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5-DEOXYFLAVONES FROM *PARKIA CLAPPERTONIANA*

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Key Word Index—*Parkia clappertoniana*; Leguminosae; 5-deoxyflavone; 7-hydroxy-3,8,4′-trimethoxyflavone; 2′-hydroxy-3,7,8,4′,5′-pentamethoxyflavone; molluscicide.

Abstract—Two new 5-deoxyflavones, 7-hydroxy-3,8,4′-trimethoxyflavone and 2′-hydroxy-3,7,8,4′,5′-pentamethoxyflavone, were isolated from leaves from *Parkia clappertoniana* during an investigation of the plant for molluscicidal compounds. The structures were elucidated by spectroscopic methods (IR, UV on addition of shift reagents, mass spectrometry, ^{1}H NMR, ^{13}C NMR and ^{1}H NOE). The molluscicidal activity of the two compounds were tested against *Biomphalaria glabrata*, at a concentration of 25 ppm. The first compounds was inactive and the latter was molluscicidal.

INTRODUCTION

The tree *Parkia clappertoniana* Keay or West African Locust Bean is distributed from Ghana to the Sudan [1]. During a systematic screening, the leaf material from this tree showed molluscicidal activity towards the schistosomiasis transmitting snail *Biomphalaria pfeifferi* and it was selected for investigation of the active compounds. Molluscicidal compounds are used for killing the intermediate snail host transmitting the tropical parasitic disease schistosomiasis [2] and molluscicides of plant origin might be developed into cheap, locally produced, alternatives to the expensive synthetic molluscicides available today [3]. Development of a safe and affordable molluscicide from the berries of *Phytolacca dodecandra* is in progress [4–6], but screening of new plant material is still justifiable, because the snails might develop resistance against the molluscicides in use after some time.

Previously, anthocyanidins and flavonoids have been isolated from the seeds of *P. clappertoniana*, e.g. the 5-deoxyflavone 3,7,3′,4′-tetrahydroxyflavone (fisetin) [7, 8]. Whilst searching for the active constituent in this plant, two new 5-deoxyflavones were isolated, 7-hydroxy-3,8,4′-trimethoxyflavone (**1**) and 2′-hydroxy-3,7,8,4′,5′-pentamethoxyflavone (**2**), the latter with an oxygenation pattern not previously found in natural flavonoids. The molluscicidal activity of the two pure compounds was tested against *B. glabrata*.

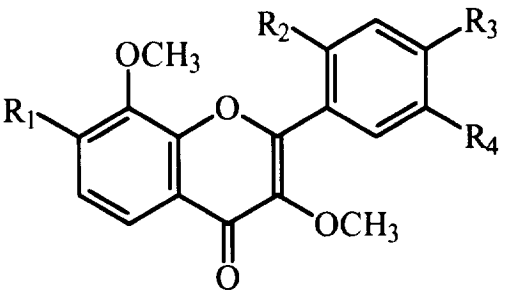
RESULTS AND DISCUSSION

TLC analysis of an ethanolic leaf extract of *P. clappertoniana* revealed a very complex mixture of compounds of different polarity. The extract was partitioned into five phases by successive distribution between non-miscible solvents. All phases except the heptane phase showed molluscicidal activity at 400 ppm. The two new compounds (**1** and **2**) were isolated from the methylene dichloride phase and purified by repeated column chromatography and crystallization.

The flavonoid structure of **1** was indicated by its yellow colour in UV light and UV spectral maxima at 260 and 335 nm, corresponding to band II (240–285 nm) and band I (300–400 nm), respectively [9]. Addition of $AlCl_3$ caused no change in the UV spectrum and thus **1** had no free hydroxyl group at C-3 or at C-5. Addition of sodium methoxide and of sodium acetate caused bathochromic shifts of both maxima, which indicated a free hydroxyl group at C-7 and/or at C-4′. The ^{13}C NMR spectrum of **1** showed three peaks at δ 55.4, 60.0 and 61.9. Between δ 113.7 and 161.5, 12 peaks were seen, of which two showed double intensity, and in addition one peak was seen at δ 174.6. Based on these data it was concluded that the molecule contained 18 carbon atoms, including one carbonyl group and three aliphatic carbons with an oxygen function. In the ^{1}H NMR spectrum three singlets at δ 3.88, 3.90 and 4.11 were interpreted as arising from three methoxyl groups. A signal corresponding to one proton at δ 6.78 disappeared on addition of D_2O, indicating that a phenolic group was present. A pair of doublets at δ 7.05 and 8.13, corresponding to two protons each, was interpreted as arising from a *para* substituted ring, which could only be ring B. The

chemical shift of the protons was in accordance with the range for the protons C-2′, C-6′ (7.9–8.1 ppm) and C-3′, C-5′ (6.5–7.1 ppm) in 4′-oxygenated flavonoids [9]. A pair of doublets, at δ 7.06 and 7.93, corresponding to one H each ($J = 8.8$ Hz), indicated two protons in the *ortho* position, most likely at positions C-5 and C-6, as the chemical shift range for H-5 and H-6 in 5-deoxyflavones with oxygen at C-7 are 7.9–8.2 and 6.7–7.1 ppm, respectively [9]. A combination of these data and the molecular ion, appearing at *m/z* 328 in the EI mass spectrum, led to the tentative formulae **1** and **3**. In the EI mass spectrum, *m/z* 328 was a prominent peak, second only to the base peak $[M-H]^+$ at *m/z* 327. The ions at *m/z* 167 and 135 supported structure **1**, since they corresponded to fragmentation pathways leading to the ions $[A_1+H]^+$ and B_2^+, containing ring A and B [9, 10]. A structure with interchanged substitution pattern, as in **3**, would be expected to give rise to ions at *m/z* 181 and 121, which were not found. The structure **3** can thus be excluded.

Compound **2** appeared yellow in UV light with two UV spectral maxima at 249 and 346 nm. Addition of $AlCl_3$ caused no change and therefore there was no free hydroxyl group at C-3 or C-5. Addition of sodium methoxide caused a bathochromic shift of band I which indicated a free phenolic group and a slightly hypsochromic shift of band II. Addition of sodium acetate gave no change in the UV spectrum, and thus free phenolic groups can only be placed at C-6, C-8, C-2′ (C-6′) and C-3′ (C-5′). The ^{13}C NMR spectrum showed 20 signals of which five in the range δ 56–62 were interpreted as arising from methoxyl groups. Between δ 102.8 and 156.6, 14 signals were seen, corresponding to aromatic or vinylic carbons. A signal at δ 173.3 indicated the presence of a carbonyl group. The ^1H NMR spectrum of **2** showed five singlets near δ 4, each integrating for thee protons, corresponding to five methoxyl groups. A signal at δ 8.20, disappearing on exchange with D_2O and integrating for one proton, indicated a free phenolic group. The remaining signals were a pair of doublets at δ 7.08 and 8.01 ($J = 8.0$ Hz), corresponding to one proton each, and two singlets at δ 6.64 and 7.32, also corresponding to one proton each. The four protons at the unsubstituted positions in the flavonoid, which is substituted with five methoxyl groups and one phenolic group, are thus located such that two protons are in a vicinal position, and two protons are isolated or in a *para* position. This gives several structure possibilities. In the EI mass spectrum the molecular ion at *m/z* 388 was a prominent peak, second only to the base peak at *m/z* 373 $[M-CH_3]^+$. Calculations of the possible ions obtainable from the fragmentation pathways leading to ions containing rings A and B, respectively, and comparison with the EI mass spectrum of **2**, resulted in the tentative structures **2** and **4**. The ion present at *m/z* 181 corresponds to $[A_1+H]^+$ or B_2^+ and that at *m/z* 208 corresponds to B_1^+. Furthermore, the ion at *m/z* 371, corresponding to $[M-17]^+$, has been found as a characteristic ion for 2′-hydroxyflavonoids [9, 10]. Final proof of the structure of **2** was obtained by a ^1H NOE NMR spectrum. On irradiation of the signal at δ 8.20, arising from a phenolic group, a much greater effect was observed for the signal at δ 6.64 than for that at δ 7.32. This proves that the structure is **2**.



	R_1	R_2	R_3	R_4
1:	OH	H	OCH_3	H
2:	OCH_3	OH	OCH_3	OCH_3
3:	OCH_3	H	OH	H
4:	OCH_3	OCH_3	OCH_3	OH

5-Deoxyflavonoids are common in the family Leguminosae [11]. The oxygenation pattern in **1** is not new [12–14], but the combination 7-hydroxy-3,8,4′-trimethoxy has not previously been found in nature. The oxygenation pattern in the B ring of the structure **2** is common in natural flavonoids [15], but has not previously been found in combination with 7,8-dioxygenation.

The molluscicidal activity of **1** and **2** was tested against *B. glabrata*, but, due to shortage of material, at only one concentration. At 25 ppm, **1** was inactive, and 80% of the snails were killed in a 25 ppm solution of **2**, indicating only weak activity. The leaf extract of *P. clappertoniana* probably contains other molluscicidal compounds, but as there seems to be no significantly active major constituents, the potential use of this plant in schistosomiasis control is limited.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at 200 and 50.3 MHz, respectively, with $CDCl_3$ as solvent and TMS as int. standard. EIMS were recorded at 70 eV on a V670-250SE instrument. Mps are uncorr.

Plant material. The plant material was collected in Ipetumodu, Nigeria, in June 1990. Plant material was identified by Mr A. Adesakin, and herbarium specimens are deposited at the herbarium of the Faculty of Pharmacy, Obafemi Avolowo University, Ile-Ife, Nigeria.

Extraction and purification. Leaves of *P. clappertoniana* (390 g) were extracted with EtOH (3500 ml) for 72 hr at room temp. Evapn of solvent left 25.3 g, which was distributed between CH_2Cl_2 and H_2O. The H_2O phase was extracted with EtOAc. The residue from the CH_2Cl_2 phase was defatted by distribution between MeOH–H_2O (9:1) and heptane. Finally, the defatted residue was distributed between CH_2Cl_2 and MeOH–H_2O (3:2). The residues from the H_2O phase (13.8 g), EtOAc phase (1.1 g), CH_2Cl_2 phase (3.4 g) and MeOH–H_2O phase (1.0 g) showed molluscicidal activity at 400 ppm, while the heptane phase (5.3 g) was inactive at 400 ppm. The CH_2Cl_2 phase was fractionated by CC on silica gel eluted with CH_2Cl_2, to which was added EtOAc (2 → 50%), and molluscicidally active frs were further purified to give **1** (25 mg) and **2** (200 mg). On crystallization, 13 mg **1** and 70 mg **2** were obtained. The fractionation was monitored by TLC on silica gel with $CHCl_3$–MeOH (9:1) and detected by UV light.

7-Hydroxy-3,8,4′-trimethoxyflavone (**1**). Yellow crystals, mp 187.5–188°. IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1640, 1600 (C=O, arom., *o* and/or *p* to OH). UV λ_{max}^{MeOH} nm (log ε): 260 (4.01), 317 (4.02), sh, 335 (4.07). λ_{max}, on addition of NaOMe: 272, 309, 373 nm. λ_{max}, on addition of NaOAc: 272, 311, 358 nm. On addition of $AlCl_3$ no change of the UV spectrum. ^1H NMR (200 MHz, $CDCl_3$): δ 8.13 (*d*, $J = 9.1$ Hz, H-2′, H-6′), 7.93 (*d*, $J = 8.8$ Hz, H-5), 7.06 (*d*, $J = 8.8$ Hz, H-6), 7.05 (*d*, $J = 9.1$ Hz, H-3′, H-5′), 6.78 (OH), 4.11, 3.90, 3.88 (3 × OMe), lit. [9]. ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.6 (C-4), 161.5 (C-4′), 149.0 (C-2), 155.0, 153.2, 140.4 (C-7, C-8, C-9), 134.1 (C-3), 130.0 (C-2′, C-6′), 123.3 (C-1′), 121.5, 118.7, 113.7 (C-5, C-6, C-10), 114.1 (C-3′, C-5′), 61.9, 60.0, 55.4 (3 × OMe), lit. [15, 16]. EIMS (direct inlet) *m/z* (rel. int.): 328 $[M]^+$ (75), 327 $[M - H]^+$ (100), 312 (13), 270 (16), 167 (5) $[A_1 + H]^+$, 150 (7), 135 (22) B_2^+, 119 (11), lit. [9, 10].

2′-Hydroxy-3,7,8,4′,5′-pentamethoxyflavone (**2**). Yellow crystals, mp 183.5–184°. IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1600, 1620 (C=O, arom., *o* and/or *p* to OH). UV λ_{max}^{MeOH} nm (log ε): 248 (4.28), 307 (3.93), 347 (3.92). λ_{max}, on addition of NaOMe: 237, 305, 400 nm. No shifts were observed on addition of $AlCl_3$ and NaOAc. ^1H NMR (200 MHz, $CDCl_3$): δ 8.20 (OH), 8.02 (*d*, $J = 8.0$ Hz, H-5), 7.32 (*s*, H-6′), 7.08 (*d*, $J = 8.0$ Hz, H-6), 6.64 (*s*, H-3′), 4.05, 4.01, 3.95, 3.94, 3.90 (5 × OMe). ^{13}C NMR (50 MHz, $CDCl_3$): δ173.3 (C-4), 156.6, 155.6, 138.1 (C-7, C-8, C-9), 153.6 (C-4′), 151.3 (C-2′), 149.9 (C-2), 143.5 (C-5′), 136.7 (C-3), 121.1, 118.7, 110.1 (C-5, C-6, C-10), 110.8 (C-6′), 109.0 (C-1′), 102.8 (C-3′), 61.9, 61.5, 56.5, 56.3, 56.1 (5 × OCH_3), lit. [15, 16]. EIMS (direct inlet) *m/z* (rel. int.) 388 $[M]^+$ (76), 373 $[M - Me]^+$ (100), 371 (34) $[M - 17]^+$, 357 (64), 345 (17), 343 (11), 208 (4) B_1^+, 193 (9), 181 (10) $[A_1 + H]^+$ or B_2^+, lit [9, 10].

Determination of molluscicidal activity. The molluscicidal activity of **1** and **2** was assessed against *B. glabrata* snails according to ref. [17], modified according to ref. [18]. The number of snails, usually recommended to be 20 for each concn, was reduced to 5, due to shortage of the compounds. Stock solns of the compounds were prepd using DMSO as solvent. The resulting concn of DMSO was 0.5% (v/v) in all tests. Controls with the same DMSO concn were included. The molluscicidal activity of **1** and **2** were assessed at 25 ppm.

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