PERGAMON

Phytochemistry 52 (1999) 1705–1709

PHYTOCHEMISTRY

Prenylated flavones from *Neoraputia paraensis*☆

José Pio I. de Souza, Alberto C. Arruda*, Gaspar D. Muñoz, Mara S. P. Arruda, Adolfo H. Müller

Departamento de Química, Centro de Ciências Exatas e Naturais, Universidade Federal do Pará, Campus Universitário, 66075-970 Belem, Para, Brazil

Received 22 October 1998; received in revised form 2 March 1999

Abstract

Three new prenylated flavones were isolated from the aerial parts of *Neoraputia paraensis*. On the basis of spectral data these flavones were identified as 5,7,5′-trimethoxy-6-(3″-hydroxy,3″-methyl-*trans*-but-1″-enyl)-3′,4′-methylenedioxy-flavone; 5,4′-dihydroxy-3′,5′-dimethoxy-6,7-(2″,2″-dimethylpyran)flavone and 5,4′-dihydroxy-8,3′,5′-trimethoxy-6,7-(2″,2″-dimethylpyran)-flavone.

Keywords: *Neoraputia paraensis*; Rutaceae; Prenylated flavones

1. Introduction

The genus *Neoraputia* (Rutaceae), containing six species, was described in 1978 by Emmerich during the study of the tribe Cusparieae (Emmerich, 1978). Some species previously described in *Raputia*, like *R. paraensis* and *R. alba*, were moved to the new created genus *Neoraputia*. The species *Neoraputia alba* was chemically investigated and ten flavones and one flavanone were reported (Arruda, Vieira, Fernandes & Silva, 1993; Arruda et al., 1991). The main structural features of these flavonoids were the highly methoxylated substitution pattern and the presence in some structures of a 2,2-dimethylpyran moiety attached to the A-ring of the flavone skeleton. In a previous paper (Souza, Arruda & Arruda, 1995), we reported the isolation of seven flavones from *Neoraputia paraensis*, six highly methoxylated flavones and one pyranoflavone. In a continuation of our chemical investigation on this species, we now report the isolation and structural determination of three new prenylated flavones (**1**–**3**).

2. Results and discussion

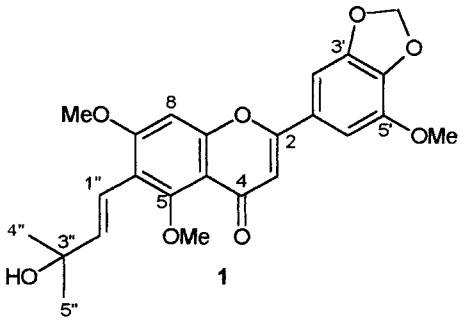
Stems and leaves of *N. paraensis* were percolated separately with hexane and dichloromethane, successively. The dichloromethane extract of leaves, when submitted to chromatography on silica gel columns, afforded compounds **1** and **2**. The dichloromethane extract of stems, using similar techniques, furnished compound **3**. These compounds (**1**–**3**) were identified as flavones mainly via the characteristic ^{13}C NMR signals of C-2, C-3 and C-4 (Table 1) (Markham & Chari, 1982), together with their UV spectra (see Experimental) (Markham & Mabry, 1982). Unlike **1**, compounds **2** and **3** have hydroxyl groups at C-5 positions (δ_H 13.05 and 12.75, respectively) supported by the lower field signals of carbon C-4 (δ_C 182.4 and 183.8, respectively).

Compound **1** was isolated as a colorless solid with a molecular ion peak at m/z 440 (EIMS) corresponding to the formula $C_{24}H_{24}O_8$. The ^1H NMR spectrum showed two doublets at δ_H 6.74 and 6.84 (1H each, J = 16.4 Hz) and a singlet at δ_H 1.41 (6H, a pair of magnetically equivalent methyl groups) which is consistent with a 3-hydroxy-3-methyl-*trans*-but-1-enyl moiety (Khalid & Waterman, 1981). The mass spectral fragments at m/z 381 $[M\text{-}C_3H_7O]^+$ and 59 $[C_3H_7O]^+$

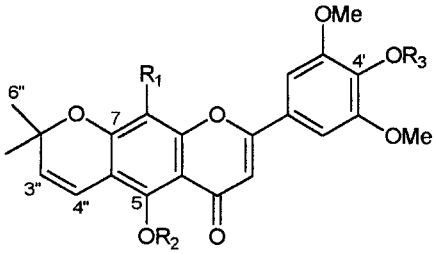
☆ Based in part on the M.Sc. dissertation that was presented by J.P.I.S. to the Universidade Federal do Pará, Belém, Pará, Brazil.

* Corresponding author.

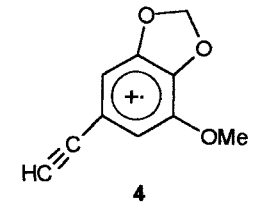
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00197-1



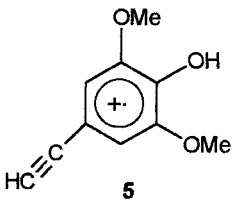
1



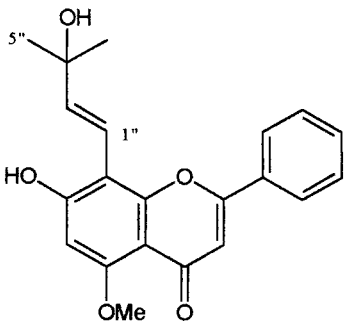
	R_1	R_2	R_3
2	H	H	H
2a	H	$COCH_3$	$COCH_3$
3	OMe	H	H
3a	OMe	$COCH_3$	$COCH_3$



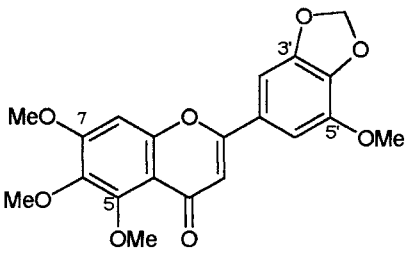
4



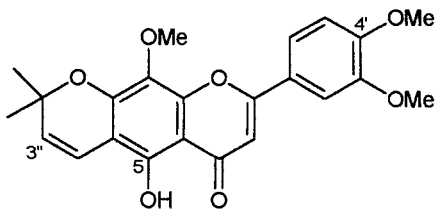
5



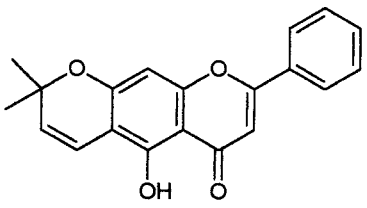
6



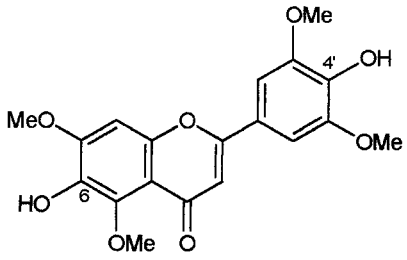
7



8



9



10

confirmed this group. Further examination of the ^{1}H NMR spectrum revealed the presence of one methylenedioxy group [δ_H 6.07 (*s*, 2H)], three methoxyls [δ_H 3.83, 3.97 and 4.00 (*s*, 3H each)] and an AB coupling system [δ_H 7.07 and 7.11 (J=1.3 Hz)]. The AB coupling system and fragment ion at *m/z* 176 (**4**), produced by C-ring RDA fission, indicated that the B-ring was substituted at 3′, 4′ and 5′ positions with a methylenedioxy and methoxyl group. The absence of a $[M\text{-}15]^+$ peak in the mass spectrum suggested that there was no methoxyl group attached to C-6 or C-8 on the A-ring (Mabry & Markham, 1975). Moreover, the signals observed at δ_C 96.8 and δ_H 6.80 are typical of a non-substituted C-8 A-ring (Markham & Chari, 1982; Markham & Mabry, 1982), therefore the 3-hydroxy-3-methyl-*trans*-but-1-enyl substituent was assigned to C-6 and the remaining methoxyls at C-5 and C-7. This was confirmed by the presence of a methoxyl signal at δ_C 61.1 (OMe-5), which is characteristic of an *ortho*-disubstituted methoxyl group (Panichpol & Waterman,

Table 1
^{13}C NMR spectral data for **1** and **2** (100 MHz, in $CDCl_3$), **3** (75 MHz, in CD_3OD) and model compounds **6**, **7**, **8** and **9**

C	1	2	3	6[a]	7[b]	8[b]	9[c]	10[c]
2	161.8	163.8	166.0					
3	107.6	104.5	108.9					
4	175.8	182.4	183.8			182.3	181.8	
5	157.2	156.4	150.7			151.5	155.3	
6	117.6	105.4	105.6			105.7	104.7	
7	159.8	159.5	152.4			152.1	158.1	
8	96.8	95.1	129.8			128.3	94.8	
9	159.8	157.0	150.3			149.1	156.3	
10	111.9	105.7	105.3			104.7	104.7	
1′	125.4	122.4	120.0		125.8			120.4
2′	107.0	103.5	103.3		106.5			104.2
3′	143.7	147.4	153.4		143.7			148.1
4′	149.4	138.5	145.0		149.4			139.7
5′	138.0	147.4	153.4		140.3			148.1
6′	102.4	103.5	103.3		102.2			104.2
1″	144.5			143.7				
2″	114.3	78.0	79.4	114.7		78.0	77.8	
3″	69.9	128.1	129.5	70.4		128.0	128.8	
4″	30.3	115.5	116.3	30.2		115.5	114.2	
5″	30.3	28.3	28.4	30.2		28.0	27.7	
6″		28.3	28.4			28.0	27.7	
OMe	56.7	56.5	62.1		56.8	61.4		
OMe	56.9	56.5	58.8		56.2			
OMe	61.1		58.8					
OCH_2O	100.4				100.3			

[a] Data from Khalid and Waterman (1981) (DMSO-d_6 as solvent).
[b] Data from Arruda et al. (1993) ($CDCl_3$ as solvent).
[c] Data from Agrawal and Rastogi (1981) ($CDCl_3$ as solvent).

1978). Finally, the ^{13}C NMR spectral data supported the proposed structure by comparison with values for related compounds **6** and **7** (Khalid & Waterman, 1981; Arruda et al., 1993). From these spectral data, **1** was identified as 5,7,5′-trimethoxy-6-(3″-hydroxy,3″-methyl-*trans*-but-1″-enyl)-3′,4′-methylenedioxy-flavone.

NMR, UV and EIMS data of compounds **2** and **3**, obtained as yellow prisms and yellow pellets respectively, suggested very similar structures, with compound **3** having an additional methoxyl group {$[M]^+$ *m*/*z* 396 (**2**) and 426 (**3**)}. UV showed bands at λ_{max} 359 nm (**2**) and 357 nm (**3**), typical of flavones, the bathochromic shifts (54 and 55 nm, respectively) induced by 2 M NaOH solution, indicating a 4′-OH (Mabry, Markham & Thomas, 1970). The symmetrical B-ring system for both compounds, with methoxyl groups at C-3′ and C-5′ [δ_H 4.00 (*s*, 6H) for **2** and 3.99 (*s*, 6H) for **3**], was confirmed by the ^1H NMR spectra showing a 2H singlet peak at δ_H 7.09 (**2**) and 7.19 (**3**) which corresponded to the H-2′/6′. This was supported by the C-ring RDA fragment ion at *m*/*z* 178 (**5**) in its mass spectrum. The presence of a 2,2-dimethylpyran ring was deduced from the 6H singlet at δ_H 1.47 (**2**) and 1.60 (**3**) due to the *gem*-dimethyl group and two doublets (J = 10.0 Hz) at δ_H 5.62 and 6.71 for (**2**) and 5.64 and 6.74 for (**3**), corresponding to the two *cis*-coupled olefinic protons. The 2,2-dimethylpyran ring at 6- and 7-positions on A-ring was deduced on the basis of ^1H NMR spectra of the diacetylated derivatives **2a** and **3a** which exhibited upfield shift for H-4″ (0.21 and 0.23 ppm, respectively) and downfield shift for H-3″ (0.15 and 0.16 ppm, respectively). The acetylation of the 5-OH group is observed to cause a marked diamagnetic shift ($\Delta\delta_{acetyl} = +0.25$ to $+0.4$) of the *peri*-H (H-4″) and a small paramagnetic shift ($\Delta\delta_{acetyl} = -0.1$ to -0.15) of H-3″, attributed to steric effects, while the acetylation of an angular system (2,2-dimethylpyran ring at 7- and 8-positions) does not show any appreciable effect on H-4″ (Arnone, Cardillo, Merlini & Mondelli, 1967). Therefore, the remaining signals in the ^1H NMR spectrum of **2** δ_H 6.54 and 6.44 (*s*, 1H each) were attributed to H-3 and H-8, respectively. The absence of a singlet around δ_H 6.4 in the ^1H NMR spectrum of **3** was indicative of the presence of a methoxyl group [δ_H 3.95 (*s*, 3H) and δ_C 62.1] at C-8. The ^{13}C NMR spectral data for **2** and **3** were assigned by comparison with data from literature (**8**–**10**) (Agrawal & Rastogi, 1981; Arruda et al., 1993). From the above data, the structures **2** and **3** were characterized as 5,4′-dihydroxy-3′,5′-dimethoxy-6,7-(2″,2″-dimethylpyran)flavone and 5,4′-dihydroxy-8,3′,5′-trimethoxy-6,7-(2″,2″-dimethylpyran)flavone, respectively.

3. Experimental

3.1. General

Mps uncorr. UV: MeOH; IR: KBr discs; EIMS: 70 eV, Finnigan-INCOS-X; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz): Bruker-ARX 400, $CDCl_3$ with TMS as int. standard; ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz): Varian-GEMINI-300, $CDCl_3$ and CD_3OD, δ 7.26 for ^1H and δ 48.7 for ^{13}C as int. references; TLC: Silica gel 60H (Merck 7736); CC: Silica gel (Merck 7734).

3.2. Plant material

Leaves and stems of *Neoraputia paraensis* (Ducke) Emmerich were collected in Paragominas, State of Pará, Brazil in December 1991 and identified by Dr. Elisabeth van den Berg. A voucher specimen (code number 8822) is deposited in the herbarium of the Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.

3.3. Extraction and isolation

The dried and powdered leaves (1.53 kg) of *N. paraensis* were successively extracted with hexane, CH_2Cl_2 and MeOH at room temp. A part of the residue (10

g), obtained after evaporation of the CH_2Cl_2 extract (38 g), was subjected to silica gel CC and mixts of hexane–CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2–MeOH of increasing polarity were used as eluents affording 40 frs. Fr. 14 eluted with CH_2Cl_2 was obtained as amorphous yellow solid. This was purified by prep. TLC (CH_2Cl_2–MeOH, 99:1) and after recrystallization (MeOH–CH_2Cl_2) yielded **2** (45 mg). Frs 26–28 eluted with a mixt. of CH_2Cl_2–MeOH were washed with MeOH to afford **1** (29 mg) after recrystallization (CH_2Cl_2–MeOH).

The dried and pulverized stems (2 kg) were treated in the same manner as described above for the leaves. A part of the residue (10 g), obtained after evaporation. of the CH_2Cl_2 extract (18 g), was chromatographed over silica gel, eluted first with hexane followed by hexane–CH_2Cl_2, CH_2Cl_2, CH_2Cl_2–EtOH mixts. Frs 32–33 eluted with a mixt. of CH_2Cl_2–EtOH were submitted to a selective dissolution with hexane–CH_2Cl_2 7:3 and the remaining solid was purified by recrystallization (CH_2Cl_2–MeOH) yielding **3** (15 mg).

*3.4. 5,7,5′-Trimethoxy-6-(3″-hydroxy,3″-methyl-trans-but-1″-enyl)-3′,4′-methylenedioxy-flavone (**1**)*

Colorless solid, mp 200–202°C (MeOH–CH_2Cl_2); UV λ_{max}^{MeOH} nm (log ϵ): 342 (4.50), 253 sh (4.49), 235 (4.59); IR ν_{max}^{KBr} cm^{-1}: 3400, 1640, 1590, 1510, 1460, 1200, 1080; ^1H NMR (400 MHz, $CDCl_3$): δ 6.54 (1H, *s*, H-3), 6.80 (1H, *s*, H-8), 7.07 (1H, *d*, $J=1.3$, H-2′), 7.11 (1H, *d*, $J=1.3$, H-6′), 6.74 (1H, *d*, $J=16.4$, H-1″), 6.84 (1H, *d*, $J=16.4$, H-2″), 6.07 (2H, *s*, OCH_2O), 3.83, 3.97 and 4.00 (3H each, *s*, 5-OMe, 7-OMe and 5′-OMe), 1.41 (6H, *s*, 3″-Me_2); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 440 [M^+] (2), 381 [M-59]$^+$ (23), 351 (15), 264 (9), 217 (10), 176 (8), 59 (3), 57 (100).

*3.5. 5,4′-dihydroxy-3′,5′-dimethoxy-6,7-(2″,2″-dimethylpyran)flavone (**2**)*

Yellow prisms, mp 228–229°C (MeOH–CH_2Cl_2); UV λ_{max}^{MeOH} nm (log ϵ): 359 (4.45), 310 sh (4.27), 297 (4.33), 242 (4.48); IR ν_{max}^{KBr} cm^{-1}: 3430, 1640, 1580, 1500, 1460, 1200, 1080; ^1H NMR (400 MHz, $CDCl_3$): δ 6.54 (1H, *s*, H-3), 6.44 (1H, *s*, H-8), 7.09 (2H, *s*, H-2′/6′), 5.62 (1H, *d*, $J=10.0$, H-3″), 6.71 (1H, *d*, $J=10.0$, H-4″), 4.00 (6H, *s*, 3′/5′-OMe), 1.47 (6H, *s*, 2″-Me_2), 6.00 (1H, *s*, 4′-OH), 13.05 (1H, *s*, 5-OH); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 396 [M^+] (30), 381 [M-15]$^+$ (100), 365 (15), 203 (24), 181 (3), 178 (3), 69 (29).

*3.6. Diacetylated derivative **2a***

Acetylation (Ac_2O-pyridine 1:1, room temp.) of **2** (7 mg) furnished, after work-up, **2a** (6 mg). ^1H NMR (400 MHz, $CDCl_3$): δ 6.65 (1 H, *s*, H-3), 6.84 (1H, *s*, H-8), 7.05 (2H, *s*, H-2′/6′), 5.77 (1H, *d*, $J=10.0$, H-3″), 6.50 (1H, *d*, $J=10.0$, H-4″), 3.90 (6H, *s*, 3′/5′-OMe), 1.49 (6H, *s*, 2″-Me_2), 2.37 and 2.48 (3H each, *s*, $2\times CH_3CO$).

*3.7. 5,4′-dihydroxy-8,3′,5′-trimethoxy-6,7-(2″,2″-dimethylpyran)flavone (**3**)*

Yellow pellets, mp 191–192°C (MeOH–CH_2Cl_2); UV λ_{max}^{MeOH} nm (log ϵ): 357 (4.34), 324 sh (4.29), 304 (4.33), 239 (4.38); IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1580, 1510, 1460, 1210, 1040; ^1H NMR (300 MHz, $CDCl_3$): δ 6.57 (1H, *s*, H-3), 7.19 (2H, *s*, H-2′/6′), 5.64 (1H, *d*, $J=10.0$, H-3″), 6.74 (1H, *d*, $J=10.0$, H-4″), 3.99 (6H, *s*, 3′/5′-OMe), 3.94 (3H, *s*, 8-OMe), 1.53 (6H, *s*, 2″-Me_2), 12.72 (1H, *s*, 5-OH); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 426 [M^+] (79), 411 [M-15]$^+$ (100), 396 (17), 381 (13), 365 (24), 181 (19), 178 (5), 69 (50).

*3.8. Diacetylated derivative **3a***

Acetylation (Ac_2O-pyridine 1:1, room temp.) of **3** (6 mg) furnished, after work-up, **3a** (6 mg). ^1H NMR (300 MHz, $CDCl_3$): δ 6.56 (1H, *s*, H-3), 7.15 (2H, *s*, H-2′/6′), 5.80 (1H, *d*, $J=10.0$, H-3″), 6.51 (1H, *d*, $J=10.0$, H-4″), 3.98 (3H, *s*, 8-OMe), 3.90 (6H, *s*, 3′/5′-OMe), 1.55 (6H, *s*, 2″-Me_2), 2.37 and 2.46 (3H each, *s*, $2\times CH_3CO$).

Acknowledgements

The authors are grateful to the Department of Chemistry UFSCar, São Carlos, Brazil for NMR spectra (400 and 100 MHz). We also thank to Emílio Goeldi Museum, Pará, Brazil for the plant identification and MS spectra and CNPq and CAPES Brazilian agencies for financial support.

References

Agrawal, P. X., & Rastogi, R. P. (1981). *Heterocycles*, *16*, 2181.

Arnone, A., Cardillo, G., Merlini, L., & Mondelli, R. (1967). *Tetrahedron Letters*, *43*, 4201.

Arruda, A. C., Vieira, P. C., Fernandes, J. B., & Silva, M. F. G. F. (1993). *Journal of the Brazilian Chemical Society*, *4*, 80.

Arruda, A. C., Vieira, P. C., Fernandes, J. B., Silva, M. F. G. F., Francisco, R. H. P., Rodrigues, A. M. G. D., & Lechart, J. R. (1991). *Phytochemistry*, *30*, 3157.

Emmerich, M. (1978). *Rodriguesia*, *45*, 233.

Khalid, S. A., & Waterman, P. G. (1981). *Phytochemistry*, *20*, 1719.

Mabry, T. J., & Markham, K. R. (1975). Mass spectrometry of flavonoids. In J. B. Harborne, K. R. Mabry, & H. Mabry, *The flavonoids* (p. 88). New York: Academic Press (Part 1).

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). *The systematic identification of flavonoids*. Berlin: Springer.

Markham, K. R., & Chari, V. M. (1982). Carbon-13 NMR spectroscopy of flavonoids. In J. B. Harborne, & T. J. Mabry, *The flavonoids: advances in research* (p. 19). London: Chapman and Hall.

Markham, K. R., & Mabry, T. J. (1975). Ultraviolet–visible and proton magnetic resonance spectroscopy of flavonoids. In J. B. Harborne, K. R. Mabry, & H. Mabry, *The flavonoids* (p. 45). New York: Academic Press (Part 1).

Panichpol, K., & Waterman, P. G. (1978). *Phytochemistry*, *17*, 1363.

Souza, J. P. I., Arruda, A. C., & Arruda, M. S. P. (1995). *Fitoterapia*, *LXVI*, 465.