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ALKALOIDS OF *PERGULARIA PALLIDA*‡

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Key Word Index—*Pergularia pallida*; Asclepiadaceae; phenanthroindolizidine alkaloids; isolation; structural determination.

Abstract—Five phenanthroindolizidine alkaloids namely tylophorine, tylophorinidine, pergularinine, desoxypergularinine and an unidentified base (M^+ 409) have been isolated from the roots of *Pergularia pallida* plants.

INTRODUCTION

Phenanthroindolizidine* alkaloids have been isolated from *Tylophora* species (Asclepiadaceae) [2] and a few of them also occur in *Antitoxicum funebre* Boise and Kotschy [3] (Asclepiadaceae), *Ficus septica* [4] (Moraceae), *Vincetoxicum officinale* [5] (Asclepiadaceae) and *Cyananchum vincetoxicum* [6] (L.) Pers (Asclepiadaceae). These alkaloids have been the subject of great interest because of their reported anticancer properties [7].

Recently a new phenolic alkaloid, tylophorinidine, was isolated from *Tylophora asthmatica* by one of us (N.B.M.) [8a,b] and a search for new members of this group of alkaloids in other plants has led us to the isolation of five such alkaloids from *Pergularia pallida* (Asclepiadaceae) plant which is hitherto univestigated.

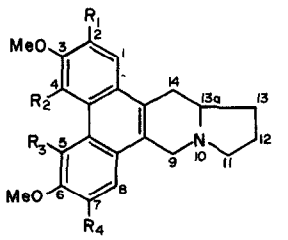
RESULTS AND DISCUSSION

The total alkaloidal mixture isolated from the roots of this plant in 0.08% yield was triturated with dry EtOH to yield an insoluble basic residue with some of the bases being retained in the solution. The latter on concentration yielded a crystalline base, $C_{22}H_{23}NO_4$, which was found to be identical with tylophorinidine (**4**) by mp, mmp, TLC, UV and IR with an authentic sample isolated from *T. asthmatica*. Its identity was further confirmed from comparisons of the diacetate (**7**) and monoMe ether (**5**) derivatives.

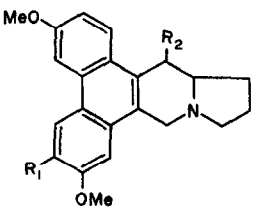
The EtOH-insoluble mixture was dissolved in $CHCl_3$ and chromatographed on alumina with solvents of increasing polarity. Alkaloids A and D were obtained pure in initial and later fractions respectively while a mixture of alkaloids B and C was obtained in the intermediate fractions. The mixture of alkaloids B and C was later separated on a column of Florisil.

Alkaloid A, $C_{23}H_{25}NO_3$, had properties characteristic of a phenanthroindolizidine derivative and this was further confirmed from the MS fragment at *m/e* 294 arising from cleavage of pyrrolidine moiety. The MS also indicated the presence of three OMe groups. It was found to be identical with 3,6,7-trimethoxyphenanthroindolizidine (**6**), a desoxy base obtained from alkaloid C (**5**). Its IR spectrum was almost the same as that of DL-3,6,7-trimethoxyphenanthroindolizidine obtained from DL-tylophorinine (**5**).

Alkaloid B was found to be tylophorine (**1**) on the basis of its mp UV, IR, MS and TLC behaviour, when compared with an authentic sample isolated from *T. asthmatica*.



(**1**) $R_1 = R_4 = OMe$; $R_2 = R_3 = H$
(**2**) $R_1 = R_3 = OMe$; $R_2 = R_4 = H$
(**3**) $R_1 = R_3 = H$; $R_2 = R_4 = OMe$



(**4**) $R_1 = R_2 = OH$
(**5**) $R_1 = OMe$; $R_2 = OH$
(**6**) $R_1 = OMe$; $R_2 = H$
(**7**) $R_1 = R_2 = OAc$
(**8**) $R_1 = OMe$; $R_2 = OAc$

Alkaloid C, $C_{23}H_{25}NO_4$, was also a phenanthroindolizidine derivative. Its IR spectrum (KBr) showed absorption at *ca* 3175 cm^{-1} indicating the presence of a bonded OH. The absence of any bathochromic shift in the UV spectrum with alkali confirmed that the OH group was not phenolic. The MS showed a base peak at *m/e* 310 arising by cleavage of the pyrrolidine ring by a retro-Diels–Alder reaction characteristic of phenanthroindolizidine alkaloids. A strong peak at *m/e* 281 arising from that at 310 by loss of CHO was indicative of the presence of an OH at C-14.† The IR spectra of alkaloid C and of the monoMe ether of tylophorinidine (**5**) were nearly superimposable except that the latter revealed two additional absorptions at 871 and 735 cm^{-1} while an extra peak at 851 cm^{-1} was seen in alkaloid C. However, *O*-methyltylophorinidine possessed a (+) rotation but the alkaloid C showed a (−) rotation. This led to the conclusion that alkaloid C and *O*-methyltylophorinidine (**5**) were related to each other as diastereoisomers. Since this new stereoisomer has been obtained from a natural source, it has been named as pergularinine.

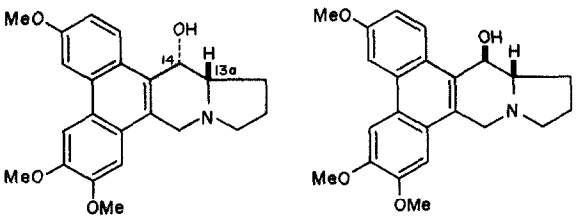
* 9,11,12,13,13a,14-Hexahydrodibenzo (f,h)-pyrrolo (1,2-b) isoquinoline.

† In some cases M^+-28 due to loss of C=O ion is also observed.

‡ See ref. [1].

The IR spectra of pergularinine and *O*-methyltylophorinidine showed the presence of a band at 3175 cm^{-1}. This can be attributed to the C14-OH where the OH group of one molecule is bonded with the lone pair of electrons on the nitrogen of the other. This was confirmed by the preparation of the corresponding methiodides in which well defined OH absorption could clearly be seen at 3450 cm^{-1} because the lone pair of electrons were no longer available on the nitrogen for bonding.

O-Methyltylophorinidine with (+) rotation has been shown to exist as **9A** in which the C14-OH and C13a-H are *trans* diaxially disposed. The OH is below the plane of the molecule and the C13a-H above it [8b]. Therefore, diastereoisomeric pergularinine with a (−) rotation could possess the structure **9B** or its mirror image. ORD studies of desoxypergularinine (**6**) in $CHCl_3$ showed a negative Cotton effect of the same order of magnitude in the region 270 nm as observed in tylophorine (**1**). This indicated that the two compounds possessed the same absolute configuration at C13a. In tylophorine C13a-H has been shown to be above the plane [9]. Therefore, pergularinine itself could be assigned the configuration shown in **9B**. (−)-Tylophorinine (**5**) having the same substitution has been shown to be racemic [10].



9A **9B**

One interesting observation has been made during hydrogenolysis of (−)-pergularinine and (+)-*O*-methyltylophorinidine. The latter gives the racemic desoxy base while (−)-pergularinine furnishes the (−)-desoxy base.

In alkaloid D, $C_{24}H_{27}NO_5$, the alcoholic OH was located at C14 since the loss of 29 amu (CHO) was observed at *m/e* 311 in its MS from a fragment arising by the cleavage of pyrrolidine ring (*m/e* 340). This group was not involved in intermolecular H bonding since the OH absorption in its IR is quite pronounced unlike that in *O*-methyltylophorinidine and pergularinine. Alkaloid D contains four OMe groups and its UV values resembled those of tylophorine (**1**) more than those of tylocrebrine (**2**) and isotylocrebrine (**3**), the alkaloids with four OMe groups already known. Tylophorine itself has been isolated from this plant. Since the yield of this compound was extremely low, the exact substitution pattern could not be confirmed at this stage.

EXPERIMENTAL

Mp's (uncorr) were determined using a Fisher–John's block. UV spectra were run in MeOH. The various fractions isolated by column chromatography were examined by TLC on alumina G using $CHCl_3$–EtOAc (9:1).

Preliminary separation of alkaloids. Air-dried roots (1 kg) of *Pergularia pallida* plants grown at Experimental Field Station, Trombay collected in May, were macerated (×3) with EtOH (3 l.) containing 2% HOAc at room temp. Combined extracts were concentrated under red. pres. to yield a brown viscous material which was treated with H_2SO_4 (0.5 N, 1 l.) and the acidic extract washed with Et_2O until free of neutral components. The aq. acidic layer was then made alkaline (pH 9) with NH_4OH (30% soln) and repeatedly extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried and evaporated *in vacuo* to yield a brownish material (0.8 g, 0.08% yield) containing total bases. This was triturated with dry EtOH and the insoluble bases (500 mg) filtered off. The alcohol-soluble portion was concentrated and left overnight. A pale yellow solid separated out which was filtered off. The mother liquor was further concentrated and left overnight to yield a significant amount of a crystalline base (80 mg). Repetition of the above process yielded more of the compound (20 mg). It was crystallized from dry EtOH as shining plates, mp 213–214° (decomp), $[\alpha]_D^{25}$ + 125° (*c* 0.1, MeOH) UV: λ_{max}^{MeOH}258, 286.5, 310, 339.5, 355 nm (log ε 4.7, 4.43, 3.9, 3.26, 2,8); with KOH λ_{max}^{MeOH} 258, 296.5, 328.5, 352.5, 368 nm (log ε 4.64, 4.45, 3.99, 3.79, 3.47); IR: ν_{max}^{KBr} 3448, 1613, 1538, 1515, 1250, 1200 cm^{-1}; MS *m/e* 365 (M^+, $C_{22}H_{23}NO_4$, 22%), 296 (100), 281 (10), 268 (10), 267 (16), 253 (9), 225 (13), 210 (6), 209 (8), 182.5 (6), 181 (15), 165 (16), 163 (10), 152 (17), 70 (55) identified as tylophorinidine (**4**). *O*-Methyltylophorinidine (**5**). A soln of tylophorinidine (25 mg) in MeOH was treated with excess ethereal CH_2N_2. Removal of the solvent *in vacuo* yielded a pale yellow solid (15 mg), which was crystallized from $CHCl_3$–dry EtOH, mp 215–216° (decomp); $[\alpha]_D^{25}$ + 116° (*c* 0.25, $CHCl_3$); UV: λ_{max}^{MeOH} 260, 287, 313, 340, 357 nm (log ε 4.7, 4.4, 3.8, 3.3, 2.7); IR: ν_{max}^{KBr} 3175, 1613, 1527, 1515, 1250 cm^{-1}; MS *m/e* 379 (M^+, $C_{23}H_{25}NO_4$ 80%), 310 (100), 295 (15), 281 (18), 267 (6), 251 (6), 237 (12), 224 (20), 223 (13), 208 (10), 196 (10), 195 (11), 189.5 (15), 181 (10), 177 (8), 176 (12), 165 (25), 163 (15), 152 (24), 70 (40). Diacetyltylophorinidine (**7**). Tylophorinidine (39 mg) was refluxed in dry C_6H_6 with Ac_2O (0.9 ml) and C_5H_5N (0.3 ml) for 26 hr in an oil bath. Evaporation of the solvent *in vacuo* yielded a pale brown mass which was crystallized from MeOH to give a colourless solid (18 mg), mp 191–193° (decomp); $[\alpha]_D^{25}$ + 151.3° (*c* 0.75, $CHCl_3$); UV: λ_{max}^{MeOH} 254, 271, 287, 310, 336, 350 nm (log ε 4.6, 4.4, 4.2, 3.9, 3.3, 3.1); IR: ν_{max}^{KBr} 1757, 1725, 1613, 1515, 1235 cm^{-1}; MS *m/e* 449 (M^+, $C_{26}H_{27}NO_6$), 389 (26), 347 (15), 320 (32), 292 (9), 278 (100), 263 (7), 235 (6), 220 (2), 192 (2), 163 (5).

Separation of alcohol-insoluble alkaloids. The alcohol-insoluble mixture of bases (500 mg) was chromatographed on a column of alumina using solvents of increasing polarity. Alkaloids A (15 mg) and B (30 mg) in pure form were eluted using C_6H_6–$CHCl_3$ (9:1) and (17:3) respectively. A mixture of alkaloids B and C (250 mg) was obtained in latter fractions. Further elution with C_6H_6–$CHCl_3$ (4:1) yielded pure alkaloids C (20 mg) and D (10 mg). Tylophorinidine (15 mg) was finally eluted with $CHCl_3$–EtOAc (1:1). Fractions containing mixture of alkaloids B and C were pooled and the solvent evaporated *in vacuo*. The residue (250 mg) was chromatographed on a column of Florisil. Elution with C_6H_6–EtOAc (7:3) yielded alkaloid C (80 mg) and alkaloid B (40 mg) was eluted with EtOAc–MeOH (9:1).

Identification of alkaloids. 3,6,7-Trimethoxyphenanthroindolizidine (**6**). Alkaloid A crystallized as pale yellow globules from $CHCl_3$–dry EtOH mp 208° (decomp); $[\alpha]_D^{25}$ − 13.6° (*c* 0.25, $CHCl_3$); UV: λ_{max}^{MeOH} 258, 286, 311, 341, 360 nm (log ε 4.3, 3.9, 3.5, 3.1, 2.6); IR: ν_{max}^{KBr} 1610, 1530, 1515, 1250 cm^{-1}; MS *m/e* 363 (M^+, $C_{23}H_{25}NO_3$, 85%), 294 (100), 279 (20), 251 (12), 236 (10), 220 (9), 208 (20), 189 (12), 181.5 (34), 165 (26), 70 (5). This was identified as the desoxy form of alkaloid C.

Tylophorine (**1**). Alkaloid B crystallized from $CHCl_3$–dry EtOH had mp 287–288° (decomp): $[\alpha]_D^{25}$ − 21.45° (*c* 1.1, $CHCl_3$); UV: λ_{max}^{MeOH} 259, 291, 340, 357 nm (log ε 4.6, 4.4, 3.2, 2.8); IR: ν_{max}^{KBr} 1612, 1538, 1514, 1250 cm^{-1}; MS *m/e* 393 (M^+, $C_{24}H_{27}NO_4$, 73%), 325 (60), 324 (100), 309 (20), 281 (10), 266 (12), 251 (11), 223 (8), 196.5 (12), 196 (7), 189 (12), 181 (17), 162 (29), 152 (15), 70 (6). Alkaloid B was found to be identical with tylophorine.

Pergularinine (**5**). Alkalid C crystallized from $CHCl_3$–dry EtOH as pale yellow globules, mp 233–235° (decomp);

$[\alpha]_D^{25}$ − 16° (*c* 0.25, $CHCl_3$); UV: λ_{max}^{MeOH} 260, 287, 313, 341, 357 nm (log ε 4.7, 4.4, 3.9, 3.2, 2.8); IR: ν_{max}^{KBr} 3175, 1613, 1539, 1515, 1255 cm^{-1}; MS *m/e* 379 (M^+, $C_{23}H_{25}NO_4$, 83%) 310 (100), 295 (19), 281 (21), 267 (8.5), 251 (6), 237 (14.6), 224 (24), 223 (15), 208 (10), 196 (12), 195 (11), 189.5 (17), 181 (10), 177 (10), 176 (12), 165 (27), 163 (15), 152 (27), 70 (46). Alkaloid C was characterized as the diastereoisomer of *O*-methyltylophorinidine.

O-Acetylpergularinine (**8**). Alkaloid C (30 mg) was heated at 50–60° for 4 hr with Ac_2O (0.5 ml) and C_5H_5N (0.2 ml). The residue obtained after removal of the solvent was diluted with H_2O, the soln made alkaline with NH_4OH (30% soln) and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried and evaporated *in vacuo* to yield a product which crystallized from MeOH to give white globules (15 mg), mp 177–178° (decomp); $[\alpha]_D^{25}$ − 17.4° (*c* 0.112, $CHCl_3$); UV: λ_{max}^{MeOH} 258, 286, 313, 340 nm (log ε 4.5, 4.2, 3.7, 3.9); IR: ν_{max}^{KBr} 1725, 1615, 1539, 1510, 1250 cm^{-1}; MS *m/e* 421 (M^+, $C_{25}H_{27}NO_5$, 5%), 361 (100), 360 (83), 352 (42), 344 (8), 333 (52), 324 (53), 310 (75), 309 (37), 294 (20), 281 (10), 251 (3), 237 (6), 223 (6), 181 (8), 180.5 (20), 165 (10), 163 (13), 152 (8), 150 (9), 124 (9), 70 (8).

Desoxypergularinine (**6**). A soln of alkaloid C (80 mg) in AcOH (15 ml) containing $HClO_4$ (60–70%, 0.3 ml) was shaken with H_2 at 3 kg/cm^2 in the presence of Pd–C (10%, 60 mg) for 6 hr at 50–60°. After filtration and evaporation of the solvent *in vacuo*, the residue was made alkaline with NH_4OH (30%) and extracted with $CHCl_3$. Removal of solvent yielded the desoxy base which was crystallized from $CHCl_3$–dry EtOH as white globules (30 mg), mp 208–209° (decomp); $[\alpha]_D^{25}$ − 13.6° (*c* 0.25, $CHCl_3$); UV: λ_{max}^{MeOH} 257, 286, 311, 340, 360 nm (log ε 4.2, 3.9, 3.4, 3.0, 2.6); IR: ν_{max}^{KBr} 1610, 1530, 1515, 1250 cm^{-1}; MS *m/e* 363 (M^+, $C_{23}H_{25}NO_3$, 90%), 294 (100), 279 (18), 251 (10), 236 (8), 220 (8), 208 (20), 189 (9), 181.5 (30), 165 (25), 70 (5). This compound was found to be identical with alkaloid A.

Pergularinine methiodide. Alkaloid C (25 mg) was refluxed in $CHCl_3$ with excess MeI for 2 hr. The solvent was removed *in vacuo*, the residue (15 mg) washed with MeOH and crystallized from MeOH–H_2O to yield pale yellow needles, mp 200–205° (decomp); UV: λ_{max}^{MeOH} 258, 285, 310, 341, 357 nm (log ε 4.7, 4.4, 3.9, 3.2, 2.8); IR: ν_{max}^{KBr} 3450, 1615, 1530, 1510, 1250 cm^{-1}.

Alkaloid D. The base was obtained as pale yellow globules from $CHCl_3$–dry EtOH, mp 210–212° (decomp); $[\alpha]_D^{25}$ − 9.4° (*c* 0.04, $CHCl_3$); UV: λ_{max}^{MeOH} 258, 287, 302, 339, 355 nm (log ε 4.4, 4.2, 3.6, 3.1, 2.9); IR: ν_{max}^{KBr} 3480, 1614, 1535, 1510, 1250 cm^{-1}; MS *m/e* 409 (M^+, $C_{24}H_{27}NO_5$, 19%), 340 (100), 325 (11), 311 (8), 310 (15), 309 (9), 297 (6), 282 (7), 254 (8), 239 (6), 211 (4), 196 (3), 168 (3), 204.5 (5), 70 (45). Alkaloid D was not further characterized.

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