Anti-HIV Prenylated Flavonoids from *Monotes africanus*[1]

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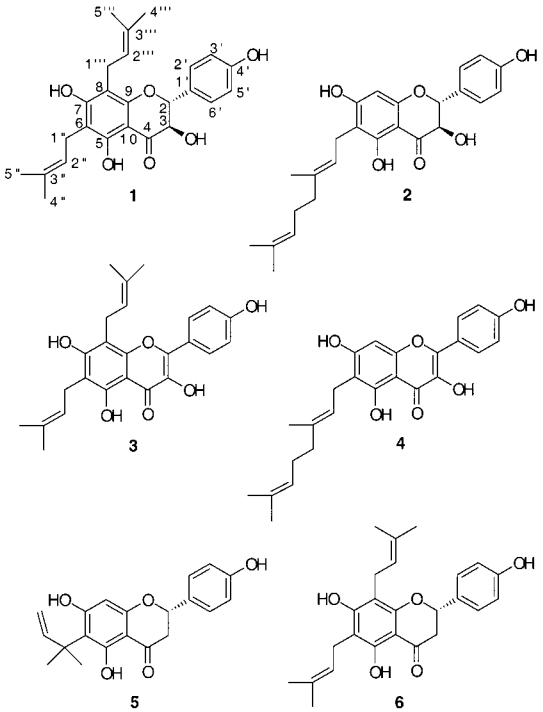
Six flavonoids, among them a new dihydroflavonol, 6,8-diprenylaromadendrin (**1**), and the flavonol 6,8-diprenylkaempferol (**3**), have been isolated from the organic extract of *Monotes africanus*. The isolated compounds containing a 5,7-dihydroxy-6,8-diprenyl system in the A ring of the flavonoid (**1**, **3**, and **6**) exhibited HIV-inhibitory activity in the XTT-based, whole-cell screen. In addition, several ^{13}C NMR assigments of lonchocarpol A (**6**) were corrected.

The genus *Monotes* A. DC. (Dipterocarpaceae) consists of approximately 36 species, distributed in tropical Africa and Madagascar.[2] Only *Monotes engleri* has been studied, yielding cytotoxic prenylated flavanones[3,4] and a coumarin.[5] Flavonoids are known to exhibit several biological activities,[6] including anti-HIV activity.[7,8]

The organic extract of *Monotes africanus* was active in the XTT-based, anti-HIV in vitro primary screen.[9] The bioassay-guided purification of this extract led to a new dihydroflavanol, 6,8-diprenylaromadendrin (**1**), and the known flavonoids bonanniol A (**2**),[10] 6,8-diprenylkaempferol (**3**),[11,12] macarangin (**4**),[13] 6-(1,1-dimethylallyl)naringenin (**5**),[3] and lonchocarpol A[14] (senegalensin)[15] (**6**). Compound **3** has been obtained from prenylation of kaempferol, but has never been reported as a natural product.[11,12]



The most abundant of the isolated compounds, **1**, was obtained as a yellow solid. Its molecular formula was determined as $C_{25}H_{28}O_6$ by HRFABMS (*m/z* 424.1885, calcd 424.1889). The UV spectrum (MeOH) showed the characteristic absorbances for a dihydroflavonol [λ_{max} = 297.5 and 351.0 (sh) nm].[16] The ^1H NMR spectrum (Table 1) contained signals for two exchangeable protons, one of which was chelated (δ 11.49, OH-5), two AB systems at δ 4.53 (1H, d, J = 12.0 Hz) and 4.97 (1H, d, J = 12.0 Hz) characteristic of H-2 and H-3 in the axial conformation of a 2,3-*trans*-dihydroflavonol, and δ 6.83 (2H, d, J = 8.0 Hz) and 7.39 (2H, d, J = 8.0 Hz) indicative of a *para* substituent in the B ring of the flavonoid, and signals for two prenyl groups [δ 1.76 (3H, s), 1.83 (3H, s), 3.36 (2H, d, J = 6.5 Hz), and 5.24 (1H, br t, J = 6.5 Hz) and δ 1.67 (3H, s), 1.71 (3H, s), 3.26 (2H, d, J = 7.0 Hz), and 5.18 (1H, br t, J = 7.0 Hz)]. The ^{13}C NMR spectrum (Table 1) contained signals for all 25 carbons. Fifteen signals were accounted for by the flavonoid skeleton, among them four signals belonging to aromatic carbons bearing an oxygen atom (δ 156.4, 157.8, 158.8, and 163.2). Also present were the signals belonging to the 10 carbons of the two prenyl side chains. These data indicated that compound **1** is a dihydroflavonol with three hydroxyl and two prenyl substituents. HMBC correlations between δ 156.4 (C-4′) and H-2′, H-3′, H-5′, and H-6′

Table 1. NMR Assignments of 6,8-Diprenylaromadendrin (**1**)[a]

position	C δ_C mult	H δ_H mult (J in Hz)	HMBC
2	82.9 d	4.97 d (12.0)	H-3, H-2′, H-6′
3	72.6 d	4.53 d (12.0)	H-2
4	196.3 s		H-2, H-3
5	158.8 s		OH-5, H-1″
6	107.9 s		OH-5, H-1″, H-2″
7	163.2 s		H-1″, H-1‴, OH-7
8	107.0 s		H-2‴
9	157.8 s		H-1‴
10	100.4 s		OH-5
1′	128.4 s		H-3, H-3′, H-5′
2′	128.9 d	7.39 d (8.0)	H-2
3′	115.5 d	6.83 d (8.0)	H-2′
4′	156.4 s		H-2′, H-3′, H-5′, H-6′
5′	115.5 d	6.83 d (8.0)	H-6′
6′	128.9 d	7.39 d (8.0)	H-2
1″	21.3 t	3.36 d (6.5)	
2″	121.4 d	5.24 t (6.5)	H-1″
3″	134.9 s		H-1″, CH_3-4″, CH_3-5″
4″	25.81 q	1.76 s	H-2″, H-5″
5″	17.9 q	1.83 s	H-2″, H-4″
1‴	21.7 t	3.26 d (7.0)	
2‴	121.5 d	5.18 t (7.0)	H-1‴
3‴	134.4 s		H-1‴, CH_3-4‴, CH_3-5‴
4‴	25.79 q	1.71 s	H-2‴, H-5‴
5‴	17.8 q	1.67 s	H-2‴, H-4‴
OH-5		11.49 s	
OH-7		6.51 s	

[a] Spectra recorded in $CDCl_3$.

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indicated that the *para* substituent in the B ring was a hydroxyl group. This suggested the presence of two hydroxyl and two prenyl groups in the A ring of the dihydroflavonol. The presence of an exchangeable proton at low field (δ 11.49) indicated that one of the hydroxyl groups was at C-5 and was hydrogen-bonded to the carbonyl group at C-4. This substitution was confirmed by the HMBC correlations between OH-5 and C-5 (δ 158.8), C-6 (δ 107.9), and C-10 (δ 100.4). The other hydroxyl group was placed at C-7 by the HMBC correlations between this carbon (δ 163.2) and the protons of the methylene groups of both prenyl chains (H-1″ and H-1‴) and the proton of the hydroxyl group (δ 6.51). The positions of both prenyl groups at C-6 and C-8 were confirmed by the HMBC correlations H-1″ from C-5, C-6, C-7; H-2″ from C-6; H-1‴ from C-7, C-9; and H-2‴ from C-8.

Compound **3** was defined as 6,8-diprenylkaempferol. This compound has been reported as a synthetic product, but only limited spectroscopic data were described.[11,12] This represents the first isolation of 6,8-diprenylkaempferol as a naturally occurring metabolite. A standard battery of NMR experiments, including COSY, HSQC, and HMBC, provided assignments for all signals in the ^{1}H and ^{13}C NMR spectra (Experimental Section).

Lonchocarpol A (**6**), also named senegalensin, was isolated previously from *Lonchocarpus minimiflorus*[14] and *Erythrina senegalensis*[15] (Leguminosae). On the basis of careful analysis of the NMR data of compound **6**, including DEPT, HSQC, and HMBC, some of the ^{13}C NMR signals were reassigned (Experimental Section). The ^{13}C NMR spectrum of compound **6** showed signals for all 25 carbons, among them 10 signals belonging to the two prenyl groups. DEPT experiments indicated that the signals at δ 21.2 and 21.9 belong to methylene groups, while the signals at δ 17.80, 17.85, 25.79, and 25.80 belong to methyl groups. Originally for lonchocarpol A, the four signals at higher field (δ 17.80, 17.85, 21.33, and 21.93) were assigned to methyl groups, while the signal at δ 25.76 was assigned to both methylene groups.[14] The ^{13}C NMR spectrum showed also four signals belonging to carbons bearing an oxygen atom (δ 156.0, 157.9, 159.3, and 162.4). The positions of these carbons were determined by HMBC correlations. The signal at δ 156.0 was assigned to C-4′ by the correlations between this carbon and the signals of the AB system in the B ring (δ 7.31, H-2′, H-6′ and 6.88, H-3′, H-5′). The signal at δ 162.4 was identified as C-7 by the correlations between this carbon and the protons of the methylene groups of both prenyl side chains and the signal for the exchangeable proton at δ 6.40 (OH-7). The signal at δ 159.3 was assigned to C-5 by the correlations between this carbon and the protons of the methylene group of one of the prenyl chains (δ 3.35, C-1″) and the proton of the chelated hydroxyl group (δ 12.30, OH-5). The remaining oxygen-bearing carbon (δ 157.9) was C-9 on the basis of the correlation between it and the protons of the methylene group of the other prenyl side chain (δ 3.30, C-1‴). The stereochemistry at C-2 was determined to be *S* by analysis of the CD spectrum, in which a positive Cotton effect was observed at 350 nm along with a negative Cotton effect at 292 nm.[3,17]

The spectral data for the known flavonoids **2**,[10] **4**,[13] and **5**[3] were in agreement with those reported in the literature.

The isolated compounds containing a 5,7-dihydroxy-6,8-diprenyl system in the A ring, 6,8-diprenylaromadendrin (**1**), 6,8-diprenylkaempferol (**3**), and lonchocarpol A (**6**), were active (EC_{50} 2.1, 2.4, and 1.3 μg/mL, and IC_{50} 4.7, 5.8, and 2.7 μg/mL, for **1**, **3**, and **6**, respectively) in the anti-HIV screen with 60−100% maximum protection. The other three flavonoids, bonanniol A (**2**), macarangin (**4**), and 6-(1,1-dimethylallyl)naringenin (**5**), were inactive. These compounds represent the first prenylated flavonoids with a 2-prenylchroman skeleton for which anti-HIV data have been reported. There has, however, been a prenylated isoflavanone[18] for which anti-HIV data have been reported. Interestingly, kaempferol and naringenin have been reported to be inactive against HIV,[19] which, when coupled with the fact that compounds **2**, **4**, and **5** are also inactive, may suggest that the presence of prenyl groups on the flavonoid core is important for anti-HIV activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. CD spectra were obtained on a JASCO J-720 spectropolarimeter. UV spectra were run on a Beckman DU 640 spectrophotometer, and IR spectra on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. NMR spectra were recorded on a Varian Inova Unity VXR-500 spectrometer in $CDCl_3$ or DMSO-d_6 as solvent. Mass spectra were obtained with a JEOL SX102 mass spectrometer.

Plant Material. Leaves of *M. africanus* Welw. & Kirk ex A. DC. were collected in Iringa region, Mufindi District, Sadani, Tanzania, in December 1988, by J. Lovett under contract with the National Cancer Institute. Voucher specimens (Q66T-2735) have been deposited at Missouri Botanical Garden and the Smithsonian Institution. The taxonomy was determined by R. Gereau.

Extraction and Isolation. Leaves of *M. africanus* (508 g) were air-dried, ground, and sequentially extracted with CH_2Cl_2−MeOH (1:1) and MeOH. The extracts were combined and evaporated to generate 49.57 g of organic extract. A portion (0.99 g) of the extract was subjected to solvent−solvent partitioning.[20] The anti-HIV activity was concentrated in MeOtBu and EtOAc fractions. The MeOtBu fraction was subjected to gel permeation on Sephadex LH-20 (hexane−CH_2Cl_2−MeOH, 2:5:1), to give 6,8-diprenylaromadendrin (**1**) (30.1 mg, 3.04% of extract), macarangin (**4**)[13] (4.4 mg, 0.44% of extract), 6-(1,1-dimethylallyl)naringenin (**5**)[3] (13.6 mg, 1.37% of extract), lonchocarpol A (**6**)[14] (27.1 mg, 2.74% of extract), and two impure fractions. Further purification of these fractions by Sephadex LH-20 (hexane−CH_2Cl_2−MeOH, 2:5:1) afforded bonanniol A (**2**)[10] (1.6 mg, 0.16% of extract) and 6,8-diprenylkaempferol (**3**)[11,12] (1.0 mg, 0.10% of extract). Fractionation of the EtOAc fraction by Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) afforded impure fractions containing 6,8-diprenylaromadendrin (**1**) (14.3 mg), 6-(1,1-dimethylallyl)naringenin (**5**)[3] (7.4 mg), and lonchocarpol A (**6**)[14] (2.7 mg).

6,8-Diprenylaromadendrin (1): yellow solid; $[\alpha]^{25}_D$ +8.3° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 223.5 (4.47), 297.5 (4.20), 351.0 (sh) (3.65) nm; IR (NaCl) ν_{max} 3368, 2969, 2918, 1625, 1515, 1448, 1370, 1265, 1227, 1170, 1108 cm^{-1}; ^{1}H and ^{13}C NMR in $CDCl_3$, see Table 1; HRFABMS (m−b) *m*/*z* 424.1885, calcd for $C_{25}H_{28}O_6$, 424.1889; FABMS (m−b) *m*/*z* 447 $[M + Na]^+$ (27), 425 $[M + H]^+$ (34), 424 $[M]^+$ (25), 369 $[M - C_4H_7]^+$ (100), 313 $[M - C_4H_7 - C_4H_8]^+$ (28), 177 (74), 107 $[B_4]^+ = [HOC_6H_4CH_2]^+$ (42).[21]

6,8-Diprenylkaempferol (3): ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.66 (1H, s, OH-5), 10.15 (1H, s, OH-4′), 9.62 (1H, s, OH-7), 9.32 (1H, s, OH-3), 8.00 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.90 (2H, d, J = 8.5 Hz, H-3′, H-5′), 5.11 (1H, t, J = 6.5 Hz, H-2‴), 5.14 (1H, t, J = 6.5 Hz, H-2″), 3.50 (2H, d, J = 6.5 Hz, H-1‴), 3.31 (2H, d, J = 6.5 Hz, H-1″), 1.73 (3H, s, CH_3-5‴), 1.72 (3H, s, CH_3-5″), 1.61 (6H, s, CH_3-4″, CH_3-4‴); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 176.6 (s, C-4), 159.2 (s, C-4′), 158.7 (s, C-7), 155.3 (s, C-5), 151.7 (s, C-9), 146.8 (s, C-2), 135.4 (s, C-3), 130.9 (2C, s, C-3″ and C-3‴), 129.5 (2C, s, C-2′ and C-6′), 122.7 (d, C-2‴), 122.3 (d, C-2″), 121.9 (s, C-1′), 115.4 (2C, s, C-3′ and C-5′), 110.9 (s, C-6), 106.2 (s, C-8), 103.2 (s, C-10), 25.4 (2C, q, C-4″ and C-4‴), 21.3 (2C, t, C-1″ and C-1‴), 17.9 (2C, q, C-5″ and C-5‴); FABMS (m−b) *m*/*z* 423 $[M + H]^+$ (48), 422 $[M]^+$

(20), 367 [M − C_4H_7]$^+$ (15), 311 [M − C_4H_7 − C_4H_8]$^+$ (22), 309 (27), 251 (25).

Lonchocarpol A (6): [α]$^{25}_D$ −26.2° (*c* 0.08, MeOH); CD (*c* 1.2 × 10^{-3} M, MeOH) Δε (nm) +3.4 (251), −37.2 (292), +4.0 (350); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.7 (s, C-4), 162.4 (s, C-7), 159.3 (s, C-5), 157.9 (s, C-9), 156.0 (s, C-4′), 134.7 (s, C-3″), 133.9 (s, C-3‴), 130.9 (s, C-1′), 127.9 (2C, d, C-2′ and C-6′), 121.9 (d, C-2‴), 121.7 (d, C-2″), 115.5 (2C, d, C-3′ and C-5′), 107.2 (s, C-6), 106.4 (s, C-8), 102.8 (s, C-10), 78.5 (d, C-2), 43.1 (t, C-3), 25.80 (q, C-4‴)*, 25.79 (q, C-4″)*, 21.9 (t, C-1‴), 21.2 (t, C-1″), 17.85 (q, C-5‴)†, 17.80 (q, C-5″)† (*,† assignments bearing the same superscript may be interchanged).

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