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SYNTHESIS OF ANTIPARASITIC LICORICE CHALCONES

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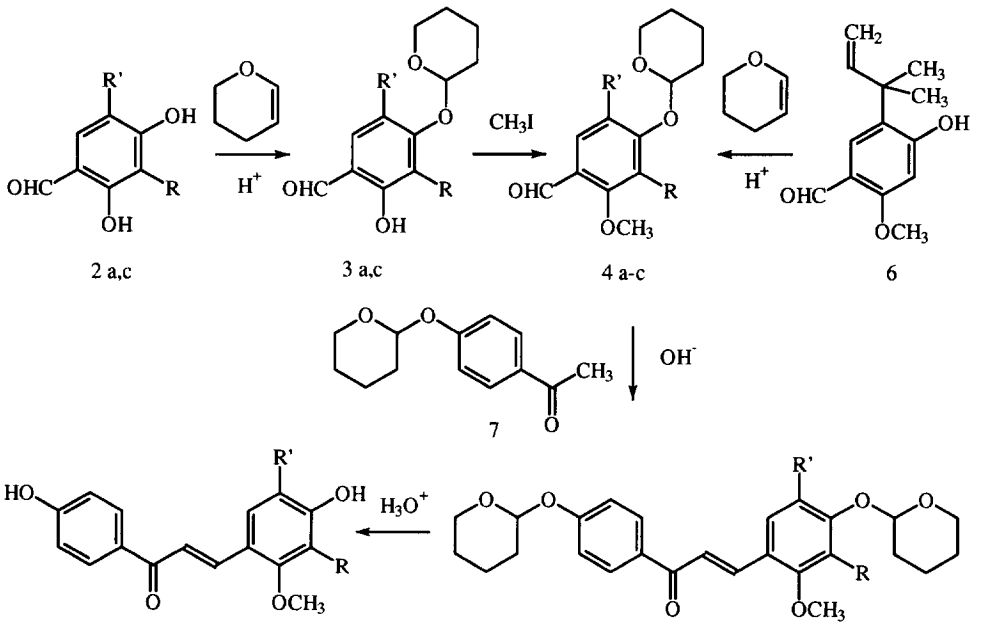
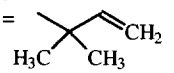
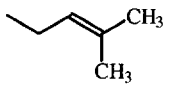
Abstract. A simple, inexpensive high yield method for preparation of echinatin and licochalcone C is described. Echinatin inhibits the growth of Leishmania parasites in concentrations, in which the proliferation of lymphocytes is only slightly affected, indicating that the compound might be used for treatment of leishmaniasis.

Roots of *Glycyrrhizae inflata* are a source for retrochalcones[1] *e.g.* echinatin (**1a**), licochalcone A (**1b**) and C (**1c**).[2] Recently we have observed that licochalcone A very potently inhibits the growth of Leishmania parasites (*Leishmania major* and *L. donovani*) *in vitro*.[3] Furthermore **1b** has been found to be a very efficient agent for controlling infections of *L. major* in mice and *L. donovani* in hamsters.[4] These observations have encouraged us to investigate the utilities of retrochalcones as potential drugs for the treatment of leishmaniasis. At the present the drugs of first choice are pentavalent antimonials such as Pentostam, which are inconvenient to administer and potentially toxic. The appearance of drug resistant Leishmania parasites and the spreading of the diseases have made WHO state that the situation is alarming and there is desperate need for new drugs.[5] In this paper we report the antiparasitic potencies of the retrochalcones **1a**-**1c** and simple and efficient syntheses, which only involve inexpensive starting materials.

A general procedure for synthesizing chalcones is a sodium hydroxide catalyzed condensation of an appropriately substituted acetophenone with an appropriate benzaldehyde. This method, however, only proceeds in good yield if no phenolic hydroxy group is present in either of the two oxo compounds. The presence of hydroxy groups in all the three target chalcones **1a** - **1c** rendered the use of a protecting group advantageous. Since our experiences with other protecting groups were disappointing we focused our attention on the tetrahydropyranyl group. An attractive feature of this group was, that 2,4-dihydroxyacetophenone was reported to form selectively the 4-tetrahydropyranyl ether by reaction with one equivalent of 3,4-dihydro-2*H*-pyran.[6] Analogously 2,4-dihydroxybenzaldehyde (**2a**) and 3-(3-methyl-2-butenyl)-2,4-dihydroxybenzaldehyde (**2c**)[7] selectively reacted with 3,4-dihydro-2*H*-pyran to give the 4-tetrahydropyranyl ethers **3a** and **3c** respectively.[8] The crude reaction products were methylated to give the two 2-methoxy derivatives **4a** and **4c**,[9] which were



a, R = R' = H
b, R = H, R' = 

c R = 

R' = H

condensed with 4-tetrahydropyranyloxyacetophenone (**7**) to give **5a** and **5c**, respectively. The target molecules **1a** and **1c** were obtained after hydrochloric acid provoked hydrolysis of the tetrahydropyranyl ethers in overall yields (based on **2a** and **2c**) of 84% and 50%, respectively.[10,11] The identity of the products were established by ^{1}H NMR, ^{13}C NMR spectroscopy and by combustion analysis. Attempts to synthesize licochalcone A (**1b**) by the same procedure using **6**[12] as a starting material also succeeded, although the overall yield in this case was only 30%, in spite of one less step in this reaction sequence. Apparently the bulkiness of the side chain in the 5 position hinders the tetrahydropyranyl ether formation.

Table 1. Inhibition of growth of promastigotes of *L. major* and phytohaemaglutinin A induced proliferation of human lymphocytes.

Compound	100 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml
1a	86.8±2.9 **26.4±10.8**	69.3±8.8 **18.0±9.3**	50.3±8.5 **10.4±8.3**	30.0±16.3 **9.2±5.9**	16.4±13.2 **7.8±5.9**
	20 µg/ml	10 µg/ml	5 µg/ml	1 µg/ml	
1b	96.0±7.5 **65.3±7.0**	91.9±8.4 **39.7±5.4**	63.9±13.0 **20.8±4.8**	3.6±11.5 **4.7±5.6**	
1c	93.8±1.5 **73.2±17**	51.5±4.8 **30.4±24**	20.7±6.3 **14.8±13**	3.9±2.1 **5.5±9.7**	

The effect of the chalcones on the promastigotes as assessed by a method previously described.[3] Briefly promastigotes were incubated at 26°C in the presence of the chalcones or in the medium alone. After 2h 1 µCi of [^{3}H]thymidine was added to each well. Parasites were harvested 18h later and [^{3}H]thymidine incorporation measured. The effect of the chalcones on the PHA induced proliferation of human peripheral lymphocytes was determined according to the conventional method.[13] The data in the Table represent the decreased uptake of [^{3}H]thymidine in the cells incubated with chalcone in percentages of the uptake in the non treated cells. The upper numbers represent the effect on *L. major* and the lower (bold) numbers the effect on lymphocytes. The results are the mean of at least five experiments±standard deviation each performed in triplicate. Pentostam in the same system inhibited the parasites 95±2% at 100 µg/ml, 35±5% at 10 µg/ml, and 18±4% at 1 µg/ml.

The antiparasitic activity was estimated from the ability of the chalcones to inhibit the *in vitro* growth of *L. major*[3] measured by the decreased uptake of [^{3}H]thymidine. As is shown in Table 1 licochalcone C (**1c**) inhibited the growth of the parasites to the same extent as licochalcone A (**1b**). In contrast to **1c** the two other retrochalcones inhibited the proliferation of lymphocytes to a small extent in concentrations in which they inhibited the growth of parasites more than 80 %. Encouraged by these findings we tested whether echinatin (**1a**) like licochalcone A (**1b**)[3] also had effect on the intracellular infective stage of the parasites, the amastigotes. An illustrative experiment performed as previously described [3] showed inhibition of [^{3}H]thymidine uptake in amastigotes of *L. donovani* in infected human peripheral blood monocyte derived macrophages of 94 %, 92 %, 62 %, and 57 %, of controls in concentrations of 10, 5, 1, and 0.5 µg/ml of echinatin, respectively. In the same concentrations the percentages of infected macrophages and the average number of amastigotes per macrophage were determined to 0 % and 0, 2 % and 1, 3 % and 5, and 5 % and 3.3 whereas 24 % of macrophages in the control were infected with an average of 5.9 amastigotes. Three additional experiments using both pentostam resistant and pentostam sensitive strains of the parasites showed similar results. These results indicate that licochalcone A and echinatin might be developed into non-toxic drugs for controlling

infections caused by Leishmania parasites.

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8. A solution of **2a** (2.76 g, 20 mmol), pyridinium *p*-toluenesulfonate (100 mg, 0.4 mmol), and 3,4-dihydro-2*H*-pyran (2.7 ml, 30 mmol) in methylene chloride (30 ml) was stirred for 4 h at room temperature. The solution was washed with *M* sodium carbonate (20 ml), dried ($MgSO_4$) and concentrated *in vacuo* to give 4.3 g of an oil, which according to ^1H NMR consisted of almost pure **3a**.

9. The crude **3a**, sodium hydroxide (3.2 g) and iodomethane (2.5 ml, 40 mmol) was suspended in DMSO (15 ml), stirred for 60 min at room temperature, and added water (150 ml). The mixture was extracted four times with methylene chloride (150 ml), and the organic phase was washed three times with water (50 ml), dried ($MgSO_4$), and concentrated *in vacuo* to give 4.5 g of an oil, which according to ^1H NMR consisted of almost pure **4a**.

10. A solution of crude **4a** (1.18 g), 4-tetrahydropyranyloxyacetophenone (1.10 g, 5 mmol) and sodium hydroxide (50 mg) in dry ethanol (10 ml) was stirred for 18 h at room temperature. The solution was added 4 M hydrochloric acid (2 ml), stirred for additional 15 min, and added water (40 ml). The mixture was extracted four times with ether (50 ml). The combined organic phases was dried ($MgSO_4$) and concentrated *in vacuo* to give crystals, which were recrystallized from ethanol-water to give 1.2 g of **1a**, m.p. 209-212°C. The ^1H NMR and ^{13}C NMR data were consistent with those of echinatin.

11. The synthesis of licochalcone C (**1c**) proceeded analogous to that described for **1a** (notes 9-11), except for a final purification by column chromatography, since **1c** has not been obtained in a crystalline state.

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