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PHYTOCHEMICAL STUDY OF MURRAYA EXOTICA L. CULTIVATED IN EGYPT

III- COUMARINS AND CYCLOARTENOLS OF THE LEAVES

D.W. Bishay, S.M. El-Sayyad, M.A. Abdel-Hafiz, H. Achenbach and E.K. Desoky*

PHarmacognosy Department, Faculty of Pharmacy,

Assiut University, Assiut, Egypt.

**Institut für Angewandte Chemie, Friedrich-Alexander*

Universität, Erlangen, West Germany

ABSTRACT

Two coumarins (osthol and aurapten), two cycloartenols (lupeol and 3-epi-cycloartenol), besides cis-nerolidol were isolated from cyclohexane fraction of the methanolic extract of the leaves of Murraya exotica L.

For the first time, we report the presence of aurapten in the plant, cis-nerolidol and lupeol in this species, and 3-epi-cyclolaudenol in this family.

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

INTRODUCTION

Family Rutaceae comprises many genera of important economic and medicinal uses on account of their volatile oil and medicinally active constituents[1-3].

Many plants belonging to this family have extensive folk uses especially in South Africa, Australia and Asia[4,12]. In addition, extracts of certain species from the Rutaceae are among the most active against cancer[5]. Murraya exotica L. is considered as one of these species exhibiting anticancer activity[5].

Murraya exotica L. is reported to contain several coumarins[6-12], carbazol alkaloids[13,14] and flavonoids[15-17].

In our previous publications, we reported the isolation of six methoxylated flavonoids[18] from carbon tetrachloride fraction and five coumarins from methylene chloride fraction of the methanolic extract of the leaves of *M. exotica* L.[19]

In a continuation of the studies on *M. exotica* L. we report the isolation of other coumarins, as well as, cycloartenols from hexane extract of the leaves of *M. exotica* L. cultivated in Egypt.

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, Mass spectra were recorded in Finnigan 4000, E.I. 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 and alumina (E.Merck) were used , TLC : silica gel G with the solvent systems listed in Table 1.

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 dark containers.

Phytochemical study of Murraya Exotica L. Cultivated in Egypt
III-Coumarins and Cycloartenols of the leaves

Extraction :-

The concentrated methanolic extract of 3 Kg. of the air-dried powdered leaves was extracted with cyclohexane. The solvent-free cyclohexane extract was used in this investigation.

TLC of this extract using silica gel G and cyclohexane-ethyl acetate (9 : 1) as a developer revealed at least 10 substances. 10 g. of the concentrated extract was fractionated on alumina column (4.3 x 72 cm, 300 g) using cyclohexane-ethyl acetate (9:1). Three components were isolated (A.B and C). Trials for isolation of substances with R_f 0.48, 0.44 and 0.36 were unsuccessful. So, the fraction containing them was acetylated and the acetate product was subjected to HPLC : reversed phase preparative R_p-18 column ; methanol-ethanol (4:1) as developer, rate of flow 1.6 ml/minute; UV-detector at 215 nm, range 1.28 : Two components D & E were isolated.

Their physical and chromatographic characters are given in Table 1 and ^{13}C-NMR spectral analyses are shown in Table 2.

Compound A :-

Obtained by repeated crystallisation from acetone-pet. ether as colourless needles, m.p. 83-4^{o}C, shows an intensive blue violet fluorescence in UV light and gives a violet colour with anisaldehyde - sulphuric acid reagent. UV and IR spectra of the compound show the characteristic features of 7-alkoxycoumarins[20].

The ^{1}H NMR spectrum exhibits the following signals : δ6.18 (1 H,d, J= 10 Hz, H-3), 7.66 (1 H, d, J= 10 Hz, H-4), 7.34 (1H,d, J = 8.3 Hz, H-5), 6.82 (1H,d, J = 8.3 Hz, H-6), 3.92 (3H,s, OCH_3), 3.54 (2H,d, J = 7 Hz, H-1)5.23 (1H,m, H-2), 1.67 (3H,s, CH_3-4), 1.84 (3H,s, CH_3-5).

MS spectrum shows M^+ at m/z 244 (100), 229 (61), 201 (37), 189 (36) and 176(4).

Compound B :

Obtained by recrystallisation from acetone as colourless needles, m.p. 60°C., has a greenish-blue fluorescence under UV light and gives a green colour when sprayed with anisaldehyde/ sulphuric acid reagent. UV λ_{max} (logϵ) (MeOH) nm : 322 (2.98), 296 sh (2.82), 253 (2.60), 243 sh (2.70) and 218(3.05) Not affected by alkalies.

IR $\bar{\nu}_{max}$ (KBr) cm^{1} : 1710 (coumarinic CO), 1601, 1500, 880, 750 and 700 (substituted benzene ring), 1357 (=C $(CH_3)_2$) and 1210 (ar-O-C-alkyl)[21].

The ^{1}H NMR spectrum shows the following signals : δ 6.23 (1H,d, J = 10 Hz, H-3), 7.65 (1 H,d, J = 10 Hz, H-4), 7.38 (1 H,d, J = 8.5 Hz, H-5), 6.85 (1 H, dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, H-6), 6.8 (1 H, d, J = 2.5 Hz, H-8), 4.65 (2H, d, J = 6 Hz, H - 1), 5.50 (1 H, t, J = 6 Hz, H - 2), 1.8 (3 H, s, H-4),2.1 (4 H,m, $-CH_2-CH_2-$ at C-5 and C-6), 5.1 (1 H, m, H-7), 1.6 (3 H, s, CH_3-9), 1.63 (3 H, s, CH_3-10).

MS spectrum shows M^+ at m/z 298 (7), The other significant peaks are at m/z 162 (89), 136 (26) and a base peak at m/z 69 (100). MS spectrum of the hydrogenated product of the compound shows M^+ at m/z 302 .

Compound C :

Oily substance, $[\alpha]_D^{20}$ = + 1.475 (C = 0.07, $CHCl_3$), shows no fluorescence under UV light and gives blue colour when sprayed with anisaldehyde/ sulphuric acid reagent.

The ^{1}H NMR spectrum gives the following data :δ5.9 (1 H, dd, J = 10 Hz, H-2), 5.2 (1 H, dd, J = 2.5 Hz, J_2 = 17 Hz) and 5.05 (1 H, dd, J_1 = 2.5, J_2 = 10 Hz), two protons of C - 1, 5.12 (2H, m, H - 7 and H - 12), 1.3 (3 H, s, CH_3 - 4), 1.7 (3 H, s, CH_3 - 15), 1.47 (6 H, s, CH_3 - 9 and CH_3 - 14) 2.0 (8 H, m, 4x CH_2 at C-5, C-6, C-10 and C-11).

MS : shows a molecular ion peak at m/z 222 and a base peak at m/z 69. The other significant peaks are found at m/z 204 (5), 189)19), 123 (11), 81 (18), 71 (38), 55 (23) and 41 (50).

Phytochemical study of Murraya Exotica L. Cultivated in Egypt
III-Coumarins and Cycloartenols of the leaves

Compound D :

Isolated by HPLC, and purified by recrystallisation from acetone as colourless needles, m.p. 214 C and $[\alpha]_D^{20} = +$ 30 (c = 0.4 in $CHCl_3$), shows no fluorescence under UV light and gives a violet red colour when sprayed with anisaldchyde sulphuric acid reagent.

The ^1H NMR spectrum disclosed the following signals :δ0.84 (3H, s, CH_3-23), 0.86 (3H, s, CH_3 -24), 0.93 (3H, s, CH_3 - 25), 1.03 (3H, s, CH_3 - 26), 0.84 (3H, s, CH_3 - 27), 0.79 (3H, s, CH_3 - 28), 1.68 (3H, broad s, CH_3 - 29), 2.05 (3H, s, $COCH_3$), 4.69 (2H, d, J = 2 Hz, H-30).

El-MS : shows the parent peak at m/z 426 (18) and peaks at m/z 207 (70) and 189(100) which are characteristic for triterpenes[22]. The other significant peaks are similar to that of lupeol[23].

El-MS of the acetate of the isolated compound shows that it has a molecular ion peak at m/z 468 and this confirmed that the isolated compound has only one hydroxyl group in the molecule[11,13].

Compound E :-

Compound E was isolated by HPLC as mentioned before. Crystallisation from acetone-pet. ether (3:1) as fine needles of m.p. 132 C. It shows no fluorescence under UV light and gives a violet colour when sprayed with anisaldehyde/ sulphuric acid reagent.

IR spectrum of the acetylated compound shows these characteristic bands : 3040 (methylene group of cyclopropane ring), 1715 (CO-group), 1375, 1360 (geminal dimethyl)[25] and 880 cm^{-1} (Terminal methylene group).

^1H NMR spectrum showed the following signals :δ0.32 and 0.55 (2H, AB quartet, J = 5 Hz, cyclopropane ring)[23] 2.01 (3H, s, CO - CH_3), 4.65 (1 H, bd, J = 2.3 Hz) and 4.71 (1 H, bd, J = 2.3 Hz) = CH_2, 0.85 (3 H, d, J = 7.5 Hz, CH_3-29), 0.84 (3 H, s, CH_3 - 30), 1.0 (3 H, d, J = 6.5 Hz, CH_3 - 21), 0.92 (6 H,s, CH_3 - 18 and CH_3 - 31), 0.97 (3 H, s, CH_3 - 28), 1.62 (3 H, s, CH_3 - 27).

El-MS of the acetylated compound shows a molecular ion peak at m/z 482 (7) and a base peak at m/z 69. Other significant peaks appear at m/z 467 (4), 422 (47), 407 (12), 379 (7), 357 (3), 353 (1), 300 (20), 203 (31), 187 (30), 175(67), 162 (67), 147 (50), 133 (47), 121 (49), 107 (65), 95 (90),81 (52) and 43 (95) (Fig. 1).

Hydrolysis of the isolated compound E and spectral analysis of the hydrolsate.

Compound E was hydrolysed by alcoholic KOH to perform extra IR, NMR and MS to confirm its identity. The hydrolysed product was purified by repeated crystallisation from acetone to give needles (7 mg) of m.p. 141 C and $[\alpha]_D^{20}$ = - 9.9 (c = 0.94, $CHCl_3$). The hydrolysed product gives a violet pink colour with anisaldehyde / sulphuric acid reagent and no fluorescence under UV light.

A - IR spectrum shows the following characteristic bands :
3040, 1375, 1360, 880 and 3400 cm^{-1} (OH group).

B - ^1H NMR spectrum shows the following differences :
1- 0.55 ppm and 0.85 (2H, AB quartet, J = 5 Hz (Cyclopropane ring)[25].
2- Disappearance of the acetate protons at 2.01 ppm.
3- 3.31 ppm (1H, dd, J = 6.6, 13 Hz, C_3 - H).

C - MS spectrum shows M^+ at m/z 440 and a base peak at m/z 95. The other significant peaks are at m/z 425 (12), 422 (4), 300 (15), 297 (6), 203 (21), 187 (24), 175 (69) 162 (41), 161 (49), 147 (50), 133 (46), 121 (59), 107 (71), 81 (56), 69 (90), 55 (66) and 43 (42).

RESULTS AND DISCUSSION

The concentrated cyclohexane fraction of methanolic extract of the leaves of M. exotica L. was chromatographed on silica gel column to yield five pure compounds designated A -E.

Compound A :-

In the El-MS : the molecular ion peak appears as the base peak at m/z 244. The peak at m/z 201 can be rationalized by an expected loss of CO from the ion at m/z 229. Loss of the $(CH_3)_2$-

-C = CH give rise to the peak at m/z 189 (37). The peak at m/z 176 (4) would result from the loss of dimethyl allyl group from the original compound.

The physical and chemical properties as well as spectral studies of the compound indicate its identity as Osthol(Fig. 2), which has been isolated from the leaves of M. exotica L. growing abroad[26].

Compound B :-

On the basis of physical, chemical and spectral properties (UV, IR, ^{1}H NMR, ^{13}C NMR and MS) compound B was found to be Aurapten (Fig. 2) which was isolated from M. elongata A.DC.[27] and by Gray et al[28]. Furthermore, this is the first report of aurapten in M. exotica L. Also this is the first ^{13}C NMR assignment of this compound (Table 2).

Compound C :-

From its physical and chemical properties as well as spectral data Compound C was proved to be cis-3-7-11-trimethyl-1,6,10-dodecatrien-3-Ol (cis-nerolidol)[29] (Fig. 2), which has been isolated from the flowers of Melaleuca viridflora saland (Myrtaceae) and Myroxylon pereire (Leguminoseae)[30-35]. This is the first report of cis-nerolidol in Murraya species.

Compound D :-

The physical constants, chemical properties and spectroscopic data of compound D were identical with the reported data of lupeol[23] (Fig. 2).

Lupeol was also isolated from Teclea grandifolia[36] (Rutaceae), Sonchus asper (Compositae)[37] Rauwolfia psychotriodes[38] (Apocyanaceae) and Arbutus andrachme[39] (Ericaceae). This is the first report of this compound in Murraya species.

Compound E :-

Physical, chemical and spectral analyses of the isolated compound indicated 3-epi-cyclolaudenol (Fig. 2) which was isolated for the first time from Euphorbia caudicifolia (Euphorbiaceae)[25].

This is also the first report of this class of compounds (cycloartenols) in Rutaceae. The structure elucidation of the compound in its acetate form was proved for the first time by studying the IR, ^{13}C NMR and MS spectra. The isolation of this compound from M. exotica L. is considered of great importance from the chemotaxonomical point of view, since this class of compounds was not isolated from any plant belonging to family Rutaceae.

Antimicrobial Activity of Cyclohexane Extract and Aurapten :

The antimicrobial activity of cyclohexane extract and aurapten was studied. Both antifungal and antibacterial properties were determined using four species of fungi and one species of bacteria by the usual agar-cup assay method using Sabourand's agar medium[29]. The results cited in Table 3 show that the cyclohexane fraction and aurapten have no inhibitory effect against Bacterium subtilis after 24 hours and a very weak effect against cartain species of tested fungi.

Cytotoxic Activity of Cyclonexane Extract and Some Isolated Substances of Murraya Exotica L.

In vitro cytotoxic tests were carried out on some of the isolated substances of M. exotica L. The effects of these substances against P_{388} and KB cells is shown in (Table 4).

The results obtained showed that Osthol and aurapten have a moderate effect against P_{388}, while aurapten has a moderate effect against KB cells. The cyclohexane extract showed no effect on both systems.

Phytochemical study of Murraya Exotica L.Cultivated in Egypt III-Coumarins and Cycloartenols of the leaves.

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Table 1 : Characters of the Isolated Compounds :

No.	R_F	Solvent system*	Colour		Crystal form	m.p.
			UV	anisaldhyde/ H_2SO_4		
A	0.30 0.40	I II	blue violet	violet	colourliss needles	83-4°C
B	0.45 0.20	II III	greenish blue	green	colourless needles	66°C
C	0.33 0.26	IV V	---	blue	oily	---
D	0.44	VI	---	violet red	colourless needles	214°C
E	0.30	VI	---	blue	fine needles	132°C

* Solvent system I : Benzene - ethyl acetate (4:1)
II : Pet. ether - acetone (3:2)
III: Cyclohexane - ethyl acetate (6:1)
IV : Methylene chloride
V : Cyclohexane : ethyl acetate (82:8)
VI : Cyclohexane : ethyl acetate (9:1)

Phytochemical Study of Murraya Exotica L. Cultivated in Egypt
III-Coumarins and Cycloartenols of the leaves

Table 2: ^{13}CNMR data of the isolated compounds :

Carbon	Comp. B	Carbon	Comp. C	Comp. D	Comp. E
C_2	162.3	C-1	111.60	38.4	29.8
C_3	112.5	C-2	145.23	23.7	26.3
C_4	143.2	C-3	73.42	81.0	79.1
C-4a	113.0[a]	C-4	27.89	37.8	38.7
C-5	128.6	C-5	42.20	55.4	49.1
C-6	113.2[a]	C-6	26.70	18.2	20.9
C-7	161.0	C-7	124.30	34.3	28.0
C-8	101.8	C-8	135.56	40.9	45.2
C-8a	156.0	C-9	16.02	50.4	20.1
C-1'	65.6	C-10	39.70	37.1	25.7
C-2'	118.6	C-11	22.77	21.0	25.6
C-3'	142.2	C-12	124.30	25.1	36.0
C-4'	16.7	C-13	132.00	38.1	42.2
C-5'	39.5	C-14	25.60	42.9	48.9
C-6'	26.3	C-15	17.65	27.5	32.9
C-7'	123.7	C-16		35.6	26.3
C-8'	131.9	C-17		43.0	52.2
C-9'	17.8	C-18		48.0	18.0
C-10'	25.9	C-19		48.3	29.8
		C-20		150.9	36.1
		C-21		29.9	18.3
		C-22		40.0	34.0
		C-23		28.0	26.0
		C-24		16.5	41.6
		C-25		16.2	150.2
		C-26		16.0	109.3
		C-27		14.0	18.6
		C-28		18.0	19.4
		C-29		19.3	28.6
		C-30		109.4	25.4

Table 3 : Antifungal activity of cyclohexane extract and aurapten.

Compound	Coprinus cinereus	Botrytis cinerea	Rhizoctonia salania	Sparolegnia
Cyclohexane extract	(+)	(+)	O	(+)
Aurapten	(+)	O	(+)	O

O = inactive

(+) very weak

Table 4 : Cytotoxic activity of cyclohexane extract and some isolated compounds of M. exotica L.

Compound	LD_{50} (Mg/ml) P_{388}	LD_{50} (Mg./ml) KB cells
Osthol	7.0	24.1
Aurapten	9.6	9.2
Cis-Nerolidol	>50.0	11.0

Phytochemical Study of Murraya Exotica L. Cultivated in Egypt
III-Coumarins and Cycloartenols of the leaves.

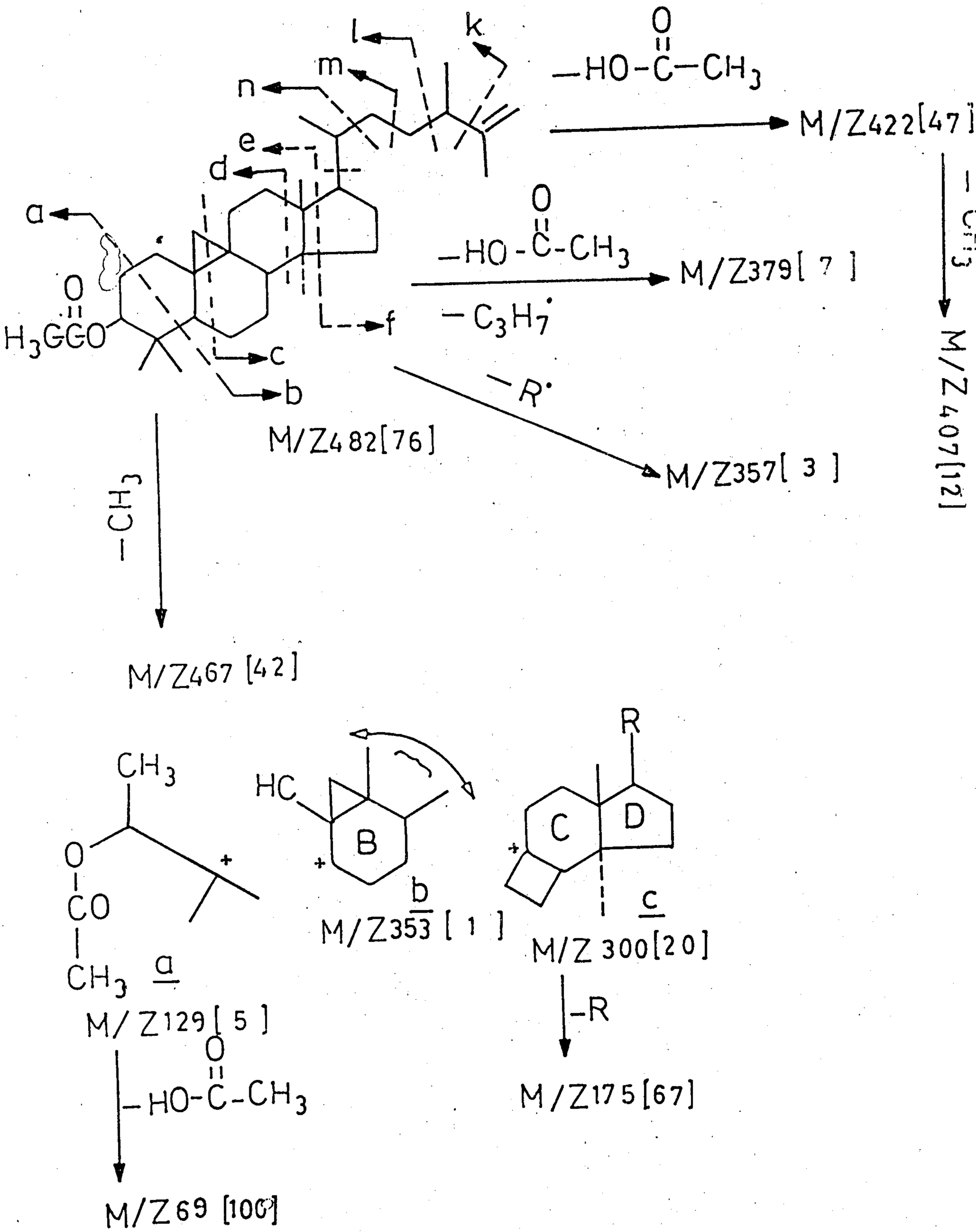
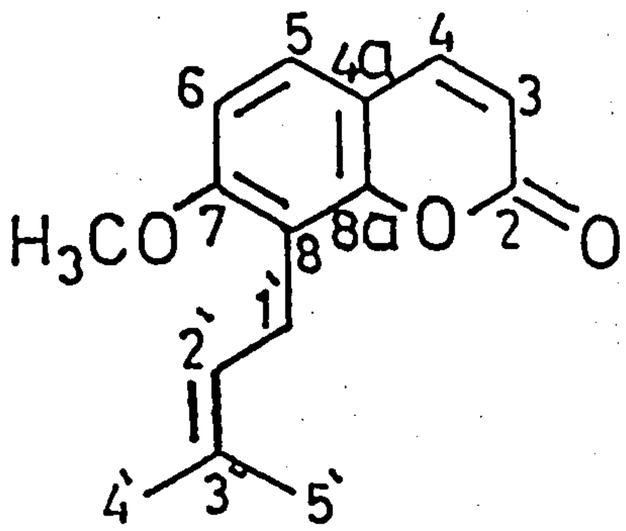


Fig. 1 : Fragmentation pattern of 3-Epi-cyclolaudenol acetate



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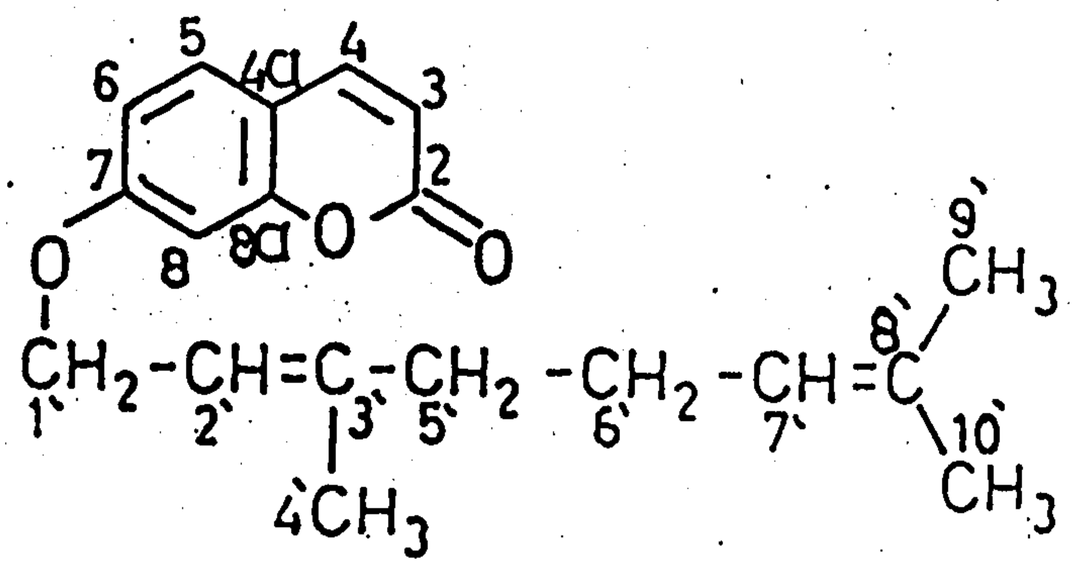

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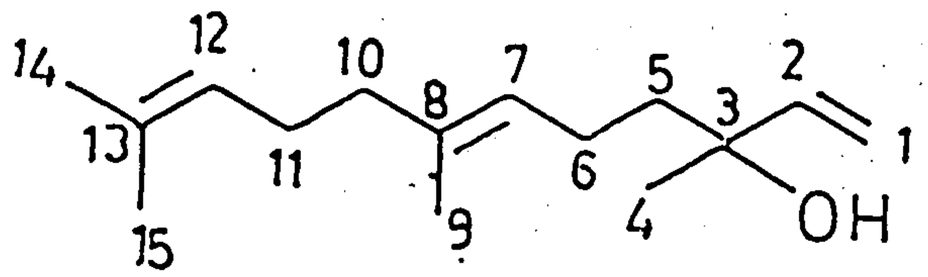
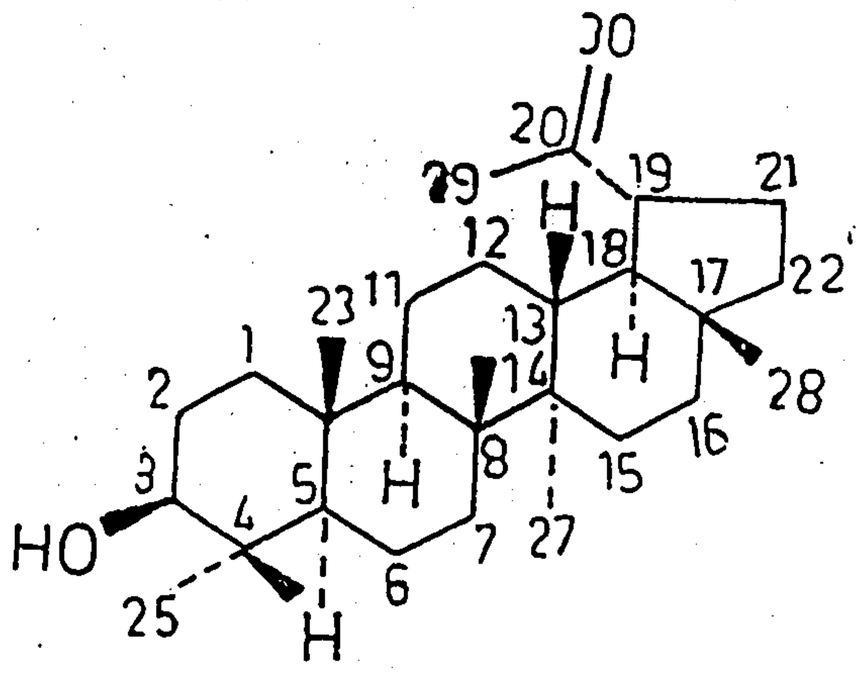
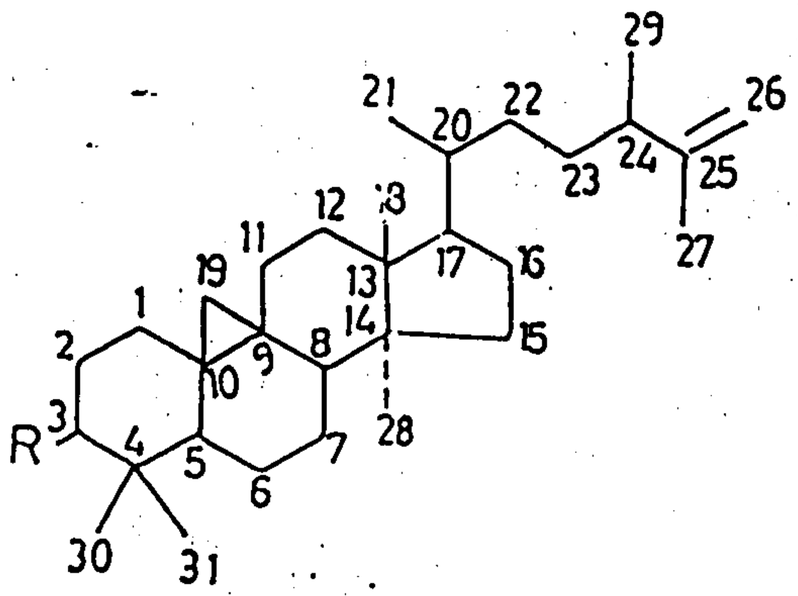


CIS-NEROLIDOL



LUPEOL



3-EPI-CYCLOLAUDENOL

Fig. 2 : The isolated compounds of cyclohexane extract.

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" دراسة كيميائية لنبات الموراليا اكسوتيكال المنزرع فى مصر
٣ - الكومارينات ومركبات السيكلوارتينول فى الاوراق

داود ونيس بشاى - ساميه محمد الصياد - محمد عبدالمطلب عبدالحافظ - هـ . اخنباخ*

و عـز الدين قاسم دسوقى

قسم العقاقير - كلية الصيدلة - جامعة أسيوط *ومعهد الكيمياء أيرلانحن - المانيا الغربية*

فى هذا البحث تمت دراسة خلاصة الهكسان الحلقى لاوراق نبات الموراليا اكسوتيكال وأمكن فصل خمس مركبات بواسطة كل من كروماتوجرافيا العمود وكروماتوجرافيا السائل عالى الضغط وهم :

أ - ٧ - ميثوكس - ٨ (٢ بنتينيل) كومارين : أثول

ب - ٧ أوكس (٣ ، ٨ ثنائى ميثيل - ٧ - نونانينال) كومارين : أورابتين

ج - ٣ ، ٧ ، ١١ ثلاثى ميثيل - ١ ، ٦ ، ١٥ - دود يكاترين - ٣ - هيدروكسيل سيس - نيروليدول

د - لوبيول

هـ - ٣ - أبى - سيكلولاودينول

وقد تم التعرف على التركيب الكيميائى لهذة المركبات بواسطة دراسة الاشعة دون الحمراء والاشعة فوق البنفسحية والرنين النووى المغناطيسى بنوعيه الهيدروجينى والكربونى ومطياف الكتله .

وأثبتت التجارب المعملية أن خلاصة الهكسان الحلقى والاوربتين ليس لهما فاعلية ضد البكتريم سوبتيليز وذات تأثير ضد بعض الفطريات المختبرة .

وقد ثبت أن مركبى الاوثول والاوربتين لهما تأثير محدود ضد الخلايا السرطانية (خلايا ب ٣٨٨ ومركب الاوربتين له تأثير محدود على خلايا السرطان (ك ى) .
بينما أثبتت خلاصة الهكسان الحلقى عدم فاعليتها ضد الخلايا السرطانية بنوعيها .

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