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PRENYLATED FLAVONOIDS FROM SEEDS OF *CALOPOGONIUM MUCUNOIDES*

MARÍLIA O. DA S. PEREIRA, EDNA C. FANTINE and JOSÉ R. DE SOUSA

Departamento de Química da Universidade Federal de Minas Gerais, 30000—Belo Horizonte, MG, Brazil

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Key Word Index—*Calopogonium mucunoides*; Leguminosae; seeds; prenylated isoflavone; pyranoflavanone; isoflavone.

Abstract—The seeds of *Calopogonium mucunoides* furnished 7-*O*-γ,γ-dimethylallyl-8-methoxy-3′,4′-dioxymethylene-isoflavone, 7-*O*-γ,γ-dimethylallyl-3′-hydroxy-4′-methoxyisoflavone, 7-*O*-γ,γ-dimethylallyl-3′,4′-dimethoxyisoflavone and 2*S*-di[6″,6″-dimethylpyrano(2″,3″:7,8; 2″,3″: 4′,3′)]-flavanone whose structures were established by spectroscopic means involving the use of 400 MHz ^{1}H NMR with double irradiation and INDOR techniques.

INTRODUCTION

Five isoflavones and three pyranoisoflavones have been isolated from seeds of *Calopogonium mucunoides* by Vilain and Jadot [1–3]. In the present study we describe the isolation and structural determination of three new isoflavones and a new pyranoflavanone from the seeds of the same plant grown in Brazil, none of them identical with those obtained by Vilain. However, the compounds isolated in each case have similar structural features, being mainly isoflavones but the Brazilian seeds contain compounds mainly with *O*-prenylation and no oxygenation at C-5 whereas the isoflavones isolated by Vilain have *C*-prenylation and some have C-5 oxygenation.

RESULTS AND DISCUSSION

CC on Si gel of the solid and oily material obtained from the concentrated C_6H_6 extract of *C. mucunoides* each gave, on elution with hexane containing increasing amounts of $CHCl_3$ and then $CHCl_3$ with increasing amounts of MeOH, two pure compounds together with other constituents not fully studied.

The first component eluted by CC of the solid material analysed for $C_{22}O_6H_{20}$, M^{+} *m/z* 380. Both the ^{1}H NMR and UV spectra were typical of 7-oxygenated isoflavones showing the H-2 proton at δ 7.8,*s*, and coupled resonances ($J = 9$ Hz) at δ 8.05 and 7.05 for the C-5 and C-6 protons, respectively. The UV spectrum exhibited two absorption maxima at 256 (*s*) and 295 (*w*) nm [4]. Complete 400 MHz ^{1}H NMR spectrum analysis revealed the presence of two hydrogens on ring A (H-5 and H-6, by use of double irradiation and INDOR techniques) and three hydrogens on ring B (H-2′, H-5′ and H-6′ by use of the same techniques), a dioxymethylene group (δ 6.0, *s*), a methonyl group (δ 4.0, *s*) and a *O*-prenylated group (δ 1.58, 1.62, 4.71 and 6.52). The MS showed fragments indicating that the prenyl group was on ring A, the oxygen at position 7 (*m/z* = 311, 100%) and the dioxymethylene on ring B (*m/z* = 146, 8%). From these data structure **1** was assigned to this compound and IR, UV, MS and ^{1}H NMR spectra were in complete agreement.

The second and more polar compound analysed for $C_{21}O_5H_{20}$, M^{+} *m/z* 352 and was also a 7-oxygenated isoflavone with one hydroxy (formation of a monoacetate) as shown by UV and ^{1}H NMR (δ 7.91, *s*, associated with H-2 and λ_{max} 265, strong, 296 nm, weak) [4]. The OH was located on C-3′ as indicated by its UV spectrum in the presence of NaOAc and NaOH and by the distinctive chemical shift of the acetate on H-6′ and H-2′. The 400 MHz ^{1}H NMR spectrum showed additionally three protons on ring A (H-5, H-6 and H-8 by double irradiation technique), three protons on ring B (H-2′, H-5′ and H-6′ by the same technique), one methoxyl (δ 3.92, *s*, 3 H) and one *O*-prenylated group (δ 1.79, 1.83, 4.62 and 5.52). The prenyl group was located on the oxygen at position 7 by MS (*m/z* = 284, 45%) and by analogy with **1**. From these data structure **2** was assigned to this compound and IR, UV, MS and ^{1}H NMR spectra were in accordance.

The first component eluted by CC of the oily material analysed for $C_{25}O_4H_{24}$, M^{+} *m/z* 388, was named xambioona. Both the 400 MHz ^{1}H NMR (δ 2.78, 3.02 and 5.36 respectively H-3 *cis* and H-3 *trans* to H-2, and H-2, by use of double irradiation technique and coupling constants) and UV spectra (λ_{max} 227, strong; 315, weak, and inflections at 255 and 268 nm) were typical of a flavanone. The 400 MHz ^{1}H NMR spectrum showed additionally a set of four one-proton doublets typical of the chromene ring system associated with ring A [1, 2, 5, 6] at 5.57 (H-5″, $J = 10$ Hz), 6.5 (H-4″, $J = 10$ Hz), 6.81 (H-6, $J = 8.5$ Hz) and 7.75 (H-5, $J = 8.5$ Hz); and a set of five one-proton doublets associated with a dimethylchromene ring involving ring B [7] at 5.67 (H-5‴, $J = 10$ Hz), 6.64 (H-4‴, $J = 10$ Hz), 6.34 (H-5′, $J = 9.0$ Hz), 7.08 (H-2′, $J = 2.5$ Hz), 7.21 (H-6′, *dd*, $J = 9.0$ and $J = 2.5$ Hz). The presence of two dimethylchromene rings, one on ring A and one on ring B, was supported by MS fragments (*m/z* 187 and 171). From these data structure **3** was assigned to xambioona. The absolute stereochemistry is 2*S* as illustrated because of the similarity of the CD curve in the n, π* and π,π* region to that of flavanones of established absolute configuration [8]. Xambioona was refluxed for 48 hr with base and gave the chalcone **4**. The IR, UV, MS and ^{1}H NMR spectra were in complete agreement with the structure **4**.

The second compound eluted by CC of the oily material analysed for $C_{22}O_5H_{22}$, $M^{\ddagger}$ m/z 366 and was a 7-oxygenated isoflavone as shown by UV and ^{1}H NMR (δ 7.80, associated with H-2 and $\lambda_{\max}$ 264, strong, and 292 weak [4]. Its M^+ was 14 mu higher than that of **2** and its UV spectrum was also almost identical to that of **2** except that it was not affected by NaOH. The ^{1}H NMR spectrum at 60 MHz showed two OMe groups (δ 3.78, *s*, 6 H), the absence of a –O–H proton (no change with D_2O) and the same proton aromatic proton pattern as that of **2**. From these data structure **5** was assigned to this compound.

EXPERIMENTAL

Seeds of *C. mucunoides* Desv. were collected from Xambioa region, north Goiás, Brazil. The whole, dried, finely pulverized seeds (1.1 kg) were successively extrd in a Soxhlet with C_6H_6 and EtOH. After evapn the C_6H_6 extract (96 g) afforded a yellow solid (5.6 g) in mixture with an oily material (90 g). The EtOH extract had free amino acids and was not examined in the present work. The solid C_6H_6 extract was submitted to Si gel CC and afforded isoflavones **1** and **2** eluted with $CHCl_3$–hexane (1:4) and MeOH–$CHCl_3$ (1:19), respectively. **1** after several purifications by TLC showed a single spot ($CHCl_3$–hexane, 2:3); 50 mg, white needles (hexane), mp 142°; $C_{22}O_6H_{20}$. (Found: C, 69.27; H, 5.5 requires: C, 69.5; H, 5.3 %.) $\nu_{\max}^{\text{KBr}}$ cm^{-1} 1640, 1610, 1595, 1560, 1500; $\lambda_{\max}^{\text{EtOH}}$ nm (ε) 229 (10 800), 256 (15 200) and 295 (9300) which remained unaffected on the addition of NaOH, HCl, NaOAc, $AlCl_3$; ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, TMS int. standard): 1.58 (3 H, *s*, γ-Me), 1.62 (3 H, *s*, γ-Me), 4.0 (3 H, *s*, Me-O), 4.71 (2 H, *d*, $J = 8$ Hz, α-CH_2), 5.52 (1 H, *t*, $J = 8$ Hz, β–CH=), 6.0 (2 H, *s*, –OCH_2O–), 6.88 (1 H, *d*, $J = 8$ Hz, H-5′), 6.99 (1 H, *dd*, $J = 8$ and 1.75 Hz, H-6′), 7.05 (1 H, *d*, $J = 9$ Hz, H-6), 7.10 (1 H, *d*, $J = 1.75$ Hz, H-2′), 7.98 (1 H, *s*, H-2), 8.05 (1 H, *d*, $J = 9$ Hz, H-5); MS m/z (%): 380 (1) ($M^{\ddagger}$, $C_{22}O_6H_{20}$), 379 (8), 311 (100), 296 (3), 267 (2), 266 (2), 265 (2), 146 (8), 69 (60).

2. Several purifications by TLC (MeOH–$CHCl_3$, 1:19) gave 100 mg **2**; pale yellow crystals, (hexane), mp 139°; $C_{21}O_5H_{20}$. (Found: C, 71.20; H, 5.5; requires: C, 71.8; H, 5.6 %.) $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3510, 1625, 1590, 1560, 1510; $\lambda_{\max}^{\text{EtOH}}$ nm (ε) 225 (13 000), 237 (21 500), 252 (11 900), 265 (11 800), 296 (9500) which remained unaffected on addition of HCl, NaOAc, $AlCl_3$, $\lambda_{\text{nm}}^{\text{EtOH + NaOH}}$ (ε): 240 (28 100) 280 (*infl.*, 16 800), 305 (16 500); ^{1}H NMR (400 MHz, $CDCl_3$, δ, TMS int. standard): 1.79 (3 H, *s*, γ-Me), 1.83 (3 H, *s*, γ-Me), 3.92 (3 H, *s*, Me-O), 4.62 (2 H, *d*, $J = 6.8$ Hz, α-CH_2), 5.52 (1 H, *tt*, $J = 6.8$ and 1.2 Hz, β–CH=), 5.72 (1 H, *s*, OH), 6.86 (1 H, *d*, $J = 2.5$ Hz, H-8), 6.93 (1 H, *d*, $J = 9$ Hz, H-5′), 7.02 (1 H, *dd*, $J = 8.8$ and 2.5 Hz, H-6), 7.13 (1 H, *dd*, $J = 9$ and 2 Hz, H-6′), 7.14 (1 H, *d*, $J = 2$ Hz, H-2′), 7.91 (1 H, *s*, H-2), 8.2 (1 H, *d*, $J = 8.8$ Hz, H-5); MS m/z (%): 352 (24) ($M^{\ddagger}$ $C_{21}O_5H_{20}$), 284 (45), 269 (8), 241 (3), 213 (1), 148 (7), 85 (75), 83 (100), 69 (65).

Acetate. White crystals, (hexane), mp 172°; ^{1}H NMR (400 MHz, $CDCl_3$, δ, TMS int. standard): 1.79 (3 H, *d*, $J = 1$ Hz, γ-Me), 1.83 (3 H, *d*, $J = 1$ Hz, γ-Me), 2.32 (3 H, *s*, Me–COO), 3.87 (3 H, *s*, MeO), 4.62 (2 H, *d*, $J = 7$ Hz, α-CH_2), 5.52 (1 H, *tq*, $J = 7$ and 1 Hz, β–CH=), 6.86 (1 H, *d*, $J = 2.5$ Hz, H-8), 7.0 (1 H, *dd*, $J = 9$ and 2.5 Hz, H-6), 7.03 (1 H, *d*, $J = 8.5$ Hz, H-5′), 7.32 (1 H, *d*, $J = 2.3$ Hz, H-2′), 7.44 (1 H, *dd*, $J = 8.5$ and 2.3 Hz, H-6′), 7.94 (1 H, *s*, H-2), 8.2 (1 H, *d*, $J = 9$ Hz, H-5). $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1760, 1630, 1600, 1580, 1515, 1270, 1210 cm^{-1}; $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 237 (8000), 252 (9600), 262 (10 000), 306 (4100) which remained unaffected on addition of NaOAc and $AlCl_3$; $\lambda_{\max}^{\text{EtOH + NaOH}}$ nm (ε): 247 (8300), 252 (8400), 280 (3500), 307 (5100); $\lambda_{\max}^{\text{EtOH + NaOH + HCl}}$ nm (ε): 227 (9600), 239 (8900), 252 (9500), 266 (9900), 295 (9200); MS m/z (%): 394 (5), 352 (8), 284 (55), 162 (8), 69 (100).

The oily benzene extract was submitted to Si gel CC and afforded the flavanone **3** and isoflavone **5**.

3. Several purifications by TLC ($CHCl_3$–C_6H_6, 3:7) gave 300 mg **3**; white crystals, (hexane), mp 138°; $C_{25}O_4H_{24}$. (Found: C, 77.5; H, 6.1; requires: C, 77.3; H, 6.18 %.) $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1683 1630, 1578, 1490; $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 230 (20 000), 255 (10 700), 268 (12 300), 315 (3900) which remained unaffected on the addition of NaOAc, $AlCl_3$; $\lambda_{\max}^{\text{EtOH + NaOH}}$ nm (ε): 235 (9700), 279 (8200), 360 (7800); $\lambda_{\max}^{\text{EtOH + NaOH + HCl}}$ nm (ε): 230 (11 000), 290 (9000); ^{1}H NMR (400 MHz, $CDCl_3$, δ, TMS int. standard): 1.54 (3 H, *s*, α–Me), 1.56 (6 H, *s*, α–Me), 1.58 (3 H, *s*, α–Me), 2.78 (1 H, *dd*, $J = 16.5$ and 2.8 Hz, H-3 *cis* to H_2), 3.02 (1 H, *dd*, $J = 16.5$ and 13 Hz, H-3 *trans* to H_2), 5.36 (1 H, *dd*, $J = 13$ and 2.8 Hz, H-2), 5.57 (1 H, *d*, $J = 10$ Hz, H-5″), 5.67 (1 H, *d*, $J = 10$ Hz, H-5‴), 6.34 (1 H, *d*, $J = 9$ Hz, H-5′), 6.5 (1 H, *dd*, $J = 10$ Hz and 0.8 Hz, H-4″), 6.64 (1 H, *dd*, $J = 10$ and 0.8 Hz, H-4‴), 6.8 (1 H, *d*, $J = 8.5$ Hz, H-6), 7.08 (1 H, *d*, $J = 2.5$ Hz, H-2′), 7.21 (1 H, *dd*, $J = 9$ and 2.5 Hz, H-6′), 7.75 (1 H, *d*, $J = 8.5$ Hz, H-5); CD curve (MeOH): $[\theta]_{343}$ + 10 230, $[\theta]_{307}$ – 25 600, $[\theta]_{282}$ – 10 500, $[\theta]_{268}$ – 20 400, $[\theta]_{244}$ + 4300, $[\theta]_{236}$ – 7600; MS m/z (%): 388 (23) ($M^{\ddagger}$, $C_{25}O_4H_{24}$), 373 (70), 187 (100), 171 (85), 57 (48), 43 (48), 41 (33).

4. Xambioona was refluxed with 10 % KOH for 48 hr, and extrd with EtOAc. The EtOAc extract was dried, concd and submitted to Si gel CC affording 17 mg chalcone **4**; yellow crystals, (hexane), mp 128°; $C_{25}O_4H_{24}$. (Found: C, 77.2; H, 6.2; requires: C, 77.3; H, 6.18 %.) $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1625, 1600, 1580, 1480; ^{1}H NMR (60 MHz, $CDCl_3$, δ, TMS int. standard): 1.4 (12 H, *s*, α-Me), 5.4–5.8 (2 H, *m*, H-5″ and H-5‴), 6.2–6.5 (2 H, *m*, H-4″ and H-4‴), 6.7–6.9 (2 H, $^{H}\!\diagdown\!\!=\!\!\diagdown_{H}$), 7.2–7.8 (5 H, *m*, aromatic Hs); $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 292 8700), 390 (12 800) which remained unaffected on the addition of $AlCl_3$, NaOAc; $\lambda_{\max}^{\text{EtOH + NaOH}}$ nm (ε): 283 (8700), 365 (7900), 435 (3100); MS m/z (%): 388 (6), 373 (18), 203 (5), 187 (100), 179 (25), 171 (32), 159 (7), 144 (6), 131 (7), 115 (8), 103 (6), 77 (6).

5. Several purifications by TLC (EtOAc–C_6H_6, 7:93) gave 15 mg **5**; white powder, (hexane), mp 112°; $C_{22}O_5H_{22}$. (Found: C, 72.3; H, 6.1; requires: C, 72.1; H, 6.0 %.) $\nu_{\max}^{\text{KBr}}$ 1638, 1623, 1600. 1562, 1438; $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 257 (16 000), 264 (16 100), 292 (9600) which remained unaffected on the addition of NaOH, HCl, NaOAc, $AlCl_3$; ^{1}H NMR (60 MHz, CCl_4, δ, TMS int. standard): 1.81 (6 H, *s*, γ-CH_3), 3.78 [6 H, *s* (*br*), O–Me], 4.55 [2 H, *d*, (*br*), $J = 7$ Hz, –CH_2–O–], 5.4 (1 H, *t*, $J = 7$ Hz, $^{H}\!\diagdown$C=C), 7.0 (5 H, *m*, H-8, H-5′, H-6, H-6′, H-2′), 7.8 (1 H, *s*, H-2), 8.0 (1 H, *d*, $J = 8$ Hz, H-5); MS m/z (%): 366 (10), 298 (30), 69 (100).

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