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Effects of Phenyl Ring Substituents on the Fungicidal Activity of O-Ethyl-n-Phenyl Carbamate

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Abstract: Series of O-Ethyl-N-phenyl carbamates and O-Ethyl-N-substituted phenyl carbamates were prepared by reaction of ethyl chloroformate with aniline or substituted aniline. The preparation compounds were characterized by elemental analyses, Fourier-transform Infrared, ^{1}H and ^{13}C nuclear magnetic resonance spectroscopic techniques. *In-vitro* fungicidal assay of this class of carbamates against *Rhizopus stolonifer, Aspergillus flavus, Fusarium oxysporum* and *Aspergillus niger,* showed that they were effective. O-Ethyl-N-(4-methylphenyl) carbamate, with minimal inhibitory concentration of 50ppm and IC_{50} of 20 ppm, was found to be most active, while O-ethyl-N-(2-nitrophenyl) carbamate showed the least activity, with minimal inhibitory concentration of 2000ppm and IC_{50} of 1000ppm. Very little variations in activity were observed with the tested four fungi species.

Key words: Preparation · elemental analyses · TLC · FTIR · ^{1}H-NMR and ^{13}C-NMR spectroscopic techniques · fungicidal assay

INTRODUCTION

Fungicides are effective and reliable means of controlling pests and diseases that could lead to loss of crops in the field and during storage [1]. The earliest fungicides were inorganic materials like sulphur (or lime sulphur), copper in various forms and mercury. The development of purely organic fungicides started with the discovery of fungicidal activity of the dithiocarbamates [2] originally produced as vulcanization agents in the rubber industry. Metal salts such as ferbam and the corresponding zinc salt, ziram, are powerful fungicides; the oxidation product of the unstable N, N-dimethyldithiocarbamic acid known as thiram is effective as seed dressing against foot rot of beans as well as peas and botrytis mould on lettuce and strawberry. Salts of the related ethylenebisdithiocarbamate, such as the sodium (nabam), the zinc (zineb) and the manganese (maneb) salts are also used as agricultural fungicides. The toxicity to fungi of the dithiocarbamates is probably associated with their ability to chelate with certain essential metal cations, like copper. However, the ethylenebisdithiocarbamates have a different mode of action: nabam itself is not itself fungicidal, but under the influence of air it decomposes into ethylene isothiocyanate, which is probably the active toxicant by virtue of its reaction with essential thio groups [3].

Another important group of organosulphur fungicides are the trichloromethylthio derivatives [4]. The best known example is captan or orthocide widely used to control black spot (roses), botrytis (strawberry), scab (apple and pear) and foot rot (peas and beans). The majority of antifungal materials behave as protectant fungicides; such compounds can be gradually removed from the plant by wind and rain and all new growth will be protected and so will be liable to immediate fungal attack. These difficulties could be overcome by application of a systemic fungicide [5]. The development of systemic fungicides has been considerably slower than that of systemic insecticides and herbicides because of the much greater problems arising from the close similarity between the host plant and the fungus [6], but there is now little doubt that in the near future we shall have a range of effective commercial systemic fungicides available for use against economically fungal pathogens.

With growing world population, there is an urgent need for the discovery and development of safer and more efficient pesticides. This is necessary both to contend with the continuing problem of insect resistance [7] and to move the toxic hazards presented by some of currently used pesticides, like organochlorine insecticides and organ mercury seed dressings [8].

Methyl carbamates are known for their high toxicity on mammals. Severe poisoning by methyl carbamates in

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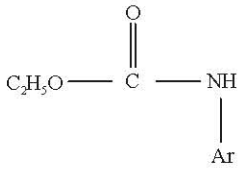
mammals has been reported [9]. Less toxic and effective phenyl carbamates could be a substitute for methyl carbamates.

As a result of the fungicidal activity shown by O-ethyl-N-phenyl carbamate [10], it is of interest to examine O-ethyl-N-substituted phenyl carbamates for their fungicidal activity. This report is concerned with the synthesis and fungicidal activity of O-Ethyl-N-phenyl carbamate of the general structure I (where Ar = C_6H_5, p-ClC_6H_4, p-MeC_6H_4, m-$NO_2C_6H_4$, o-$NO2C_6H_4$).



The inhibitory effect of the synthesized compounds on the growth of the four fungi species were expressed through Minimal Inhibitory Concentration (MIC) and 50% inhibitory concentration (IC_{50}) [11].

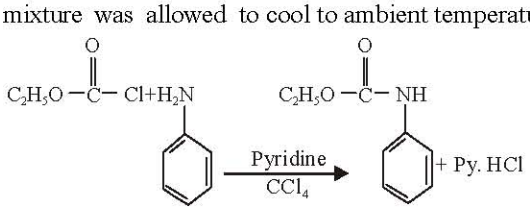
The goal is clearly attainable that it is feasible to design chemical pesticides capable of selectively controlling a specific pest with little harm to natural predators and other forms of wild life.

MATERIALS AND METHODS

Synthesis of the compounds: O-Ethyl-N-phenyl carbamate and O-Ethyl-N-substituted phenyl carbamates were synthesized by the reaction between ethyl chloroformate and aniline (or substituted aniline) in the presence of pyridine which was used for accepting the liberated hydrogen chloride [12, 13]. The following procedure for the synthesis of O-Ethyl-N-phenyl carbamate (II) according to scheme 1 is typical.

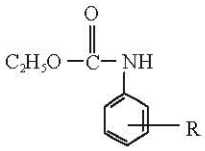
To a mixture of re-distilled aniline (9.3g, 9.1ml, 0.1mol) and excess pyridine in carbon tetrachloride (40ml) was added ethyl chloroformate (10.9 g, 9.6 ml, 0.1 mol) dropwise. The reaction mixture was heated under reflux for 1 hour.

Pyridine hydrochloride is separated out within a few minutes after addition of ethyl chloroformate. The reaction mixture was allowed to cool to ambient temperature and



Scheme 1: Synthesis of O-ethyl-N-phenyl carbamates

Table 1: Analytical data for O-Ethyl-N-substituted phenyl carbamates



			Elemental analysis			
			Calculated		Found	
	MP°C	TLC	C	N	C	N
1. O-Ethyl-N-(2-nitrophenyl) carbamate	90-91	0.75	51.43	13.32	51.97	13.66
2. O-Ethyl-N-(3-nitrophenyl) carbamate	39-40	0.83	51.43	13.32	51.02	13.08
3. O-Ethyl-N-(4-nitrophenyl) carbamate	129-13	00.75	51.43	13.32	51.68	13.50
4. O-Ethyl-N-(4-chlorophenyl) carbamate	40-41	0.76	54.14	7.01	54.09	6.92
5. O-Ethyl-N-(4-methylphenyl) carbamate	49-50	0.75	67.02	7.81	67.20	7.98

finally stirred overnight. The dark brown reaction mixture was poured onto distilled water (200ml) acidified with concentrated hydrochloric acid (20cm^3) and stirred mechanically. The wine-coloured organic layer was separated and washed with water (3x25 ml). After the final washing, the organic layer was dried over anhydrous Sodium Sulphate. Upon filtration, the volatile solvents were removed at a reduced pressure to give an oily product, which crystallized on cooling. The wine-coloured crude product was re-crystallized from ethanol to give the desired product, O-ethyl-N-phenyl carbamate, II (12.4 g, 75.0%); (Found: C,65.75; N, 8.94. Calc. for $C_9H_{11}NO_2$: C, 65.44; N,8.47%); m.p 52-53°C (lit. 53°C). TLC (ethanol/DMSO, 3:1) on silica gel gave a single spot, R_f = 0.74. The diagnostic infrared of carbonyl stretching band at 1700 cm^{-1} was very strong. Secondary amide band appeared at 3300 cm^{-1} for N-H stretching. ^{1}H-NMR spectrum showed the following absorptions δ_H (DMSO); 1.2 (CH_3CH_2, t, 3H), 4.1 (CH_3CH_2, q, 2H), 6.8 - 7.5 (Ar-H, m, 5H), 9.6 (N-H, b.s, 1H); ^{13}C-NMR spectrum showed the following absorptions δ_C (DMSO): 15 (CH_3CH_2), 61 (CH_3CH_2), 119 (Ar C-4), 123 (Ar C-3,5), 129 (Ar C-2, 6), 140 (Ar C-1), 155 (C = 0).

The melting points, elemental analyses and TLC values of O-Ethyl-N-substituted phenyl carbamates are shown in Table 1. ^{1}H-and ^{13}C-NMR spectra were obtained from a varian Mercury Ft-NMR spectrometer (200 MHz). Results of the infrared, ^{1}H-NMR and ^{13}C-NMR spectra of this class of carbamates are presented in Table 2-4 respectively.

Table 2: Observed infrared bands of O-Ethyl-N-phenyl carbamate and O-Ethyl-N-Substituted phenyl carbamates

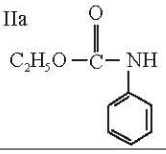
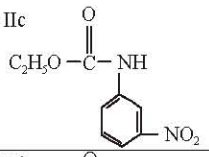
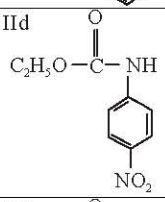
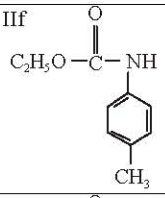
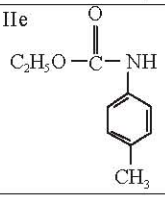
Structure of carbamate	Name of carbamate	IR (cm^{-1})
1. IIa 	O-Ethyl-N-phenyl carbamate	3300 (m), N-H_{str} (sec. Amide); 3100 (w), 3050 (w), = C-H_{str} (aryl); 2800 (w) - C-H_{str} (alkyl; 1700(s), C = O_{str}; 1600(s), C= C_{str} (aryl); 1450 (m); 1240(s), O-C = O_{str} (ester); 780 (m), 680 (m), monosubstituted benzne
2. IIc 	O-Ethyl-N-(3-nitrophenyl) carbamate	3300 (m), N-H_{str} (sec. Amide); 3100 (w), = C-H_{str} (aryl); 2990 (w), 2900 (w), -C-H_{str} (alkyl); 1740 (vs), 1700 (vs), C = Ostr; 1350 (vs), -NO_2; 1240 (vs), O-C=O_{str} (ester)
3. IId 	O-Ethyl-N(4-nitrophenyl) carbamate	3350 (m), N-H_{str} (sec. Amide); 2900(w), = C-H_{str}; 1690 (vs), C = O_{str}; $1585_{(s)}$ $1580_{(s)}$, C = C_{str} (aryl); $1300_{(vs)}$ -NO_2; $1200_{(vs)}$ O - C str (ester)
4. IIf 	O-Ethyl-N-(4-Methytolylphenyl) carbamate	3340(m), N-H_{str} (sec. amide); $3100_{(w)}$, =C-H_{str} (aryl); $2980_{(w)}$, $2800_{(w)}$ -C-H_{str}; $1400_{(m)}$, $1300_{(m)}$; -C-H_{str} (alky); $1200_{(vs)}$, $1100_{(vs)}$, O - C = O_{str} (ester); $800_{(s)}$, 1,4-disubstituted benzene.
5. IIe 	O-Ethyl-N-(4-Chlorophenyl) carbamate	$3350_{(m)}$, N-H_{str} (sec. amide); $3100_{(w)}$, $3050_{(w)}$, = C - H_{str} (aryl); $2900_{(w)}$, $2800_{(w)}$ - C-H_{str} (alkyl); $1700_{(vs)}$,; C=O_{str}; $850_{(s)}$; 1,4-disubstituted benzene

Table 3: 1H-NMR data of O-Ethyl-N-substituted phenyl carbamate

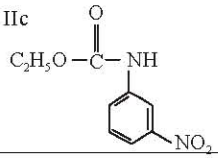
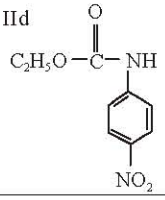
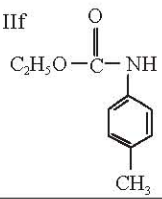
Structure of compound	Name of compound	δ (ppm)	Multiplicity
1. IIc 	O-Ethyl-N-(3-nitrophenyl) carbamate	1.2 (3H) $C\underline{H}_3CH_2$ 4.1 (2H) $CH_3C\underline{H}_2$ 7.5 (4H) 8.3 (1H) N-$\underline{H}$	t q m bs
2. IId 	O-Ethyl-N-(4-nitrophenyl) carbamate	1.3 (3H) $C\underline{H}_3CH_2$ 4.2 (2H) $CH_3C\underline{H}_2$ 6.1 (1H) N - $\underline{H}$ 7.4.(4H) AB-system (Ar)	t q bs 2d
3. IIf 	O-Ethyl-N-(4-methytolylphenyl) carbamate	1.3 (3H) $C\underline{H}_3CH_2$ 2.30 (3H) $C\underline{H}_3$ Ar 4.2 (2H) CH_3CH_2 7.4.(4H) AB-system 8.5 (1H) N - $\underline{H}$ (Ar)	t s q 2d bs

Table 3: Continued

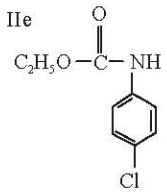
4.		O-Ethyl-N-(4-chlorophenyl) carbamate	1.2 (3H) $C\underline{H}_3CH_2$	t
			4.1 (2H) $CH_3C\underline{H}_2$	q
			7.35 (4H) AB-system (Ar)	2d
			9.7 (1H) N-$\underline{H}$	bs

Table 4: 13C-NMR data of some O-ethyl-N-substituted phenyl Carbamates

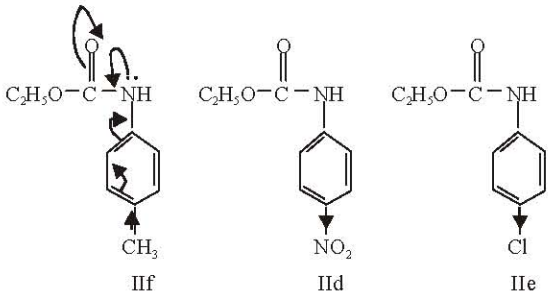
		δ/ppm								
	Compound	$\underline{C}H_3$	$\underline{C}H_2O$	$\underline{C}=O$	ArC-1	ArC-2	ArC-3	ArC-4	ArC-5	ArC-6
Iie	O-Ethyl-N-(4-chloro-phenyl) carbamate	15	61	154	127	120	127	139	127	120
Iic	O-Ethyl-N-(3-nitro-phenyl) carbamate	15	61	154	141	130.5	149	124.5	117.5	112.5

Biological screening: Potato Dextrose Agar (PDA) plates were flooded with spore suspension of each fungus. About 6mm diameter filter paper discs were sterilized in Petri dishes at 160°C for 2 hours. With the aid of sterilized pair of forceps, filter paper discs that have been soaked in solutions of various concentrations of each new compound were put on the surface of inoculated PDA plates. Filter paper discs were also soaked in the standard and the control,then placed on the surface of inoculated PDA plates. All the PDA plates were put in an incubator at room temperature. The growth diameter of the fungal spores was measured every 24 hours until there was complete growth of fungus on the control plate. The minimum concentration of each synthesized compound that inhibits the growth of the fungus was taken as the Minimal Inhibitory Concentration (MIC) of the compound. The IC_{50} (inhibitory concentration of the new compound at 50% inhibition of the fungus population) was extrapolated from the curve of percentage inhibition (% I) of fungus against concentration of the new compound [11].

RESULTS AND DISCUSSION

Table 5 shows the inhibitory effect of the synthesized compounds on the growth of fungi species expressed through the MIC and IC_{50} (ppm).

The type of constituent attached to the phenyl ring of the synthesized compounds affected their activity. All the substituted phenyl carbamates (except O-ethly-N-(2- nitrophenly) carbamete were found to be more active than the unsubstituted O-ethly-N-phenyl carbamate (Table 5). The compound containing methyl substituent showed greater inhibition than those containing either chloro-or nitro substituent. This observation may be correlated to do with the inductive effects of these substituents. Methyl ($-CH_3$) substituent has + I effect whereas, nitro ($-NO_2$) and chloro (-Cl) substituents have-I effect. The attachment of electron releasing groups like methyl ($-CH_3$) to the phenyl ring (III) would lead to increase in electron density of the carbonyl oxygen of the carbamate and the nucleophilic property of the carbamate is thereby increased. Fungicidal activity of this type of carbamate will be increased since fungicides act in the opposite direction to that of methyl substituent, by withdrawing electrons from the phenyl ring. This will reduce the electron density of the carbonyl (IV and V).

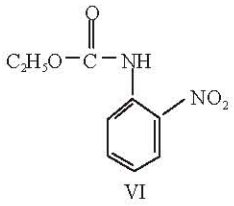


IIf IId IIe

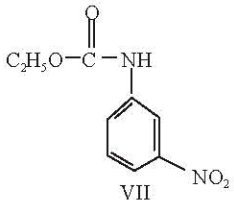
The position of substituents on the bezene ring also affected the fungicidal activity of the synthesized carbamates. When the activities of the three isomers of O-ethyl-N-nitrophenyl carbamate were compared, the *meta* isomer (VII), has the highest activity, while the *ortho* isomer (VI) has the least activity (Table 5).

Table 5: Inhibitory effect of the synthesized compounds on the growth of fungi species expressed through MIC and IC_{50} (in ppm)

		Percentage inhibition (% 1)							
		Rhizopus	*Stolonifer*	*Aspergillus*	*Flavus*	*Fusarium*	*Oxysporum*	*Aspergillus*	*Niger*
	Compounds	MIC	$1C_{50}$	MIC	$1C_{50}$	MIC	$1C_{50}$	MIC	$1C_{50}$
1.	O-Ethyl-N-phenyl carbamate	1000	500	1000	450	1000	470	1000	490
2.	O-Ethyl-N(2-nitrophenyl) carbamate	2000	1000	2000	950	2000	1000	2000	950
3.	O-Ethyl-N(3-nitrophenyl) carbamate	100	60	100	60	100	50	100	40
4.	O-Ethyl-N(4-nitrophenyl) carbamate	250	100	250	80	250	100	250	80
5.	O-Ethyl-N(4-chlorophenyl) carbamate	250	120	250	150	250	110	250	100
	O-Ethyl-N(4-methytolylphenyl) carbamate	50	35	50	30	50	40	5020	

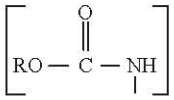


VI



VII

The low activity of the *ortho* isomer could be due to the steric hindrance as a result of its nearness to the bulky carbamoyl group.



It is then possible that advantage of the nucleophilic effects of the substituents on the electron density in the phenyl ring as well as the consequent effect of this on the electron availability of the oxygen of the carbonyl group, can be utilized to improve the fungicidal activity of the carbamates.

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