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A NEW ALKALOID FROM OXANDRA XYLOPIOIDES DIELS

M.A. El-Shanawany

Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

D.J. Slatkin, P.L. Schiff and A. El-Shabrawy
Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pa., U.S.A.

ABSTRACT

A new alkaloid, Oxylopine(II). was isolated from the stem-bark and twigs of Oxandra xylopioides Diels and its structure was proved. In addition, an oxoaporphine alkaloid(Liriodenine) (I) was isolated. The isolated alkaloids were identified by chemical evidence and spectral analysis

INTRODUCTION

Oxandra xylopioides Diels (Annonaceae), is a tree distributed throughout the tropical regions of America and the West Indies[1]. It was reported that many members of family Annonaceae are of economic importance

and have many domestic uses[1,2,3,4]. Fruits of Annona squamosa, are commonly eaten, and the seeds possess poisonous and insecticidal properties[2]. Recently, it has been reported that Liriodenine (alkaloid isolated from some Annonaceae plants) has antitumor, antifungal and antimicrobial activities[5,6].

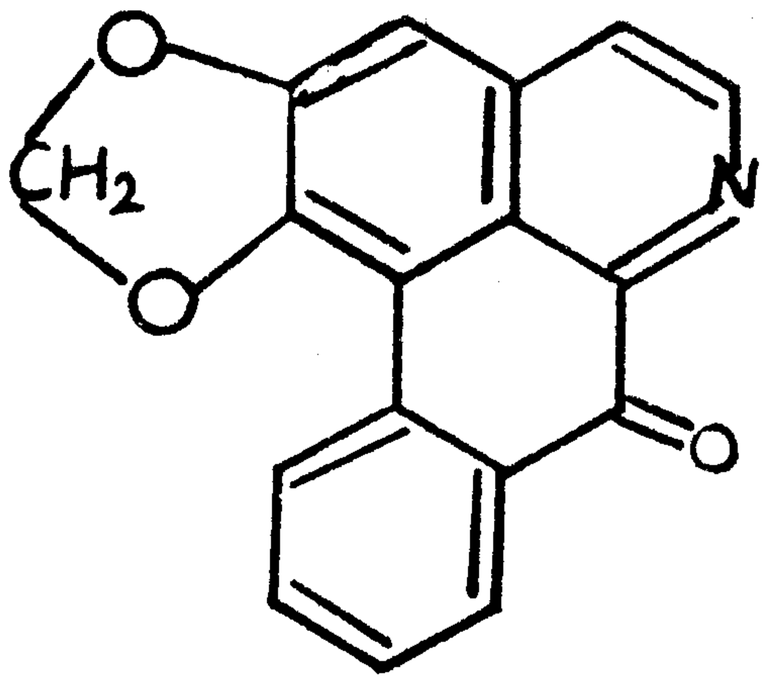
A search of the literature indicated that non of the species of Oxandra has been studied before.

RESULTS AND DISCUSSION

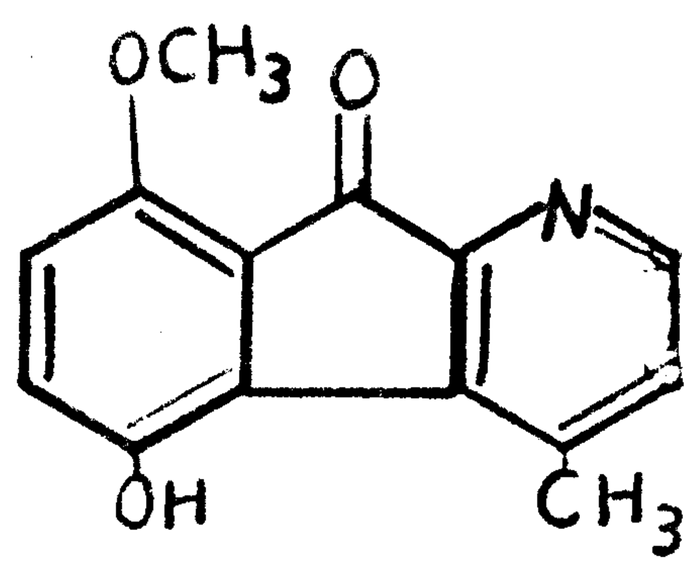
Dried ground stem-bark and twigs from O.xylopioides Diels, were extracted by percolation with 95% ethanol. The ethanolic extract was subjected to acid base fractionation using chloroform for extraction. The chloroform extract residue was fractionated into phenolic and non-phenolic alkaloid fractions. The non-phenolic fraction on chromatographing over silicic acid column afforded a crystaline compound (compound A), its mp, UV, Ir, ^{1}H-nmr and mass were found to be identical with those reported for Liriodenine (I)[7]. This represents the first reported isolation of this alkaloid

from Oxandra species.

The phenolic fraction on chromatographing over silicic acid column afforded yellowish crystals(compound B) and identified by mp, chemical evidence and spectral analysis as, Oxylopine (II). (1-aza-4-methyl fluorenone derivative)[8]



(I)



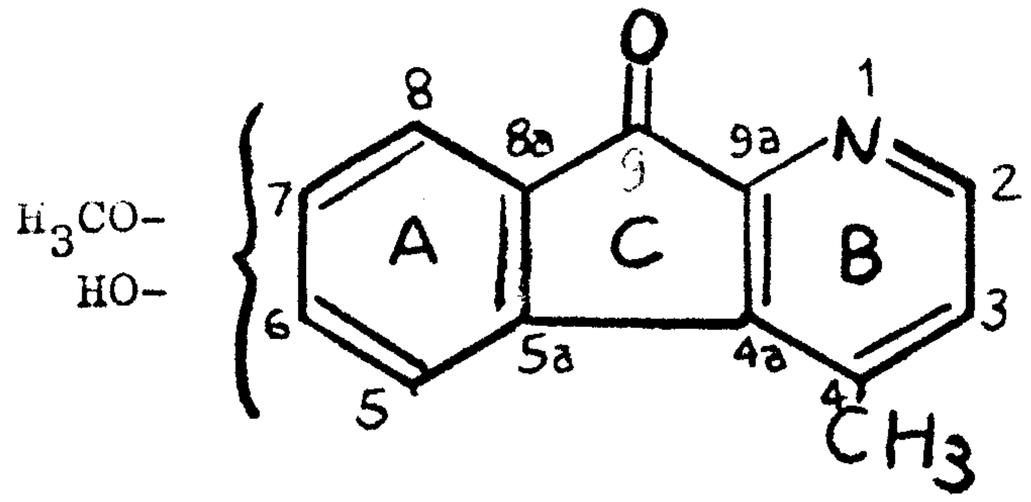
(II)

Compound B (Oxylopine) (II):

This compound (R_f 0.38 $CHCl_3$-MeOH-NH_4OH 90:10:0.1) was isolated from the phenolic alkaloid fraction. It was obtained as yellowish-white prisms (71 mg) mp 140-142°C. The UV spectrum showed a bathochromic shift on the addition of base indicating the phenolic character of the alkaloid, and a bathochromic shift with

acid indicating the presence of imine function[9]. The ir spectrum of compound B confirmed the presence of phenolic OH-group (3500 and 1275 cm^{-1}) and C=O group (1710 cm^{-1}). The ir spectrum of the O-acetyl dertivative confirmed the presence of phenolic OH-group which became an aromatic ester (1765 cm^{-1}).

The ^{1}H-nmr spectrum ($CDCl_3$) indicated the presence of one aromatic methyl group at δ 2.63 (3H,S), one methoxyl group at δ 4.21 (3H,S), and four aromatic protons. Two aromatic protons at δ 6.9 and δ 7.45, showed ortho coupling (J=7.9 Hz), while the other two aromatic protons, 6.94 and 8.47 were also ortho coupled (J=5.7 Hz). These two last aromatic protons represented the C-3 and C-2 protons (ring B). Thus, ring B had no additinal substitution, and the methoxyl and hydroxyl groups must be located in ring A as indicated (III)



(III)

Since the two aromatic protons in ring A appeared as an ortho coupled doublet, the methoxyl and hydroxyl groups attached to that ring A are either ortho or para oriented.

Irradiation of δ4.21 (OCH_3) caused a visual inhancement of the aromatic proton at δ6.94. This proved that the position ortho to the methoxyl group was unsubstituted. The absence of perihydroxy proton signal in ^{1}H-nmr and the downfield position of the methoxyl signal, indicated that it was located near and resonated with the C=O group as illustrated in Fig. 1.

The ^{1}H-nmr of the O-methyl derivative of compound B showed another methoxyl group at δ 3.96 (3H, S). An NOE experiment on this derivative supported the proposal structure (II) for compound B.

The ^{13}C-nmr ($CDCl_3$), showed that the carbon skeleton of compound B consisted of 14 carbons and the downfield chemical shift of the methoxyl group (δ 62.6) confirmed that it was located at C-8 and resonated with the carbonyl group (Fig. 1). The ^{13}C-chemical shift assignments are shown in Table 1.

The mass spectrum of compound B showed molecular ion at m/z 241 (73%) and other important fragments are illustrated in Table 2 and Fig. 2.

The UV, ir, ^{1}H-nmr, ^{13}C-nmr and mass spectral data were consistent with proposed structure (II) for compound B which is given the trival neme oxylopine. Since this new alkaloid is phenolic, it might be expected to possess antimicrobial and/or antifungal activities.

EXPERIMENTAL

Plant Material:

The plant material used in this study consisted of the air-dried stem-bark and twigs of Oxandra xylopioids Diels which was collected and identified in May, 1979 (in Peru). A herbarium specimen is deposited at the National Arboretum, U.S.D.A., (No. PR-51790). The sample was reduced to fine Powder.

Extraction and Isolation:

The plant material (stem-bark (1.51 kg), and twigs (1.07 kg) was extracted to exhaustion with 95% ethanol. The ethanol extract of the stem bark and of twigs, showed essentially identical composition upon TLC. Thus the extracts were combined together (934 g) (concentrated under reduced pressure), digested with 5% citric acid, filtered and the filtrate was then washed

with chloroform. The acidic aqueous solution was rendered alkaline with NH_4OH (PH 8) and exhausted with chloroform . The chloroform extract was concentrated under reduced pressure. The obtained residue (27 g) was treated with 5% aqueous NaOH and extracted with chloroform to get the non-phenolic alkaloid fraction (22 g). The aqueous solution was treated with HCl(PH 3-5) and extracted with chloroform to get the phenolic alkaloid fraction (3.3 g).

The non-phenolic alkaloid fraction (22 g)was fractionated over silicic acid (300 g) (100 mesh Mallinckrodt). Elution was started with chloroform. then chloroform-methanol gradient. Fractions (500 ml each) were analysed for the alkaloidal content by TLC screening.

Isolation cf Compound A (Liriodenine):

Fractions (12:14) eluted with chloroform-methanol (99:2) showed single spot on TLC screening. The fractions were collected and concentrated under reduced pressure where a greyish residue was obtained. The residue was dssolved in methanol and left for crystallization where white crystals were obtained (compound A) and proved to be Liriodenine through the following:

Compound A, R_f 0.41 ($CHCl_3$-MeOH 90:10), mp. 282-283°C, UV λ_{max} (MeOH) 247 nm (log ε 4.22), 268(4.15) and 303 (3.7); λ_{max} (MeOH + 0.1N HCl) 256 nm (log E 4.32) 278 (4.24) and 334 (3.7); ir (KBr) 2940 cm^{-1}, 1651, 1600, 1580, 1490, 1042, 953 and 650 cm^{-1}; ^1H-nmr (90 MHz) (CD_3OD,) 6.72 (2 H,S $-O-CH_2-O$), 7.61(1 H, S, Ar-H), 7.69-8.79 (6 Ar-H,m); ms, M^+ m/z 275 (100%), 246 (35%), 219 (9%), 188 (53%), 162 (20%), 113 (16%), 94 (28%) and other peaks.

The phenolic alkaloid fraction (3.6 g) was dissolved in chloroform-methanol (1:1) and adsorbed silicic acid (3 g) and chromatographed over silicic acid column (100 mesh Mallinckrodt). Elution was started with chloroform, and chloroform-methanol gradient as shown in Table 3. One compound was isolated, compound B, Oxylopine. The remaining of the fractions was kept for further study.

Isolation of Compound B (Oxylopine):

Fraction 4 (Table 3) afforded a yellowish residue (0.071 g), which upon crystallization from petroleum-ether gave pale yellow to yellowish white prisms of Oxylopine. R_f 0.38 ($CHCl_3$-MeOH) (90:10) and 0.62 ($CHCl_3$-MeOH) (80:20), mp 140-142°.

UV spectrum λ_{max} (MeOH) 204 nm (log ε 4.04), 243(sh) (4.05), 250 (4.11), 280 (sh) (3.77), 289 (3.79), 300

(3.73), and 355 (2.73) λ_{max}(MeOH + OH^-) 206 nm (logϵ 4.43), 252 (4.06), 300 (3.66), and 320 (3.69); λ_{max} (MeOH + HCl) 206 nm (log 4.43), 243 (3.86), 250 (sh) (3.83), and 317 (3.88); ir ν_{max}(KBr) 3450, 1710, 1600, 1565, 1490, 1440, 1380, 1320, 1275, 1255, 1235, 1200, 1115, 1075, 1010, 940, 880, 840, 815, 800, 740 and 720 cm^{-1}; ^{1}H-nmr (90 MHz) ($CDCl_3$, δ) 2.63 (3H, S, Ar-CH_3), 4.21 (3H, S, OCH_3), 6.94 (1H, d, J= 7.9 Hz, Ar-H), 6.95 (1H, d, J=5.7, Ar-H), 7.45 (1H, d, J=7.9 Hz, Ar-H), and 7.47 (1H, d, J=5.7, Ar-H); {NOE experiments: irradiation of the methyl signal (δ 2.63), while monitoring the signal of the aromatic proton at δ 6.95, and irradiation of the methoxyl signal (δ 4.2), while monitoring the signal of the aromatic proton at δ 6.94). ^{13}C-nmr ($CDCl_3$, δ) (INEPT) 17.2, and 62.57 (2 x 1^o), 116.3, 121.5, 125.4 and 152.3(4 x 3^o), 127, 128.7, 133.5, 142.5, 147, 155.9, 163.5, and 191.5(8 x q);ms, M^+ m/z 241 (73%.). 223 (84%), 212 (67%), 198 (29%), 195 (60%), 183 (74%), 167 (29%), 154 (49%). 140 (30%), 127 (30%), 115 (34%), 91 (25%), 90 (7%) and 77 (100%).

Preparation of O-Acetyl Compound B (O-Acetyloxylopine):

Acetic anhydride (1 ml) was added to a solution of Compound B (10 mg) in pyridine (1 ml). The mixture was allowed to stand at room temperature for 24 hours. The solution was chilled and diluted with cold distilled

water (2 ml). The resulting aqueous solution was basified with concentrated ammonium hydroxide to pH 8 and extracted with chloroform (5 x 10 ml). The chloroform extracted were combined, dried over anhydrous sodium sulfate, filtered and evaporated ro afford an amorphous residue (7 mg), ir λ_{max}(KBr) 2930, 2860, 1765, 1710, 1595, 1560, 1470, 1430, 1375, 1280, 1270, 1250, 1208, 1160, 1075, 1020, 960, 940, 900, 875, 840, 810, and 725 cm^{-1}; ^{1}H-nmr (60 MHz) ($CDCl_3$, δ)2.38 (3H, s,O-C(=O)-CH_3); 2.64 (3H, s, Ar-CH_3), 6.95 (1H, d, J=5.8 Hz, Ar-H), 7.05 (1H, d, J=7.5 Hz, Ar-H), 7.48(1H,d, J=7.5 Hz, Ar-H), and 8.48 (1H, d, J=5.8 Hz, Ar-H); ms, M^+ m/z 283 (4%), 241 (100%), 223 (98%), 212 (67%), 195 (37%), and 183 (50%).

Preparation of O-Methyl Compound II(O-Methyloxylopine):

Ethereal diazomethane (5 ml) was added to compound II (10 mg), and the mixture kept at room temperature overnight. Following evaporation, the residue was crystallized from methanol to afford yellow needles (7 mg) (R_f 0.67) (chloroform-methanol-ammonium hydroxide)(90:10:0.1); mp 148-150°; uv λ_{max}(MeOH) 204 nm (log ε 3.58),244 (sh)(3.65), 250 (3.71), 277 (sh) (3.36), 287 (3.37), 299 (3.30), and 350 (3.03); λ_{max} (MeOH + OH^-) no change; λ_{max}(MeOH + HCl) 205 nm (log ε 3.63), 225(3.51), 250 (3.45), 314(3.51) and 370 (sh) (2.88); ir ν_{max}(KBr)

2930, 2850, 1710, 1635, 1610, 1555, 1500, 1490, 1470, 1435, 1365, 1290, 1270, 1242, 1200, 1168, 1140, 1080, 1050, 1030, 1005, 960, 915 , 870 , 845 , 825 , 790 , and 725 cm^{-1}; ^{1}H-nmr (90 MHz)($CDCl_3$, δ) 2.72 (3H, s, Ar-CH_3), 3.97 (3H, s, OCH_3), 4.22 (3H, s, OCH_3), 6.94 (1H, d, J=7.9 Hz, Ar-H), 7.17 (1H, d, J=5.7 Hz, Ar-H), 7.48 (1H, d, J=7.9 Hz, Ar-H), and 8.71 (1H, d, J= 5.7 Hz, Ar-H) {NOE experiment: irradiation of the methoxy signals (δ 4.0) while monitoring the aromatic signals at δ 6.94 and 7.48}. ms, M^+ m/z 255 (32%), 254(33%), 241 (7%), 240 (59%), 238 (18%), 226 (83%), 224 (47%), 211 (42%), 209 (100%), 196 (31%), 195 (69%), 183 (55%), 166 (56%), 154 (28%), 141 (26%), 129 (54%), 115 (11%), 92 (6%), 90 (29%) and 77 (18%).

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Table 1: ^{13}C-nmr chemical shift assignments for compound II (Oxylopine).

Carbon Atom	*Chemical Shift ($CDCl_3$, δ)*	*Carbon*	*Chemical Shift ($CDCl_3$, δ)*
2	152.3	3	125.4
4	142.5	5	147
6	121.5	7	116.3
8	155.9	9	191.5
4a	133.5	5a	128.7
8a	127	9a	163.5
C_8-OCH_3	62.5	C_4-CH_3	17.2

Table 2: Mass spectral fragmentation of compound II (Oxylopine).

m/z (Intensity)	*Assignment*
223 (84 %)	$M^+ - H_2O$
213 (9 %)	$M^+ - CO$
195 (60 %)	$M^+ - H_2O - CO$
183 (74 %)	$M^+ - CO - OCH_2$
91 (25 %)	$M^+ - CO - C_7H_6O_2$
90 (7 %)	$M^+ - CO - C_7H_7O_2$

Table 3: Column chromatography of the phenolic fraction (3.36 g).

Fraction	*Solvent*	*Volume (L)*	*Weight (mg)*
1	Chloroform	0.60	52
2	Chloroform	0.10	--
3	Chloroform	0.10	265
4	Chloroform	0.10	71
5	Chloroform-methanol(99.5:0.5)	0.30	125
6	Chloroform-methanol(99.5:0.5)	0.30	140
7	Chloroform-methanol(99.5:0.5)	0.30	42
8	Chloroform-methanol(99:1)	0.40	466
9	Chloroform-methanol(99:1)	0.40	100
10	Chloroform-methanol(99:1)	0.20	90
11	Chloroform-methanol(98:2)	0.40	220
12	Chloroform-methanol(98:2)	0.40	64
13	Chloroform-methanol(96:4)	0.30	159
14	Chloroform-methanol(96:4)	0.25	104
15	Chloroform-methanol(96:4)	0.30	231
16	Chloroform-methanol(94:6)	1.00	276
17	Chloroform-methanol(92:8)	1.00	109
18	Chloroform-methanol(90:10)	1.00	197
19	Chloroform-methanol(85:15)	1.00	102

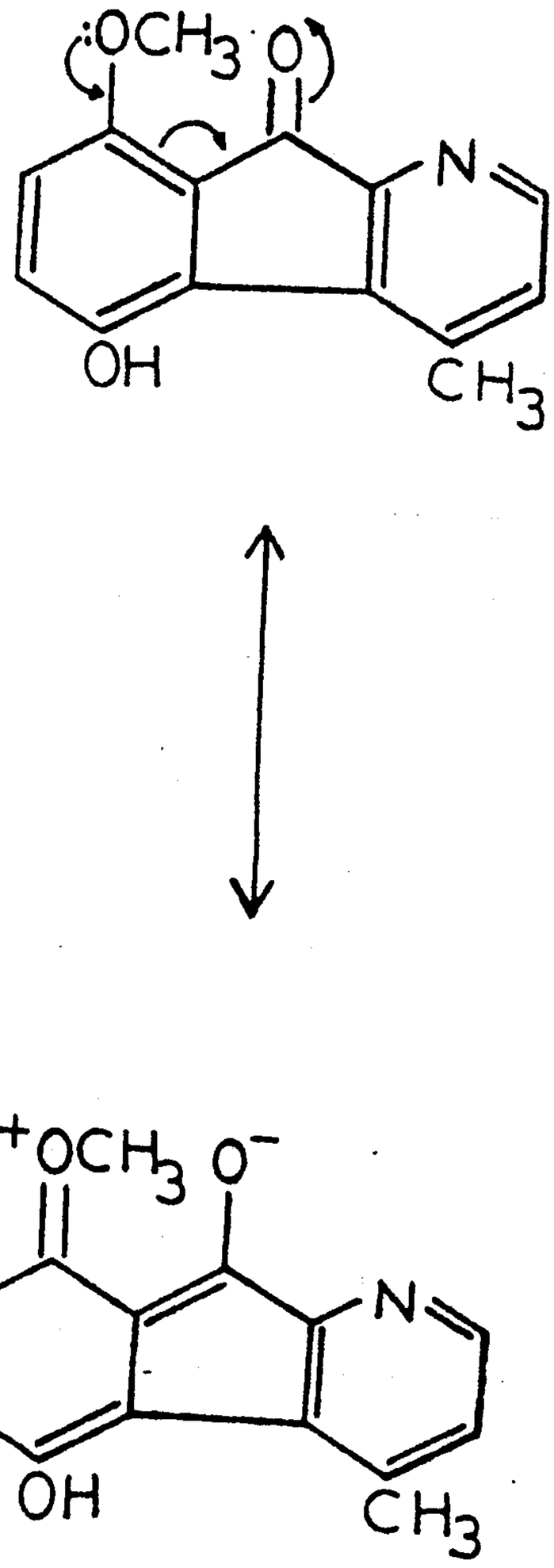

Fig. 1: A proposed resonance structure for Compound B

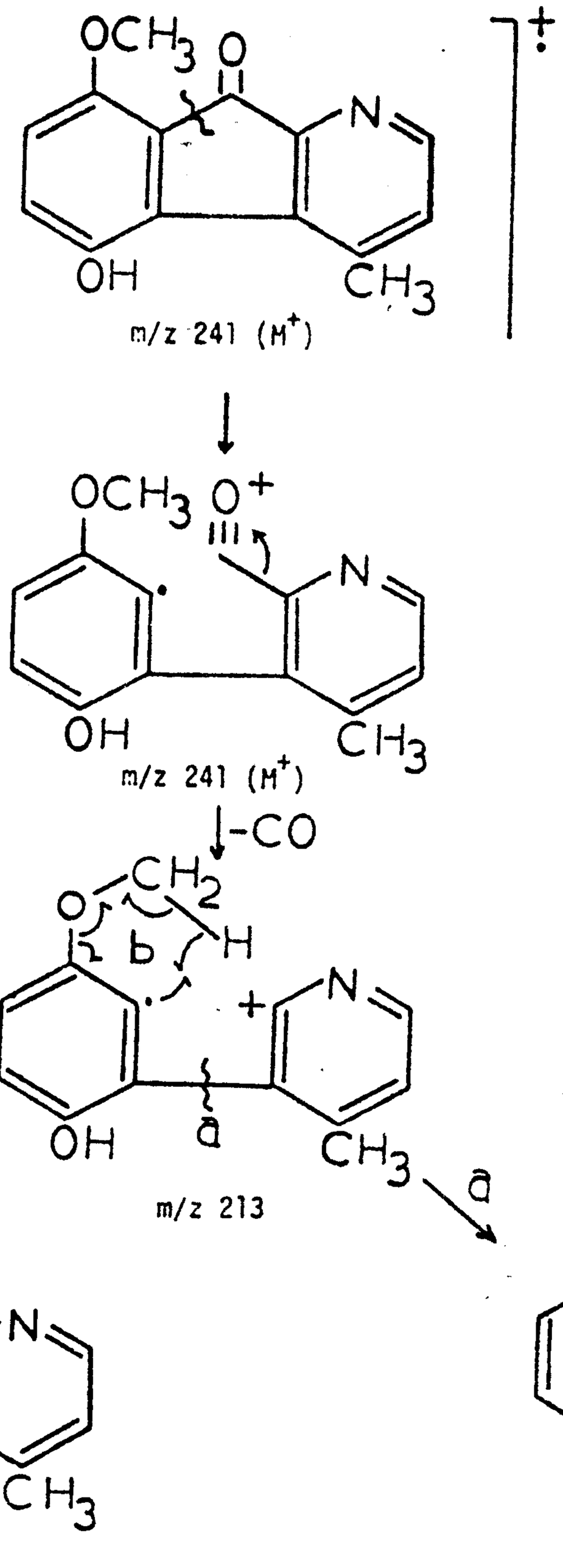

Fig. 2: A proposed mechanism for mass spectral fragmentation of Compound B (Oxylopine)

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قلويد جديد من نبات اوكساندرازيلوبويدر

محمد أحمد الشنوانى
قسم العقاقير - كلية الصيدلة - جامعة أسيوط
ديفيد اسلاتكن - بول شيف - وعبد الرحمن الشبراوى
قسم العقاقير - كلية الصيدلة - جامعة بتسبرج - بنسلفانيا

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