JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2010. Vol. 4(1), p. 63-68

Antifungal vs. Antibacterial Activity of α,β-unsaturated Carbonyl Compound

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(Received: 07 August 2009; accepted: 26 September 2009)

Antimicrobial activity of two α,β-unsaturated carbonyl compounds, 9-(propan-3-ene-1-methyl-2-one) anthracene (5) and *trans, trans* –dibenzylidene acetone (7) were investigated using the Agar diffusion and Poison plate methods. Compounds were synthesized via the aldol route in very good yields and were characterized via ^{1}H NMR, ^{13}C NMR, DEPT-135 and IR spectroscopy. Both compounds were found to be antimicrobial. However, their mode of action was more antibacterial than antifungal.

Key words: Antimicrobial activity, α,β-unsaturated carbonyl compounds, Agar well diffusion, poison plate methods and spectroscopy.

Over the years, plant extracts[1,4,5-9] and isolated pure natural products[2,3] have been used for their antimicrobial activities. However, there are few reports of synthesized compounds such as α,β-unsaturated carbonyl compounds as antimicrobial agents[10-13]. In our continuing search for novel, potent and selective antimicrobial agents, we report here the investigation of antimicrobial activity of α,β-unsaturated carbonyl compounds (5) and (7) via Agar diffusion and Poison plate techniques[15]. Compounds (5), *9-(propan-3-ene-1-methyl-2-one)anthracene* and (7), *trans, trans –dibenzylidene acetone* were obtained via base catalysed condensation of acetone with benzaldehyde and acetone with anthrone followed with dehydration of each respectively. After work up, compounds (5) and (7) were obtained as a yellow and pale yellow solids in 85 and 92% yield respectively[14].

Both compounds were characterized fully via ^{1}H NMR, ^{13}C NMR, DEPT-135 and IR spectroscopy[14].

Below is a synthetic scheme of compound (5) with a mechanistic overview.

MATERIAL AND METHODS

Reagents and materials

α,β-unsaturated carbonyl compounds (5) and (7) were synthesized and solvents purchased from Aldrich. Antibiotic Ciprofloxacin and nystatin, Mueller Hinton agar, agar plates and microbial discs were purchased from the International Pharmacy Association in Guyana.

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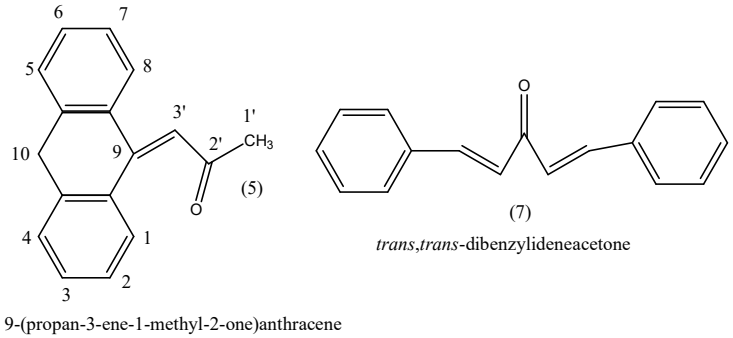
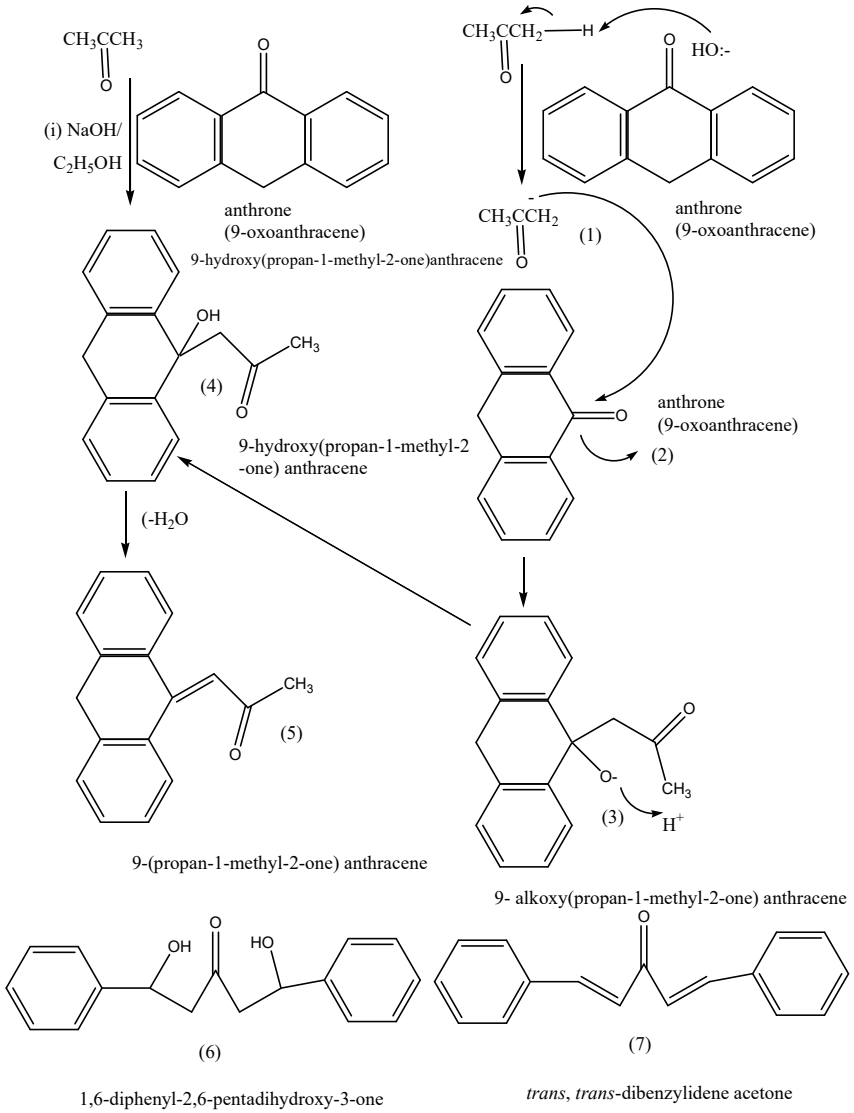


Fig. 1. α,β unsaturated carbonyl compounds (5) and (7)



Scheme. 1. Syntheses of a,b-unsaturated carbonyl compounds (5) and (7), a mechanistic overview

Bacterial and fungal culture were obtained at John's campus, Berbice, University of Guyana.

Preparation of α,β-unsaturated carbonyl compounds solution

α,β unsaturated carbonyl compounds (5) and (7) were made up to the appropriate concentration of 10mg in 10ml (1mg in 1 ml) of dichloromethane in a 25 ml round bottom flask and were stored under aseptic conditions.

Source of microorganisms

For the bacterial organisms, gram negative bacteria used was *Staphylococcus aureus* (ATCC 25923). For the fungi, yeast of the *Candida albicans* (ATCC 1023) species was investigated. These microorganisms were stored in a refrigerator at the microbiology laboratory at John's Science Campus, Berbice.

Reference and Control

The references were antibiotic in nature. *Ampicillin* and *Nystatin*. *Ampicillin* was choosen as the reference for all bacterial species used: *E.coli* and *S.aureus*. *Nystatin* was used as the reference for the fungus, *Candida albicans.* The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion[21].

Antimicrobial tests

Compounds (5) and (7) were investigated for their antimicrobial activity using the Agar diffusion[15] and Poison Plate techniques[15] under aseptic conditions.

Aseptic conditions

The aseptic chamber consists of a wooden box (1m x1m x 0.5m) with a door which was cleaned with 70% ethanol and irradiated with short wave UV light for 1 hour.

Mother plates

These were made by culturing *C.albicans* on PDA (Potato dextrose Agar). A sterilized 9 cm cork borer was used to cut agar discs in the plate.

Potato dextrose agar (PDA)

This is an agar medium on which the fungi was cultured.The potato was peeled and 200g weighed, finely chopped and boiled to a

Table 1. Random check (1 mg in 1 ml) (Reference experiments)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* ED_{50} (mm)	Area of inhibition* (mm^2)	(Average of Triplicate)* ED_{50} (mm)	Area of inhibition (mm^2)*	(Average of Triplicate)* ED_{50} (mm)	Area of inhibition (mm^2)*
5	6.30	158.29	7.10	158.29	4.30	58.06
7	5.80	57.19	6.6	136.78	5.3	88.20

*Mean value

Table 2. Controlled experiment (1 mg in 1 ml)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* ED_{50} (mm)	Area of inhibition* (mm^2)	(Average of Triplicate)* ED_{50} (mm)	Area of inhibition (mm^2)*	(Average of Triplicate)* ED_{50} (mm)	Area of inhibition (mm^2)*
5	< 5	< 5 mm^2	< 5	< 5 mm^2	< 5	< 5 mm^2
7	< 5	< 5 mm^2	< 5	< 5 mm^2	< 5	< 5 mm^2

*Mean value

mash in distilled water.The dextrose was weighed (12.5g) and placed in a 1L measuring cylinder. Agar was weighed (12.5g) and added to the measuring cylinder (with the dextrose).The potato mash was stirred and strained into the cylinder. Distilled water was added to make up the solution to 500mL.The contents was continuously stirred until consistency was achieved and was then poured into a conical flask, plugged with cotton wool, over which aluminium foil was tightly wrapped. The flask was then autoclaved at 121 °C, 15psi for fifteen minutes.

Agar diffusion Technique

The spore suspension of pathogens was seeded into a molten PDA medium or poured into petri plates. When the medium solidified, a 9 cm well was made at the centre of the plate with the help of a sterile 9 cm cork borer. A solution of the test compound, α,β-unsaturated carbonyl compounds (5) and (7) at a concentration of 1mg in 1ml was transferred into the well and incubated for three days. The zone of inhibition in mm^2 was measured for the test compound and recorded. From these values, the area of inhibition was calculated.

Poison Plate technique

Under aseptic conditions, the test compound was seeded into molten PDA medium and poured into Petri plates. The plates were covered and allowed to cool. As soon as the agar was solidified, a 9 cm sterile cork borer was used to make a disc on the pathogen plate (Mother plate). A pathogen disc was taken from pathogen plate (mother plate) and kept at the centre of test compound seeded plate with help of a sterile innoculum needle and was incubated for 3 to 4 days. The innoculum needle was sterilized with alcohol and flame before each application.

The zone of inhibition was measured for pathogenecity of test compound. The experiment was repeated thrice. Triplicate were maintained for each test compound at every 24 hours interval.

Table 3. Diffusion Plate (1 mg in 1 ml)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition* (mm^2)	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)*	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)*
5	3.3	34.0	4.2	55.39	3.5	38.47
7	2.6	21.23	2.9	26.40	4.2	55.39

*Mean value

Table 4. Poison Plate (1 mg in 1 ml)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition* (mm^2)	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)*	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)*
5	4.2	55.38	5.3	88.20	3.3	34.19
7	3.2	32.15	3.7	42.99	4.2	55.39

*Mean value

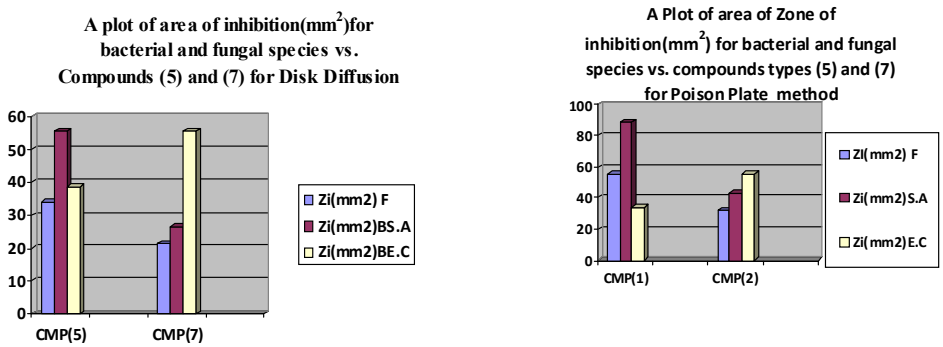


Fig. 2. A plot of zone of inhibition for bacterial and fungal species vs. compounds type for diffusion and poison plate, methods

The diameter of mycelium growth was measured and the average value taken.

DISCUSSION

Compounds (5), 9-(propan-3-ene-1-methyl-2-one)anthracene and (7), trans, trans – dibenzylidene acetone α,β-unsaturated carbonyl compounds were obtained via the base catalysed condensation of acetone with anthrone (9-oxo-anthracene) and acetone with benzaldehyde respectively to yield aldols (4) and (6) followed by dehydration, Scheme 1. The mechanism, Scheme 1 involves the abstraction of an a-proton from acetone to yield carbanion (1) followed by nucleophilic attack of that carbanion on benzaldehyde or anthrone (which doesn't bear an a-hydrogen) to yield addition adducts (3). The adduct abstract a proton from the protic solvent to furnish aldols (4), 9-hydroxy(propan-1-methyl-2-one)anthracene and (6) 1,6-diphenyl-2,6-pentadihydroxy-3-one which are further dehydrated to yield a,b –unsaturated carbonyl compounds (5) and (7).

Antimicrobial activity of both compounds were investigated using the Agar diffusion and poison plate methods[15]. The zone of inhibition (mm) is quoted at the ED_{50} value and as the area of inhibition (mm^2). ED_{50} is the effective dose concentration of the sample required to kill 50% of the pathogen growth. The zone of inhibition in mm at ED_{50} was calculated and converted into area of inhibition, mm^2. First, a random check of both compounds antimicrobial activity was investigated using both methods, Table 1. These served as the reference experiments. Ampicillin was used as the reference for bacterial species and Nystatin as the reference for the fungal species. A controlled experiment was also investigated using the pure solvent with the microorganism as the innoculant[18]. It was found that the pure solvent induced negligible zone of inhibition on the agar medium, Table 2. Thus, the zone of inhibition are indeed due to the plant extracts rather than to the pure solvents. This was followed with careful microbial triplicate experiments for both techniques against microbial strains: *Candida albicans*, *E.coli* (gram negative bacteria), *Staphylococcus aureus* (gram positive). Results indicate under similar aseptic conditions, compounds (5) and (7) induce a larger zone of inhibition against the bacteria, *Staphylococcus aureus* and *E.coli* than against the fungal strains, *Candida albicans* for both diffusion and poison plate methods. For example, for the diffusion plate method, Table 3, compound (5) induce zone of inhibition of 34.0 mm^2 (ED_{50}= 3.3 mm) against fungal strain, *Candida albicans* compared with a zone of inhibition of 55.39 mm^2 (ED_{50} = 4.2 mm^2) and 38.47 mm2(ED_{50} = 3.5 mm) for the bacterial species, *Staphylococcus aureus* and *E.coli* respectively. A similar antimicrobial trend was shown for compound (7) using the poison plate technique. For example, for compound (7), zone of inhibition of 32.15 mm^2 (ED_{50} = 3.2 mm) was noted for *Candida albicans* versus values of 42.99

mm^2 (ED_{50} = 3.7 mm) and 55.39 mm^2(ED_{50} = 4.2 mm) for bacterial species, *S.aureus* and *E.coli* respectively, Table 4.

Thus, both compounds (5) and (7) were found to be more antibacterial than antifungal in their activities i.e selective. Further antimicrobial selective activities are shown in the results. For example, compound (5) showed a larger zone of inhibition against *S.aureus* vs *E.coli* for both methods. For example, for disk diffusion, (5) exhibits zone of inhibition of 55.39 mm^2 (ED_{50} = 4.2 mm) and 38.47 mm^2 (ED_{50} = 3.5 mm) against *S.aureus* vs. *E.coli* respectively. However, a reversal is noted for compound (7) against *E.coli.* Compound (7) showed a larger zone of inhibition for *E.coli* vs *S.aureus* for both methods. For example, for disk diffusion, (7) exhibits zone of inhibition of 55.39 mm^2 (ED_{50} = 4.2 mm and 26.40 mm^2 (ED_{50} = 2.9 mm^2) for *E.coli* and *S.aureus* respectively.

ACKNOWLEDGEMENTS

This research was financially supported by a small grant to Dr. R.C. Jagessar from the Royal Society of England and the University of Guyana, Turkeyen. The use of the microbiology laboratory at John's Science Centre, Berbice Campus, University of Guyana is acknowledged.

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