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Unusual indole alkaloid–pyrrole, –pyrone, and –carbamic acid adducts from *Alstonia angustifolia*

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ABSTRACT

Three unusual natural products, viz., alstopirocine, a macroline alkaloid incorporating a substituted pyrrole moiety, pleiomaltinine, an alkaloid–pyrone adduct, and pleiomalicine, an alkaloid–carbamic acid adduct, were isolated from the Malayan *Alstonia angustifolia*. The alkaloid moiety in alstopirocine was alstomicine while that in pleiomaltinine and pleiomalicine was pleiocarpamine. The structures were determined by spectroscopic methods and in the case of pleiomaltinine, confirmed by X-ray diffraction analysis. Possible biogenetic pathways to these unusual compounds are presented.

1. Introduction

Plants of the genus *Alstonia*[1] (Apocynaceae) are well known as rich sources of structurally novel as well as biologically active alkaloids.[2–4] A prominent feature of the *Alstonia* alkaloids is the predominance of the macroline unit, which abounds in the alkaloids found in plants of the genus.[2–14] A number of the *Alstonia* bisindoles are known for displaying significant in vitro antiamoebic activity against *Plasmodium falciparum* (the causative agent of Malaria), as well as cytotoxic activity against several human cancer cell lines.[4,15–20] Recently, a novel indole alkaloid, actinophyllic acid from an Australian *Alstonia* (*Alstonia actinophylla*), was reported to be an effective inhibitor of carboxypeptidase U (CPU), and hence a potential facilitator of fibrinolysis.[21] We previously reported the structure of the novel *Alstonia* alkaloid, bipleiophylline, a cytotoxic bisindole constituted from the bridging of two indole moieties by an aromatic spacer unit.[15] In continuation of our studies of biologically active alkaloids from Malaysian *Alstonia*[5–10,15] we wish to report the structure of three unusual alkaloids, viz., an alkaloid–pyrrole, an alkaloid–pyrone, and an alkaloid–carbamic acid adduct from the stem-bark extract of the Malayan *A. angustifolia* Wall.

2. Results and discussion

Alstopirocine (**1**) was obtained in minute amount as a colorless oil, with $[\alpha]_D^{25}$ +146 (*c* 0.29, $CHCl_3$). The UV spectrum showed bands at 229 and 309 nm, which suggested a superposition of indole and pyrrole chromophores, while the IR spectrum showed bands at 3366 and 1614 cm^{-1}, due to OH/NH and conjugated ketone functionalities, respectively. The EIMS showed an M^+ ion at *m*/*z* 451, the odd mass indicating the presence of a third nitrogen. This was confirmed by HREIMS measurements, which gave the molecular formula $C_{26}H_{33}N_3O_4$. Other notable fragments include those due to loss of one and two molecules of water (*m*/*z* 433 and 415) and hydroxyethanal (*m*/*z* 391) from a McLafferty fragmentation, while the mass fragments observed at *m*/*z* 197, 182, 181, 170, and 144 are typical of macroline derivatives.[22]

The ^{13}C NMR spectral data (Table 1) showed the presence of 26 carbon resonances (three methyls, five methylenes, ten methines, and eight quaternary carbons). In addition to the resonance due to a conjugated ketone function at δ 188.4, the ^{13}C NMR spectrum also indicated the presence of two oxymethylenes (δ 65.7, 65.9) and one oxymethine (δ 69.4), corresponding to the presence of two primary and one secondary OH groups. This was supported by the observed oxymethylene (δ 3.67, 3.74; 3.53, 3.64) and oxymethine (δ 4.18) resonances in the ^1H NMR spectrum (Table 2). The presence of the three OH functions was supported by acetylation which provided a triacetate derivative, **2** (M^+, *m*/*z* 577; δ 1.97, 1.99, 2.07, see Table 2).

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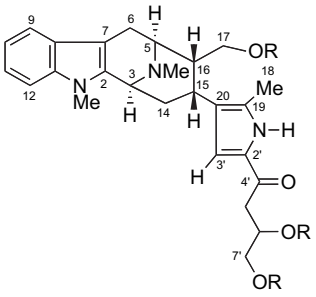

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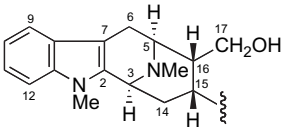

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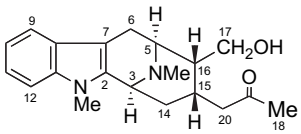

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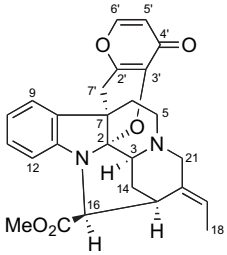

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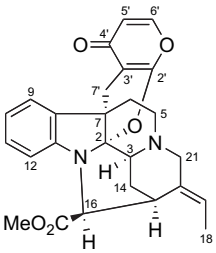

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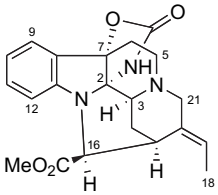

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In addition to the aromatic resonances associated with the indole moiety, four more aromatic resonances are present, which are associated with the presence of a pyrrole unit.[23] Of these, three are quaternary carbons, suggesting the presence of a trisubstituted pyrrole ring. While the alcohol OH resonances were not detected, a downfield one-H broad singlet was seen at δ 9.99 (which undergoes exchange with D_2O), which was attributed to an intramolecularly H-bonded NH of the pyrrole moiety.[23] This was confirmed by the three-bond correlations from the pyrrole NH to the pyrrole C-3′ and C-20 in the HMBC spectrum (Fig. 1). The ^{1}H NMR spectrum showed the presence of three methyl groups. Two of these at δ 3.60 and 2.41 were readily assigned to the indolic and *N*(4)-methyl groups of a macroline alkaloid,[5–9] while the third at δ 1.98 (δ_C 11.4) was assigned to a methyl substituent of the pyrrole moiety.[24] The ^{1}H NMR spectrum also showed the presence of an unsubstituted indole aromatic ring from the signals due to four contiguous aromatic hydrogens. In addition, a one-H signal due to a lone aromatic resonance was observed at δ 6.99. This signal was assigned to the lone pyrrole hydrogen and was split into a broad doublet (J=2 Hz) as a result of long-range *W*-coupling to the pyrrole NH (as confirmed by homonuclear decoupling).[25]

Analysis of the COSY and HMQC data revealed partial structures, which are characteristic of a macroline skeleton. Examination of the NMR data revealed that the macroline unit present corresponds to the partial structure **3**. This is evident from comparison of the NMR data with that of the known ring-opened macroline alkaloid, alstomicine (**4**),[6] which revealed a close correspondence of the ^{13}C NMR shifts of C-2 through to C-17 of **1** with those of **4**, except for the C-15 resonance, which was shifted downfield by ca. 2 ppm, suggesting that this carbon is the site of substitution (Table 1). In addition, the signals due to the CH_2COCH_3 side chain corresponding to C(20)–C(19)–C(18) of **4** are absent. The ^{1}H NMR spectrum (Table 2) showed the same behavior, retaining all the signals corresponding to alstomicine with the exception of the H-20 signals.

Table 1
^{13}C (100 MHz) NMR spectral data of **1**, **2**, **4**, **5**, and **7** in $CDCl_3$[a]

C	1	2	4[b]	5	7
2	132.6	133.6	132.5	95.7	77.8
3	53.5	53.4	53.2	50.3	54.3
5	58.2	53.7	59.1	47.4	47.4
6	22.5	22.5	22.2	31.6	23.8
7	105.9	106.9	105.9	44.9	86.7
8	126.1	126.5	126.3	134.1	131.7
9	118.1	118.1	118.1	120.5	123.0
10	118.9	118.9	119.0	119.8	120.1
11	121.1	121.0	121.0	127.6	129.2
12	108.9	108.9	108.9	110.6	110.1
13	137.1	137.1	137.2	146.7	144.9
14	33.2	31.3	34.3	27.2	29.8
15	27.0	26.3	25.1	31.6	31.6
16	45.7	45.4	43.6	57.3	58.3
17	65.7	63.7	66.0	170.0	169.3
18	11.4	11.9	30.5	12.2	12.5
19	134.3	134.0	207.5	119.1	121.2
20	123.9	123.8	46.2	134.9	134.0
21	—	—	—	52.9	52.0
CO_2Me	—	—	—	51.8	52.1
2′	129.5	129.2	—	146.3	—
3′	118.6	117.0	—	142.5	—
4′	188.4	184.3	—	171.6	—
5′	40.2	38.4	—	115.8	—
6′	69.4	68.7	—	152.6	—
7′	65.9	64.8	—	26.6	—
N(1)-Me	28.9	29.1	28.9	—	—
N(4)-Me	41.4	42.3	41.4	—	—
N′COO	—	—	—	—	158.9
17-OAc	—	21.1 171.3	—	—	—
6′-OAc	—	21.1 170.3	—	—	—
7′-OAc	—	20.9 170.8	—	—	—

[a] Assignments based on COSY, HMQC, HETCOR, and HMBC.
[b] From Ref. 6.

Table 2
^{1}H (400 MHz) NMR spectral data of **1**, **2**, **5**, and **7** in $CDCl_3$[a]

Position	1	2	5	7
3	4.18 (m)	4.06 (m)	3.19 (m)	3.15 (m)
5	3.60 (m)	3.29 (d, 7)	2.86 (m)	2.96 (m)
			3.15 (m)	3.12 (m)
6	2.59 (d, 17)	2.51 (d, 17)	1.45 (dt, 14.4, 2)	2.21 (m)
	3.39 (dd, 17, 7)	3.74 (dd, 17, 7)	2.32 (td, 14.4, 4)	2.21 (m)
9	7.53 (d, 8)	7.54 (br d, 8)	7.08 (br d, 7.8)	7.28 (br d, 7.8)
10	7.12 (t, 8)	7.12 (td, 8, 1)	6.82 (td, 7.8, 1)	6.82 (br t, 7.8)
11	7.21 (t, 8)	7.19 (td, 8, 1)	7.06 (td, 7.8, 1)	7.10 (td, 7.8, 1)
12	7.29 (d, 8)	7.29 (br d, 8)	6.30 (br d, 7.8)	6.14 (br d, 7.8)
14	1.69 (br d, 11)	1.63 (m)	1.74 (dt, 13, 3.6)	2.07 (dt, 13, 3.6)
	2.89 (m)	2.41 (td, 13, 4)	2.83 (m)	2.17 (m)
15	2.92 (m)	2.97 (dt, 9, 5)	3.37 (q, 3.6)	3.30 (q, 3.6)
16	1.58 (m)	2.02 (m)	4.79 (d, 3.6)	4.31 (d, 3.6)
17	3.67 (m)	3.85 (dd, 10.6, 3.7)	—	—
	3.74 (m)	4.51 (t, 10.6)		
18	1.98 (s)	2.08 (s)	1.59 (dd, 6.8, 2)	1.56 (dd, 6.8, 2)
19	—	—	5.42 (qd, 6.8, 2)	5.46 (qd, 6.8, 2)
21	—	—	3.04 (d, 12.4)	2.95 (d,12)
			4.27 (dt, 12.4, 2)	4.01 (dt, 12, 2)
CO_2Me	—	—	3.73 (s)	3.67 (s)
3′	6.99 (br d, 2)	6.66 (br d, 2)	—	—
5′	2.83 (dd, 16, 8)	2.92 (dd, 16, 7)	6.20 (d, 5.6)	—
	2.95 (m)	3.10 (dd, 16, 7)		
6′	4.18 (m)	5.50 (m)	7.53 (d, 5.6)	—
7′	3.53 (dd, 11, 6)	4.17 (dd, 12, 5.5)	3.20 (m)	—
	3.64 (dd, 11, 4)	4.35 (dd, 12, 3.2)	3.20 (m)	
N(1)-Me	3.60 (s)	3.61 (s)	—	—
N(4)-Me	2.41 (s)	2.35 (s)	—	—
N(1′)H	9.99 (br s)	9.51 (br s)	—	6.86 (br s)
17-OAc	—	1.97 (s)	—	—
6′-OAc	—	1.99 (s)	—	—
7′-OAc	—	2.07 (s)	—	—

[a] Assignments based on COSY, HMQC, HETCOR, and HMBC.

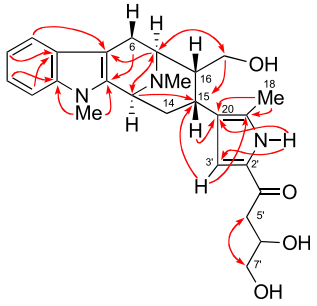


Figure 1. Selected HMBCs of **1**.

A methyl signal seen at δ_H 1.98 is distinguished from the methyl ketone resonances of **4** (δ_H 2.04, δ_C 30.5) by its observed δ_C at 11.4. This signal is assigned to the pyrrole methyl substituent. One of the three pyrrole substituents therefore corresponds to a C(15)-substituted ring E-opened, seco-macroline, derived from alstomicine, as shown in **3**, while the other substituent is a methyl group.

After discounting the macroline and methyl substituents, the remaining substituent on the trisubstituted pyrrole unit corresponds to a four-carbon side chain of partial formula $C_4H_7O_3$. Since one of the oxymethylenes corresponds to the hydroxymethyl side chain attached to C-16 of the macroline unit, the remaining hydroxymethylene and hydroxymethine, as well as the conjugated ketone, are associated with the pyrrole four-carbon side chain. The conjugated ketone function has to be directly attached to the pyrrole ring while the primary alcohol must be at the other end of the side chain. The large coupling constant observed for the methylene unit (J=16 Hz) and its ^{1}H and ^{13}C NMR shifts indicate that it is α to the carbonyl function. The structure of the side chain unit is also in agreement with the COSY spectrum (which showed the presence of a $COCH_2CH(OH)CH_2OH$ fragment) as well as the observation of the m/z 391 McLafferty fragmentation peak in EIMS.

Having identified the three substituents, it remains to determine the regiochemistry of the substitution on the pyrrole moiety. The NOE observed between the pyrrole methyl (H-18) and the pyrrole NH (Fig. 2) requires the methyl to be proximate and hence attached at one of the α-carbons, which is also consistent with the observed shift of this carbon at δ 134.3.[24]

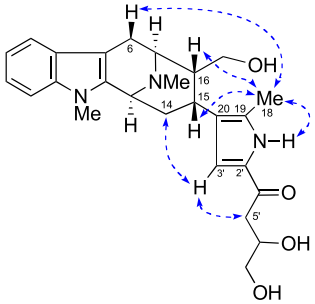


Figure 2. Selected NOEs of **1**.

Although there are in principle a total of six regioisomeric structures that can be considered for the trisubstituted α-methylpyrrole (**A**–**F**, Fig. 3), only one (A) is in complete agreement with the NMR data (chemical shift, COSY, HMBC, NOE) as we shall proceed to demonstrate.

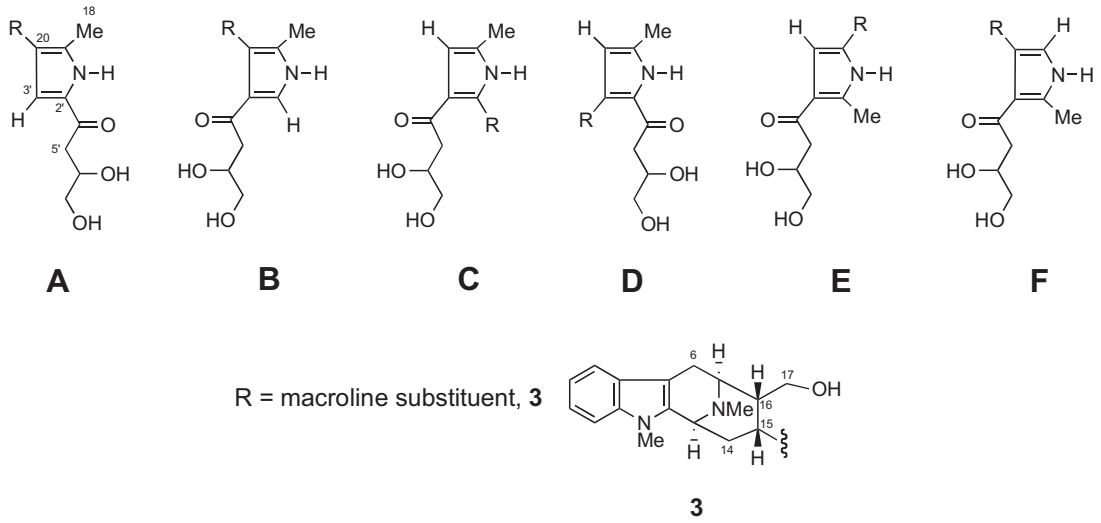


Figure 3. Six regioisomers of the trisubstituted α-methylpyrrole (**A**–**F**).

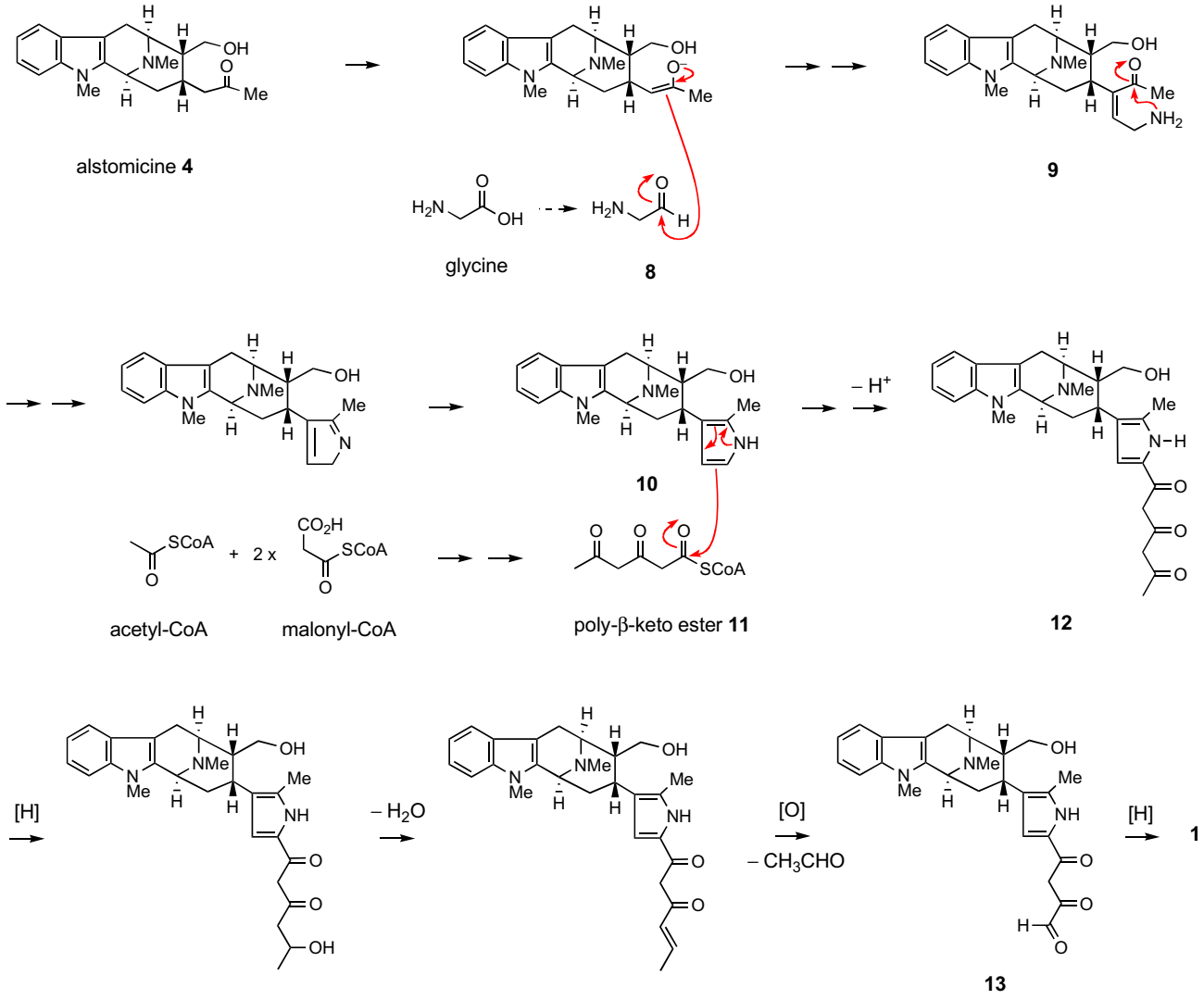
Elucidation of the correct regioisomer can in fact be achieved based on NOE data (Fig. 2) alone, although the other NMR data provided additional support for the assignment. First, as mentioned above, the observed NOE between the pyrrole NH and the pyrrole methyl substituent requires placement of the methyl substituent on one of the α carbons of the pyrrole unit. Next, the observed NOE between the pyrrole methyl and H-15 and H-16 of the macroline moiety requires substitution of the macroline at the β-carbon (C-20) adjacent to the pyrrole methyl at C-19. The attachment from this β-carbon (C-20) is to C-15 of the macroline, from the observed three- and two-bond correlations, respectively, from the pyrrole-H to C-15 and from H-15 to the pyrrole C-20 in the HMBC spectrum (Fig. 1). The observed NOE between the macroline H-14 with the lone pyrrole-H in turn places the pyrrole hydrogen on the other β carbon (i.e., C-3′), vicinal to the site of macroline substitution on the pyrrole moiety at C-20. This is also consistent with observation of long-range *W*-coupling (J=2 Hz) from this hydrogen to the pyrrole NH, requiring the lone pyrrole hydrogen to be attached to one of the β-carbons.[25] This leaves the remaining α-carbon (C-2′) as the site of attachment of the ketone containing four-carbon side-chain substituent, which is also confirmed by the observed NOE between the pyrrole H-3′ and H-5′ of the side chain unit, requiring these two units to be vicinal to each other. The location of the ketone-containing side chain on the other α carbon (C-2′) of the pyrrole unit is also consistent with observation of the pyrrole NH as a downfield signal at δ 9.99, suggesting the involvement of intramolecular H-bonding,[23] requiring the ketone function to be proximate. These assignments are also entirely consistent with the HMBC data, for instance, the observed three-bond correlations from the pyrrole NH to the macroline-substituted C-20 and the pyrrole methine C-3′, and from the pyrrole H-3′ to the methyl-substituted C-19 and the macroline C-15 (Fig. 1). These observations, leading to the correct regioisomer (A), reveal the complete structure of alstopirocine as shown in **1**.

A possible pathway to alstopirocine is shown in Scheme 1 from the ring-opened macroline alkaloid, alstomicine (**4**), which also co-occurs with **1** in the stem-bark extract and has also been recently isolated from another *Alstonia* species.[6] An aldol condensation between the enolate derived from **4** and the glycine-derived amino-aldehyde **8**, gives the conjugated aminoketone **9**, which on subsequent cylization and dehydration yield the pyrrole **10**. Acylation of **10** by the poly-β-keto ester **11**, which is in turn formed from one acetate and two malonate chain extension units, furnishes after deprotonation, the 2-substituted pyrrole **12**. Selective reduction of the acetyl carbonyl, followed in succession by dehydration and oxidative scission of the terminal two-carbon unit leads to the diketobutanal-substituted pyrrole **13**. A final selective reduction of the aldehyde and keto functions gives alstopirocine (**1**). This proposed pathway leads to the numbering system adopted for **1**.

Pleiomaltinine (**5**) was isolated as a minor compound (yield, ca. 6 mg kg^{-1}) after repeated chromatographic fractionation of the basic fraction from the EtOH extract and subsequent crystallization from EtOAc as colorless plates, mp 180–182 °C, $[\alpha]_D^{25}$ +94 (*c* 0.48, $CHCl_3$). The UV spectrum showed bands at 214, 240, and 284 nm, which suggested the presence of a dihydroindole chromophore. The IR spectrum showed in addition to an ester carbonyl absorption at 1756 cm^{-1}, three other bands at 1650, 1614, and 1576 cm^{-1}, which are characteristic of a γ-pyrone moiety.[26] The EIMS showed an M^+ ion at m/z 446, which analyzed for $C_{26}H_{26}N_2O_5$. Notable fragments were observed at m/z 387, 322, and 263 (base peak), due to loss of CO_2Me, $C_6H_4O_3$, and (CO_2Me+$C_6H_4O_3$), respectively. The ^{13}C NMR data (Table 1) showed the presence of 26 carbon resonances comprising two methyls, five methylenes, ten methines, and nine quaternary carbons. In addition to the methyl ester function indicated by the carbon resonance at δ 170.0, a conjugated ketone signal was seen at δ 171.6, while a downfield signal at δ 95.7 indicated the presence of a quaternary carbon linked to an oxygen and a nitrogen atom.[14,15,27–29]

The ^1H NMR spectrum (Table 2) showed signals due to an unsubstituted indole aromatic ring from the signals due to the four aromatic hydrogens (δ 6.30 to 7.08), a methyl ester group (δ 3.73), an ethylidene side chain (δ 1.59; 5.42), and a methine doublet at δ 4.79. In addition, a pair of olefinic AB doublets were observed at δ 6.20 and 7.53 with J=5.6 Hz. The corresponding ^{13}C shift for the lower field doublet was seen at δ 152.6, which indicated oxygen substitution. The methine doublet at δ 4.79 with J=3.6 Hz and δ_C 57.3 is reminiscent of H-16 of pleiocarpamine derivatives (δ_H 5.21, δ_C 61.6)[18] and examination of the ^1H and ^{13}C NMR data confirmed the presence of a pleiocarpamine moiety.

The 2-D COSY and HETCOR data showed in addition to the four aromatic hydrogens, the presence of the following partial structures, viz., an NCH_2, NCH_2CH_2, $NCHCH_2CHCH$, $CHCH_3$, OCH=CH, and an isolated CH_2. The first four partial structures correspond to the C(21), C(5)–C(6), C(3)–C(14)–C(15)–C(16), and C(18)–C(19) fragments, respectively, of a dihydropleiocarpamine moiety.



Scheme 1. Possible biogenetic pathway to **1**.

Substracting the pleiocarpamine contribution ($C_{20}H_{22}N_2O_2$) from the molecular formula gives a six-carbon unit corresponding to the formula $C_6H_4O_3$. The residual ^{13}C signals after discounting the pleiocarpamine moiety, comprises two olefinic methines (δ 115.8, 152.6), one linked to an oxygen, two olefinic quaternary carbons (δ 146.3, 142.5), both linked to oxygen, one conjugated ketone carbonyl (δ 171.6), and one CH_2 (δ 26.6).

The olefinic AB doublets at δ 6.20 and 7.53 with characteristic coupling constant of 5.6 Hz,[30] together with the IR absorptions at 1650, 1614, and 1576 cm^{-1}, as well as the other carbon resonances, are strongly suggestive of a disubstituted γ-pyrone unit. Examination of the NMR data indicated that in common with *Alstonia* bisindoles incorporating pleiocarpamine, branching from the pleiocarpamine moiety in **5** is from C-2 and C-7.[14,15,27–29] The connection from C-2 to the pyrone is mediated by an ether oxygen as indicated by the observed C-2 shift of δ 95.7, while C-7 (δ 44.9) is attached to the pyrone unit by a methylene bridge (C-7′). This conclusion is supported by the observed three-bond correlations from H-7′ to C-2, C-6, C-8, and C-3′ (Fig. 4).

Examination of the NMR data of the γ-pyrone unit leads to two possible structures, **5** and **6**, differing in the regiochemistry of pyrone substitution. In this instance the HMBC (Fig. 4) as well as the NOE data (Fig. 4) do not provide a sufficiently clear distinction.

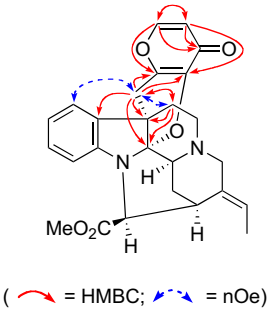


Figure 4. Selected HMBCs and NOEs of **5**.

Based on ^{13}C chemical shift grounds however (by comparison of the ^{13}C shifts of the pyrone moiety in pleiomaltinine with the appropriate γ-pyrone containing model compounds, such as zanthopyranone,[31] elysiapyrone A,[32] and nortridachidione[33]), structure **5** is preferred over **6** principally on the grounds that C-2′, which is linked to two oxygen atoms in **6** should show a ^{13}C shift in the region of ca. δ 160, while the C-4′ carbonyl signal should be at ca. δ 182, which were not the case in pleiomaltinine ($\delta_{C\text{-}2'}$ 146.3; $\delta_{C\text{-}4'}$ 171.6). The structure of pleiomaltinine is therefore as represented

by **5**. In order to secure unambiguous confirmation of the structure, X-ray diffraction analysis was carried out (Fig. 5), which confirmed the structure deduced based on analysis of the spectroscopic data.

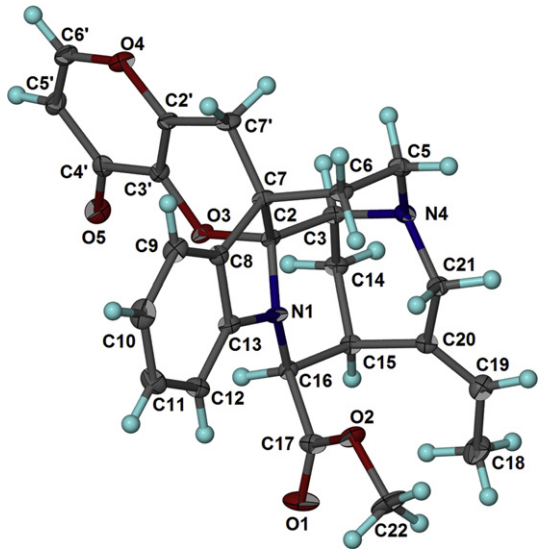


Figure 5. X-ray crystal structure of **5**. Thermal ellipsoids are shown at the 50% probability level. Three molecules of H_2O per molecule of **5** observed in the crystal lattice are omitted for clarity.

Pleiomalicine (**7**) was also isolated in minor amount (yield 0.1 mg kg^{-1}) as a colorless oil, with $[\alpha]_D^{25}$ +68 (*c* 0.009, $CHCl_3$). The UV spectrum showed bands at 230, 248, and 293 nm, which was somewhat similar to that of **5**. The IR spectrum showed bands at 3256 and 1756 cm^{-1}, suggesting the presence of NH and ester/carbamate functionalities, respectively. The EIMS showed an M^+ peak at *m*/*z* 381, the odd mass indicating the presence of a third nitrogen. This was confirmed by HREIMS, which revealed the molecular formula $C_{21}H_{23}N_3O_4$.

In common with pleiomaltinine **5**, the NMR data (Tables 1 and 2) clearly indicated the presence of a 2,7-dihydropleiocarpamine moiety, with the characteristic H-16 and C-16 resonances observed at δ_H 4.31, J=3.6 Hz and δ_C 58.3. In addition to the methyl ester carbonyl resonance at δ 169.2, the ^{13}C NMR spectrum of **7** also showed a carbamate signal at δ 158.9. Discounting the pleiocarpamine component from the molecular formula leaves a residual unit of formula CO_2NH. Attachment of this unit is from C-7 via the carbamate oxygen, as indicated by the ^{13}C chemical shift value of C-7 of 86.7, which in turn requires connection of the NH to C-2 (δ 77.8). The attachment of C-2 to the carbamate NH was also supported by the observed NOE between the carbamate NH and H-3, H-14β, H-16 (Fig. 6). The structure is consistent with the HMBC data (Fig. 6).

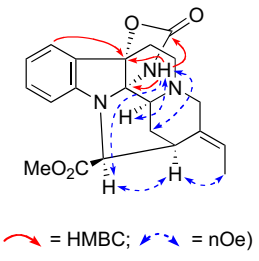


Figure 6. Selected HMBCs and NOEs of **7**.

Pleiomaltinine (**5**) and pleiomalicine (**7**) represent two unusual natural products, the former an alkaloid–pyrone conjugate, while the latter an alkaloid–carbamic acid adduct. The alkaloid moiety in both adducts was pleiocarpamine, which is fused to a γ-pyrone unit via a dihydropyran ring in pleiomaltinine (**5**), while in pleiomalicine (**7**), the alkaloid moiety is fused to a carbamic acid unit to forge a new oxazolidone ring.

Possible biogenetic pathways to these unusual adducts are shown in Scheme 2 from the indole alkaloid **14**, which co-occurs with **5** and **7** in the stem-bark extract. In the case of pleiomaltinine (**5**), the sequence is initiated by a lone-pair assisted conjugate addition of **14** through its nucleophilic C-7 onto the oxidized, conjugated, 1,2-diketone form (**15**) of the naturally-occurring hydroxypyrone, maltol. This is then followed by intramolecular capture of the resultant iminium ion by the enol OH to give **5**. In the case of pleiomalicine (**7**), oxidation of pleiocarpamine (**14**) provides the 1,2-dihydroxy-derivative **16**, which is known in another *Alstonia*.[34] Attack by the 7-OH on the phosphorylated carbamic acid derivative, carbamoyl phosphate, gives the carbamate, **17**. Subsequent protonation and dehydration provide the iminium ion **18**, which on intramolecular addition by the amino group yields the adduct **7**. To the best of our knowledge, this is the first isolation of such adducts from plants.

Alstopirocine (**1**) was evaluated for cytotoxic effects as well as reversal of drug-resistance in human KB cells but was found to be ineffective (IC_{50}>25 μg/mL). Pleiomaltinine (**5**) did not show any appreciable cytotoxicity against both drug-sensitive as well as vincristine-resistant KB cells (IC_{50}>25 μg/mL) but showed moderate ability to reverse multidrug-resistance in vincristine-resistant KB (VJ300) cells (IC_{50} 12 μg/mL in the presence of 0.1 μg/mL of vincristine).

3. Experimental

3.1. General

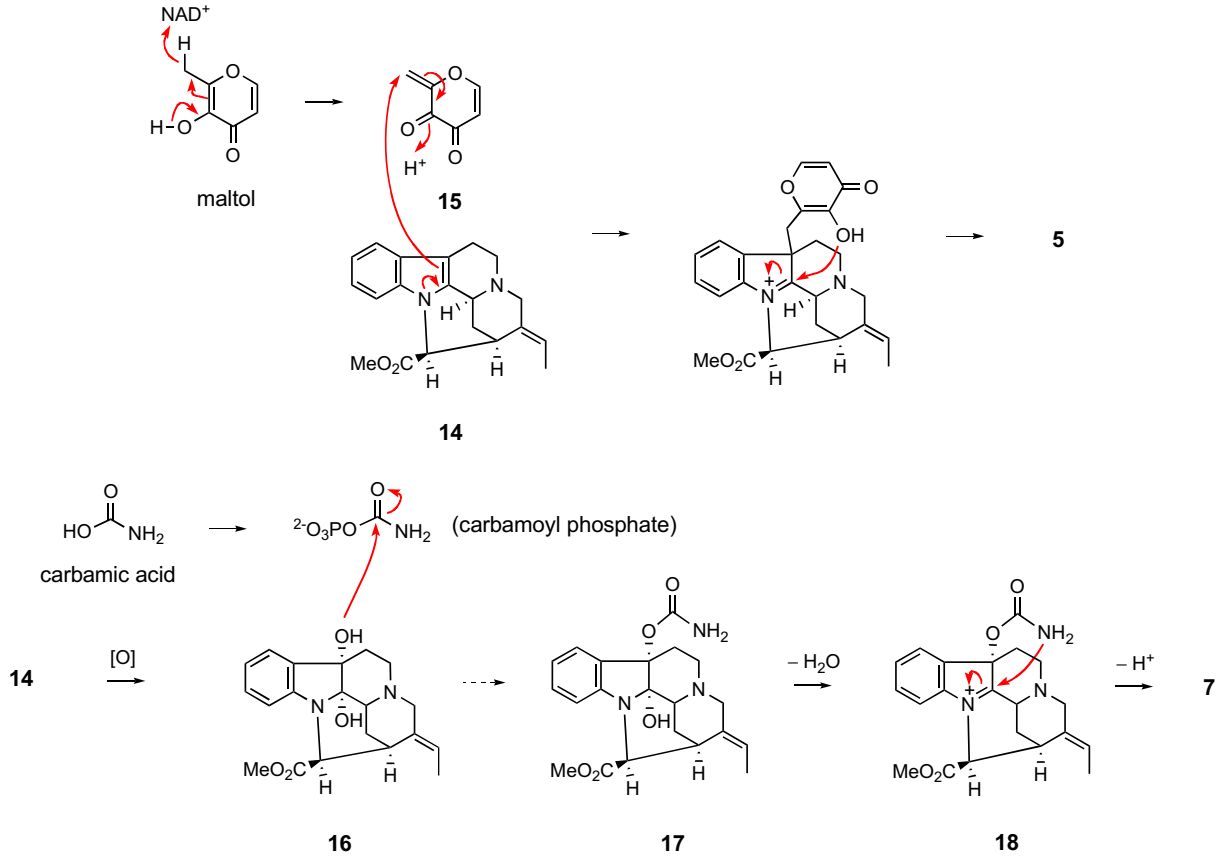
Optical rotations were determined on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as an internal standard on JEOL JNM-LA 400 and JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. X-ray diffraction analysis was carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube (λ=0.71073 Å). LSIMS, EIMS and HREIMS were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia.

3.2. Plant material and extraction of alkaloids

Plant material was collected in Johor, Malaysia (June 2003) and was identified by Dr. K.M. Wong, Institute of Biological Sciences, University of Malaya, Malaysia. Herbarium voucher specimens (K 605) are deposited at the Herbarium of the University of Malaya. The ground stem-bark material was extracted with EtOH and the concentrated EtOH extract was then partitioned with dilute acid to provide a basic fraction, as has been described in detail elsewhere.[35]

3.3. Isolation

The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH followed by rechromatography of the appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were Et_2O/MeOH (50:1) (NH_3-saturated), $CHCl_3$/hexane (4:1),



Scheme 2. Possible biogenetic pathways to **5** and **7**.

$CHCl_3$/hexane (6:1), $CHCl_3$, $CHCl_3$ (NH_3-saturated), $CHCl_3$/MeOH (100:1) (NH_3-saturated), $CHCl_3$/MeOH (50:1) (NH_3-saturated), and $CHCl_3$/MeOH (20:1) (NH_3-saturated). The yields (mg kg^{-1}) of the alkaloids were as follows: **1** (1.9), **5** (6.0), and **7** (0.1).

*3.3.1. Alstopirocine (**1**).* Colorless oil; $[\alpha]_D^{25}$ +146 (*c* 0.29, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (4.31), 309 (4.31) nm; IR (dry film) ν_{max} 3366, 1614 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 451 $[M]^+$ (9), 433 $[M-H_2O]^+$ (8), 415 $[M-H_2O-H_2O]^+$ (48), 391 (37), 364 (18), 346 (15), 328 (75), 304 (70), 261 (14), 245 (7), 215 (20), 197 (100), 182 (24), 181 (13), 170 (82), 144 (10), 94 (2), 70 (3), 42 (14); HREIMS *m*/*z* 451.2476 (calcd for $C_{26}H_{33}N_3O_4$ $[M]^+$, 451.2471).

*3.3.2. Acetylation of alstopirocine (**1**).* Alstopirocine (**1**) (6 mg, 0.013 mmol) was added to a mixture of acetic anhydride/pyridine (1:1; 1 mL) and the mixture stirred under N_2 at room temperature for 2 h. The mixture was then poured into saturated Na_2CO_3 solution (5 mL) and extracted with CH_2Cl_2 (3×5 mL). Removal of the solvent, followed by purification by centrifugal chromatography over SiO_2 ($CHCl_3$, NH_3-saturated) afforded 4 mg (50%) of the triacetate derivative **2** as a colorless oil; $[\alpha]_D^{25}$ +81 (*c* 0.14, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (4.36), 309 (4.08) nm; IR (dry film) ν_{max} 3435, 1740, 1629 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 577 $[M]^+$ (8), 517 (38), 448 (22), 418 (3), 388 (100), 328 (17), 261 (12), 225 (7), 197 (52), 170 (52), 149 (7), 43 (10); HREIMS *m*/*z* 577.2805 (calcd for $C_{32}H_{39}N_3O_7$ $[M]^+$, 577.2788).

*3.3.3. Pleiomaltinine (**5**).* Colorless plates from EtOAc; mp 180–182 °C; $[\alpha]_D^{25}$ +94 (*c* 0.48, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 214 (4.15), 240 (3.86), 284 (3.81) nm; IR (dry film) ν_{max} 1752, 1650, 1614, 1576 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; LSIMS *m*/*z* (rel int) 447 $[MH]^+$ (100),391(23), 360 (9), 323 (15), 273 (35), 235 (54); EIMS *m*/*z* (rel int) 446 $[M]^+$ (28), 387 $[M-CO_2Me]^+$ (12), 339 (6), 322 (81), 263 (100), 248 (23), 232 (35), 218 (19), 180 (72), 135 (34), 108 (19), 96 (8), 71 (8), 43 (8); HREIMS *m*/*z* 446.1842 (calcd for $C_{26}H_{26}N_2O_5$ $[M]^+$, 446.1842).

*3.3.4. Pleiomalicine (**7**).* Colorless oil; $[\alpha]_D^{25}$ +68 (*c* 0.01, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 230 (3.59), 248 (3.60), 293 (3.21) nm; IR (dry film) ν_{max} 3256, 1756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 381 $[M]^+$ (32), 366 $[M-NH]^+$ (10), 338 (28), 322 (63), 309 (16), 279 (14), 265 (18), 249 (7), 230 (36), 197 (9), 172 (16), 158 (14), 144 (10), 121 (100), 93 (17), 79 (10), 57 (10), 43 (24); HREIMS *m*/*z* 381.1691 (calcd for $C_{21}H_{23}N_3O_4$ $[M]^+$, 381.1689).

3.4. X-ray crystallographic analysis of 5

A single crystal of **5** was obtained from EtOAc; $C_{26}H_{26}N_2O_5 \cdot 3H_2O$, M_r=500.54, monoclinic, space group $P2_1$, *a*=9.3450 (17) Å, *b*=7.5774 (14) Å, *c*=16.845 (3) Å, $\alpha=\gamma=90°$, β=99.459 (3)°; *V*=1176.6 (4) $Å^3$, Z=2, D_{calcd}=1.413 g cm^{-3}. The structure was solved by direct methods and refined by the least squares method. The final *R* value is 0.0439 (R_W=0.0884) for 2154 reflections [$I>2\sigma(I)$]. Further details of crystal structure including final atomic parameters have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 777389). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

3.5. Cytotoxicity assays

Cytotoxicity assays were carried out following the procedure that has been described in detailed previously.[36,37]

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.07.079. These data include MOL files and InChIKeys of the most important compounds described in this article.

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