SYNTHESIS AND PRELIMINARY BIOASSAYS OF SOME BIS - 1,3 - BENZOXAZINES

A. Rivera*, J. Calle, M. Zamudio, F. Aristizábal
Departamento de Química, Facultad de Ciencias,
Universidad Nacional de Colombia, A.A. 14490 Bogotá, Colombia
P. Joseph - Nathan
Centro de Investigación y de Estudios Avanzados del
Instituto Politécnico Nacional, México D.F., México.

Keywords: 1,3-benzoxazines, bioassays, teratogeny, synthesis, Diptera, Lepidoptera.

RESUMEN

Se hicieron ensayos preliminares con miras a establecer la posible actividad insecticida de cinco bis-1,3-benzoxazinas del tipo 3,3-etilen-bis(3,4-dihidro-6R-2H-1,3-benzoxazina). De cuatro de ellas (R = -F, -Cl,-Br,-I) se conocía su síntesis, mientras que la del compuesto R = -H se describe por primera vez. Los insectos de control fueron larvas de *M. domestica* (Diptera) y de *C. carye* (Lepidoptera). No se observó ninguna acción tóxica y aunque todas las larvas empuparon sin novedad, los insectos adultos mostraron malformaciones internas o externas.

ABSTRACT

Four previously synthesized (R =-F,-F,-Cl,-Br,-I) and one new (R =-H) 3,3' -ethylene-bis(3,4-dihydro-6R-2H-1,3-benzoxazine) were preliminary tested for insecticidal activity against *M. domestica* (Diptera) and *C. cary* (Lepidoptera) larvae. Under laboratory conditions no toxic action was observed. All larvae continued development and pupated successfully but internal or external malformations were observed in adults when hatched.

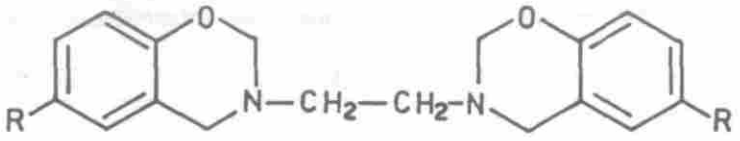
INTRODUCTION

1,3-Benzoxazines (BOAs) have long been recognized for their wide range of biological activities, including fungistatic, bacteriostatic and insecticidal activities (1). A method for the preparation of N-substituted-3,4-dihydro-2H-1,3-benzoxazines by a Mannich - type condensation of appropriate phenol with primary amines and two equivalents of formaldehyde was developed by Burke (2). Using this method with substantial modifications which will be published elsewhere we

have synthesized a variety of 3,3 - ethylene-bis(3,4-dihydro-6-substituted-2H-1,3-benzoxazine) (R-BISBOAs).

In the present work we report the preparation of compounds Ia-e searching for enhanced biological activity. These compounds were tested against a dipterous and a lepidopterous larvae. The synthesis of compounds I *b-e* has been reported earlier (3) (4) while to the best of our knowledge, Ia was unknown so far. On the other hand, it is very important to mention that the National Cancer Institute (USA) has requested us to provide them with additional material of compound Ib for AIDS - antiviral evaluation.

a = H
b = F
c = Cl
d = Br
e = I



EXPERIMENTAL

Identification. Both proton and carbon-13 NMR spectra were recorded with a Varian XL-300 apparatus; Infrared spectrum was obtained from KBr pellets using a Perkin-Elmer 467 model spectrophotometer; mass spectrum was run on a Shimadzu 9020 spectrometer.

General procedure for BISBOA s synthesis.

A mixture of dioxane (50 ml) and formaldehyde 37% (3.2 mL, 0.04 mol) cooled in an ice bath was stirred for 15 min and ethylenediamine (0.6 g, 0.01 mol) was then added slowly dropwise. After 5 min a solution of the appropriate phenol (0.02 moles) in dioxane (10 mL) was added and the reaction mixture was left at 40°C until completion. Solvent was removed and the solid compound was separated by vacuum filtration and purified by recrystallization.

3,3'-ethylene-bis(3,4-dihydro-2H-1,3-benzoxazine) (Ia)
IR (KBr): $\lambda_{max.}$ 3018, 3041, 3072 (= C-H); 2959, 2852, 1456 (R-CH_2-N=); 1605, 1582, 1488 (= C-C= arom.); 1227, 1023 (Ar-O-CH_2); 758 cm^{-1} (Benz 1,2-disubst.)

^1H NMR ($CDCl_3$); δ: 2.98 (4H, s, CH_2-N); 4.04(4H, s, H-C4); 4,90 (4H, s H-C2); 6.78 (2H, dd, ABCD-system, J_{ab} = 1.10 Hz, J_{ac} = O Hz, J_{ad} = 8.15 Hz, H-C8); 6.87 (2H, m, ABCD-system, J_{ba} = 1.22 Hz, J_{bc} = 7.46 Hz, J_{bd} = 7.89 Hz, H-C6); 6.95 (2H, dd, ABCD—system, Jcb=.54 Hz, J_{cd} = 1.68 Hz, H-C5); 7.12 (2H, m, ABCD-system, J_{da} = 8.15 Hz, J_{db} = 8.13 Hz, Jdc= 1.80 Hz, H-C7)ppm.

^{13}C NMR ($CDCl_3$); δ (Assignation): 49.61 (-CH_2-N); 50.39 (4); 82.73 (2); 116.43 (8); 119.98 (10); 120.59 (6); 127.56 (5); 127.75 (7) 154.14 (9) ppm.

MS (EI) (70 eV), m/z (%): 296 (M^+, 26); 190 (4); 189 (22); 162 (16); 148 (100); 134 (14); 107 (22); 106 (10); 79 (7); 78 (54); 77 (19); 56 (24); 52 (8); 51 (8); 42 (69).

Bioassays.

Insects. **Musca domestica** larvae (third instar) were obtained from a laboratory colony maintained for several years by Dr. Renato Ripa, INIA, Estación Experimental La Cruz, Chile. *Cynthia carye* larvae were from a colony reared in 400 mL colorless plastic cages containing leaves of *Malva nicaeencis* at room temperature.

Natural diets were changed daily. This colony was originally started from field-collected larvae which were reared as above until the pupae stage was reached. Adults hatched after nine days and were then transferred to a net-covered cage. Butterfilies were reared with honey bee and *M. nicaeencis* flowers.

Forced-contact test. Solution of BISBOA s were prepared in acetone, aliquots of them were pipetted into Petri dishes (9 cm diam) and the solvent was allowed to evaporate. Solutions at eight different concentrations of each test compound were used within a range from 2.0 to 100 $\mu g/cm^2$. Twenty-five *M. domestica* larvae were placed in each dish and four dishes were assayed in each experiment (total of 100 larvae for each concentration). Records of mortality were taken every 3 h during the initial 12 h and then every 12 h.

Ingestion test. They were conducted in plastic cages. Two fifth instar larvae of *Cynthia carye* were placed in each cage. A dose of approximately 7 mg/day of each compound evenly spread over the leaves of *M. nicaeencis* was provided daily during 3 days. After this time the larvae pupated. When adults emerged they were examined and then dissected.

RESULTS AND DISCUSSION

In all reactions, once the equilibrium was reached and no more BISBOA was formed, the solvent (dioxane) was removed by reduced pressure distillation. Further isolation and purification of the different substances was performed by recrystallization from adequate solvents. Except for the compound with R= H (H-BISBOA), whose structure was spectroscopically solved as it had not been previously reported (3) (4). Then, for purpose of this report, special emphasis will be put on the preparation of compound H-BISBOA since much work has already been carried out on the synthesis of BISBOAs with substituent at the 6-position.

Our results show that this reaction is nort restricted to *para*-substitud phenols; since when phenol was used the reaction took place to give the corresponding BISBOA. Best parameters were estabhished for this reaction.

The spectral anaysis unambiguously proved the structure and the purity of this compound. In the proton NMR spectrum, aromatic protons show a complex

splitting pattern of four interacting protons. Chemical shift assignments were made on the basis of coupling patterns and aromatic substituent effects (5). The chemical shifts and coupling constants are listed in esperimental. Full assignment of the ^{13}C-NMR spectrum was donde by means of theoretical calculations of the proton decoupled ^{13}C spectrum and by analogy with those of related BISBOAs (6). The 70 eV electron impact mass spectrum clearly indicates a mol. wt. 296 and showed the ussually diagnostic peaks as expected. Siscussion of the fragmentation path, mecanisms and structures of the presumed gragments formedad will be published later.

Results from a forced-contact test with *M. domestica* showed that none of the compounds tested has effect on larval mortality neither on their capacity to pupate (table 1) at the tested concentrations.

Table 1 Efect of BISBOA's on larvae of *Musca domestica*

BISBOA assayed (R=)	Death (%)*	Pupation (%)	Deformation (%)
H	0	100	40
F	0	100	60
Cl	0	100	65
Br	0	100	70
I	0	100	60

Doses tested: 2.0 - 5.0 - 7.5 - 10 - 25 - 50 - 75 - 100 mg/cm^2 Four replicates each dose.
25 *Musca domestica* larvae/Petri dish
*Results as given correspond to the highest dose used. Standard erros of the 4 replicates were less than 10%.

However, an important percentage of the hatched adults presented developmental disorders such as not well defined abdominal segments,colorless sclerites being thinner than the control insect and some of them had long legs and/or atroprhied wings, Moreover, adults houseflies showed some feeding inapetency.

As the results of these tests might be depressed by adsoption problems, ingestion tests were performed. *Cynthia carye* fifth instar larvae were considered appropriate since they show voraceus apetite, are easily handled and are polyphagous. Again, it was noted that doses of up to 21 mg of BISBOA during a 3-day period had no effect on larval survival neither on their capacity to pupate. However, when larvae were subjected to tactile stimulation, they became highly irritable, specially with the H-BISBOA compound, These behavioral abnormalities could be indicative of disruption to the nervous system (7). Adults looked externally normal; but, upon dissection, both males and famales had damaged sexual organs.

The described results point to BISBOAs as potentially interesting compounds affecting development of insects. More detailed studies on the site of their

deleterous action at the tissue, cellular, and/or biochemical level have or to be determined.

ACKNOWLEDGEMENTS

We wish to thank Prof. Jan Pettersson and Mr. Agustin Martos for entomological advise.

We thank the CINDEC (Universidad Nacional de Colombia) for partial financial support.

AR and JC are grateful to IFS and IPICS (Sweden) and to Dr. H.M. Niemeyer for fellowships and to Universidad de Chile.

Grateful thanks are due to Dra. Myriam de Gómez for facilities typing of this work.

REFERENCES

1. Arteaga M.; Rincón, J.; Maldonado M. and Robayo, E., *Rev. Col. Ciens. Quim. Farm.*, **1980**, 3, 63.

2. Burke, W.J., *J. Am. Chem. Soc.*, **1950**, 72, 4691.

3. Rivera, A.; Ospina, E.; Sánchez, A. and Joseph-Nathan, P., *Rev. Col. Quim.*, **1985**, 14, 77.

4. Rivera, A.; Aguiar, Z.; Clavijo, D. and Joseph-Nathan, P., *Anales de Química* **serie C. 1989**, 85, 9.

5. Jovanovic, M.V. and Biehl, E.R., *Org. Magn. Reson.*, **1984**, 22, 491.

6. Rivera, A.; Ospina, E.; Sánchez A. and Joseph-Nathan, P., *Heterocycles*, **1986**, 24, 2507.

7. Samuels, R. and Knox, P., *J. Chem. Ecol.*, **1989**, 15, 855.