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SYNTHESIS AND BIOLOGICAL ACTIVIVITY OF JUVENILE HORMONE ANALOGUES (JHA) FOR *TRYPANOSOMA CRUZI*

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ABSTRACT: Several derivatives of geraniol, geranylacetone, and farnesol bearing carbonate and thiolcarbonate functional groups as well as several derivatives of 4-phenoxyphenol were synthesized and tested for their respective biological activity as growth inhibitors for *Trypanosoma cruzi*, and for inhibition of tritium-labelled thymidine incorporation in *T. cruzi* cells. The results indicated that some JHA showed important activity against the development of the cells.

Juvenile hormone analogues (JHA) have shown effects on the development of eggs and nymphal stages of the insect *Triatoma infestans*, Chagas' disease vector.[1-6] It has been also established that *T. infestans* treated with JHA are less susceptible to gut infection with *Trypanosoma cruzi* than normal untreated vectors.[7] We have recently reported [8] the biological activity of several JHA for microorganism *Trypanosoma cruzi*; some of them showed important activity values towards growth inhibition when tested *in vitro*. Those positive results encouraged the search for more active compounds against *T. cruzi*, and we wish to inform the preparation of additional JHA and their activities as growth inhibitors for the microorganism that causes the Chagas' disease.

The tested products were prepared as shown in the Schemes. All the products were purified by flash chromatography (silica gel) and HPLC (Altex Ultrasphere ODS-2, 5 μm, 250 x 10 mm column), and characterized by spectroscopic methods (^{1}H-, ^{13}C-NMR, MS, IR).

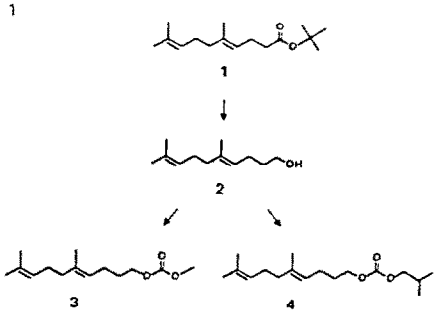
Treatment of compound **13** with ethyl triphenylphosphorane under controlled stereospecific conditions to yield almost exclusively the Z isomer [9,10] afforded, a 1:1 mixture of **14** and **15**, both having the expected stereoisomeric arrangement; the production of an equimolecular mixture of free alcohol and acetoacetate ester could be explained by a partial formation of the enolate ion of the acetate followed by a Claisen ester condensation.

On the other hand, the reaction of **13** with *sec*-butyl-triphenylphophorane produced the expected Z isomer of the acetate **17** without hydrolysis of the ester group. In both cases, after purification, the stereochemical purity was established by ^{13}C-NMR analysis.[11,12]

When required, regioselective epoxidation was performed on compounds **7**, **9**, **22** and **24**; this step was conducted by reaction of the substrate with *m*-chloroperbenzoic acid in stoichiometric relation followed by purification of the epoxyderivative.[13]

The biological assays were performed on *Trypanosoma cruzi* (epimastigotes, tulahuen strain, Tul-2 stock) growing in the culture medium detailed elsewhere.[8] Experimental

SCHEME 1



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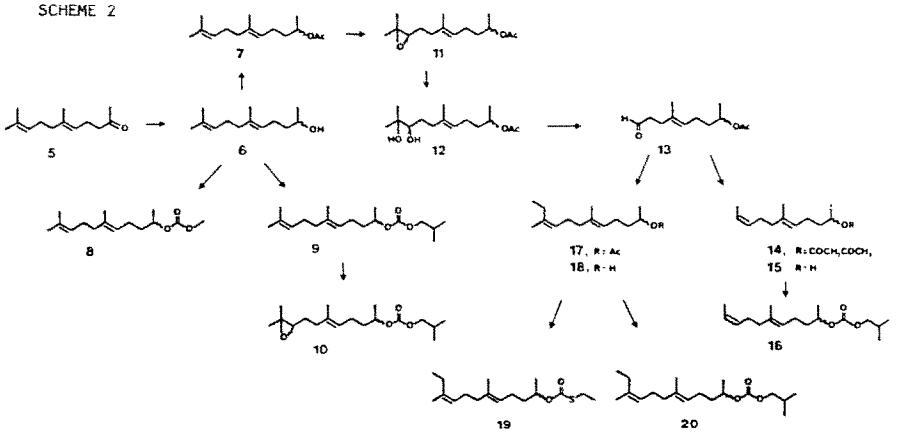

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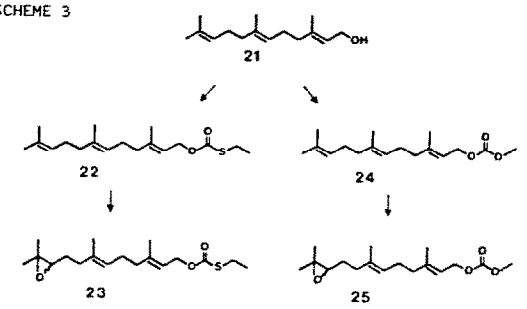
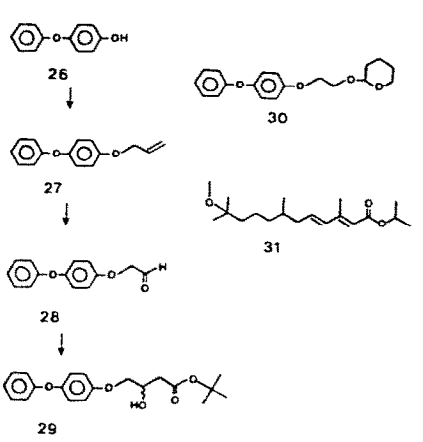


SCHEME 4



cultures were started with inocula from exponentially dividing cultures growing in the same medium. The synthetic JHA were tested in the concentration indicated below (see Table). The cells were counted in a Neubauer chamber (three replicates, each counted twice) and the figures compared against the amount of cells growing in untreated cultures.

Synthesis of DNA was assessed by determining the incorporation of [methyl-^{3}H]-thymidine into the parasites growing in the same conditions. At the end of the incubation period the remaining radiactivity localized into trifluoroacetic acid insoluble material was counted on a scintillation counter.[8]

In the present work, compounds **30** [8] and **31** (commercial Methoprene) were used as standard controls. The results are shown in the Table.

The results show that several JHA, within a certain range of concentrations, are very active in inhibiting the growth and thymidine incorporation for *T. cruzi* without killing the cells. Although the effects of JHA are unspecific, the differences in level of activity among the tested compounds indicate that **23**, having a sulfur atom and a terminal epoxide function in an open chain structure, is more active than those analogues bearing just oxygen as heteroatoms. Likewise, in the case of aromatic JHA it is important to remark that synthetic analogue **29** shows higher level of activity than Fenoxycarb.[8]

Considering that juvenilized *Triatoma infestans* insects are less susceptible to natural infection with *T. cruzi* than untreated insects, a probable relation between the mentioned result and these here reported could be postulated.

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Table. Influence of juvenile hormone analogues (JHA) on the inhibition of growth and of thymidine incorporation for *Trypanosoma cruzi* at 25°C.

JHA	Concentration µmol/l	Inhibition of growth (%)	Inhibition of thymidine incorporation (%)
3	50	41.3 ± 3.5	66.3 ± 8.5
	100	56.0 ± 6.4	95.3 ± 7.4
4	17	---	50.6 ± 5.4
	50	57.3 ± 7.5	88.4 ± 3.6
	100	94.0 ± 4.0	100.1 ± 1.0
8	50	51.0 ± 4.7	75.6 ± 6.5
	100	97.5 ± 0.5	91.6 ± 3.9
9	50	70.8 ± 5.0	73.3 ± 6.4
	100	89.6 ± 7.6	95.8 ± 3.9
10	50	33.4 ± 5.9	50.8 ± 9.2
	100	68.0 ± 6.5	70.0 ± 2.6
16	50	47.6 ± 5.4	70.1 ± 8.6
	100	75.4 ± 7.2	89.6 ± 3.3
19	50	50.7 ± 5.4	65.3 ± 2.6
	100	79.4 ± 6.1	75.2 ± 1.7
20	50	56.6 ± 3.8	62.8 ± 0.3
	100	68.0 ± 2.7	70.8 ± 2.0
22	50	69.3 ± 7.4	57.9 ± 3.1
	100	94.2 ± 3.8	72.6 ± 7.6
23	50	90.7 ± 1.9	80.7 ± 2.6
	100	99.0 ± 1.0	95.6 ± 2.9
25	50	44.8 ± 6.4	25.7 ± 10.8
	100	62.8 ± 5.2	46.3 ± 2.9
27	50	80.8 ± 9.3	60.9 ± 5.9
	100	95.4 ± 3.0	83.9 ± 6.7
29	50	67.2 ± 4.3	70.6 ± 9.6
	100	98.8 ± 1.3	93.5 ± 6.0
30	50	57.0 ± 7.2	40.8 ± 9.5
	100	90.6 ± 3.4	86.2 ± 3.8
31 (Methoprene)	50	60.0 ± 5.8	70.5 ± 9.8
	100	92.5 ± 7.2	86.5 ± 3.4