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THE ANTHRAQUINONES OF *HETERODERMIA OBSCURATA*

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Key Word Index—*Heterodermia obscurata*; Physciaceae; lichen; emodin; hypericin; anthraquinone; bianthrones.

Abstract—Three anthraquinones, two bianthrones and a phenanthroperylenequinone were isolated from the lichen *Heterodermia obscurata* (Nyl.) Trevis. (Physciaceae). The structures were inferred from their spectra (UV, mass, ^{1}H and ^{13}C NMR): emodin, 7-chloroemodin, 5,7-dichloroemodin, flavoobscurin A, flavoobscurin B and 7,7′-dichlorohypericin, with the latter being recently discovered as a lichen anthraquinone. 7-Chloroemodin and 5,7-dichloroemodin were synthesized by a new method, and characterized spectroscopically. The synthetic and natural products were found to be identical in all respects.

INTRODUCTION

Anthraquinones are important constituents of lichens [1]. They are particularly prominent in the families Nephromataceae, Physciaceae and Teloschistaceae [2–5]. The genus *Heterodermia* (Physciaceae) consists of about 80 species, mostly tropical and sub-tropical in distribution [6–8]. Approximately 20 species of *Heterodermia* are known to contain anthraquinones; only one of these, *H. obscurata*, occurs in temperate areas of the world [8, 9]. The chemistry of *H. obscurata* was first described, in 1940, by Asahina and Yosioka, when they isolated the well known lichen substances atranorin and zeorin [10]. They also described an orange–yellow substance that turned red–violet in alkali. Although unable to determine the structure of the compound, they suggested that it was probably a hydroxyanthraquinone, identical to the one described by Hesse in 1898 [11, 12]. A systematic chemical examination of *H. obscurata*, collected in Japan, was undertaken by Yosioka *et al.* in 1968 [13–15]. Four novel and two known compounds were ultimately isolated and identified by IR, UV, mass and ^{1}H NMR spectra.

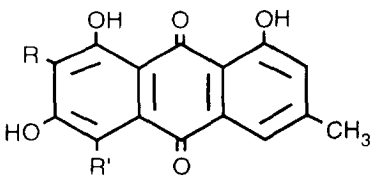
Heterodermia obscurata is abundant in deciduous forests of the eastern United States. Several samples were collected from Cedarville State Forest in southern Maryland. Extraction of the dried lichen afforded a crude mixture of anthraquinones, anthrones and atranorin. Purification by column chromatography and preparative TLC resulted in the isolation of six compounds: emodin (**1**), 7-chloroemodin (**2**), 5,7-dichloroemodin (**3**), flavoobscurin A (**4**), flavoobscurin B (**5**) and 7,7′-dichlorohypericin (**6**).

Compounds **1**–**5** were reported to be present in *H. obscurata* from Japan [13, 14]. Although **6** was not identified in the Japanese lichen, several other substances, possibly anthrones, were found by TLC of the lichen extracts [15].

RESULTS AND DISCUSSION

The freeze-dried lichen was extracted successively with cold diethyl ether, acetone and methanol. All the extracts were concentrated to minimal volumes and examined by TLC. Compound **2** was the main constituent of the three extracts. The ether extract indicated the presence of several yellow pigments, believed to be anthrones. Their identities, however, could not be confirmed, as they were present in only minute amounts.

Ultimately, six compounds were identified in the three extracts, using TLC with chloroform–methanol (4:1): **1** (R_f 0.88), **2** (R_f 0.72), **3** (R_f 0.32), **4** (R_f 0.42), **5** (R_f 0.20) and **6** (R_f 0.30).

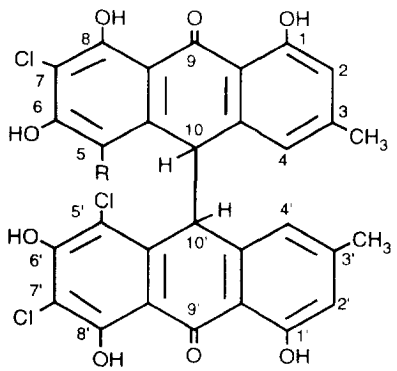


1 R = R′ = H

2 R = Cl, R′ = H

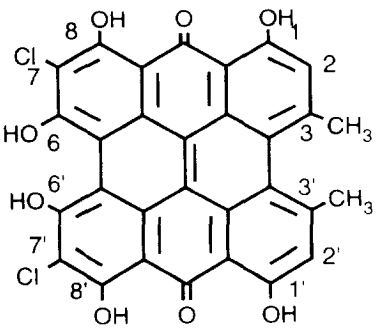
3 R = R′ = Cl

*Author to whom correspondence should be addressed.



4 R = H

5 R = Cl



6

The identity of **2** was confirmed by analysis of the chemical ionization mass spectrometry (CIMS) and 2D COSY ^{1}H NMR spectra. The positive-ion CIMS spectrum shows peaks at 307 and 305; the negative-ion CIMS spectrum shows peaks at 306 and 304. The 2D COSY ^{1}H NMR spectral data are shown in Table 1. The CIMS and 2D COSY ^{1}H NMR data are consistent with those previously reported [2, 13, 16]. A series of NOE experiments confirmed the identity of **2**. Irradiation of the methyl protons resulted in the enhancement of signals of H-2 and H-4. Irradiation of the hydroxyl proton at C-6 resulted in a signal intensity enhancement of H-5. Carbon-13 NMR assignments were obtained from APT and HETCOR experiments, and are shown in Table 3.

Compound **3** proved to be 5,7-dichloroemodin on the basis of MS and 2D COSY ^{1}H NMR spectra. The LSIMS spectrum shows peaks at 341, 339 and 337 (relative intensity 1:6.3:8.7). This ratio of relative intensities is characteristic for the presence of two chlorine atoms in the compound. The 2D COSY ^{1}H NMR spectral data are shown in Table 1. The data agree well with earlier results [13, 17]. A NOE experiment confirmed the identity of **3**. Irradiation of the methyl protons produced a corresponding increase in the signal intensities of H-2 and H-4.

Compound **1** was characterized by UV, CIMS and 2D COSY ^{1}H NMR spectra (Table 1). Several NOE experiments confirmed that **1** was emodin. Irradiation of the methyl protons resulted in increases in the signal intensities of H-2 and H-4. Irradiation of the hydroxyl proton at C-8 also produced an increase in the signal intensity of H-7.

Compounds **4** and **5** are chlorinated bianthrones, originally isolated by Yosioka *et al.* [14]. Non-halogenated bianthrones have been found in a crinoid, *Lamprometra palmata* [18]. The structures of **4** and **5** were proved by LSIMS, CIMS and 2D COSY ^{1}H NMR spectra. The two compounds produce characteristic fragmentation patterns in their mass spectra. The negative-ion LSIMS spectrum of **4** shows peaks corresponding to the parent bianthrone structure, as well as the two non-symmetrical monomers. The negative-ion LSIMS spectrum of **5** also shows the parent peak, along with a single set of monomeric fragments. Only the monomeric fragments can be seen in the CIMS spectra of the two compounds; they do

Table 1. ^{1}H NMR data for emodin (**1**), 7-chloroemodin (**2**) and 5,7-dichloroemodin (**3**)*

H	1†	2†	3†
2	7.19 *s*	7.18 *s*	6.95 *s*
4	7.57 *s*	7.61 *s*	7.43 *s*
5	7.26 *d* (2.5)	7.43 *s*	
7	6.69 *d* (2.5)		
OH-1		11.97 *s*	11.72 *s*
OH-6			13.66 *s*
OH-8		12.20 *s*	12.80 *s*
Me-3	2.48 *s*	2.48 *s*	2.40 *s*

* Chemical shifts (δ) are reported in ppm from TMS internal standard. The coupling constants are given in Hz.

† The spectra were recorded in Me_2CO-d_6 at 400 MHz.

Table 2. ^{1}H NMR data for flavoobscurin A (**4**), flavoobscurin B (**5**) and 7,7′-dichlorohypericin (**6**)*

H	4†	5†	6‡
2,2′	6.54 *s*	6.68 *s*	7.42 *s*
4,4′	5.11 *s*	5.74 *s*	
5,5′	6.96 *s*		
7,7′			
10,10′	4.66 *d* (3.0)§	4.95 *s*§	
10,10′	4.72 *d* (3.0)§		
OH-1,1′			
OH-6,6′			
OH-8,8′			
Me-3,3′	2.32 *s*	2.32 *s*	2.65 *s*

* Chemical shifts (δ) are reported in ppm from TMS internal standard. The coupling constants are given in Hz.

† The spectra were recorded in Me_2CO-d_6 at 400 MHz.

‡ The spectrum was recorded in DMSO-d_6 at 400 MHz.

§ No attempt has been made to assign stereochemistry (*cf.* ref. [14]).

Table 3. ^{13}C NMR data for emodin (**1**), 7-chloroemodin (**2**), 5,7-dichloroemodin (**3**) and flavoobscurin B (**5**)*

C	1	1†	2	2†	3	3†	5
1	161.9	158.7	161.4	158.7	160.0	158.7	160.4
1′							160.4
2	124.8	116.2	124.2	116.2	124.6	116.2	122.1
2′							122.1
3	149.3	142.4	148.3	142.4	145.4	142.4	146.0
3′							146.0
4	121.2	125.2	120.4	125.2	119.4	125.2	117.2
4′							117.2
5	109.7	111.8	108.5	113.2	119.3	119.5	115.4
5′							115.4
6	167.0	162.8	163.1	163.2	168.5	163.6	166.6
6′							166.6
7	108.6	106.6	121.0	112.9	122.7	114.3	117.3
7′							117.3
8	165.9	160.2	162.8	160.6	160.4	158.7	161.6
8′							161.6
9	191.9	201.6	189.0	199.7	210.9	197.8	188.8
10	182.2	173.9	180.8	173.9	182.4	175.3	188.8
1a							116.0
1b‡							139.5
4a§	134.0	131.3	132.8	131.3	127.0	129.4	139.5
8a‖	110.0	102.5	110.8	103.9	111.0	105.3	112.9
8b‡							139.6
9a‖	114.4	107.0	113.4	107.0	113.6	107.0	116.0
9b‡							139.5
9c							30.8
10a§	136.6	132.8	132.6	130.9	133.6	131.3	112.9
10b							139.6
10c							30.8
3-Me	21.8		21.6		21.5		21.6
3′-Me							21.6

*Chemical shifts (δ) are reported in ppm from TMS internal standard. The spectra were recorded in DMSO-d_6 at 125 MHz.

†The calculated values are based on the reported effect of various substituents on the ^{13}C-chemical shifts of monosubstituted benzenes (*cf.* ref. [31]) as applied to the reference compound, **1**.

‡Values for 1b/9b and 8b/10b can be interchanged.

§Values for 4a and 10a can be interchanged.

‖Values for 8a and 9a can be interchanged.

display, however, the same overall patterns seen in the two LSIMS spectra. The 2D COSY ^{1}H NMR chemical shifts for **4** and **5** are shown in Table 2. Table 3 lists the ^{13}C NMR shifts for **5**.

Compound **6** was recently isolated from the lichen *Nephroma laevigatum* [19]. It also appears to be a minor constituent in *H. obscurata*. The spectral data for **6** from the different lichen sources are comparable. Thus, **6** from *H. obscurata* gave a negative-ion LSIMS spectrum with parent peaks at 575, 573 and 571 (relative intensity 1:6:8). This ratio of relative intensities is indicative of two chlorine atoms in the compound. This conclusion was supported by the 2D COSY ^{1}H NMR data (Table 2). An NOE experiment also confirmed the identity of **6**; irradiation of the methyl protons produced an increase in the signal intensity of H-2 (H-2′).

The biogenesis of several anthraquinones has been established in higher plants and fungi. Compound **1** and related compounds are derived from acetate and malonate by way of the polyketide pathway [20–22]. The mechanism and stage of formation of the chlorinated anthraquinones is unknown at present.

The formation of bianthrones and phenanthroperylenequinones in higher plants (*Hypericum* spp.) has been discussed by Brockman and Sanne [23]. The biogenesis is believed to involve the primary linkage of two emodin anthrone units leading initially to a bianthrone structure. This coupling is followed by several consecutive oxidation steps, ultimately producing hypericin.

Compound **6** is related to the well known antiviral compound hypericin, found in *Hypericum* species [24, 25] and in a basidiomycete, *Dermocybe austroveneta* [26]. Hypericin is the subject of intensive medical scrutiny because of its antiviral activity [27]. Meruelo and colleagues, in 1988, showed that hypericin inhibited the spread of the Friend and radiation leukaemia viruses

in vitro and *in vivo* [28]. The same group also reported the inactivation of the human immunodeficiency virus (HIV), by hypericin, when measured by reverse transcriptase activity. The purified enzyme, however, does not appear to be the principal target of hypericin activity [29]. Kraus and co-workers have also shown that hypericin has anti-retroviral activity, when tested against HIV [30]. Thus, the mode of action of hypericin still remains a topic of debate [30].

Many lichen genera produce anthraquinones, and anthrones have been detected in several species (by TLC). Bianthrones are probably formed by oxidative coupling of anthrones. A TLC, performed a few hours after sample collection, clearly showed the presence of bianthrones and **6**. Thus, we consider it unlikely that our natural products are exclusively artefacts generated during extraction and sample manipulation.

EXPERIMENTAL

Mps (uncorr.) were recorded on a Kofler hot stage microscope. ^{1}H NMR (2D COSY and NOE experiments): 400 MHz; APT and HETCOR ^{13}C NMR: 125 MHz. Me_2CO-d_6 and DMSO-d_6 were used in the NMR experiments, with TMS as int. standard; LSIMS: thioglycerol matrix; CIMS: 70 eV (probe); prep. TLC: Merck Kieselgel 60 GF_{254} layers (0.1 × 20 × 20 cm) on glass plates; Lichen material: *H. obscurata* (Nyl.) Trevis. (Physciaceae) was deposited (2 reference samples) in the Botany Department Herbarium at the University of British Columbia.

Isolation of compounds. Lichen thalli were collected in Cedarville State Forest, MD (U.S.A.) in September, 1994. The dried lichen (10 g) was extracted successively with Et_2O (200 ml), Me_2CO (200 ml) and MeOH (200 ml). Following TLC examination, the orange extracts were combined, filtered and concd to a solid. The material remaining on the filter proved to be atranorin (180 mg, 1.8% yield) based on TLC comparison with commercial atranorin [R_f 0.85 for both substances; $CHCl_3$–petrol–MeOH (12:12:1)] and mixed mp (198–200°; no depression). The mixt. of pigments (40 mg) was submitted to CC on Sephadex LH-20 (100 g). A gradient of $CHCl_3$–MeOH (4:1) to MeOH was used for elution.

Compounds **3**, **4**, and **5** were purified by prep. TLC using $CHCl_3$–MeOH (4:1) as the eluent. The immobile purple layer remaining on the column was extruded and extracted with 100 ml pyridine for 16 hr. The extract was concd, and dried *in vacuo*, to give pure **6**.

Syntheses of **2** *and* **3**. Compound **1** (40 mg, 0.15 mM) was added to 50 ml dry DMF. The red solution was stirred at ambient temp. until all the **1** had dissolved (40 min). *N*-chlorosuccinimide (40 mg, 0.30 mM) was added, in one portion, to the stirred soln. The reaction mixt. was maintained at ambient temp. for 24 hr. The solvent was removed, under reduced pressure to give an orange solid, which was dried, *in vacuo*, for 24 hr. CC of the crude mixt. on Sephadex LH-20 in $CHCl_3$–MeOH (4:1) afforded (in order of elution) **1** (8 mg from EtOAc, 20% recovery), **2** (22 mg from EtOAc, 48% yield) and **3** (12 mg from MeOH, 24% yield).

Emodin (**1**). Compound **1** (3 mg, 0.03% dry wt) was obtained as orange crystals (EtOAc); mp 255–256°; UV (EtOH) λ_{max} nm (log ε): 262 (4.30), 288 (4.34), 434 (4.20); CIMS *m/z* (rel. int.): 270 $[MH]^-$ (100); ^{1}H NMR: Table 1; ^{13}C NMR: Table 3.

7-Chloroemodin (**2**). Compound **2** (12 mg, 0.12% dry wt) was obtained as orange crystals (EtOAc); mp 280–282°; UV (EtOH) λ_{max} nm (log ε): 257 (4.24), 315 (4.22), 325 (4.08), 437 (3.88), 504 (3.70); CIMS *m/z* (rel. int.): 307 $[MH]^+$ (38), 305 (100); CIMS *m/z* (rel. int.): 306 $[MH]^-$ (33), 304 (100); ^{1}H NMR: Table 1; ^{13}C NMR: Table 3.

5,7-Dichloroemodin (**3**). Compound **3** (1 mg, 0.01% dry wt) was obtained as red crystals (MeOH); mp 268–270°; UV (EtOH) λ_{max} nm (log ε): 262 (4.34), 320 (4.20), 457 (4.00), 524 (3.90); LSIMS *m/z* (rel. int.): 341 $[M - H]^-$ (12), 339 (25), 337 (35); CIMS *m/z* (rel. int.): 343 $[MH]^+$ (7), 341 (38), 339 (55); CIMS *m/z* (rel. int.) 342 $[MH]^-$ (12), 340 (68), 338 (100); ^{1}H NMR: Table 1; ^{13}C NMR: Table 3.

Flavoobscurin A (**4**). Compound **4** (2 mg, 0.02% dry wt) was obtained as lemon-yellow crystals (AcOH); mp >350°; UV (EtOH) λ_{max} nm (log ε): 273 (4.25), 410 (4.30); LSIMS *m/z* (rel. int.): 615 $[M - H]^-$ (2), 613 (4), 611 (5), 326 (19), 324 (75), 322 (100), 290 (9), 288 (18); CIMS *m/z* (rel. int.): 329 $[MH]^+$ (17), 327 (80), 325 (100), 293 (47), 291 (86); CIMS *m/z* (rel. int.): 327 $[MH]^-$ (7), 325 (27), 323 (33), 290 (40), 288 (66); ^{1}H NMR: Table 2.

Flavoobscurin B (**5**). Compound **5** (3 mg, 0.03% dry wt) was obtained as lemon-yellow crystals (AcOH); mp >350°; UV (EtOH) λ_{max} nm (log ε): 276 (4.20), 403 (4.26); LSIMS *m/z* (rel. int.): 651 $[M - H]^-$ (2), 649 (6), 647 (12), 645 (9), 327 (7), 325 (26), 323 (32); CIMS *m/z* (rel. int.): 329 $[MH]^+$ (11), 327 (54), 325 (79); CIMS *m/z* (rel. int.): 327 $[MH]^-$ (8), 325 (31), 323 (40); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

7,7′-Dichlorohypericin (**6**). Compound **6** (0.8 mg, 0.008% dry wt) was obtained as purple crystals (AcOH); mp >350°; UV (EtOH) λ_{max} nm (log ε): 259 (4.70), 292 (4.60), 332 (4.51), 485 (4.08), 552 (4.26), 594 (4.56); LSIMS *m/z* (rel. int.): 575 $[M - H]^-$ (19), 573 (39), 571 (50); ^{1}H NMR: Table 2.

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