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CYCLOPEPTIDE ALKALOIDS: FURTHER STUDIES ON MAURITINE-C AND SATIVANINE-C

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ABSTRACT

The 14-membered cyclopeptide alkaloid muritine-C and the 13-membered cyclopeptide alkaloid sativanine-C were isolated from Zizphus spinea-christi and Zizyphus sativa commonly used in the Saudi Folklor medicine. The N-formyl derivatives of these compounds were prepared and their corresponding spectral data was analyzed. Fundamental differences were observed in the mass spectrometric fragmentation of the newly formed derivatives as compared to the parent compounds. High resolution mass spectrometry was found a useful tool to substantiate the fragmentation pattern proposed for these potential natural products.

Introduction

The cyclopeptide alkaloids are polyamide plant bases composed of amino acid residue in common and highly modified forms. All so far known cyclopeptide alkaloids with the exception of Lasiodine-A are cyclic, containing 13-,14- or 15-membered ring systems. The 14-membered ring system containing alkaloids represent the largest known group of these compounds. Earlier structural studies of these natural compounds were difficult due to their complexity and many of the proposed structures were later revised (Tschesche and Kaussmann 1975; Shah and Pandey, 1985). The advancement of high resolution mass spectrometry accelerate the structure elucidation of these bases. Integrerrine and frangulanine type of cyclopeptide alkaloids were verified by mass spectrometry according to Fehlhaher's scheme (Fehlhaber, 1968). Their fragmentation pattern was subsequently used as a model to determine the structure of similar as well as new type of cyclopeptide alkaloids. Recently, the novel alkaloids nummularine-G (Tschesche *et. al*, 1977), sativanine-ß (Shah *et. al.*, 1985) and sativanine-F (Shah *et al*, 1985) have been established. So far no N-formyl derivative of 14-membered cyclopeptide alkaloid ring system has been found in nature, although 13-membered counterparts have recently been described (Shah *et. al*, 1987).

In continuation of our work on the chemical and biological studies of the plants used in Saudi Folk Medicine, here we wish to report the preparation and structure elucidation of the potential natural alkaloids N-formylmauritine-C (2) and N-formylsativanine-C (4). The biological activity of these N-formyl derivatives comparable to other natural alkaloids of this group still need to be explored.

Experimental

The optical rotation as obtained on Perkin-elmer 141 electric polarimeter and melting points were determined on Weigand microscope stage and are uncorrrected. Pye Unicam SP 8-100 (UV), Perkin-Elmer 580 B(IR) and Broker-HX 90 (NMR) instruments were used. Mass spectra were measured on MS-50 (Kratos) at 70eV with evaportion of the sample in the ion source at 200°.

Material

Zizyphus spina-chnsti was collected from King Saud University campus, Riyadh, Saudi Arabia while *z. saliva* was collected from Hazara District, Pakistan.

Method

Mauritine-C (1): About 5 Kg bark of *Z. spina-christi* was repeatedly extracted with a mixture of benzene-ethanol- ammonia (100:5:1) and the solvent evaporated in vacuum. The residue was dissolved in 7% citric acid aqueous solution and filtered. Now the pH of this aqueous solution was brought to 10 with ammonia and the crude bases extracted with chloroform. On evaporation of chloroform 2.1g of brownish mixture of alkaloids was obtained which was subjected to column chromatography eluting with increasing polar chloroform: methanol mixtures. Finally, by prep. TLC 13.2mg of mauritine-C were obtained.

Sativanine-C (3)

From the 10Kg bark of *Z. saliva* 6.6g of crude base were obtained as described earlier (Shah *et. al,* 1984). Pure compound (3)4 mg was isolated by TLC using dichloromethanemethanol (20:1), tetrahydroforan-cyclohexane-acetone (10:5:1) and cyclohexane-ethylacetate-methanol (35:15:1) as solvent system.

N-formylation

A few mg of the compound (1) and (3) were reacted with mixture of formic acid/acetic anhydride and left overnight (Ugi, 1971). The solvent was evaporated and the product purified.

N-formylmauritine-C (2)

After N-formylation the pure compound (2) was obtained by preparative TLC using cyclohexane-acetone (3:2) and cyclohexane-acetone (7:10) as solvent systems. Chloroform-methanol mixture was used to elute the alkaloidal band. Compound (2) could not be crystalized and gave no sharp melting point. It possessed an $[\alpha]D=-311°$ (c=0.2, $CHCl_3$). UV (MeOH) λ_{max} nm: strong end absorption with shoulders at 252 and 280 nm. IR ($CHCl_3$): 3360 (HN), 2985-2850 (CH), 2775 (NCH_3), 1680 (amide-CO), 1616 (c=c),

1590 + 1490 (Aromat.), 1228 cm-1 + 1018 cm^{-1} (aryl ether).^{1}H-NMR ($CDCl_3$/TMS): δ=0.83 dd, 6H $(CH_3)_2$-CH, 3.05 (s, 3H, CHO-N-CH_3). 4.09 (d, J = 5.8Hz, 1 H, 3-Hypro-2-H), 6.28 (d, J = 7.5Hz, 4.09, -CH = CH), 6.2-7.5 (Complex multiplets of olefinic, aromatic, and NH). 8.12 (s, 1H, CHO-N). MS: 585.3163; calcd. for $C_{29}H_{34}N_4O_5$: 518.2530.

N-FOrmylsativatine-C (4):

Fine colourless crystals were obtained upon crystallization the reaction product from ether. Mp.130-131°. UV (MeOH) λ_{max} nm: 260 and 230nm. IR ($CHCl_3$): 3370 (NH), 3000-825 (CHH), 1670 (amide), 1632 (c-c, 1590+1445 (aromat.), 1230+1040 cm^{-1}(aryl ether). ^{1}H-NMR ($CDCl_3$/TMS). complex methyl reasonances 0.51 - 1 ppm of isoleucine and valine groups; 1.23 (d, J=7.3Hz 3H,CH_3 -C) of end amino acid, 2.88 (s, 3H, CHO-N-CH_3). 3.77 (s, 3H, -OCH_3),6.0 (d, J=9Hz, 1H, -CH=C-N) styrylamine, 7.0 (d, J=9Hz , 1H, -CH=CH-N), 7.8 (s, 1H, N-CHO), 7.7 (s, br. 1H, NH) of interniediate amino acid, 8.9ppm (d, br, 1H, NH) of styrylamine. δ 6.6-7.5 (signals for aromatic, olefinic and HN protons). MS: 5853163: calcd. for $C_{30}H_{43}N_4O_7$: 585.3177.

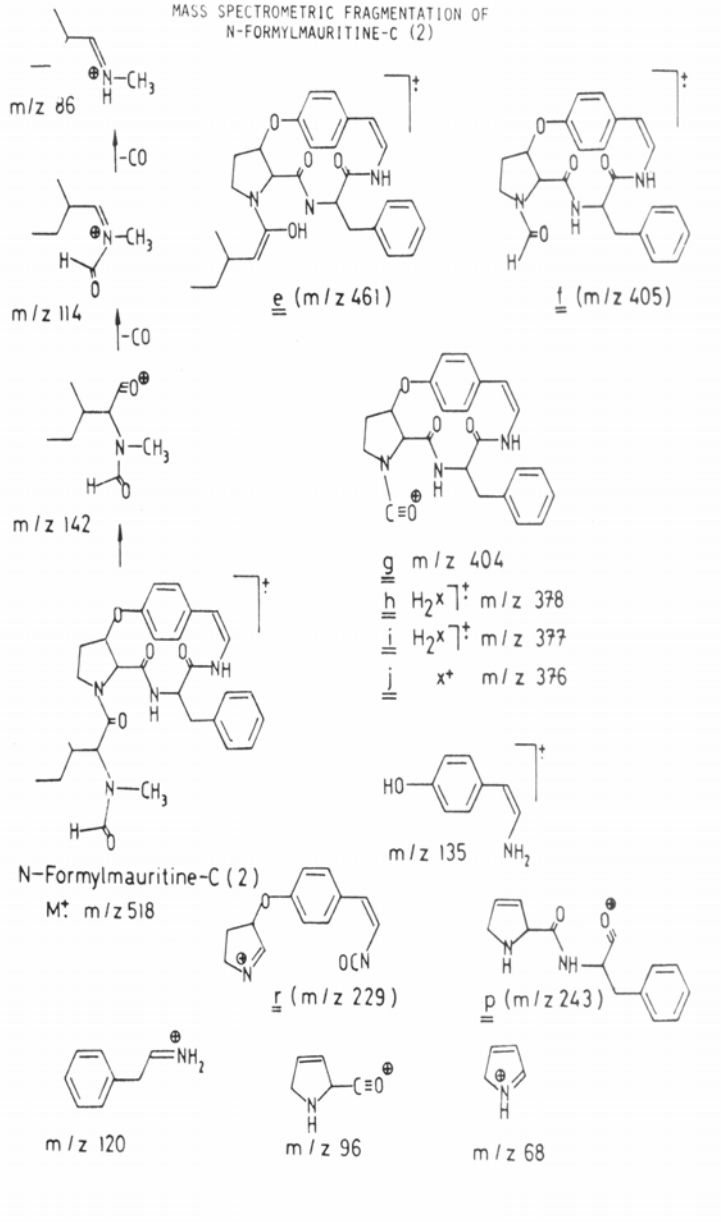
Results and Discussion

The UV absorption characteristic to N-formylmauritine-C (2) showed an end absorption with shoulders at 252 and 280 nm a pattern diognastic for styrylaminc moiety in 14- membered cyclopeptide alkaloids (Tschesche, Ammermann, 1974). The IR spectrum (2) showed similar vibrations to the parent compound mauriline-C(1) except the N-formyl absorption region was complexed with several amide bands. Compound (2) also differed in PMR spectrum from the parent compound (1) in processing 0.7 ppm down field shift of the - NCH_3 protons due to the anisotropy of formyl group. The N-formyl proton resonates as a singlet at 8.12 ppm in (2) while rest of the protons appear at the same place as reported for mauritine-C (Tschesche *et. al.,* 1974).

The elementry composition of (2) was determined by high resolution mass spectrometry as $C_{29}H_{34}N_4O_5$ (Table-1). A systematic presentation of the proposed fragmentation of this molecule upon electron impact is given in Scheme-l. Careful analysis of different fragment ions of the spectrum revealed a fundamental deviation of cleavage pattern from all so far known 14-membered cyclopeptide alkaloids. Since (2) contains no basic end amino acid, the ionized molecule is fairly stable and gives an intence molecular ion at m/z 518 (81.86%). The characteristically known ‘-cleavage ions a and b are completely absent. An alternate cleavage has taken place where the peptide band between the side chain amino acid and proline is broken yielding m/z 142 ($C_7H_{12}NO_2$). It then eleminates twice CO to give ions at to m/z 14 ($C_6H_{12}NO$, 91.16%) and M/Z 86 ($C_5H_{12}N$, 25.8%).

Scheme - 1

MASS SPECTROMETRIC FRAGMENTATION OF
N-FORMYLMAURITINE-C (2)



Other fragment ion $(M\text{-}CO)^+$ appeared at m/z 490 with 6.1% relative intensity. The composition and linkage of the side chain is clear from the fragment ion m/z 142 and fragment e at m/z 461. The ion at m/z 142 forms the base peak of the spectrum. Hydroxyproline is joined to styrylamine unit by an ether linkage as indicated by ion r at m/z 229 and s at m/z 186 and on the other side, to phenylalanine by an amide bonding as shown by fragment P at mz 243. The fragment ions f, g, h, i and j represent the whole macrocylic ring system and consequently the whole structure of N-formyhmauritine-C (2). The elementry composition of all the fragments was substantiated by high resolution mass spectrometry (Table-I).

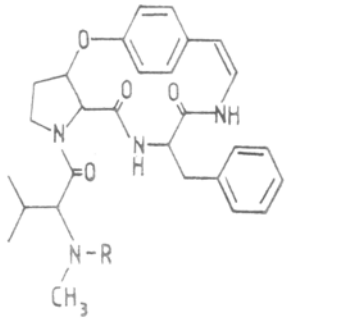
N-formylsativanine-C (4) unlike the parent alkaloid (3) gave no response to Dragendorff's reagent. It was found less polar than sativanine-C. However, both the IR and UV spectra were closely related to (3). The ^{1}H-NMR spectrum of (4) exhibited some differences previously observed in compound (2). An N-methyl down-field shift of 0.45 ppm was noticed in comparison with (3). A proton singlet at 7.8 ppm was observed corresponding to the newly introduced N-formyl group proton. It is interesting to note that one amide proton signal was shifted 0.6 ppm down-field in the spectrum of (4). Though decoupling experiments it was proved to be the amide proton of styrylamine unit. Molecular model studies on this molecule indicated a possible interaction between the end N-formyl group with this NH of the 13-membered ring system.

Table-I: Highh resolution mass spectrometry of N-formylmauritine-C (2)

Ion	Formula	Div.	Found	Intensity (%)
M^+	$C_{29}H_{34}N_4O_5$	±0	518.2530	81.86
$(M\text{-}CO)^+$	$C_{28}H_{34}N_4O_4$	4.8	490.2628	6.07
e	$C_{27}H_{31}N_3O_4$	2.1	461.2335	1.06
f	$C_{23}H_{23}N_3O_4$	0.6	405.1694	2.80
g	$C_{23}H_{22}N_3O_4$	-1.3	404.1597	3.41
h	$C_{22}H_{24}N_3O_3$	-3.3	378.1784	1.59
i	$C_{22}H_{23}N_3O_3$	-2.3	377.1737	4.82
j	$C_{22}H_{22}N_3O_3$	2.3	376.1684	4.57
P	$C_{14}H_{15}N_2O_2$	-1.4	243.1120	1.08
r	$C_{13}H_{13}N_2O_2$	-0.4	229.0973	1.37
	$C_7H_{12}NO_2$	-0.3	142.0865	100
	$C_6H_{12}NO$	0.1	114.0920	91.16
	$C_5H_{12}N$	0.9	86.0978	25.80
	C_8H_9NO	0.7	135.0691	23.83
	$C_6H_{10}N$	0.1	120.0815	10.63
	C_4H_6N	0.7	68.0507	6.23
	C_5H_6NO	-0.2	96.0447	2.14

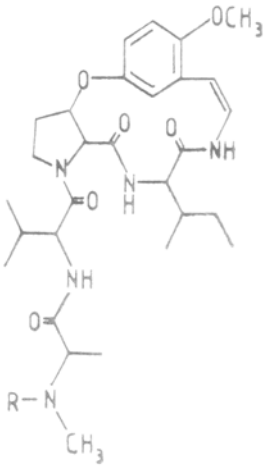
Due to the short side-chain fragments k, l, m and n not possible.

The mass spectrum of (4) was found completely different from other known cyclopcptide alkaloids, however, it was similar to the spectrum of (2). The molecular ion of m/z 585 of (4) was fairly stable showing high intensity (37.7%) in the mass spectrum. The usual '-cleavage fragments a - d were fully missing. Instead of this, the base peak of the mass spectrum was formed by the N-formyl group carrying fragment at m/z 114 with the elemetry composition of $C_5H_8NO_2$. It eliminated twice CO unit and gave rise to the ions m/z 86 and m/z 58 respectively. The counterpart of the fragment m/z 114 is the ion m/z 472. The linkage of the side chain intermediate amino acid valine with N-formylmonomethylalanine was represented by fragment 1 (m/z 227). Fragment 1 was formed by the total cleavage of the side chain from the macrocyclic ring system of the molecule. On elimination of one of unit from a new fragment designated as m is formed (m/z 185). The fragment ions n (m/z 195) and o (m/z 221) demonstrate the linkage of the side chain with hydroxyproline of the main ring system. The attachment of hydroxyproline with styrylamine moiety on one side and with phenylalanine on the other side can be deduced by the ions r (m/z 259),s (m/z 233), t (m/z 216), p(m/z 243) and q (m/z 215). Fragment u (m/z 304) demonstrates the linkage of phenylalanine with styrylamine unit; thus establishing the full macrocyclic ring system. This whole 13-membered ring system is shown by the ions e (m/z 457), f (m/z 401), g (m/z 400), h (m/z 374), i (m/z 373) and j (m/z 372). The elementry composition of each fragment was substantiated by high resolution mass spectrometry. The high resolution mass spectrometry data is given in table 2 and the assignments are depicted in scheme 2.



MAURITINE-C R=H (1)

N-FORMYL-MAURITINE-C R=CHO (2)



SATIVANINE-C R=H (3)

N-FORMYL-SATIVANINE-C R=CHO (4)

Scheme 2

MASS SPECTROMETRIC FRAGMENTATION OF N-FORMYLSATIVINE-C (4)

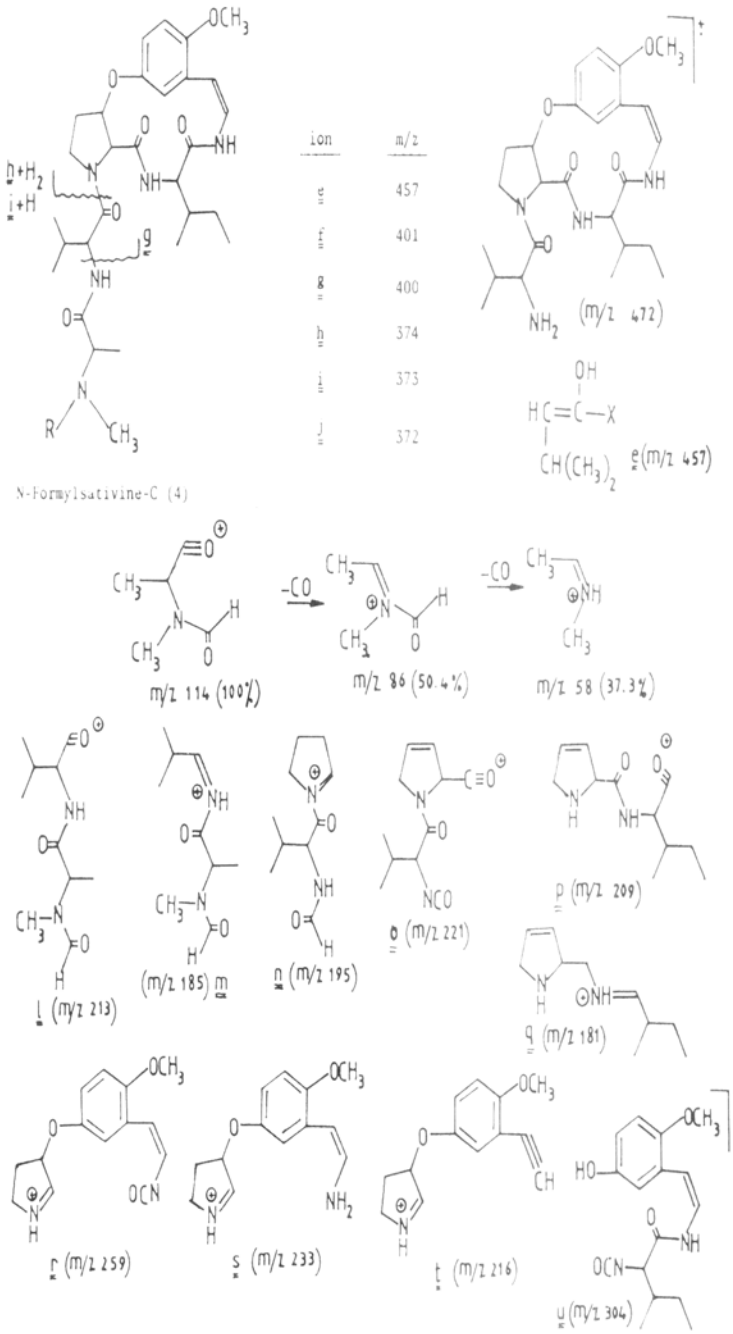


Table-II: High Resolution Mass Spectrometry of N-Formyl-Sativanine-C (4)

Ion	Formula	Div.	Found	Intensity (%)
M^+	$C_{30}H_{43}N_5O_7$	1.4	585.3177	27.7
$(M\text{-}CO)^+$	$C_{29}H_{43}N_5O_5$	-	557	< 1
e	$C_{25}H_{35}N_3O_5$	-1.3	457.2563	8.32
f	$C_{21}H_{27}N_3O_5$	-	401	< 0.5
g	$C_{21}H_{26}N_3O_5$	-	400	< 0.5
h	$C_{20}H_{28}N_3O_4$	-2.6	374.2053	0.60
i	$C_{20}H_{27}N_3O_4$	1.1	373.2012	1.76
j	$C_{20}H_{26}N_3O_4$	-2.7	372.1897	0.88
l	$C_{10}H_{17}N_2O_3$	0.8	213.1247	6.1
m	$C_9H_{17}N_2O_2$	-1.3	185.1276	3.2
n	$C_{10}H_{15}N_2O_2$	-2.0	195.1114	4.43
p	$C_{11}H_{17}N_2O_2$	-0.8	209.1282	1.86
r	$C_{14}H_{15}N_2O_3$	-0.3	259.1080	0.64
s	$C_{13}H_{17}N_2O_2$	-1.3	233.1277	0.56
t	$C_{13}H_{14}NO_2$	-1.2	216.1013	2.45
	$C_9H_{11}NO_2$	0.1	165.079	24.2
	$C_5H_8NO_2$	0.5	114.0560	100
	C_4H_8NO	0.6	86.0612	50.4
	C_3H_8N	1.7	58.0674	37.3

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