Chemotherapeutic Activity of Polyhalogenated Terpenes from Spanish Algae¹

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Key Word Index:

Algae; Polyhalogenated Terpenes; Cytostatic and Antibacterial Activity.

Abstract

A study of the cytostatic and antibacterial activity of four halogenated alicyclic monoterpenes obtained from *Plocamium cartilagineum* L. and of six polyhalogenated sesquiterpenes isolated from two species of *Laurencia*; *L. obtusa* HUDS. and *L. caespitosa* LAMX. is presented. The first monoterpene series was proved to possess higher cytostatic activity. This greater inhibitory activity would seem to be related to a greater presence of halogens in the molecule.

The antibacterial activity of both series may be considered as noticeable although the sesquiterpene compounds isolated from the genus *Laurencia* proved more active in the tests reported here.

POLYHALOGENATED SESQUITERPENES FROM LAURENCIA CAESPITOSA AND L. OBTUSA

POLYHALOGENATED MONOTERPENES FROM PLOCAMIUM CARTILAGINEUM

Introduction

Numerous symposia, congresses, scientific papers, reviews and books are a testimony to the growing importance of chemical and pharmacological research into marine natural products [1, 5]. Despite intense work on the part of investigators devoted to the different taxonomic, ecological, chemical and pharmacological aspects of this field, its possibilities are, as yet, unpredictable. Convinced of the importance of the subject and compelled by geographical circumstances, the Institute of Organic Natural Products of the C. S. I. C. at the University of La Laguna established, some ten years ago, a line of investigation directed towards a deeper knowledge of the chemo-biological possibilities of the benthonic flora of Spain.

¹ A report on this work was presented at the International Research Congress on Natural Products as Medicinal Agents. Strasbourg, July, 1980.

In this paper we present a chemotherapeutic study of the major components of three species of Rhodophyceae found in abundance in the inter-tidal zones of the Spanish coasts.

Material and Methods

a) *Plocamium cartilagineum* (L.), gathered on the Playa de la Victoria, Cadiz, in June 1978, classified by Prof. J. SEOANE of the Faculty of Pharmacy, University of Barcelona and from which were extracted four new halogenated alicyclic monoterpenes, represented in Fig. 1, the structures of which were established by spectroscopic methods and by chemical transformation and were confirmed by x-ray diffraction studies [6]. Compound P₁-B proved to be a bromine analogue of an identical chloro-compound isolated from *Plocamium violaceum* [7].

b) Two species of the genus *Laurencia*: *L. obtusa* (HUDS.) (LAMOUROUX) and *L. caespitosa* (LAMX.), both collected in September 1978 along the north coast of Tenerife and identified by the Department of Botany of the University of La Laguna. From these algae, four new polyhalogenated sesquiterpenes [5, 6, 8 and 9] were mostly isolated, the structures of which were established by the above-mentioned methods [8, 9] and are represented in Fig. 2. It should be noted that the acetylated derivatives [7] and [10] were isolated, in high yield, from the digestive gland of the sea-hare, *Aplysia dactylomela*, an opisthobranch mollusc very frequently found in the zone and which feeds on this type of algae, confirming the works of IRIE et al. [10].

Measurement of cytostatic activity

The cytostatic activity was determined by measuring the inhibition of development of a single-layer culture of HeLa 229 cells 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 after 72 h of incubation.

Measurement of antibacterial activity

The method used to determine antibacterial activity was that of CHABBERT [12], which, briefly, consists in seeding the different organisms over previously sterilised Agar Muller-Hilton 3.5 % medium 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 and 5 mg/ml. The measurement of results was taken after 24 h of incubation at 37° C.

Results and Discussion

The results obtained for cytostatic activity are set out in Table I, together with the yields of the tested compounds. This data suggests that the monoterpenes from *Plocamium cartilagineum* present an interesting inhibitory activity with ID₅₀ = 1–10 µg/ml, values inferior to or near those recommended by the protocols of the National Cancer Institute of U.S.A. [11] to be followed by more sophisticated experiments.

The series under study being limited, it is difficult to establish any structure-activity relationship, although it may be deduced that greater richness in Br induces a greater cytostatic activity. We are experimenting at present with these products (P₁-B and P₁-D) on several types of murine tumours in order to evaluate their possibilities on systems "in vivo."

Table I

Cytostatic Activity on He La Cells

A Monoterpenes from *Plocamium Cartilagineum*

Compound	Molecular Formula	Yield (%) [*]	ID ₅₀ (µg/ml)
1 P ₁ -B	C ₁₀ H ₁₄ Br ₂ Cl ₂	0.01	1
2 P ₁ -D	C ₁₀ H ₁₄ Br ₂ Cl ₂	0.008	1
3 P-X ₁	C ₁₀ H ₁₃ BrCl ₂	0.001	10
4 P-X ₂	C ₁₀ H ₁₃ BrCl ₂	0.001	10

B Sesquiterpenes from *Laurencia Obtusa* - *L. Caespitosa*

5	Caespitol	C ₁₅ H ₂₄ O ₂ Br ₂ Cl	0.03	100
6	Isocaespitol	C ₁₅ H ₂₄ O ₂ Br ₂ Cl	0.06	50
7	Isocaespitol Acetate	C ₁₇ H ₂₆ O ₃ Br ₂ Cl	—**	10
8	Obtusol	C ₁₅ H ₂₃ OBr ₂ Cl	0.02	50
9	Isoobtusol	C ₁₅ H ₂₃ OBr ₂ Cl	0.03	10
10	Isoobtusol Acetate	C ₁₇ H ₂₅ O ₂ Br ₂ Cl	—**	4.5
	-6 Mercapto			
	Purine-	-	-	0.1

* Referring to dry alga

** From digestive gland of APLYSIA DACTYLOMELA

Table II

Antimicrobial Testing of Monoterpenes from *Plocamium Cartilagineum*

Compound	Bacterial Test				
	Gram +	Gram -			
	Bacillus Sphaericus	Staphylococcus Aureus	Streptococcus Faecalis	Pseudomonas Aeruginosa	Proteus S.P
1 P ₁ -B	P* (5)**	P (5)	R	P (5)	R
2 P ₁ -D	P (5)	S (5)	P (5)	P (5)	R
3 P-X ₁	R	P (5)	R	R	R
4 P-X ₂	R	P (5)	R	R	R

* Zone of growth Inhibition in M.M.

R (Resistant) ≤ 11 m.m.; P (Partially sens.) (12–18 m.m.);

S (Sensitive) ≥ 19 m.m.

** Disc loading concentration in MG/ML.

The polyhalogenated chamigrene-skeleton sesquiterpenes from species of *Laurencia* proved to be less active with ID₅₀ = (10–100 µg/ml), with the exception of the isoobtusol acetate (ID₅₀ = 4.5). In this series, it may be deduced, firstly, that the acetylation of the hydroxyl group increases cytotoxicity and, secondly, that the compounds with halogens in *trans* are slightly more active than their corresponding *cis* isomers.

The results obtained on both Gram + and Gram - bacteria are shown in Tables II and III. Activity may

Table III

Antimicrobial Testing of Sesquiterpenes from
Laurencia Caespitosa and *L. Obtusa*

Compound		Bacterial Test				
		Bacillus Sphaericus	Staphylo- coccus Aureus	Strepto- coccus Faecalis	Pseudo- monas Aeruginosa	Proteus Sp.
5	Caespitol	P(5)	P(5)	R	P(5)	R
6	Isocaesitol	P(5)	P(1)	R	S(5)	R
7	Isocaesitol Acetate	P(1)	S(0.5)	P(5)	P(5)	P(5)
8	Obtusol	P(5)	P(5)	P(5)	P(5)	R
9	Isoobtusol	P(5)	P(1)	P(5)	P(1)	R
10	Isoobtusol Acetate	P(5)	S(0.5)	P(5)	P(1)	P(5)

globally be classified as noticeable, since those compounds isolated from *Plocamium cartilagineum* are only partially active at the highest dose tested (5 mg/ml) and singling out, if one must, a greater effectiveness against *Staphylococcus aureus*. The sesquiterpenes obtained from the genus *Laurencia* proved more active, pointing out once more both acetylated derivatives (compounds 7 and 10) against which the five organisms tested proved, at least partly, to be sensitive.

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References

- (1) González, A. G., J. Darías, J. D. Martín, V. S. Martín, M. Norte and C. Pérez: Marine Natural Products from the Atlantic Zone series, Tetrahedron Letters 21, 1151 (1980).
- (2) Hoppe, H. A., T. Levring and Y. Tanaka: Marine Natural Products, Berlin 1979, Walter de Gruyter.
- (3) Kaul, P. N.: Food and Drugs from the Sea, Norman 1978, The University of Oklahoma Press.
- (4) Scheuer, P. J.: Marine Natural Products: Chemical and Biological Perspectives New York, 1978, Academic Press Inc.
- (5) Webber, H. H. and G. D. Ruggieri: Proceeding of the Fourth Food-Drugs from the Sea Conference, Washington 1976, Marine Technological Society.
- (6) González, A. G., J. M. Arteaga, J. D. Martín, M. L. Rodríguez, J. Fayos and M. Martínez-Ripoll: Phytochemistry, 17, 947 (1978).
- (7) Mynderse, J. S. et al.: Tetrahedron Letters 2175 (1975).
- (8) González, A. G., J. D. Martín, V. S. Martín, M. Martínez-Ripoll and J. Fayos: Tetrahedron Letters 29, 2717 (1978).
- (9) González, A. G., J. Darías, J. D. Martín, C. Pérez, J. J. Sims, G. H. Y. Lin and R. M. Wing: Tetrahedron 31, 2449 (1975).
- (10) Irie, T. et al.: Bull. Chem. Soc. Japan 42, 843 (1969).
- (11) Geran, R. I. et al.: Cancer Chemotherp. Rep. 33 2 (1972).
- (12) Chabbert, Y. A.: Microbiología e Inmunología de las Enfermedades Infecciosas, Madrid, 1976, Ed. Marban.

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