Synthesis of a New Polycyclic Quinone by Reduction of a Dihydrobenzo[a]naphthacenequinone

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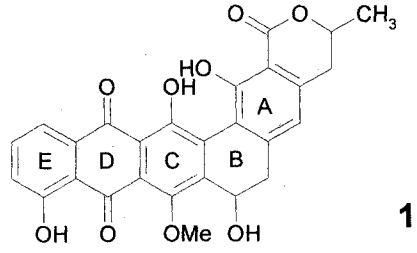
Presently, more than 20 compounds have been reported containing the dihydrobenzo[a]naphthacenequinone skeleton such as the pradimicins[1], benanomicins[2], WS79089-compounds[3], ericamycin[4] and others. Previously we isolated benaphthamycin and WS79089A (**1**) from *Streptomyces* sp. HKI-0057[5]. During our chemical derivatization program for naturally occurring compounds we subjected **1** to a series of reduction procedures using complex hydrides ($NaBH_4$, $NaAlH_4$, $LiAlH_4$, $(t\text{-Butyl})_2AlH$) and catalytic hydrogenation (Pt/H_2, Pd/H_2). The initial aim was to open the lactone ring and to introduce an aldehyde or a primary alcohol structure at C-1. With the exception of $NaAlH_4$ the above reductions furnished mixture of products. However, reduction by $NaAlH_4$ in excess and subsequent hydrolysis under acidic conditions afforded a violet compound showing a lower Rf on TLC than **1**. The product was purified in 30 % yield by preparative TLC, and its structure was unambiguously assigned by mass spectrometry and NMR spectroscopy (**2**; Fig. 1).

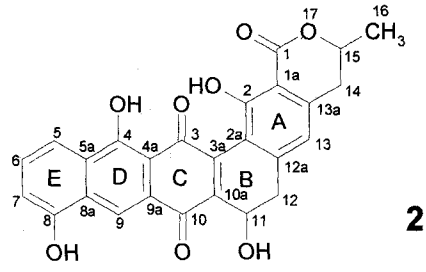
A molecular weight of 458 and the formula $C_{26}H_{18}O_8$ were readily suggested by HREI-MS (*m/z* 440.0908 ($[M-H_2O]^+$; calcd. 440.0913) and ESI-MS (*m/z* 457.3 $[M-H]^-$). The ^1H NMR spectrum displayed signals of a methyl group (H-16, 1.45 ppm, d), two methylene (H-12, 2.86 ppm; H-14, 3.01 and 3.12 ppm) and two methine groups (H-11, 5.08 ppm; H-15, 4.85 ppm). In addition, six aromatic protons were visible. Two appeared as singlets (H-9, 8.41 ppm; H-13, 6.92 ppm). The others (H-5, 7.81 ppm; H-6, 7.55 ppm; H-7, 7.2 ppm) displayed the typical coupling pattern of three neighboured aromatic protons. Signals at 11.0, 11.8, 13.2 and 5.22 ppm were attributable to the hydroxyl protons. The ^{13}C NMR spectrum showed 26 signals, attributable to the quinone (186.3 and 181.0 ppm) and lactone carbonyls (169.6 ppm), three phenolic (158.7, 159.6, 155.9 ppm), two aliphatic oxygen-bonded (57.5 and 76.2 ppm) and a series of aromatic and aliphatic carbons (see Experimental). For the assignment of the quaternary carbons the C, H long-range coupled NMR spectra (HMBC) were particularly helpful. Thus, the visible ^2J and $^3J_{C,H}$ long-range correlations (Fig. 2) and weak $^4J_{C,H}$ correlations between H-9 and C-2, and H-13 and C-1 supplied supporting evidence.

In comparison with **1** the new quinone structure **2** (2,4,8,11-tetra-hydroxy-15-methyl-11,12,14,15-tetrahydro-17-oxa-hexaphene-1,3,10-trione) harbours the quinone in the C ring instead in D ring. Moreover, during $NaAlH_4$ reduction and subsequent acidification under aerobic conditions one molecule of methanol was eliminated. The mechanism of this reductive rearrangement-elimination reaction is presently under discussion. It is remarkable that solely $NaAlH_4$ provoked the formation of **2**. Moreover, the observed rearrangement reaction was not observed when madurahydroxylactone was used as another representative of the dihydrobenzo[a]naphthacenequinones[6]. Compound **2** displayed moderate activity against Gram-positive bacteria

Fig. 1. Chemical constitutions of WS79089 A (**1**) and new quinone (**2**).



1



2

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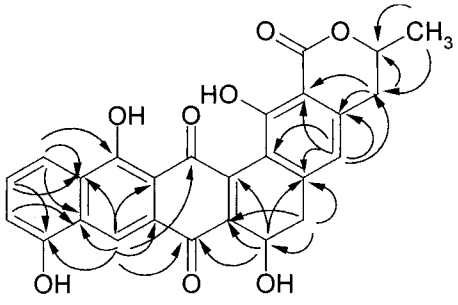
Fig. 2. Observable C, H long-range correlations in the HMBC spectrum of **2**.



such as *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* SG511 during standardized agar diffusion assay[7]. Thus 50 μg **2** dissolved in 50 μl methanol afforded inhibition zones with 16 mm and 13 mm diameter, respectively. No activity was found against Gram-negative bacteria and fungi. The IC_{50} values of **2** were determined with 72 hours L929, K562, and HeLa cells (IC_{50}) to amount to 1.9, 1.7 and 5.4 μg/ml, respectively. Comparable biological activities were found with WS79089A (**1**)[3].

Experimental

Mass spectra were recorded with a high-resolution sector-field spectrometer AMD 402 (AMD Intectra, Bremen, Germany) and triple-quadrupole instrument Quattro equipped with an electrospray ion source (VG Biotech, Altrincham, England). 1D and 2D NMR spectra (^{1}H, ^{13}C, ^{1}H, ^{1}H-COSY, HSQC, NOESY, HMBC) were recorded with a 500 MHz NMR spectrometer Bruker Avance DRX 5000. Determination of cytotoxicity using the analyzer system CASY 1 was described earlier[8].

Synthesis of **2**

20 mg **1** dissolved in 50 ml dry tetrahydrofurane was stirred in a 100 ml three-necked vessel under nitrogen. The solution was cooled to −78°C, and 50 mg $NaAlH_4$ was added in small portions. After 5~6 hours stirring at −78°C the reaction mixture was added to a concentrated aqueous solution of $NaHSO_3$ and, thereafter, the pH was adjusted to 1.0. Subsequently the mixture was extracted with diethylether, the ether phase dried over Na_2SO_4 and concentrated *in vacuo*.

The residue was chromatographed on silica gel plates (Merck, $CHCl_3$/MeOH, 97 : 3). In addition to the starting material running with Rf 0.8, a violet zone with Rf 0.35 was visible. It was extracted with $CHCl_3$/MeOH (1 : 1) and the solution was evaporated. Yield: 7.5 mg (30%), violet solid, mp. 250~252°C (decomposition).

HREI-MS: *m/z* 440.0908 ($[M-H_2O]^+$, calcd. 440.0913 for $C_{26}H_{16}O_7$). ^{1}H NMR (500 MHz in DMSO-d_6, δ in ppm, TMS as internal standard): 1.45 (H-16, d, 7.0 Hz), 2.86 (H-12, dd, 16.0 Hz, 3.0 Hz), 3.00 (H-14_A, d, 2.5 Hz), 3.12 (H-14_B, d, 2.5 Hz), 4.85 (H-15, m), 5.08 (H-11, d, t, br), 7.2 (H-7, d, 8.2 Hz), 7.55 (H-6, dd, 8.3 Hz, 8.2 Hz), 7.81 (H-5, d, 8.3 Hz), 8.41 (H-9, s), 5.22 (11-OH, br), 11.0 (8-OH, s), 11.8 (2-OH), 13.2 (4-OH). ^{13}C NMR (125 MHz, δ in ppm, in DMSO-d_6, TMS as internal standard): 20.2 (C-16), 33.5 (C-14), 37.1 (C-12), 57.8 (C-11), 76.2 (C-15), 107.2 (C-13a), 109.0 (C-4a), 114.3 (C-5), 114.4 (C-7), 115.1 (C-9), 116.0 (C-2a), 119.5 (C-13), 125.9 (C-9a), 125.9 (C-8a), 128.5 (C-5a), 130.5 (C-6), 140.7 (C-3a), 142.8 (C-1a), 144.6 (C-10a), 147.0 (C-12a), 155.9 (C-9), 158.7 (C-2), 159.6 (C-4), 169.6 (C-1), 181.0 (C-10), 186.3 (C-3).

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