Bull. Pharm. Sci., Assiut University
Vol. 8, Part 1, pp. 172-191

OXYLOPIDINE AND OXYLOPININE NEW ALKALOIDS FROM OXANDRA XYLOPIOIDES DIELS

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ABSTRACT

Two new alkaloids, Oxylopidine(I) and Oxylopinine (II), were isolated from the stem-bark and twigs of Oxandra xylopioides Diels. The structures of the two alkaloids were proved.

INTRODUCTION

In a previous paper[1], we reported the isolation and elucidation of structure of a new alkaloid oxylopine(1-aza-4-methyl-5-hydroxyfluorenone) as well as the oxoaporphine alkaloid liriodenine.

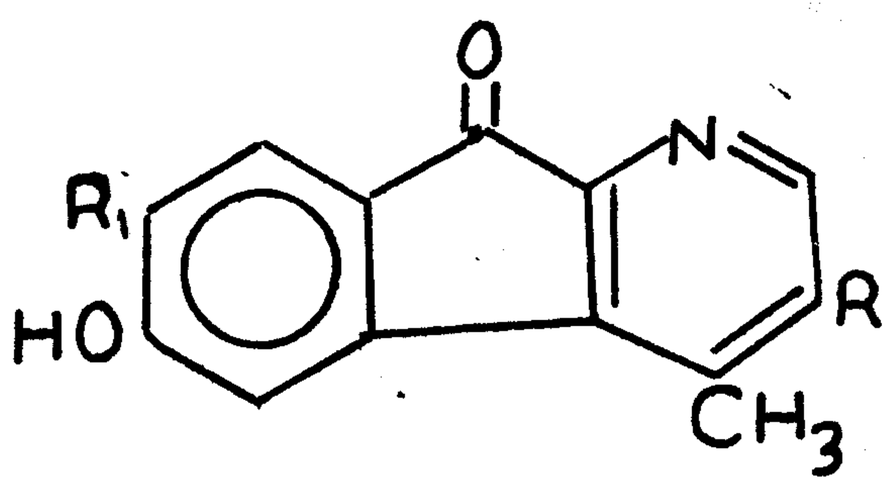
This work is a continuation to the phytochemical study of O.xylopiodes Diels.

Oxylopidine and oxylopinine, new alkaloids from Oxandra xylopioides Diels

RESULTS AND DISCUSSION

The ethanol extract of the air-dried powdered stem-bark and twigs of O.xylopioides Diels was subjected to acid–base fractionation using chloroform for extraction. The chloroform–free residue was fractionated into phenolic and non-phenolic alkaloid fractions as mentioned before[1]).

The phenolic fraction on chromatographing over silicic acid column, using chloroform and chloroform-methanol gradient afforded orange-red crystals(compound C)and yellow needles (Compound D). The two alkaloids(C andD) were found to be 1-aza-4-methylfluorenone) derivatives and given the trival names oxylopidine (I) and oxylopinine (II) respectively.



(I) R= R_1 = OCH_3

(II) R= R_1 =H

Compound C (Oxylopidine):

This compound (R_f 0.40; $CHCl_3$-MeOH-NH_4OH; 90:10:0.1) was isolated from the phenolic non-quaternary alkaloid fraction, as orange-red prisms (15 mg), mp 271-274°. The UV spectrum λ_{max} (MeOH) 223 nm (logξ 3.41), 252 (3.58), 267 (sh) (3.40), 300 (3.70), 334 (3.03), and 350 (sh) (2.88); bathochromic shift occurred upon the addition of base and acid suggested that the alkaloid was also similar to 1-aza-4-methylfluorenone derivative[2].

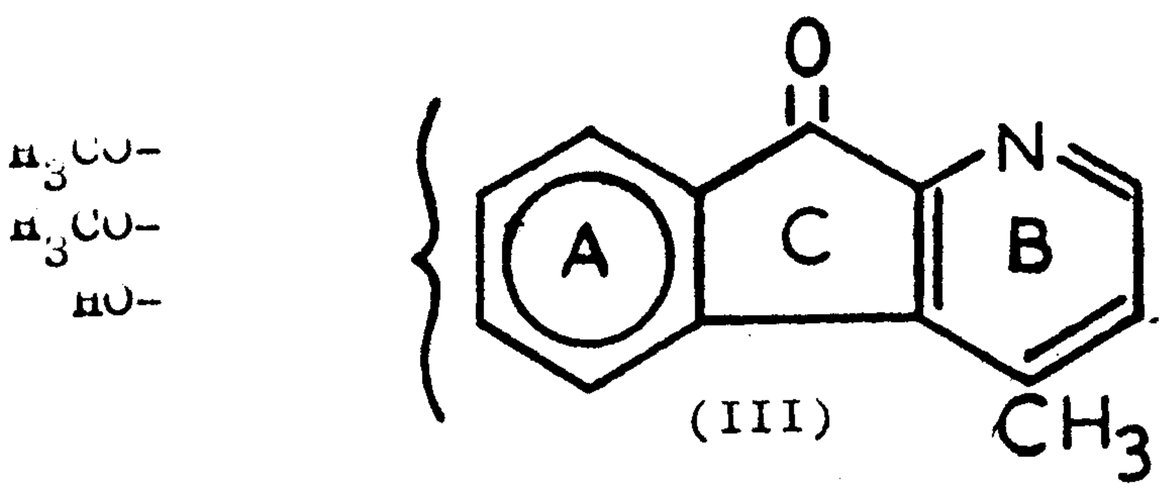
The infrared spectrum of Compound C indicated the presence of a phenolic hydroxy group (3440, and 1290 cm^{-1}), methoxyl groups (2840 and 1065 cm^{-1}), carbonyl function (1710 cm^{-1}), and aromaticity (1600, 1575,1485, and 1460 cm^{-1}).

The ^1H-nmr spectrum ($CDCl_3$ + CD_3OD) showed the presence of one aromatic methyl group at ∂ 2.50(3H,s), two metnoxyl groups appearing as one singlet at ∂ 3.94 (6H, s) and three aromatic protons at ∂ 7.15 (1H,s), ∂ 7.18 (1H,s), and ∂ 7.83 (1H,s), as listed in Table 1.

The mass spectrum of Compound C showed a molecular ion at m/z 271 (88%), and other significant fragments at m/z 256 (100%), 241 (11%), 228 (56%), 213 (29%), 212 (5%), 200 (6%), and 106 (11%) as shown in Table

2, and Fig. 1.

The above data suggested the presence of two methoxyl and one phenolic hydroxyl group substituted onto a 1-aza-4-methyl-fluorenone nucleus (III).



(III)

The ^{1}H-nmr spectrum showed three aromatic protons each occurring as singlet at 7.15, 7.18 and 7.83, suggesting that two substituents are possibly attached to ring A and to ring B.

Since the C-2 proton is reportedly the most downfield proton (imine proton), and the C-3 proton chemical shift did not exceed ∂ 7.2 (3,4), the signal located at ∂ 7.83 represented the C-2 proton. The presence of either a methoxyl or hydroxyl group on C-3, shielded the proton on the ortho position. Thus, the C-2 proton occurred relatively upfield (∂ 7.83) as compared to the other compounds in this series[4].

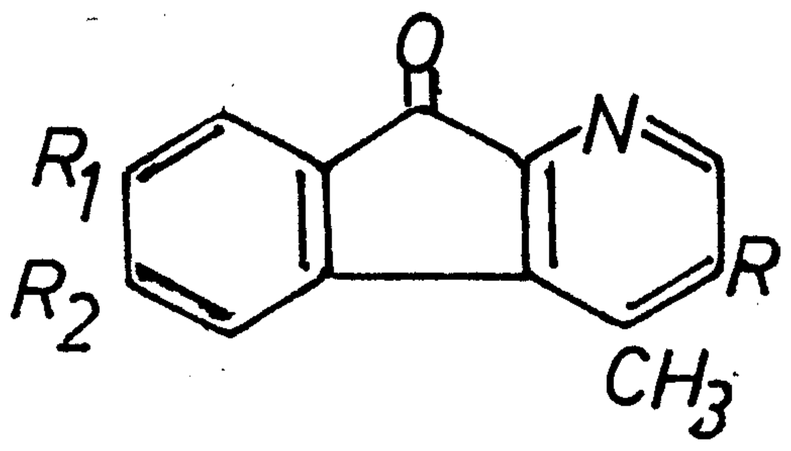
The above data indicated that Compound C had one

of three possible structures (IV), (V), (VI).

(IV) R_1 = OH, R=R_2 = OCH_3

(V) R_2 = OH, R=R_1 = OCH_3

(VI) R = OH, R_1=R_2 = CCH_3



An NOE experiment was helpful in locating the positions of the two methoxyl and one hdyroxyl group. Irradiation of the methoxyl signals (∂ 3.93), caused a visual enhancement of the aromatic protons at ∂7.18 and ∂7.83 (C-2). This indicated that these two protons were ortho to the methoxyl groups. Since the signal at ∂ 7.18 is the most downfield proton in ring, A, it was assigned to C-8. This is due to its proximity to the carbonyl group as compared to the C-5 proton (7.15). Thus, one methoxyl should be located at C-7, and the other methoxyl at C-3 position. Consequently, Compound C should be represented by proposed structure (I).

Compound D (oxylopinine) (II):

This compound (R_f 0.37; $CHCl_3$-MeOH-NH_4OH; 90:10:0.1)was isolated from the phenolic non-quaternary fraction. It was obtained as yellow needles (15 mg), mp 245-248°. The UV spectrum λ_{max}(MeOH) 203 nm (log ξ 3.45), 238(sh) (3.52), 246(3.59), 270 (sh) (3.41), 282(3.45), 293(3.38),

328(2.80), and 344 (2.57); bathochromic shift occurred upon addition of base or acid suggested that the Compound was phenolic, and was similar to 1-aza-4-methylfluorenone derivative[2].

The infrared spectrum of Compound D showed the presence of a phenolic hydroxyl group (3400, and 1290 cm^{-1}), a carbonyl function (1710 cm^{-1}), and aromaticity (1613, 1603, 1575 and 1480 cm^{-1}). The infrared spectrum of dihydro-Compound D(VII) showed the presence of broad band at 3420 cm^{-1} (OH), and the disappearance of a band at 1710 cm^{-1} (C=O). This was indicative of the conversion of a carbonyl function to an alcoholic hydroxyl group upon reduction, as shown in Fig. 2.

The mass spectrum of Compound D, showed the molecular ion at m/z 211 (100%). In addition, other important fragments were found at m/z 194 (1%), 183(17%), 155 (8%), 92(2%), 91(1%) and 76(2%), Fig. 3.

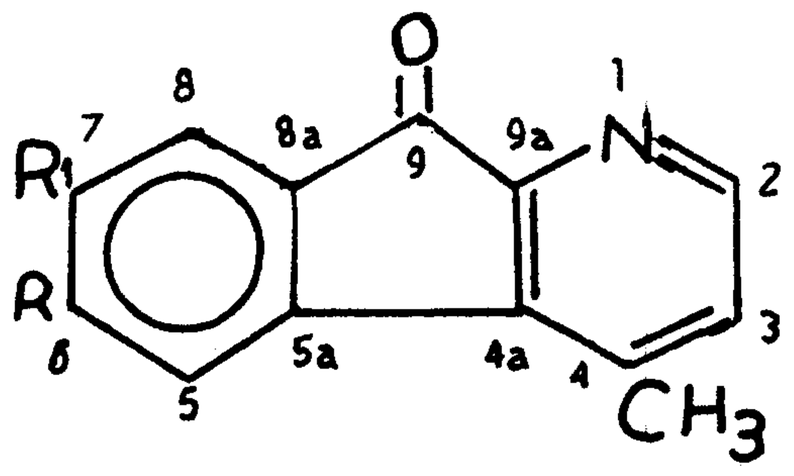
The $^{1}_{H}$-nmr spectrum of Compound D (Table 3) showed the presence of one aromatic methyl group at ∂ 2.60 (3H, s), and five aromatic protons. Two aromatic protons were ortho coupled and centered at ∂ 8.33 and ∂ 7.21 (J= 5.3 Hz), The downfield position for the proton at ∂ 8.33 and the coupling constant, indicated that these two aromatic protons were attached to C-2 and C-3 in Compound D, respectively[2]. Thus the three remaining

aromatic protons belonged to ring A. They showed a characteristic AMX pattern, one proton which showed ortho coupling was centered at δ 7.54 ($J_{8,7(XA)}$=7.9 Hz), the second which showed meta coupling was centered at δ 7.21 ($J_{5,7(MA)}$=2.2 Hz), and the third proton showing ortho and meta coupling was centred at δ 6.79 ($J_{7,8(AX)}$= 7.9 Hz and $J_{7,5(AM)}$=2.2 Hz) (Table 3).

The ^{1}H-nmr chemical shifts and multiplicity(AMX system) of the three aromatic protons, indicated that the phenolic hydroxyl group was located at one of two possible postitions (C-6 or C-7) in Compound D; as represented by the following two structures:(VIII),(IX).

(VIII) R=H,R_1 = OH

(IX) R=OH,R_1 = H



The ^{1}H-nmr spectrum of the dihydro-Compound (VII) (Table 4), indicated that the ortho coupled proton in ring A of Compound D (δ 7.54, d, J_{XA}=7.9 Hz) showed additional splitting in dihydro - Compound D(7.42, dd, J= 7.2, 2.6 Hz). In addition, a new signal appeared at

δ 3.62(1H, d, J= 2.6 Hz, C_9-H). These changes indicated that the aromatic proton at δ 7.42 in the dihydro-derivative was at C-8 and was ortho coupled to the C-7 proton (δ 6.79, d, $J_{7,8}$= 7.2 Hz) and allylic coupled to the C-9 proton (3.62, d, $J_{8,9}$=2.6 Hz) produced as a result of the reduction process; as shown in dihydro-Compound D (Fig. 2).

The chemical shift of the C-8 proton in Compound D (δ 7.54) was shifted moderately upfield (δ 7.42) in dihydro-Compound D. This was due to the prevention of a resonance effect upon the conversion of a carbonyl function to alcoholic function, as shown in Fig. 2. Finally, the C-5 proton in Compound D (δ 7.21), was shifted downfield (δ 7.67) in dihydro-Compound D. A structural model showed that this could be caused by the tilting of the cyclopentadienol ring (ring C), which moves the C-5 proton away from the shielding zone of the sigma bond between the carbon of the methyl group, and C-4 of ring B.

These data indicated that the hydroxyl group was present at C-6, and thus Compound D should be represented by structure (II).

EXPERIMENTAL

Plant material:

The plant material used in this study was prepared and identified as mentioned before[1].

Extraction and Isolation:

The plant material (stem-bark (1.51 kg), and twigs (1.07 kg) was extracted and fractionated as mentioned by the authors in a previous paper[1].

Isolation of Compound C (Oxylopidine):

Fraction 6 eluted with chloroform-methanol (99.5: 0.5) afforded an orange-red residue (0.140 g), which upon crystallization from methanol gave orange-red round crystals of oxylopidine. R_f 0.40 ($CHCl_3$-MeOH-NH_4OH) 90:10: 0.1) mp 271-274°, UV λ_{max}(MeOH) 223(log ε3.41), 252 (3.58), 267 (sh) (3.40), 300 (3.70), 334 (3.03), and 350 (sh) (2.88); λ_{max}(MeOH + OH^-) 230 nm (log ε3.49) 255 (3.60), 270 (sh)(3.47), 330 (3.70), and 370 (sh) (3.13) λ_{max} (MeOH + HCl) 252 nm (log ε3.58), 307(sh) (3.48), 320 (3.54), and 375 (3.27); ir ν_{max}(KBr) 3440, 2940, 2840, 1710, 1600, 1575, 1485, 1460, 1440, 1365, 1240, 1215, 1180, 1140, 1065, 1023, 960, 870, 798,753, 700, and 640 cm^{-1}; ^{1}H-nmr (90 MHz)($CDCl_3$ + $CDOD_3$, δ) 2.50 (3H, s, Ar-CH_3), 3.94 (6H, s,2xOCH_3), 7.15(1H,s,

Ar-H), 7.18 (1H,s, Ar-H), and 7.83 (1H, s, Ar-H)(NOE experiment: irradiation of the methoxyl signal 3.9,while monitoring the aromatic signals at δ 7.18 and δ 7.83).

ms, M^+ m/z 271 (88%), 257(12%), 256 (100%), 241(11%) 228 (56%), 213 (29%), 212 (5%), 200 (6%), 198(12%), 185 (21%), 170 (10%), 157 (13%), 136 (39%) 129 (21%), 115 (17%), 114 (25%), 106 (11%), 101 (28%) and 77 (15%).

Isolation of Compound D (Oxylopinine):

Fraction 10 eluted with chloroform-methanol (99:1) afforded a residue (0.090 g), which upon crystallization from methanol gave yellow needles (15 mg) of oxylopinine. R_f 0.37 ($CHCl_3$-MeOH-NH_4OH) 90:10:0.1) mp 245-248; λ_{max} (MeOH) 203 nm (log ε 3.45), 238 (sh) (3.52), 246(3.59), 270 (sh) (3.41), 282 (3.45),293 (3.38), 328 (2.80) and 344 (2.57); λ_{max}(MeOH + OH^-) 204 nm (log ε 3.72), 247 (3.56), 280 (3.15), 295(sh) (3.21), 303 (3.37),and 354 (2.98); λ_{max}(MeOH + HCl) 203 nm (log ε 3.50),240 (3.54), 246 (3.54) 284 (sh) (3.30), 296 (3.41), 300 (3.40), 343 (3.07), and 354 (3.08); ir ν_{max} (KBr) 3400, 3100, 3000, 1718, 1613, 1603, 1575, 1480, 1380, 1370, 1325, 1290, 1270, 1250, 1185, 1090, 908, 852, 802, 765, 753, 680, and 645 cm^{-1}; ^{1}H-nmr (90 MHz) ($CDOD_3$; δ) 2.6 (3H, s, Ar-CH_3), 6.79 (1H, dd, J_1 =2.2 Hz, J_2 = 8.3 Hz, Ar-H) 7.11 (1H, d, J=5.28 Hz. Ar-H), 7.21(1H,

d, J_1= 2.2 Hz, Ar-H), 7.54 (1H, d, J_2=8.3 Hz, Ar-H), and 8.33 (1H, d, J=5.28 Hz, Ar-H) (NOE experiments : Irradiation of the aromatic signals δ 6.79, or δ 8.33, while sharpened and monitoring the aromatic signals at δ 7.21 and δ 7.11; ms, M^+ m/z 211 (100%), 194 (1%) 183 (17%), 182 (7%), 155 (8%), 154 (17%), 153 (3%), 129 (3%) 127 (9%), 105 (2%), 101 (3%), 100 (3%), 92 (2%), 91 (1%), 77 (7%), and 76 (2%).

Preparation of Dihydro-oxylopinine:

Compound D (2.5 mg), was dissolved in dry ethanol (2 ml) and hydrogenated over 10% Pd/C (5 mg) at atmospheric pressure for three hours. The solution was filtered, and the Pd/C residue was washed with ethanol (2 ml x 3). The filtrate was evaporated to afford a yellowish-white residue (1.5 mg); R_f0.5 ($CHCl_3$-MeOH-NH_4OH) (90:10:0.1); λ_{max}(MeOH) 205 nm (log ξ 4.68) , 280 (sh) (3.98), 289 (3.86), and 315 (3.75); λ_{max} (MeOH + OH^-) 205 nm (log ξ 5.01), 243 (sh) (4.14), 289 (3.92), 300 (3.84), and 350; (3.50); λ_{max}(MeOH + HCl) 205 nm (log ξ 4.62), 220 (sh) (4.41), 308 (3.78), and 350 (3.65), ir ν_{max} (KBr) 3420 (br), 2930, 2860, 1610, 1510, 1465, and 1385; ^{1}H-nmr (90 MHz) ($CDOD_3$, δ) 2.47 (3H, s, Ar-CH_3), 3.62 (1H, d, J=2.6 Hz, C_9-H), 6.89 (1H, dd, J_1= 2.2 Hz, J_2= 7.2 Hz , Ar-H), 7.16 (1H, d, J_3= 5.2 Hz, Ar-H), 7.42 (1H,dd, J= 2.6 Hz, J_2= 7.2 Hz, Ar-H), 7.67 (1H, d, J_1= 2.2

Hz, J_2= 7.2 Hz, Ar-H), 7.16 (1H, d, J_3= 5.2 Hz, Ar-H), 7.42 (1H, dd, J= 2.6 Hz, J_2= 7.2 Hz, Ar-H), 7.67 (1H, d, J_1= 2.2 Hz, Ar-H) and 8.35 (1H, d, J_3= 5.2 Hz, Ar-H).

ACKNOWLEDGMENT

This investigation was supported by a Peace Fellowship grant from America-Mideast Education and Training Survices.

Table 1: ^{1}H-NMR chemical shift assignments for Compound C (Oxylopidine).

Proton	*Chemical Shift (δ)*
C-2	7.83
C-5	7.15
C-8	7.18
C_3-OCH_3	3.94 (6H, S)
C_3-OCH_3	
C_7-CH_3	2.50 (3H, S)
C_4-CH_3	

Table 2: Mass spectral fragmentation of Compound C (Oxylopidine)

m/z	*(Intensity)*	*Assignment*
271	(88%)	M^+
256	(100%)	$M^+ - CH_3$
241	(11%)	$M^+ - CH_2O$
228	(56%)	$M^+ - CH_3 - CO$
213	(29%)	m/z 241 - CO
212	(5%)	$M^+ - OCH_3 - CO$
200	(6%)	m/z 228 - CO
106	(11%)	$M^+ - CO - C_6H_9O_2$

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Table 3: ^{1}H-NMR chemical shift assignment of Compound D (Oxylopinine)

Proton	*Chemical Shift (δ)*
C_2	8.33 (d, J=5.30 Hz)
C_3	7.11 (d, J=5.30 Hz)
C_4-CH_3	2.60 (3H, s)
C_5	7.21 (d, J=2.2 Hz)
C_7	6.79 (dd, J=2.2Hz, J_1= 7.9 Hz)
C_8	7.54 (d, J_1=7.9Hz)

Table 4: ^{1}H-NMR chemical shift assignment of reduced Compound D (Dihydro-Oxylopinine).

Proton	*Chemical Shift (δ)*
C_2	8.35(1H,d, $J_{2,3}$=5.2Hz)
C_3	7.16(1H,d, $J_{3,2}$=5.2Hz)
C_4-CH_3	2.47(3H,s)
C_5	7.67(1H,d,$J_{5,7}$=2.2Hz)
C_7	6.89(1H,dd,$J_{7,5}$=2.2Hz, $J_{7,8}$=7.2 Hz)
C_8	7.42(1H,dd,$J_{8,7}$=7.2Hz, $J_{8,9}$=2.6 Hz)
C_9	3.62(1H,d,$J_{9,8}$=2.6Hz)

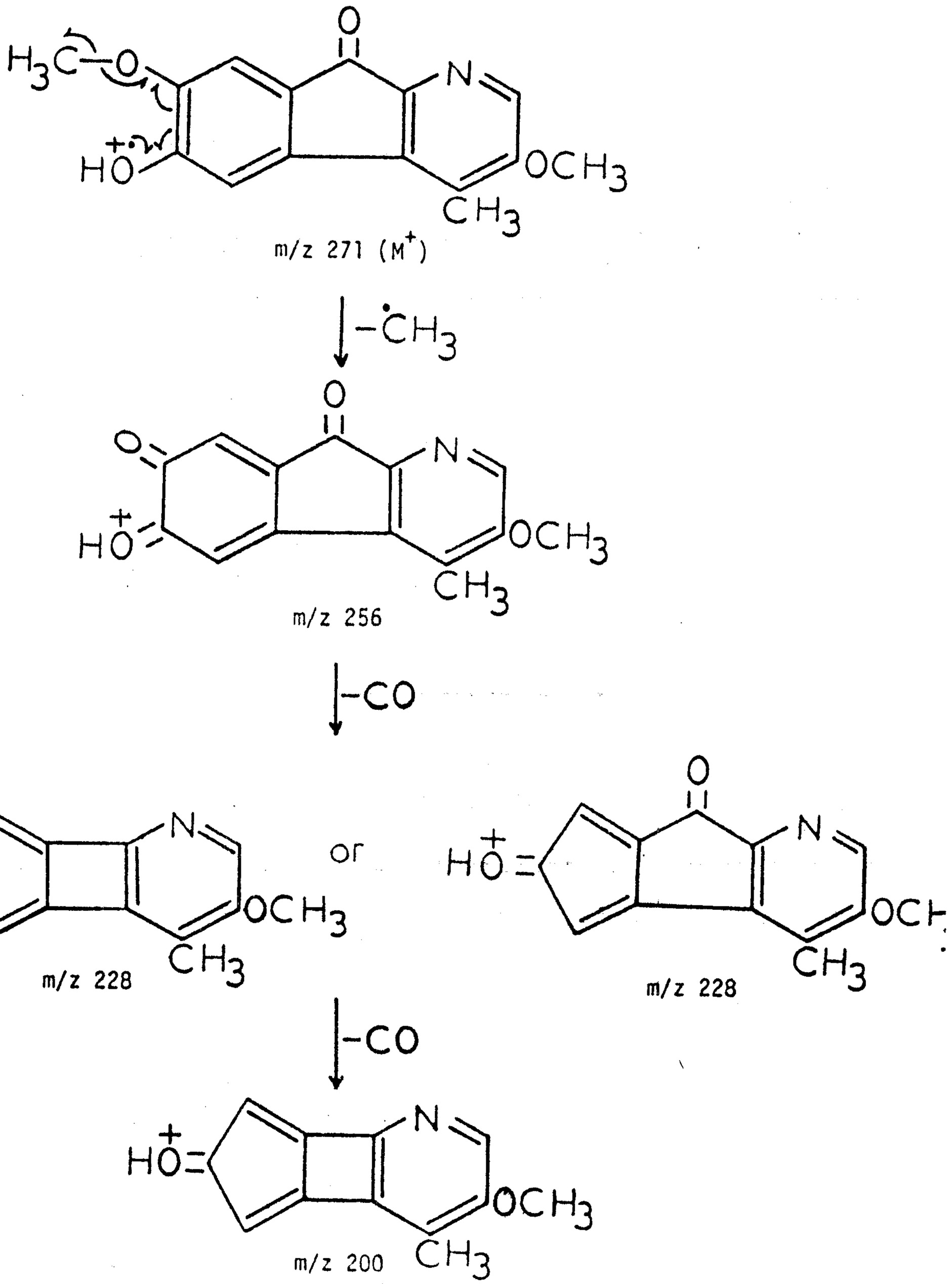
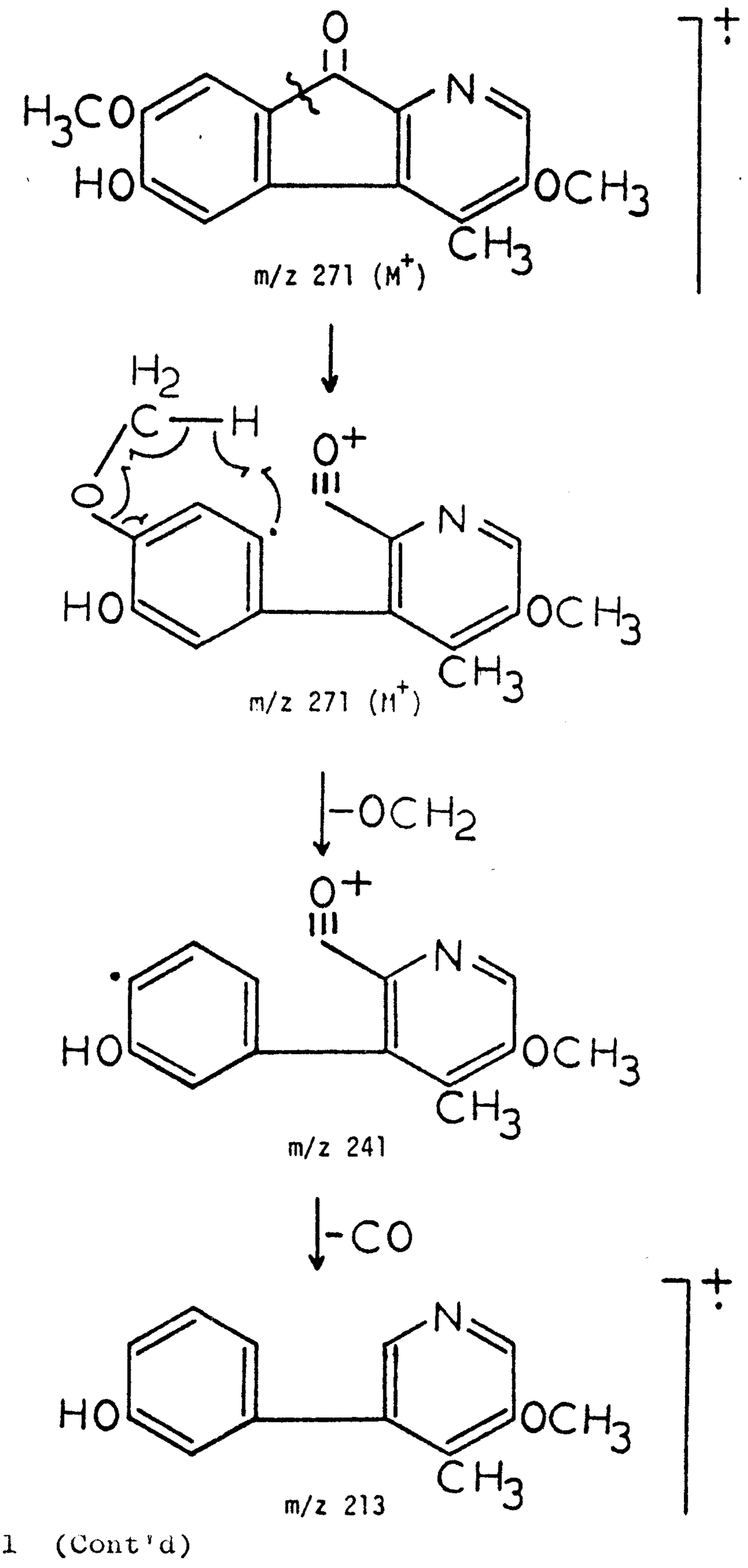


Fig. 1: A proposed mechanism for mass spectral fragmentation of compound C (Oxylopidine)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H3CO
HO
N
OCH3
CH3
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H2
C
H
O+
O
HO
N
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CH3
m/z 271 (M+)
-OCH2
O+
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m/z 241
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HO
N
OCH3
CH3
m/z 213

Fig. 1 (Cont'd)

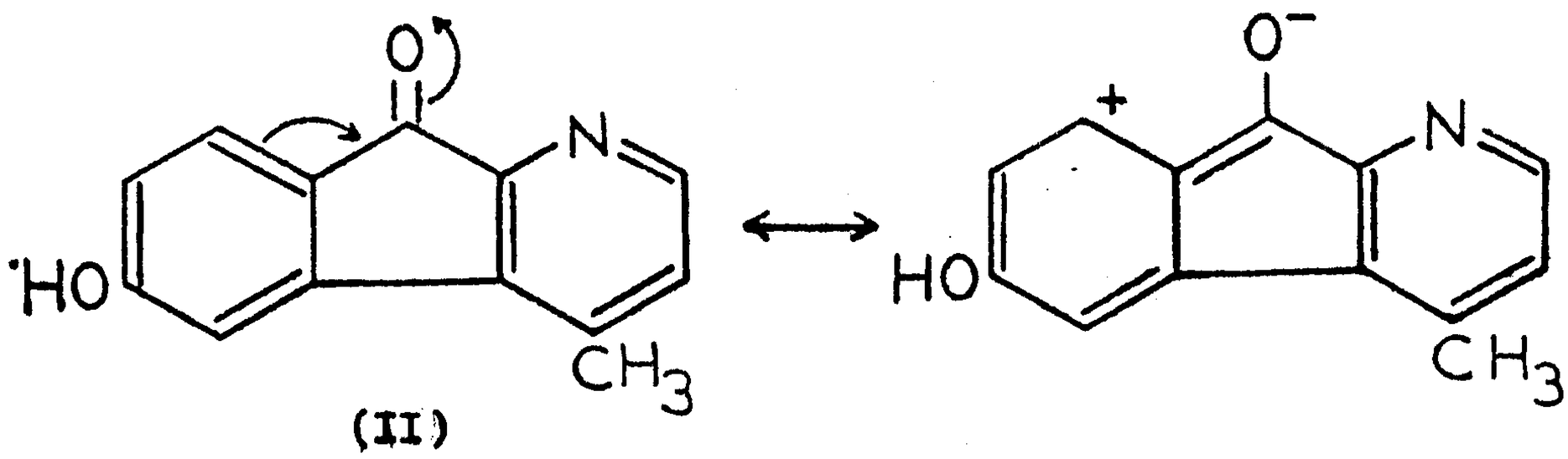

Fig. 2: Reduction of the compound D (Oxylopinine) (II)

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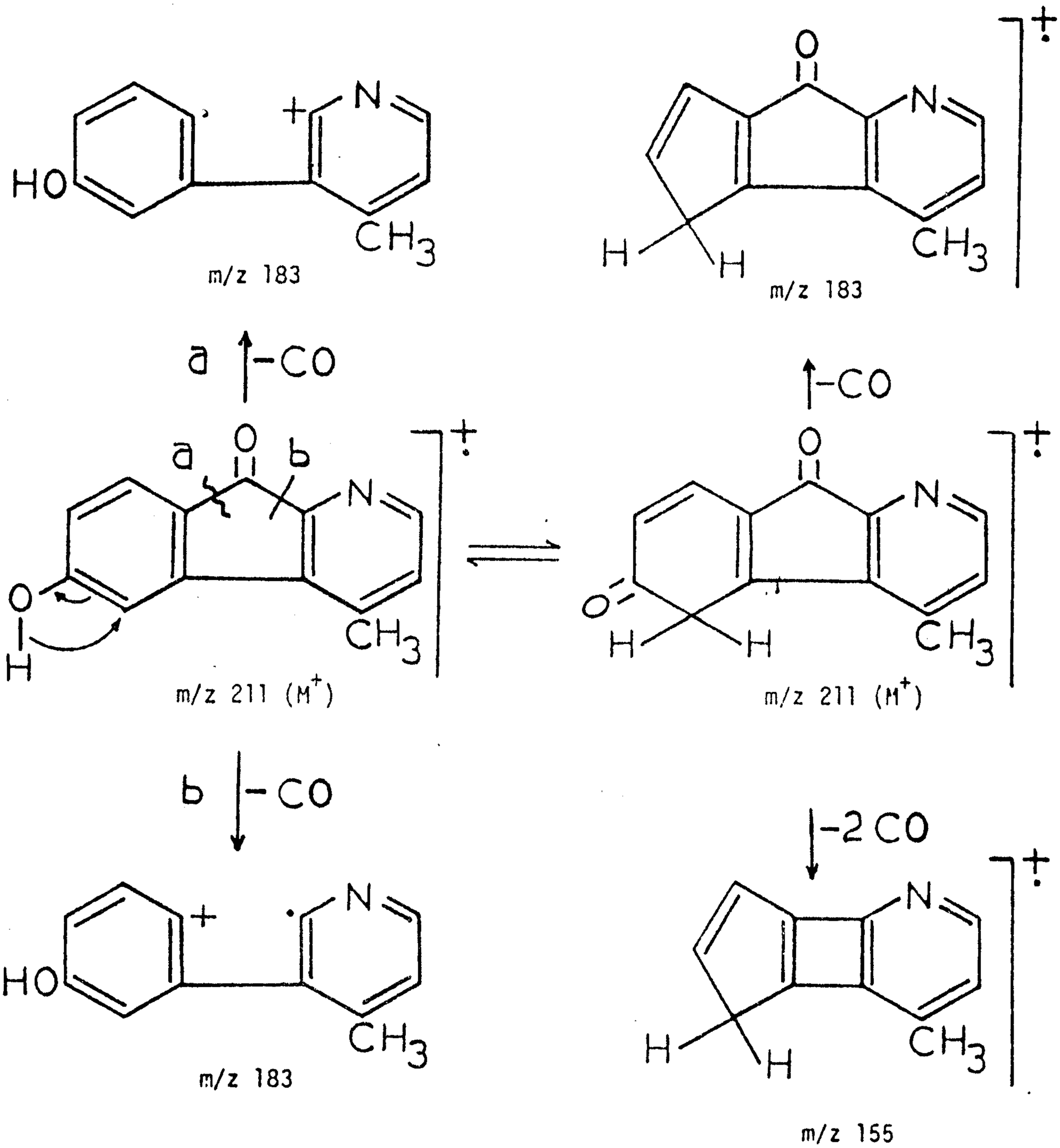

Fig. 3: A proposed mechanism of mass spectral fragmentation of compound D(Oxylopinine)

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اوكسيلوبيدين و اكسيلوبينين
قلويدات جديدة من نبات اوكساندرازيلوبيدز

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فى هذا البحث تم فصل قلويدين جديدين (اوكسيلوبيدين و اكسيلوبينين) من الجزء الفينولى من الخلاصة الكحولية لقلف السيقان و الافرع الصغيرة للنبات .

وتم فصل و التعرف و اثبات التركيب الكيميائى لهذين القلويدين باستعمال كروماتوجرافيا العمود و الطرق الكيميائية وكذلك طرق التحليل الالى المختلفة .

received in 11/5/1985 & accepted in 2/1/1986