Phytochemistry, Vol. 25, No. 2, pp. 429–431, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Press Ltd.

ACRIDONE ALKALOIDS FROM *PLEIOSPERMIUM ALATUM* (RUTACEAE)

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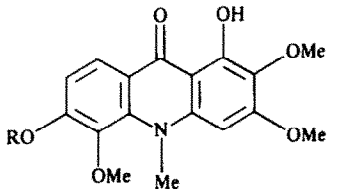
Key Word Index—*Pleiospermium alatum*; Rutaceae; chemotaxonomy; acridone alkaloids.

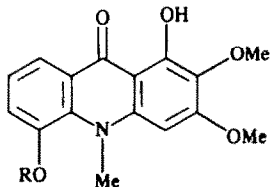
Abstract—The stem of *Pleiospermium alatum* has yielded two acridone alkaloids, 1,6-dihydroxy-2,3,5-trimethoxy-10-methyl-9-acridone and 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone. The significance of their occurrence in this genus is discussed.

INTRODUCTION

The genus *Pleiospermium*, according to Swingle [1], consists of five species. He comments: "This genus includes some of the most puzzling species of the subtribe Citrinae. These species have been referred to several different genera usually with a question mark". The genus is of interest in throwing light on the origin and development of the unique and highly specialized pulp-vesicle organs which are found only in the Citrinae and which reach their highest specialization in the genus *Citrus*. Very little information is available, however, on the phytochemistry of the genus. *Pleiospermium sumatranum* is reported [2] to contain eight flavonoids in the leaves, whilst from the leaves of *P. alatum* two alkamides have been isolated [3]. In this present work, however, we have not found significant amounts of secondary metabolites in the leaves but from the stems we have isolated two acridone alkaloids (**1** and **3**).



1 R = H
2 R = Me



3 R = H
4 R = Me
5 R = Ac

RESULTS

The leaves and stem of *Pleiospermium alatum* Wight et Arnold were separately and successively extracted with petrol, chloroform and methanol. TLC studies of the petrol extract of the stem revealed the presence of two compounds **1** and **3**, **3** being present in a very small quantity. The chloroform extract contained a small quantity of **1** and a much larger amount of **3**, nothing being detected in the methanol extract. The petrol and chloroform extracts of the leaves contained a yellow compound which seemed to be steroidal but which could not be isolated in sufficient amounts to allow characterization. None of the stem constituents appeared in the leaf extracts.

Compound **1**, isolated from the petrol and chloroform extracts of the stem, crystallized from chloroform–petrol (2:1) as yellow needles, mp 181–182°. The UV and IR spectra were typical of a 9-acridone [4–6], the IR spectrum showing bands at 3400 (OH) and 1630 cm^{-1} (CO) indicating hydrogen bonding and the presence of a *peri* (C-1) hydroxyl group [7]. The UV spectrum showed bathochromic shifts on addition of sodium methoxide. The accurate mass (331.1052) of **1** corresponded to a molecular formula of $C_{17}H_{17}NO_6$. The ^1H NMR spectrum indicated the presence of three methyls from OMe and one NMe group, represented by three singlets at δ3.79 (6H), 3.88 (3H) and 3.98 (3H). The spectrum also showed a downfield resonance at δ14.19 confirming the presence of a 1-hydroxyl group, strongly bonded to the carbonyl group. A singlet at δ6.4 (1H) was considered to be at too high a field to be due to a C-2 proton in a noracridone and was therefore attributed to a proton at C-4. In order to better characterize the (12 proton) three methyl singlets, comparisons of **1** and the known chemical shifts for OMe and NMe of 1,5-dihydroxy-3-methoxy-10-methyl-9-acridone (isolated from *Atalantia ceylanica* [8]) and 5-hydroxyarborinine (see compound **3**) and arborinine were made.

From these comparisons, the 6-proton singlet at δ3.79 was attributed to OMe at C-2 and the NMe group. The singlets at δ3.88 and 3.98 were assigned to OMe at C-5 and C-3 respectively. Addition of TFA [9] and observation of the methyl shifts in the NMR spectrum was not employed for **1** (isolated in very small quantity) as the process is destructive.

An examination of the NMR spectrum for the aromatic

proton resonance due to ring A showed two doublets centred at δ6.95 (J = 9 Hz, 1H) and δ7.95 (J = 9 Hz, 1H). The latter downfield resonance is characteristic for the C-8 proton due to the adjacent (*peri*-) carbonyl group. For this proton to appear as a doublet with a J-value of 9 Hz suggests an *ortho* coupling and therefore the doublet at δ6.95 was attributed to the C-7 proton. Thus the C-5 and C-6 positions are occupied by OH and OMe and in order to determine their relative positions a double resonance (NOE) experiment was carried out. Irradiation of the C-5/C-6 OMe group at δ3.88 did not result in any enhancement of the C-7 proton signal. The OMe group in ring A was therefore assigned to C-5 and the OH to C-6. During these experiments it was also observed that irradiation of the signal at δ3.98 due to C-3 OMe group, resulted in an enhancement of the C-4 proton, thereby further supporting the assignment of the singlet at δ3.98 to a C-3 OMe. Compound **1** was therefore identified as 1,6-dihydroxy-2,3,5-trimethoxy-10-methyl-9-acridone (**1**). The MS supported this assignment, with major peaks occurring at $[M-15]^+$, $[M-30]^+$ and $[M-29]^+$ due to two successive losses of Me and CHO.

Methylation of **1** with methyl iodide (reflux 4 hr) yielded yellow needles of the monomethyl derivative (**2**). The ^{1}H NMR spectrum showed an additional signal at δ4.05 (3H, *s*) due to the new methoxy group at C-6.

Acridone alkaloids with substitution at both C-5 and C-6 positions have recently been isolated from *Citrus depressa* [10] and a comparison of their NMR chemical shift values for the protons of ring A with those of **1** shows close agreement, supporting the assignment proposed for the OMe and OH groups of ring A in **1**.

Compound **3** also displayed all the characteristics of an acridone and seemed to be similar to 1,5-dihydroxy-2,3-dimethoxy-10-methylacridone (or 5-hydroxyarborinine) previously isolated from *Glycosmis bilocularis* [11] and *Atalantia monophylla* [12] although reported mp values for the compound and its derivatives varied somewhat. The 5-methyl ether (**4**) of **3** was therefore prepared and a comparison made of the physical and spectral characteristics of the alkaloid **3** from the three botanical sources. TLC of the parent compound and the monomethyl derivative of the three samples suggested they were similar. Mixed melting points of the samples of **3** showed no depression but the lower melting point of the *A. monophylla* sample was confirmed. On TLC, this sample was observed to be slightly improved, thus accounting for the deviation from the true melting point. The mixed melting points of the mono-methyl derivatives of **3** also showed no depression and the NMR values of the parent compounds and monomethyl derivatives were all in agreement with the structure proposed. We suggest therefore that the reported melting points for 5-hydroxyarborinine from *Glycosmis bilocularis* and *Atalantia monophylla* (and the monomethyl derivatives) be amended to read as herein quoted for **3**. The MS of **3** supported this assignment, with major peaks occurring at $[M-15]^+$, $[M-28]^+$, $[M-43]^+$ and $[M-58]^+$ due to losses of Me, CO, CH_2N and CH_2O. The fragment $[M-15]^+$ would be expected, being characteristic for *N*-methylacridones. Acetylation of **3** (acetic anhydride–pyridine) gave the monoacetate (**5**), its NMR spectrum showing an Me (acetate) peak at δ2.49.

DISCUSSION

1,6-Dihydroxy-2,3,5-trimethoxy-10-methyl-9-acridone is a new compound and, together with 5-hydroxyarborinine, forms the first report of the presence of acridone alkaloids in the genus *Pleiospermium*. As stated earlier, the leaves of *P. alatum* have been studied by Chaterjee *et al.* [3] and they reported the presence of the alkamides alatamide and *N*-benzoyltyramine methyl ether which we could not detect in our leaf extracts. In a study of a second sample of leaves they isolated only one alkamide, *N*-benzoyltyramine methyl ether, in a low yield but did not state whether the second sample was collected at the same time as the first or at a different time of the year; nor are there any reports of work on the stems of the plant. It is possible that there may be a seasonal and or geographical variation in the nature and quantity of the secondary metabolites of *Pleiospermium alatum*.

The presence of 5-hydroxyarborinine in both *Atalantia* and *Pleiospermium* suggests the possibility of an evolutionary connection between the two, especially as there are also morphological similarities between members of the two genera. This common presence of 5-hydroxyarborinine certainly supports both Swingle's and Engler's placing of *Atalantia* and *Pleiospermium* within the same subtribe and gives added support for Swingle's Citrinae as a natural group. Although acridones are fairly widely distributed within the three major subfamilies of the Rutaceae, those with substituents in both rings A and C are of more restricted occurrence. Most examples of this latter type have been isolated from the Aurantioideae, four of the five reports being from genera in the subtribe Citrinae (*Atalantia* [8, 12], *Pleiospermium*, *Severinia* [13], *Citrus* [10]). The fifth genus, *Glycosmis* [11], is in the subtribe Clauseninae (tribe Clauseneae) and it is not possible, therefore, to draw firm taxonomic conclusions from these data. The Aurantioideae as a whole would appear to be a complex group with many interrelationships and it is perhaps worth noting the observation of Scora [14] that the essential oil pattern of *Pleiospermium* suggests it to be closely related to the remote citroids of the Clauseneae.

EXPERIMENTAL

In all cases, petrol = petroleum bp 60–80°. Air dried powdered stem of *Pleiospermium alatum* Wight et Arnold (collected near Harbarane, Sri Lanka, Voucher No. 9E8, P.S.G.B. Museum, University of Bradford, Yorks) (1.36 kg) was extracted to exhaustion successively with petrol, $CHCl_3$ and MeOH in a Soxhlet. Flash chromatography [15] was performed on the petroleum extract using gradient elution from petroleum 100% to acetone 100% (increments of 5% Me_2CO).

Compounds **1** and **3** eluted together in the solvent system petrol–Me_2CO (3:1). Both gave visible yellow spots they were separated using prep. TLC with $CHCl_3$ and developing the plates twice. Flash chromatography [15] of the $CHCl_3$ extract using petrol–Me_2CO (7:3) also yielded **1** and **3** again eluting together. Their elution was monitored by TLC as before and they were separated by flash chromatography using $CHCl_3$–Me_2CO (9:1). Further purification of **1** was achieved by prep. TLC using $CHCl_3$–Me_2CO (9:1). Compound **3** eluted from the column in a pure state and did not require further purification. Fractions containing **3** were combined and reduced to dryness under vacuum to yield a yellow residue which was repeatedly washed with cold MeOH.

The air dried powdered leaves of *Pleiospermium alatum* (1.34 kg) were extracted to exhaustion successively with petrol, $CHCl_3$ and MeOH in a Soxhlet. TLC of the extracts revealed the

presence of a yellow coloured compound which gave a green colour with Dragendorff's reagent and a positive Liebermann–Burchardt reaction. Compounds **1** and **3** could not be detected in the leaf extracts. Repeated flash chromatography and prep. TLC failed to yield sufficient of the yellow compound to allow further characterization.

Compound **1** (1,6-dihydroxy-2,3,5-trimethoxy-10-methyl-9-acridone) crystallized from $CHCl_3$–petrol (2:1) as yellow needles (18 mg, 0.0013%) mp 181–182°. R_f ($CHCl_3$–MeOH, 19:1) 0.59, (petrol–Me_2CO, 4:1) 0.08. UV λ_{max}^{MeOH} nm (log ε): 220 (3.54), 258 (3.66), 267 (sh, 3.64), 330 (3.12). $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 224 (3.55), 258 (3.63), 272 (sh, 3.44), 336 (3.09). $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 222 (3.64), 258 (3.54), 267 (3.57), 333 (3.09). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2960, 2940, 1630, (CO), 1590, 1568, 1495, 1459, 1428, 1392, 1350, 1285, 1249, 1200, 1175, 1137, 1103, 1064, 1010, 899. ^1H NMR (60 MHz FT, Me_2CO-d_6): δ3.79 (*s*, 6H, C-2 OMe and NMe), 3.88 (*s*, 3H, C-5 OMe), 3.98 (*s*, 3H, C-3 OMe), 6.40 (*s*, 1H, C-4), 6.95 (*d*, 1H, *J* = 9 Hz, C-7), 7.95 (*d*, 1H, *J* = 9 Hz, C-8), 14.19 (*s*, 1H, C-1 OH, D_2O exchangeable). MS *m/z* (rel. int.): 332 (10%), 331 $[M]^+$ (52), 317 (20), 316 (100), 302 (9), 301 (45), 300 (19), 286 (4), 258 (4), 166 (9), 150 (8). Accurate mass 331.1052; $C_{17}H_{17}NO_6$ requires 331.1052.

Methylation of **1**. Compound **1** (10 mg) K_2CO_3 (100 mg) and MeI (1 ml) in dry Me_2CO (10 ml) were refluxed for 4 hr with the addition of 0.5 ml MeI every 30 min. The reaction mixture was poured into dil. HCl and the derivative extracted with EtOAc, washed repeatedly with water and evaporated to dryness to leave a yellow residue. TLC of the residue showed the reaction to have gone to completion and the derivative was purified by prep. TLC ($CHCl_3$–MeOH, 19:1; R_f 0.43). The compound was crystallized from $CHCl_3$–petrol (1:1) as yellow needles, mp 170°, and was characterized as the monomethyl ether of **1** (1-hydroxy-2,3,5,6-tetramethoxy-10-methyl-9-acridone, **2**). UV λ_{max}^{MeOH} nm (log ε): 220 (3.99), 258 (4.29), 267 (4.28), 332 (3.84), 396 (3.32). $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 222 (3.97), 258 (4.27), 268 (4.24), 333 (3.81), 405 (3.32). $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 240 (4.11), 265 (sh, 4.13), 279 (4.24), 356 (4.02). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1634 (CO). ^1H NMR (60 MHz FT, $CDCl_3$): δ3.77 (*s*, 6H, C-2, OMe and NMe), 3.86 (*s*, 3H, C-6 OMe), 3.94 (*s*, 3H, C-3 OMe), 4.01 (*s*, 3H, C-5 OMe), 6.37 (*s*, 1H, C-4), 6.96 (*d*, 1H, *J* = 9 Hz, C-7), 8.11 (*d*, 1H, *J* = 9 Hz, C-8), 14.09 (*s*, 1H, C-1 OH, D_2O exchangeable). MS *m/z* (rel. int.): 345 $[M]^+$ (54%), 330 (100%), 315 (26), 300 (15). $[M]^+$ 345 = $C_{18}H_{19}NO_6$.

Compound **3** (*1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone*) crystallized from $CHCl_3$–petrol (1:1) as yellow needles (237 mg, 0.017%), mp 202–203°. R_f ($CHCl_3$–MeOH, 19:1) 0.48 (petrol–Me_2CO, 4:1) 0.06. Accurate mass 301.0942. $C_{16}H_{15}NO_5$ requires 301.0950. The spectral data of **3** were similar to the authentic sample [11, 12, 16].

Methylation of **3** (*monomethyl*). Compound **3** (50 mg), K_2CO_3 (anhydrous, 500 mg) and MeI (1 ml) in Me_2CO (25 ml) was refluxed for 3 hr, with the addition of 0.5 ml MeI every 30 min. The reaction mixture was poured into dil. HCl and the derivative extracted with EtOAc, washed repeatedly with H_2O and evaporated to dryness to leave a yellow residue. The monomethyl derivative was purified using flash chromatography and crystallized from $CHCl_3$–petrol (2:1) as yellow needles, mp 134–135°, identical with authentic 1-hydroxy-2,3,5-trimethoxy-10-methyl-9-acridone [11, 12, 16] (**4**).

Acetylation of **3** (*monoacetate*). Compound **3** (30 mg), pyridine (1 ml) and HOAc (3 ml) were refluxed and the reaction monitored by TLC until complete (15 hr). The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. Prep. TLC was carried out on this extract using the solvent system petrol–Me_2CO (3:2) to purify the monoacetate, which crystallized as yellow needles from petrol–Et_2O (1:1), mp 182–183°, identical with authentic 5-acetyl-2,3-dimethoxy-1-hydroxy-10-methyl-9-acridone [16] (**5**).

Acknowledgements—We thank Dr. J. R. Lewis (Aberdeen University) for provision of 220 MHz NMR and MS data, and Prof. M. U. S. Sultanbawa and Dr. N. Balasubramaniam (University of Sri Lanka) for collection and authentication of plant material and Dr. J. S. Shah (Bombay) for samples of **3** and **4**.

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