ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. ANTIMICROBIAL AGENTS FROM *PEGANUM HARMALA* SEEDS

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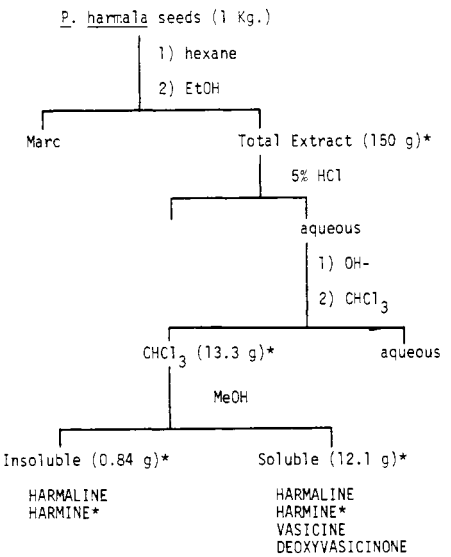
In the course of a screening of Iraqi plants for antimicrobial agents, ethanolic extracts of the seeds of *Peganum harmala* (fam. Zygophyllaceae) were found to be particularly potent and showed a broad spectrum of activity against the screening organisms (1). Subsequent fractionation of the defatted seeds according to a general scheme (2) showed that the bioactivity was restricted to the non-phenolic tertiary alkaloid fraction (Scheme).

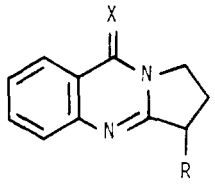


Scheme. Bioassay-directed Fractionation of P. harmala Seeds

To facilitate analysis, the active fractions were analyzed by hplc on a Perkin-Elmer Series 2/2 Chromatograph and a 4.6 mm i.d. x 250 mm Lichrosorb reverse-phase column, 10μ-type 415089. A linear gradient was used, beginning with 10% methanol in aqueous perchloric acid (pH 1.24) and increasing methanol by 2%/min.; a flow rate of 2.0 ml/min was employed. The retention time of known alkaloids were: vasicine (**1**), 420 secs.; harmalol (**2**), 830 secs.; harmol (**3**), 910 secs.; harmane (**4**), 1080 secs.; harmaline (**5**), 1175 secs.; harmine (**6**), 1220 secs.; and deoxyvasicinone (**7**), 1680 secs. Using this procedure and spiking chromatograms with authentic specimens showed the methanol insoluble alkaloid fraction to contain harmaline (**5**) and harmine (**6**) (figure 1), while the methanol-soluble alkaloid fraction was shown to contain harmaline (**5**), harmine (**6**), vasicine (**1**) and deoxyvasicinone (**7**) (figure 2).

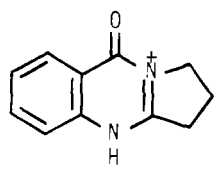
Samples of the various alkaloids were made available for *in vitro* antibacterial evaluation by preparative silica gel (70–230 mesh) column chromatography; the elutants were chloroform-methanol mixtures. The identities of the various alkaloids were confirmed by mixture mp, hplc, and spectroscopic comparisons with authentic samples. Interestingly, deoxyvasicinone (**7**) was isolated, in part, as its crystalline pseudohydrochloride salt (**8**).

This salt (**8**) has ir, uv and pmr (but not ms) spectra substantially different from that of deoxyvasicinone because of the tautomerism involved, and this confused its identification for a while: mp 187–189°; ir, ν ($CHCl_3$) 2992, 2886, 1660, 1620, 1600, 1550, 1445, 1400 (sh), 1360, 1310, 1130, 1080, 990, 950 (sh) and 880 cm^{-1}; uv λ max (MeOH) 210.5 nm (ϵ 31500), 265 (10600), 282 (8700), 290 (4700) and 312.5 (4100); pmr δ ($CDCl_3$) 1.56 (2H, s), 2.45 (2H, s), 3.99 (2H, s), 7.13–8.04 (4H, m), 8.73 (1H, d), 8.78 (1H, m); and eims *m/e* 186, 185 (100%), 160, 144, 130, 119, 103, 102,

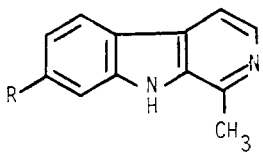


(1), X=H_2, R=OH

(7), X=O, R=H



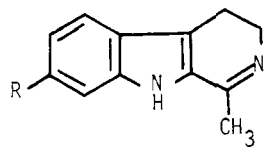
(8)



(6), R=OCH_3

(3), R=OH

(4), R=H



(2), R=OH

(5), R=OCH_3

93, 90, 82, 76 and 75. Deoxyvasicinone: mp 104–6°; ir, ν (KBr) 2600, 2150, 1825, 1700, 1660, 1610 (sh), 1560, 1475, 1410, 1380, 1335, 1300, 1280, 1170, 1065, 1010, 935 (sh), 880, 850 (sh), 750 and 670 cm^{-1}; uv λ max (EtOH) 222.5 nm (ϵ 33000), 262.5 (9600), 271 (9590), 303 (4800) and 314 (3900); pmr, δ ($CDCl_3$) 2.29 (2H, m), 3.43 (2H, t), 4.20 (2H, t), 7.30–7.80 (3H, m) and 8.00 (1H, d); and eims *m/e* 186, 185 (100%), 160, 144, 130, 119, 103, 102, 93, 90, 82, 76, and 75. Deoxyvasicinone, synthesized for comparison (3), could be converted to the pseudohydrochloride salt by treatment with 5% ethanolic HCl, from which it crystallized upon cooling, and the pseudohydrochloride could be converted to deoxyvasicinone by basification (10% NH_4OH) and methylene chloride extraction.

From these results it is clear that the broad spectrum activity of Iraqi *P. harmala* is primarily due to harmine (**6**). The other alkaloids found, harmaline (**5**), vasicine (**1**), and deoxyvasicinone (**7**), are inactive at the top level tested (100 mcg/ml). It is interesting to note that the quinazoline bases (**1** and **7**) are not active. That the pyridine ring may not be reduced if activity is to be retained is shown by the excellent activity of harmol (**3**) and the inactivity of harmalol (**2**). Bases **2**, **3**, and **4** were not detectable in our extracts by careful hplc examination.

P. harmala has an ancient reputation as an antiseptic and for treatment of skin diseases (4). The smoke of the plant has been used as a disinfectant (4). Nevertheless, the active

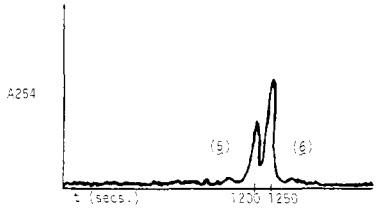


Figure 1. HPLC of MeOH-insoluble alkaloids of P. harmala.

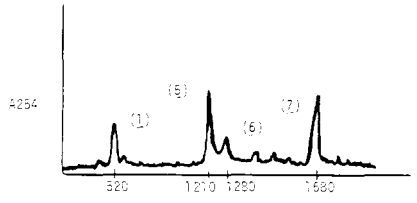


Figure 2. HPLC of MeOH-soluble alkaloids of P. harmala.

agent (evidently harmine) has not previously been identified. The finding that such a relatively simple and well-known β-carboline alkaloid possesses antimicrobial activity and that this is shared by related simple bases is of some interest and recalls the previous finding that the canthin-6-one series also possessed activity of this type (5).

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