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A chlorinated amide and piperidine alkaloids from *Aloe sabaea*

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Abstract

Phytochemical investigations of *Aloe sabaea* afforded a new chlorinated amide, *N*-4′-chlorobutylbutyramide, and the toxic piperidine alkaloids coniine, γ-coniceine and the quarternary *N*,*N*-dimethylconiine. This is the first report of the occurrence of a chlorinated compound in the Aloeaceae family. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Aloe sabaea*; Aloeaceae; *N*-4′-chlorobutylbutyramide; Piperidine alkaloids

1. Introduction

Aloe species (Aloeaceae) have long been known as medicinal plants (Cheney, 1970). *Aloe* exudates were also used as arrow poison and in meat baits to kill animals in the African continent (Neuwinger, 1998). Investigations of approximately 300 *Aloe* species native to Africa and Arabia have shown that six of them contain toxic hemlock alkaloids such as coniine, γ-coniceine and conhydrine (Dring et al., 1984; Nash et al., 1992). The presence of the toxic piperidine alkaloids in *Aloe* species readily available for potential medical use by local inhabitants sounds a note of caution against the ignorant use of these plants.

Herein, we report the isolation and structural elucidation of a new chlorinated natural product named *N*-4′-chlorobutyl-butyramide (**1**) from *Aloe sabaea*, which has not been investigated phytochemically before. Furthermore, the piperidine alkaloids coniine, γ-coniceine and the hitherto novel *N*,*N*-dimethylconiine (**2**), were also identified.

2. Results and discussion

The 95% MeOH extract of dried and powdered leaves was partitioned successively with *n*-hexane, EtOAc and $CHCl_3$/EtOH (2:1). The $CHCl_3$/EtOH extract was concentrated and subjected to a Sephadex LH-20 column followed by reverse phase column chromatography to yield compounds **1** and **2** as well as coniine and γ-coniceine.

N-4′-Chlorobutylbutyramide (**1**), detected by LC-electrospray(ESI)-MS in the crude 95% MeOH extract, was isolated as a colourless oil. Both the $[M+H]^+$ ion in the ESI mass spectrum at *m*/*z* 178/180 and the $[M]^+$ ion in the EIMS at *m*/*z* 177/179 show an abundance ratio of about 3:1 indicating the presence of one chlorine atom in the molecule. The molecular formula was determined as $C_8H_{16}NOCl$ from HR-EIMS. The mass spectral fragmentation of **1** is mainly characterized by C–C-cleavages in the carbon chain (ions at *m*/*z* 128, 114, 100) also displaying the position of the chlorine atom (Fig. 1). Thus, the key ions at *m*/*z* 149 and 87, originating from a McLafferty rearrangement, present evidence for both an amide bond and the butyr moiety. Furthermore, the ion pair at *m*/*z* 91/93 being complementary to *m*/*z* 87 also displays the chlorine isotopic pattern. The key ion at *m*/*z* 91, also occurring in the collision induced dissociation (CID) mass spectrum of the $[M+H]^+$ ion (*m*/*z* 178) under ESI conditions, is shifted to *m*/*z* 93 in the corresponding CID-MS of the ^{37}Cl-containing ion at *m*/*z* 180. The $^{13}C\{^1H\}$ and the ^{13}C APT NMR spectra showed signals of one methyl, six methylenes and one quaternary carbon of the carbamide function. The ^{1}H NMR spectrum showed signals of four triplets and two multiplets. The CH_2–Cl protons exhibit the most downfield resonances at δ 3.58 followed by CH_2 protons neighbouring the NH-group at δ 3.19. The ^{1}H and ^{13}C

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NMR assignment of **1** was attained by combined use of homo- and heteronuclear shift correlation experiments, including APT, ^{1}H- ^{1}H-COSY, HSQC and HMBC and confirmed the structure of **1**. Thus, in the HMBC spectrum all of the 19 possible correlations via $^2J_{CH}$ and $^3J_{CH}$ were observed.

This is the first report of the occurrence of a chlorinated compound in the Aloeaceae family. About 750 chlorinated natural constituents from 275 species are described in the literature until 1992 (Naumann, 1993). A series of chlorinated compounds could also be found in higher plants (Engvild, 1986). However, in most of the described natural constituents, chlorine is bound to a sp^2-carbon, preferably at aromatic ring systems. It is known that fungi and plants can incorporate bioavailable chloride into low molecular weight compounds (Engvild, 1986; Neidelman and Geigert, 1986).

Besides the piperidine alkaloids coniine and γ-coniceine already characterized by ^{1}H, ^{13}C NMR and EIMS (Asensio et al., 1993; Moody et al., 1997) the quarternary *N,N*-dimethylconiine (**2**) was also isolated. The ^{1}H NMR spectrum of **2** showed one methyl triplet (δ 1.01, CH_3-9) and two methyl singlets (δ 2.96, CH_3–10, and δ 3.16, CH_3–11). The ^{13}C chemical shifts of CH_3–10 and CH_3–11 (δ 42.8 and 54.1, respectively, from the HSQC spectrum) and the HMBC correlations of the ^{1}H signals of both methyl groups with C–2 (δ 73.7) and C–6 (δ 66.9) as well as with the ^{13}C signals of CH_3–11 and CH_3–10, respectively, clearly indicated the dimethyl immonium structure of coniine. The structural elucidation was completed by the combined use of ^{1}H, ^{1}H-COSY, HSQC and HMBC experiments. The *equatorial* position of the side chain as well as the assignment of *axial* and *equatorial* oriented *N*-methyl groups and ring methylene protons, respectively, was deduced from the NOESY spectrum. Thus, *N*–Me–10 showed NOESY cross peaks with H–3$_{ax}$, H-5$_{ax}$ and H–6$_{eq}$, whereas *N*–Me–11 showed NOESY correlation swith H–2$_{ax}$, H–6$_{ax}$ and H–6$_{eq}$. The ^{13}C chemical shifts of *N*–Me–10$_{ax}$ and *N*–Me–11$_{eq}$ are in good agreement with those reported for gentrymine B (Hallock et al. 1995). The ^{1}H and ^{13}C NMR data of 2 are summarized in Table 1.

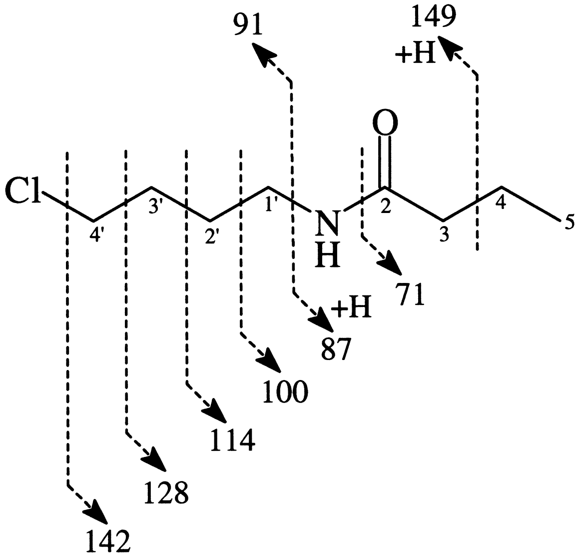
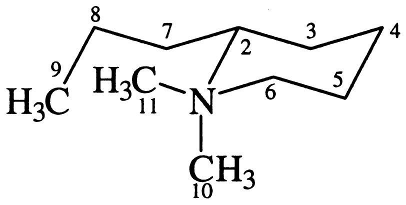


Fig. 1. Mass spectral fragmentation of *N*-4′-chlorobutylbutyramide (**1**).

The identity of all piperidine alkaloids were confirmed by positive ion ESI-MS and ESI-MS/MS. Generally, the ESI mass spectra display an ion of type $[M+H]^+$. The collision induced dissociation (CID) mass spectra of the $[M+H]^+$-ion show a characteristic fragmentation pattern. Fragments at *m*/*z* 69, 55 and 41 reflect parts of the basic skeleton in coniine and compound **2**. The detected mass shift of the key fragment **a** is an indication of the position of the additional CH_2-unit at the nitrogen (Fig. 2).

N,N-Dimethylconiine (**2**) has been described as a synthetic compound (Cocker et al., 1984), but it was firstly isolated as a naturally occurring constituent to our knowledge. Quarternary *N,N*-dimethyl alkaloids are often described as naturally occurring compounds (Domínguez et al., 1974; Kametani, 1974; Marsaioli et al., 1979; Hallock et al., 1995; Barbosa-Filho et al., 1997). The detection of toxic piperidine alkaloids in *A. sabaea* again indicate that caution must be exercised in the use of this plant species.



N,N-dimethylconiine (**2**)

Table 1
^{13}C chemical shifts (75.50 MHz, CD_3OD) and ^{1}H NMR data (500 MHz, CD_3OD) of compound **2**

	^{13}C	^{1}H
2	73.7	3.28[a] *dddd* (11.1/10.4/2.8/2.8)
3	26.6	1.67[a] *m*/2.04[b] *br d* (15.4)
4	22.9	1.58[a] *dtt* (12.9/12.8/4.0)/1.88[b] *br d* (14.0)
5	21.5	1.96[a,c]/1.82[b] *br d* (15.2)
6	66.9	3.38[a] *ddd* (12.9/12.8/3.3)/3.51[b] *dddd* (12.8/3.8/2.8/1.8)
7	31.8	1.96[c]/1.43 *m*
8	20.8	1.51 *m*/1.35 *m*
9	14.1	1.01 *t* (7.2)
10	42.8	2.96[a] *s*
11	54.1	3.16[b] *s*

[a] *Axial.*
[b] *Equatorial.*
[c] Chemical shift of HSQC correlation peak.

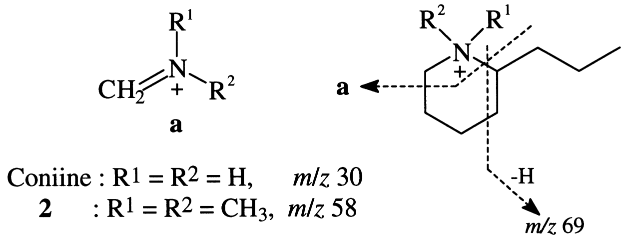


Fig. 2. Main fragmentation of the $[M+H]^+$ ion of coniine and compound **2**.

Possibly, a biosynthetic relation between γ-coniceine and *N*-4′-chlorobutyl-butyramide (**1**) exists. In both molecules the nitrogen is attached to two C-4 carbon units. A theoretically biosynthetic pathway might start with a hydroxylation at the double bond. After opening of the six-membered ring and oxidation of the hydroxyl the chlorine can be inserted (Fig. 3).

Interestingly, leaves of *A. sabaea* can accumulate remarkable amounts of potassium chloride representing probably both the chlorine source for *N*-4′-chlorobutyl-butyramide (**1**) as well as the counterion of the hitherto novel *N,N*-dimethylconiine (**2**).

3. Experimental

3.1. General experimental procedures

^1H and 2D NMR spectra: Varian UNITY 500, 499.82 MHz, solvent CD_3OD; $^{13}C\{^1H\}$ and ^{13}C APT spectra: Varian GEMINI2000 300BB, 75.50 MHz, solvent CD_3OD; chemical shifts were referenced to internal TMS (δ 0, ^1H) and CD_3OD (δ 49.0, ^{13}C), respectively; HR-EIMS (resolution 7000) and EIMS (DIS): 70 eV, AMD 402, AMD Intectra. GCMS: MD-800 (Fisons Instruments), EI 70 eV; source temp. 200°C; column DB-5MS (J&W, 15 m × 0.32 mm, 0.25 μm film thickness), injection temp. 260°C, interface temp. 300°C, carrier gas He, flow rate 1.3 ml/min, splitless injection, column temp. program: 170°C for 1 min, then raised to 290°C at a rate of 30 grd/min and held on this temp. for 20 min; positive ion electrospray (ESI) mass spectra and the ESI-MS/MS: TSQ 7000 (Finnigan, electrospray voltage 4.5 kV), heated capillary temp. 220°C; sheath gas: nitrogen) coupled with a Micro-Tech Ultra-Plus MicroLC system, equipped with a RP18-column (5 μm, 1×100 mm, SEPSERV), HPLC conditions: gradient system from H_2O:CH_3CN 4:1 (each contained 0.2% HOAc) to 1:9 within 15 min, then held at this value for 10 min; flow rate 70 μl min^{-1}. The collision-induced dissociation (CID) mass spectra during the HPLC run were performed with a collision energy of—25 eV; collision gas: argon, collision pressure: 1.8×10^{-3} Torr. All mass spectra are averaged and background substracted. $[\alpha]_D$: JASCO DIP 1000 polarimeter. CC: reverse silica gel RP-18 (Merck, C_{18}; 40–63 μm) and Sephadex LH 20 (Fluka); TLC: percoated silica plates gel 60 F_{254}, 20 × 20 cm, thickness 1 mm (Merck).

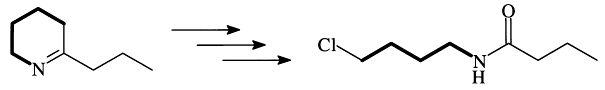


Fig. 3. Putative biosynthetic pathway of *N*-4′-chlorobutylbutyramide.

3.2. Plant material

Leaves of *Aloe sabaea* were collected near Taiz'z, Yemen, in August 1997. The plant material was identified and deposited by Prof. A. K. Nasher in the Department of Biology, Faculty of Science, Sanáa University, Sanáa, Republic of Yemen.

3.3. Extraction and purification of the alkaloids

A mass of 271 g of dried and powdered leaves were extracted 3× with aq. MeOH (MeOH/water 95:5; 600 ml). After solvent evaporation the residue (26 g) was diluted in H_2O and extracted with *n*-heptane (5×100 ml) and EtOAc (5×100 ml) followed by an extraction with $CHCl_3$/EtOH (2:1, 5×100 ml). The combined and evaporated $CHCl_3$/EtOH extract yielded 3.6 g of a dark syrup. A part (2.1 g) was chromatographed on Sephadex LH-20 with MeOH (750 ml) yielding a crude basic extract (1.4 g), a part of which (1 g) was rechromatographed twice on reversed silica gel with water/MeOH (98:2).

3.4. N-4′-Chlorobutylbutyramide (*1*)

Fifteen milligrams (0.055%) of a colourless oil; ^1H NMR (500 MHz, CD_3OD): δ 0.93 (*t*, 3H, *J* = 7.4 Hz, 5-CH_3), 1.62 (*m*, 2H, 4-CH_2), 1.63 (*m*, 2H, 2′-CH_2), 1.78 (*m*, 2H, 3′-CH_2), 2.15 (*t*, 2H, *J* = 7.4 Hz, 3-CH_2), 3.19 (*t*, 2H, *J* = 7.0 Hz, 1′-CH_2), 3.58 (*t*, 2H, *J* = 6.6 Hz, 4′-CH_2); ^{13}C NMR (75.50 MHz, CD_3OD): δ 13.9 (C-5), 20.4 (C-4), 27.8 (C-2′), 31.1 (C-3′), 39.0 (C-3), 39.5 (C-1′), 45.3 (C-4′), 176.2 (C-2); EIMS (GC–MS) 70 eV, *m*/*z* (rel. int.): 179 (1.2), 177 $[M]^+$ (4), 162 (6), 149 ($[M-C_2H_4]^+$, 30), 142 ($[M-Cl]^+$, 30), 128 ($[M-CH_2Cl]^+$, 22), 114 ($[M-CH_2CH_2Cl]^+$, 37), 100 ($[M-CH_2CH_2CH_2Cl]^+$, 35), 93 (6), 91 ($C_4H_8Cl^+$, 17), 87 (15), 71 ($C_3H_7CO^+$, 75), 58 (20), 55 (59), 43 (100); HR-EIMS (probe): 177.0914 (calc. for $C_8H_{16}NO^{35}Cl$ 177.0920), 149.0621 (calc. for $C_6H_{12}NO^{35}Cl$ 149.0606), 100.0769 (calc. for $C_5H_{10}NO$ 100.0763); LC-ESI-MS: RT = 12.45 min, ESI-MS, *m*/*z* (rel. int.): 180 (33), 178 $[M+H]^+$ (100); ESI-CIDMS of *m*/*z* 178: *m*/*z* (rel. int.): 178 $[M+H]^+$ (5), 91 (22), 88 (17), 71 (10), 55 (100), 43 (24).

3.5. Coniine

Forty-eight milligrams (0.017%); ESI-MS *m/z* (rel. int.): 128 $[M+H]^+$ (100); ESI-CIDMS *m/z* (rel. int.): 69 (100), 55 (61), 41 (47), 30 (a, 23).

3.6. γ-Coniceine

Sixty-seven milligrams (0.025%); ESI-MS *m/z* (rel. int.): 126 $[M+H]^+$ (100); ESI-CIDMS *m/z* (rel. int.): 98 $[M+H\text{-}C_2H_4]^+$ (63), 97 (100), 96 (50), 84 (11), 70 (14), 56 (21), 55 (16).

3.7. N,N-Dimethylconiine (**2**)

Twenty-six milligrams (0.0094%); $[\alpha]_D^{24.5}$ + 7.5° (MeOH; c 0.1); ESI-MS *m/z* (rel. int.): 156 $[M]^+$ (100); ESI-CIDMS *m/z* (rel. int.): 69 (100), 58 (**a**, 90), 55 (48), 46 (23), 41 (19). ^1H and ^{13}C NMR data see Table 1.

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