Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 711–720
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Characterisation of alkaloids from some Australian *Stephania* (Menispermaceae) species

Joanne T. Blanchfield[a,*], Donald P.A. Sands[b], Colin H.L. Kennard[a], Karl A. Byriel[a], William Kitching[a]

[a]*Department of Chemistry, School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, Qld. 4072, Australia*
[b]*CSIRO, Division of Entomology, Private Bag 3, Indooroopilly, Qld. 4068, Australia*

Received 5 January 2003; received in revised form 30 March 2003; accepted 4 April 2003

Abstract

Chemical investigations of some *Stephania* species native to Australia and reportedly employed by Aboriginal people as therapeutic agents, are described. The alkaloids from the forest vines *Stephania bancroftii* F.M. Bailey and *S. aculeata* F.M. Bailey (Menispermaceae) have been isolated and characterised. The major alkaloids in the tuber of the former species are (−)-tetrahydropalmatine and (−)-stephanine, whereas these are minor components in the leaves, from which a C-7 hydroxylated aporphine has been identified. The major tuber alkaloids in *S. aculeata* are (+)-laudanidine, and the morphinoid, (−)-amurine, whose absolute stereochemistry has been established by X-ray structural analysis of the methiodide derivative. No significant levels of alkaloids were detected in *S. japonica*. Complete and unambiguous ^{1}H and ^{13}C NMR data are presented for these alkaloids.

Keywords: Alkaloid; Menispermaceae; *Staphania bancroftii*; *Stephania aculeata*; *Stephania japonica*; NMR data

1. Introduction

The genus *Stephania* Lourdiro is a member of the Menispermaceae plant family which is a rich source of alkaloids (Tomita, 1952; Thornber, 1970). Some of the most extensively scrutinised members of this family belong to the genus, *Stephania*, and are native to Africa, India, South-East Asia and the northern and eastern parts of Australia. In these countries, the *Stephania* species feature prominently in the traditional medicine of the native peoples. (Kozuka et al., 1985; Anon, 1976; Tomita et al., 1966; Tomita and Kazuka, 1966; Doskotch et al., 1967). Investigations of *Stephania* species from other countries have been reported and a wide variety of alkaloids described (Chen and Chen, 1935; Schiff, 1987).

The vast literature, on the genus *Stephania* Lourdiro, justifies chemical examination of Australian species, which have a history of medicinal use by the Aboriginal people. The Australian native forest creeper, *Stephania bancroftii* Bailey (1910) was the most thoroughly studied in this investigation. The plant once appeared across a large area in Central and Northern Queensland, but its distribution has contracted to small pockets, mainly in the monsoonal, deciduous forests of Cape York. A characteristic feature of the plant is its large tuber from which the long stem of the climber emerges. The tubers can grow to 0.5 m in diameter, and are partly submerged (Bailey, 1909). The Aboriginal people of central Queensland and the Cape York region used this tuber to form a poultice that was applied to wounds and joints as an anti-inflammatory agent, and the crushed leaves were used as a fish poison (Halfpap, 1992). In addition, *S. bancroftii* is an interesting host plant for the larvae of the fruit piercing moth, *Othreis fullonia* Clerck, in Northern Australia. The rain-forest vine, *Stephania aculeata* F.M. Bailey, has a natural range extending from the Clarence River in northern New South Wales to Nambour in Southern Queensland, but is becoming increasingly rare. Unlike *S. bancroftii*, *S. aculeata* does not possess a large tuber, but has a complex, far reaching root system, extracts of which are extremely poisonous (Bailey, 1899). The third native species examined

* Corresponding author. Fax: +61-7-3365-4273.
E-mail address: j.blanchfield@mailbox.uq.edu.au (J.T. Blanchfield).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00240-1

was *Stephania japonica* (Thunb) Miers *var*. discolor, previously identified as *S. hernandiifolia* Walp (Webb, 1948) the roots of which are very toxic.

2. Results and discussion

2.1. Stephania bancroftii

Samples (~1 kg) of the tuber of *S. bancroftii* were cut from the plant, without effecting its growth. The material, similar in texture to a common turnip, was processed as described in the Section 3. The extracts at pH 1 and 10 yielded no significant organic compounds, indicating the absence of organic acids and strong bases. The ether extract at pH 7, however, yielded a complex mixture of organic compounds.

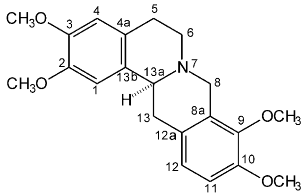
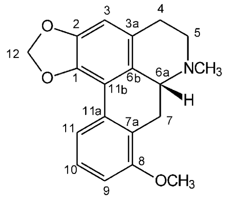
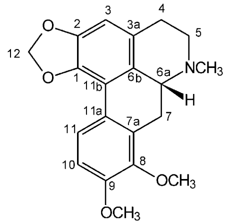
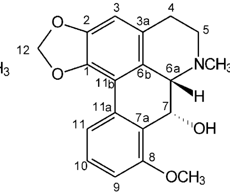
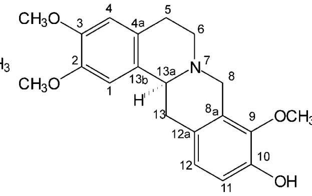
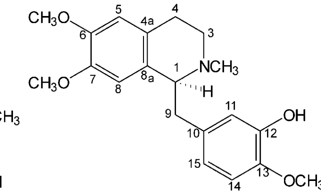
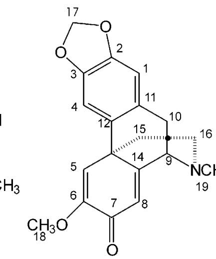
Column chromatography provided the major component as an off-white solid, mp 139–141 °C. The molecular ion (EIMS) corresponded to $C_{21}H_{25}NO_4$ and the ^{1}H and ^{13}C NMR spectra indicated the presence of two aromatic rings, four methoxyl groups and four CH_2 groups. There was no evidence for a carbonyl function. The aromatic region of the ^{1}H NMR spectra displayed a pair of doublets (''AB'' pattern) suggesting the presence of two *ortho* protons on one of the aromatic rings and two singlets consistent with two *para* related hydrogens in the second aromatic ring. The aliphatic region of the ^{1}H NMR spectrum displayed a pair of ''AB'' doublets at δ 4.20 and δ 3.49. The large coupling between these signals ($J_{H-H}=15$ Hz) suggested *geminal* protons and the direct bond C-H correlation experiment (HMQC) confirmed this. The presence of this deshielded, isolated methylene group is characteristic of the berberine bridge position (C8) of the protoberberine alkaloids. The spectra also revealed the presence of an isolated CH–CH_2 moiety. The considerable deshielding of the ^{13}C signal of the CH group indicated this carbon atom was adjacent to the nitrogen atom and so, the group was likely to correspond to the C13a and C13 positions. The signal for the *axial* proton in the methylene group (δ 2.70) appeared as a doublet of doublets with a *geminal* $J_{H-H}=15.6$ Hz, and vicinal $J_{H-H}=11.4$ Hz. This indicated that the dihedral angle between this proton and the adjacent CH proton must be very close to 180°. Similarly, the signal for the *equatorial (geminal)* proton (δ 3.23) was a doublet of doublets, with a much smaller vicinal coupling ($J_{H-H}=3.7$ Hz), suggesting a dihedral angle closer to 90°. This was further evidence for the protoberberine system, and these coupling constants require an *axially* oriented H13a. Other aliphatic signals were multiplets arising from the CH_2–CH_2 spin system. The deshielding of one of the corresponding ^{13}C signals (δ 51.3) confirmed that this was the third group attached to nitrogen. This analysis indicated this alkaloid was (−)-tetrahydropalmatine (**1**), and our data matched those reported (Southon and Buckingham, 1989a, b). The optical rotation ($[\alpha]_D^{25}=-276.6°$, ethanol), was in good agreement with that reported for the S-enantiomer (−290.8) (Southon and Buckingham, 1989a). (−)-Tetrahydropalmatine (**1**) constituted approximately 70% of the alkaloid content, and is a common component of many plants, particularly *Stephania* species. The purified compound is distributed in China as an analgesic (Hussain et al., 1989) and has been reported to exhibit bradycardial, hypotensive and sedative activities (Wang and Lui, 1985; Hsu and Kin, 1964).

The available literature describing alkaloids of the benzylisoquinoline class, often lacks accurate spectral data. This is partly attributable to the number of non-protonated carbons and electronically similar methoxyl groups in most molecules that, until recently, have been impossible to assign unambiguously. Hussain et al., published the ^{13}C NMR assignments of five biologically active protoberberine alkaloids, one of which was tetrahydropalmatine, (Hussain et al., 1989) but the assignments of the four methoxyl signals were uncertain. These have now been assigned using the conventional DQF–COSY and HMQC experiments, in addition to the HMBC experiment. These assignments are summarised in Table 1.

The second most abundant alkaloid in the *S. bancroftii* tuber was obtained as fine yellow crystals, mp 142–145 °C, and molecular formula (HRMS) $C_{19}H_{18}NO_3$. This constituted about 28% of the alkaloid content of the tuber extract, with approximately 0.4 g being isolated from ~800 g of tuber. The distinctive signals observed in the ^{1}H NMR spectra include the ''AB pattern'' at δ 5.98 and δ 5.83, $J=1.4$ Hz and the ^{13}C signal at δ 100.3, characteristic of the methylenedioxy functionality. The spectra also indicated the presence of an *N*-methyl group, one methoxyl but no hydroxyl groups. The aromatic region displayed a doublet at δ 7.71 coupled to a triplet at δ 7.24, in turn coupled to a doublet at δ 6.80. These aromatic multiplets were accompanied by one aromatic singlet. These data, which account for the three oxygen atoms (attached to aromatic rings) suggested that the two benzyl rings may be directly linked at one point. This suggested that the alkaloid may belong to the **aporphine** class [see (−)-stephanine (**2**)]. The aliphatic region of the spectrum supported this, with isolated CH_2–CH_2 and CH–CH_2 systems accounting for the proton signals. The chemical shifts of one of the ethano group carbons and the CH group (δ 53.3, δ 61.5) implied that both carbon atoms were attached to the nitrogen atom. These NMR data and the optical rotation $[\alpha]_D^{24}=-100.5°$ ($CHCl_3$) indicated that the alkaloid was (−)-stephanine (**2**). [Reported $[\alpha]_D^{24}=-92.5°$ ($CHCl_3$) of *R*-enantiomer] (Kunitomo et al., 1982).

A HMBC experiment was performed on (**2**), and the signals corresponding to C1 and C2 were identified by

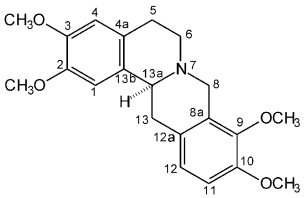
Table 1
^{1}H and ^{13}C NMR data for isolated alkaloids

(−)-Tetrahydropalmatine (**1**)			(−)-Stephanine (**2**)			(−)-Crebanine (**3**)			(−)-Ayuthianine (**4**)			(−)-Corydalmine (**5**)			(+)-Laudanidine (**8**)			(−)-Amurine (**9**)		
Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)	Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)	Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)	Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)	Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)	Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)	Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
1	6.7 *s*	108.5	1		142.4	1		142.1	1		143.0	1	6.69 *s*	108.6	1	3.67 *dd* J = 5.2, 7.8 Hz	64.7	1	6.52 *s*	107.3
2		147.4	2		146.3	2		146.8	2		147.1	2		147.6	3	3.2–3.14 *m*[a] 2.78–2.73 *m*[a]	46.5	2		146.7[b]
3		147.3	3	6.51 *s*	107.3	3	6.50 *s*	106.8	3	6.53 *s*	107.8	3		147.8	4	2.86–2.80 *m*[a] 2.57 *dt* J = 4.5, 16 Hz	25.0	3		146.6[b]
4	6.58 *s*	111.2	3a		126.2	3a		128.8	3a		128.4	4	6.59 *s*	111.4	4a		125.5	4	6.77 *s*	105.0
4a		126.6	4	3.13 *m* 2.54 *brd*	28.9	4	2.62 *t,d* J = 3.5 *Hz* 3.11 *brt*	28.9	4	3.10 *t* 2.60 *d*	28.8	4a		126.5	5	6.53 *s*	111.9	5	6.23 *s*	122.1
5	ax. 3.10 *m* eq. 2.65 *m*	28.9	5	3.00 t J = 5.7 Hz 2.46 *td* J = 3.5, 11.8 Hz	53.3	5	2.54 *t,d* J = 3.5, 11.6 Hz 3.06 *m*	53.5	5	3.1 *d* 2.69 *td* J = 3.8, 13.9 Hz	53.3	5	ax.3.15 *m* eq.2.70 *d*	28.6	6		147.2	6		151.1
6	ax. 2.59 *m* eq. 3.16 *m*	51.3	6a	3.05 *m*	61.5	6a	3.15 *brd*	61.8	6a	3.20 *brd*	65.9	6	ax.2.69 *m* eq.3.21 *m*	51.3	7		146.2	7		181.3
8	ax. 3.49 *d* J = 15 Hz eq. 4.20 *d* J = 5.5 Hz	53.8	6b		126.5	6b		126.4	6b		122.3	8	ax.3.59 *d* J = 15.4 Hz eq 4.23 *d* J = 15.4 Hz	53.6	8	6.03 *s*	111.0	8	6.25 *s*	118.7
8a		127.6	7	3.70 *dd* J = 4.6. 15 Hz 2.26 *dd* J = 14.3 Hz	25.7	7	3.66 *dd* J = 4.3, 14.6 Hz 2.31 *dd* J = 14.3	26.7	7	5.44 *d* J = 2.9 Hz	58.8	8a		126.3	8a		129.2	9	3.60 *d* J = 6 Hz	60.5
9		144.9	7a		123.4	7a		129.6	7a		123.1	9		143.3	9	3.11 *dd* J = 5.2 13.7 Hz 2.68 *dd* J = 7.9 13/7	40.73	10	3.24 *d* J = 18 Hz 2.92 *dd* J = 6, 18 Hz	32.7
10		150.1	8		155.9	8		152.0	8		157.2	10		146.8	10		133.2	11		129.2
11	6.74 *d* J = 8.3 Hz	110.8	9	6.80 *d* J = 8 Hz	109.3	9		145.8	9	6.88 *d* J = 8 Hz	110.2	11	6.78 *d* J = 8.2 Hz	114.7	11	6.75 *d* J = 2.07 Hz	115.8	12		130.7
12	6.83 *d* J = 8.4 Hz	123.7	10	7.24 *dd* J = 8.8Hz	126.8	10	6.85 *d* J = 8.6 Hz	110.3	10	7.32 *dd* J = 8 Hz, 8Hz	129.7	12	6.70 *d* J = 8.2 Hz	124.7	12		145.5	13		41.4

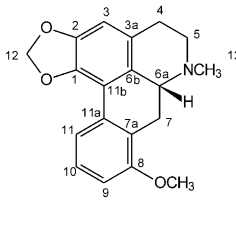
(*continued on next page*)

Table 1 (*continued*)



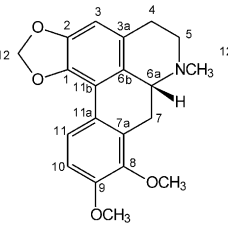
(−)-Tetrahydropalmatine (**1**)

Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
12a		128.5
13	ax. 2.79 *dd* J=11.4 15.6 Hz eq. 3.23 *dd* J=3.7 15.6 Hz	36.1
13a	3.49 *dd*	59.1
13b		129.5
2OCH_3	3.84 *s*	55.9
3OCH_3	3.81 *s*	55.7
9OCH_3	3.82 *s*	60.0
10OCH_3	3.80 *s*	55.6



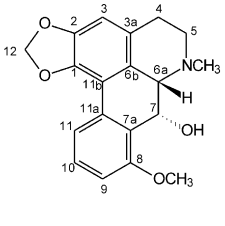
(−)-Stephanine (**2**)

Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
11	7.71 *d* J=8 Hz	119.2
11a		131.9
11b		116.2
12	5.98 *d* J=1.4 Hz 5.83 *d* J=1.4Hz	100.3
N-CH_3	2.56 *s*	43.7
8OCH_3	3.82 *s*	55.2



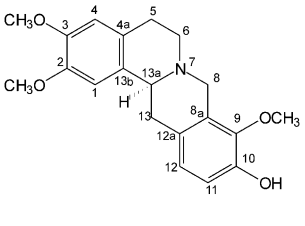
(−)-Crebanine (**3**)

Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
11	7.78 *d* J=8.6 Hz	123.1
11a		124.5
11b		116.5
12	6.04 *d* J=1.5Hz 5.89 *d* J=1.5Hz	100.6
N-CH_3	2.53 *s*	43.4
8OCH_3	3.79 *s*	60.7
9OCH_3	3.87 *s*	55.7



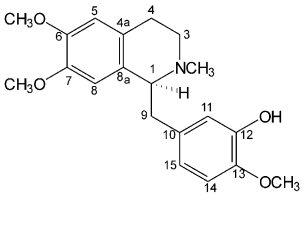
(−)-Ayuthianine (**4**)

Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
11	7.71 *d* J=8 Hz	119.8
11a		131.5
11b		115.5
12	5.88 *d* J=1.3 Hz 6.04 *d* J=1.3 Hz	100.7
N-CH_3	2.61 *s*	43.4
8-OCH_3	3.90 *s*	55.7



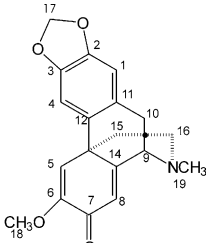
(−)-Corydalmine (**5**)

Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
12a		127.1
13	ax 2.82 *dd* J=15.3 15.0 Hz eq 3.23 *d* J=11.8 Hz	35.7
13a	3.64 *m*	59.3
13b		128.8
2OCH_3	3.85 s 3H	56.1
3OCH_3	3.83 s 3H	55.9
9OCH_3	3.76 s 3H	60.5



(+)-Laudanidine (**8**)

Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
13		145.1
14	6.70 *d* J=8.17 Hz	110.4
15	6.50 *dd* J=2.07, 8.17 Hz	121.2
6-OCH_3	3.80 *s*	55.69
7-OCH_3	3.54 *s*	55.24
13-OCH_3	3.82 *s*	55.94
11-CH_3	2.49 *s*	42.4



(−)-Amurine (**9**)

Position Number	^{1}H (ppm) ($CDCl_3$)	^{13}C (ppm)
14		161.4
15	1.85 *td* J=6.4, 11.5 Hz 1.75 *dt* J=2.5 12.6 Hz	40.8
16	2.5 d J=2.5 2.52 *m*	45.4
17	5.86 *d* J=1.3 Hz 5.83 *d* J=1.3 Hz	101.0
6-OCH_3	3.71 *s*	54.9
N-CH_3	3.37 *s*	42.2

[a] Overlapping.
[b] Coincident.

the three-bond couplings to the methylenedioxy proton and distinguished from each other by the three-bond coupling between the C3 protons (δ 6.51) and the C1 signal (δ 142.4). The long range couplings to the C3 proton singlet also identified the ^{13}C signal at δ 126.5 as belonging to C6b which was confirmed by coupling of this ^{13}C signal to the C7 signals. Similarly, the ^{13}C signal at δ 126.2 was assigned to position 3a on the basis of long range couplings to the H5a and H6a protons. The ^{13}C signal for C7a was identified at δ 123.4 by the presence of a three-bond coupling to this signal from both H9 and H11. The higher field doublet, δ 6.80, showed only one other 3-bond coupling to the protonated aromatic carbon at δ 119.2. This implied that this ^{1}H doublet corresponds to position C9. The lower field doublet, δ 7.71 showed two additional 3-bond couplings, one to position C9 and the other to the non-protonated ^{13}C signal at δ 116.2. This confirmed that the lower field ^{1}H doublet represents H11 and the ^{13}C signal at δ 116.2 corresponds to C11b. The complete assignment of the ^{1}H and ^{13}C NMR spectra of (−)-stephanine (**2**) appears in Table 1.

Two minor alkaloids were separated from (−)-stephanine by exhaustive column chromatography. The first of these was a yellow solid, ($M^+ = 338.1395$), corresponding to $C_{20}H_{20}NO_4$. The ^{13}C and ^{1}H spectra of this compound indicated a structure similar to stephanine with the addition of an extra methoxyl group on the D-ring. Analysis of the 1 and 2-D NMR spectra implicated the *aporphine* alkaloid crebanine (**3**). The optical rotation (−55.2° ($CHCl_3$) matches that reported for the (*R*)-enantiomer (−61.0°) (Kunitomo et al., 1982). Analysis of the HMBC spectrum of (**3**) and comparison of the results with those for (−)-stephanine (**2**) permitted the complete assignment of the ^{1}H and ^{13}C NMR spectra, including definite assignment of the two methoxyl groups.

Stephanine was isolated from *Stephania japonica* (Thunb.) Miers earlier this century [Kondo and Sanada, 1924, 1928)]. Crebanine was isolated from *Stephania sasakii*, and co-occurs with stephanine in *S. capitata* (Tomita, 1939; Shira, 1944; Kunitomo et al., 1981; Charles et al., 1987; Shamma, 1972; Pharadai et al., 1981). The (*R*)-configurations of (−)-stephanine and (−)-crebanine were determined by X-ray analysis by Kunitomo et al. (1982). Many of the plants that yield (−)-stephanine and (−)-crebanine, including *Stephania bancroftii*, are used in traditional medicine, and the major physiological effects appear to lie in their interaction with receptors in the mammalian central nervous system [Chen et al., 1987; Liu et al., 1989a, b; Han and Liu, 1988; Ma et al., 1990; Li, 1989)].

An additional minor alkaloid isolated from the tuber of *Stephania bancroftii* corresponded to $C_{19}H_{19}O_4N$ (HRMS). The NMR spectra were remarkably similar to those of (−)-stephanine, having 12 aromatic ^{13}C signals, methylene dioxy signals and the same aromatic ^{1}H spin system. The ^{1}H spectrum indicated that the aromatic substitution pattern was the same as in (−)-stephanine, but the molecular formula indicated an additional oxygen atom. The HMQC experiment revealed the C4–C5 ethano group but that C7 was a methine centre. The H7 signal is a doublet ($J = 2.9$ Hz) at δ 5.44 and the C7 signal is shifted to lower field (δ 58.8). The spectra indicated that this alkaloid was (−)ayuthianine (**4**), which is effectively 7-hydroxylated (−)-stephanine (Guinaudeau et al., 1982) and possesses the (6a*R*)(7*R*)-configuration, on the basis of the generalisation developed by Guinaudeau (Guinaudeau et al., 1982). The small coupling constant between H7 and H6a indicates a *cis* relationship and therefore the 7-OH group is *axial*, as deduced for the alkaloid from *Stephania venosa* (Guinaudeau et al., 1982). To date, the C7-hydroxylated aporphine alkaloids appear to be limited to the Annonaceae, Magnoliaceae, Lauraceae and Menispermaceae families of plants and all have the (6a*R*)(7*R*)-configuration (Guinaudeau et al., 1982).

2.2. Chemical investigation of the leaves and stems of *Stephania bancroftii Bailey*

The tuber of *Stephania bancroftii* was used by Aboriginal people for medicinal purposes, and the leaves as a fish poison, (Kunitomo et al., 1981) and hence these parts of the plant were examined separately. While (−)-ayuthianine (**4**) was a minor alkaloid from the tuber constituting ~1% of the total alkaloid fraction, it was a major alkaloid in the leaves and stems. From the extraction of 1kg of leaf and stem material, 10 mg of (−)-ayuthianine was isolated, and this was accompanied in roughly equal amounts by one other alkaloid that was identified as (−)-corydalmine (**5**) (see later). There was no evidence for (−)-tetrahydropalmatine (**1**), (−)-stephanine (**2**), or (−)-crebanine in the leaf extract. This was very surprising considering the large amount of (−) tetrahydropalmatine isolated from the tuber. Trace amounts of a third compound were detected in the leaf extract, but useful spectral data were unobtainable. The leaves of *Stephania bancroftii* have a far lower alkaloid content than the tuber and it appears that the compounds are stored in the tuber and selected metabolites are transported to the leaves.

The second alkaloid was of the tetrahydroberberine class, (−)-corydalmine (**5**). The spectra were strikingly similar to those of (−)-tetrahydropalmatine (**1**) but indicated the presence of three methoxyl and one hydroxy groups, rather than four methoxyl groups. The position of the hydroxyl group was determined from the HMBC experiment. The ^{13}C signals corresponding to positions C2 and C3 (δ 147.6 and δ 147.8, respectively) were identified by their three bond couplings to H4 and H1. Each of these ^{13}C signals displayed a long range

coupling to one of the methoxyl proton singlets, indicating that both positions C2 and C3 bore a methoxyl group. The third methoxyl ^{1}H singlet exhibited a three bond coupling to the ^{13}C signal at δ 143.3 and this signal also coupled to the aromatic proton doublet (δ 6.78) assigned to H11. This indicated that the third methoxyl group was attached at C9 rather than C10, and was supported by careful analysis of the mass spectrum (Richter and Brochmann-Hanssen, 1975). The optical rotation ($[\alpha]_D^{20} = -156.8^\circ$ ethanol) was lower than that reported (Cava et al., 1968) rotation ($[\alpha]_D^{20} = -310^\circ$ ethanol), and this is attributable in part to the small sample and purification problems. The enantiomeric (+)-corydalmine had been isolated previously (Imaseki and Taguchi, 1962; Kametani et al., 1970; Gellert and Rudzats, 1972; Brochmman-Hanssen and Chiang, 1977).

Stephania bancroftii resembles other *Stephania* species, in being an abundant source of various alkaloids. The tuber and leaves have yielded protoberberin and aporphine alkaloids as well as the less common C-7 hydroxylated aporphines (Blanchfield et al., 1993). An independent examination of the tuber of *Stephania bancroftii* by Bartley et al. (1994) concurred with the above findings, but in addition, the minor alkaloids, (+)-sebiferine (**6**) and (+)-stepharine (**7**) were identified (see Fig. 1).

2.2.1. Chemical investigation of the roots of Stephania aculeata Bailey

The root system of *Stephania aculeata* was processed in the manner described for *S. bancroftii*. The organic extract (pH = 7) appeared to contain alkaloidal material, and when dried under vacuum, and then resuspended in methanol:dichloromethane (1:1), off-white crystals precipitated from the brown solution. HRMS measurement (343.1789) indicated a molecular formula of, $C_{28}H_{25}NO_4$, (calc. 343.1784). The ^{13}C spectrum exhibited 12 aromatic and 8 aliphatic signals, indicating the presence of two aromatic rings, as present in the benzylisoquinoline system. The ^{1}H NMR spectrum confirmed the presence of three methoxyl and an *N*-methyl group. The aromatic region displayed 5 signals: two singlets, one doublet with a large coupling constant of 8.2 Hz, another doublet with a small coupling constant of 2.1 Hz and a doublet of doublets ($J = 2.1$, 8.2 Hz). The latter three signals are consistent with one of the aromatic rings possessing two *ortho* protons and a third proton *meta* to one of these. DQFCOSY and HMQC data confirmed the presence of CH_2–CH_2 and CH–CH_2 moieties and overall the data suggested the benzylisoquinoline alkaloid, laudanidine, (**8**).

Analysis of the HMBC spectrum provided unambiguous assignment of the ^{1}H and ^{13}C NMR spectra (Table 1). These assignments and the optical rotation ($[\alpha]_D^{20} = +90^\circ$, $CHCl_3$) confirmed the compound as (+)-laudanidine (**8**). (Reported for the (*S*)-enantiomer $[\alpha]_D^{20} = +87^\circ$, $CHCl_3$) (Southon and Buckingham, 1989b). Laudanidine has been isolated from several species of plant including the opium poppy, *Papaver somniferum* L (Proksa et al, 1979) and the bark of *Cryptocarya amygdalina*, (Borthakur et al., 1981) and several *Machilus* and *Thalictrum* species (Southon and Buckingham, 1989b).

One other alkaloid was isolated from this root extract, and its ^{1}H NMR spectrum revealed the presence of a methylene dioxy moiety, one methoxyl group and an *N*-methyl group. The ^{13}C NMR spectrum indicated profound structural differences when compared with (+)-laudanidine, (**8**) and the benzylisoquinoline alkaloids from *Stephania bancroftii*. A carbonyl group (δ 181.3) and 11 signals below 100 ppm, were observed. This discounted the possibility of two aromatic rings in the molecule. The ^{1}H NMR spectrum exhibited four low field signals with the lowest two of these (δ 6.52 and δ 6.77) displaying three-bond couplings to the ^{13}C signals at δ 146.6 and δ 146.7 respectively, which in turn showed long range couplings to the methylenedioxy protons in the HMBC experiment. This series of connectivities implied that the one aromatic ring present in the molecule bore the methylene dioxy group and only two aromatic protons. The remaining long range couplings to the aromatic protons were used to assign the two nonprotonated positions to the ^{13}C signals at δ 129.2 and δ 130.7, leaving only five low field ^{13}C signals, including the carbonyl signal, to be assigned. One of these signals (δ 151.1) displayed a three-bond coupling to the methoxyl proton signal, confirming that the methoxyl group is directly attached to the carbon corresponding to this signal. The methoxyl bearing carbon at δ 151.1 also showed long range couplings to both the olefinic ^{1}H signals at δ 6.23 and δ 6.25. The signal at δ 6.23 also displayed 3-bond couplings to the carbonyl carbon and the final nonprotonated low field signal at δ 161.4. The signal at δ 6.25, however, showed a three-bond coupling to a higher field ^{13}C signal at δ 41.4. These data indicated the presence of a dienone moiety and the substitution pattern suggested the morphinane alkaloid amurine (**9**). Accurate mass measurement (measured mass, 325.1315. $C_{19}H_{19}NO_4$ requires 325.1314) supported the postulated structure.

Further analysis of the NMR spectra including DQFCOSY and HMQC and comparison with the NMR data available (Gozler et al., 1990; Roblot et al, 1984) confirmed the structure as amurine (**9**). This alkaloid was nicely crystalline and its confirmation as amurine followed from X-ray structural analysis.

The optical rotation $[\alpha]_D^{20} = -8^\circ$ ($CHCl_3$), indicated the (9S)-configuration. [Reported for the (*S*)-enantiomer, ($[\alpha]_D^{20} = -10^\circ$ $CHCl_3$)] (Gozler et al., 1990). The crystalline methiodide was used for X-ray analysis and the "heavy" iodide enabled the Flack parameter (Flack, 1983) to be determined with high accuracy, (0.04), and confirmed the absolute configuration as (9S) (Flack,

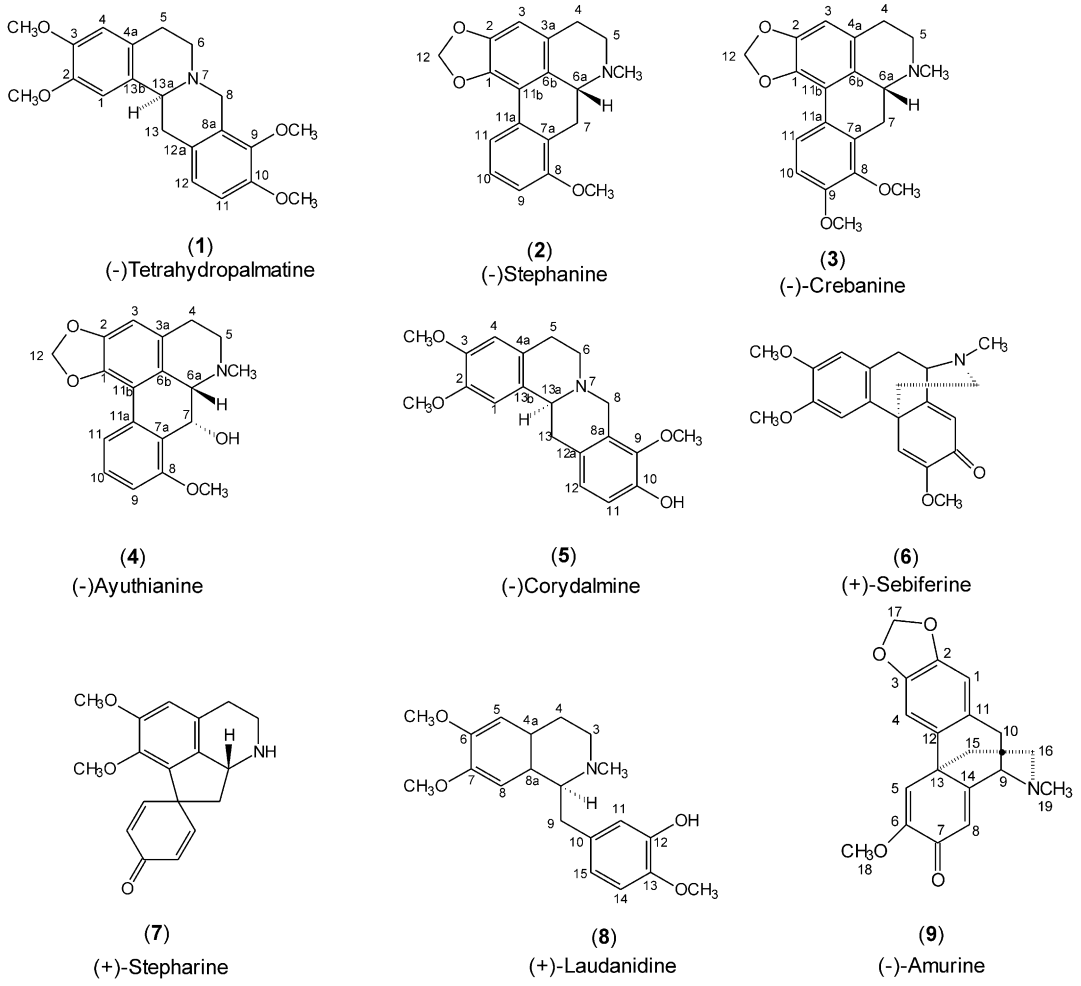


Fig. 1. Alkaloid structures **1**–**9**.

1983) (see Fig. 2).[1] (+)-Amurine was first isolated from *Papaver amurense* Hart in 1960 (Boit and Flentje, 1960) but its structure was not correctly elucidated until 1968 (Dopke et al., 1968) Since then (+)-amurine has been isolated from several *Papaver* species, (Oztekin et al, 1984; Veznik et al, 1987) and is often the major alkaloid present. (−)-Amurine, (**9**), however, is a much less common natural product (Hemingway et al, 1981; Gozler et al., 1990). Alkaloids incorporating the dienone moiety are known to be formed during the biogenesis of morphanoid alkaloids. The isolation of (−)-amurine from the roots of Stephania aculeata, a Menisperm, represents a significant extension of the sources of this alkaloid.

2.2.2. Roots of Stephania japonica Miers var. discolor

Several species of *S. japonica* occur throughout Japan, China, northern Australia, Polynesia and Papuasia (Johns, 1987). The species have been identified under a number of synonyms and reported to be efficacious towards a variety of ailments. There has been interest in the Japanese varieties of this plant and a number of alkaloids characterised (Matsui et al, 1982). However, our examination of the roots of *stephania japonica* (Thunb.) Miers var discolor (40 Mile Scrub National Park in North Queensland) revealed no significant level of alkaloids.

2.3. Experimental

Chromatography: All TLC was performed on aluminium backed pre-coated silica gel 60 F_{254} sheets. All column chromatography was performed on Silica gel 60, 230–400 mesh in glass columns using compressed air for Flash column chromatography. Prep. TLC plates were prepared using Merck 7477 silica gel with 1% $CaSO_4$ binder.

NMR spectroscopy: NMR spectra were measured on a Bruker AMX 500 spectrometer using a 5 mm Inverse ^1H/broad band Probe. The spectra were run as $CDCl_3$ solutions and were referenced to $CHCl_3$ as the internal standard ($^1H = \delta$ 7.24; $^{13}C = \delta$ 77.0).

Mass spectrometry: The High Resolution spectra and accurate mass measurements were obtained with a Kratos MS 25 instrument using an ionising potential of 70 eV.

[1] X-Ray Crystal structure data submitted to the Cambridge Crystallographic Data Centre, CCDC deposit number: CCDC 200100.

Polarimeter: Optical rotations were recorded on a Perking-Elmer 241 MC Polarimter using a sample concentration of 10 mg/ml, unless otherwise specified.
Plant material: The plant material was supplied and identified by Dr. Donald Sands, Department of Entomology, CSIRO, Long Pocket, Brisbane, Australia.

2.3.1. Chemical investigation of the tuber of Stephania bancroftii Bailey

A portion of the large tuber of a mature plant was excised (835 g) and ground to a fine pulp in a blender. The material was continuously extracted (Soxhlet) into boiling ethanol for several days. The extract was concentrated and acidified using aqueous HCl. The acidic suspension was filtered and the filtrate extracted into ether (3×100 ml). The ether was removed to yield 0.2 g of yellow residue. NMR analysis showed no sugnificant compounds present. The pH of the aqueous solution was adjusted to 7 by the addition of 20% Na_2CO_3. This solution was filtered and the filtrate extracted exhaustively into ether, which was then concentrated. TLC (100% CH_2Cl_2/drop of NH_3) of the viscous residue indicated that it contained a complex mixture of organic compounds. The aqueous fraction was again adjusted to pH 10. No solid material formed. The solution was extracted into ether (3×100 ml). NMR analysis revealed a negligible level of organic compounds.

2.3.2. Chromatography of pH = 7 extract

The residue from the ether extract at pH = 7 was chromatographed (silica) and eluted with CH_2Cl_2. Four fractions were collected and these appeared, by NMR and TLC analysis, to contain alkaloids. The first fraction contained a single compound that was identified as (−)-*tetrahydropalmatine* (**1**). The other three fractions from the above column contained varying amounts of three minor alkaloids as well as tetrahydropalmatine. Extensive flash chromatography of these fractions eventually provided a second compound identified as (−)*stestephanine* (**2**). The remaining mixture contained a small amount of (−)-*stephanine* (**2**) but predominantely a third compound and trace amounts of a fourth. This mixture was separated by preparative TLC to provide the compounds (−)*crebanine* (**3**) and (−)-ayuthianine, (**4**).

*2.3.2.1. (−)-Tetrahydropalmatine (**1**).* One gram was obtained from 835 g of tuber and constituted 70% of the total alkaloid content of the tuber extract (pH = 7). HREIMS: *m/z* obs. 355.1784. $C_{21}H_{25}NO_4$ requires 355.1784. $[\alpha]_D^{25} = -276°$ (ethanol) (Reported for *S*-enantiomer = −290.8°) (Southon and Buckingham, 1989a) mp: 139–141 °C (Reported 144 °C) (Southon and Buckingham, 1989a). Mass spectrum: *m/z* 355 (M^+ 40%), 354 (26), 340 (4), 324 (7), 190 (23), 165 (23), 164 (100), 149 (74), 121 (18), 104 (20). ^1H and ^{13}C NMR: see Table 1.

*2.3.2.2. (−)-Stephanine (**2**).* A. 0.4 g of stephanine was obtained and constituted ∼28% of the total alkaloid content of the tuber extract (pH = 7). HREIMS: *m/z* obs. 309.1365. $C_{19}H_{18}NO_3$ requires 309.1365. $[\alpha]_D^{25} = -100.5°$ ($CHCl_3$) (Reported for *R*-enantiomer = −92.5° $CHCl_3$) (Kunitomo et al., 1982) mp: 148–150 °C (Reported = 155–157°) (Kunitomo et al., 1981). Mass spectrum: *m/z* 309 (M^+ 29%), 308 (100), 294 (6), 292 (4), 280 (13), 279 (14), 278 (22), 266 (10), 235 (5), 208 (5), 165 (6) 149 (4). ^1H and ^{13}C NMR: see Table 1

*2.3.2.3. (−)-Crebanine (**3**).* A 0.01 g of crebanine was obtained and constituted ∼1% of the total alkaloid content of the tuber extract (pH = 7). HREIMS: *m/z* obs. 338.1394 ($M^+ - H$). $C_{20}H_{20}NO_4$ requires 338.1392. $[\alpha]_D^{25} = -55.2°$ ($CHCl_3$) (Reported for *R*-enantiomer = −61.0° $CHCl_3$) (Kunitomo et al, 1982). Mass spectrum: *m/z* 339 (M^+ 46%), 338 (89), 324 (11), 308 (13), 296 (28), 265 (17), 190 (10), 165 (10), 152 (13), 150 (13), 149 (50), 113 (11). ^1H and ^{13}C NMR: See Table 1.

*2.3.2.4. (−)-Ayuthianine (**4**).* A 0.015 g of ayuthianine was obtained and constituted ∼1% of the total alkaloid content of the tuber extract (pH = 7). HREIMS: *m/z* obs. 325.1315 (M^+). $C_{19}H_{19}NO_4$ requires 325.1314. $[\alpha]_D^{25} = -29.8°$ ($CHCl_3$). Mass spectrum: *m/z* 325 (M^+ 100%), 322 (26), 321 (26), 310 (43), 294 (29), 292 (33), 282 (38), 267 (22), 252 (25), 190 (98), 149 (46), 132 (31), 102 (27). ^1H and ^{13}C NMR: see Table 1.

2.3.3. Chemical investigation of the leaves and stems of Stephania bancroftii Bailey

Green leaves and stems (∼1 kg) from *S. bancroftii* were chopped into small pieces, and continuously extracted into boiling ethanol for 4 days. The crude extract (30.6 g) was concentrated and acidified with aqueous HCl. The residue was extracted with ether at pH = 1, 7 and 10 as described above for the tuber extract. The viscous oil obtained from the organic extract at pH = 7 was the only fraction shown to contain alkaloid material. This was chromatographed (silica), eluting with 5% methanol/95% CH_2Cl_2. The major alkaloid fraction contained two compounds with a trace of a third. These were isolated by preparative TLC with 4% methanol/96% CH_2Cl_2 and identified as (−)-*ayuthianine* (**4**) and (−)-*corydalmine* (**5**). A third compound, with a molecular weight of 341 amu, was also detected but insufficient for spectral characterisation was obtained.

*2.3.3.1. (−)-Ayuthianine (**4**).* A 0.01 g of ayuthianine was obtained from 1 kg plant material and constituted ∼50% of the total alkaloid content of the leaves and stems. NMR Spectral data were identical with those reported for the sample isolated from the tuber.

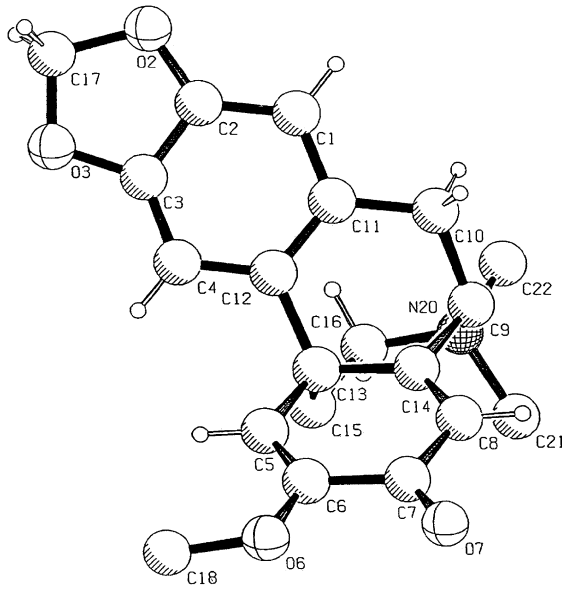


Fig. 2. A PLUTON drawing, showing the absolute configuration (−)amurine methiodide.

*2.3.3.2. (−)-Corydalmine (**5**).* A 0.01 g of corydalmine was obtained and constituted ~50% of the total alkaloid content of the leaves and stems. HREIMS: *m/z* obs. 341.1606 (M^+). $C_{20}H_{23}NO_4$ requires 341.1627. $[\alpha]_D^{25} = -157$ (ethanol) (Reported for *S*-enantiomer = −310° (ethanol) (Cava et al., 1968). Mass spectrum: *m/z* 341 (M^+ 38%)m 349 (28), 310 (M–OCH_3) (6), 193 (12), 192 (100), 190 (31), 151 (11), 150 (36), 149 (25), 135 (33), 121 (8), 107 (11). ^1H and ^{13}C NMR: see Table 1.

2.3.4. Chemical investigation of the Roots of Stephania Aculeata Bailey

Root material (186 g) from a wild vine was collected in Chapel Hill, Brisbane. The ground material was continuously extracted into boiling ethanol for 4 days. The extract was treated in the manner described for the extraction of the *S. bancrofti* tuber. NMR analysis of the ether extracts revealed that the pH7 extract only contained alkaloids. This was treated with 50% methanol/50% CH_2Cl_2 (5 ml) and the precipitated yellow crystals were identified as (+)-*laudanidine* (**8**). The filtrate from this collection was chromatographed on silica (50% methanol:50% CH_2Cl_2) and provided a second alkaloid identified as (−)-*amurine* (**9**).

*2.3.4.1. (+)-Laudanidine (**8**).* Approximately 20 mg was obtained from 186 g of root material. HREIMS: *m/z* obs. 343.1789. $C_{20}H_{25}NO_4$ requires 343.1784. $[\alpha]_D^{25} = +90.0°$ ($CHCl_3$) (Reported for *S*-enantiomer = +87° ($CHCl_3$) (Southon and Buckingham, 1989a, b). mp: 176–178 C (Reported 181–182 °C) (Southon and Buckingham, 1989a, b). Mass Spectrum: *m/z* 343 (M^+ 1%), 326 (2), 207 (16), 206 (100), 190 (15), 162 (6), 137 (5). ^1H and ^{13}C NMR: see Table 1.

*2.3.4.2. (−)-Amurine (**9**).* Approximately 200 mg was obtained from the root material (186 g). HREIMS: *m/z* obs. 325.1315. $C_{19}H_{19}NO_4$ requires 325.1314. $[\alpha]_D^{25} = -8°$ ($CHCl_3$) (Reported for S-enantiomer = −10° ($CHCl_3$) (Gozler et al., 1990). mp: 213–215 °C (Reported for *R*-enantiomer = 212–213 °C) (Dopke et al, 1968; Veznik et al., 1987). Mass spectrum: 325 (M^+ 37%), 297. (17), 282 (28), 266 (13), 240 (21), 106 (100), 190 (13), 152 (13), 149 (33), 139 (22), 113 (12). ^1H and ^{13}C NMR: see Table 1.

2.3.5. X-ray crystal structure determination of the methiodide of (−) amurine

The crystal structure analysis of (−)amurine (**9**) is described elsewhere (PhD Thesis, Dr. J.T. Blanchfield, (1996), University of Queensland). The X-Ray crystal structure analysis of the methiodide of (−)amurine presented herein has been deposited with the CCDC, deposit number: 200100. X-Ray diffraction data was collected from a crystal of the methiodide of (−)-amurine on an Enraf-Nonius CAD-4 four circle diffractometer (Frenzy, 1985) with molybdenum X-radiation ($\lambda = 71073$ Å). There were 5501 observed [$I > 2\sigma$ (I)] reflections out of 8383 collected. The scattering factors for C, H, O, I and N (Biers and Hamilton, 1974) were used. The structure was solved by Patterson heavy atom techniques in SHELXS-86 (Sheldrick, 1990) and using full matrix least-squares refinement (Sheldrick, 1993) on F^2s. The position of the hydrogen atoms were calculated using the SHELXL 93 (Sheldrick, 1993) program. The Flack parameter was determined to be 0.04 (**4**) (Flack, 1983). The plots were generated using the Pluton-94 program for display of crystal structures (Spek, 1994).

2.3.6. Chemical investigation of the roots of Stephania japonica *var* discolor *Blume*

Root material (150 g) from a wild *S. japonica* var *discolor* (Blume) was taken from 40 Mile Scrub National Park in North Queensland. The root material was processed as already described but NMR analyses of the extracts revealed no significant level of alkaloid components.

References

Anon., 1976. The Wealth of India - Raw Materials. CSIR, New Delhi.
Bailey, F.M., 1899. The Queensland Flora. Queensland Government Printers, Australia.
Bailey, F.M., 1909. Comprehensive Catalogue of Queensland Plants. Queensland Government Publication, Australia.
Bailey, F.M., 1910. Queensland Agricultural Journal 24, 221.
Bartley, J.P., Baker, L.T., Carvalho, C.F., 1994. Phytochemistry 36, 1327.
Biers, J.A., Hamilton, W.C., 1974. Scattering Factors: International Tables for X-Ray Crystallography. Kynoch press, Birmingham.
Blanchfield, J.T., Kitching, W., Sands, D.P.A., Thong, Y.H., Kennard, C.H.L., Byriel, K.A., 1993. Natural Product Letters 3, 305.
Boit, H.G., Flentje, H., 1960. Naturwiss 47, 180.
Borthakur, N., Mahanta, P.K., Rastogi, R.C., 1981. Phytochemistry 20, 501.

Brochmann-Hanssen, E., Chiang, H., 1977. Journal Organic Chemistry 42, 3588.
Cava, M.P., Nomura, K., Talapatra, S.K., Mitchell, M.J., Schlessinger, R.H., Buck, K.T., Beal, J.L., Douglas, B., Raffauf, R.F., Weisback, J.A., 1968. Journal of Organic Chemistry 33, 2785.
Charles, B., Bruneton, J., Pharadai, K., Tantisewie, B., Guinaudeau, H., Shamma, M., 1987. Journal of Natural Products 50, 133.
Chen, K.K., Chen, A.L., 1935. Journal Biological Chemistry 109, 681.
Chen, S., Lui, G., Min, Z., 1987. Yaoxue Xuebao 22, 341.
Dopke, W., Flentje, H., Jeffs, P.W., 1968. Tetrahedron 24, 4459.
Doskotch, R.W., Malik, M.Y., Beal, J.L., 1967. Journal Organic Chemistry 32, 3253.
Flack, H.D., 1983. Acta Cryst. A39, 876.
Frenzy, G.A., 1985. Entraf-Nonius Structure Determination Package Delft, The Netherlands.
Gellert, E., Rudzats, R., 1972. Australian Journal Chemistry 25, 2477.
Gozler, B., Ozic, P., Freyer, A., Shamma, M., 1990. Journal Natural Products 53, 986.
Guinaudeau, H., Shamma, M., Tantisewie, B., Pharadai, K., 1982. Journal Natural Products 45, 355.
Halfpap, K., 1992. Personal Communication. QDPI, PO Box 1054, Mareeba, Q. 4880.
Han, B.Y., Liu, G., 1988. Yaoxue, Xuebao 23, 806.
Hemingway, S.R., Phillipson, J.D., Verpoorte, R., 1981. Journal of Natural Products 44, 67.
Hsu, B., Kin, K.C., 1964. Int. Journal Neuropharmacology 2, 283.
Hussain, R.A., Kim, J., Beecher, C.W.W., Kinghorn, A.D., 1989. Heterocycles 29, 2257.
Imaseki, I., Taguchi, J., 1962. Journal Pharmaceutical Society Japan 82, 1214.
Johns, R.J., 1987. The Flowering Plants of Papuasia Part 1. Papua New Guinea University of Technology, Lae, Papua New Guinea.
Kametani, T., Ihara, M., Honda, T., 1970. Journal Chemical Society (C) 1060.
Kondo, H., Sanada, T., 1924. Journal Pharmaceutical Society Japan 45, 1034(a).
Kondo, H., Sanada, T., 1928. Journal Pharmaceutical Society Japan 48, 1141(b).
Kozuka, M., Miyaji, K., Sawada, T., Tomita, M., 1985. Journal Natural Products 48, 342.
Kunitomo, J., Oshikata, M., Adasu, M., 1981. Journal Pharmaceutical Society Japan 101, 951.
Kunitomo, J., Satoh, M., Watanabe, Y., Matsui, M., Inoue, M., Ishida, T., 1982. Heterocycles 19, 1883.
Li, S., Yang, X., Wang, F., 1989. Zhongcayao 20, 422.
Liu, G., Han, B., Wang, E., 1989a. Zhongguo Yaoli Xuebao 10, 302.
Liu, G., Hou, Y., Pan, L., Lu, Y., 1989b. Zhongguo Yaoke Daxue Xeubao 20, 114.
Ma, Z., Liu, G., Hou, X., 1990. Zhonggue Yaoke Daxue Xuebao 21, 107.
Matsui, M., Kabashima, T., Ishida, K., Tadebayashi, T., Watanabe, Y., 1982. Journal Natural Products 45, 497.
Oztekin, A., Hocquemiller, R., Cave, A., 1984. Journal Natural Products 47, 560.
Pharadai, K., Tantisewie, B., Ruchirawat, S., Hussain, S.F., Shamma, M., 1981. Heterocycles 15, 1067.
Proksa, B., Cerny, J., Putek, J., 1979. Pharmazie 34, 194.
Richter, W.J., Brochmann-Hanssen, E., 1975. Helv. Chim. Acta 58, 203.
Roblot, F., Hocquemiller, R., Cave, A., 1984. Bull. Soc. Chim. Fr. 3-4, Pt 2, 139.
Schiff, P.L., 1987. Journal Natural Products 50, 529.
Shamma, M., 1972. The Isoquinoline Alkaloids. Academic Press, New York.
Sheldrick, G.M.. , 1990. Acta Cryst. A46 467.
Sheldrick, G.M., 1993. SHELXL 93. Program for Crystal Structure Determination. University of Cambridge, UK.
Shira, H., 1944. Journal of Pharmaceutical Society Japan 64, 44.
Southon, I.W., Buckingham, J., 1989a. Dictionary of Alkaloids. Chapman and Hall, New York, p. 1044.
Southon, I.W., Buckingham, J., 1989b. Dictionary of Alkaloids. Chapman and Hall, New York, p. 632.
Spek, A.L., 1994. Program for the Display and Analysis of Crystal and Molecular Structures. Pluton-94. University Utrecht, The Netherlands.
Thornber, C.W., 1970. Phytochemistry 9, 157.
Tomita, M., 1952. Fortschritte der Chemie Organischer Naturstoffe 9.
Tomita, M., 1939. Journal Pharmaceutical Society Japan 59, 207.
Tomita, M., Kozuka, M., 1966. Journal Pharmaceutical Society of Japan 86, 871.
Tomita, M., Kozuka, M., Uyeo, S., 1966. Journal Pharmaceutical Society of Japan 86, 406.
Veznik, F., Taborska, E., Bochorakova, H., Turecek, F., Hanus, V., Slavik, J., 1987. Coll. Czechoslovak Chem. Commun. 52, 1634.
Wang, Z., Lui, G., 1985. Trends Pharmacological Science 6, 423.
Webb, L.J., 1948. Guide to the Medicinal and Poisonous Plants of Queensland. Council for Scientific and Industrial Research, Melbourne, Australia.