# Cytostatic Activity of Aeroplysinin-1 against Lymphoma and Epithelioma Cells

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(±)-Aeroplysinin-1, an optically active 1,2-dihydroarene-1,2-diol, was isolated from the marine sponges *Verongia aerophoba* (+-isomer) and *Ianthella ardis* (−-isomer). For the experiments presented we used the +-isomer from *Verongia aerophoba*. Here we describe the hitherto unknown biological and pharmacological property of this compound to display pronounced anticancer activity against L5178y mouse lymphoma cells ($ED_{50}$: 0.5 μM), Friend erythroleukemia cells ($ED_{50}$: 0.7 μM), human mamma carcinoma cells ($ED_{50}$: 0.3 μM) and human colon carcinoma cells ($ED_{50}$: 3.0 μM) *in vitro*. Furthermore, aeroplysinin caused a preferential inhibition of [$^3$H]thymidine (dThd) incorporation rates in L5178y mouse lymphoma cells if compared with murine spleen lymphocytes *in vitro*. At concentrations between 1.1 and 28.5 μM, the [$^3$H]dThd incorporation rates in L5178y cells were suppressed to 28%–0% but only to 78%–18% in murine spleen lymphocytes. The same differential effect *in vitro* was found with the following epithelial cells: 14.70 μM of the compound were required to inhibit normal human fibroblasts to 50%, but only 2.9 μM in the assays with human malign keratinocytes or malignant melanoma cells to observe the same inhibitory effect. Moreover, aeroplysinin-1 displayed antileukemic activity *in vivo* using the L5178y cell/NMRI mouse system; administered at a dose of 50 mg/kg for five consecutive days, the T/C (%) value was determined to be 338. Preliminary toxicology studies revealed an acute $LD_{50}$ of 202 mg/kg and a subacute $LD_{50}$ of 150 mg/kg. Aeroplysinin-1 is neither a direct mutagen nor a premutagen in the *umu*/*Salmonella typhimurium* test system.

## Introduction

Aeroplysinin-1, an 1,2-dihydroarene-1,2-diol with the chemical structure of [1-(3′,5′-dibromo-1′,2′-dihydroxy-4′-methoxycyclohexa, 3′,5′-dien-1′-yl)-methylcyanide] (Fig. 1) is a naturally occurring tyrosine metabolite from the marine sponge *Verongia aerophoba* [1–3]. This compound has been described to display at a concentration of 20–100 μg/ml some antibacterial activity *in vitro* [1, 3]; administered *in vivo* at a dose of 128 mg/kg the compound did not protect mice against bacterial infections [3].

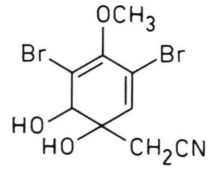


Fig. 1. Structure of aeroplysinin-1.

Now we describe that aeroplysinin-1 displays a pronounced cytostatic activity against a series of transformed cells, but not against the related normal cells *in vitro*. Moreover, we show that aeroplysinin-1 has an antileukemic activity in the L5178y cell/NMRI mouse system *in vivo*.

*Abbreviations:* ConA, concanavalin A; dThd, thymidine; $ED_{50}$, concentration which causes a 50% inhibition of cell growth *in vitro* when compared with the controls; ILS, increase in median life span; $LD_{10}$, 10% lethal dose; $LD_{50}$, 50% lethal dose; LPS, lipopolysaccharide; M.D.D., median day of death.

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## Materials and Methods

Concanavalin A [ConA] (No. C5275), lipopolysaccharide [LPS] (No. L4130), benzo[a]pyrene and 4-nitroquinoline-1-oxide were obtained from Sigma, St. Louis, Mo. (U.S.A.); [6-$^3$H]thymidine [dThd] (spec. activity 15 Ci/mmol) from New England Nuclear, Boston, Mass. (U.S.A.).

### *Aeroplysinin-1*

The compound (mol. wt. 339.6; ref. [3]) was isolated from the sponge *Verongia aerophoba* collected in the Bay of Kotor (Yugoslavia) as follows. The fresh sponge (1 kg) was homogenized in a Waring Blender in the presence of acetone, evaporated at 50 °C under reduced pressure. The aqueous material obtained was extracted three times with diethylether. The organic extracts were collected and evaporated. The oil-like residue obtained was chromatographed on a column of silica gel [1]. After recrystallization for two times from chloroform–diethylether (1:1) 1.5 g of aeroplysinin-1 was obtained from 100 g of dried material. The material was spectroscopically (ultra-violet, infra-red) and chromatographically (thin layer chromatography) pure. The identity with the described compound [1–3] was established on the basis of spectroscopic examinations ($^1$H NMR; $^{13}$C NMR; MS [E/I Cs Fab], UV and IR).

For the *in vitro* tests, the compound was dissolved in 0.1% dimethyl sulfoxide (final concentration); at this concentration dimethyl sulfoxide had no influence on cell growth. For the application *in vivo* aeroplysinin-1 was suspended in methylcellulose [4].

### *Cell culture*

L5178y mouse lymphoma cells [5] were grown in Eagle's minimum essential medium supplemented with 10% horse serum in roller tube cultures [6]. For the dose-response experiments, 5-ml cultures were initiated by inoculation of $5 \times 10^3$ cells/ml and were incubated at 37 °C for 48 or 72 h; the controls showed a population doubling time of 10.5 h. The cell growth was determined by cell count with a Cytocomp counter (128-channel counter, system Michaelis; Mainz, West Germany) with a 32-channel size-distribution plotter; calibration of the counter was performed with paper mulberry pollen (diameter: 13.5 µm; Hollister-Stier, Lab.; Coulter). The volume distribution of L5178y cells for treated and untreated cultures was determined as described [7]. For the determination of the effect of aeroplysinin-1 on the [$^3$H]dThd incorporation into DNA, 5-ml suspensions of exponentially growing cells at 100,000 cells/ml were treated for 24 h with the compound. [$^3$H]dThd (2 µCi/ml; 134 nM) was added 2 h prior to harvest of the cultures. Samples of 1 ml were analyzed for acid-insoluble radioactivity [8]. The incorporation rate of the controls was $6.69 \times 10^3$ dpm/$10^6$ cells × 2 h.

Friend erythroleukemia cells derived from a clone of Friend virus-transformed 745A cells [9] were grown in Joklik minimal essential medium supplemented with 10% fetal calf serum. For dose-response experiments, 5-ml cultures ($3 \times 10^3$ cells/ml) were incubated at 37 °C for 48 or 72 h; the controls showed a population doubling time of 11.8 h.

Human malign melanoma cells (derived from a 31 years old male European; ATCC CRL 1424) [10] were grown in plastic flasks in RPMI-1640 medium supplemented with 10% calf serum and cultured in a fully humidified atmosphere of 5% $CO_2$ and air at 37 °C. The population doubling time was 24 h. For the determination of the cytostatic activity, caused by aeroplysinin-1, the cells were seeded at a density of $8 \times 10^5$ cells/cm$^2$ and were incubated for 24 h. Then the compound was added and the incubation was continued for additional 48 or 72 h. During the last 2 h 0.1 µCi of [$^3$H]dThd/ml (= 6.7 nM) was added. Then the acid precipitable radioactivity was determined [8]. Incorporation rate of the controls: $1.2 \times 10^3$ dpm/$10^6$ cells/2 h.

Spontaneously transformed human keratinocytes, kindly provided by Prof. N. E. Fusenig (Heidelberg) [11], were grown in plastic flasks in Dulbeccos minimal essential medium in the presence of 10% fetal calf serum in a 10% $CO_2$/air atmosphere. The population doubling time was determined to be 48 h. The influence of aeroplysinin-1 on the incorporation rate of [$^3$H]dThd was determined as described above (control: $1.2 \times 10^3$ dpm/$10^6$ cells/2 h).

Normal human fibroblasts were obtained from the skin of an upper arm as follows. Small pieces (0.5 cm$^2$) were placed onto chicken plasma coated microtiter plates (Difco) and incubated in plastic flasks in RPMI-1640 medium with 20% fetal calf serum at 37 °C in 5% $CO_2$/air. After 1 week the outgrown fibroblasts were collected and dissociated with trypsin/ethylenediaminetetraacetic acid [12]. Cells from passages 5–10 were used for the experiments. The population doubling time was 4.3 days. The in-

corporation studies, using [$^3$H]dThd, were performed as described above (control: $0.2 \times 10^3$ dpm/$10^6$ cells/2 h).

Mouse spleen cells were prepared and cultured essentially as described [13]. They were suspended at a density of $4 \times 10^6$ cells/ml in Dulbeccos minimal essential medium, supplemented with 10% fetal calf serum. Where indicated the cultures were incubated either in the absence or presence of mitogen (2 μg/ml of ConA or 20 μg/ml of LPS). The cells were incubated for 48 or 72 h in the absence or presence of aeroplysinin-1; 18 h prior to the end of the incubation 0.1 μCi of [$^3$H]dThd/0.2 ml (= 33.5 nM) was added. Incorporation of [$^3$H]dThd was determined as described [7]; (controls without mitogen: $0.5 \times 10^3$ dpm/$10^6$ cells/18 h; plus ConA: $8.4 \times 10^3$ dpm/$10^6$ cells/18 h; plus LPS: $19.8 \times 10^3$ dpm/$10^6$ cells/18 h).

Human colon carcinoma cell line WiDr [14] was grown in plastic flasks in Eagles basal medium, supplemented with 10% fetal calf serum at 37 °C in 5% $CO_2$/air. The cells were seeded at a density of 200, 100 or 50 cells per 5 ml and incubated in the absence or presence of the compound for 8 or 12 d. Then the number of colonies per 5-ml assays was determined. The plating efficiencies [15] were as follows: 62 ± 7% at 200 cells/5 ml, 70 ± 8% at 100 cells/5 ml and 69 ± 8% at 50 cells/5 ml. For the inhibition studies, the cultures were initiated with 100 cells/5 ml.

Human mamma carcinoma cells (MCF-7 human breast epithelial-like cancer cells) [16] were grown in Eagles basal medium, supplemented with 10% fetal calf serum at 37 °C in 5% $CO_2$ and air. For the dose-response experiments the cells were seeded at a density of $5 \times 10^3$ cells/ml; after 24 h for adaptation the cells were incubated for 48 or 72 h with the compound. Then the cells were dissociated with trypsin/ethylenediaminetetraacetic acid [12] and counted with a hemocytometer. The controls showed a population doubling time of 23 h.

*Determination of the 50% inhibitory concentrations in vitro*

For the determination of the 50% inhibitory concentration of aeroplysinin-1 the following growth parameters were used: (i) For the dose-response experiments the number of cells (for L5178y mouse lymphoma cells, Friend erythroleukemia cells and human mamma carcinoma cells) was determined at 7 different concentrations, causing a 10% to 90% inhibition of cell growth; (ii) 5–7 different concentrations were selected which caused 10% to 90% inhibition of [$^3$H]dThd incorporation rate (for L5178y cells, normal human fibroblasts, human peripheral lymphocytes, human malign melanoma cells, human keratinocytes) or (iii) 4–6 different concentrations, causing a 10% to 90% inhibition of plating efficiencies (human colon carcinoma cells). The control values (growth in the absence of compound) were set at 100%. The $ED_{50}$ concentrations were estimated by logit regression [17]. The data from 5 parallel experiments each were evaluated; the means (± SD) are given. The slopes of the dose-response curves at the $ED_{50}$ values were calculated [17].

*Tumor passage in vivo*

The L5178y mouse leukemic cells [5] were maintained by serial transplantations as an ascites tumor in 31 to 35 g male outbred NMRI mice. The appropriate concentration of leukemic cells for i.p. inoculation ($1.8 \times 10^8$ cells/mouse) was obtained from ascites fluid by dilution in 0.9% NaCl solution. Under these conditions, 95–100% of the mice developed a palpable ascites and 50% of the animals died between days 13 and 17 post inoculum. The increase in body weight after this period of time was 45 ± 14%; the number of leukemic cells increased to $1.3-1.9 \times 10^9$/animal.

*Chemotherapy*

For the evaluation of the anti-tumor effect of aeroplysinin-1 on L5178y leukemia, mice were made leukemic by i.p. injection of $1.8 \times 10^8$ cells. One day later the animals were divided into groups of 10 mice. One group was used as a control (treated with the methylcellulose solution only), whereas the other groups were treated from day two on, once a day i.p. with the drug for 5 consecutive days.

The antitumor activity was assessed according to the following criteria: the weight changes reflect the differences between the mean weights on the fifth and first days of treatment. The percentage increase in median life span over the control (ILS (%) was calculated [18] and ranked [19] as described). The life span (in percent) was estimated according to the formula, $(T:C) \times 100$, [18] whereby T is the median day of death (M.D.D.) of the treated leukemic mice and C the M.D.D. of untreated mice. M.D.D. was

estimated by the method appropriate for grouped observations [17]. Tumor growth delay (T-C value) was estimated according to the published procedure [18]; as the predetermined number of tumor cells in the mice, we chose twice the number of L5178y cells initially used for inoculation ($3.6 \times 10^8$). This number was reached in tumor-bearing control mice after 8.9 days; for details see ref. [4]. The $\log_{10}$ cell kill values (per dose) as well as the gross and net cell kill values were calculated from the formula published [18]; the procedure was outlined previously [4]. The $\log_{10}$ kill values were converted to an arbitrary activity rating as described [18]. The increase of mean weights of treated and untreated controls as well as leukemia bearing mice was determined during an 18-days observation time. In case of mice with L5178y cells, day 1 is set the day of inoculation.

*Toxicity*

The toxicity studies were performed as follows. Mice (groups of 10 animals per cytostatic dose) were treated either once (acute toxicity), for 5 days (subacute toxicity), or for 30 consecutive days (subacute toxicity). Signs of a possible toxicity, *e.g.* loss of weight and hair or nail destruction were checked throughout the period of treatment with the compound. 40 days after the last injection an autopsy was conducted to determine possible morphological alterations.

*Mutagenicity testing*

The potential mutagenic activity of aeroplysinin-1 was measured with the *umu*-test [20] using *Salmonella typhimurium* TA1535 strain, carrying the fused gene *umuC'-'lacZ*. The levels of *umu* operon expression were determined by measuring the β-galactosidase activity in the cells produced by the fusion gene. β-Galactosidase was measured by the procedure described by Miller [21]. Where indicated S-9 fraction was added to the bacterial culture. This fraction was isolated from the immature carp liver 48 h after i.p. treatment with 3'-methylcholanthrene (50 mg/kg), as described [22, 23].

Table I. Influence of aeroplysinin-1 on growth of selected cells in culture. The degree of aeroplysinin-1-caused inhibition is shown by the respective $ED_{50}$ values. All $ED_{50}$ determinations (means ± SD) were evaluated from results of growth inhibition experiments or of incorporation studies using [$^3$H]dThd (as indicated); the drug exposure period was 48 or 72 h with the exception of the experiments with human colon carcinoma cells (period: 8 or 12 d). The slopes of the dose-response curves at the $ED_{50}$ values are given.

| Cells | Incubation time [h/d] | $ED_{50}$ concentration [μM]/slopes at $ED_{50}$ Growth inhibition | Slopes | Inhibition of [$^3$H]dThd incorporation | Slopes |
|---|---|---|---|---|---|
| L5178y mouse lymphoma cells | 72 h | 0.47 ± 0.04 | 1.69 | | |
| | 48 h | 0.68 ± 0.06 | 1.57 | | |
| Friend mouse erythroleukemia cells | 72 h | 0.74 ± 0.04 | 2.09 | | |
| | 48 h | 1.04 ± 0.06 | 1.88 | | |
| Human mamma carcinoma cells | 72 h | 0.39 ± 0.06 | 1.07 | | |
| | 48 h | 1.12 ± 0.19 | 0.91 | | |
| Human colon carcinoma cells | 12 d | 2.96 ± 0.28 | 0.99 | | |
| | 8 d | 4.31 ± 0.41 | 0.76 | | |
| Normal human fibroblasts | 72 h | | | 14.70 ± 1.71 | 1.91 |
| | 48 h | | | 23.86 ± 2.94 | 1.60 |
| Murine spleen lymphocytes | 72 h | | | 5.06 ± 1.14 | 0.73 |
| | 48 h | | | 17.74 ± 1.91 | 0.51 |
| plus ConA | 72 h | | | 6.32 ± 1.30 | 0.69 |
| | 48 h | | | 9.10 ± 1.74 | 0.49 |
| plus LPS | 72 h | | | 4.36 ± 0.82 | 0.77 |
| | 48 h | | | 6.53 ± 1.27 | 0.71 |
| Human melanoma cells | 72 h | | | 2.94 ± 0.34 | 0.88 |
| | 48 h | | | 9.47 ± 0.91 | 0.67 |
| Human transformed keratinocytes | 72 h | | | 2.99 ± 0.25 | 1.21 |
| | 48 h | | | 7.57 ± 0.74 | 1.04 |

## Results

*Cytostatic activity in vitro*

Table I summarizes data showing that aeroplysinin-1 displays a cytostatic activity especially against cultured L5178y mouse lymphoma cells, Friend erythroleukemia cells, human mamma carcinoma cells and human colon carcinoma cells. Weaker was the cytostatic effect on normal murine spleen lymphocytes and human malign melanoma cells. Normal human fibroblasts were determined to be almost insensitive against the compound. To compare the effect of aeroplysinin-1 on normal mouse spleen lymphocytes with that on mouse L5178y cells, [$^3$H]dThd incorporation studies were performed (Table II). The results show that higher concentrations of the compound are required to influence the incorporation rates in lymphocytes than in L5178y cells; *e.g.* at a concentration of 2.9 μM the incorporation rates in lymphocytes are reduced only by 40–50% while those in L5178y cells are diminished by almost 100%. In order to rule out the possibility that the compound displayed its cytostatic activity only after a longer period of incubation, two periods were chosen (48 h and 72 h for all cell types, with the exception of human colon carcinoma cells for which the incubation time was 8 or 12 d). In all experiments a 1.4–3.2-times higher concentration was required to inhibit cell growth by 50% after a 48 h-incubation period, compared to the concentration necessary to reach the 50% inhibition after a 72 h-incubation.

The slopes of the dose-response curves for the experiments with all cell types chosen are steep (Table I), resulting in the fact that at a 2–3-fold $ED_{50}$ concentration of the compound for the respective cell type an at least 80% inhibition of growth was measured.

Aeroplysinin-1 did not change the mean size of the L5178y cells significantly; the mean value (32 channel-analysis) of the controls was 14.37 ± 5.72 corresponding to 1172 ± 467 μm$^3$, of cells treated with $1 \times ED_{50}$ concentration, 1134 ± 559 μm$^3$ and of cells, treated with $2 \times ED_{50}$, 1221 ± 486 μm$^3$. The reversibility of the effect of the compound on cell growth was determined as follows. The cells were incubated in the standard assay for 24 h in the presence of different concentrations of aeroplysinin-1; then the compound was washed out and the assays were brought to a cell concentration of $5 \times 10^4$ cells/ml and incubation was continued for additional 24 h. It was found that up to 3 times the $ED_{50}$ concentration, the growth inhibitory effect was completely reversible. At concentrations of $4 \times ED_{50}$ and $5 \times ED_{50}$ the cell density at the end of the incubation period was 15.7% and 43.1%, respectively lower than that of the controls.

*Cytostatic activity in vivo*

The median life span of L5178y lymphoma-bearing control mice was 14.8 d. Aeroplysinin-1 was determined to increase the life span of tumor bearing mice considerably (Table III). The animals were given i.p. injections of the compound for 5 consecutive days, starting at day 1. At the highest dosage chosen (50 mg/kg/day) the increase of the life span was 3.4-fold compared to the untreated controls. The lower dosage of 30 mg/kg/day likewise increased the life span 2.0-fold.

The compound significantly affected the growth of

Table II. Effect of aeroplysinin-1 on [$^3$H]dThd incorporation into DNA of normal murine lymphocytes and L5178y cells. The incorporation rates were determined as described under Materials and Methods. The controls were set to 100%.

| | Incorporation rates of [$^3$H]dThd (in %) using the following systems | | | |
|---|---|---|---|---|
| | Mouse spleen lymphocytes | | | |
| Aeroplysinin-1 concn. [μM] | without mitogen | plus LPS | plus ConA | L5178y cells |
| 0 | 100 | 100 | 100 | 100 |
| 1.1 | 72.1 | 77.1 | 78.3 | 28.2 |
| 2.9 | 56.3 | 58.1 | 61.1 | ≤ 2.0 |
| 28.5 | 17.5 | 10.9 | 18.2 | ≤ 2.0 |

Table III. Effect of aeroplysinin-1 on L5178y mouse leukemia *in vivo*. The assessment of the antitumor activity which is indicated by the parameters median day of death (M.D.D.) and life span (T/C; %) was performed as described under Materials and Methods.

| Compound | Dose [mg/kg × day] | Total dose [mg/kg in 5 days) | M.D.D. | T/C [%] |
|---|---|---|---|---|
| Aeroplysinin-1 | 0 | 0 | 14.8 ± 1.4 | 100 |
| | 10 | 50 | 21.2 ± 2.4 | 143.2 |
| | 30 | 150 | 29.8 ± 4.3 | 201.4 |
| | 50 | 250 | 50.0 ± 10.2 | 337.8 |

L5178y cells *in vivo* (Fig. 2). While in untreated leukemic mice the number of L5178y cells increased from $1.8 \times 10^8$ cells to $1.09 \pm 0.16 \times 10^9$ cells/animal (at day 15 after inoculation), the increase in compound-treated animals was significantly reduced to $1.4 \pm 0.2 \times 10^8$ cells (10 mg/kg for 5 days) and $8.0 \pm 1.2 \times 10^7$ cells/animal (30 mg/kg). The inhibition of tumor cell growth by the compound *in vivo* is also reflected in a reduction of the percental increase of the mean body weight in compound-treated leukemic mice (Table IV). At a daily dose of 30 mg/kg, the increase of body weight of leukemic mice is as high as the one of non-leukemic mice.

For the evaluation of the activity rating two dosages (10 or 30 mg aeroplysinin-1/kg/day) were chosen (Table V). Based on the determinations of the parameters tumor growth delay (T-C value), the ILS and the $\log_{10}$ kill values, the activity rating was found to be as high as ++ → +++ (10 mg/kg/day) and +++ (30 mg/kg/day), respectively.

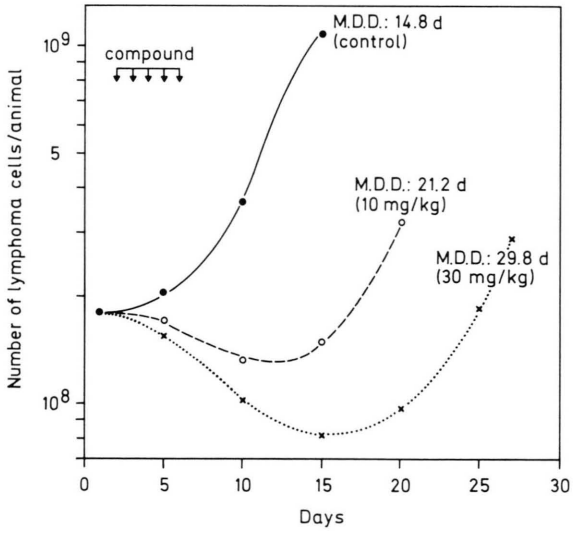


Fig. 2. Influence of aeroplysinin-1 on growth of L5178y cells in mice. Mice were made leukemic at day 1 and treated from day 2 to day 6 daily with 0 mg/kg (●——●), 10 mg/kg (○– –○) or 30 mg/kg of aeroplysinin-1 (x·····x). At the days indicated the total numbers of lymphoma cells per animal were determined electronically, using a Cytocomp counter. Groups of 5 animals each were used for the calculations of the respective mean values; the SD were less than 15% and are not included in the figure. The M.D.D. are also indicated.

*Toxicity in vivo*

Compared to the antitumor effect of aeroplysinin-1 *in vivo*, the toxic effect of this compound in mice is considerably weaker (Table VI). *E.g.*, the most effective antitumor activity was observed at 50 mg/kg/d, a dosage which is 2.7 times lower than the 10% subacute lethal dose ($LD_{10}$). Moreover, it was determined that a daily dose of 30 mg/kg for 30 consecutive days, neither caused any toxicity nor a change of body weight compared to the controls in a group of 10 mice.

Table IV. Influence of aeroplysinin-1 on mean weight of L5178y cell bearing as well as of control mice. Aeroplysinin-1 was given daily at the doses indicated for 5 days, starting one day after inoculation of the tumor. The mean weights of the groups (5 animals each) were determined after a total observation time of 18 days. The mean weight of the control mice at day 1 was 33 ± 2 g.

| Dose of Aeroplysinin-1 [mg/kg] | Increase of mean weight [%] Non-leukemic | Leukemic |
|---|---|---|
| 0 | 15 | 42 |
| 3 | 14 | 39 |
| 10 | 15 | 19 |
| 30 | 14 | 15 |

Table V. Evaluation of activity rating of aeroplysinin-1. All mice were given i.p. inoculations of L5178y cells on day 1. Treatment was i.p. for 5 days as indicated; one injection was given per day. The assessment of the antitumor activity was performed according to the criteria summarized under Materials and Methods.

| Aeroplysinin-1 [mg/kg/day] | ILS [%] | Weight change [g] | T-C value | Log kill/dose | Total log kill gross | net | Activity rating |
|---|---|---|---|---|---|---|---|
| 10 | 43 | +4.5 | 10.5 | 0.30 | 1.51 | 0.80 | ++ → +++ |
| 30 | 101 | +0.5 | 18.0 | 0.52 | 2.60 | 1.86 | +++ |

Table VI. Toxicity of aeroplysinin-1 for male mice. Mice (groups of 5 animals each) were treated either once (acute toxicity) or daily for 5 days (subacute toxicity) with aeroplysinin-1. The number of surviving animals was determined after 40 days. The $LD_{50}$ and $LD_{10}$ values were determined by logit regression [17].

| | Toxicity [mg/kg] | | | |
|---|---|---|---|---|
| | Acute | | Subacute | |
| Compound | $LD_{50}$ | $LD_{10}$ | $LD_{50}$ | $LD_{10}$ |
| Aeroplysinin-1 | | | | |
| daily dose | 202.0 | 160.0 | 150.0 | 133.3 |
| total dose | – | – | 750.0 | 666.5 |

40 days after the last injection the animals were sacrificed for autopsy reasons. In no case were any morphological abnormalities detected in the peritoneal cavity.

*Mutagenicity*

The potential mutagenic activity of aeroplysinin-1 was determined in the *umu*-test, described under Materials and Methods. The data summarized in Table VII show that the indirect mutagen benzo[a]-pyrene caused an expression of β-galactosidase (in the presence of S9) of 362 or 732 units at a concentration of 0.3 μg/ml and 3.0 μg/ml, respectively. The direct mutagen 4-nitroquinoline-1-oxide caused (both in the absence or presence of S9 fraction) an enzyme induction of 882 or 1539 units at 0.3 μg/ml and 1.0 μg/ml, respectively. Using this system both aeroplysinin-1 (at concentrations between 0.01 μg/ml and 10 μg/ml) and its solvent dimethyl sulfoxide (1000 μg/ml) did not cause any measurable enzyme induction, irrespectively of the presence of the S9 fraction. In the absence of any compound the enzyme level varied between 180 and 250 units.

## Discussion

Since the discovery that sponges live in a species-specific symbiosis with microorganisms [24] a justified search for antibiotic and cytostatic agents from sponges became possible. Two sponge secondary metabolites, 1-β-D-arabinofuranosylthymine [25, 26] and Avarol [4, 27] are already used in clinics or are under clinical investigation. It is impossible to pre-

Table VII. Evaluation of the potential mutagenicity of aeroplysinin-1 by the *umu*-test.

| Compound [μg/ml] | β-Galactosidase (unit) Minus S9 | Plus S9 |
|---|---|---|
| Aeroplysinin-1; 0.01 | 214, 250, 169 | 162, 249, 173 |
| 0.1 | 175, 214, 213 | 275, 209, 193 |
| 1.0 | 257, 205, 148 | 206, 187, 169 |
| 10.0 | 190, 224, 202 | 221, 195, 217 |
| Dimethyl sulfoxide; 1000 | 208, 218, 151 | 236, 203, 198 |
| Benzo[a]pyrene; 0.3 | 215, 184, 236 | 371, 322, 394 |
| 3.0 | 244, 271, 200 | 746, 613, 837 |
| 4-Nitroquinoline-1-oxide; 0.3 | 940, 856, 915 | 872, 794, 916 |
| 1.0 | 1532, 1322, 1572 | 1680, 1656, 1473 |
| None | 192, 239, 245 | 187, 183, 228 |

dict the probable pharmacological activity of a putative drug in a given sponge; therefore the selection of the test systems applied in a screening program is essential for a future success in the preclinical work.

In the present work we demonstrate that the secondary metabolite from the marine sponge *Verongia aerophoba,* aeroplysinin-1 [1, 3], is a potent cytostatic agent *in vitro* against L5178y mouse lymphoma cells and mouse erythroleukemia cells and, even more interestingly against human mamma carcinoma and human colon carcinoma cells. The $ED_{50}$ values of aeroplysinin-1 determined for these cell lines (0.47–2.96 μM) are significantly lower ($P \leq 0.001$) than those obtained in cultures with non-transformed lymphocytes (4–5 μM). The mode of action of aeroplysinin-1 is apparently not on the level of DNA synthesis, as can be deduced from the lack of unbalanced growth [28] of treated cells. In comparison to other cytostatic agents, the inhibitory activity of aeroplysinin-1 in the L5178y cell system is high ($ED_{50}$: 0.47 μM, 72 h incubation): 9-β-D-arabinofuranosyladenine, $ED_{50}$ = 2.9 μM [29]; 1-β-D-arabinofuranosylthymine, $ED_{50}$ = 9.8 μM [30]; bleomycin, $ED_{50}$ = 0.9 μM [31] and distamycin, $ED_{50}$ = 13.1 μM [32].

Aeroplysinin-1 is also shown to be a potent antitumor agent *in vivo,* using the L5178y cell/NMRI mouse system. The excellent T/C (%) value of 338, obtained after treating the mice with a total dosage of 250 mg/kg, is the combined result of the antitumor effect and the low toxicity. Following the arbitrary activity rating proposed [18], aeroplysinin-1 must be classified to the markedly active (++ → +++) to highly active anticancer agents (+++).

Using the *umu*-test system, which has been demonstrated to detect many types of DNA-damaging agents, such as base change mutagens, frameshift mutagens or oxidative mutagens [20], aeroplysinin-1 turned out to be neither a direct nor an indirect mutagen.

The present study strongly indicates the potential usefulness of aeroplysinin-1 as a cytostatic agent.

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