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Chemotherapeutic Study of Chromones from Spanish Cneoraceae[1]

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Abstract

This paper deals with the cytostatic and antibacterial tests applied to a series of ten natural chromones isolated from two Spanish Cneoraceae, *Cneorum tricoccum* and *C. pulverulentum*. The results obtained suggest that these two species and, in particular, some of the compounds studied, may be of potential interest as antitumorals, more complete assays being recommended.

Introduction

Within our line of research devoted to demonstrating the exact therapeutical possibilities of natural products isolated from the Spanish flora, we have carried out a study of the cytostatic and antibacterial activities of a series of new chromones obtained from two species of Cneoraceae, these structures and this family being infrequently studied.

Although chromonic structures appear quite frequently in nature, particularly flavones (2-phenyl chromones) and iso-flavones (3-phenyl chromones), the therapeutical possibilities of which have been amply investigated, the simple chromones and their 2-methyl- and 2-hydroxymethylene-derivatives are less frequent and, consequently, their pharmacology is scarcely known [1, 2].

Our study has been carried out on 10 chromones of this last type which were isolated from two species of Cneoraceae to be found in Spain.

The small Cneoraceae family, whose chemistry has scarcely been investigated, is made up of only one genus, i.e. gen. Cneorum, consisting of three species. These are *C. trimerum* (URB.) of the Cuban flora [3], of which we have been unable to find any chemical or pharmacological report, *C. tricoccum* L. from the western mediterranean flora, commonly known in Spain as "rapuilt" or "olivillo común", of very potent purging properties and occasionally used as a rubefacient [4]; and *C. pulverulentum* (VENT), also named *Neochamaelea pulverulenta* (ERNDT.) by other authors who classify it as a different genus (5), an endemism of the Canary flora, extensively found in the coastal zones of the central and westerly islands, where it is commonly known as "leñablanca" or "leñabuena" [6]. It was often used in the burial rites of the pre-Hispanic inhabitants of the Canaries, known as the Guanches, and is occasionally used today as an antifebrifuge in rural communities [7].

Experimental

Plant material

The products studied were isolated from the aerial parts of samples of *C. tricoccum*, gathered in February at La Herradura (Granada) and in July in Ibiza (Balearic Islands) and from *C. pulverulentum*, collected in December in several areas of South Tenerife (Canary Islands), mainly on the Mountain of Guaza.

Extraction and isolation procedure

The dried and powdered aerial part was continuously extracted with ethanol in a Soxhlet. The extract was cooled, filtered and concentrated in vacuo. The concentrated extract was twice dissolved in benzene and later in $CHCl_3$ and chromatographed on silica gel columns using eluents of increasing polarity. The products isolated were identified by physical and spectroscopical methods and by comparison with authentic samples. For further details see [9, 10].

Measurement of cytostatic activity

The cytostatic activity was determined by measuring the inhibition of the development of a single-layer culture of HeLa 229 cells

[1] A report on this work was presented at the XVII Rencontres Internationales de Chimie Therapeutique – Besançon, September 1981.

in Difco's "TC Minimal Medium Eagle Dried" according to the method described by GERAN et al. [11]. The products tested were suspended in Tween 80 and a blank control and 6-mercaptopurine as positive control were employed under identical conditions. The measurements of the tests were carried out by microscopic methods after 72 h of incubation.

Measurement of antibacterial activity

The method used to determine the antibacterial activity was that of CHABERT [12] which, briefly, consists in sowing the different germs over Agar Muller-Hilton in 3.5 %, after previous sterilization and observing the zones of growth inhibition around discs of Whatman no. 1 paper impregnated with different concentrations of the compounds to be tested. These concentrations were 0.25, 0.5, 1 and 5 mg/ml. The measurement of results was taken after 24 h of incubation at 37° C.

Results and Discussion

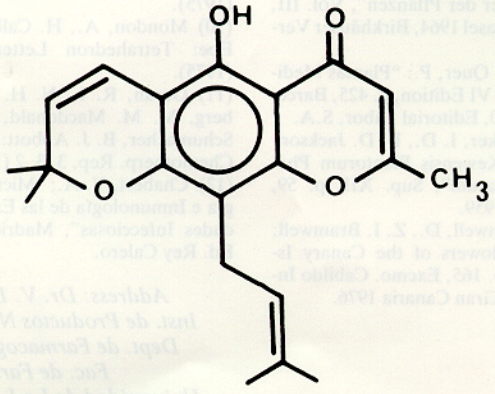
Cytostatic activity

The results obtained are set out in Table I. This data suggests that some chromone derivatives of *C. tricoccum* and *C. pulverulentum* (compounds 1, 2, 9 and 10) show an interesting inhibitory activity on HeLa cells with ID_{50} between 1–10 μg/ml, values inferior to or near those recommended by the Protocols of the National Cancer Institute of USA [11], to be followed by more sophisticated experiments.

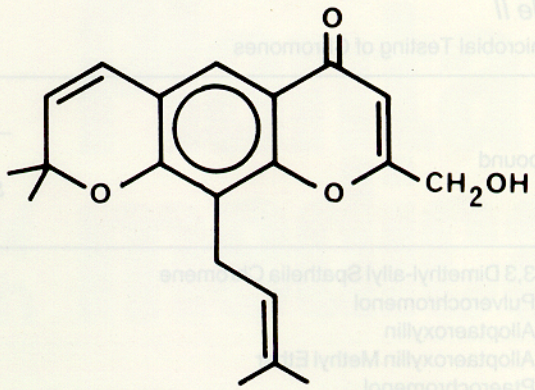
Table I

Cytostatic Activity on HeLa Cells

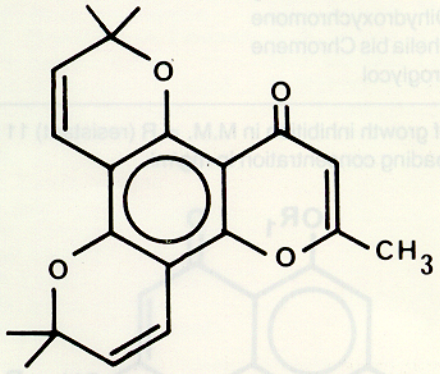
Compound	Name	ID_{50} (μg/ml)
1	3,3 Dimethyl-Allyl-Spathelia Chromene	5
2	Pulverochromenol	1
3	Alloptaeroxyllin	100
4	Alloptaeroxyllin Methyl Ether	100
5	Ptaerochromenol	10–100
6	Ptaerochromenol Methyl Ether	10–100
7	Ptaerochromenol Methyl Ether Acetate	100
8	5,7-Dihydroxychromone	10–100
9	Spathelia bis Chromene	10
10	Ptaeroglycol	5
6 MP	6-Mercaptopurine	0.1



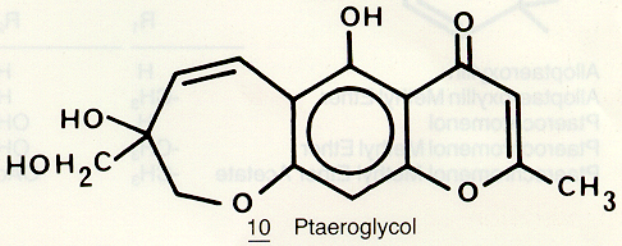
1 3,3-Dimethyl-Allyl-Spathelia Chromene



2 Pulverochromenol



9 Spathelia bis Chromene



10 Ptaeroglycol

The series under study is limited so it is difficult to establish firmly any structure-activity relationship, although it seems that those compounds with linear chromene-chromone structures present a higher activity than is the case with angular chromene-chromone structures. The linear structure of the oxepin-chromone studied might justify the high activity of ptaeroglycol. In this type of linear structure it would be interesting to pinpoint the possible importance of the 3,3-dimethyl-allyl radical in 8. In angular chromones the presence of a second 2,2-dimethyl-chromene ring would seem to give them a greater cytostatic potency (9). On the other hand it seems that compounds with a hydroxymethylene radical in 2 possess a greater inhibitory capacity than their homologues with a methyl radical (2, 5 and 6 versus 1, 3 and 4).

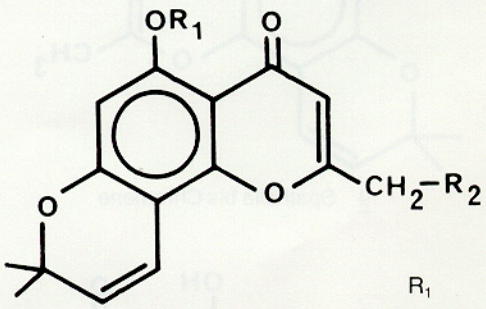
Table II

Antimicrobial Testing of Chromones

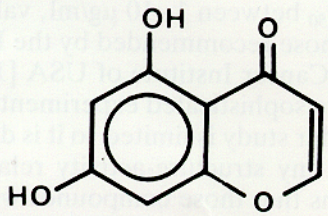
Compound	Gram positive			Gram negative	
	Bacillus Sphaericus	*Staphylo-coccus aureus*	*Strepto-coccus faecalis*	*Pseudomonas aeruginosa*	*Proteus S.P.*
I 3,3 Dimethyl-allyl Spathelia Chromene	P* (5)**	P (5)	P (5)	R	R
II Pulverochromenol	P (5)	S (5)	P (5)	P (5)	R
III Alloptaeroxyllin	R	R	R	R	R
IV Alloptaeroxyllin Methyl Ether	R	R	R	R	R
V Ptaerochromenol	P (5)	P (5)	R	R	R
VI Ptaerochromenol Methyl Ether	P (5)	P (5)	R	R	R
VII Ptaerochromenol Methyl Ether Acetate	R	R	R	R	R
VIII 5,7 Dihydroxychromone	P (5)	P (5)	R	R	R
IX Spathelia bis Chromene	R	P (5)	R	R	R
X Ptaeroglycol	P (5)	P (5)	R	R	R

* Zone of growth inhibition in M.M. = R (resistant) 11 m.m., P (partially senst.) (12–18 m.m.), S (senst.) (19 m.m.)

** Disc. loading concentration in mg/ml.



		R_1	R_2
3	Alloptaeroxyllin	H	H
4	Alloptaeroxyllin Methyl Ether	$-CH_3$	H
5	Ptaerochromenol	H	OH
6	Ptaerochromenol Methyl Ether	$-CH_3$	OH
7	Ptaerochromenol Methyl Ether Acetate	$-CH_3$	OAc



8 5,7-Dihydroxychromone

The results obtained on gram + and gram – bacteria are shown in Table II. Activity may globally be classified as noticeable on gram positive bacteria at the highest doses studied (5 mg/ml) and almost nil on gram negative. It was found that in this type of test compounds with linear structures were also the more potent (1 and 2).

These results are sufficiently interesting to justify further tests with compounds (2) pulverochromenol, (1) 3,3-dimethyl-allyl-spathelia chromene and (10) ptaeroglycol, which have the highest inhibitory powers, in order to determine the extent of the chemotherapeutical possibilitites of this type of compound, which have been glimpsed in this report.

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