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J. Appl. Sci. Environ. Manage. *December*, 2008
Vol. 12(4) 5 - 9

Synthesis and fungicidal activity of some alkyl – 2,4 – dinitrobenzenesulfenate esters

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ABSTRACT: 2,4-dinitrobenzenesulfenyl chloride was prepared by reacting 2,4-dinitrochlorobenzene with benzyl mercaptan and subsequently cracking the formed 2,4-dinitrophenyl benzyl sulphide with sulfuryl chloride. The prepared 2,4-dinitrobenzenesulfenyl chloride reacted with series of primary alcohols in the presence of pyridine to give alkyl-2,4-dinitrobenzenesulfenate esters. The synthesized compounds were characterized by elemental analysis, thin layer chromatography (TLC), fourier-transform infrared, ^1H and ^{13}C nuclear magnetic resonance spectroscopic techniques. In vitro fungicidal assay of these alkyl sulfenate esters against *Aspergillus flavus, Rhizopus stolonifer* and *Fusarium oxysporum* showed that all the synthesized sulfenate esters were fungicidal. Increase in chain length of the alkyl substituents was found to reduce the fungicidal activity of the alkyl sulfenate esters. Presence of an hydroxyl group increased the fungicidal activity of ethyl-2,4-dinitrobenzenesulfenate by 5-to 10-fold. @ JASEM

The development of purely organic fungicides started with the discovery of the fungicidal activity of the dithiocarbamates which had been originally developed as vulcanization agents for the rubber industry (Cremlyn, 1979). The dithiocarbamates and their derivatives are one of the most important groups of organic fungicides for controlling plant diseases. The economically important fungicides that played a significant role in fruit growing were discovered through the work of Kittleson (Hassall, 1983). An example of this class of fungicides is captan, used as a foliar fungicide for the control of Venturia spp. in fruit and as a seed dressing and soil treatment against pythium spp. Captan is used widely today in fruits, vegetables, cereal, rice, grape, citrus, and ornamentals (Lamberth, 2004). Heterocyclic mercaptans are highly fungicidal. Omadine, an heterocyclic mercaptan has been used as a fungicide in soaps and shampoos. It also has a wide spectrum of activity and systemic properties against numerous foliar diseases such as apple scab and peach leaf curl *Taphrina deformans* (Buchel, 1983). Of all the derivatives of the thiocyanic acid, only the aromatic thiocyanates have been found to be fungicidal. For example, 2, 4-dinitrophenyl thiocyanate is active against *plasmopara viticola* on grape vines scab and rust on orchard fruit, while ethyl (4-thiocyanatophenyl) carbonate is used against powdery mildews on ornamentals Ayodele, *et al* 2005). Sodium tetrathiocarbonate, a new soil fumigant, recently under field development by Union Oil Co. of California, gives a broad spectrum control of nematodes, nitrifying bacteria, and a wide range of soil fungi Ayodele,*et al,* 2000).

Today, research is still going on with the discovery of new organic fungicides that are less toxic, non-persistent and highly active with curative effect. These groups of fungicides are generally referred to as 'synthetic organic fungicides' Sulphur–containing types of fungicides, continue to have new variations, more especially in the direction of increased activity towards target organism.

EXPERIMENTAL

General Procedure

The 2,4 –dinitobenzenesulfenyl chloride used in this study was synthesized in our laboratory according to the method of Kharasch *et al* (1973). Solid reagents were re-crystallized while liquid reagents were re-distilled. Thin layer chromatography (TLC) was carried out on pre-coated silica gel plates (0.2 mm thick). Spots were detected with iodine vapour. Melting prints were determined on a Gallenkamp apparatus. Fourier – transform infrared spectrophotometer, (with KBr discs) was used for the IR. ^1H NMR data were recorded on a Varian Mercury FT-NMR equipment (199.9 MH_Z), using TMS as internal reference and C_3D_6O as solvent. ^{13}C NMR spectra were recorded on a Brucker WM-300 instrument operating at 121.4 MH_2, using $CDCl_3$ as solvent.

Synthesis

2,4-Dinitrobenzenesulfenyl chloride reacted with series of alcohols in the presence of pyridine, which was used for trapping the generated HCl (Zubair and Oladosu, 2006). The following procedure for the synthesis of ethyl 2,4-dinitrobenzenesulfenate is as follow: To a dry 100cm^3 erlenmeyer flask was added 20cm^3 of ethylene chloride and 2,4-dinitrobenzenesulfenyl chloride. The mixture was stirred with a glass rod and heated gently on an oil bath at 30^{0}C for 10 minutes to effect dissolution. This was followed by addition of mixture of ethanol (0.9g, 1.2cm^3, 0.02mol) and pyridine (1.6g, 1.6cm^3, 0.02mol.) White fumes were observed with a distinct deepening of the yellow colour of the reaction mixture. The reaction mixture was further stirred at

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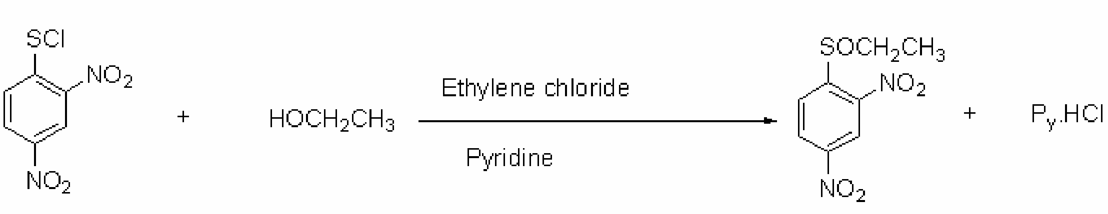
room temperature for 30 minutes (Scheme 1). The solid pyridine hydrochloride was removed by filtration. The reaction mixture was washed with 10% hydrochloric acid ($100cm^3$) solution in a separatory funnel. The organic layer was separated and washed with distilled water ($3x100cm^3$). The organic layer was separated from the aqueous layer, dried with anhydrous sodium sulphate and filtered under suction. Volatile solvents were removed by means of a rotary evaporator to leave an oily residue which solidified on cooling. The crude product was re-crystallized from methanol to give the desired product, ethyl 2,4-dinitrobezenesulfenate, (1) (3.45g, 71%) as orange crystals of m.p. 124-126^{0}C; (Found: C, 39.45; N, 11.28; S, 12.93. Calc. for $C_8H_8N_2O_5S$: C, 39.34, N, 11.47; S, 13.13%)TLC (ethanol/DMSO, 3:1) gave a single spot with R_f = 0.78; ^{1}H (C_3D_6O) : 2. 2 (-$SOCH_2$ $C\underline{H}_3$, t, 3H), 4.4 (-$SOC\underline{H}_2CH_3$, S,2H), 7.9 (Ar-H, M, 3H). 13C ($CDCl_3$). 20 ($\underline{C}H_3CH_2O$), 48 ($CH_3\underline{C}H_2O$), 80 (ArC-3), 95 (ArC-5) 100 (ArC-6), 130 (ArC-4), 136 (ArC-2), 140 (ArC-1).



Scheme 1: Synthesis of ethyl 2,4-dinitrobenzenesulfenate ester

Synthesis of methyl 2, 4- dinitrobenzenesulfenate: 2,4-dinitrobenzenesulfenyl chloride (4.70g, 0.02mol) and redistilled methanol (0.64g, $0.8cm^3$ 0.02mol.) gave methyl 2,4-dinitrobenzenesulfenate (2.9g, 63%) as a fine yellow crystalline solid on recrystallizaton from hot ethanol, (Found: C,36.23; N, 12.41; S, 14.19. Calc. for $C_7H_6N_2O_5S$: C, 36.52; N, 12.17;S, 13.93%); m.p. 120-122^{0}C. TLC (ethanol/DMSO, 3:1) on silica gel gave a single spot, R_f = 0.80. ^{1}H (C_3D_6O): 3.5(-$SOC\underline{H}_3$, s, 3H), 7.5 (Ar-H,m,3H). ^{13}C ($CDCl_3$): 50 (CH_3O), 85 (ArC-3), 98 (ArC-5), 102 (ArC-6), 132 (ArC-4) 138 (ArC-2), 141 (ArC-1).

Synthesis of n – propyl 2,4-dinitrobenzenesulfenate:
2,4-dinitrobenzenesulfenyl chloride (4.70g, 0.02mol) and redistilled n-propanol (1.20g, $1.5cm^3$, 0.02mol) gave n-propyl 2,4-dinitrobenezesulfenate (4.5g, 86%) as a bright yellow crystalline solid on re-crystallization from hot ethanol; (Found: C, 42.14; N,11.01; S,12.16. Calc. for $C_9H_{10}N_2O_5S$: C, 41.85; N, 10.85; S, 12.41%); m.p. 75-76^{0}C. TLC (ethanol/DMSO, 3:1) on silica gel gave a single spot, R_f = 0.75, ^{1}H (C_3D_6O):1.4 (-$SOCH_2$CH2$C\underline{H}_3$, t, 3H), 2.4 (-$SOCH_2C\underline{H}_2CH_3$, q, 2H), 2.8 (-$SOC\underline{H}_2CH_2CH_3$, S, 2H), 7.8 (Ar-H, m, 3H).

Synthesis of n-butyl 2,4-dinitrobenzenesulfenate: 2,4-dinitrobenzenesulfenyl chloride (4.70g, 0.02mol) and n-butanol (1.48g, $1.8cm^3$, 0.02mol) gave n-butyl 2,4-dinitrobezenesulfenate, (3.9g, 83%) as a yellow crystalline solid on recrystallization from hot ethanol; (Found: C,44.45; N, 9.98; S, 11.41. Calc. for $C_{10}H_{12}N_2O_5S$: C, 44.10; N, 10.29; S, 11.77%) m.p. 53 -54^{0}C. TLC (ethanol/DMSO, 3:1) on silica gel gave a single spot, R_f = 0.72. ^{1}H (C_3D_6O): 1.3 (-$SOCH_2CH_2$ $CH_2C\underline{H}_3$, t, 3H), 2.2 (-$SOCH_2$ $CH_2CH_2C\underline{H}_2CH_3$, q, 2H), 4.4 (-$SOCH_2$ $C\underline{H}_2CH_2CH_3$, q, 2H), 4.8 (-$SOC\underline{H}_2$ $CH_2CH_2CH_3$, S, 2H), 7.5 (Ar-H, m, 3H).

Synthesis of 2- hydroxyethyl, 2,4-dinitobenzenesulfenate:
2,4-dinitrobenzenesulfenyl chloride (4.70g, 0.02mole) and ethylene glycol (1.24g, $1.1cm^3$, 0.02mole)gave 2-hydroxy ethyl 2,4-dinitrobenzene sulfenate, (4.5g,87%) as a yellow crystalline solid on recrystallization from hot ethanol; (Found: C, 37.16; N10.94; S, 12.59. Calc. for $C_8H_8N_2O_6S$: C, 36.92; N, 10.77; S, 12.32%); m.p. 112-114^{0}C. TLC (ethanol/DMSO, 3:1) on silica gel gave a single spot. R_f = 0.71. ^{1}H (C_3D_6O): 2.89 ($SOCH_2CH_2O\underline{H}$, s, 1H), 3.71 (-$SOCH_2C\underline{H}_2OH$, t, 2H), 3.86 (-$SOC\underline{H}_2CH_2OH$, t, 2H), 7.3 (Ar – H, m, 3H)

The infrared spectra of the synthesized compounds showed bands of C-H and C-C saturated bonds and C-H, C-C and C=C of aromatic bonds. In addition to these, 2,4-dinitrobenzenesulfenate showed a strong hydroxyl stretching of free O-H bond at $3300cm^{-1}$.

Biological screening: Potato Dextrose Agar (PDA) plates were flooded with spore suspension of each fungus. About 6 mm diameter filter paper discs were sterilized in petri dishes at 160^{0}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 synthesized compound were put on the surface of inoculated PDA plates. Filter paper discs were also soaked in the standard and the control, and 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 at every 12 hours until when there was a complete growth of fungus on

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the control plate. Each value is the mean of three measurements of the colony diameter and the percentage of growth inhibition was calculated from the mean value according to the equation below (Reyes *et al*, 1997):

$$\%\, inhibition = \frac{d_c - d}{d_c}\, x\, 100$$

where:

d_c = diameter of mycelial growth in control

d = diameter of mycelial growth in synthesized compound.

The minimum concentration of each synthesized compound that gave 100% inhibition of fungus growth was taken as the 'Minimal Inhibitory Concentration' (MIC) of the compound. While the IC_{50} (Inhibitory concentration of the synthesized compound at 50% inhibition of the fungus population) was extrapolated from the graph of percentage inhibition (%I) of fungus against concentration of the synthesized compound (Tabakora, and Dodoff, 1995).

RESULTS AND DISCUSSION

The percentage of growth inhibition of all fungi species increased with increase in concentration of the synthesized compounds. The results of the percentage of growth inhibition of *Aspergillus flavus* using various concentrations of the synthesized compounds is a typical example (Table 1). This is in agreement with the results of other workers (Tanaka *et al,* 1978). The synthesized compounds showed activity towards the three fungi species. Figure 1 is the graph of percentage inhibition of *Aspergillus flavus* by various concentrations of ethyl 2,4-dinitrobenzenesulfenate, showing the extrapolation of IC_{50} at 50% inhibitory concentration. The results of the MIC and IC_{50} of the synthesized compounds on the growth of fungi species showed that the sulfenate ester having a methyl group was most active. The fungicidal activity of methyl 2,4-dinitrobenzenesulfenate measured in terms of MIC against *Aspergillus flavus* was 50-fold greater than that of ethyl 2, 4-dinitrobenze sulfenate and 100-fold greater than that of n-propyl 2, 4-dinitrobenzesulfenate and n-butyl 2, 4-dinitrobenzesulfinate (Table 2) . The above observations probably have to do with the electron releasing ability of the alkyl groups to the reaction site of the fungi. The electron releasing ability of alkyl groups decreases with increase in the number of carbon (Buchel,1983). Fungicides are able to bind covalently with the thiol group of the fungus cell through donation of electrons to the reaction site of the fungus (Lukens, 1971). Donations of electrons to the reaction site has been related to activity (Hetnarski, and O' Brien, 1975). Thus alkyl groups with greater electron releasing ability would bind more to the fungus (Finar, 1980). The presence of a methyl group (an alkyl group with the highest electron releasing ability) in the structure of methyl 2,4-dinitrobenzene sulfenate has made it to exhibit the highest fungicidal activity towards all the fungi species.

All the synthesized compounds showed greater activity towards Aspergillus flavus. For instance, the fungicidal activity of methyl 2, 4-dinitrobenzene sulfenate (in terms of IC_{50}) against Aspergillus flavus was about 4 to 5-fold greater than that of the other two fungi species (table 2). Aspergillus flavus is one of the most potent fungal pathogens commonly found in soil, air and in decaying organic matter. It infests products such as groundnut meal and dried foods, producing a carcinogenic toxin called aflatoxin known to cause diseases such as ergotism, alimentary-toxialuki (ATA) and liver cancer in man (Xavier *et al*, 2003). Reducing the activity of this fungus by chemical means serves as a way of preventing diseases caused by it. This will lead to a more healthy living for man and also reduce the environmental pollution caused by the production of aflatoxin by this fungus. 2-hydroxy ethyl 2, 4-dinitrobenzenesulfenate showed greater fungicidal activity when compared with ethyl-2, 4-dinitrobenzenesulfenate. The presence of the hydroxyl group in the former has made the activity (in terms of MIC) towards Aspergillus flavus to increase by 10-fold. With the presence of lone pairs of electrons on oxygen, the hydroxyl group of 2-hydroxyethyl 2,-4-dininitrobenzenesulfenate could act as a ligand and therefore capable of forming a chelate with heavy metals present in the fungus (Lukens, 1971). Heavy metals such as manganese, zinc, cobalt, nickel and copper are required in trace amount by fungus enzymes for catalyzing metabolic functions. Formation of metal – chelates by fungus have been found to enhance the fungicidal activity of the fungus (Xavier *et al*, 2003).

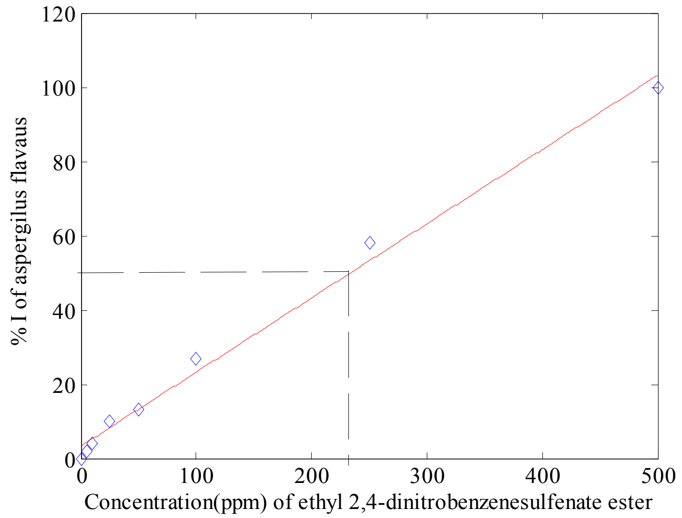

Fig.1: Determination of 50% inhibitory concentration (IC_{50}) of ethyl 2,4-dintrobenzenesulfenate ester

Table 1: Percentage growth inhibition (% I) of *Aspergilus flavus* by various concentrations of the synthesized compounds

Synthesized compounds	1000ppm	500ppm	250ppm	100ppm	50ppm	25ppm	10ppm	5ppm	0ppm
Methyl 2,4-dinitrobenzenesulfenate	100	100	100	100	100	100	100	100	0
Ethyl 2,4-dinitrobenzenesulfenate	100	100	58	27	13	10	4	2	0
n-Propyl 2,4-dinitrobenzenesulfenate	100	60	22	8	3	1	0	0	0
n-Butyl2,4-dinitrobenzenesulfenate	100	55	32	11	5	2	0	0	0
2-Hydroxyethyl2,4-dinitrobenzenesulfenate	100	100	100	100	100	65	20	5	0
Phenylmercury acetate (standard)	100	100	100	100	100	100	100	100	0
DMSO/H_2O (8.2) (Control)	0	0	0	0	0	0	0	0	0

Table 2: Inhibitory effect of the synthesized compounds on the fungi species through MIC and IC_{50} in parts per million (ppm)

	Aspergilus flavus		*Rhizopus stolonifer*		*Fusariam oxysporum*	
Synthesized compounds	MIC (ppm)	IC_{50} (ppm)	MIC (ppm)	IC_{50} (ppm)	MIC (ppm)	IC_{50} (ppm)
Methyl 2,4-dinitrobenzenesulfenate	10	5	50	23	50	20
Ethyl 2,4-dinitrobenzenesulfenate	500	225	1000	370	500	225
n-Propyl 2,4-dinitrobenzenesulfenate	1000	470	1000	400	1000	435
n-Butyl 2,4-dinitrobenzenesulfenate	1000	485	1000	460	1000	385
2-Hydroxyethyl 2,4-dinitrobenzenesulfenate	50	24	100	49	100	43

Conclusion: A considerable quantity of agricultural products is lost due to the infestation of food crops before and after harvesting by the fungal attack of *Aspergillus flavus, Rhizopus stolonifer and Fusarium Oxysporum*. It is therefore very encouraging that this research has shown that the synthesized compounds showed promising activities against these economic agricultural fungi.

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