Bulgarian Journal of Agricultural Science, 18 (No 6) 2012, 929-933
Agricultural Academy

SYNTHESIS OF CYCLOPENTANESPIRO-5-(2,4-DITHIOHYDANTOIN) AND *IN VITRO* INSECTICIDAL ACTIVITY AGAINST *CLADIUS PECTINICORNIS*

M. MARINOV[1], D. GANCHEV[1], P. MARINOVA[2], ST. KRUSTEV[1], N. ATANASOVA[1], M. ZLATEVA[1] and N. STOYANOV[3]
[1] *Agricultural University, Faculty of Plant Protection and Agroecology, BG - 4000 Plovdiv, Bulgaria*
[2] *University of Plovdiv, Faculty of Chemistry, BG - 4000 Plovdiv, Bulgaria*
[3] *University of Ruse, Branch Razgrad, BG - 7200 Razgrad, Bulgaria*

Abstract

MARINOV, M., D. GANCHEV, P. MARINOVA, ST. KRUSTEV, N. ATANASOVA, M. ZLATEVA and N. STOYANOV, 2012. Synthesis of cyclopentanespiro-5-(2,4-dithiohydantoin) and *in vitro* insecticidal activity against *Cladius pectinicornis. Bulg. J. Agric. Sci.,* 18: 929-933

This paper present a method for synthesis of cyclopentanespiro-5-(2,4-dithiohydantoin), which lead to obtaining product of higher yield, compared to already known data in the literature. The compound was prepared by reaction of the cyclopentanespiro-5-hydantoin with P_4S_{10} and hexamethyldisiloxane. The structure of obtained product was verified via ^{1}H, ^{13}C NMR, IR and UV-Vis spectroscopy. Standard *in vitro* filter paper discs test with *Cladius pectinicornis* were conducted in order to be investigated eventual insecticidal activity of compound against this pest on oil yielding roses (*Rosa damascena Mill*).

Key words: cyclopentanespiro-5-(2,4-dithiohydantoin), insecticidal activity, *Cladius pectinicornis, Rosa damascena*

Abbreviations: cpsdth – cyclopentanespiro-5-(2,4-dithiohydantoin); HMDO – hexamethyldisiloxane

Introduction

The hydantoins (imidazolidines) and their derivatives are organic compounds with a wide range of applications in various fields. The most significant application of them is in the area of medicine and the clinical practice as aldose reductase inhibitors (Schnur, 1979), (Sarges et al., 1988), which makes them useful in the treatment of chronic complications of diabetes. They also possess antitumor (Deen et al., 1979), anticonvulsant, antiepileptic (Oldfield and Cashin, 1965) and antiarrhythmic action (Kiec-Kononowicz et al., 2003), as well as inhibitory activity towards muscle and liver glycogen phosphorylase (Somsak et al., 2001).

On the other hand, there is evidence of use of such compounds in the field of agricultural sciences as potential herbicides (Sano et al., 1995a; Sano et al., 1995b) and fungicides (Klauke et al., 1963; Rentzea et al., 1980).

The purpose of this paper is to present a method for synthesis of cyclopentanespiro-5-(2,4-dithiohydantoin), which is significantly shorter compared to already known techniques (Marinov et al., 2005) and lead to obtaining product of higher yield.

Cladius pectinicornis is a typical pest on oil yielding rose which appears incidentally during some years and can cause significant leaves damage, respectively loss of rose-oil (Nikolova, 1969). In Bulgaria however there is no registered plant protection products against this pest although, *Rosa damascena* in economic important culture for the region.

The purpose of this investigation was to reveal eventual insecticidal action of the product on base of

E-mail:danyo@abv.bg

its chemical stricture (structure activity relations) and know action as antitumor, anticonvulsant and antiarrhythmic remedy of such compounds.

Materials and Methods

All used chemicals were purchased from Merck and Sigma-Aldrich. Melting point temperature of cyclopentanespiro-5-(2,4-dithiohydantoin) (**2**) was determined by an SMP-10 digital melting point apparatus. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The product analyzed gave results within ± 0.2 % of the calculated values. The purity of the compound was checked by thin layer chromatography on Kieselgel 60 F_{254}, 0.2 mm Merck plates. eluent systems (vol. ratio): (A) chloroform : acetone = 9 : 1 and (B) ethyl acetate : petroleum ether = 1 : 5. IR spectrum was taken on spectrometer Bruker-113 in KBr disc. Electronic spectrum was taken on a Specord UV-Vis spectrometer. NMR spectra were taken on a Bruker DRX-250 spectrometer, operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. Chemical shifts were referenced to tetramethylsilane (TMS). Measurements in DMSO-d_6 solution were carried out at ambient temperature (300 K). Typical conditions for 1D ^{1}H spectrum were: pulse width 30º, 1 s relaxation delay, 16K time domain points, zero-filled to 64K, hard pulse with 90º pulse width of 11.8 μs; 1D ^{13}C spectrum: pulse width 30º, 1 s relaxation delay, 16K time domain points, zero-filled to 32K, hard pulse with 90º pulse width of 6.4 μs at a power level of 3 dB below the maximum output.

In vitro insecticidal tests

Filter paper disc test was performed for *in vitro* examination of insecticidal activity (Paranagama et al., 2004). The product was tested with 10 different concentrations to be determined LC_{05} (NOEL), LC_{50} and LC_{90}. Each product concentration was tested on 10 larvae placed at the bottom of standard 20 mm Petri dishes covered with layer of filter paper forming one test replication. Each variant consisted of five replications.

The individuals of each variant were sprayed with solution of tested product in tested concentration using laboratory sprayer with delivery rate 1000 l ha^{-1}. The Petri dishes were covered and mortality was observed after 24, 48 and 72 h. The effectiveness was calculated using formula of Abbot (Abbot, 1925).

Synthesis of cyclopentanespiro-5-(2,4-dithiohydantoin) (cpsdth, 2)

The synthesis of the target compound was carried out in accordance to Figure 1. The initial cyclopentanespiro-5-hydantoin (**1**) was synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb, 1934).

A mixture of 4.62 g (0.03 mol) of cyclopentanespiro-5-hydantoin (**1**), 4.89 g (0.011 mol) of P_4S_{10}, 21 ml (0.1 mol) of hexamethyldisiloxane (HMDO) and 60 ml of xylene was refluxed for an hour and a half. After cooling, the obtained crystalline product (**2**) was filtered off and recrystallized from methanol/water solution.

Yield: 92 %; M. p.: 245–246 °C; Rf_A = 0.68; Rf_B = 0.34.

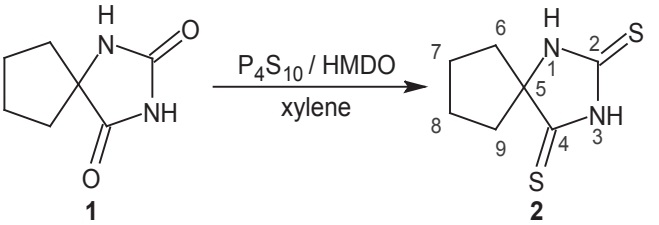

Fig. 1. Synthesis of cyclopentanespiro-5-(2,4-dithiohydantoin)

Spectral data for **2**:

UV-Vis (EtOH) λ_{max} / nm: 398, 302, 223.

IR (KBr) ν_{max} / cm^{-1}: 3184, 3125, 2947–2806, 1542, 1435, 1157–1133.

^{1}H NMR (DMSO-d_6) δ / ppm: 2.00–2.49 (m, 8H), 10.9 (s, 1H), 13.11 (s, 1H).

^{13}C NMR (DMSO-d_6) δ / ppm: 25.3 (C7, C8), 41.8 (C6, C9), 83.3 (C5), 179.3 (C2), 212.5 (C4).

Mathematical manipulation of data

The received data from conducted *in vitro* insecticidal test with *Cladius pectinicornis* were statistically manipulated with R language for statistical computing (R Development Core Team, 2011) and drc R language package (Ritz and Streibig, 2005) in order to be calculated values of LC_{05} (NOEL), LC_{50} and LC_{90}. In

Figure 2 is illustrated R language IDE (Integrated Development Environment) output results with conducted general model fitting function for concentration/dose/time-response models:

Figure 3 presents calculation of LC_{05}, LC_{50} and LC_{90} by function ED.

The statistical manipulation was conducted at 95 % confidence level. P values are under 0.05 which proves the reliability of the test and method for mathematical analysis.

The calculated values of LC_{05}(NOEL), LC_{50} and LC_{90} are respectively: 0.00058489, 0.00315592 and 0.01110208.

The three – parameter logistic function is given by following formulas (Ritz and Streibig, 2005):

With lower limit 0 – Figure 4.

Or in another parameterization – Figure 5.

With upper limit 1 – Figure 6.

Or in another parameterization – Figure 7.

$$f(x) = 0 + \frac{d-0}{1+\exp(b(\log(x)-\log(e)))}$$

Fig. 4. Three –parameter logistic function with lower limit 0

$$f(x) = 0 + \frac{d-0}{1+\exp(b(\log(x)-e))}$$

Fig. 5. Three –parameter logistic function in another parameterization

$$f(x) = c + \frac{1-c}{1+\exp(b(\log(x)-\log(e)))}$$

Fig. 6. Three –parameter logistic function with lower limit 1

$$f(x) = c + \frac{1-c}{1+\exp(b(\log(x)-e))}$$

Fig. 7. Three –parameter logistic function in another parameterization with lower limit 1

```
> summary (drm(eff~conc, data= cpsdth, fct=LL.3()))

Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0 (3 parms)

Parameter estimates:

                 Estimate  Std. Error     t-value p-value
b:(Intercept) -1.74680563  0.37038365 -4.71620613  0.0022
d:(Intercept) 98.87539428  5.94012140 16.64534908  0.0000
e:(Intercept)  0.00315592  0.00045125  6.99368320  0.0002

Residual standard error:

 6.80493 (7 degrees of freedom)
```

Fig. 2. General model fitting function for concentration/dose/time-response models

```
> ED(drm(eff~conc, data= cpsdth, fct=LL.3()), c(5, 50, 90), interval="delta")

Estimated effective doses
(Delta method-based confidence interval(s))

       Estimate Std. Error      Lower  Upper
1:5  0.00058489 0.00017301 0.00017579 0.0010
1:50 0.00315592 0.00045125 0.00208888 0.0042
1:90 0.01110208 0.00410728 0.00138990 0.0208
```

Fig. 3. Lethal dose/concentration calculation

Results and Discussion

The main method for synthesis of hydantoins and spirohydantoins is the Bucherer-Lieb method (Bucherer and Lieb, 1934) based on the interaction between the corresponding ketone, sodium or potassium cyanide, ammonium carbonate and ethanol. In our case, we applied the above-mentioned procedure to cyclopentanone. Because of this interaction, the cyclopentanespiro-5-hydantoin (**1**) was obtained. The compound **1** was thionated with the reagent combination of P_4S_{10} and HMDO, following a modification of a procedure developed by Curphey (Curphey, 2002). It is important to note, that we used this technique for the first time for spirohydantoin thionation. The combination of P_4S_{10} and HMDO lead to a significant reduction of the reaction time, and to obtaining product with higher yield, compared to previously used thionation methods (Marinov et al., 2005).

The obtained compound (**2**) was characterized by IR and NMR spectral data, which confirmed the suggested structure. The physicochemical parameters and the spectral data cited in this study (see the experimental section) are identical with the previously published data (Marinov et al., 2005) for the cyclopentanespiro-5-(2,4-dithiohydantoin).

The dose-response curve describing conducted insecticidal test and respectively insecticidal activity of the product towards to *Cladius pectinicornis* is shown in Figure 8.

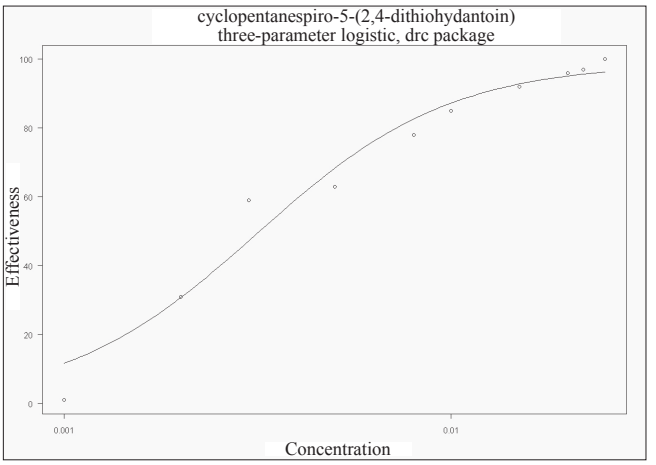

Fig. 8. Dose – Response Curve of cyclopentanespiro-5-(2,4-dithiohydantoin)

Conclusion

The results show the extremely high effectiveness of tested compound which was able to induce insecticidal effect on the tested pest at 0.011 % concentration as LC_{90} (0.0031 % as LC_{50}), which is completely comparative with the insecticidal effect produced by active substances of most commonly used commercial insecticides on the market. These give grounds, the product (**2**) to be developed in the future as promising plant protection product with insecticidal activity.

Acknowledgements

Financial support by the Agricultural University – Plovdiv, Bulgaria (Contract 06-12) is gratefully acknowledged.

References

Abbot, S., 1925. A method for computing the effectiveness of an insecticide. *Journal of Economoc Enthomology*, **18:** 367-271.

Bucherer, H. T. and V. Lieb, 1934. Über die Bildung substituierter Hydantoine aus Aldehyden und Ketonen. Synthese von Hydantoinen. *J. Prakt. Chem.*, **141:** 5-43.

Curphey, T. J., 2002. Thionation with the Reagent Combination of Phosphorus Pentasulfide and Hexamethyldisiloxane. *J. Org. Chem.*, **67:** 6461-6473.

Deen, D. F., T. Hoshino, M. E. Williams, K. Nomura and P. M. Bartle, 1979. Response of 9L tumor cells in vitro to spirohydantoin mustard. *Cancer Res*., **39:** 4336-4340.

Kiec-Kononowicz, K., K. Stadnicka, A. Mitka, E. Pekala, B. Filipek, J. Sapa and M. Zygmunt, 2003. Synthesis, structure and antiarrhythmic properties evaluation of new basic derivatives of 5,5-diphenylhydantoin. *Eur. J. Med. Chem.*, **38:** 555-566.

Klauke, E., E. Kuehle and F. Greve, 1963. *Belgian Patent 631731*.

Marinov, M., S. Minchev, N. Stoyanov, G. Ivanova, M. Spassova and V. Enchev, 2005. Synthesis, spectroscopic characterization and ab initio investigation of thioanalogues of spirohydantoins. *Croat. Chem. Acta.*, **78:** 9-16.

Nikolova, W., 1969. Die Hymenopteren und Dipterenfauna in Pflanzungen dep Ölhaltigen Rose (*Rosa damascena* Mill) in Bulagarien. *Anzeiger für Schädlingskunde*, **42** (12): 177-184.

Oldfield, W. and C. H. Cashin, 1965. The Chemistry and Pharmacology of a Series of Cycloalkanespiro-5'-hydantoins. *J. Med. Chem.*, **8:** 239-249.

Paranagama, P., K. Abeysekera, L. Nugaliyadde and K. Abeywickrama, 2004. Repellency and toxicity of four essentials oils to *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *J. Natn. Sci. Foundation Sri Lanka,* **32** (3&4): 127-138.

R Development Core Team, 2011. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Rentzea, C., E.-H. Pommer, C. J. Mappes and B. Zeeh, 1980. *U. S. Patent 4198423.*

Ritz, C.,and J. C. Streibig, 2005. Bioassay Analysis using R. *J. Statist. Software*, **12**

Sano, H., S. Mio, J. Kitagawa, M. Shindou, T. Honma and S. Sugai, 1995. Synthesis of spirothiohydantoin analogues of hydantocidin. *Tetrahedron*, **51:** 12563-12572.

Sano, H., S. Mio, M. Hamura, J. Kitagawa and M. Shindou, 1995. Synthesis and Herbicidal Activity of Hydantocidin Analogues : Modification of the Carbonyl Groups in Spirohydantoin. *Biosci, Biotechncol. Biochem.*, **59:** 2247-2250.

Sarges, R., R. C. Schnur, J. L. Belletire and M. J. Peterson, 1988. Spiro hydantoin aldose reductase inhibitors. *J. Med. Chem.*, **31:** 230-243.

Schnur, R. C., 1979. *U. S. Patent 4176185.*

Somsak, L., L. Kovacs, M. Toth, E. Osz, L. Szilagyi, Z. Gyorgydeak, Z. Dinya, T. Docsa, Toth B., P. Gergely, 2001. Synthesis of and a Comparative Study on the Inhibition of Muscle and Liver Glycogen Phosphorylases by Epimeric Pairs of d-Gluco- and d-Xylopyranosylidene-spiro-(thio)hydantoins and *N*-(d-Glucopyranosyl) Amides. *J. Med. Chem.*, **44:** 2843-2848.

Received February, 2, 2012; accepted for printing September, 22, 2012