THE NEW CYTOTOXIC ANTIBIOTIC CYTORHODIN X, AN UNUSUAL ANTHRACYCLINONE-9α-GLYCOSIDE

U. HEDTMANN[1], H.-W. FEHLHABER[1], D. A. SUKATSCH[1], M. WEBER[1], D. HOFFMANN[2] and H. P. KRAEMER[2]

[1] Pharma Research, Hoechst AG, P.B. 800320, D-6230 Frankfurt 80, Federal Republic of Germany
[2] Research Laboratories of Behringwerke AG, D-3550 Marburg/Lahn, Federal Republic of Germany

(Received for publication January 6, 1992)

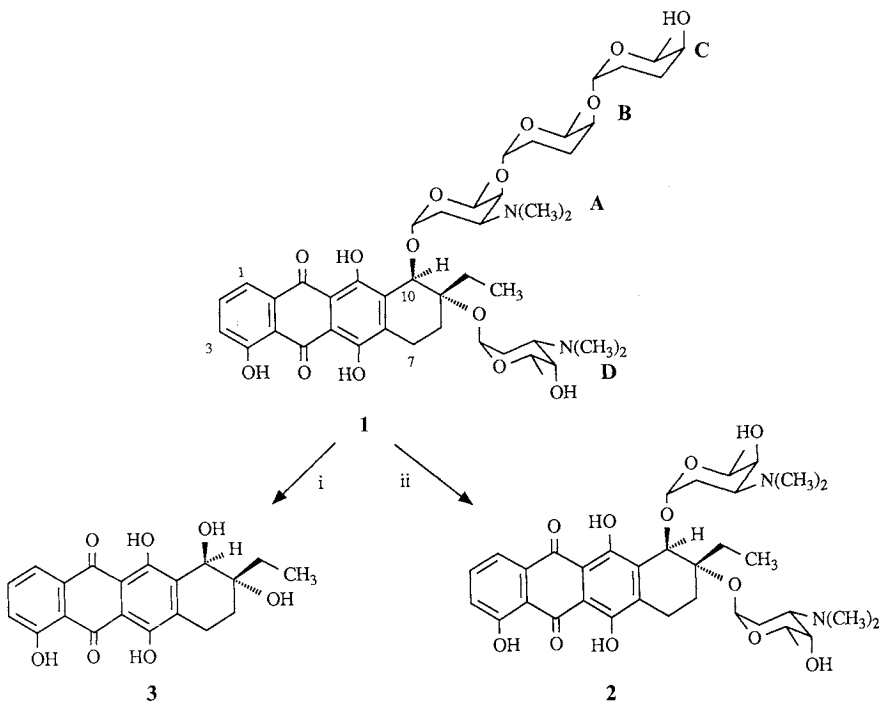
From our screening program for novel antitumor antibiotics several anthracyclines (cytorhodins A~W) belonging to the rhodomycin-group have been isolated from the *Streptomyces* species HPL Y-11472 (DSM 2658)[1~3].

Very recently a new cytotoxic compound was isolated from this strain, which was found to have a new and unusual structure, and which we named cytorhodin X[4]. Based on NMR, mass spectral analysis and chemical degradation cytorhodin X was identified as a heretofore unknown γ-rhodomycinone glycoside containing a common trisaccharide chain at C-10 and an additional rhodosamine unit at C-9 (**1**). Compound **1** and its partial hydrolysis product **2** inhibited the growth of L1210 murine leukemia cells showing ED_{50} values of 0.36 μM and 0.51 μM, respectively. Here, we report the isolation procedure and structure elucidation of this new 9α-glycoside of the γ-rhodomycin-group and its hydrolysis products. The biological properties of the new compounds and the remarkable high-field shift of some sugar proton signals in the ^1H NMR spectrum of **1** are also briefly discussed.

Streptomyces species, HPL Y-11472 (DSM 2658), was fermented in 1,800 liters of medium containing (g per liter H_2O) starch sugar (40), yeast extract (4), glucose syrup (2), and KNO_3 (0.5), in a 2,000-liter fermenter at 26°C. The fermentation time was about 160~170 hours, depending on the course of fermentation. Antibiotic activity was detected in both mycelium and extracellular medium.

A complex cytorhodin mixture was isolated by the following steps: The mycelial cake (41 kg)

Scheme 1. Cytorhodin X and its hydrolysis products.



i: 6 N H_2SO_4, 90°C, 2 hours; ii: 0.1 N HCl, 23°C, 2 hours.

obtained by filtration was extracted with acetone, the extract was concentrated *in vacuo* and the resulting aqueous residue lyophilized. The culture filtrate (about 1,750 liters) was adjusted to pH 7.5, extracted with ethyl acetate, and the organic layer evaporated *in vacuo*. Both dried residues were combined (230 g).

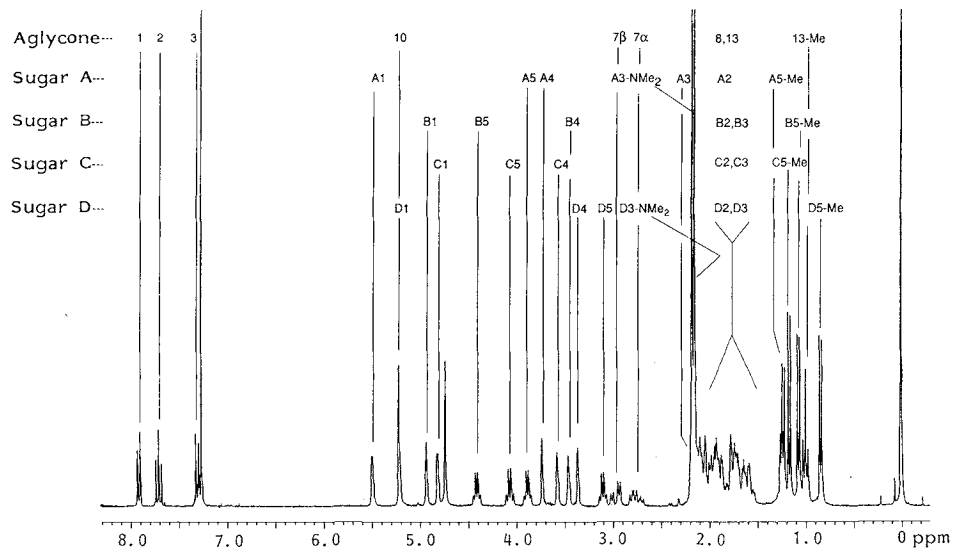
A portion of this material (120 g) was dissolved in sodium acetate buffer, pH 3.5, and extracted with ethyl acetate. The aqueous phase was adjusted to pH 7.0 and to pH 8.5, respectively, and extracted with ethyl acetate in each case. The organic layers were evaporated *in vacuo* to yield three fractions (70, 22 and 14 g, respectively) with different contents of cytorhodins. Isolation of cytorhodin X was achieved by chromatography of 4.8 g of the pH 7.0 extract, using a reverse phase column (LiChroprep RP-18, 1.21 column volume, elution with methanol - pH 7.0 phosphate buffer (93 : 7)) to give a semipure material in 40% yield, which was further purified by silica gel chromatography ($CHCl_3$ - MeOH - glacial acetic acid - water (50 : 25 : 7 : 4)) to afford 660 mg pure cytorhodin X (**1**), purity > 97% by HPLC.

Compound **1** was obtained as an orange powder, the molecular formula ($C_{48}H_{68}N_2O_{15}$) of which was determined by high-resolution FAB mass spectrometry ($(M+H)^+$ found *m/z* 913.4695, calcd 913.4698). Its spectral data are as follows: IR ν_{max} ($CHCl_3$) cm^{-1} 1605, 1440, 995; UV λ_{max} (MeOH - 1 N HCl (9 : 1)) nm (log ε) 236 (4.57), 256 (4.45), 296 (3.79), 493 (4.22), 526 (4.03); Rf 0.50 (silica gel, $CHCl_3$ - MeOH - glacial acetic acid - water (75 : 16 : 10 : 7)). The ^1H NMR spectrum is shown in Fig. 1.

Using weak hydrolysis conditions (0.1 N HCl, 23°C, 2 hours) we were able to isolate from **1** the partial hydrolysis product **2**, the molecular formula of which was determined as $C_{36}H_{48}N_2O_{11}$ (calcd for $(M+H)^+$ 685.3336, found 685.3340 by HR-FAB-MS). Total hydrolysis (6 N H_2SO_4, 90°C, 2 hours) of **1** gave the aglycone **3**, which was identified as γ-rhodomycinone† by ^1H NMR and HPLC comparison with an authentic sample[5,6]. Spectral data of compound **2**: IR ν_{max} ($CDCl_3$) cm^{-1} 1600, 1440, 995; UV λ_{max} (MeOH - 1 N HCl (9 : 1)) nm (log ε) 236 (4.54), 255 (4.44), 295 (3.87), 493 (4.19), 526 (4.03); Rf 0.27 (silica gel, $CHCl_3$ - MeOH - glacial acetic acid - water (75 : 16 : 10 : 7)). ^1H NMR δ ($CHCl_3$, TMS) 0.83 (3H, d, J=6 Hz, D5-CH_3), 1.01 (3H, t, J=8 Hz, CH_3 (ethyl)), 1.35 (3H, d, J=7 Hz, A5-CH_3), 1.53 to 2.39 (10H, m, 4 × CH_2 and 2 × CH), 2.14 (6H, s, $N(CH_3)_2$), 2.19 (6H, s, $N(CH_3)_2$), 2.55 (1H, m, 7α-H), 2.97 (1H, m, 7β-H), 3.09 (1H, m, D5-H), 3.36 (1H, m, D4-H), 3.64 (1H, m, A4-H), 3.91 (1H, m, A5-H), 5.22 (2H, m, 10-H and D1-H), 5.49 (1H, m, A1-H), 7.31 (1H, d, J=8 Hz, 3-H), 7.71 (1H, pseudo-t, J=8 Hz, 2-H), 7.91 (1H, d, J=8 Hz, 1-H).

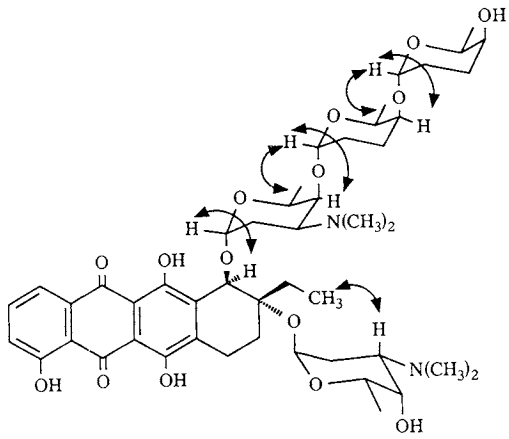
Fig. 1. ^1H NMR spectrum of cytorhodin X.

400 MHz, 0.5% solution in $CDCl_3$, shaken with a 5% Na_2CO_3 solution in D_2O.



† A sample of γ-rhodomycinone was kindly provided by Dr. H. G. BERSCHEID, Hoechst AG.

Scheme 2. NOE's observed in **1**.



Number, type and sequences of the hexoses in **1** were determined from NMR data (H, H COSY; NOE) and mass spectrometric fragmentation. The molecular formula, NMR data and mass fragmentation showed the presence of two rhodosamine (RhN) and two rhodinose moieties (Rh). A fragment ion *m/z* 685, corresponding to γ-rhodomycinone plus two rhodosamine units, indicated the presence of two sugar chains, each linked to the aglycone by rhodosamine. The complete sugar sequences were determined by NOE to be RhN-Rh-Rh and RhN, respectively (NOE's are shown in Scheme 2). The absolute configurations of the sugar components were not determined. However, all sugar components are presumed to have the same configuration (L-) as that of the hexoses of the known anthracycline antibiotics[7].

Fig. 1 shows the ^{1}H NMR spectrum of cytorhodin X together with the complete signal assignments. Interestingly, most of the 9α-rhodosaminyl signals are shifted to higher field as compared to the signals of the rhodosamine moiety linked to C-10. This could indicate that the 9α-RhN unit lies behind the plane of the aromatic nucleus and mainly 5-H, 4-H and 5-CH_3 of this sugar are in the anisotropic region. The same effect can be seen in the ^{1}H NMR spectrum of compound **2**.

Cytorhodin X and compound **2** show cytotoxic activity as determined by a proliferation assay using L1210 cells. The results are summarized in Table 1. The ED_{50}'s of **1** and **2** are almost comparable whereas the acute intravenous toxicity in mice after single application appeared to be lower in case of compound **2**.

Table 1. Cytotoxic activity of **1** and **2***.

	ED_{50} (μM)	LD_{50} (mg/kg)
1	0.36	10～25
2	0.51	25～50
Doxorubicin	0.036	10

* Determined using L1210 cells from ascites of DBA/2 mice (strain purchased from Flow Laboratories, Irvine, Ayrshire, Scotland).

Acknowledgments

We thank Dr. H. G. BERSCHEID for several reference samples and for helpful discussions, and Dr. E. EHLERS for large scale work-up.

References

1) REDDY, G. C. S.; R. SAHAI, H. W. FEHLHABER & B. N. GANGULI: Isolation and structure of a new ε-rhodomycin compound produced by a *Streptomyces* species HPL Y-11472. J. Antibiotics 38: 1423～1425, 1985
2) BERSCHEID, H. G.; W. ARETZ, D. BÖTTGER, H.-W. FEHLHABER, G. HUBER, B. N. GANGULI, J. GHANDI, R. S. SOOD, R. SAHAI, G. C. S. REDDY, K. R. DESIKAN & H. P. KRAEMER: Cytorhodins, novel anthracycline antibiotics. I. Taxonomy, fermentation, production, biological properties, and structure characterization of aglycones. J. Antibiotics, in preparation.
3) ARETZ, W.; H. G. BERSCHEID, G. HUBER, H.-W. FEHLHABER, H. P. KRAEMER, H. H. SEDLACEK, B. N. GANGULI, R. S. SOOD, J. GANDHI & G. C. REDDY (Hoechst AG): Microbiological production of anthracycline derivatives, and their cytostatic use. EP 0 131 181, Mar. 7, 1990 [U.S. 4,713,371]
4) HEDTMANN, U.; H.-W. FEHLHABER, D.-A. SUKATSCH, H. P. KRAEMER & D. HOFFMANN (Hoechst AG): Neue Anthracyclin Derivate, ein Verfahren zu ihrer Herstellung und ihre Verwendung als Cytostatika. EP 0 403 963, Dec. 27, 1990 [Jpn. Kokai 43093 ('91), Feb. 25, 1991]
5) BROCKMANN, H. & K. BAUER: Rhodomycin, ein rotes Antibioticum aus Actinomyceten. Naturwissenschaften 37: 492～493, 1950
6) MATSUZAWA, Y.; A. YOSHIMOTO, T. OKI, T. INUI, T. TAKEUCHI & H. UMEZAWA: New anthracycline antibiotics. Roseorubicins A and B. J. Antibiotics 32: 420～424, 1979
7) STRAUSS, D. G.: Strukturen der Anthracyclin-Tumoristatica. Pharmazie 42: 289～300, 1987