Ipvelutine, 7β-Acetoxy-2α-(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 [7β-acetoxy-2α-(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 • 7β-Acetoxy-2α-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
3α-tropanol (basic component of the well-known atropine and other esters), and 3β-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 C_{15}H_{23}NO_{4} (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 | ¹H-NMR (in MeOD) | ¹³C-NMR* (in MeOD) |
|------|-----------------|-------------------|
| 1    | 3.55 br d       | 3.2 Hz            | 72.9 |
| 2a   | 5.02 ddd        | 2.2 Hz; 5.9 Hz; 11.3 Hz | 68.9 |
| 3e   | 1.98 m          |                   | 22.8 |
| 3a   | 1.49 dtd        | 6.4 Hz; 12.1 Hz; 12.8 Hz |       |
| 4a   | 1.89 m          |                   |       |
| 4e   | 1.68 ddd        | 2.3 Hz; 6.7 Hz; 13.7 Hz | 27.5 |
| 5    | 3.82 br t       | 5.2 Hz            | 64.7 |
| 6n   | 2.36 dd         | 8.0 Hz; 14.6 Hz   | 37.8 |
| 6x   | 2.27 ddd        | 3.5 Hz; 6.3 Hz; 14.7 Hz |       |
| 7n   | 4.61 dd         | 3.4 Hz; 7.9 Hz    | 70.8 |
| N−CH₃| 2.91 s          |                   | 40.9 |
| 1'   |                 |                   | 167.8|
| 2'   |                 |                   | 129.0|
| 3'   | 6.96 dq         | 1.2 Hz; 6.9 Hz    | 139.3|
| CH₃−4' | 1.83 d      | 7.1 Hz            | 11.9 |
| CH₃−5' | 1.84 d      | 0.9 Hz            | 14.1 |
| 1''  |                 |                   | 176.7|
| CH₃−2'' | 1.93 s     |                   | 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
Ipvelutine, an Unusual Tropane Alkaloid from *Ipomoea velutina* R. BR. (Convolvulaceae)

(δC 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 [M – X–COO–CH=CH₂]⁺ 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 7β-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 (δH 5.02) showed correlations to H-1 (δH 3.55), to the equatorial H-3e (δH 1.98) and to the axial H-4a (δH 1.89) which is only possible if H-4a and H-2 are both axial [11]. H-7 (δH 4.61) was correlated to H-1 (δH 3.55) and – only enabled by its endo-position – to the axial H-3a (δH 1.49) and H-6n (δH 2.36).

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

![Structure of ipvelutine](image)

**Fig. 1.** Structure of ipvelutine [7β-acetoxy-2α-(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. sagitattus*, 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 15N-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 alkalinized (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).
Ipvelutine, an Unusual Tropane Alkaloid from Ipomoea velutina R. BR. (Convolvulaceae)

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.

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