

With respect to the clinically investigated combined HIV-therapy with two PI's [16] both cage and *syn* dimeric HIV-1 protease inhibitors may attract of great interest as their potential Pgp inhibitory activities would suggest an increased intestinal absorption of the peptidic PI's as Pgp substrates in a combined administration as has been demonstrated for the Pgp inhibitor quinindine in the *in vitro* model [4].

Experimental

1. Preparations of P-glycoprotein [5]

Pgp expression was induced in Caco-2 cell lines (passage 72) with vinblastine. Cells were grown in 225 cm³ flasks at 37 °C in a 5% CO₂ atmosphere using DMEM containing 10 mM vinblastine, 16.5% fetal calf serum, 1% of non-essential amino acids, 100 U/ml penicillin, 100 µg/ml streptomycin and 1% L-glutamine. Cells were seeded at an initial density of 0.8×10^6 cells per flask and medium was changed every other day. Monolayers were trypsinized at 90–95% confluence and the cell suspension was then used for radioligand-binding studies.

2. Radioligand-binding assay

Incubations were performed at 37 °C under mild shaking conditions in Hanks Balanced Salt Solution (HBSS) containing 10 mM morpholino ethane sulfonic acid (MES) at pH 7.0 and Pgp preparation of 1.25×10^6 cells per ml with cell membranes permeabilized with lysolecithine-solution (0.01%) as described [5], 24 different dilutions of H 17, H 19 and verapamil within a concentration range of 7.82 nM to 2500 µM in DMSO/HBSS-MES – 1 : 3 and ³H-Verapamil as radioligand in a total volume of 250 µl. The incubation medium was supplemented as described [5]. Incubation was stopped after 30 min by vacuum filtration. Filters were washed twice with ice-cold HBSS containing MES, incubated with scintillation fluid for 12–16 h at RT, and total radioactivity on the filters was counted by liquid scintillation counting. For all calculations Excel 5.0, (Microsoft, USA) was used.

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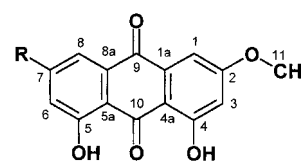
2-Methoxy-4,5,7-trihydroxy-anthraquinone, a new lichen metabolite produced by *Xanthoria parietina*

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Lichens have been shown, in the past, as a rich source of novel natural products with promising properties for applications as drugs, agricultural agents and cosmetics [1, 2]. Thus, special habitats promoting the growth of lichens such as the Canary Islands were investigated and various lichen compound have been reported [3].

In the course of screening for new natural products we recently investigated an extract of the lichen *Xanthoria parietina* and found it to contain **1** as a new anthraquinone structure. **1** is formed in addition to physcion (**2**) and fallacinal (**3**) as related metabolites [4]. Here we report the isolation and structure elucidation of the new lichen metabolite.

The lichen *Xanthoria parietina* was collected from rocks near Los Llanos (La Palma, Canary Islands, Spain). Compound **1** was isolated by several subsequent chromatographic steps from the evaporated residue of the methanolic extract of this lichen as a yellowish amorphous solid. 250 g of the material were extracted three times by 1 l methanol. The combined extracts were evaporated to dryness on a rotary evaporator. The residue was dissolved in chloroform and chromatographed on a silica gel column (5 cm × 30 cm, silica gel 60, 0.063–0.1 mm, stepwise elution by CHCl₃ and CHCl₃-MeOH 9 : 1, each 10 column volumes). Thereby **1** was separated from several other anthraquinone components such as physcion (**2**) [4] and fallacinal (**3**) [4]. Final purification was carried out by preparative TLC using silica gel sheets RP₁₈ (Merck, acetonitrile/water, 83 : 17, 0.1% trifluoroacetic acid; R_f 0.85) and preparative HPLC on silica gel RP₁₈ (1.5 × 25 cm; 5 µm, gradient 95% water to 95% acetonitrile, 25 min, R_t: 17 min).



	R
1	–OH
2	–CH ₃
3	–CH ₂ OH

Structures of 2-methoxy-4,5,7-trihydroxy-anthraquinone (**1**, R = OH) and coproduced physcion (**2**, R = CH₃) and fallacinal (**3**, R = CH₂OH).

The molecular weight and the elemental composition of **1** were determined by HREI-MS (M⁺: m/z 286.1270; calcd. 286.1241 for C₁₅H₁₀O₆). The VIS spectrum displayed an absorbance maximum at 435 nm which is the same as was found with **2** and **3**.

The IR spectrum attested to the presence of quinone carbonyles and, respectively, hydroxyl groups due to absorbances at 1704 cm⁻¹, 1736 cm⁻¹ and 3438 cm⁻¹.

Conclusive evidence for the structure of **1** was furnished by 1 D and 2 D NMR spectroscopy (¹H, ¹³C, DEPT,

COSY, HSQC, HMBC). The ^1H NMR spectrum and the COSY spectrum displayed two pairs of meta-coupled aromatic protons (6.75 and 7.45 ppm ($^4J = 2.54$ Hz), and respectively, 7.76 and 8.29 ppm ($^4J = 1.53$ Hz)) and one methoxyl group (3.97 ppm, singlet). In addition, the signals of the three hydroxyl protons (10.11, 12.18, 12.25 ppm) were visible.

The ^{13}C NMR spectrum showed the presence of 15 carbon atoms: two quinone carbonyles (181.0 and 190.3 ppm), twelve aromatic carbons and one methoxyl group (56.3 ppm). The structure of **1** and the assignment of NMR signals (see Experimental part) was confirmed by the 2 D C,H-long-range coupled NMR spectra (HMBC). HMBC was useful, too, for the identification of **3** as fallacinol [4] despite of the unusual low coupling constants (0.5 Hz, in CDCl_3) of H-6 and H-8 in this compound. Compound **1** thus appears as a new anthraquinone occurring in a lichen, but was reported previously as the product of chemical decomposition of anthraquinone structures isolated from a marine animal [5].

Moderate cytotoxicity of **1** with $\text{LD}_{50} = 50 \mu\text{g/ml}$ was determined with Hela cells, L-929 and K562 cells. Weak antibacterial activity was found against *Bacillus subtilis* ATCC 6633 ($\text{MIC} > 200 \mu\text{g/ml}$).

Experimental part

1. Instruments

HRESI-MS was carried out on an AMD 402 sector field mass spectrometer (AMD Intectra, Harpstedt, Germany). UV-VIS- and IR-spectra were recorded on a Beckman DU 601 and Shimadzu IR scanning spectrophotometers.

NMR spectra were recorded on a Bruker Avance DRX 500 instrument.

2. 2-Methoxy-4,5,7-trihydroxy-anthraquinone (**1**)

Yield 5 mg from 250 g lichen material; yellowish amorphous mass, TLC: R_f : 0.6 (silica gel 60, Merck; $\text{CHCl}_3/\text{MeOH}$; 9:1; v/v. MS (70 eV) m/z (M^+): 286.1270, UV-VIS (MeOH, λ_{max} (ϵ): 266 (24 000), 292 (23 100), 435 (16 300). IR (KBr, cm^{-1}): 3430, 2920, 1736, 1704, 1620, 1476, 1302, 1321, 1266, 1219, 1167, 1098, 1034, 980, 902, 757. ^1H NMR (500 MHz, CDCl_3 ; δ , ppm): 3.97 (s, 3H; H-11), 6.75 (d, $^4J = 2.54$ Hz; 1H, H-3), 6.45 (d, $^4J = 2.54$ Hz; 1H, H-1), 7.76 (d, $^4J = 1.54$ Hz, 1H, H-6), 8.29 (d, $^4J = 1.54$ Hz, 1H, H-8), 10.11 (s, 1H, OH-7), 12.18 (s, 1H, OH-4), 12.25 (s, 1H, OH-5). ^{13}C NMR (125 MHz, CDCl_3 ; δ , ppm): 56.3 (C-11), 107.0 (C-3), 109.2 (C-1), 115.0 (C-4a), 117.5 (C-5a), 119.9 (C-8), 124.5 (C-6), 128.1 (C-8a), 134.6 (C-1a), 161.5 (C-7), 162.5 (C-5), 165.5 (C-4); 167.5 (C-2), 181.1 (C-9), 190.3 (C-10).

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