Two New Aromatic Constituents from the Rootwood of *Aeschynomene mimosifolia*

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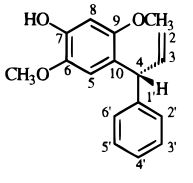
The rootwood of *Aeschynomene mimosifolia* Vatke (Leguminosae) has yielded a new neoflavonoid, mimosifoliol (**1**), and an unusual C_{16}-styrylcycloheptenone derivative, mimosifolenone (**2**). The structures of these compounds were determined on the basis of spectral analysis. Compound **1** demonstrated weak activity in DNA-strand scission assay, while compound **2** was found to be inactive. Mimosifoliol (**1**) was inactive toward several human cell lines, while **2** was moderately active against the KB cell line.

The genus *Aeschynomene* has not been subjected to phytochemical investigation previously, except the species *Aeschynomene indica* from which the flavonoid reynoutrin has been isolated.[2] Some biological studies on this genus have been reported. For example, it has been claimed that a 50% EtOH/H_2O extract and a saponin fraction obtained from the entire plant of *A. indica* are spermicidal.[3−5] An unspecified part of *Aeschynomene sensitiva* has been reported to possess insecticidal activity,[6] while a 95% EtOH/H_2O extract of the entire plant of *Aeschynomene stolzii* has been found to be cytotoxic.[7]

In our continuing search for antineoplastic agents from plants, we have investigated the rootwood of *Aeschynomene mimosifolia* Vathe (Leguminosae). No phytochemical or biological study on this plant has been reported in the literature. An aqueous MeOH extract (see Experimental Section) obtained from a $CHCl_3$ extract of *A. mimosifolia* showed significant DNA-strand scission activity when assayed according to a procedure described previously.[8,9] Repeated Si gel column chromatography of this extract and final preparative TLC purification afforded a weakly active new neoflavonoid designated as mimosifoliol (**1**) and an inactive but unusual C_{16}-aromatic compound designated as mimosifolenone (**2**). In this paper, the structure elucidation and biological evaluation of **1** and **2** are reported.



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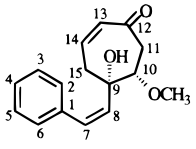

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Results and Discussion

The numbering system utilized for compound **1** in this paper is that recommended by Donnelly,[10] and Agrawal and Bansal.[11] The HREIMS of mimosifoliol (**1**) revealed a molecular ion at *m/z* 270.1258, assignable to a molecular formula of $C_{17}H_{18}O_3$. The IR spectrum displayed absorption bands for hydroxyl (3590 cm^{-1}) and $-CH{=}CH_2$ (1648, 990 cm^{-1}) groups. In the ^{1}H-NMR spectrum of **1**, the nonaromatic portion presented signals representing a typical coupling pattern for an ABCX spin system of the type $>CH_ACH_X{=}CH_BH_C$ at δ 4.91 (1H, d, J = 6.6 Hz), 5.01 (1H, dt, J = 1.5, 17.2 Hz), 5.28 (1H, dt, J = 1.5, 10.2 Hz), and 6.31 (1H, ddd, J = 6.6, 10.2, 17.2 Hz). Furthermore, signals occurred for two methoxyl groups at δ 3.75 and 3.77, two aromatic protons at δ 6.45 and 6.59, and five other aromatic protons (δ 7.24−7.32). Consistent with the ^{1}H-NMR data, the ^{13}C- and DEPT NMR spectra of **1** displayed signals for two methoxyl, one methylene, nine methine, and four quaternary carbons. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those reported for other neoflavonoids indicated **1** to be a member of the 3,3-diarylpropene-type subclass of neoflavonoids.[10−13] Analysis of the HMQC spectrum of **1** confirmed the presence of an allylic methine carbon at δ 49.2 (C-4, δ_H 4.91), a terminal methylene carbon at δ 117.0 (C-2, δ_H 5.01, H_a-2 and 5.28, H_b-2), and an olefinic carbon at δ 139.4 (C-3, δ_H 6.31). The aromatic singlet proton resonances at δ 6.45 (H-8) and 6.59 (H-5) suggested that the other positions in ring A were substituted. The upfield ^{13}C-NMR chemical shift of C-8 (δ 101.7) indicated that this carbon was flanked by the oxygenated carbons C-7 (δ 147.6) and C-9 (δ 148.7). Long-range HMBC correlations of H-4 (δ 4.91), with C-9 (δ 148.7), and *Me*O-9 (δ 3.77) with C-9 established the position of one of the methoxy groups at C-9. A series of differential NOE experiments confirmed the substitution pattern of ring A; irradiation of the methoxyl signal at δ 3.77 enhanced to signal at δ 6.45 (H-8) only, and reciprocal irradiation of the H-8 signal gave enhancement of only CH_3O-9. Likewise, NOE effects were observed between CH_3O-6 (δ 3.75) and H-5 (δ 6.59). Thus, the positions of the substituents in ring A were proven to be as shown in the structure of **1**. The ^{1}H- and ^{13}C-NMR data of **1** (Table 1) clearly revealed that ring B was not substituted. Comparison of the optical rotation of **1**, $\{\alpha\}_D$

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Table 1. NMR Data of Mimosifoliol (**1**) and Mimosifolenone {**2**}[a]

Mimosifoliol (**1**)				Mimosifolenone (**2**)			
position	δ_H	δ_C	HMBC correl	position	δ_H	δ_C	HMBC correl
2a	5.01 dt (1.5, 17.2)	117.0	H-3	1		136.8	
2b	5.28 dt (1.5, 17.2)			2, 6	7.27−7.41[b]	128.8	
3	6.31 ddd (6.6, 10.2, 17.2)	139.4	H-2a, H-2b, H-4	3, 5	7.27−7.41[b]	128.6	
4	4.91 d (6.6)	49.2	H-2a, H-2b, H-3, H-5	4	7.27−7.41[b]	126.2	
5	6.59 s	113.4	H-4	7	5.99 d (10.2)	127.8	
6		143.1	H-5, *Me*O-6	8	6.77 d (10.2)	152.5	H-15α
7		147.6	H-5	9		73.7	H-7, H-10, H-11α, H-11*β*, H-15α, H-15*β*
8	6.45 s	101.7		10	3.74 dd (4.3, 10.0)	82.3	H-11α, H-11*β*, MeO-10
9		148.7	H-4, MeO-9	11α	2.93 dd (4.3, 17.0)	39.5	H-10
10		119.5	H-3, H-8, H-5	11*β*	2.49 dd (10.0, 17.0)		
1′		141.4	H-4	12		197.2	H-11α, H-11*β*
2′, 6′	7.24−7.32[b]	128.5	H-4	13	6.52 d (14)	135.3	H-15α, H-15*β*,
3′, 5′	7.24−7.32[b]	128.7		14	6.32 dt (7.2, 14.0)	123.3	H-15α, H-15*β*
4′	7.24−7.32[b]	126.8		15α	2.82 ddd (1.3, 7.2, 14.0)	39.3	H-10
6-OMe	3.75 s	56.6		15*β*	2.52 ddd (1.3, 7.2, 14.0)		
7-OH	4.67 br s			10-OMe	3.47 s	57.8	
9-OMe	3.77 s	55.9					

[a] Values recorded in $CDCl_3$ at 500 MHz for proton and 125 MHz for carbon; values in δ (ppm); coupling constants (Hz) in parentheses. [b] Proton signals are overlapped.

+22°, $CHCl_3$, with similar data reported for (*R*)-(+)-dalbergiphenol, a 6-hydroxy-7-methoxy isomer of **1**,[12] suggested an *R*-configuration for mimosifoliol (**1**). The structure of mimosifoliol was, thus, as shown in **1**.

Mimosifolenone (**2**) was isolated as a colorless gum, $[\alpha]_D$ +81.2° (*c* 0.25, $CHCl_3$). The HREIMS of **2** showed $[M]^+$ at *m/z* 258.1254, corresponding to the molecular formula $C_{16}H_{18}O_3$. The IR spectrum showed absorptions at 3620 (OH) and 1675 (α,*β*-unsaturated ketone) cm^{-1}. The ^{1}H-NMR spectrum of **1** presented signals for five aromatic (δ 7.27−7.41), four olefinic (δ 5.99, 6.32, 6.52, and 6.77), one methine (δ 3.74), one methoxyl (δ 3.47), and two methylene groups, each consisting of two nonequivalent protons (δ 2.49, 2.52, 2.82, and 2.93). The connectivity pattern was inferred from ^{1}H−^{1}H COSY NMR correlations between H-7 (δ 5.99) and H-8 (δ 6.77); H-10 (δ 3.74) and $H_{2\alpha}$-11 (δ 2.93) and $H_{2\beta}$-11 (δ 2.49); H-13 (δ 6.52) and H-14 (δ 6.32); and H-14 and $H_{2\alpha}$-15 (δ 2.82) and $H_{2\beta}$-15 (δ 2.52). The corresponding ^{13}C-NMR resonances of the protonated carbons were assigned with the aid of an HMQC experiment. The long-range HMBC correlation of CH_3O-10 (δ 3.47) with C-10 (δ 82.3) established the methoxyl group at C-10. Other HMBC NMR correlations are shown in Table 1. The *cis*-geometry of the C-7/8 double bond was deduced from the coupling constant of H-7 with H-8 (J = 10.2 Hz). The large coupling constant of $H_{2\beta}$-11 with H-10 (10.0 Hz) required pseudoaxial orientations of these protons. Furthermore, in the NOESY spectrum of **2**, H-10 showed correlation with $H_{2\beta}$-15. The more downfield signal of $H_{2\alpha}$-15 (δ 2.82) as compared with that of $H_{2\beta}$-15 (δ 2.52) suggested that the α-proton was on the same side of **2** as the C-9 hydroxyl group. The structure and relative stereochemistry of mimosifolenone are thus as shown in **2**.

Evaluation of compounds **1** and **2** for DNA-scission activity indicated that **1** was weakly active (activity ratio of 0.49) when assayed at 25 μg/mL and compared with a positive control (bleomycin sulfate at 0.1 μg/mL), while **2** was inactive.[8] Mimosifoliol (**1**) was evaluated in a panel of human cell lines,[14] including breast cancer (BC1), lung cancer (Lu1), oral epidermoid carcinoma (KB), prostate cancer (LNCaP), glioma (U373), and hormone-dependent breast cancer (ZR-75-1) and was found to be inactive. Compound **2** was evaluated only in the KB cell type and found to be moderately active (ED_{50} 3.3 μg/mL).

Experimental Section

General Experimental Procedures. NMR experiments were performed on a Bruker 500 MHz spectrometer operating at 500 MHz for proton and 125 MHz for carbon. $CDCl_3$ was used as solvent and TMS as internal standard. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. UV and IR spectra were recorded on a Varian 2290 UV−vis spectrometer and a Shimadzu IR-460 spectrometer, respectively. Ms were measured on a VG ZAB-E magnetic sector instrument. Column chromatography was carried out on Si gel 60 (70-230 mesh, E. Merck, Darmstadt, Germany). Fractions were monitored by TLC using aluminum-backed TLC sheets (Si gel 60 F254, 0.25-mm thick) with visualization under UV (254 and 365 nm) and using phosphomolybdate spray reagent (5% phosphomolybdic acid in EtOH). Preparative TLC was carried out on Merck Si gel F254 plates (0.5-mm thick).

Plant Material. The rootwood of *Aeschynomene mimosifolia* was collected in Nov. 1992, in Zimbabwe. A voucher specimen (A1842) is on deposit in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. Dried and ground rootwood of *A. mimosifolia* (285 g) was extracted with 50% MeOH/$CHCl_3$ (2 × 1 L) in a Soxhlet apparatus. Extracts were concentrated *in vacuo* at 40 °C and partitioned between 20% MeOH/$CHCl_3$ and H_2O. The organic portion was concentrated, and the residue was partitioned between 90% MeOH/H_2O and hexane. The aqueous MeOH portion (3.6 g) was chromatographed over Si gel (250 g) using $CHCl_3$ and increasing percentages of MeOH/$CHCl_3$. Fractions were combined based on TLC profile to give four pooled fractions (F1-F4). Fraction F1 (0.4 g) eluted with 1% MeOH/$CHCl_3$ was further chromatographed over Si gel (50 g) using a solvent gradient of 90% $CHCl_3$/hexane to 5% MeOH/$CHCl_3$. A total of seven pooled fractions (F1a−F1g) were obtained. Fractions F1a (50 mg) and F1d (30 mg) were finally purified by preparative TLC (EtOAc/$CHCl_3$,

1:1) to afford mimosifoliol (**1**) (24 mg) and mimosifolenone (**2**) (10 mg), respectively.

Mimosifoliol (1): light brown oil; $[\alpha]_D$ +22° (*c* 0.64, $CHCl_3$); UV λ_{max} (EtOH) (log ϵ) 231 (3.84), 293 (3.69) nm; IR ν max (film) 3590 (OH), 2955, 2850 (OMe), 1648 ($-CH=CH_2$), 1506, 1460, 1301, 1218, 1179, 999, 924, 789, 753 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; EIMS (70 eV) *m/z* 270 $[M]^+$ (100), 255 (19), 243 (16), 223 (13), 195 (15), 166 (13), 152 (7), 141 (6), 128 (7), 115 (14), 91 (11), 67 (7); HREIMS *m/z* found $[M]^+$ 270.1258 ($C_{17}H_{18}O_3$ requires 270.1256).

Mimosifolenone (2): colorless gum; $[\alpha]_D$ +81° (*c* 0.25, $CHCl_3$); UV λ_{max} (MeOH) (log ϵ) 250 (3.96), 283 (2.78), 293 (2.24) nm; IR ν_{max} (film) 3620 (OH), 3000, 2825, 1673 (α,β-unsaturated C=O), 1458, 1374, 1326, 1229, 1207, 1093, 1024 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; EIMS (70 eV) *m/z* 258 $\{M\}^+$ (30), 242 (17), 240 (10), 172 (6), 141 (63), 117 (100), 109 (44), 91 (50); HREIMS *m/z* found $[M]^+$ 258.1254 ($C_{16}H_{18}O_3$ requires 258.1256).

DNA Strand-scission Assay. The DNA-strand scission assay was carried our according to a procedure described previously.[8,9] Compounds **1** and **2** were tested at a concentration of 25 μg/mL, and the activity was compared with that of bleomycin sulfate at 0.1 μg/mL.

Cytotoxicity Assay Procedures. Compounds **1** and **2** were tested for cytotoxic activity using established protocols.[14]

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References and Notes

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(2) Morita, N.; Arisawa, M.; Nagaes, M.; Hsu, H. Y.; Chen, Y. P. *Shoyakugaku Zasshi* **1977**, *31*, 172−174.
(3) Setty, B. S.; Kamboj, V. P.; Khanna, N. M. *Ind. J. Expt. Biol.* **1977**, *15*, 231−232.
(4) Dhawan, B. N.; Patnaik, G. K.; Rastogi, R. P.; Singh, K. K.; Tandon, J. S. *Ind. J. Expt. Biol.* **1977**, *15*, 208−230.
(5) Setty, B. S.; Kamboj, V. P.; Garg, H. S.; Khanna, N. M. *Contraception* **1976**, *14*, 571−578.
(6) Sievers, A. F.; Archer, W. A.; Moore, R. H.; McGovran, B. R. *J. Econ. Entomol.* **1949**, *42*, 549−551.
(7) Suffness, M.; Abbot, B.; Statz, D.; Wonilowicz, E.; Spjut, R. *Phytother. Res.* **1988**, *2*, 89−97.
(8) Chaudhuri, S. K.; Huang, L.; Fullas, F.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Tucker, J. C.; Beecher, C. W. W.; Kinghorn, A. D. *J. Nat. Prod.*, in press.
(9) Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869−877.
(10) Donnelly, D. M. X. In *The Flavonoids*; Mabry, T. J.; Mabry, H., Eds.; Academic Press: New York, 1975; Vol. 2, pp 801−865
(11) Agrawal, P. K.; Bansal, M. C. In *Carbon-13 NMR of Flavonoids*; Agrawal, P., Ed.; Elsevier: New York, 1989; pp 236−243.
(12) Muangnoicharoen, N.; Frahm, A. W. *Phytochemistry* **1982**, *21*, 767−772.
(13) Barnes, M. F.; Ollis, W. D.; Sutherland, I. O.; Gottlieb, O. R.; Magalhães, M. T. *Tetrahedron* **1965**, *21*, 2770−2715.
(14) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 30−38.

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