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ARISTOLACTAMS AND 4,5-DIOXOAPORPHINES FROM *PIPER LONGUM*

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Key Word Index—*Piper longum*, Piperaceae, root alkaloids; aristolactams; dioxoaporphines; piperolactam A, piperolactam B; piperadione; NMR.

Abstract—Nine alkaloids were isolated from the cold ethanol extract of *Piper longum* roots, of which six known compounds were identified as cepharadione B, cepharadione A, cepharanone B, aristolactam AII, norcepharadione B, and 2-hydroxy-1-methoxy-4H-dibenzo[*de*, *g*]quinoline-4,5(6H)-dione. The three new alkaloids were characterized as 10-amino-4-hydroxy-3-methoxyphenanthrene-1-carboxylic acid lactam [piperolactam A], 10-amino-4-hydroxy-2,3-dimethoxyphenanthrene-1-carboxylic acid lactam [piperolactam B] and 2-hydroxy-1-methoxy-6-methyl-4H-dibenzo[*de*, *g*]quinoline-4,5(6H)-dione [piperadione]

INTRODUCTION

The roots of *Piper longum*, commonly known as 'Piplimool' are used in the indigenous system of medicine [1]. The presence of some amides and lignans has been reported earlier from this plant [2–4]. In the present study, an investigation of the cold ethanol extract of the roots yielded four aristolactams, viz. cepharanone B (**1**), aristolactam AII (**2**), 10-amino-4-hydroxy-3-methoxyphenanthrene-1-carboxylic acid lactam [piperolactam A] (**3**) and 10-amino-4-hydroxy-2,3-dimethoxyphenanthrene-1-carboxylic acid lactam [piperolactam B] (**4**). The five 4,5-dioxoaporphines were characterized as cepharadione B (**5**), cepharadione A (**6**), norcepharadione B (**7**), 2-hydroxy-1-methoxy-6-methyl-4H-dibenzo [*de*, *g*] quinoline-4,5(6H)-dione [piperadione] (**8**) and 2-hydroxy-1-methoxy-4H-dibenzo [*de*, *g*]quinoline-4,5-(6H)-dione (**9**). Of these, compounds, **3**, **4** and **8** are new natural products. The known compounds, viz. **1** [5], **2** [5], **5**, [6], **6**, [6], **7** [7], and **9** [8] were identified by comparing their physical and spectroscopic properties with those reported in the literature. So far, the presence of **5** and **6** in *P. auritum* [9] and *P. sanctum* [10] form the only examples of aporphine alkaloids in the Piperaceae. This is the first report dealing with the isolation of aristolactams from the Piperaceae.

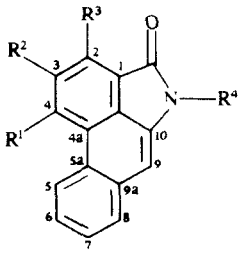
RESULTS AND DISCUSSION

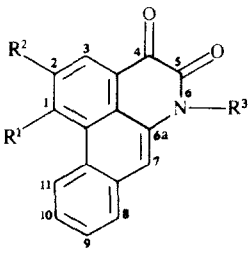
The cold ethanol extract on CC gave piperlongumine as the major compound, subsequent prep. TLC of the polar fractions yielded aristolactams (**1–4**) and 4,5-dioxoaporphines (**5–9**) as minor components. The structural determination of the new alkaloids **3**, **4** and **8** only is described below.

Compound **3**, mp 303–306° decomposition, ([M]$^+$ *m/z* 265), shared many structural features in common with cepharanone B (**1**) and aristolactam AII (**2**). Its IR spectrum indicated the presence of OH (3320 cm^{-1}), NH (3220 cm^{-1}) and conjugated CO (1710 cm^{-1}) groups. It displayed UV absorption nm (log ε) [234 (4.61), 263 (4.50), 276 (4.56), 286 (4.56), 320 (4.01) and 384 (3.96)] characteristic of a phenanthrene chromophore. The ^{1}H NMR spectrum, which revealed the presence of an aromatic methoxyl at δ 4.02 (3H), was strikingly similar to that of aristolactam AII (**2**) (Table 1). The only exception was the H-5 proton which appeared rather upfield at δ 9.12 (1H), in comparison to the similar proton in **2** (δ 9.64) This could be due to the shielding effect of a hydroxyl group at C-4. This was confirmed by the fact that both **2** and **3** on treatment with diazomethane yielded a compound identical (co-TLC, mass spectrum) with cepharanone B. On the basis of above observations the structure of compound **3** was elucidated to be 10-amino-4-hydroxy-3-methoxyphenanthrene-1-carboxylic acid lactam. This has beeen named by us as piperolactam A

A similar approach was adopted to determine the structure of aristolactam **4**, mp 212–214°, ([M]$^+$ *m/z* 295) indicating one more methoxyl group in the molecule than in **3**. It had UV nm (log ε) [248 sh (4.58), 254 sh (4.67), 260 (4.72), 285 (4.51), 296 sh (4.42), 335 (4.01), 379 (3.89), 402 (3.75)] and IR absorptions at OH,NH (3360 cm^{-1}) and CO (1650 cm^{-1}). A study of the its ^{1}H NMR spectrum indicated it to be a 2,3,4-trisubstituted aristolactam (Table 1). The signal at δ 8.96 which was assigned to H-5 appeared upfield as compared to that in **4a** and **4b** (δ 9.20 and 9.10, respectively). This was suggestive of the shielding effect of a C-4 hydroxyl (Table 1). The two singlets at δ 3.90 (3H) and δ 4.03 (3H) were attributed to two aromatic methoxyls which were placed at C-2 and C-3, respectively, since the signal for H-2 was conspicuously absent in **4**. Finally, the position of these aromatic methoxyl groups were unambiguously established by the application of 2D NOE (Fig. 1). As expected no effect could be observed between H-5 and any of the methoxyl groups On the basis of above results the structure of **4** was established as 10-amino-4-hydroxy-2,3-dimethoxyphenanthrene-1-carboxylic acid lactam. This was named as piperolactam B

Compound **8**, mp 273–276° decomposition, ([M]$^+$ *m/z* 307), had UV (245, 290 sh, 302, 314 and 440 nm) absorptions similar to that of 4,5-dioxoaporphines **5–7** and **9**.





1 $R^1 = R^2 = OMe$, $R^3 = R^4 = H$
2 $R^1 = OMe$, $R^2 = OH$, $R^3 = R^4 = H$
3 $R^1 = OH$, $R^2 = OMe$, $R^3 = R^4 = H$
4 $R^1 = OH$, $R^2 = R^3 = OMe$, $R^4 = H$
4a $R^1 = OAc$, $R^2 = R^3 = OMe$, $R^4 = Ac$
4b $R^1 = R^2 = R^3 = OMe$, $R^4 = H$
5 $R^1 = R^2 = OMe$, $R^3 = Me$
6 $R^1 + R^2 = OCH_2O$ $R^3 = Me$
7 $R^1 = R^2 = OMe$ $R^3 = H$
8 $R^1 = OMe$, $R^2 = OH$ $R^3 = Me$
8a $R^1 = OMe$, $R^2 = OAc$, $R^3 = Me$
9 $R^1 = OMe$, $R^2 = OH$ $R^3 = H$

Table 1 ^{1}H NMR chemical shifts of aristolactams

Compound	H-2	H-5	H-6, H-7	H-8	H-9	MeO-2	MeO-3	MeO-4	AcO-4	N-H	N-Ac
1	7·90*	9·16, *m*	7·61, *m*	7·98, *m*	7·18	-	- 4·08	—	- -	10·83	
	7·88†	9·13, *d*	7·59, *m*	7·98, *d*	7·16		4·05	4·06	--	10·87	
2	7·58*	9·64, *m*	7·47, *m*	7·89, *m*	7·08	—	-	4·00	—	10·32	--
3	7·62†	9·12, *d*	7·58, *m*	7·95, *d*	7·10	-	4·02	—	—	10·81	-
4	-*	8·98, *m*	7·48, *m*	7·94, *m*	7·30	3·94	4·08	--		10·77	
	--†	8·96, *d*	7·42, *m*	7·88, *d*	7·15	3·90	4·03	—		10·47	
4a	—*	9·20, *d*	7·73, *m*	8·17, *d*	8·46	3·98	4·21	—	- ‡		2·69
	--§	9·23, *d*	7·66, *m*	7·99, *d*	8·50	4·02	4·23	--	2·55	-	2·80
4b	—†	9·10, *d*	7·57, *m*	7·98, *d*	7·27	3·93	4·12	4·39	—	11·00	

Values reported in δ (ppm) from TMS, singlets not mentioned, *d*, $J = 8$ Hz
*100 MHz (DMSO-d_6)
†500 MHz (DMSO-d_6)
‡Signal masked in DMSO peak
§500 MHz ($CDCl_3$)

An alkali-induced bathochromic shift suggested the presence of at least one phenolic group, which was further confirmed by the presence of hydroxyl (3160 cm^{-1}) in its IR Conjugated carbonyls were seen at 1670 and 1650 cm^{-1} Its mass spectrum showed a $[M]^+$ at *m/z* 307 and an $[M-28]^+$ indicating a facile loss of a carbonyl group Further fragment ions at *m/z* 264 $[M-CO-Me]^+$ and 236 $[M-CO-Me-CO]^+$ were also observed This particular fragmentation pattern is characteristic of 4,5-dioxoaporphine type of compounds [8] Methylation of **8** with diazomethane yielded the known compound cepharadione B (**5**) (co-TLC, IR, mass spectrum) This indicated that in all probability **8** was the phenolic analogue of **5**. This was evident from a study of its ^{1}H NMR spectrum (Table 2), The two singlets at δ 3.73 (3H) and 4 04 (3H) were attributed to *N*-methyl and aromatic methoxyl groups, respectively A comparison of the chemical shift of H-3 in **8** (δ 8.07) and **8a** (δ 8.38) strongly supported the presence of a hydroxyl group at C-2 but not at C-1 [11] This was further confirmed by the application of 2D NOE (Fig 2) Thus, the C-11 aromatic proton at δ 9 44 shows NOE with the signal (δ 4 04) of the methoxyl group. This proves beyond doubt that the methoxyl group is attached to C-1 and not to C-2 Thus, taking into consideration all the above data, the structure of compound (**8**) was elucidated as a 2-hydroxy-1-methoxy-6-methyl-4H-dibenzo[*de*, *g*]quinoline-4,5(6H)-dione, named by us as piperadione

EXPERIMENTAL

Mps uncorr ^{1}H NMR (100 and 500 MHz) $CDCl_3$, DMSO-d_6 In the 2D NOE expt the mixing time was 1 2 sec and 16 transients were accumulated for 256 values of the evolution period, a delay of 2 5 sec was employed MS 70 eV direct insertion UV MeOH IR KBr Chromatographic sepns were carried out on silica gel, TLC on silica gel G TLC spots were detected under UV (254 and 365 nm), exposing the plates to I_2 vapour and heating to 100° after spraying with 10% H_2SO_4

Extraction and separation of compounds Fresh, crushed roots (1 1 kg) of *P longum* plants cultivated at The Trombay Experimental Field station were extracted repeatedly with EtOH (4 × 2 l) Removal of solvent under red pres furnished a resinous mass (38 g) This on repeated CC over silica gel G, using increasing polarity of solvents from petrol to MeOH yielded compounds **1**–**9** in crude form Compounds **1**–**6** and **9** were purified by normal phase prep TLC ($CHCl_3$–MeOH, 19 1) However, compounds **7** and **8**, were purified by reversed phase TLC (5%

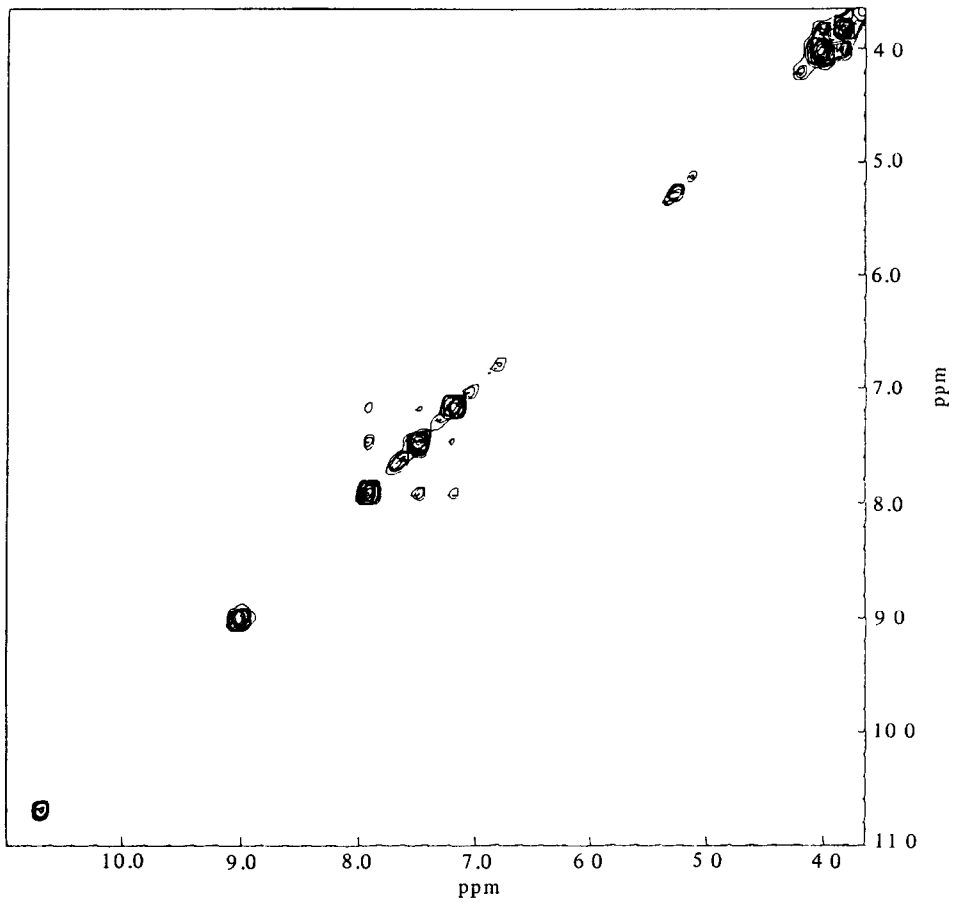


Fig 1 2D NOE spectrum of piperolactam B (**4**) after symmetrization

paraffin impregnation, MeOH–H_2O, 13:7) The relative yields and R_f values of the alkaloids were as follows: **1**, 9 mg 0.72; **2**, 8 mg, 0 52, **3** 2 mg, 0.36, **4** 10 mg, 0.06, **5** 10 mg, 0.82; **6**, 14 mg, 0.76, **7** 3 mg, 0.44; **8** 7 mg, 0 43; **9** 2 mg, 0.19.

10-*Amino*-4-*hydroxy*-3-*methoxyphenanthrene*-1-*carboxylic acid lactam*, [*piperolactam A*] (**3**). $C_{16}H_{11}NO_3$, mp 303–306° decomp (C_6H_6–MeOH), UV and IR: see text, ^{1}H NMR· see Table 1; MS *m/z* (rel int.) 265 $[M]^+$ (100), 250 $[M-Me]^+$ (60), 222 $[M-Me-CO]^+$ (25), 193 (4), 181 (4), 166 (25), 155 (6), 151 (19), 139 (6)

10-*Amino*-4-*hydroxy*-2,3-*dimethoxyphenanthrene*-1-*carboxylic acid lactam* [*piperolactam B*] (**4**). $C_{17}H_{13}NO_4$; mp 212–214° (MeOH), UV and IR see text, ^{1}H NMR· see Table 1, MS *m/z* (rel int.) 295 $[M]^+$ (100), 280 $[M-Me]^+$ (23), 277 (28), 267(14), 262 (15), 252 $[M-Me-CO]^+$ (57), 239 (19), 234 (17), 225 (16), 211 (18), 209 (28), 197 (16), 183 (23), 169 (28), 155 (32), 153 (30), 141 (39), 127 (55), 125 (53)

10-*Amino*-4-*acetoxy*-2,3-*dimethoxyphenanthrene*-1-*carboxylic acid lactam*, [*diacetate of piperolactam B*] (**4a**). $C_{21}H_{17}NO_6$, mp 260–262° (C_6H_6); UV λ_{max}^{MeOH} nm (log ε) 228 (4 60) 249 (4.54), 285 (4.45), 325 sh (3.86), 339 sh (3.84), 364 (3 77), 381 (3 81), IR ν_{max}^{KBr} cm^{-1}. 3040, 2980, 2840, 1800, 1750 (OAc),1730 (NAc) 1700, 1650, 1620, 1510, 1470, 1450, 1410, 1390, 1320, 1310, 1280, 1215, 1180, 1115, 1080, 1050, 1010, ^{1}H NMR. see Table 1; MS *m/z* (rel int) 379 $[M]^+$ (24), 337 $[M-CH_2CO]^+$ (82), 295 $[M-2\times CH_2CO]^+$ (100), 280 $[295-Me]^+$ (26), 277 (15), 262 (10), 252 $[295-Me-CO]^+$ (38), 236 (19), 209 (21), 180 (17), 153 (21), 152 (22), 129 (15)

10-*Amino*-2,3,4-*trimethoxyphenanthrene*-1-*carboxylic acid lactam*, [*methyl ether of piperolactam B*] (**4b**). $C_{18}H_{15}NO_4$, mp 187–188° (C_6H_6–MeOH), UV λ_{max}^{MeOH} nm (log ε) 228 (4 47), 233 (4.47), 254 (4 52), 259 sh (4 91), 289 sh (4 30), 335 (3 96), 360 (3 93), 378 (3.89), IR ν_{max}^{KBr} cm^{-1} 3170 (NH), 3040, 2960, 2860, 1710 (CO), 1670, 1600, 1500, 1470, 1400, 1330, 1280, 1210, 1170, 1090, 1025, ^{1}H NMR see Table 1; MS *m/z* (rel. int) 309 $[M]^+$ (100), 294 $[M-Me]^+$ (49), 280 (33), 266 $[M-Me-CO]^+$ (12), 262 (11), 251 (38), 236 (46), 179 (34), 163 (11), 151 (37), 128 (16), 124 (15)

2-*Hydroxy*-1-*methoxy*-6-*methyl*-4*H*-*dibenzo* [de, g] *quinoline*-4,5(6*H*)-*dione*, [*piperadione*] (**8**) $C_{18}H_{13}NO_4$, mp 273–276° decomp ($CHCl_3$–MeOH), UV λ_{max}^{MeOH} nm (log ε)· 245 (4 63), 290 sh (4 07), 302 (4 16), 314 (4 17), 440 (4 10), UV $\lambda_{max}^{MeOH+NaOH}$ nm (log ε)· 240 (4 61), 314 (4.14), 325 (4.13), 396 (3 85), 493 (3 96), IR see text, ^{1}H NMR see Table 2, MS *m/z* (rel int). 307 $[M]^+$ (100), 279 $[M-CO]^+$ (49), 264 $[M-CO-Me]^+$ (70), 236 $[M-CO-Me-Co]^+$ (35), 181 (22), 152 (13), 140 18)

2-*Acetoxy*-1-*methoxy*-6-*methyl*-4*H*-*dibenzo* [de, g] *quinoline*-4,5 (6*H*)- *dione*, [*acetate of piperadione*] (**8a**) $C_{20}H_{15}NO_5$, mp 244–248° decomp (C_6H_6–MeOH), UV λ_{max}^{MeOH} nm (log ε) 237 (4 36), 270 sh (4 04), 301 (4 0) 312 (3.99), 427 (3 65), IR ν_{max}^{KBr} cm^{-1} 2980, 2940, 2860, 1750 (OAc), 1680 (CO), 1540, 1480, 1385, 1275, 1210, 1030, ^{1}H NMR see Table 2; MS *m/z* (rel int) 349 $[M]^+$ (58), 307 $[M-CH_2\ CO]^+$ (100), 279 $[M-CH_2\ CO-CO]^+$ (56), 264 $[279-Me]^+$ (42), 236 $[279-Me-CO]^+$ (17), 184 (9), 181 (12), 165 (6), 153 (9), 140 (9)

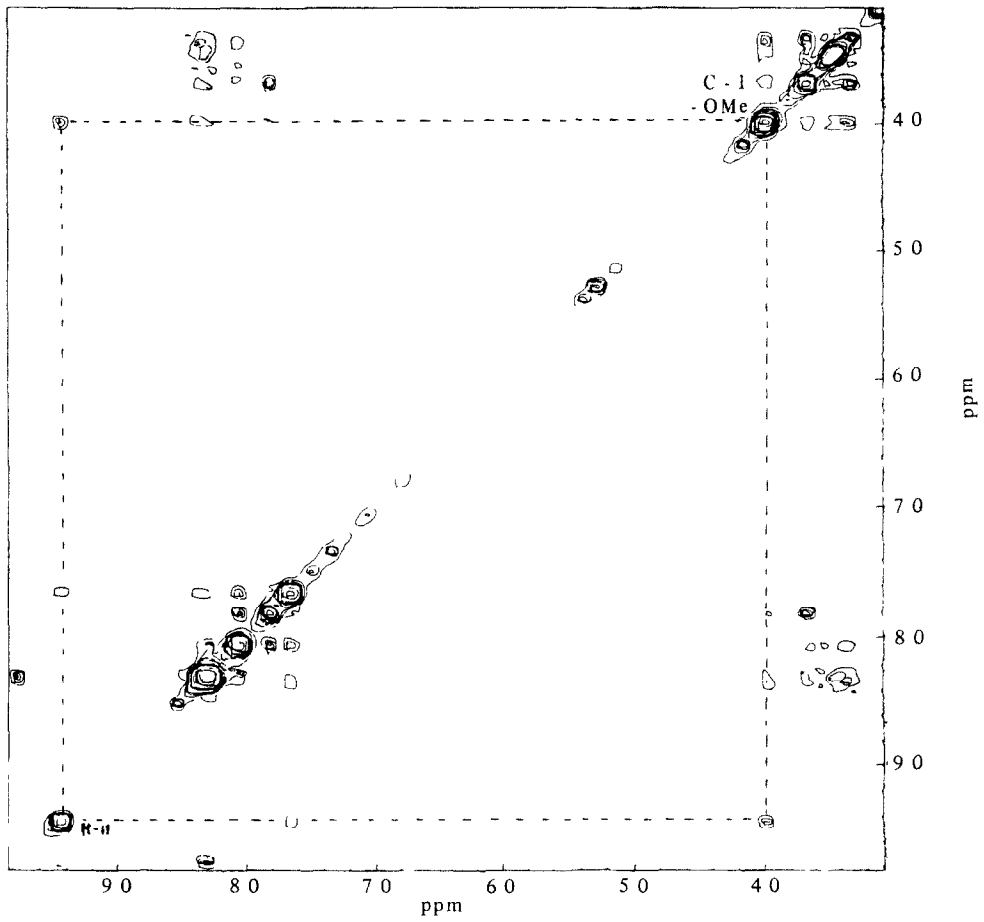


Fig 2. 2D NOE spectrum of piperadione (**8**) after symmetrization

Table 2 ^{1}H NMR chemical shifts of 4,5-dioxoaporphines

Compound	H-7	H-3	H-8	H-9,H-10	H-11	MeO-1	MeO-2	AcO-2	N-H	N-Me
5	7 88*	8 16	8 10, *d*	7 71, *m*	9 44, *d*	—4 06,	4.10	—	—	3 74
7	7 58†	8 22	7 96, *m*	7 69, *m*	9 46, *m*	—4 10,	4 14	—	12 1	—
8	7 88†	–	8 10, *br s*	7 71, *m*	9 50, *m*	4 09	–	—	—	3 79
	7 82*	—	8 07, *d*	7 68, *m*	9.37, *d*	4 05	—	—	—	3.73
8a	8 04*	8 38	8 17, *d*	7 75, *m*	9 37, *d*	4 05	—	2 49	—	3 76
9	7.50†	8 10	7 95, *d*	7 66, *m*	9 47, *d*	4 06	—	–	12 1	—

Values in δ (ppm) from TMS, DMSO-d_6 solutions, singlets not mentioned, *d*, $J = 8$ Hz
*500 MHz
†100 MHz

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