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ARISTOLACTAMS OF *OROPHEA ENTEROCARPA**

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Key Word Index—*Orophea enterocarpa*; Annonaceae; aristolactams; phenanthrene alkaloids; enterocarpam-I (10-amino-3-hydroxy-4,8,9-trimethoxyphenanthrene-1-carboxylic acid lactam); enterocarpam-II (10-amino-3-hydroxy-4,8-dimethoxyphenanthrene-1-carboxylic acid lactam).

Abstract—Two new aristolactams named as enterocarpam-I and enterocarpam-II have been isolated and characterized from the stem bark of *Orophea enterocarpa*.

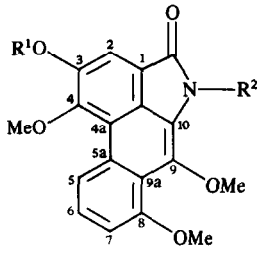
INTRODUCTION

Orophea enterocarpa is a small tree, *ca* 7 m tall which grows in Malaysia and parts of South east Asia. The genus *Orophea* [1] has been known to possess some medicinal properties. For example, the roots of *O. setosa* is chewed to cure coughs or ground into a powder which is administered externally to remove fever. *Orophea polycarpa* is sudorific and bechic. No previous work has been reported on *O. enterocarpa*; in our present investigation on the constituents of this plant we report the isolation and characterization of two new aristolactams from the stem bark extract.

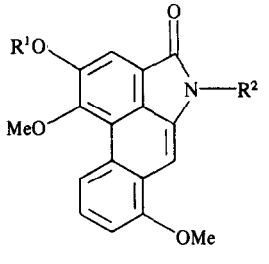
RESULTS AND DISCUSSION

Extraction of the stem bark of *O. enterocarpa* and separation of the ether-soluble constituents yielded two fluorescent components, which were identified as the new enterocarpam-I (**1**) and enterocarpam-II (**3**). Enterocarpam-II (**3**), mp 268–272°, ($C_{17}H_{13}NO_4$; $[M]^+$ *m/z* 295) showed a UV spectrum characteristic of a phenanthrene chromophore [2, 3], while the bathochromic shift by alkali treatment suggested the presence of a phenolic hydroxyl group in the molecule. The IR spectrum of **3** supported this structure and showed that it possessed OH, NH and CO absorption bands. The ^{1}H NMR spectrum verified the NH (δ10.70, 1H), OH (δ10.00, 1H) functions which disappeared upon addition of D_2O and acetylation to acetoxyl (δ2.51, 3H, *s*) and N–Ac (δ2.81, 3H, *s*) and further indicated two methoxyl groups at δ4.03(3H) and 4.00(3H). Two singlets of uncoupled aromatic protons at δ7.42(1H) and 7.83(1H) could be ascribed to H-2 and H-9, while H-5, 6, 7 appeared as an ABC coupling pattern at δ8.62 (1H, *d*, J = 8 Hz), 7.36 (1H, *t*, J = 8 Hz) and 7.06 (1H, *dd*, J = 1, 7 Hz) respectively. That the hydroxyl group was located at C-3 was suggested by the downfield shift [3,4] of H-2 from δ7.42 to 8.41 in enterocarpam-II acetate (**4**). The mass fragmentation pattern showed characteristic peaks of two methoxyl degradation [5]. On the basis of these results, the structure of **3** was established as 10-amino-3-hydroxy-4,9-dimethoxyphenanthrene-1-carboxylic acid lactam.

Enterocarpam-I (**1**), mp 214° ($C_{18}H_{15}NO_5$; $[M]^+$ *m/z*



Enterocarpam-I (**1**) $R^1 = R^2 = H$
Enterocarpam-I acetate (**2**) $R^1 = R^2 = Ac$



Enterocarpam-II (**3**) $R^1 = R^2 = H$
Enterocarpam-II acetate (**4**) $R^1 = R^2 = Ac$

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325) was obtained from **2** by deacetylation with ammonia in methanol. Its UV spectrum showed the characteristics of a phenanthrene chromophore [2, 3] and its IR spectrum showed that it possessed OH, NH and CO groups. The ^{1}H NMR spectrum verified the above spectral data, OH (δ10.02, 1H) and NH (δ10.85, 1H) and in addition indicated the presence of three methoxyl groups (δ3.91, 4.08 and 4.38). The newly appeared methoxyl group at δ4.38 could be located at C-9, since H-9 of **3** had disappeared in **1**. H-5, 6, 7 showed the same ABC coupling pattern as in **3**. The remaining singlet aromatic proton at δ7.53 (1H) was assigned to H-2 and this signal in the acetate of enterocarpam-I (**2**) had shifted downfield [4] from δ7.53 to 8.34 so that the hydroxyl group should be located at C-3.

Therefore, the structure of **1** was elucidated as 10-amino-3-hydroxy-4,8,9-trimethoxyphenanthrene-1-carboxylic acid lactam.

EXPERIMENTAL

All mps are uncorr. ^{1}H NMR spectra were recorded at 80 MHz with TMS as int. standard. MS were obtained on a GC/MS system.

Extraction. Pulverized dried stem bark (1.5 kg) of *O. enterocarpa* Maing was extracted with boiling MeOH (2 × 6 l.) for 3 hr. The MeOH extract was evapd *in vacuo* to afford a dark brown syrup (146 g) that was suspended in H_2O (750 ml) and extracted with Et_2O (3 × 1 l.). Resinous material (undissolved in H_2O) was left behind (*ca* 60 g). The Et_2O extracts were combined, dried (Na_2SO_4) and evapd to a residue (7.3 g).

*Isolation of enterocarpam-*I (**1**) *and* -II (**3**). The Et_2O extract (7.3 g) was subjected to silica gel flash CC eluting with $CHCl_3$. The polarity was progressively increased by the addition of MeOH. The $CHCl_3$–MeOH (10 : 1) soluble fraction was left to stand overnight and a yellow crystalline solid was collected (80 mg, enterocarpam-II). The filtrate was evapd and the residue (1 g) showed a single spot by TLC (silica gel; $CHCl_3$–MeOH, 10 : 1); however, acetylation [Ac_2O (10 ml) + pyridine (10 ml), at room temp. for 16 hr followed by heating at 90° for 3 hr] showed it to be a mixture of two components, when subjected to TLC (silica gel; cyclohexane–Me_2CO–MeOH, 35:15:1, R_f 0.42 and 0.38). The two components were resolved by multiple development prep. TLC (hexane–EtOAc, 3:1).

*Enterocarpam-*I *acetate* (**2**). The solid residue obtained from the R_f 0.42 band was crystallized from MeOH as pale yellow needles (350 mg); mp 186°; UV λ_{max}^{MeOH} nm (log ε): 249 (4.68), 293 (4.36), 340 (3.96), 358 (3.86), 380 (3.96); IR ν_{max}^{KBr} cm^{-1}: 1750 (OAc), 1720 (NAc), 1700 (C=O); ^{1}H NMR (DMSO-d_6): δ2.46 (3H, *s*, OAc), 2.69 (3H, *s*, NAc), 3.97 (3H, *s*, 8-OMe), 4.18 (3H, *s*, 4-OMe), 4.32 (3H, *s*, 9-OMe), 7.43 (1H, *dd*, J = 1.1, 8 Hz, H-7), 7.69 (1H, *t*, J = 8, 1 Hz, H-6), 8.34 (1H, *s*, H-2), 9.05 (1H, *d*, J = 8.1 Hz, H-5). ^{13}C NMR ($CDCl_3$): see Table 1; MS *m/z* (rel. int.): 409 $[M]^+$ (20.6), 367 $[M-CH_2CO]^+$ (63.1), 325 $[M-2\times CH_2CO]^+$ (21.3), 310 $[325-Me]^+$ (21.3), 282 $[325-CO-Me]^+$ (0.72), 267 $[325-Me\times 2-CO]^+$ (5), 252 $[325-2\times Me+CO]^+$ (12.0).

*Enterocarpam-*I (**1**). Compound **2** (46 mg) was suspended in MeOH (20 ml) and conc NH_3 soln (20 ml). After 2 hr the solvent was removed under red. pres. The residue was purified by mini silica gel flash CC using $CHCl_3$–MeOH (20 : 1). Enterocarpam-I was obtained in crystalline form from $CHCl_3$–MeOH (35 mg), mp 214°; UV λ_{max}^{MeOH} nm (log ε): 239 (4.12), 253 (4.36), 268 (4.06), 298 (3.80), 397 (3.66), in NaOH: 261 (4.42), 319 (3.62), 448 (3.66); IR ν_{max}^{KBr} cm^{-1}: 3340 (OH, NH), 1680 (C=O), 1615 (C=C); ^{1}H NMR (DMSO-d_6): δ3.91 (3H, *s*, 8-OMe), 4.08 (3H, *s*, 4-OMe), 4.38 (3H, *s*, 9-OMe), 7.01 (1H, *dd*, J = 1.1, 8 Hz, 7-H), 7.35 (1H, *t*, J = 8 Hz, 6-H), 7.53 (1H, *s*, H-2), 8.58 (1H, *d*, J = 8 Hz, 5-H), 10.02 (1H, *br s*, OH), 10.85 (1H, *br s*, NH); MS *m/z* (rel. int.): 325 $[M]^+$ (89.3), 310 $[M-Me]^+$ (29.7), 296 $[M-2\times Me+H]^+$ (31.7), 295 $[M-2\times Me\times 2]^+$ (22.7), 282 $[M-Me-CO]^+$ (11.4), 267 $[M-2\times Me-CO]^+$ (98.4), 252 $[M-2\times Me-2\times CO)^+$, (100).

Table 1. ^{13}C NMR data of enterocarpam-I acetate (**2**) and enterocarpam-II (**3**)

Carbon Number	Chemical shifts (δ ppm) 2	3
C-1	125.5	125.6
2	107.1	110.0
3	147.9	154.1
4	146.8	150.7
4a	107.8	124.0
5a	116.2	118.0
5	124.0	121.5
6	126.2	123.4
7	119.7	112.1
8	154.8	153.6
9a	128.2	127.1
9	158.4	98.7
10	132.4	133.8
10a	124.2	120.2
C=O	164.3	168.3
O ‖ NC–Me	169.3	
O ‖ O–C–Me	171.0	
O ‖ N–C–Me	26.1	
O ‖ O–C–Me	20.9	
4-OMe	61.6	59.8
8-OMe	60.8	57.0
9-OMe	62.8	

*Enterocarpam-*II *acetate* (**4**). From the R_f 0.38 band a solid residue was obtained which was recrystallized from MeOH (400 mg), mp 219–220°; UV λ_{max}^{MeOH} nm (log ε): 220 (4.66), 251 (4.66), 279 (4.38), 289 (4.39), 320 (3.92), 375 (3.70), 393 (3.79); IR ν_{max}^{KBr} cm^{-1}: 1735 (OAc), 1700 (NAc), 1635 (C=O); ^{1}H NMR ($CDCl_3$): δ2.51 (3H, *s*, OAc), 2.81 (3H, *s*, NAc), 4.07 (3H, *s*, 8-OMe), 4.12 (3H, *s*, 4-OMe), 7.43 (1H, *dd*, J = 1, 8 Hz, H-7), 7.63 (1H, *t*, J = 8 Hz, H-6), 7.79 (1H, *s*, H-9), 8.41 (1H, *s*, H-2), 9.17 (1H, *d*, J = 8 Hz, H-5); MS *m/z* (rel. int.): 379 $[M]^+$ (40.7), 337 $[M-CH_2CO]^+$ (63.8), 295 $[M-2\times CH_2CO]^+$ (44.3), 280 $[M-2\times CH_2CO-Me]^+$ (4.9), 252 $[M-2\times CH_2CO-Me-CO]^+$ (12.5), 237 $[M-2\times CH_2CO-2\times Me-CO]^+$ (7.7), 209 $[M-2\times CH_2CO-2Me-2CO]^+$ (6.7).

*Enterocarpam-*II (**3**). Mp 268–272°; UV λ_{max}^{MeOH} nm (log ε): 246 (4.8), 255 (4.47), 293.5 (4.4), 405 (4.18); in NaOH: 255 (4.80), 323 (4.05), 450 (4.00); IR ν_{max}^{KBr} cm^{-1}: 3220 (OH, NH), 1710, 1670 (C=O), 1605 (C=C); ^{1}H NMR (DMSO-d_6): δ4.00 (3H, *s*, 8-OMe), 4.03 (3H, *s*, 4-OMe), 7.06 (1H, *dd*, J = 1, 7 Hz, H-7), 7.36 (1H, *t*, J = 8 Hz, H-6), 7.42 (1H, *s*, H-2), 7.83 (1H, *s*, H-9), 8.62 (1H, *d*, J = 8 Hz, H-5), 10.0 (1H, *br s*, OH), 10.70 (1H, *br s*, NH). ^{13}C NMR (DMSO-d_6): see Table 1. MS *m/z* (rel. int.): 295 $[M]^+$ (17.6), 280 $[M-Me]^+$ (2.1), 252 $[M-Me-CO]^+$ (10.5), 237 $[M-2\times Me-CO]^+$ (9.2), 209 $[M-2Me-2CO]^+$ (6.7).

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REFERENCES

1. Perry, L. M. and Metzger, J. (1980) *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses.* MIT Press. Cambridge, MA.
2. Sangster, A. W. and Stuart, K. L. (1965) *Chem. Rev.* **65**, 69.
3. Crohare, R., Priestap, H. A., Farina, M., Cedola, M. and Ruveda, E. A. (1974) *Phytochemistry* **13**, 1957.
4. Haynes, L. J., Stuart, K. L., Barton, D. H. R. and Kirby, G. W. (1966) *J. Chem. Soc.* (*C*) 1976.
5. Akasn, M., Itokawa, H. and Fujita, M. (1974) *Tetrahedron Letters* 3609.

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SISUNINE, A GLYCOALKALOID FOUND IN HYBRIDS BETWEEN *SOLANUM ACAULE* AND *SOLANUM* × *AJANHUIRI*

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Key Word Index—*Solanum acaule*; *Solanum ajanhuiri*; hybrids; glycoalkaloids; sisunine.

Abstract—Clones of hybrids of *Solanum acaule* and *Solanum* × *ajanhuiri* contain two glycoalkaloids, the previously identified commersonine and an unknown glycoalkaloid, sisunine, which is considered to be the tomatidine O(3)-β-commertetraoside.

INTRODUCTION

The diversity of glycoalkaloids found in *Solanum* species [1] is, in part, the result of the independent inheritance of the aglycone and glycosidic moieties [2]. This inheritance pattern can be helpful in determining parents of a particular hybrid. Such is the case for a group of clones referred to as sisu which are cultivated by the Aymara people in the 'altiplano' of Western Bolivia. These clones are believed to be hybrids between *Solanum acaule* × *Solanum* × *ajanhuiri* [3]. The wild parent of *S.* × *ajanhuiri* (*S. megistacrolobum*) contains glycoalkaloids that have the demissidine aglycone (**1**) and the commertetraose saccharide unit (**3**) [3]; *S. acaule* contains the glycoalkaloids demissine, which is composed of the aglycone demissidine and the tetrasaccharide lycotetraose (a terminal glucose in **3** replaced by xylose), and tomatine [tomatidine (**2**) as aglycone, lycotetraose as saccharide moiety] [4]. The sisu hybrids would be expected to contain a glycoalkaloid that has tomatidine as aglycone (from *S. acaule*) and commertetraose as saccharide moiety (from *S.* × *ajanhuiri*). In this communication we report the glycoalkaloid composition of the sisu hybrid and the characterization of a new glycoalkaloid which results from the independent inheritance of aglycone and saccharide.

RESULTS

All the 'sisu' clones contained two major ammonia precipitable components as determined by TLC. One of these compounds was readily identified as commersonine by comparison of its TLC behavior, GC/MS of the aglycone and GC analysis of the monosaccharides with an authentic sample of commersonine. The unknown glycoalkaloid named sisunine, yielded on hydrolysis tomatidine (determined by GC/MS) and the sugars galactose and glucose (determined by GC of aldononitrile derivatives). The fast atom bombardment mass spectrum (FAB-MS) (Table 1) confirms the M_r of sisunine to be 1063 and has a fragmentation pattern which is consistent with the sequential loss of four hexoses from a tomatidine aglycone [5] as shown in structure **3**. Permethylation analysis indicated hexoses with three different substitution patterns: unsubstituted, 4-substituted and 2,3-disubstituted which is again consistent with the structure **3**. According to these results, sisunine is considered to be the tomatidine O(3)-β-commertetraoside.

EXPERIMENTAL

For FAB-MS the sample was added to glycerol on the probe with 1 μl N oxalic acid. GC/MS were obtained using a 20 m OV-