Flavonoids from Three Ethiopian Species of *Premna*

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Z. Naturforsch. **47b,** 144–147 (1992); received June 26, 1991

Verbenaceae, *Premna*, Polyoxygenated Flavonoids

Two novel hepta-oxygenated flavonols have been isolated from the aerial parts of *Premna oligotricha* and identified, primarily on the basis of NMR spectroscopy, as 3,5,5′-trihydroxy-6,7,3′,4′-tetramethoxyflavone (**2**) and 3,5,7,5′-tetrahydroxy-6,3′,4′-trimethoxyflavone (**3**). Known flavonoids, with lower levels of oxygenation were obtained from two other *Premna species, P. schimperi* and *P. recinosa*.

Introduction

We have recently reported the isolation and characterization of a number of labdane and clerodane diterpenes, some with antibacterial activity, from the ethanolic extracts of the above ground parts of two Ethiopian *Premna species, P. oligotricha* Baker and *P. schimperi* Engl. [1–3]. In our continuing investigation of the chemistry of *Premna* species we have isolated, from these two species and from *Premna recinosa* Schaur, a number of flavanones, flavonols and flavones. In this paper we report the characterization of two new 3,5,6,7,3′,4′,5′-hepta-oxygenated flavonols and seven known compounds.

Result and Discussion

The two new flavonoids were isolated from an ethanolic extract of the above ground parts of *P. oligotricha* by vacuum liquid chromatography.

The high resolution electron impact MS of the new compounds revealed empirical formulae of $C_{19}H_{18}O_9$ and $C_{18}H_{16}O_9$ respectively. ^{1}H NMR spectra, run in deuteriopyridine (Table I), revealed signals for four and three methoxyl resonances, respectively, together with a single aromatic proton, a pair of aromatic protons showing *meta* coupling and a deshielded H-bonded hydroxyl resonance typical of the 5-OH of a flavonoid.

The data suggested the two compounds were partially methylated flavonols carrying oxygenation at C-5, C-7, C-3′, C-4′, C-5′ and two other positions (from C-3, C-6, C-8). An extensive NMR

Table I. ^{1}H NMR chemical shift data for **2, 3** and **10.**

	2	**2**-Ac_3	**3**	**3**-Ac_4	**10**
H-8	6.80 s	6.88 s	6.89 s	7.25 s	6.66 s
H-2′	8.26 d(2)	7.24 d(2)	8.21 d(2)	7.24 d(2)	7.82 dd(8,2)
H-5′					7.38 d((8)
H-6′	7.85 d(2)	7.15 d(2)	7.82 d(2)	7.16 d(2)	7.82 d(2)
OMe-3					3.89 s
OMe-6	3.99 s	3.89 s	3.95 s	3.86 s	3.99 s
OMe-7	3.82 s	3.99 s			3.87 s
OMe-3′	3.88 s	3.92 s	3.88 s	3.91 s	
OMe-4′	3.99 s	3.91 s	3.97 s	3.91 s	
OH-5	13.12 s		13.30 s		12.12 s
Ac		2.32		2.32	
		2.36		2.35	
		2.37		2.37	
				2.48	

J values in brackets; **2, 3** and **10** run in C_5D_5N, acetates of **2** and **3** in $CDCl_3$, all at 400 MHz.

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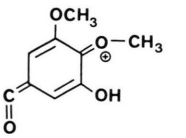
Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0932–0776/92/0100–0144/$ 01.00/0

study of the tetramethoxy flavonol aided assignment of the methoxyl resonances. The ^{13}C NMR spectrum of the peracetylated derivative (Table II) revealed two methoxyl carbons resonating at *ca.* 56 ppm and two at *ca.* 61 ppm. The former methoxyls must have at least one *ortho* position unsubstituted while the latter must have them both substituted [4]. A NOESY spectrum further revealed enhancement between one methoxyl and a *meta*-coupled (B-ring) proton and between a second methoxyl and the aromatic singlet. Therefore one methoxyl must be placed at C-7 (adjacent to an isolated H-6 or H-8 proton) and the other at C-3′ adjacent to the *meta*-coupled H-2′ proton. Therefore C-3 must be oxygenated and C-5 and C-5′ must carry hydroxyls, in the latter case this being established by the absence of any enhancement of H-6′ in the NOESY spectrum. The remaining two methoxyls must therefore be at C-3, C-6/C-8 or C-4′.

Placement of one methoxyl at C-4′ followed from the electron impact MS which revealed a fragment at *m/z* 181 (**1**) which requires two methoxyl groups in ring-B. A further feature of the electron impact MS was the occurrence of M^+ as base peak. This suggest a 6-methoxy substituent as in 8-methoxyl flavonols M^+-15 is characteristically the base peak [5].



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The assignment of the methoxyl to C-6 was finally established by a long-range $^1H-^{13}C$ coupling study on the peracetate using the HMBC technique [6]. The results of this are outlined in Table III. The key features of this experiment were the ^2J and ^3J coupling of H-8 to the carbons at 158.1 and 139.8 ppm; these two carbons also show ^3J coupling to the protons of two of the methoxyl resonances. This can occur only if the methoxyls are placed at C-7 and C-6 and not at C-7 and C-3.

Table II. ^{13}C NMR chemical shift values for **2** and **3** peracetates and **10.**

C	**2**-Ac	**3**-Ac	**10**
2	153.7	153.4	156.9
3	133.8	133.2	138.7
4	170.1	170.1	179.9
5	142.0	142.4	152.6
6	139.8	142.4	132.8
7	158.1	151.7	159.4
8	98.1	110.2	91.3
9	153.4	148.6	153.3
10	111.2	115.7	106.9
1′	124.6	124.2	122.1
2′	110.0	109.8	121.7
3′	153.5	153.5	116.9
4′	143.5	143.7	151.0
5′	143.9	143.8	147.4
6′	115.7	115.9	116.8
3-OMe			59.9
6-OMe	61.5	60.8	60.7
7-OMe	56.5		56.5
3′-OMe	56.2	56.2	
4′-OMe	60.9	61.8	
Ac	169.7, 168.8	168.9, 168.7	
	168.0	167.8, 167.8	
	20.9, 20.7	20.9, 20.6	
	20.5	20.6, 20.6	

2 and **3** run in $CDCl_3$, **10** run in C_5D_5N; all at 400 MHz.

Table III. $^1H-^{13}C$ correlations in **2**-Ac from HMBC.

	^1J	^2J	^3J
H-8	98.1	158.1, 153.4	139.8, 111.2
H-2′	110.0		143.5
H-6′	115.7	153.5, 124.6	143.5
OMe-6	61.5		139.8
OMe-7	56.5		158.1
OMe-3′	56.2		153.5
OMe-4′	60.9		143.5

On this basis the flavonoid was characterized as 3,5,5′-trihydroxy-6,7,3′,4′-tetramethoxyflavone (**2**), which appears to be novel.

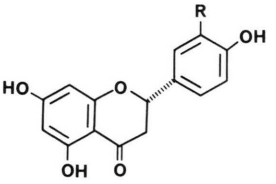
The ^{13}C NMR spectrum of the second flavonol differed from that of **2** by loss of one of the shielded methoxyl resonances. Both ^1H and ^{13}C NMR spectra showed marked changes in resonance positions for H-8/C-8 and addition of sodium acetate led to a small bathochromic shift in band II of the UV spectra: all features suggesting a free hydroxyl at C-7. This was confirmed by the HMBC analysis of the peracetate (Table IV) which still showed ^3J coupling between H-8 and a carbon (142.4 ppm) carrying a methoxyl but not the comparable ^2J coupling. The second flavonol must therefore be 3,5,7,5′-tetrahydroxy-6,3′,4′-trimethoxyflavone (**3**).

Table IV. $^{1}H-^{13}C$ correlations in **3**-Ac from HMBC.

	^{1}J	^{2}J	^{3}J
H-8	110.2	151.7, 148.6	142.4
H-2′	109.8	153.5, 124.2	143.7
H-6′	115.9	143.8, 124.2	109.8
OMe-6	60.8		142.4
OMe-3′	56.2		153.5
OMe-4′	61.8		143.7

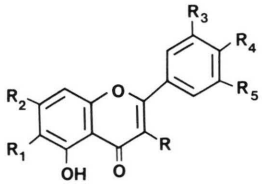
Column chromatography and PTLC of the extract of *P. schimperi* afforded only luteolin (**4**), quercetin (**5**) and kaempferide (**6**), all of which were readily characterized on the basis of spectral data. Confirmation of the position of the methyl ether in **6** came from an NOE study.

Similar treatment of an extract of *Premna recinosa* also gave **4** and **5** together with the common flavanones naringenin (**7**) and eriodictyol (**8**). In addition pachypodol (**9**), a trimethyl ether of quercetin was isolated and the position of the methyl ethers confirmed by NOE and electron impact MS.



7 R = H
8 R = OH

Finally *P. recinosa* yielded a hexa-oxygenated flavone trimethyl ether which was characterized as chrysosplenol-D (**10**). The ^{1}H and ^{13}C NMR spectra of this rather rare flavonol are recorded in Table I and II respectively. The HMBC study (Table V) again substantiated the presence of the methoxyl at C-6.



	R	R_1	R_2	R_3	R_4	R_5
2	OH	OMe	OMe	OMe	OMe	OH
3	OH	OMe	OH	OMe	OMe	OH
4	H	H	OH	OH	OH	H
5	OH	H	OH	OH	OH	H
6	OH	H	OH	H	OMe	H
9	OMe	H	OMe	OMe	OH	H
10	OMe	OMe	OMe	OH	OH	H

All three *Premna* species have yielded flavonols but in only two, *P. recinosa* and *P. oligotricha* has C-6 oxygenation been observed and only *P. oligotricha* has given 3′,4′,5′-oxygenated products. *Premna* appears to be an interesting source of poly-oxygenated flavonols.

Tab. V. $^{1}H-^{13}C$ correlation in **10.**

	^{1}J	^{2}J	^{3}J
H-8	91.3	159.4, 152.6	132.8, 106.9
H-2′	116.8	147.4	156.9, 151.0, 121.7
H-5′	116.9	151.0	147.4, 122.1
H-6′	121.7	116.9	156.9, 151.0
OMe-3			138.7
OMe-6			132.8
OMe-7			159.3

Experimental

General

Mps: Uncorr., IR: KCl, MS: 70 eV direct inlet, ^{1}H, ^{13}C, NOESY and HMBC NMR studies: 250 and 400 MHz spectrometer. UV spectra of flavonoids were recorded using a standard procedure [7]. Acetylation of **2** and **3** were performed at room temperature by adding 2 ml of acetic anhydride to 20 mg of the sample dissolved in 1 ml of pyridine.

Plant material

The leaves of *Premna schimperi* (voucher: SHM-13) were collected in September 1989 from the Menagesha Forest (*ca.* 2000 m), Menagesha Administrative region, Shoa Province, Ethiopia; *Premna oligotricha* (voucher: SHM-12) in September 1989 from beside the Yabellow-Mega Road at *ca.* 1600 m, Sidamo Province, Ethiopia; and *Premna recinosa* (voucher: Sebsebe 2535) in April 1990 from an *Acacia/Commiphora* woodland forest (*ca.* 870 m), 2 km from Melkaguba towards Negele, Sidamo Province, Ethiopia. Voucher specimens have been deposited at the National Herbarium of Ethiopia, Addis Ababa University.

Isolation of flavonoids

Powdered leaves of *Premna oligotricha* were placed in a glass percolator and continually extracted with EtOH for two weeks. Removal of the solvent yielded a dark gum (150 gm) which was

subjected to vacuum liquid chromatography (silica gel) eluting with petroleum ether and then EtOAc mixtures of increasing polarity and then EtOAc. The EtOAc VLC fraction was further subjected to column chromatography over silica gel eluting with $CHCl_3$:MeOH mixtures of increasing polarity. The fraction eluted with 80% $CHCl_3$ afforded **3** (20 mg) while the 40–60% $CHCl_3$ eluents gave impure **2** which was recrystallized from MeOH to give **2** (50 mg).

Powdered leaves of *Premna recinosa* (750 g) were extracted as described for *Premna oligotricha*. Removal of the solvent yielded 55 g of residue which was subsequently fractionated using VLC (silica gel) eluting with petroleum ether and EtOAc mixtures of increasing polarity. The 10% EtOAc eluent was applied on a Sephadex LH-20 column (solvent, $CHCl_3$:MeOH 1:1) and yielded **9** (25 mg) and impure **7** which was purified by PTLC (silica gel, petroleum ether:$CHCl_3$:EtOAc, 4:6:4) to yield **7** (10 mg). The 20% EtOAc fraction afforded **8** (20 mg). The 50–70% EtOAc fractions were bulked and subjected to column chromatography (Sephadex LH-20, as above) to give pure **5** (10 mg) and mixture of two flavonoids. PTLC of this mixture (silica gel, petroleum ether:$CHCl_3$:EtOAc, 4:6:6) yielded **10** (25 mg) and **4** (8.7 mg).

Leaves of *Premna schimperi* (700 g) were extracted with 80% ethanol as described for *Premna oligotricha*. Removal of solvent under reduced pressure yielded 96 g of residue which was subsequently partitioned into $CHCl_3$ and water. Column chromatography (Sephadex LH-20) of the $CHCl_3$ fraction (15 g) gave impure **6** from the last fraction which was recrystallized from MeOH to yield **6** (6 mg). PTLC (silica gel, $CHCl_3$:MeOH 7:3) of the first column fraction afforded **5** (150 mg) and **4** (12 mg).

Luteolin (**4**), *quercetin* (**5**), *kaempferide* (**6**), *naringenin* (**7**), *eriodictyol* (**8**) *and pachypodol* (**9**)

All gave UV, IR and ^{1}H NMR data in close agreement with that reported or with authentic samples.

Chrysosplenol-D (**10**)

Orange needles from MeOH, m.p. 241–245 °C (lit. [8] 214–216 °C). UV λ_{max}, nm: MeOH 256 sh, 281, 360; ($AlCl_3$) 284, 384, 428; (NaOMe) 284, 410; (NaOAc) 280, 360, 422; (NaOAc + H_3BO_3) 274, 360. IR ν_{max}, cm^{-1}: 3450, 2920, 1660, 1600, 1490, 1430, 1350, 1230, 1210, 1160. ^{1}H NMR – see Table I, ^{13}C NMR – see Table II. MS (rel. int.): *m/z* 360 (100), (calcd for $C_{18}H_{16}O_8$ 360.0845 found 360.0804), 359 (19), 345 (76), 137 (26).

3,5,5′-Trihydroxy-6,7,3′,4′-tetramethoxyflavone (**2**)

Yellow needles, 211 °C. UV λ_{max}, nm (logε): MeOH 260 (4.15), 356 (4.39), 422 (3.61); ($AlCl_3$) 266, 416; (NaOMe) 260, 410; (NaOAc) 260, 338 sh, 368, 416 sh; (NaOAc + H_3BO_3) 260, 356. IR ν_{max}, cm^{-1}: 3450, 3300, 2950, 1650, 1590, 1490, 1210. ^{1}H NMR – see Table I. ^{13}C NMR – see Table II. MS (rel. int.): *m/z* 390 (100) (calcd $C_{19}H_{18}O_9$ for 390.0951 found 390.0927), 347 (74), 329 (9), 181 (3).

3,5,7,5′-Tetrahydroxy-6,3′,4′-trihydroxyflavone (**3**)

Amorphous solid, 253 °C. UV ν_{max}, nm (logε): MeOH 245 (4.22), 266 (4.25), 362 (4.35); ($AlCl_3$) 270, 362, 418; (NaOMe) 270, 398; (NaOAc) 272, 380; (NaOAc + H_3BO_3) 254, 166, 362. IR ν_{max}, cm^{-1}: 3380, 3310, 2950, 1655, 1630, 1600, 1550, 1490, 1400, 1210, 800. ^{1}H NMR – see Table I. ^{13}C NMR – see Table II. MS (rel. int.) *m/z* M^+ not seen but M^+ of peracetate 544 (4.3) (calcd $C_{26}H_{24}O_{13}$ 544.1217 found 544.1249).

One of us (S. H.) thanks the University of Strathclyde for the award of a scholarship. Dr. Sebsebe Demissew, National Herbarium of Ethiopia, is thanked for collecting and identifying *Premna recinosa*. NMR studies were performed in the University of Strathclyde NMR laboratory.

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