J.Serb.Chem.Soc. 65(8)555-560(2000)
JSCS-2776

UDC 547.673:582.29
Original scientific paper

Isolation and antimicrobial activity of anthraquinones from some species of the lichen genus *Xanthoria*

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(Received 20 December 1999, revised 3 May 2000)

The isolation of six anthraquinones, erythroglaucin, physcion, xanthorin, emodin, fallacinal and teloschistin, from three species of the lichen genus *Xanthoria (X. fallax, X. elegans* and *X. policarpa)* is reported. Physcion is the dominant anthraquinone in all species. The anthraquinones showed broad-spectrum antifungal activity and selective activity against some phytopathogenic bacterial species.

Keywords: anthraquinones, *Xanthoria*, antibacterial activity, antifungal activity.

Xanthoria is one of the larger lichen genera within the family *Teloschistaceae*. The apothecia are usually yellow to dark brown red, the thallus in many cases possessing a similar but usually lighter color.[1] The *Xanthoria* species usually grow on rocks and trees. Some species of this genus are mainly distributed in the Durmitor region (Montenegro) in Yugoslavia.

Anthraquinones, as colored compounds, are widely spread in the lichen of the genus *Xanthoria* and are responsible for their color.[2] Natural anthraquinones are distinguished by a large structural variety, a wide range of biological activity, and low toxicity. They possess astringent, purgative, anti-inflammatory, antiviral, moderate antitumour, and bactericide effects.[2]

The isolation, separation, and antimicrobial activity of six anthraquinones from three *Xanthoria* species are reported herein. Some of the anthraquinones are identified in these species for the first time.

RESULTS AND DISCUSSION

Extraction of the dried thallus of the lichen *Xanthoria elegans* collected from Durmitor with benzene gave a deep red solution, which after concentration and column chromatograph with benzene and benzene-acetone (10:1) yielded six crystalline fractions.

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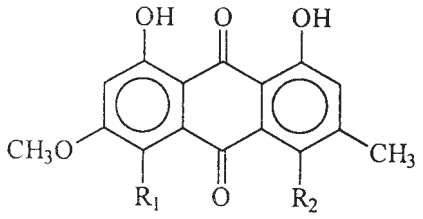
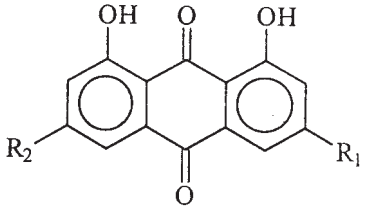


erytroglaucin: R_1=H; R_2=OH
xanthorin: R_1=OH; R_2=H



physcion: R_1= CH_3; R_2= OCH_3
fallacinal: R_1= CHO; R_2= OCH_3
teloschistin: R_1= CH_2OH; R_2= OCH_3
emodin: R_1= CH_3; R_2= OH

Fig. 1. Chemical structures of the isolated anthraquinones.

The first isolated anthraquinone was erythroglaucin **1**,[3] (red needles, m.p. 204–206 °C). Erithroglausin was isolated in a relatively small amount (0.17 % of lichen dry weight). The ^{1}H-NMR spectrum of **1** contained eight signals that integrated to a total of 12 protons and suggested a pentasubstituted anthraquinone type structure for **1**. The signals at δ 12.37, 12.46, 13.36 (hydroxyl protons), δ 6.71 (1H, *d*, *J* = 2.6 Hz), 7.14 (1H, *br.s*) and 7.41 (1H, *d*, *J* = 2.6 Hz) (three aromatic protons) in the spectrum confirmed the structure of anthraquinone **1**. Erytroglaucin has previously been reported from higher fungi and lichen. This pigment occurs in various species of the genera, *e.g.*, *Cortinarius, Xanthoria* and *Dermocybe*.[4,5]

The most abundant anthraquinone was the orange pigment, physcion[6] **2** (1.85 % of lichen dry weight). Physcion (parietin) had long been supposed to occur only in the lichen species belonging to the family *Teleschistaceae*. Physcion has lately been found in some species usually included in the families *Lecanidiaceae* and *Stereocaulaceae*. In addition to lichens, physcion has also been found,[9] *e.g.*, in *Penicillium, Aspergillus, Cassia, Polugonum, Rhamnus, Rheum* and *Rumex*.[7]

The second red anthraquinone (m.p. 251 – 253 °C) was identified as xanthorin **3** (0.02 %) from its spectral data and by comparison with a synthetic sample prepared from emodin.[8] It is feasible that physcion is the immediate precursor of erytroglaucin and xanthorin which is derived by hydroxylation in position C-4 and C-5.

The anthraquinones fallacinal **4**, teloschistin (fallacinol) **5** and emodin **6** (all yellow pigments) were isolated from thallus in yields of 0.09, 0.37 and 0.28 %, respectively. These anthraquinones have been known for a long time in the literature and have been isolated from a variety sources like *Xanthoria*.[9–11] In addition to lichens, emodin,[12] a compoud present in pharmaceutical preparations, has been found, *e.g.*, in *Rhamnus, Casia* and *Rhumnex*. The purified compounds were identified by comparison of their physical constants and spectral data with those published earlier.[3–12]

X. fallax contains significant amounts of physcion (main pigment), fallacinal, teloschistin, emodin and small amounts of erytroglaucin. From the lichen *X. poli-*

carpa collected at Durmitor, the anthraquinones physcion, emodin, teloschistin and fallacinal (minor pigment) were isolated by the same procedure as mentioned above. The basic pattern is very similar in all these species. Physcion is the dominant compound in all the species examined (1.35–1.85 % of lichen dry weight). Xanthorin was isolated from the lichen *X. elegans*, but was not found in the species *X. fallax* and *X. policarpa*.

After isolation and identification, the anthraquinones were tested for antifungal and antibacterial activities. All the isolated anthraquinones were active in counterpoint inoculation disk assay[13] against non-lichenized fungi *Aspergillus niger, Doratomyces stemonitis, Trhichoderma viride* and *Penicillium verucosum* causing a between 10–65 % reduction in the radial growth rates of these fungal competitors at 100 μg/disk.

The anthraquinones also displayed antibacterial activity[14] (at 25 μg/disk) against *Pseudomonas fluorescens* (5–15 %), *Pseudomonas glicinea* (5–25 %), and *Pseudomonas phaseolicola* (5–8 %). They were found to be inactive against *Bacillus mycoides* at a concentration of 50 μg/disk and lower.

The results of these assays are summarized in Table I. The preliminary testing of the anthraquinones against microorganisms showed that the activity depends on the structure of the compound. Antagonistic interaction among lichen fungi, non-lichen fungi and bacteria often involve the production of chemical agents by one species that inhibits the growth of the other. Due to this fact, lichens have proven antimicrobial properties, which may account for their resistance to attack by bacterial or fungal pathogens in nature.

TABLE I. The inhibition effect of the isolated anthraquinones against different fungi (at 100 μg/disk) and bacteria (at 25 μg/disk)

Fungi	Anthraquinones					
	1	**2**	**3**	**4**	**5**	**6**
A.niger	13 %	20 %	28 %	38 %	25 %	25 %
T.viride	25 %	15 %	10 %	25 %	18 %	15 %
P.verucosum	60 %	30 %	45 %	65 %	35 %	35 %
D.stemonitis	38 %	10 %	65 %	45 %	25 %	15 %
Bacteria	Anthraquinones					
	1	**2**	**3**	**4**	**5**	**6**
P. glicinea	25 %	15 %	5 %	–	–	5 %
P. fluorescens	15 %	–	8 %	–	5 %	10 %
P. phaseolicola	–	5 %	5 %	–	8 %	8 %
B. mycoides	–	–	–	–	–	–

EXPERIMENTAL

General

The melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer Grating Spectrophotometer Model 197. The UV spectra were run on a Varian Super Scan 3. The NMR spectra were measured on a Varian Gemini 200 MHz spectrometer (^{1}H at 200 MHz, ^{13}C at 50 MHz) in $CDCl_3$ and DMSO-d_6 using TMS as the internal standard; δ are given in ppm; *J* in Hz. Abbreviations: *s*-singlet, *d*-doublet, *t*-triplet, *q*-quartet, *m*-multiples and *br.*-broad. Column chromatography was performed on silica gel G 60 (Merck). Thin layer chromatography (TLC) was carried out on silica gel G (Merck) plates.

Material

Lichens of the *Xanthoria species*, collected from the Durmitor mountain in August 1997, were used for extraction.

Extraction and fractionation

The air-dried thalluses (100 g) were extracted with benzene for 35 h in a Soxhlet apparatus, after which the extract was concentrated under treduced pressure. The resulting solids were chromatographed on a silica gel 60 column using benzene as the eluent. The slower moving anthraquinones were eluted with benzene-acetone (10:1), the eluents being collected in fractions.

Xanthoria elegans (weight of extract = 2.79 g) contains physcion (major pigment; 1.85 g), erythroglaycin (0.18 g), fallacinal (0.09 g), emodin (0.28 g), xanthorin (0.02 g) and teloschistin (0.37 g). Erythroglaucin and xanthorin were separated from physcion by column and TLC chromatography on silica gel. Physcion was eluted with benzene, while erythroglaucin and xanthorin were eluted with benzene-ethyl acetate (7:3).

Xanthoria fallax (weight of extract = 1.91 g) contains physcion (1.57 g), erythroglaucin (0.02 g) fallacinal (0.09 g), teloschistin (0.08 g), and emodin (0.15 g).

Xanthoria policarpa (weight of extract = 1.61 g) contains physcion (1.35 g), emodin (0.08 g), fallacinal (0.06 g), and teloschistin (0.12 g).

Antibacterial and antifungal assay

The disk diffusion assay[13,14] was used for screening of the antibacterial (at 25 μg/disk) and antifungal (at 100 μg/disk) activity of the anthraquinones. The plates were inoculated with the microorganisms before placing the extract-impregnated paper disks on the plates.

Erythroglaucin; m.p. 204–206 °C; red needles (from EtOAc); ^{1}H-NMR ($CDCl_3$):2.36 (3H, *d, J* = 0.9 Hz, Me), 3.95 (3H, *s*, OMe), 6.71 (1H, *d, J* = 2.6 Hz, H-7), 7.14 (1H, *br.s*, H-2) 7.41 (1H, *d, J* = 2.6 Hz, H-5), 12.37 (1H, *s*, OH-1), 12.46 (1H, *s*, OH-8) and 13.36 (1H, *d, J* = 0.9 Hz, OH-4). ^{13}C-NMR ($CDCl_3$): 22.3 (CH_3), 56.2 (CH_3O), 106.9 (C-2), 108.0 (C-4), 158.4 (C-5), 123.9 (C-13), 137.3 (C-11), 125.3 (C-7), 136.3 (C-14), 164.9 (C-1), 166.5 (C-3), 109.8 (C-12), 130.5 (C-6), 155.0 (C-8), 182.4 (C=O), 184.3 (C=O). IR (KBr): ν = 2945, 2840, 1645, 1605, 1595, 1435, 1160; UV ($CHCl_3$): λ (log ε) 223 (4.28), 256 (4.05), 308 (3.85), 466 (3.89); 478 (3.95), 493 (4.05), 513 (3.88), 522 (3.81); Anal. calcd. for $C_{16}H_{12}O_6$ (300.27): C, 64.00; H, 4.03. Found: C, 64,07; H, 4.09.

Physcion; m.p. 208–210 °C; dark orange needles (from $CHCl_3$); ^{1}H-NMR ($CDCl_3$): 2.45 (3H, *s*, Me), 3.94 ((3H, *s*. OMe), 6.68 (1H, *d, J* = 2.5 Hz, H-7), 7.09 (1H, *br.s*, H-2), 7.37 (1H, *d, J* = 2.5 Hz, H-5), 7.63 (1H, *br.s*, H-4), 12.12 (OH-1) and 12.32 (OH-8). ^{13}C-NMR ($CDCl_3$): 22.1 (CH_3), 56.0 (CH_3O), 106.7 (C-2), 108.0 (C-4), 121.3 (C-5), 121.9 (C-13), 123.9 (C-11), 124.1 (C-7), 135.8 (C-14), 164.9 (C-1), 166.5 (C-3), 136.1 (C-12), 147.7 (C-6), 161.9 (C-8), 182.3 (C=O), 184.2 (C=O). IR(KBr): ν = 2945, 2840, 1625, 1595, 1490, 1160; UV (ethanol): λ (log ε) = 224 (4.41), 254 (4.14), 264 (4.16), 286 (4.14) 433 (3.99); Anal. calcd. for $C_{16}H_{12}O_5$ (284.267): C, 67.60; H, 4.26. Found: C, 67.68; H, 4.29.

Xanthorin; m.p. 250–251 °C; red needles (from $CHCl_3$-petrol) ^{1}H-NMR ($CDCl_3$): 2.43 (3H, *s*. Me), 3.99 (3H, *s*, OMe), 6.68 (1H, *d, J* = 2.5 Hz, H-7), 6.99 (1H, *s*, H-2), 7.39 (1H, *d, J* = 2.5 Hz, H-5).

^{13}C-NMR ($CDCl_3$): 22.1 (CH_3), 56.4 (CH_3O), 108.2 (C-2), 144.8 (C-4), 121.3 (C-5), 125.3 (C-13), 135.8 (C-11), 123.8 (C-7), 123.4 (C-14), 156.9 (C-1), 153.8 (C-3), 121.8 (C-12), 147.7 (C-6), 162.3 (C-8), 181.3 (C=O). IR(KBr): ν = 2945, 2835, 1645, 1605, 1435, 1155; UV (ethanol): λ (log ε) = 235 (4.40), 258 (4.55), 306 (4.10), 462 (4.05), 4.85 (4.16), 490 (4.25), 5.11 (4.06), 525 (4.06). Anal. calcd. for $C_{16}H_{12}O_6$ (300.266): C, 64.00; H, 4.03. found: C, 63.93; H, 3.97.

Fallacinal; m.p. 227–228 °C; yelow needles (from $CHCl_3$); ^{1}H-NMR ($CDCl_3$): 3.97 (3H, *s*, OMe), 6.74 (1H, *d* = 2.5 Hz, H-7), 7.44 (1H, *J* = 2.5 Hz, H-5), 7.76 (1H, *br.s,* H-2), 8.29 (1H, *br.s,* H-4), 12.19 (1H, *s*, OH-1), 12.17 (1H, *s*, OH-8). ^{13}C-NMR ($CDCl_3$): 56.1 (CH_3O), 121.1 (C-2), 123.2 (C-4), 107.9 (C-5), 123.8 (C-13), 123.5 (C-11), 106.9 (C-7), 136.2 (C-14), 164.0 (C-1), 139.7 (C-3), 135.8 (C-12), 166.7 (C-6), 164.7 (C-8), 182.2 (C=O), 184.7 (C=O), 194.8 (CHO). IR(KBr): ν = 2850, 2835, 2740, 1715, 1635, 1600, 1440, 1155; UV(ethanol): λ (log ε) = 237 (4.45), 260 (4.60), 310 (4.20), 445 (4.05). Anal. calcd. for $C_{16}H_{10}O_6$ (198.27): C, 64.43; H, 3.38. Found: C, 64.49; H, 3.41.

Teloschistin; m.p. 236–237 °C, yelow needles (from EtOH); ^{1}H-NMR (DMSO-d_6): 3.98 (3H, *s*, OCH_3), 4.82 (1H, *s*. CH_2), 6.70 (*d*, 1H, *J* = 2.5 Hz, H-7), 7.39 (1H, *br. s*, H-2), 7.93 (1H, *d, J* = 2.5 Hz, H-5), 7.97 (1H, *br. s,* H-4), 12.20 (1H, *s*, OH-1), 12.30 (1H, *s*, OH-8). ^{13}C-NMR (DMSO-d_6): 56.1 (CH_3O), 65.1 (CH_2), 120.1 (C-2), 121.8 (C-4), 108.1 (C-5), 124.1 (C-13), 124.1 (C-11), 106.9 (C-7), 136.2 (C-14), 164.5 (C-1), 148.9 (C-3), 122.1 (C-12), 166.7 (C-6), 165.1 (C-8), 181.7 (C=O), 184.3 (C=O). IR(KBr): ν = 2840, 1635, 1600, 1440, 1155; UV(ethanol) λ (log ε) = 227 (4.41), 257 (4.14), 268 (4.16), 289 (4.14), 437 (3.99); Anal. calcd. for $C_{16}H_{12}O_6$ (300.266): C, 64.00; H, 4.03. Found: C, 64.29; H, 4.21.

Emodin; m.p. 255–257 °C, yellow needles (from EtOH); ^{1}H-NMR (DMSO-d_6): 2.35(3H, *s*, CH_3), 6.45 (1H, *d, J* = 2.5 Hz, H-7), 6.95 (2H, *d, J* = 2.5 Hz and 1H, *s*, H-2 and H-5), 7.25 (1H, *s*, H-4), 12.1 (1H, *s*, OH-1) and 12.2 (1H, *s*, OH-8). ^{13}C-NMR ($CDCl_3$): 22.2 (CH_3), 108.0 (C-2), 109.1 (C-4), 121.2 (C-5), 136.2 (C-13), 120.0 (C-11), 124.0 (C-7), 124.0 (C-14), 165.3 (C-1), 163.7 (C-3), 136.0 (C-12), 147.7 (C-6), 162.1 (C-8), 181.4 (C=O), 190.2 (C=O). IR (KBr): ν = 3280, 2945, 2840, 1680, 1635, 1595, 1440, 1380, 1155; UV (ethanol): λ (log ε) = 255 (4.57), 269 (4.16), 290 (3.27), 436 (3.99); Anal. calcd. for $C_{15}H_{10}O_5$ (270.24): C, 66.67; H, 3.73. Found: C, 66.73; H, 3.83.

И З В О Д

ИЗОЛОВАЊЕ И АНТИМИКРОБНА АКТИВНОСТ АНТРАХИНОНА НЕКИХ ВРСТА ЛИШАЈЕВА РОДА *Xanthoria*

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Из три врсте лишајева рода *Xanthoria* (*X. fallax, X. elegans* и *X. policarpa*) изоловани су следећи антрахинони: еритроглауцин, фисцион, ксанторин, емодин, фалацинал и фалацинол. Најзаступљенији антрахинон у свим врстама је фисцион. Тестирани антрахинони показују широк спектар антифунгалне активности и селективну активност према неким врстама фитопатогених бактерија.

(Примљено 20. децембра 1999. ревидирано 3. маја 2000)

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