ALKALOIDS FROM *Peschiera laeta* MART.

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Affinine, akuammidine, condurine, tombozine, voacamine and vobasine were isolated from leaves and twigs of *Peschiera laeta* MART.; the occurrence of the alkaloids evidences the chemotaxonomical appurtenance of this plant species to the genus *Tabernaemontana*. In addition to the above-mentioned alkaloid geissoschizol was identified; its presence in the genus *Tabernaemontana* has not been reported as yet.

The tribe *Apocynaceae*, to which the genus *Tabernaemontana* with the species *Peschiera* belongs, is a wealthy source of indole alkaloids. So far, alkaloids from *Peschiera affinis* (MEULL.-ARG.) MIERS.[1] and *P. lundii* (D. C.) MIERS.[2] have been isolated and their structure elucidated. This paper deals with the isolation and identification of alkaloids from *P. laeta* MART. collected in Brasil.

The mixture of total alkaloids, from which the tarry biopolymers were removed, was gradually extracted with McIlvain buffer solutions to give 5 fractions. In the pH 6·5 portion alkaloids tombozine, affinine and geissoschizol were identified; the latter has already been found in *Rauwolfia vomitoria* AFZ.[3] and *Aspidosperma oblongum* SCHL.[4] Conodurine was identified in the pH 6·0 fraction, voacamine and akuammidine in the pH 5·0 portion. All the above-mentioned alkaloids were also present in a little amount in portions of lower pH. Vobasine was found in pH 4 and 3 portions. The extract obtained with 2% hydrochloric acid contained a complex mixture of basic compounds and dyes and has not been worked up.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage, optical rotations with a Perkin-Elmer 141 apparatus in 1 cm cells. Mass spectra were measured with an MCh 1306 spectrometer (USSR) adapted for a direct introduction of the sample to the ionization chamber at the ionizing electron energy 70 eV and 1 mA trap current. Infrared spectra were recorded with a Perkin-Elmer 457 spectrophotometer in KBr discs, ultraviolet spectra with an ORD/UV-5 Jasco apparatus in ethanol and the ^{1}H-NMR spectra (in ppm on the δ scale) with a Varian HA-100 spectrometer

at 100 MHz in $CDCl_3$–hexadeuteriodimethyl sulphoxide 1 : 1, tetramethylsilane being the internal reference substance. The alumina for column chromatography (Reanal), neutral, was of activity grade II; the purity of alkaloids was monitored by thin-layer chromatography using alumina Woelm G according to Stahl in solvent systems chloroform–n-heptane 7 : 3 (S_1); chloroform–n-heptane–ethanol 6 : 3·6 : 0·4 (S_2); 6 : 3 : 1 (S_3); 6 : 2·5 : 1·5 (S_4); ethyl acetate–benzene–ethanol 5 : 4·2 : 0·8 (S_5). The chromatographic spots were vizualized with Dragendorf reagent.

Isolation of Alkaloids

The ethanolic extract of the drug (twigs and leaves of *Peschiera laeta* MART., 33 kg) was concentrated under diminished pressure to a syrup (2800 g) and diluted with methanol–chloroform 1 : 1 (5600 ml), Kieselgur (Hyflo Super Cel, 2800 g) was added to this solution and the solvent was distilled off *in vacuo*. The free-flowing residue (5700 g) was eluated with an aqueous solution of citric acid (1 . 5%, 1 . 1%, 4 . 0·5%) in 15 l portions, the extract was evaporated under reduced pressure to a 1/20 of the original volume, made alkaline with ammonium hydroxide and extracted with chloroform. The usual work-up gave the mixture of alkaloids (106 g) from which the phenolic bases (3·9 g) were separated by extraction with 2% sodium hydroxide. The aqueous solutions, which still exhibited a positive reaction with Dragendorf reagent, was acidified to pH 3 and the quaternary bases (4·26 g) were precipitated with Mayer reagent. The chloroform solution of the alkaloid mixture was fractionated into buffer solutions (5 . 200 ml each); the work-up afforded portions of pH 6·5 (5·59 g), 6·0 (2·92 g), 5·0 (6·85 g), 4·0 (8·25 g), 3·0 (13·12 g) and a part soluble in 2% hydrochloric acid (12·05 g). The individual fractions were separated by chromatography on alumina (30—50-fold excess) using an eluotropic series of eluants.

Characterization of the Isolated Alkaloids

Tombozine[5] (normacusine B (ref.[6]), vellosiminol[7]): m.p. 245°C (dec.) (dichloromethane–n-heptane), 381 mg, eluant ether–chloroform 3 : 1, $[\alpha]_D^{24}$ +35·5° (*c* 0·97, ethanol), R_F 0·51 (S_3). This alkaloid is reported[6] to have m.p. 246—275°C, $[\alpha]_D$ +35° (methanol). Peaks in the mass spectrum at *m/e* 294 (M^+), 263 (M — CH_2OH), 249, 169 and 168 were characteristic of a tetrahydro-β-carboline backbone; the low intensity of the peak at *m/e* 249 was indicative of a monosubstitution pattern at $C_{(16)}$ (ref.[8]). The UV spectrum showed absorption bands at 222, 282 and 290 nm (log ε 4·70, 3·90 and 3·75) typical of a simple indole chromophore[9], the IR spectrum revealed vibration bands of a primary hydroxyl group (1035 cm^{-1}) and an ethylidene grouping (1640, 845 cm^{-1}).

Affinine: m.p. 264—265°C (dec.) (dichloromethane–n-heptane, 152 mg, eluant chloroform, $[\alpha]_D^{24}$v —108° (*c* 0·78, ethanol), R_F 0·65 (S_5); ref.[10]: m.p. 265°C (dec.). The displayed peaks in its mass spectrum at *m/e* 324 (M^+), 293 (M — CH_2OH), 158, 152, 122, and 108 were coincident with those reported[11]. The absorption in the UV region at 238 and 318 nm (log ε 4·18 and 4·34) was indicative of a vobasine type of alkaloids and the vibration bands in the IR range of the spectrum evidenced the presence of a primary hydroxyl group (1050 cm^{-1}) and a carbonyl group (1660 cm^{-1}).

Geissoschizol[12]: m.p. 216°C (methanol–chloroform–n-heptane 1 : 3 : 0·3), 103 mg, eluant chloroform–ether 1 : 1, $[\alpha]_D^{24}$ —68° (*c* 0·5 pyridine), R_F 0·48 (S_3); ref.[3]: m.p. 224—226°C (dec.), $[\alpha]_D$ —70° (pyridine). Its mass spectrum showed, in addition to the peak of molecular radical ion at *m/e* 296 peaks at *m/e* 295, 281 (M — 15), 265 (M — CH_2OH), 251 (M — CH_2CH_2OH), 249, 169 and 156 diagnostic of alkaloids of akuammidine type[13]. The UV spectrum (220, 279, 286, 312 nm, log ε 6·70, 5·86, 5·87, 5·96) indicated an unsubstituted indole chromophore. The pro-

minent vibration bands in the IR spectrum were assigned the primary hydroxyl group (1035 cm^{-1}) and an ethylidene grouping (1640 and 840 cm^{-1}). The ^{1}H-NMR spectrum with signals at 10·60 (s, NH), 7·26 (d, J = 9 Hz, $C_{(12)}$—H), 6·82 (d, J = 8·5 Hz, $C_{(11)}$—H), 6·75 (s, $C_{(10)}$—H) 6·73 (s, $C_{(9)}$—H), 5·63 (*q*, J = 7 Hz, $C_{(19)}$—H), 5·27 (m, $C_{(3)}$—H), 1·60 (d, J = 7 Hz, $C_{(18)}$—3 H) corresponded to the structure of this type of compounds[14].

Conodurine: m.p. 221—224°C (dichloromethane–n-heptane), 761 mg, eluant ether, $[\alpha]_D^{23}$ —96° (*c* 0·72, $CHCl_3$), R_F 0·63 (S_1); ref.[15]: m.p. 222—225°C, $[\alpha]_D^{22}$ —101°. Its YV, UR and mass spectra were superimposable with those of the authentic specimen, with which no depression of the m.p. on admixture of the sample was observed.

Voacamine: m.p. 223°C (dichloromethane–n-heptane), 485 mg, eluant ether, $[\alpha]_D^{24}$ —50° (*c* 0·94, $CHCl_3$), R_F 0·49 (S_1); ref.[16]: m.p. 223—224°C, $[\alpha]_D$ —52° ($CHCl_3$). Its identity with the authentic specimen was proved by comparison of their UV, IR and mass spectra and by the mixed m.p. without depression.

Akuammidine: m.p. 240°C (benzene), 192 mg, eluant dichloromethane), $[\alpha]_D^{26}$ +16° (*c* 0·62, methanol), R_F 0·40 (S_5); ref.[17]: m.p. 249°C, $[\alpha]_D^{18}$ +21 (ethanol). The mass spectrum showed the peak of the molecular radical ion at *m/e* 352 and other peaks at *m/e* 351, 337 (M — 15), 335 (M — 17), 321 (M — 31), 293 (M — 59) and a fragmentation pattern *m/e* 249, 182, 169 and 168 characteristic of this type of alkaloids. (The $C_{(16)}$-epimer of akuamidine polyneuridine has an intense M — 18 peak[18]). The UV spectrum with maxima at 228 and 281 nm (log ε 4·51 and 3·80) evidenced the presence of an indole chromophore and the IR spectrum the presence of a carbonyl group (1710 cm^{-1}).

Vobasine: m.p. 118—119°C (n-hexane), 289 mg, eluant chloroform, $[\alpha]_D^{24}$ —149° (*c* 1·8, $CHCl_3$), R_F 0·34 (S_1); ref.[19]: m.p. 111—113°C, $[\alpha]_D^{22}$ —159° ($CHCl_3$). The hydrobromide m.p. 249°C (dec.) (ethanol). The IR and UV spectra were virtually identical with those of the authentic specimen. The mixed m.p. did not show depression.

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