# 14-Epidihydrocochlioquinone B and 14-Epicochlioquinone B, Antibiotics from Fermentations of the Ascomycete *Neobulgaria pura:* Structure Elucidation and Effects on Platelet Aggregation

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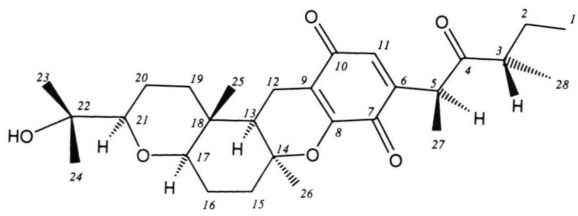
Ascomycetes, Helotiales, Platelet Aggregation, Antibiotics, Cochlioquinones

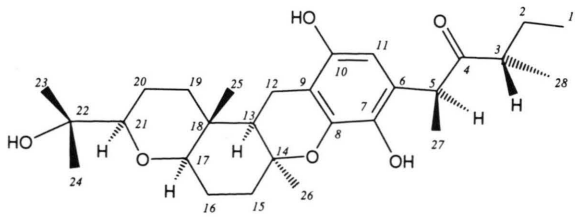
14-Epicochlioquinone B (**1**) and 14-epidihydrocochlioquinone B (**2**) were isolated from submerged cultures of *Neobulgaria pura* (Pers. ex Fr.) Petrak. 14-Epicochlioquinone B is a potent inhibitor of human and bovine platelet aggregation stimulated by different inducers. 14-Epidihydrocochlioquinone B does not inhibit the aggregation of platelets. In addition, both **1** and **2** exhibited cytotoxic and antimicrobial activites.

## Introduction

The frequent occurrence of vascular disorders and arterial thrombosis (Heusel, 1986) and the side effects of some medications used for their treatment (Smith and Willis, 1971; Hiller and Riess, 1988) make a search for new inhibitors of platelet aggregation seem worthwile. Platelets are multiresponding cell fragments with a complex structured signal transmission (Holmson, 1982) and therefore offer several targets for inhibitors. In the course of a search for fungal metabolites inhibiting the aggregation of platelets induced by either collagen or ADP several hundred extracts derived from cultures of basidiomycetes and ascomycetes were tested. 2-Methoxy-4-methyl-1,4-benzoquinone, a metabolite of *Lentinus adhaerens (basidiomycetes)* interfering with human platelet aggregation by binding to the thromboxane $A_2$ receptor has been described previously (Lauer, 1990). Among the ascomycetes, *Neobulgaria pura* was choosen as a promising fungus because in the primary screening culture extracts showed a strong inhibitory effect on the aggregation of bovine platelets induced by either collagen or ADP.



Formel 1



Formel 2

In the following we wish to describe the fermentation, isolation, identification, and biological properties of 14-epicochlioquinone B (**1**) and 14-epidihydrochochlioquinone B (**2**) from *Neobulgaria pura*.

## Experimental

### *General experimental procedures*

Melting points were determined on a Büchi 510 apparatus and are uncorrected. IR spectra (KBr) were determined with a Bruker IFS 48 spectrometer. UV spectra were recorded in MeOH with a Perkin-Elmer Lambda 16 UV/VIS spectrophotometer. For analytical HPLC a Hewlett Packard 1090 instrument series II was used. Mass spectra were measured with a Finnigan MAT CH 7A

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$^{13}C$ NMR ($CDCl_3$, 126 MHz) δ 212.80 (C-4), 186.35* (C-10), 180.97* (C-7), 153.19 (C-8), 145.46 (C-6), 133.64 (C-11), 119.04 (C-9), 84.57 (C-21), 83.60 (C-17), 79.07 (C-14), 71.81 (C-22), 47.37 (C-3), 43.68 (C-13), 43.14 (C-5), 36.95 (C-19), 36.90 (C-15), 36.73 (C-18), 27,00 (C-26), 26.07** (C-23), 25.77 (C-2), 23.76** (C-24), 23.66 (C-16), 21.67 (C-20), 16.60 (C-28), 16.28 (C-12), 14.82 (C-27), 11.79 (C-1), 11.65 (C-25) ppm.

*14-Epidihydrocochlioquinone B* (**2**)

This compound was obtained as slightly off-white amorphic solid, soluble in organic solvents; $R_f = 0.39$ [toluene:acetone (7:3), silica gel], UV (MeOH) $\lambda_{max}$: 294 nm, $\varepsilon_{294\ nm} = 7153$, $[\alpha]_D^{20} = +0.02$ (c = 1, MeOH), IR (KBr) $\upsilon_{max}$: 3431, 2970, 2935, 1701, 1633, 1607 and 1451 $cm^{-1}$, Eims, MS, *m/e* 474 ($M^+$) ($C_{28}H_{42}O_6$).

$^1H$ NMR ($CDCl_3$, 500 MHz), δ 6.11 (s, H-11), 4.29 (qu, 6.9 Hz, H-5), 3.18 (dd, 2.8, 11.9 Hz, H-21), 3.12 (dd, 3.4, 11.9 Hz, H-17), 2.78/2.68 (dd, 18 Hz/dd, 18.0, 8.1 Hz, H-12), 2.62 (sx, 6.7 Hz, H-3), 2.19/1.67 (dt, 3.3/14.3 Hz/m), 1.97/not determined (dt, 12.1, 3.8 Hz/n.d.), 1.89/appr. 1.57 (m/m), 1.73/1.37 (m), 1.64/appr. 1.45 (m/m), 1.42 (d, 7.2 Hz), 1.31 (d, 6.9 Hz, H-27), 1.20 (s), 1.18 (s), 1.15 (s), 0.88 (d, 6.8 Hz), 0.85 (t, 7.5 Hz, H-1), 0.68 (s) ppm.

$^{13}C$ NMR ($CDCl_3$, 126 MHz) δ 216.42 (C-4), 146.37, 141.33, 136.65, 122.71, 108.62 (C-6-10), 104.58 (C-11), 84.63, 83.98 (C-17, C-21), 76.30 (C-14), 71.94 (C-22), 46.01 (C-3), 44.24 (C-13 and C-5), 37.59, 37.09, 36.93 (C-19, C-15, C-18), 26.93 (C-26), 26.83, 26.05, 23.83, 23.72 (C-24, C-2, C-23, C-16), 21.80 (C-20), 17.04, 16.27, 16.04 (C-28, C-12, C-27), 11.77, 11.63 (C-1, C-25) ppm.

*Biological tests*

*Platelet aggregation assay*

9 volumes of fresh bovine slaugther blood were mixed with 1 volume of citrate buffer (93 mM sodium citrate, 140 mM glucose, pH adjusted to 7.4 with 1 M citric acid). To obtain platelet rich plasma (PRP), the anticoagulated blood was centrifuged at 150 × *g* for 15 min at 22 °C. In general the PRP contained $3-4 \times 10^5$ platelets/μl. Platelet poor plasma (PPP) was obtained by centrifugation of the anticoagulated blood at 1000 × *g* for 10 min.

To test the effect of thrombin bovine platelets were washed two times with citrate buffer pH 7.4 and stored at a density of $1 \times 10^6$ platelets/μl in PBS buffer (g/l: NaCl 8; KCl 0.2; $Na_2HPO_4 \times 2\ H_2O$ 1.44; $KH_2PO_4$ 0.2; glucose 1; aqua bidest 1 l, pH 7.4).

The aggregation assay was carried out in a spectrophotometer (Hitachi, model 100-60) with temperated (37 °C) and stirred plastic cuvettes. After preincubation for 10 min with **1** or **2** the aggregation was stimulated with different inducers like collagen, ADP or others. The change of transmittance was monitored at 600 nm with PPP or PBS buffer as blanks, respectively. The aggregation of human platelets was measured in a platelet aggregation profiler (BIO DATA Corporation) with preincubation (10 min). For screening purposes the assay was scaled down to 150 μl in 96-well microtiterplates (Greiner) and the change of transmittance measured at 600 nm by using a BIO-RAD-EIA-Reader.

*Further biological tests*

The plate diffusion assay, the test for cytotoxicity, hemolytic activity, and mutagenicity were carried out as described previously (Lauer, 1990; Lorenzen, 1993).

## Results and Discussion

*Fermentation*

Fig. 1 shows a typical fermentation of *Neobulgaria pura*. The production of 14-epicochlioquinone B (**1**) as determined by analytical HPLC started early and increased significantly after the 4th day. The maximal concentration of 5.1 mg/l was reached after 7 days.

*14-Epicochlioquinone B* (**1**) *and 14-epidihydrocochlioquinone B* (**2**)

**1** and **2** were isolated as described in the experimental section.

* Assignments based on the assumption that $^3J$ (H-16, C-14) > $^2J$ (H-16, C-17).

** Stereochemically not assigned.

spectrometer. Proton and carbon spectra were recorded on a Bruker AMX 500 spectrometer operating at 500.14 MHz for $^1H$. The concentration of the sample of **1** was 34 mM in $CDCl_3$. That of **2** was 21 mM in $CDCl_3$. All spectra were recorded at 305 K and TMS was used as reference. For the DQF-H,H-COSY (Aue *et al.*, 1976; Marion *et al.*, 1983), 512 experiments of 16 scans each (2 dummy scans before the experiment) were recorded with a relaxation delay of 3 s, an acquisition time of 0.2 s and 4 K of data size. The heteronuclear inverse experiments were recorded with 512 experiments of 2 K data size and an acquisition time of 0.26 s each (16 dummy scans before the experiment). HMQC (Bax and Subramanian, 1986) and HMQC-TOCSY (Lerner and Bax, 1986) were carried out with BIRD-filter. For the latter a 42 ms spin-lock of 10 kHz with DIPSI2 (Shaka *et al.*, 1988) and two 2.5 ms trim-pulses were applied. The delay for evolution of the long range couplings in the HMBC (Bax and Summers, 1986) was 25 ms. For the ROESY (Bothner-By *et al.*, 1984; Kessler *et al.*, 1987) spectrum a mixing time of 400 ms was used ($T_1^{min}$ appr. 420 ms). The spin-lock of 2 kHz was produced by 15° pulses with an inter-puls delay of 19.3 µs.

The models were build with Alchemy III, V 2.0 (Tripos Associates, Inc.). Energy minimizations were carried out during the build up of the molecule, as well as on the complete molecule, in order to assure a proper overall structure.

*Neobulgaria pura* strain A 4588 was isolated by H. Anke from spore prints of a fruiting body collected in the Bavarian Alps. The strain is deposited in the culture collection of the LB Biotechnologie, University of Kaiserslautern.

### *Fermentation*

For maintenance on agar slants the fungus was grown on YMG medium (yeast extract 0.4%, malt extract 1.0%, glucose 0.4%, pH 5.5).

Fermentations were carried out in a Biolafitte C 6 or Braun-Melsungen fermentor containing 20 l of GP medium (composed of g/l: Glucose, 20; mashed potato, 4; pH 5.5) at 22 °C with aeration (2 l air/min) and agitation (130 rpm). 250 ml of a well grown culture in YMG medium were used as inoculum. The on-line data were recorded using a Braun Melsungen MFCS system. $O_2$- and $CO_2$-measurements were performed using a Hartmann & Braun Magnos 4 G instrument and an ADC Carbon Dioxide Analyzer. During fermentation 14-epicochlioquinone B was assayed using the platelet aggregation assay. Quantitative measurements were achieved by analytical HPLC.

### *Isolation of 14-epicochlioquinone B and 14-epidihydrocochlioquinone B*

After 7 days of fermentation, the mycelia were separated from the culture fluid by centrifugation (1000 × *g*, 10 min). 14-Epicochlioquinone B and 14-epidihydrocochlioquinone B were removed from the culture fluid (18 l) by adsorption to HP 21 resin (Mitsubishi) from which they were eluted with 1.5 l of acetone. The crude extract (1.2 g) was applied to a column (15 × 15 cm) containing silica gel (0.063–0.2 mesh, Merck 60) and eluted with 1 l toluene:acetone 70:30. Further purification was achieved by preparative HPLC on MerckLiChrogel PS 1, 10 µm (column 250 × 25 mm), elution with isopropanol and then LiChrosorb DIOL, 7 µm (column 250 × 25 mm), elution with cyclohexane:tert. butylmethylether 1:1. Yields: 90 mg 14-epicochlioquinone B (**1**) and 36 mg of 14-epidihydrocochlioquinone B (**2**). During purification the compounds were detected by TLC and the platelet aggregation assay as described below.

### *14-Epicochlioquinone B* (**1**)

This compound was obtained as yellow needles, soluble in organic solvents, $R_f = 0.54$ [toluene:acetone (7:3), silica gel], m.p. 161–162 °C, UV (MeOH) $\lambda_{max}$: 398, 260, 303 (sh) nm, $\varepsilon_{260\ nm} = 10528$, $[\alpha]_D^{20} = +0.003$ (c = 1, MeOH), IR (KBr) $\upsilon_{max}$: 3439, 2938, 1714, 1675, 1652, 1637, 1609 and 1457 $cm^{-1}$, Hreims, MS, *m/e* 472.2814 ($M^+$) ($C_{28}H_{40}O_6$ requires 472.2824).

$^1H$ NMR ($CDCl_3$, 500 MHz) δ 6.51 (s, H-11), 4.10 (q, 8.1 Hz, H-5), 3.16 (dd, 3.0, 12.0 Hz, H-21), 3.09 (dd, 3.5, 12.0 Hz, H-17), 2.73 (sx, 6.8 Hz, H-3), 2.60/2.42 (d, 19.8 Hz, dd 8.4, 19.8 Hz, H-12), 2.35/1.67 (dt, 3.2, 14.7 Hz, m, H-15), 1.91/1.12 (dt, 3.9, 12.8 Hz, m, H-19), 1.80/1.55 (m, m, H-16), 1.73/1.39 (m, m, H-2), 1.167 (s, H-24)**, 1.61/1.44 (m/m, H-20), 1.41 (d, 8.4 Hz, H-13), 1.134 (s, H-23)**, 1.21 (s, H-26), 1.30 (d, 7.2 Hz, H-27), 1.172 (d, appr. 6.7 Hz, H-28), 0.88 (t, 7.5 Hz, H-1), 0.69 (s, H-25) ppm.

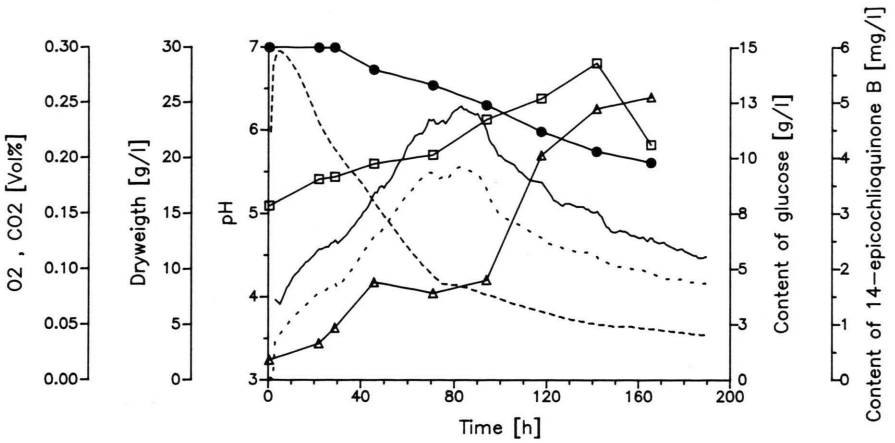


Fig. 1. Fermentation of *Neobulgaria pura* strain A4588 in 20 l of GP-medium. -------- pH; – – – – $O_2$ [Vol%]; —— $CO_2$ [Vol%]; ●—● Glucose [g/l]; △—△ content of 14-epicochlioquinone B [mg/l]; □—□ dryweigth of solids [g/l].

### *$^1H$ and $^{13}C$ NMR spectral assignments of 14-epicochlioquinone B* (**1**)

The structures of cochlioquinones A and B have been elucidated by chemical, spectroscopic, and crystallographic methods some years ago (Carruthers *et al.*, 1971; Canonica *et al.*, 1976; Canonica *et al.*, 1980). The structure of the closely related compound stemphone has also been described previously (Huber *et al.*, 1974). Spectroscopic data of the title compound **1** strongly indicated the relation with cochlioquinones in very early stages of our investigations. However, distinct differences made a detailed determination of the structure inevitable.

Several 2 D-NMR techniques such as DQF-H,H-COSY (Aue *et al.*, 1976; Marion *et al.*, 1983), HMQC (Bax Subramanian, 1986) HMQC-TOCSY (Lerner and Bax, 1986), HMBC (Bax and Summers, 1986), were used for the assignment of the proton and carbon signals of **1.**

The classification of several carbon atoms according to their chemical shift is straight forward. However, the major goal is the distinction of similar spin-system of carbons 19, 20, 21 and 15, 16, 17 and the assignment of the quaternary carbons. The individual proton sub-spin-systems were mainly assigned through the HMQC-TOCSY spectrum, where the COSY yielded the neighbours and the HMQC the directly bound carbons.

One of the most prominent signals is the doublet of one of the H-$12^{pro\text{-}R}$ only showing a coupling of 19.8 Hz with is geminal neighbour. That proton shows three strong correlations to carbon atoms in the HMBC which can be assigned to be C-14, C-9 and C-8. The latter can be distinguished by their chemical shifts. Further, protons of a methyl group and of a methylene group show correlations to C-14. Henceforth, the methyl group has to be 26 and the methylene group is 15, where the distinction from methylene 16 is made in the COSY spectrum. The strong cross-peak between the methyl group at highest field and a methylene carbon assigns H-25 and C-19. In the HMQC-TOCSY spectrum, C-19 and C-15 can clearly be distinguished. Additionally, methyl group 23 and 24 protons show weak cross-peaks with C-22 and C-21.

From H-11, one strong cross-peak can be found to a carbonyl carbon at 180.97 ppm. There is no cross-peak with the other carbonyl at 186.35 ppm. It is reasonable to assume to $3J$ (H-11, C-7) to be larger than the $2J$ (H-11, C-10). Therefore, the carbonyl at 180.97 ppm is assigned as C-7.

Disregarding the stereochemistry, the elucidated structure is identical with cochlioquinone B. However, there is a striking difference of 26.72 ppm between the chemical shifts of C-12 of cochlioquinone B and **1** (Canonica *et al.*, 1980) (both measured in $CDCl_3$). Some other chemical shifts in this part of the molecule show differences as well. In

order to establish the complete stereo structure, a ROESY (Bothner-By *et al.*, 1984; Kessler *et al.*, 1987) spectrum was recorded to measure the inter-proton distances. The peaks in the ROESY spectrum were integrated and offset-corrected. The corrected volume integrals of the cross-peaks were treated in a simplified manner as being proportional to $d^{-6}$ (Kessler *et al.*, 1990). For the calibration, the distance between the geminal protons H-19 was used as a reference. The significant differences between cochlioquinone B and **1** are a few inter-proton distances. For that reason, it was necessary to measure distances between protons and methyl groups. The distance from a single proton to a methyl group in the model was calculated by summing up over the individual distances, in an energetically preferred conformation. From the model of cochlioquinone B, the distance between H-16$^{pro\text{-}S}$ (the acial proton) and the protons of methyl group 26 ($H_3$-26) can be calculated to be equivalant to a distance of about 230 pm. However, no effect could be found in the ROESY spectrum. In the same way, a cross-peak should be found between H-12$^{pro\text{-}S}$ and $H_3$-25 but only a weak signal can be found. It the chirality of C-14 is inverted, rings B and C are cis-connected. In that case the distances mentioned above become much longer and are in accordance with the ROESY of the title compound **1.** Additionally, the distances between the H-15 protons and the methyl protons H-26 are more alike, whereas in cochlioquinone B these distances are strongly different. All other available distances of **1** are in good accordance with the model. Therefore, we conclude that the compound is 14-epicochlioquinone B. In Fig. 2 the two epimers are compared by space filling models of cochlioquinone B (left) and 14-epicochlioquinone B (right), Fig. 3 shows the stereo view of 14-epicochlioquinone B (**1**).

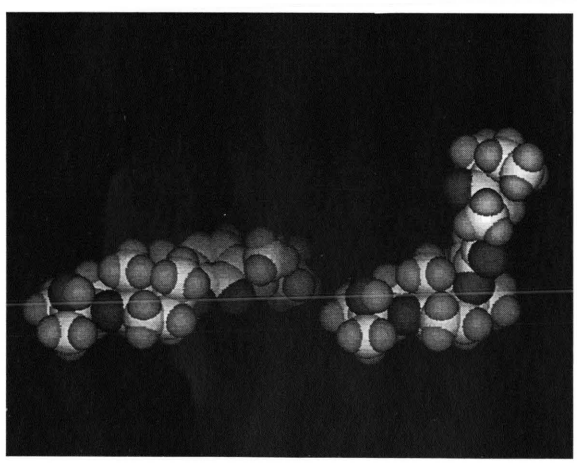

Fig. 2. Space filling models of cochlioquinone B (left) and 14-epicochlioquinone B (right).

### *$^1H$ and $^{13}C$ NMR spectral assignments of 14-epidihydrocochliochinon B* (**2**)

The absence of the quinone structure is clearly indicated by the lack of carbon signals in the 180's ppm range. Strong differences in the aromatic/double-bond region and only little differences in the other regions of the carbon spectrum are clear indicators for the dihydro nature of compound **2.**

Cochlioquinone B was isolated first from Beljak (Beljak *et al.*, 1971) from the fungus *Cochliobolus miyabeanus* in 1971. One year later Barrow and Murphy (1972) described the occurrence of cochlioquinone A and its hydroquinone in extracts of *Heminthosporium leersii.* Stemphone, a related structure, was isolated from *Stemphylium sarcinaeforme* (Scott and Lawrence, 1968). *Neobulgaria pura* thus is a new producer of cochlioquinone-type compounds. Although the cochlioquinones A and B have been known for about 20 years very few biological activities have been reported so far. Only recently Schaeffer *et al.* (1990) described the

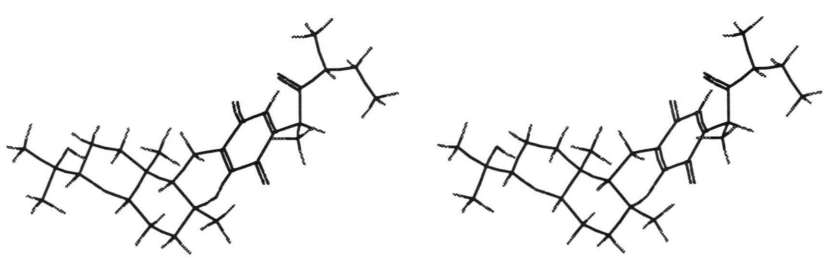

Fig. 3. Stereo view of 14-epicochlioquinone B.

nematicidal activity of cochlioquinone A by binding at the same binding sites as ivermectin.

*Biological activities*

*Effect of* **1** *and* **2** *on platelet aggregation*

The influence of **1** on the collagen-induced aggregation of bovine platelets is shown in Fig. 4I. 14-Epicochlioquinone B inhibits the collagen-induced aggregation at concentrations of 5 µg/ml. Fig. 4II shows the influence of various concentrations of **1** on the ADP-induced aggregation of bovine platelets. The inhibition started at 7.5 µg/ml. At concentrations above 20 µg/ml the inhibition became irreversible. The influence of various concentrations of **1** on the aggregation of human platelets induced with collagen and ADP is shown in Fig. 4III and 4IV.

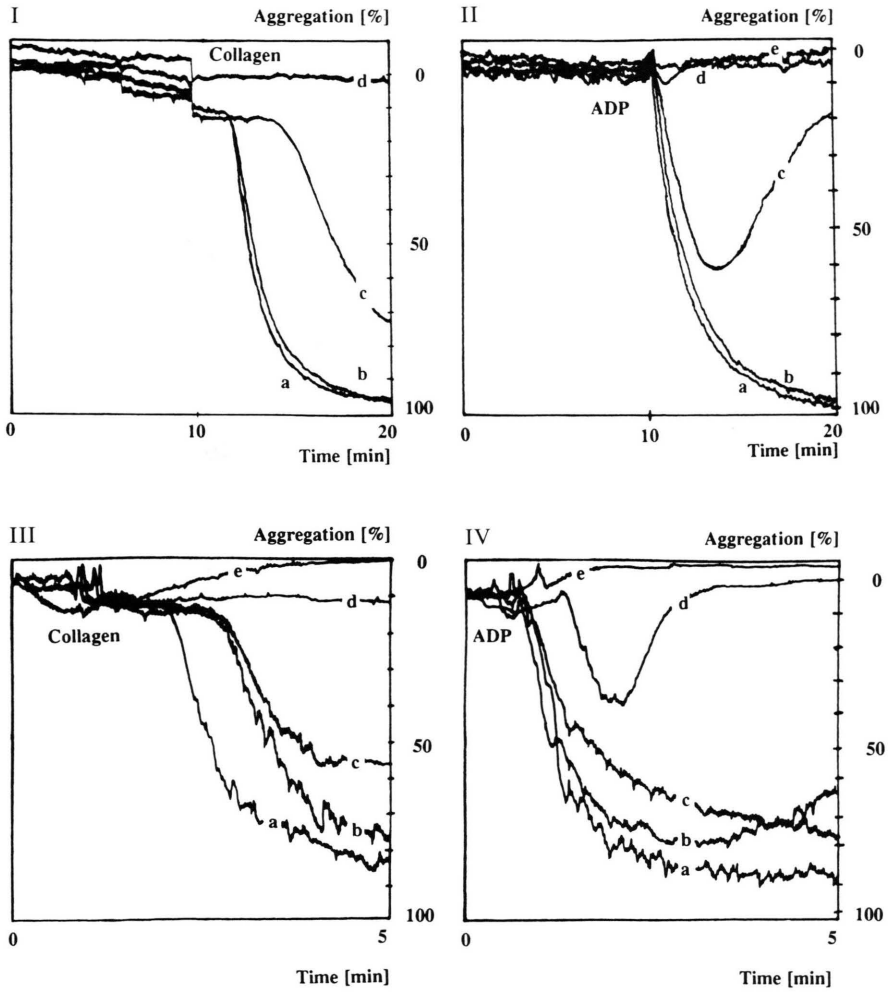


Fig. 4. I. Effects of **1** on collagen-induced bovine platelet aggregation; a – control; b – 2 [µg/ml]; c – 5 [µg/ml]; d – 7.5 [µg/ml].
II. Effects of **1** on ADP induced bovine platelet aggregation; a – control; b – 5 [µg/ml]; c – 7.5 [µg/ml]; d – 20 [µg/ml]; e – 30 [µg/ml].
III. Effects of **1** on collagen-induced human platelet aggregation; a – control; b – 5 [µg/ml]; c – 7.5 [µg/ml]; d – 25 [µg/ml]; e – 50 [µg/ml].
IV. Effects of **1** on ADP-induced human platelet aggregation; a – control; b – 20 [µg/ml]; c – 37.5 [µg/ml]; d – 50 [µg/ml]; e – 75 [µg/ml].

Table I summarizes the inhibitory effects ($IC_{30}$) of 14-epicochlioquinone B on human and bovine platelets stimulated with different inducers. 14-Epicochlioquinone B interfered with the platelet aggregation in the presence of all inducers. This lack of specificity with regard to the different signal pathways might be due to an interference with several basis metabolic processes, a view which is supported by the biological activities of **1** in other biological systems. 14-Epidihydrocochlioquinone (**2**) did not interfere with platelet aggregation.

Table I. $IC_{30}$ values (30% inhibition of aggregation) of 14-epicochlioguinone B (**1**).

| Inducer (Concentration) | $IC_{30}$ [μM] Bovine platelets | Washed bovine platelets | Human platelets |
|---|---|---|---|
| ADP (2.5 μM) | 16–42 | 2–5 | 80–160 |
| Collagen (100 μl) | 10–16 | 10–16 | 16– 53 |
| Thrombin (15 μl) | – | 21–26 | 52– 79 |
| Arachidonic acid (40 μg/ml) | – | – | 106–160 |
| U 46619* (0.45 μM) | – | – | 132–212 |

* Thromboxane A2 analogue, (Upjohn).
– Inducers not suitable for platelets from this source.

*Cytotoxic effects of* **1** *and* **2**

The cytotoxic activities of **1** and **2** were tested as described previously (Lauer, 1990; Lorenzen, 1993). The growth of Balb 3T3 cells was inhibited at concentrations of 5 μg/ml of **1.** Lysis of cells was observed at 25 μg/ml. **1** and **2** strongly inhibited BHK cells (complete lysis) at concentrations of 12.5 μg/ml, at concentrations of 6.5 μg/ml the morphology of cells was altered. At concentrations higher than 20 μg/ml both quinones showed weak cytostatic effects on the growth of L 1210 cells. Quite contrary to the effects on bovine and human platelets both cochliquinone and its hydroquinone exhibit the same cytotoxic effects on Balb 3T3 and BHK cells. This effect was due to metabolic processes and not to a direct lytic action on the cytoplasmic membranes as could be observed in the microscope shortly after the addition of the compounds. This view is supported by a complete lack of hemolytic activity of both compounds on bovine erythrocytes at concentrations of 100 μg/ml.

Table II. Cytotoxic effects of 14-epicochlioquinone B (**1**) and 14-epidihydrocochlioquinone B (**2**).

| Cells | $IC_{(100)}$* [μg/ml] **1** | **2** |
|---|---|---|
| Balb 3T3 | 25 | 25 |
| BHK | 12.5 | 12.5 |
| L1210 | 75 | >100 |

* $IC_{100}$: complete lysis of cells.

*Antibiotic effects of* **1** *and* **2**

In the plate diffusion assay **1** and **2** inhibited the growth of bacteria and fungi (Table III). It is interesting to note that the hydroquinone **2** did not inhibit the growth of *Bacillus subtilis*. Both compounds exhibited weak antifungal activities. As shown in Table III, *Mucor miehei* and *Venturia cerasi* were the fungi most sensitive to **1,** whereas *Epicoccum purpurascens* was preferentially inhibited by **2.** It is therefore concluded that **2** was not di-

Table III. Antimicrobial effects of 14-epicochlioquinone B (**1**) and 14-epidihydrocochlioquinone B (**2**).

| Strain | Diameter inhibition zone [mm] μg/disk* **1** 10 | **1** 100 | **2** 10 | **2** 100 |
|---|---|---|---|---|
| Bacteria: | | | | |
| *Acinetobacter calcoaceticus* | 7 | 9 | 7 | 11 |
| *Arthrobacter citreus* | 8 | 9 | 7 | 11 |
| *Bacillus brevis* | 12 | 15 | 8 | 12 |
| *Bacillus licheniformes* | 9 | 15 | 9 | 15 |
| *Bacillus subtilis* | 9 | 16 | – | – |
| *Corynebacterium insidiosum* | – | – | – | – |
| *Escherichia coli K12* | – | – | – | – |
| *Micrococcus luteus* | 8 | 9 | 8 | 19 |
| *Mycobacterium phlei* | 10 | 19 | 7 | 9 |
| *Proteus vulgaris* | – | 12 | – | – |
| *Pseudomonas fluorescens* | – | – | – | – |
| Fungi: | | | | |
| *Epicoccum purparascens* | – | – | – | 20 |
| *Mucor niehei* | – | 11 | – | – |
| *Phytophthora infestans* | – | 10 | – | – |
| *Venturia cerasi* | 8 | 9 | – | – |

* Diameter: 6 mm.

rectly oxidized to **1** during the assays. In the spot-test for mutagenicity according to Ames (Ames *et al.*, 1975) no induction of revertants of *Salmonella thyphimurium* TA 98 and TA 100 could be observed with 100 µg of **1** and **2**/disk.

**1** and **2** exhibited a variety of biological activities as has been reported for other quinones and hydroquinones (Wilson, 1971). Of these some have been reported to inhibit platelet aggregation by binding selectively to the thromboxane $A_2$ receptor (Lauer, 1990; Mitsura, 1989). The cytotoxic and antibiotic activites of 14-epicochlioquinone B and its hydroquinone however, indicate a lack of specificity with regard to this target and an interference with other metabolic activities.

*Acknowledgements*

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