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ALKALOIDS FROM *ALSTONIA MACROPHYLLA*

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Key Word Index—*Alstonia macrophylla*; Apocynaceae; alkaloids; (−)-strictaminolamine, (−)-1,2-dihydro-*N*-methylstrictamine; 2D-NMR

Abstract—The leaves of *Alstonia macrophylla* have yielded a new alkaloid, (−)-strictaminolamine. In addition to this the known alkaloid, (−)1,2-dihydro-*N*-methylstrictamine previously found in *Catharanthus longifolius*, was also obtained.

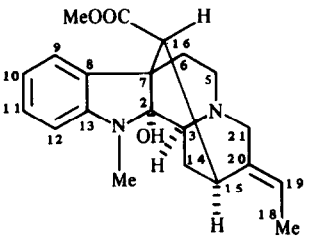
INTRODUCTION

Alstonia macrophylla Wall. has considerable physiological importance and is reputed for its medicinal and poisonous properties. Chemical investigations of *A. macrophylla* have resulted in the isolation of many monomeric and dimeric indole bases [1–10]. The present publication describes the isolation of a new dihydroindole alkaloid, (−)-strictaminolamine (**1**), and a known indoline base, (−)-1,2-dihydro-*N*-methylstrictamine (**2**) which has been isolated for the first time from this plant. Their structures have been established and identified on the basis of 2D NMR and other spectroscopic studies.

RESULTS AND DISCUSSION

The crude alkaloids were isolated from the concentrated alcoholic extracts of the leaves of *A. macrophylla* by extraction at different pH values. The fraction obtained at pH ~9.0 afforded the alkaloids **1** and **2**.

The UV spectrum of (−)-strictaminolamine (**1**) was characteristic for the dihydroindole chromophore with absorption maxima at 207, 224 and 292 nm and minima at 227 and 268 nm [11]. The IR spectrum of compound **1** displayed strong absorptions at 1720 (ester C=O) and 1595 (C=C).



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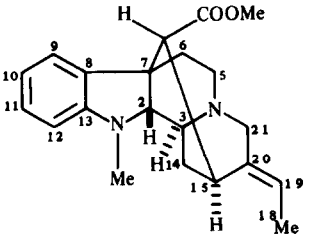

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The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of **1** bore a distinct similarity to that of (−)-1,2-dihydro-*N*-methylstrictamine (**2**) [13]. A three-proton double doublet resonating at δ1.45 ($J_{18,19}$=7.2 Hz, $J_{18,21}$=1.9 Hz) was assigned to the methyl group of the ethylidine side chain showing homoallylic coupling with C-15 and C-21 protons. The C-19 olefinic proton appeared as a split quartet at δ5.42 showing vicinal coupling with the Me-18 protons ($J_{19,18}$=7.2 Hz) and allylic coupling with the C-15 and C-21 protons ($J_{19,15}=J_{19,21}\sim$1 Hz). A three-proton singlet at δ2.69 was due to the indolic N-CH_3 group, while another 3H singlet at δ3.51 could be assigned to the carbomethoxy methyl protons. The C-12 aromatic proton was found to resonate as a doublet at δ6.58 ($J_{12,11}$=7.3 Hz) while the C-11 aromatic proton appeared as a doublet of double doublet at δ6.75 ($J_{11,12}$=7.3 Hz, $J_{11,10}$=7.6 Hz, $J_{11,9}$=0.9 Hz) The vicinal C-10 aromatic proton resonated as another doublet of double doublet centred at δ7.13 ($J_{10,11}$=7.6 Hz, $J_{10,9}$=7.3 Hz, $J_{10,12}$=1.3 Hz). A double doublet at δ7.08 ($J_{9,10}$=7.3 Hz, $J_{9,11}$=1.2 Hz) was assigned to the C-9 proton. The presence of four signals each integrating for one-proton, corresponded to the four aromatic protons of the dihydroindole nucleus.

Two dimensional NMR experiments (COSY 45°, NOESY) [14,15] were carried out to verify the assignments. The coupling interactions were established through the COSY 45° spectrum, which showed a strong cross-peak between the C-18 methyl (δ1.45) and C-19 olefinic protons (δ5.42). COSY interactions between C-6 protons (δ2.10) with C-5α and β protons were observed. The C-3 proton appeared as a doublet at δ3.91 ($J_{3,14}$=4.8 Hz), while the C-15 proton resonated as a multiplet at δ3.35. The C-16 bridgehead proton appeared as a

multiplet at δ2 63 ($J_{16,15}$=4 5 Hz) The C-21α proton resonated at δ2 96 as a doublet ($J_{21\alpha,21\beta}$= 16.2 Hz) while a multiplet at δ3 87 was assigned to the C-21β proton ($J_{21\beta,21\alpha}$=16 2 Hz) The multiplets at δ3 44 and 3.89 were assigned to the C-5α and β protons respectively The downfield shift reflecting the electron withdrawing α nitrogen function The C-6 protons appeared at δ2 10 as a 2H multiplet The absence of the characteristic low field signal for the C-2 proton (around δ4 0) suggested the presence of a C-2 substituent (hydroxyl group) The C-3 proton (δ3 91) showed cross-peaks only with the C-14 protons This also suggested that no proton is present at C-2 Similarly the cross-peaks between C-15H (δ3 35) and C-16H (δ2.63) were also observed in the COSY-45 spectrum The geminal coupling interaction between C-21αH (δ2 96) and C-21βH (δ3 87) helped to confirm their assignments The assignments of aromatic protons were also confirmed by their respective cross-peaks in the COSY-45 spectrum

The NOESY spectrum of **1** established the relative stereochemistry at various asymmetric centres The cross peak between the C-18 methyl protons (δ1 45) and C-15H (δ3 35) suggested '*E*' configuration for the 19,20 double bond Similarly the cross-peak between N-Me protons (δ2 69) and the C-12 aromatic proton (δ6 48) confirmed the presence of a methyl group and the dihydroindole nitrogen atom Cross-peaks between C-6H and C-5αH, as well as between C-15H and C-16H were also observed in the NOESY spectrum

The mass fragmentation pattern of the alkaloid was characteristic of picraline bases [16]. The high resolution mass spectrum of compound **1** showed the molecular ion at *m/z* 354 1947 corresponding to the molecular formula $C_{21}H_{26}N_2O_3$ indicating the presence of 10 double bond equivalents A peak at *m/z* 336 arose by the loss of water from the molecular ion The base peak at *m/z* 122 corresponding to the formula $C_8H_{12}N$ was attributed to the substituted piperidine ion Other prominent peaks were found at *m/z* 307, 277, 263, 167, 149, and 121 The mass spectrum of compound **1** after deuterium exchange with CD_3OD showed an increase of one mass unit in M^+ confirming the presence of one exchangeable proton (hydroxyl group).

The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of the alkaloid confirmed the presence of a dihydroindole nucleus and its picraline-type skeleton The multiplicity assignments were made by GASPE experiments The methyl carbon of the ethylidine side chain appeared at δ13 53, while its C-19 olefinic carbon resonated at δ128 63 The signals at δ29 72 and 51 53 corresponded to *N*-methyl carbon and ester methyl carbon resonances respectively while the ester carbonyl carbon appeared at δ171 25 The C-15 and C-16 methine carbons, and the C-7 quaternary carbon resonated at δ31 52, 52 15 and 52 33 respectively suggesting the presence of a picraline nucleus The C-3 carbon appeared at δ56.96, its upfield chemical shift indicating the absence of an indolenine double bond to it. The aromatic carbons also showed their respective resonances consistent to the structure proposed for (−)-strictaminolamine (**1**). The ^{13}C chemical shifts are presented in Table 1 On the basis of above spectroscopic studies, structure **1** is assigned to (−)-strictaminolamine

The second alkaloid was identified as (−)-1,2-dihydro-*N*-methylstrictamine (**2**) by comparison of its spectral data, (MS, NMR, UV and IR) with those reported in the literature [13] The ^1H NMR spectral assignments were confirmed by 2D NMR experiments Alkaloid **2** had previously been found in *C. longifolius* [13]

Table 1 ^{13}C NMR chemical shift assignments for (−)-strictaminolamine (**1**)

C	δ	C	δ
2	97 00*	18	13 53
3	56 96	19	128 63†
5	49 46	20	130 00
6	28 57	21	56 40
7	52 33	N-Me	29 72
8	128 84	COOMe	51 53
9	126 61†	COOMe	171 25
10	123 86		
11	120 58		
12	109 93		
13	148 50		
14	21 93		
15	31 52		
16	52 15		

†Values are interchangeable
*Weak signal

EXPERIMENTAL

The leaves of *A. macrophylla* (dry weight 30 kg) were collected from Colombo district, Sri Lanka in Nov 1985 The plant was identified by Prof S Balasubramonium (Department of Botany, University of Paradeniya)

Extraction and purification Extraction was with EtOH at room temp The solvent was evapd *in vacuo* to afford a gum (30 g) which was taken up in 10% HCl The pH was then adjusted by addition of 20% NH_4OH The fraction obtained by extraction with $CHCl_3$ at pH ~ 9 0 was evapd and subjected to CC on silica gel Elution was with increasing polarities of $CHCl_3$–MeOH Two important fractions were obtained fractions A (9 1) and B (19 1)

(−)-*Strictaminolamine* (**1**) Fraction A was again subjected to CC (silica gel) and eluted with $CHCl_3$–MeOH The fraction obtained using $CHCl_3$–MeOH (9 1) was purified on TLC (alumina, C_6H_{14}–MeOH 99 1) to afford **1** (4 mg), yellow amorphous, $[\alpha]_D$ = −30 ($CHCl_3$) UV λ_{max}^{MeOH} nm 207, 244, 292, λ_{min} nm 227, 268, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1722 (ester carbonyl), 1598 (C=C), 1280 (O-H), ^1H NMR (300 MHz, $CDCl_3$) δ 1 45 (3H, *dd*, $J_{18,19}$=7 2 Hz, $J_{18\,21}$=1 9 Hz, H-18), 2 01 (1H, *m*, H-14α), 2 10 (2H, *m*, H-6), 2 54 (1H, *dd*, $J_{14\beta\,14\alpha}$=13 5 Hz, $J_{14\beta\,15}$=9 2 Hz, H-14β), 2 63 (1H, *d*, $J_{16\,15}$=4 5 Hz, H-16), 2 69 (3H, *s*, N-Me), 2 96 (1H, *d*, $J_{21\alpha,21\beta}$=16 2 Hz, H-21α) 3 35 (1H, *m*, H-15), 3 51 (3H, *s*, COOMe), 3 87 (1H, *d*, $J_{21\beta,21\alpha}$=16 2 Hz, H-21β), 3 44 (1H, *m*, H-5α), 3 89 (1H, *m*, H-5β), 3 91 (1H, *d*, $J_{3\,14}$=4 8 Hz, H-3), 5 42 (1H, *q*, $J_{19\,18}$=7 2 Hz, H-19), 6 58 (1H, *d*, $J_{12,11}$=7 3 Hz, H-12), 6 75 (1H *ddd*, $J_{11,12}$=7 3 Hz, $J_{11\,10}$=7 6 Hz, $J_{11\,9}$=0 9 Hz, H-11), 7 13 (1H, *ddd*, $J_{10\,11}$=7 6 Hz, $J_{10\,9}$=7 3 Hz, $J_{10\,12}$=1 3 Hz, H-10), 7 08 (1H, *dd*, $J_{9,10}$=7.3 Hz, $J_{9,11}$=1 2Hz, H-9), MS *m/z* (rel int) 354 1947 (M^+ $C_{21}H_{26}N_2O_3$, calcd 354 1943), 339 (5), 336 (20), 307 (8), 277 (26), 263 (17), 167 (13), 149 (21), 122 (100), 121 (58)

(−)-1,2-*Dihydro*-N-*methylstrictamine* (**2**) Fraction B was loaded on a silica gel column and eluted with increasing polarities of $CHCl_3$–MeOH (49 1) It was purified further by prep TLC (alumina) to afford alkaloid **2**, (5 mg), $[\alpha]_D$ = −45° ($CHCl_3$), UV λ_{max}^{MeOH} nm 207, 244, 292, λ_{min} nm 227 267, IR $\nu_{max}^{CHCl_3}$ cm^{-1}

1722 (ester carbonyl), 1598 (C=C), 1280 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (3H, *dd*, $J_{18,19}=5.3$ Hz, $J_{18,21}=1.8$ Hz, H-18), 2.59 (3H, *s*, N-Me), 3.60 (3H, *s*, COOMe), 5.42 (1H, *dq*, $J_{19,18}=5.8$ Hz, $J=1.0$ Hz, H-19), 6.58 (1H, *d*, $J_{12,11}=5.9$ Hz, H-12), 6.75 (1H, *ddd*, $J_{11,12}=5.9$ Hz, $J_{11,10}=5.8$ Hz, $J_{11,9}=0.7$ Hz, H-11), 7.14 (1H, *ddd*, $J_{10,11}=5.8$ Hz, $J_{10,9}=5.5$ Hz, $J_{10,12}=1.0$ Hz, H-10), 7.08 (1H, *dd*, $J_{9,10}=5.5$ Hz, $J_{9,11}=0.7$ Hz, H-9), MS *m/z* (rel int) 338.2107 (M^+, $C_{21}H_{26}N_2O_2$, calcd 338.2072, 100), 323 (20), 279 (23), 144 (30), 122 (21), 121 (19), 69 (62), 58 (92)

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