Synthesis of some new amides and their toxicological effect on an insect *Spodoptera litura*

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Abstract- Some novel organic amides were synthesized by the reaction of glycine/cystiene and ethylene diamine with respective organic acids (succinic acid, maleic acid, tartaric acid and oxalic acid). The newly synthesized compounds were isolated as crystalline solid and have sharp melting point. The compounds were characterized by their elemental, I.R., NMR spectral analysis along with their activity against an insect responsible for the damage of Indian crops *Spodoptera litura*. It was found that the compounds show moderate efficacy against the insect.
Key Wards: Glycine, ethylenediamine, succinic acid, maleic acid, oxalic acid, tartaric acid, insecticidal activity, *Spodoptera litura*.

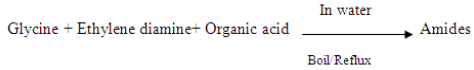
Introduction

In recent year literature survey revealed that nitrogen and sulfur containing compounds [1-3] are potentially active against cancer, viral and fungal disease [4, 5]. Amines in general have been known to be biologically active [6] and the effect of presence of various constituents in the amines increases their antimicrobial and insecticidal activity, has been investigated [7, 8]. The compounds having amino acid have proven to be potentially active against various bacterial and fungal strains and many of them got wide acceptance in clinical trials [9, 10]. The differential inhibition of cytochrome P-450 between pathogenic bacterial and fungal strains and human beings is the basis for the clinically important amino acid as antimicrobial agents. It may be found that the inhibition can be determined by the differential complementarities between the structures of antimicrobial agent and the active sites of enzymes responsible for microbial activity.

Insects are found in almost all types of environment. They affect man's interest in a number of ways; insects like mosquitoes and housefly spread large number of diseases like malaria, dengue and cholera in addition to painful bites. Further, the compounds containing amide moiety has also attracted attention due to their important role as insecticides and pesticides to save our Indian crops [11]. In view of the, important behavior of amines and amino acid, the present communication deals with the synthesis, characterization and insecticidal activity of some new amide of amino acid.

Results and discussion

The amide was generally prepared by using following method



The newly prepared compounds were crystallized after vacuum distillation. The compounds have sharp melting point. The further characterization of these entire compounds was done by elemental analysis, I.R and NMR spectral data followed by antimicrobial activity.

I.R. spectra

The infrared spectra of newly synthesized amides of amino acids were recorded in KBr/CsI pellets in the range of 4000-200cm^{-1}. The I.R. spectra of the entire compound clearly exhibit absorption bands due to amides and methylene groups. The absorption frequencies due to carbonyl groups in amide have been fully assigned.

^{1}H-NMR spectra

The ^{1}H-NMR spectra of the amide of amino acids were recorded in $CDCl_3$ at room temperature using TMS as standard. The peaks values suggested the presence of secondary amides group along with methylene groups in the compound. The peak's located at δ8.0 ppm (for secondary amides) while the peaks value appears at δ2.46, δ3.46 and δ4.09 in case of compound (A) suggested the presence of six methylene group in the compound. The peaks values for other compounds are very close to the first compound indicating the presence of secondary amides and methylene proton.

Contact toxicity

The contact toxicity of the compounds is given in Table-2. It was found that approximately all these compounds are active against the insect larvae of *Spodoptera litura* in different concentrations. The compounds 3, 4 and 5 show higher toxicity against the larvae while the rest of the compounds show moderate toxicity. These compounds are generally used as emulsion by adding tween 20 emulsifier in the dissolved compounds. The emulsion of the compounds then applies on the dorsal surface of the insect which affect the nervous systems of the insect larvae and ultimately causing death of the insects.

Stomach toxicity

The stomach toxicity of the compounds was given in Table-3. The toxicity of these

compounds was tested against the larvae of *Spodoptera litura* using different concentrations of the test compounds. The corrected mortality data was calculated for detection of LC_{50}/LD_{50} values of these compounds. It was found that the compound 1,2,5,6 and 7 shows low value of toxicity while the rest of the compounds 3 and 4 show high toxicity against the larvae of insect.

Anti-feedant activity

The antifeedant activity of these compounds is given in Table-4. It was found that approximately all these compounds were found to be active against the insect larvae of *Spodoptera litura* in different concentrations. The compounds 5,2,1,6 and 4 shows high value of antifeedant activity respectively while the remaining compounds shows moderate to light activity.

Experimental

The synthesis of amides of amino acid was carried out by the simple reaction of glycine, ethylenediamine with respective carboxylic acid in water followed by refluxing the reaction mixture for about 24 hrs. The vacuum distillation of reaction mixture afforded an off white color crystalline solid. The newly synthesized compounds have sharp melting point. The general method of preparation of some representative compounds is as follows.

Reaction of glycine and ethylenediamine with succinic acid (Synthesis of decacyclo 1,3,6,8, tetracarboxy tetraamide)

Glycine (2 mole, 0.2M) and ethylenediamine (1 mole, 0.1M) were taken in a flask fitted with an air condenser. Since the reaction is exothermic therefore the flask was kept in ice bath for half an hour before refluxtion. Now the reaction mixture refluxed for 3-4 hour on a water bath followed by slow addition of an aqueous solution of succinic acid (1 Mole 0.02M). The reaction content was further refluxed again for 8-10 hours. The resulting mixture was reduced to half of its volume and kept over night; white shining crystals are obtained which was further recrystallized in ethanol.

Reaction of glycine and ethylenediamine with oxalic acid (Synthesis of octacyclo 1,3,4,6, tetracarboxy tetraamide)

Glycine (2 mole, 0.2M) and ethylenediamine (1 mole, 0.1M) were taken in a flask fitted with an air condenser. Since the reaction is exothermic therefore the flask was kept in ice bath for half an hour before refluxtion. Now the reaction mixture refluxed for 3-4 hour on a water bath followed by slow addition of an aqueous solution of oxalic acid (1 Mole 0.02M). The reaction content was further refluxed again for 8-10 hours. The resulting mixture was reduced to half of its volume and kept over night; white shining crystals are obtained which was further recrystallized in ethanol.

Reaction of cysteine and ethylenediamine with oxalic acid (Synthesis of octacyclo 2, 5, dimethylene mercapto 1,3,4,6, tetracarboxy tetraamide)

Cysteine (2 mole, 0.2M) and ethylenediamine (1 mole, 0.1M) were taken in a flask fitted with an air condenser. Since the reaction is exothermic therefore the flask was kept in ice bath for half an hour before refluxtion. Now the reaction mixture refluxed for 3-4 hour on a water bath followed by slow addition of an aqueous solution of oxalic acid (1 Mole 0.02M). The reaction content was further refluxed again for 8-10 hours. The resulting mixture was reduced to half of its volume and kept over night resulting light yellow crystal which was further recrystallized in ethanol.

Reaction of cysteine and ethylenediamine with tartaric acid (Synthesis of decacyclo 4,5, dihydroxy 2,7, dimethylene mercapto, 1,3,6,8, tetracarboxy tetraamide)

Cysteine (2 mole, 0.2M) and ethylenediamine (1 mole, 0.1M) were taken in a flask fitted with an air condenser. Since the reaction is exothermic therefore the flask was kept in ice bath for half an hour before refluxtion. Now the reaction mixture refluxed for 3-4 hour on a water bath followed by slow addition of an aqueous solution of tartaric acid (1 Mole 0.02M). The reaction content was further refluxed again for 8-10 hours. The resulting mixture was reduced to half of its volume and kept over night resulting light yellow crystal which was further recrystallized in ethanol.

Contact toxicity

The contact toxicity of these compounds was carried out by topical application method (12) against larvae of *Spodoptera litura*, which is harmful for Indian crops. First the given compounds were dissolved in acetone and different concentrations were prepared *viz*., 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. Now each concentration was applied on the dorsal surface of the larvae of insect. About 10 µl of each concentration was applied on each larvae. Some of the larvae of insect was treated by acetone alone, were works as control. Now the mortality data was recorded after 24 hrs, and the treated mortality was corrected with control morality. These corrected mortality data was used for calculation of LC_{50}/LD_{50}.

Stomach toxicity

The stomach toxicity of these compounds was carried out by leaf dip method (13). In this method we used fourth instar larvae of *Spodoptera litura* of an insect. Ten larvae were used for each replication and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared *viz*. 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. The leaf disc were prepared out of caster leaf and dipped in various concentrations of the test compounds for thirty seconds. Now air dried the leaf discs to evaporate the excess acetone. (The leaf discs dipped only in acetone were

served as control). The mortality data was recorded after 24 hrs. and the treatment mortality was corrected with control mortality. These mortality data was used for calculation of LC_{50}/LD_{50}.

Anti-feedant activity

The antifeedant activity of these compounds was also carried out by leaf dip method (13) using fourth instar larvae of *Spodoptera litura*. There are ten larvae were used for each replications and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared *viz*. 0.06%, 0.12%, 0.25%, 0.50% and 1.00%. The leaf discs of about 25 cm^2 were prepared and dipped for thirty seconds in various concentrations of the test compounds. Now air-dried the leaf discs to evaporate the excess acetone and the leaf discs offered for feeding. The insects were allowed to feed for 24 hrs. After 24 hrs leaf area uneaten was measured by using leaf area meter. The differences between leaf area provided and the leaf area uneaten is taken as amount of leaf area consumed. The feeding inhibition was calculated and used for calculation of effective concentration (EC_{50}/LD_{50}).

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Table 1- Analytical data of amides of amino Acid

S.N.	Formula of Compound	Melting Point, 0^0C	Color	Elemental Analysis			Formula Weight
				C%	H%	N%	
1	$C_{10}H_{16}N_4O_4$	247	White	46.87	6.29	21.86	256.12
2	$C_{10}H_{14}N_4O_4$	175	White	47.24	5.55	22.04	254.10
3	$C_{10}H_{16}N_4O_5$	210	White	44.12	5.92	20.58	272.11
4	$C_{10}H_{16}N_4O_6$	171	White	41.67	5.59	19.44	288.11
5	$C_8H_{12}N_4O_4$	200	White	42.10	5.30	24.55	228.09
6	$C_{10}H_{16}N_4O_4S_2$	215	Yellow	37.49	5.03	17.49	320.06
7	$C_{12}H_{20}N_4O_4\ S_2$	198	Yellow	41.36	5.79	16.08	348.09
8	$C_{12}H_{18}N_4O_4\ S_2$	210	Yellow	41.60	5.24	16.17	364.08
9	$C_{12}H_{20}N_4O_5\ S_2$	190	Yellow	39.55	5.53	15.37	364.09
10	$C_{12}H_{20}N_4O_6\ S_2$	196	Yellow	37.88	5.30	14.73	380.08

Table 2- Contact Toxicity of amides

S. No.	Compounds	Fiducial Limits	Slop ±	Chi. Sq. (3)	LC_{50}/LD_{50} At 24 hrs.
1.	$C_{10}H_{16}N_4O_4$	1.87–12.07	1.09±0.19	1.62 (3)	3.53
2.	$C_{10}H_{14}N_4O_4$	1.57–9.32	1.07±0.17	0.72 (3)	2.83
3.	$C_{10}H_{16}N_4O_5$	0.28–0.40	1.96±0.16	4.39 (3)	0.33
4.	$C_{10}H_{16}N_4O_6$	0.39–0.59	1.67±0.15	5.62 (3)	0.46
5.	$C_8H_{12}N_4O_4$	0.43–0.75	1.63±0.16	2.94 (3)	0.58
6.	$C_{10}H_{16}N_4O_4S_2$	1.87–12.07	1.09±0.19	1.63 (3)	3.52
7.	$C_{12}H_{20}N_4O_4\ S_2$	0.56–1.05	1.32±0.15	0.63 (3)	0.73
8.	$C_{12}H_{18}N_4O_4\ S_2$	1.42–3.89	1.32±0.16	2.37 (3)	2.12
9.	$C_{12}H_{20}N_4O_5\ S_2$	1.61–9.30	1.07±0.17	0.67 (3)	2.83
10.	$C_{12}H_{20}N_4O_6\ S_2$	0.72–1.46	1.71±0.18	3.32 (3)	0.97

Table 3- Stomach toxicity of amides

S. No.	Compounds	Fiducial Limits	Slop ±	Chi. Sq. (3)	LC_{50}/LD_{50} At 24 hrs.
1.	$C_{10}H_{16}N_4O_4$	1.61–9.55	1.45±0.17	0.68 (3)	2.97
2.	$C_{10}H_{14}N_4O_4$	0.86–1.99	1.28±0.16	0.80 (3)	1.20
3.	$C_{10}H_{16}N_4O_5$	0.49–0.76	1.57±0.16	2.78 (3)	0.60
4.	$C_{10}H_{16}N_4O_6$	0.55–0.90	1.48±0.16	3.37 (3)	0.67
5.	$C_8H_{12}N_4O_4$	0.56–0.97	1.33±0.15	0.63 (3)	0.75
6.	$C_{10}H_{16}N_4O_4S_2$	0.85–1.82	1.22±0.16	0.72 (3)	1.12
7.	$C_{12}H_{20}N_4O_4\ S_2$	0.55–0.97	1.32±0.15	0.69 (3)	0.73
8.	$C_{12}H_{18}N_4O_4\ S_2$	1.33–3.99	1.42±0.20	2.38 (3)	2.01
9.	$C_{12}H_{20}N_4O_5\ S_2$	1.61–9.39	1.01±0.17	0.69 (3)	2.93
10.	$C_{12}H_{20}N_4O_6\ S_2$	0.74–1.32	1.62±0.18	3.24 (3)	0.94

Table 4- Antifeedant activity of amides

S. No.	Compounds	Fiducial Limits	Slop ±	Chi. Sq. (3)	LC_{50}/LD_{50} At 24 hrs.
1.	$C_{10}H_{16}N_4O_4$	0.82–3.41	0.81±0.14	0.43 (3)	1.35
2.	$C_{10}H_{14}N_4O_4$	0.68–1.72	1.03±0.14	0.66 (3)	0.98
3.	$C_{10}H_{16}N_4O_5$	0.43–0.87	1.03±0.14	0.34 (3)	0.58
4.	$C_{10}H_{16}N_4O_6$	0.62–1.42	1.06±0.14	1.07 (3)	0.86
5.	$C_8H_{12}N_4O_4$	0.83–2.33	1.08±0.15	0.79 (3)	1.24
6.	$C_{10}H_{16}N_4O_4S_2$	0.72–2.41	0.93±0.14	0.22 (3)	1.13
7.	$C_{12}H_{20}N_4O_4\ S_2$	0.30–0.47	1.28±0.14	3.42 (3)	0.39
8.	$C_{12}H_{18}N_4O_4\ S_2$	0.33–0.61	1.00±0.13	0.68 (3)	0.43
9.	$C_{12}H_{20}N_4O_5\ S_2$	0.45–1.09	0.87±0.13	1.71 (3)	0.64
10.	$C_{12}H_{20}N_4O_6\ S_2$	0.49–0.76	1.52±0.16	2.59 (3)	0.58