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# Fungal metabolites. Part 45:☆ The sesquiterpenes of *Collybia maculata* and *Collybia peronata*

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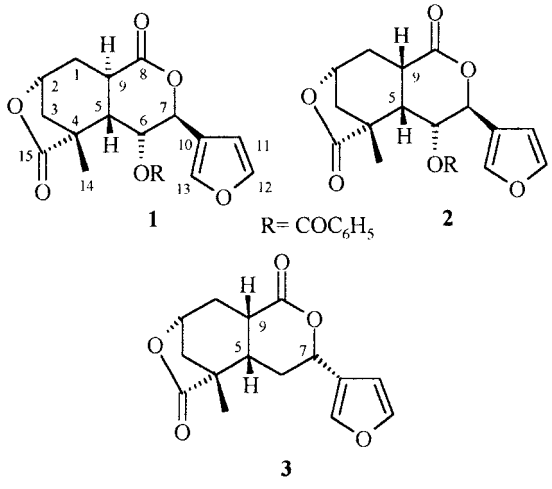
**Abstract**—The sesquiterpenes of *Collybia maculata* and *C. peronata* (Basidiomycetes) have been reinvestigated. In addition to collybolide, isocollybolide and deoxycollybolidol, five new structurally related sesquiterpene lactones have been isolated. The structures have been established by spectroscopic methods. Stereochemical assignments required a detailed conformational investigation, which has been carried out at the AM1 level for all compounds, and at the B3LYP level for some of them. This led to the inversion of the previously proposed C-7 configuration of deoxycollybolidol. The absolute configuration of collybolides could tentatively be inferred from CD studies. 

## 1. Introduction

Collybolides **1**–**3** constitute an unusual class of sesquiterpenes, which so far have been isolated only from two *Collybia* species (Basidiomycetes), the first two compounds from *C. maculata*[1] and the third one from *C. peronata*.[2] The structures **1**–**3** consist of a tricyclic nucleus bearing both a γ- and a δ-lactone group, and a benzoate and a furan ring as substituents. The junction between the two six-membered rings is *trans* in collybolide **1** and *cis* in **2** and **3**. The structure of compound **2** was firmly established by X-ray analysis.[3] The molecular formula of compound **1**, already recognized as a stereoisomer of **2**, was determined by $^1$H NMR spectroscopy and epimerization of **2** to **1** in the presence of trifluoroacetic acid.[1] Deoxycollybolidol was assigned structure **3**, in which the C-6 hydroxy group is missing and the configuration of the C-7 stereocentre is inverted with respect to **2**.[2]

In the course of screening higher fungi as a source of new bioactive compounds, we decided to reinvestigate the secondary metabolites of *C. maculata* and *C. peronata*. Indeed, the authors of previous investigations extracted the two species with acetone[1] or a mixture of $CHCl_3$–MeOH,[2] which very often promote formation of artifacts in contact with mushroom fruiting bodies.[4]




**1** **2** **3**

For this reason, in the present investigation fresh and undamaged fruiting bodies of the two *Collybia* species were frozen at −20°C and then minced and extracted with EtOAc at the same temperature, following a standard procedure which has proved to be harmless.[4] Deoxycollybolidol was again the only collybolide-like sesquiterpene isolated from *C. peronata*. Collybolide and isocollybolide were both present in the extract of *C. maculata*; however, contrarily to the previous investigation when partial epimerization of **2** probably occurred during the extraction or isolation step, isocollybolide was found to be more abundant than collybolide. Moreover, five new sesquiterpenes **4a**, **4b**, **4c**, **5** and **6** were isolated and their structures have been assigned, mainly by means of spectroscopic methods.

☆ For Part 44 in this series, see ref. 20.

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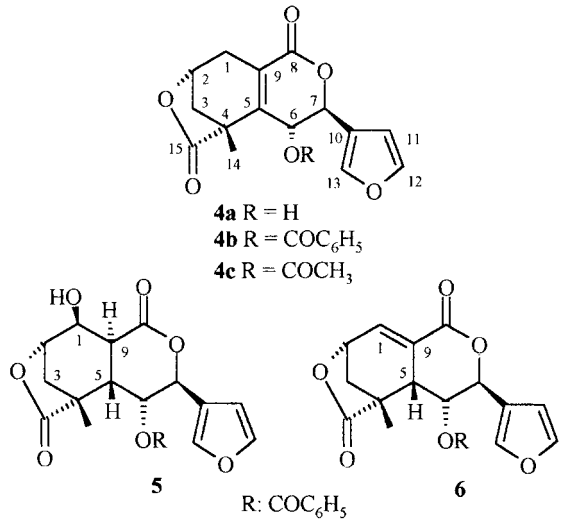



## 2. Results and discussion

Multiple silica gel preparative chromatographic separations of the crude extract of *C. maculata* allowed the isolation, in increasing order of polarity, of benzoate **4b**, collybolide and compound **6**, acetate **4c**, 1-hydroxycollybolide **5**, isocollybolide and compound **4a**. Chromatographic fractionation of the extract of *C. peronata* gave deoxycollybolidol.

Collybolide, isocollybolide and deoxycollybolidol showed spectroscopic data consistent with the literature.[1,3] The three lactones **4a–c** had NMR spectra closely related to each other and to **1** and **2**, with which they share the same tricyclic dilactone structure carrying a 3-substituted furan ring as an appendage. In addition, the structures of **4a–c** have a double bond between the two six-membered rings as indicated by the disappearance of the resonances of protons H-5 and H-9 and the presence, in the $^{13}C$ NMR spectrum of **4a**, of two quaternary carbons at $\delta_C$ 149.2 and 125.9 ppm, which were assigned to C-5 and C-9, respectively. The most polar of the three compounds was the free alcohol **4a**, while **4b** and **4c** were the corresponding benzoate and acetate derivative, respectively. In fact, in the $^1H$ NMR spectrum of **4a** the signals of protons H-6 and H-7 were shifted upfield with respect to the corresponding resonances of **4b** and **4c**, in accordance with the presence of a free hydroxy group at C-6. The O*H* proton was assigned to the broad singlet at 2.78 ppm, exchangeable with $D_2O$, while the resulting molecular formula $C_{15}H_{14}O_6$ was confirmed by HRMS and the presence of the expected pseudomolecular ion $[M+H]^+$ at *m/z* 291 in the CI-MS spectrum. In the $^1H$ NMR spectrum of **4b** the proton at C-6 geminal to a benzoate group was shifted downfield with respect to the corresponding signals of **1** and **2** ($\delta$ 5.85 vs ca 5.6–5.7 ppm) upon becoming allylic to the double bond between C-5 and C-9, while in the $^1H$ NMR spectrum of **4c** a singlet at $\delta$ 2.15 of an *Me*CO group replaced the multiplets between 7.4 and 8.1 ppm assigned to the protons of the benzoate moiety of **4b**. The molecular formulae of **4b** and **4c** were confirmed by HRMS and the presence of a pseudo-molecular ion $[M+H]^+$ at *m/z* 395 and 333, respectively, in the corresponding CI-MS spectra, in agreement with an $M_W$ equal to 394 and 332, respectively.

The well resolved ($C_6D_6$ and not $CDCl_3$ was the solvent of choice) $^1H$ NMR spectrum of the new alcohol **5** clearly revealed that the compound corresponded to a hydroxy derivative of collybolide **1**, the additional free hydroxy group then being placed at C-1 by a comparative study of the coupling constants of protons H-1, H-2 and H-9 in the $^1H$ NMR spectra of **5** and **1**. The corresponding O*H* proton was assigned to a signal at $\delta$ 2.88 ppm that readily exchanged with $D_2O$. Compound **6** was chromatographically inseparable from collybolide **1**; therefore, the structure, including the stereochemistry, could be assigned unambiguously only when **6** was found to be identical to the product of dehydration of alcohol **5** (vide infra). Compared to **1**, sesquiterpene **6** contained an additional trisubstituted double bond and was thus isomeric to **4a**. The position of the olefin at C-1–C-9 was firmly established by the following evidence: (a) the downfield shift ($\delta$ 7.67) of H-1 in the $^1H$ NMR of **6**, indicating that the proton was placed on the β carbon of an α,β-unsaturated carbonyl group; (b) the disappearance of the resonance of H-9, C-9 becoming a quaternary centre, and (c) the resonance of the allylic proton H-5, which moved downfield with respect to the corresponding signal of lactone **1** ($\delta$ 3.05 vs 2.14).

### 2.1. Stereochemical assignments

None of the five new sesquiterpenes **4–6** gave suitable crystals for X-ray analysis, while the poor amounts of isolated compounds hindered most derivatization reactions; therefore, we tried to shed light on the stereochemistry (both relative and absolute) of tricyclic compounds **4–6** and on the still unknown absolute configurations of **1–3** through a combination of NMR studies, molecular modelling calculations and CD spectra analysis. A search of the conformational space of isocollybolide **2** by AM1 calculations revealed the great mobility of the compound; in fact, four conformations of the tricyclic skeleton were located, each being doubled as the furan ring can assume two almost isoenergetic orientations. Interestingly, the X-ray conformation of **2**[3] did not correspond to the calculated global minimum, instead it resulted about 3 kcal $mol^{-1}$ higher in energy. By contrast, **1** showed a single conformation for the decalin system, most molecular mobility being restricted to an easy rotation of the furan ring and to a certain mobility of the benzoate moiety. Moreover, the calculated $^3J$ coupling constants were in full agreement with those reported for **1**.[1] The stereochemical analysis was then extended to 1-hydroxycollybolide **5**. The experimentally determined $^3J$ coupling constants for proton H-9 of compound **5** were 14.0 ($J_{5-9}$) and 3.5 Hz ($J_{1-9}$), respectively, indicating a *trans* collybolide-type fusion of the decalin system (for collybolide **1** $J_{5-9}$ is equal to 13.5 Hz, while for the *cis*-fused isocollybolide **2** $J_{5-9}$ is equal to 9.0 Hz)[1] and an axial orientation of the hydroxy group at C-1. The latter assignment was also corroborated by comparing the $^3J_{1-2}$ coupling constants of compounds **5** and **1** ($J_{1-2}$=4.5 Hz vs $J_{1ax-2}$=0.7 Hz and $J_{1eq-2}$=4.5 Hz, respectively). As a matter of fact, molecular modelling calculations established that the stereoisomeric structure **7**, having opposite stereochemistry at C-2 and C-4,

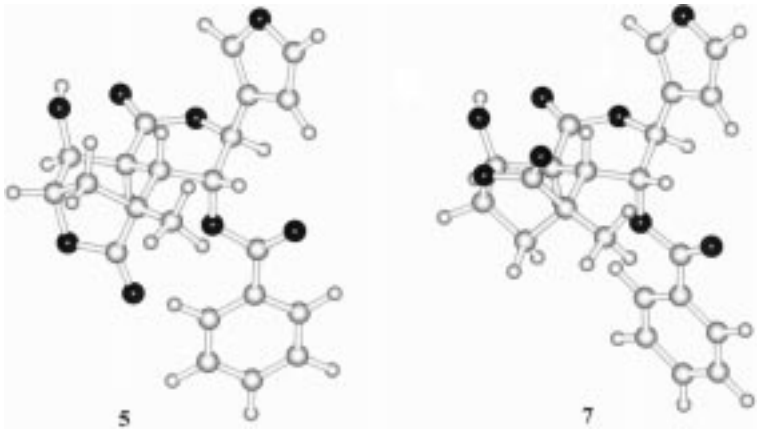


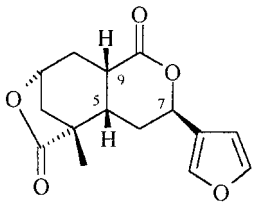

**Figure 1.** 3D Plots of the AM1 calculated minimum energy conformation of compounds **5** and **7**.

also had a geometry compatible with the measured coupling constants (Fig. 1).

The actual geometry could be safely assigned after the observation of an NOE effect between proton H-$3_{ax}$ and H-5, which are spatially close (calculated distance 2.23 Å) in structure **5** but not in **7**. On the other hand, no NOE effect was observed between H-$3_{ax}$ and H-9, which become closer (calculated distance 2.04 Å) in structure **7**.

In addition, a sample of **5** was treated with $Ac_2O$ under standard conditions, in order to obtain the corresponding acetate, thus confirming the proposed structure. Not unexpectedly, instead of the desired compound, the reaction afforded directly olefin **6**, possibly occurring through an easy base promoted elimination of HOAc from the short-lived acetate of **5**. Formation of the α,β-unsaturated lactone **6** acted as the driving force for the elimination reaction; moreover, the geometry of **5** possessed the required *trans*-diaxial orientation of the two groups involved in the reaction, i.e. proton H-9 and the acetate unit.

In the light of the close stereochemical similarities existing between sesquiterpenes **1**, **2**, **5** and **6**, the configuration at C-7 of deoxycollybolidol appeared quite ambiguous and it, unique among collybolides, was proposed to have structure **3** having the furan group *cis* oriented to the γ-lactone ring.[2] We decided, therefore, to reinvestigate this configurational assignment by performing a detailed modelling study at the B3LYP level, taking into consideration both structure **3** and its C-7 epimer **8**. As already pointed out for isocollybolide **2**, the tricyclic system in both stereoisomers presents a great mobility and, as for **2**, exists in four different conformations, each doubled by a different orientation of the furan ring.




**8**

We found that all the eight conformers of **8** do not give a negligible contribution to the overall population, as their energy spans a range of 2.5 kcal $mol^{-1}$ above the global minimum. Quite interestingly, the structure of the global minimum (Fig. 2) that only accounts for about 35% of the overall population resembles the X-ray conformation of isocollybolide **2**. By contrast, the strong steric interaction of the α-oriented furan ring with the γ-lactone moiety heavily destabilizes (by more than 7 kcal $mol^{-1}$) this type of conformation of epimer **3**. Moreover, for the latter stereoisomer, one arrangement of the tricyclic system largely prevails with a contribution of more than 90% to the overall population; the minimum energy conformer is reported in Fig. 2.

In order to correlate these results with the experimental data,[2] the $^1H$ NMR vicinal coupling constants were calculated for each conformation with the Haasnoot et al. equation.[5] The weighted average values are reported in

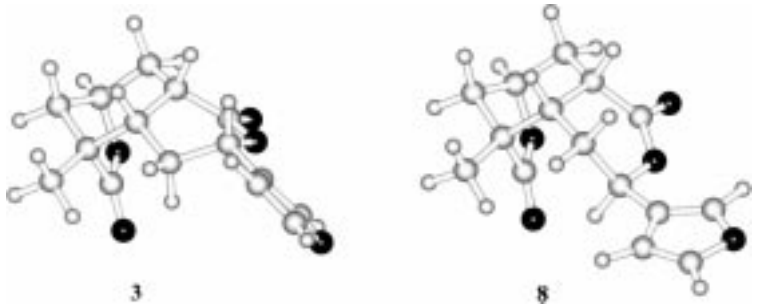



**Figure 2.** 3D Plots of the B3LYP calculated minimum energy conformation of compounds **3** and **8**.

**Table 1.** $^1$H NMR data of collybolides—$\delta_H$ in ppm (mult) ($J$ in Hz)

| No. | **1**[a] | **2**[b] | **4a**[b] | **4b**[b] |
|---|---|---|---|---|
| 1α | 1.80 (14.0–14.2, 4.5, 1.6, 6.0) | 2.74 ddt (15.5, 8.0, 1.5) | 2.95 ddd (19.5, 2.5, 0.8) | 3.10 ddd (19.5, 2.5, 1.0) |
| 1β | 2.69 (14.0–14.2, 11.5–11.7, 0.7) | 2.51 ddd (15.5, 9.0, 4.8) | 2.65 ddd (19.5, 2.5, 1.5) | 2.70 ddd (19.5, 2.5, 1.5) |
| 2 | 4.79 (6.0, 4.5, 0.7) | 4.89 ddd (5.0, 4.8, 1.5) | 4.93 dt (6.0, 2.5) | 5.00 dt (5.5, 2.5) |
| 3α | 2.27 (12.0, 6.0, 1.6) | 2.23 ddd (12.0, 5.0, 1.5) | 2.34 ddd (11.5, 6.0, 0.8) | 2.35 ddd (11.5, 5.5, 1.0) |
| 3β | 1.79 (12.0) | 2.08 d (12.0) | 1.96 d (11.5) | 2.01 d (11.5) |
| 5 | 2.14 (13.5–13.7, 2.0) | 2.63 dd (9.0, 2.5) | – | – |
| 6 | 5.71 (2.0, 1.0–1.2) | 5.63 dd (5.0, 2.5) | 4.50 br s | 5.85 t (1.5) |
| 7 | 5.63 (1.0–1.2, 1.2) | 5.87 dd (5.0, 1.0) | 5.62 dd (2.3, 1.0) | 5.75 br s |
| 9 | 3.38 (6.0, 11.5–11.7, 13.5–13.7) | 3.24 dt (8.0, 9.0) | – | – |
| 11 | 6.51 (1.0, 1.7) | 6.43 dd (1.8, 1.0) | 6.30 dd (1.8, 1.0) | 6.45 dd (2.0, 1.0) |
| 12 | 7.3–7.5 | 7.40–7.55[c] m | 7.40 t (1.8) | 7.40–7.60[c] m |
| 13 | | | 7.35 dt (1.8, 1.0) | |
| 14 | 1.20 | 1.30 s | 1.50 s | 1.40 s |
| 2′, 6′ | | 8.02 br d | – | 8.05 br d |
| 3′, 5′ | | 7.40–7.55[c] m | | 7.40–7.60[c] m |
| 4′ | | 7.60 br t | | 7.59 br t |
| OH | – | – | 2.78 br s | – |
| $COCH_3$ | – | – | – | – |

| No. | **4c**[b] | **5**[d] | **6**[b] | **8**[b] |
|---|---|---|---|---|
| 1α | 3.03 ddd (19.5, 2.5, 1.0) | 4.35 m | 7.67 ddt (5.5, 3.0, 0.8) | 3.05 ddt (15.0, 4.2, 1.8) |
| 1β | 2.65 ddd (19.5, 2.5, 1.3) | – | – | 1.78 ddd (15.0, 8.6, 1.5) |
| 2 | 4.95 dt (6.0, 2.5) | 4.06 dd (6.0, 4.5) | 4.95 t (5.5) | 4.80 ddd (6.4, 4.2, 1.5) |
| 3α | 2.34 ddd (11.5, 6.0, 1.0) | 1.13 ddd (12.0, 6.0, 0.8) | 2.40 ddd (12.0, 5.5, 0.8) | 2.32 ddd (11.8, 6.4, 1.8) |
| 3β | 1.95 d (11.5) | 1.78 d (12.0) | 2.19 d (12.0) | 1.83 br d (11.8) |
| 5 | – | 2.23 dd (14.0, 2.0) | 3.05 dd (3.0, 2.0) | 2.40–2.51[c] m |
| 6 | 5.75[c] t (1.3) | 5.65 br dd (2.0, 1.5) | 5.78 td (2.0, 0.8) | 6α: 2.40–2.51[c] m<br>6β: 2.23 dt (14.0, 6.4) |
| 7 | 5.60[c] t (1.3) | 5.25 t (1.5) | 5.72 dd (2.0, 1.5) | 5.65 ddd (6.4, 4.2, 0.5) |
| 9 | – | 3.30 dd (14.0, 3.5) | – | 2.91 td (8.6, 1.8) |
| 11 | 6.36 dd (1.9, 1.3) | 6.20 dd (2.0, 1.0) | 6.50 dd (2.0, 1.0) | 6.40 t (1.5) |
| 12 | 7.45 t (1.9) | 6.91 t (2.0) | 7.30–7.60[c] m | 7.42 d (1.5) |
| 13 | 7.40 dt (1.9, 1.3) | 7.28 ddd (2.0, 1.5, 1.0) | | |
| 14 | 1.35 s | 0.72 s | 1.42 s | 1.30 s |
| 2′, 6′ | – | 8.15 br d | 7.95 br d | – |
| 3′, 5′ | – | 7.05–7.15 m | 7.05–7.15[c] m | – |
| 4′ | – | | | – |
| OH | – | 2.88 d (5.0) | – | – |
| $COCH_3$ | 2.15 s | – | – | – |

[a] Taken from Ref. 1.
[b] Recorded in $CDCl_3$ at 300 MHz.
[c] Overlapping signals.
[d] Recorded in $C_6D_6$ at 300 MHz.

Table 3 together with the experimental constants of deoxycollybolidol. The close agreement of the experimental data with the values calculated for the structure **8** ensures that this stereochemistry must be attributed to deoxycollybolidol; therefore, the C-7 configuration must be inverted with respect to the previous assignment[2] and becomes identical to the other collybolides **1**, **2**, **5** and **6**.

Finally, the stereochemistry of compounds **4a–c** was examined through modelling and NMR spectroscopy. Actually, the 5–9 double bond separates the molecular structure common to **4a–c** into two distinct portions that conformationally are almost independent. Most molecular mobility is confined to rotations around the C(6)–O and C(7)–furan bonds and to the interconversion between two half-chair conformations of the δ-lactone ring. Indeed, complete exploration of the conformational space of lactones **4a–c**, and of the three stereoisomers having the alternative possible configurations at C-6 and/or at C-7, resulted, for each compound, in the location of several conformers whose $^3J_{6-7}$ coupling constants were individually calculated. Unfortunately, they were very close to each other and the experimental values of **4a–c**, thus not allowing a clear discrimination between the possible structures. Equally inconclusive were the NOE experiments performed on lactone **4a**. On irradiation of H-6, clear NOE interactions were indeed noticed for H-7, the furan protons H-11 and H-13, and the methyl group at C-4; however, these enhancements could occur in at least one conformer of each of the different diastereomers at C-6 and C-7. Eventually, the stereostructures of sesquiterpenes **4a–c** were assigned on the basis of biogenetic considerations, as it seemed reasonable that they are either precursors or derivatives of collybolides **1** and **2**. It is, therefore, highly likely that all collybolides isolated from *C. maculata* and *C. peronata* exhibit the same stereochemistry of the substituents linked to the common bicyclic structure.

## 2.2. CD study

In an attempt to determine the still unknown absolute configuration of collybolides, we selected to examine 1-hydroxycollybolide **5**, since the relative configuration was firmly established, and both AM1 and B3LYP

**Table 2.** $^{13}C$ NMR data of collybolides—$\delta_C$ (mult)

| No. | **1**[a] | **2**[a] | **4a**[a] | **5**[b] | **8**[a] |
|---|---|---|---|---|---|
| 1 | 29.6[c] t | 31.1[c] t | 30.8[c] t | 80.6[d] d | 28.5 t |
| 2 | 68.2[d] d | 69.4[d] d | 64.5[d] d | 65.4[d] d | 74.3 d |
| 3 | 44.6[c] t | 40.3[c] t | 39.7[c] t | 30.9 t | 43.9 t |
| 4 | 42.6 s | 40.5 s | 43.1 s | 41.9 s | 43.1 s |
| 5 | 34.3[e] d | 34.1[e] d | 149.2 s | 38.8[e] d | 39.1 d |
| 6 | 73.8[d] d | 73.7[d] d | 73.1[d] d | 69.6[d] d | 28.9 t |
| 7 | 79.7[d] d | 75.7[d] d | 77.7[d] d | 75.8[d] d | 71.2 d |
| 8 | 170.1 s | 170.3 s | 161.8 s | 170.1 s | 171.1 s |
| 9 | 42.2[e] d | 40.2[e] d | 125.9 s | 41.8[e] d | 35.4 d |
| 10 | 123.5[f] s | 122.4[f] s | 122.4 s | 124.9[f] s | 124.1 s |
| 11 | 107.6 d | 107.3 d | 108.1 d | 108.7 d | 108.5 d |
| 12 | 139.8[g] d | 144.4[g] d | 144.0[g] d | 145.2[g] d | 139.1 d |
| 13 | 144.5[g] d | 138.8[g] d | 139.6[g] d | 140.7[g] d | 144.1 d |
| 14 | 17.4 q | 20.3 q | 15.6 q | 17.5 q | 18.8 q |
| 15 | 176.4 s | 178.5 s | 176.8 s | 176.8 s | 178.3 s |
| $COC_6H_5$ | 165.5 s | 165.5 s | – | 166.7 s | – |
| 1′ | 128.5[f] s | 128.5[f] s | – | 127.8[f] s | – |
| 4′ | 133.7 d | 133.6 d | – | 134.3 d | – |
| 2′, 3′, 5′, 6′ | 128.5 d, 130.0 d | 128.5 d, 129.8 d | – | 129.5 d, 131.3 d | – |

Multiplicity inferred from DEPT experiments.
[a] Recorded in $CDCl_3$ at 75.47 MHz.
[b] Recorded in $C_6D_6$ at 75.47 MHz.
[c] Assignments may be interchanged.
[d] Assignments may be interchanged.
[e] Assignments may be interchanged.
[f] Assignments may be interchanged.
[g] Assignments may be interchanged.

**Table 3.** Calculated $^1H$ NMR vicinal coupling constants for stereoisomers **3** and **8**; for comparison the experimental values of deoxycollybolidol are reported

| | $J_{2-3\alpha}$ | $J_{2-3\beta}$ | $J_{1\alpha-2}$ | $J_{1\beta-2}$ | $J_{1\alpha-9}$ | $J_{1\beta-9}$ | $J_{5-9}$ | $J_{5-6\alpha}$ | $J_{5-6\beta}$ | $J_{6\alpha-7}$ | $J_{6\beta-7}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **3** (calcd) | 6.9 | 0.9 | 4.5 | 1.8 | 1.2 | 8.4 | 8.1 | 9.9 | 7.6 | 11.2 | 1.4 |
| **8** (calcd) | 6.8 | 0.9 | 4.5 | 2.0 | 1.7 | 7.9 | 7.5 | 6.8 | 6.1 | 3.9 | 5.9 |
| Deoxycollybolidol (exp) | 6.4 | <1 | 4.2 | 1.5 | 1.8 | 8.6 | 8.6 | 7.2[a] | 6.4 | 4.2 | 6.4 |

[a] Taken from Ref. 2.

calculations indicated the same conformation as the global minimum that accounts, according to B3LYP, for about 85% of the overall population.

Since paucity of material prevented any derivatization aimed at preparing Mosher-like esters, we decided to resort to CD calculations in the independent systems approximation. In fact, the absolute configurations of molecules containing both the benzoate and furan ring chromophores have been assigned by Gawronski et al. on the basis of chiroptical considerations.[6] The authors examined the interaction between the benzoate long-axis transition (at 230 nm), and the long-wavelength furan transition (at 215 nm), which produces a non-degenerate exciton couplet in the spectral region at 210–240 nm. We were, however, aware that chromophore complexity of sesquiterpene **5** could complicate this interpretation, since the CD bands associated with the $n-\pi^*$ transitions of the two saturated lactones could overlap, at least partly, in the same frequency region. Not surprisingly, the experimental CD spectrum of **5** showed, indeed, a non-conservative shape and the absence of couplets in the 210–245 nm region (Fig. 3, solid line).

In calculating the CD spectrum of **5**, taken in the lowest energy B3LYP conformation, we used a computer program based on the De Voe coupled oscillator theory,[7–9] and we

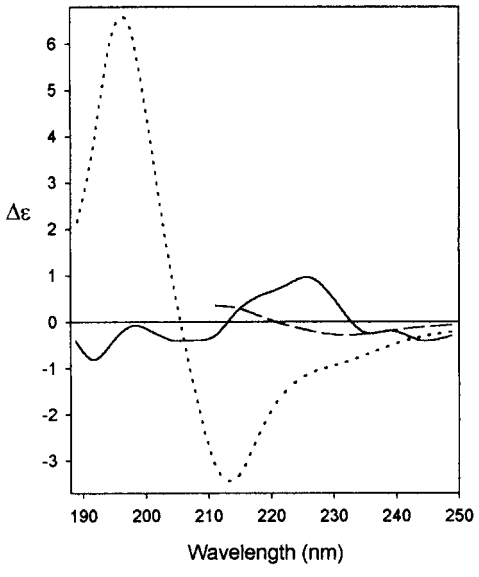



**Figure 3.** Comparison between the experimental and the calculated CD curves for compound **5**. Solid line: experimental (cyclohexane). Dashed line: calculated by the De Voe method (only long-wavelength furan and benzoate transitions). Dotted line: calculated by the De Voe method (all oscillators).

**Table 4.** Signs of the lactone ring chirality and substituents' anti-octant contributions to the $n-\pi^*$ CD band (ca 225 nm) of compound **5**

| Chromophore skewness[a] (δ-lactone) | Chromophore skewness[a] (γ-lactone) | Ring chirality of δ-lactone[b] | Ring chirality of γ-lactone[b] | CD sign resulting from substituents dissymmetry[c] |
|---|---|---|---|---|
| +40° | +25° | − | − | + |

[a] The value of the dihedral angle O–C(=O)–C–C is reported.
[b] According to Refs. 11 and 12.
[c] Considering a pure anti-octant contribution, see Ref. 13.

considered the above benzoate and furan electric dipole transitions. In the resulting CD spectrum (Fig. 3, dashed line), we took into account only the longest-wavelength side of the exciton couplet, which should not suffer from overlaps with the lactone $n-\pi^*$ transitions (occurring at ca 220 nm). Comparison of the CD calculated value ($\Delta\epsilon_{233}=-0.29$) with the experimental one ($\Delta\epsilon_{235}=-0.22$) thus allowed the absolute configuration depicted in formula **5** to be inferred.

It should be noted, however, that a more exhaustive calculation, also including the benzoate short-wavelength transitions at ca 200 nm,[10] provided the wrong sign in the high-energy region and a much higher intensity too. Not even the inclusion of the furan 190 nm transition[†] and the lactone $\pi-\pi^*$ transitions[†] improved the match between the calculated and experimental CD curves at shorter wavelengths (Fig. 3, dotted line).

Additional stereochemical information for compound **5** could in principle be obtained from the CD band of the lactone $n-\pi^*$ transition, which has been related to the sign of the O–C(=O)–C–C dihedral angle by a semiempirical rule, known as the 'ring-chirality' rule.[11,12] The $n-\pi^*$ transitions of the two saturated lactones of collybolide **5** could firmly be assigned to the system of positive bands occurring at 215–230 nm since, as expected for such a transition, the maximum observed at ca 226 nm in cyclohexane was shifted to 221 nm in MeOH. However, we promptly realized that the sign of this band could not be directly connected to the absolute configuration of **5**. In fact, two mechanisms actually operate in determining the Cotton Effect of the lactone $n-\pi^*$ transitions of compound **5**: one is due to the chromophore skewness[11,12] (ring chirality), and the other one to the dissymmetric arrangement of substituents that in lactones gives an anti-octant contribution.[13]

Given the presence of strong chromophoric groups, the latter contribution cannot be neglected. It was thus calculated by means of a computer program based on the dynamic coupling mechanism,[14] which provides an octant-like division of the space around the chromophore, with the signs being those of the familiar saturated ketones octant rule. As a first approximation, to account for the anti-octant behaviour of lactones, the sign obtained from the calculation was simply inverted.[‡] The result is reported in Table 4 (where only the sign is given), together with the sign of the CE resulting from ring chirality. As can be seen, the contribution of the dissymetric arrangement of substituents is unfortunately opposite to that of the lactone helicity. Moreover, the overlap of the CE due to the lactone $n-\pi^*$ transitions, with that arising from the coupling of electric dipoles, should also be considered. Therefore, interpretation of the CD curve of compound **5** through simple semi-empirical rules is highly ambiguous.

In conclusion, interpretation of the CD spectra of compound **5** is hampered by the complexity of the chromophoric system and assignation of the absolute configuration is merely based on the sign of the CD band at ca 235 nm. Note also that the reported CD of collybolide **1** is negative at 230–240 nm,[1] indicating that collybolides **1** and **2** (that was converted into **1** by epimerization at C-9)[1] are likely to have the same absolute stereochemistry as compound **5**.

## 3. Conclusions

The sesquiterpenes of *C. maculata* and *C. peronata* have been reinvestigated extracting the fruiting bodies with EtOAc which, contrary to solvents used in previous studies, should not promote the formation of artefacts. In addition to collybolide, isocollybolide and deoxycollybolidol, five new sesquiterpenes **4a**–**c** and **5** were isolated. Their structures have been assigned by spectroscopic methods and molecular modelling. This led to a revision of the stereochemistry of deoxycollybolidol, previously formulated as **3**[2] which, instead, must have the C-7 configuration inverted, as shown in structure **8**. The absolute configuration of collybolides could tentatively be inferred from a study of the CD spectrum of 1-hydroxycollybolide **5**.

Deoxycollybolidol and isocollybolide showed no activity in the brine shrimp lethality assay.[15]

## 4. Experimental

### 4.1. Extraction and isolation

Fruiting bodies of *C. maculata* were collected in the province of Pavia in October 1995. Immediately after the collection, the mushrooms were frozen at −20°C and then extracted with EtOAc. The raw extract was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. 75 g of fruiting bodies gave 255 mg of crude extract. A preliminary partition of polar and lipophilic compounds between hexane and $CH_3OH-H_2O$, 9:1, was attempted; however, this procedure was soon discarded

[†] The directions of the relative transition moments were taken from a CNDOS/CI calculation.
[‡] We did not take into account the distortion of the nodal planes which are calculated theoretically in Ref. 13. Again, according to this reference, the direction of the magnetic dipole moment of the $n-\pi^*$ transition employed in our calculation was chosen as almost collinear with the C=O bond.

since in the hydroalcoholic phase some compounds rapidly decomposed. Multiple chromatographic separations of the extract on silica gel columns eventually led to isolation of sesquiterpenes **1**–**6**. In addition, ergosterol, a mixture of free fatty acids and triglycerides were identified. EtOAc/hexane and EtOAc/toluene gradient mixtures, and $CH_2Cl_2$ were employed as eluants in the chromatographic separations.

Fruiting bodies of *C. peronata* were extracted according to the procedure described above. 13.5 g fresh mushrooms gave a raw extract (47.5 mg) which was fractioned on a silica gel column (3.5 g). Elution with EtOAc–hexane, 2:3, afforded deoxycollybolidol (**8**).

### 4.2. Materials and methods

$^1$H NMR (300 MHz) and $^{13}$C NMR (75.47 MHz) spectra were recorded in $CDCl_3$ solution unless otherwise indicated, using a Bruker CXP 300 spectrometer. Chemical shifts are reported in $\delta$ units with $Me_4Si$ as the internal standard; the abbreviations s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad are used throughout. Coupling constants (*J*) are in Hz. The multiplicity (in parentheses) of each carbon atom was determined by DEPT experiments. Mass spectra (direct inlet system) were recorded at 70 eV (0.5 mA) with a Finnigan MAT 8222 instrument. IR spectra (film) were obtained on a Perkin–Elmer 881 Infrared spectrometer. Optical rotations were determined on a Perkin–Elmer 241 polarimeter at 20°C. UV spectra ($\epsilon$ in $mol^{-1}$ L $cm^{-1}$) were recorded on a Perkin–Elmer Lambda 5 instrument and CD spectra ($\Delta\epsilon$ in $mol^{-1}$ L $cm^{-1}$) on a Jasco J-710 spectropolarimeter, employing 0.1 cm optical cuvettes. Analytical TLC was carried out on 0.25 mm glass-supported silica gel plates and visualization was effected with short-wavelength UV light (254 nm) or with 0.5% vanillin solution in $H_2SO_4$–EtOH (4:1) followed by heating. Flash column chromatography was accomplished with 230–400 mesh silica gel. All commercial reagent grade solvents were dried by standard techniques just before use.

### 4.3. Molecular modelling

Molecular modelling calculations were performed with the TITAN package[16] at the semiempirical level using the AM1 method[17] or within the DFT framework using the B3LYP method at the 6-31G* level.[18,19] The conformational space of compounds **1**–**5**, **7** and **8** was explored through the following strategy. First of all, the tricyclic moieties without the benzoate and the furan substituents were modelled by optimizing all the possible starting geometries, i.e. by considering the half-chair and twist-boat conformations of the δ-lactone ring and the different arrangements at the junction of this ring with the carbocyclic one. Four geometries were located in the case of the *cis* structure, only one in the case of the *trans* one, and two for the tricyclic moiety of compounds **4a**–**c**. The benzoate and furan substituents were then added and the energy profiles were determined for the rotation, in 10° steps, around the single bond connecting each substituent to the δ-lactone ring. The energy profiles due to furan rotation usually presented two almost isoenergetic minima with opposite orientation of the furan ring and very low energy barriers (1–3 kcal $mol^{-1}$). The barriers for the rotation of the benzoate substituent that usually presented one largely preferred orientation (split into two close almost isoenergetic minima in the case of compounds **1**, **5** and **7**) were much higher. All the geometries corresponding to the minima in the energy profiles were used as starting geometries and fully optimized. The so obtained conformers were ranked with respect to energy and the population of each conformer was determined considering a Boltzmann distribution at 298 K. Calculated $^1$H NMR vicinal coupling constants were determined as weighted averages taking into account these conformer populations and the theoretical coupling constants determined for each conformer with the Haasnoot et al. equation.[5]

### 4.4. Methods of CD calculations

CD calculations based on the De Voe theory[7,8] were performed according to Ref. 9 and those based on the dynamic coupling mechanism according to Ref. 14.

**4.4.1. Collybolide 1.** 3 mg; $^1$H NMR spectrum (Table 1) identical to the literature;[1] $^{13}$C NMR spectrum reported in Table 2.

**4.4.2. Isocollybolide 2.** White crystals (11 mg); $^1$H NMR (Table 1), UV and IR spectra identical to the literature;[1] CD ($CH_3CN$): $\lambda_{max}$ ($\Delta\epsilon$): 234 (−1.0), 206 (−1.7), 192 (−15.0); $^{13}$C NMR spectrum reported in Table 2.

**4.4.3. Deoxycollybolidol 8.** 2.5 mg; IR, $^1$H and $^{13}$C NMR spectra (Tables 1 and 2, respectively) identical to the literature.[2]

**4.4.4. Compound 4a.** 4.5 mg, $[\alpha]_D$=+41.5 (*c*=2, $CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ ($\epsilon$): 240 nm (5035); CD (MeOH) $\lambda_{max}$ ($\Delta\epsilon$): 215 (−15.8), 240 (+16.7); IR $\nu$ ($cm^{-1}$): 3650–3150 (OH), 1780 (γ-lactone), 1720 (δ-lactone); CI-MS ($CH_4$) *m/z*: 291 $[M+H]^+$; EI-MS *m/z* (rel. int.): 233 (9), 203 (9), 194 (40), 167 (19), 149 (100), 133 (20), 119 (30), 105 (59), 91 (61), 71 (67), 57 (89), 43 (79); HRCI-MS *m/z* 291.0865 $[M+H]^+$ (calcd for $C_{15}H_{15}O_6$ 291.0869); $^1$H NMR and $^{13}$C NMR spectra reported in Tables 1 and 2, respectively.

**4.4.5. Compound 4b.** 1 mg, $[\alpha]_D$=+11.43 (*c*=0.35, $CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ ($\epsilon$): 236 nm (5449); CD (MeOH) $\lambda_{max}$ ($\Delta\epsilon$): 212 (−7.6), 240 (+2.2), 247 (+2.7); IR $\nu$ ($cm^{-1}$): 1780 (γ-lactone), 1725 (δ-lactone), 1715 (benzoate); CI-MS ($CH_4$) *m/z*: 395 $[M+H]^+$, EI-MS *m/z* (rel. int.): 272 $[M^+-C_6H_5COOH]$ (78), 228 (35), 213 (15), 200 (26), 185 (11), 171 (10), 149 (40), 105 (100), 95 (45), 77 (69), 57 (45), 43 (38); HRCI-MS *m/z* 395.1131 $[M+H]^+$ (calcd for $C_{22}H_{19}O_7$ 395.1130); $^1$H NMR spectrum reported in Table 1.

**4.4.6. Compound 4c.** 2 mg, $[\alpha]_D$=+9.33 (*c*=0.75, $CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ ($\epsilon$): 240 nm (4245); CD (MeOH) $\lambda_{max}$ ($\Delta\epsilon$): 217 (−22.2), 242 (+14.6); IR $\nu$ ($cm^{-1}$): 1780 (γ-lactone), 1740 (δ-lactone) and 1720 (acetate); CI-MS ($CH_4$) *m/z*: 333 $[M+H]^+$, EI-MS *m/z* (rel. int.): 272 $[M^+-CH_3COOH]$ (73), 228 (59), 213 (30), 200 (50), 194 (46), 150 (85), 105 (40), 95 (77), 91 (42), 77 (45), 43 (100); HRCI-MS *m/z* 333.0974 $[M+H]^+$ (calcd for $C_{17}H_{17}O_7$ 333.0980); $^1$H NMR spectrum reported in Table 1.

**4.4.7. Compound 5.** 1.5 mg, UV (MeOH) $\lambda_{max}$ ($\epsilon$): 230 nm (6252); CD (MeOH) $\lambda_{max}$ ($\Delta\epsilon$): 206 (−1.16), 221 (+0.25), 237.5 (−0.7); IR $\nu$ ($cm^{-1}$): 3600–3100 (OH), 1790 (γ-lactone), 1780 (δ-lactone), 1720 (benzoate); CI-MS ($CH_4$) *m/z*: 413 $[M+H]^+$, EI-MS *m/z* (rel. int.): 381 (16), 290 $[M^+-C_6H_5COOH]$ (58), 204 (32), 191 (19), 163 (27), 149 (13), 105 (100), 85 (20), 77 (45), 69 (34), 57 (44), 43 (31); HRCI-MS *m/z* 413.1231 $[M+H]^+$ (calcd for $C_{22}H_{21}O_8$ 413.1236); $^1H$ NMR and $^{13}C$ NMR spectra reported in Tables 1 and 2, respectively.

**4.4.8. Conversion of 5 into 6.** A solution of **5** (1.5 mg) in pyridine was cooled to 0°C and 50 μl of $Ac_2O$ were added. The reaction mixture was stirred at rt for 1 h and then the excess of $Ac_2O$ was quenched with excess MeOH. The solvent was removed under reduced pressure and the residue (2.5 mg) was purified on silica gel (elution with toluene–EtOAc) to furnish a pure sample of **6** (1 mg). Compound **6** was also isolated as an inseparable mixture with collybolide **1** from the EtOAc extract of *C. maculata*. CI-MS ($NH_3$) *m/z*: 412 $[M+NH_4]^+$, 429 $[M+NH_4+NH_3]^+$; HRCI-MS *m/z* 412.1392 $[M+NH_4]^+$ (calcd for $C_{22}H_{22}NO_7$ 412.1396); $^1H$ NMR spectrum reported in Table 1.

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