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5,7-Dihydroxy-5,6,7,8-tetrahydro-1*H*-azocin-2-one from a Marine-derived *Streptomyces* sp.[¶]

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[¶]Dedicated to Prof. Dr. Dr. h.c. mult. H. W. Roesky on the occasion of his 70th birthday.

In the course of a screening program, we have isolated the new natural product, 5,7-dihydroxy-5,6,7,8-tetrahydroazocin-2(*1H*)-one (**1**), from the staurosporine producing marine-derived *Streptomyces* sp. strain QD518. Here we report the isolation and structure elucidation of **1** and the artifacts **3** and **4** resulting from **1** by acid catalyzed intra- and inter-molecular reactions.

Keywords: marine Streptomycetes, fermentation, natural products, azocine.

Marine bacteria are presently subject of many natural product studies, demonstrating that these organisms are a prolific source of unique secondary metabolites. Among these, compounds having the tetrahydro-1*H*-azocin-2-one skeleton are very rare in comparison to the homologous hexahydro-1*H*-azepin-2-one substructure (caprolactam), but are found in some plant metabolites like the indole alkaloids decursivine [2], moschamide [3], moschaminindolol and moscham-indole isolated from the seeds of *Centaurea moschata* [4]. We isolated now the new natural product 5,7-dihydroxy-5,6,7,8-tetrahydro-1*H*-azocin-2-one (**1**), which undergoes a rapid cyclization and trimerization. Related azocinones may be the precursors also of various other microbial pyrrolizidines.

During a previous investigations of the marine-derived *Streptomyces* sp. QD518, among other compounds, staurosporins and various indole derivatives were obtained [1], however, we were not able to isolate substantial amounts of **1** due to its instability and rapid decomposition. Repeated fermentations led to the isolation of sufficiently pure samples, and immediate measurements allowed us now to determine the structure of this labile metabolite and also of its follow-up products.

Compound **1** was obtained as an oil by preparative TLC from a strongly UV absorbing spot, which turned to green on spraying with anisaldehyde /sulfuric acid. The ^{1}H NMR spectrum of **1** was very simple and exhibited two doublets of doublets in the aromatic region at δ 7.26 and 6.07 and two methine protons connected to oxygen at δ 4.83 (t) and 4.77 (ddm). In addition, two pairs of signals assigned to methylene groups appeared at δ 4.05/3.51 and 2.39/1.64, respectively. The EI-MS suggested a molecular weight of *m/z* 157, and the molecular formula $C_7H_{11}NO_3$ (from EI-HRMS) indicated three double bond equivalents. The ^{13}C NMR spectrum showed in accordance with the molecular formula 7 carbon signals, which were assigned by HMQC to an amide carbonyl, two sp^2 methines, two oxygen-bound sp^3 methines and two methylene carbons. By systematic interpretation of H,H COSY and HMBC spectra (see Figure 1), compound **1** was identified as

5,7-dihydroxy-5,6,7,8-tetrahydro-1*H*-azocin-2-one, which is reported here for the first time.

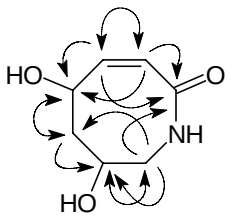
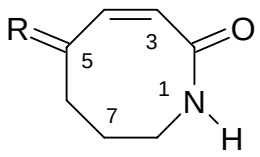


Figure 1: Structure and selected H,H COSY (↔) and HMBC (→) correlations of **1**.

Azocin-2-one derivatives are very rare in nature, and in microorganisms no compound of this type has been found, however, some homologous hexahydro-1*H*-azepin-2-ones (caprolactams) are known. Caprolactin A and B have been isolated from an unidentified Gram-positive marine bacterium and are cytotoxic towards human epidermoid carcinoma cells and human colorectal adenocarcinoma and also exhibit antiviral activity towards *Herpes simplex* type II virus [5]. Although the chemistry of caprolactam and its synthetic unsaturated derivatives has been explored extensively, very few reports appeared regarding the eight-membered ring homologues, the 1*H*-azocin-2-ones [6]. An example is the description of the synthetic 5,6,7,8-tetrahydro-1*H*-azocin-2-one (**2a**) by Ridley *et al*. [6,7].



2a: R = H, H
2b: R = H, OH
2c: R = O

The unusual reactivity of tetrahydro-1*H*-azocin-2-one (**2a**) has been attributed to ring conformational effects, which promote an intramolecular cyclization in suitably substituted tetrahydro-1*H*-azocin-2-ones [7]. Obviously, this happened also with **1**. During the NMR measurements in $CDCl_3$, a complete decomposition was observed when the sample was allowed to stand in the NMR tube resulting in two artifacts **3** and **4**.

Compound **3** was obtained as minor component and showed in its ^{1}H NMR spectrum only four signals. Three protons of an ABC system at δ 7.02, 6.43 and δ 5.94 were assigned to a five-membered ring due to the small coupling constants, and a broad 4H singlet at δ 3.00 was attributed to two methylene groups. The molecular weight was deduced from EI MS to be *m/z* 121. The ^{13}C NMR spectrum of **3** indicated the presence of seven carbon atoms as in **1**, a carbonyl, a quaternary carbon at δ 139.6, three sp^2 methines and two aliphatic methylenes. Interpretation of the HMBC spectrum (Figure 2) led to the identification of the rearrangement product as 1,2-dihydro-pyrrolizin-3-one (**3**). The structure was fully confirmed by comparison with synthetic **3**, which we obtained following a procedure of Flitsch *et al*. [8].

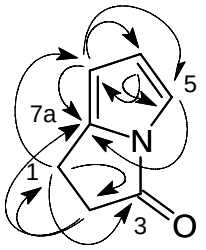


Figure 2: Structure and selected HMBC couplings of compound **3**.

The molecular formula of the more polar decomposition product **4** was established as $C_{21}H_{22}N_3O_3$ by high-resolution ESI-MS of the peak at *m/z* 364 ($[M+H]^+$). The EI-MS delivered a fragment at *m/z* 242, which corresponds to $[M-C_7H_7NO]$ and may be a hint, that compound **3** was polymerized. The ^{1}H NMR spectrum of **4** showed some similarities with that of **3**, however, revealed 21 protons including four pairs of doublets for olefinic protons at δ 6.26, 6.14, 5.84 and δ 5.78. In addition, to a methine group connected to a hetero atom at δ 3.95, there are eight methylene groups, of which two at δ 3.01 and 2.93 appeared as broad singlet as in the spectrum of **3**. The ^{13}C NMR spectrum also exhibited signals for eight methylene groups in the range of δ 46.8-19.0, of which the resonances at δ 46.8 and δ 44.6 may be due to CH_2-N groups. An aliphatic and four sp^2 methine groups, four signals of quaternary sp^2, one of a quaternary sp^3 and three of amide carbonyl C atoms suggested that **4** may be a trimer of **3**. Based on comparison of the data with those of **3** followed by intensive interpretation of H,H COSY and HMBC spectra, three fragments were constructed (Figure 3).

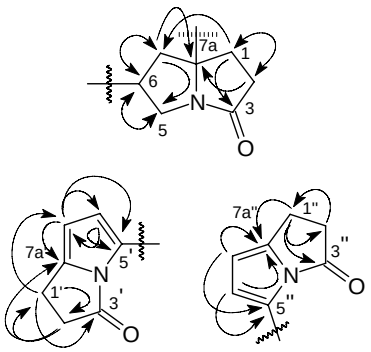


Figure 3: Fragments of compound **4** derived from H,H COSY (↔)and HMBC spectra (→).

There is only one way to connect these parts, resulting in **4**, which was further confirmed by HMBC correlation of the 6-methine signal at δ_H3.95 (δ_C 37.2, the two methylene groups 5-H_2 and 7-H_2 at δ_H 2.78, 2.10 (δ_C 44.6 and δ_H3.63 (δ_C 46.8), respectively, to the carbon atom C-5' at (δ_C 128.5. Additionally, the 1- and 7-methylene signals indicated cross-peaks to the quaternary carbon atoms C-7a (δ 70.6) and C-5" (δ 130.3). The trimer **4** decomposed slowly at –20 °C under formation of insoluble products.

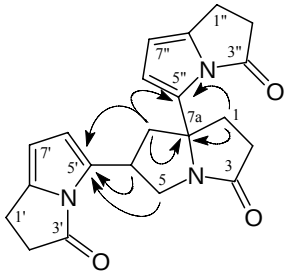
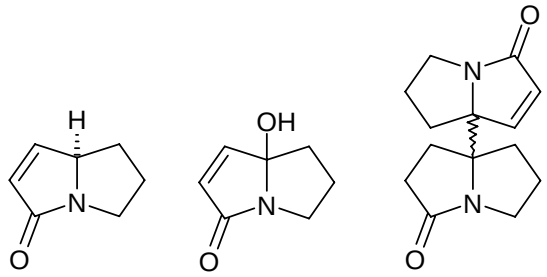


Figure 4: Structure of compound **4** with important HMBC correlations between the sub-structures

Compound **3** and its congener **4** are having the 1-azabicyclo[3.3.0]octane skeleton (pyrrolizidine), which is found frequently in plants [9] and insects [10] and is part of pheromones, defensive agents, or growth determinants. Mammals convert many of these pyrrolidizine alkaloids into dehydro-pyrrolizidines, which exhibit e.g. hepatotoxic, mutagenic, and carcinogenic activities [11]. The pyrrolams (e.g. A, **5**) or 5,6,7,7a-tetrahydro-3*H*-pyrrolizin-7a-ol-3-one (**6**) are some of the very few pyrrolizidine derivatives from microorganisms [12,13]. Also in the case of **5**, a dimerization forming **7** was observed when a solution of the former was kept aside for 3 weeks. In contrast, compound **1** rearranged within a few hours.



5 **6** **7**

It can be assumed that the cyclization of **1** in alcohol-free chloroform was catalyzed by a light-induced liberation of HCl. Protonation of 5-OH will allow a transannular attack of the nitrogen atom on C-5 under cyclization and elimination of water. A second loss of water and rearrangement of the double bond delivers **3**. It can be speculated that the *Streptomyces* metabolite **5** is formed in a similar way from the hypothetical **2b**, and **6** from the corresponding ketone **2c**. A similar reaction may responsible for the formation of epohelmins A and B [14].

Based on the fact that **5** dimerized slowly in solution under formation of **7** [12], an alcohol-free chloroform solution of **3** was put aside for a while, however, no reaction occurred. This observation led us to assume that the trimer **4** may result from an intermolecular reaction of **1** or between **1** and **3**. Mechanistic details of the rearrangement of **1** are, however, still under investigation.

Compounds **3** and **4** showed no activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus*, and *Escherichia coli*, the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*, and the fungi *Mucor miehei* and *Candida albicans* at a concentration of 80 µg/paper disk; **1** was not tested because of its small amount.

Experimental

ESI mass spectra were recorded on a Finnigan LCQ spectrometer with quaternary pump Rheos 4000 (Flux Instrument). ESI HR mass spectra were recorded on A Bruker FTICR 4.7 T mass spectrometer. EI MS spectra were recorded on a Finnigan MAT 95 spectrometer at 70 eV. Further materials & methods were used as described earlier [15].

The marine *Streptomyces* sp. QD518 was cultivated on a 25 L scale on meat extract medium at 28 °C for 7 days on a linear shaker (110 rpm), and worked up as described previously [1]. Column chromatography of the extract delivered a fraction B_1, which was further purified by PTLC (CH_2Cl_2/3% MeOH) to yield a sesquiterpene (4 mg) and the azocinone **1** (5 mg). The other fractions delivered 15 known compounds and two new staurosporine derivatives [1].

During the NMR measurement in $CDCl_3$, a sample of **1** (6 mg) get decomposed. Separation of the mixture by preparative TLC with CH_2Cl_2/MeOH (93:7) afforded **3** (2 mg), **4 (**1.5 mg), and remaining **1 (**1.1 mg).

5,7-Dihydroxy-5,6,7,8-tetrahydro-1*H*-azocin-2-one (**1**)

Colorless oil.
R_f: 0.34 (CH_2Cl_2 -3% MeOH).
IR (KBr) ν: 3650, 2926, 2855, 2361, 2343, 1691cm^{-1}.
UV λ_{max} (MeOH) nm (log ε): 233 (sh), 206 (3.80).
^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.26 (1H, dd, *J* = 5.8, 1.8 Hz, 4-H), 6.07 (1H, dd, *J* = 5.8, 1.6 Hz, 3-H), 4.83 (1H, t br, *J* = 5.8 Hz, 7-H), 4.77 (1H, ddm, *J* = 10.8, 5.6 Hz, 5-H), 4.05 (1H, dd, *J* = 13.2, 5.9 Hz, 8-H_β), 3.51 (1H, *d*, *J* = 13.2 Hz, 8-H_α), 2.39 (1H, dd, *J* = 13.3, 5.6 Hz, 6-H_α), 1.64 (1H, ddd, *J* = 13.3, 10.8, 5.4 Hz, 6-H_β).
^{13}C NMR (150.8 MHz, $CDCl_3$) δ: 175.6 (C-2), 149.2 (CH-4), 128.6 (CH-3), 65.6 (CH-5), 62.8 (CH-7), 53.2 (CH_2-8), 40.1 (CH_2-6).
EIMS (70 eV): *m/z* (%) 157 ([M$^+$], 86), 141 (16), 129 (20), 122 (34), 99 (26), 95 (100), 67 (78), 41 (48); EI HRMS: *m/z* [M]$^+$ calcd for $C_7H_{11}NO_3$: 157.0734; found: 157.0740.

1,2-Dihydro-pyrrolizin-3-one (**3**)

Colorless oil.
R_f: 0.83 ($CHCl_3$-3% MeOH).
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.02 (1H, d, *J* = 2.9 Hz, 5-H), 6.43 (1H, dd, *J* = 2.9, 2.8 Hz, 6-H), 5.94 (1H, brd, *J* = 2.8 Hz, 7-H), 3.00 (4H, s, 1,2-H_2).
^{13}C NMR (150.8 MHz, $CDCl_3$): δ 175.1 (C-3), 139.6 (C-7a), 119.0 (CH-6), 104.5 (CH-7), 110.9 (CH-5), 34.8 (CH_2-2), 19.3 (CH_2-1).
EI MS (70 eV): *m/z* (%)121 ([M$^+$], 22), 111 (6), 93 (12), 83 (12), 69 (16), 57 (44), 41 (28).

Trimer 4

Colorless oil.
R_f: 0.31 (CH_2Cl_2-3% MeOH).
IR (KBr): 2912, 1659, 1642, 1502 cm^{-1}.
UV λ_{max} (MeOH) nm (log ε): 269 (3.70), 230 (4.14), 209 (4.20).
^{1}H NMR (300.0 MHz, $CDCl_3$): δ 6.26 (1H, *d*, *J* = 3.1 Hz, 6"-H), 6.14 (1H, dd, *J* = 3.1, 0.9 Hz, 6'-H), 5.84 (1H, d, *J* = 3.1 Hz, 7"-H), 5.78 (1H, d, *J* = 3.1 Hz, 7'-H), 3.95 (1H, *m*, 6-H), 3.63 (2H, *m*, 5-H_2), 3.01 (4H, *s* br, 1",2"-H_2), 2.93 (4H, s br, 1',2'-H_2), 2.78 (1H, dd, *J* = 12.3, 6.6 Hz, 7-H_α), 2.70 (1H, m, 2-H_α), 2.50-2.30 (3H, m, 2-H_β, 1-H_2), 2.10 (1H, t br, *J* = 12.3 Hz, 7-H_β).
^{13}C NMR (150.8 MHz, $CDCl_3$): δ 175.4 (C-3), 172.6 (C-3'), 172.4 (C-3"), 141.2 (C-7a"), 139.9 (C-7a'), 130.3 (C-5"), 128.5 (C-5'), 116.8 (CH-6"), 115.3 (CH-6'), 103.6 (CH-7'), 103.5 (CH-7"), 70.6 (C-7a), 46.8 (CH_2-5), 44.6 (CH_2-7), 37.2 (CH-6), 35.1 (CH_2-2'), 35.0 (CH_2-2"), 34.8 (CH_2-1), 33.5 (CH_2-2), 19.2 (CH_2-1"), 19.0 (CH_2-1').
(+)-ESIMS: *m/z* 364 ([M+H]$^+$, 11), 386 ([M+Na]$^+$, 14), 749 ([2M+Na]$^+$, 100).
EIMS (70 eV): *m/z* 363 ([M$^+$], 14), 242 (24), 216 (61), 203 (57), 187 (36), 160 (25), 147 (20), 118 (22), 83 (30), 70 (40), 57 (73), 43 (100).
(+)-ESI-HRMS: *m/z* [M+Na]$^+$ calcd for $C_{21}H_{21}N_3O_3Na$: 386.14752; found: 386.14763.

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