BUTYL-*META*-CYCLOHEPTYLPRODIGININE —A REVISION OF THE STRUCTURE OF THE FORMER *ORTHO*-ISOMER

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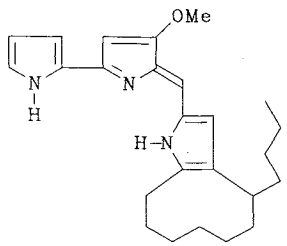
A red pigment produced by the actinomycete strain B 4358 was identified as butyl-*meta*-cycloheptylprodiginine (**4**) by ^{1}H, ^{13}C and correlation *via* long range coupling NMR spectra. It shows striking spectroscopic similarities to butyl-*ortho*-cycloheptylprodiginine (**1**), the structure of which needs to be revised.

Results and Discussion

The actinomycete strain B 4358, produces an antibiotic of unknown structure and a prodigiosin $C_{25}H_{33}N_3O$. So far three pigments of this formula have been described in the literature: butyl-*ortho*-cycloheptylprodigiosin[1~3] (**1**), ethyl-*meta*-cyclononylprodigiosin[4,5] (**2**, "*meta*-cycloprodigiosin"), and methylcyclodecylprodigiosin[6] (**3**). UV and IR spectra and the chromatographic behavior of the new pigment are similar to ethyl-*meta*-cyclononylprodigiosin (**2**), but ^{13}C NMR data in the aliphatic region correspond to those of butyl-*ortho*-cycloheptylprodiginine (**1**) only. In striking contrast to formula **1**, **2** or **3** is the ^{1}H NMR spectrum of the B 4358 prodigiosin by a multiplet at unusual high field (δ −1.55).



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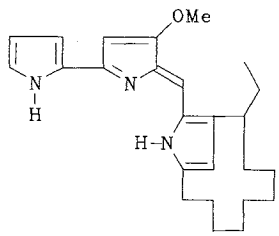

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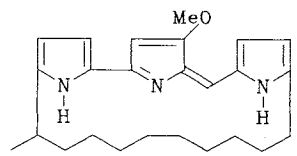

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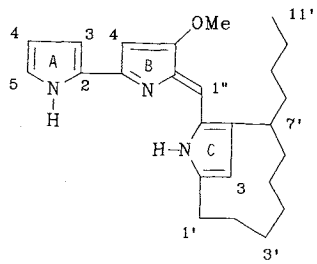

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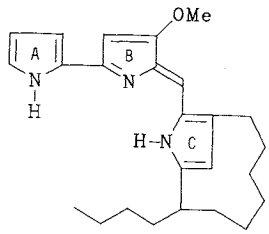

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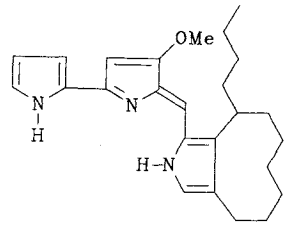

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Table 1. ^{1}H and ^{13}C NMR data of butylcycloheptylprodiginine (**4**) in $CDCl_3$.

	^{1}H (500 MHz)	^{13}C (50.3 MHz)		^{1}H (500 MHz)	^{13}C (50.3 MHz)
1_A	12.71	— —	1′	3.34, 2.54	29.90 t
2_A	—	122.39 s	2′	1.98～1.89,	31.56 t
3_A	6.90	116.57 d		1.42～1.32	
4_A	6.34	111.55 d	3′	1.82～1.76,	29.07 t
5_A	7.22	126.68 d		0.83～0.78	
1_B	12.58	— —	4′	1.89～1.11,	27.64 t
2_B	—	120.31 s		−1.55	
3_B	—	165.45 s	5′	1.70～1.50,	25.36 t
OCH_3	4.02	58.62 q		0.97～0.90	
4_B	6.10	92.71 d	6′	1.89～1.82,	30.88 t
5_B	—	147.05 s		1.70～1.50	
1″	7.12	112.54 d	7′	3.10	37.34 d
1_C	12.67	— —	8′	1.85～1.11,	38.85 t
2_C	—	154.73 s		1.76～1.69	
3_C	6.52	116.77 d	9′	1.42～1.32	30.55 t
4_C	—	150.63 s	10′	1.42～1.32	22.84 t
5_C	—	125.05 s	11′	0.97～0.90	14.08 q

δ in ppm relative to internal TMS; multiplicities by DEPT analysis.

Irradiation into the NH-signals reveals an aromatic three-spin system with coupling constants expected for a 2-monosubstituted pyrrole[7]. Thus, structure **3** is to be eliminated, as also shown by the aliphatic ^{13}C and ^{1}H NMR data clearly deviating from the literature[1],†. On irradiation three singlets which are clearly different only become sharper, they correspond to 1″-H, 4_B-H and a proton in ring C, if the spectroscopically proved methoxy group here also is localized at C-3_B like in all other prodigiosins. Therefore all aliphatic C-atoms must be attached to pyrrole-ring C like in **1** or **2**. Allyl-couplings from the aliphatics to the pyrrole ring are absent like in other prodiginines[8,9], and also the allylic coupling from 1″-H to 4_C-H expected for **1**; so the linkage of the aliphatics to the heterocycle remained uncertain.

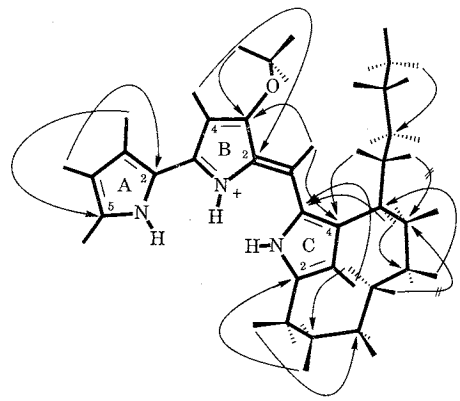
Six signals of tertiary and seven signals of quarternary pyrrole-C-atoms were indicated by the ^{13}C NMR-DEPT spectrum. C-3_B (δ 165.45), which was assigned by ^{3}J-coupling to the methoxy group, absorbs at lowest field. Shifts for the remaining 11 aliphatic signals correspond to the literature of **1**[9]. They can be assigned to 9 methylene, 1 methine and 1 methyl group by multiplet selection, CH-correlation and signal intensities. The methine proton at δ 3.10 shows only vicinal coupling to two methylene groups and therefore must be, as confirmed by the shift of the ^{13}C-signal (δ 37.34), bound to the aromatic ring C. 2D NMR ^{1}H, ^{1}H-correlation experiments revealed a sequence of six methylene units beginning at the first methylene group (C-6′) at δ 30.88, which closes the ring to the aromatic at the C-1′-methylene (δ 29.90). The second methylene group at the methine carbon (C-8′, δ 38.85) couples into a butyl side-chain as in **1**, excluding an aliphatic part as in structure **2**: also comparison with authentic **2** indicated this.

Especially striking in the aliphatic area of the ^{1}H NMR spectrum is the high-field signal at δ −1.55. According to 2D NMR ^{1}H,^{1}H-correlations it belongs to one of the diastereotopic C-4′-protons and is to be assigned to the methylene group just in the middle of the macrocycle. The extreme shift can only be explained by positioning the concerned proton directly into the anisotropy cone of pyrrole ring C. From

† Differences of the aromatic signals may be explained by the fact that we measured the hydrochloride, but GERBER had used the free base.

force field calculations† this is less plausible for C-4′ in **1** or **6**, and only to be expected for the isomers **4** and **5**. Also the anisochronism of the methylene signals of the aliphatic ring supports such a sterically fixed structure.

Fig. 1. Long range (^3J) CH-couplings in **4** from COLOC-experiments.



Further insight was obtained by long-range coupling NMR experiments (correlation *via* long range coupling: COLOC). The methine proton at δ 3.10 correlates with two quaternary pyrrole-C-atoms by ^2J- and ^3J-couplings. One of these C-atoms (δ 150.63) couples with a proton at δ 7.12 which shows a ^3J-coupling to C-3_B and is assigned to 1″-H. So the attachment of the aliphatic to C-4_C as well as the position of the butyl chain are clarified. Its *endo*-position relative to the *ansa*-system is finally elucidated by an Overhauser effect (Difference NOE) of the methoxy group to C-9′/C-10′. So, also structures **1** and **5** are to be excluded.

Whether the aliphatic ring ends at C-2_C, like in **4**, or the assignment of atoms C-2_C (δ 154.73) and C-3_C (δ 116.77) might be exchanged giving **6**, is neither shown by $^{13}C,^1H$-long-range-correlation, nor by the NH-coupling of the pyrrole H in ring C ($J=1.5$ Hz). From molecular orbital calculations however, a much smaller charge density is to be expected at C-2_C than at C-3_C resulting in a distinct low-field-shift of the ^{13}C NMR-signal of C-2_C. This and the multiplicity of the ^{13}C NMR-signals excludes **6**.

That the pigment from B 4358 indeed is butyl-*meta*-cycloheptylprodiginine (**4**), is also supported by the conformational stability of the *ansa*-system, which shows no signal-broadening up to 120°C (barrier > 17 kcal); for structure **1** or **6**, a considerably smaller barrier would be expected from molecular modelling. So the structure of the *ortho*-isomer **1** (butylcycloheptylprodiginine, streptorubin B[11]) described earlier by GERBER[1], which shows striking similarities to **4** in it's spectroscopic data, must be revised. GERBER came to the same result as formula **4** was mentioned some time ago[9], yet without any further discussion.

Experimental

IR spectra: Perkin-Elmer, model 297; (KBr); ^1H and ^{13}C NMR spectra, Varian XL 200, VXR 200, VXR 500 (TMS as internal standard), COLOC delay 30 and 60 mseconds. MS: low resolution, Varian MAT 311 A (70 eV); HR, Varian MAT 731 (peak-matching with perfluorokerosene; resolution 10,000); FAB: Finnigan 8200; matrix glycerol. UV spectra: Beckman DB-G (Beckman Instruments, München). Fermentation: Fermenter B. Braun Melsungen AG type 883720/1 721. TLC: Polygram SIL G/UV_{254}; Macherey-Nagel & Co. Preparative thick layer chromatography (PLC): a slurry of 55 g silica gel (silica gel P/UV_{254}; Macherey-Nagel & Co.) in 120 ml water was poured onto 20 × 40 cm glass plates, air-dried and activated by heating for 3 hours at 130°C. Column chromatography (CC): Silica gel 60 (0.05 ~ 0.2 mm; Macherey-Nagel & Co.).

Artificial seawater (add water to 1 liter): Ferrous citrate 0.1 g, NaCl 19.45 g, $MgCl_2 \cdot 6H_2O$ 8.8 g, Na_2SO_4 3.24 g (dissolve separately), $CaCl_2$ 1.8 g, Na_2HPO_4 0.008 g, sodium silicate solution 0.015 ml, solution A 1 ml, solution B 10 ml. Solution A (add water to 1 liter): H_3BO_3 0.611 g, $MnCl_2$ 0.389 g, $CuSO_4$

† Force field program PCModel and MMX calculations using ALLINGER's parameters[10].

0.056 g, $ZnSO_4 \cdot 7H_2O$ 0.056 g, $Al_2(SO_4)_3 \cdot 6H_2O$ 0.056 g, $NiSO_4 \cdot 6H_2O$ 0.056 g, $Co(NO_3)_3 \cdot 6H_2O$ 0.056 g, TiO_2 0.056 g, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.056 g, LiCl 0.028 g, $SnCl_2$ 0.028 g, KI 0.028 g. Solution B (add water to 1 liter): KCl 55 g, $NaHCO_3$ 16 g, KBr 8 g, $SrCl_2 \cdot 6H_2O$ 3.4 g (dissolve separately), H_3BO_3 2.2 g, NaF 0.24 g, NH_4NO_3 0.16 g. Fermentation medium (before sterilization adjusted to pH 7.8 with 2 N NaOH): Malt extract (Difco) 10 g, glucose 4 g, yeast extract (Difco) 4 g, seawater 500 ml, water 500 ml.

Actinomycete B 4358

The actinomycete strain B 4358 has been isolated from a glacier near Longyearbyen, Spitzbergen, using cellulose medium[12] containing 25% natural seawater with incubation at 18°C. The pure culture was maintained an yeast extract-malt extract medium[13]. The strain forms extensive grey aerial mycelia, with spiral spore chains. Spores are oval having a smooth surface. A wide range of organic compounds can be utilized by the strain as sole sources of carbon for energy and growth and a number of biopolymers can be degraded. The strain is susceptible to lysozyme. B 4358 exhibits antibiotic activity against a variety of Gram-positive and Gram-negative bacteria as well as against fungi. Red soluble pigment is formed especially in media containing colloidal chitin[13]. The cell wall of B 4358 contains *meso*-diaminopimelic acid (*meso*-DAP), arabinose, and galactose (chemotype IV) but lacking mycolic acids. Due to its chemical and morphological features B 4358 can be assigned to the suprageneric group *Micropolyspora*. Additional studies are needed to establish its generic status. The taxonomic description will be given in a separate paper.

Fermentation

The fermenter was sterilized with 40-liter fermentation medium *in situ* for 30 minutes at 120°C. After cooling, it was inoculated with 4 liters of a 60-hour old shaking-culture (24 × 0.2 liter fermentation medium in 1-liter Erlenmeyer flasks) and incubated at 28°C, 200 rpm and 3 m^3 air/hour for 60 hours. Foam was suppressed by Niax solution (10%) in EtOH.

Isolation

The mycelium was separated by centrifugation, suspended in 0.6 liter acetone-EtOAc (1:1), homogenized with an Ultraturrax and filtered by aid of Celite. The filtrate was concentrated *in vacuo* to the aqueous residue, and extracted three times with EtOAc. On evaporation, 6 g mycelial extract were obtained. CC of 1.26 g extract (column 26 × 600 mm: EtOAc) yielded crude **4** from the red main zone, which was further purified by CC on acidic silica gel (0.1 N HCl; 40 × 300 mm; EtOAc) and PLC (3 plates 20 × 20 cm; CH_2Cl_2-MeOH, 97:3). Addition of pentane to a concentrated solution in CH_2Cl_2 gave 11.5 mg **4**-hydrochloride as red brown to black, metallic glistening crystals. IR (KBr) cm^{-1} 2935, 2865, 1627, 1604, 1550, 1518, 1458, 1417, 1379, 1265, 1250, 1230, 1145, 1136, 1038, 964. MS *m/z* 392 (100%, $(M+1)^+$), 376 (5, $M-CH_3$), 348 (7, $M-CH_3-C_2H_2$). UV $\lambda_{max}^{EtOH-HCl}$ nm (ε) 293 (3.84), 3.61 (3.72), 510 (4.57), 537 (4.92), $\lambda_{max}^{CHCl_3,qual.}$ nm 514, 544. ^1H NMR ($CDCl_3$, 500 MHz): δ 12.71 (1H, br s, N-H_A), 12.67 (1H, br s, N-H_C), 12.58 (1H, br s, N-H_B), 7.22 (1H, ddd, $^3J=3$ Hz, $^4J=1.5$ Hz, $^3J=1.7$ Hz, 5_A-H), 7.12 (1H, s, 1″-H), 6.90 (1H, ddd, $^3J=3.5$ Hz, $^4J=1.5$ Hz, $^4J=2$ Hz, 3_A-H), 6.52 (1H, d, $^4J=1.5$ Hz, 3_C-H), 6.34 (1H, ddd, $^3J=3.5$ Hz, $^4J=2$ Hz, $^3J=3$ Hz, 4_A-H), 6.10 (1H, $^4J=2$ Hz, 4_B-H), 4.02 (3H, s, OCH_3), 3.34 (1H, m, 1′-H), 3.10 (1H, m, 7′-H), 2.54 (1H, m, 1′-H), 1.98~1.89 (1H, m, 2′-H), 1.89~1.82 (1H, m, 6′-H), 1.89~1.11 (1H, m, 4′-H), 1.85~1.11 (1H, m, 8′-H), 1.82~1.76 (1H, m, 3′-H), 1.76~1.69 (1H, m, 8′-H), 1.70~1.50 (2H, m, 5′-H, 6′-H), 1.42~1.32 (5H, m, 2′-H, 9′-CH_2, 10′-CH_2), 0.97~0.90 (4H, m, 5′-H, 11′-CH_3), 0.83~0.78 (1H, m, 3′-H), −1.55 (1H, m, 4′-H). COLOC NMR ($CDCl_3$, delay 50 mseconds, 500 MHz) δ 7.22 (5_A-H, ^3J→C-2_A), 7.12 (1″-H, ^3J→C-3_B and ^3J→C-4_C), 6.90 (3_A-H, ^3J→C-5_A and ^2J→C-2_A), 6.52 (3_C-H, ^3J→C-5_C, ^2J→C-4_C and ^2J→C-2_C), 6.34 (4_A-H, ^3J→C-2_A and ^2J→C-5_A), 6.10 (4_B-H, ^3J→C-2_B and ^2J→C-5_B), 4.02 (OCH_3, ^3J→C-3_B), 3.34 (1′-H, ^3J→C-3′, ^2J→C-2′ and ^2J→C-2_C), 3.10 (7′-H, ^3J→C-5_C and ^2J→C-4_C), 1.92 (2′-H, ^3J→C-2_C), 1.70 (8′-H, ^3J→C-4_C and ^2J→C-7′), 0.92 (11′-H, ^2J→C-10′), 0.92 (11′-H, ^3J→C-9′ or 5′-H and ^2J→C-4′). COLOC NMR ($CDCl_3$, delay 30 mseconds 500 MHz). δ 3.10 (7′-H, ^3J→C-5′, ^2J→C-6′ and ^2J→C-6′), 1.35 (9′-H, ^2J→C-10′), 1.35 (10′-H, ^2J→C-9′), 1.15 (8′-*H*, ^3J→C-6′ or 8′-H, ^2J→C-9′ or 4′-H and ^3J→C-4′), 1.15 (8′-H, ^2J→C-7′ or 4′-H and ^4J→C-7′), 0.90 (11′-H, ^4J→C-8′), −1.55 (4′-H, ^3J→C-2′). Calcd 391.26236, found 391.2624 (MS).

Acknowledgments

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