Pergamon

0031-9422(95)00564-1

Phytochemistry, Vol. 41, No. 2, pp. 647–649, 1996

0031-9422/96 $15.00 + 0.00

ALKALOIDS AND COUMARINS FROM *ESENBECKIA* SPECIES

FERNANDO M. OLIVEIRA, ANTÔNIO EUZÉBIO G. SANT'ANA, LUCIA M. CONSERVA,* JOSÉ GUILHERME S. MAIA† and GISELLE M. P. GUILHON‡

Departamento de Química, Universidade Federal de Alagoas, 57072 – 970 Maceió/AL, Brazil; †Departamento de Ecologia, Museu Paraense Emílio Goeldi, 66060 – 060 Belém/PA, Brazil; ‡Departamento de Química, Universidade Federal do Pará 66060 – 060 Belém/PA, Brazil

(*Received in revised form* 14 *June* 1995)

Key Word Index—*Esenbeckia almawillia*; *E. grandiflora*; Rutaceae; trunk bark; roots; quinolin-2-one; 2-arylquinolin-4-one; furoquinolines; furocoumarins; 3-(1′,1′-dimethylallyl)dihydrofurocoumarins.

Abstract—Investigation of two species of *Esenbeckia*, *E. almawillia* and *E. grandiflora*, has led to the isolation and identification of kokusaginine, maculine, maculosidine, flindersiamine, xanthotoxin, pimpinellin, 4-methoxy-1-methylquinolin-2-one, two new 2-arylquinolin-4-one alkaloids and two 3-(1′,1′-dimethylallyl)dihydrofurocoumarins derivatives. Structures of all compounds were elucidated by spectroscopic methods.

INTRODUCTION

Esenbeckia is a genus of *ca* 30 species, native to tropical America [1]. Species of this genus are known as a source of a variety of typical rutaceous secondary metabolites [1–8].

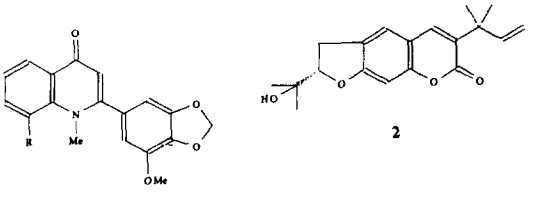
Recently, we have described from the hexane extract of the trunk bark of *E. almawillia*, the isolation and identification of 2-alkylquinolin-4-one alkaloids, together with simple cinnamic acid derivatives and isopimpinellin [9]. In the present study, we describe, from the $CHCl_3$ extract of the same material, the isolation of two new 2-arylquinolin-4-one alkaloids, **1a** and **1b**, and chalepin **2**, which have not been previously reported from *Esenbeckia* species, besides flindersiamine and maculosidine. Isolation of these interesting groups of compounds stimulated an investigation into the $CHCl_3$ extract of the roots of *E. grandiflora* which afforded three known furoquinoline, kokusaginine, maculine and flindersiamine, two furocoumarins, xanthotoxin and pimpinellin, a 4-methoxy-1-methylquinolin-2-one, isolated for the first time from this genus, and 3-(1′,1′-dimethylallyl)columbianetin, **3**, previously known as a synthetic compound [10] but a new natural product.

RESULTS AND DISCUSSION

The four known furoquinoline alkaloids [11] and 4-methoxy-1-methylquinolin-2-one [12] were identified by spectral properties and comparison with those of the corresponding compounds recorded in the literature. The positions for the methoxyl groups were established from mass spectral and ^{1}H NMR evidence.

The structures of xanthotoxin [13] and pimpinellin [14] were deduced on the basis of their spectral data, which were confirmed by comparative analysis with literature values, in addition to mmp and co-TLC with authentic samples.

The new compounds **1a** and **1b** had spectroscopic characteristics of other alkaloids of the 2-arylquinolin-4-one series. The presence of a quinolin-4-one carbonyl group was evidenced by IR absorption (**1a** 1630; **1b** 1624 cm^{-1}) and downfield ^{13}C and ^{1}H NMR signals [**1a** δ_H: 8.49 *dd*, $J = 8$; 1.5 Hz; δ_C: 177.63; **1b** δ_H 8.03 (*dd*, 8; 1.4 Hz; δ_C: 178.01)] (Table 1). In addition, the NMR spectra showed signals for an olefinic proton [**1a** δ_H: 6.30



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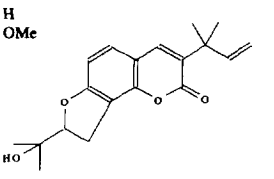
R
1a H
1b OMe



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*Author to whom correspondence should be addressed.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1a** and **1b**

H	1a	1b	C	1a	1b
3	6.30	6.36	2	154.27	150.77
	s	*s*	3	112.68	112.21
5	8.49	8.03	4	177.63	178.01
	dd(8; 1.5)	*dd*(8; 1.4)	4a	127.02	126.64
6	7.42	7.34	5	126.88	118.07
	dt(8; 0.9)	*t*(8)	6	123.68	124.29
7	7.71	7.17	7	132.33	113.65
	dt(8; 1.5)	*dd*(8; 1.4)	8	115.91	157.56
8	7.54	—	8a	142.06	135.39
	br d(8)		1′	130.02	130.25
2′	6.57	6.72	2′	103.04	103.17
	d(1.5)	*d*(1.5)	3′	149.19	149.07
6′	6.52	6.74	4′	136.46	136.69
	d(1.5)	*d*(1.5)	5′	143.83	143.08
NMe	3.64	3.65	6′	109.15	108.82
	s	*s*	NMe	37.26	44.29
OCH_2O	6.07	6.08	OCH_2O	102.02	102.04
	s	*s*	MeO-3′	56.95	56.78
MeO-8	—	3.98	MeO-8	—	56.08
		s			
MeO-3′	3.93	3.96	—	—	—
	s	*s*			

^{1}H (300 MHz, $CDCl_3$); ^{13}C (**1a**: 75 MHz, $CDCl_3$; **1b**: 50.3 MHz, $CDCl_3$). Chemical shifts (δ) expressed in ppm from internal TMS, coupling constants (J) in Hz.

Table 2. ^{1}H and ^{13}C NMR data for compounds **2** and **3**

H	2	3	C	2	3
4	7.45	7.44	2	162.2 C	162.8
	s	*s*	3	130.8 C	130.9
5	7.17	7.16	4	138.0 CH	138.2
	s	*d*(8)	4a	113.1 C	113.2*
6	—	6.65	5	123.2 CH	128.4
		d(8)	6	124.5 C	106.3
8	6.68	—	7	160.2 C	159.8
	s		8	97.1 CH	113.5*
2′	6.14	6.09	8a	154.6 C	150.4
	dd(18; 10)	*dd*(17.6; 10)	1′	40.3 C	40.4
3′	5.07–5.0	5.12–5.03	2′	145.6 CH	145.6
	m	*m*	3′	112.1 CH_2	112.1
2″	4.69	4.77	2″	90.8 CH	91.2
	t(8.6)	*t*(9)	3″	29.6 CH_2	27.6
3″	3.18	3.30	4″	71.7 C	71.8
	br d(8.6)	*dd*(9; 3)	Me-1′	26.1 CH_3	26.1
Me-1′	1.44	1.44	Me-4″	26.1 CH_3	28.2
	s	*s*		24.2 CH_3	23.8
Me-4″	1.34, 1.20	1.29, 1.16	—	—	—
	s	*s*			

^{1}H (200 MHz, $CDCl_3$); ^{13}C (50.3 MHz, $CDCl_3$); *may be interchanged. Chemical shifts (δ) expressed in ppm from internal TMS, coupling constants (J) in Hz.

(*s*, 1H); δ_C: 112.68; **1b** δ_H: 6.36 (*s*, 1H); δ_C: 112.21], an *N*-methyl group [**1a** δ_H: 3.64 (*s*, 3H); δ_C: 37.26; **1b** δ_H: 3.65 (*s*, 3H); δ_C: 44.29)] and a 3-methoxy-4,5-methylenedioxyphenyl group [**1a** δ_H: 6.57 (*d*, J = 1.5 Hz, H-2′, 1H), 6.59 (*d*, J = 1.5 Hz, H-6′, 1H); δ_C: 103.04 (C-2′), 109.15 (C-6′); **1b** δ_H: 6.72 (*d*, J = 1.5 Hz, H-2′, 1H), 6.74 (*d*, J = 1.5 Hz, H-6′, 1H); δ_C: 103.17 (C-2′), 108.82 (C-6′)]. The additional 30 mu which characterize **1b** (*m/z*) 339 $[M]^+$, 100) in relation to **1a** (*m/z* 309 $[M]^+$, 100) in the low resolution mass spectra, must be associated with a methoxyl group at C-8. This assumption was evidenced by a diamagnetic shift ($\Delta\delta$–0.46 ppm) of the H-5 signal when compared with **1a** and by the presence of signals at δ7.34 (1H, *t*, J = 8 Hz) and δ7.17 (1H, *dd*, J = 8 and 1.4 Hz) due to H-6 and H-7, respectively. All these assignments were based on the application of usual shift parameters and comparison with literature data [17].

Spectral comparison between **2** and **3** showed that they were coumarins. Characteristic coumarin pyrone doublets in the ^{1}H NMR spectra (Table 2) of both compounds were replaced by the H-4 singlet at about the usual value for this proton [**2** δ 7.45 (*s*, 1H); **3** δ 7.44 (*s*, 1H)]. This locates the position of the 1′,1′-dimethylallyl group at C-3. The main difference observed between the two was in a 6,7-disubstituted coumarin nucleus, as evidenced by two aromatic proton singlets [δ7.17 (*s*, H-5, 1H); δ6.78 (*s*, H-6, 1H)] in the case of **2**, replaced in **3** by a7,8-disubstituted one [δ7.16 (*d*, 8 Hz, H-5, 1H); δ6.65 (*d*, 8 Hz, H-6, 1H)]. Additionally, all spectroscopic data, except for the previously unreported DEPT-135 for **2** and ^{13}C NMR for the compound **3** (Table 2), are in agreement with those described for chalepin [15, 16] and for synthetic 3-(1′,1′-dimethylallyl)columbianetin [10].

EXPERIMENTAL

General. Mp: uncorr. IR: KBr pellets. ^{1}H and ^{13}C NMR were recorded in $CDCl_3$ solns, using TMS as int. standard, employing a Varian FM-360 (60 MHz), Bruker AC-200 (^{1}H, 200 MHz; ^{13}C, 50.3 MHz) and Varian Gemini 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz). Low resolution MS were obtained at 70 eV. TLC was carried out on silica gel (PF_{254}).

Plant material. Collection and identification of *E. almawillia* are reported in [9]. Roots of *E. grandiflora* were collected at the Municipality of Marechal Deodoro, Alagoas State. The specimen was identified by Rosangela P. L. Lemos and a voucher (Number MAC 8426) is deposited in the Herbarium of the Instituto do Meio Ambiente, Maceió-AL, Brazil.

Isolation of constituents from trunk bark of E. almawillia. After drying, trunk bark was reduced to powder (1.2 kg) and extracted in a Soxhlet apparatus successively with *n*-hexane and EtOH. Solvents were evapd and the EtOH residue (77 g) obtained was dissolved in MeOH-H_2O (3:2) and extracted with $CHCl_3$ and EtOAc. The $CHCl_3$ extract (3 g) was chromatographed on a silica gel column using *n*-hexane containing increasing amounts of EtOAc. The less polar frs after repeated silica gel CC and recrystallization from Me_2CO and *n*-hexane-EtOAc yielded, respectively, flindersiamine (8 mg) and maculosidine (9 mg) and by prep. TLC on silica gel (*n*-hexane-EtOAc,

4:1) gave **2** (15 mg). More polar frs were purificated by recrystallization from EtOAc and MeOH yielding **1a** (28 mg) and **1b** (10 mg), respectively.

Isolation of constituents from roots of E. grandiflora. Ground roots (1.7 kg) were extracted in a Soxhlet apparatus with EtOH. The EtOH residue (72 g) was dissolved in MeOH-H_2O (3:2) and extracted sucessively with *n*-hexane, $CHCl_3$ and EtOAc. Solvents were evapd and the $CHCl_3$ extract (20 g) was chromatographed on a silica gel column. Elution with *n*-hexane-EtOAc mixts of gradually increasing polarities gave frs A (0.13 g), B (1.4 g), C (0.8 g) and D (2.24 g). Fr. A was recrystallized from EtOH yielding pimpinellin (80 mg). Fr. B was chromatographed on a silica gel column and elution with benzene-EtOAc (97:3) afforded **3** (70 mg), besides pimpinellin (15 mg) and xanthotoxin (45 mg). Fr. C after successively recrystallized from EtOH yielding kokusaginine (70 mg). Fr. D was submitted to silica gel CC using benzene-EtOAc (19:1). After repeated recrystallization from MeOH and EtOH maculine (25 mg) and flindersiamine (5 mg) were obtained. Rechromatography (silica gel, benzene–EtOAc, 7:3) gave 4-methoxy-1-methylquinolin-2-one (12 mg).

2-3-*methoxy*-4,5-*methylenedioxyphenyl*-1-*methylquinolin*-4-*one* (**1a**). Amorphous solid, mp 193–194° (EtOAc). IR (KBr) cm^{-1}: 2921, 2852, 1630, 1598, 1577, 1494, 1360, 1257, 1101, 930, 865, 839, 823. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): Table 1. MS *m/z* (rel int.): 309 $[M]^+$ (100), 281 (58), 250 (2), 238 (10), 222 (2), 208 (3), 180 (6), 141 (13).

8-*methoxy*-2-(3′-*methoxy*-4,5-*methylenedioxyphenyl*)-1-*methylquinolin*-4-*one* (**1b**). Amorphous solid, mp 201–203° (MeOH). IR (KBr) cm^{-1}: 2968, 2847, 1624, 1598, 1571, 1429, 1346, 1267, 1100, 971, 894, 844. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 1. MS *m/z* (rel. int.): 339 $[M]^+$ (100), 311 (58), 281 (11), 266 (43), 238 (12), 223 (43), 206 (8), 180 (6), 170 (37).

Chalepin (**2**). Crystals, mp 122–123° (*n*-hexane-EtOAc) [15] 118–119°. IR (KBr) cm^{-1}: 3450, 1712, 1622, 1578, 1495, 1372, 1240, 1120, 980, 860, 815. ^1H NMR (200 MHz, $CDCl_3$) and ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. MS *m/z* (rel. int.): 314 $[M]^+$ (90), 299 (100), 281 (30), 271 (26), 255 (56), 241 (25), 227 (20), 213 (33), 199 (40), 128 (30), 59 (81).

3-(1′.1′-*dimethylallyl*)*columbianetin* (**3**). Oil $[\alpha]^{21}$ + 52.9° ($CHCl_3$; ¸0.44). IR (KBr) cm^{-1}: 3433, 1715, 1617, 1487, 1464, 1378, 1358, 1247, 1073, 963, 923, 785, 757. ^1H NMR (200 MHz, $CDCl_3$) and ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. MS *m/z* (rel. int.): 314 $[M]^+$ (100), 299 (65), 281 (25), 271 (8), 255 (68), 241 (42), 227 (33), 213 (27), 199 (41), 59 (62).

Acknowledgements—This work was supported by CNPq and FAPEAL. The authors are grateful to the Instituto de Química, Universidade de São Paulo and Universidade Federal do Ceará for acquisition of NMR spectra, and Dr Domingos Sávio Nunes, Universidade Federal do Pará, for providing a sample of 5-methoxypsoralen.

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