Larvicidal Effects of Fungal Meroterpenoids in the Control of *Aedes aegypti* L., the Main Vector of Dengue and Yellow Fever

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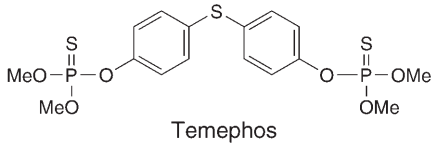
The mosquito *Aedes aegypti* is an increasing problem of public health, being the vector responsible for dengue and Yellow Fever in tropical and subtropical regions. The aim of this work was to determine the potential larvicidal activity of a series of meroterpenoids, compounds **1**–**7**, previously obtained fungal secondary metabolites from *Penicillium* sp., against the third-instar larvae of *A. aegypti*. The lethal concentrations (LC_{50} and LC_{90}) of **1**–**7** were evaluated 24 h after exposure. Dehydroaustin (**4**) was the most active meroterpenoid in the series, with an LC_{50} value of 2.9 ppm, making it an attractive natural insecticide.

Introduction. – *Aedes aegypti* (LINNAEUS, 1762) is a mosquito associated to human, and the vector for infection with dengue and Yellow Fever, endemic diseases covering tropical and subtropical regions [1][2]. Although Yellow Fever has been reasonably brought under control, no vaccine is available against dengue [3], its control being restricted to combating the vector by attacking the larval breeding places [4]. The non-systemic organophosphorus insecticide temephos is most often used for control, but the inefficiency of this drug and growing resistance in the dengue vector has been reported in several municipalities in Brazil [5].



Temephos

The spread of resistant mosquito have raised the need for new drugs to overcome this problem. Moreover, growing human conscientiousness about environmental risks associated with chemical pesticides have stimulated the search for new methods of vector control. Thus, natural products represent ideal insect-control agents since they are readily biodegradable [6]. Plant extracts have been recognized to have a variety of properties, including insecticidal activities such as repellent, antifeedant, and growth-regulating properties [3][7][8]. Besides, biolarvicides have been used for biocontrol of

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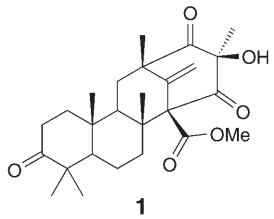
insect pests and vectors such as the fungus *Beauveria bassiana*, the nematode *Romanomermis culicivorax*, the bacterium *Bacillus thuringiensis* var. *israelensis*, and the crustacean *Chlamydoteca* sp. on anopheles larvae [9]. Among the *Bacillus* genus, *Bacillus subtilis* is known to produce a broad spectrum of bioactive lipopeptides [10].

Some compounds produced by fungi also show insecticidal and antimicrobial activities [11–13]. Recently, we have isolated the fungus *Penicillium* sp. from *Melia azedarach* roots [14]. Our chemical studies on the constituents of this microorganism, when cultivated over sterilized rice, led to the isolation and structural elucidation of a series of meroterpenoids: preaustinoid A (**1**), preaustinoid B (**2**), preaustinoid A2 (**3**), dehydroaustin (**4**), acetoxydehydroaustin (**5**), and neoaustin (**6**), as reported earlier [15–17]. Based on chemical-structure comparison, these compounds could be precursors of the meroterpenoid austin (**7**), the general biosynthesis route including a forel *Baeyer–Villiger* oxidation and other structural rearrangements [15] that resemble, in part, the biosynthetic steps leading to the production of some limonoids, which, in turn, are well-known for their insecticidal properties [8a].



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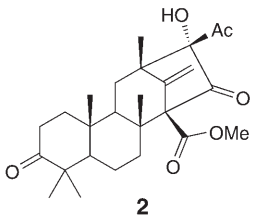

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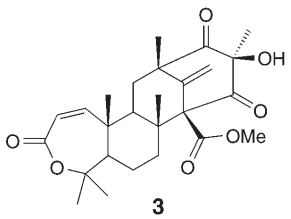

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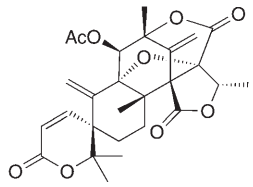

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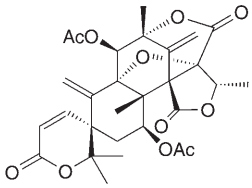

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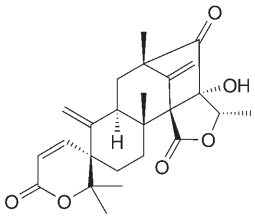

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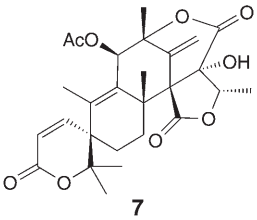

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Meroterpenoids are natural products of mixed biosynthetic origin that are partially derived from terpenoids [18] and exhibit important biological activities. Austin-related meroterpenoids are not active themselves, but have been shown to enhance the convulsive activity of verruculogen in a silkworm bioassay [19]. In addition, preaustinoids A and B exhibited moderate bacteriostatic effects [15]. Since their

larvicidal activities against the dengue vector have never been tested before, we investigated the activity of the meroterpenoids **1**–**7** against the third-instar larvae of *A. aegypti*.

Results and Discussion. – The third-instar larvae of *A. aegypti* were exposed to the meroterpenoids **1**–**7** at a concentration of 500 ppm each. Compounds **4** and **5** exhibited *in vitro* larvicidal activities of 100 and 70%, respectively, after 24 h of exposure. Compound **7** displayed a very low larval mortality, the other congeners being inactive. DMSO used as co-solvent ($<1.3\%$) was shown to have no effect on the larvae (solvent control). As positive control, temephos (1 ppm) was used under the same conditions, in combat programs against *A. aegypti* performed in Goiânia (Brazil), showing a larvicidal potency of 100%.

Statistical analysis was used to determine the lethal-concentration (LC) values of the bioactive compounds. As can be seen from the *Table*, dehydroaustin (**4**) and acetoxydehydroaustin (**5**) exhibited LC_{50} values of 2.9 and 7.3 ppm, respectively. Notably, compound **4** caused larval mortality almost instantaneously, *i.e.*, within less than 10 min.

Table. *Larvicidal Effects of the Meroterpenoids* **4** *and* **5** *of Fungal Origin against Third-Instar Larvae of* A. aegypti. LC_{50} and LC_{90} refer to the concentration causing death of 50% or 90% of the exposed larvae, resp.

No.	Name	LC_{50} [ppm][a])	LC_{90} [ppm][a])
4	Dehydroaustin	2.9 (1.6–3.8)	10.5 (8.9–14.3)
5	Acetoxydehydroaustin	7.3 (6.4–8.5)	25.1 (19.5–35.9)

[a]) In parentheses, the confidence intervals at 95% probability are given.

Some studies have shown the great potential of natural products for the control of *A. aegypti*, the active substances having LC_{50} values typically in the range 0.1–49 ppm. Sesquiterpenoids such as (*E*)-nerolidol and farnesol showed LC_{50} values of 17 and 13 ppm, respectively [20]. Ocimenone, a monoterpenoid isolated from *Tagetes minuta* oil, exhibited a higher LC_{50} value of 40 ppm [21], and a triterpene from *Azadirachta indica* showed an LC_{50} value of 21 ppm [22].

Since the meroterpenoid dehydroaustin (**4**) is much more active that the above natural insecticides, it seems to have great potential for the control of *A. aegypti* larvae. However, it will be necessary first to more deeply investigate the larvicidal mode-of-action and possible effects on non-target organisms before it can be practically used as a natural mosquito-control agent.

The larvicidal activity displayed by the meroterpenoids **4** and **5** is probably related to the δ-spirolactone system. Moreover, the additional AcO group in **5** seems to significantly reduced the larvicidal activity. Further, the very low activity of **7** compared to **4** and **5** suggests that the additonal bridging furan ring in the latter two compounds also significantly influences activity. This could indicate a hydrophobic binding/reactivity site in this part of the molecule. Finally, compounds **1**–**3** with 'intact' (non-spiro) *A*-rings or an *A*-ring ε-lactone (as in **3**) did not show any larvicidal effect, further supporting the significance of a spiro structure.

Intensive screening of microorganisms for substances of value in medicine or agriculture has revealed a wide range of biologically active secondary metabolites. The reason for secretion of these compounds by microorganisms is not exactly understood [23]. In fact, competition for the habitat and nutrients causes many species of fungi to excrete substances that inhibit growth, or may even cause the death of organisms in their vicinity, such as bacteria, fungi, and insects [23]. Nowadays, microbial products are being used in crop protection against invaders [24]. Moreover, certain fungi have entomopathogenic activity, infecting and killing insects *via* production of secondary metabolites. One such compound is bassianolide, a cyclodepsidipeptide produced by the fungus *Beauveria bassiana*, which elicits atonic symptoms in silkworm larvae [25].

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Experimental Part

General. Preaustinoid A (**1**), preaustinoid B (**2**), preaustinoid A2 (**3**), dehydroaustin (**4**), acetoxydehydroaustin (**5**), and neoaustin (**6**) were obtained from the fungus *Penicillium* sp., as previously described [15–17]. The meroterpenoid austin (**7**) was kindly supplied by Prof. *Thomas James Simpson*, School of Chemistry, Bristol, UK. Temephos (*Temefós Fersol 1G*, granulated, 1%) in dist. H_2O was used at a concentration of 1 ppm as pos. control. DMSO was used as initial solvent for stock solns., and dist. H_2O was added to produce durg concentrations of 500 ppm. The final DMSO concentration in the test was below 1.3%, which was non-toxic to the larvae (solvent control).

Insects. Third-instar larvae of *A. aegypti* were collected from a mosquito colony available at the Laboratório de Biologia e Fisiologia de Insetos of the Instituto de Patologia Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás (UFG), Goiânia, Brasil. The larvae were obtained from a cyclic colony maintained for more than 10 years at a temp. of $28 \pm 1°$ in $80 \pm 5\%$ relative humidity, with alternating 12-h light/darkness cycles [26].

Larvicidal Bioassay. The larvae toxicity assay was performed according to *Guimarães et al.* [27]. All experiments were carried out in an environmental chamber kept under the same conditions as the colony. Replicates ($n = 2$) of 20 larvae were used for each drug concentration tested. Each group of larvae was exposed to test soln., and their mortality, indicated by torpor and darkening of the cephalic capsule, was recorded after 24 h.

Statistical Method. The 50 and 90% lethal concentrations (LC_{50} and LC_{90}, resp.) and their respective confidence intervals were determined by Probit analysis, by plotting mortality *vs.* concentration, using the Statistical Analysis Product and Service Solution (SPSS) program.

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