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DIMERIC TROPANE ALKALOIDS FROM *ERYTHROXYLUM MOONII*

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Key Word Index—*Erythroxylum moonii*; Erythroxylaceae; leaves; tropane alkaloids; cinnamate dimers.

Abstract—Two new dimeric tropane alkaloids, mooniines A and B were identified from the leaves of *Erythroxylum moonii.* Their structures were determined by mass spectrometry and multi-dimensional NMR spectroscopy. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Erythroxylum moonii is a small shrub from Sri Lanka, which has not been previously chemically investigated. This plant has been used as an effective anthelmintic for round worms and its leaves have also been used externally for the suppuration of boils and abscesses. The alcoholic extract showed strong antifungal activity against *Candida albicans*, *Aspergillus niger*, *Curvularia lunata*, *Penicillium notatum*, *Alternaria solani*, *Epidermophyton floccosum*, *Dutarium rotatum*, *Microsporum canis* and *Ascomycetes* sp. but was found inactive against *Fusarium solani.* The extract did not exhibit antibacterial activity against *Staphylococcus aureus*, *Streptococcus epidermidis*, *Bacillus subtilis*, *Corynebacterium hofmanni*, *Corynebacterium diphtheriae*, *Pseudomonas aeruginosa*, *Pseudomonas pseudomalliae*, *Aeromonas sobriae*, *Shigella boydii* and *Escherichia coli* at concentrations of 100 and 200 μg ml^{-1}.

The dimeric tropane alkaloids form a small group of natural products, comprising two tropane nuclei combined with one, two or three carboxylic acid moieties, which, in most cases, is a dibasic acid. Such alkaloids have been reported from the Solanaceae, Erythroxylaceae and Convolvulaceae. The first dimeric tropane alkaloid was reported as early as the last century [1]. Up till now ca 15 dimeric tropane alkaloids have been reported [2]. We now report two new dimeric tropane alkaloids, mooniine A (**1**) and mooniine B (**2**) from leaf extracts of *E. moonii.*

RESULTS AND DISCUSSION

The methanolic extract of powdered leaves afforded a mixture of tropane alkaloids which were fractionated by column and thin layer chromatography. Two new dimeric alkaloids **1** and **2** thus obtained were characterized by IR, UV, mass spectrometry and one- and two-dimensional NMR spectroscopy [3, 4].

The HREI mass spectrum of **1** showed the $[M]^+$ at m/z 600.3194 corresponding to the molecular formula $C_{36}H_{44}N_2O_6$ (calc. 600.3199). The EI mass spectrum showed that the alkaloid fragments into two units, **1a** and **1b**, corresponding to m/z 329 (3α-cinnamoyloxy, 6β-propanoyloxynortropane) and m/z 271 (3α-cinnamoyloxytropane), respectively. Unit **1a** ($C_{19}H_{23}NO_4$) further fragmented at C-3 giving rise to two peaks at m/z 182 (propanoyloxynortropane moiety) and at m/z 147 ($C_9H_7O_2$, cinnamoyloxy moiety). The presence of the propanoyloxy group was also indicated by the peaks at m/z 73 ($C_3H_5O_2$) and m/z 101 [C(7)H_2-C(6)HOCOCH_2CH_3], resulting from cleavage of the heterocyclic ring at C-1/C-7 and at C-5/C-6. The peaks at m/z 73, 101 and 182 indicated that **1a** possessed a 6- or 7-substituted nortropane nucleus, which was further supported by the appearance of the base peak at m/z 110.0953 ($C_7H_{12}N$, calc. 110.0969). Further fragmentation of **1b** [m/z 271 ($C_{17}H_{21}NO_2$)] at C-3′ led to the formation of fragments at m/z 124 ($C_8H_{14}N$) and 147 (PhCHCHCOO). The fragments at m/z 140 ($C_8H_{14}NO$) and 168 ($C_9H_{14}NO_2$) resulted from the loss of PhCHCHCO and PhCHCH ions from **1b**, respectively. The ions at m/z 77 (C_6H_5), 103 (C_8H_7), 131 ($C_9H_7O_2$) and 147 ($C_9H_7O_2$) were consistent with the sequential cleavage of the cinnamoyloxy moiety. The mass spectrum showed that both the phenyl groups were located on the same side of the cyclobutane ring, as indicated by the peak at m/z 180, corresponding to the ion PhCH=CHPh [5–7]. The ion at m/z 180 ($C_{14}H_{12}$, PhCH=CHPh) can mainly give rise to two major daughter ions, namely, m/z 77 (C_6H_5) and 103 (C_8H_7, PhCH=CH). Both

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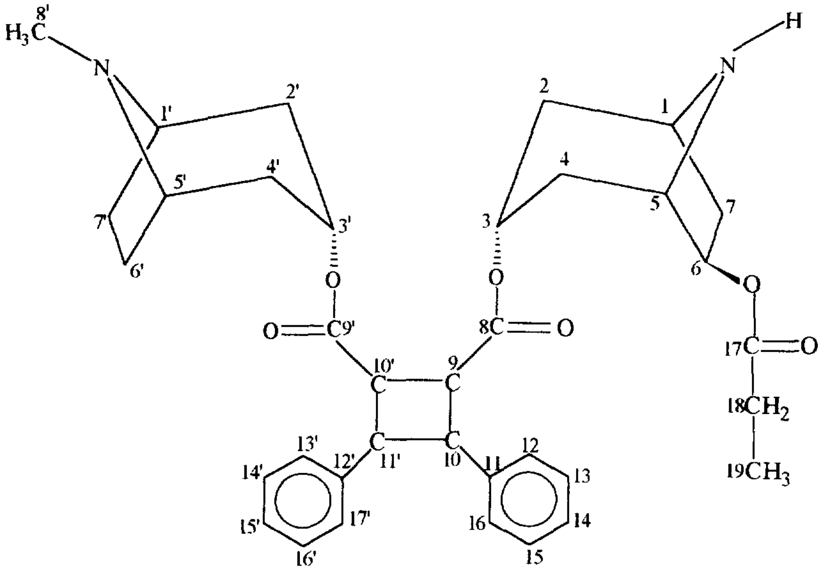

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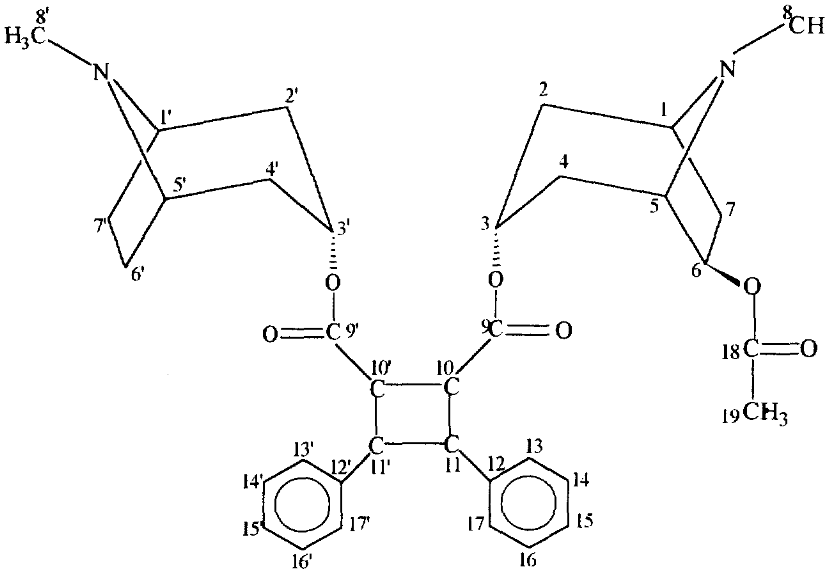

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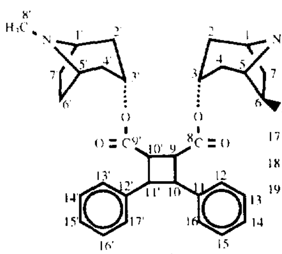
these peaks were observed in the mass spectrum of compound **1**; the fragmentation pattern is shown in Fig. 1.

The ^1H NMR ($CDCl_3$, 400 MHz) spectral assignments for **1** were carried out using a combination of 1D- and 2D-NMR techniques (Table 1). The spectrum showed two downfield double doublets at δ 8.06 and δ 7.99, which were assigned to the *ortho*-protons of the two phenyl rings. The *para*-protons appeared at δ 7.58 as a triple triplet, while a triplet centered at δ 7.45 was assigned to the *meta*-protons. Three overlapping multiplets centered at δ 5.29, 5.31 and 5.35 were ascribed to the H-3′ β, H-3β, and H-6α protons α to the oxygen atom of the ester substitutent on the tropane nucleus, respectively. The bridgehead protons (H-1, H-1′, H-5 and H-5′) showed resonances in the region δ 4.11-4.16 as a broad singlet. Another distinguishable signal was a broad doublet at δ 2.78 which was assigned to the C-7 *exo*-proton, while the *N*-methyl protons appeared at δ 2.38 as a singlet.

In the COSY 45° spectrum, the C-6 methine proton (δ 5.35) showed shift correlations with the C-7 methylene protons resonating at δ 2.78 and 2.13, while one of the C-7 methylene proton (*exo*) at δ 2.13 in turn showed a cross-peak with the bridgehead proton (H-1) at δ 4.13. The C-3 proton resonating at δ 5.31 showed interactions with the methylene protons of C-2 and C-4 (δ 2.33, 2.30 and 2.10). The cross-peaks of



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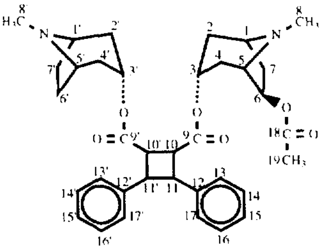

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Fig. 1. Mass fragmentation pattern of compound **1**.

Table 1. ^1H NMR assignments of compounds **1** and **2**

Hydrogen	**1**	**2**
H-1	4.13 (*br s*)	3.72 (*br m*)
$H\text{-}2_{ax,eq}$	*2.33 (*m*),* 2.10 (*m*)	2.21 (*m*), 2.15 (*m*)
H-3	5.31 (*m*)	4.67 (*m*, $W_{1/2} = 16$ Hz)
$H\text{-}4_{ax,eq}$	*2.30 (*m*), *2.10 (*m*)	2.40 (*m*), 1.75 (*m*)
H-5	4.14 (*br s*)	3.90 (*br m*)
$H\text{-}6_n$	5.35 (*m*)	5.16 (*dd*, $J_1 = 7.5$ Hz, $J_2 = 2.8$ Hz)
$H\text{-}7_x$	2.78 (*br d*, $J = 14$ Hz),	2.75 (*br d*, $J = 14.6$ Hz),
$H\text{-}7_n$	2.13 (*m*)	2.15 (*m*)
H-1′ H-5′	4.11–4.16 (*br s*)	*3.42, (*br s*), *3.52 (*m*)
$H\text{-}2'_{ax,eq}$ $H\text{-}4'_{ax,eq}$	*2.40 (*m*), *2.20 (*m*), *1.83 (*m*)	*2.15 (*m*), *1.80 (*m*), *1.76(*m*)
H-3′	5.29 (*m*)	4.76 (*m*, $W_{1/2} = 15$ Hz)
$H\text{-}6'_{x,n}$, $H\text{-}7'_{x,n}$	*2.30 (*m*), *2.00 (*m*),	*2.20 (*m*), *2.00 (*m*),
H-8′	2.38 (*s*)	*2.65 (*s*),
H-8′		*2.75 (*s*)
H-9, H-10′	*2.34 (*m*), *2.30 (*m*)	
H-10, H-11′	*2.15(*m*), *2.05 (*m*)	
H-10, H-10′		*2.34, (*m*), *2.10 (*m*)
H-11, H-11′		2.15 (*m*),
H-12, H-13′, H-16, H-17′	*8.06 (*dd*, $J_1 = 8.40$, $J_2 = 1.36$) *7.99 (*dd*, $J_1 = 8.47$ $J_2 = 1.30$),	
H-14, H-15′	*7.58 (*tt*, $J_1 = 7.40$, $J_2 = 1.10$)	
H-13, H-14′, H-15, H-16′	*7.45 (*t*, $J = 7.64$)	
H-13, H-13′, H-17, H-17′		*8.15 (*dd*, $J_1 = 8.40$, $J_2 = 1.37$)
H-15, H-15′		*7.57 (*tt*, $J_1 = 7.31$, $J_2 = 1.34$)
H-14, H-14′, H-16, H-16′		7.40–7.45 (2*t*, overlapping)
H-18	2.10 (*m*)	
H-19	1.25 (*m*)	2.13 (*s*)

Spectra were recorded at 300 and 400 MHz for **1** and **2** in $CDCl_3$ solution, respectively; chemical shifts are in units. ax = axial, eq = equatorial, x = exo, n = endo.

* Assignments uncertain and may be interchangeable.

the bridgehead H-1 and H-5 (δ 4.13 and 4.14) with the C-2 and C-4 methylene protons, respectively, were also observed (Fig. 2).

The ^{13}C NMR values of **1** were assigned on the basis of DEPT experiments, direct (HMQC) and long-range (HMBC) $^1H/^{13}C$ correlations (Fig. 3) and by comparison with the values of related alkaloids reported in the literature [8] (Table 2). The ester linkages to the tropane residue were determined by correlation of the skeletal and acyl protons to the corresponding ester carbonyl group in 2D long-range ^{13}C-^1H chemical shift correlation experiments. Thus, the carbonyl carbon (C-17) of the propanoyloxy group resonating at δ 166.5 showed shift correlation with the protons at δ 2.10 (H-18, propanoyl methylene protons). The carbonyl carbons (C-8, C-9′) of the cinnamoyl moieties resonating at δ 172.1 and 177.6 showed interactions with the cyclobutane methine protons (H-9 and H-10′, interchangeable) resonating at δ 2.34 and 2.30, respectively. The protons resonating at δ 2.15, 2.05 (H-10, H-11′) showed cross-peaks with the *ipso*-carbons of the phenyl rings which appeared at δ 136.0 and 137.2, respectively (Fig. 3).

Alkaloid **2** indicated the $[M]^+$ at *m/z* 600.3223 ($C_{36}H_{44}N_2O_6$) and showed peaks at *m/z* 329 (**2a**) and *m/z* 271 (**2b**). The fragment at *m/z* 329 was further cleaved to the ions at *m/z* 147 (cinnamoyloxy) and *m/z* 182, as in the case of **1a** of alkaloid **1**, but the absence

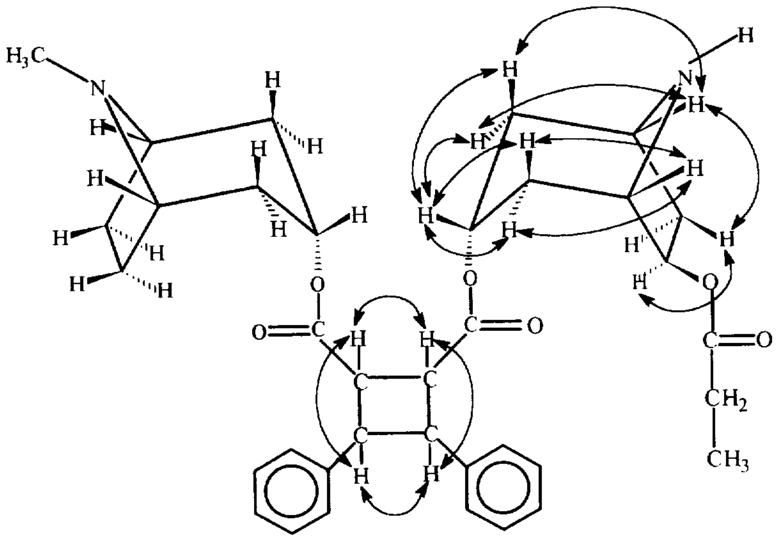

Fig. 2. COSY interactions of compound 1.

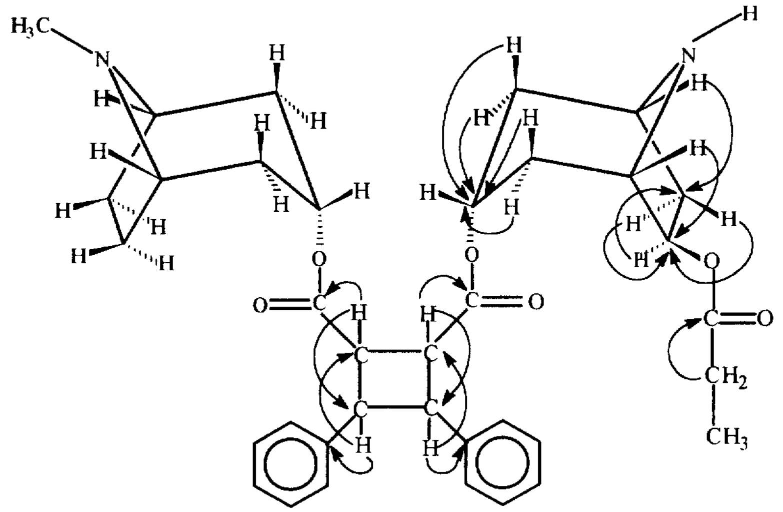

Fig. 3. HMBC interactions of compound 1.

of the peak at m/z 73 (propanoyloxy) and the presence of peaks, at m/z 43 (CH_3CO), 59 (CH_3COO) and 86 (C[7]H_2-C[6]$HOCOCH_3$), suggested the linkage of the acetoxyl group to C-6. The peaks at m/z 557 $[M-43]^+$ and 514 $[M-86]^-$ further substantiated the presence of the C-6 acetoxy-substituted tropane nucleus. Compound **2**, like compound **1**, exhibited an ion with m/z 180, establishing that both aromatic rings were located on the same side of the cyclobutane ring. The fragmentation pattern of **2** is shown in Fig. 4.

The ^1H NMR spectrum of compound **2** showed a double doublet at δ 8.15, which was ascribed to the *ortho*-protons. A triple triplet at δ 7.57 appeared due to the *para*-aromatic protons, while two overlapping triplets in the range δ 7.40–7.45 were assigned to the *meta*-protons. The downfield signals at δ 5.16 (*dd*, $J_1 = 7.50$, $J_2 = 2.80$ Hz), δ 4.76 (*m*, $W_{1/2} = 15$ Hz) and δ 4.67 (*m*, $W_{1/2} = 16$ Hz) were ascribed to H-6, H-3′β and H-3β protons geminal to the ester moieties, respectively. The bridgehead H-1 and H-5 protons appeared at δ 3.72 and 3.90, respectively, as multiplets, while the H-1′ and H-5′ protons showed resonances as multiplets at δ 3.42 and δ 3.52 (interchangeable). A broad doublet at δ 2.75 was assigned to the C-7 *exo*-proton (Table 1).

The COSY 45° spectrum (Fig. 5) showed interactions of H-6 (δ 5.16) with the C-7 *exo*-(δ 2.75) and *endo*-(δ 2.15) protons. The shift correlations of the bridgehead protons resonating at δ 3.72 (H-1) and 3.90 (H-5) were also observed with the C-2 (δ 2.21, 2.15) and C-4 (δ 2.40, 1.75) methylene protons, respectively, which, in turn, showed connectivities with the C-3 proton (δ 4.67). The assignments (Table 2) of the ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) were based on direct and long-range $^1H/^{13}C$ correlation experiments (Fig. 6). The HMBC experiment showed interaction

Table 2. ^{13}C NMR assignments of compounds **1** and **2**

Carbon	**1**	**2**
C-1	62.5	62.8
C-2	33.8	33.6
C-3	66.0	65.5
C-4	33.8	36.0
C-5	59.7	62.5
C-6	77.7	74.2
C-7	34.5	40.0
C-1′, C-5′	*55.2, *60.3	*61.0, *63.5
C-2′, C-4′	*33.4, *34.5	34.0
C-3′	63.0	65.5
C-6′, C-7′	26.0	29.8
N-CH$_3$	41.6	*40.2, *39.3
C-8, C-9′	*172.1, *177.6	
C-9, C-9′	*175.0, *177.2	
C-9, C-10′, C-10, C-11′	*34.0, *33.0	
C-10, C-10′, C-11, C-11′	*31.0, *34.0, *33.5	
C-11, C-12′ (ipso-C)	*136.0, *137.2	
C-12, C-12′ (ipso-C)	132.0	
Other aromatic C	137.2–128.0	133.0–127.7
C-17	166.5	
C-18	34.5	165.9
C-19	25.0	29.0

Spectra were recorded at 75 MHz for **1** and 125 MHz for **2** in CDCl$_3$ solution.

* Assignments uncertain and may be interchangeable.

of the acetoxyl methyl protons (H-19) resonating at δ 2.13 with the carbonyl carbon (C-18) of the acetoxyl group at δ 165.9. Long-range heteronuclear interactions were also observed between the methine protons (H-10, H-10′) of the cyclobutane ring, resonating at δ 2.10 and 2.34, with the carbonyl carbons (C-9 and C-9′) of the cinnamoyl moieties, which appeared at δ 175.0 and 177.2, respectively. The *ipso*-carbons resonating at δ 132.0 showed connectivity with the H-11, H-11′ protons resonating at δ 2.15 (Fig. 6).

EXPERIMENTAL

Plant material

Fresh leaves of *E. moonii*, a glabrous shrub, were collected from Ja-ela, 12 km from Colombo, Sri Lanka, in November 1992.

General

^{1}H NMR were recorded in CDCl$_3$ at 400 MHz, using TMS as int. standard. ^{13}C NMR were recorded at 75 and 125 MHz, respectively, in CDCl$_3$. 2D (COSY, HMQC and HMBC) expts were performed using standard microprograms. IR were recorded in CHCl$_3$. Purities of samples were checked by TLC on silica gel (Merck); spots were detected by UV or Dragendorff's reagent.

Isolation of the alkaloids

Leaves (4 kg) were ground and extracted with MeOH at room temp. for 2 weeks. The MeOH extract was concd under vacuum at 27°. The crude gum (160 g) thus obtained was dissolved in H$_2$O and defatted with hexane (1 l). The defatted aq. extract was then extracted with CHCl$_3$ (1.5 l, each) at pH 7, 8 and 4, respectively. The CHCl$_3$ extract (1.2 g) obtained at pH 7 was subjected to CC on silica gel (100 g), using mixts of hexane–CHCl$_3$ and CHCl$_3$–MeOH as eluents, respectively. The fr. collected on elution with CHCl$_3$–MeOH (19 : 1) was subjected to TLC on silica gel using CHCl$_3$–MeOH (24 : 1) and NH$_3$ vapour as solvent, resulting in the isolation of compounds **1** and **2**.

Mooniine A (**1**)

Colourless solid (3.9 mg). Yield 0.0024%. R^f. 0.34. IR ν_{max} (CHCl$_3$) cm^{-1} 1710 (ester C=O), 1600 (CH=CH). UV λ_{max} (CHCl$_3$): 272.4, 227.4, 201.4 nm.

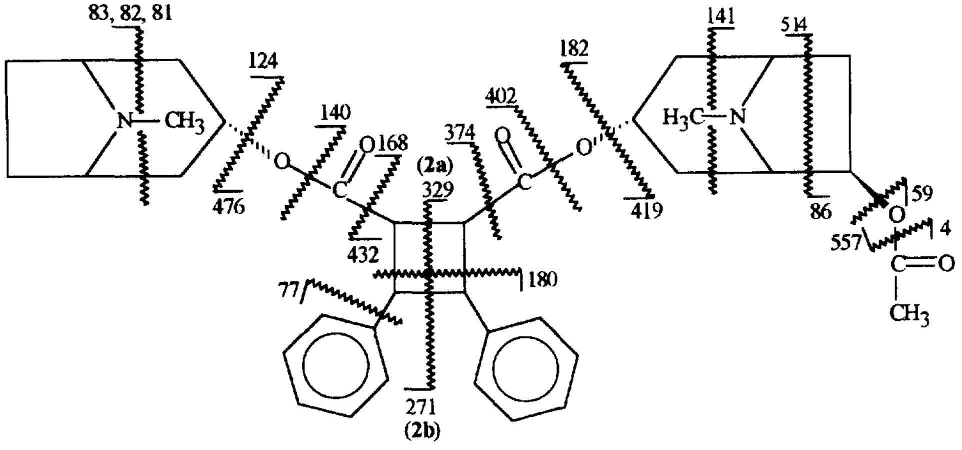

Fig. 4. Mass fragmentation pattern of compound **2**.

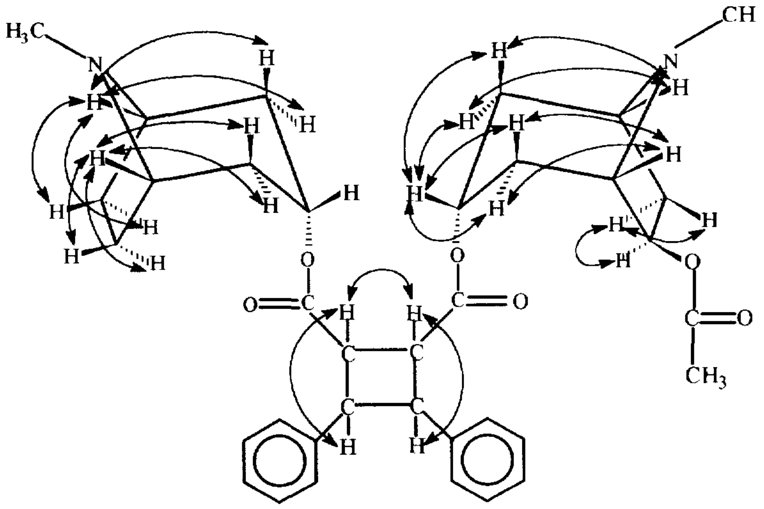

Fig. 5. COSY interactions of compound **2**.

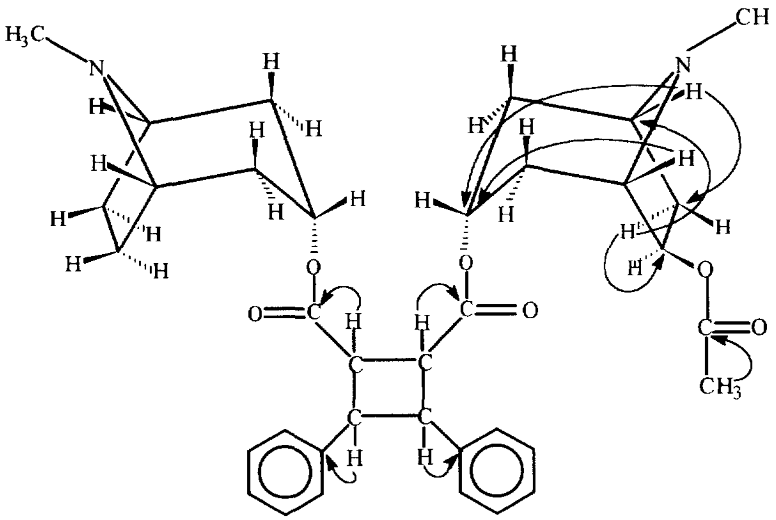

Fig. 6. HMBC interactions of compound **2**.

^{1}H NMR (400 MHz, $CDCl_3$): Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): Table 2. EIMS (70 eV) *m/z* (rel. int.): 600 (4), 432 (4), 419 (4), 374 (3), 373 (2), 278 (2), 270 (1), 257 (1) 234 (1), 232 (6), 205 (1) 198 (2) 181 (13), 167 (4), 149 (6), 140 (6), 139 (2), 138 (3), 131 (8) 124 (44) 122 (16), 111 (11), 110 (100), 105 (22) 96 (12), 95 (31), 94 (33), 83 (23), 82 (24), 81 (19), 77 (25), 73 (6), 57 (13); HREIMS observed (fragment, Calc.) 600.3194 ($C_{36}H_{44}N_2O_6$, 600.3199), 110.0953 ($C_7H_{12}N$, 100.0969).

Mooniine B (**2**)

Colourless solid (4.6 mg). Yield 0.0028 %. R^f 0.29. IR ν_{max} ($CHCl_3$) cm^{-1} 1715 (ester, C=O), 1615 (CH=CH). UV λ_{max} ($CHCl_3$) 272.4, 220.8, 200.4 nm. ^{1}H NMR (300 MHz, $CDCl_3$): Table 1. ^{13}C NMR (125 MHz, $CDCl_3$): Table 2. EIMS (70 eV) *m/z* (rel. int.): 600 (33), 557 (3), 514 (1), 476 (7), 432 (3), 419 (10), 402 (1), 374 (9), 329 (1), 271 (3), 226 (4), 182 (31), 180 (1), 168 (1), 141 (2), 140 (9), 124 (100), 96 (5), 95 (82), 94 (74), 83 (30), 82 (32), 81 (14), 86 (2), 67 (13), 59 (3), 55 (16). HREIMS observed (fragment, Calc.) 600.3223 ($C_{36}H_{44}O_6N_2$, 600.3199), 271.1579, ($C_{17}H_{21}NO_2$, 271.1572), 182.1162 ($C_{10}H_{16}NO_2$, 182.1180), 131.0483 (C_9H_7O, 131.04968).

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