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ALKALOIDS AND COUMARINS FROM *TICOREA LONGIFLORA**

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Key Word Index—*Ticorea longiflora*; Rutaceae; stem; leaves; coumarins; anthranilate alkaloids.

Abstract—A new dihydropyranocoumarin, (−)-6-methoxy-*trans*-khellactone, has been isolated from the stems of *Ticorea longiflora*, in addition to the known coumarins, osthol, *O*-methylcedrelopsin and braylin, the anthranilate alkaloids, skimmianine, γ-fagarine, dictamnine, evolitrine and 4-methoxy-*N*-methyl-2-quinolone, and trimethoxyphloroglucinol. From the leaves, we have isolated the coumarin, braylin, the furoquinoline alkaloids, skimmianine, evolitrine and γ-fagarine, and the steroids, stigmasterol and β-sitosterol. The structures of the isolated compounds were elucidated on the basis of spectral data.

INTRODUCTION

The genus *Ticorea* belongs to the family Rutaceae, sub-family Rutoideae and the tribe Cusparieae [1]. Only one species from this genus, *T. pedicellata*, collected in French Guyana, has been studied before, where, from the leaves, compounds of the paracotoin-type were reported [2].

The present work, part of our phytochemical and chemotaxonomic study on members of the Cusparieae of the Amazon region, describes the isolation and structural elucidation of steroids, anthranilate alkaloids, coumarins and a phloroglucinol derivative from *T. longiflora*. During the present investigation, a new dihydropyranocoumarin (**2**) was isolated.

RESULTS AND DISCUSSION

Stems and leaves of *T. longiflora* were percolated with hexane, chloroform and methanol, successively. From the hexane extract of the leaves, we isolated the coumarin, braylin (**1**) [3], the furoquinoline alkaloids, evolitrine (**8**) [4], γ-fagarine (**9**) [5] and skimmianine (**10**) [6], and a mixture of stigmasterol and β-sitosterol. From the hexane and chloroform extracts of the stems, we isolated the coumarins, braylin, (−)-6-methoxy-*trans*-khellactone (**2**), osthol (**5**) [7] and *O*-methylcedrelopsin (**6**) [8], the anthranilate alkaloids, dictamnine (**7**) [4], evolitrine, γ-fagarine, skimmianine and 4-methoxy-*N*-methyl-2-quinolone (**11**) [9], and trimethoxyphloroglucinol (**12**) [10]. Known compounds were identified by comparison of their spectral data with those reported in the literature. The structure of *O*-methylcedrelopsin (**6**) was confirmed by NOE difference spectroscopy. The phloroglucinol derivative (**12**) has been reported as a constituent of the essential oil of *Zieria chevalieri* (Rutaceae) [11].

The novel dihydropyranocoumarin (**2**), $C_{15}H_{16}O_6$ (EI mass spectrometry) was isolated as an amorphous solid. The IR spectrum showed absorptions for a hydroxyl (3388 cm^{-1}) and a carbonyl group (1719 cm^{-1}). The UV spectrum showed maximal absorptions at 203, 228 sh, 255 sh, 301 sh and 341 nm, indicative of the presence of a 6,7-dioxygenated coumarin moiety [12]. The ^1H NMR spectrum (see Experimental) showed the signals of the lactone ring protons as a pair of doublets (J = 9.3 Hz) at δ_H 6.28 (H-3) and 7.64 (H-4). The chemical shift for H-4 indicated the absence of any oxygenation at C-5 [12]. Therefore, the aromatic singlet at δ_H 6.81 must be assigned to H-5. The location of the methoxyl group, associated with the signal singlet at δ_H 3.89 (3H) at C-6, was confirmed by NOE difference spectroscopy, where irradiation of the signal at δ_H 6.81 enhanced only the signals corresponding with H-4 and to the methoxyl group. The remaining signals, two methyl singlets at δ_H 1.35 (3H) and 1.58 (3H), and a pair of doublets (J = 6.9 Hz) of two vicinal protons at δ_H 3.86 (H-3′) and 4.99 (H-4′), were attributed to a 7,8-dihydropyran moiety. The relative configuration of this khellactone was indicated *trans*, because of the coupling constant $J_{3',4'}$ = 6.9 Hz [13]. These data, together with those

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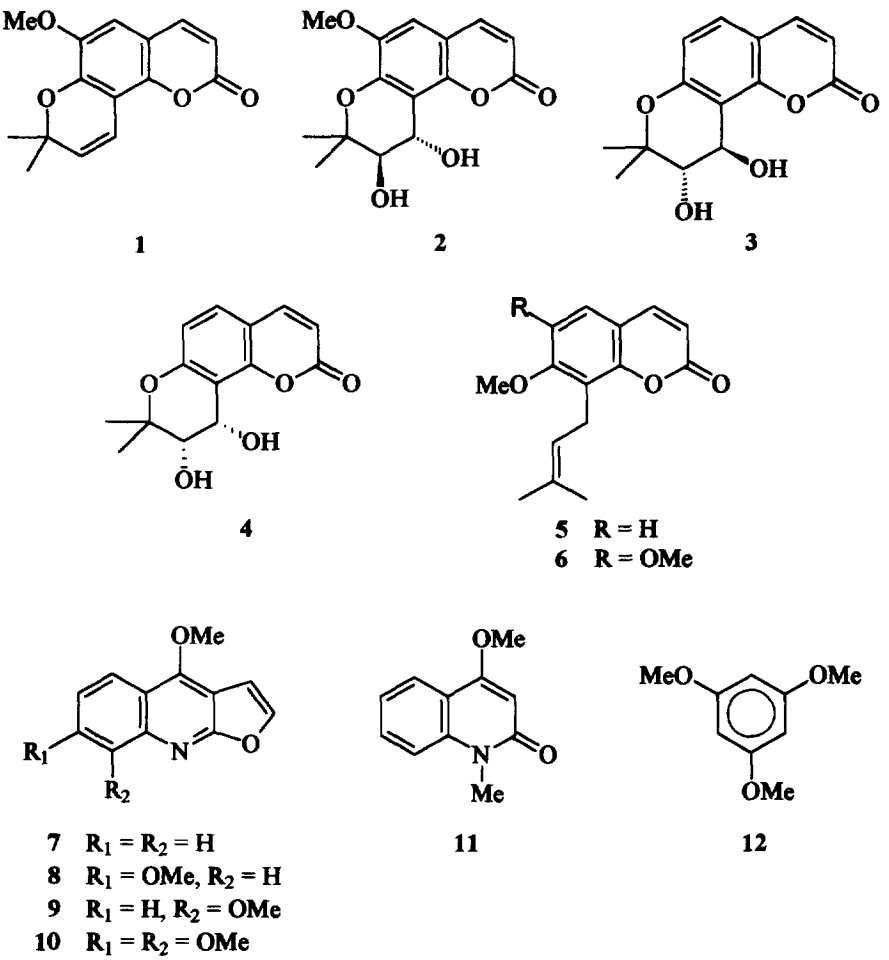

1 2 3

4

5 R = H
6 R = OMe

7 $R_1 = R_2 = H$
8 R_1 = OMe, R_2 = H
9 R_1 = H, R_2 = OMe
10 $R_1 = R_2$ = OMe

11 12

of ^{13}C NMR (Table 1), when compared with those reported for (+)-*trans*- (**3**) and (−)-*cis*-khellactone (**4**) [14], allowed us to propose for **2** the structure, (−)-6-methoxy-*trans*-khellactone.

EXPERIMENTAL

General. Mps: uncorr. IR and UV: $CHCl_3$ and EtOH, respectively. ^1H and ^{13}C NMR: 300 and 75 MHz, respectively, in $CDCl_3$. EIMS: probe, 70 eV. Silica gel 60 GF_{254} (Merck 7730) was used for TLC, silica gel 60 PF_{254} (Merck 7747) for prep. TLC and silica gel 60 (Merck 7734) for VLC and CC.

Plant material. Ticorea longiflora was collected in Salinópolis, State of Pará, Brazil, in April 1994. A voucher specimen is deposited at the Herbarium of the Museu Paraense Emilio Goeldi, Belém, PA, Brazil.

Extraction and isolation. After drying, stems (1.2 kg) and leaves (1 kg) were ground and percolated with hexane, CH_2Cl_2 and MeOH, successively. The hexane extract of stems (6 g) was submitted to VLC over silica gel using hexane (A), hexane–CH_2Cl_2 (4:1) (B), hexane–CH_2Cl_2 (1:1) (C) and CH_2Cl_2 (D) as eluents. Fr. C was purified by prep. TLC (silica gel, hexane–CH_2Cl_2–MeOH, 10:10:1) to yield osthol (5 mg). Fr. D after CC over silica gel eluting with hexane–CH_2Cl_2–MeOH (20:20:1) afforded 17 frs. Fr. D_2 was purified by prep. TLC (silica gel, hexane–CH_2Cl_2–MeOH, 10:10:1) to yield braylin (50 mg). Fr. D_3 was purified

Table 1. ^{13}C NMR spectral data of compounds **2–4**

C	2*	3†	4†
2	162.5 *s*	161.5 *s*	161.2 *s*
3	112.8 *d*	112.0 *d*	112.1 *d*
4	143.9 *d*	144.4 *d*	144.3 *d*
5	108.1 *d*	128.4 *d*	128.6 *d*
6	146.3 *s*	114.8 *d*	114.9 *d*
7	153.3 *s*	156.4 *s*	156.6 *s*
8	111.5 *s*	111.8 *s*	111.1 *s*
4a	112.9 *s*	112.5 *s*	112.2 *s*
8a	151.5 *s*	154.3 *s*	154.6 *s*
2′	79.8 *s*	79.5 *s*	79.1 *s*
3′	74.7 *d*	74.8 *d*	71.2 *d*
4′	66.9 *d*	66.4 *d*	61.1 *d*
gem-Me	19.9 *q*	20.3 *q*	21.6 *q*
	25.4 *q*	25.4 *q*	25.3 *q*
6-OMe	56.5 *q*	—	—

* Spectrum obtained at 75.4 MHz. Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 77.20. Multiplicity of carbons determined by DEPT experiments.

† Spectra obtained at 100 MHz in $CDCl_3$ [14].

by prep. TLC (silica gel, hexane–CH_2Cl_2–MeOH, 10:10:2) to yield dictamnine (16 mg). The CH_2Cl_2 extract of stems (12 g) was submitted to VLC over silica gel using hexane–CH_2Cl_2 (4:1) (E), hexane–CH_2Cl_2 (1:1) (F), CH_2Cl_2 (G) and CH_2Cl_2–EtOAc (3:2) (H) as eluents. Fr. F after CC over silica gel with hexane–CH_2Cl_2 (4:5) afforded 50 frs. Frs F_{19}, F_{20}, F_{23} and F_{42} were purified by prep. TLC (silica gel, hexane–CH_2Cl_2–MeOH, 10:10:1) to give evolitrine (5 mg), skimmianine (10 mg), 4-methoxy-*N*-methyl-2-quinolone (5 mg) and trimethoxyphloroglucinol (10 mg), respectively. Fr. G, treated as described for F, furnished γ-fagarine (15 mg). Fr. H was washed with hexane and purifed by prep. TLC (silica gel, hexane–EtOAC, 1:2) to yield *O*-methylcedrelopsine (5 mg) and (−)-6-methoxy-*trans*-khellactone (6 mg). The hexane extract of the leaves (20 g), after similar chromatographic treatment as described for the hexane extract of stems, gave β-sitosterol + stigmasterol (205 mg), evolitrine (203 mg), γ-fagarine (10 mg), skimmianine (200 mg) and braylin (14 mg).

(−)-6-*Methoxy*-trans-*khellactone* (**2**). Amorphous solid. $[\alpha]_D^{20} -26.36°$ ($CHCl_3$; *c* 0.11). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3388, 2919, 2850, 1719, 1655, 1460, 1376, 1034. ^1H NMR (300 MHz, $CDCl_3$): δ 1.35 and 1.58 (each 3H, *s*, *gem*-Me_2), 3.86 (1H, *d*, *J* = 6.9 Hz, H-3′), 3.89 (3H, *s*, 6-OMe), 4.99 (1H, *d*, *J* = 6.9 Hz, H-4′), 6.28 (1H, *d*, *J* = 9.3 Hz, H-3), 6.81 (1H, *s*, H-5), 7.64 (1H, *d*, *J* = 9.3 Hz, H-4). ^{13}C NMR: Table 1. EIMS *m/z* (rel. int.): 292 $[M]^+$ (8), 274 $[292-H_2O]^+$ (100), 246 (40), 243 (50), 231 (38), 229 (16), 221 (15), 205 (44), 175 (15), 167 (21), 149 (86), 111 (25), 97 (41), 83 (56), 71 (73), 69 (62).

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REFERENCES

1. Engler, A. Z., *Die Natürlichen Pflanzenfamilien*, 2nd edn. Engelmann, Leipzig, 1931.
2. Bévalot, F., Fournet, A., Moretti, C., Vaquette, J., Waterman, P. G. and Young, S. A., *Phytochemistry*, 1983, **22**, 2875.
3. Silva, M., Cruz, M. A. and Sammes, P. G., *Phytochemistry*, 1971, **10**, 3255.
4. Dreyer, D. I., *Phytochemistry*, 1980, **19**, 941.
5. Robertson, A. V., *Australian Journal of Chemistry*, 1963, **16**, 451.
6. Ahond, A., Picot, F., Potin, P., Poupat, C. and Sévenet, T., *Phytochemistry*, 1978, **17**, 166.
7. Ito, C. and Furukawa, H., *Chemistry and Pharmacology Bulletin*, 1987, **35**, 4277.
8. Borges-del-Castillo, J., Martinez-Martir, A. I., Rodriguez-Luis, F., Rodriguez-Ubis, J. C. and Vazquez-Bueno, P., *Phytochemistry*, 1984, **23**, 859.
9. Hifnawy, M. S., Vaquette, J., Sévenet, T., Pousset, J. L. and Cavé, A., *Phytochemistry*, 1977, **16**, 1035.
10. Sasaki, S., *Handbook of Proton-NMR Spectra and Data*, Vol. 3. Asahi Research Center Company, Tokyo, 1985, p. 355.
11. Flynn, T. M. and Southwell, I. A., *Phytochemistry*, 1987, **26**, 1673.
12. Murray, R. D. H., Méndez, J. and Brown, S. A., *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*. Wiley, Chichester, 1982.
13. Chen, I. S., Chang, C. T., Shen, W. S., Teng, C. M., Tsai, I. L., Duh, C. Y. and Ko, F. N., *Phytochemistry*, 1996, **41**, 525.
14. Ikeshiro, Y., Mase, I. and Tomita, Y., *Phytochemistry*, 1992, **31**, 4303.