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Occurrence of loline alkaloids in *Argyreia mollis* (Convolvulaceae)☆

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Abstract

N-Formylloline was isolated from roots of *Argyreia mollis*. This is the first identification of a 1-aminopyrrolizidine alkaloid (loline alkaloid) in a species of the Convolvulaceae. Lolines are only known from the genus *Adenocarpus* (Fabaceae) and certain grasses (e. g. *Festuca*) infected with endophytic fungi. A GC–MS analysis revealed *N*-formylloline to be present in roots and aerial vegetative plant parts. It is accompanied by three congeners (i. e. loline, *N*-methylloline and *N*-propionylnorloline) and simple pyrrolidine alkaloids such as hygrine and its derivatives as well as tropan-3β-ol. Lolines could not be detected in *Argyreia capitata*, *A. hookeri*, *A. nervosa* and numerous species of 14 other convolvulaceous genera.

Keywords: *Argyreia capitata*; *Argyreia hookeri*; *Argyreia mollis*; *Argyreia nervosa*; Convolvulaceae; GC–MS; Pyrrolizidine alkaloids; Loline alkaloids; *N*-Formylloline; Pyrrolidine alkaloids; Tropan-3β-ol (=pseudotropine)

1. Introduction

The genus *Argyreia* (Convolvulaceae) comprises approximately 90 species, tropical lianas mainly indigenous to South and Southeast Asia. Ergoline alkaloids, both clavines and lysergic acid amides, have been identified in the seeds of 14 out of 20 *Argyreia* species examined so far, while the seeds of the remaining six species contained a number of unidentified compounds, probably also ergolines (Chao & Der Marderosian, 1973a, 1973b). This frequent occurrence of ergoline alkaloids in the genus *Argyreia* shows their significance as a chemotaxonomical marker for this taxon. However, apart from one report that no ergoline alkaloids could be detected in the vegetative parts *of A. nervosa* (Burm. f.) Boj., no phytochemical investigations concerning the occurrence of alkaloids in the vegetative parts of this genus have been published so far (Chao & Der Marderosian, 1973a).

This prompted us to investigate the alkaloid pattern of the roots and the aerial vegetative parts of *A. capitata* (Vahl) Choisy, *A. hookeri* Clarke, *A. mollis* (Burm. f.) Choisy and *A. nervosa*.

2. Results and discussion

In contrast to the seeds no ergoline alkaloids could be detected in the vegetative parts of all four *Argyreia* species. This is surprising since we could detect such compounds not only in the seeds, but also in the aerial vegetative parts of three other convolvulaceous species, *Ipomoea pes-caprae* (L.) R. BR. (Kayser, 1994), *Ipomoea piurensis* O'Donell (Jenett-Siems, Kaloga, & Eich, 1994) and *Stictocardia campanulata* (L.) Merrill (Schimming, in preparation). However, we observed one compound both in the aerial vegetative parts and

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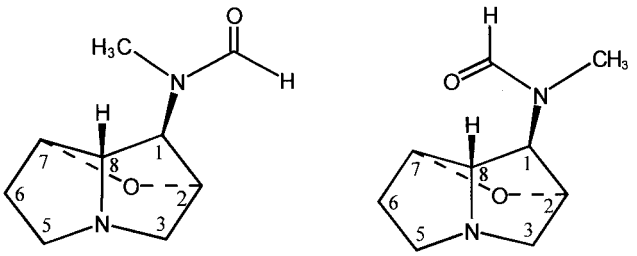

1a **1b**

Fig. 1. Rotamers of *N*-formylloline.

in the roots of *Argyreia mollis*, which gave a strong orange–red colour with Dragendorff's reagent on TLC. This compound (**1**) was isolated from the alkaloid extract of the aerial vegetative parts by column chromatography on silica gel followed by ion exchange chromatography. Its structure was elucidated by EIMS, HRMS, FABMS, ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and NOE experiments.

All spectral data and assignments of **1** were in complete agreement with the structure of *N*-formylloline, first isolated from *Festuca arundinacea* Schreb. (Poaceae) (Robbins, Sweeny, Wilkinson, & Burdick, 1972). The presence of a mixture of two rotational isomers (Fig. 1), detected by differing shifts and a different signal size in the ^{1}H NMR and ^{13}C NMR spectra, has been observed by the same authors, too.

With the exception of the results of the NOE experiments the spectral data have already been published in detail (Petroski, Yates, Weisleder, & Powell, 1989). In the rotational isomer **1a** (65% of the mixture) NOE interactions could be observed between the formyl proton and H-8, H-2 and H-1, but no NOE effect between C*H*O and N–CH_3 could be detected. Thus the formyl proton and the methyl group had to possess *trans*-configuration. On the other hand rotamer **1b** (35% of the mixture) showed an NOE effect between the formyl proton and N–CH_3, indicating a *cis*-configuration. NOE interactions could also be observed between H-7/H-8, H-1/H-8 and H-1/H-7 for both rotamers and were in agreement with the configurations at C-1 and C-8 as given in Fig. 1. *N*-Formylloline from *A. mollis* showed a specific optical rotation of +43.4°. A very similar result was obtained for *N*-formylloline from *Lolium cuneatum* (Poaceae) (Batirov, Khamidkhodzhaev, Malikov, & Yunusov, 1976). Therefore our compound should also have the same absolute configuration as loline isolated from *F. arundinacea* Schreb. (Bates & Morehead, 1972; Knoch, Wiedenfeld, & Roeder, 1993).

GC–MS analysis of the vegetative parts of *A. mollis* showed that *N*-formylloline is accompanied by three further 1-aminopyrrolizidine alkaloids (Table 1). The alkaloids are present in plant materials of different provenances collected in the wild in Indonesia as well as in greenhouse plants regrown from seeds. The presence of 1-aminopyrrolizidine alkaloids in a member of the Convolvulaceae is surprising since these rare structures are only known from two other sources (Powell, 1992; Porter, 1994; Hartmann, 1995). Lolines are found in certain grasses such as *Festuca arundinacea* and *F. pratensis* (Poaceae). Most interestingly, the alkaloids are only detectable in grass specimens which are associated with endophytic fungi of the genus *Acremonium* (Clavicipitaceae). The second source is the fabaceous genus *Adenocarpus*.

Within the Convolvulaceae the occurrence of lolines in *Argyreia mollis* is rather isolated. Lolines are absent from *A. capitata*, *A. hookeri*, *A. nervosa* and numerous other species so far analysed belonging to the genera *Aniseia*, *Bonamia*, *Calystegia*, *Convolvulus*, *Falkia*, *Hewittia*, *Ipomoea*, *Iseia*, *Jacquemontia*, *Merremia*, *Odonellia*, *Operculina*, *Stictocardia* and *Turbina* (Jenett-Siems, 1996; Henrici, 1996; Mann, 1997;

Table 1
Alkaloids identified in *Argyreia mollis* by GC–MS

Compound	RI	$[M]^{+}$ (m/z)	Aerial vegetative parts[a]	Roots[a]
Hygrine	1060	141	+	+
Tropan-3β-ol (=pseudotropine)	1185	141	+	+
Loline	1252	154	+[b]	+
N-Methylloline	1275	168	+[bc]	+
Nicotine	1312	162	+[bc]	–
2′,4-*N*-Methylpyrrolidinylhygrine	1570	224	–	+
2′,3-*N*-Methylpyrrolidinylhygrine	1580	224	–	+
N-Formylloline	1600	182	+[bc]	+
Cuscohygrine	1650	224	+	+
N-Propionylnorloline (decorticasine)	1660	196	+	–

[a] Plant material obtained from the greenhouse.
[b] Also detected in leaves collected near Surabaya, Java (Indonesia).
[c] Also detected in leaves collected on Madura Island, Indonesia.

Tofern, in preparation; Schimming, in preparation). As already mentioned the lolines are 1-aminopyrrolizidine alkaloids and thus should be distinguished from the 1-hydroxymethylpyrrolizidine alkaloids discovered in other convolvulaceous species, e. g. the platynecine diesters of *Ipomoea hederifolia* L. (Jenett-Siems, Kaloga, & Eich, 1993; Jenett-Siems et al., 1998) and the lycopsamine-type compounds of *Merremia quinquefolia* (L.) Hall. f. (Mann, 1997).

In endophyte infected grasses the lolines often occur together with ergoline alkaloids (i. e. clavines and lysergic acid amides). These alkaloids which are produced by the fungal endophyte are responsible for the fescue toxicosis in cattle (Hemken & Bush, 1989). In this respect the abundance of clavines and lysergic acid amides in seeds of *Argyreia* species (see Introduction) is interesting. Although it seems unlikely that endophytic fungi are involved in loline and ergoline formation in *Argyreia*, we cannot exclude this possibility by sure.

The GC–MS analysis further showed that *A. mollis* is able to synthesize quite a few pyrrolidine derivatives including tropan-3β-ol (=pseudotropine) and nicotine (Table 1). In contrast to the lolines those types of alkaloids could also be observed in the aerial vegetative parts and in the roots of *A. capitata*, *A. hookeri* and *A. nervosa*. Their presence in the genus *Argyreia* is reported here for the first time and is quite in line with reports on the occurrence of hygrines, tropanes and nicotine in species of other convolvulaceous genera, e. g. *Convolvulus*, *Ipomoea and Merremia* (Jenett-Siems & Eich, 1994; Henrici, 1996).

3. Experimental

3.1. TLC

TLC was carried out on silica gel 60 F_{254} with $CHCl_3$–MeOH 25% aq. NH_3 (8:2:0.2) or EtOAc–*iso*-PrOH 25% aq. NH_3 (45:35:15) as solvent systems. Dragendorff's reagent or van Urk's reagent were used for the detection.

3.2. GC–MS

GC–MS spectra were obtained with a Carlo Erba 5160 GC equipped with a 30 m × 0.32 mm fused silica capillary column coated with the methyl silicone stationary phase DB-1. Carrier gas: He, 1 ml min^{-1}. Conditions during split injection: injector 250°, split 1:20, temperature program 70–300° at 6° min^{-1}. The capillary column was directly coupled to the quadrupole mass spectrometer Finnigan MAT 4515. The identities of the alkaloids were confirmed by comparing retention indices and mass spectra with those of authentic compounds (Witte, Müller, & Arfman, 1987; Witte, Rubiolo, Bicchi, & Hartman, 1993; Justus, Witte, & Hartmann, 1997).

3.3. Spectroscopic methods

EIMS, HRMS and FABMS spectra were obtained using Varian MAT CH_7A, Finnigan MAT 711 and Finnigan MAT CH_5DF spectrometers. All NMR spectra were recorded in CD_3OD on a Bruker AC 400 spectrometer, using TMS as internal standard.

3.4. Plant material

Aerial vegetative parts and roots of *A. capitata*, *A. nervosa* and *A. mollis* were obtained from plants cultivated in the greenhouse (Berlin). They were grown from seeds collected in the wild near Chiang Mai, Thailand (*A. capitata*), at Bogor, Java (*A. nervosa*) and on Madura Island, Indonesia (*A. mollis*). Leaves of *A. hookeri* collected in the wild near Purwodadi, Java and leaves of *A. mollis* collected on Madura Island and near Surabaya, Java were also analysed. Voucher specimens are deposited at the Institut für Pharmazie II (Pharmazeutische Biologie), Freie Universität Berlin, Germany.

3.5. Extraction of alkaloids

Ground dried plant material was extracted with MeOH for 24 h at room temp. After evaporation the residue was dissolved in 1% aq. tartaric acid and extracted with petrol, CH_2Cl_2 and EtOAc. The pH of the aqueous phase was adjusted to pH 9 with 25% aq. NH_3 and extracted with CH_2Cl_2–*iso*-PrOH (3:1) to obtain the crude basic fraction, which was analysed by TLC and GC–MS.

3.6. Isolation of *1*

Ground dried aerial vegetative parts from the greenhouse (350 g) were extracted as described above. The crude basic fraction contained one main alkaloid (**1**), which was isolated by column chromatography on silica gel 60, eluting with a $CHCl_3$–MeOH gradient, which contained 1% aq. NH_3. For the final purification the alkaloid (**1**) was dissolved in 0.6% aq. tartaric acid solution and applied to a small column with a strongly acidic cation exchange resin (Adsorbex®, SCX-columns, 400 mg). The column was washed with water, and the alkaloid (**1**) was eluted with 5% aq. NH_3.

3.7. N-Formylloline (*1*)

Yellow–brown oil, 21 mg. $[\alpha]_D^{20}$ +43.4° ($CHCl_3$, *c* 0.4), (Lit.: $[\alpha]_D^{20}$ +47.9°, $CHCl_3$, *c* 0.73; Batirov et al.,

1976). EIMS 70 eV, *m/z*: 182 $[M]^+$. (+)-FABMS *m/z*: 183 $[M+H]^+$. HRMS 80 eV, *m/z*: $[M]^+$ 182.1059 (calc. for $C_9H_{14}N_2O_2$; 182.1013).

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