

Short Communication

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Ipvelutine, 7 β -Acetoxy-2 α -(tigloyloxy)tropane, an Unusual Tropane Alkaloid from *Ipomoea velutina* R. BR. (Convolvulaceae)

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Abstract

Convolvulaceae provide a rich source of tropane alkaloids, however, 2-substituted tropanes have been described for only few species of this taxon. In this note, 2,7-diesters such as ipvelutine [7 β -acetoxy-2 α -(tigloyloxy)tropane] isolated from the vegetative parts of the Australian *Ipomoea velutina* R. BR. are described as a new group of tropane diesters.

Keywords

Ipomoea velutina • Convolvulaceae • Ipvelutine • 7 β -Acetoxy-2 α -tigloyloxytropane • 2,7-Disubstituted Tropanes • Structure Elucidation

Introduction

During our continuous studies on secondary metabolites of the Convolvulaceae, this plant family has been shown to produce a plethora of tropane alkaloids, especially 3-tropanols and their esters (e. g. [1, 2]), as well as some 3,6-disubstituted tropanes [3] or the polyhydroxylated calystegines [4]. This underlines the chemotaxonomic relationship with their sister family Solanaceae where the biosynthetic pathway of tropane alkaloids is well investigated. The main route leads to two stereoisomeric 3-hydroxytropanes, namely

3 α -tropanol (basic component of the well-known atropine and other esters), and 3 β -tropanol which is also precursor of the calystegines. 2-Substituted tropane alkaloids could only be found as a by-product in the Solanaceae [5]. Accordingly, amongst the tropane alkaloids of the Convolvulaceae 2-substituted ones are extremely rare, too, and could only be detected in some *Calystegia*, *Erycibe*, and *Ipomoea* species [6].

Results and Discussion

In the alkaloidal screening of Convolvulaceae via GC-MS analysis the basic extracts of the Australian *Ipomoea velutina* R. BR. revealed the presence of several unknown substances. In the basic extract of the vegetative parts seven unknown nitrogen-containing compounds were detected: one main alkaloid and six minor ones (0.7–18.7% of the main alkaloid by integration of the corresponding GC-MS peaks). The molecular formula of the main compound (**1**) is consistent with C₁₅H₂₃NO₄ (*m/z* 281).

The ¹H-NMR (Table 1) in combination with HSQC and HMBC experiments showed two acyclic residues: a C₅-acid containing a double bond, namely tiglic acid, as well as acetic acid. Both were confirmed by fragmentation ions in the EIMS as products of α -cleavage neighbouring the ester carbonyls: *m/z* 83 (C₄H₇-CO⁺; HRMS: [C₅H₇O]⁺ as 83.04959, calcd. 83.04969) and *m/z* 43 (CH₃-CO⁺).

Tab. 1. ¹H- and ¹³C-NMR data of ipvelutine (in MeOD)

| atom | ¹ H-NMR (in MeOD) | ¹³ C-NMR* (in MeOD) |
|----------------------|---------------------------------|-----------------------------------|
| 1 | 3.55 <i>br d</i> | 72.9 |
| 2a | 5.02 <i>ddd</i> | 68.9 |
| 3e | 1.98 <i>m</i> | 22.8 |
| 3a | 1.49 <i>dtd</i> | 27.5 |
| 4a | 1.89 <i>m</i> | 27.5 |
| 4e | 1.68 <i>ddd</i> | 64.7 |
| 5 | 3.82 <i>br t</i> | 64.7 |
| 6n | 2.36 <i>dd</i> | 37.8 |
| 6x | 2.27 <i>ddd</i> | 37.8 |
| 7n | 4.61 <i>dd</i> | 70.8 |
| N-CH ₃ | 2.91 <i>s</i> | 40.9 |
| 1' | | 167.8 |
| 2' | | 129.0 |
| 3' | 6.96 <i>dq</i> | 139.3 |
| CH ₃ -4' | 1.83 <i>d</i> | 11.9 |
| CH ₃ -5' | 1.84 <i>d</i> | 14.1 |
| 1'' | | 176.7 |
| CH ₃ -2'' | 1.93 <i>s</i> | 21.9 |

*...taken from HSQC/HMBC.

The HSQC spectrum revealed a characteristically downfield shifted N-CH₃ (δ_C 40.9, δ_H 2.91) as well as three methylene signals (δ_C 37.8, 27.5, and 22.8) and four methine groups

(δ_C 72.9, 70.8, 68.9, and 64.7). From the ^1H - ^1H -COSY, the complete coupling sequence could be deduced. As a result, **1** (Fig. 1) could be identified as a 2,7-disubstituted tropane.

The substitution pattern of the tropane diester was derived from the mass spectrometric data on the basis of the specific mass fragmentation in bridge-substituted tropanes. The most important fragment is $[\text{M} - \text{X}-\text{COO}-\text{CH}=\text{CH}_2]^+$ after expulsion of the ethylene bridge C-6–C-7 including its substituent; this allows a prediction of the substituents' positions in 3,6/7-disubstituted tropanes [7, 8]. Regarding **1**, there are two possible key ions: in case of acetylation in position 7 m/z 195 or in case of acetylation in position 2 m/z 155. As there is only a veritable peak at m/z 195, **1** has to be acetylated in position 7 of the tropane.

The relative stereochemistry of **1** was deduced from characteristic coupling constants: H-7 showed a doublet-doublet with coupling constants of 3.4 Hz and 7.9 Hz that can also be observed in the 7 β -substituted schizanthines C-E [9]. This corresponds with the experience that, for steric reasons, bridge substituents usually are *exo*-orientated. H-2 showed a *trans*-diaxial coupling constant $J = 10$ Hz which is – according to [10] and [11] – specific for α -orientated substituents at C-2. These conclusions were also confirmed by NOE measurements: H-2 (δ_H 5.02) showed correlations to H-1 (δ_H 3.55), to the equatorial H-3e (δ_H 1.98) and to the axial H-4a (δ_H 1.89) which is only possible if H-4a and H-2 are both axial [11]. H-7 (δ_H 4.61) was correlated to H-1 (δ_H 3.55) and – only enabled by its *endo*-position – to the axial H-3a (δ_H 1.49) and H-6n (δ_H 2.36).

Thus, **1** (ipvelutine) was identified as 7 β -acetoxy-2 α -(tigloyloxy)tropane.

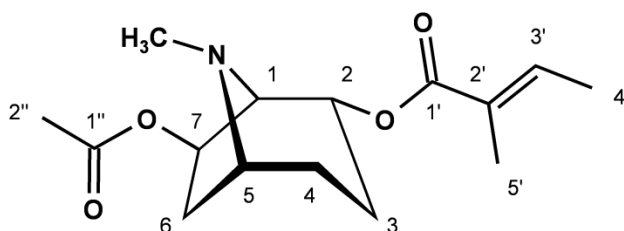


Fig. 1. Structure of ipvelutine [7 β -acetoxy-2 α -(tigloyloxy)tropane], main alkaloid from the vegetative parts of *Ipomoea velutina* R. BR.

In the vegetative parts and/or roots, eight minor compounds related to ipvelutine could be detected by GC-MS analysis. They were identified by their fragmentation patterns; characteristic base peaks of those 2,7-disubstituted tropanes are m/z 95 and m/z 82 or m/z 81 together with a prominent peak at m/z 156, and of their *nortropane* derivatives m/z 125 and m/z 81 including a half-maximal peak at m/z 108. An additional result of the systematic GC-MS screening is the detection of ipvelutine (appearing as deacetylated derivative in GC-MS analysis) in vegetative parts of *Convolvulus graminetinus*, *C. sagittatus*, and *Ipomoea abrupta*. Both *Convolvulus* species afforded similar structures, as well, and, additionally, the corresponding *nortropanes* in the roots. Ipvelutine-related substances were also found in *Ipomoea asarifolia* and *I. plebeia*. The mass fragmentation patterns obtained by GC-MS analysis show that these variations include differences in the stereostructure at C-2 or/and C-7, alternation of the position of the substituents, methylbutyric and hydroxymethylbutyric acid as diverging acyl components, change of the

bridge substituents' position from C-7 to C-6 and a hydroxy group as additional substituent (for details see [12]).

2,7-Dihydroxynortropane showing the same substitution pattern as ipvelutine is also synthesized by root cultures of *Calystegia sepium* (Solanaceae). Incorporation experiments with ^{15}N -labelled 3-tropanone revealed that, unless 2,7-dihydroxynortropane derives the regular tropane alkaloid pathway, it is not an intermediate in calystegine biosynthesis, but can be seen as a by-product [5].

From the pharmacological point of view, the finding of ipvelutine and derivatives is of interest since they show structural similarity to bao gong teng A [13] obtained from the vegetative parts of *Erycibe obtusifolia* (Convolvulaceae). Bao gong teng A is characterized by strong miotic properties and therefore used as an antiglaucoma agent in medicinal products. This pharmacological effect is contradictory to that of atropine/hyoscyamine having significance as a mydriatic in ophthalmology and being one of the most commonly used tropanes of natural origin.

Experimental

General procedures

^1H -NMR and ^1H - ^1H -COSY spectra were obtained on a Bruker AMX 400 MHz, HSQC and HMBC spectra on a Bruker DRX 500 MHz (TMS as internal standard). EIMS and HR-EIMS were recorded on a Varian MAT 711 (80 eV), FABMS on a Varian MAT CH₅DF. The GC-MS system consisted of a Fisons GC 8060 coupled to a quadrupole mass spectrometer Fisons MD 800c.

Plant material

Roots and vegetative parts of *Ipomoea velutina* R. BR. grown from seeds collected in the wild at Florence Falls, Litchfield National Park, Northern Territory/Australia, were harvested in the greenhouse of the Institut für Pharmazie, Freie Universität Berlin. A voucher specimen is deposited at the herbarium of the Berlin-Dahlem Botanical Garden – Botanical Museum (BGBM), Freie Universität Berlin, Germany.

Extraction and isolation of ipvelutine

235 g dried and ground vegetative parts of *Ipomoea velutina* were extracted 4 h with 3 L MeOH three times and once with a mixture of 2.4 L MeOH and 600 mL 2% aqueous tartaric acid. After evaporation of the MeOH (50°C i. V.), the residue was redissolved in 600 mL 2% aqueous tartaric acid and extracted with petrol ether, CH₂Cl₂, and EtOAc, respectively (3 x 500 mL each). Then, the aqueous layer was alkalized (pH 10) with aqueous NH₃ (25%) and extracted with 4 x 500 mL CH₂Cl₂. The united alkaline CH₂Cl₂ fractions gave 172 mg crude alkaloid fraction which was dissolved in 50 mL 2% aqueous tartaric acid again and extracted with petrol ether, CH₂Cl₂, and EtOAc (3 x 50 mL each). After addition of aqueous NH₃ (pH 10), the aqueous layer was extracted with 4 x 50 mL CH₂Cl₂. After drying over Na₂SO₄ and evaporation of CH₂Cl₂ (40°C i. V.), the alkaline fractions were united and 10 mg ipvelutine were gained (81% purity according to NMR spectra).

7β-Acetoxy-2α-(tigloyloxy)tropane [(1S,2S,5R,7R)-7-(acetyloxy)-8-methyl-8-azabicyclo[3.2.1]oct-2-yl (2E)-2-methylbut-2-enoate, ipvelutine, 1]

Yellow oil. ¹H-NMR (400 MHz, MeOD): see Table 1. ¹³C-NMR (100.6 MHz, MeOD): see Table 1. MS (EI, 80 eV, 110°C): *m/z* (%) = 281 (2) [M]⁺, 239 (83), 195 (7), 156 (100), 142 (60), 140 (35), 112 (11), 98 (46), 96 (84), 95 (91), 94 (50), 85 (41), 84 (31), 83 (27), 55 (22), 43 (20). (+)-FAB MS (80 eV): *m/z* = 282 [M+H]⁺. HR MS (80 eV): *m/z* = 281.16256 (calcd. 281.16271 for C₁₅H₂₃NO₄), 239.15283 (calcd. 239.15214 for C₁₃H₂₁NO₃), 156.10254 (calcd. 156.10245 for C₈H₁₄NO₂⁺), 142.08678 (calcd. 142.08681 for C₇H₁₂NO₂⁺), 140.10749 (calcd. 140.10754 for C₈H₁₄NO⁺), 98.062524 (calcd. 98.06059 for C₅H₈NO⁺), 95.072728 (calcd. 95.073499 for C₆H₉N).

GC-MS analysis

Ground plant parts (50 g) were extracted three times with 500 mL MeOH (80%). After evaporation the residue was dissolved in 2% aqueous tartaric acid and extracted with petrol ether, CH₂Cl₂, and EtOAc. The aqueous layer was alkalinized and extracted with CH₂Cl₂. To purify the extracts obtained, this procedure was repeated with corresponding smaller amounts of the solvents. The resulting extracts were subjected to GC-MS analysis. Samples were injected at 240°C (split 1:20) and separated on a DB-1 column (0.32 mm x 30 m, J&W Scientific, California) by raising temperature from 70°C to 300°C at 6°C/min. Helium was used as carrier gas. Retention indices (RI): Kovats indices [14] were calculated in relation to a set of co-injected hydrocarbons.

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

References

- [1] Ott SC, Jenett-Siems K, Pertz HH, Siems K, Witte L, Eich E. Bonabiline A, a monoterpene 3α-acyloxytropane from the roots of *Bonamia spectabilis* showing M₃ receptor antagonist activity. *Planta Med.* 2006; 72: 1403–1406. <http://dx.doi.org/10.1055/s-2006-951728>
- [2] Ott SC, Tofern-Reblin B, Jenett-Siems K, Siems K, Müller F, Hilker M, Onegi B, Witte L, Eich E. Unusual tropane alkaloid pattern in two African Convolvulaceae species. *Phytochemistry and chemotaxonomy of the Convolvulaceae*, part 20. *Z Naturforsch.* 2007; 62b: 285–288.
- [3] Jenett-Siems K, Weigl R, Böhm A, Mann P, Tofern-Reblin B, Ott SC, Ghomian A, Kaloga M, Siems K, Witte L, Hilker M, Müller F, Eich E. Chemotaxonomy of the pantropical genus *Merremia* (Convolvulaceae) based on the distribution of tropane alkaloids. *Phytochemistry.* 2005; 66: 1448–1464. <http://dx.doi.org/10.1016/j.phytochem.2005.04.027>

- [4] Schimming T, Jenett-Siems K, Mann P, Tofern-Reblin B, Milson J, Johnson RW, Deroin T, Austin DF, Eich E.
Calystegines as chemotaxonomic markers in the Convolvulaceae.
Phytochemistry. 2005; 66: 469–480.
<http://dx.doi.org/10.1016/j.phytochem.2004.12.024>
- [5] Scholl Y, Höke D, Dräger B.
Calystegines in *Calystegia sepium* derive from the tropane alkaloid pathway.
Phytochemistry. 2001; 58: 883–889.
[http://dx.doi.org/10.1016/S0031-9422\(01\)00362-4](http://dx.doi.org/10.1016/S0031-9422(01)00362-4)
- [6] Eich E.
Solanaceae and Convolvulaceae: secondary metabolites.
Berlin – Heidelberg: Springer, 2008: 127–132.
<http://dx.doi.org/10.1007/978-3-540-74541-9>
- [7] Lounasmaa M.
Sur les alcaloïdes mineurs de *Knightia deplanchei*.
Planta Med. 1975; 27: 83–88.
<http://dx.doi.org/10.1055/s-0028-1097765>
- [8] Evans WC, Ramsey KPA.
Tropane alkaloids from *Anthocercis* and *Anthotroche*.
Phytochemistry. 1981; 20: 497–499.
[http://dx.doi.org/10.1016/S0031-9422\(00\)84174-6](http://dx.doi.org/10.1016/S0031-9422(00)84174-6)
- [9] San-Martín A, Labbé C, Muñoz O, Castillo M, Reina M, de la Fuente G, González A.
Tropane alkaloids from *Schizanthus grahamii*.
Phytochemistry. 1987; 26: 819–822.
[http://dx.doi.org/10.1016/S0031-9422\(00\)84794-9](http://dx.doi.org/10.1016/S0031-9422(00)84794-9)
- [10] Johns SR, Lamberton JA, Sioumis AA.
New tropane alkaloids, (+)-(3R,6R)-3 α -acetoxo-6 β -hydroxytropane and (+)-2 α -benzoyloxy-3 β -hydroxynortropane, from *Peripentadenia mearsii* (Euphorbiaceae).
Aust J Chem. 1971; 24: 2399–2403.
<http://dx.doi.org/10.1071/CH9712399c>
- [11] Asano N, Yokoyama K, Sakurai M, Ikeda K, Kizu H, Kato A, Arisawa M, Höke D, Dräger B, Watson AA, Nash RJ.
Dihydroxynortropane alkaloids from calystegine-producing plants.
Phytochemistry. 2001; 57: 721–726.
[http://dx.doi.org/10.1016/S0031-9422\(01\)00131-5](http://dx.doi.org/10.1016/S0031-9422(01)00131-5)
- [12] Ott SC.
Neuartige Tropanalkaloide und andere stickstoffhaltige Sekundärstoffe in Windengewächsen (Convolvulaceae).
Dissertation, Freie Universität Berlin, Fachbereich Biologie, Chemie, Pharmazie (to be published).
- [13] Yao T, Chen Z, Yi D, Xu G.
[Chemical study on Bao Gong-teng (*Erycibe obtusifolia* BENTH.). II. Structure of bao gong teng A – a new myotic agent].
Yao Xue Xue Bao (Acta Pharm Sin). 1981; 16: 582–588.
<http://www.ncbi.nlm.nih.gov/pubmed/7324958>
- [14] Kovats E.
Gas chromatographic characterization of organic compounds. I. Retention indices of aliphatic halides, alcohols, aldehydes, and ketones.
Helv Chim Acta. 1958; 41: 1915–1932.
<http://dx.doi.org/10.1002/hlca.19580410703>