Quinolizidine Alkaloids of *Platycelphium vöense* (Engl.) Wild (Leguminosae)

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13 Alkaloids were identified in leaves and twigs of *Platycelphium vöense* (a monotypic genus in the tribe Sophoreae) by capillary GLC and GLC-MS. Cytisine, N-methylcytisine, anagyrine and 5,6-dehydrolupanine figured as major and N-formylcytisine, N-acetylcytisine, baptifoline, thermopsine, isolupanine, and rhombifoline as minor alkaloids in both leaves and twigs. Lupanine occurred in considerable amount in the leaves and as a minor component in the twigs. The rare alkaloid, 6β-hydroxylupanine, was detected only in the leaves while N-ethylcytisine was shown to be a minor constituent of the twigs only.

Introduction

Platycelphium vöense (Engl.) Wild represents a monotypic genus occurring in the drier regions of Ethiopia, Somalia, Kenya and Tanzania (Thulin, 1983). It has violet flowers, characteristic flat indehiscent pods which contain only one seed and it grows as a small deciduous tree or shrub (height 1.5–7.5 m) (Thulin, 1989).

According to Polhill (1981), the genus is placed in the *Sophora* group of plants within the tribe Sophoreae (family Leguminosae). Although many genera belonging to this group have been investigated for their alkaloidal constituents, there is no previous report in the literature concerning the genus *Platycelphium*. In view of the importance of quinolizidine alkaloids as chemotaxonomic markers, the present investigation was carried out to analyse the alkaloid patterns of the leaves and the twigs of *P. vöense* by highly sensitive techniques *viz*. GLC and GC-MS.

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Materials and Methods

Plant material

The plant was collected in June 1995 in Sidamo region of Ethiopia 52 km north-west of Bokol Mayo on the road from Dolo to Filtu and Negele. Its identity was confirmed by Dr Insermu Kelbesa (The National Herbarium, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia).

Alkaloid extraction

Dried powdered leaves (250 g) were defatted with *n*-hexane in a Soxhlet apparatus for 48 hr and extracted with 80% MeOH for 72 hr. The dark green residue remaining after removal of the aqueous MeOH under reduced pressure was taken up in 2% H_2SO_4 (50 ml) and filtered. The acidic aqueous extract was washed with Et_2O until the washings were colourless, basified with conc. NH_4OH (pH 9) and extracted with CH_2Cl_2 (4 x 50 ml). The combined CH_2Cl_2 extracts were dried (anhydrous Na_2SO_4) filtered and concentrated *in vacuo*. The acid base purification procedure was repeated three times to give a light reddish oil (0.325 g, 0.13%). A similar procedure was adopted for the isolation of alkaloids from the twigs to yield a light yellowish oil (0.275 g, 0.11%). Preperative TLC [silica gel F_{254}, $CHCl_3$-MeOH-NH_4OH (25%), 90:10:1] of the alkaloid extracts of the leaves and the twigs yielded alkaloids **1**,**3**,**5**,**7** and **11**.

Alkaloid analysis

Alkaloid extracts were subjected to high resolution gas chromatography under the following conditions: DB1 (J & W) fused silica capillary column (15 m x 0.25 mm), carrier gas He; FID detector, det. temp. 300°; inj. temp. 250°; split 1:5; oven temp. prog.: initial temp. 150° 2 min isothermal, 150–250° at 15° min^{-1}, 250–300° at 25° min^{-1}, 300° 15 min isothermal. Capillary GC-MS was performed on a Ohio Valley OV1 (15 m x 0.25 mm) column coupled directly to a quadrupole Finnigan Mat 4500 mass spectrometer. EIMS were recorded

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at 45 eV. Conditions: carrier gas He; splitless, inj. temp. 250°, oven 120° 2 min isothermal, 120–250° at 10° min^{-1}, 250–300° at 15° min^{-1}. RIs were calculated using cochromatographed standard hydrocarbons.

Results and Discussion

The alkaloid extracts of the leaves and twigs of *Platycelphium vöense* were analysed by capillary GC and GC-MS. A total of thirteen alkaloids were detected and unequivocally identified (Table I) by comparison of their mass spectral data and/or retention indices with those reported in the literature (Wink, 1993; Wink *et al.*, 1995). The alkaloid profile of the genus appears to be characterized by tri- and tetracyclic α-pyridone and 2-oxosparteine-type quinolizidine alkaloids. The alkaloid contents of the leaves and twigs were found to be similar both qualitatively and quantitatively with some minor differences. The common alkaloids cytisine, N-methylcytisine, anagyrine, 5,6-dehydrolupanine and lupanine occurred as major alkaloids in the leaves. These alkaloids were also the major components of the twigs with the exception of lupanine which occurred in the twigs as a minor component. N-Formylcytisine, N-acetylcytisine, baptifoline, thermopsine, isolupanine and rhombifoline were detected as minor components in both the leaves and the twigs. N-Ethylcytisine was detected only in the twigs while 6β-hydroxylupanine occurred only in the leaves. The quantitative alkaloid pattern of both leaves and twigs is given in Table II.

Table II. Alkaloid profile of *Platycelphium vöense* (Engl.) Wild leaves and twigs as determined by GLC; total alkaloid = 100%.

	Alkaloids	% Leaves	Twigs
1	N-Methylcytisine	9.7	25.6
2	N-Ethylcytisine	–	trace
3	Cytisine	43.6	59.4
4	α-Isolupanine	trace	trace
5	5,6-Dehydrolupanine	20.5	2.8
6	Rhombifoline	trace	trace
7	Lupanine	8.2	0.9
8	Thermopsine	trace	trace
9	N-Formylcytisine	[a]2.4	[b]1.9
10	N-Acetylcytisine	[a]2.4	[b]1.9
11	Anagyrine	14.0	6.9
12	6β-Hydroxylupanine	trace	–
13	Baptifoline	1.6	2.5

[a,b] Alkaloids coeluted. The value is the sum of the two compounds.

The unstable alkaloid, 6β-hydroxylupanine, did not show a molecular ion in its EIMS and also its RI was not reported previously. However, its characterization was based on its peculiar chromatographic properties (i.e. its rapid conversion to 5,6-dehydrolupanine) and MS fragmentation pattern which were closely similar to the literature data (Asres *et al.*, 1986). Furthermore, solid probe mass spectrometry of the total alkaloidal extract using chemical ionization technique has revealed the presence of a prominent peak at m/z = 265 which was attributed to $[M^+ + 1]$ of 6β-hydroxylupanine.

It is worthwhile to note that previously it was proposed that 6β-hydroxylupanine could be an in-

Table I. Alkaloid profiles of the leaves and twigs of *Platycelphium vöense* (Engl.) Wild as analysed by GC-MS.

	Alkaloid	RI	M^+	Abundant ions (relative abundance %)				
1	N-Methylcytisine	1952	204	204(15)	160(4)	146(5)	73(5)	58(100)
2	N-Ethylcytisine	1997	218	218(15)	160(13)	146(19)	72(100)	58(52)
3	Cytisine	2005	190	190(70)	160(23)	147(70)	146(100)	134(20)
4	α-Isolupanine	2100	248	248(58)	247(37)	149(66)	136(100)	98(32)
5	5,6-Dehydrolupanine	2128	246	246(34)	148(6)	134(10)	98(100)	97(39)
6	Rhombifoline	2154	(244)	203(58)	160(17)	146(54)	73(44)	58(100)
7	Lupanine	2160	248	248(45)	150(10)	149(61)	136(100)	98(25)
8	Thermopsine	2310	244	244(38)	203(12)	160(25)	146(34)	98(100)
9	N-Formylcytisine	2335	218	218(59)	190(10)	160(19)	147(54)	146(100)
10	N-Acetylcytisine	2343	232	232(42)	190(10)	160(17)	147(83)	146(100)
11	Anagyrine	2391	244	244(35)	160(10)	146(14)	136(10)	98(100)
12	6β-Hydroxylupanine	2460	(264)	246(9)	148(11)	147(13)	134(28)	98(100)
13	Baptifoline	2690	260	260(56)	160(16)	146(31)	114(100)	96(26)

termediate between lupanine and 5,6-dehydrolupanine in the biosynthesis of quinolizidine alkaloids. 5,6-Dehydrolupanine was also implicated as a likely precursor of the α-pyridone-type alkaloids such as anagyrine. It appears that bridgehead hydroxyl groups are rare in nature The only previous occurrence of bridgehead hydroxylation at C-6 among 2-oxosparteine-type alkaloids is in *Bolusanthus speciosus* (Asres *et al.*, 1986) and *Lygos raetam* var. *sarcocarpa* (Abdel-Halim, 1995), although 6-hydroxyaphxylline which was isolated from *Anabasis aphylla* (family Chenopodiaceae) (Sadykov and Nurriddinov, 1960) has also been implicated as a precursor of aphyllidine in the 10-oxosparteine series of alkaloids (Arslanian *et al.*, 1990).

The occurrence of 6β-hydroxylupanine in the leaves of *P. vöense* not only supports the previously proposed key sequence that leads to the array of α-pyridone type alkaloids, but also substantiates the strong botanical affinities between the two genera, *Bolusanthus* and *Platycelphium*, both of which belong to the *Sophora* group of plants in the tribe Sophoreae of the family Leguminosae.

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