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PHYTOCHEMICAL STUDY OF MURRAYA EXOTICA L. CULTIVATED IN EGYPT

II-Coumarins of the Leaves.

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ABSTRACT

Five coumarins were isolated from the methylene chloride fraction of the methanol extract of the leaves of Murraya exotica L. Cultivated in Egypt and identified as; (±) erythro-murrangatin, (-) minumicrolin, Murralongin, 7-methoxy-8 (2-hydroxy-3-ethoxy-3-methyl-butyl) coumarin and murraxocin.

The identification and structure determination are based on physical, chemical and spectral studies including UV, IR, ^{1}HNMR, ^{13}CNMR and MS analyses. Three of them were shown to be either enantiomer to known derivatives or new. The other two coumarins were identified as murralongin and murraxocin.

The antimicrobial and cytotoxic activities of the isolated compounds were studied.

INTRODUCTION

The roots of Murraya exotica L. (Rutaceae) are used as a pain-killer and the leaves and bark of the plant in the treal-ment of diarrhoea and dysentery[1]. The plant is known to cont-ain several coumarins[1-12], carbazoles[13-14] and flavonoids[15,16]. The coumarins isolated from this species are : 5,7-dimethoxy-6-(2',3'-dihydroxy-isopentyl) coumarin; mexoticin[2], 8-isopentyllimettin[3] ;5,7-dimethoxy-8- (2'- isopentyl) coumarin;coumurryin[4], 7-methoxy-8- (3'-butenyl-3'-methyl-2'-oxo) coumarin[5], Murralongin[6],

murrangatin[6] , meranzin hydrate[6] ,7-methoxy-8-(1',2'-epoxy-3'-methyl -3'-butenyl) coumarin; phebalosin[7] , imperatorin[8], 7-methoxy-8-(1'-methoxy-2'-hydroxy-3'-methyl- Δ^3 butenyl) coumarin[9] , 7-methoxy -8-(2'-methoxy-3'-hydroxy-3'-methyl butyl) coumarin[9] , 7-methoxy-8-(2',3'-dihydroxy-3'-methyl butyl) coumarin[9] , 7-methoxy-8-(2'-isovaleryloxy-3'-hydroxy-3'-methyl butyl) coumarin; murrayatin[10]; 7-methoxy-8- (1'-acetoxy-2'-oxo-3'-methyl butyl) coumarin; hainanmurpanin[11], 7-methoxy-8-(1'-ethoxy-2'-hydroxy-3'-methyl-but-3'-enyl) coumarin; murraxocin[1], and auraptenol[12].

In a previous paper we reported the isolation of several methoxylated flavonoids from the carbon tetrachloride fraction of the methanolic extract of the leaves[17].

The present communication deals with the isolation and structure elucidation of coumarins from methylene chloride fraction of the methanolic extract of the leaves and evaluation of their biological activity.

EXPERIMENTAL

General experimental procedures:

Melting points are uncorrected, IR spectra were taken as KBr pellets with a Perkin Elmer (Model 457) instrument. NMR spectra were determined in $CDCl_3$ at 400 MHz with a Brucker (Model GX 400) instrument, the chemical shifts are given in ppm. Mass spectra were recorded in Finnigan 4000, E.L. spectrometer operating at 70 ev. ^{13}C NMR spectra were carried out in DMSO or $CDCl_3$ using TMs as internal standard with a Brucker-Physik (Model WP 80) instrument. $[\alpha]_D$ values were taken in $CHCl_3$ (Perkin-Elmer polarimeter, Model 241). Column chromatography : silica gel (E.Merck) was used : TLC : silica gel G with the solvent systems listed in Table 1.

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Plant Material :

The leaves of M. exotica L. were collected in October 1984 from plants growing in the Botanic Island in Aswan. Identity of the plant was confirmed by Mr. Ismail A. Mousa, the director of the Botanic Island of Aswan. The leaves were air-dried, reduced to No. 40 powder and kept in well-closed containers.

Extraction :

The concentrated methanolic extract of (3 Kg.) of the defatted air-dried powdered leaves was successively extracted with cyclohexane, carbon tetrachloride, methylene chloride, ethyl acetate and n-butanol.

The concentrated methylene chloride fraction (35 g.) was tested for coumarins[2,18] and showed positive results.

TLC of this extract using silica gel G and benzne-acetone (3:2) as a developer, revealed the presence of 13 spots.

10 g. of the dried methylene chloride fraction were chromatographed over silica gel column (4.3. x83, 420 g.) and eluted with benzene-methanol (85 : 15). Fractions of 10 ml separately were collected and tested by TLC as mentioned before. Each group of similar fractions containing a single spot was concentrated under vacuum. Fractions (82-100; R_f 0.75) gave 117mg. residue, crystallised from pet. ether-acetone (1:1) as needle-shaped crystals, labelled compound 1 . Fractions (38-50, 0.87) gave 68 mg. residue, crystallised from acetone as needle-shaped crystals, labelled compound 2. Fractions (114-136, Rf 0.68) gave 84.6 mg. oily creamy residue labelled compound 3. Fraction (64-81) gave 1.21 g. residue showed on TLC two major compounds besides traces of other two, was subjected to a series of small silica gel columns, using chloroform, chloroform-methanol in increasing polarities when two compounds were isolated as needle-shaped crystalline material from pet. ether, labelled compound 4, (Rf 0.82) and as prisms from methanol labelled compound 5 (Rf 0.78).

The isolated compounds (1-5) are characterised by:

a) No bathochromic shift on addition of NaOH solution.

b) Did not produce any colouration with ferric chloride.

This indicates the absence of a phenolic (OH) function in the isolated compounds. The physical and chromatographic characters are given in Table 1.

^{1}H NMR and ^{13}C NMR data are given in Tables 2 & 3 respectively,

$[\alpha]_D^{20}$, UV , IR and MS spectra of the isolated compounds :

COMPOUND 1 :

$[\alpha]_D^{20}$ = + 3 (C = 0.62, $CHCl_3$)

UV λ_{max} (log ϵ) (MeOH) nm 323;(4.24). 258 (3.27), 248 (3.28). 231 (3.28) and 220 (3.80).

$\bar{\nu}$ max 3490, 1713, 1603-1595 and 910 cm^{-1}

MS spectrum showed significant peaks at m/z 206 (24), 205 (100) 191 (6), 175 (10) and 162 (6).

COMPOUND 2 :

$[\alpha]_D^{20}$ = -17 (C = 0.25, $CHCl_3$).

UV λ_{max} (log ϵ) (MeOH) nm:323 (4.24), 258 (3.27), 248 (3.28) 231 (3.28) and 220 (3.80).

$\bar{\nu}$ max 3490, 1713, 1605, 1565, and 910 cm^{-1}.

MS spectrum showed significant peaks at m/z 206 (18), 205 (100) 191 (3), 175 (8), 162 (5), 72 (48), 53(65) and 43 (50).

COMPOUND 3 :

$[\alpha]^{20}$ = -52 (C = 0.90 , $CHCl_3$).

UV λ_{max} (log ϵ) (MeOH) nm 323 (4.02), 258 (3.57). 248 sh. (3.49) and 220 (3.92).

$\bar{\nu}$ max 3560, 1710, 1603 and 1560 cm^{-1}.

MS : M^+ at m/z 306, 261 (2), 220(65), 219 (15), 87 (100) and 59(84).

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COMPOUND 4 :

UV λ_{max} (log ϵ) (MeOH) nm:318 (4.16). 235 (4.17) and 229(4.15).
$\bar{\nu}$ max 1725, 1665, 1605, 1575, and 1500 cm^{-1}.
MS : M^+ at m/z 258 (100), 215 (67) and 205 (91).

COMPOUND 5 :

$[\alpha]_D^{20}$ = + 44 (C = 0.65, $CHCl_3$).
UV λ_{max} (log ϵ) (MeOH) nm:322 (4.25) , 258 (3.75).
248 (3.71), 234 (3.67) and 220 (4.16).
$\bar{\nu}$ max 3490, 1723, 1603, 1565, and 915 cm^{-1}.
MS : 234 (22), 233 (75) and 205 (100).

RESULTS AND DISCUSSION

Chromatographic investigation of the methylene chloride extract of the leaves of Murraya exotica L revealed the presence of at least 13 spots. Chromatographing the methylene chloride extract over silica gel column succeeded in the isolation of five coumarins (with alcoholic potassium hydroxide give positive test for coumarins)[2]. The structures of the isolated compounds were established by physical, chemical and spectral analyses (UV, IR, MS, ^{1}HNMR and ^{13}CNMR).

COMPOUND 1.

It showed UV absorption maxima characteristic of a 7 - oxygenated -8- substituted coumarin moiety[19,20].

It did not produce any colouration with ferric chloride, indicating the absence of a phenolic (OH) function.

The IR spectrum (KBr) exhibited strong absorption at 3490 cm^{-1} (intramolecular hydrogen bond diol), 1713 cm^{-1} (coumarinic CO), 1603-1595 cm^{-1} (aromatic nucleus), 1565 (α-pyrone double bond) and 910 cm^{-1} (gem-disubstituted methylene group).

From the NMR study of the isolated compound, two doublets each with J=10 Hz centered at δ 6.20 and 7.60 were noticed. These are characteristic for the δ3 and 4 positions in the coumarin ring system. Another two doublets with J= 8.5 Hz at δ 7.39 and 6.86 characteristic of 5 and 6 protons in the same coumarin ring system, indicating that these positions were unsubstituted. A singlet at δ 3.97 is due to three protons suggested the presence of one methoxy group attached to 7-position of the aromatic ring. Another signal appears at 1.75 ppm (singlet,3H) indicating the presence of methyl group carried on double bond. Two broad signals at δ 3.2 and 3.75 ppm (1H) assigned the presence of two hydroxyl groups in the molecule. There are two doublets at 4.55 and 4.63 ppm (1H) attributed to the gem protons of the double bond. The other two doublets at 5.25 and 4.45 ppm correspond to the proton at C_1 and C_2 respectively.

In the mass spectrum of compound 1 (Fig.1) the parent peak is very unstable and hard to detect due to the easy fission of C_1 - C_2 bond. In addition, the two fragment ions, formed by cleavage, are stabilised oxonium ions and one of them which contains the aromatic part of the molecule forms the base peak at m/z 205. The other significant peaks are at m/z 206 (24) which are due to the ion formed by H-transfer from the eliminated side chain to the coumarin moiety during C_1 - C_2 bond cleavage, m/z 191 (206-CH_3) (6), 175 (205-CH_2O) (10) and 162 (205-CH_3-CO) (6).

The abovementioned physical, chemical and spectral properties coincide with those reported by Talapatra et al[21] for murrangatin which was isolated from Murraya elongata. However, compound 1 isolated from M.exotica L. growing in Egypt showed $[\alpha]_D^{20}$ = +3 ($CHCl_3$), while murrangatin isolated by Talapatra et al has $[\alpha]_D^{20}$ = -3 ($CHCl_3$) and was proved to have an erythro-configuration[21].

Therefore compound 1 is an enantiomer of murrangatin and this is the first report of (+) erythro-murrangatin in nature (Fig.4).

Murrangatin was also isolated from M. exotica by Barik et al[12] but the absolute configuration at the C-2 position could not be confirmed.

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COMPOUNDS 2 :

The physical, chemical and spectroscopic properties show great similarity with those of compound 1, but differences were found and are summarised in Table 4.

From Table 4, it is clear that there are significant differences concerning melting point, optical rotation as well as the position of OH groups in ^{1}HNMR and for C_1 - C_2 and C_3 in C^{13}NMR. It is concluded that compound 2 is a threo - isomer of compound 1. A similar compound, minumicrolin was isolated by Das et al[22] from Micro melun minutum (Futaceae) and proved to have a threo-configuration. Minumicrolin with $[\alpha]_D^{20} = +17$ ($CHCl_3$), while that of compound 2 is $[\alpha]_D^{20} = -17$ ($CHCl_3$). Therefore it is suggested that compound 2 is an enantiomer of minumicrolin, and this is the first report of (-) minumicrolin in nature (Fig 4).

COMPOUND 3 :

UV spectrum of compound 3 is similar to that of the previously isolated compounds.

IR is characterised by bands at 3560 (OH), 1710 (coumarinic CO), 1603 (aromatic nucleus) and 1560 cm^{-1} (C- pyrone double bond).

The ^{1}HNMR (90 MHZ, $CDCl_3$) showed four aromatic protons of coumarin nucleus similar to those of compound 1 and 2. The presence of OCH_3 group is indicated by the signal at δ3.91 (3H) (S), while a singlet at δ1.29 due to 6 protons showed the presence of two methyl groups probably the gem-dimethyls of a terminal side chain at C_3. A broad singlet located at δ2.99 was due to the hydroxyl proton. The CH_3 at 1.18 and CH_2 signal at δ 3.54 constituted the terminal ethyl group. The chemical shifts of these protons suggest the presence of $O\text{-}CH_2\text{-}CH_3$ arrangement in the side chain.

Generally, the ^{1}HNMR of this compound showed some of the characteristics of the spectrum of meranzin hydrate[12].

The MS spectrum (Fig.2) shows intense peaks at m/z 306 (M^+) which readily looses 15 and 45 (OC_2H_5) mass units. Other significant peaks are found at m/z 200 (65), 219(15) and a base peak at m/z 87 due to cleavage of ethoxyisopropyl group $(CH_3)_2$ C-OCH_2CH_3 and this is a key ion for placing the ethoxy group at C_3.

Trials to find a similar compound with the same characters in the literature failed. However, meranzin hydrate which was isolated from M. exotica L.[12] growing abroad has a related structure but lacking- CH_2CH_3.

It, therefore, seems probable that compound 3 (Fig 4) is an artefact formed from meranzin hydrate (is a known leaf constituents of M. exotica L.[12]) and the residual ethyl alcohol possibly present in certain solvents used in separation processes.

The isolation of compound 3 indicates that meranzin hydrate is originally present in M. exotica L. cultivated in Egypt.

COMPOUND 4 :

Was found to be similar in structure with murralongin which was isolated by Talapatra et al[23] . From M.elongata Alph . DC leaves and by Raj et al[6] from M. exotica L. leaves. Furthermore[13], ^{13}CNMR assignment (Table 3) which were carried out for the first time are in agreement with the structure of murralongin(Fig.4).

COMPOUND 5 :

Uv spectrum of compound 5 is similar to that of the previously isolated compounds from the titled plant. It did not produce any colouration with Ferric chloride, indicating the absence of a phenolic OH function.

The IR spectrum (KBr) has bands at 3480 (broad (OH),1723 (cOumarinic CO), 1603 (aromatic system), 1565 (C-pyrone double bond) and 915 Cm^{-1} (gem double bond).

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The ^{1}HNMR spectrum (Table 2) of the isolated compound confirmed the presence of the 7-methoxy -8-substituted coumarin system[9,10] as mentioned before under compound 1 and 2. The remaining signals due to the side chain at C-8 comprise a finely single peak at δ1.85 (3H, 3-CH_3), a broad signal atδ3.15 (1H, 2-OH, disappearing on deuteration), a clear doublet at δ 4.90 (H - 1), another doublet at δ 5.20 (H - 2) and a third doublet at δ 4.85 (terminal double bond). The presence of double quartet at δ 3.47 (2H) and a triplet at δ 1.19 (3H) indicate the presence of ethoxy group in the side chain and this is confirmed by both MS and ^{13}CNMR.

The side chain is thus identified as 1'- ethoxy- 3'- methyl but- 3'- ene - 2'- ol- leading to the ssructure 5 (Fig. 4) .

It was found to be similar in structure with nurraxocin which was recently isolated by Barik et al. From M.exotica L. leaves. However the mass spectrum (Fig. 3) and ^{13}CNMR assignment which were carried out for the first time fully conform with its structure.

Antimicrobial and Cytotoxic Screening of the Isolated Compounds

The antimicrobial and cytotoxic activities of the isolated coumarins were studied. Compound 4 only showed an inhibitory action against Bacterium subtilis after 24 hours.

On the other hand the isolated compounds showed no cytotoxic effect.

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Table 1: Characters of the Isolated Compounds.

Compound No.	R_f	Solvent system*	Colours UV	Colours anisaldhyde H_2SO_4	Crystals	m.p
1	0,75	1	blue violet	violet	colourless	133°C.
	0,28	11			fine needles	
2	0,87	1	blue violet	violet	colourless	168°C.
	0,13	111			needles	
3	0,68	1	blue violet	violet	oily	____
	0.60	11				
4	0.82	1	blue violet	violet	heavy needles	134-135°C
	0.26	111				
5	0.32	111	blue violet	violet	needles	129°C.
	0.36	11				
	0.78	1				

* Solvent system :

1 : benzene - methanol (85:15)

11 : benzene - acetone (1 :1)

111: pet.ether - acetone (3 :2)

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Table 2 : ^{1}H- NMR data (400 MHZ) of the isolated compounds

	1	2	3	4	5
H-3	6.21(1H)(d) (J=10HZ)	6.22(1H)(d) (J=10HZ)	6.20(1H)(d) (J=10HZ)	6.21(1H)(d) (J=9.6HZ)	6.23(1H)(d) (J=10HZ)
H-4	7.60(1H)(d) (J=10HZ).	7.62(1H)(d) (J=10HZ)	7.63(1H)(d) (J=10HZ)	7.69(1H)(d) (J=9.6HZ)	7.63(1H)(d) (J=10HZ)
H-5	7.39(1H)(d) (J=8.5HZ)	7.40(1H)(d) (J=8.5HZ)	7.5(1H)(d) (J=8.5HZ)	7.49(1H)(d) (J=8.5HZ)	7.40(1H)(d) (J=8.5HZ)
H-6	6.86(1H)(d) (J=8.5HZ)	6.89(1H)(d) (J=8. HZ)	6.86(1H)(d) (J=8.5HZ)	6.84(1H)(d) (J=8.5HZ)	6.86(1H)(d) (J=8.5HZ)
C_7-OCH_3	3.97(3H)(S)	3.97(3H)(S)	3.91(3H)(S)	3.81(3H)(S)	3.98(3H)(S)
H-1¯	5.25(1H)(d) (J=8.5 HZ)	5.43(1H)(d)	3.07(2H)(m)	2.42(3H)(S)	4.9(1H)(d) (J=9.5 HZ)
H-2¯	4.45(1H)(d) (J=8.5 HZ).	4.5(1H)(d) (J = 8.5 Hz)	3.43(1H)(m)	1.78(3H)(s)	5.2(1H)(d) (J=9.5HZ)
H-3¯	———	———	———	———	———
H-4¯	4.55(1H)(d) (J=2.5HZ) 4,63(1H)(d) (J=2.5HZ)	5.0(2H)(m) exomethylenic double bond	———	———	4.85(2H)(d) exomethylenic double bond
H-5¯	1.75(3H)(s)	1.9(3H)(s)	1.29(6H)(S) ———	———	1.85(3H)(s)
H-6¯	———	———	3.54(2H)(m)	———	3.47(2H,q) (J=7Hz)
H-7¯	———	———	1.18(3H)(T) (J=7.5Hz)		1.19(3H)(T) (J=7. HZ)
C_1-OH	3.20(1H), broad,OH	1.7(1H), broad, OH	———	———	———
C_2-OH	3.75(1H), broad,oH.	3.6(1H) broad,oH	2.99(1H), broad,OH.	———	3.15 H) broad,oH
CHO	———	———	———	10.22(1H) (s)cHO	———

Table 3 : ^{13}C NMR Spectral Analyses of the Isolated Compounds $CDcl_3$, TMS as Internal standard .

Carbon âton	Chemicâl Shift , PPm				
	Comp.1	Comp. 2	Comp.3	Comp. 4	Comp.5
C - 2	160.2â	160.7â	161.0â	160.2â	161.0â
C - 3	113.5â	112.5â	112.8â	113.3b	112.6b
C - 4	143.9b	144.5â	143.6	143.4b	143.6b
C - 4â	113.1â	112.1â	112.6a	113.1b	113.4b
C - 5	128.5	128.1	126.4	128.5c	128.7
C - 6	107.8	108.4	107.2	107.7c	107.8
C - 7	152.9	152.9	153.6	152.7	153.4
C - 7-OCH_3	56.2	56.2	56.5	56.2	55.9
C - 8	116.9	118.3	116.1	113.5b	114.0a
C - 8â	159.8	159.3	160.5	160.9a	160.3a
C - 1'	69.6	66.2	24.9	129.3	76.1
C - 2'	78.4	75.4	76.9b	107.7c	75.6
C - 3'	143.5b	147.0â	76.4b	——	143.3b
C - 4'	113.5â	112.0â	20.7	——	112.6b
C - 5'	17.4	17.5	21.3	----	16.9
C - 6'	---	---	55.9	----	64.7
C - 7'	---	---	15.9	----	14.8
C1' - CH_3	---	---	---	19.8	---
C2' - CH_3	---	---	---	24.2	---
CHo	---	---	---	188.6	---

* - a , b and C Assignments may be interchanged .

Table 4 : Characteristic Differences between compound 1 and 2 .
Compound 2 .

Character	Compound 2	Compound 1
Crystal shape	heavy needles (acetone)	fine needles (pet.ether-acetone)
M.p	168°C	133°C
$[\alpha]_D^{Rt}$ ($CHCl_3$)	-17	+3
^{1}HNMR: C-1'-OH	1.70 ppm	3.20 ppm
C-2'-OH	3.60 ppm	3.75 ppm
C=CH_2	5.00 ppm	4.63, 4.55 ppm
^{13}CNMR: C-1'	66.2 ppm	69.6 ppm
C-2'	75.4 ppm	78.4 ppm
C-3'	147.8 ppm	143.4 ppm

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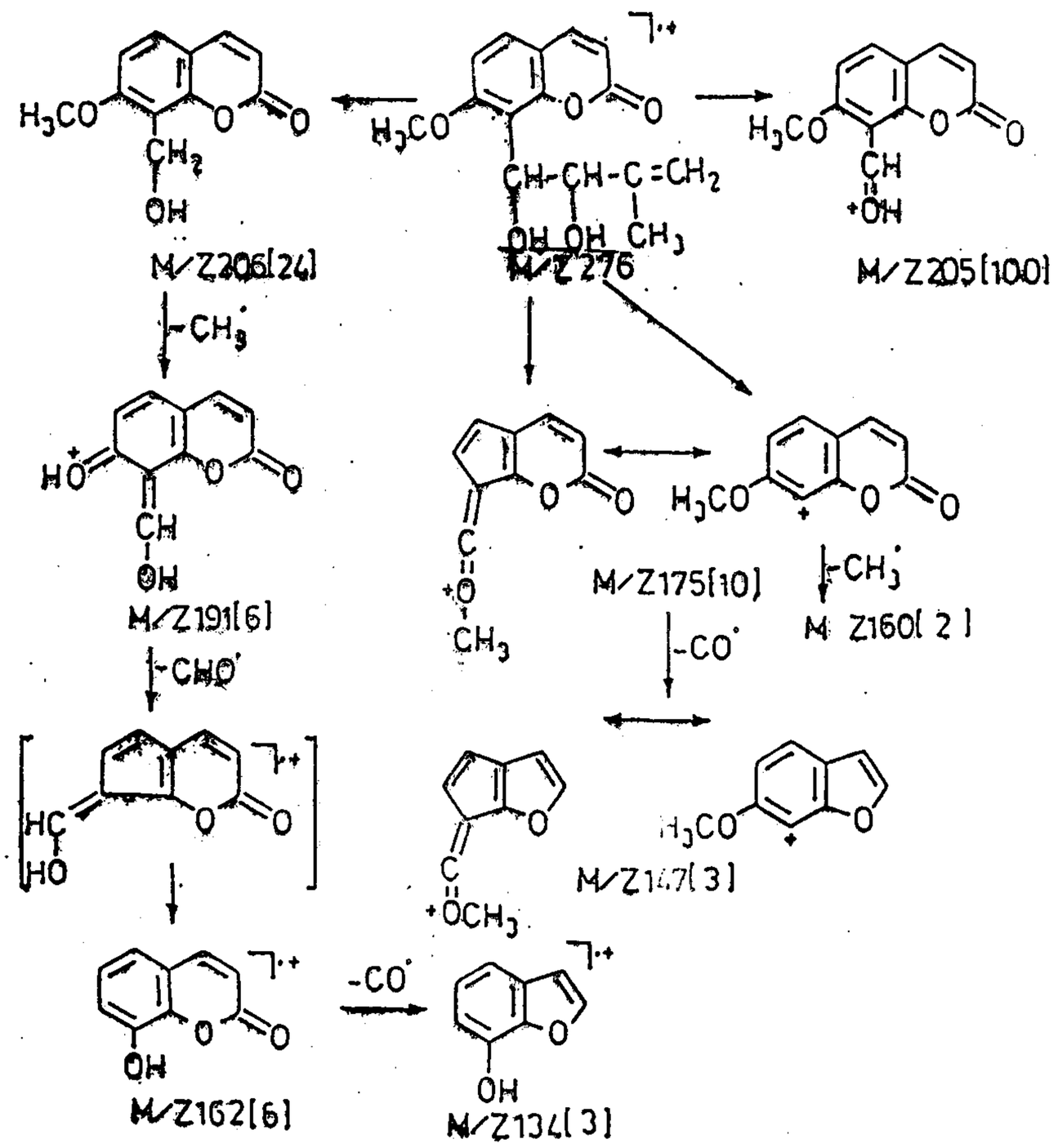


Fig. 1: Fragmentation pattern of (+) Erythro-Murrangatin

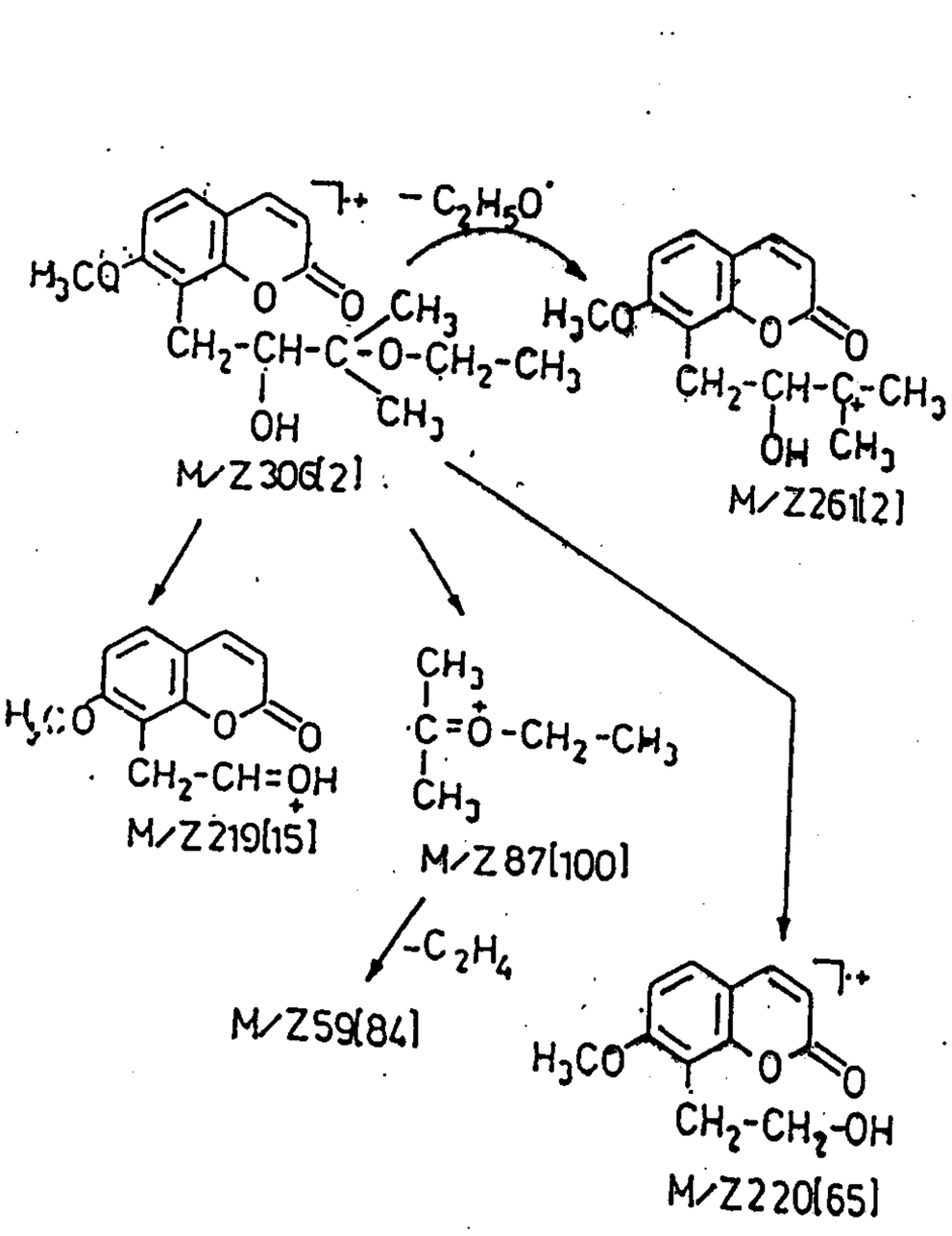


Fig. 2: Fragmentation pattern of Compound 3

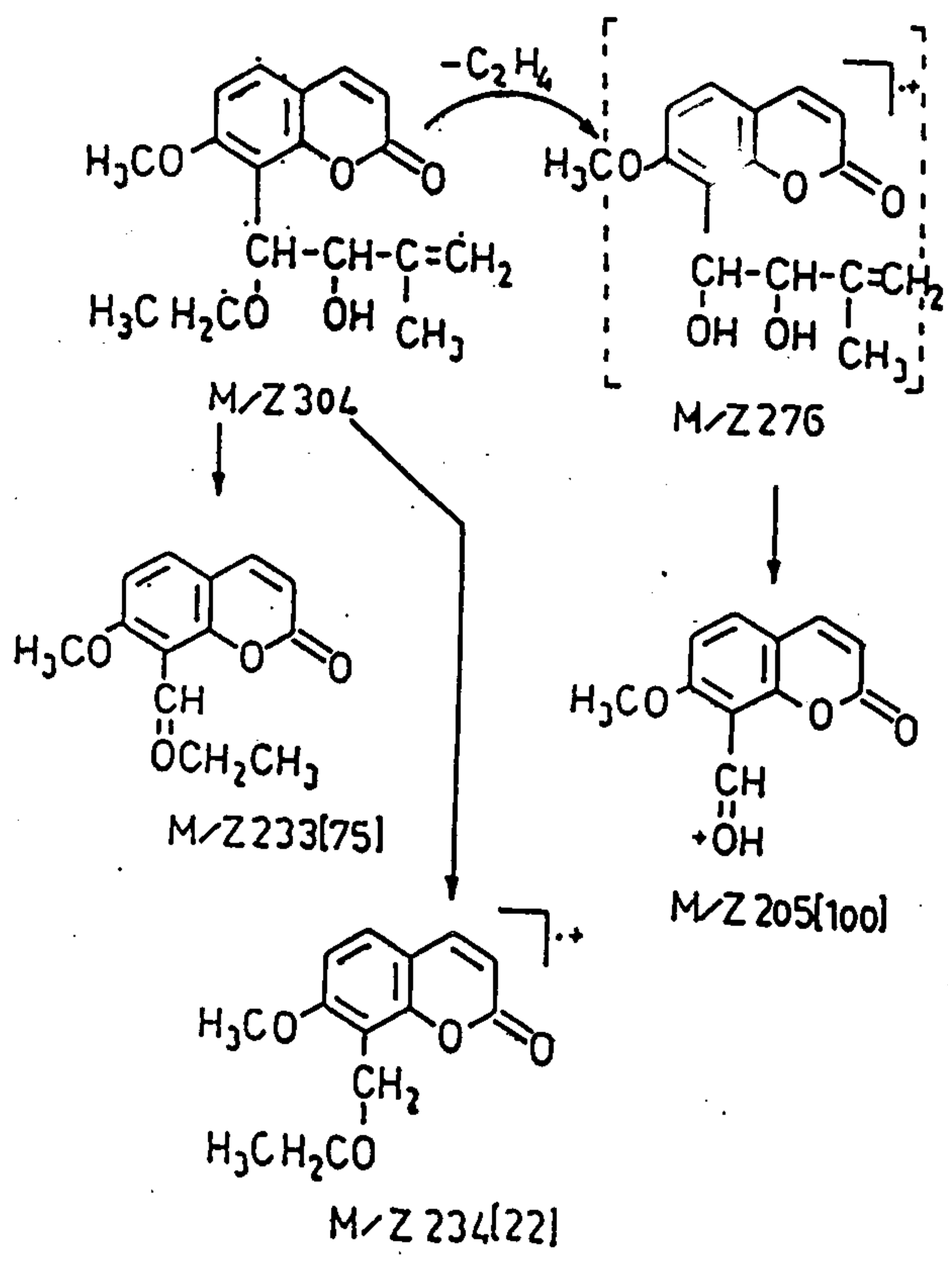
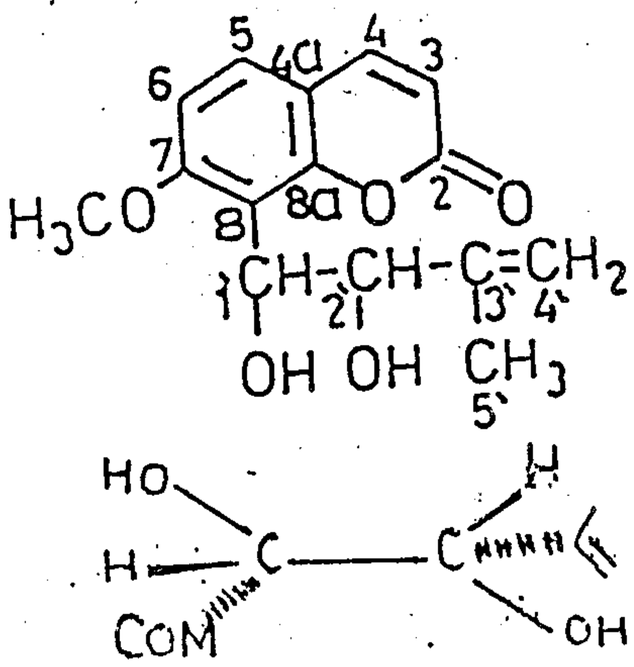
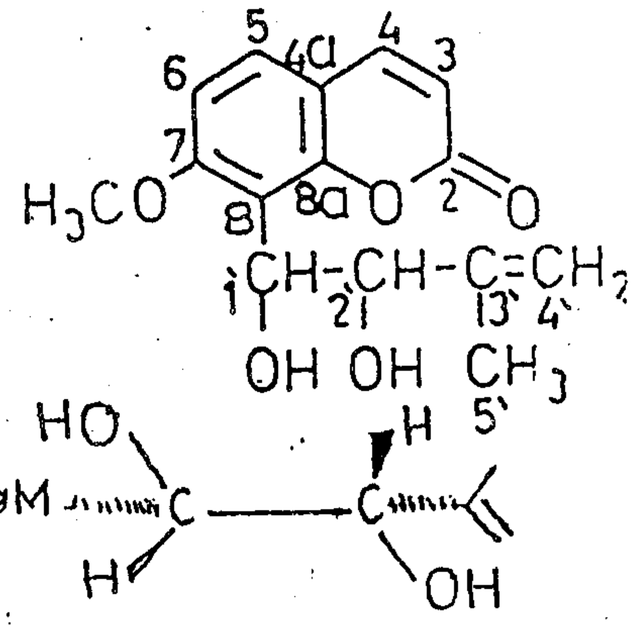


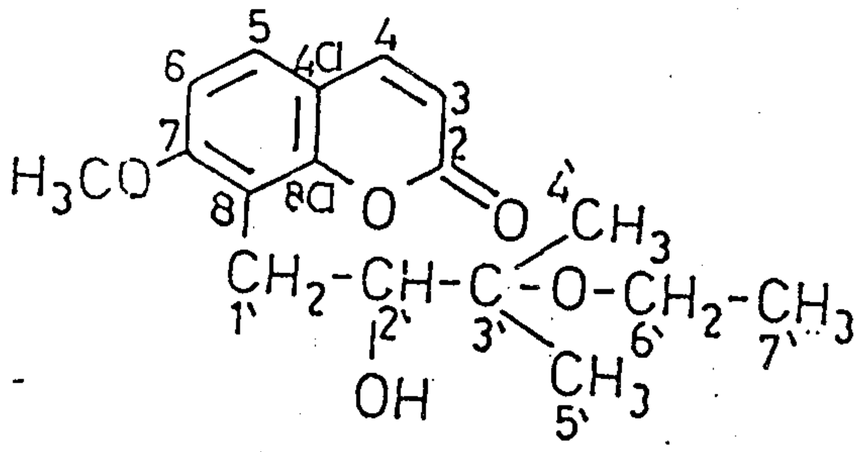
Fig. 3: Fragmentation pattern of Compound 5



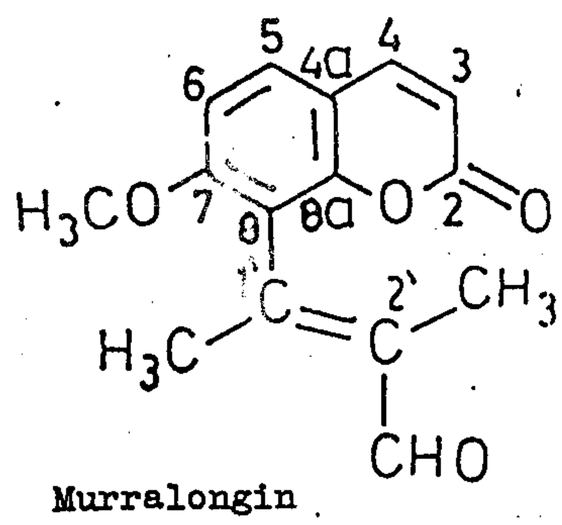
(+) Erythromurrangatin



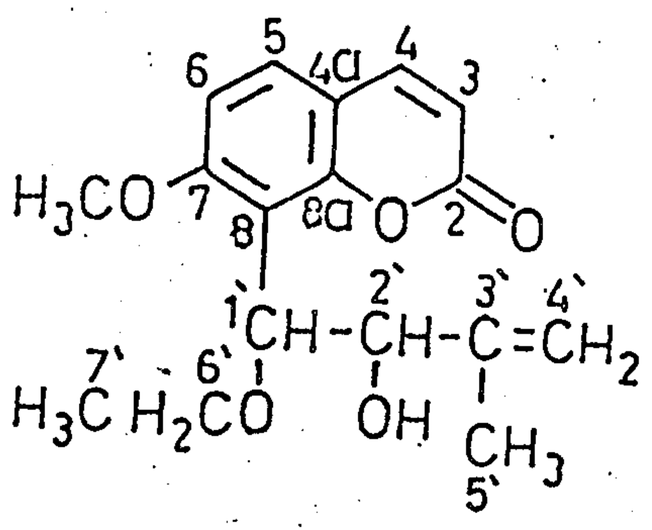
(-)minumicrolin



Compound 3



Murralongin



Murraxocin

Fig 4 : The isolated compounds of M. exotica L.

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دراسة كيميائية لنبات الموراياً اكسوتيكال المنزرع فى مصر
٢ - الكومارينات الموجودة بالاوراق .

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تم فصل خمس مركبات كومارينية من خلاصة كلوريد الميثيلين لاوراق نبات الموراياً أكسوتيكا وهى :

١ - (+) اريثرو - مورانجتين

٢ - (-) مينو ميكرولين

٣ - ٧- ميثوكسى - ٨ (٢' - هيدروكسى - ٣ - ايسوكسى - ٣ - ميثيل - بيوتانيل)كومارين.

٤ - مورالنجين .

٥ - موراكسوسين .

وتم التعرف على التركيب الكيميائى لهذة المركبات بواسطة الطرق الفيزيائية والكيمائية والتحليل الطيفى بالاشعة دون الحمراء والاشعة فوق البنفسجية والرنين النووى المغناطيسى البروتونى والكربونى وكذلك مطياف الكتلة .

وتمت دراسة التأثير البيولوجى للمركبات التى تم فصلها بدراسة تأثير كل منها على أنواع خاصة من البكتريا والفطريات وكذلك دراسة التأثير القاتل على نوعين من خلايا السرطان وقد أظهرت الدراسة أن مركب مورالنجين له تأثير قاتل على بكتريا سوبتليز .

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