Antimicrobial Activities of Synthesized 3-Acetyl Coumarin and Benzo-4-Methyl Coumarin

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ABSTRACT

3-Acetyl coumarin and Benzo-4-methyl coumarin were synthesized and confirmed for their chemical structures by their melting points; carbon and hydrogen analysis in addition to their spectroscopy measurements By U.V, I.R , H^1-NMR and M.S. These two compounds were evaluated for their fungicidal activities against *Alternaria solani* and *Fusarium oxysporum* in comparison with mancozeb as standard fungicide using radial growth method. The bactericidal of the two compounds were determined against *Erwinia amylovora* and *Ralstonia solanacearum* in comparison with streptomycin using agar disk-diffusion method. The results indicated that the synthesized benzo-4-methyl coumarin and 3-Acetyl coumarin have fungicidal and bactericidal activities but less than standard (mancozeb and streptomycin) respectively. It also was found that Benzo-4-methyl coumarin was more active against the tested fungi *Alternaria solani* and *Fusarium oxysporum*, and bacteria *Erwinia amylovora* and *Ralstonia solanacearum* than 3-acetyl coumarin.

Key words: coumarin derivatives, Pechmann condensation, Fungicide, Bactericide.

INTRODUCTION

Coumarins and their derivatives are very large groups have attracted considerable attention due to their wide range of biological activites such as antibacterial (Modranka *et al* , 2006), antifungal (Sardari *et al*, 1997); antitumor (Jaipathi, *et al* 2012); anti-HIV therapy (Selvam *et al*, 2011); anti-inflammatory (Selvam et al 2010), antioxidant (Tygai *et al* 2003) anticoagulant (Ruszat *et al* 2006) in addition to anticonvulant (Bhat *et al* 2009). Patel *et al*(2013) investigated in vitro antimicrobial activity of some coumarin derivatives against several bacteria (*S. aureus, B. cercus, E. coli, P. aeruginosa, k. Pneumonia, S. typhi, P. vulgaris, S. flexneri*); fungi (*A. fumigatus, A. clavatus, C. albicans*) and antimycobacterial activity against (*Mycobacterium tuberculosis*). Sheikh *et al* (2016) evaluated some coumarin derivatives for their antitubercular activity in vitro against *Mycobacterum tuberculosis* H37Ra; antioxidant activity by 1.1 diphenyl-1-picrylhydrazy (DPPH) radical scavenging assay; antimicrobial activity invitro against three gram-positive bacteria (*Staphylococcus aureus, Micrococcus luteus and Bacillus cereus*) and three gram-negative bacteria (*Escherichia coli, Pseudomonas fluorescens* and *Flavobacterium devorans*) as well as three fungi (*Aspergillus niger, Penicillium chrysogenum* and *Curvularia lunata*). They indicated that some synthesized coumarin triazole derivatives displayed better antitubercular, antioxidant, antibacterial and antifungal efficacy in comparison with reference drugs. So, two compounds of coumarin derivatives 3-Acetyl coumarin, Benzo-4-methyl coumarin were synthesized and confirmed for their chemical structures by their melting point; carbon and hydrogen elemental analysis in addition to their spectroscopy identification measurements by U.V, I.R, H^1-NMR, and M.S. These two compounds were evaluated for their fungicidal effects against *Alternaria* effects on *Erwinia amylovora* and *Ralstonia solanacearum* in comparing with streptomycin as standard bactericide.

MATERIALS AND METHODS

Two compounds of coumarin derivatives were synthesized and there melting points were determined on kofler-block instrument and were uncorrected. Micro analysis of C,H and spectroscopy measurements were carried out at Micro-analytical Center, Faculty of Science, university of Cairo, Giza, A.R.E. U.V measurements were conducted on Absorbance U.V-1600 series Normal S/R Exchange. I.R spectra were measured on Shimadzu FT/IR4100 Instrument. H^1-NMR spectra were recorded on Mercury-300 BB "NMR300" instrument. Mass Spectra (MS) were determined on DI Analysis, Shimadzu Qp-2010 plus.

I- Experimental procedure; mechanism and identification.

1) 3-Acetyl coumarin

3- Acetyl coumarin was prepared according to Siddiqui *et al* (2009) with few modification. A mixture of salicyaldehyde (0.4mol) and ethyl acetoacetate (0.4mol) was stirred and cooled. Sodium ethoxide (5gm), (piperidine used by Siddiqui *et al,* 2009) was add gradullay with shaking. The reaction mixture was maintained at freezing (-13Cº) for 5 hours and resulting in a yellow colored precipitate was separated out and recrystallized from ethanol producing 3-Acetyl coumarin, m.p 122 Cº (125-128Cº) by Siddiqui et al (2009),Calculated %C=70.2 %H=4.2 Founded %C=71.8, %H=4.36 The mechanism of reaction can be elucidated as follows:

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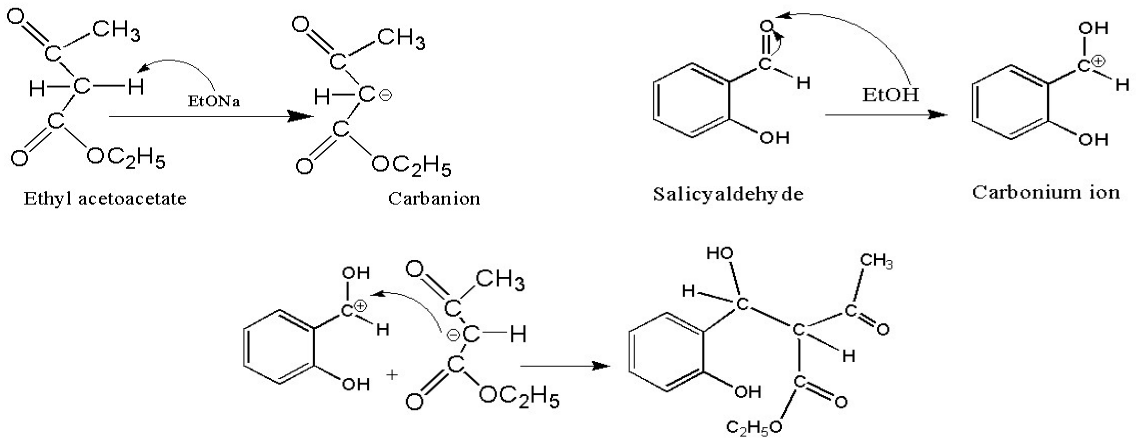
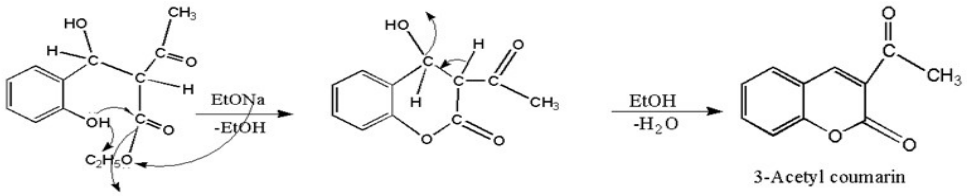
A- Aldol condensation between salicyaldehyde and ethyl acetoacetate



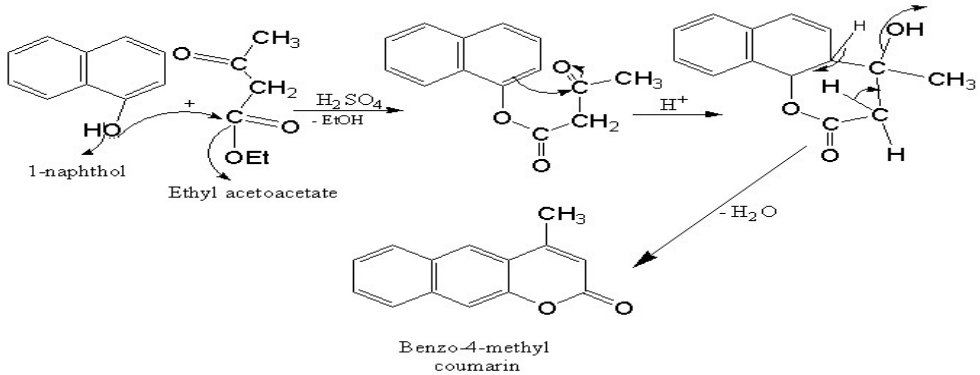
B- Ester hydrolysis and cyclization



U.V (EtOH): $\pi \rightarrow \pi^*$ and $n \rightarrow \pi$ transition due to C-O-C, C=O and phenyl rings, $\pi \rightarrow \pi^*$ at 267.5 nm. Forbidden $n \rightarrow \pi$ transition due to C=O in –O-CO-CH-CH_3 part at 297 nm. I.R , C=C (benzenoid ring), 1677.8 – 1610.3 Cm^{-1},CH_3CO 3433 Cm^{-1}, C-O-C 1359.6 – 1295.9 Cm^{-1}. H^1NMR (DMSO) δ(ppm) = ph-H 7.38 – 7.69 δ (ppm); H-C=C-C=O in α-pyrone ring 8.65 δ(ppm), H-methyl group 3.3 δ ppm. M.S: m/z 188$(M)^+$, 173, 145, 117, 94, and 65.

2) Benzo-4-methyl coumarin

Preparation of Benzo-4-methyl coumarin, a solution of 1-naphthol (0.05mol) dissolved in hot concentrated sulphuric acid (35ml) was placed in round-bottomed flask, then ethyl acetoacetate (0.1 mol) was gradually added (drop by drop) with intermittent shaking. The reaction mixture was maintained at freezing temperature (-5 Cº) with continuous stirring for 4 hours. The precipitate recrystallized from hot ethanol producing Benzo-4-methyl coumarin m.p 144 Cº.Calculated %C=80 ,%H=4.6, founded %C= 78.7, %H= 4.47, the mechanism of reaction can be elucidated as follows:



The mechanism of reaction depends on pechman condensation reaction which performed under acidic condition involving ester hydrolysis by acid catalyzed acyl-oxygen cleavage uni-molecular (A-acyl-O-1) producing stable carbonium ion which coordinated by the pair electrons of hydroxyl oxygen atom followed by attack of the activated carbonyl naphtyl ring to generate the new 3-methyl α-pyrone ring.

U.V (EtOH): λ= 278, 267 and 214 nm for $\pi \rightarrow \pi^*$ due to poly aromatic rings; 306.0 and 319.5 nm for $\sigma \rightarrow \pi^*$ due to the hyper-conjugation of σ-electrons of a methyl C-H bond in resonance with pyranone ring. I.R C=C of phenyl rings 1634.4 – 1609.3 Cm^{-1}; lactone carbonyl 1715.4 Cm^{-1} ; C-O-C 1373.1 - 1171 Cm^{-1} , CH_3 group 3060.5 – 2919.7 Cm^{-1}. H^1NMR (DMSO) δ(ppm) = H-aromatic of phenyl rings 7.69 – 8.3 δ(ppm),

H-C=C- 6.46 δ(ppm), H-CH_3 3.327 δ(ppm). M.S: m/z 210$(M)^+$, 182, 143, 115, 76 and 51.

II- Biological Parts:

The two synthesized and identified coumarin derivatives were tested for their fungicidal effects against Early blight pathogen (*Alternaria solani*) and Damping-off pathogen (*Fusarium oxysporum*), and bactericidal effects on Fire blight pathogen (*Erwinia amylovora*) and Bacterial wilt pathogen (*Ralstonia solanacearum*).

1) Fungicidal effects measurements:

The fungicidal effects of tested coumarin derivatives 3-Acetyl coumarin and Benzo-4-methyl coumarin were evaluated against *Alternaria solani* and *Fusarium oxysporum* by regular radial growth method, which belongs to poisoned food technique. The tested fungi were allowed to grow on Czapek-Dox agar medium for 7-days before using the test. The results were recorded by measuring two vertical radii of the growth in each Petri-dish. The percentage inhibition (%I) calculated according to Topps and wain (1957) formula, when the hyphal growth of untreated fungi (control) filled the Petri-dish.

$$\%1 = \frac{A-B}{A} \times 100$$

Where:

A= diameter of untreated fungus.

B= diameter of treated fungus.

A regression line between %I values and the tested concentrations was conducted on log-probit paper from which the concentration caused 50% inhibition (EC_{50}) in the hyphal growth was determined for each compound.

2) Bactericidal effects measurements:

The bactericidal effects of tested coumarin derivatives 3-Acetyl coumarin and Benzo-4-methyl coumarin were evaluated against *Erwinia amylovora* and *Ralstonia solanacearum* by agar disk-diffusion method according to Balouiri *et al*(2016). The diameters of inhibition growth zones of germinated bacteria in Petri-dish are measured. The minimum inhibitory concentrations (MIC) are determined.

RESULTS AND DISCUSSION

A) Chemistry results

The synthesis procedures of the two tested compounds and their structures confirmation by melting points, elemental analysis and the spectrometric measurements by U.V; I.R; H^1-NMR and Mass spectroscopy were represented and discussed in the previous chemistry part I (in Materials and Methods).

B) Biological results:

1- Fungicidal activities measurements:

The fungicidal activities of the tested 3-Acetyl coumarin and Benzo-4-methyl coumarin in addition to mancozeb as standard fungicide against *Alternaria solani* and *Fusarium oxysporum* are recorded in **table** (1) and (2) ,respectively.

a) Fungicidal activities on *Alternaria solani*:

From table (1), it was found that 3-acetyl coumarin inhibited the growth of *Alternaria solani* at all the tested concentrations with range of inhibition percents 38.8 – 93.3% with IC_{50} value equal $4x10^{-4}$ M. The inhibition percents increased with increasing the tested compound concentration. Benzo-4-methyl coumarin proved to be highly effective to inhibit the hyphal growth of *Alternaria solani* with inhibition percents 45.6 – 98.2% range with IC_{50} value equal $2x10^{-4}$ M. the percents of inhibition increased with increasing the tested compounds concentrations. The results indicated that Benzo-4-methyl coumarin was twice more effective to inhibit the hyphal growth of *Alternaria solani* than 3-acetyl coumarin. So, the methyl substituent in addition the fused benzene ring in Benzo-4-methyl coumarin may be required to increase the activity against *Alternaria solani.* Mancozeb as standard fungicide was found to be more effective to inhibit the hyphal growth of *Alternaria solani* with IC50 value equal $0.07x10^{-4}$M. So, the activity of the tested compounds can be arranged in descending order as follows:

Mancozeb >> Benzo-4-methyl coumarin > 3-Acetyl coumarin

Table 1. Effect of the tested compounds on *Alternaria solani* , shown as inhibition percents of the hyphal growth and their IC_{50} values

Tested compounds	% Inhibition					EC_{50}
	$2x10^{-4}$ M	$5x10^{-4}$ M	$1x10^{-3}$ M	$2x10^{-3}$ M	$1x10^{-2}$ M	
3-Acetyl coumarin	38.78	50.07	62.81	78.22	93.33	$4x10^{-4}$ M
Benzo-4- methyl coumarin	54.59	65.48	73.26	83.48	98.15	$2x10^{-4}$ M
Mancozeb	67.84	76.44	84.14	92.87	98.56	$0.07x10^{-4}$M

b) Fungicidal activities on *Fusarium oxysporum*:

Table (2) showed the inhibition percents rang of 3-acetyl coumarin to be 32.4 – 77.2% and IC50 value equal $4x10^{-4}$M on *Fusarium oxysporum*. The Benzo-4-methyl coumarin was found to be more active to inhibit the hyphal growth of *Fusarium oxysporum* with 45.2 – 91.6 % inhibition range and $5x10^{-4}$M. So, Benzo-4-methyl coumarin was more effective to inhibit the hyphal growth of *Fusarium oxysporum* with 3.2 times than 3-Acetyl coumarin which revealed that the chemical structure of Benzo-4-methyl coumarin was ideal model to be effective against the hyphal growth of *Fusarium oxysporum* . However, mancozeb as standard fungicide was highly effective with IC_{50} equal $0.36x10^{-4}$M against *Fusarium oxysporum*, the hyphal growth of *Fusarium oxysporum* highly effected in descending order as follows Mancozeb >> Benzo-4-methyl coumarin > 3-Acetyl coumarin. It can be concluded that although mancozeb as standard fungicide was highly effective against both fungi, the tested coumarin compounds proved to be active to inhibit the hyphal growth of *Alternaria solani* and *Fusarium oxysporum*. *Alternaria solani* was found to be more sensitive to the tested compounds.

2- Bactericidal activities measurements:

The effects of the tested 3-acetyl coumarin and benzo-4-methyl coumarin in addition to streptomycin as a standard antibacteria on the growth of *Erwinia amylovora* and *Ralstonia solanacearum* are recorded in table (3) and (4) as zone of inhibition diameter (mm) and MIC (minimum inhibitory concentration)

a) Effect on *Erwinia amylovora*

Table (3) showed that the diameter of inhibition zone in *Erwinia amylovora* caused by 3-acetyl coumarin was 11mm at the highest tested concentrartion which revealed that MIC was $1x10^{-2}$ M for this compound. The diameters of inhibition zone of growth *Erwinia amylovora* in case of benzo-4-methyl coumarin were 9.1, 16.6 and 23.2 mm at $1x10^{-3}$, $2x10^{-3}$ and $1x10^{-2}$ M , respectively. So, the MIC value caused by benzo-4-methyl coumarin was $1x10^{-3}$ M.

The results indicated that benzo-4-methyl coumarin proved to be more active ten times than 3-acetyl coumarin to inhibite the growth of *Erwinia amylovora*. Streptomycin as standard antibacteria was higher effective against this bacteria, it MIC was less than the lowest tested concentration.

a) Effect on *Ralstonia solanacearum*

Table (4) exhibited the inhibition zone diameter of *Ralstonia solanacearum* by 3-acetyl coumarin were 5 and 16.4 mm in case of $1x10^{-3}$ M and $1x10^{-2}$ M which gave MIC equal $2x10^{-3}$ M , whereas the diameters of inhibition zone of *Ralstonia solanacearum* were 4.7, 17.7 and 25.5 mm caused by $1x10^{-3}$, $2x10^{-3}$ and $1x10^{-2}$ M, respectively producing MIC equal $1x10^{-3}$ M. Streptomycin was also strongly effective against *Ralstonia solanacearum* with MIC < $2x10^{-4}$ M.

The results showed that benzo-4-methyl coumarin was more effective on both tested bacteria but more less than streptomycin it can be concluded that the synthesized benzo-4-methyl and 3-acetyl coumarin have fungicidal and bactericidal properties but less than the used standard.

Table 2. Effect of the tested compounds on *Fusarium oxysporum*, shown as inhibition percents of the hyphal growth and their IC_{50} values

Tested compounds	% Inhibition					EC_{50}
	$2x10^{-4}$ M	$5x10^{-4}$ M	$1x10^{-3}$ M	$2x10^{-3}$ M	$1x10^{-2}$ M	
3-Acetyl coumarin	32.37	35.70	38.37	47.67	77.19	$14x10^{-4}$ M
Benzo-4- methyl coumarin	45.22	48.67	54.37	64.59	91.63	$5x10^{-4}$ M
Mancozeb	59.59	67.4	75.9	84.48	98.67	$0.36\ x10^{-4}$M

Table 3. effect of the tested compounds on the growth of *Erwinia amylovora* shown as zone of inhibition diameter and MIC value.

Tested compounds	Zone of Inhibition diameter (mm)					MIC
	$2x10^{-4}$ M	$5x10^{-4}$ M	$1x10^{-3}$ M	$2x10^{-3}$ M	$1x10^{-2}$ M	
3-Acetyl coumarin	0.00	0.00	0.00	0.00	11.0	$1x10^{-2}$ M
Benzo-4- methyl coumarin	0.00	0.00	9.10	16.60	23.20	$1x10^{-3}$ M
Streptomycin	7.65	19.45	31.25	40.46	46.13	$< 2x10^{-4}$M

Table 4. Effect of the tested compounds on the growth of *Ralstonia solanacearum* shown as zone of inhibition diameter and MIC value

Tested compounds	Zone of Inhibition diameter (mm)					MIC
	$2x10^{-4}$ M	$5x10^{-4}$ M	$1x10^{-3}$ M	$2x10^{-3}$ M	$1x10^{-2}$ M	
3-Acetyl coumarin	0.00	0.00	0.00	5.00	16.4	$2x10^{-3}$ M
Benzo-4- methyl coumarin	0.00	0.00	4.70	17.70	25.5	$1x10^{-3}$ M
Streptomycin	9.10	21.75	40.14	44.80	49.76	$< 2x10^{-4}$M

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الملخص العربي

التاثير الابادي الميكروبي ل٣-استيل كومارين وبنزو-٤ميثيل كومارين المحضرين

محمد عبد الفتاح دشيش، سعد رشاد الزميتي، عزت امين قادوس، ماجدة محمد فهمي، عماد السيد توفيق

تم تحضير مركبين معمليا ٣-استيل كومارين وبنزو٤-ميثيل كومارين والتأكد من التركيب الكيميائى عن طريق تقدير نقطه الانصهار وتحليل نسبه الكربون والهيدروجين بالاضافه الى القياسيات الطيفيه (الاشعه فوق البنفسجيه، الاشعه تحت حمراء، جهاز الرنين المغناطيسى والكتله الطيفيه). وتم تقييم كفاءه المركبين كمضادات فطريه ضد فطر الفيوزاريم اكسوسبورم وفطر ألترناريا سولانى، بمقارنه المبيد الفطري القياسي (المانكوزب) باستخدام طريقه الطعم السام فى البيئه الصلبه. وتم تقييم كفاءه المركبين كمضادات بكتيريه ضد بكتيريا ايروينا اميلوفورا ورالستونيا سولانا سيريم بمقارنه المضاد البكتيرى القياسيى (الاستربتوميسين) باستخدام طريقه انتشار الديسك. والنتائج المتحصل عليها ان بنزو٤-ميثيل كومارين اكثر فعاليه من ٣-استيل كومارين على فطريات ألترناريا سولانى والفيوزاريم اكسوسبورم وبكتيريا ايروينا اميلوفورا ورالستونيا سولانا سيريم.