	4'	145.0—147.0 C		145.0—147.0 C	
	5'	115.7—116.4 CH	6.80 d (8.0)	115.7—116.4 CH	6.76 d (8.2)
	6'	121.0 CH	6.86 br, dd (8.0, 2.0)	121.0 CH	6.83 dd (8.2, 2.1)

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] M. K. Valsakumari and N. Sulochana (1991). Chemical examination of the *Acacia leucophloea* Willd., *J. Indian Chem. Soc.* **68**, 673–674.

[2] K. C. Joshi and T. Sharma (1977). Chemical constituents of the root of Dichrostochys Macb. and the stem bark and heart wood of *Acacia leucophloea* Willd., *J. Indian Chem. Soc*. **54**, 649-650.

[3] A. Perales, M. Martinez-Ripoll, J. Fayos, R. K. Bansal, K. C. Joshi, R. Patni and B. Rodriguez (1980). Leucoxol, A new Diterpenoids from *Acacia leucophloea*. X-ray structure determination, *Tetrahedron Lett.* **21**, 2843–2844.

[4] R. K. Bansal, M. C. Garcia-Alvarez, K. C. Joshi, B. Rodriguez and R. Patni (1980). Diterpenoids from *Acacia leucophloea*. *Phytochemistry*, **19**, 1979–1984.

[5] L. Bennie, J. Coetzeea, E. Malana and D. Ferreira (2002). Structure and stereochemistry of dimeric proteracacinidins possessing the rare C-4(C) → C-5(D) interflavanyl linkage. *Phytochemistry*, **59**, 673–678.

[6] L. Bennie, J. Coetzeea, E. Malana and D. Ferreira (2002). (4→6)-Coupled proteracacinidins and promelacacinidins from *Acacia galpinii* and *Acacia caffra*, Phytochemistry, **60**, 521–532.

[7] L. Bennie, J. Coetzeea, E. Malana, D. Slade, J. P. J. Marais and D. Ferreira (2004). Trimeric proteracacinidins and a (6→6)-bis-leucoteracacinidin from *Acacia galpinii* and *Acacia caffra*, *Phytochemistry*, **65**, 215–220.

[8] R. Kusano, S. Ogawa, Y. Matsuo, T. Tanaka, Y. Yazaki and I. Kouno (2011) α-Amylase and lipase inhibitory activity and structural characterization of Acacia bark. Proanthocyanidins, *J. Nat. Prod.* **74**, 119–128.

[9] J. J. Botha, P. M. Viviers, D. A. Young, I. C. Preez and D. Ferreira (1982). Synthesis of condensed tannins. Part 5. The first angular [4,6 : 4,8]-triflavanoids and their natural counterparts, *J. Chem. Soc. Perkin Trans.* 1. 527–533.

[10] P. M. Viviers, H. Kolodziej, D. A. Young, D. Ferreira and D. G. Roux (1983). Synthesis of condensed tannins. Part 11. Intramolecular enantiomerism of the constituent units of tannins from the anacardiaceae: stoicheiometric control in direct synthesis: derivation of ^{1}H nuclear magnetic resonance parameters applicable to higher oligomers *J. Chem. Soc., Perkin Trans.* 1. **10**, 2555–2562.

[11] X. M. Liu, J. P. Wei, F. S. Tan, S. Zhou, G. Wurthwein, P. Rohdewald, (2004). Antidiabetic effect of Pycnogenol® French maritime pine bark extract in patients with diabetes type II. *Life Sci.* **75**, 2505–2513.

[12] A. Schafer and P. Hogger (2007). Oligomeric procyanidins of French maritime pine bark extract (Pycnogenol®) effectively inhibit α-glucosidase, *Diabetes Res. Clin. Pr*, 77, 41–46.