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AN AROMATIC HYDROCARBON FROM THE FOLIAR EPICUTICULAR WAX OF *PILOCARPUS JABORANDI*

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Key Word Index—*Pilocarpus jaborandi*; Rutaceae; foliar epicuticular wax; hydrocarbon; 1-phenyl-5-vinyl-5,9-dimethyl-decane.

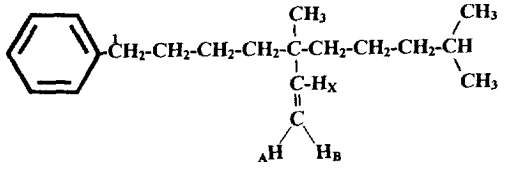
Abstract—1-phenyl-5-vinyl-5,9-dimethyl decane was obtained from the foliar epicuticular wax of *Pilocarpus jaborandi*. The hydrocarbon fraction from the leaf wax of *P. jaborandi* can be distinguished by TLC from those of other species of *Pilocarpus*, based on the presence of this hydrocarbon. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Pilocarpus is a genus of neotropical plants of great medical importance due to presence of pilocarpine in their leaves. Several species are presently being used as sources of this alkaloid. Although *P. microphyllus* is presently the main source of pilocarpine, *P. jaborandi*, commercially designated as Pernambuco jaborandi, is still mentioned in pharmacopeias [1] and used for extraction of the alkaloid [2]. In the course of an investigation examining the taxonomic distribution of alkanes from the foliar epicuticular waxes of Brazilian species of *Pilocarpus*, we came across a C_{20} compound present in the hydrocarbon fraction, with different MS characteristics than the long chain *n*-alkanes normally found on plant surfaces [3]. The substance enables the chemical characterization of *P. jaborandi*, distinguishing it from at least 10 other *Pilocarpus* species. In addition to representing a new chemical character, probably useful for the taxonomy of *Pilocarpus*, the quoted substance has a practical value in terms of authenticity evaluation of foliar material commercially marketed for the extraction of pilocarpine.

RESULTS AND DISCUSSION

From the total hydrocarbons from the foliar wax, 1-phenyl-5-vinyl-5,9-dimethyl decane (**I**) occurred with amounts of 71% in both samples obtained from wild populations, and 22% in the cultivated sample. This implies that either environmental or cultivation conditions influence the amount of the compound in the foliar wax. No information is available whether the cultivated sample is an offspring of breeding programs. **I** was not detected in any sample of the other species of *Pilocarpus* tested, either by TLC or GC-MS analysis.



I

Compound **I** has the molecular formula $C_{20}H_{32}$ as established by its EI-mass spectrum. The ^1H NMR spectrum showed the presence of a phenyl group and three vinylic protons. The coupling constants for a vinylic system are characteristic, the trans coupling being large than the cis, with the geminal coupling being very small. Although ABX systems with three coupling constants are not first order, these patterns are frequently recognized if distortions are not too severe. Protons A and B are magnetically not equivalent, each being represented by a pair of doublets. Proton A (δ 4.86) is deshielded about 9.4 Hz compared with proton B. Proton X (δ 5.68) is strongly deshielded and split by protons A (J_{trans} = 17.5 Hz) and B (J_{cis} = 10.8 Hz). The proton A signal is split by protons X (J_{trans} = 17.5 Hz) and B (J_{gem} = 1.35 Hz), whereas the proton B signal (δ 4.83) is split by protons X (J_{cis} = 10.8 Hz) and A (J_{gem} = 1.35 Hz). Mass spectra fragmentation was consistent with structure **I**, showing a base peak ion at *m/z* 137 $(M-135)^+$, corresponding to a loss of $(Ph(CH_2)_4–H_2)$ fragment, as

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well as peaks at m/z 139 $(M-133)^+$, corresponding to a loss of $Ph(CH_2)_4$ fragment, and at m/z 187 due to a loss of a C_6H_{13} fragment. The presence of a phenyl group is also indicated by peaks at m/z 91 (cation tropylium), at m/z 92 $(C_7H_8)^+$, at 119 $(C_9H_{11})^+$ and at m/z 133 $(C_{10}H_{13})^+$. The ^{13}C NMR spectrum showed a phenyl group at δ 129.3 and δ 137.5, and a vinyl group at δ 110.0 (C-14) and δ 149.2 (C-13). B (benzenoid) bands are characteristic of the spectra of aromatic molecules. Benzene shows a broad absorption band containing multiple peaks in the UV range (i.e., 230 and 270 nm). Although carbon 5 is asymmetric, the compound has no optical activity, probably because the asymmetrical centre is flanked on both sides by voluminous groups [4]. Structure **I** is biogenetically consistent with the phenyl group and carbons 1–4 stemming from acetate and the ten remaining carbons corresponding to two isoprene units [5].

Thin layer chromatograms of the hydrocarbon fraction, when treated with Carr-price reagent (recommended for visualization of isoprenoid compounds [6]), gives rise to magenta spots only with *P. jaborandi* wax. TLC analysis of the separated hydrocarbon fraction may be viewed as a simple and rapid procedure for chemical characterization of *P. jaborandi* and its distinction from other *Pilocarpus* species.

EXPERIMENTAL

Plant material

Leaves of *Pilocarpus alatus* Joseph ex Skorupa sp. nov. (L. A. Skorupa and G. P. da Silva 1024), *P. carajaensis* Skorupa sp. nov. (L. A. Skorupa and Elsamar 996), *P. giganteus* Engler (L. A. Skorupa and S. Pompéia 981), *P. grandiflorus* Engler var. *recurvus* Skorupa var. nov. (L. A. Skorupa and A. Amorim 992), *P. jaborandi* Holmes (L. A. Skorupa and L. M. Barros 1000), *P. jaborandi* Holmes (L. A. Skorupa and Ivanildo 1991), *P. jaborandi* Holmes (Neto, s.n.), *P. microphyllus* Stapf. ex Wardleworth (L. A. Skorupa 999), *P. pauciflorus St. Hil. subsp. clavatus* Skorupa subsp. nov. (L. A. Skorupa *et al.* 991), *P. riedelianus* Engler (L. A. Skorupa *et al.* 988), *P. spicatus* St. Hil. subsp. *spicatus* var. *spicatus* (L. A. Skorupa *et al.* 983), *P. sulcatus* Skorupa sp. nov. (L. A. Skorupa 1012) and *P. trachylophus* Holmes (L. A. Skorupa 1010) were collected from natural populations, mostly from North-Northeast Brazil. Voucher specimens are deposited in the Herbarium of the Institute of Biosciences, University of São Paulo (SPF).

Extraction and analysis of the foliar epicuticular waxes

The leaves were immersed in $CHCl_3$ three times for 20 sec each [7]. The pooled extracts were evaporated under reduced pressure and dissolved in a small volume of $CHCl_3$. The solutions of wax were chromatographed in columns (2 cm × 30 cm) of silica gel (Mesh 70–230) with *n*-hexane. The first 30 ml of eluant corresponded to hydrocarbons. These were analysed by GC-EIMS at 70 eV. An HP Ultra 1 capillary column (25 m × 0.25 mm) was used with He as carrier gas at a flow of 1 cm min^{-1}. The temperatures of injector and detector were 280°C and of the column ranged from 110°C–280°C at 6°C min^{-1}.

Isolation and structure determination of **I**

Foliar wax samples of *P. jaborandi* were analyzed by preparative layer chromatography using silica gel G 60 impregnated with 0.02% sodium fluoresceine and *n*-hexane: $CHCl_3$ (73:27), using the technique of stepwise development. Five runs (3 cm, 6 cm, 9 cm, 12 cm and 15 cm) were carried out. The plates were visualized under long wave length UV. The desired hydrocarbon exhibited a lower R_f in comparison with the *n*-alkanes fraction. The structure of compound **I** was determined by means of MS, ^1H NMR, ^{13}C NMR, UV spectrophotometry and its optical activity was also measured.

1-Phenyl-5-vinyl-5,9-dimethyl decane. EIMS: m/z 272 $(M)^+$ (32), 257 $(M-CH_3)^+$ (53), 229 $(M-CH(CH_3)_2)^+$ (4), 187 $(M-(CH_2)_3CH(CH_3)_2)^+$ (7), 139 $(M-Ph(CH_2)_4)^+$ (1), 138 $(M-Ph(CH_2)_4-H)^+$ (11), 137 $(M-Ph(CH_2)_4-H_2)^+$ (100), 133 $(Ph(CH_2)_4)^+$ (26), 119 $Ph(CH_2)_3)^+$ (32), 105 $(Ph(CH_2)_2)^+$ (45), 91 $(PhCH_2)^+$ (74), 77 $(Ph)^+$ (31), 57 $(C_5H_9)^+$ (14), 55 $(C_5H_7)^+$ (53), 43 $(C_3H_7)^+$ (22), 41 $C_3H_5)^+$ (84). ^1H NMR (300 Mz, CCl_4/TMS/D_2O): δ 0.91 (6H, *d*, $J = 9$ Hz, 2 × Me), 1.05 (3H, *s*, Me), 1.3 (12H, *s*, 6 × CH_2), 1.33 (1H, *m*, $J = 9$ Hz, 9-H), 2.3 (2H, CH_2), 4.83 (1H, *dd*, $J = 10.8$ and 1.35, 14-H_B), 4.86 (1H, *dd*, $J = 17.5$ and 1.35, 14-H_A), 5.68 (1H, *q*, $J = 17.5$ and 10.8, 13-from H_X), 7.2 (5H, *m*, Ph). ^{13}C NMR (300 29.1 (C-2 and C-9), 39.5 (C-1, C-4 and C-6), 42.0 (C-5 and C-8), 110.0 (C-14), 129.3 and 137.5 (carbons of benzene ring) and 149.2 (C-13). UV: 235 nm, 253.5 nm (sh), 274.5 nm (sh), 300 nm (sh) and 322 (sh). The compound showed no optical activity.

TLC of the hydrocarbon from foliar epicuticular waxes of species of Pilocarpus

The hydrocarbon fraction of waxes of all samples of *Pilocarpus* collected were chromatographed on silica gel G plates using *n*-hexane: $CHCl_3$ (73:27). The chromatograms were visualized by spraying with Carr Price [6] reagent followed by heating at 105°C.

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