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Spectral Assignments and Reference Data

NMR spectral assignments of a new chlorotryptamine alkaloid and its analogues from *Acacia confusa*

Malcolm S. Buchanan, Anthony R. Carroll, David Pass and Ronald J. Quinn*

Natural Product Discovery, Eskitis Institute, Griffith University, Nathan, Queensland 4111, Australia

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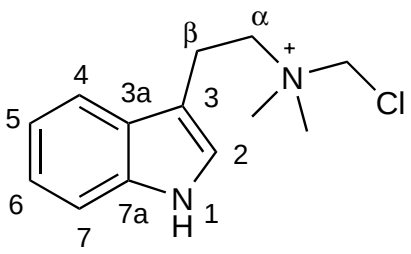
A new chlorotryptamine alkaloid, *N*-chloromethyl-*N*,*N*-dimethyltryptamine, was isolated from a methanol extract of the Chinese shrub *Acacia confusa* Merr., together with its known hallucinogenic analogues, *N*-methyltryptamine, *N*,*N*-dimethyltryptamine and *N*,*N*-dimethyltryptamine-*N*-oxide. The new compound was an artefact of the isolation conditions. The complete ^{1}H and ^{13}C NMR assignments for these compounds were carried out using ^{1}H, ^{13}C, DEPT, gCOSY, gHSQC and gHMBC NMR experiments. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H; ^{13}C; tryptamine alkaloids; *N*-chloromethyl-*N*,*N*-dimethyltryptamine; *Acacia confusa*



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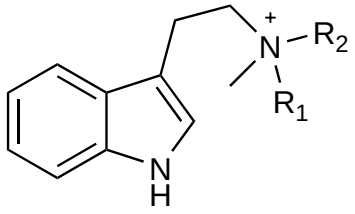

2 R_1 = H, R_2 = H
3 R_1 = H, R_2 = Me
4 R_1 = Me, R_2 = O^-

1

INTRODUCTION

Both serotonin (5-hydroxytryptamine) and tryptamine (in lower concentrations) are endogenous constituents of the CNS and possess important functions in central neurotransmission.[1] In our investigations on bioactive secondary metabolites from the shrub *Acacia confusa* Merr., several tryptamine alkaloids related to these were isolated. A new chlorotryptamine alkaloid, *N*-chloromethyl-*N*,*N*-dimethyltryptamine **(1)** was isolated together with its known analogues, *N*-methyltryptamine **(2)**,[2] *N*,*N*-dimethyltryptamine **(3)**[2] and *N*,*N*-dimethyltryptamine-*N*-oxide **(4)**.[3] Compounds **2–4** are among several tryptamine derivatives which are recognized hallucinogens.[4,5] The compounds studied here were isolated on a screening campaign to find inhibitors of Signal Transducer and Activator of Transcription 6 (STAT6) for the treatment of asthma.[6] However, although these compounds were active against STAT6 in this screen, they were also active on the artefact assay and thus did not progress any further. There have been a number of reports describing the constituents of *Acacia confusa*, including tryptamine alkaloids, which exhibit significant phytotoxicity.[7] As far as we know, a complete set of ^{1}H and ^{13}C NMR data for the known compounds **2–4**, has not been published previously; furthermore there have been no previously published NMR data for *N*,*N*-dimethyltryptamine-*N*-oxide **(4)** as far as we can see. In this paper, we report the full chemical shift assignments for tryptamines **1–4**.

RESULTS AND DISCUSSION

Acacia confusa Merr. was collected in the Zi Yuan County of Guang Xi Province, South West China and a MeOH extract prepared. The MeOH extract was then acidified before passing through strong acidic cation exchanger (SCX). The $MeOH/NH_3$ (4 : 1) and CH_2Cl_2 eluents were combined and passed through Polyamide Gel (PAG) and the resulting $MeOH/H_2O$ eluent gave an alkaloid extract, which was purified by C_{18} semi-preparative HPLC to yield the tryptamine alkaloids **1–4**.

Compound **1** was isolated in a yield of 0.007% dry weight. The LRESIMS revealed molecular ion peaks at $[M]^+$ (m/z 237) and $[M+2]^+$ (m/z 239) in a 3 : 1 ratio suggesting the presence of chlorine in the molecule. The HRESIMS revealed a molecular formula of $[C_{13}H_{18}ClN_2]^+[CF_3COO]^-$ {m/z 237.11535 $[C_{13}H_{18}ClN_2]^+$ (calcd 237.11530), $\Delta = +0.21$ ppm}. Compound **1** had a UV spectrum λ_{max} (MeOH) 290 sh, 280 and 217 nm, typical for an indole chromophore.[8] This was further confirmed by a broad strong IR absorption at 3412 cm^{-1}, characteristic of the N–H vibration in the indole moiety. The downfield region of the ^{1}H NMR spectrum (Table 1) showed the typical aromatic resonance pattern for 3-substituted indole alkaloids. Thus, there was the ABCD system of the aromatic ring {δ_H 7.61 (brd, J = 8.0 Hz), 7.04 (td, J = 8.0, 1.0 Hz), 7.12 (td, J = 8.0, 1.0 Hz), 7.39 (brd, J = 8.0 Hz)} and the signal at δ_H 7.28 (H-2), which showed a 2.4 Hz coupling to δ_H 11.03 (H-1). The side chain attached at C-3 of the indole unit consists of two methylene groups, two *N*-methyls, and a chloromethyl group. The methylene at δ_H 3.20 displayed gHMBC correlations to C-2 (δ_C 123.7), C-3 (δ_C 107.7) and C-3a (δ_C 126.5), showing that it was directly attached to the indole at C-3. It also had a gCOSY correlation to the methylene at δ_H 3.67, which had gHMBC correlations to the *N*-methyls (δ_C 48.9) and the chloromethyl (δ_C 68.2). Thus, the structure of **1** was established as *N*-chloromethyl-*N*,*N*-dimethyltryptamine. The crude MeOH extract of *Acacia confusa* Merr. was examined by MS and ^{1}H NMR. There were no observable ion peaks at m/z 237 and 239 in the MS, while the ^{1}H NMR showed no sign of the isolated chloromethyl signal at δ_H 5.48. This evidence supports the assumption that **1** is an artefact of the isolation conditions. Presumably CH_2Cl_2 underwent nucleophilic substitution with *N*,*N*-dimethyltryptamine to give **1**.

The structures of the known compounds *N*-methyltryptamine **(2)**, *N*,*N*-dimethyltryptamine **(3)** and *N*,*N*-dimethyltryptamine-*N*-oxide **(4)** were elucidated using ^{1}H, ^{13}C, DEPT, gCOSY, gHSQC and gHMBC NMR data, independently of previously published partial NMR data.[2,9] Tables 1 and 2 list a complete set of ^{1}H and ^{13}C NMR data for these compounds.

*Correspondence to: Ronald J. Quinn, Natural Product Discovery, Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia.
E-mail: r.quinn@griffith.edu.au

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Spectral Assignments and Reference Data

Table 1. ^{1}H (500 MHz) NMR data (δ_H, m, *J* Hz) for tryptamine alkaloids **1–4** in DMSO-d_6

Position	1	2	3	4
1	11.03 (brs)	10.98 (brs)	10.99 (brs)	11.02 (brs)
2	7.28 (d, 2.4)	7.23 (brd, 2.4)	7.25 (brd, 2.4)	7.26 (brd, 2.4)
4	7.61 (brd, 8.0)	7.57 (brd, 8.0)	7.60 (brd, 8.0)	7.62 (brd, 8.0)
5	7.04 (td, 8.0, 1.0)	7.01 (td, 8.0, 1.0)	7.02 (td, 8.0, 1.0)	7.02 (td, 8.0, 1.0)
6	7.12 (td, 8.0, 1.0)	7.10 (td, 8.0, 1.0)	7.10 (td, 8.0, 1.0)	7.11 (td, 8.0, 1.0)
7	7.39 (brd, 8.0)	7.37 (brd, 8.0)	7.37 (brd, 8.0)	7.38 (brd, 8.0)
β	3.20 (m, 2H)	3.03 (m, 2H)	3.09 (m, 2H)	3.27 (m, 2H)
α	3.67 (m, 2H)	3.18 (m, 2H)	3.34 (m, 2H)	3.89 (m, 2H)
NH	–	8.59 (m, 2H)	9.81 (m)	–
N–CH_2Cl	5.48 (s, 2H)	–	–	–
N-Me	3.26 (s, 6H)	2.61 (brd, 5.5)	2.87 (brd, 3.1)	3.54 (s, 6H)

Table 2. ^{13}C (125 MHz) NMR data (δ_C, m) for tryptamine alkaloids **1–4** in DMSO-d_6

Position	1	2	3	4
2	123.7 CH	123.4CH	123.3 CH	123.5 CH
3	107.7 C	109.1C	108.7 C	108.0 C
4	118.1 CH	118.1CH	118.1 CH	118.2 CH
5	118.6 CH	118.5CH	118.4 CH	118.5 CH
6	121.3 CH	121.2CH	121.2 CH	121.3 CH
7	111.6 CH	111.5CH	111.5 CH	111.6 CH
7a	136.2 C	136.3C	136.3 C	136.3 C
3a	126.5 C	126.7C	126.6 C	126.7 C
β	18.2 CH_2	21.6CH_2	20.1 CH_2	18.6 CH_2
α	62.2 CH_2	48.6CH_2	56.8 CH_2	68.1 CH_2
N–CH_2Cl	68.2 CH_2	–	–	–
N–Me	48.9 CH_3	32.5 CH_3	42.2 CH_3	55.5 CH_3
	(2C)	–	(2C)	(2C)

CONCLUSIONS

A new chlorotryptamine alkaloid, *N*-chloromethyl-*N*,*N*-dimethyl tryptamine **(1)**, together with its known analogues, *N*-methyltryptamine **(2)**, *N*,*N*-dimethyltryptamine **(3)** and *N*,*N*-dimethyltryptamine-*N*-oxide **(4)** were isolated from *Acacia confusa* Merr. Tables 1 and 2 provide a complete set of ^{1}H and ^{13}C NMR data for these biologically significant compounds.

EXPERIMENTAL

General experimental procedures

Water was Millipore Milli-Q PF filtered, while all other solvents used were Lab-Scan HPLC grade. Trifluoroacetic acid (TFA) was Fluka spectroscopic grade. Ammonia solution (about 25% NH_3, sp. gr. 0.91) was from Merck. A Betasil C_{18} 5 µm (150 × 21.2 mm i.d.) was used for semi-preparative HPLC. Waters 600 pump fitted with a 996 Photodiode Array Detector and 717 plus Autosampler was used for the semi-preparative separations. SCX was DOWEX 50WX8-400A, C_{18} was 04K-4348 Sepra C_{18} End-Capped Silica, PAG was Machery Nagel Polyamide CC6 (0.05–0.16 mm). HRESIMS were measured on a Mariner TOF mass spectrometer equipped with an electrospray ion source (TurboIonSpray). FTIR and UV spectra were recorded on a Bruker Tensor 27 FTIR spectrophotometer and an Agilent 8453 UV/vis spectrophotometer, respectively.

Plant material

The plant sample *Acacia confusa* Merr. [Kingdom-Plantae, Phylum-Spermatophyta, Class-Magnoliopsida, Order-Fabales, Family-Fabaceae] was collected on the 17th June 1999 from Zi Yuan county in Guang Xi Province, China and a voucher sample (7-JUL-1999 : 02:16_76) is lodged at the Zi Yuan Medicine company, China.

Extraction and isolation

The plant material (214 g) was ground and extracted with methanol. The methanol crude extract was then acidified with 1 M formic acid (pH 4) and put through SCX, and sequentially eluted with MeOH followed by MeOH/NH_3 (4 : 1) and finally CH_2Cl_2. The MeOH/NH_3 and CH_2Cl_2 eluents were combined and put through PAG, eluting with MeOH/H_2O to give 1.07 g of alkaloid extract. Next, 500 mg of alkaloid extract was purified by semi-preparative C_{18} HPLC. The sample was pre-absorbed on C_{18} and loaded into a refillable preparative guard column (10 × 30 mm i.d.) in line with the semi-preparative C_{18} HPLC column. The following solvent conditions were used: H_2O/1% TFA to H_2O/1% TFA: CH_3CN/1% TFA (2 : 3) in 100 min, then to CH_3CN/1% TFA in 20 min; 60 fractions collected. Four compounds were collected, *N*-chloromethyl-*N*,*N*-dimethyltryptamine **(1)** (15.26 mg, 0.007% dry wt), *N*-methyltryptamine **(2)** (11.90 mg, 0.006% dry wt), *N*,*N*-dimethyltryptamine **(3)** (11.38 mg, 0.005% dry wt) and *N*,*N*-dimethyltryptamine-*N*-oxide **(4)** (20.02 mg, 0.009% dry wt), with retention times of 60, 48, 52 and 54 min, respectively. Compounds **1–3** were isolated as their trifluoroacetate salts.

N-chloromethyl-*N*,*N*-dimethyltryptamine **(1)** was isolated as an amorphous solid (15.26 mg, 0.007% dry wt); UV (MeOH) λ_{max} (log ε) 290sh (3.37), 280 (3.44), 217 (4.15) nm; IR ν_{max} (film) 3412, 1680, 1202, 1131, 746 cm^{-1}; ^{1}H: refer Table 1; ^{13}C NMR refer Table 2; positive-HRESIMS *m*/*z* 237.11535 $[C_{13}H_{18}ClN_2]^+$ (calcd 237.11530), Δ + 0.21 ppm.

Nuclear magnetic resonance spectroscopy

NMR spectra (^{1}H, ^{13}C, DEPT, gCOSY, gHSQC and gHMBC) were recorded at 30 °C on a Varian INOVA 500 MHz spectrometer, equipped with a switchcable 5 mm inverse detection z-gradient probe. The ^{1}H and ^{13}C chemical shifts were referenced to the residual solvent peak of DMSO-d_6 at δ 2.50 and 39.5 ppm, respectively. The samples were placed in 5 mm tubes using 0.5 ml of DMSO-d_6. The ^{1}H sweep width was set at 6290 Hz for all experiments with a 90° pulse for ^{1}H of 10.7 µs and a ^{13}C 90° pulse of 5.7 µs. The gCOSY was acquired with 128 F1 increments with 16 scans per increment. A sinebell weighting was applied to each dimension and zero filled to 2 k points. The gHSQC was acquired with ^{13}C sweep width of 20 111 Hz and 128 F1 increments. Each increment was acquired with 16 transients. A Gaussian weighting was applied to both the F1 and F2 dimensions before zero filling to 2 k points and then Fourier transformation. A one-bond coupling constant delay was set using 140 Hz and ^{13}C decoupling was applied during acquisition. The gHMBC was acquired using 32 transients per increment with 400 F1 increments. A sweep width of 27 653 Hz was used for the ^{13}C dimension. The one-bond coupling constant of 140 Hz and long range coupling constant of 8 Hz were used to set the delays in the

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pulse sequence. A sinebell weighting was applied to both ^{1}H and ^{13}C dimensions and zero filled to 2 and 2 k, respectively.

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