Asian Journal of Chemistry; Vol. 23, No. 8 (2011), 3438-3440

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

A New Insecticidal Compound from *Heliotropium indicum* Linn.

A.K. DOLUI[1], M. DEBNATH[1,*] and B. DE[2]

[1]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786 004, India
[2]Regional Institute of Pharmaceutical Science and Technology, Abhoynagar, Agartala-799 005, India

*Corresponding author: E-mail: manab_d@rediffmail.com

(*Received*: 6 September 2010; *Accepted*: 20 April 2011) AJC-9821

Earlier we reported the screening results of antifeedant activities of six fractions of methanolic extract of leaves of *Heliotropium indicum*. The fraction E showed highest antifeedant activity. In the present study a new compound E (2-ethyl-1,2,3,4-tetrahydro-6-hydroxymethylisoquinoline) has been isolated from fraction E. The characterization and structure elucidation of the compound E was carried out by determination of melting point, solubility, UV, HPLC, IR, NMR (^{1}H NMR and ^{13}C NMR) and LC-MS data. The compound E showed antifeedant activity comparable with those of standard insecticides *viz.*, neemazal and endosulfan. The LC_{50} value of the compound E was found to be 2.18 %.

Key Words: Antifeedant, 2-Ethyl-1,2,3,4-tetrahydro-6-hydroxymethylisoquinoline, Neemazal, Endosulfan, LC_{50}, *Heliotropium indicum*, Insecticidal.

INTRODUCTION

Indiscriminate use of synthetic insecticides in insects control programme has led to building up of resistance in insects to insecticides, resurgence of sucking pests and destruction of natural enemy complexes leading to upsetting of the balance in the crop ecosystem[1]. Once the synthetic chemical insecticides are introduced into the eco-system, they may remain there for a very long duration or forever. This has made the scientist to think in the use of botanical insecticides in the recent years. Due to their biodegradable nature, neither they cause any harm to beneficial organisms and pollinators, nor are the derivatives injurious to mammals and other non-targets[2].

The pool of plants possessing insecticidal substance is enormous[3]. Today over 2000 species of plants are known to possess some insecticidal properties[4-6].

Earlier, we reported the antifeedant activity of different fractions of methanol extract of *Heliotropium indicum*[7]. The present study has been aimed to carry out the isolation and characterization of the active insecticidal compound from the leaves of *Heliotropium indicum*.

EXPERIMENTAL

Isolation of the active compound: Fraction E obtained by column chromatography of methanolic extract of *H. indicum*[7] was evaporated to dryness. The residue was dissolved in acetone: water mixture (1:1) and crystallized. The compound was again recrystallized from the same solvent to get a grey crystalline powder.

Characterization and structure elucidation: The melting point (uncorrected) of the compound E (2-ethyl-1,2,3,4-tetrahydro-6-hydroxymethylisoquinoline) was determined by open capillary method by using a melting point apparatus (INDO, M-AB-92). The solubility was tested by taking 10 mg of compound E in a test tube and 5 mL of solvent was added and shaken for 5 min at room temperature. The λ_{max} of the active component E was determined by using a UV-VIS spectrophotometer (EI Double beam, Model-1372). The sample was dissolved in 10 mL of methanol:water (1:1) and methanol:water (1:1) was used for baseline correction. The chemical examinations were performed to confirm the presence of functional groups and the nature of the compound E[8]. CHN Analyzer 2400 Ser II estimated nitrogen, carbon and hydrogen for the isolated active compound E. HPLC (Shimadzu) of the active compound E was performed by taking mobile phase methanol: water (1:1) and methanol:benzene (9:1) and using Khromosil C_{18} analytical column. The active compound E was subjected for IR Spectroscopy in KBr (JASCO FT/IR-5300), NMR (VARIAN 300 MHz FT NMR) [^{1}H NMR in D_2O and ^{13}C NMR in $CDCl_3$] and LC-mass spectroscopy (Shimadzu 2010A).

Comparison of bioactivity of compound E with those of standard insecticides: The antifeedant activity of the compound E was compared with those of standard botanical

insecticides *viz.* neemazal (0.15 % azadirachtin) and synthetic insecticides *viz.* endosulfan (35 % EC) at different concentrations.

LC_{50} Determination: Contact toxicity was carried out by the method of Patil *et al.*[9] with slight modification to evaluate the LC_{50} dose. One mL of different concentrations of the compound was spread on 9 cm diameter filter paper at a bottom in 10 cm diameter petri dishes and air dried for 0.5 h. Ten numbers of insect (*H. theivora*) were released directly to the treated filter paper and triplicate sets were setup for each concentration. Mortality was recorded after 48 h. The LC_{50} value of the compound was calculated converting the observed mortality percentage into probit[10]. The values thus obtained were plotted against log concentration. The log concentration of 50 % mortality was determined from the graph using the regression equation and antilog value gave LC_{50} of the compound.

RESULTS AND DISCUSSION

The melting point of compound E after recrystallization was recorded as 255 ºC in open capillary method. The colour of the compound was found to be grey. The solubility tests were then carried out for compound E at room temperature. The compound was found to be soluble in water and ammonia solution. The λ_{max} of the compound E was determined as 200 nm, where the absorbance was 0.165. The chemical examination indicates that the compound E contains tertiary amine and aliphatic primary alcohol. The elemental analysis reveals that the compound E contains nitrogen 7.34 %, hydrogen 8.89 % and carbon 75.39 %. The HPLC study (Shimadzu) in two different mobile phases indicates the purity of the compound. It showed a sharp peak at 4.397 min in methanol:water (1:1) mobile phase and the peak area indicates the purity of the compound as 99.96 %. A peak was obtained at 4.225 min in methanol:benzene (9:1) mobile phase and the peak area indicates the purity of the compound as 99.91 %.

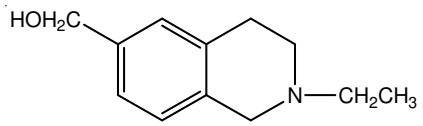
IR spectrum shows a broad band at 3398 cm^{-1} for -OH group and 1645 cm^{-1} for C=C (aromatic), 2851 cm^{-1} for -N-CH_2-, 1026 cm^{-1} for  .

^{1}H NMR shows only two peaks for a methyl group at δ 1.03 (triplet) and a methylene group at δ 1.29 and at δ 3.39 and 4.2 indicating that these protons are adjacent to a heteroatom.

^{13}C NMR shows three signals in the aliphatic region at δ 54.85, 56.26 and at 58.10 indicating three carbon atoms attached to nitrogen atom. The signal at δ 68.29 may be due to a -CH_2OH group attached to a benzene ring. The two signals at 23.70 and 30.42 may be due to presence of an ethyl group. In addition to this signals in the aromatic region for six carbon atoms at δ 125.92, 126.02, 128.06, 128.18, 128.91 and 144.12 indicates the presence of a benzene ring.

From the data available from the IR, ^{1}H NMR, ^{13}C NMR the probable structure of compound E may be 2-ethyl-1,2,3,4-tetrahydro-6-hydroxymethylisoquinoline. Further the LC-MS showed a single peak at 254 nm with an absorbance 0.525, indicating its purity.

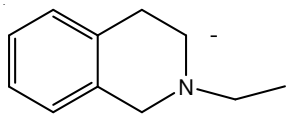
The molecular mass peak (M+2) is found in 193 (m/z).



2-Ethyl-1,2,3,4-tetrahydro-6-hydroxymethylisoquinoline

m/z = M+2

The base peak of the compound (M-1) is recorded in m/z 160.



m/z 160

Most potent insect antifeedants are isoquinoline, indole alkaloids, sesquiterpene lactones, diterpinoids and triterpinoids as reported by Schoonhoven[11]. In the present study the isolated active compound E is isoquinoline derivative and supports the view.

The antifeedant activities of the compound E at various concentrations were compared with standard botanical and synthetic insecticides *viz.* neemazal and endosulfan. The results are presented in the Table-1. It has been found that the mean number of feeding spots at 0.015 % concentration with neemazal treatment was 18.33, with that of endosulfan treatment (at 0.5 % conc.) was 13.33 and with compound E treatment (0.5 % conc.) was 17.33. This observation indicates that compound E has a comparable feeding inhibitory activity when compared with that of the standard insecticide.

TABLE-1
COMPARISON OF ANTIFEEDANT ACTIVITY OF COMPOUND E WITH THOSE OF STANDARD BOTANICAL AND SYNTHETIC INSECTICIDES

Treatment	Concentrations (%)	Number of spots produced after 24 h treatment (Mean ± SE)
Compound E	0.2500	27.67 ± 0.67
	0.5000	17.33 ± 0.33
Neemazal (Azadirachtin)	0.0015	26.33 ± 0.33
	0.0150	18.33 ± 0.33
Endosulfan	0.2500	23.33 ± 0.88
	0.5000	13.33 ± 0.88
Control	0.0	103.00 ± 1.00
CD at 0.05	–	2.06
CD at 0.01	–	2.81

Each figure is the mean ± SE for 3 replicates. CD denotes critical difference.

Insect mortality by the treatment with compound E and evaluation of LC_{50} are shown in the Table-2 and Fig. 1. The LC_{50} value was determined as 2.18 %. As the 100 % mortality rate of the compound E is above the 4 % concentration and at 0.5 % concentration the number of feeding spots of the insect shows significant reduction (17.33) as compared to control (103.00), it indicates that the compound E has feeding inhibitory effect against *H. theivora*. It also seems that the compound E is comparatively less toxic at low concentration. With the increase of concentration, the toxicity is increased. So it can be assumed that the compound E acts as an antifeedant

TABLE-2
MORTALITY OF *H. theivora* AFTER TREATMENT WITH DIFFERENT CONCENTRATIONS OF COMPOUND E AND DETERMINATION OF LC_{50}

Conc. (%)	Total No. of insects	No. of dead insects	Mortality (%)	Corrected mortality	log dose	Probit
1.00	30	5	16.66	17	0	4.05
1.25	30	7	23.33	23	0.097	4.26
1.50	30	9	30.00	30	0.176	4.48
1.75	30	10	33.33	33	0.243	4.56
2.00	30	12	40.00	40	0.301	4.75
2.25	30	16	53.33	53	0.352	5.08
2.50	30	18	60.00	60	0.397	5.25
2.75	30	20	66.67	67	0.439	5.44
3.00	30	21	70.00	70	0.477	5.52
3.25	30	23	76.67	77	0.512	5.74
3.50	30	24	80.00	80	0.544	5.84
3.75	30	27	90.00	90	0.570	6.28
4.00	30	29	96.67	97	0.602	6.88

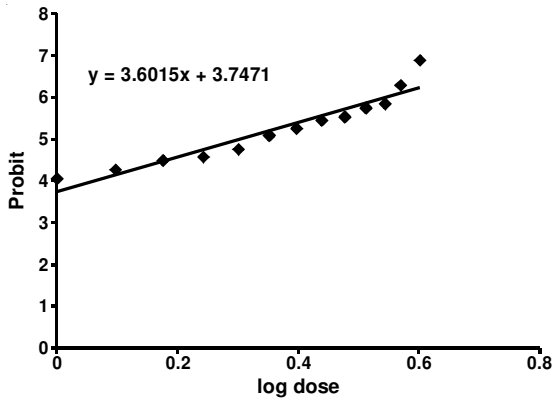


Fig. 1. log dose and probit curve for determination of LC_{50} value of compound E; The calculated LC_{50} value is 2.188 %

agent which allows the insect to take less amount of food (tea leaves) and ultimately the insect died due to starvation *i.e.* the compound E indirectly killing the insects. This is the reason that the antifeedant agent may be used as an ideal insecticide in agriculture.

In the present study the insect did not consume the treated filter paper. Therefore, contact toxicity seems to be main reason for mortality. Insecticidal contact activity of emodin extracted from *Rhamnus dispermus* was also reported against adult peach trunk aphid[12]. Pronounced antifeedant and insecticidal activity of two isolated compounds piperitone and *trans*-ethyl cinnamate from the essential oil of *Artemisia judaica* was reported against third instar larvae of *S. littoralis* in a concentration dependent manner[13].

It may be concluded that the newly isolated compound E can be used as a powerful tool for the management of *H. theivora* population in tea plantation. However the field trials are necessary before commercial exploration can be achieved.

ACKNOWLEDGEMENTS

The authors thanks the Indian Association of Cultivation of Science, Kolkata for providing CHN-analyzer facility.

REFERENCES

1. G. Jilani and R.C. Saxena, *J. Econ. Entomol.*, **83**, 629 (1990).
2. U. Bhattacharya, *The Assam Rev. Tea News*, **83**, 16 (1994).
3. M. Jacobson, Agricultural Hand Book, U.S. Department of Agriculture, Washington D.C., p. 138 (1975).
4. M.F. Balandrin, *Science*, **228**, 1154 (1985).
5. R.L. Rawls, *Chem. Eng. News*, **22**, 2124 (1986).
6. K. Sukamar, M.J. Perich and L.R. Boobar, *J. Am. Mosq. Control Assoc.*, **7**, 210 (1991).
7. A.K. Dolui, M. Debnath and B. De, *Asian J. Chem.*, **22**, 5331 (2010).
8. A.A. Siddiqui and M. Ali, Practical Pharmaceutical Chemistry, CBS Publishers and Distributors, New Delhi, India, p. 79 (1997).
9. G.S. Patil, M.G. Patil, P.S. Mendki, V.L. Maheswari and R.M. Kothari, *Pestology*, **24**, 5 (2000).
10. D.J. Finney, Probit Analysis, Cambridge University Press, Cambridge, England (1952).
11. L.M. Schoonhoven, *Ent. Exp. Appl.*, **31**, 57 (1982).
12. M.A. Ateyyat and M.S. Abu-Darwish, *Span. J. Agric. Res.*, **7**, 160 (2009).
13. S.A.M. Abdelgaleil, M.A. Abbassy, A.S.H. Belal and M.A.A. Rasoul, *Bioresour. Tech.*, **99**, 5947 (2008).