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1-Phenylisoquinoline larvicidal activity against *Culex quinquefasciatus*

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The larvicidal potential of several dopamine 1-phenylisoquinoline derivatives against *Culex quinquefasciatus* third instar larvae was investigated in a rational search for insecticides. The results showed that these isoquinolines presented moderate larvicidal activity, that the presence of a substituent is needed on carbon 1 for such activity to be presented and that it may be possible to develop novel insecticidal compounds having potential use in controlling insects by appropriate structural modification of 1-phenylisoquinolines. This article presents a possible structure–larvicidal activity relationship for isoquinolinic compounds.

Keywords: Pictet–Spengler; isoquinoline; alkaloid; *Culex quinquefasciatus*; dopamine; *Berberis*

1. Introduction

The continued use of synthetic insecticides generating serious environmental problems in water, air and soils and contamination of foods and animals, is ecologically inacceptable because it produces adverse effects on beneficial organisms and leads to the development of resistance in insects, fungi and bacteria. Developing resistance consequently brings the application of ever-increasing doses, accompanied by a greater risk of human intoxication. In addition to environmental problems, public health becomes seriously affected by diseases such as filariasis, malaria, dengue, yellow fever and leishmaniasis which are transmitted by insect vectors. These types of disease cause a great economic and social impact, especially in regions with insanitary conditions and deficient potable water supply and in overpopulated regions such as Latin America and the Caribbean. The *Culex quinquefasciatus* mosquito is amongst the vectors which are most frequently associated with urban and rural human habitat (Alvarez, Briceño, & Oviedo, 2006; Bisset, Rodriguez, Diaz, & Soca, 1998).

Botanical insecticide-oriented studies have been carried out during the past few years in the search for alternatives to synthetic insecticides. These are of great interest because they are natural, biodegradable, have low toxicity and can be used as

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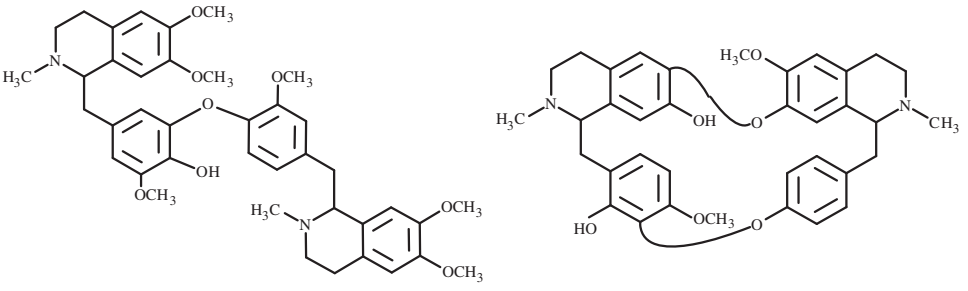


Figure 1. Compound **1** shows tabienine structure and **2** the tabienine b structure.

insecticides, larvicides, antifeedants, repellents or oviposition deterrents and growth inhibitors (Isman, 2006; Mandal, 2010).

On the other hand, alkaloid isoquinolines have received considerable attention as they are genetic precursors for a large variety of natural compounds arousing great biological interest due to their potential usefulness in treating many diseases (Fajardo, Podesta & Urzúa, 1986; Leet, Fajardo, Freyer, & Shamma, 1983) and controlling insects (Baird, Taylor, & Brayden, 1997; Wright et al., 2000).

The Berberidaceae family is a recognised source of isoquinoline alkaloids having novel structures. Studies concerning the chemical composition of an active fraction ($LC_{50} = 8\,\mu g\,mL^{-1}$) against *C. quinquefasciatus* third instar larvae obtained from *Berberis tabiensis* (Berberidaceae) stems (a native species from the Colombian Andes) have led to the two bisbenzylisoquinoline alkaloids being obtained: tabienine **1** and tabienine b **2** (Figure 1) (Quevedo, Núñez, & Moreno, 2011; Quevedo, Valderrama, Moreno, Laverde, & Fajardo, 2008).

In spite of the promising larvicidal activity of the fraction where tabienine **1** and tabienine b **2** were isolated, the low amount of compound which was isolated did not permit bioassays to be carried out with pure compounds; it can only be suggested that these two compounds having isoquinolinic nuclei are responsible for larvicidal activity. Some simple isoquinolines were synthesised as their structure would lead to the establishment of the possible structure–activity relationships which would orient the search for isoquinolines having larvicidal activity so as to determine whether the isoquinolinic nucleus was responsible for larvicidal activity and observe the influence of the substituent on carbon 1 (Table 1).

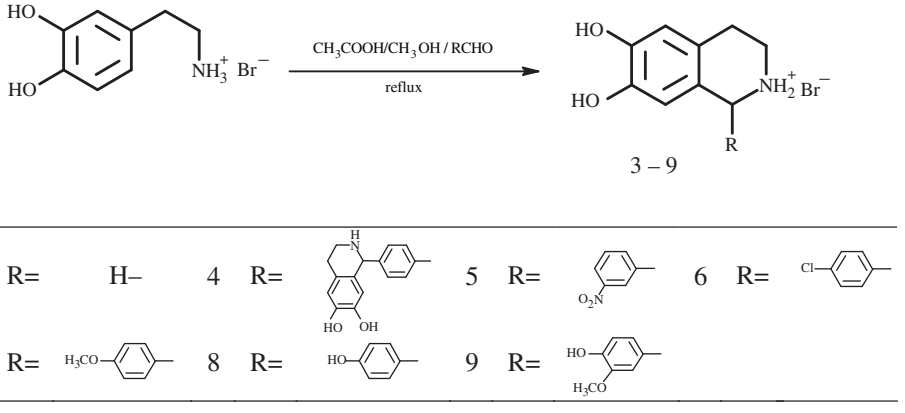
2. Results and discussion

It is known that some plant and essential oil extracts have larvicidal activity against different mosquito species; thus, the use of their active constituents in controlling insects is promising due to their selectivity, biodegradability and low toxicity.

Chemical composition and bioactivity studies of alkaloidal extracts from species from the *Berberis* genus which grow in the Colombian Andes led to the proposal that their benzylisoquinoline-type constituents present potential usefulness in controlling insect plagues (Moreno, Morgensztern, Luque, & Fajardo, 1995;

Table 1. Larvicidal activity of 1-phenylisoquinolines.

Compound	Larvicidal activity, LC_{50} ($\mu g\,mL^{-1}$)	Compound	Larvicidal activity, LC_{50} ($\mu g\,mL^{-1}$)
3	>2000	**7**	179 ± 23
4	>2000	**8**	612 ± 71
5	1035 ± 89	**9**	479 ± 49
6	460 ± 39	**10**	1624 ± 171
Caffeine	450 ± 29	Tabienine fraction	78

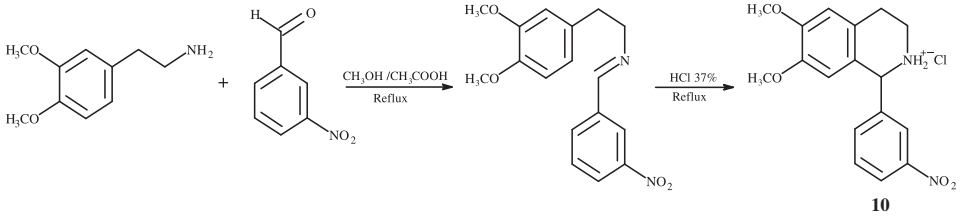


Scheme 1. Isoquinoline synthesis from dopamine.

Quevedo et al., 2011). A study of the larvicidal activity of non-natural 1-phenylisoquinoline compounds was thus begun due to the structural characteristics of these alkaloids, their low percentage found in nature and the availability of less knowledge regarding their potential use in controlling insects. Such compounds were synthesised by means of Pictet–Spengler cyclisation between dopamine or 3,4-dimethoxyphenylethylamine and aldehydes using methanol as solvent and acetic acid as catalyser (Schemes 1 and 2) (Cox & Cook, 1995; Quevedo, Baquero, & Rodríguez, 2010).

Dopamine- and formaldehyde-derived isoquinoline (**3**) was synthesised first to establish whether the presence of the isoquinolinic nucleus itself only presented larvicidal activity (Table 1). The activity assay gave an LC_{50} value greater than $2000\,\mu g\,mL^{-1}$, thereby indicating that the presence of a single isoquinolinic nucleus was not sufficient for the molecule to be bioactive. A molecule possesses two isoquinolinic units in its structure **4** by means of dopamine reaction with terephthaldehyde; the obtained product was synthesised, giving an LC_{50} value greater than $2000\,\mu g\,mL^{-1}$. This result showed that the number of isoquinolinic nuclei was not a determinant factor in bioactivity.

After establishing that the number of isoquinolinic nuclei did not significantly influence larvicidal activity, it was necessary to explore the influence of the



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Scheme 2. Isoquinoline synthesis from 3,4-dimethoxyphenylethylamine.

substituent on carbon 1. The compound 1-(3-nitrophenyl)-6,7-dihydroxytetrahydroisoquinoline (**5**) was thus synthesised; this compound had low larvicidal activity ($1035\,\mu g\,mL^{-1}$), but was higher than that for both previously assayed isoquinolines. The following stage was to assay 1-(3-nitrophenyl)-6,7-dimethoxytetrahydroisoquinoline (**10**) to observe the influence of substituents in positions 6 and 7. This compound presented lower larvicidal activity than that of **5** ($1624\,\mu g\,mL^{-1}$). This result led to the conclusion that the activity was greater when positions 6 and 7 were hydroxylated.

Finally, an attempt was made to ascertain the influence of substituents on the aromatic ring located on carbon 1. The compound 1-(4-chlorophenyl)-6,7-dihydroxytetrahydroisoquinoline **6** was thus synthesised; its activity ($460\,\mu g\,mL^{-1}$) improved in the case of the isoquinolines which are not present in carbon 1 or in rings having strong electroattractor substituents. This result suggested that the substituents on this ring were the determinants for this type of molecule to present larvicidal activity. The compound 1-(4-methoxy)-6,7-dihydroxytetrahydroisoquinoline (**7**) was then evaluated; this compound presented the best LC_{50} value ($179\,\mu g\,mL^{-1}$), indicating that the presence of this electrodonor group favoured larvicidal activity. LC_{50} rose to $612\,\mu g\,mL^{-1}$ when the methoxy group was replaced by a hydroxy group as in 1-(4-hydroxyphenyl)-6,7-dihydroxytetrahydroisoquinoline (**8**). Likewise, the presence of a hydroxyl group and a methoxyl group on ring **9** caused a reduction in bioactivity ($479\,\mu g\,mL^{-1}$) when compared to compound **7**. It was interesting to see that compound **9** presented an intermediate LC_{50} value compared to that for compounds **7** and **8** which have similar substituents. The bioactivity observed for compounds **7**, **8** and **9** did not present a lineal relationship with the number of electrodonor groups present on the aromatic ring.

The results obtained led to the proposal of a preliminary structure–larvicidal activity relationship for 1-phenylisoquinolin type compounds: (1) larvicidal activity was independent of the number of isoquinoline units present in the molecule; (2) larvicidal activity was lower when oxygen in positions 6 and 7 was methylated; (3) the presence of a substituent on carbon 1 was a necessary condition for larvicidal activity to be presented; and (4) the substituents in the aromatic ring (C ring) had a direct effect on larvicidal activity.

Few studies are known to have been carried out regarding the larvicidal activity of alkaloids. It has been reported that some isobutylamide alkaloids present potent larvicidal activity against *Culex pipiens pullens* third instar larvae having LC_{50} values ranging from 3.2 to $0.028\,\mu g\,mL^{-1}$ (Park, Lee, Shin, Park, & Ahn, 2002); aporphine alkaloid (+)-dicentrine has been shown to have a $30.2\,\mu g\,mL^{-1}$ LC_{50} value against *Aedes aegypti* larvae.

The activity observed for synthesised 1-phenylisoquinoline alkaloids in all cases was lower than that observed for the alkaloidal fraction where tabienine and tabienine b were isolated and reported for isobutylamide alkaloids and (+)-dicentrine. This pattern indicated that some additional structural aspects to those considered in this study are important in natural isoquinolines.

3. Experimental

3.1. *General procedure for dopamine reaction with aldehydes*

The 1-phenyltetrahydroisoquinolines studied were obtained by means of Pictet–Spengler regioselective cyclisation between dopamine or 6,7-dimethoxyphenylethylamine and aldehydes.

Acetic acid (0.1 mol) was added to a solution of dopamine hydrobromide (*X* mol) and the respective aldehyde (*X* mol) in methanol; the mixture was refluxed for 48 h. The precipitated product was filtered and washed with methanol (Quevedo et al., 2010).

3.2. *Procedure for 6,7-dimethoxyphenylethylamine reaction with 3-nitrobenzaldehyde*

A solution of 6,7-dimethoxyphenylethylamine (*X* mol) and 3-nitrobenzaldehyde (*X* mol) in methanol was refluxed for 72 h. The solvent was evaporated and the crude product treated with 37% HCl; the resulting mixture was refluxed for 1 h. The precipitated product was filtered and washed with methanol (Quevedo et al., 2010).

3.3. *Larvicidal activity bioassay*

Culex quinquefasciatus third instar larvae were used for the bioassays; these came from standardised breeding colonies kept in the Entomology laboratory of Universidad Nacional de Colombia. The larvicidal activity assays followed the methodology proposed by McLaughlin, Lingling, Rogers and Anderson (1998) using multiwell plates (96 well), 250-μL unit working volume, five concentrations ranging from 1000 to 10 $\mu g\,mL^{-1}$, at laboratory temperature ($25^{\circ}C \pm 2$) and one larva per well. The pure compound solutions were prepared by serial dilution with dechlorinated water from a stock solution of the sample in water containing 0.5% dimethylsulphoxide. The larvicidal activity of each sample was determined at 1000, 500, 100, 50 and 10 $\mu g\,mL^{-1}$. Water was used as absolute control and caffeine as positive control. No mortality was observed in any absolute control. All treatments were replicated three times using 20 larvae per replicate. LC_{50} data represented the mean values of each separate experiment (Table 1).

The effects were measured as larval mortality after 48 h of exposure. Larvae were considered to be dead if appendages did not move when prodded with a wooden dowel. Probit analysis was used to establish the lethal concentration for 50% mortality (LC_{50}) and 95% confidence intervalvalues for the respective compounds (Finney & Stevens, 1948).

4. Conclusions

It may thus be concluded that the larvicidal activity observed in some isoquinolinic compounds did not depend on the number of isoquinolinic nuclei present in the molecule, that activity was less when positions 6 and 7 had methoxyl groups and that the presence of a substituent on carbon 1 was necessary for larvicidal activity, activity being greater when the substituent on carbon 1 was an aromatic ring having electrodonor substituents.

The activity observed in all cases was lower than that observed for the alkaloidal fraction where tabienine and tabienine b were isolated. This pattern indicated that some additional structural aspects to those considered in this study are important in natural isoquinolines.

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