*Journal of Natural Products*
*Vol. 51, No. 6, pp. 1277-1280, Nov-Dec 1988*



# STUDIES OF SWEDISH MARINE ORGANISMS, PART X. BIOLOGICALLY ACTIVE COMPOUNDS FROM THE MARINE SPONGE *GEODIA BARETTI*

GÖRAN LIDGREN,* LARS BOHLIN,

*Faculty of Pharmacy, Uppsala Biomedical Centre, Department of Pharmacognosy, University of Uppsala, Box 579, S-751 23 Uppsala, Sweden*

and CARSTEN CHRISTOPHERSEN

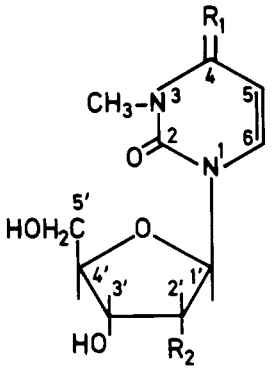
*Marine Chemistry Section, Department of General and Organic Chemistry, University of Copenhagen, Universitetsparken 5, DK 2100 Copenhagen, Denmark*

*Geodia baretti* (Bowerbank) Lamarck is predominant on the deep bottoms in the Koster fjord on the northern Swedish west coast. Chemical investigations of the genus *Geodia* (phylum Porifera, order Tetraactinellidae, class Demospongiae, family Geodidae) are very limited (1–17).[1]

In a screening of crude extracts of marine organisms, aqueous and MeOH extracts of *G. baretti* exhibited strong contractile activity in the isolated guinea-pig ileum assay (18). The aqueous extract was devoid of lectinlike activity (19).

The EtOH extracts of freshly collected material, on LH-20 cc, gave rise to seven fractions (Table 1) all exhibiting either contractile activity or inhibition of electrically stimulated guinea-pig ileum. The nature of these fractions was investigated by $^1$H nmr and tlc. Fraction 1 contained phthalates, presumably artifacts from the isolation procedure. Fractions 2, 3, and 4 lack aromatic protons and uv (254 nm) absorption but have intense signals at δ 2.9–3.4 ppm indicating low mol wt hydrophilic compounds. Dragendorff-positive reactions attested to nitrogenous constituents like betaines and taurine. Fraction 7 apparently contained minor amounts of aromatic compounds.

Only fractions 5 and 6 were further investigated and resulted in the identification of barettin, adenosine (fraction 6), histamine (responsible for the main activity of the crude extract), and inosine (fraction 5), identified by comparison with authentic samples. Fraction 5, in addition, yielded three *N*-methylated nucleosides, namely 3-methylcytidine (3mCyd) [**1**], 3-methyl-2′-deoxycytidine (3mdCyd) [**2**], and 3-methyl-2′-deoxyuridine (3mdUrd) [**3**]. The biological activities of the isolated compounds and some reference nucleosides are given in Table 2. The structures of the three nucleosides were inferred from comparison of fabms, $^1$H-nmr, and $^{13}$C-nmr data with values reported for the synthetic compounds (see Experimental). The presence of 3mdUrd [**3**] is curious, because




**1** $R_1=\overset{+}{N}H_2$, $R_2$=OH

**2** $R_1=\overset{+}{N}H_2$, $R_2$=H

**3** $R_1$=O, $R_2$=H

[1]The sponge referred to as *Geodia gigas* by Ackermann and List (4,5) must be *G. baretti*, and *Geodia cynodium* of DeRosa *et al.* (10) is actually *Geodia cydonium* (personal communication, Dr. O.S. Tendal, Zoological Museum, University of Copenhagen).

TABLE 1. Effect of Chromatographic Fractions in the Guinea-Pig Ileum Assay.

| Fraction no. | Volume (ml) | Yield (%) | Concentration (μg/ml)[a] | Contraction (%)[b] | Inhibition (%)[c] |
|---|---|---|---|---|---|
| 1 | 500 | $10^{-3}$ | 20 | 0 | 50[d] |
| 2 | 135 | 3 | 200 | 44 | 0 |
| 3 | 106 | 15 | 200 | 60 | 0 |
| 4 | 117 | 29 | 200 | 79 | 0 |
| 5 | 440 | 30 | 2.5 | 100 | 0 |
| 6 | 542 | 8 | 20 | 0 | 35 |
| 7 | 1500 | 3 | 200 | 0 | 52 |
| EtOH extract | | | 12 | 95 | 0 |

[a]Concentration of the fraction in the organ bath (5 ml).
[b]Contraction (%) as compared with 0.2 μg/ml histamine (100%). The values are means of two injections.
[c]Inhibition (%) of electrically induced contractions. The values are means of two injections.
[d]Effect due to contaminants (phthalates).

deoxyuridine is not naturally occurring. This compound could be formed by mild hydrolysis of 3mdCyd [**1**] during the isolation procedure. Although methylation of polynucleotide and oligonucleotide sequences is a well known phenomenon, methylation of DNA-bound deoxycytosine is only known to occur in position 5 (20). 3mCyd [**1**] has been isolated from yeast RNA hydrolysates (21) and human urine (22). We believe that the present conditions of extraction and isolation are far too mild to produce nucleosides by hydrolysis of polymeric material. Hence, at least 3mCyd [**1**] and 3mdCyd [**2**] and possibly also 3mdUrd [**3**] are natural products present as the free nucleosides. This need not be the case with the purine bases isolated by Ackermann and co-workers (2,3,6), as extreme hydrolytic conditions were used.

To the best of our knowledge, 3mdCyd [**2**] and 3mdUrd [**3**] have never been found as free natural products before.

Unusual nucleosides are not unprecedented in marine organisms. Sponges (23–27), nudibranchs (28,29), an acorn worm (30), and a red alga (31) all gave unusual nucleosides. Most of these nucleosides have potent biological or physiological effects. It is noteworthy (Table

TABLE 2. Effect of Isolated Metabolites and Reference Nucleosides in the Guinea-Pig Ileum Assay.

| Nucleoside | Concentration (μg/ml)[a] | Contraction (%)[b] | Inhibition (%)[c] |
|---|---|---|---|
| Adenosine | 15 | 0 | 85 |
| Inosine | 200 | 0 | 23 |
| 3mCyd [**1**] | 67 | 60 | 0 |
| 3mdCyd [**2**] | 232 | 60 | 0 |
| 3mdUrd [**3**] | 50[d] | 0 | 0 |
| Cytidine | 1200 | 0 | 0 |
| Uridine | 1200 | 0 | 83 |
| Deoxycytidine | 1200 | 0 | 27 |
| Barettin | 15 | 0 | 62 |

[a]Concentration of the fraction in the organ bath (5 ml).
[b]Contraction (%) as compared with 0.2 μg/ml histamine (100%). The values are means of two injections.
[c]Inhibition (%) of electrically induced contractions. The values are means of two injections.
[d]Only tested in this concentration due to limited amounts of material.

2) that only **1** and **2** exhibit strong contractile activity in the ileum assay, whereas most other nucleosides have either no effect or inhibitory effect on the electrically stimulated preparation. The products described here are present only in minute amounts: inosine $4 \times 10^{-5}$%, 3mdCyd $6 \times 10^{-4}$%, 3mdCyd $2 \times 10^{-4}$%, 3mdUrd $6 \times 10^{-5}$%, histamine $2 \times 10^{-4}$%, barettin $1 \times 10^{-4}$%, and adenosine $3 \times 10^{-5}$%, all based on wet wt. Normally such low abundances are taken as an indication of an exogenous source of metabolites. It is an open question whether these products originate with the sponge or with some associated microorganisms.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Tlc was carried out on either 0.25 mm (Merck no. 5729) or (in preparative scale, ptlc) 0.5 mm (Merck no. 5744) silica 60 $F_{254}$ plates. Cc was carried out on Si gel (Merck no. 9385) using the flash technique or on Biogel P-2 (Biorad 200–400 mesh) using a low pressure pump and uv detection at 280/206 nm. Nmr spectra were recorded on a JEOL FX 90Q MHz instrument in the solvents specified. Eims and fabms were taken on a JEOL DX303 instrument. Fabms are uncorrected for background. The tests for guinea-pig ileum contracting effect (32) as well as the inhibition of electrically induced contractions (33) were performed as described.

ISOLATION PROCEDURES.—*G. baretti* was collected by dredging at 40–60 m in July, 1985 in Koster fjord off the Swedish west coast; a voucher specimen is deposited in the Department of Pharmacognosy, University of Uppsala. Organisms were frozen for transportation to Uppsala where freshly thawed material (5.3 kg wet wt) was extracted at room temperature (12 h) with 95% EtOH (2 × 15 liters). Evaporation in vacuo left a residue (120 g). MeOH extraction and centrifugation left insoluble matter (67 g) and, after evaporation of the supernatant, 50 g soluble material.

Chromatography of MeOH-soluble material (25 g) on a Sephadex LH-20 (400 g) open bed column with MeOH as mobile phase gave rise to seven fractions, numbered 1–7 (Table 1). The fractions were detected by tlc analysis using two mobile phases: (A) EtOH (95%)-aqueous $NH_3$ (25%) (8:2) and (B) $CHCl_3$-MeOH (1:1). Visualization of the tlc's was effected by uv (254 and 366 nm) and by ninhydrin and vanillin/$H_2SO_4$ spraying. Fractions 5 and 6 were further purified on Si-60 columns with $CHCl_3$/MeOH gradient (10% steps) elution using tlc as described above for detection.

Selected fractions originating from fraction 5 were separated on Biogel P-2 columns with distilled $H_2O$ as eluent (flow 2 ml/min) using uv monitoring. Almost pure histamine and inosine were obtained. Ptlc using system A gave 9.1 mg pure histamine, which after conversion to the dihydrochloride proved identical with an authentic sample. Ptlc with solvent system C [iPrOH-$H_2O$-aqueous $NH_3$ (25%) (18:1:1)] yielded 2.2 mg pure inosine, identical ($R_f$, $^1$H nmr, eims) with an authentic sample.

Ptlc using system C served to purify the *N*-methylated nucleosides from two other Biogel P-2 fractions. One yielded 32 mg 3mCyd [**1**] and the other 8.8 mg 3mdCyd [**2**] and 3.2 mg 3mdUrd [**3**]. The identity of the anions associated with **1** and **2** was not investigated, but they are likely to be chloride ions.

The identity of the nucleosides was established from fabms data and from comparison of nmr ($^1$H and $^{13}$C) chemical shifts with reported values. For compound **1**, nmr ($D_2O$, DSS internal standard) only exhibited chemical shifts compatible ($^{13}$C signals identical within 0–0.3 ppm) with reported values (34). Fabms showed abundant signals at *m/z* 258 (23%) for $[M]^+$, *m/z* 126 (50%) for the protonated heterocyclic base $[BH]^+$, and *m/z* 110 (15%) for $[BH - NH_3]^+$ in agreement with sims experiments (36). For compound **2**, nmr (DMSO-$d_6$) results were virtually identical with $^{13}$C- and $^1$H-nmr data reported (34,35). Fabms gave signals at *m/z* 242 (2%) for $[M]^+$, *m/z* 126 (7%) for $[BH]^+$ and *m/z* 110 (100%) for $[BH - NH_3]^+$ in agreement with reported sims data (36). For compound **3**, nmr ($D_2O$) gave almost identical ($^{13}$C signals identical within 0–0.3 ppm and $^1$H signals within 0–0.03 ppm) chemical shifts to those reported (37). Fabms exhibited signals at *m/z* 243 (7%) for $[MH]^+$, *m/z* 127 (20%) for $[B + 2H]^+$ and *m/z* 117 (7%) for the sugar moiety $[S]^+$.

Fraction 6 gave crude barettin (7.5 mg, 80% pure) identified by comparison (tlc and $^1$H nmr) with an authentic sample. Another fraction from the Si-60 column gave crude adenosine, which after further purification (ptlc system C) gave 1.6 mg adenosine identical (eims, $^1$H nmr, $R_f$ value, and biological activity) with an authentic sample.

## ACKNOWLEDGMENTS

Part of this work has been financed by grants from Apotekare Gunnar Hyltens minnesfond, The Bank of Sweden, Tercentenary Foundation, and from the Nordic Council. We thank Eva Kumlin, Helena Odqvist, and Marie Allen for skillful technical assistance. Thanks are due to Dr. Lars Afzelius, Tjärnö Marine Biology Laboratory, for taxonomic identification of the material.

## LITERATURE CITED


1. F. Holtz, *Z. Biol. (Munich)*, **81**, 65 (1923).
2. D. Ackermann, F. Holtz, and H. Reinwein, *Z. Biol. (Munich)*, **82**, 278 (1924).
3. D. Ackermann and P.H. List, *Z. Physiol. Chem.*, **308**, 270 (1957).
4. D. Ackermann and P.H. List, *Z. Physiol. Chem.*, **308**, 274 (1957).
5. D. Ackermann and P.H. List, *Z. Physiol. Chem.*, **309**, 286 (1957).
6. D. Ackermann, P.H. List, and H.G. Menssen, *Z. Physiol. Chem.*, **312**, 210 (1958).
7. D. Ackermann and P.H. List, *Z. Physiol. Chem.*, **317**, 78 (1958).
8. D. Ackermann and P.H. List, *Z. Physiol. Chem.*, **323**, 192 (1961).
9. J.F. Kingston, E. Benson, B. Gregory, and A.G. Fallis, *J. Nat. Prod.*, **42**, 528 (1979).
10. M. DeRosa, L. Minale, and G. Sodano, *Comp. Biochem. Physiol.*, **46B**, 823 (1973).
11. P.A. Voogt, *Neth. J. Zool.*, **26**, 84 (1976).
12. G. Pettit, J.A. Rideout, and J. Hasler, *J. Nat. Prod.*, **44**, 588 (1981).
13. W.E.G. Mueller, J. Conrad, R.K. Zahn, M. Gramzow, B. Kurelec, and G. Uhlenbruck, *Mol. Cell. Biochem.*, **67**, 55 (1985).
14. B. Diehl-Seifert, R.K. Zahn, G. Uhlenbruck, A. Maidhof, and W.E.G. Mueller, *Basic Appl. Histochem.*, **29**, 7 (1985).
15. G. Lidgren, L. Bohlin, and J. Bergman, *Tetrahedron Lett.*, **27**, 3283 (1986).
16. A. Lieberknecht and H. Griesser, *Tetrahedron Lett.*, **28**, 4275 (1987).
17. W.R. Chan, F.T. Winston, P.S. Manchand, and L.J. Todaro, *J. Org. Chem.*, **52**, 3091 (1987).
18. L. Andersson, G. Lidgren, L. Bohlin, L. Magni, S. Ögren, and L. Afzelius, *Acta Pharm. Suec.*, **20**, 401 (1983).
19. L. Andersson, G. Lidgren, L. Bohlin, P. Pisa, H. Wigzell, and R. Kiessling, *Acta Pharm. Suec.*, **23**, 91 (1986).
20. A. Razin, H. Cedar, and A.D. Riggs, in: "DNA Methylation." Ed. by A. Razin, H. Cedar, and A.D. Riggs, Springer Verlag, New York, 1984, p. 2.
21. R.H. Hall, *Biochemistry*, **4**, 661 (1965).
22. G.C. Mills, F.C. Schmalstieg, and R.M. Goldblum, *Biochem. Med.*, **34**, 37 (1985).
23. W. Bergmann and D.C. Burke, *J. Org. Chem.*, **20**, 1501 (1955).
24. W. Bergmann and D.C. Burke, *J. Org. Chem.*, **21**, 226 (1956).
25. W. Bergmann and R.J. Freeney, *J. Org. Chem.*, **16**, 981 (1951).
26. A.J. Weinheimer, C.W.J. Chang, J.A. Matson, and P.N.J. Kant, *J. Nat. Prod.*, **41**, 488 (1978).
27. R.J. Quinn, R.P. Gregson, A.F. Cook, and R.J. Bartlet, *Tetrahedron Lett.*, **21**, 567 (1980).
28. T. Kim, R.J. Nachman, L. Pavelka, H.S. Mosher, F.A. Fuhrman, and G.J. Fuhrman, *J. Nat. Prod.*, **44**, 206 (1981).
29. F.A. Fuhrman, G.J. Fuhrman, R.J. Nachman, and H.S. Mosher, *Science*, **212**, 557 (1981).
30. S. Sakemi and T. Higa, *Comp. Biochem. Physiol.*, **82B**, 107 (1985).
31. R. Kazlauskas, P.T. Murphy, R.J. Wells, J.A. Baird-Lambert, and D.D. Jamieson, *Aust. J. Chem.*, **36**, 165 (1983).
32. S. Rosell, U. Björkroth, D. Chang, I. Yamaguchi, Y.P. Wan, G. Rackur, G. Fisher, and K. Folkers, in: "Substance P." Ed. by U.S. Euler and B. Pernow, Raven Press, New York, 1977, p. 2.
33. S. Rosell and S. Agurell, *Acta Physiol. Scand.*, **94**, 142 (1975).
34. D.J. Ashworth, C. Chang, S.E. Unger, and G.R. Cooks, *J. Org. Chem.*, **46**, 4770 (1981).
35. C. Chang, J. DaSilva Gomes, and S.R. Byrn, *J. Org. Chem.*, **48**, 5151 (1983).
36. S.E. Unger, A.E. Schoen, R.G. Cooks, D.J. Ashworth, J. DaSilva Gomes, and C. Chang, *J. Org. Chem.*, **46**, 4765 (1981).
37. B.D. Allore, A. Queen, W.J. Blonski, and F.E. Hruska, *Can. J. Chem.*, **61**, 2397 (1983).


*Received 6 May 1988*