BIOCELL
2004, 28(1): 31-34

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Bacteriostatic action of synthetic polyhydroxylated chalcones against *Escherichia coli*

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Key words: chalcones, bacteriostatic activity, hydroxylation

ABSTRACT: In previous work the bacteriostatic action of trihydroxylated chalcones against *Staphylococcus aureus* ATCC 25 923 was investigated. In this work the action of 2´,4´,2-$(OH)_3$-chalcone, 2´,4´,3-$(OH)_3$-chalcone and 2´,4´,4-$(OH)_3$ –chalcone against *Escherichia coli* ATCC 25 922 was evaluated. Growth kinetic curves of *E.coli* were made in nutritive broth added with increasing drug concentrations. The specific growth rates of the microorganisms were calculated by a kinetic turbidimetric method, which was previously probed and the minimal inhibitory concentrations (MIC´s) were evaluated by a mechanism of action proposed. The MICs of 2´,4´,3-$(OH)_3$-chalcone and 2´,4´,2-$(OH)_3$-chalcone were 46 µg/ml and 122 µg/ml, respectively. The 2´,4´,4-$(OH)_3$–chalcone was inactive. The MIC value of 2´, 4´, 3-$(OH)_3$-chalcone (46 µg/ml), more active than 2´, 3-$(OH)_2$-chalcone (72.2 µg/ml) may be due to the introduction of an electron donating group (-OH) at position 4´ in the aromatic A- ring, which activates the region that includes the 2´-hydroxyl neighbur group and the α,β- unsaturated carbonyl group.

Introduction

A great number of natural flavonoids with various biological activities have been identified in the last years (Czinner *et al.*, 1999; Hernández and Prieto, 1999; Wächter *et al.,* 1999). In this family, several compounds show biological activities such as: antimicrobial (Gafner *et al.*, 1996; Li *et al.*, 1998; Ravha *et al.*, 2000; Olivella *et al.*, 2001), antiviral (Kurokawa *et al.*, 1995; Sindabiwe *et al.*, 1999), anti-inflammatory (Wiseman and Hallywell, 1996; Middleton *et al.*, 2000) and other therapeutic applications (So *et al.*, 1996; Zi and Agarwal, 1999; Evans, 2000). The increase of bacteriostatic action due to the presence of free hydroxyl groups into the chalcone molecule has been previously demonstrated (Pappano *et al.*, 1990, 1994). It was found that the presence of hydroxyl groups, especially at 4 and 4´ positions of 2´- hydroxychalcone enlarges the bacteriostatic activity against *Staphylococcus aureus* ATCC 25 923 (Pappano *et al.*, 1990; Devia *et al.*, 1998). In the present work we investigate whether trihydroxylated chalcones possess greater bacteriostatic action against *Escherichia coli* ATCC 25 922 than the dihydroxylated ones previously tested (Pappano *et al.*, 1994). Minimal inhibitory concentrations (MICs) of 2´,4´,2-trihydroxychalcone (**I**), 2´,4´,3-trihydroxychalcone (**II**) and 2´,4´,4-trihydroxychalcone (**III**) against the microorganism were evaluated using a kinetic turbidimetric method (Pappano *et al.*, 1990).

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Received on May 16, 2003. Accepted on September 19, 2003.

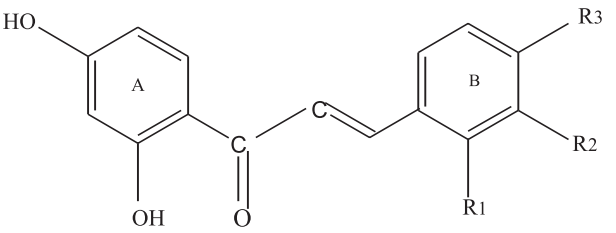
Materials and Methods

Microbial strain:

E. coli ATCC 25 922 (acquired in American Type Culture Collection) maintained by successive subcultures in trypticase soy agar (BBL) at 4ºC and by liofilization.

Chemicals:

High purity compounds were employed: 2´,4´,2-trihydroxychalcone (**I**) 2´,4´,3-trihydroxychalcone (**II**) and 2´,4´,4-trihydroxychalcone (**III**) were prepared by Claisen-Schmidt condensation (Dhar, 1981). Their structures (Fig. 1) were determined by the chromatographic and spectroscopic data: Rf (TLC): 0.174; UVλmax (MeOH) nm: 368; 242; 205; ^{1}H NMR and ^{13}C NMR (Devia *et al.*, 1998).



R_2=R_3=H; R_1=OH: 2´,4´,2-trihydroxychalcone
R_1=R_3=H; R_2= OH: 2´,4´,3-trihydroxychalcone
R_1=R_2=H; R_3= OH: 2´,4´,4-trihydroxychalcone

FIGURE 1. Structure of compounds.

Turbidimetric kinetic method:

A 24 h culture of *E.coli* ATCC 25 922 in slant agar were transferred to 30 ml of Müller- Hinton broth (Oxoid) and incubated for 18 h at 35ºC with permanent stirring, in order to be used as inoculum. Erlenmeyers containing 100 ml of culture medium with increasing drug concentrations to be tested were inoculated with 2 ml of inoculum and stirred in a Rosi 1000 culture chamber at 35ºC and 180 rpm, including one without drug as control. Aliquots were extracted at 20 min intervals during 5 h and transmittance (T) was registered in a UV-Visible recording spectrophotometer Shimadzu 160 A. T values were related to the number cfu/ml (colony forming units/ml) N_t, through the expression for *E. coli* (Pappano *et al*., 1990):

$$\ln N_t = 27.1 - 8.56 \cdot T \qquad (1)$$

Results

Compounds assayed were efficient against *E. coli* ATCC 25 922. The number of cfu/ml at different times was obtained by the expression of the turbidimetric kinetic method.

Considering the microbial growth law

$$\ln N_t = \ln N_o + \mu \cdot t \qquad (2)$$

where t: time in min; N_o: cfu/ml at t = 0; N_t : cfu/ml at time t; μ: specific growth rate in min^{-1}, values for *E. coli* specific growth rates in media with increasing drug concentrations were obtained from the ln N_t vs. t plot in the exponential growth phase. Results from the *E. coli* growth tests in presence of 2´,4´,3 - trihydroxychalcone are shown in Figure 2.

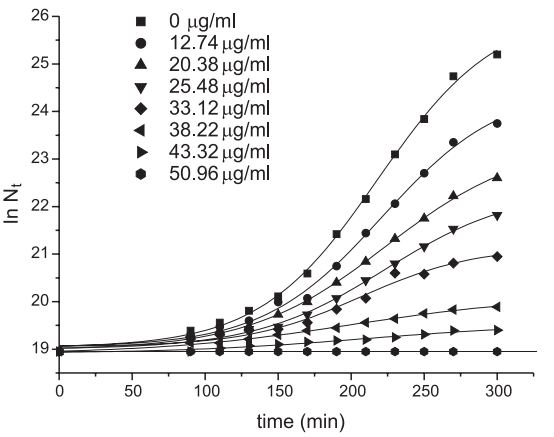


FIGURE 2. Growth of *Escherichia coli* ATCC 25 922 in media containing 2´,4´,3-trihydroxychalcone, at the indicated concentration.

In Table 1 values of T, ln N_t (evaluated from equation 1) and t are informed.

Table 2 exhibits values for the microbial specific growth rates and the drug concentrations added to the culture media.

TABLE 1.

***E. coli* ATCC 25 922 growth in presence of 2′,4′, 3 – trihydroxychalcone at 35ºC**

	2′,4′,3-trihydroxychalcone concentrations (μg/ml of nutritive broth)															
	50.96		43.32		38.22		33.12		25.48		20.38		12.74		0	
Extraction Time (min)	**T**	**ln N_t**	**T**	**ln N_t**	**T**	**ln N_t**	**T**	**ln N_t**	**T**	**ln N_t**	**T**	**ln N_t**	**T**	**ln N_t**	**T**	**ln N_t**
0	0.95	18.95	0.95	18.95	0.95	18.95	0.95	18.95	0.95	18.95	0.95	18.95	0.95	18.95	0.95	18.95
90	0.95	18.95	0952	18.95	0.931	19.13	0.922	19.21	0.922	19.21	0.911	19.3	0.911	19.3	0.901	19.39
110	0.95	18.95	0.944	19.02	0.931	19.13	0.911	19.3	0.911	19.3	0.901	19.39	0.891	19.47	0.881	19.56
130	0.95	18.95	0.938	19.07	0.922	19.21	0.901	19.39	0.897	19.42	0.891	19.47	0.876	19.6	0.852	19.81
150	0.95	18.95	0.935	19.1	0.911	19.3	0.897	19.42	0.891	19.47	0.861	19.73	0.831	19.99	0.817	20.11
170	0.95	18.95	0.929	19.15	0.901	19.39	0.881	19.56	0.861	19.73	0.831	19.99	0.821	20.07	0.761	20.59
190	0.95	18.95	0.925	19.18	0.891	19.47	0.848	19.84	0.821	20.07	0.783	20.4	0.742	20.75	0.664	21.42
210	0.95	18.95	0.923	19.2	0.881	19.56	0.821	20.07	0.777	20.45	0.731	20.84	0.661	21.44	0.577	22.16
230	0.95	18.95	0.917	19.25	0.871	19.64	0.759	20.6	0.736	20.8	0.675	21.32	0.589	22.06	0.467	23.1
250	0.95	18.95	0.910	19.31	0.859	19.75	0.762	20.58	0.694	21.16	0.625	21.75	0.514	22.7	0.381	23.84
270	0.95	18.95	0.901	19.39	0.849	19.83	0.735	20.81	0.651	21.53	0.570	22.22	0.438	23.35	0.276	24.74
300	0.95	18.95	0.900	19.4	0.842	19.89	0.718	20.95	0.617	21.82	0.526	22.6	0.391	23.75	0.222	25.2

T: Transmittance; $\mathbf{N_t}$: number of *E. coli* ufc/ml in nutritive broth (equation 1).

TABLE 2.

Specific growth rate of *E.coli* ATCC 25 922 as a function of the concentration of chalcones I, II and III

I	C	**0**	**11.39**	**18.23**	**25.07**	**29.62**	**34.18**	**41.02**	**45.58**
	μ x 10^3	41.50	38.56	37.00	32.85	31.12	29.40	27.20	25.10
II	**C**	0	12.74	20.38	25.48	33.12	38.22	43.32	50.96
	μ x 10^3	41.5	32.85	22.30	18.00	12.05	5.27	2.45	0
III	**C**	0	12.66	17.72	22.79	30.38	35.45	43.04	48.11
	μ x 10^3	41.5	38.20	37.30	35.80	35.50	34.50	32.70	31.70

C: drug concentration (μg/ml); μ: specific growth rate (min^{-1}); **I**: 2,′4,′2′-trihydroxychalcone; **II**: 2′,4′,3-trihydroxychalcone and **III**: 2′,4′,4- trihydroxychalcone.

Discussion

The results were interpreted satisfactorily by means of the bacteriostatic inhibition mechanism previously proposed for dihydroxylated chalcones (Pappano *et al*., 1994). Thus, the variation of the specific growth rate (μ) with the drug concentration follows the relation

$$\mu = \mu_T - k \cdot C \qquad (3)$$

where μ: specific growth rate (min^{-1}); μ_T: specific growth rate in medium without drug (min^{-1}) (control); k: specific inhibition rate (ml /(μg.min)) and C: drug concentration (μg/ml).

The graphical representation of equation (3) is shown in Figure 3 for the assayed compound and minimal

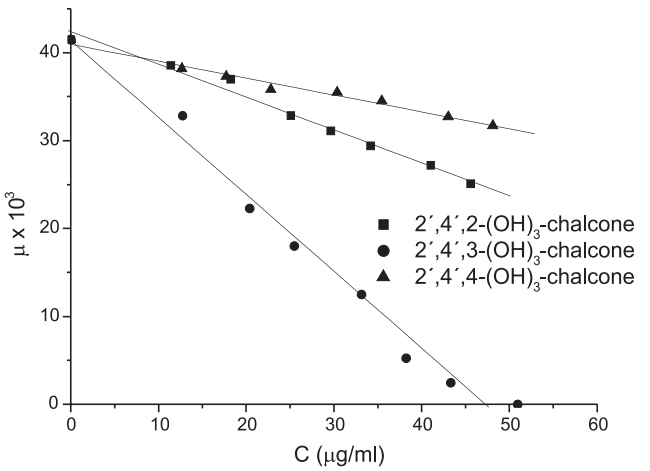


FIGURE 3. Graphical determination of MICs (minimal inhibitory concentrations) by extrapolation at the abcis when μ = 0

inhibitory concentrations were evaluated by extrapolation at μ = 0.

The minimal inhibitory concentration (MIC) values obtained for the compounds tested in our study were as follows: 2′,4′,3-trihydroxychalcone 46 μg/ml <<< 2′,4′,2-trihydroxychalcone 122 μg/ml <<< 2′,4′,4-trihydroxychalcone inactive.

These values allowed us to conclude that the presence of a new OH group at 4′ position in the structure of 2′, 3-dihydroxychalcone (MIC = 72.2 μg/ml) increases the bacteriostatic activity of the base compound. The increase of efficacy on the part of 2′,4′,3-trihydroxychalcone could be attributed to the presence of a OH group at 4′ position in connection with the active region that comprises the –OH at 2′ position and the α,β unsaturated carbonyl group. On the other side, it was found that the presence of a OH group in 4 position of 2′,4′,4-trihydroxychalcone, in contrast to the results of the same chalcones against *Staphylococcus aureus* (Devia *et al*., 1998), annuls the action of the same one. The similar behavior was observed for 2′,4′,2-trihydroxychalcone regarding 2′,4′-dihydroxychalcone. Different wall structure of Gram positive and Gram negative bacteria could be the reason of those results.

Acknowledgements

The present work was supported by the National University of San Luis.

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