The Isolation of 2,2'-Biindoline-3,3'-diones from Injured Fruit Bodies of Collybia peronata and Tricholoma scalpturatum

Zljie Pang, and Olov Sterner

J. Nat. Prod., **1994**, 57 (6), 852-857• DOI:
10.1021/np50108a030 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 6, pp. 852-857, June 1994

THE ISOLATION OF 2,2′-BIINDOLINE-3,3′-DIONES FROM INJURED FRUIT BODIES OF *COLLYBIA PERONATA* AND *TRICHOLOMA SCALPTURATUM*

ZIJIE PANG and OLOV STERNER*

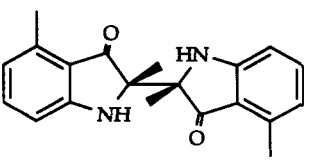
Division of Organic Chemistry 2, University of Lund, P.O. Box 124, S-22100 Lund, Sweden

ABSTRACT.—Several 3-indolinone dimers have been isolated from EtOAc extracts of the fruit bodies of *Collybia peronata* {peronatin A [**1**] and peronatin B [**2**]} and *Tricholoma scalpturatum* {7-hydroxy-7′-methoxyperonatin B [**3**] and 7,7′-dimethoxyperonatin B [**4**]}, and their structures have been determined by mass spectrometry and nmr spectroscopy. The compounds are formed in the fruit bodies as a response to injury, although small amounts may be present also in intact specimens. It is suggested that they are probably formed by oxidative coupling of the corresponding 3-indolinones.

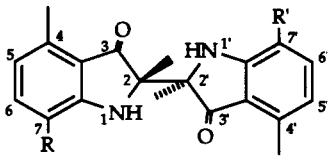
The fruit bodies of *Collybia peronata* (=*Marasmius peronata*) are common in the beech woods of Sweden in autumn. They are characterized by a pungent taste that is sensed after chewing a piece of fresh fruit body for a few seconds and are not considered to be edible. Pungent metabolites previously isolated from the fruit bodies of other species (e.g., *Lactarius*) have been proposed to be repellents or antifeedants in a chemical defense system (1,2). The possibility that the same or similar compounds are also formed in the fruit bodies of *C. peronata* prompted us to investigate this species. It has frequently been observed that metabolites are formed in the fruit bodies as a response to injury, in both pungent (2) and nonpungent species (3,4). Intact as well as injured fruit bodies of *C. peronata* were therefore investigated. The pungent principle(s) of *C. peronata* appear to be difficult to extract, as both EtOAc and EtOH extracts lacked pungency (verified by tasting). Furthermore, young, undamaged fruit bodies that were left for a couple of days in the refrigerator lost their pungency. It may be that the pungent principle(s) are too hydrophilic to be extracted even with EtOH, or are unstable, or are formed by a very sensitive enzymatic system. A comparison by tlc of the EtOAc extracts of the intact and injured fruit bodies indicated that metabolic conversions do take place as a response to injury, as two compounds were consistently present in larger amounts (see below) in the extracts of the injured specimens. These two metabolites were isolated and their structures determined.

The fruit bodies of *Tricholoma scalpturatum* are not considered to be pungent, but instead are edible, although not very tasty. Comparison by tlc of the EtOAc extracts of intact and injured fruit bodies of this species showed that two compounds not present in the intact fruit bodies are formed as a response to injury. The two metabolites formed in *T. scalpturatum* are structurally related to the compounds isolated from *C. peronata*. The isolation and structure determination of these compounds are reported herein.

The two compounds, peronatin A [**1**] and peronatin B [**2**], isolated from *C. peronata* were not found exclusively in the extracts of the injured specimens, although the quantities in the extracts of the intact specimens varied in different experiments (harvests) from nearly 0% to ca. 30% of those present in the extracts of



1



2 R=R′=H
3 R=OH, R′=OCH_3
4 R=R′=OCH_3

the injured specimens (as determined by the size of the resolved tlc spots). This may reflect the fact that the fruit bodies of *C. peronata* are somewhat fragile, and it is possible that the process of collection and transportation to the laboratory caused undetected injuries and hence initiated the formation of these compounds. The two compounds isolated from *T. scalpturatum* were found to be hydroxylated and/or methoxylated derivatives of peronatin B and were given the names 7-hydroxy-7′-methoxyperonatin B [**3**] and 7,7′-dimethoxyperonatin B [**4**].

The structure determination of the peronatins was based on high-resolution nmr and ms, and the ^{1}H- and ^{13}C-nmr data are given in Tables 1 and 2. 2D COSY, NOESY, HMQC, and HMBC nmr spectra (of compounds **2**, **3**, and **4**) facilitated the assignment of the nmr signals. The interpretation of the ms was complicated by the fact that all compounds gave weak molecular ions both in the ei and ci modes. Compounds **1**, **2**, and **4** appeared to be readily cleaved symmetrically in the ion source, where one half takes up a hydrogen and gives the base peak of (M/2)+1 with ei ionization, and (M/2)+2 with ci. In the ei spectrum of the unsymmetrical metabolite 7-hydroxy-7′-methoxyperonatin B [**3**], it is the methoxylated portion that gives rise to the base peak at *m/z* 191, which becomes *m/z* 192 in the ci spectrum. This first observation suggested that the symmetrical dimers in fact are monomeric 2,4-dimethylindolin-3-ones, but the fact that C-2 appeared as a singlet in the coupled ^{13}C-nmr spectra of all four compounds as well as the recurrent presence of small M (ei) and M+1 (ci) ions in the ms, proved that they actually are dimers.

TABLE 1. ^{1}H- (500 MHz) Nmr Data for Peronatin A [**1**], Peronatin B [**2**], 7-Hydroxy-7′-methoxyperonatin B [**3**], and 7,7′-Dimethoxyperonatin B [**4**].[a]

Proton	Compound			
	1	**2**	**3**	**4**
	δ, mult., *J*	δ, mult., *J*	δ, mult., *J*	δ, mult., *J*
1	4.38, s	6.12, s	6.02, s	6.15, s
5	6.53, dd, 0.9, 7	6.52, dd, 0.8, 7.3	6.39, dd, 0.9, 7.6	6.44, dd, 0.8, 7.8
6	7.21, dd, 7, 8	7.30, dd, 7, 7	6.85, d, 7.6	6.78, d, 7.8
7	6.50, dd, 0.9, 8	6.71, dd, 0.5, 7.3	—	—
2-CH_3	1.66, s	1.14, s	1.22, s	1.15, s
4-CH_3	2.56, s	2.56, s	2.41, s	2.50, d, 0.8
7′-OH	—	—	8.40, br s	—
7-OCH_3	—	—	—	3.87, s
1′	4.38, s	6.12, s	6.14, s	6.15, s
5′	6.53, dd, 0.9, 7	6.52, dd, 0.8, 7.3	6.48, dd, 0.9, 7.7	6.44, dd, 0.8, 7.8
6′	7.21, dd, 7, 8	7.30, dd, 7, 7	6.94, d, 7.7	6.78, d, 7.8
7′	6.50, dd, 0.9, 8	6.71, dd, 0.5, 7.3	—	—
2′-CH_3	1.66, s	1.14, s	1.23, s	1.15, s
4′-CH_3	2.56, s	2.56, s	2.42, d, 0.9	2.50, d, 0.8
7′-OCH_3	—	—	3.87, s	3.87, s

[a]The spectra of compounds **1**, **2**, and **4** were recorded in $CDCl_3$, and the spectrum of compound **3** in CD_3COCD_3. The coupling constants *J* are given in Hz, and the solvent signals (7.26 ppm in $CDCl_3$ and 2.05 ppm in CD_3COCD_3) were used as references.

TABLE 2. ^{13}C- (125 MHz) Nmr Data for Peronatin A [**1**], Peronatin B [**2**], 7-Hydroxy-7′-methoxyperonatin B [**3**], and 7,7′-Dimethoxyperonatin B [**4**].[a]

Carbon	Compound			
	1	**2**	**3**	**4**
	δ, mult.	δ, mult.	δ, mult.	δ, mult.
2	68.8, s	68.4, s	70.0[b], s	68.8, s
3	204.3, s	204.9, s	204.5[c], s	204.7, s
3a	118.4, s	118.0, s	120.4, s	118.4, s
4	140.1, s	140.1, s	129.9, s	130.4, s
5	120.5, d	119.9, d	120.2, d	118.9, d
6	136.5, d	137.5, d	116.7, d	115.8, d
7	110.0, d	109.4, d	142.4, s	144.2, s
7a	161.6, s	161.6, s	152.5, s	153.2, s
2-CH_3	19.5, q	18.5, q	18.8[d], q	18.5, q
4-CH_3	18.2, q	18.3, q	17.5, q	17.5, q
7-OCH_3	—	—	56.0, q	55.5, q
2′	68.8, s	68.4, s	69.8[b], s	68.8, s
3′	204.3, s	204.9, s	204.5[c], s	204.7, s
3′a	118.4, s	118.0, s	119.8, s	118.4, s
4′	140.1, s	140.1, s	130.4, s	130.4, s
5′	120.5, d	119.9, d	120.4, d	118.9, d
6′	136.5, d	137.5, d	121.2, d	115.8, d
7′	110.0, d	109.4, d	145.4, s	144.2, s
7′a	161.6, s	161.6, s	153.4, s	153.2, s
2′-CH_3	19.5, q	18.5, q	19.0[d], q	18.5, q
4′-CH_3	18.2, q	18.3, q	17.4[e], q	17.5, q
7′-OCH_3	—	—	—	55.5, q

[a]The spectra of compounds **1**, **2**, and **4** were recorded in $CDCl_3$, and the spectrum of compound **3** in CD_3COCD_3. The solvent signals (77.0 ppm in $CDCl_3$ and 29.8 ppm in CD_3COCD_3) were used as references.
[b,c,d,e]Interchangeable.

The structure determination of peronatin B [**2**] is summarized in Figure 1, in which pertinent NOESY and HMBC nmr correlations are indicated. The corresponding correlations were also observed in the spectra of compounds **3** and **4**. The ortho aromatic protons 5-H and 6-H as well as 5′-H and 6′-H are still present in compounds **3** and **4** coupled to each other (J=8 Hz), and H-5 and H-5′ give NOESY correlations to the corresponding aromatic methyl groups (CH_3-4 and CH_3-4′). HMBC correlations between CH_3-4 and C-3a, C-4, and C-5; CH_3-4′ and C-3a′, C-4′, and C-5′; 5-H and C-3a and C-7; 5′-H and C-3a′ and C-7′; 6-H and C-4 and C-7a as well as between 6′-H and C-4′ and C-7a′, permitted the unambiguous assignment of the aromatic carbon signals for compounds **3** and **4**. Compared with peronatin B [**2**], the chemical shifts for C-7 and C-7′ were shifted downfield, indicating that this carbon has been hydroxylated or

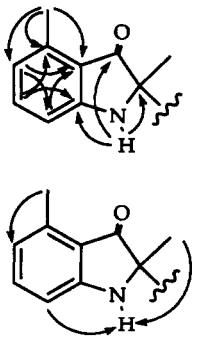


FIGURE 1. Significant HMBC (top) and NOESY (bottom) Correlations for Peronatin B [**2**]. (The corresponding correlations were also observed for compounds **3** and **4**).

methoxylated. An HMBC correlation was also noted between OCH_3-7′ and C-7′ in 7-hydroxy-7′- methoxyperonatin B (3) and OCH_3-7/7′ and C-7/7′ in 7,7′-dimethoxyperonatin B [**4**].

Peronatin A [**1**] and B [**2**] are obviously closely related, as their ^{13}C-nmr spectra and ms were almost identical, which led to the conclusion that they are diastereoisomers. When left in $CDCl_3$ overnight at room temperature, peronatin A [**1**] is partly transformed to peronatin B [**2**], identified by tlc and ^{1}H nmr, and small amounts of a large number of unidentified yellow by-products. Peronatin B [**2**] is the more stable isomer, and when the structures of **1** and **2** are compared, it is evident that **2** should benefit from lower steric energy in the conformation where the carbonyl oxygens are hydrogen bonded to the amino hydrogen. In **1**, a similar hydrogen bonding would be destabilized by steric interactions which would distort the molecule. This difference is seen in the ^{1}H-nmr spectra, where the signals for 1-H and 1′-H are upfield in **1** (due to the loss of hydrogen bonding) as compared to **2**, from 6.12 ppm to 4.38 ppm. The signals for CH_3-2 and CH_3-2′ are shifted downfield from 1.14 ppm to 1.66 ppm due to anisotropic effects. None of the isolates [**1**–**4**] showed optical activity. It should be noted that compounds **1** and **3** were of insufficient quantity to determine if a weak optical activity existed. Based on the lack of optical activity, it is suggested that peronatin A [**1**] is the *d,l*-pair (no optical activity), while peronatin B [**2**] is in the meso form. The slow conversion of **1** into **2** in solution may be caused by a homolytic cleavage of the C(2)-C(2′) bond forming two relatively stable captodative radicals (5). These may then recombine in the solvent cage to form peronatin A [**1**] and B [**2**], and to also form the unidentified trace by-products.

The two compounds isolated from *T. scalpturatum* showed 1-H and 2-CH_3 ^{1}H-nmr shifts comparable with those of **2**, and are consequently suggested to be 7-hydroxy-7′-methoxyperonatin B [**3**] and 7,7′-dimethoxyperonatin B [**4**].

The presence of a number of unidentified yellow compounds of various polarities in trace amounts in the extracts supports the suggestion that radicals are involved in the formation of the peronatins. It is possible that the pungency of the fruit bodies of *C. peronata* is caused by the generation of reactive and short-lived radicals as a response to injury, which would explain why the causative principles are difficult to extract and isolate. If 2,4-dimethyl-3-indolinone is formed or liberated in the fruit bodies of *C. peronata* as a response to injury, it would probably easily be oxidized to peronatin A [**1**] and B [**2**]. 2-Methyl-3-indolinone has previously been shown to be oxidized by, for instance, oxygen to the corresponding 2,2′-biindolinone (6). The apparent lack of pungent taste of the fruit bodies of *T. scalpturatum* could simply be due to the fact that the amounts of the indolinones in its fruit bodies are considerably smaller (<10%) than those in *C. peronata*. If dimers **3** and **4** are formed from oxidative coupling of the corresponding monomers, one would expect to find 7,7′-dihydroxyperonatin B as well in the fruit bodies of *T. scalpturatum*. However, as only 3 mg of **3** was isolated, as compared to 13 mg of compound **4**, the ratio of hydroxylated and methoxylated monomers would be approximately 1 to 10. Less than 1 mg of the dihydroxylated compound would therefore be expected, and such small amounts may have escaped our attention.

The chemistry of the dimers isolated here to some extent resembles that of indigo and tyrian purple, two well-known dyes originally isolated from plants and molluscs, respectively (see Figure 2). Indigo [**6**] occurs as the precursor glucoside indican [**5**] in the leaves of *Indigofera tinctoria*, while tyrian purple [**9**] is formed from the sulphate ester **7** via tyriverdin [**8**] (7). Compound **7** is hydrolzed by a

sulfatase to the corresponding 3-hydroxyindole (or 3-indolinone), which by oxidative coupling forms the dimeric tyriverdin [**8**] (8) [as a mixture of stereoisomers (9)]. The oxidation of indoxyl (3-indolinone) to indigo [**6**] has been shown to involve the indoxyl radical (10). Neither tyriverdin [**8**] nor tyrian purple [**9**] were originally present in the hypobranchial glands of the mollusc *Thais clavigera*; only tyrindoxyl sulphate [**7**] (9) was found. It is thus possible that the dimers [**1–4**] isolated in the present investigation are formed from similar precursor glucosides or sulphate esters (corresponding to compounds **5** and **7**). This, however, remains to be investigated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra (Tables 1 and 2) were recorded with a Bruker ARX500 spectrometer; the uv spectra with a Cary 219; the ir spectra with a Perkin-Elmer 257; and the mass spectra (ci with NH_3) with a JEOL SX102 spectrometer. Optical rotations were measured with a Perkin-Elmer 1541 automatic polarimeter (10 cm cell), and mps (uncorrected) were determined with a Reichert microscope.

FUNGAL MATERIALS.—Fruit bodies of *Collybia peronata* (Bolt. ex Fr.) Sing. and *Tricholoma scalpturatum* (Fr.) Quél. were collected in the vicinity of Lund, Sweden. Both species belong to the Tricholomataceae family of the Basidiomycotina subdivision of fungi. The fruit bodies were identified by Prof. Germund Tyler, department of plant ecology, and Prof. Börje Wickberg, department of Chemistry, of the University of Lund.

EXTRACTION AND ISOLATION.—The fruit bodies were extracted on the same day. Some specimens were ground in a meat grinder together with EtOAc or EtOH (2 liters per kg fruit bodies) in order to investigate the original contents (i.e., the preparation of extracts of intact fruit bodies). Other specimens were ground in a meat grinder without solvent, in order to injure the fruit bodies, and left as a mush for 15–30 min before they were extracted with EtOAc or EtOH. The extracts were compared by tlc analysis, and the compounds were isolated by chromatography on SiO_2 columns eluted with different mixtures of EtOAc and heptane. Fruit bodies of *C. peronata* (200 g) yielded 2 mg of peronatin A [**1**] and 15 mg of peronatin B [**2**], while 2500 g of *T. scalpturatum* yielded 3 mg of 7-hydroxy-7′-methoxyperonatin B [**3**], and 13 mg of 7,7′-dimethoxyperonatin B [**4**].

Peronatin A [**1**].—Compound **1** was obtained as a yellow oil; uv (EtOH) λ max (ε) 237 (27900), 260 (6100), and 387 nm (5100); ir ν max (KBr) 3340, 2940, 1685, 1620, 1510, 1310, 970, and 780 cm^{-1}; eims (70 eV) *m/z* 320 (M^+, 3), 161.0825

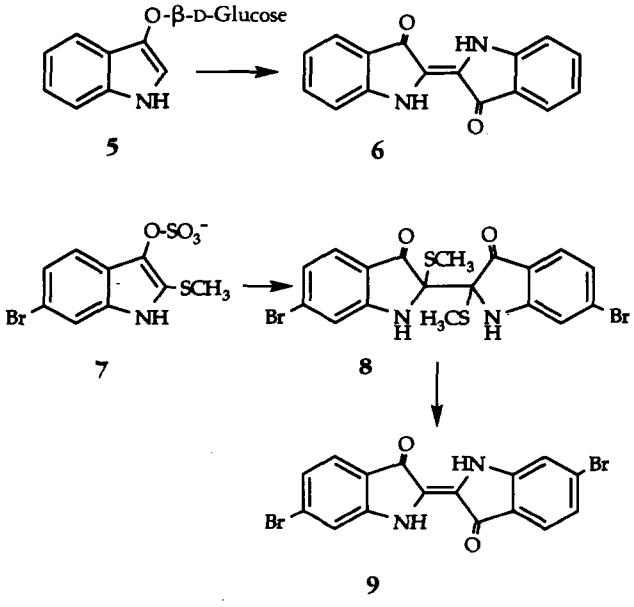


FIGURE 2. Oxidative Coupling Formation of Structurally Related 3-Indolinones Indigo [**6**] and Tyrian Purple [**9**].

(M/2+1, 100, $C_{10}H_{11}NO$ requires 161.0840), 131 (11), 90 (10). For nmr data, see Tables 1 and 2.

Peronatin B [**2**].—Compound **2** was obtained as yellow crystals, mp 213° (dec); uv (EtOH) λ max (ε) 238 (29400), 265 (7800), and 389 nm (5300); ir ν max (KBr) 3390, 2970, 2930, 1680, 1610, 1510, 1300, 1140, 970, and 780 cm^{-1}; eims (70 eV) *m/z* 320 (M^+, 4), 161.0823 (M/2+1, 100, $C_{10}H_{11}NO$ requires 161.0840), 131 (10), 90 (10). For nmr data, see Tables 1 and 2.

7-Hydroxy-7'-methoxyperonatin B [**3**].—Compound **3** was obtained as a yellow solid, mp 203–204°; uv (EtOH) λ max (ε) 226 (42100), 238 (33600), 278 (13300), and 401 nm (7100); ir (KBr) ν max 3360, 2920, 1685, 1620, 1530, 1265, 1170, and 970 cm^{-1}; eims (70 eV) *m/z* 366 (M^+, 2), 191.0950 (M/2+1, 100, $C_{11}H_{13}NO_2$ requires 191.0946), 176 (60), 147 (22), 106 (33), 90 (9). For nmr data, see Tables 1 and 2.

7,7'-Dimethoxyperonatin B [**4**].—Compound **4** was obtained as a yellow solid, mp 224–225°; uv (EtOH) λ max (ε) 227 (30900), 239 (27600), 277 (8600), and 401 nm (5100); ir ν max (KBr) 3400, 2920, 1680, 1620, 1530, 1250, 1170, 1060, and 970 cm^{-1}; eims 70 eV *m/z* 380 (M^+, 2), 191.0947 (M/2+1, 100, $C_{11}H_{13}NO_2$ requires 191.0946), 176 (13), 148 (15), 90 (10). For nmr data, see Tables 1 and 2.

ACKNOWLEDGMENTS

Financial support from the Swedish National Science Research Council is gratefully acknowledged. The authors thank Prof. Germund Tyler and Prof. Börje Wickberg for identifying the fruit bodies.

LITERATURE CITED

1. S.M. Camazine, J.F. Resch, T. Eisner, and J. Meinwald, *J. Chem. Ecol.*, **9**, 1439 (1983).
2. O. Sterner, R. Bergman, J. Kihlberg, and B. Wickberg, *J. Nat. Prod.*, **48**, 279 (1985).
3. M. De Bernardi, G. Vidari, and P. Vita-Finzi, *Tetrahedron*, **48**, 7331 (1992).
4. Z. Pang and O. Sterner, *J. Org. Chem.*, **56**, 1233 (1991).
5. A.L.J. Beckwith, *Chem. Soc. Rev.*, **22**, 143 (1993).
6. E. Giovannini, F. Farkas, and J. Rosales, *Helv. Chim. Acta*, **46**, 1326 (1963).
7. J.T. Baker and C.C. Duke, *Aust. J. Chem.*, **26**, 2153 (1973).
8. C. Christophersen, F. Wätjen, O. Buchardt, and U. Anthoni, *Tetrahedron*, **34**, 2779 (1978).
9. Y. Fujise, K. Miwa, and S. Ito, *Chem. Lett.*, 631 (1980).
10. G.A. Russell and G. Kaupp, *J. Am. Chem. Soc.*, **91**, 3851 (1969).

Received 21 December 1993