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ALKALOIDS FROM THE STEM OF *GLYCOSMIS BILOCULARIS* (RUTACEAE)

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Key Word Index—*Glycosmis bilocularis*; Rutaceae; alkaloids; 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone; skimmianine; kokusaginine; dictamnine.

INTRODUCTION

We have previously reported [1] the isolation, from the leaves of *Glycosmis bilocularis* Thw. [2], of a mixture of quinazoline, furoquinoline and acridone alkaloids, including the novel alkaloid 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone (**1a**). We now report the isolation of alkaloids from the young stems of this same species and describe a synthesis of 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone. Its identity with the natural product provides final proof for the structure **1a**.

RESULTS AND DISCUSSION

The powdered young stems of *Glycosmis bilocularis* were successively extracted with petrol (bp 60–80°) and chloroform and the extracts concentrated. On standing 24 hr, a solid separated from the petrol extract, and preparative TLC of this solid yielded three alkaloids which were identical in all respects with authentic samples of skimmianine (**2a**), kokusaginine (**2b**) and 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone (5-hydroxyarborinine, **1a**), all previously isolated from the leaves [1]. Conventional acid/base extractions of the petrol mother liquor and the chloroform extract yielded the same three alkaloids and an additional furoquinoline alkaloid which we have identified as dictamnine (**2c**). We did not find any trace of quinazoline alkaloids in the stem.

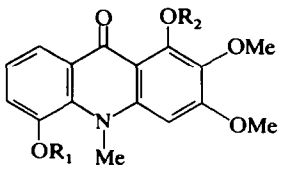
Although furoquinolines are common in the Rutaceae, they have been found in only six genera of the subfamily Aurantioideae, viz. *Glycosmis* [3], *Aegle* [4], *Afreagle* [5], *Citrus* [4], *Poncirus* [4] and *Murraya* [4]. It is also interesting to note that all reports of the occurrence of dictamnine in this subfamily have involved isolations from either the roots or the stems. Its absence from the leaves would indicate that the root is possibly the site of biosynthesis of this alkaloid. The relative lack of furoquinolines in the Aurantioideae might be considered as an advanced feature of the subfamily over the other two major subfamilies (Rutoideae and Toddalioideae) in which furoquinolines have been more frequently found.

The synthesis of 5-hydroxyarborinine (**1a**) was achieved by the Ullmann condensation of an aromatic amine and a halogen compound to yield a diphenylamine carboxylic acid which was subsequently cyclized to an acridone. This is a well-established route to the acridones [6, 7]. Condensation of 3-hydroxy-2-iodo-benzoic acid (**3a**) [8] with 3,4,5-trimethoxyaniline (**4**) gave 6-hydroxy-3′,4′,5′-trimethoxy-diphenylamine-2-carboxylic acid (**5a**) which cyclized on warming with conc sulphuric acid to yield a mixture of 5-hydroxy-1,2,3-trimethoxy-9-acridone (**6a**) and 1,5-dihydroxy-2,3-dimethoxy-9-acridone (**6b**), the latter compound being formed by the facile 1-demethylation of **6a** under the reaction conditions employed. Methylation of **6a** (MeI) produced a mixture of 5-hydroxy-1,2,3-trimethoxy-10-methyl-9-acridone (**1b**) and 1,2,3,5-tetramethoxy-10-methyl-9-acridone (**1d**), whilst similar treatment of **6b** produced three compounds, viz. 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone (**1a**), 1-hydroxy-2,3,5-trimethoxy-10-methyl-9-acridone (**1c**) and the fully methylated derivative **1d**. Compounds **1c** and **1d** were found to be identical with the mono- and di-methyl derivatives of the naturally occurring alkaloid which was itself identical with **1a**. Compound **1b** is interesting as it cannot easily be produced by methylation of the natural alkaloid which would preferentially methylate at the less resistant 5-hydroxyl (giving **1c**) rather than in the 1-position. In an alternative cyclization of **5a** by phosphorus oxychloride, hydrolysis of the resulting 9-chloroacridine (**7**) yielded **6a** with only traces of the demethylated product **6b**. Commencing the synthesis with 2-iodo-3-acetoxybenzoic acid (**3b**) produced the acetylated diphenylamine **5b** but during cyclization with acid the acetate group was hydrolysed (as expected) and the same mixture as before (**6a** and **6b**) resulted with no improvement in overall yield.

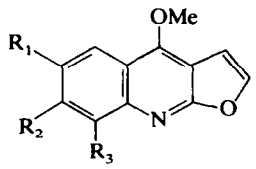
EXPERIMENTAL

All TLC separations were carried out on Si gel.

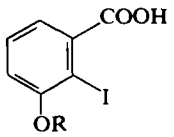
Extraction and isolation of the alkaloids. The air-dried, powdered young stems of *Glycosmis bilocularis* (410 g) were extracted successively (Soxhlet, 32 hr) with petrol (60–80°) and $CHCl_3$ (7 l. of each). The extracts were concd to 250 ml and left in a refrigerator for 24 hr. A brownish-yellow solid (1.6 g) separated from the petrol extract and PLC of this solid (C_6H_6–EtOAc–MeOH, 40:40:1; or $CHCl_3$–MeOH, 19:1) yielded three alkaloids, viz. skimmianine, kokusaginine (trace) and 5-hydroxyarborinine. The petrol mother liquor



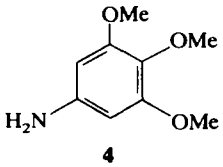
1a $R_1 = R_2 = H$
1b $R_1 = H^2$; $R_2 = Me$
1c $R_1 = Me$; $R_2 = H$
1d $R_1 = R_2 = Me$



2a $R_1 = H$; $R_2 = R_3 = OMe$
2b $R_1 = R_2 = OMe$; $R_3 = H$
2c $R_1 = R_2 = R_3 = H$



3a R = H
3b R = Ac



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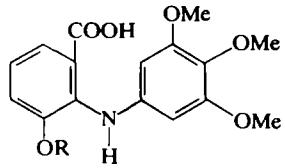
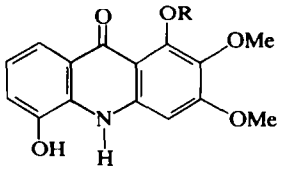
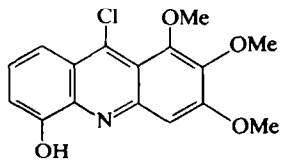


5a R = H
5b R = Ac



6a R = Me
6b R = H



7

was extracted with N HCl which was neutralized ($NaHCO_3$) and extracted with $CHCl_3$. The evapd $CHCl_3$ extract was separated by PLC ($CHCl_3$–MeOH, 19:1) and yielded skimmianine, kokusaginine and dictamnine. The residual petrol extract was extracted with N NaOH which was acidified (HCl) and extracted with $CHCl_3$. PLC of the evapd $CHCl_3$ soln gave 5-hydroxyarborinine. The $CHCl_3$ extract of the stems was treated in a similar manner to the petrol mother liquor and yielded the same four alkaloids; similar alkaloid fractions were combined and purified by TLC (C_6H_6–EtOAc, 3:2). The alkaloids 5-hydroxyarborinine (**1a**, 32 mg, 0.008%), skimmianine (**2a**, 32 mg, 0.008%) and kokusaginine (**2b**, 12 mg, 0.003%) were identical in all respects with the samples previously isolated from the leaves [1]. Dictamnine (**2c**, 21 mg, 0.005%) was identical (mp, mmp, spectral data, R_f) to an authentic sample.

Synthesis of 5-hydroxyarborinine (**1a**). *6-Hydroxy-3′,4′,5′-trimethoxy-diphenylamine-2-carboxylic acid* (**5a**). A mixture of 3-hydroxy-2-iodobenzoic acid [8] (1.5 g), 3,4,5-trimethoxyaniline (2.5 g), K_2CO_3 (dry, 1.0 g), freshly prepared Cu bronze (150 mg) and *i*-amyl alcohol (20 ml) was refluxed gently (oil bath, 150°) for 8 hr. The solvent was removed by steam distillation and the residual aq. soln clarified by boiling with charcoal followed by hot filtration. The filtrate was cooled (4°) and acidified (HCl) when a brown solid separated which was collected (filtration), washed (H_2O), dried and crystallized (hexane) as pale brown needles (280 mg), mp 146–147°; ν_{max}^{Nujol} cm^{-1}: 3320 (NH), 1660 (C=O), 1610, 1595, 1515, 1380, 1240, 1140, 1020, 1000, 820. M^+ at 319 for $C_{16}H_{17}NO_6$.

5-Hydroxy-1,2,3-trimethoxy-9-acridone (**6a**) *and 1,5-dihydroxy-2,3-dimethoxy-9-acridone* (**6b**). 6-Hydroxy-3′,4′,5′-trimethoxy-diphenylamine-2-carboxylic acid (**5a**) (140 g) was heated with conc H_2SO_4 on a H_2O bath for 2 hr. The H_2SO_4 soln was cooled, poured onto ice, and extracted with EtOAc which on evapn yielded a yellow-green powder. PLC (toluene–EtOAc, 3:2) yielded 5-hydroxy-1,2,3-trimethoxy-9-acridone (**6a**, 4 mg), mp 262–264°, M^+ at 301 for $C_{16}H_{15}NO_5$, and 1,5-dihydroxy-2,3-dimethoxy-9-acridone (**6b**, 11 mg), mp 298–299°, M^+ at 287 for $C_{15}H_{13}NO_5$.

5-Hydroxy-1,2,3-trimethoxy-9-acridone (**6a**): R_f (toluene–EtOAc, 3:2) 0.48; brown colour with $FeCl_3$; λ_{max}^{EtOH} nm: 275, 312, 425; $\lambda_{max}^{EtOH-NaOMe}$ nm: 280, 325, 438; ν_{max}^{KBr} cm^{-1}: 3460, 1660, 1610, 1590, 1540, 1530; MS *m/e* (rel. int.): 301 (M^+, 92%), 286 (100), 273 (58), 271 (45), 258 (30), 243 (92), 227 (28), 215 (22), 214 (26).

1,5-Dihydroxy-2,3-dimethoxy-9-acridone (**6b**): R_f (toluene–EtOAc, 3:2) 0.72, brown colour with $FeCl_3$; λ_{max}^{EtOH} nm: 269 (log $\sum$ 4.28), 333 (4.02), 347 (sh, 3.42), 362 (sh, 3.05); $\lambda_{max}^{EtOH-NaOMe}$ nm: 272 (4.42), 318 (sh, 4.36), 334 (3.28); $\lambda_{max}^{EtOH-HCl}$ nm: 276 (4.62), 318 (4.30), 332 (3.98), 349 (3.42), 367 (3.28); ν_{max}^{KBr} cm^{-1}: 3480 (NH), 3000 (br), 1640 (C=O); MS (*m/e*) 287 (M^+, 67%), 284 (62), 272 (100), 258 (15), 257 (75), 242 (8), 224 (28), 212 (80), 198 (65), 186 (60), 160 (42), 134 (98), 98 (45).

1,5-Dihydroxy-2,3-dimethoxy-10-methyl-9-acridone (**1a**) *and its mono* (**1c**) *and di-methyl* (**1d**) *derivatives*: A mixture of **6b** (6 mg), ignited K_2CO_3 (300 mg), MeI (4 ml) and dry Me_2CO (8 ml) was refluxed on a H_2O bath for 4 days. The reaction mixture was filtered and evapd to give a yellow solid

which was purified by PLC (toluene–EtOAc, 3:2). Two main products (**1c** and **1d**) were isolated in pure form and found to be identical (mp, mmp, TLC, spectral data) with the mono- and di-methyl derivatives of the naturally occurring alkaloid. A minor product (**1a**) was found to be identical (mmp, TLC, spectral data) with the natural product (**1a**, 5-hydroxyarborinine) itself.

5-*Hydroxy*-1,2,3-*trimethoxy*-10-*methyl*-9-*acridone* (**1b**). A mixture of **6a** (0.5 mg), ignited K_2CO_3 (30 mg), MeI (1 ml), dry Me_2CO (2 ml), was refluxed on a H_2O bath for 4 days. The reaction mixture was filtered, evapd and examined by TLC (toluene–EtOAc, 3:2). Two alkaloids were present, one of which (R_f 0.19) was identical with the fully methylated derivative **1d**. The second alkaloid (R_f 0.86) we tentatively identified as 5-hydroxy-1,2,3-trimethoxy-10-methyl-9-acridone (**1b**) which would be expected as an intermediate between **6a** and **1d**, but it was produced in too small an amount to be fully characterized.

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