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AN IMIDAZOLE ALKALOID AND OTHER CONSTITUENTS FROM *PILOCARPUS TRACHYLLOPHUS*

MANOEL ANDRADE-NETO, PAULO HENRIQUES MENDES* and EDILBERTO ROCHA SILVEIRA*†

Curso de Pós-Gradução em Química Orgânica, Departamento de Química Orgânica e Inorgânica, Laboratório de Produtos Naturais, Universidade Federal do Ceará, Cx. Postal 12.200, Fortaleza, CE, 60.021-970, Brazil; *Laboratório de Pesquisas Fitoquímicas, Merck S.A. Indústrias Químicas, Rio de Janeiro, Brazil

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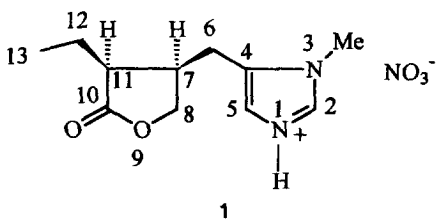
Key Word Index—*Pilocarpus trachyllophus*; Rutaceae; Jaborandi; roots; alkaloids; lignan; flavonoid glycoside; aliphatic ketones; isobutylamide.

Abstract—Roots of *Pilocarpus trachyllophus* yielded (−)-episesamin, hesperidin, tridecan-2-one, 1-hydroxy-tridecan-2-one, *N*-isobutyl-deca-2(E),4(E)-dienamide, pilocarpine and a new imidazole alkaloid, 13-nor-7(11)-dehydropilocarpine. Except for pilocarpine and tridecan-2-one, this is the first report of these substances in the genus *Pilocarpus*. Structural determinations were accomplished by spectroscopic analysis, particularly two-dimensional NMR, chemical interconversion and, where appropriate, comparison with literature data.

INTRODUCTION

The generic taxon *Pilocarpus* comprises 13 neotropical species, all inhabiting the region between the tropics of Cancer and Capricorn [1]. Nine are found in Brazil where they receive a general designation of 'Jaborandi' and are used in folk medicine for the treatment of several diseases [2]. *P. microphyllus* is currently the major source of pilocarpine (**1**), an imidazole alkaloid commercially relevant because of its use in the treatment of glaucoma. We have reported on a phytochemical study of *P. spicatus* leaves yielding two novel triterpenes [3].

We now report the phytochemical analysis of the roots of *P. trachyllophus* from Bahia State, northeast Brazil. Pilocarpine (**1**), tridecan-2-one (**2**), 1-hydroxy-tridecan-2-one (**3**), (−)-episesamin (**4**), *N*-isobutyldeca-2(*E*), 4(*E*)-dieneamide (**5**), hesperidin (**6**), and a new alkaloid, 13-nor-8(11)-dihydropilocarpine (**7**) have been isolated and characterized by spectroscopic analysis, including 2D-NMR techniques, such as COSY, HETCOR, INADEQUATE and HMBC, and comparison with literature data.



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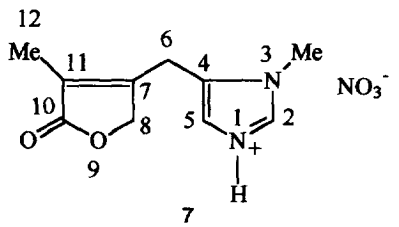

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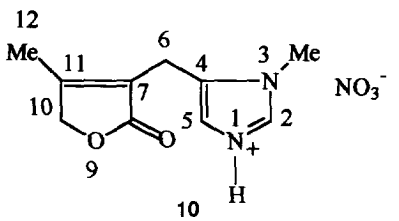

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RESULTS AND DISCUSSION

Fractional distillation of the oily extract from the root bark of *P. trachyllophus* (PTRCH) yielded a distilled fraction designated PTR-D_2 (80–81°), homogeneous by TLC, and a residue designated PTR-R. GC–mass spectrometric analysis of PTR-D_2 showed one major compound, M_r 198, whose NMR data were compatible with tridecan-2-one (**2**). PTR-R was submitted to column chromatography over silica gel to yield three major fractions after TLC comparison: R-FA, R-FB and R-FC.

R-FA, a wax-like compound showed a $[M]^+$ at *m/z*

†Author to whom correspondence should be addressed.

214 compatible with a molecular formula of $C_{13}H_{26}O_2$, and a fragmentation pattern similar to **2**. IR analysis indicated the presence of hydroxyl groups. Comparison of the ^{13}C NMR data of **2** and R-FA revealed the structure of 1-hydroxy-tridecan-2-one **3** for R-FA [4].

R-FB, an amorphous solid, gave a $[M]^+$ at *m/z* 354 that, in conjunction with the NMR data, suggested the molecular formula $C_{20}H_{18}O_6$. Its ^{13}C NMR spectrum showed 19 signals but the higher intensity of peak δ 108.3 suggested overlapping of two signals. Both ^{13}C and ^1H NMR data of R-FB were compatible with a lignan moiety and a search of the literature indicated co-identity with the laevorotatory enantiomer of episesamin, **8** [5].

R-FC, colourless needle crystals, showed a $[M]^+$ at *m/z* 223, suggesting the presence of nitrogen. Its ^{13}C NMR spectrum showed 13 signals. ^1H NMR analysis (see Experimental) in conjunction with a 2D-INADEQUATE experiment confirmed the structure of R-FC as the *N*-isobutyl-2(*E*),4(*E*) decadienamide (**5**), common name pellitorine, previously isolated from *Anacyclus pyrethrum* [6] and having insecticidal properties [7]. A literature survey revealed that $\alpha,\beta,\gamma,\delta$ unsaturated amides are very common in the genus *Piper* [8], but as far as we know this is the first report of this kind of substance in the genus *Pilocarpus*, although it has been reported in the Rutaceae [7]. The presence of this type of compound is responsible, at least in part, for the strong pungent taste of the hexane extract of jaborandi.

Ethanol extraction of the marc, obtained after hexane extraction, yielded a brown resinous extract designated PTRCE. Aqueous H_2SO_4 (3%) extraction, followed by usual work-up yielded a mixture of two alkaloids homogeneous on TLC but separated by preparative HPLC. The one with greater retention time (23.8 min) was identified as pilocarpine (**1**) by co-injection with an authentic sample. The other, retention time 16.4 min, was analysed as its nitrate salt, mp 156.1–156.3°. Its ^{13}C NMR spectrum showed 10 signals—one less than pilocarpine—corresponding to six unsaturated and four saturated carbons. DEPT and APT techniques showed two saturated methylenes at δ 72.7 and δ 22.2, and two

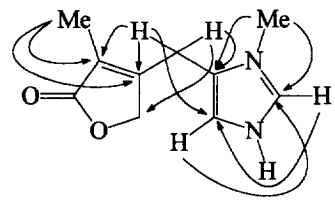

Fig. 1. Observed ^1H–^{13}C long-range correlation through inverse-detected NMR experiment (HMBC) for compound **7**.

methyl groups at δ 34.0 and δ 8.6, two hydrogenated sp^2 carbons at δ 137.7 and δ 119.1, three nonhydrogenated sp^2 carbons at δ 155.6, 132.0 and 126.8, and a carbonyl at δ 176.8. From its ^1H and ^{13}C NMR the presence of the imidazole moiety was observed and the absence of the ethyl group present in pilocarpine. The absence of any saturated methyne, as in pilocarpine, and the presence of two unsaturated carbons besides the imidazole moeity suggested dehydrogenation of C_7–C_{11} of pilocarpine, in agreement with a conjugated double bond (δ 155.6, C-7; δ 126.8, C-11) to the γ-lactone carbonyl now at δ 176.8, comparable to δ 180.3 in pilocarpine (Table 1) [9]. At this point can argue that the data presented could fit either one of structures **7** or **10**. Long-range ^1H–^{13}C correlations observed through HMBC analysis (Fig. 1) suggested the structure 13-nor-7(11)-dehydropilocarpine, **7**, particularly by the correlation of proton H-6 with C-8, impossible to be observed for structure **10**.

Direct from the ethanol extract of the root heartwood a precipitate was formed. Acetylation, ^1H and ^{13}C NMR analysis and comparison with an acetylated authentic sample, characterized it as hesperidin, **6**, [10]. Hesperidin is very common in Rutaceae, particularly in the *Citrus* genus [11], but this is the first report of it in *Pilocarpus*.

EXPERIMENTAL

General. Mps, uncorr. IR: KBr pellets (solids) or NaCl disc (oil films). ^1H NMR: 300 or 400 MHz. ^{13}C

Table 1. ^1H and ^{13}C NMR data of PTRCE-NB1 (MeOD, 100 MHz) and PTRCE-NB2 (MeOD, 100 MHz), in comparison with pilocarpine (D_2O, 50 MHz) [10]

	PTRCE-NB2.HNO_3 (**7**)		PTRCE-NB1.HNO_3 (**1**)		Pilocarpine-HCl	
C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	119.16	8.87	118.27	8.84	116.9	8.49
4	131.98	—	134.41	—	132.7	—
5	137.67	7.42 (*s*)	137.17	7.45	135.4	7.17
6	22.17	3.99 (*s*)	22.01	2.78 (H-6a); 2.62 (H-6b)	20.7	2.74 (H-6a); 2.51 (H-7b)
7	155.6		37.64	3.03	36.2	2.96
8	72.70		71.12	4.30 (H-8a); 4.07 (H-8b)	71.3	4.24 (H-8a); 4.00 (H-8b)
10	176.78		180.29	—	182.4	
11	126.80		45.60	2.90	44.6	2.70
12	8.60	1.86 (*s*)	19.36	1.80 (H-12a); 1.62 (H-12b)	17.9	1.62 (H-12a); 1.45 (H-12b)
13	—	—	12.42	1.11	11.4	0.89
CH_3N	34.00	3.84 (*s*)	33.85	3.85	33.3	3.66

NMR: 50, 75 or 100 MHz: $CHCl_3$ δ 7.24 (^1H) and δ 77.0 (^{13}C) was used as int. reference. EIMS: 70 eV.

Plant material. Pilocarpus trachyllophus Holmes was collected in, Macaúbas County, Bahia State, Brazil. A voucher specimen (#21267) representing the collection was identified by Dr Afrânio G. Fernandes (Botanist, Departamento de Biologia, UFC) and has been deposited at Herbário Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Extraction and isolation. Roots were separated into bark and heartwood, and 1.5 kg of root bark, sun-dried and powdered, were extracted at room temp. with hexane followed by EtOH. The residues after solvent evapn were designated PTRCH (165 g) and PTRCE (83 g), respectively. PTRCH (165 g) was distilled under vacuum to yield a residue. PTR-R (68.8 g) and a fr. PTR-D_2 (10 g), homogeneous on TLC, characterized as tridecan-2-one.

PTR-R was filtered to yield a solid material, which after cc over silica gel yielded three frs designated R-FA (150 mg), R-FB (250 mg) and R-FC (130 mg). R-FA, mp 55–58°, (M_r 214, $C_{13}H_{26}O_2$) was characterized as 1-hydroxy-tridecan-2-one [4]. R-FB, mp 117–119°, $[\alpha]_D$ −110° ($CHCl_3$, *c* 6.8), was characterized as the laevorotatory isomer of episesamin (lit. 121–122°, $[\alpha]_D$ +120.0°) [5]. R-FC, mp 82–86°, (M_r 223, $C_{14}H_{25}NO$) for (354, $C_{20}H_{18}O_6$) was characterized as pellitorine (lit. 81–86°) [12].

PTRCE (82 g) was extracted (×10) with 100 ml 3% aq. H_2SO_4. The acid soln was treated with 10% NH_4OH and filtered. Both residue and alkali soln were extracted with $CHCl_3$ until the $CHCl_3$ soln gave a negative Draggendorff's reaction. The $CHCl_3$ soln residue, designated PTRCE-B, yielding 3.26 g after $CHCl_3$ evapn, was dissolved in EtOH and treated with conc. HNO_3 until pH 4.0. At this pH, a ppt formed and was filtered off. The nitrate salts by TLC analysis showed one spot, but under analytical HPLC (RP-18, 50°; MeOH–H_2O, 93:7, +KH_2PO_4 5%, pH 2.5) conditions revealed two peaks, R_t 2.28 min and 2.70 min. Prep. HPLC of 350 mg of PTRCE-B yielded 205 mg of PTRCE-NB1 (mp = 172–174°), R_t 23.80 min, that was identified as pilocarpine nitrate by HPLC co-injection with an authentic sample (lit. 173.5–174°) [11]. The other sample, 12 mg, R_t 16.37 min, mp 156.1–156.3°, was designated PTRCE-NB2 and characterized as 13-*nor*-7(11)-dehydropilocarpine. EIMS *m/z* (rel. int.): 192 $[M]^+$ (14), 164 (14), 135 (8), 110 (8), 95 (55), 83 (55), 82 (100), 65 (22), 56 (10), 55 (32), 54 (50). ^1H and ^{13}C NMR (400 and 100 MHz respectively, CD_3OD): Table 1.

From the EtOH extract of the root heartwood, a ppt. formed and was filtered, and because of solubility problems, was peracetylated yielding 50 mg of PTLRE1. Peracetylation of an authentic sample of commercially available hesperidin and co-spectrometric comparison (^1H and ^{13}C NMR) with PTLRE1 proved their co-identity. Mp 159.8–162.4°, (lit. 176–178°) [13].

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REFERENCES

1. Kaastra, R. C. (1982). *Pilocarpinae. Flora Neotropica.* Monograph number **33**. New York Botanical Garden, New York.
2. Costa, O. A. (1968) *Revista Brasil. Farm.* **6**, 324.
3. Andrade Neto, M., Silveira E. R., Braz Filho, R., Gambardela, M. T. A., Santos R. H. A. (1994) *Phytochemistry* **35**, 739.
4. Gellerrt, M. and Rozsa, Z. (1985) *Herb. Hung.* **24**, 53–65.
5. Pelter, A., Ward, R. S., Rao E. V. and Sastry, K. V. (1976) *Tetrahedron* **32**, 2783.
6. Gulland, J. M., Hopton, G. U. (1949) *J. Am. Chem. Soc.* **71**, 366.
7. Kubo, I., Matsumoto, T., Klocke, J. K. and Kamikawa, T. (1984) *Experientia* **40**, 340.
8. Sengupta, S. and Ray, A. B. (1987) *Fitoterapia* **58**, 147–165.
9. Gaggelli, E., Gaggelli, N., Valensin, G. and Vivi, A. (1993) *Can. J. Chem.* **71**, 738.
10. Markham, K. R. and Ternai, B. (1976) *Tetrahedron* **32**, 2607.
11. *The Index Merck* (1983) 10th edn. Merck and Co., Inc.
12. Reisch, J., Hussain, R. A., Adesina, S. K. and Szendrei, K. (1985) *J. Nat. Prod.* **48**, 862.
13. *Dictionary of Organic Compounds* (1982) 5th edn, vol **III**. Chapman & Hall, London.