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ALKALOIDS OF *HAPLOPHYLLUM TUBERCULATUM* FROM IRAQ

ALI AL-SHAMMA*†, NEDHAL AHMED AL-DOURI† and J. DAVID PHILLIPSON‡

† Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Iraq; and ‡ Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1N 1AX, U.K.

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Key Word Index—*Haplophyllum tuberculatum*; Rutaceae; Iraq; alkaloids; γ-fagarine; skimmianine; evoxine.

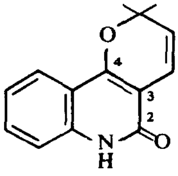
INTRODUCTION

There are 4 species of *Haplophyllum* (family Rutaceae) which are native to Iraq, namely *H. blanchei* Boiss., *H. filifolium* (Spach) Boiss., *H. tuberculatum* (Forssk.) Adr.-Juss. and *H. mesopotamicum* Boiss. [1]. The most wide-spread species is *H. tuberculatum* (syn. *H. glabrum* (DC.) Hand.-Mzt., *H. obovatum* (Steud.) Hand.-Mzt., *H. propinquum* Spach) which is known locally as juwaifah. This common perennial herb is found wild, even growing as a common weed among summer crops [2]. Decoctions of the plant are recommended by herbalists for preparations used as carminatives for children. Alkaloids have been found in *H. tuberculatum* growing in Palestine but there is no reference to the presence of alkaloids in Iraqi plants. The leaves and stems of the Palestinian species are reported to contain flindersine (1) and 3-dimethylallyl-4-dimethylallyloxy-2-quinolone (2) [3]. The present investigation was undertaken to determine the identity of any alkaloidal constituents in Iraqi *H. tuberculatum*.

RESULTS AND DISCUSSION

H. tuberculatum, from Iraq, yielded 3 known furoquinoline alkaloids, γ-fagarine (3), skimmianine (4) and evoxine (5). The pyrano-quinoline alkaloid, flindersine (1) was available as a reference compound and its presence was not detected in any of the alkaloidal extracts. The identity of γ-fagarine was established by means of IR and MS, both of which clearly indicated the furoquinoline nature of the alkaloid. Accurate mass determinations on the M^+ peak, which occurred at *m/e* 229, gave a molecular formula of $C_{13}H_{11}NO_3$. The high percentage relative abundance of the $M^+ - 15$ and the $M^+ - 43$ ions were indicative of the C-4 OMe substituent, while the presence of fragments due to $M^+ - 1$ (6) and $M^+ - 29$ were characteristic of the C-8 OMe substituent [4]. These latter two fragment ions are not found in furoquinoline alkaloids which lack a C-8 OMe substituent. Confirmation of the identity of γ-fagarine was obtained by direct TLC comparison with an authentic sample. Skimmianine (4) was also identified mainly on the basis of its MS which was identical with that previously published [4]. The MS fragmentation was generally similar to that of γ-fagarine but the M^+ ion was 30 amu larger and the base peak was the $M^+ - 15$ ion. The presence of C-4 and C-8 OMe substituents was indicated by the use of the same arguments as those given above for γ-fagarine. The additional 30 amu were thought most likely to be due to the presence of a OMe substituent which was at either C-5, C-6 or C-7. Direct TLC comparison with authentic alkaloid indicated that the alkaloid was skimmianine (4). In addition, the IR was identical with that reported for skimmianine [5] and the mp was not depressed by admixture with authentic alkaloid, thus clearly confirming the identity as skimmianine (4).

Evoxine (5) was identified on the basis of its UV which indicated the presence of the furoquinoline chromophore and by means of MS and ^1H NMR. The MS gave the M^+ at *m/e* 347 and accurate mass determinations established the molecular formula as $C_{18}H_{21}NO_6$. The



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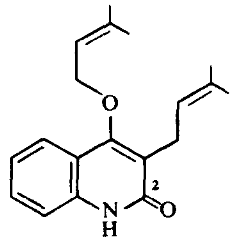

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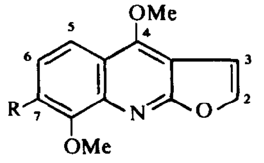
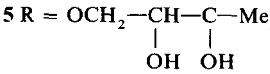
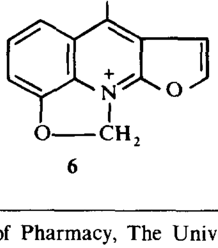


3 R = H
4 R = OMe


$$5\ R = OCH_2\underset{OH}{\underset{|}{CH}}-\underset{OH}{\underset{|}{C}}-Me_2$$



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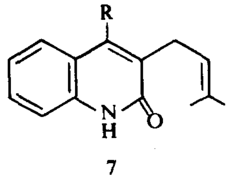

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* Present address: School of Pharmacy, The University of Kansas, Lawrence, KS 66045, U.S.A.

^{1}H NMR spectrum showed the presence of two OMe groups and the MS fragment ions at $M^+ - 15/M^+ - 43$ and at $M^+ - 1/M^+ - 29$ again indicated that the groups were located at C-4 and C-8, respectively [4]. The presence of two AB quartets in the aromatic region of the ^{1}H NMR provided confirmation of the furan protons (δ 7.03 and 7.57, $J = 2.5$ Hz) and of two adjacent aromatic protons (δ 7.18 and 8, $J = 10$ Hz). Hence the additional substituent must be located at either C-5 or C-7, the latter being preferred on biogenetic grounds [6]. ^{1}H NMR indicated that there were two tertiary Me groups, a CHOH and O—CH_2 group in the molecule. This additional substituent possessed an additional 118 amu more than γ-fagarine and fragmented in MS by loss of 59 amu (*m/e* 288) which could be accounted for by loss of $Me_2\overset{|}{C}$—OH and then by subsequent loss of 30 amu ($M^+ - 89$) possibly due to loss of —CHOH. This type of fragmentation is similar to that reported for the coumarin myrsellinol [7] and is due to a 3′-methyl-2′,3′-dihydroxybutyloxy-substituent. The alkaloid was therefore either the known evoxine (**5**) or an analogue with a C-5 substituent. Confirmation of the identity as evoxine was obtained by direct TLC comparison with authentic evoxine. The alkaloids from two other collections of *H. tuberculatum* collected in different parts of Iraq were also identified as γ-fagarine, skimmianine and evoxine.

The 3 alkaloids reported in this paper have been reported previously in other species of *Haplophyllum*, γ-fagarine in 4 species, skimmianine in 13 species and evoxine in 4 species [6]. However, the unusual feature reported here is that these alkaloids are distinctly different from those previously reported from the same species growing in Palestine. Apparently there are at least two different chemical strains of *H. tuberculatum*. The common precursors of both types of these alkaloids, the furoquinolines (Iraqi plants) and the angular pyranoquinoline, flindersine, (Palestinian plants) are substituted 3-dimethylallyl-2-quinolines (**7**). In the Palestinian plants, cyclization can presumably occur via coupling of the 3-dimethylallyl side chain and a OH at C-4. If prior methylation of the C-4 OH occurs in Iraqi plants then cyclization can only take place at C-2 giving rise to the furoquinoline-type alkaloids [6].

EXPERIMENTAL

Plant material was collected in the flowering stage during April 1975 some 10 km east of Fallujah. Herbarium material is retained in the College of Pharmacy, University of Baghdad and the samples were authenticated by the staff of the National Herbarium of Iraq. Smaller samples of *H. tuberculatum* were collected in April 1975, 5 km north of Kirkuk and from Al-Khalis Agricultural Garden, Baghdad. Extraction of these latter two samples by the methods described below and examination by TLC in several systems indicated that all 3 samples were identical in their alkaloid composition. All mps are uncorr. MS were determined by the Morgan–Schaffer Corporation and by the Mass Spectrometry Service, School of Pharmacy, University of London, at 70 eV. ^{1}H NMR spectra were recorded at 90 MHz.

Isolation of alkaloids. Dried, powdered aerial parts (1 kg) were extracted with hot EtOH until complete extraction had taken place. The filtered EtOH extract was concd to dryness (170 g) under red. pres. and an aliquot (100 g) suspended in $CHCl_3$–5% NH_4OH. The evapd alkaline layer was repeatedly extracted with $CHCl_3$ and the combined $CHCl_3$ extracts washed with H_2O, dried (Na_2SO_4), filtered and concd to low vol. under red. pres. The conc $CHCl_3$ extract was shaken with 5% HCl until no further alkaloid could be extracted. The combined acid extracts were made alkaline with conc NH_4OH, extracted repeatedly with $CHCl_3$ which was washed with H_2O, dried (Na_2SO_4), filtered and concd to dryness under red. pres. to yield total crude alkaloid (11 g, 1.8%). An aliquot of the total alkaloid (8.5 g) was fractionated on neutral Al_2O_3 (850 g, BDH) and eluted with $CHCl_3$–MeOH (99:1). A major alkaloid fraction (2.5 g) was chromatographed on a Si gel column (250 g; BDH) and eluted with $CHCl_3$. Alkaloids were obtained from 3 major fractions as follows: (1) The main alkaloid crystallized as yellow needles from EtOAc–hexane and was identified as γ-fagarine (0.3 g, 0.06%). (2) The main alkaloids were separated by PLC with Si gel GF_{254} (Merck) using $CHCl_3$–MeOH (99:1) into γ-fagarine (0.1 g) and skimmianine (0.72 g, 0.15%) which was further purified on a small column of neutral Al_2O_3 and obtained as yellow prisms from MeOH. (3) The main alkaloid was separated by PLC (as above) to yield evoxine (0.034 g, 0.06%) which crystallized from EtOAc.

Identification of alkaoids. γ-Fagarine, mp 138° (lit. 138–140°) [8]: TLC R_f values on Si gel G (Merck), 0.37 ($CHCl_3$–MeOH, 99:1), 0.28 ($CHCl_3$–C_6H_6, 4:1), 0.1 (toluene–EtOAc–HCOOH; 5:4:1) identical with authentic sample. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3155 and 3125 (C—H of furan), 1621 (CH=CH), 1261 (ether), 868 and 816 (C—H out of plane bending of furan ring), identical with lit. [9]. MS *m/e* (%): 229 (M^+; 100), 228 ($M^+ - 1$; 80), 214 ($M^+ - 15$; 29), 200 ($M^+ - 29$; 72), 184 ($M^+ - 45$; 27), 156 ($M^+ - 73$; 25) [4]. Metastable peak *m/e* 174.7 (*m/e* 229 → 200). Accurate mass determination, found 229.0744; $C_{13}H_{11}NO_3$ calculates for 229.0739. *Skimmianine*, mp 170–172° (lit. 169–171°) [5], not depressed by admixture with authentic alkaloid. TLC R_f values on Si gel G, 0.3 ($CHCl_3$–MeOH, 99:1), 0.16 ($CHCl_3$–C_6H_6, 4:1), 0.06 (toluene–EtOAc–HCOOH, 5:4:1), identical with authentic sample. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3120, 2970 (C—H), 1620 (CH=CH), 1270 (ether), 873 and 827 (C—H out of plane bending of furan ring), identical with authentic alkaloid [5, 9]. MS *m/e* (%): 259 (M^+; 93), 258 ($M^+ - 1$; 29), 244 ($M^+ - 15$; 100), 230 ($M^+ - 29$; 52), 216 ($M^+ - 43$; 26), 201 ($M^+ - 58$; 20). Accurate mass determination, found 259.0844; $C_{14}H_{13}NO_4$ calculates for 259.0844. *Evoxine*, mp 146–148° (lit. 153°) [10]. TLC R_f values on Si gel G, 0.92 (EtOAc–*iso*-PrOH–5% NH_4OH, 13:3:24), 0.27 ($CHCl_3$–Me_2CO, 5:4), 0.42 ($CHCl_3$–MeOH, 99:1), identical with authentic sample. UV (MeOH) nm: 250, 320–335 (sh) [10]. MS *m/e* (%): 347 (M^+; 77), 346 ($M^+ - 1$; 60), 332 ($M^+ - 15$; 17), 318 ($M^+ - 29$; 13), 288 ($M^+ - 59$; 20), 246 ($M^+ - 89$; 47), 245 (67), 230 (37), 228 (33), 227 (100), 216 (33), 215 (27), 202 (23), 199 (23), 59 (90), 43 (30). Accurate mass determination, found 347.1368; $C_{18}H_{21}NO_6$ calculates for 347.1369. ^{1}H NMR ($CDCl_3$): δ 1.25 and 1.27 (2 × 3H, *s*, Me_2—C—O—), 3.79 (1H, *m*, C$\underline{H}$ OH), *ca* 4.3 (2H, *m*, partly hidden, —C$\underline{H}_2$—O—Ar), 4.13 and 4.41 (2 × 3H, *s*, 2 × OMe), 7.03 and 7.57 (2H, AB *q*, $J = 2.5$ Hz, *s* C-2 and -3H), 7.18 and 8 (2H, AB *q*, $J = 10$ Hz, *s* C-5 and -6H).

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REFERENCES

1. Townsend, G. C. (1964) in *Flora of Lowland Iraq* (Rechinger,

K. H., ed.) p. 409. J. Cramer, Weinheim.
2. Guest, E. and Al-Rawi, A. (1966) *Flora of Iraq*, Vol. 1, pp. 93, 99, 102, 103. Ministry of Agriculture of the Republic of Iraq.
3. Lavie, D., Danieli, N., Weitman, R. and Glotter, E. (1968) *Tetrahedron* **24**, 3011.
4. Clugston, D. M. and Maclean, D. B. (1965) *Can. J. Chem.* **43**, 2516.
5. Chang, P. T. O., Cordell, G. A., Aynilan, G. H., Fong, H. H. S. and Farnsworth, N. R. (1976) *Lloydia* **39**, 134.
6. Waterman, P. G. (1975) *Biochem. Syst. Ecol.* **3**, 149.
7. Hifnawy, M. S., Vaquette, J., Pousset, J. L. and Cave, A. (1977) *Planta Med.* **31**, 156.
8. Grundon, M. F. and McCorkindale, N. J. (1957) *J. Chem. Soc.* 2177.
9. Briggs, L. H. and Colebrook, L. D. (1960) *J. Chem. Soc.* 2458.
10. Holubek, J. and Strouf, O. (1965) *Spectral and Physical Constants of Alkaloids*, Vol. 1, p. 115. Heyden, London.

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ALKALOIDS FROM *RAUWOLFIA PSYCHOTRIOIDES*

HERNÁN E. CÓRDOVA B. and CARMEN ALICIA PEÑA
Departamento de Química, Universidad de Oriente, Cumaná, Venezuela

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Key Word Index—*Rauwolfia psychotrioides*; Apocynaceae; alkaloids; harman; ethyl harman; rauvoxinine; triterpenes.

Rauwolfia psychotrioides H. B. & K. (Apocynaceae) is a perennial tree found in the northern part of South America, where it grows in a temperate climate.

The alkaloidal fraction of the leaves,* extracted with EtOH and isolated with 10% HOAc, showed the presence of many compounds by TLC, some of which were separated by preparative TLC. Compounds A and B showed UV spectra characteristic of non-oxygenated indole alkaloids [1] λ_{max}(EtOH) 347, 334, 287 (sh), 239 (sh), 234 and 212 nm. The IR spectra of these alkaloids showed bands for the N-H function (ν_{max}(KBr) 3125 cm^{-1}) and for the aromatic system (ν_{max}(KBr) 3060, 1625, 1560 and 1500 cm^{-1}). The mass spectral fragmentation for both alkaloids was relatively simple, A showing its molecular ion at *m/e* 182 and B at *m/e* 210. A was identified as harman based on the UV spectrum and the mass spectral fragmentation [2] showing the following ions: 182 (100, M$^+$), 181 (12, M$^+$ − H), 167 (12, M$^+$ − Me), 154 (18, M$^+$ − H − HCN), 127 (11, M$^+$ − H − 2 HCN). B was identified as ethyl harman because of the typical fragmentation pattern showing the following ions: 210 (24, M$^+$), 195 (18, M$^+$ − Me), 182 (100, M$^+$ − C_2H_4), 181 (24, M$^+$ − C_2H_5), 154 (18, M$^+$ − C_2H_5 − HCN), 127 (11, M$^+$ − C_2H_5 − 2 HCN). Another eluate from the TLC showed the same characteristics as harman, but its mass spectrum showed an additional small fragment at *m/e* 196, which seems to be due to some methyl harman impurity. The UV spectrum of alkaloid C showed absorptions at 216 and 280 nm, characteristic for the oxindole moiety [3] and was identified as rauvoxinine based mainly on its mass spectrum [4] which showed ions at *m/e* 428 (68, M$^+$), 413 (7, M$^+$ − Me), 411 (6, M$^+$ − OH), 397 (6, M$^+$ − OMe), 223 (67), 219 (25), 208 (26). The ion at 223 belonging to the alicyclic moiety of the molecule and by further loss of C-9 Me gives the fragment at *m/e* 208; one typical oxindole fragment occurs at *m/e* 219, which retains the triptamine bridge; the indole fragment occurring at *m/e* 206, 190 and 204.

From the neutral fraction, after column chromatography using silica gel and eluting with petrol, C_6H_6 and $CHCl_3$, lupeol, sitosterol and stigmasterol were isolated and identified according to their physical properties, spectroscopic evidence and comparison with authentic compounds.

It is noteworthy that while the *Rauwolfia* genus and the Apocynaceae family are very rich in indole complex alkaloids [5], it seems that harmine-type alkaloids, found in several families, i.e. Styraceae, Rubiaceae, Eleagnaceae, Malpighiaceae, Passifloraceae, Leguminosae, Loganiaceae and Polygonaceae, do not occur widely in this genus and family, *Rauwolfia psychotrioides* being one of the few species of this genus containing harmine-type alkaloids.

EXPERIMENTAL

Isolation of bases. The air-dried leaves (1.15 kg) were extracted by refluxing with 95% EtOH, HOAc was added and extracted with C_6H_6. The acid soln was basified with 10% NH_4OH precipitating a gummy material which was extracted with $CHCl_3$ after decanting. The alkaline soln was extracted with $CHCl_3$ and the chloroformic solns were combined, dried over Na_2SO_4 and evapd leaving 10.2 g of solid which were chromatographed over Si gel (1:25) and eluted with EtOH–HOAc–C_6H_6 (20:3:80; 20:3.6:80; 20:8:80) and EtOH. Partially separated alkaloids were obtained after alkalinizing with NH_4OH and were purified by preparative TLC using Si gel and eluting with a mixture of $CHCl_3$–MeOH (3.8:0.2). The separated bands were scraped off and extracted with Et_2O–MeOH (9:1) to recover the pure alkaloids. *Compound A.* Band VII (R_f 0.55) crystallizing as brownish crystals with mp 236–7° (Me_2CO)

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* Source: San Juan de Marcarapana, South of Cumaná (voucher specimen No. Cumana 0070 deposited in the University).