

Mass Spectrometric Fragmentation of Sativanine-A and -B^{*1}

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Summary: Detailed mass spectrometric fragmentation pattern and structure of 14-membered cyclopeptide alkaloids; sativanine-A (1) and sativanine-B (2) are discussed.

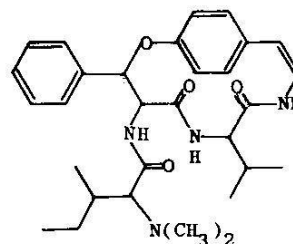
Introduction

The cyclopeptide alkaloids are an expanding group of polyamide plant bases which incorporate an alkoxy-styrylamine unit as an integral part of 13-, 14-, or 15-membered heterocyclic rings. The size of the rings has been adopted as the basis for the classification of this group of natural products. The larger group containing 14 members is again separated via the contained β -hydroxyamino acid; namely, those with β -hydroxyleucine (frangulanine type), with β -hydroxyphenylalanine (integerrine type), and with trans-3-hydroxyproline (amphibine-B-type). Cyclopeptide alkaloids are commonly found in the plants of family Rhamnaceae [2].

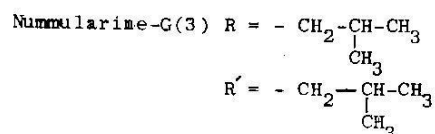
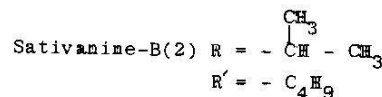
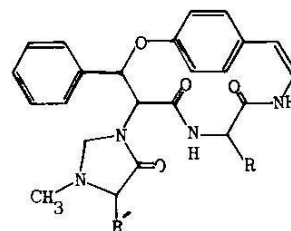
Results and Discussion

In continuation of our work on cyclopeptide alkaloids from the plants of family Rhamnaceae [1], we have been able to isolate and report two new compounds of this class containing 14-membered ring system from *Zizyphus sativa*; namely sativanine-A (1) and -B (2) [3]. (1) belongs to integerrine type whilst the structure of (2) is related to nummularine-G (3) [4]; so providing the second example of the

occurrence of this type of alkaloid in plants. Here we wish to discuss the structures and details of mass spectrometric fragmentation of these compounds.



Sativanine-A (1)



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Sativanine-A (1)

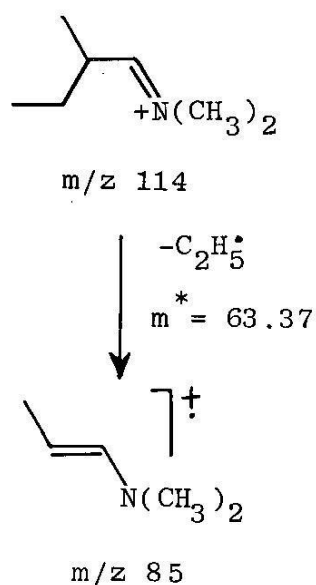
Sativanine-A was isolated from the polar fraction by thin layer chromatography on silica gel plates as colourless substance. It melted at 80° and was easily soluble in methanol, chloroform and dichloromethane. The IR spectrum of (1) displayed characteristic secondary amide bands, conjugated C=C double bond, an aryl ether and N-methyl groups. The UV spectrum showed end absorption with shoulders at 250 and 280 nm which revealed the presence of 14-membered ring system [5].

The elementary composition of (1) was determined by high resolution mass spectrometry as C₃₀H₄₀N₄O₄. The mass spectrum of (1) follows the typical fragmentation pattern for the 14-membered cyclopeptide alkaloids [6].

The ions a (m/z 114), i (m/z 131) and the amine fragment [H₂N⁺CH-CH(CH₃)₂] show the presence of N,N-dimethylleucine or isoleucine, p-hydroxystyrylamine, β-phenylserine and valine. The fragment c (m/z 229) indicates that p-hydroxystyrylamine unit is linked with β-phenylserine. On the other hand ions f (m/z 224) and h (m/z 260) demonstrate that p-hydroxystyrylamine unit is bound on one side through oxygen with β-phenylserine and on the other side with valine through nitrogen. The ions k (m/z 230) and l (m/z 202) represent the direct linkage between β-phenylserine and valine; thus proving the structure of the 14-membered ring system.

The fragment ions a (m/z 114) and b (m/z 463) are of high intensity. They are formed due to the breakage of C-C bond adjacent to the N,N-dimethylamino group (α-cleavage). The ion a analysed as C₇H₁₆N forms

the base peak of the spectrum. This gave a daughter ion at m/z 85 by the loss of ethyl radical with a strong meta stable ion for that process at m/z 63.37.

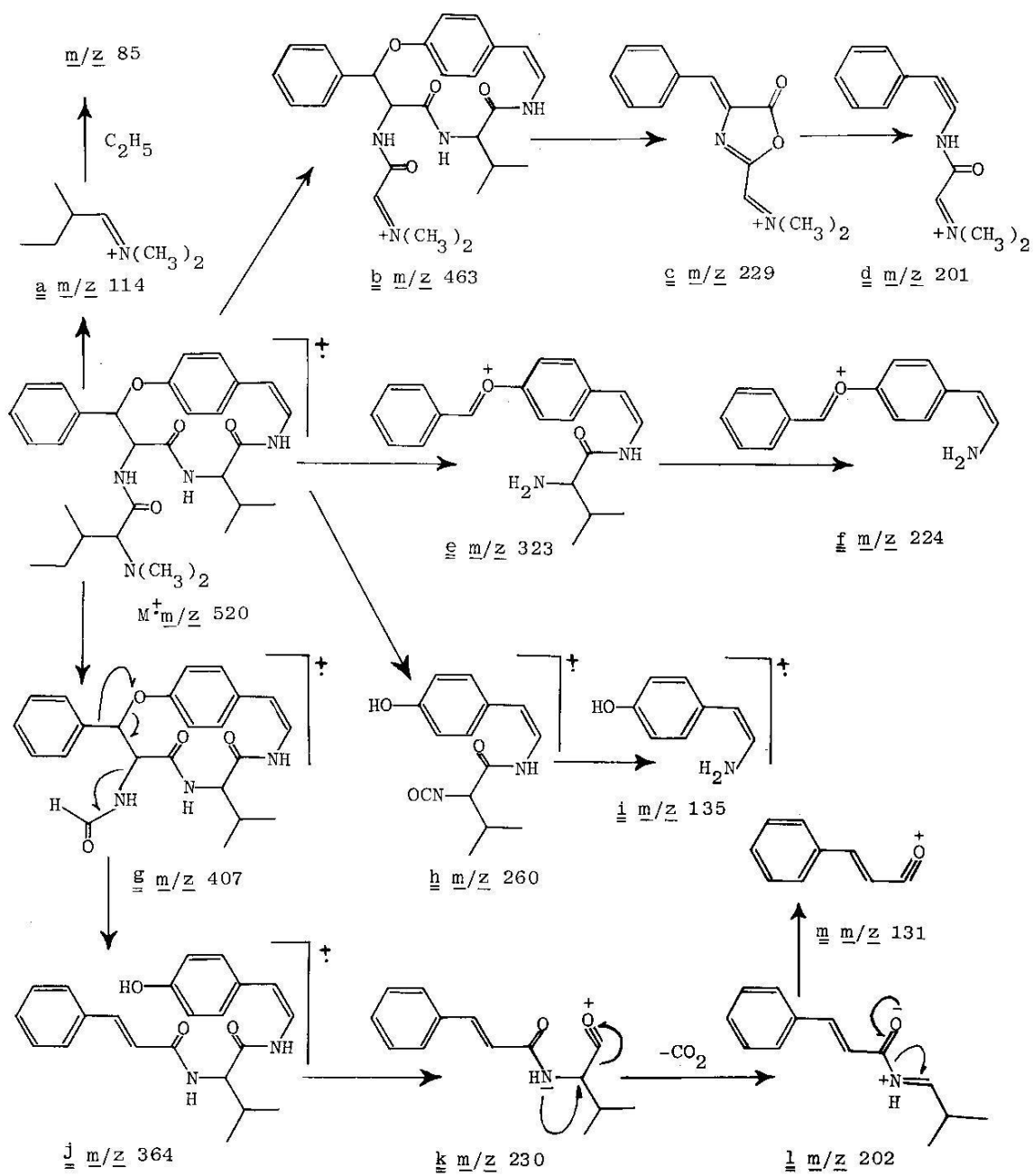


The fragmentation pattern is encountered for N,N-dimethylisoleucine but not for leucine [6]. The principal fragmentations observed are listed in Table-1 and assignments are depicted in Scheme-1. The elementary composition of all fragments was substantiated by high resolution mass measurements. The mass spectrum of (1) resembles that of integerrine [5].

Sativanine-B (2)

Sativanine-B (2) is a colourless amorphous substance which is easily soluble in methanol and chloroform. Its IR spectrum gave the usual bands for cyclopeptide alkaloids. The UV spectrum of (2) revealed the presence of 14-membered ring cyclopeptide alkaloid [4].

By high resolution MS the elementary composition of (2) was determined as C₃₀H₃₈N₄O₄ (M⁺ 518). It shows 14



Scheme-I: Mass spectrometric fragmentation of sativanine-A (1).

double bond equivalents which indicates the presence of an additional ring in the side chain. The principal fragments observed and assignments are exhibited in Table-II and Scheme-II. The mass spectrum of (2) differs greatly from those of 14-membered cyclopeptide alkaloids but resembles closely that of nummularine-G (3) [4] which carries an additional ring in the side chain.

Through the breakage of butyl group on the terminal amino acid (α -cleavage) of (2) the primary fragment ion m/z 461 is formed. Because of the very small amount of the material it was not possible to confirm whether this amino acid was the derivative of leucine or isoleucine.

On the breakage of the cyclic side chain, the ions m/z 155 ($C_8H_{15}N_2O$) and j (m/z 364) are formed. The ion m (m/z 131), i (m/z 135) and m/z 72 indicate the presence of phenylserine, *p*-hydroxystyrylamine and valine. The fragment i forms the base peak of the spectrum. The ions f (m/z 224) and h (m/z 260) shows that *p*-hydroxystyrylamine unit is bound on one side with phenylserine and on the other side with valine. The linkage of the side chain with phenylserine is confirmed by fragments c (m/z 227) and d (m/z 201). The identity of each fragment was proved by high resolution mass spectrometry.

Experimental

Plant material was collected from Hazara district of Pakistan. Mps were determined on Weigand microscope stage. MS analyses were performed on MS-30 and MS-50 (Kratos) at 70 eV with evaporation of the sample in the ion source at ca 200°. TLC, unless otherwise specified was carried out on Si gel Merk 60 F₂₅₄. Perkin-Elmer 221 (IR) and Carry-14 (UV) spectrometers were used.

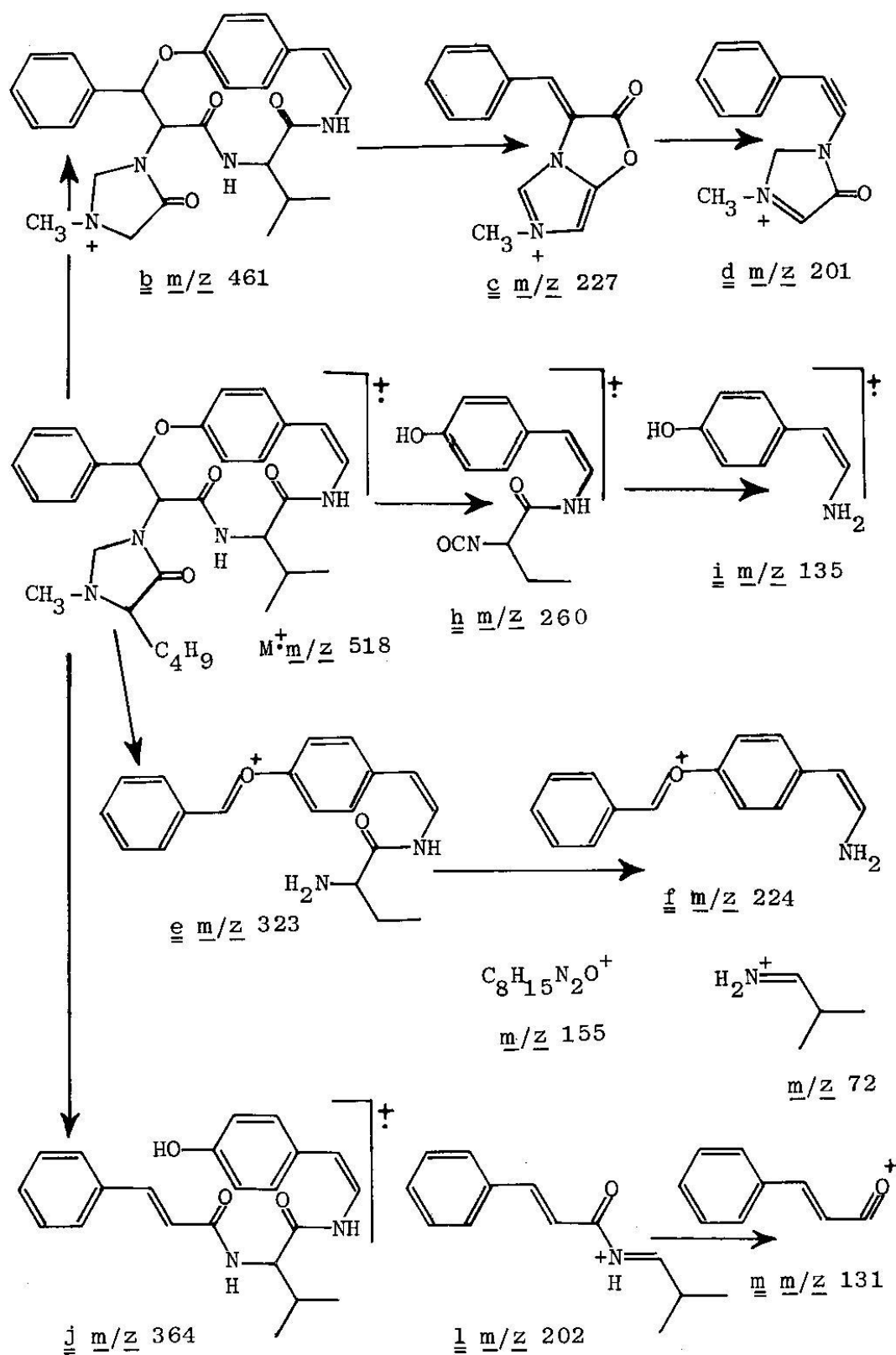
Table 1: High resolution mass spectrometry of sativanine-A (1)

Ion	Formula	Div.	Found	Intensity %
M^+	$C_{30}H_{40}N_4O_4$	-0.5	520.3050	0.06
\underline{a}	$C_7H_{16}N$	-0.7	114.1275	1.00
\underline{b}	$C_{26}H_{31}N_4O_4$	0.8	463.2353	1.45
\underline{c}	$C_{13}H_{13}N_2O_2$	2.6	229.1003	0.15
\underline{d}	$C_{12}H_{13}N_2O$	-0.3	201.1025	0.32
\underline{e}	$C_{20}H_{23}N_2O_2$	-0.2	323.1758	0.56
\underline{f}	$C_{15}H_{14}NO$	0.5	224.1081	0.98
\underline{g}	$C_{23}H_{25}N_3O_4$	-	407	0.5
\underline{h}	$C_{14}H_{16}N_2O_3$	0.4	260.1165	1.53
\underline{i}	C_8H_8NO	0.3	135.0687	13.59
\underline{j}	$C_{22}H_{24}N_2O_3$	0.1	364.1788	0.68
\underline{k}	$C_{14}H_{16}NO_2$	-1.6	230.1165	0.34
\underline{l}	$C_{13}H_{16}NO$	-2.2	202.1210	0.68
\underline{m}	C_9H_7O	-0.3	131.0494	7.66
\underline{n}	$C_4H_{10}N$	2.4	72.0837	3.54

Table-2: High resolution mass spectrometry of sativanine-B (2)

Ion	Formula	Div.	Found	Intensity (%)
M^+	$C_{30}H_{38}N_4O_4$	-0.5	518.2888	2.84
\underline{b}	$C_{26}H_{29}N_4O_4$	1.2	461.2201	11.84
\underline{c}	$C_{13}H_{11}N_2O_2$	1.3	227.0834	32.03
\underline{d}	$C_{12}H_{13}N_2O$	-0.3	201.0124	12.90
\underline{e}	$C_{20}H_{23}N_2O_2$	-	323	0.5
\underline{f}	$C_{15}H_{14}NO$	0.2	224.1078	1.29
\underline{h}	$C_{14}H_{16}N_2O_3$	-0.2	260.1159	6.27
\underline{i}	C_8H_9NO	-0.6	135.0678	100
\underline{j}	$C_{22}H_{24}N_2O_3$	0.5	364.1788	7.7
\underline{l}	$C_{12}H_{15}NO$	-1.1	202.1176	4.50
\underline{m}	C_9H_7O	-3.7	132.0493	1.81
\underline{n}	$C_4H_{10}N$	-0.2	72.0811	7.32

SATIVANINE-A AND B



Scheme-II: Mass spectrometric fragmentation of sativanine-B (2).

Extraction of the powdered bark (10 Kg) was carried out with a mixture of benzene-ethyl alcohol-ammonia (100:1:1) [7]. The filtrate was evaporated to almost half of its volume and extracted repeatedly with 5% aqueous citric acid. After filtration the aqueous layer was made alkaline with ammonia to pH 10; and extracted extensively with chloroform. After the evaporation of chloroform brownish alkaloidal mixture 6.6 g was obtained. It was fractionated on a 900 g Si gel (Gebr.Herrmann/Koln) column, eluting with increasingly polar $\text{CH}_2\text{Cl}_2/\text{MeOH}$ mixture into 20 fractions. The chromatographic separation was followed by LKB Uvicord, and the collected fractions were analysed by TLC, which were purified using PLC or column chromatography.

Sativanine-A (1)

1.2 mg were obtained from fraction 8 by repeated chromatography on Si gel using cyclohexane-acetone-methanol (35:15:1) and cyclohexane-EtOAc-methanol (30:15:4) as solvent systems; mp 80° (uncorr.); IR (CHCl_3) 3370 (NH), 2990-2820 (CH), 2775 (NCH_3), 1675 (amide), 1618 ($\text{C}=\text{C}$), 1590+1490 (aromat.) $1225+1040\text{ cm}^{-1}$ (aryl ether); UV (MeOH) strong end absorption and shoulders at 250 and 280 nm. Mol.wt (MS) 520.3055; calcd for $\text{C}_{30}\text{H}_{40}\text{N}_4\text{O}$, 520.3050.

Sativanine-B (2)

1.1 mg were obtained from fraction 9 using benzene-EtOAc-methanol (25:15:4) and benzene-acetone-methanol (25:30:4) as solvent systems;

mp: amorphous; IR (CHCl_3) 3330 (NH), 2960-2870 (CH), 2800 (NCH_3), 1675 (amide), 1610 ($\text{C}=\text{C}$), 1585-1485 (aromat.), $1220, 1025\text{ cm}^{-1}$ (aryl ether); UV (MeOH) strong end absorption with shoulder at 280 nm. Mol.wt. (MS) 518.2888; calcd for $\text{C}_{33}\text{H}_{38}\text{N}_4\text{O}_4$, 518.2893.

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