Pergamon

Biochemical Systematics and Ecology 30 (2002) 259–262

biochemical systematics and ecology

www.elsevier.com/locate/biochemsyseco

Nucleosides and nucleobases from *Ophiactis asperula*, *Ophiacantha vivipara* and *Gorgonocephalus chilensis*

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Received 3 November 2000; accepted 1 May 2001

Keywords: Ophiactis asperula; *Ophiacantha vivipara*; *Gorgonocephalus chilensis*; Ophiuroidea; Echinodermata; Nucleobases; Nucleosides

1. Subject and source

In continuation of our studies on the metabolites of echinoderms of cold waters of the South Atlantic (Roccatagliata et al. 1994, 1995, 1996, 1998) we have investigated the ethanolic extract of three ophiuroid species, *Ophiactis asperula* (Philippi, 1858), *Ophiacantha vivipara* (Ljungman, 1870) and *Gorgonocephalus chilensis* (Philippi, 1858). Specimens of *O. asperula* and *O. vivipara* were collected off San Antonio Oeste on the Argentine Patagonian coast. Specimens of *G. chilensis* were collected at different locations around the South Georgia Islands. The ophiuroids were identified by Dr. Alejandro Tablado from the Museo de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina where voucher specimens (*O. asperula*, MACN 33885; *O. vivipara*, MACN 34376 and *G. chilensis*, MACN 31242) are preserved.

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PII: S0305-1978(01)00091-6

2. Previous work

None.

3. Present study

The ethanolic extract of each ophiuroid was evaporated and the residue was partitioned between H_2O and cyclohexane. This aqueous residue was then extracted with *n*-BuOH. The glassy material obtained after evaporation of the *n*-BuOH extract was chromatographed on a Sephadex LH20 column (50 cm × 2.5 cm i.d., MeOH). Fractions were analysed by TLC on SiO_2 in *n*-BuOH–HOAc–H_2O (12:3:5) and detected by spraying with *p*-anisaldehyde. Fractions containing the nucleosides and nitrogenated bases were subjected to vacuum-dry column chromatography on C_{18} reversed-phase using H_2O, H_2O–MeOH mixtures with increasing amounts of MeOH and finally MeOH. Nucleosides and bases were eluted with H_2O. Final purification of these fractions was accomplished by HPLC on a Phenomenex AQUA 5 μ C_{18} 125A column with H_2O–MeOH (98:2, flow rate 2 ml/min) to give the pure nucleosides and bases. The compounds were identified by comparison of their ^1H and ^{13}C NMR spectra with published data (Jones et al., 1970; Dematté et al., 1985; Pretsch et al., 1989).

From *O. asperula*, inosine (19.0%), 2′-deoxyuridine (10.6%), thymidine (14.9%), and the bases uracil (17.4%), cytosine (15.9%) and thymine (22.2%) were obtained. *O. vivipara* afforded inosine (7.1%), 2′-deoxyinosine (13.4%), uridine (3.8%), 2′-deoxyuridine (7.1%), 2′-deoxycytidine (1.4%), and thymidine (4.6%) with the bases uracil (8.1%), cytosine (4.8%), thymine (31.9%) and hipoxanthine (17.8%). The nucleosides inosine (2.5%), 2′-deoxyinosine (5.7%), uridine (2.1%), 2′-deoxyuridine (3.6%), cytidine (2.3%), 2′-deoxycytidine (2.5%), and thymidine (10.3%) as well as the bases uracil (15.5%), cytosine (22.8%), thymine (22.7%) and hipoxanthine (10.3%) were separated and identified from the ethanolic extract of *G. chilensis*.

4. Chemotaxonomic significance

Ophiuroids are characterized by their content of polar sulfated steroidal polyols (D'Auria et al., 1993). Surprisingly, the content of these secondary metabolites proved to be very low in the three species studied. On the other hand, a mixture of nucleosides and nucleobases was found to be the major component of the three ethanolic extracts. Nucleosides and nucleobases composition of the three studied ophiuroid species are reported here for the first time. Ribonucleosides have been isolated in free form from marine organisms. Thus, adenosine and cytidine were isolated from *C. crypta* (Porifera) (Cohen, 1963) and inosine from *Tapes japonica* (Mollusca) (Baker and Murphy, 1981, pp. 83–88). As these ophiuroids proved to contain various known 2′-deoxyribonucleosides, it is appropriate to mention that in the marine environment, this type of nucleosides have only been isolated from two

starfish, *Acanthaster planci* and *Luidia maculata* (Komori et al. 1978, 1980), and from an ascidian (Dematté et al., 1985).

The possibility that the nucleosides which have been isolated here are the result of degradation of DNA during work up can be discarded. Firstly, the resistance of DNA to selective hydrolysis is well known (Blackburn, 1979). Secondly, 2′-deoxyuridine is not expected to be a component of DNA since uracil is normally associated only with ribose. Finally, no adeninedeoxyriboside or guaninedeoxyriboside was detected in spite of there being among the eight common nucleosides (Walker, 1979; Bergmann et al., 1957).

It has been proposed that as Tunicata and Echinodermata are the only marine phyla where 2-deoxynucleosides have been detected, this fact might support a phylogenetic link between Chordata (and therefore Tunicata) and Echinodermata (Dematté et al., 1986). As ophiuroids belong to the echinoderms, our findings support this idea. However, the use of 2′-deoxynucleosides as systematic markers must be viewed with care in light of the reports on the isolation of new 2′-deoxynucleosides from two different marine sponge species (Kondo et al., 1992; Searle and Molinski, 1994).

Acknowledgements

This work was supported by the International Foundation for Science, Stockholm, Sweden, and the Organization for the Prohibition of Chemical Weapons, The Hague, The Netherlands, through a grant to M.S. Maier. We also wish to thank CONICET, ANPCyT and the Universidad de Buenos Aires for financial support of this work. We are indebted to UMYMFOR (CONICET-FCEN, UBA) for NMR spectra and LANAIS-EMAR (CONICET-FCEN, UBA) for mass spectra. We thank Lic. Enrique Marschoff (Instituto Antártico Argentino), Dr. Daniel Nahabedian and Dr. Javier Calcagno (Departamento de Ciencias Biológicas, FCEN, UBA) for collection of *G. chilensis* and the scientific team and crew of the BIP Dr. Holmberg for their collaboration. We are also indebted to Lic. Mario Lasta and Lic. Claudia Bremec (INIDEP, Mar del Plata, Buenos Aires) for collection of *O. asperula* and *O. vivipara*. M.S.M. and A.M.S. are Research Members of the National Research Council of Argentina (CONICET).

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