Pergamon

Phytochemistry, Vol. 49, No. 5, pp. 1339–1342, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

PII: S0031-9422(98)00067-3

NON-POLAR CONSTITUENTS FROM LEAVES OF *PIPER LHOTZKYANUM*

Davyson de L. Moreira*†, Elsie F. Guimarães‡ and Maria Auxiliadora C. Kaplan†

†Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, CEP:21941-590, Brazil; ‡Botânica Sistemática, Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil

(*Received* 10 *November* 1997)

Key Word Index—*Piper lhotzkyanum*; Piperaceae; leaves; chromene; benzoic acid derivatives.

Abstract—The hexane extract from leaves of *P. lhotzkyanum* yielded lhotzchromene (2-methyl-2-[4′-methyl-3′-pentenyl]-2H-1-benzopyran-6-carboxylic acid) together with the known (*E*) and (*Z*) isomers of 4-hydroxy-3-(3′,7′-dimethyl-1′-oxo)-2′,6′-octadienylbenzoic acid and a mixture of six hydroxylated sesquiterpenes along with phytol. Spectroscopic methods were used to identify these compounds. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Piper lhotzkyanum Kunth (syn. *P. inversum* C.DC., *P. aromaticum* C.DC., *P. damazii* C.DC., *P. bennetianum* C.DC., *P. santa-barbaranum* C.DC.) belongs to the family Piperaceae and it is commonly found in humid forests with low incidence of light in Southeast Brazil [1, 2]. Recently, many chromenes and benzoic acid derivatives have been isolated and identified in Piperaceae species. These kind of compounds have shown interesting insecticidal properties [3–10]. The hexane extract from leaves of *P. lhotzkyanum* was purified by chromatographic methods resulting in isolation of a new chromene: lhotzchromene (**1**); two known prenylated benzoic acid derivatives isomers, previously isolated from leaves of *P. murrayanum*: (*E*) and (*Z*) of 4-hydroxy-3-(3′,7′-dimethyl-1′-oxo)-2′,6′-octadienylbenzoic acid (**2**, **3**) [8]; and a mixture of hydroxylated sesquiterpenes: spathulenol, guaiol, *epi*-γ-eudesmol, hinesol, β-eudesmol, α-eudesmol along with the acyclic diterpene phytol (Table 2). The isolated substances were identified using spectroscopic analysis. The mixture was analysed by GC/MS and the substances were identified by comparison of the mass spectra and retention indices (RI) with literature records [11, 12]. ^{1}H NMR and IR analyses were also used to confirm the major substances in this mixture.

RESULTS AND DISCUSSION

The EI mass spectrum of compound **1** showed the molecular ion at m/z 272 which is in agreement with the molecular formula $C_{17}H_{20}O_3$. The ^{1}H NMR spectrum (Table 1) showed a set of three coupled aromatic protons (δ 6.80, 7.74 and 7.88, $J_{a,b} = 8.0$ Hz, $J_{b,c} = 2.0$ Hz), indicating a 1,3,4-trisubstituted benzene ring; two coupled protons in an AB system (δ 5.62 and 6.42, $J_{a,b} = 10.0$ Hz) suggesting a *cis*-olefine; a singlet at δ 1.44, corresponding to a methyl group attached to an oxygen-bearing carbon. The mass spectral fragmentation pattern showed a base peak at m/z 189 $[C_{11}H_9O_3]+$ and a signal due to a fragment at m/z 257 [M-15]+ that results from a loss of a methyl group (Scheme 1). These data are expected for a 2-methyl substituted chromenoic acid nucleus. The ^{1}H NMR spectrum also showed signals of an olefinic proton at δ 5.10 (1H, *m*), of four coupled aliphatic protons at δ 1.80 (2H, *t*, 7.4; 2H) and 2.10 (2H, *m*) besides two methyl groups at δ 1.58 and 1.68 (6H, *s*). The data suggested a 4′-methyl-3′-pentenyl substitution in the position C-2 of the chromene nucleus. The ^{13}C NMR spectral assignments of **1** were in agreement with the chromene proposed and confirmed the presence of an acid carbonyl (δ 171.67). Comparative phytochemistry using piperochromenoic acid [6] as a model, indicated the position C-6 for the carboxyl group location Table 1.

EXPERIMENTAL

General

UV: MeOH sols.; IR: film; EIMS 70 eV; ^{1}H NMR (200 MHz) and ^{13}C NMR (50.2 MHz): $CDCl_3$ using TMS as int. standard.

---

* Author to whom correspondence should be addressed. E-mail: moreiradl@nppn.ufrj.br;.

Table 1. Non-polar terpenes from leaves of *P. lhotzkyanum*.

Compound	RT*	RI**	(%)	Identification
Spathulenol	26.74	1572	7.66	MS, RI
Guaiol	28.79	1626	11.39	MS, RI
epi-γ-Eudesmol	28.89	1628	6.23	MS, RI
Hinesol	29.11	1634	3.51	MS, RI
β-Eudesmol	29.69	1649	22.59	MS, RI, IR, ^{1}HNMR
α-Eudesmol	29.83	1653	17.09	MS, RI, IR, ^{1}HNMR
Phytol	45.56	1990	20.82	MS, RI, IR, ^{1}HNMR

* RT = Retention Time measure in minutes.

** RI = Retention Indices obtained from a homologous hydrocarbons series within C_8 to C_{24}.

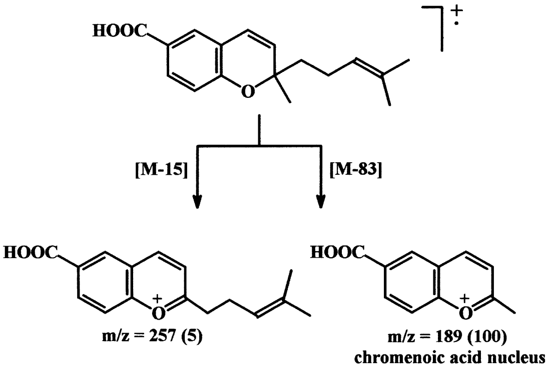
Table 2. NMR data for compound **1** compared to NMR data of piperochromenoic acid **A** [6], ($CDCl_3$, *J* values in Hz in parentheses).

Position	**1** ^{13}C	 ^{1}H	**A** ^{13}C	 ^{1}H*
2	79.90		80.1	
3	129.73	5.62 *d* (10.0)	130.0	5.61 *d* (10.0)
4	122.02	6.42 *d* (10.0)	122.2	6.42 *d* (10.0)
4a	120.35		120.5	
5	128.60	7.74 *d* (2.0)	128.8	7.72 *d* (2.0)
6	121.30		121.4	
7	131.73	7.88 *dd* (8.0, 2.0)	131.9	7.87 *dd* (8.0, 2.0)
8	115.84	6.80 *d* (8.0)	116.1	6.79 *d* (8.0)
8a	158.17		158.4	
1′	41.57	1.80 *t* (7.4)	41.8	
2′	22.51	2.10 *m*	22.5	
3′	123.62	5.10 *m*	123.6	5.11 *m*
4′	131.73		135.5	
5′	25.45	1.68 *s*	25.7	
6′	17.42	1.58 *s*	17.7	
7′	26.99		27.2	
8′			131.3	
9′			25.7	
10′			17.7	
11′			16.0	
1″	26.99	1.44 *s*	27.2	1.43 *s*
COOH	171.67	9.20 *bs*	171.8	

* Main ^{1}H NMR signals of piperochromenoic acid [6].

Separation

Silica gel (Merck); GC: was performed using a Varian Star 3400 gas chromatograph, fused silica capillary column (DB-5, 30 m × 0.20 mm), hydrogen as carrier gas and temp. programming from 60°C to 270°C (3°C min^{-1}); GC-MS: was performed using a HP5890 SII gas chromatograph coupled to a VG Autospect mass spectrometer at 70 eV, fused silica capillary column (DB-1, 30 m × 0.20 mm), helium as carrier gas and temp. programming from 60°C to 270°C (3°C min^{-1}). Retention Indices: were obtained



Scheme 1. Mass spectral fragmentation of compound **1**.

from a series of linear hydrocarbons within C8 to C24, performed in the same condition of GC analyses.

Plant material

Leaves of *P. lhotzkyanum* were collected near Terezopolis, Rio de Janeiro State, Brazil, in September 1996. Voucher samples (RB 323487) are deposited in the Herbarium of Rio de Janeiro Botanical Garden, Rio de Janeiro, Brazil.

Extraction and isolation

Dried and powdered leaves of *P. lhotzkyanum* (280 g) were submitted to successively extraction with hexane and MeOH at room temp. The extracts were evaporated to dryness under red. pres. The hexane extract (7.5 g) was fractionated on a silica gel column eluted with hexane-EtOAc and EtOAc-MeOH gradients. A fraction eluted with pure hexane afforded the mixture of terpenes (240 mg) which were analysed and identified by GC/MS Table 2. Compounds **1** (90 mg), **2** (220 mg) and **3** (65 mg) were eluted from the silica gel column with a mixture of hexane-EtOAc and were purified by recrystalization in CH$_2$Cl$_2$.

Compound

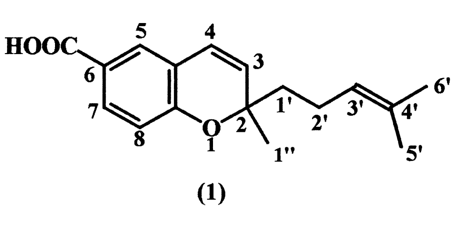
1. Brown amorphous solid (hexane-EtOAc). IR $\upsilon^{film}_{max} cm^{-1}$: 3400, 1720, 1690, 1610, 1270, 1300. UV λ^{MeOH}_{max} nm (log ϵ): 245 (3.98), 278 (0.81), 318 (0.47). EIMS *m/z* (rel. int.): 272 [M]+ (8), 257 [M-15]+ (5), 189 [M-83]+ (100), 115 (7), 105 (5), 91 (5), 69 (10). ^{1}H (200 MHz) and ^{13}C (50.2 MHz) NMR: Table 1.

Compound

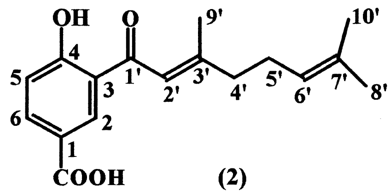
2. Plates (hexane-EtOAc). IR, UV and EIMS, in agreement with lit. values [8]. ^{1}H NMR (200 MHz, CDCl$_3$): δ 1.69 (3H, *s*, H-10′), 1.76 (3H, *s*, H-8′), 2.28 (3H, *d*, $J = 1.0$ Hz, H-9′), 2.29 (2H, *m*, H-5′), 2.38 (2H, *t*, $J = 7.0$ Hz, H-4′), 5.15 (1H, *m*, H-6′), 6.90 (1H, *bs*, H-2′), 7.06 (1H, *d*, $J_{a,b} = 8.0$ Hz, H-5), 8.20 (1H, *dd*, $J_{a,b} = 8.0$ Hz, $J_{b,c} = 2.0$ Hz, H-6), 8.60 (1H, *d*, $J_{b,c} = 2.0$ Hz, H-2), 9.20 (1H, *bs*, COO*H*), 13.45 (1H, *s*, O*H*-4). ^{13}C NMR (50.2 MHz, CDCl$_3$): δ 17.60 (C-10′), 20.20 (C-9′), 25.48 (C-8′), 26.00 (C-5′), 41.75 (C-4′), 118.47 (C-2′), 118.64 (C-5), 119.44 (C-3), 119.93 (C-1), 122.55 (C-6′), 132.00 (C-7′), 132.80 (C-2), 136.81 (C-6), 163.76 (C-3′), 167.55 (C-4), 171.07 (*C*OOH), 195.45 (C-1′).

Compound

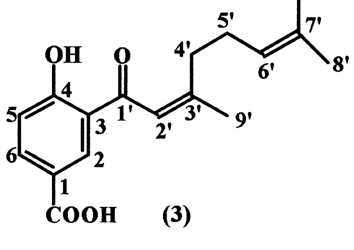
3. Plates (hexane-EtOAc). IR, UV and EIMS, in agreement with lit. values [8]. ^{1}H NMR (200 MHz, CDCl$_3$): δ 1.68 (3H, *s*, H-10′), 1.66 (3H, *s*, H-8′), 2.10 (3H, *d*, $J = 1.0$ Hz, H-9′), 2.24 (2H, *m*, H-5′), 2.66 (2H, *t*, $J = 7.0$ Hz, H-4′), 5.15 (1H, *m*, H-6′), 6.88 (1H, *bs*, H-2′), 7.06 (1H, *d*, $J_{a,b} = 8.0$ Hz, H-5), 8.20 (1H, *dd*, $J_{a,b} = 8.0$ Hz, $J_{b,c} = 2.0$ Hz, H-6), 8.60 (1H, *d*,



(1)



(2)



(3)

$J_{b,c} = 2.0$ Hz, H-2), 13.45 (1H, *s*, O*H*-4). ^{13}C NMR (50.2 MHz, CDCl$_3$): δ 17.45 (C-10′), 25.49 (C-9′), 26.23 (C-8′), 26.69 (C-5′), 34.58 (C-4′), 118.66 (C-5), 119.23 (C-2′), 119.55 (C-3), 119.93 (C-1), 123.22 (C-6′), 132.53 (C-7′), 132.91 (C-2), 136.81 (C-6), 163.93 (C-3′), 167.55 (C-4), 170.70 (*C*OOH), 195.18 (C-1′).

Acknowledgements—We thank CNPq for the financial support.

REFERENCES

1. Yunker, T. G., *Hohenia*, 1972, **2,** 147.
2. Guimarães, E. F., Mautone, L., Magalhães, H. G., Guimarães, L. A., *Daphne*, 1992, **2**(3), 7.
3. Filho, A. M. O., Pinchin, R., Santos, C. E., *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1980, **74**(4), 545.
4. Burke, B., Nair, M., *Phytochemistry*, 1986, **25,** 1427.
5. Diaz, P. P., Arias, T., Joseph-Nathan, P., *Phytochemistry*, 1987, **26**(3), 809.
6. Amporfo, S. A., Roussis, V., Wiemer, D. F., *Phytochemistry*, 1987, **26**(8), 2367.
7. Orjala, J., Erdelmeier, C. A. J., Wright, A. D., Rali, T., Sticher, O., *Phytochemistry*, 1993, **34,** 813.
8. Seeram, N. P., Jacobs, H., McLean, S., Reynolds, W. F., *Phytochemistry*, 1996, **43**(4), 863.
9. Pereda-Miranda, R., Bernard, C., Durst, T., Arnason, J. T., Sanches-Vindas, P., Poveda, L., Romàn, L. S., *J. Nat. Prod.*, 1997, **60**(3), 282.
10. Moreira, D. L., Guimarães, E. F. and Kaplan, M. A. C. A Chromene from *P. aduncum* L., *Phytochemistry*, 1998, **48**(6), 1075.
11. McLafferty, F. W. and Stanffer, D. B., *Registry of Mass Spectral Data*, Vol I–II, Willey-Interscience Pub., New York, 1989.
12. Adams, R. P., *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, Allured Pub. Co., Carol Stream, USA, 1995.