168. Novel, Racemic or Nearly-Racemic Antibacterial Bromo- and Chloroquinols and γ-Lactams of the Verongiaquinol and the Cavernicolin Type from the Marine Sponge *Aplysina* (= *Verongia*) *cavernicola*[1])

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Summary

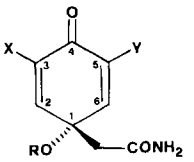
Butanolic extracts of the Mediterranean sponge *Aplysina* (= *Verongia*) *cavernicola* have given, by reverse-phase HPLC, the antibacterial quinols (±)-3-bromoverongiaquinol (= (±)-3-bromo-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetamide; **1d**) and (±)-3-bromo-5-chloroverongiaquinol (= (±)-3-bromo-5-chloro-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetamide; **1c**) besides the products of their formal cyclization 5-chlorocavernicolin (= 5-chloro-3,3a,7,7aβ-tetrahydro-3aβ-hydroxy-2,6(1*H*)-indoledione; **6**), the C(7)-epimerizing 7β-bromo-5-chlorocavernicolin (= 7β-bromo-5-chloro-3,3a,7,7aβ-tetrahydro-3aβ-hydroxy-2,6(1*H*)-indoledione; **4a**) and 7α-bromo-5-chlorocavernicolin (**4b**), and the C(7)-epimerizing 5-bromo-7β-chlorocavernicolin (= 5-bromo-7β-chloro-3,3a,7,7aβ-tetrahydro-3aβ-hydroxy-2,6(1*H*)-indoledione; **5a**) and 5-bromo-7α-chlorocavernicolin (**5b**). The latter four were isolated as mixtures of C(7)-epimerizing monoacetates **4a'**/**4b'** and **5a'**/**5b'**. Both **1d** and **1c** proved to be racemic from NMR examination of their esterification products with (−)-menthyloxyacetic acid, whilst **6** had a *ca.* 6% enantiomeric purity as shown by a ^{1}H-NMR study of its monoacetate **6'** in the presence of a chiral shift reagent. These chiroptical data of the first chiral quinols from the Verongida and of **6** suggest phenol oxidative routes from tyrosine precursors for their formation. In view of their bioactivities, **1d** and **1c** have been synthesized from (*p*-hydroxyphenyl)acetic acid by phenol oxidative routes.

1. Introduction. – Marine sponges of the order Verongida are of much current biological [1] and organic-chemical interest [2]. Unusual fatty acids [3], sterols [4], carotenoids [5], and amino acids [6] have been isolated from members of this order. However, what makes these sponges unique organisms is their extraordinary ability to give a vast array of dibrominated metabolites of either proven, in certain cases [7], or likely origin from 3,5-dibromotyrosine. These dibrominated metabolites have been recently reviewed from both the chemical [2] and the chemotaxonomical [8] points of view. To

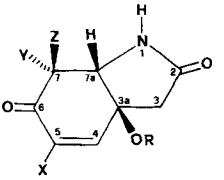
[1]) Presented in preliminary form in a lecture by *F. P.* at the 7th International Diving Science Symposium, Padova, September 15–18th, 1983.

these lists [2] [8] have been recently added some spirocyclohexadienylisoxazoles [9] related to aerothionin [10][2]).

The Verongida probably also elaborate both 3-bromotyrosine and 3,5-dichlorotyrosine, which may be seen as biogenetic precursors of 3,5-dichloroverongiaquinol (**1a**) [12], of some macrocyclic bastadins [13], of 5-bromocavernicolin (**2**) [14], and of an incompletely defined bromohomogentisamide [15]. Bromocavernicolin **2**, which has been isolated from *A. cavernicola* [14] is unusually interesting since it is the first discovered marine natural product having low (*ca.* 6%) enantiomeric purity [14]. As this may have an important bearing on biogenetic problems for natural products of the Verongida, we deemed interesting to investigate further the chiroptical properties of the Verongida products. In particular, quinols such as **1b** have been assumed to play a central [7], though obscure [16] role in the biogenesis of the tyrosine metabolites of these sponges. Unfortunately, all quinols so far isolated from these sponges such as **1b** [2] [17] and **1a** [12] are achiral, thus depriving us of fundamental informations. Following our recent discovery of **2** [14], we have now further investigated *A. cavernicola* in the hope of isolating a monobromo, and thus chiral, quinol. Our expectation has been fulfilled, and we have also isolated verongiaquinols [12] and cavernicolins [2] bearing both bromine and chlorine in the same molecule and thus representing further cases of chiral verongiaquinols.



	X	Y	R
1a	Cl	Cl	H
1b	Br	Br	H
1c	Br	Cl	H
1c′	Br	Cl	(−)-menthyloxyacetyl
1d	Br	H	H
1d′	Br	H	(−)-menthyloxyacetyl



	X	Y	Z	R
2	Br	H	H	H
3a	Br	H	Br	H
3b	Br	Br	H	H
4a	Cl	H	Br	H
4a′	Cl	H	Br	Ac
4b	Cl	Br	H	H
4b′	Cl	Br	H	Ac
5a	Br	H	Cl	H
5a′	Br	H	Cl	Ac
5b	Br	Cl	H	H
5b′	Br	Cl	H	Ac
6	Cl	H	H	H
6′	Cl	H	H	Ac

[2]) The chemotaxonomists have rendered a very useful service by pointing out several cases of erroneous classification within the Verongida, or of sponges erroneously taken to be Verongida, in organic-chemical papers [8]. This has served to rationalize previously puzzling results [8]. Unfortunately, a report according to which some Brazilian *Verongia* spp. do not produce brominated metabolites [11a] has escaped the chemotaxonomist's attention [8]. An appraisal of this point would have been useful. It is also relevant to mention here that the correction of Verongida classification in the chemical literature continues. This is the case of a recent statement that previous work in Naples on *Aplysina aerophoba* SCHMIDT should rather be referred to *Aplysina cavernicola* VACELET [9b], while previous organic-chemical work on a French collection of *A. cavernicola*, as identified by Dr. *J. Vacelet* himself [2], has unfortunately escaped the attention of these authors [9b]. It would also be useful to know whether collections of *Aplysina aerophoba* sent from Naples to Brazilian chemists for study [11b] should rather be referred to as of *A. cavernicola*.

2. Isolations and Bioassays. – Reverse-phase HPLC of the same fraction which previously gave **1a** [12] gave now with H_2O/MeOH 82:18 (5 ml/min) 3-bromo-5-chloroverongiaquinol (**1c**; 0.02% of dry sponge weight) 10.5 min (**1a** at 8.3 min). We have then examined the same fraction which previously gave **3a** and **3b** [2] by reverse-phase HPLC and gradient elution with H_2O/MeOH from 4:1 to 1:1 within 12 min (5 ml/min). We thus obtained the mixture **4a**/**5a** (6.3 min), **1c** (8.1 min), and the mixture **4b**/**5b** (9.6 min)[3]). Both mixtures **4a**/**5a** and **4b**/**5b** equilibrated immediately to the mixture **4a**/**4b**/**5a**/**5b** of the bromochlorocavernicolins. However, acetylation of such a mixture, followed by chromatography *(Exper. Part, Section 9)*, cleanly led to the acetates **4a'**, **4b'**, **5a'**, and **5b'**. Both **4'** and **5'** were observed to epimerize at C(7) so quickly as to prevent recording their individual spectra, which were rather obtained for the equilibrated mixtures **4a'**/**4b'** and **5a'**/**5b'** of C(7)-epimers ([**4a'**]/[**4b'**] = 1.6; 0.0027% overall yield of dry sponge weight; [**5a'**]/[**5b'**] = 1.6; 0.0006% overall yield of dry sponge weight).

The same fraction which previously gave **3a** and **3b** [2] was also examined by reverse-phase HPLC with MeCN/H_2O 1:9 (5.5 ml/min). This allowed us to separate the rather unstable 3-bromoverongiaquinol (**1d**) (0.004% of dry sponge weight) at 5.6 min, besides **2** [14] at 6.0 min.

Finally, we examined the last fractions from the previously described '*Merck*-Kieselgel-*60*' column [2]. Reverse-phase HPLC with H_2O/tetrahydrofuran 98:2 (5 ml/min) gave 5-chlorocavernicolin (**6**; 0.008% of dry sponge weight) at 8.9 min, 3-bromoverongiaquinol (**1d**) at 10.9 min, and **2** [14] at 11.6 min.

The quinols **1c** and **1d** have been assayed by the *Petri*-disc zonal inhibition technique. The bromochloro compound **1c** proved to strongly inhibit the gram-positive bacterium *Sarcina lutea* and the gram-negative bacteria *Alcaligena foecalis* and *Proteus vulgaris*. The bromo compound **1d** proved to strongly inhibit the gram-positive bacteria *Streptococcus foecalis* and *Bacillus subtilis*, whilst it failed to inhibit *A.foecalis* and *P.vulgaris* as well as *Trichothecium roseum*. These are the only bioassays which have been carried out. It is interesting that 3,5-dibromoverongiaquinol (**1b**) [2] [17] has been recently shown to possess cytotoxic activity [18].

3. Structure Elucidations and Synthesis. – 3.1. *3-Bromoverongiaquinol* (**1d**). MS and ^{13}C-NMR data give the composition $C_8H_8BrNO_3$. UV and IR data suggest a quinol with an acetamide side chain [2] [12]. ^{1}H-NMR spectra reveal two non-equivalent enone β-H-atoms, one of which is also coupled to a higher-field enone α-H-atom. All this points to the chiral structure **1d**, which is also in accordance with ^{13}C-NMR multiplicities.

However, unusual for a chiral natural product, **1d** failed to show optical activity. Considering the meager molecular dissymmetry, we checked whether the failure to show optical acitivity is due to intrinsically low optical activity or to racemic mixtures. Since **1d** proved not soluble in low-polarity solvents suitable for chiral shift reagents, **1d** was esterified with (−)-menthyloxyacetic acid to the mixture **1d'** of diastereoisomers which could not be separated by HPLC. The ^{1}H-NMR spectrum of **1d'** clearly shows the H−C(2) and H−C(6) resonances for the two diastereoisomers in a 1:1 ratio, as expected for racemic **1d**.

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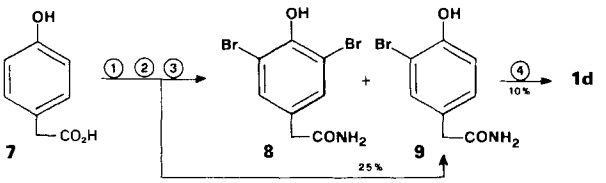
[3]) Besides **3a** [2]/**1a** [12] (7.3 min), **1b** [2] (8.8 min), and **3b** [2] (10.2 min).

Scheme 1



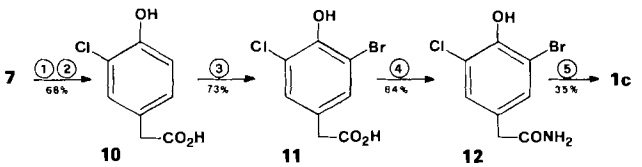
① Br_2, AcOH, dark, r.t., 60 h. ② Urea, 140°, 2 h. ③ SiO_2-column chromatography. ④ $Tl(ClO_4)_3$/60% $HClO_4$, 2 h.

Because of the strong, wide-spectrum antibacterial activity, racemic **1d** was synthesized starting from (*p*-hydroxyphenyl)acetic acid (**7**) according to *Scheme 1* (→**8** + **9**). This is of interest also in connection with the recently discovered cytotoxic activity of the related quinol **1b** [18].

3.2. *3-Bromo-5-chloroverongiaquinol* (**1c**). MS (EI; high resolution) and ^{13}C-NMR data agree for the composition $C_8H_7BrClNO_3$. UV and IR data suggest a quinol with an acetamide side chain, as above for **1d**. In the ^{1}H-NMR spectrum, two non-equivalent enone β-H-atoms are revealed as two very low-field *AB* systems. This suggests the chiral structure **1c**, in accordance also with ^{13}C-NMR multiplicities. However, as in the case of **1d**, **1c** failed to show optical activity. For reasons stated above for **1d**, reinforced by the fact that CH_2CONH_2 shows up as a sharp *s* even at 300 MHz, in spite of the chiral structure, **1c** was reacted with (–)-menthyloxyacetyl chloride to give the mixture **1c′** of diastereoisomers which could not be separated by HPLC. The two diastereoisomers were revealed in a 1:1 ratio by ^{1}H-NMR, as in the case of **1d′**.

Racemic **1c** was synthesized, for the same reasons as **1d**, along phenol oxidative routes, as shown in *Scheme 2* (**7**→**10**→**11**→**12**→**1c**).

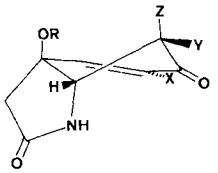
Scheme 2

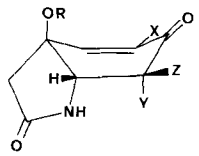


① Cl_2, AcOH, dark, r.t., overnight. ② HPLC. ③ Br_2, AcOH, dark, r.t., 60 h. ④ Urea, heating. ⑤ $Tl(ClO_4)_3$/60% $HClO_4$, 0°, 2 h.

3.3. *5-Chlorocavernicolin* (**6**). The MS shows the molecular ion at *m/z* 201 and 203 requiring 1 Cl-atom and primary losses from M^+ of OH, H_2O, CO, ketene, and $C_3H_2O_2$, which is reminiscent of the pattern for the cavernicolins [2] [14]. Both the ^{1}H-NMR and the ^{13}C-NMR spectra are quite similar to those of 5-bromocavernicolin (**2**) [14], when due account is taken of the relative effects of the Cl- and Br-atom.

In fact, Br has a stronger deshielding effect than Cl on both H–C(4) and C(4). In contrast, Cl has a stronger deshielding effect than Br on C(5), as shown by data for **1c**. Also, the *H*–C(7a)/*H*–C(4) W-coupling is typical of both cavernicolin-2 (= 5,7-dibromocavernicolin; **3b**) [2] and 5-bromocavernicolin (**2**) [14]. This suggests a quasi-chair conformation for **6** (as well as, on the same grounds, for **6′**; see below) *(Fig.)*. This is similar to the cases of both **3b** [2] and **2** [14]. In the hypothetical case of a quasi-boat conformation (of the type of **4a** and **5a**, *Fig. 1*) for **6**, no coupling between H–C(7a) and H–C(4) would have been expected [2].





	X	Y	Z	R
4b	Cl	Br	H	H
5b	Br	Cl	H	H
6	Cl	H	H	H
6′	Cl	H	H	Ac

	X	Y	Z	R
4a	Cl	H	Br	H
5a	Br	H	Cl	H

Figure. *Preferred quasi-boat conformation for* **4a** *and* **5a** *and quasi-chair conformation for* **4b**, **5b**, **6**, *and* **6′**

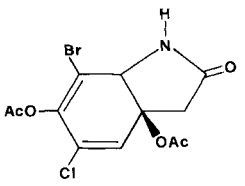
The weak optical rotation of **6** suggested a low enantiomeric purity like in the case of 5-bromocavernicolin (**2**) [14]. The low solubility of **6**, in low-polarity solvents preventing a direct use of chiral shift reagents, **6** was acetylated to give **6′**, which was isolated after repeated HPLC (no recrystallizations). Addition of the chiral shift reagent $Yb(HFBC)_3$ to a solution in $CDCl_3$ led to low-field shifts for all signals and a splitting for those of OAc and H–C(3), each into two broad *s* integrating for 87:77 (after repeated pulses, allowing for relaxation). This leads to a *ca.* 6% enantiomeric purity, *i.e.* to the same situation as for 5-bromocavernicolin (**2**) [14]. In this case too, either repeated extractions of **6**, or prolonged acetylation times led to the same result, thus ruling out racemizations during either the extraction or the acetylation. This allows to estimate $[\alpha]_D^{23} \approx -15°$ for (–) – **6′**[4]).

3.4. *7-Bromo-5-chlorocavernicolins* **4a** *and* **4b** *and 5-Bromo-7-chlorocavernicolins* **5a** *and* **5b**. HPLC only allowed us to separate **4a**/**5a** from **4b**/**5b** and from other components, but both mixtures, were immediately equilibrated to a mixture of all four bromochlorocavernicolins (see *Section 2*, above). Therefore, only spectra for the total mixture could be recorded. However, owing to our previous experience with **3a** and **3b** [2], we could extract from these spectra the ^{1}H-NMR data for all individual components and the ^{13}C-NMR data for the more abundant component **4a** (see *Exper. Part, Section 8*). Arguments similar to those used above for **6** and previously [2] for **3a** and **3b** suggest a quasi-chair conformation for both **4b** and **5b** and a quasi-boat conformation for both **4a** and **5a** *(Fig.)*.

Separate spectra could be run for the monoacetates **4a′**/**4b′** and **5a′**/**5b′**. Moreover, from the acetylation mixture the diacetylation product **13** of the more abundant couple of epimers (**4a** and **4b**), was isolated, where problems of equilibration are no more present. ^{1}H-NMR data for **13** in the presence of a chiral shift reagent indicate that **13**, and thus **4a** and **4b**, are racemic.

4. Conclusions. – It has been shown by biosynthetic experiments *in vivo*, that the Caribbean sponge *Aplysina fistularis* is able to transform phenylalanine into 3,5-dibromotyrosine and **1b** without deamination [7]. The intermediacy of, in the given sequence, (3,5-dibromo-4-hydroxyphenyl)pyruvic acid oxime, (3,5-dibromo-4-hydroxyphenyl)acetonitrile, and (3,5-dibromo-4-hydroxyphenyl)acetamide has also been sug-

[4]) No absolute-configuration significance is to be attached to any of the structural formulae given.



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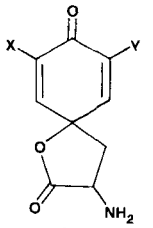

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gested [7] from the isolation of the last two compounds from the same sponge [7] and of (4-hydroxyphenyl)pyruvic acid oxime from the unrelated sponge *Hymeniacidon sanguinea* (Halichondrida) [19]. This is consistent with our findings as to the occurrence of the chiral quinols **1c** and **1d** as racemates in *Aplysina cavernicola,* provided that the change of the appropriate (halo-4-hydroxyphenyl)acetamide into the quinol is seen as a phenol oxidation [20]. In fact, natural products deriving from phenol oxidations occur in nature in both optically active forms or as racemates, or even in nearly-racemic forms [21].

The suggestion of an arene epoxide as a biogenetic precursor of **1b** in a *Verongia* sp. of the Gulf of California [22] is not consistent with the above findings for our sponge, as such a precursor would have demanded enantiomerically pure **1d** and **1c**. Admittedly, arene epoxides are formed at low enantiomeric purity in foreign compound metabolism [23]. However, this might be simply the result of the operation of enzymes which could not have been exactly designed for the metabolization of the particular foreign compound. The case of natural products is different, as the enzymes do not lack their usual enantiospecificity in giving arene epoxides [23b].

The occurrence of racemic (**3** [2], **4**, and **5**) and nearly-racemic (**2** [14] and **6**) cavernicolins also suggests phenol oxidative pathways for the same reasons as above. Concerning this it must be recalled that the persulfate oxidative cyclization of certain amides to either γ-or δ-lactams has been performed [24]. However, a way to rationalize the formation of the cavernicolins in full respect of the classical *ortho-para* orientation rules for the oxidative coupling of phenolic compounds [21] is to postulate a racemic or nearly-racemic spirolactone **14** as intermediate derived from a halotyrosine precursor. There is, in fact, ample precedent for cyclohexadienone spirolactones in oxidative couplings of phenols [21]. Hydrolysis of the spirolactone, followed by conjugate attack of the amino-acid N-atom and decarboxylative oxidation may then be seen to lead to the cavernicolins. As to the halotyrosine precursors, it is relevant that free 3-chlorotyrosine, 3-bromotyrosine, and 3-chloro-5-bromotyrosine have been detected in the marine crab *Limulus polyphemus* [25].

We thank Dr. *R. De Clauser* for the bioassays and *MPI, CNR* (Roma), and the *Provincia Autonoma di Trento, Assessorato Agricoltura* (project on the biological control of phytopathogenesis), for financial support.

Experimental Part

1. *General Remarks.* Reverse-phase HPLC was carried out on a *Merck-LiChrosorb-RP-18* (7 μm) column (25 × 1 cm), except for mediumpressure HPLC, which was carried out on *Merck-LiChrosorb-RP-18* (25–40 μm) using a *Jobin-Yvon Miniprep* with a 2 × 30 cm column. NMR spectra were obtained with *Varian-CFT20* (^{13}C-NMR at 20 MHz with a microprobe, ^{1}H-NMR at 80 MHz) or *Bruker-CXP-300* (^{1}H-NMR at 300 MHz)

spectrometers; chemical shifts δ are given in ppm with respect to internal Me_4Si and coupling constants J in Hz. Multiplicities for ^{13}C-NMR spectra are from off-resonance decoupling. MS (EI) were taken with either a home-made spectrometer based on the *ELFS-4-162-8-Extranuclear* quadrupole or a *VG ZAB2F* spectrometer, the latter allowing us to carry out linked-scans (B/E). IR and UV spectra are from a *Perkin-Elmer-337* and *Beckman-DB-4* spectrometer. Optical rotations were measured with a *Jasco-DIP-181* polarimeter (10-cm cell). Isolations: see *General Part*.

2. *(±)-3-Bromoverongiaquinol (= (±)-3-Bromo-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetamide;* **1d**). Sticky semi-solid; no [α] from 589 to 365 nm (c = 0.45 g/100 ml, MeOH). UV (MeOH): 235 (5500). IR (film): 3300*s*, 3150*s*, 1660*s*, 1610*s*, 1580*m*, 1460*s*, 1380*m*. ^{1}H-NMR (80 MHz, $(CD_3)_2CO$): 7.52 (*d*, J = 2.8, 1H, H–C(2)); 7.08 (*dd*, $J_{5,6}$ = 10.0, $J_{6,2}$ = 2.8, 1H, H–C(6)); 6.21 (*d*, J = 10.0, 1H, H–C(5)); 3.0 (br., 3H, OH, 2NH); 2.68 (*s*, 2H, CH_2); double irradiations fully support the assignments. MS: 248, 246 (4 each, $(M+1)^+$); 247, 245 (4 each, M^+); 230, 228 (8 each, $M^+ - 17$); 202, 200 (6 each); 189, 187 (10 each); 166 (7, $M^+ - Br$); 161, 159 (10 each); 149 (10); 133, 131 (3 each); 123, 121 (4 each).

3. *3-Bromoverongiaquinol (–)-Menthyloxyacetates (= 3-Bromo-1-menthyloxyacetoxy-4-oxo-2,5-cyclohexadiene-1-acetamide, Mixture of Diastereoisomers;* **1d′**). According to [26], to naturally occurring **1d** (0.002 g) in 0.5 ml of dried benzene were added (–)-menthyloxyacetic acid (*Fluka*, $[\alpha]_D^{20} = -93 \pm 3°$ (c = 10, EtOH); 20 µl, 0.1 mmol) and 4-(dimethylamino)pyridine (0.003 g). The mixture was cooled in an ice bath and then, under N_2 with stirring, was added dicyclohexylcarbodiimide (0.020 g). After 3 h stirring at r.t., the mixture was flash-chromatographed on *Merck LiChrosorb Si-60* with, stepwise, Et_2O (4 ml), acetone (2 ml), and 95% EtOH (2 ml). The residue from evaporation of the acetone fraction was further purified by TLC on silica gel with benzene AcOH 75:25 to give oily **1d′** in 95% yield. ^{1}H-NMR (80 MHz, $CDCl_3$): 7.55, 7.54 (2 *d*, J = 2.9, 1H each, H–C(2)); 7.14, 7.13 (2 *dd*, $J_{5,6}$ = 10.1, $J_{5,3}$ = 2.9, 1H each, H–C(6)); 6.43 (2 coincident *d*, J = 10.1, 1H each, H–C(5)); 4.15, 4.08 (two coincident *AB*, J = 16.0, 2H each, $CH_2COOC(1)$); 2.81, 2.79 (2 *s*, 2H each, CH_2CONH_2); 3.2 (2 coincident *m*, 1H each, H–C(3′)); 0.92 (or 0.91) (2 coincident *d*, J = 6.0, 3H each, CH_3–C(1′)); 0.91 (or 0.92) (2 coincident *d*, J = 7.1, 3H each, CH_3CHCH_3; 0.79 (2 coincident *d*, J = 6.9, 3H each CH_3CHCH_3).

4. *Synthesis of* **1d**. To a solution of (*p*-hydroxyphenyl)acetic acid (**7**; 1.5 g, 0.01 mmol) in 50 ml of glacial AcOH was added Br_2 (1.6 g, 0.01 mmol). The mixture was stirred in the dark for 60 h, then evaporated *in vacuo*. Urea (1.2 g) was added and the mixture heated at 140° under N_2 for 2 h. Addition of H_2O and column chromatography on *Merck* 'Kieselgel' 70–230 mesh with benzene/AcOH 95:5 led to, in the order of increasing elution time, *(3,5-dibromo-4-hydroxyphenyl)acetic acid* [17] (0.95 g), *(3-bromo-4-hydroxyphenyl)acetic acid* (0.5 g) m.p. 105–107°, unreacted **7** (0.03 g), *(3-bromo-4-hydroxyphenyl)acetamide* (**9**; 0.1 g), *(3,5-dibromo-4-hydroxyphenyl)acetamide* (**8**; 0.58 g, 25%), m.p. 181–182°, and *(4-hydroxyphenyl)acetamide*. To a suspension of **8** (0.1 g) in dioxane/H_2O 1:3 (2 ml) at 0° were added, dropwise, 1.7 ml of 0.26M $Tl(ClO_4)_3$ in 60% perchloric acid. After 2 h, H_2O (100 ml) was added and the mixture repeatedly extracted with 1-BuOH. The org. phase was evaporated *in vacuo* and the residue was subjected to reverse-phase chromatography with H_2O/MeOH 85:15 (5 ml/min) to get **1d** (0.01 g, 10%) at 6.4 min (identical with the naturally occurring compound in any respect) besides unreacted **8** (0.003 g) at 9.3 min.

5. *(±)-3-Bromo-5-chloroverongiaquinol (= (±)-3-Bromo-5-chloro-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetamide* **1c**). Colourless crystals, m.p. 175–77°, no [α] from 589 to 365 nm (c = 0.4 g/100 ml, MeOH). IR (nujol): 3400*s*, 3150*m*, 1695*m*, 1590*w*. ^{1}H-NMR ($(CD_3)_2CO$, 300 MHz): 7.58, 7.33 (*AB*, J = 2.6, 1H each H–C(2), H–C(6), resp.); 7.1, 6.6 (br., 1H each, H_2N); 5.92 (br. *s*, 1H, OH); 2.78 (*s*, 2H, CH_2). ^{13}C-NMR ($(CD_3)_2SO$): 172.6 (*s*, C(4)); 169.4 (*s*, $CONH_2$); 153.2 (*d*, C(2)); 148.8 (*d*, C(6)); 127.6 (*s*, C(5)); 119.9 (*s*, C(3)); 70.8 (*s*, C(1)); 45.1 (*t*, CH_2). MS: 283 (1), 281 (4), 279 (3, M^+; calc. for $C_8H_7{}^{79}Br^{35}ClNO_3$ 278.9298; found 278.9292 ± 0.001); 266 (4), 264 (17), 262 (13, $M^+ - OH$); 239 (9); 236 (34); 234 (22, 262 – CO); 202 (6); 200 (8, $M^+ - Br$); 53 (100).

6. *3-Bromo-5-chloroverongiaquinol (–)-Menthyloxyacetates (= 3-Bromo-5-chloro-1-menthyloxyacetoxy-4-oxo-2,5-cyclohexadiene-1-acetamide, Mixture of Diastereoisomers;* **1c′**). Standard acylation of **1c** with (–)-menthyloxyacetyl chloride (*Erba*, $[\alpha]_D^{20} = -107 \pm 2.5°$) and pyridine for 15 min, followed by reverse-phase HPLC with MeOH/H_2O 8:2 (5 ml/min) gave, at 11.0 min, oily **1c′** as a single peak (50% yield) besides little unreacted **1c** at 2.8 min. **1c′**: ^{1}H-NMR (80 MHz, $(CD_3)_2CO$): 7.79, 7.78 (2 *d* (1:1), J = 2.7, 1H each, H–C(2)); 7.56, 7.55 (2 *d*, J = 2.7, 1H each, H–C(6)); 4.18 (2 coincident *s*, 2H each, $CH_2COOC(1)$); 2.96 (2 coincident *s*, 2H each, CH_2CONH_2); 0.91 (or 0.87) (2 coincident *d*, J = 5.9, 3H each, CH_3–C(1′)); 0.87 (or 0.91) (2 coincident *d*, J = 7.0, 3H each, CH_3CHCH_3); 0.77 (2 coincident *d*, J = 7.0, 3H each, CH_3CHCH_3).

7. *Synthesis of* **1c**. A solution of **7** (3.0 g in 0.33M Cl_2 in AcOH (60 ml) was stirred overnight at r.t. in the dark. The mixture was evaporated, and the residue was subjected to medium-pressure reverse-phase HPLC with

MeOH/5% AcOH 1:3 (11 ml/min) to get *(3-chloro-4-hydroxyphenyl)acetic acid* (**10**), m.p. 108–109° (68%), at 11.5 min besides little *(3,5-dichloro-4-hydroxyphenyl)acetic acid*, m.p. 180–183°. To a solution of **10** (2.5 g) in 70 ml of glacial AcOH was added Br_2 (0.8 ml), and the mixture was stirred for 60 h in the dark. Evaporation of the mixture *in vacuo* left a residue which was washed with benzene/Et_2O 6:4 to give *(3-bromo-5-chloro-4-hydroxyphenyl)acetic acid* (**11**; 2.6 g, 73%), m.p. 174–177°. A mixture of **11** and urea was heated, under N_2 with stirring, until the evolution of NH_3 from the melt ceased. Partition between 5% aq. $NaHCO_3$ and AcOEt gave *(3-bromo-5-chloro-4-hydroxyphenyl)acetamide* (0.36 g, 84%), m.p. 180–183° after HPLC. To a suspension of **12** (0.1 g) in H_2O/dioxane 1:3 (2 ml) at 0° were added, dropwise, 1.5 ml of 0.26M $Tl(ClO_4)_3$ in 60% perchloric acid. After 2 h, the mixture was diluted with H_2O (100 ml), repeatedly extracted with AcOEt, and the org. layer evaporated to leave a residue which was subjected to reverse-phase HPLC with H_2O/MeOH 6:4: **1c** (0.035 g, 35%), identical with the naturally occurring product.

8. *Mixture of 7β-Bromo-5-chlorocavernicolin (= 7β-Bromo-5-chloro-3,3a,7,7aβ-tetrahydro-3aβ-hydroxy-2,6(1H)-indoledione;* **4a**), *7a-Bromo-5-chlorocavernicolin* (**4b**), *5-Bromo-7β-chlorocavernicolin (= 5-Bromo-7β-chloro-3,3a, 7,7aβ-tetrahydro-3aβ-hydroxy-2,6(1H)-indoledione* (**5a**) *and 5-Bromo-7α-chlorocavernicolin* (**5b**). Sticky semi-solid, isolated as described in the *General Part, Section 2*. UV (MeOH): 250 (2,500), 312 (60). ^{1}H-NMR ($(CD_3)_2CO$, 80 MHz): for **4a**: 7.21 (*s*, 1H, H–C(4)); 5.15 (*d*, *J* = 9.8, 1H, H–C(7)); 5.5 (br. *s*, 1H, NH); 4.19 (br. *d*, *J* = 9.8, 1H, H–(7a)); 2.90, 2.50 (*AB*, *J* = 16.9, 2H, 2H–C(3)); for **4b**: 7.07 (br. *s*, 1H, H–C(4)); 5.5 (br. *s*, 1H, NH); 5.24 (*d*, *J* = 4.1, 1H, H–C(7)); 4.8 (br. *d*, *J* = 4.1, 1H, H–C(7a)); 2.74, 2.59 (*AB*, *J* = 16.9, 2H, 2H–C(3)); for **5a**: 7.43 (*s*, 1H, H–C(4)); 5.6 (br. *s*, 1H, NH); 5.06 (*d*, *J* = 10.4, 1H, H–C(7)); 4.03 (br. *d*, *J* = 10.4, 1H, H–C(7a)); 2.90, 2.48 (*AB*, *J* = 16.9, 2H, CH_2); for **5b**: 7.32 (br. *s*, 1H, H–C(4)); 5.6 (br. *s*, 1H, NH); 5.26 (*d*, *J* = 3.8, 1H, H–C(7)); 4.48 (br. *d*, *J* = 3.8, 1H, H–C(7a)); 2.74, 2.56 (*AB*, *J* = 16.9, 2H, 2H–C(3)). ^{13}C-NMR ($(CD_3)_2SO$, 20 MHz): only the signals for the more abundant **4a** could be clearly identified: 183.4 (C(6)); 173.6 (C(2)); 146.3 (C(4)); 127.4 (C(5)); 74.4 (C(3a)); 67.9 (C(7a)); 58.0 (C(7)); 42.4 (C(3)). MS: 283 (4), 281 (14), 279 (11) (four M^+); fragmentations are only shown for the acetates, see below.

9. *Acetylation of the Mixture* **4a**/**4b**/**5a**/**5b**. The mixture **4a**/**4b**/**5a**/**5b** (0.011 g) was treated with 3 drops of Ac_2O and 2 drops of dry pyridine at 0° for 2 h. Reverse-phase HPLC of the mixture under gradient-elution with H_2O/MeOH from 8:2 to 1:1 within 12 min led to **5a′** (11.0 min), **5b′** (11.5 min), **4a′** (12.3 min), **4b′** (12.9 min), and **13** (14.0 min). The yield of the sticky semi-solid **4a′**/**4b′** was 26%, that of the sticky semi-solid diacetate **13** 50%, while only a very minute amount of **5a′**/**5c′** was obtained only allowing the MS to be recorded. *7β-Bromo-5-chlorocavernicolin Acetate (= 3aβ-Acetoxy-7β-bromo-5-chloro-3,3a,7,7aβ-tetrahydro-2,6(1H)-indoledione;* **4a′**)/*7a-Bromo-5-chlorocavernicolin Acetate* (**4b′**). ^{1}H-NMR (80 MHz, C_6D_6): for **4a′**: 6.66 (*s*, 1H, H–C(4)); 5.9 (br. 1H, NH); 3.84 (*dd*, $J_{7a,7}$ = 8.8, $J_{7a,3a}$ = 1.2, H–C(7a)); 3.45 (*d*, *J* = 8.8); 2.51, 2.28 (*AB*, *J* = 17.5, 2H, 2H–C(3)); 1.56 (*s*, 3H, CH_3); for **4b′**: 6.48 (*d*, *J* = 0.9, 1H, H–C(4)); 5.9 (br., 1H, NH); 4.42 (*d*, *J* = 4.3); 3.60 (br. *J* = 4.3, 1H, H–C(7a)); 2.53, 2.16 (*AB*, *J* = 18.0, 2H–C(3)); 1.55 (*s*, 3H, CH_3); **4a′**/**4b′** = 1.6. MS: 325 (0.5), 323 (1.3), 321 (1, M^+); 266 (1), 264 (3), 262 (2, M^+–AcO); 265 (6), 263 (24), 261 (17, M^+–AcOH); 184 (13), 182 (36, 261–Br); 185 (13), 183 (36, 262–Br); 156 (9), 154 (26, 182–CO); 237 (1), 235 (4), 233 (3, 261–CO); 157 (5), 155 (14, 183–CO); 43 (100).

5-Bromo-7β-chlorocavernicolin Acetate (= 3aβ-Acetoxy-5-bromo-7β-chloro-3,3a,7,7aβ-tetrahydro-2,6(1H)-indoledione; **5a′**)/*5-Bromo-7α-chlorocavernicolin Acetate* (**5b′**). MS: 325 (0.3), 323 (1.2), 321 (1, M^+); 266 (0.8), 264 (2.9), 262 (1.6, M^+–AcO); 265 (4), 263 (15), 261 (12, M^+–AcOH); 228 (6), 226 (4, 261–Cl); 229 (25), 227 (25, 262–Cl); 201 (6), 199 (6, 227–CO); 200 (4), 198 (4, 226–CO); 184 (2), 182 (4, 261–Br); 185 (2), 183 (6, 262–Br); 156 (2.5), 154 (7, 182–CO); 120 (12, 199–Br); 43 (100, $OCNH^+$).

7-Bromo-5-chlorocavernicolin Diacetate (= 3aβ,6-diacetoxy-7-bromo-5-chloro-1,3,3a,7aβ-tetrahydro-2-indolone; **13**). ^{1}H-NMR (C_6D_6, 80 MHz): 6.17 (*s*, 1H, H–C(4)); 5.95 (br. 1H, NH); 4.19 (br. *s*, 1H, H–C(7a)); 2.52, 2.43 (*AB*, *J* = 17.7, 1H, 2H–C(3)); 1.73, 1.35 (2 *s*, 3H each, 2 Ac). Addition of $Yb(HFBC)_3$: all signals shifted to lower field; Ac signals split into 2 *s* each with a separation much lower than in the case of **6′** and of the cavernicolin-1 and -2 diacetate [2], approximate integration 1:1, pointing to racemic **13**.

10. *5-Chlorocavernicolin (= 5-Chloro-3,3a,7,7aβ-tetrahydro-3aβ-hydroxy-2,6(1H)-indoledione;* **6**). Sticky semi-solid UV (MeOH): 238 (4,100). ^{1}H-NMR ($(CD_3)_2$ CO/little D_2O; 80 MHz): 7.03 (*d*, *J* = 0.9, 1H, H–C(4)); 4.14 (*ddd*, $J_{7a,7\alpha}$ = 6.0, $J_{7a,7\beta}$ = 5.1, $J_{7a,4}$ = 0.9, 1H, H–C(7a)); 3.04, 2.80 (*AB*, *J* = 16.4, 2H, H_β– and H_α–C(7), resp.); 2.70 (*s*, 2H, 2H–C(3)). ^{13}C-NMR (CD_3OD, 20 MHz): 183.1 (C(6)); 176.3 (C(2)); 145.4 (C(4)); 133.0 (C(6)); 74.8 (C(3a)); 60.8 (C(7a)); 45.0 (C(3)); 41.0 (C(7)). MS: 203 (3), 201 (9, M^+); 186 (1), 184 (3, M^+–17, also from B/E); 185 (1), 183 (3, M^+–H_2O, also from B/E); 175 (0.8), 173 (2, M^+–CO, also from B/E); 166 (51, M^+–Cl, also from B/E); 161 (15), 159 (46, M^+–CH_2CO, also from B/E); 134 (5), 132 (15, probably M^+–71); 133 (5), 131 (15, M^+–$C_3H_2O_2$, also from B/E); 96 (21, probably 131–Cl); 70 (95); 43 (100, $OCNH^+$).

Acetylation of **6**. At 0°, pyridine (2 drops) and Ac_2O (3 drops) where added to **6** (0.005 g) and stirred for 4 h. Reverse-phase HPLC with H_2O/MeCN 95:5 led to **6′** (0.0035 g, 73%) at 7.9 min besides unreacted **6** (0.001 g) at 6.7 min. *5-Chlorocavernicolin Acetate (= 3aβ-Acetoxy-5-chloro-3,3a,7,7aβ-tetrahydro-2,6(1H)-indoledione; 6′):* $[\alpha]_D^{23} = -0.93°$ ($c = 0.75$, MeOH), calculated as if **6′** were enantiomerically pure. With *ca.* 6% (measured) enantiomeric excess *(Sect. 3.3)* we obtain $[\alpha]_D^{23} \simeq -15°$ for enantiomerically pure (−)-**6′**. **6′**: ^{1}H-NMR ($CDCl_3$, 80 MHz): 7.14 (*d*, $J = 1.3$, 1H, H–C(4)); 5.96 (br. *s*, 1H, NH); 4.33 (*ddd*, $J_{7a,7\alpha} = 4.7$, $J_{7a,7\beta} = 4.3$, $J_{7a,4} = 1.3$, 1H, H–C(7a)); 3.16, 2.78 (*AB*, $J = 17.0$, H_α– and H_β–C(7), resp.); 2.97 (*s*, 2H, 2H–C(3)); 2.11 (*s*, 3H, CH_3); data with **4b** $(HFBC)_3$ in *Sect. 3.3*.

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