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ARISTOLOLACTAMS OF *GONIOTHALAMUS SESQUIPEDALIS* WALL. REVISED STRUCTURES OF THE 2-OXYGENATED ARISTOLOLACTAMS

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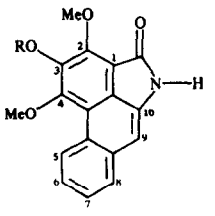
Key Word Index—*Goniothalamus sesquipedalis*; Annonaceae; aristololactams; goniopedaline; ^{13}C NMR; revised structures of 2-oxygenated aristololactams.

Abstract—Goniopedaline, a new phenanthrene lactam, aristololactam A-II and its *N,O*-diacetyl derivative, taliscanine (second natural occurrence), aurantiamide acetate and β-sitosterol and its β-D-glucoside have been isolated from the leaves and twigs of *Goniothalamus sesquipedalis*. The structure of goniopedaline has been established from its spectral evidence as 10-amino-3-hydroxy-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam. The structures of aristololactam CII, CIII, DII and DIII have been revised from the previously suggested 3-hydroxymethyl-2-methoxy derivatives and the 3-carboxy-2-hydroxy derivatives to the 2-hydroxymethyl-3-methoxy derivatives and the 2-carboxy-3-hydroxy derivatives respectively based on a careful analysis of their ^{13}C NMR spectral data. Thus, goniopedaline is the only 2-oxygenated aristololactam known so far. This constitutes the first report of the phenanthrene lactams from the *Goniothalamus* genus.

INTRODUCTION

Recently we reported the isolation of four new 5,6-dihydro-α-pyrones [1] and pinocembrin [2] from the leaves and twigs of two Indian *Goniothalamus* species [3], *G. sesquipedalis* and *G. grifithii*, collected from the states of Manipur and West Bengal respectively. From the leaves and twigs of *G. sesquipedalis* some other chemical constituents including one new and two known phenanthrene lactams have been isolated. To our knowledge, this is the first report of the occurrence of phenanthrene lactams in the genus *Goniothalamus* and the third in the family Annonaceae [4].



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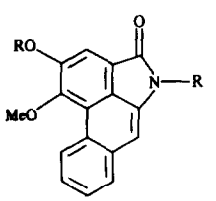
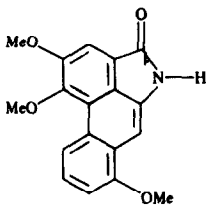
4 R = COMe



2 R = H
5 R = COMe

RESULTS AND DISCUSSION

Extensive chromatography of the remaining fractions of the petrol (60–80°) extract [1] and the methanol extract of *G. sesquipedalis* afforded a new phenanthrene lactam designated goniopedaline (**1**) along with two known ones, aristololactam A-II (**2**) isolated as its *N,O*-diacetyl derivative (**5**), and taliscanine (**3**), a modified dipeptide aurantiamide acetate (**6**) and β-sitosterol and its β-D-glucoside.



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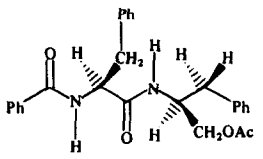

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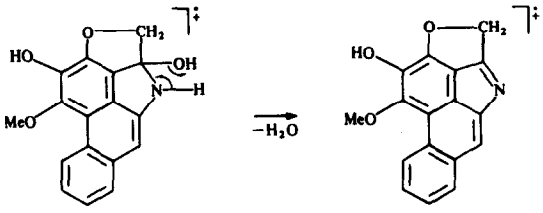
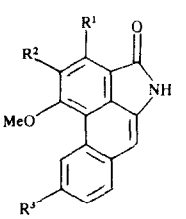
Goniopedaline, $C_{17}H_{13}O_4N$ (M^+ 295), exhibited a UV spectrum characteristic of a phenanthrene chromophore and the bathochromic shift of the maxima produced by the addition of alkali suggested the presence of a phenolic hydroxyl group in the molecule. The appearance of bands at 3492, 3178, 1695 and 1655 cm^{-1} in its IR spectrum revealed the presence of hydroxyl, imine and lactam carbonyl groups. The structure was deduced from the chemical shifts and signal multiplicities of its proton resonances and from double irradiation experiments. The 250 MHz ^{1}H NMR spectrum ($CDCl_3$) of **1** confirmed the presence of an imine (δ7.79, 1H, *br*, exchangeable with D_2O), a hydroxyl (δ6.24, 1H, *s*, exchangeable with D_2O)



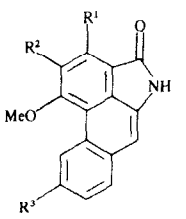
a **b**

and two methoxyl groups (δ4.17 and 4.62, 3H *s* each). The aromatic region of the spectrum was quite revealing: it closely resembled those of other 5,6,7,8,9-unsubstituted aristololactams, therefore both methoxyl groups had to be in ring A. A singlet at δ7.15 was ascribed to H-9 while H-8, H-7, H-6 and H-5 appeared as a complex ABCX coupling pattern. Thus H-5 appeared as the characteristic most downfield 1H multiplet at δ9.21, the 2H multiplet at δ7.56 was assigned to H-6 and H-7 and the 1H multiplet at δ7.84 was due to H-8. The methoxyl groups at C-3 and C-4 in phenanthrene lactams (≡ aristololactams) have been reported [5, 6] to resonate between δ 4.0–4.1. Both methoxyl groups in goniopedaline had to be in ring A; the one appearing at δ4.17 was allocated to C-4 from the 7–8% NOE of its signal intensity upon irradiation of H-5 at δ9.21 which converted the H-8 multiplet to a clear *dd* (J = 8.0 and 2.5 Hz). Again, upon irradiation of the multiplet at δ7.56 (H-6 and H-7) both multiplets at δ9.21 (H-5) and 7.84 (H-8) collapsed to singlets. The second methoxyl appeared at an unexpectedly downfield region of the spectrum (δ4.62) and could only be located at C-2, being significantly deshielded under the influence of the *peri* carbonyl of the lactam group. Goniopedalin is thus shown to be 10-amino-3-hydroxy-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam (**1**). The only other alternative location of the methoxyl at C-9 (keeping C-3 and C-4 dioxygenated and hence the hydroxyl group at C-3) was ruled out since aristololactams having 4-methoxyl and 3-hydroxyl groups are found to have the H-2 singlet at nearly δ7.6, whereas the observed singlet (δ7.15) agreed well with the H-9 singlet appearing between δ7.00 and 7.15 in the case of all known 2,3-oxygenated aristololactams (see Table 1 in ref. 6). Even in the new aristololactam enterocarpam-I, the methoxyls at C-4, C-8 and C-9 appeared at δ4.08, 3.91 and 4.38 respectively [7]. Recently, two more aristololactam alkaloids, aristololactam CII (10-amino-3-hydroxymethyl-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam) (**7**) and aristololactam CIII (10-amino-3-hydroxymethyl-2,4,6-trimethoxyphenanthrene-1-carboxylic acid lactam) (**8**) have been reported [8] from *Aristolochia argentina*. In each case the 4-methoxyl group resonated at δ4.14 whereas the 3H singlet at around δ4.00 was assigned to the 2-methoxyl group. In goniopedaline (**1**) possessing a 3-hydroxyl group, the 2-methoxyl protons were deshielded significantly to δ4.62 which brought into question the validity of the structures of aristololactams CII and CIII with a methoxyl group at C-2. The pronounced deshielding of the 2-methoxyl protons in an environment as in **1** seems to be unprecedented and no parallel situation could be found in the literature.

The *ortho* disposition of the two methoxyls in ring A of aristololactam CII and the usual 3,4-dioxygenated pattern were ruled out [8] based on the carbon shifts of C-2 and C-4 and of the *O*-methyls attached to them. In view of the ambiguity that arose regarding the allocation of the second methoxyl group at C-2, the resonances of the oxyaromatic carbons of this and other 2-oxygenated aristololactams were carefully reviewed in terms of the additivity rules [9] and the observation [10, 11] that the *ortho* (and *para*) substituent effects of the out-of-plane methoxyl group flanked by two substituents are almost half of their normal values and that a strong γ-shielding effect (2–3 ppm) is exerted by such non-planar methoxyl groups on the gauche aromatic carbon. The C-3 and C-4 resonances of the 3,4-dimethoxyaristololactams have been correctly assigned to the signals in the δ154.2 and 150.5 regions respectively [6] (since nearly 4–5 ppm less shielding effect than usual will operate on C-3 due to the nonplanar 4-methoxyl group). However, the C-3 and C-4 resonance assignments [7] in enterocarbam II (≡ 3-hydroxy-4,8-dimethoxyphenanthrene lactam) to the signals at δ154.1 and 150.7 respectively are incompatible with the calculated values and should be interchanged since the hydroxylated C-3 should have about 4.5 ppm less deshielding effect and the 3-hydroxyl group should have about 2 ppm less *ortho* shielding effect on C-4, compared to the 3,4-dimethoxyaristololactams. Furthermore, in aristololactam CII although the C-2 resonance assignment to the signal at δ150.7 is more or less in agreement with the suggested 2,4-dimethoxy-3-hydroxymethyl structure, calculating from δ110 value for C-2 in enterocarpam II, the C-4 resonance assignment at δ155.1 is far from being in agreement with the suggested structure for which C-4 is expected to resonate at a much lower field ($\delta > 164$) due to about 12.7 ppm less shielding by the *ortho*-CH_2OH which has replaced the *ortho*-methoxyl group at C-3 and about 0.4 ppm deshielding by the *meta*-methoxyl group at C-2. On the contrary, the δ150.7 and 155.1 signals are both found to be in fair agreement with the C-3 and C-4 methoxyl-bearing carbon resonances in the revised 3,4-dimethoxy-2-hydroxymethylphenanthrene lactam structure **9** for aristololactam CII, assuming that, here, compared to 3,4-dimethoxyaristololactams the *ortho*-CH_2OH group at C-2 exerts nearly 1.7 ppm shielding influence on C-3 [11], and the nonplanar 3-methoxyl group exerts about 5–6 ppm less shielding influence and the *meta*-CH_2OH (at C-2) 0.4 ppm shielding [11] influence on C-4. In these systems, the shielding influence of the methoxyl and CH_2OH groups on *ortho* and *meta* carbons may however deviate to some extent from the literature values which however will not affect the difference of carbon shifts of the oxyaromatic carbons significantly. Thus the revision of the structures of aristololactam CII and hence aristololactam CIII as 2-hydroxymethyl-3,4-dimethoxy derivative (**9**) and 2-hydroxymethyl-3,4,6-trimethoxy derivative (**10**) respectively is warranted. On similar grounds, the structures of the congener aristololactams DII and DIII should also be revised from the assigned 2-hydroxy-4-methoxy-3-carboxylic acid derivatives **11** and **12** [8] to 3-hydroxy-4-methoxy-2-carboxylic acid derivatives **13** and **14** respectively. In these compounds or their derivatives the oxyaromatic carbon resonances assigned to C-2 and C-4 were also found to differ by only about 5 ppm



	R¹	R²	R³
7	OMe	CH_2OH	H
8	OMe	CH_2OH	OMe
9	CH_2OH	OMe	H
10	CH_2OH	OMe	OMe



	R¹	R²	R³
11	OH	CO_2H	H
12	OH	CO_2H	OMe
13	CO_2H	OH	H
14	CO_2H	OH	OMe

thereby suggesting the aforesaid revised structures. Thus these aristololactams also are expected to possess 3,4-dioxygenation.

The mass spectrum of goniopedaline is also consistent with the structure **1**. It showed $[M]^+$ at *m/z* 295 (base peak) and other important peaks at *m/z* (rel. int.) 280 $[M-Me]^+$ (13.3), 277 $[M-H_2O]^+$ (17.0), 252 $[M-H_2O-Me]^+$ (29.9), 249 $[M-H_2O-CO]^+$ (11.3), 243 (19.8) and 221 (17.5). The loss of elements of water to form the ion *b* at *m/z* 277 through the ion *a*, isomeric to $[M]^+$, supported the presence of a methoxyl group at C-2, *peri* to the CO group [12]. On the other hand, aristololactam A-II (**2**) did not exhibit any peak corresponding to loss of H_2O, thus supporting the above contention. The MS fragmentations of aristololactams CII and DII are also in agreement with the revised structures. Upon acetylation (Ac_2O – pyridine) **1** formed an acetate (**4**). Its mass spectrum showed that the parent ion underwent loss of ketene to generate the base peak at *m/z* 295 which in turn underwent fragmentation to form most of the major peaks.

Goniopedaline thus appears to be the first 2-oxygenated as well as the first 2,3,4-trioxygenated aristololactam alkaloid. Moreover, so far no aporphine, the biogenetic precursor of aristololactams, has been reported with oxygenation at C-2 of ring A. Thus the revised structures of aristololactams C II, C III, D II and D III are biogenetically more satisfying, since these alkaloids need not be derived from the non-protein amino acids such as 3-carboxy- or 3-hydroxymethylphenylalanine [6]. Goniopedaline is the twentieth member of aristololactam alkaloids which were found to occur mostly in *Aristolochia* species (Aristolochiaceae): 15 alkaloids in *A. argentina* [6, 8]), two in *Stephania cepharantha* (Menispermaceae) [13], one in *Schefferomitra subaequalis* [14] and two in *Orophea enterocarpa* [7]—the latter two being Annonaceae plants. Only these three families are known to produce aristololactams which is of taxonomic significance. All aristololactams known so far have 3,4-oxygenation and only oxygenation at C-5 and C-7 is yet to be encountered in nature.

The sticky gum obtained from the petrol–EtOAc (1:1) eluate fractions of the methanol extract showed two spots but no pure compound could be isolated by chromatography. Acetylation of the sticky gum followed by chromatography resulted in the isolation of *N,O*-diacetylaristololactam A-II (**5**), $C_{20}H_{15}O_5N$ (M^+ 349), which on hydrolysis furnished aristololactam A-II (**2**), identified by its spectral data and finally by direct comparison with an authentic sample. The two spots of the sticky mass corresponded to aristololactam A-II (major spot) and *N,O*-diacetyl aristololactam A-II (minor spot) thus bearing testimony to their natural occurrence. Another phenanthroid lactam isolated from the methanol extract was identified as taliscanine (**3**) from its IR, UV, ^{1}H NMR spectral features [15]. The mass spectral fragments, not reported earlier, are mentioned in the Experimental. This is the second report of the natural occurrence of taliscanine.

Aurantiamide acetate (**5**), β-sitosterol and its β-D-glucoside were isolated from the petrol extract.

EXPERIMENTAL

General. Mps: uncorr.; IR: KBr; UV: 95% EtOH; ^{1}H NMR, TMS as int. stand.; MS: 70 eV; Chromatography: silica gel (60–120 mesh), spots visualized in UV light and on exposure to I_2 vapour.

Extraction. Dried and powdered leaves and twigs (5.6 kg) of *Goniothalamus sesquipedalis* was extracted exhaustively in a Soxhlet apparatus with petrol (bp 60–80°) [1] and $CHCl_3$ successively. The marc left was extracted with cold MeOH. The chromatography of the petrol extractives was continued [1] with solvent mixtures of increasing polarity. The MeOH extract was chromatographed over silica gel using solvents and solvent mixture of increasing polarities. Fractions of similar composition (as indicated by TLC) were combined.

Isolation of β-sitosterol. Chromatography of the residue obtained from the petrol–EtOAc (19:1) eluate fractions over silica gel resulted in the isolation of β-sitosterol (240 mg), mp 137°, identical in all respects (mmp, co-TLC) with an authentic sample.

Isolation of goniopedaline (**1**). The residue obtained from the earlier petrol–EtOAc (4:1) eluate fractions on rechromatography over silica gel followed by crystallization from $CHCl_3$–petrol furnished goniopedaline (**1**) as fine greenish-yellow crystals (30 mg), mp 218°, UV λ_{max}^{EtOH} nm (log ε): 216 (4.24), 246 (4.51), 248 (4.51), 282 (4.37), 291 (4.37), 352 (3.82), 371 (3.90), 389 (3.99); $\lambda_{max}^{EtOH+KOH}$ nm (log ε): 296 (3.99), 307 (3.92), 330 (3.49), 343 (3.82), 353 (3.79), 370 (3.56), 418 (4.03). (Found: C, 68.72; H, 4.29; N, 4.52. Calc. for $C_{17}H_{13}O_4N$: C, 69.15; H, 4.41; N, 4.74%).

Goniopedaline acetate (**4**). A soln of goniopedaline (6 mg) in C_5H_5N (0.5 ml) was treated with Ac_2O (1 ml, room temp., 24 hr). The residue obtained after usual work-up of the reaction mixture, on chromatography over silica gel afforded the acetate **4** from the petrol–EtOAc (9:1) eluate fractions, crystallizing from $CHCl_3$–petrol as fine greenish-yellow needles, mp 250°, IR ν_{max}^{KBr} cm^{-1} 3200 (>NH), 1765 and 1210 (–OCOMe), 1690 and 1655 (lactam CO); ^{1}H NMR (80 MHz, $CDCl_3$): δ2.31 (3H, *s*, –OCOMe), 4.02 (3H, *s*, 4-OMe), 4.42 (3H, *s*, 2-OMe), 7.13 (1H, *s*, H-9), 7.49 (2H, *m*, H-6 and H-7), 7.72 (1H, *br s*, exchangeable with D_2O, NH), 9.03 (1H, *m*, H-5); MS: *m/z* (rel. int.): 337 $[M]^+$ (36), 295 $[M-CH_2=C=O]^+$ (100)—this base peak being same as the $[M]^+$ of **1**; the other fragments were the same as those of **1**: 280 (36), 277 (63), 252 (43), 249 (39), 234 (29), 221 (26), 209 (22).

Isolation of aurantiamide acetate (**6**). The residue obtained from the later petrol–EtOAc (4:1) eluate fractions on rechromatography over silica gel followed by crystallization from $CHCl_3$–petrol furnished aurantiamide acetate (**6**) (12 mg), mp 181°, IR ν_{max}^{KBr} cm^{-1}: 3330, 1728, 1660, 1632, 1260, 750, 700.

Isolation of β-sitosterol-β-D-glucoside. The brown jelly-like residue containing a white solid obtained from the petrol–EtOAc (3:7) eluate fractions on rechromatography over silica gel afforded β-sitosterol-β-D-glucoside as a white amorphous solid, mp 270°; acetate mp 168°; identical in all respects (mmp, co-TLC, IR) with an authentic sample.

Isolation of taliscanine (**3**). The residue obtained from the earlier petrol–EtOAc (1:1) eluate fractions on rechromatography over silica gel afforded from the petrol–EtOAc (1:1) eluate fractions taliscanine (**3**), (6 mg), crystallizing from $CHCl_3$–petrol in fine yellow needles, mp 270° (lit. [7] 272–273°), identified by its spectral (IR, UV, ^{1}H NMR) data [7]; UV λ_{max}^{EtOH} nm: 246, 255 and 299; IR ν_{max}^{KBr} cm^{-1}: 3200 (>NH), 1705 and 1660 (γ-lactam CO), 1552, 1475, 1380, 1320, 1275; ^{1}H NMR ($CDCl_3$, 270 MHz): δ4.11 (3H, *s*, 4-OMe), 4.03 and 4.07 (3H, *s*, each, 3-OMe and 8-OMe), 7.08 (1H, *d*, $J_{7,6}=9$ Hz, H-7), 7.53 (1H, *t*, $J_{6,5}=J_{6,7}=9$ Hz, H-6), 7.64 (1H, *s*, H-9), 7.83 (1H, *s*, H-2), 8.75 (1H, *d*, $J_{5,6}=9$ Hz), 10.70 (1H, *s*, NH, exchangeable with D_2O): MS: *m/z* (rel. int.); 309 $[M]^+$ (40), 294 $[M-Me]^+$ (21), 279 $[M-CH_2O]^+$ or $[M-2Me]^+$ (100), 264 $[M-CH_2O-Me]^+$ (21), 251 [*m/z* 279 $-CO]^+$ (12), 236 [*m/z* 264–$CO]^+$ (19), 221 [*m/z* 236–$Me]^+$ (12) and 193 [*m/z* 221–$CO]^+$ (16). Non-availability of an authentic sample, however, precluded direct comparison.

Isolation of N,O-*diacetylaristololactam A-II* (**5**). The sticky gum obtained from the later petrol–EtOAc (1:1) eluate fractions exhibited two spots corresponding to *N*-acetyl aristololactam A-II and *N,O*-diacetylaristololactam A-II. Attempted isolation of the lactams from a part of the sticky gum by CC did not succeed, possibly due to insufficient amounts being present. The total gummy residue was acetylated (Ac_2O–Py, room temp, 20 hr) and the product obtained after usual work-up, was repeatedly chromatographed over silica gel to afford *N,O*-diacetylaristololactam A-II, (R_f 0.9, silica gel, $CHCl_3$), crystallizing from $CHCl_3$–petrol in fine colourless needles (16 mg), mp 178°, UV λ_{max}^{EtOH} nm: 228, 247, 284, 323; IR ν_{max}^{KBr} cm^{-1}: 1760, 1725, 1700, 1600, 1490, 1450, 1390, 1300, 1245; ^1H NMR ($CDCl_3$, 80 MHz): δ2.47 (3H, *s*, –OCOMe), 2.81 (3H, *s*, NCOMe), 4.10 (3H, *s*, –OMe), 7.65 (2H, *m*, H-6 and H-7), 7.74–7.98 (1H, *m*, H-8), 7.91 (1H, *s*, H-9), 8.45 (1H, *s*, H-2), 9.08 (1H, *m*, H-5); MS: *m/z* (rel. int.): 349 $[M]^+$ (26), 307 (41), 265 (100), 250 (38), 222 (12), 193 (6), 165 (6) and 149 (12).

Hydrolysis of N,O-*diacetylaristololactam A-II*: *isolation of aristololactam A-II* (**2**). A soln of *N,O*-diacetylaristololactam A-II (**2**) (6 mg) in a 5% aq. EtOH soln of NaOH (3 ml) was refluxed for 1 hr. The residue obtained after neutralization of alkali (with 2 m HCl) and removal of alcohol was filtered and chromatographed over silica gel. The petrol–EtOAc (4:1) eluate fractions afforded aristololactam A-II (3 mg) R_f 0.5(silica gel G, $CHCl_3$–MeOH 9:1), identical (mmp, co-TLC and IR) with an authentic sample.

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