Phytochemistry, Vol. 24, No. 11, pp. 2771–2773, 1985.
Printed in Great Britain.

0031-9422/85 \$3.00+0.00

SCHOLARICINE, AN ALKALOID FROM *ALSTONIA SCHOLARIS*

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(*Revised received* 11 *March* 1985)

Key Word Index—*Alstonia scholaris*; Apocynaceae; leaves; alkaloid; scholaricine; ^{13}C NMR.

Abstract—A new alkaloid, scholaricine, has been isolated to which structure **2** (demethylscholarine) has been assigned.

INTRODUCTION

Alstonia scholaris is a large ornamental tree widely cultivated in Pakistan. The bark of the tree is used in China and the Pacific islands as a bitter tonic, febrifuge and as an antimalarial. It is also commonly used in the treatment of diarrhoea, dysentery and for snake-bite [1]. The alcoholic extracts of the stem bark have shown anti-cancer activity in human sarcoma in embryonated eggs [1].

RESULTS AND DISCUSSION

The crude alkaloids (30 g) were isolated by the usual procedure from leaves of *A. scholaris* and subjected to preliminary separation on the basis of differential solubilities. Scholaricine (**2**) was isolated by a combination of CC and prep. HPLC.

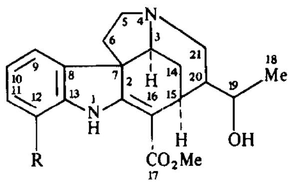
The UV spectrum of scholaricine was characteristic of an anilino-acrylate chromophore, $\lambda_{\max}^{\mathrm{MeOH}}$: 210, 235, 285 and 335 nm; on addition of base the maxima shifted to $\lambda_{\max}^{\mathrm{MeOH}}$: 217, 247, 299 and 358 nm. This suggested the presence of a phenolic OH group in the molecule. The IR spectrum gave absorptions at 3500 cm^{-1} (OH), 3400 cm^{-1} (NH) and 1660 cm^{-1} (α,β unsatd ester, C=O).

The mass spectrum afforded a $[\mathrm{M}]^+$ at m/z 356 while other major fragments appeared at m/z 338, 257, 139 and 94. The exact mass obtained by high resolution measurement was found to be m/z 356.1736 corresponding to the molecular formula $C_{20}H_{24}N_2O_4$ (calcd. 356.1735). The major fragment at m/z 257 (100%) afforded the exact mass 257.1299 which agreed with the formula $C_{15}H_{17}N_2O_2$. The ion is formed by loss of 99 mu which corresponds to cleavage of a fragment bearing the conjugated ester group. In addition prominent peaks were found at m/z 139 and 94 which are diagnostic of the akuammicine-type strychnos alkaloids [2]. A comparison of the mass spectrum with that of echitamidine [3] and scholarine (**1**) [2] established their close similarity and indicated that scholaricine is probably a hydroxy-echitamidine derivative.

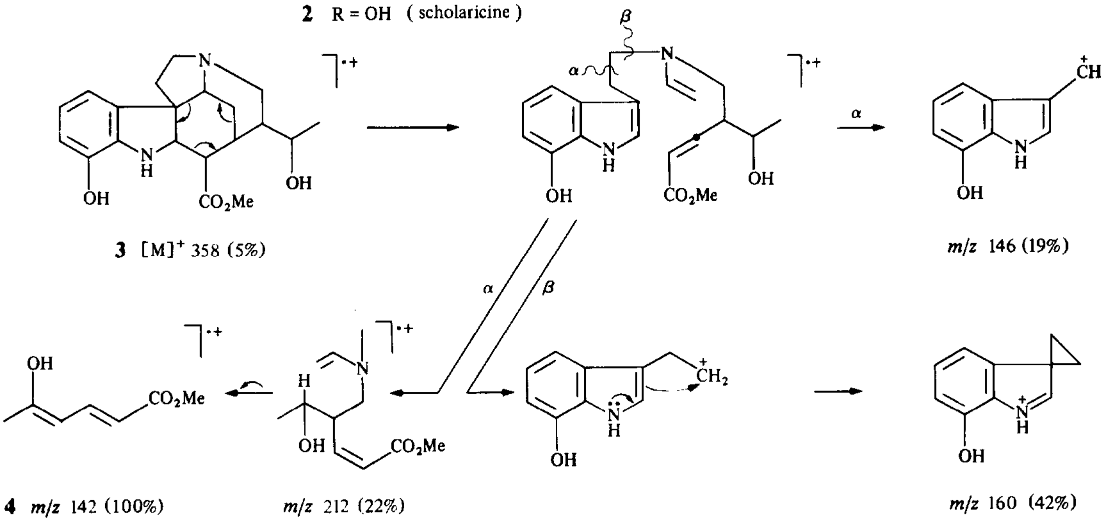
The ^{1}H NMR spectrum showed a three-proton singlet at δ3.83 which is assigned to the ester Me group. The NH proton appeared as a singlet at δ8.5. The Me group afforded a doublet at δ1.12 (J = 6.0 Hz) suggesting the presence of a –CH–Me moiety as in scholarine [2]. Integration of the aromatic region showed the presence of only three protons, which indicated the existence of a substituent in the benzene ring. The aromatic protons gave a complex ABC type multiplet in the region δ6.63 to δ7.12. This suggested that the phenolic OH group was present at C-9 or C-12, as location of the OH group at C-10 or C-11 would have afforded a readily recognizable ABX pattern.

The above conclusions were supported by the ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of scholaricine. The Me group of the –CH(OH)Me moiety resonated at δ19.74 while the OH bearing methine carbon appeared at δ68.53. A corresponding quartet and doublet were observed in the off-resonance spectrum. The peaks at δ51.77 and δ172.24 are assigned to the Me and carbonyl carbons of the ester group. Three aromatic methine carbons at δ111.32, δ122.35 and δ115.80 were discernible in the spectrum. These were assigned to C-9, C-10 and C-11, respectively. The chemical shifts were consistent with the location of the phenolic OH group at C-12 rather than at C-9. If the OH had been located at C-9, then an upfield shift would have been expected at C-10 and C-12 in comparison to the unsubstituted compound [4]. Comparison with the chemical shift values obtained for scholarine showed the expected shifts on transformation of an aromatic OMe group to a phenolic OH group. The assignments were confirmed by off-resonance, gated spin echo and polarisation transfer (DEPT) experiments. The ^{13}C NMR spectrum of scholaricine is recorded in Table 1.

Reduction of scholaricine with zinc–methanolic sulphuric acid afforded a slower moving compound. The reduction product showed a UV spectrum characteristic of the dihydroindole chromophore with absorption maxima at $\lambda_{\max}$ 245, 290 nm and $\lambda_{\min}$ 257 nm. The mass spectrum afforded an $[\mathrm{M}]^+$ at 358.1885 (calcd. for $C_{20}H_{26}N_2O_4$ 358.1892). Intense peaks were present at m/z 212, 160 and 146 corresponding to the m/z 212, 174, 160, respectively, observed in 2,16-dihydroscholarine [2]. The fragments at m/z 160 and 146 contain the indole moiety and are seen to be shifted to a lower mass by 14 mu in (**3**), (Scheme 1), confirming the presence of an OH group (instead of an OMe group, as in scholarine) on the benzene ring.



1 R = OMe (scholarine)
2 R = OH (scholaricine)



Scheme 1. Mass spectral fragmentation of **3**.

EXPERIMENTAL

IR were recorded as KBr discs, ^1H NMR at 300 MHz and ^{13}C NMR at 75 MHz with TMS as int standard. DEPT expts were carried out with $\theta = 45°, 90°$ and $135°$, quaternary carbons being determined by substraction from the broad-band ^{13}C spectrum. MS were recorded with a direct inlet system. HPLC was carried out on a 30 cm × 2.5 cm i.d. column packed with Merck 15–25 mu silica gel.

Table 1. ^{13}C NMR spectrum of scholaricine

Carbon	Chemical shifts	Multiplicity
2	169.08	*s*
3	60.17	*d*
5	53.88	*t*
6	43.37	*t*
7	57.96	*s*
8	132.15	*s*
9	111.32	*d*
10	122.35	*d*
11	115.08	*d*
12	136.98	*s*
13	141.82	*s*
14	31.02	*t*
15	28.94	*d*
16	96.67	*s*
17	172.24	*s*
18	19.74	*q*
19	68.53	*d*
20	45.96	*d*
21	48.16	*t*
COOMe	51.77	*q*

Plant material. Leaves of *A. scholaris* were collected from Karachi, Pakistan in June 1981 and identified by Prof. S. I. Ali, Department of Botany, University of Karachi.

Extraction and isolation. Leaves (40 kg) were cut into small pieces and finely ground in an Ultra-turrax in 95% EtOH. The extract was concd under vacuum to a gum. This was dissolved in 10% HOAc and the non-alkaloidal portion removed by extraction with EtOAc. The aq. acidic fraction was basified with dil NH_3 to pH 9 and extracted exhaustively with EtOAc to afford the alkaloids (30 g). These were subjected to initial fractionation by solvent extraction at room temp, first with petrol, then with EtOAc and finally with MeOH.

The EtOAc fraction (20 g) was further fractionated by CC over silica gel (F-254, mesh 60). Elution was carried out with increasing polarities of petrol, petrol–EtOAc, EtOAc and EtOAc–MeOH. The fraction obtained on elution with EtOAc–MeOH (9:1) (250 mg) was subjected to prep. HPLC. Elution with petrol–EtOAc and finally with EtOAc afforded the new alkaloid, scholaricine (**2**), $C_{20}H_{24}N_2O_4$, mp 180° (decomp), yield 250 mg $[\alpha]_D$ $-200°$ ($CHCl_3$); UV λ_{max}^{MeOH}: 210, 235, 285 and 335 nm, λ_{min}: 225, 255, 299 nm; IR (KBr): 3500 (OH), 3400 (NH), 1660 (α,β unsatd. ester) cm^{-1}; ^1H NMR ($CDCl_3$): δ1.12 (*s*, 3H, *J* = 6.0 Hz, H-18), 3.83 (*s*, 3H, COOMe), 8.5 (*s*, 1H, NH), 6.63 to 7.12 (*m*, 3H, aromatic protons). MS *m/z* (rel. int.) 356.1736 (40) $[M]^+$ ($C_{20}H_{24}N_2O_4$ calc. 356.1735) 311 (20) 257 (100) 196 (40) 139 (30) 94 (70).

2,16-*Dihydroscholaricine* (**3**). Zn dust (1 g) was added in small portions to a soln of scholaricine (4 mg) in 10% H_2SO_4 in MeOH

(10 ml). The mixture was refluxed under N_2 for 5 hr. The soln was then filtered, the residue washed with MeOH and the filtrates combined with the washings and concd. It was then partitioned between an ice-cold aq. NH_3 soln and $CHCl_3$. The $CHCl_3$ extract was washed (H_2O), dried (Na_2SO_4) and evapd to give 2,16-dihydroscholaricine as a gum (1 mg) which was then purified by prep. TLC on silica gel using MeOH–$CHCl_3$–NH_3 (1:9:0.1); UV λ_{max}^{MeOH}: 245, 290 nm; λ_{min}: 257 nm; MS: m/z 358.1885, (calc. for $C_{20}H_{26}N_2O_4$ 358.1892), 286, 257, 212, 160, 146, 129, 97.

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Phytochemistry, Vol. 24, No. 11, pp. 2773–2775, 1985.
Printed in Great Britain.

0031-9422/85 $3.00 + 0.00

THREE QUINICINE DERIVED ALKALOIDS FROM *GUETTARDA TRIMERA**

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(*Received* 25 *February* 1985)

Key Word Index—*Guettarda trimera*; Rubiaceae; trunk bark; alkaloids; quinicine derivatives.

Abstract—Three alkaloids, isolated from the trunk bark of *Guettarda trimera* have been identified as derivatives of quinicine on the basis of mass spectrometry, ^1H and ^{13}C NMR data.

INTRODUCTION

Extracts of *Guettarda* species have been extensively studied in our laboratory as they represent an interesting source of biosynthetic intermediates in the indole alkaloid series [1]. Moreover, it appears that each species produces alkaloids of only one structural type. This unusual situation is important for the chemotaxonomic classification of the Rubiaceae.

We wish now to report the isolation of three quinicine derivatives **1**, **3** and **4** from the trunk bark of *G. trimera*. This type of alkaloid was previously prepared by partial synthesis from quinine but was not known as natural products.

RESULTS AND DISCUSSION

Air dried bark of *G. trimera* was powdered and extracted following a classical method. Alkaloid **1** was obtained directly from the crude extract by crystallization from hot acetone. Alkaloids **3** and **4** were subsequently isolated by CC of the mother liquors on alumina and prep. TLC separation of alkaloid enriched fractions on silica gel.

Compound **1**, colourless crystals (mp 156°; acetone), $[\alpha]_D^{20} -84°$ ($CHCl_3$, c 1.2), $[M]^+$ at m/z 342, displayed a UV spectrum identical to that of quinine (λ_{max}^{EtOH} 233, 271, 281, 291, 323, 333). Fragments were observed in the mass spectrum at m/z 189 and 160 which suggested a methoxyquinoline derivative bearing a secondary hydroxyl group at C-9. However the fragments at m/z 138, 110 and 82 characteristic of the quinuclidine ring of quinine were absent.

In the ^1H NMR spectrum of **1** resonances were observed for a N-Me group (s, $\delta 2.2$) and a methoxyl group (s, $\delta 3.9$), the terminal methyl hydrogens of an ethyl side chain (t, 3H, $\delta 0.85$) and five aromatic protons. The signal at $\delta 5.32$ in **1** was observed to shift downfield to 6.41 in its

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*Part 98 in the series "Plants from New Caledonia". For part 97 see Razafinbelo, T., Langlois, N. and Andriamialisoa, R. Z. (1985) *C. R. Acad. Sci.* (in press).

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