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Aporphine-Type Alkaloids from *Piper auritum*

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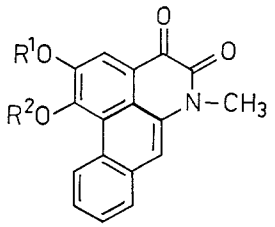
In the course of studies on the lipophilic aromatic compounds of some New World *Piper* species our attention was drawn to strongly fluorescent constituents present in the woody roots of *Piper auritum* HBK. Monitoring by tlc, we isolated by column chromatography on silica gel and by re-chromatography on Celite–polyamide (1:1), cepharadione A(**2**) and **B**(**1**). These compounds were obtained in very small yields; 5 mg of cepharadione B and 2 mg of cepharadione A from 2.5 kg air-dried plant material. These two substances have recently been isolated from *Stephania cepharantha* (Menispermaceae) (1). Identity was obtained by comparison of the ir, uv, nmr and mass spectra and by mmp determination.



1 $R^1 = R^2 = CH_3$

2 $R^1 + R^2 = CH_2$

To our knowledge, this is the first time "true" alkaloids have been isolated from a *Piper* species; so far only amides of the piperine-type have been obtained. Cepharadione A and B may be regarded as oxidation products of corresponding aporphine bases, and as biosynthetic precursors of the aristolactones and the aristolochic acids (1). Thus, remarkably, the order Piperales shares a chemotaxonomic feature of synthesizing and accumulating benzyltetrahydroisoquinoline alkaloids with the Magnoliales, Aristolochiales, Ranunculales and Papaverales, all orders belonging to the same subclass Magnoliidae (2).

EXPERIMENTAL

PLANT MATERIAL.—*Piper auritum* HBK is a widespread species occurring in many areas in tropical America. Collections were made in the region of Los Tuxtlas in Veracruz (Mexico), in a tall evergreen rain forest area where the species is abundant as an early secondary tree (3). The vouchers of this study are: Gomez-Pompa 4879 bis, collected Oct. 12, 1970; R. Hernandez 1010, collected Nov. 2, 1971; and R. Hernandez s. n. 27, collected in May 1971. These collections are deposited at the National University of Mexico Herbarium (MEXU). Plant parts under study were the woody roots of the plant.

FRACTIONATION CONTROL BY TLC.—Silica gel plates (aluminium sheets Merck, 20 x 20 cm; layer thickness 0.25 mm) were spotted with 10 to 50 μl of each fraction, and developed 10 cm in chloroform–methanol (9:1). Two spots visible in daylight with $R_F = 0.74$ (yellow fluorescent; cepharadione B), and with $R_F = 0.67$ (under uv, yellow; cephara-

dione A), were seen with a detection limit of less than 0.1 μg per spot.

EXTRACTION AND ISOLATION.—The dried woody roots (2.5 kg) were continously extracted with methylene chloride (Soxhlet apparatus) for about 70 hr, and the extract was concentrated *in vacuo* leaving 50 g residue. The extract was purified by column chromatography (using 4 columns [4.5 cm] each of 500 g silica gel). Fractions (20 ml each) were collected, beginning with cyclohexane–ethyl acetate (7:3) followed by cyclohexane–ethyl acetate mixtures of increasing polarity (3:2, 1:1, 2:3, 3:7, 1:4). Fractions containing one or both of the fluorescent compounds (from the eluates containing 60 to 80% ethyl acetate) were collected, pooled and dried to give 1.1 g of dark brown residue. This material was dissolved in a minimal volume of ethyl acetate–EtOH (3:2), impregnated on 8.0 g Celite, and after drying placed on top of a column (2.0 cm) filled with a mixture of polyamide (45 g) and celite (45 g). The column was eluted first with 500 ml cyclohexane–EtOH(3:2), then with solvent mixtures of increasing polarity (1:1, 2:3, 3:7, 1:9, 500 ml each). Fractions shown to contain a single compound by tlc were combined. With cyclohexane–EtOH (1:1) cepharadione B was eluted to give, after evaporating to approximately 5 ml, 5 mg of orange needles. After an intermediate fraction (cyclohexane–EtOH, 2:3), containing both fluorescent compounds, cepharadione B was eluted with cyclohexane–EtOH (3:7). Evaporation to approximately 3 ml gave 2 mg of orange needles.

CEPHARADIONE B (**1**).—Orange needles of moderate solubility in $CHCl_3$, mp 255–262° (Kofler); ms, (mol. wgt. calcd for $C_{19}H_{15}NO_4$, 321.1001; found 321.1001) *m/e* (%) 321 (100), 293 (75), 278 (24), 250 (40), 235 (22), 222 (15), 207 (8), 179 (37) and 150 (17); nmr, (d_6DMSO at 105°) δ 6.45 (3H, s, NCH_3), 6.09 (6H, s, 2 OCH_3), 2.4–2.7 (1 H, s+2 H, m; arom. protons), 2.1–2.3 (1H, m, arom. protons); 2.21 (1H, s, arom. protons) and 0.65–0.9 (1H, m, arom. protons); ir, ν max (KBr) 1640–1670 cm^{-1} (overlapping bands, CO); uv, λ max ($CHCl_3$) (log ϵ) 245 (4.60), 304 (4.17), 316 (4.22) and 440 nm (4.12).

CEPHARADIONE A (**2**).—Orange-red needles of moderate solubility in $CHCl_3$, mp 338–342° (decomp); ms, *m/e* (%) 305 (M^+, 90), 278 (22), 277 (100), 276 (22), 260 (15), 248 (13), 220 (12), 219 (18), 192 (20), 191 (21), 190 (21), 164 (20), 163 (30), 150 (14) and 138 (18); ir, ν max (KBr) 1667 and 1650 (CO) cm^{-1}; uv, λ max ($CHCl_3$) (log ϵ) 239 (4.51), 278 (3.97), 303 (4.17), 316 (422) and 434 nm (4.12).

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