Research Article

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Larvicidal activity of synthetic tropane alkaloids against *Ascia monuste orseis* (Lepidoptera: Pieridae)

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Abstract

BACKGROUND: Tropane alkaloids are known to play a role in plant defence. By blocking acetylcholine receptors, they exert insecticidal and deterrent effects against herbivore insects. Carbamates are an important class of chemical insecticides that also inhibit acetyl cholinesterase. The objective of this work was to synthesise a series of tropane alkaloids bearing a carbamate group, and to evaluate their effects against the pest *Ascia monuste*. The effects of the most active compounds were evaluated on the *A. monuste* predator *Solenopsis saevissima* and on the pollinator *Tetragonisca angustula*.

RESULTS: The synthesis of carbamate-tropane alkaloids was accomplished in 4–5 steps from commercially available ketones. Results from bioassays showed that compounds 6a, 10a and 14a presented higher activities against second-instar larvae of *A. monuste*, with LD_{50} values of 1.01, 3.76 and 1.92 µg substance mg^{-1} insect, and TL_{50} values of 7.0, 15.0 and 5.0 h respectively. These compounds were also tested for their selectivity in favour of *S. saevissima* and *T. angustula*. Compound 6a, which showed the highest activity against *A. monuste*, also showed lower toxicity against *S. saevissima*.

CONCLUSION: Tropane alkaloid derivatives bearing a carbamate group show potential for the development of novel insecticides against *A. monuste*.

Supporting information may be found in the online version of this article.

Keywords: insecticide activity; tropane alkaloids; carbamate; *Ascia monuste*; *Solenopsis saevissima*; *Tetragonisca angustula*

1 INTRODUCTION

Insect pests constitute a major cause of agricultural losses worldwide,[1–3] and for several decades the use of chemical insecticides has been the most adopted method for effective control of such pests in crop areas. However, factors such as the development of resistance to commercial insecticides, as well as the needs for more environmentally friendly pesticides, makes the development of new insecticides an important and continuous task.[4,5]

Plants produce a plethora of secondary metabolites that play a role in plant defences, and the search for natural products that can be used either as natural pesticides or as models for the development of novel synthetic molecules has been regarded as a promising strategy.[6–9] In line with this tendency, our research group has been using natural products as a model for the development of new synthetic compounds as potential herbicides[10,11] or insecticides.[12,13]

In the present paper, we report the synthesis and evaluation of the insecticidal properties of a series of tropane alkaloids bearing a carbamate group. Tropane alkaloids constitute an important class of secondary metabolites produced by various plant species and are known to play an important role in plant defence against insect herbivory.[14–19] Chemically, the tropane skeleton is characterised by a 8-azabicyclo[3.2.1]octane skeleton (Fig. 1), a bicyclic structure in which a piperidine ring and a pyrrolidine ring share two carbon atoms and one nitrogen atom.[20,21] Their deterrent and insecticidal activities are due to the ability to block acetylcholine receptors, thus preventing the binding of acetylcholine and resulting in interruption of insect neuromuscular function.[22,23] Moreover, carbamates constitute one of the main classes of synthetic insecticides[24] that also act by interfering with acetylcholine neurotransmission. This prompted us to produce hybrid molecules presenting both a tropane skeleton and a carbamate in an attempt to obtain new, potent insecticidal compounds. The obtained compounds were tested against the agricultural insect pest *Ascia monuste* (Godart) (Pieridae). Also known as Great Southern White butterfly, this insect is widely found in the American continent, being

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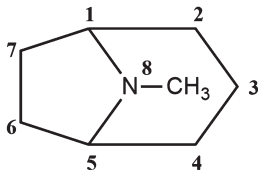


Figure 1. The chemical structure of the tropane alkaloid skeleton.

responsible for loses of up to 100% in production of Brassicaceae crops.[25] Besides, the selectivity of the compounds was accessed by testing against *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae), a natural enemy that prey larvae and pupae of *A. monuste*[26] and against *Tetragonisca angustula*, a pollinator species of extreme importance for agriculture.[27]

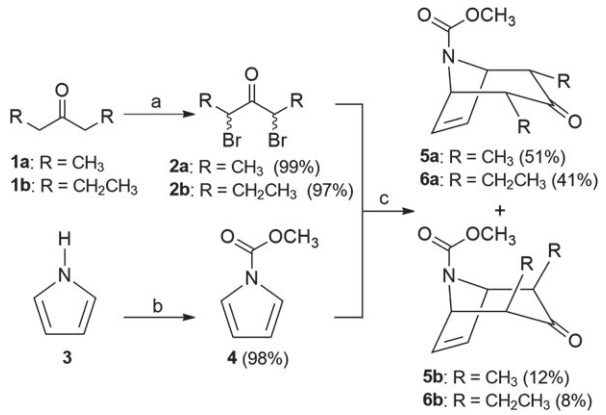
2 MATERIALS AND METHODS

2.1 General chemical procedures and synthesis

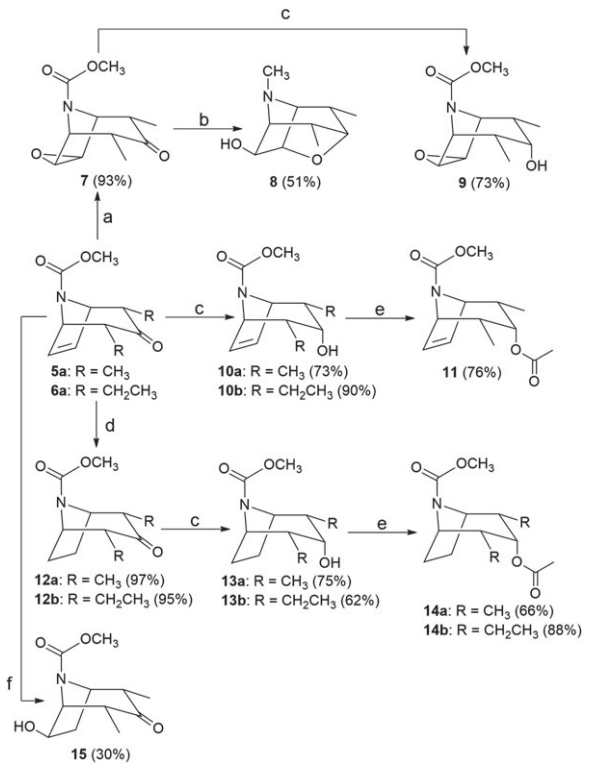
The synthetic pathways for compounds **2** to **6** are presented in Scheme 1. The transformation of compounds **5a** and **6a** into derivatives **7** to **15** is shown in Scheme 2. The details of all experimental procedures and the physical and spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, HRMS) used for the complete structural characterisation of all compounds are included in the supporting information.

2.2 Bioassays

The synthesised compounds were tested for their insecticidal activities against second-instar larvae of *A. monuste orseis* obtained from an established laboratory strain. This population was collected from a commercial cabbage field from Viçosa County (20° 44′ 52.5″ S, 42° 50′ 29.8″ W, Minas Gerais State, Brazil) and reared in the laboratory for at least five generations before the start of the experiments. Moreover, the selectivity of the compounds was assessed by testing on adults of *S. saevissima*, a natural enemy of *A. monuste*, and of *T. angustula*, an important pollinator in agricultural ecosystems. Adults of the non-target species were collected from nests located around the campus of Universidade Federal de Viçosa. In all assays, malathion (Malathion 500 EC; Cheminova, São Paulo, Brazil) was used as positive control. Malathion is a widely used contact insecticide and is registered for the control of *A. monuste* in Brassicaceae crops. The experimental design was completely randomised with three replications. Each experimental unit consisted of ten insects kept on a glass petri dish (9 cm diameter × 2 cm height) covered with organza. The average weight of each insect species was obtained by measuring, on an analytical balance, the mass of ten groups containing ten insects each. In all cases, the petri dishes were placed in an incubator at 25 ± 0.5 °C and 75 ± 5% relative humidity, with a 12:12 h light/dark photoperiod. Bioassays were conducted by topical application. A 10 µL Hamilton microsyringe was employed to apply, on the thoracic tergite of each individual insect, 0.5 µL of a solution of the test compound dissolved in acetone. In a control experiment carried out under the same conditions, 0.5 µL of acetone was used as negative control. After application, insects were kept in individual petri dishes and supplied with appropriate food as follows: discs of cabbage for *A. monuste orseis* and moistened cotton and a plastic container (1.5 cm diameter × 1 cm height) with candy (85% sugar and 15% honey) for *S. saevissima* and *T. angustula*.



Scheme 1. Reagents and conditions: (a) Br2, HBr 48% v/v, room temperature for 1.5 h; (b) NaH, methyl chloroformate, THF, 50 °C for 24 h; (c) Cu, NaI, CH_3CN, 50 °C for 24 h.



Scheme 2. Reagents and conditions: (a) *m*-CPBA, dichloromethane, room temperature for 14 h, reflux for 7 h; (b) DIBAH (10 equiv.), toluene, −78 °C for 2 h, room temperature for 22 h; (c) $NaBH_4$, ethanol, reflux for 1 h; (d) H_2, cat. Pd/C, ethyl acetate, room temperature for 2 h; (e) acetic anhydride, cat. BF_3.MeOH, dichloromethane, room temperature for 19 h; (f) (i) BH_3, tetrahydrofuran, 0 °C for 30 min, room temperature for 3 h, (ii) H_2O_2, NaOH, ethanol, reflux for 1 h.

2.2.1 Toxicity of the compounds against *A. monuste*

The insecticidal activity against *A. monuste* was initially assessed by testing all substances at a fixed dose (10 µg mg^{-1} insect). Mortality was evaluated after 48 h of exposure to treatments. Mortality data were subjected to analysis of variance,[28] and the averages were compared by the Scott–Knott grouping analysis test ($P < 0.05$). Compounds that caused mortality equal to or greater than 80% were considered to be satisfactorily active and were selected for further experiments.[29]

2.2.1.1 Determination of the dose–mortality curves for the active compounds. Dose–mortality curves were constructed for some selected compounds according to their activities in the first bioassay. The experimental design, experimental unit, procedure and evaluations of these experiments were similar to the previous bioassay. The treatments consisted of doses of three alkaloids selected in the previous bioassay, malathion and the negative control (acetone). The compounds were tested at concentrations ranging from 0.05 to a maximum of 20 µg mg^{-1} insect. The insect mortality was assessed 48 h after treatment application. Dose–mortality data were corrected by Abbott's method[30] and then subjected to probit analysis[31] using the PROC PROBIT procedure in SAS[28] to estimate dose–mortality curves. The curves that presented probabilities greater than 0.05 by the χ^2 test[32] were accepted. The lethal doses that caused 50 and 90% mortality (LD_{50} and LD_{90}) were also estimated.

2.2.1.2 Determination of survival curves of the most toxic alkaloids against *A. monuste*. The treatments were the LD_{90} of selected alkaloids and the control (acetone). In each treatment, 60 insects were used, and the mortality rate was monitored over a period of 0–48 h. Time–mortality data were subjected to survival analysis ($P < 0.05$) with a non-parametric Kaplan–Meier estimator[33] using the LIFETEST procedure.[28] The survival curves constructed were compared by log-rank test ($P < 0.05$), and the median survival times (LT_{50}) of the larvae were estimated.

2.2.2 Selectivity of alkaloids in favour of predatory ant *S. saevissima* and pollinator bee *T. angustula*

S. saevissima and *T. angustula* were exposed to the LD_{90} of the most active compounds against *A. monuste* in order to evaluate their selectivity. The experimental design was completely randomised, with six replicates. Insect mortality was recorded after 48 h of treatment. To evaluate selectivity, mortality of non-target species was compared with pest mortality by Student's *t*-test for independent samples ($P < 0.05$).

3 RESULTS AND DISCUSSION

3.1 Synthesis

The synthetic strategy adopted for the preparation of tropane alkaloids bearing a carbamate group is depicted in Scheme 1. In the first step, the required starting materials α,α-dibromoketones were produced by halogenation of commercial carbonyl compounds using elemental bromine to afford **2a** and **2b**. Both ketones were obtained as isomeric mixtures and were used without purification.[34] Moreover, *N*-carboxymethylpyrrole **4** was produced through a reaction between pyrrole anion and methyl chloroformate. The required products were obtained in excellent yields. In the second step, cycloaddition reactions of **2a** and **2b** with **4** in the presence of NaI/Cu to generate the necessary oxyallyl cation intermediates[35] afforded the tropane alkaloid-carbamate hybrids **5a**, **5b**, **6a** and **6b** in 51, 12, 41 and 8% yield respectively. In this type of cycloaddition, the products with the groups at equatorial to the carbonyl group are the major ones (**5a**, **6a**) owing to the stability of the transition state for this reaction.[35] The yields obtained were not optimised, but compound **5a** and **6a** were produced in a sufficient amount for further transformations.

Having obtained compounds **5a** and **6a** in large quantities, they were then further modified into new tropane-carbamate molecules for an initial biological screening (Scheme 2). Initially,

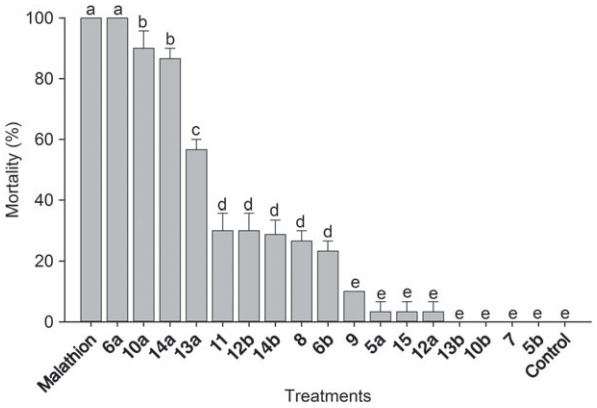


Figure 2. Contact toxicity of the treatments at a concentration of 10 µg compound mg^{-1} insect against *A. monuste* 48 h after topical application. Means followed by the same letter are not significantly different by the Scott–Knott grouping analysis test at $P > 0.05$. Only acetone was used in the control.

the epoxidation of **5a** with *m*-chloroperoxybenzoic acid afforded **7** in 93% yield, which was then subjected to reduction by two different methods. The first reduction used diisobutylaluminium hydride in dry toluene to produce **8** (51% yield), a compound structurally similar to the natural alkaloid scopine.[36] Further treatment of compound **7** with sodium borohydride in ethanol afforded the epoxide **9** in 73% yield.

Hydrogenation of the compounds **5a** and **6a** was carried out under a hydrogen atmosphere using Pd/C as catalyst, affording products **12a** and **12b** in 97 and 95% yield respectively. Compounds **5a**, **6a**, **12a** and **12b** were then subjected to reduction with $NaBH_4$ to give the reduced **10a**, **10b**, **13a** and **13b** with yields of 73, 90, 75 and 62% respectively. Hydroxylated compounds **10a**, **13a** and **13b** were subjected to acylation with acetic anhydride using BF_3.MeOH as catalyst, affording **11**, **14a** and **14b** in 76, 66 and 88% yield respectively. Finally, compound **15** was obtained in 30% yield from **5a** through a hydroboration/oxidation reaction. The poor yield of compound **15** can be explained by the formation of byproducts from reduction of the carbamate group.[37]

The structures of all compounds were elucidated through spectroscopic analysis, as shown in the supporting information.

3.2 Biological activity

3.2.1 Toxicity of alkaloids against A. monuste

The toxicity of synthesised tropane alkaloid derivatives against *A. monuste* was assessed through three different bioassays. Firstly, all synthesised compounds were assayed against larvae of *A. monuste* at 10 µg substance mg^{-1} insect. The results showed a significant difference in mortality of the larvae of *A. monuste* by the treatments ($F_{18,38} = 162.20$, $P < 0.001$). Alkaloid derivatives **6a**, **10a** and **14a** caused higher mortalities (86.7–100%). Compounds **6b**, **8**, **11**, **12b**, **13a** and **14b** showed intermediate activity (23.3–56.7%), while compounds **5a**, **5b**, **7**, **9**, **10b**, **12a**, **13b** and **15** resulted in low mortality (0–10%) and did not differ statistically from the negative control (Fig. 2). In view of these results, the most active alkaloid derivatives **6a**, **10a** and **14a** were selected for further experiments.

From the data obtained, a preliminary structure–bioactivity analysis revealed that the stereochemistry of the alkyl groups alpha to the carbonyl is relevant for the activity, as the isomer **6a** was active and the isomer **6b** showed low activity. The same trend was observed for compounds **5a**/**5b**, although they both had very little activity. On the other hand, the size of the groups alpha to

the carbonyl (methyl versus ethyl) has a dramatic influence on the activity, as isomer **6a** was the most active compound. The reduction of the carbonyl group to axial OH had a great effect on insect toxicity, as compound **10a** was 27.0 times more active than **5a**. On the other hand, the alcohol **10b** was 100 times less active than the corresponding starting ketone **6a**. Reduction of the double bond of **10a**, producing **13a**, caused a 1.6-fold decrease in activity, while reduction of the double bond of **10b**, producing **13b**, did not influence the activity. Removal of the double bond of compound **6a** lowered activity considerably, as **12b** was 3.3 times less active than **6a**. The effect of acetylation on insecticidal activity was negative in the case of **10a** (**11** was 3.0 times less active than **10a**) and positive in the cases of **13a** (**14a** was 1.5 times more active than **13a**) and **13b** (**14b** was 28.7 times more active than **13b**). Removal of the double bond from **5a** (producing **12a**) and introduction of a OH group at position 6 (compound **15**) did not change the activity. In general, the epoxides were not active. In fact, compound **9**, the corresponding epoxide of **10a**, was 9.2 times less active. The same effect was observed in the epoxidation of **5a**.

Compounds such as carbaryl,[38] methomyl,[39] carbofuran[40] and methiocarb[41] are among the carbamate insecticides commercially registered. The natural products cocaine,[42] scopolamine[22] and atropine[43] are examples of tropane alkaloids that exhibit insecticidal activity. It is worth pointing out that the scopolamine analogue **8**, lacking the carbamate group, had some insecticidal activity. As observed from the above discussion, the association of a tropane and carbamate group seems to have a positive effect on insecticidal activity. The toxicity level of the compounds is very sensitive to the functionalities present on the tropane skeleton; however, no correlation was observed with their polarities. The log *P* values for all compounds were calculated, and they varied between 0.18 and 2.08. For the two most active compounds **6a** and **10a**, the log *P* values were 2.08 and 1.26 respectively, while for compound **14a** the log *P* value was 1.98. Despite this lack of correlation, it is known that the toxicity level of an insecticide depends on factors such as rate of penetration, decomposition and excretion, which are influenced by the physicochemical properties of the insecticide, including its lipophilicity.[44–46]

Based on the results obtained, we selected the most active compounds **6a**, **10a** and **14a** for further assays. These three compounds were tested at various doses on larvae of *A. monuste*. Dose–mortality curves were constructed (Fig. 3), allowing calculation of the corresponding LD_{50} and LD_{90} values (Table 1). As indicated in the preliminary tests, derivative **6a** was the tropane alkaloid-carbamate with the highest toxicity, presenting an LD_{50} of 1.01 μg mg^{-1} insect. The LD_{50} values for **10a** and **14a** were 3.76 and 1.92 μg mg^{-1} insect respectively. Finally, the insecticide malathion, used as positive control, showed a LD_{50} value 3.2 times lower than that of **6a**. However, it is worth mentioning that malathion is a commercial formulation containing additives that facilitate drug

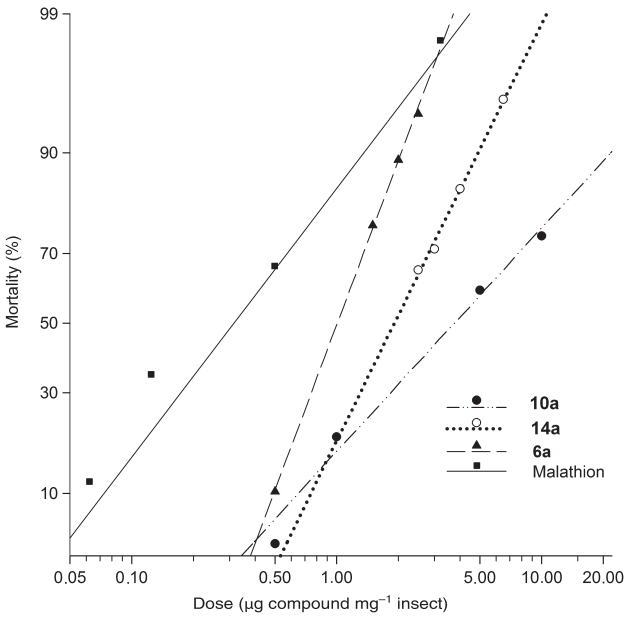


Figure 3. Dose–mortality curves for substances **6a**, **10a** and **14a** for second-instar larvae of *A. monuste*.

penetration into the cuticle of the insect. By contrast, all compounds synthesised and tested in this work were administered using only acetone as solvent and without any additive, which may restrict their penetration into the cuticle and thereby lower their toxicity to the insect. Thus, we can consider that tropane alkaloid-carbamate hybrids may constitute a promising model for the development of new insecticides.

The speeds of action of compounds **6a**, **10a** and **14a** against *A. monuste* were also determined (log-rank test, $\chi^2 = 6.621$, df = 2, $P = 0.036$). Compounds **6a**, **10a** and **14a** caused 88, 75 and 83% of mortality after 48 h of exposure respectively. No mortality was produced by the negative control (Fig. 4). The lethal times of **6a** and **14a** to half the population of larvae of *A. monuste* (LT_{50}) were similar [7.00 (5.52–8.48) and 5.00 (3.96–6.04) h respectively] and significantly higher than that of compound **10a** [15.00 (11.75–18.25) h].

3.2.2 Selectivity of alkaloids in favour of predatory ant *S. saevissima* and pollinator bee *T. angustula*

Natural enemies play a key role in controlling insect pests in agricultural ecosystems. Thus, it is highly desirable that new insecticides present selective action, capable of controlling target pests but with the lowest possible toxicity against non-target species, including natural enemies.[23,24] The ant *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) is an important natural enemy of *A. monuste*, preying on both larvae and pupae of this

Table 1. Results of probit analysis on mortality of *A. monuste* after 48 h of exposure to compounds malathion, **6a**, **10a** and **14a**[a]

Compound	*Y*	χ^2	df	*P*	LD_{50} (μg mg^{-1})	LD_{90} (μg mg^{-1})
6a	4.98 + 4.11*x*	0.10	2	0.95	1.01 (0.90–1.11)	2.06 (1.86–2.34)
10a	4.03 + 1.68*x*	1.63	2	0.55	3.76 (3.09–4.64)	21.72 (15.33–34.92)
14a	4.11 + 3.14*x*	0.26	2	0.88	1.92 (1.38–2.27)	4.90 (4.27–6.28)
Malathion	1.02 + 2.02*x*	2.54	2	0.28	0.32 (0.24–0.42)	1.35 (0.92–2.43)

[a] *Y* = curve equation; χ^2 = chi-square test; df = degrees of freedom; *P* = probability; LD_{50} LD_{90} = lethal doses that caused 50 and 90% mortality, with 95% fiducial limits.

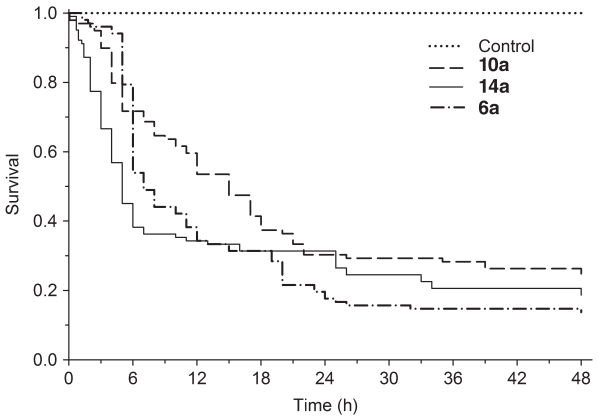


Figure 4. Survival curves of second-instar larvae of *A. monuste* submitted to the LD_{90} of substances **6a**, **10a** and **14a** and the control. Only acetone was used in the control.

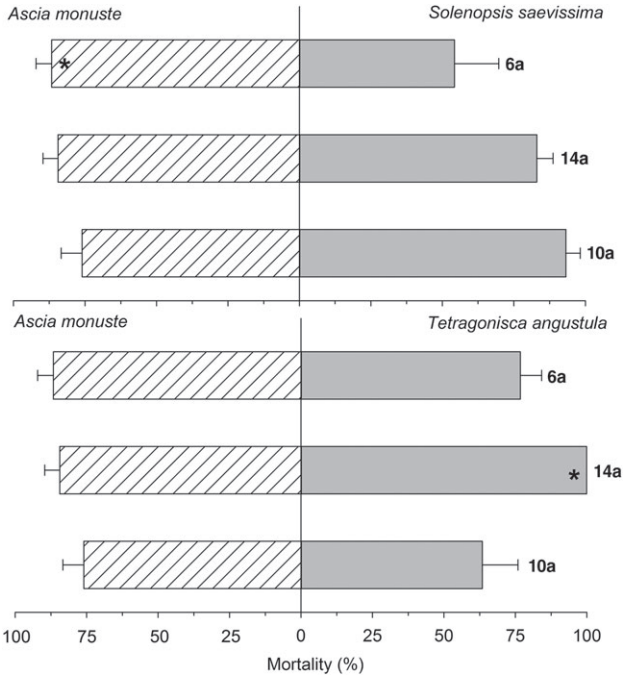


Figure 5. Comparison of mortality (mean ± standard error) caused by substances **6a**, **10a** and **14a** to the larvae of *A. monuste*, the ant *S. saevissima* and the bee *T. angustula*. The asterisk (*) indicates that the mortality caused by the treatment was greater for one histogram by *t*-test with $P < 0.05$. The dose of alkaloids used was the LD_{90} for larvae of *A. monuste*. Only acetone was used in the control.

pest.[26] Another important group of beneficial insects associated with ecosystems are bees (Hymenoptera: Apidae). Pollination by bees is extremely important in agricultural crops and accounts for 90% of the reproductive success of flowering plants.[27] A decline in bee populations is currently being observed by researchers in various regions of the planet,[47,48] and one hypothesis is the use of neonicotinoid insecticides.[49] Among the species of bees is *Tetragonisca angustula* (Latreille) (Meliponinae), popularly known in Brazil as jataí. *T. angustula* is one of the stingless bee species more important in the Neotropics.[50]

Thus, we also investigated the selectivity of compounds **6a**, **10a** and **14a** by testing on both *S. saevissima* and *T. angustula*. The results are presented in Fig. 5. Compound **6a** caused 86.7% mortality of larvae of *A. monuste*, and 54.2 and 76.7% mortality of *S. saevissima* and *T. angustula* respectively. Compound **10a** caused 76.0% mortality of larvae of *A. monuste*, and 93.2 and 63.4% mortality of *S. saevissima* and *T. angustula* respectively. The mortalities produced by compound **10a** on larvae of *A. monuste* and on *T. angustula* did not differ statistically. Compound **14a** caused 84.4% mortality of larvae of *A. monuste*, and 83.0 and 100.0% mortality of *S. saevissima* and *T. angustula* respectively. Therefore, only compound **6a** showed selectivity in favour of the ant *S. saevissima*, and none of the tested compounds showed selectivity in favour of the pollinator *T. angustula*.

According to the recommendations of the Brazilian Health Surveillance Agency for tests of efficacy on pest control products,[29] for the selection of new lead compounds as potential insecticides, only those causing 90 ± 10% insect mortality can be considered. Thus, the high mortality values observed for compounds **6a** (100%), **10a** (90.0%) and **14a** (86.7%) make them model candidates for future development of new agrochemicals for controlling the insect pest *A. monuste*. It can be observed that the dose–mortality curve has a higher slope for compounds **6a** (4.11) and **14a** (3.14) in comparison with compound **10a** (1.68), indicating a more homogeneous response of the *A. monuste* population exposed to compounds **6a** and **14a**. Both compounds showed fast-acting control (less than 24 h) in the survival analysis, a desirable quality for chemical control of large infestations.

The selectivity of insecticides in relation to its natural enemy may be physiological or ecological.[44] If the insecticide is more toxic to the pest than towards a non-target organism, it is considered physiologically selective. In addition, ecological selectivity is characterised by minimisation of contact between pesticide and non-target organisms. Only compound **6a** was physiologically selective in favour of the predator *S. saevissima* and non-selective to the pollinator *T. angustula*. The compounds **10a** and **14a** were non-selective to the predator *S. saevissima* and the pollinator *T. angustula*.

4 CONCLUSION

In this work, 17 tropane alkaloids bearing a carbamate group were synthesised and tested for their toxicity against the agricultural insect pest *A. monuste*. Three compounds (**6a**, **10a** and **14a**) caused high mortalities on larvae of *A. monuste*. These most active compounds were also tested for their toxicity against *S. saevissima*, a natural enemy of *A. monuste*, and against *T. angustula*, an important pollinator in agricultural systems. Compound **6a** was physiologically selective in favour of the predator *S. saevissima*, but none of the tested compounds was selective to the pollinator *T. angustula*. Thus, in the case of further development of such compounds, if no selectivity is found towards the pollinator, the application on agricultural areas should only occur during the period of no activity of such insects. These results demonstrate that the new hybrid compounds with a tropane skeleton bearing a carbamate group are promising for further development of new insecticides.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Painia DR, Shepparda AW, Cookc DC, De Barroe PJ, Wornerf SP and Thomas MB, Global threat to agriculture from invasive species. *Proc Natl Acad Sci USA* **113**:7575–7579 (2016).
2 Oliveira CM, Auad AM, Mendes SM and Frizzas MR, Crop losses and the economic impact of insect pests on Brazilian agriculture. *Crop Prot* **56**:50–54 (2014).
3 Singh A and Gandhi S, Agricultural insect pest: occurrence and infestation level in agricultural fields of Vadodara, Gujarat. *Int J Sci Res Publ* **2**:1–5 (2012).
4 Suckling DM, Stringer LD, Stephens AEA, Woods B, Williams DG, Baker G *et al.*, From integrated pest management to integrated pest eradication: technologies and future needs. *Pest Manag Sci* **70**:179–189 (2014).
5 Lamberth C, Jeanmart S, Luksch T and Plant A, Current challenges and trends in the discovery of agrochemicals. *Science* **341**:742–746 (2013).
6 Cantrell CL, Dayan FE and Duke SO, Natural products as sources for new pesticides. *J Nat Prod* **75**:1231–1242 (2012).
7 Isman MB, A renaissance for botanical insecticides? *Pest Manag Sci* **71**:1587–1590 (2015).
8 Farooq M, Jabran K, Cheema ZA, Wahid A and Siddique KHM, The role of allelopathy in agricultural pest management. *Pest Manag Sci* **67**:493–506 (2011).
9 Sparks TC, Insecticide discovery: an evaluation and analysis. *Pest Biochem Physiol* **107**:8–17 (2013).
10 Barbosa LCA, Alvarenga ES, Demuner AJ, Figueiredo R and Silva AA, Synthesis of new aliphatic and aromatic phytotoxic derivatives of 2α,4α-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one. *Pest Manag Sci* **59**:1043–1051 (2003).
11 Teixeira RR, Pinheiro PF, Barbosa LCA, Carneiro JWM and Forlani G, QSAR modeling of photosynthesis-inhibiting nostoclide derivatives. *Pest Manag Sci* **66**:196–202 (2010).
12 Paula VF, Barbosa LCA, Demuner AJ, Piló-Veloso D and Picanço MC, Synthesis and insecticidal activity of new amide derivatives of piperine. *Pest Manag Sci* **56**:168–174 (2000).
13 Paula VF, Barbosa LCA, Teixeira RR, Picanço MC and Silva GA, Synthesis and insecticidal activity of new 3-benzylfuran-2-yl *N*,*N*,*N*′,*N*′-tetraethyldiamidophosphate derivatives. *Pest Manag Sci* **64**:863–872 (2008).
14 Shonle I and Bergelson J, Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution* **54**:778–788 (2000).
15 Ali A, Ahmad F, Biondi A, Wang Y and Desneux N, Potential for using *Datura alba* leaf extracts against two major stored grain pests, the khapra beetle *Trogoderma granarium* and the rice weevil *Sitophillus oryzae*. *J Pest Sci* **85**:359–366 (2012).
16 Mithöfer A and Boland W, Plant defense against herbivores: chemical aspects. *Annu Rev Plant Biol* **63**:431–450 (2012).
17 War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, S Ignacimuthu *et al.*, Mechanisms of plant defense against insect herbivores. *Plant Signal Behav* **7**:1306–1320 (2012).
18 Chowanski S, Adamski Z, Marciniak P, Rosinski G, Büyükgüzel E, Büyükgüzel K *et al.*, A review of bioinsecticidal activity of *Solanaceae* alkaloids. *Toxins* **8**:1–28 (2016).
19 De Simone R, Margarucci L and De Feo V, Tropane alkaloids: an overview. *Pharmacologyonline* **1**:70–89 (2008).
20 Humam M, Shoul T, Jeannerat D, Muñoz O and P Christen, Chirality and numbering of substituted tropane alkaloids. *Molecules* **16**:7199–7209 (2011).
21 Schmeller T, Sporer F, Sauerwein M and Wink M, Binding of tropane alkaloids to nicotinic and muscarinic acetylcholine receptors. *Pharmazie* **50**:493–495 (1995).
22 Schule MA, The role of cytochrome P450 monooxygenases in plant–insect interactions. *Plant Physiol* **112**:1411–1419 (1996).
23 Fernandes FL, Bacci L and Fernandes MS, Impact and selectivity of insecticides to predators and parasitoids. *EntomoBrasilis* **3**:1–10 (2010).
24 Martin EA, Reineking B, Seo B and Steffan-Dewenter I, Natural enemy interactions constrain pest control in complex agricultural landscapes. *Proc Natl Acad Sci USA* **110**:5534–5539 (2013).
25 Picanço MC, Oliveira IR, Rosado JF, Silva FM, Gontijo PC and Silva RS, Natural biological control of *Ascia monuste* by the social wasp *Polybia ignobilis* (Hymenoptera: Vespidae). *Sociobiology* **55**:1–10 (2010).
26 Ramos RS, Picanço MC, Santana PA, Jr, Silva EM, Bacci L, Gonring AHR *et al.*, Natural biological control of lepidopteran pests by ants. *Sociobiology* **59**:1389–1399 (2012).
27 Shipp JL, Whitfield GH and Papadopoulos AP, Effectiveness of the bumblebee, *Bombus impatiens* Cr. (Hymenoptera: Apidae), as a pollinator of greenhouse sweet pepper. *Sci Hortic* **57**:29–39 (1994).
28 *SAS/STAT User's Manual, Version 9.4.* SAS Institute, Cary, NC (2013).
29 *Manual de Protocolo para Testes de Eficácia de Produtos Desinfestantes*. Agência Nacional de Vigilância Sanitária, Brasília, Brazil (2004).
30 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
31 Finney DJ, *Probit Analysis.* Cambridge University Press, Cambridge/London, UK, 333 pp. (1971).
32 Young LJ and Young JH, Goodness-of-fit tests, in *Statistical Ecology: a Population Perspective.* Kluwer Academic Publishers, Boston, MA, pp. 42–70 (1998).
33 Kaplan EL and Meier P, Nonparametric estimation from incomplete observations. *J Am Statist Ass* **53**:457–481 (1958).
34 Costa AV, Barbosa LCA, Demuner AJ and Silva AA, Synthesis and herbicidal activity of 2α,4α-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one derivatives. *J Agric Food Chem* **47**:4807–4814 (1999).
35 Demuner AJ, Barbosa LCA and Veloso DP, [3 + 4] Cycloadditions via oxyallyl cations: applications in organic synthesis. *Quim Nova* **20**:17–29 (1997).
36 Mann J and Barbosa LCA, An efficient route to tropane alkaloids. *J Chem Soc Perkin Trans I* **7**: 787–790 (1992).
37 Alcântara AFC, Barroso HS and Piló-veloso D, Reduction of amides by boranes. *Quim Nova* **25**:300–311 (2002).
38 Nithyakalyani V, Kannan M and Anandan R, Insecticide and salt tolerance of plant growth promoting root nodule bacteria. *Int J Curr Microbiol Appl Sci* **5**:942–956 (2016).
39 Baek SH, Kang JH, Hwang YH, Ok KM, Kwak K and Chun HS, Detection of methomyl, a carbamate insecticide, in food matrices using terahertz time-domain spectroscopy. *J Infrared Milli Terahz Waves* **37**:486–497 (2016).
40 Mayakaduwa SS, Vithanage M, Karunarathna A, Mohan D and Ok YS, Interface interactions between insecticide carbofuran and tea waste biochars produced at different pyrolysis temperatures. *Chem Spec Bioavail* **28**:110–118 (2016).
41 Inam R and Bilgin C, Square wave voltammetric determination of methiocarb insecticide based on multiwall carbon nanotube paste electrode. *J Appl Electrochem* **43**:425–432 (2013).
42 Nathanson JA, Hunnicutt EJ, Kantham L and Scavone C, Cocaine as a naturally occurring insecticide. *Proc Natl Acad Sci USA* **90**:9645–9648 (1993).
43 Shields VDC, Smith KP, Arnold NS, Gordon IM, Shaw TE and Waranch D, The effect of varying alkaloid concentrations on the feeding behavior of gypsy moth larvae, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). *Arthropod Plant Interact* **2**:101–107 (2008).
44 Wright DJ and Verkerk RHJ, Integration of chemical and biological control systems for arthropods: evaluation in a multitrophic context. *Pestic Sci* **44**:207–218 (1995).
45 Rao H, Huangfu C, Wang Y, Wang X, Tang T, Zeng X *et al.*, Physicochemical profiles of the marketed agrochemicals and clues for agrochemical lead discovery and screening library development. *Mol Inf* **34**:331–338 (2015).
46 Gerolt P, Insecticides: their route of entry, mechanism of transport and mode of action. *Biol Rev* **58**:233–274 (1983).
47 Bernal J, Garrido-Bailón E, Del-Nozal MJ, González-Porto AV, Martín-Hernández R, Diego JC *et al.*, Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. *J Econ Entomol* **103**:1964–1971 (2010).
48 Neumann P and Carreck NL, Honey bee colony losses. *J Apic Res* **49**:1–6 (2010).
49 Goulson D, An overview of the environmental risks posed by neonicotinoid insecticides. *J Appl Ecol* **50**:977–987 (2013).
50 Moure JS, A preliminary supra-specific classification of the Old World Meliponine bees (Hymenoptera, Apoidae). *Studia Entomol* **4**:181–242 (1961).

