Investigations on the Reactivity of Fascaplysin

Part I

Aromatic Electrophilic Substitutions Occur at Position 9

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Electrophilic aromatic substitutions, such as halogenation and sulfonation, occur primarily *para* to the indole N-atom at C(9) of the unique 12*H*-pyrido[1,2-*a*:3,4-*b′*]diindole ring system of fascaplysin.

Introduction. – Fascaplysin (**1a**; *Scheme 1*) is a red pigment that was isolated in 1988 from the Fijian sponge *Fascaplysinopsis* BERGQUIST sp. [1], and more recently from its relative *Fascaplysinopsis reticulata* as a complex with dehydroluffariellolide diacid monoanion [2]. Antibiotic properties of this natural product were reported, such as growth inhibition of several microbes, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Saccharomyces cerevisiae*, and suppression of proliferation of mouse leukemia cells L-1210 with an ED_{50} of 0.2 > g/ml [1]. After proliferative or mitogenic stimulation, fascaplysin strongly inhibited incorporation of labeled thymidine, uridine, and leucine into DNA, RNA, and proteins, respectively, at low concentrations of up to 1 μg/ml [3]. *Soni et al.* recently reported that fascaplysin specifically inhibits cyclin-dependent kinase 4 (Cdk4), causing a G1 arrest of tumor (U2-OS, HCT-116) and normal (MRC-5) cells [4a][1]). Furthermore, DNA binding and intercalating properties of fascaplysin could be demonstrated [5]. Apart from these interesting biological properties, we were also attracted by the unique chemical structure. Several successful approaches towards the total synthesis of fascaplysin have been undertaken [6–9]. So far, no reports concerning reactivity and stability of this 12*H*-pyrido[1,2-*a*:3,4-*b′*]diindole ring system have been reported.

The structure of fascaplysin was elucidated by NMR-spectroscopic and X-ray analyses [1]. A fully aromatic system was proposed due to ^{1}H- and ^{13}C-NMR spectra displaying signals in the aromatic region at δ 7.5–9.0 ppm for 10 H-atoms bound to sp^2 C-atoms and at δ 116–150 ppm for 17 C-atoms. Finally, the X-ray model confirmed the planarity of the molecule. We were interested in the chemical reactivity of fascaplysin when subjected to various reaction conditions. In this study, we describe the products formed under electrophilic aromatic substitution conditions, including halogenation, sulfonation, nitration, *Friedel-Crafts* alkylation, and acylation.

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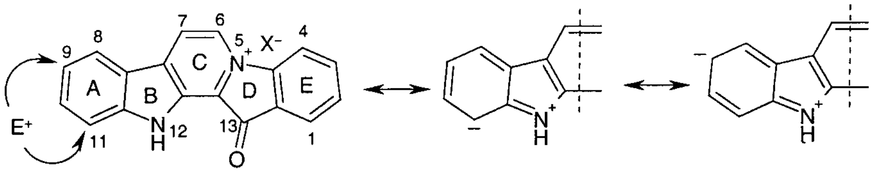
[1]) Parts of the results were presented at the annual meeting of the American Association of Cancer Research, April 1–5, 2000, in San Francisco, CA [4b].

Scheme 1. *Structure of Fascaplysin* (**1a**), *with Its Canonical Forms* **A** *and* **B**, *and Attacking Electrophile* E^+



1a $X^- = Cl^-$ (Fascaplysin)
1b $X^- = NO_3^-$

A

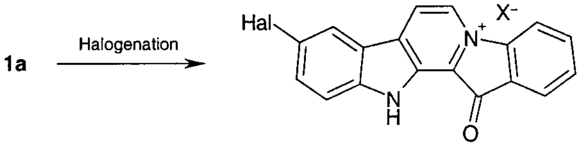
B

Results and Discussion. – Fascaplysin (**1a**) was assumed to undergo aromatic electrophilic substitutions predominantly at positions 9 or 11 of ring A (*Scheme 1*). The positive pyridinium atom N(5) is *meta*-directing to positions 1, 3 (ring E), and 7 (C), concomitantly deactivating positions 2, 4 (E), and C (6). The carbonyl group directs to the *meta* positions 2 and 4 (E), with a deactivating effect on positions 1 and 3 (E). The indole atom N(12), with a deactivating effect on position 7 (C), strongly activates *ortho* and *para* positions 11 and 9 (A). The canonical forms **A** and **B** also imply that aromatic electrophilic substitution would occur predominantly at positions 9 or 11. These considerations were further supported by *ab initio* calculations and by the observation that carbazoles undergo electrophilic substitutions at the 3-position [10].

Synthetic fascaplysin was used throughout the experiments described in this work. The material was obtained in a five-step synthesis according to the procedure of *Radchenko et al.* [6]. The main spectral characteristics (IR, UV, MS, ^{1}H- and ^{13}C-NMR) of synthesized fascaplysin **1a** and **1b** were identical to those published for the natural product [1].

Halogenation of **1a** could be accomplished under various reaction conditions. Complete conversion to the 9-bromo derivative **2** was easily achieved with Br_2 or *N*-bromosuccinimide (NBS) as brominating agents (*Scheme 2*). Two equivalents of NBS were required to drive the reaction to completion. Chlorination with Cl_2 or ^{t}BuOCl yielded exclusively **3**, whereas no reaction was observed with *N*-chlorosuccinimide

Scheme 2



2 Hal = Br
3 Hal = Cl

	Reaction conditons	Yield [%]
Bromination	Br_2, AcOH, 70°	41
	NBS, AcOH, 65°	65
Chlorination	Cl_2, AcOH, 70°	73
	t-BuOCl, AcOH, CH_2Cl_2, 40°	20[a])
	65% HNO_3 or $NO_2^+BF_4^-$, AcOH, 80°	42

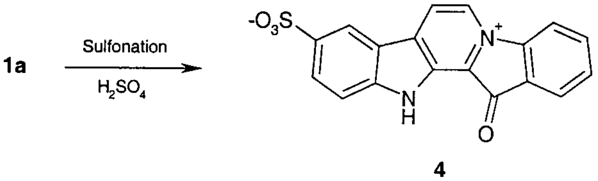
[a]) Transformation incomplete.

(NCS). Structural evidence for the substitution at C(9) in **2** and **3** was obtained by extensive NMR spectroscopy, including 1D-NOE, 2D-heteronuclear single-quantum coherence (HSQC), and 2D-heteronuclear multiple-bond coherence (HMBC) experiments.

The ^{1}H-NMR spectra of **2** and **3** contained nine aromatic proton signals and at δ 13.66 a D_2O-exchangeable NH proton for N(12). Irradiation of the NH signal induced an enhancement of the H–C(11) signal at δ 7.78 and 7.83, respectively, excluding a substituent at this position. In the ^{13}C-NMR spectrum, resonances were observed for nine quarternary and nine H-bearing C-atoms in the aromatic region from δ 115–147, and a single signal for the carbonyl C-atom at δ 180. Direct ^{1}H,^{13}C correlations were provided by HSQC experiments, whereas long-range ^{1}H,^{13}C correlations from HMBC experiments confirmed the proposed connectivities.

Sulfonation of **1a** was accomplished under classical conditions in conc. H_2SO_4, yielding zwitterionic 9-sulfofascaplysin **4** (*Scheme 3*). The compound was fully characterized, and the substitution pattern was confirmed by NMR spectroscopy as described for the halogen-substituted derivatives.

Scheme 3



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Fascaplysin (**1a**) could be nitrated with neither 65% HNO_3 nor 65% HNO_3 in conc. H_2SO_4 solution. Decomposition of the starting material was observed with 65% HNO_3 solution at elevated temperature without formation of a nitro derivative. A single fascaplysin derivative, substituted at C(9), was obtained under milder conditions with nitrating agent 65% HNO_3/AcOH or $NO_2^+BF_4^-$/AcOH. Eventually, the product was identified as 9-chlorofascaplysin (**3**). This material was shown to be identical with the product obtained under the previously described chlorination conditions (*Scheme 2*). We assume that the Cl^- ion of **1a** might be the Cl-source for this unprecedent conversion. To prevent chlorination, fascaplysin derivative **1b** containing NO_3^- as anion was prepared and subjected to nitrating conditions. Indeed, no nitration took place by means of any of the above-mentioned reaction conditions.

No acylation or alkylation products were observed under classical *Friedel-Crafts* reaction conditions, catalyzed either with $AlCl_3$ or proton acids. Obviously, an Al complex of fascaplysin was formed after treatment with $AlCl_3$. This complex was not further characterized; however, hydrolysis liberated unchanged fascaplysin.

Conclusions. – This work demonstrates that electrophilic aromatic substitutions, such as halogenation and sulfonation, occur primarily *para* to the indole N-atom at C(9) of the unique 12*H*-pyrido[1,2-*a*:3,4-*b*′]diindole ring system of fascaplysin. An unprecedented conversion to Cl-substituted fascaplysin was observed under nitrating conditions with its Cl^- form. No nitration took place under various nitrating conditions and no alkylated or acylated products were obtained by *Friedel-Crafts* reactions, which is probably attributable to the unusual aromatic ring system.

Experimental Part

1. G*eneral*. All reagents and solvents were commercially available and were used without further purification. The reactions were monitored and the products analyzed by reversed-phase HPLC: *Kontron* system, consisting of an autosampler model *465*, pump model *420*, detector model *430*; *Nucleosil C_{18}* column (250 × 4.6 mm, 5 µm, 100 Å; from *Macherey-Nagel*, Düren, FRG); linear gradient of 0.09% CF_3COOH/MeCN (*A*) and 0.1% CF_3COOH/H_2O (*B*) from 20 to 100% *B* within 20 min, flow rate 1 ml/min; detection at 216 nm. The products were isolated by reversed-phase MPLC: *Büchi B-688* pump, detector; *Merck Lichroprep RP-18* column (46 × 3.6 cm, 15 – 26 µm, from *Merck*, Darmstadt, FRG); gradient from 20 to 35% *B* within 45 min, flow rate 53 ml/min; detection at 216 nm. NMR Spectra (^{1}H, ^{13}C, NOE, HMBC, HSQC): *Bruker-DPX-400* spectrometer; at 300 K, (D_6)DMSO soln.; δ in ppm downfield from $SiMe_4$ with the residual solvent signal (δ(H) 2.49 and δ(C) 39.5 for (D_6)DMSO) as an internal standard; coupling constants *J* in Hz. Electrospray mass spectra (ESI-MS): *Fisons Instruments VG Platform II*; *m*/*z*.

2. *Halogenation of Fascaplysin*. 2.1. *Bromination*. 2.1.1. Br_2 (60 µl, 1.2 mmol) was added to a stirred suspension of fascaplysin (**1a**; 122 mg, 0.4 mmol) in AcOH (5 ml). The mixture was stirred for 18 h at 70° and then evaporated. The residue was subjected to MPLC purification: **2** ($X = CF_3COO$, 74 mg, 41%). Red solid.

2.1.2. A mixture of **1a** (76.5 mg, 0.25 mmol) and NBS (88.7 mg, 0.5 mmol) in AcOH (2 ml) was stirred for 18 h at 65°. Workup and purification as described in *2.1.1* yielded **2** ($X = CF_3COO$; 81 mg, 65%). Red solid.

2.1.3. *9-Bromo-12,13-dihydro-13-oxopyrido[1,2-*a*:3,4-*b*′]diindol-5-ium Trifluoroacetate* (**2**; $X = CF_3COO$). M.p. 186 – 190°. R_f (CH_2Cl_2/MeOH 4 : 1) 0.30. t_R 8.96 min, single peak (98.9%). ^{1}H-NMR (400 MHz, (D_6)DMSO): 13.66 (*s*, NH(12), exchangeable with D_2O); 9.67 (*d*, *J* = 6.3, H−C(6)); 9.19 (*d*, *J* = 6.3, H−C(7)); 8.90 (*d*, *J* = 2.0, H−C(8)); 8.47 (*d*, *J* = 8.2, H−C(4)); 8.09 (*d*, *J* = 7.4, H−C(1)); 8.04 (*dd*, *J* = 8.2, 7.4, H−C(3)); 8.03 (*dd*, *J* = 8.6, 2.0, H−C(10)); 7.78 (*d*, *J* = 8.6, H−C(11)); 7.76 (*dd*, *J* = 7.4, H−C(2)). ^{13}C-NMR (100.57 MHz, (D_6)DMSO): 182.1 (C(13)); 147.0 (C(4a)); 145.5 (C(11a)); 139.2 (C(7a)); 137.1 (C(3)); 136.5 (C(10)); 131.5 (C(2)); 131.2 (C(12a)); 126.9 (C(6)); 125.7 (C(1)); 124.0 (C(13a)); 126.8 (C(8)); 123.5 (C(12b)); 121.1 (C(7)); 121.1 (C(7b)); 115.7 (C(4)); 115.7 (C(11)); 114.9 (C(9)). 1D-NOE (400 MHz, (D_6)DMSO): irrad. at 13.66 → NOE at 7.78 (5%); irrad. at 9.67 → NOE at 9.19 (8%) and 8.47 (12%); irrad. at 9.19 → NOE at 9.67 (6%) and 8.90 (5%). ESI-MS (pos. mode): 349 and 351 (M^+).

2.2. *Chlorination*. 2.2.1. Cl_2 was bubbled through a stirred suspension of **1a** (76.5 mg, 0.25 mmol) in AcOH (2 ml). The mixture was stirred for 18 h at 70° in a sealed tube. The product was filtered off, washed with Et_2O, and dried: **3** ($X = Cl$; 62 mg, 73%). Red solid. M.p. > 200°.

2.2.2. A mixture of **1a** (76.5 mg, 0.25 mmol) and ^{t}BuOCl (34 µl, 0.3 mmol) in AcOH (2 ml) and CH_2Cl_2 was stirred for 64 h at 40° and then evaporated. The residue was subjected to MPLC purification: **3** ($X = CF_3COO$).

2.2.3. A stirred suspension of **1a** (122 mg, 0.4 mmol) in AcOH (4 ml) was treated either with 65% HNO_3 (90 µl, 2 mmol) or with $NO_2^+BF_4^-$ for 14 h at 80°. At r.t., the crude crystalline product was filtered off and subjected to MPLC purification: **3** ($X = CF_3COO$; 68 mg, 42%). Red solid, identical (m.p., IR, TLC, HPLC, MS, ^{1}H- and ^{13}C-NMR) with **3** ($X = CF_3COO$) from *Exper. 2.2.2.*

2.2.4. *9-Chloro-12,13-dihydro-13-oxopyrido[1,2-*a*:3,4-*b*′]diindol-5-ium Trifluoroacetate* (**3**, $X = CF_3COO$): M.p. 198 – 201°. R_f (CH_2Cl_2/MeOH 4 : 1) 0.28. t_R 7.79 min, single peak (100%). ^{1}H-NMR (400 MHz, (D_6)DMSO): 13.66 (*s*, 1 NH(12), exchangeable with D_2O); 9.67 (*d*, *J* = 6.2, H−C(6)); 9.19 (*d*, *J* = 6.2, H−C(7)); 8.76 (*d*, *J* = 1.8, H−C(8)); 8.48 (*d*, *J* = 8.1, H−C(4)); 8.09 (*d*, *J* = 7.4, H−C(1)); 8.04 (*dd*, *J* = 8.1, 7.7, H−C(3)); 7.92 (*dd*, *J* = 8.8, 1.8, H−C(10)); 7.83 (*d*, *J* = 8.8, H−C(11)); 7.76 (*dd*, *J* = 7.7, 7.4, H−C(2)). ^{13}C-NMR (100.57 MHz, (D_6)DMSO): 182.1 (C(13)); 147.0 (C(4a)); 145.3 (C(11a)); 139.3 (C(7a)); 137.1 (C(3)); 133.9 (C(10)); 131.5 (C(2)); 131.4 (C(12a)); 127.2 (C(9)); 126.8 (C(6)); 125.7 (C(1)); 124.0 (C(13a)); 123.7 (C(8)); 123.5 (C(12b)); 121.1 (C(7)); 120.6 (C(7b)); 115.7 (C(4)); 115.4 (C(11)). 1D-NOE (400 MHz, (D_6)DMSO): irrad. at 13.66 → NOE at 7.83 (5%); irrad. at 9.67 → NOE at 9.19 (8%) and 8.48 (13%); irrad. at 9.19 → NOE at 9.67 (6%) and 8.76 (5%). ESI-MS (pos. mode): 305 and 307 (M^+).

3. *Sulfonation of Fascaplysin. 12,13-Dihydro-13-oxopyrido[1,2-*a*:3,4-*b*′]diindol-5-ium-9-sulfonate* (**4**). Conc. H_2SO_4 (1 ml) was added to **1a** (123 mg, 0.4 mmol) at r.t., and stirring was continued for 6 h. The mixture was poured on ice and the precipitate filtered off, washed with H_2O, ^{i}PrOH, and hexane, and dried *in vacuo* at 40°: **4** (77 mg, 42%). Red solid. M.p. > 350°. t_R 4.54 min, single peak (100%). ^{1}H-NMR (400 MHz, (D_6)DMSO): 13.56 (*s*, NH(12), exchangeable with D_2O); 9.64 (*d*, *J* = 6.3, H−C(6)); 9.33 (*d*, *J* = 6.3, H−C(7)); 8.87 (*d*, *J* = 2.0,

H−C(8)); 8.48 (*d*, $J = 8.2$, H−C(4)); 8.14 (*dd*, $J = 8.6$, 2.0, H−C(10)); 8.07 (*d*, $J = 7.4$, H−C(1)); 8.02 (*dd*, $J =$ 8.2, 7.4, H−C(3)); 7.77 (*d*, $J = 8.6$, H−C(11)); 7.74 (*dd*, $J = 7.4$, H−C(2)). ^{13}C-NMR (100.57 MHz, (D_6)DMSO): 138.1 (C(3)); 133.4 (C(10)); 132.4 (C(2)); 127.7 (C(6)); 126.6 (C(1)); 122.3 (C(8)); 121.7 (C(7)); 116.6 (C(4)); 114.0 (C(11)). 1D-NOE (400 MHz, (D_6)DMSO): irrad. at 9.64 → NOE at 9.33 (4%) and 8.48 (9%); irrad. at 8.87 → NOE at 9.33 (4%). ESI-MS (pos. mode) 351 (M^+), 373 ($[M + \mathrm{Na}]^+$).

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