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Hanishin, a Semiracemic, Bioactive C_9 Alkaloid of the Axinellid Sponge *Acanthella carteri* from the Hanish Islands. A Shunt Metabolite?

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***Abstract*:** The C_9 alkaloid hanishin (**2**), isolated from a collection in the Hanish Islands (Red Sea) of the highly polymorphic sponge *Acanthella carteri*, has low enantiomeric purity and may be viewed as a shunt metabolite, albeit biologically active, from co-occurring oroidin (**1**). © 1997 Elsevier Science Ltd.

Alkaloids of the oroidin family from marine sponges in the orders Axinellida and Halichondrida are most varied in cyclization modes, but were never observed to deviate from the C_{11} composition.[1] We describe here the first example of degraded alkaloid in this family, hanishin, which is also peculiar as to the chirality properties and shows an interesting bioactivity. Thus, continuing the examination of the axinellid sponge *Acanthella carteri* Dendy, 1889 (= *Acanthella aurantiaca* Keller, 1889) from the northern coast of the Hanish Islands (Yemen, South Red Sea[2]), we have now isolated by extensive reversed-phase HPLC[3] the known C_{11} alkaloid oroidin (**1**)[4] and

1 **2**

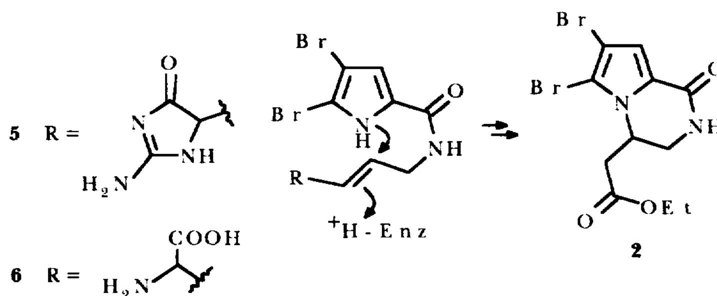
3 R=Br
4 R=H

amide **3**,[5] besides the novel C_9 alkaloid hanishin (**2**) and the new amide **4**. Oroidin (**1**)[4] and amide **3**[5] were identified by matching of spectral data with the literature. Identification of amide **4**[6] rests on the composition $C_5H_5N_2OBr$ (HR-EI-MS and NMR atom count) as well as on NMR shift and coupling data: 1-H as a broad signal at δ_H 10.95; 3-H and 5-H as dd at δ_H 6.87 and 7.03, coupled to their carbon atoms at δ_c 122.22 and 112.65,

respectively; $CONH_2$ as two broad signals at δ_H 6.49 and 7.20 for NH_2 and a s at δ_C 158.10 for C=O; C-2 and C-4 as s at δ_C 127.82 and 96.57 (upfield shift by Br), respectively. The composition $C_{11}H_{12}N_2O_3Br_2$ for hanishin (**2**)[7] was similarly derived while the pyrrole moiety rests on singlet signals at δ_C 106.15, 99.55 and 127.48 and a doublet signal at 115.17, where C-4 and C-2 assignments were made by ^1H-^{13}C correlation and inverse-detection HMBC[8] correlation of the pyrrole-fitting δ_H 6.86 s with δ_C 115.17 d and 106.15 s, respectively, while C-3 was assigned from high-field shift (δ_C 99.55) by bromine. The amide group was assigned from δ_C 158.54 s (C=O) and a δ_H 6.95 broad signal (H-7), the latter coupled, as indicated by selective irradiations, with H_2-8, which showed up as δ_H 4.00 ddd and δ_H 3.68 ddd signals, both correlated with δ_C 36.58 t. The methine group was revealed as a δ_H 4.82 dt coupled with δ_C 51.68 d as well as with both H_a-8 (δ_H 2.96 dd) and H_2-10 (δ_H 2.64 ddd). The latter was also correlated with δ_C 43.65 t. Accounting for the unsaturation due to the ester group - fully supported by 1D and 2D NMR data - this required N-1/C-9 ring closure. The connectivity C-9/C-10 was confirmed by HMBC[8] correlation of H_a-10 (δ_H 2.96 dd) with C-8 (δ_C 36.58 t).

Hanishin (**2**), although giving no optical rotation across a range of wavelengths, showed a weak Cotton effect.[7] Since no model compound was available to relate the CD elongation to the optical purity, experiments with the chiral shift reagent $Eu(tfc)_3$ were carried out, revealing the splitting of δ_H 2.94 dd (10-H) into two signals in 3:2 integration ratio, which represents also the enantiomeric excess for **2**.

Since alkaloids in this group often show therapeutically promising bioactivity[1] or have ecological relevance,[5b,9] compounds **1-4** were screened *in vitro* against NSCLC-N6 human non-small-cell-lung carcinoma, recording IC_{50} 11.2, 9.7, 4.8 and 9.4 µg/ml, respectively. This may be a case where weak biological activities are worthwhile pursuing,[10] not only because there is little treatment for this form of tumour, but also because hanishin and analogues should prove easily amenable to total synthesis, allowing screening of both enantiomers. As it is often the case with antimicrobial agents,[11] the free carboxylic acid form of hanishin could prove far more cytotoxic than the ester, although this is not our criterion of choice: we are aimed at inducing terminal differentiation of these tumour cells, which would be the basis for suppressing their neoplastic character, and low-activity compounds may give hope of no general toxicity.



Scheme 1

Oroidin type alkaloids are considered condensation products of highly modified prolines, which have been separately isolated from *Agelas mauritiana*[12a] and *Lissodendoryx* sp.,[12b] the latter belonging to an atypical order (Poecilosclerida) for these types of alkaloids. Additions to[13] or deviations from[14] this scheme have also been

proposed for related alkaloids. The biogenesis of hanishin may be viewed, as in Scheme 1, from **1**-derived aminoimidazolinone **5** or amino acid **6** intermediates *via* N(1) → C(9) (hanishin numbering) cyclization followed by oxidative breakdown of the side chain. Alternatively, breakdown of the chain may precede cyclization. Anyway, any racemization of hanishin during work up was ruled out and it can not be easily imagined for any conceivable precursor of hanishin. Thus, unlike certain cases of phenol coupling,[15] it is difficult to envision formation of hanishin other than by the aid of an enzyme or dirigent protein [16]system, like in the above routes. The low enantiomeric purity of hanishin may be related to the highly polymorphic condition of *A. carteri*, different collections of which gave different, though intact, C_{11} alkaloids of the oroidin family.[17] This suggests that *A. carteri* of the Hanish Islands saved only a memory of the ability of congeners [1,12-14,17]to bring about cyclization of oroidin or related, linear precursors. Perhaps our sponge does badly this biosynthetic function as to the stereochemistry by the way of a vestigial enzyme, as for a shunt metabolite.

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3. Fractions 20-22 of previous chromatographic (FC) work up of the ethanolic extracts of the sponge[2] were subjected to reversed-phase RP-18 HPLC with H_2O-MeCN 2:3, solvent flow 5 ml min^{-1} under UV monitoring at λ 254 nm, to give **4** (t_R 4.0 min, 3.0 mg, 0.022% on raw extract), **3** (t_R 5.5 min, 1.2 mg, 0.009%) and **2** (t_R 12.0 min, 2.5 mg, 0.087%). Amine-HPLC (EtOAc-MeOH 8:2 with added Et_3N 5%, λ 280 nm) of fraction 25 from the above FC gave oroidin **1** (t_R 6.5 min, 4.3 mg, 0.031%).
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6. Data for **4**: semisolid; UV (MeOH) λ_{max} 267 nm (ϵ 16200 l $mol^{-1}cm^{-1}$), 230 (9800), 202 (13800); ^1H NMR ($(CD_3)_2CO$, *J*/Hz, 299.94 MHz, Me_4Si) δ 10.95 (br.s, 1-H), 6.87 (dd, $J_{3,1}$ 2.7, $J_{3,5}$ 1.5, 3-H), 7.03 (dd, $J_{5,1}$ 2.7, $J_{5,3}$ 1.5, 5-H), 6.49 and 7.20 (two br.s, H_2NCO); ^{13}C NMR ($(CD_3)_2CO$, 75.43 MHZ) δ 127.82 (s, C-2), 122.22 (d, C-3), 96.57 (s, C-4), 112.65 (d, C-5), 158.10 (s, C=O). EI-MS *m/z* (%) 190, 188 (M^+, 100/100), 174, 172 ($[M - NH_2]^+$, 22/22), 146, 144 (16/16), 119, 117 (10/10), 109 (6), 65 (21). HR-EI-MS *m/z* 187.957±0.004, calc. for $C_5H_5N_2O^{79}Br$ 187.958).
7. Data for **2**: semisolid; $[\alpha]^{25}$ = 0.0 at λ 589, 546 and 435 nm (MeOH, *c* 0.1 g per 100 ml); CD (MeOH) Δϵ (λ) -0.4 (300), 0.1 (262), -0.6 (212); UV (MeOH) λ_{max} 280 nm (ϵ 6500 l mol^{-1} cm^{-1}), 230 (10000), 203 (17800); ^1H NMR ($(CD_3)_2CO$, *J*/Hz, 299.94 MHz, Me_4Si) δ 6.86 (s, 4-H), 6.95 (br.s, 7-H), 4.00 (ddd, J_{gem} 13.6, $J_{8a,9}$ 4.3, $J_{8a,NH}$ 1.5,8-H_a), 3.68 (ddd, J_{gem} 13.6, $J_{8b,NH}$ 5.3, $J_{8b,10b}$ 1.5, 8-H_b), 4.82 (dt, $J_{9,10a}$ 10.1, $J_{9,8a}$ ≈ $J_{9,10b}$

3.9, 9-H), 2.96 (dd, J_{gem} 16.0, $J_{10a,9}$ 10.1, 10-H_a), 2.64 (ddd, J_{gem} 6.0, $J_{10b,9}$ 3.9, $J_{10b,8b}$ 1.5, 10-H_b), 4.25 (q, J 7.2, O-CH_2), 1.23 (t, J 7.2, CH_3); working in $CDCl_3$, to **2** was added 0.010M $Eu(tfc)_3$ in 10-μl portion until, at 0.2 molar equivalent of $Eu(tfc)_3$ added, δ_H 2.94 dd (10-H) was split into two signals in *ca.* 3:2 integration ratio; this can be taken with qualitative confidence as the integration is for the same proton in the diastereomeric complexes and relaxation was amply allowed; ^{13}C NMR ($(CD_3)_2CO$, 75.43 MHz) δ 106.15 (s, C-2), 99.55 (s, C-3), 115.17 (d, C-4), 127.48 (s, C-5), 158.54 (s, C-6), 36.58 (t, C-8), 51.68 (d, C-9), 43.65 (t, C-10), 170.65 (s, C-11), 61.50 (t, OCH_2), 14.32 (q, CH_3). EI-MS *m/z* (%) 382, 380, 378 (M^+, 17/34/17), 337, 335, 333 ($[M - C_2H_5O]^+$, 4/8/4), 309, 307, 305 (1/2/1), 295, 293, 291 (37/74/37), 214, 212 (9/9), 183 (3), 144, 142 (8/8); HR-EI-MS *m/z* 377.921±0.004, calc. for $C_{11}H_{12}N_2O_3{}^{79}Br_2$ 377.921); FAB-MS (H^+,3-nitrobenzyl alcohol) *m/z* (%) 383, 381, 379 ($[M + H]^+$, 20/40/20).

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