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CASSINOPIN, A KAEMPFEROL TRIRHAMNOSIDE FROM *CASSINOPSIS MADAGASCARIENSIS**

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Key Word Index—*Cassinopsis madagascariensis*; Icacinaceae; phenylpropanoid glycosides; cassinopin; ^{13}C NMR.

Abstract—A new glycoside, 3-[α-L-rhamnosyl-(1→4)-*O*-α-L-rhamnopyranosyl]-7-α-L-rhamnosylkaempferol, cassinopin, was isolated from the leaves of *Cassinopsis madagascariensis*, together with the known phenylpropanoid glycosides calceolarioside A, B and C, and verbascoside. The structure of cassinopin was established by spectroscopic and chemical methods.

INTRODUCTION

Cassinopsis madagascariensis Baill. (Icacinaceae) is a shrub known as 'bemafaitra', which grows in the humid western forest of Madagascar. The leaves are used as a traditional remedy against malaria [2]. Boiteau [3] claimed that the decoction of the aerial parts is effective for the treatment of the side-effects of malaria.

RESULTS AND DISCUSSION

The methanolic extract of the air-dried leaves of *C. madagascariensis* (30%) tested *in vitro* against *Plasmodium* showed weak activity (Majori, G., personal communication). Four known phenylpropanoid glycosides (identified by direct comparison), calceolarioside A, B and C [4, 5], and verbascoside [6], were isolated from the methanolic extract, together with a new flavonol glycoside, **1**, named cassinopin. This substance, mp 202–204° (EtOH), $[\alpha]_D$ −230.4° (EtOH), corresponds to the formula $C_{33}H_{40}O_{18}$, $[M+H]^+$ at *m/z* 725 (FABMS). The UV absorbance (see Experimental) and the ^{1}H and ^{13}C NMR data (see Tables 1 and 2) suggested it to be a flavonol glycoside. Hydrolysis with cellulase afforded kaempferol (3,5,7,4′-tetrahydroxyflavone) and L-rhamnose, as the sole monose, in agreement with the presence in the ^{1}H NMR spectrum of three secondary methyl signals at δ0.98, 1.24, and 1.31. Treatment of **1** with pyridine and acetic anhydride gave the decaacetyl derivative **2**, mp 247–250°. Its ^{1}H NMR spectrum showed that two rhamnose moieties are arranged in a biose unit with a 1→4 linkage, since the signal, which remains upfield at δ3.53 (*t*, *J* = 9.5 Hz), is coupled with H-5 (δ3.20, *dq*, *J* = 6.0 and 9.5 Hz). In the ^{13}C NMR spectrum of **1** the signal at δ79.8, characteristic for C-4 in 4-substituted rhamnose units, was in agreement with this assignment [7].

Methylation of cassinopin with ethereal diazomethane gave the monomethyl ether **3**, mp 240–243°. The bathochromic shift by addition of aluminium trichloride observed in the UV spectra of **1** and **3** indicated that the

*Part 19 in the series 'Research on African Medicinal Plants'. For Part 18 see ref. [1].

Dedicated to Professor G. B. Marini-Bettolo on the occasion of his 75th birthday.

Table 1. ^{1}H NMR chemical shift assignments of compounds **1–3**

H	**1** (CD_3OD)	**2** ($CDCl_3$)	**3'** (DMSO-d_6)
6	6.46 *d* (2 0)	6 76	6 35
8	6 70 *d* (2 0)	7 06	6 61
2', 6'	7 78 *d* (9 0)	7 89	7 73
3', 5'	6 97 *d* (9 0)	7 29	7 05
OMe	—	—	3 80
Rh			
1''	5 49[a] *d* (1 5)	5.46[a]	5 47[a]
2''	4 20[b] *dd* (1.5, 3 0)	5 61[b]	3 77[b]
3''	3 88[c] *dd* (3.0, 9 5)	5.45	3 6–3.7
4''	3.42[d] *t* (9 5)	5.1–5 2	3.22[c]
5''	3 6–3 7 *m*	3.84[c]	3 50
Me	1 31[e] *d* (6 0)	1 22[d]	1.16[d]
Rh-Rh			
1'''	5 15 *d* (1 5)	4.85	5 00
2'''	4 08[b] *dd* (1 5, 3 0)	5.05[b]	4.00[b]
3'''	3 85[c] *dd* (3 0, 9 5)	5 1–5 2	3 6–3 7
4'''	3.46[d] *t* (9 5)	3 53*	3 30[c]
5'''	3 23 *dq* (6.0, 9 5)	3 20*	3.07
Me	0 98 *d* (6 0)	1 00*	0 84
1''''	5 60[a] *d* (1.5)	5.54[a]	5 35[a]
2''''	3 99[b] *dd* (1 5, 3 0)	5 06[b]	3 90[b]
3''''	3 6–3 7 *m*	5 1–5 2	3 3–3.4
4''''	3.53[d] *t* (9 5)	5 1–5 2	3 3–3.4
5''''	3 6–3 7 *m*	3 90[c]	3.3–3 4
Me''''	1 24[e] *d* (6 0)	1.13[d]	1 09[d]
OCOMe	—	2 40, 2.27, 2 18, 2 09 2.06, 2.04, 2 01, 1 99, 1 96	—

[a–e]Assignments may be interchanged in the same column
*Assigned by decoupling experiments
J in Hz parentheses

C-5 hydroxy group of kaempferol is free in both compounds, also in agreement with the resonances of the carbonyls in the ^{13}C NMR spectrum (δ 179.2 and 179 4, respectively), typical of H-bonded aromatic carbonyl groups. The C-7 hydroxy group in cassinopin is glycosylated because its UV spectrum does not undergo a bathochromic shift on addition of sodium acetate. The downfield shift of H-3' and H-5' observed in **2** after acetylation (δ7.29 against 6 97 in **1**) indicated that the C-4' hydroxy group is free in **1**, whereas it is methylated in **3** in agreement with the typical ^{13}C NMR resonance of the *ortho* free methoxy group (δ56.5) [8]. The 3- and 7-hydroxyl groups are therefore the positions to which the rhamnose and rhamnose-1 → 4-rhamnose units are attached. Mild acidic hydrolysis (0 0125 M H_2SO_4) gave a monorhamnosyl derivative which on the basis of its NMR data in DMSO-d_6 was identified as 7-α-L-rhamnosylkaempferol [9] (isolated from *Prunus spinosa*), because H-6 and H-8 remained downfield (at δ6.41 and 6.81, respectively) whereas C-2 shifted upfield (δ147 4) with respect to **1** and **3**. The monose unit was thus assigned to the OH-7, and consequently the biose unit to the OH-3. The observed upfield shift in the ^{1}H NMR spectrum of **2** of H-5 (δ3 20) as well as of the vicinal methyl group (δ1.00), due to the shielding effect of the B ring, is in agreement with this structure. The α-configuration was then assigned to the three glycosidic linkages on the basis of the chemical shift values of C-3 and C-5 of rhamnose, that resonate at a higher field in the α-anomer (δ72.5 and 69.4, respectively) than in the β-anomer (δ75.4 and 73 5) [7]

A trirhamnoside of kaempforol, in which the substitutions assigned at position 3 and 7 are reversed with respect to cassinopin, had been isolated from *Phaseolus atropurpureus* by Ford [10], but the physicochemical data do not allow a direct comparison between the two compounds

EXPERIMENTAL

Separations were performed by counter-current distribution (CCD) using a Craig Post apparatus (200 tubes, 10·10 ml upper and lower phase). ^{1}H and ^{13}C NMR spectra were recorded using TMS as int standard, coupling constants are given in Hz

Plant material Leaves of *Cassinopsis madagascariensis* were collected in region Mangamila (Madagascar). A voucher specimen is kept at the Herbarium of the Institut Malgache de Recherche Appliquées (Antananarivo)

Extraction and purification Dried leaves of *C madagascariensis* (400 g) were powdered and extracted ×3 with MeOH The pooled solutions were evapd and the residue (120 g) partitioned

Table 2 ^{13}C NMR chemical shift assignments of compounds **1–3**

C	**1** (DMSO-d_6/CD_3OD)	**2** ($CDCl_3$)	**3** (DMSO-d_6/CD_3OD)
2	159 3[a]	157 6[a]	159 1
3	135 8	137 0	136 0
4	179 2	172 1	179 4
4a	107 2	112 9	107 8
5	162 3[b]	156 1[a]	163 3[a]
6	99 8	109 4	100 1
7	163 2[b]	159 2	163 3[a]
8	95 7	102 0	95 9
8a	157 5[a]	151 0	157 8
1′	122 0	127 6	123 5
2′	131 8	130 2	131 9
3′	116 5	122 3	115 3
4′	161 4[b]	152 9	162 6[a]
5′	116 5	122 3	115 3
6′	131 8	130 2	131 9
OMe			56 5
Rhamnose moieties			
1	102 6 × 2, 100 5	99 0, 98 2, 95 9	102 7 × 2, 100 8
2–5	79 8, 73 6, 73 3, 72 5, 72 1, 71 9, 71 3, 71 2, 70 1	77 7, 71 5, 71 2, 70 6, 70 3, 69 6, 69 2, 69 0, 68 7, 68 6, 68 0, 67 3	79 8, 73 7, 73 4, 72 5, 72 4, 72 0, 71 4, 70 3
Me	18 6, 18 5, 18 2	17 7, 17 5, 17 2	18 8, 18 6, 18 4
OCOMe	–	169 9, 169 5, 168 7	
OCOMe	–	21 1, 21 0, 20 8	

[a] [b]Assignments may be interchanged in the same column

between H_2O–EtOH–EtOAc–cyclohexane (5 2 1 6)* The upper phase, mainly containing chlorophylls, was discarded, whereas the aqueous phase, after evapn of EtOH, was extracted twice with *n*-BuOH The buthanolic residue (20 g) submitted to CCD with the biphase system H_2O–EtOAc–*n*-BuOH (5.4.1), gave three fractions The fraction at K_r 1 contained pure cassinopin, **1**, the fraction at K_r 1 5, further purified by the solvent system H_2O–EtOAc–*n*-BuOH (10 9 1), gave calceolarioside B (K_r 1), calceolarioside C (K_r 0 6), and verbascoside (K_r 0.85). The most mobile fraction, purified with H_2O–$CHCl_3$–MeOH (4 5 6), gave calceolarioside A (K_r 4) Calceolarioside A, B, C and verbascoside were identified by comparison with authentic samples [4, 5]

Cassinopin (**1**) Crystals from EtOH, mp 202–204°, $[\alpha]_D$ −230 4 (EtOH, *c* 0 4), UV λ_{max}^{MeOH} nm (log ε) 343 (4 21), 315sh (4 16), 264 (4 37), 205 (4 53), +NaOAc 343, 315sh, 264, 205, +$AlCl_3$ 390, 349, 300, 273, 235, 204 ^1H and ^{13}C NMR see Tables 1 and 2, respectively FABMS, *m/z* 725 $(M+H)^+$ (Found C, 54 54, H, 5 60, calcd for $C_{33}H_{40}O_{18}$ C, 54 69, H, 5 52%)

Enzymatic hydrolysis of cassinopin The hydrolysis of **1** was carried out with cellulase Fluka (weight ratio 2 1) containing α-rhamnosidase activity at 33° in a soln of acetate buffer at pH 4 5 and MeOH (1 1) After a night, the hydrolysis was complete Some drops of HOAc were added, MeOH was eliminated *in vacuo* from the soln which was extracted with EtOAc In the residue of the organic phase kaempferol was identified by comparison with an authentic sample The aq soln was then extracted with *n*-BuOH and percolated through a column of Dowex 50W (H^+) In the dried residue L-rhamnose was identified by direct comparison (TLC and rotatory power).

Partial hydrolysis of cassinopin A solution of compound **1** (100 mg) in 0 0125 M H_2SO_4 (50 ml) was refluxed for 1 hr The reaction mixture was extracted with EtOAc, and the residue of the organic phase after CCD (H_2O–EtOH–EtOAc–cyclohexane, 5.2 3 4) gave pure 7-α-L-rhamnosylkaempferol (20 mg) UV λ_{max}^{MeOH} nm 366, 324, 265, 252, +NaOAc 407, 324, 260

Decaacetylcassinopin, **2** Cassinopin was acetylated with a mixture of pyridine and Ac_2O (1 1) After one day the reagents were evapd *in vacuo* and the residue crystallized from EtOAc, mp 247–250° $[\alpha]_D$ −151 5 ($CHCl_3$, *c* 0 5) (Found C, 55 46, H, 5 33, calcd for $C_{53}H_{60}O_{28}$, C, 55 59, H, 5 24%)

Methylcassinopin **3** Cassinopin **1**, dissolved in MeOH was methylated with an ethereal solution of CH_2N_2 After one day, the solvents were evapd and the residue purified by CCD between H_2O–EtOAc–*n*-BuOH (10 9 1) Crystals from EtOH, mp, 240–243° $[\alpha]_D$ −218 5 (EtOH, *c* 0 3), UV λ_{max}^{MeOH} nm (log ε) 340 (4 17), 312 (4 17), 265 (4 36), 205 (4 54), +$AlCl_3$ 395 (4 11), 342 (4 22), 299 (4 09), 274 (4 38), 234 (4 26), 207 (4 60)

*During the partition procedure a solid separated Recovered by filtration, washed with water and dried (10 g), it resulted an omogeneous substance, identical to compound **1**

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3-HYDROXY-4′-METHOXYFLAVONE FROM *MILLETTIA ZECHIANA*

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Key Word Index—*Millettia zechiana*; Leguminosae, flowers, 3-hydroxy-4′-methoxyflavone, flavonoids.

Abstract—3-Hydroxy-4′-methoxyflavone was identified for the first time from flowers of *Millettia zechiana* and its structure established from its chemical characteristics and from its synthesis. In addition known glycosides of kaempferol, quercetin, malvidin, cyanidin and pelargonidin were found.

INTRODUCTION

Millettia zechiana Harms. (Ir. 1957) found in secondary forests and coastal grassy plains from Guinea to Cameroons, bears reddish purple, silky brown flowers (October–December) [1] The bark pulp, with sea water and Guinea grains diluted with warm water, is used as a gargle for rhinopharyngal and bronchial troubles and the purple leaves are rubbed on painful parts [2].

As part of a continuing chemotaxonomic study of the Nigerian flora, [3–5] we report the isolation, identification and synthesis of the 4′-methyl ether of 3,4′-dihydroxyflavone, in addition to six known glycosides from the flowers.

RESULTS AND DISCUSSION

Extraction and chromatography of the flowers of *M. zechiana* afforded six known glycosides, namely the 3-*O*-rhamnoside and 3-*O*-glucoside of kaempferol and of quercetin, the 7-*O*-glucoside of 8-hydroxyquercetin, the 3,5-diglucoside of malvidin and of cyanidin and the 3-*O*-rhamnoside of pelargonidin; these were identified according to standard procedures [6, 7]. 3-Hydroxy-4′-methoxyflavone was obtained as a light yellow crystalline solid, purified by preparative TLC (20×20 cm glass plates coated with 0.5 mm layer of silica gel $60PF_{254}$). It appeared purple on ID-PC with and without UV+NH_3 indicating a methoxy group at C-4′ [8, 9], R_f values: BAW, *n*-BuOH–HOAc–H_2O (4 1.5, top layer) 92, HOAc–conc. HCl–H_2O, 85, PhOH, 90, BAFW, *n*-BuOH–HOAc–HCO_2H–H_2O (5.1·1 3) 90; BFTW, *n*-BuOH–HCO_2H–C_6H_5Me–H_2O (3:1:1:5) 92 The absence of a colour with 2-aminoethyldiphenyl borate and magnesium acetate indicated that free aromatic hydroxyl groups were not present [8–10]. The yellow fluorescence with UV+5% $AlCl_3$, the purple colour with sodium carbonate and 30% sodium hydroxide and the red colour with magnesium and zinc/hydrochloric acid showed the presence of a flavonol skeleton [6, 11] with a methoxy group at C-4′ The mp was 197–199° and the M_r 268 (Found C, 71.64, H, 4.47, O, 23 94%, calc. for $C_{16}H_{12}O_4$: C, 72 0, H, 4.0, O, 24 0%) UV λ_{max} nm (95% EtOH) 253, 370; no bathochromic shift with NaOAc, (+NaOEt) 256, 409. IR ν_{max} cm^{-1}: 3400 (OH), 2916 (C–H); 1656 (C=O); 1590 (C=C); 1064 (C–O); 800–700 (*o-o-p*, bending for substituted benzene ring) These data confirm that it has the structure as indicated above

The compound was synthesized by dissolving 3.9 g (0.025 mol) *o*,*ω*-dihydroxyacetophenone in 25 ml pyridine with stirring at 50°. 7 2 g (0 025 mol) *p*-methoxybenzoic anhydride and 120 ml 1 M HCl were added. After 15 min, it was heated at 140° for 50 min The reaction mixture was poured onto ice and the product which