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A NOVEL CHROMONE-SUBSTITUTED TRIMERIC DIHYDROFLAVONOL FROM *Anogeissus pendula*

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*A new chromone-substituted dihydrotriflavonol, (2*S*,3*S*)[6-{(3*S*) 3″,5″-dihydroxy-6″-methoxydihydrochromone}5,3′,4′,5′-tetrahydroxy-7-methoxy-3*-O-*8-dihydroflavone]*$_2$ *3*-O-*8[6-{(3*S*) 3″,5″-dihydroxy-6″-methoxydihydrochromone}3,5,3′,4′,5′-pentahydroxy-7-methoxydihydroflavonol] was isolated from the leaves of* Anogeissus pendula*. The structure was determined by UV,* ^{1}*H NMR,* ^{13}*C NMR, HMBC, and CD data.*

Keywords: *Anogeissus pendula*, dihydroflavone, chromone.

Anogeissus pendula Edgew, belonging to the *Anogeissus* genus of the Combretaceae family, is a gregarious tree in dry and mixed forests of India. It is socially acceptable and economically viable, due to the fuel, timber, and fodder value of its foliage. To the best of available information, no phytochemical investigation is reported on its leaves hitherto. As part of our research program on polyphenolics of tree leaves for their efficient utilization in ruminant nutrition, we have isolated six polyphenolic derivatives of water-soluble constituents from the alcoholic extract of leaves of *A. pendula*. Out of them we report the characterization of a novel chromone-substituted dihydrotriflavonol.

Compound **1** obtained as a yellow microcrystalline substance, mp 290–292°C, is responsive to the characteristic reactions of flavonoids [1]. UV (MeOH, λ_{max}, nm): 276, 360, 378 and bathochromic shifts of bands in the presence of NaOH, NaOAc, and $AlCl_3$–HCl relative to bands in MeOH independently led us to infer the flavonoid skeleton with the galloyl B ring [2]. Its protonated FAB-MS afforded a molecular ion peak (M + H) at *m/z* 1623, consistent with $C_{78}H_{62}O_{39}$ showing molecular fragments due to RDA cleavage at *m/z* 1457, 1247, 914, 708, 374, and 167 and confirmed the presence of three flavonoid units with chromone moieties in the upper middle and terminal unit linked by C-C linkage (*m/z* 1413 and 209). The trimeric nature of **1** was further verified by its ^{13}C NMR (see Table 1). Besides the typical chemical shifts for the dihydrotriflavonoid molecule, there are additional signals for the chromone moiety in the molecule. The shifting of the signal by 20.7 ppm in the chemical shift for C-3″ indicates the presence of a free OH-group in the heterocyclic ring (E) and the terminal flavonoid unit at C-3. Another free OH at C-5″ was represented by the chemical shift of 139.3 ppm (u, m, t) [3]. The noteworthy displacement of the chemical shift by δ 28.7 (δ 124.0) and 39.4 (δ 135.8) indicated the C-C bond between C-8″ and C-6. The C-O-C ethereal linkage between C-3 (u, m) and C-8 (m, t) was assigned by the shift of the signals by δ 57.9 and 23.8 for C-3 (u, m) and C-8 (m, t) [4]. The ^{1}H NMR spectrum (DMSO-d_6) of **1** showed the presence of a singlet in the aromatic region at δ 6.45, which indicates a free single proton at C-8 as confirmed by available HMBC relationships [5]. The aliphatic protons in the heterocyclic C ring have *cis*-diaxial conformation and *S* orientation and are apparent from the double doublets at δ 4.193 (3H, J = 7.8 Hz) and 3.894 (3H, J = 6.9 Hz) corresponding to C-3 and C-2, appropriately exhibiting the negative cotton effect at 247.1 nm (CD [Medg] = –22.6744) in the CD spectrum for the absolute stereochemistry of **1**. Resonance forming AB and A_2X systems by a pair of doublets at δ 4.065 (6H, J = 9.6, 9.6 Hz) in each of the moieties and a triplet at δ 4.38 (3H, J = 7.8, 8.6 Hz) is indicative of two protons at C-2″ and C-3″ of the chromone ring also with *cis*-diaxial conformation and *S* orientation, which duly supported $J_{2''}J_{3''}$ [6]. The presence of sharp singlets at δ 4.573 (9H) and 3.725 (9H), each for the three methoxyl groups, corresponds to δ 64.1 (C-7 u, m, t) and 62.3 (C-6″ u, m. t), respectively [7]. The free proton in chromone at C-7″ is represented by the chemical shift in low field at δ 6.53 as a singlet with ^{2}J coupling with the C-6″ carbon.

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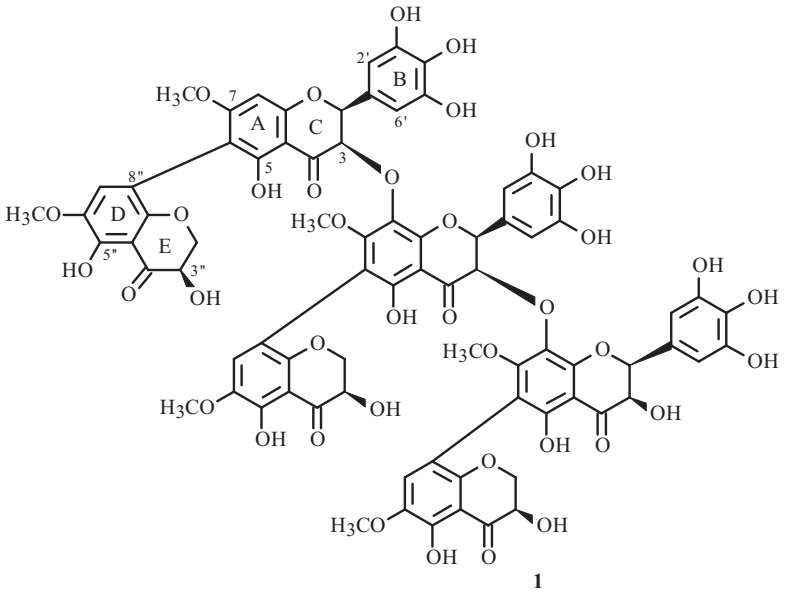
TABLE 1. ^{1}H NMR, ^{13}C NMR, and HMBC Spectral Data for Compound **1** in DMSO-d_6 (δ, ppm, J/Hz)*

C atom, u, m, t	δ_H	δ_C	HMBC	C atom, u, m, t	δ_H	δ_C	HMBC
C-2	3.894 (3H, d, J = 6.9)	77.8		C-5′		145.8	
C-3	4.193 (3H, d, J = 7.8)	108.9		C-6′	7.006 (3H, s)	123.2	1′, 5′ ^{2}J, 4′ ^{3}J
C-4		168.3		C-2″	4.065 (6H, dd, J = 9.6, 9.6)	76.5	
C-5		144.3		C-3″	4.382 (3H, t, J = 7.8, 8.4)	71.7	
C-6		135.8		C-4″		167.3	
C-7		167.0		C-5″		139.3	
C-8 u	6.455 (1H, s)	92.3	7 ^{2}J	C-6″		135.9	
C-8 m, t		118.8		C-7″	6.531 (3H, s)	115.9	6″ ^{2}J, 6 ^{3}J
C-9		165.0		C-8″		124.0	
C-10		106.2		C-9″		165.0	
C-1′		109.2		C-10″		106.2	
C-2′	6.905 (3H, s)	121.5	1′, 3′ ^{2}J, 4′ ^{3}J	OCH_3-7	4.573 (9H, s)	64.1	
C-3′		137.9		OCH_3-6″	3.725 (9H, s)	62.3	
C-4′		145.5					

*^{1}H, ^{13}C, and 2D NMR were determined on Brucker DRX-300 spectrometer.



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The cleavage of the ethereal bonds of the trimer (compound **1**) by acid hydrolysis furnished compound **2**, mp 260–262°C, with the molecular formula $C_{26}H_{22}O_{13}$ on the basis of the M^+ peak at 542. The bathochromic shift of 52 and 36 nm in band I and band II of its UV spectrum in the presence of NaOH relative to methanol permits us to infer that the free OH is at the C-3 position [2], suggesting involvement of C-3 OH in the formation the C-O-C bond in trimer formation. The positions of H and the number of OH in the molecule were ascertained by ^{1}H NMR of its acetylated derivative.

The C-C fission in **2** was achieved by its reaction with HI [8], giving compound **3** and **4** which were separated by preparative paper chromatography on 3 MM Whatman paper. Compound **3** is a colorless solid, mp 80–82°C, giving positive reactions for phenolic. It gave the molecular formula $C_{10}H_{10}O_5$, M^+ 210 on the basis of EI-MS. Compound **3** was characterized as (3*S*)-3,5-dihydroxy-6-methoxy-2,3-dihydrochromone on the basis of ^{1}H NMR data (see Experimental). Compound **4** was isolated as a yellow crystalline substance, mp 246–247°C, and was identified as (2*S*,3*S*)-3,5,3′,4′,5′-pentahydroxy-7-methoxydihydroflavonol (see Experimental).

These evidences were adequate to characterize **1** as (2*S*,3*S*)[6-{(3*S*)3″,5″-dihydroxy-6″-methoxy-dihydrochromone}5,3′,4′,5′-tetrahydroxy-7-methoxy-3-*O*-8-dihydroflavone]$_2$ 3-*O*-8[6-{(3*S*) 3″,5″-dihydroxy-6″-methoxydihydrochromone}3,5,3′,4′,5′-pentahydroxy-7-methoxy-dihydroflavonol].

EXPERIMENTAL

General Experimental Procedures. All the melting points were determined using a Bockmonoscope and were uncorrected. UV spectra were measured on an UNCAM UV/Vis spectrophotometer (Newington, USA). Mass spectra were determined on a Jeol mass spectrophotometer (Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were obtained on Bruker DRX-300 spectrophotometer (Fallanden, Switzerland) with TMS as an internal standard, and HMBC was measured using standard pulse sequence. The CD spectrum was obtained by Prof. D. Ferreira (Principal scientist), Department of Pharmacognosy, University of Mississippi, USA. TLC, column chromatography, and paper chromatography were performed on precoated Si GF^{256}, Si gel (60–120 Mesh, Merck India), Sephadex LH-20 (Sigma, USA), and Whatman paper to afford compounds **1**, **2**, and **3**.

Plant Material. Leaves of *Anogeissus pendula* were collected from the fixed tree from all side canopies at normal shoulder height during July. An identical specimen was deposited in the Grassland and Silvipasture Management Division of the Institute.

Extraction and Isolation. The defatted powder of leaves (4.5 kg) was extracted with ethanol in a Soxhlet extractor, and after removal of solvent under reduced pressure the extract was suspended in H_2O (2 L) for 12 h. The water-soluble fraction was successively partitioned with $CHCl_3$ and EtOAc. The EtOAc-soluble part was chromatographed over silica gel (60–120 mesh, 840 g), eluting with $CHCl_3$–MeOH mixture in different ratios. The fractions eluted with $CHCl_3$–MeOH (60:40) yielded a yellow solid (6.1 g), which was found to be a complex mixture. It was purified on a pre-equilibrated Sephadex LH-20 (20 g) column by eluting with H_2O–MeOH gradient (10:1) to afford a yellow crystalline compound containing two components that were resolved by preparative paper chromatography using 3 MM Whatman paper and water as irrigating solvent. The lower band was extracted with water and lyophilized to obtain compound **1**.

Compound 1: yellow microcrystalline solid, mp 290–292°C. UV (MeOH, λ_{max}, nm): 276, 360, 378; (NaOH) 284, 311, 424; ($AlCl_3$) 296, 382; ($AlCl_3$/HCl) 276, 296, 336; (NaOAc) 296, 313, 390; (NaOAc/H_3BO_3) 298, 334, 336. FAB-MS $[M + H]^+$ 1623, *m/z* 1457, 1413, 1247, 914, 708, 374, 209, and 167. ^{1}H NMR, ^{13}C NMR, and HMBC data are given in Table 1.

Acid Hydrolysis of Compound 1. 50 mg was refluxed with alc. 7% H_2SO_4 (100 mL) on a water bath for 8 h; on removal of acid from the reaction mixture, solid compound **2** was filtered off.

Compound 2: yellow microcrystalline solid, mp 276–278°C. UV (MeOH, λ_{max}, nm): 274, 358, 384; (NaOH) 280, 310, 410 nm. Acetylated derivative (compound **2** + Ac_2O in presence of C_6H_5N). ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 7.022 (1H, s, H-2′), 7.241 (1H, s, H-6′), 6.651 (1H, s, H-8), 4.512 (3H, s, OCH_3-7), 4.482 (2H, dd, J = 8.4, 8.4, H-2″), 4.281 (1H, t, J = 9.0, 9.0, H-3″), 4.318 (1H, d, J = 8.4, H-3), 3.953 (1H, d, J = 7.2, H-2), 3.899 (3H, s, OCH_3-6″), 2.01–2.28 (3 × 7H, br.s, 7OAc). EI-MS $[M^+]$ *m/z* 542.

Treatment of compound **2** with HI in the presence of phenol as per the procedure given in [8] yielded compound **3**, which was purified by PLC (20 × 20 cm, Merck, Cat. No. 1.05552), showing two spots on cellulose TLC (BAW 4:1:5). The upper band was attributed to compound **3** (R_f75) and the lower band to compound **4** (R_f65), which were separated and studied as follows.

Compound 3: colorless needles, mp 80–83°C. EI-MS $[M^+]$ *m/z* 210. ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 6.71 (1H, d, J = 6.0, H-7), 6.95 (1H, d, J = 6.0, H-8), 3.86 (2H, dd, J = 7.8, 7.8, H-2), 3.725 (1H, t, J = 7.5, 7.5, H-3), 3.560 (3H, s, OCH_3-6).

Compound 4: yellow crystalline solid, mp 246–247°C. UV (MeOH, λ_{max}, nm): 280, 308 (sh), 367, and 390; (NaOH) 315 (sh), 409 nm; ($AlCl_3$) 317, 392 nm; ($AlCl_3$/HCl) 280, 315, 349 nm; (NaOAc) 317, 385 nm; (NaOAc/H_3BO_3) 317, 362 nm. ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 4.26 (1H, d, J = 8.7, H-2), 4.95 (1H, d, J = 8.7, H-3),7.321 (1H, J = 3.0, H-8), 3.655 (3H, s, CH_3O-7), 7.245 (1H, d, J = 3.0, H-6), 6.24 (1H, d, J =2.7, H-6′), 6.264 (1H, d, J =2.7, H-2′).

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