Unusual Tropane Alkaloid Pattern in Two African Convolvulaceous Species. Phytochemistry and Chemotaxonomy of the Convolvulaceae, Part 20 [1]

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An unusual and complex tropane alkaloid pattern has been detected in the root bark of *Astripomoea malvacea* and in the whole plant of *Falkia repens* (Convolvulaceae) by GC-MS analysis. The specific profile of both species is characterized by the presence of aliphatic 3-acyloxytropanes/-nortropanes (exclusively in *A. malvacea*; predominantly in *F. repens* in co-occurrence with a few aromatic as well as arylalkyl acyl congeners). The principal alkaloid of *A. malvacea*, astrimalvine A N-oxide [3β-(3-tigloyloxy-2-methylbutyryloxy)tropane *N*-oxide], isolated and structurally elucidated by detailed spectroscopic analysis, represents the first *N*-oxide of a 3β-tropanol derivative in the Convolvulaceae. Its minor tertiary congener astrimalvine B [3β-(3-hydroxy-2-methylbutyryloxy)tropane] turned out to be a metabolite of both convolvulaceaous species. This is the first phytochemical report on the African genera *Astripomoea* and *Falkia*.

Key words: Astripomoea malvacea, *Falkia repens*, Convolvulaceae, 3-Tropanol Esters, Astrimalvine A *N*-Oxide

Introduction

The Convolvulaceae family shows as a clear characteristic the ability to synthesize a wide variety of ornithine derived alkaloids, *e. g.* pyrrolidines, pyrrolizidines, and tropanes. Esters of 3α- and 3β-tropanol, respectively, have been isolated from members of the related genera *Convolvulus* and *Merremia* [2 and ref. therein], [3].

Astripomoea malvacea (Klotzsch) Meeuse and *Falkia repens* L. f. are two convolvulaceous species both native to certain coastal zones of southern Africa [4]. *F. repens* is a small, prostrate, often matted, perennial herb, whereas *A. malvacea*, also widespread from western tropical Africa to eastern Africa, is a perennial subshrub. Both genera have not been investigated yet phytochemically apart from the detection of calystegins, hydrophilic nor-tropane derivatives in *F. repens* [5].

Results and Discussion

The GC-MS analysis of the alkaloid fraction of the root bark of *A. malvacea* revealed the presence of several tropanes. The major compound was isolated by HPLC. Using (+)-FAB MS measurements, the molecular mass of **1** (Fig. 1) could be determined as 340 $[M+H]^+$, whereas the EI MS yielded a molecular ion peak at *m/z* = 323, corresponding to a molecular formula of $C_{18}H_{29}NO_5$ (HR MS). These observations hinted to an *N*-oxide. This idea was supported by the ^{1}H NMR and ^{13}C NMR spectra (Table 1) which showed typical signals for a 3-tropanol moiety with suspicious downfield shifts of C-1/C-5 (δ_H = 4.38; δ_C = 73.4) and the *N*-methyl signals (δ_H = 3.64; δ_C = 53.0) [6]. The quintet-like multiplet at δ = 5.14 pointed to a 3β-tropanol derivative. One acyl moiety showed two methyl doublets and two additional signals at δ = 2.72 (1H, quint, J = 7.0 Hz) and δ = 5.11 (1H, m) and

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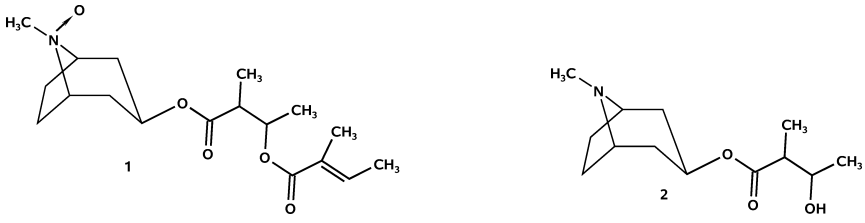

Fig. 1. Alkaloids isolated from the root bark of *A. malvacea*: Astrimalvine A *N*-oxide (**1**), astrimalvine B (**2**).

Table 1. ^{1}H NMR and ^{13}C NMR data of **1** and **2** in CD_3OD. (Chemical shifts are reported in ppm relative to TMS. *J*-Values are given in parentheses in Hz).

	1		**2**	
	δ_H[a]	δ_C	δ_H	δ_C
1	4.38 br s	73.4	3.62 br *s*	63.2
2	2.03 m	32.0	2.11 m	36.2
	2.25 m		1.88 br t (12.2)	
3	5.14 m	64.0	5.10 m	66.4
4	2.03 m	32.0	2.11 m	36.2
	2.25 m		1.88 br t (12.2)	
5	4.38 br s	73.4	3.62 br *s*	63.2
6	2.25 m	24.6	2.23 m	26.1
			1.93 d (8.0)	
7	2.25 m	24.6	2.23 m	26.1
			1.93 d (8.0)	
N-CH_3	3.64 s	53.0	2.57 s	38.8
1′		174.6		176.2
2′	2.72 quint (7.0)	46.3	2.41 quint (7.0)	33.8
3′	5.11 m	73.0	3.86 quint (7.0)	70.3
4′	1.26 d (7.0)	17.5	1.15 d (7.0)	20.5
5′	1.17 d (7.0)	13.4	1.07 d (7.0)	13.5
1″		168.4		
2″		129.7		
3″	6.83 q (6.5)	139.0		
4″	1.79 d (6.5)	12.1		
5″	1.80 br s	14.4		

[a] In $CDCl_3$.

was thus identified as a 3-hydroxy-2-methylbutyryl entity, whose stereochemistry remains to be determined. In addition, characteristic signals for a tigloyl moiety were observed [7] which had to be esterified to the 3-hydroxy-2-methylbutyryl group. Further confirmation of the structure including the linkage of the ester groups was achieved by HMBC. Thus, compound **1** which we have named astrimalvine A *N*-oxide, had to be 3β-(3-tigloyloxy-2-methylbutyryloxy)tropane *N*-oxide.

A second tertiary amine was obtained by reduction of the aqueous phase with Zn/HCl. Compound **2**, isolated by prep. TLC (Fig. 1), displayed a molecular ion peak at *m/z* = 241 in the EI MS spectrum, corresponding to a molecular formula of $C_{13}H_{23}NO_3$ (HR MS). ^{1}H and ^{13}C NMR spectra (Table 1) were similar to those of **1** revealing the presence of a 3β-tropanol moiety esterified to 3-hydroxy-2-methylbutyric acid, whereas no tigloyl residue was observed. We have named compound **2** astrimalvine B. Interestingly, the acyl components of astrimalvine A and B are similar to those occurring as constituents of certain ipangulines, unique pyrrolizidine alkaloids of the *Ipomoea* section Mina [8].

The GC-MS analysis revealed that astrimalvine A (RI 2152) is the dominating compound of the basic fraction of *A. malvacea*, minor constituents are astrimalvine B (RI 1707), nor-astrimalvine A (RI 2145), and a 3-tropanol ester with a molecular ion peak at *m/z* = 325, probably bearing a 2-methylbutyryl residue instead of the tigloyl residue of astrimalvine A (RI 2082). Presumably, all these alkaloids are occurring at least in part as *N*-oxides in the plant. Interestingly, no simple congeners like tropan-3-one or 3α/3β-tropanol were detected by GC-MS.

In contrast, the GC-MS analysis of the alkaloid fraction of *F. repens* (Table 2) revealed the occurrence of tropan-3-one, 3α/3β-tropanol and their nor-derivatives, as well as several esters of 3α- and 3β-tropanol with simple aliphatic acids, among them even astrimalvine B. In addition, contrary to *A. malvacea*, *F. repens* is able to synthesize tropanol esters with substituted benzoic and cinnamic acid derivatives as acyl residues. Another difference is the co-occurrence of nearly equal amounts of esters of both epimeric forms (3α/3β) in *F. repens* and the fact that *A. malvacea* yielded a dominant 3β-tropanol-type alkaloid. Both findings are unique for the bindweed family, since tropane-positive convolvulaceous species are usually characterized by the dominance of 3α-type forms *e. g.* [2 and ref. therein], [3].

Experimental Section

General procedures

Preparative high performance liquid chromatography (HPLC) separation was performed on a Knauer pumping system with a Knauer variable wavelength detector (225 nm) equipped with a Knauer Eurospher C-18 column (10 μm, 22 × 250 mm). CD spectra were recorded on a Jasco J-810

Table 2. GC-MS data of tropane alkaloids detected in *Falkia repens*.

Compound	Plant organ	RI	*m/z* (%)
Cyclotropine	herb, roots	1137	139 (100), 110 (75), 108 (51), 96 (94), 82 (36), 68 (88)
3α-Nortropanol	roots	1150	127 (37), 110 (38), 99 (41), 69 (41), 68 (100)
Tropan-3-one	herb, roots	1153	139 (20), 111 (5), 110 (9), 97 (12), 96 (31), 83 (44), 82 (100), 68 (10)
3β-Nortropanol	roots	1160	127 (20), 110 (58), 99 (40), 69 (49), 68 (100)
3α-Tropanol	roots	1167	141 (32), 124 (33), 113 (19), 112 (14), 97 (21), 96 (78), 83 (71), 82 (100)
3β-Tropanol	herb, roots	1183	141 (40), 124 (40), 113 (18), 112 (13), 97 (23), 96 (62), 83 (69), 82 (100)
3α-Acetoxynortropane	herb, roots	1290	169 (12), 111(18), 110 (100), 108 (18), 82 (36), 68 (59)
3α-Acetoxytropane	herb, roots	1307	183 (18), 140 (9), 124 (100), 96 (19), 95 (11), 94 (25), 83 (33), 82 (47)
3β-Acetoxytropane	herb	1317	183 (14), 140 (6), 124 (100), 96 (22), 95 (15), 94 (22), 83 (43), 82 (65)
3α-Isobutyryloxytropane	roots	1454	211 (51), 140 (33), 124 (100), 96 (37), 95 (33), 94 (67), 83 (36), 82 (68)
3α-(2-Methylbutyryloxy)tropane	herb, roots	1546	225 (12), 210 (3), 208 (5), 196 (2), 140 (17), 124 (100), 96 (29), 95 (32), 94 (24), 83 (67), 82 (58), 57 (76)
3β-(2-Methylbutyryloxy)tropane	herb, roots	1552	225 (15), 210 (2), 208 (6), 196 (1), 140 (9), 124 (100), 96 (14), 95 (18), 94 (27), 83 (40), 82 (54), 57 (27)
3β-Isovaleroyloxytropane	herb	1564	225 (28), 210 (2), 140 (5), 124 (100), 95 (11), 94 (44), 83 (11), 82 (44), 41 (33)
3α-Tigloyloxytropane	herb, roots	1645	223 (45), 208 (42), 140 (46), 124 (100), 96 (83), 94 (77), 83 (51), 82 (85), 55 (51)
3β-Tigloyloxytropane	roots	1655	223 (13), 208 (6), 140 (14), 124 (100), 96 (26), 94 (83), 83 (49), 82 (92), 55 (69)
3α-(3-Hydroxy-2-methylbutyroxy)tropane	roots	1700	241 (22), 224 (2), 196 (1), 140 (7), 124 (100), 96 (12), 94 (42), 83 (12), 82 (40), 55 (10)
3β-(3-Hydroxy-2-methylbutyroxy)tropane (astrimalvine B)	roots	1707	241 (58), 224 (5), 196 (2), 140 (25), 124 (100), 96 (28), 94 (75), 83 (35), 82 (77), 55 (22)
3-(Hydroxymethoxy-benzoyloxy)tropane	herb	2408	291 (16), 151 (13), 140 (6), 124 (100), 96 (7), 95 (8), 94 (17), 83 (14), 82 (39)
3-(Dimethoxyhydroxy-benzoyloxy)tropane	herb	2638	321 (10), 198 (6), 181 (9), 140 (9), 124 (100), 96 (6), 95 (9), 94 (16), 83 (17), 82 (31)
3α-Feruloyloxytropane	herb, roots	2725	317 (10), 194 (9), 177 (15), 140 (17), 124 (100), 96 (60), 95 (15), 94 (23), 83 (21), 82 (21)
3β-Feruloyloxytropane	herb, roots	2756	317 (16), 194 (3), 177 (4), 140 (9), 124 (100), 96 (35), 95 (17), 94 (29), 83 (51), 82 (94)
3β-Sinapoyloxytropane	herb	2980	347 (16), 224 (7), 140 (12), 124 (100), 96 (29), 95 (12), 94 (25), 83 (29), 82 (42)

instrument (0.1 cm cell, 25 °C). EIMS and HR-EIMS were recorded on a Varian MAT 711 (80 eV), FAB MS on a Varian MAT CH_5DF. ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, and HMBC spectra were obtained on a Bruker AMX 400 MHz or a Bruker DRX 500 MHz spectrometer, respectively (TMS as int. standard).

Plant material

Roots of *Astripomoea malvacea* were collected in Uganda in the wild. *Falkia repens* was grown in the Botanischer Garten und Botanisches Museum, Freie Universität Berlin, and whole plants were used for extraction. Voucher specimens are deposited at the Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin.

Extraction and isolation of compounds from Astripomoea malvacea

Ground root bark (260 g) was extracted with 3 × 3 L MeOH and once with MeOH/ 2 % aq. tartaric acid (4 : 1) at r. t. The solvent was evaporated under reduced pressure at 50 °C, the residue redissolved in 2 % aq. tartaric acid and extracted with petroleum ether, CH_2Cl_2, and EtOAc. The aq. layer was then alkalinized with aq. NH_3 (15 %) and extracted with CH_2Cl_2 again (3 × 300 mL). The crude alka-

loid fraction was separated by means of preparative reversed-phase HPLC (MeOH/0.5 % aq. H_3PO_4 25 : 75 to 55 : 45 after 80 min, flow rate = 5.0 mL/min) to afford **1** (8 mg, R_t: 57 min).

The remaining aqueous phase was reduced with Zn/HCl, alkalinized with aq. NH_3 (10 %) and extracted with CH_2Cl_2 again (3 × 300 mL). Further purification was achieved by preparative TLC ($CHCl_3$/MeOH/NH_3 conc. 40 : 10 : 1) and yielded **2** (5 mg, R_f: 0.27).

3β-(3-Tigloyloxy-2-methylbutyryloxy)tropane N-oxide (astrimalvine A *N*-oxide) ***(1)****:* Yellow oil. – CD: $\Delta\varepsilon_{218}$ = −11.6 (MeOH, *c* = 0.15). – ^{1}H NMR (400 MHz, $CDCl_3$): See Table 1. – $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD): See Table 1. – MS (EI, 80 eV): *m/z* (%) = 323 (17) $[M]^+$, 309 (7), 294 (2), 240 (4), 224 (9), 183 (2), 140 (19), 124 (100), 110 (66), 96 (14), 95 (18), 94 (16), 84 (93), 83 (61), 82 (48), 55 (34). – (+)-FAB MS (80 eV): *m/z* = 340 $[M+H]^+$. – HR MS (80 eV): *m/z* = 323.20942 (calcd. 323.20966 for $C_{18}H_{29}NO_4$).

3β-(3-Hydroxy-2-methylbutyryloxy)tropane (astrimalvine B) ***(2)****:* Yellow oil. – CD: $\Delta\varepsilon_{221}$ = −12.9 (MeOH, *c* = 0.21). – ^{1}H NMR (400 MHz, CD_3OD): See Table 1. – $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD): See Table 1. – MS (EI, 80 eV): *m/z* (%) = 241 (22) $[M]^+$, 226 (1), 224 (9), 197 (1), 196 (1), 140 (6), 124 (100), 110 (2), 96 (8), 95 (12), 94 (20), 83 (19), 82 (28), 45 (13). – (+)-FAB MS (80 eV): *m/z* = 242 $[M+H]^+$. – HR MS (80 eV): *m/z* = 241.16795 (calcd. 241.16779 for $C_{13}H_{23}NO_3$).

GC-MS Analysis

Ground plant parts (50 g) were extracted three times with 500 mL MeOH (80 %). After evaporation the extract was acidified and partitioned between water and organic solvents. The aqueous layer was alkalinized and extracted with $CHCl_3$. The extract was subjected to GC-MS analysis. The GC-MS system consisted of a Carlo Erba 5160 / Fisons 8060 GC equipped with a 30 m × 0.32 mm fused silica capillary column coated with the methyl silicone stationary phase DB 1 (J&W Scientific, California). Helium was used as carrier gas. Conditions during split injection: injector 250 °C, split 1 : 20, temperature program 70 – 300 °C, 6 °C/min. The capillary column was directly coupled to the quadrupole mass spectrometer Finnigan MAT 4515 / MD800. Retention indices (*RI*): Kovats indices [9] were calculated in respect to a set of co-injected hydrocarbons.

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[1] Part 19: T. Schimming, K. Jenett-Siems, K. Siems, L. Witte, E. Eich, *Pharmazie* **2005**, *60*, 958.
[2] K. Jenett-Siems, P. Mann, M. Kaloga, K. Siems, J. Jakupovic, L. Witte, E. Eich, *Phytochemistry* **1998**, *49*, 1449.
[3] K. Jenett-Siems, R. Weigl, A. Böhm, P. Mann, B. Tofern-Reblin, S. C. Ott, A. Ghomian, M. Kaloga, K. Siems, L. Witte, M. Hilker, F. Müller, E. Eich, *Phytochemistry* **2005**, *66*, 1448.
[4] A. D. J. Meeuse, W. G. Welman in *Flora of Southern Africa*, Vol. 28, part 1 (Ed.: G. Germishuizen), National Botanical Institute, Pretoria, **2000**.
[5] T. Schimming, K. Jenett-Siems, P. Mann, B. Tofern-Reblin, J. Milson, R. W. Johnson, T. Deroin, D. F. Austin, E. Eich, *Phytochemistry* **2005**, *66*, 469.
[6] G. L. Silva, B. Cui, D. Chavez, M. You, H. B. Chai, P. Rasoanaivo, S. M. Lynn, M. J. O'Neill, J. A. Lewis, J. M. Besterman, A. Monks, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn, *J. Nat. Prod.* **2001**, *64*, 1514.
[7] C. G. Logie, M. R. Grue, J. R. Liddell, *Phytochemistry* **1994**, *37*, 43.
[8] K. Jenett-Siems, S. C. Ott, T. Schimming, K. Siems, F. Müller, M. Hilker, L. Witte, T. Hartmann, D. F. Austin, E. Eich, *Phytochemistry* **2005**, *66*, 223.
[9] E. Kovats, *Helv. Chim. Acta* **1958**, *41*, 1915.