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Short Report

Luxenchalcone, a New Bichalcone and other Constituents from *Luxemburgia octandra*

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O fracionamento cromatográfico dos extratos de folhas e galhos de *Luxemburgia octandra* (Ochnaceae) resultou no isolamento de: isoliquiritigenina, 3'-hidroxiisoliquiritigenina, β-sitosterol, estigmasterol, lupeol, ácido betulínico, o biflavonóide, 4',5,7-triidroxiflavona-(3'→O→4'")-5",7"-diidroxiflavanona e uma nova bichalcona, 4,2',4'-triidroxichalcona-(3→O→4")-2'",4'"-diidroxichalcona (luxenchalcona). As estruturas desses compostos foram estabelecidas com base na análise dos dados espectrométricos de IV, EM e RMN incluindo experimentos 2D das substâncias naturais e do derivado metilado da luxenchalcona.

Chromatographic fractionation of leaves and branches extracts from *Luxemburgia octandra* (Ochnaceae) afforded isoliquiritigenin, 3'-hydroxyisoliquiritigenin, β-sitosterol, stigmasterol, lupeol, betulinic acid, the biflavonoid, 4',5,7-trihydroxyflavone-(3'→O→4'")-5",7"-dihydroxyflavanone and a new bichalcone 4,2',4'-trihydroxychalcone-(3→O→4")-2'",4'"-dihydroxychalcone (luxenchalcone). The structures were established from analysis of IR, MS and NMR spectral data, including 2D-NMR experiments of the natural substances and of the luxenchalcone methyl derivative.

Keywords: *Luxemburgia octandra*, Ochnaceae, flavonoids, bichalcone, terpenoids

Introduction

Luxemburgia octandra St. Hil (Ochnaceae) is a shrub or small tree distributed throughout the Brazilian Southeastern region. Ochnaceae is a large tropical family including *ca.* 40 genera and 600 species with the greatest concentration in tropical South America.[1] In the course of our phytochemical and pharmacological investigations of Brazilian plants, we have studied species of both *Ouratea* and *Luxemburgia* genera, in which the presence of biflavonoids was observed[2-5] along with their antitumoral activities.[6,7] The biflavonoid 4',5,7-trihydroxyflavone-(3'→O→4'")-5",7"-dihydroxyflavanone was isolated from *Luxemburia noilis*[4] and from *L. octandra* (present work) have shown antitumoral activities.[6] No previous work has been reported on *L. octandra* species.

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Experimental

General procedures

Melting points are uncorrected. IR spectra were recorded on Perkin-Elmer 1600/1605 FT-IR spectrophotometer using KBr disks or NaCl film. NMR spectra in D_3CCOCD_3, CD_3OD or $CDCl_3$ were recorded on Bruker (200 MHz for ^1H and 50.3 MHz for ^{13}C) and on Jeol JNM-GX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometers using tetramethylsilane (TMS) as internal standard. Electron Ionization Mass Spectra (EIMS) were taken with Gas Chromatography coupled to a Mass Spectrometry (GC/MS) on Varian Saturn 2000 and high resolution electron spray ionization mass spectra (HRESIMS) was obtained with a VG 7070E – HF spectrometer. CC: silica gel (Merck and Aldrich 0.05-0.20 mm); TLC: silica gel H or G (Merck and Aldrich) was used to analyze the fractions collected from column chromatography (CC) with visualization by UV (254 and 366 nm), $AlCl_3$-EtOH (1%) and exposure to iodine vapor.

Plant material

Luxemburgia octandra St. Hil was collected in Morro de São Sebastião, Ouro Preto, Minas Gerais, Brazil and authenticated by the botanist Jorge L. Silva. A voucher specimen (Nº 26197) is deposited at the OUPR-UFOP Herbarium, Instituto de Ciências Exatas e Biológicas of the Universidade Federal de Ouro Preto-MG, Brazil.

Extraction and isolation

Dried leaves (621g) and branches (1.865 Kg) were powdered and extracted with hexane and ethyl acetate by maceration at room temperature. The extracts were concentrated under vacuum to yield the residues A (6.1 g), and B (10.4 g) from the leaves, C (4.8 g) and D (20.7 g) from the branches, respectively, with hexane and ethyl acetate. The residues B and D were dissolved in H_2O/CH_3OH (1:1) and extracted with hexane/diethyl ether (1:1). These solvents were removed under vacuum and the hexane/diethyl ether fractions (B-1, 0.8 g) and (D-1, 3.5 g) were successively filtered on sephadex LH-20 column using methanol as mobile phase. The collected fractions of B-1 (28 fractions, 50 ml) and D-1 (25 fractions, 50 ml) were analyzed by silica gel TLC. Fractions 15-17 of the B-1 gave biflavonoid 4',5,7-trihydroxy-flavone-(3'→*O*→4''')-5'',7''-dihydroxyflavanone [**2**, 40 mg, m.p. 220 ºC, $[a]_D^{20}$ + 1.8 (Me_2CO, c = 0.33)]; fractions 20-24 yielded a mixture of chalcones isoliquiritigenin (**3**) and 3'-hydroxy-isoliquiritigenin (**4**) (20 mg, gum). The fractions collected from the column of D-1 have impure compound **1** which were crystallized in acetone/ethyl acetate do yield pure compound **1** (yellow crystals, 300 mg, mp 172 ºC). Product **1a** (yellow crystals, 50 mg, mp 168 ºC) was obtained by treating a methanol solution of **1** (50 mg) with ethereal diazomethane. The residue C was dissolved in methanol and successively submitted to column chromatography of sephadex LH-20 using methanol as mobile phase. Fraction C-10/14 yielded steroids mixture, sitosterol and stigmasterol (50 mg); fractions C-18/19 and C-22/27 gave the triterpene lupeol (20 mg, mp 202 ºC) and betulinic acid (30 mg, mp 295 ºC), respectively.

*4,2',4'-Trihydroxychalcone-(3→O→4'')-2''',4'''-dihydroxychalcone (luxenchalcone), **(1)***

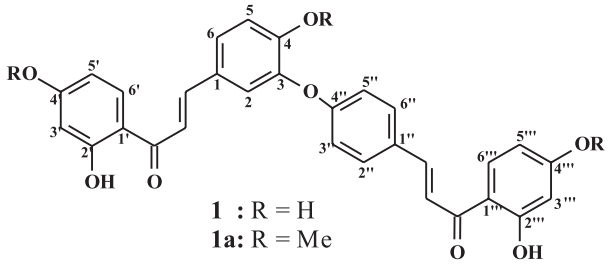
IR (KBr) ν_{max}/cm^{-1}: 3497, 1630, 1510, 1432, 1289, 1225, 974, 837, 802. ^{1}H NMR (D_3CCOCD_3, 200 MHz) and ^{13}C NMR (D_3CCOCD_3, 50.3 MHz) (Table 1). HRESIMS/MS: *m/z* (rel. int.) 510.0584 [M$^{+\bullet}$,15 (calcd 510.131467 for $C_{30}H_{22}O_8$)], 399.0673 (10), 373.0878 (100), 355.0829 (23), 135.0125 (53).

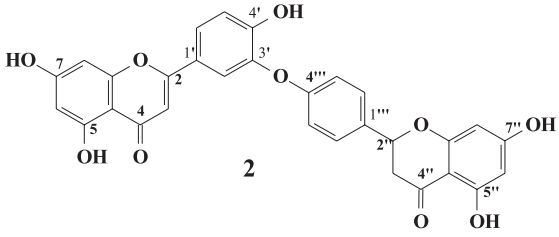
*4,4'-Dimethoxy-2'-hydroxychalcone-(3→O→4'')-2'''-hydroxy-4'''-methoxychalcone (luxenchalconetrimethylether), **(1a)***

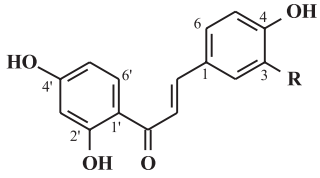
IR (KBr) ν_{max}/cm^{-1}: 3410, 2923, 2851, 1638, 1562, 1508, 1461, 1361, 1270, 1227, 1124, 972, 842. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$ 100 MHz) (Table 1). EIMS *m/z* (rel. int.) 552 (M$^+$, 33% ,$C_{33}H_{28}O_8$), 401 (65), 151 (100).

Results and Discussion

The known natural compounds β-sitosterol, stigmasterol,[10,11] lupeol,[12] betulinic acid,[13] isoliquiritigenin (**3**),[8] 3-hydroxy-isoliquiritigenin (**4**)[9] and 4',5,7-trihydroxy-flavone-(3'→O→4''')-5'',7''-dihydroxy-flavanone (**2**)[4,14] were identified mainly by ^{1}H and ^{13}C-NMR spectral analyses besides comparison with literature data.[4,5,8-14]







Compound **1** gave a positive test with $AlCl_3$-EtOH reagent, which confirmed the presence of a flavonoid. The IR spectrum showed bands at 3497 (ν_{O-H}), 1630 ($\nu_{C=O}$) and 1510, 1432 cm^{-1} attributed to aromatic ring. The 1D and 2D ^{1}H NMR spectra showed signals of three ABC systems, δ 6.34 (d, 1H), 6.36 (d, 1H), 6.46 (dd, 2H), 8.10 (d, 2H) and 7.09 (d, 1H), 7.57 (dd, 1H) and 7.69 (d, 1H) and signals of

Table 1. ^{1}H and ^{13}C-NMR spectral data (δ ppm) of compound **1** [$(CD_3)_2CO$, 200 and 50,3 MHz] and of its methyl derivative **1a** ($CDCl_3$, 400 and 100 MHz)

C	**1**		**1a**	
	δ_H, m, J (Hz)	δ_C	δ_H, m, J (Hz)	δ_C
1	-	128.4	-	128.4
2	7.69 (d, 2.3)	122.6	7.43 (d, 2.2)	121.1
3	-	144.0	-	144.0
4	-	152.8	-	153.8
5	7.09 (d, 8.3)	118.2	7.06 (d, 8.4)	112.9
6	7.57 (dd, 8.3; 2.3)	128.4	7.49 (dd, 8.4; 2.2)	127.6
1'	-	114.2	-	114.1
2'	-	165.6	-	166.2
3'	6.36 (d, 2.7)	103.7	6.47 (m)	101.1
4'	-	167.3	-	166.6
5'	6.46 (dd, 8.7; 2.7)	108.8	6.48 (dd, 9.0; 2.2)	107.7
6'	8.10 (d, 8.7)	130.9	7.81 (d, 9.0)	131.1
1"	-	129.8	-	129.3
2"	7.84 (d, 8.7)	129.8	7.62 (d, 8.4)	130.3
3"	7.00 (d, 8.7)	117.2	6.97 (d, 8.4)	116.8
4"	-	160.9	-	160.1
5"	7.00 (d, 8.7)	117.2	6.97 (d, 8.4)	116.8
6"	7.84 (d, 8.7)	129.8	7.62 (d, 8.4)	130.3
1"'	-	114.2	-	114.0
2"'	-	165.7	-	166.2
3"'	6.34 (d, 2.7)	103.7	6.47 (m)	101.1
4"'	-	167.3	-	166.6
5"'	6.46 (dd, 8.7; 2.7)	108.8	6.46 (dd, 9.0; 2.2)	107.7
6"'	8.10 (d, 8.7)	131.5	7.77 (d, 9.0)	131.1
H-α	7.85 (m)	117.2	7.42 (d, 15.4)	118.8
H-α'	7.85 (m)	117.2	7.49 (d, 15.4)	119,0
H-β	7.83 (m)	143.2	7.81 (d, 15.4)	143.2
H-β'	7.83 (m)	143.2	7.86 (d, 15.4)	143.8
HO	13.58 (brs)	-	13.49, 13.45 (brs)	-
C=O	-	192.4	-	191.7,191.5
MeO-4', 4"'	-	-	3.86, 3.85 (s)	55.5 x 2
MeO-4	-	-	3.88 (s)	56.1

AA'BB' at δ 7.00 (d, 2H) and 7.84 (d, 2H) of four aromatic rings. The additional signal at δ 13.58 (s, 2H) of two chelated hydroxyls and a multiplet at δ 7.83 and 7.85 for hydrogens connected at a and β carbons [(δ_{CH} 117.2 and 143.2 as observed in the ^{1}H,^{13}C-COSY ($^{1}J_{CH}$)] spectra, besides the $\delta_{C=O}$ at 192.4, allowed to propose the chalcone structure (Table 1). These data and ^{13}C NMR (PND and DEPT) and ^{1}H, ^{13}C-COSY ($^{n}J_{CH}$ n = 1,2,3) spectra analysis were in agreement with two chalcones reported in the literature, isoliquiritigenin (**3**)[8] and 3-hydroxy-isoliquiritigenin (**4**).[9] Treatment of **1** with diazomethane yielded **1a** with three methoxy groups, besides two chelated hydroxy groups (Table 1). These information allowed us to propose the structure of a new bichalcone 4,2',4',2"',4"'-pentahydroxy-3→*O*→4"-bichalcone (**1**, named as luxenchalcone) (Table 1). The C-3-*O*-C-4" connection between the chalcone moieties was confirmed by a NOESY and NOEDIFF experiment of the derivative **1a**. Irradiation at the methoxy groups showed NOE at δ 7.06 (d, 8.4 Hz, H-5), and at signals near δ 6.40 (H-3', H-5', H-3"', H-5"'). These experiments did not show NOE at δ 7.43 (d, H-2) and 6.97 (d, H-3", H-5"). This result indicates that the positions 3 and 4" are not substituted with OCH_3 groups. The 2D experiments [^{1}H,^{1}H-COSY, ^{1}H,^{13}C-COSY (HETCOR and COLOC of **1** and HMQC and HMBC of **1a**)] were used to confirm the ^{1}H and ^{13}C chemical shifts of **1** and **1a** (Table 1). The cross peaks observed in the HMBC spectra of **1a** show long-range couplings of C-4 (δ_C 153.8) with OCH_3 (δ 3.88), with H-2 and with H-6 ($^{3}J_{CH}$) and also of C-3 (δ_C 144.0) with H-2 ($^{2}J_{CH}$) and H-5 ($^{3}J_{CH}$) which were used to confirm the C-3-*O*-C-4" connection. The peaks at *m/z* 552 ($M^{+\bullet}$, 33%, $C_{33}H_{28}O_8$) in the LREIMS of **1a** and the *m/z* 510.0584 ($M^{+\bullet}$, 15%; $C_{30}H_{22}O_8$ requires 510.13147) in the HRESIMS of **1** were also used to confirm the proposed structure of this new bichalcone.

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References

1. Heywood, V. H.; *Flowering Plants of the World*, Oxford University Press: London, 1978, p. 82.
2. Moreira, I. C.; Sobrinho, D.C.; de Carvalho, M.G.; Braz-Filho, R.; *Phytochemistry* **1994**, *25*, 1567.
3. Moreira, I. C; Bastos, A. B.; de Carvalho, M. G.; Braz-Filho, R.; *Phytochemistry* **1999**, *51*, 833.
4. de Oliveira, M. C. C.; de Carvalho, M. G.; Silva, C. J. da; Werle, A. A.; *J. Braz. Chem. Soc*. **2002**, *13*, 119.
5. Velandia, J. R.; Carvalho, M. G. de; Braz-Filho, R.; Werle, A. A.; *Phytochem. Anal*. **2002**, *13*, 283.
6. de Carvalho, M. G.; Grynberg, N. F.; Echevarria, A. E.; de Oliveira, M. C. C. In *Recent Progress in Medicinal Plants*; Govil, J. N. ed.; Centre Res. Books (Org.): New Dehli, 2001, vol. 8, p. 77.
7. Velandia, J. R.; de Carvalho, M. G.; Grynberg, N. F.; de Oliveira, M. C. C.; Moreira, I. C.; Braz-Filho, R.; Echevarria, A. E.; *Braz. J. Med. Biol. Res*. **2002**, *35*, 819.
8. Achenbach, H.; Stocher, M.; Constenia, M. A.; *Phytochemistry* **1988**, *27*, 1835.
9. Linuma, M.; Mizuno, M.; *Phytochemistry* **1989**, *28*, 681.
10. Chaurasia, N.; Wichtl, M.; *J. Nat. Prod*. **1987**, *50*, 881.
11. Dutra, N. N.; Alves, H. de M.; de Carvalho, M. G.; Braz-Filho, R.; *Quim. Nova* **1992**, *15*, 10.
12. Sobrinho, D. C.; Hauptli, M. B.; Appolinário, E. V.; Kollenz, C. L. M.; de Carvalho, M. G.; Braz-Filho, R.; *J. Braz. Chem. Soc*. **1991**, *2*, 15.
13. Bilia, A. R.; Mendez, J.; Morelli, I.; *Pharm. Acta Helv*. **1996**, *71*, 191.
14. Geiger, H.; Quinn, C. *Biflavonoids*. In *The Flavonoids: Advances in Research*; Harborne, J. B., ed., Chapman & Hall: London, 1982, p. 505.
15. Touchstone, J. C.; Dobbins, M. F.; *Practice of Thin Layer Chromatography*, John Wiley & Sons Inc.: New York, 1983, p. 192.

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