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Quinolizidine Alkaloids from the East-African Legume *Dicraeopetalum stipulare* Harms

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Key Word Index—*Dicraeopetalum stipulare*; Leguminosae; quinolizidine alkaloids; capillary gas chromatography–mass spectrometry.

Abstract—The alkaloid composition of leaves of *Dicraeopetalum stipulare* Harms was examined by capillary gas chromatography (GLC) and GLC–mass spectrometry. 5,6-Dehydrolupanine, anagyrine and cytisine figured as major and baptifoline, *N*-methylcytisine, lupanine, ammodendrine, *N*-formylcytisine and *N*-acetylcytisine as minor alkaloids. Sparteine, rhombifoline and 6β-hydroxylupanine were detected in trace amounts. Two new alkaloids were tentatively identified as 10-oxolupanine and 17-oxolupanine. © 1997 Elsevier Science Ltd

Introduction

Dicraeopetalum stipulare Harms represents a monotypic genus of the tribe Sophoreae (family Leguminosae). The plant grows in the south-eastern province of Ethiopia and the adjoining areas of Somalia and Kenya (Polhil, 1981). Although the name of the plant has been recorded in the literature as Hangelelo in the Somali vernacular (Thulin, 1989), it is known as Saban Sable in the area from which it was collected for the present study.

D. stipulare is a tree which grows up to a height of 5–8 m and has white flowers and indehiscent flat pods which contain one or two seeds (Thulin, 1983). It belongs to the *Cadia* group of plants along with the genera *Amphimas, Acosmium, Myrocarpus, Holocalyx, Lovanafia* and *Cadia*. The genus *Acosmium* is closely related to *Dicraeopetalum* and, formerly, the latter was included in the *Acosmium* genus (Thulin, 1983). Among the chemically investigated plants belonging to this group, the genera *Acosmium* and *Cadia* were shown to produce quinolizidine alkaloids; however, no information is available in the literature regarding the chemical constituents of *D. stipulare.*

In the present study, the alkaloid composition of the leaves of *D. stipulare* was analysed by capillary GLC and GC–MS and the chemotaxonomic implication of this finding is briefly discussed.

Materials and Methods

Plant material. The plant material was collected in December 1993 from the surrounding areas of Filtu, a small village town in Sidamo region about 720 km south-east of Addis Ababa, Ethiopia. The plant was identified by Dr Insermu Kelbesa (The National Herbarium, Department of Biology, Addis Ababa University, where voucher specimens were deposited).

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Alkaloid extraction. Dried powdered leaves (150 g) were defatted with *n*-hexane in a Soxhlet apparatus for 48 h and further extracted with 80% MeOH for 72 h. The dark green residue remaining after removal of the aqueous MeOH under reduced pressure was taken up in 2% H_2SO_4 (40 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 (4×40 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 brown semi-solid (0.104 g, 0.07%).

Alkaloid analysis. Alkaloid extracts were subjected to high resolution gas chromatography under the following conditions: DB1 (J & W) fused silica capillary column (15 m×0.25 mm), carrier gas He; FID detector, detection temp., 300°; injection temp., 250°; split 1:25; 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 an Ohio Valley OV1 (15 m×0.25 mm) column coupled directly to a quadrupole Finnigan Mat 4500 mass spectrometer. EIMS were recorded at 45 eV. Conditions: carrier gas He; splitless, injection 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 profile of *D. stipulare* has been studied by the use of GLC (Fig. 1) and GC–MS (Table 1). A total of 14 quinolizidine alkaloids have been detected in the leaves of this plant. Unambiguous identification of 12 of these alkaloids was achieved by comparing their mass spectra and Kovats retention indices with those reported in the literature (Wink, 1993; Wink *et al.*, 1995). 5,6-Dehydrolupanine, anagyrine, cytisine, baptifoline and *N*-methylcytisine were shown to be the major alkaloids while lupanine, ammodendrine, *N*-formylcytisine and *N*-acetylcytisine occurred as minor components. Rhombifoline, sparteine, 6β-hydroxylupanine, RI = 2204 and RI = 2218 could only be detected in trace amounts. The quantitative pattern of these alkaloids is given in Table 2.

Further analysis of the remaining alkaloids, RI = 2204 and RI = 2218 was not possible due to their occurrence in trace amounts and also because of paucity of plant material. However, from their molecular ion at $m/z = 262$ and their respective EIMS fragmentation

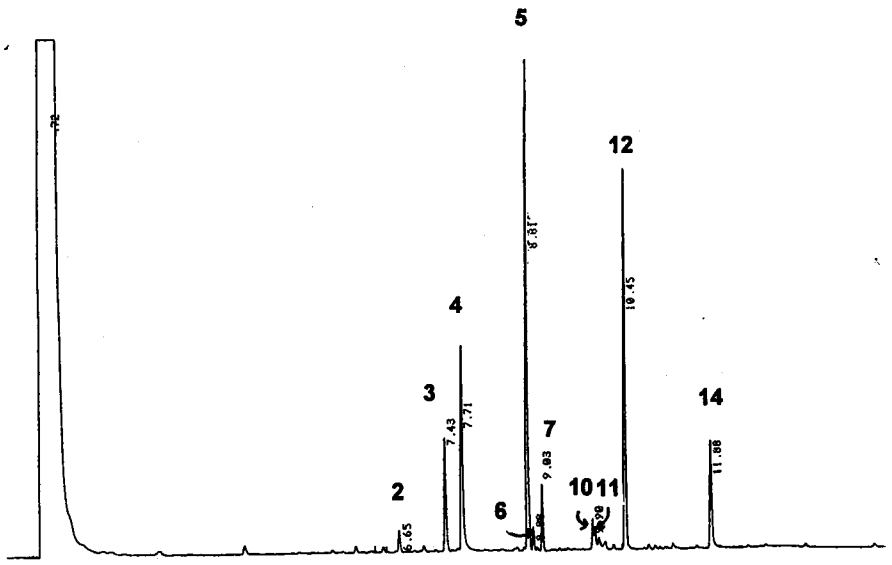

FIG. 1. SEPARATION OF QUINOLIZIDINE ALKALOIDS FROM *DICRAEOPETALUM STIPULARE* BY CAPILLARY GLC.

TABLE 1. IDENTIFICATION OF ALKALOIDS OF *DICRAEOPETALUM STIPULARE* HARMS BY GC–MS

Alkaloid		RI	M^+	Abundant ions (relative abundance %)
1	Sparteine	1780	234	234(24) 193(37) 137(100) 136(44) 98(93)
2	Ammodendrine	1873	208	208(46) 165(100) 136(56) 123(62) 109(85)
3	*N*-Methylcytisine	1959	204	204(15) 160(4) 146(5) 73(5) 58(100)
4	Cytisine	1995	190	190(70) 160(25) 147(84) 146(100) 134(22)
5	5,6-Dehydrolupanine	2132	246	246(25) 148(6) 134(9) 98(100) 97(38)
6	Rhombifoline	2155	(244)	203(84) 160(12) 146(4) 77(11) 58(100)
7	Lupanine	2165	248	248(45) 150(38) 149(58) 136(100) 98(28)
8		2204	262	262(23) 234(14) 138(51) 136(64) 84(100)
9		2218	262	262(94) 150(42) 136(83) 98(55) 97(100)
10	*N*-Formylcytisine	2334	218	218(78) 160(20) 147(47) 146(100) 134(15)
11	*N*-Acetylcytisine	2344	232	232(42) 190(10) 160(16) 147(83) 146(100)
12	Anagyrine	2390	244	244(32) 160(9) 146(13) 136(10) 98(100)
13	6β-Hydroxylupanine	2457	(264)	246(16) 148(8) 134(18) 98(100) 97(20)
14	Baptifoline	2696	260	260(46) 243(6) 160(17) 146(29) 114(100)

TABLE 2. ALKALOID COMPOSITION OF *DICRAEOPETALUM STIPULARE* HARMS AS DETERMINED BY GLC (TOTAL ALKALOIDS = 100%)

Alkaloid		%
1	Sparteine	trace
2	Ammodendrine	1.7
3	*N*-Methylcytisine	7.9
4	Cytisine	16.0
5	5,6-Dehydrolupanine	30.2
6	Rhombifoline	trace
7	Lupanine	4.1
8		trace
9		trace
10	*N*-Formylcytisine	2.5[a]
11	*N*-Acetylcytisine	2.5[a]
12	Anagyrine	25.6
13	6β-Hydroxylupanine	trace
14	Baptifoline	9.5

[a]Alkaloids coeluted. The value refers to the sum of the two alkaloids.

pattern, it can be deduced that the alkaloids are dioxosparteines. Furthermore, owing to the fact that both of these alkaloids contain a prominent ion at $m/z = 136$, the possibility of having a 10,17-dioxosparteine structure can be ruled out since such compounds do not show any fragment ion at $m/z = 136$ (Schumann *et al.*, 1968). This together with biosynthetic considerations led to the assumption that the alkaloids contain a 2,10- and/or a 2,17-dioxosparteine structure. The MS of RI = 2204 exhibits a significant peak at $m/z = 234$ ($M^+ - 28$) which arises from the elimination of a CO group typical of 17-oxosparteines (Schumann *et al.*, 1968). The presence of prominent fragment ions such as at $m/z = 150$, 136, 97 and 84 is also indicative of a 17-oxo substitution. It can therefore be proposed that RI = 2204 is a 17-oxolupanine isomer.

RI = 2218 showed a strong molecular ion peak at $m/z = 262$ (abundance 94%) indicating that it is a highly stable compound. The ions at $m/z = 234$ ($M^+ - 28$), 233

(M^+-29) and 205 (M^+-57) correspond to the elimination of CO, C_2H_5 and CH_3-CH_2-CO, respectively, characteristic of the fragmentation pattern of 10-oxosparteines (Schumann *et al.*, 1968). The MS of RI = 2218 also shows similarities with those of 10-oxosparteines in that it exhibits strong ions at $m/z = 150$ (abundance 42%), 136 (abundance 83%) and 97 (abundance 100%). Thus it is likely that the compound is one of the isomers of 10-oxolupanine.

The occurrence of the dipiperidine alkaloid, ammodendrine, in *D. stipulare* is of chemotaxonomic significance. Outside the genus *Ammodendron* (tribe Sophoreae), dipiperidine alkaloids are known to coexist with quinolizidine alkaloids mainly in plant species belonging to the tribe Genisteae. Such an observation has led to a suggestion that *Ammodendron* should be transferred to the tribe Genisteae (Mears and Mabry, 1971). However, the present finding clearly shows that plants belonging to the tribe Sophoreae already have the biosynthetic capacity to produce dipiperidine alkaloids such as ammodendrine indicating that the aforementioned view is not relevant. Furthermore, the occurrence of ammodendrine in other plants belonging to the tribe Sophoreae has also been reported previously (Wink and Witte, 1987).

It may also be worthwhile to note that among the *Cadia* group of plants that have already been investigated so far, the alkaloid profile of *D. stipulare* appears to be similar to that of *Acosmium* in that they both contain the tetracyclic sparteine and lupanine-type alkaloids supporting their botanical similarities. However, the chemistry of the two genera is different from each other by the occurrence of pentacyclic quinolizidine alkaloids in *Acosmium* (Fitzgerald *et al.*, 1964; Balandrin and Kinghorn, 1981). Thus, the present finding is consistent with the current taxonomic classification of *Dicraeopetalum* as a separate genus.

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