Phytochemistry, Vol. 22, No. 12, pp. 2847–2850, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

STRUCTURE OF A BIPYRIDINE ALKALOID FROM *BROUSSONETIA ZEYLANICA**

A. A. LESLIE GUNATILAKA†, M. UVAIS S. SULTANBAWA‡ SIVAGNANASUNDRAM SURENDRAKUMAR and RATNASWAMY SOMANATHAN† §

Department of Chemistry, University of Peradeniya, Sri Lanka; §C.R.E.G.I.T., Instituto Technologico Regional de Tijuana, Tijuana, B.C., Mexico

(*Received* 22 *November* 1982)

Key Word Index—*Broussonetia zeylanica*; Moraceae; alkaloids; 8-hydroxyquinoline-4-aldehyde; 3,4′-dihydroxy-2,3′-bipyridine; structure elucidation.

Abstract—From the benzene extract of the timber of *Broussonetia zeylanica*, 8-hydroxyquinoline-4-aldehyde, a new alkaloid and two unidentified minor alkaloids have been isolated. The spectroscopic evidence suggested the new alkaloid to be 3,4′-dihydroxy-2,3′-bipyridine.

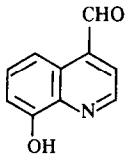
INTRODUCTION

We have previously reported [1] the isolation of 8-hydroxyquinoline-4-aldehyde (**1**), the major antimicrobial alkaloid from *Broussonetia zeylanica*, a plant species endemic to Sri Lanka [2]. In continuing our studies on medicinal and related plants of Sri Lanka [3], we have investigated the minor alkaloids present in this species and herein we report the isolation and structure elucidation of one of them, *viz.* 3,4′-dihydroxy-2,3′-bipyridine (**2**). Although neurotoxic 2,3′-bipyridyl alkaloids are known to occur in some tobacco species [4, 5] and in cigarette smoke condensate [6], this constitutes the first report of the natural occurrence of a hydroxylated 2,3′-bipyridyl alkaloid.

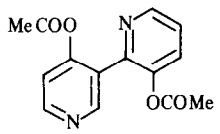
*Part 7 in the series "Studies on Medicinal and Related Plants of Sri Lanka". For Part 6 see ref. [3].

†To whom correspondence should be addressed.

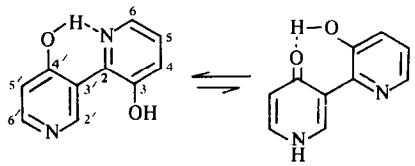
‡Present address: Department of Chemistry, University of Maiduguri, Maiduguri, Nigeria.



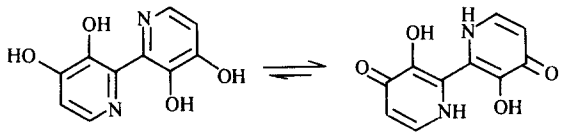
1



3



2



4

RESULTS AND DISCUSSION

The hot benzene extract of the timber of *B. zeylanica* on CC separation (see Experimental) yielded one major and three minor alkaloids. Spectral data of the least polar major alkaloid (0.011%), mp 155–156°, indicated it to be 8-hydroxyquinoline-4-aldehyde (**1**). The next polar minor yellow alkaloid (0.0034%), mp 223–224°, analysed for $C_{10}H_8N_2O_2$ and gave a green colouration with ferric chloride. The presence of a phenolic hydroxyl was indicated by a broad IR band at 3200 cm^{-1} and a bathochromic shift in the UV λ_{max} on addition of sodium hydroxide and aluminium chloride. This was further confirmed by acylation to give a colourless crystalline diacetate, mp 160–161°, which did not respond to the ferric chloride test. The UV spectrum was unchanged on addition of sodium hydroxide–boric acid, thus ruling out an *ortho*-dihydroxy structure.

Based on the spectral data (see below) we suspected the alkaloid to have a bipyridyl ring skeleton. Since the compound failed to form a red complex with ferrous sulphate (no λ_{max} in the visible region around 500 nm) a 2,2′-bipyridyl ring system was ruled out [7, 8], thus leaving 2,3′, 3,3′ and 2,4′ as possible ring attachments. The latter two were eliminated on the following spectral evidence. The 360 MHz ^{1}H NMR spectrum with double irradiation experiments revealed three adjacent protons at δ 7.14 (*d*), 7.51 (*t*) and 8.01 (*d*) (J = 7.6 Hz) in one ring and two adjacent protons at δ 7.79 (*d*) and 8.87 (*d*) (J = 4.5 Hz) in the other ring. In addition two exchangeable protons were also seen, one as a sharp singlet at δ 12.0 and the other as a broad signal at δ 9.90, probably due to a hydrogen bonded O<u>H</u> and N<u>H</u>, respectively. The broad signal at δ 9.90 strongly suggests the presence of an α- or a γ-pyridone type structure which are the preferred tautomers for α- and γ-hydroxypyridines in solution. This was further confirmed by IR which showed weak stretching at 1620 cm^{-1} in keeping with the γ-pyridone rather than the α-pyridone structure [9].

The UV, IR, ^{1}H and ^{13}C NMR (Table 1) spectra closely resembled those of a 2,3′-bipyridine ring skeleton. Having ascertained one ring to be a γ-pyridone leaves only one possible position for the second hydroxy group and that is at C-3. The upfield shifts observed for C-4, C-6 and C-5′ in ^{13}C NMR spectrum of **2** are compatible with the shifts observed for orelline (**4**) (Table 1) [10]. The foregoing evidence suggested the new alkaloid to be 3,4′-dihydroxy-2,3′-bipyridine (**2**).

It is interesting to note that both alkaloids **1** and **2** have structure capable of forming chelates with certain metal ions. Thus, they may play an important role in transport of metal ions in *B. zeylanica*.

There are only a few reports in the literature on the occurrence of alkaloids in plants belonging to the Moraceae. The significant ones are tylophorine type alkaloids in *Ficus* species [11], piperidine type in *Cannabis* [12] and *Morus* [13] species and morphine type in *Humulus* species [14].

Biosynthetically, **1** could arise from 7-hydroxytryptophan by a pathway depicted in Scheme 1, for which chemical analogies are known [15]. Biosynthesis of **2** may involve phenolic oxidative coupling of 3-hydroxy- and 4-hydroxypyridine.

EXPERIMENTAL

General. TLC was on silica gel G. Visualization was by spraying with Dragendorff reagent. CC was carried out on silica gel (30–70 mesh). Mps are uncorr. The microanalytical results were obtained from CSIRO, Microanalytical Service, Melbourne, Australia. IR were recorded in KBr discs and ^{1}H NMR spectra at 60 and 360 MHz. ^{13}C NMR were obtained at 50.11 MHz. MS were measured at 70 eV (direct insertion probe). Petrol refers to the fraction bp 60–80°.

Extraction. Dried and powdered timber (3.75 kg) of *B. zeylanica* (Thw.) Corner (= *Alleanthus zeylanicus*) collected at Hasalaka, Sri Lanka, was successively and exhaustively extracted with hot petrol, hot C_6H_6 and hot MeOH. The C_6H_6 extract (12 g) was subjected to CC on silica gel (375 g) made up in C_6H_6 and the column was eluted with increasing amounts of $CHCl_3$ in C_6H_6.

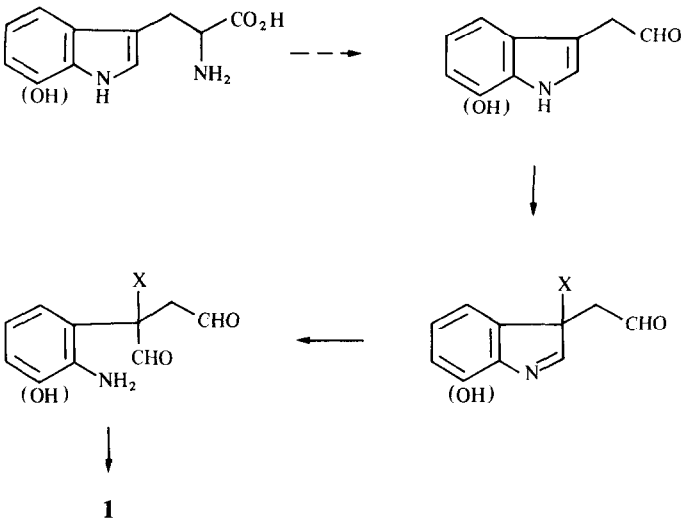
Isolation of 8-hydroxyquinoline-4-aldehyde (**1**). Elution of the column with 2% $CHCl_3$ in C_6H_6 gave **1** as a dark yellow solid (0.42 g, 0.0114%) which on recrystallization from $CHCl_3$ afforded golden yellow needles, mp and mmp 155–156° (lit. [1] 155–156°), which was identical (co-TLC, IR, ^{1}H NMR) with an authentic sample of 8-hydroxyquinoline-4-aldehyde.

Isolation of 3,4′-dihydroxy-2,3′-bipyridine (**2**). Elution of the column with 20% $CHCl_3$ in C_6H_6 gave yellow eluates, which after evaporation and recrystallization from $CHCl_3$ afforded deep yellow crystals (0.13 g, 0.0034%) of 3,4′-dihydroxy-2,3′-

Table 1. ^{13}C NMR chemical shifts (δ) of **2** and some bipyridines

Carbon No.	3,4′-Dihydroxy-2, 3′-bipyridine (**2**)	2,3′-Bipyridine	Δ*	3,3′-4′,4′-Tetrahydroxy-2,2′-bipyridine (**4**)	2,2′-bipyridine	Δ*
2	153.5 (*s*)	153.0	−0.5	—	155.4	—
3	125.5 (*s*)	121.0	−4.5	135.0	120.5	+15.5‡
4	114.4 (*d*)	135.5	+21.1†	137.0	137.2	−0.2
5	119.5 (*d*)	119.0	−0.5	112.3	124.1	+11.8‡
6	128.2 (*d*)	148.5	−20.3†	123.7	149.3	+25.6†
2′	146.3 (*d*)	146.9	+0.6	—	—	—
3′	136.8 (*s*)	133.2	−3.6	—	—	—
4′	139.1 (*s*)	132.0	−7.1	—	—	—
5′	114.4 (*d*)	122.0	+10.6‡	—	—	—
6′	147.6 (*d*)	148.5	−0.9	—	—	—

*Δ, δ parent bipyridine − δ hydroxylated bipyridine.
†Shift due to *ortho/para-N* and -OH.
‡Shift due to *ortho*-OH and *meta*-N.



Scheme 1. Possible biosynthesis of **1** from tryptophan.

bipyridine (**2**), mp 223–224°. It gave a green colouration with neutral $FeCl_3$ and exhibited an orange spot on TLC with Dragendorff spray reagent; UV λ_{max}^{MeOH} nm: 293 and 349 (log ε 4.57 and 3.53); $\lambda_{max}^{MeOH+NaOH}$ nm: 297, 307 and 408 (log ε 3.92, 3.87 and 3.49); $\lambda_{max}^{MeOH+HCl}$ nm: 302 and 393 (log ε 4.14 and 3.49); IR ν_{max}^{Nujol} cm^{-1}: 3200, 1620, 1525, 1470, 1400, 1200, 1160, 1000, 925 and 850. ^1H NMR (DMSO-*d*, 360 MHz): δ 12.05 (1H, *s*, OH), 9.90 (1H, *br s*, NH), 8.87 (1H, *d*, *J* = 4.5 Hz, H-6′), 8.82 (1H, *s*, H-2′), 8.01 (1H, *d*, *J* = 7.6 Hz, H-6), 7.79 (1H, *d*, *J* = 4.5 Hz, H-5′), 7.51 (1H, *t*, *J* = 7.6 Hz, H-5), and 7.14 (1H, *d*, *J* = 7.6 Hz, H-4); for ^{13}C NMR data see Table 1; MS *m/z* 188 $[M]^+$ (57%), 171 (96), 170 (15), 156 (14), 144 (10), 137 (16), 130 (24), 118 (22), 116 (28), 89 (17) and 69 (41). (Found: C, 63.24; H, 4.32; N, 14.61%. $C_{10}H_8N_2O_2$ requires: C, 63.83; H, 4.26; N, 14.89%.)

Acetylation of **2**. Alkaloid **2** (20 mg) was briefly warmed with Ac_2O (1 ml) and pyridine (2 ml) and kept at room temp. overnight. Pyridine was removed azeotropically with C_6H_6 and, after usual work-up, 3,4′-diacetoxy-2,3′-bipyridine (**3**) was obtained as a colourless crystalline solid (16.8 mg, 84%), mp 160–161° (from C_6H_6–petrol); ^1H NMR ($CDCl_3$, 60 MHz): δ 9.00 (1H, *d*, *J* = 5 Hz, H-6′), 8.86 (1H, *s*, H-2′), 8.33 (1H, *dd*, *J* = 6 Hz, H-5), 7.76–7.50 (3H, *m*, H-4, H-5′ and H-6), 2.46 (3H, *s*, OCOMe), and 2.26 (3H, *s*, OCOMe); MS *m/z* 230 $[M-CH_2CO]^+$ (9%), 171 (26), 170 (100), 142 (74), 114 (33) and 88 (19).

Isolation of unidentified alkaloid I. Elution of the column with 25% $CHCl_3$ in C_6H_6 afforded a pale yellow solid which, on recrystallization from $CHCl_3$, gave colourless crystals (0.42 g, 0.0112%), mp 238–239°; MS $[M]^+$, 372.1109 ($C_{22}H_{16}N_2O_4$); UV λ_{max}^{MeOH} nm: 246 and 287 (log ε 4.04 and 3.48); $\lambda_{max}^{MeOH+NaOH}$ nm: 262 (log ε 4.35); IR ν_{max}^{KBr} cm^{-1}: 3300, 1775, 1570, 1470, 1420, 1405, 1365, 1330, 1295, 1270, 1225, 1210, 1180, 1160, 1130, 1090, 1050, 1015, 980, 930, 880, 865, 835, 820, 800, 780, 750, 710, 690 and 660. ^1H NMR (DMSO-d_6, 360 MHz): δ 9.88 (1H, *br s*, OH), 9.48 (2H, *t*, *J* = 5 Hz), 8.34 (1H, *s*), 8.30 (1H, *d*, *J* = 5 Hz), 7.80 (1H, *d*, *J* = 5 Hz), 7.08–7.48 (6H, *m*), 6.78 (1H, *d*, *J* = 8 Hz), 4.88 (1H, *q*, *J* = 8 Hz), 3.04 (1H, *dd*, *J* = 8 and 2 Hz); ^{13}C NMR (DMSO-d_6): δ 42.4, 80.4, 111.2, 111.3, 112.7, 118.4, 119.1, 125.9, 127.3, 127.7, 127.9, 138.5, 143.1, 144.9, 147.8, 153.7 and 174.9.

Diacetate of alkaloid I. The alkaloid (20 mg) was acetylated with Ac_2O (1 ml) and pyridine (2 ml) overnight at room temp. After usual work-up, the diacetate (15.2 mg, 76%) was obtained as colourless crystals, mp 168–169° (from C_6H_6–petrol); ^1H NMR ($CDCl_3$, 60 MHz): δ 9.06 (1H, *d*, *J* = 2 Hz), 8.96 (1H, *d*, *J* = 2 Hz), 7.83–7.16 (8H, *m*), 6.50 (1H, *m*), 4.50 (1H, *m*), 3.09 (1H, *d*, *J* = 9 Hz), 2.83 (1H, *d*, *J* = 4 Hz), 2.53 (3H, *s*, OCOMe) and 2.50 (3H, *s*, OCOMe); MS *m/z* 414 $[M-CH_2CO]^+$ (58%), 372 (100), 171 (100), 143 (81) and 127 (53).

Isolation of unidentified alkaloid II. Elution of the column with $CHCl_3$ gave an orange gum which, when dissolved in C_6H_6 and triturated with petrol, gave a yellow amorphous solid (41.5 mg, 0.001%), mp 231–232° (from $CHCl_3$), which gave a fluorescent complex with Mg^{2+}; UV λ_{max}^{MeOH} nm: 247 and 261 (log ε 4.17 and 4.23); IR ν_{max}^{KBr} cm^{-1}: 3300, 1780, 1505, 1470, 1405, 1365, 1330, 1270, 1225, 1160, 1055, 1015 and 750. ^1H NMR (acetone-d_6, 60 MHz): δ 8.88 (1H, *s*), 8.86 (1H, *d*, *J* = 5 Hz), 8.07 (1H, *dd*, *J* = 8 and 2 Hz), 7.80 (1H, *d*, *J* = 5 Hz), 7.56 (1H, *t*, *J* = 8 Hz) and 7.17 (1H, *dd*, *J* = 8 and 2 Hz). (Found: C, 71.29; H, 4.54; N, 7.50%; $[M]^+$ 372. $C_{22}H_{16}N_2O_4$ requires: C, 70.96; H, 4.30; N, 7.53%; $[M]^+$ 372.)

Diacetate of alkaloid II. The alkaloid (16 mg) was acetylated with Ac_2O (1 ml) and pyridine (2 ml). The reaction mixture was warmed briefly and kept at room temp. overnight. Usual work-up afforded the diacetate (14 mg, 87%) as a colourless crystalline solid, mp 152–154°; ^1H NMR ($CDCl_3$, 60 MHz): δ 9.00 (1H, *d*, *J* = 5 Hz), 8.93 (1H, *s*), 8.35 (1H, *dd*, *J* = 7 and 2 Hz), 7.80 (1H, *s*), 7.70 (1H, *d*, *J* = 3 Hz), 7.57 (1H, *m*), 2.50 (3H, *s*, OCOMe) and 2.30 (3H, *s*, OCOMe).

Acknowledgements—We thank Professor S. Balasubramaniam for identification of plant material; Ms. P. H. S. S. A de Silva, D. V. Ariyapala and P. Liyanage for technical assistance; Mrs. S. C. Weerasekera for typing the manuscript; the University of Peradeniya for a Research Assistantship (to S.S.); the International Foundation for Science (Sweden) and the National Science Council (Sri Lanka) for financial assistance.

REFERENCES

1. Gunatilaka, A. A. L., Sultanbawa, M. U. S., Perera, J. S. H. Q., Brown, P. M. and Thomson, R. H. (1979) *J. Chem. Res.* (S) 61; (M), 779.
2. Bandaranayake, W. M. and Sultanbawa, M. U. S. (1969) *Proc. Ceylon Assoc. Advmt. Sci.* **25**, 90.

3. Gunatilaka, A. A. L., de Silva, A. M. Y. J. and Sotheeswaran, S. (1982) *Phytochemistry* **21**, 1751.
4. Warfield, A. H., Galloway, W. D. and Kallianos, A. G. (1972) *Phytochemistry* **11**, 3371.
5. Leete, E., Ranborn, K. C. and Riddle, R. M. (1979) *Phytochemistry* **18**, 75.
6. Brown, E. V. and Ahmad, I (1972) *Phytochemistry* **11**, 3485.
7. McInnes, A. G., Smith, D. G., Wright, J. L. C. and Vining, L. C. (1977) *Can. J. Chem.* **55**, 4159.
8. Brandt, W. W., Dwyer, F. P. and Gyarfas, E. C. (1954) *Chem. Rev.* **54**, 959.
9. Katritzky, A. R. and Jones, R. A. (1960) *J. Chem. Soc.* 2947.
10. Antkowiak, W. Z. and Gessner, W. P. (1979) *Tetrahedron Letters* 1931.
11. Russel, J. H. (1963) *Naturwissenschaften* **50**, 443.
12. Stepanyan, M. S. (1963) *Izv. Akad. Nauk. Arm. SSR. Biol. Nauki* **16**, 77.
13. Talybova, A. D. (1965) *Vopr. Vnutr. Retseptsii Fiziol. Aktivn. Veshchestv. Akad. Nauk. Azerb. SSR.* 105.
14. Orekove, A. P. (1955) *Chemistry of Alkaloids* 2nd edn. Akad. Nauk. U.S.S.R., Moscow.
15. Van Tamelen, E. E., Haarstad, V. B. and Orvis, R. L. (1968) *Tetrahedron* **24**, 687.