An Unusual Series of Thiomethylated Canthin-6-ones from the North American Mushroom *Boletus curtisii*

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Dedicated to Professor Axel Zeeck on the occasion of his 65th birthday

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A unique set of thiomethylated canthin-6-one derivatives was isolated from *Boletus curtisii*. The bright yellow color of this mushroom is caused by two optically active canthin-6-one sulfoxides for which the names curtisin and 9-deoxycurtisin are proposed. The structures of the new compounds were established by MS and NMR methods and the absolute configuration of the sulfoxides determined by quantum chemical calculations. This is the first occurrence of canthin-6-one alkaloids outside of higher plants. The chemotaxonomic implications of these findings are discussed.

Introduction

Boletus curtisii Berk. is characterized by its bright yellow appearance, a viscid cap and stalk and a white basal mycelium. This spectacular mushroom occurs in pine woods or mixed forests in the Eastern and Southern USA. We were attracted by the yellow pigments, which are located in the slimy outer layer of the fruit bodies. If the mushrooms are sprayed with methanol, the pigments dissolve completely. After this treatment, the fruit bodies remain colorless, a behavior not shown by any other bolete. Solutions of the pigments in acetone exhibit a green-yellow fluorescence in day light. In this paper we report on the isolation and structural elucidation of the pigments and related metabolites from this remarkable fungus.

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Results and Discussion

The methanolic extract of the lyophilized fruit bodies was separated into 6 fractions by preparative reversed phase HPLC. Each of these fractions was then purified further by chromatographic methods to yield several yellow pigments and structurally related colorless compounds (Scheme 1). One of the colorless compounds, $C_{14}H_8N_2O$, was identified by its spectroscopic data, including the ^{1}H-coupled ^{13}C NMR spectrum,[1] as canthin-6-one (**1**),[2] an alkaloid known from several plants of the Rutaceae, Simaroubaceae, and Amaranthaceae families.[3] Canthin-6-one exhibits antifungal[4] and cytotoxic[5] properties.

A second colorless compound **2**, $C_{15}H_{10}N_2OS$, is a methylthio derivative of canthin-6-one. Comparison of its ^{1}H NMR spectrum with that of canthin-6-one revealed a change of the AB quadruplet of the enone protons at δ = 6.91 and 7.94 ppm (J_{AB} = 9.8 Hz) to a singlet at δ_H = 6.46. Since this high-field signal can only be assigned to 5-H, the SCH_3 group (δ_H = 2.56) must be attached to C-4. 4-(Methylthio)canthin-6-one (**2**) has been isolated before from the Australian mangrove *Pentaceras australis* (Rutaceae).[6]

The most lipophilic metabolite of *B. curtisii* is isomeric with methylthio derivative **2**. The ^{1}H NMR signals of this compound are almost identical with those of **2** with the only exception that the singlet at δ_H = 6.46 has changed its position to δ_H = 7.52. This characterizes the new alkaloid as 5-(methylthio)canthin-6-one (**3**).

Two further (methylthio)canthin-6-one isomers, **4** and **5**, show in their ^{1}H NMR spectra intact AB-systems for the enone and pyridine moieties and in addition signals for

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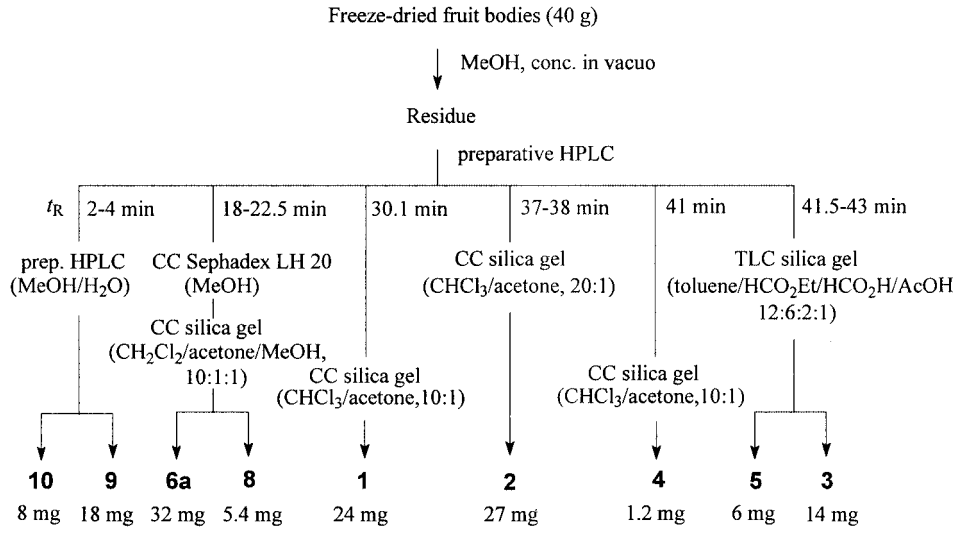
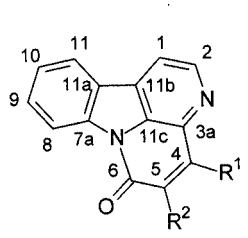
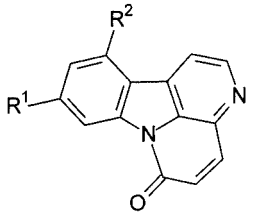


Scheme 1. Procedures outlining the isolation of alkaloids

1,2,4- or 1,2,3-trisubstituted benzene rings, respectively. The position of the methylthio substituent on the aromatic ring can easily be determined from the coupling pattern, considering that the signal for 8-H occurs at lowest field ($\delta_H \approx 8.5$) due to deshielding by the neighboring carbonyl group. Since the signal of 8-H in the spectrum of **4** exhibits only a *meta*-coupling, the compound must be 9-(methylthio)canthin-6-one. This is in accordance with NOE experiments, in which irradiation at the 8-H resonance ($\delta_H = 8.54$) enhances only the methylthio signal, whereas irradiation at the methylthio signal ($\delta_H = 2.63$) affects both neighboring protons 8-H and 10-H ($\delta_H = 7.37$). In the ^{1}H NMR spectrum of compound **5**, the 8-H signal at $\delta_H = 8.49$ appears as doublet of doublets ($^3J = 8.0$, $^4J = 0.8$ Hz), which proves its structure as 11-(methylthio)canthin-6-one (**5**). Due to steric interference with the methylthio group, the signal of 1-H is shifted from $\delta_H = 7.86$ (**1**) to 8.26 (**5**).



1, $R^1 = R^2 = H$
2, R^1 = SMe, $R^2 = H$
3, $R^1 = H$, R^2 = SMe



4, R^1 = SMe, $R^2 = H$
5, $R^1 = H$, R^2 = SMe

The major yellow pigment, curtisin (**6a**), is optically active and has the molecular formula $C_{15}H_{10}N_2O_4S$. This suggests a canthin-6-one nucleus substituted by one methylsulfinyl and two hydroxy groups. On methylation with dimethyl sulfate/K_2CO_3 curtisin yielded a dimethyl ether **6b** and with 4-bromobenzoyl chloride an oily diacyl derivative **6c**. In contrast, treatment of curtisin with acetic anhydride in the presence of concentrated sulfuric acid afforded the optically inactive *S*-deoxy derivative **7**.

In the ^{1}H NMR spectrum of curtisin signals for a 1,2,4-trisubstituted benzene ring, two singlets at $\delta_H = 8.14$ and 6.67 for isolated protons, and a singlet at $\delta_H = 3.05$ for the methylsulfinyl group are visible, indicating that each of the peripheral rings carries one substituent. The position of the substituents can be deduced from the ^{1}H-coupled ^{13}C NMR spectrum. The amide carbonyl at $\delta_C = 158.7$ displays only a single coupling $^2J_{C,H} = 2$ Hz to 5-H, whereas in canthin-6-one (**1**) an additional coupling $^3J_{C,H} = 11$ Hz to 4-H is observed. Carbon C-4, which carries the methylsulfinyl group, is split into a doublet of quadruplets, the doublet arising from the coupling with 5-H. Selective decoupling of 5-H at $\delta_H = 6.67$ changes this signal to a quadruplet and the doublet of the amide group to a sharp singlet. The positions of the two OH groups were deduced from NOE experiments with dimethyl ether **6b**. Irradiation at $\delta_H = 4.23$, the resonance of the 1-methoxy group, enhances the signal of 2-H, whereas the second methoxy group at $\delta_H = 3.97$ is connected with the neighboring protons 8-H and 10-H. From these results, structure **6a** can be proposed for curtisin. 1-Hydroxy-9-methoxycanthin-6-one, an alkaloid with the same hydroxylation pattern as curtisin has recently been isolated from roots of *Eurycoma longifolia* (Simaroubaceae).[5a]

Curtisin is optically active, $[\alpha]_D^{20} = -150$. Due to electronic interactions of the chiral sulfoxide with the canthin-6-one chromophore, the alkaloid exhibits a complex CD spectrum (Figure 1),[7] from which the (*S*)-configuration was determined by quantum mechanical calculations (see below).

The minor yellow pigment, $C_{15}H_{10}N_2O_3S$, contains one oxygen atom less than curtisin and shows ^{1}H NMR signals for a 1,2-disubstituted benzene ring. Since the remaining

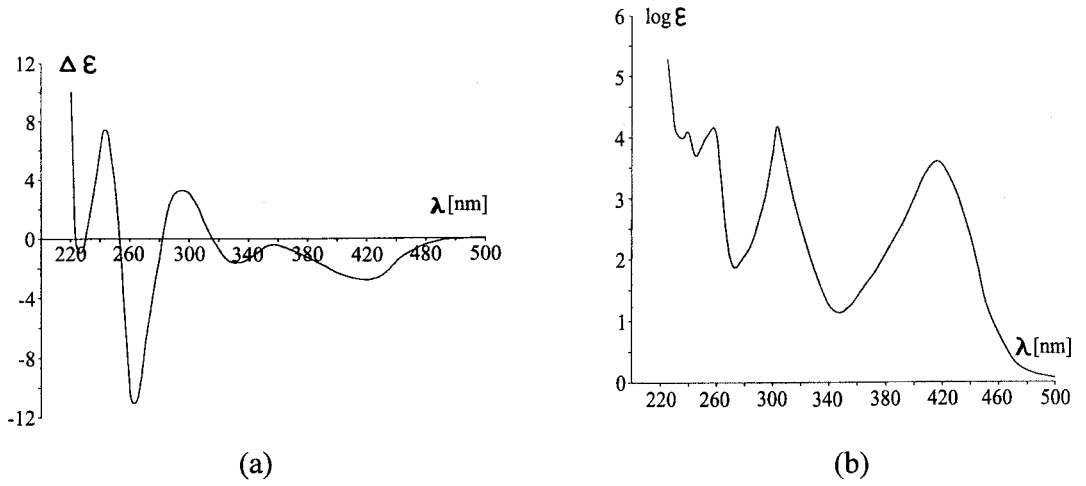
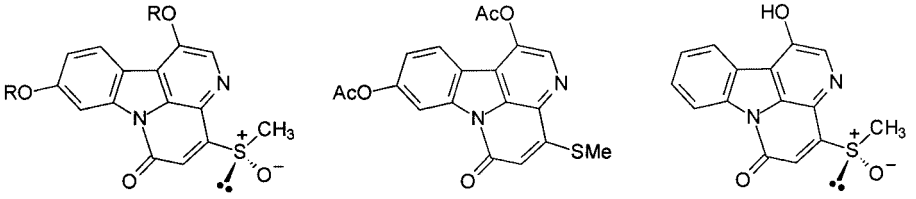


Figure 1. (a) Experimental CD spectrum and (b) UV spectrum of curtisin (**6a**) (both in MeOH)



6a, R = H
6b, R = Me
6c, R = COC_6H_4Br-(*p*)

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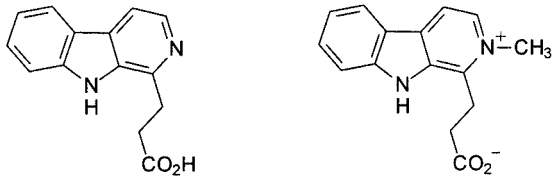
NMR signals correspond closely to those of curtisin, the structure of 9-deoxycurtisin (**8**) can be assigned to this compound. Pigment **8** exhibits bright green fluorescence in acetone and is optically active.

In the EI mass spectra of curtisin and its dimethyl ether, prominent $M^+ - SO$ peaks are visible, which are not observed with methyl phenyl sulfoxide and related compounds. To explain this fragmentation we propose a neighboring group effect exerted by the pyridine nitrogen (Figure 2). Our proposal is supported by the observation that only the mass spectrum of 2-methylthiopyridine shows a $M^+ - SO$ ion of 41% intensity, whereas in the spectra of 3- and 4-methylsulfinylpyridine this fragment is missing.[8]

The most polar constituents of *Boletus curtisii* are β-carboline-1-propanoic acid (**9**) and *N*-methyl-β-carbolinium-1-propanoate (**10**). The former is known from several plants[9] as well as the toadstool *Cortinarius infractus*,[10] the latter is a new natural product. The structures of both compounds follow from their MS and NMR spectroscopic data.



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Quantum-Chemical Calculations

We now tried to determine the absolute configuration of curtisin (**6a**) by comparison of its experimental CD spectrum with the one calculated for its (*S*)-isomer by means of the quantum-chemical time-dependent density functional theory (TDDFT).[11] To obtain molecular geometries to be

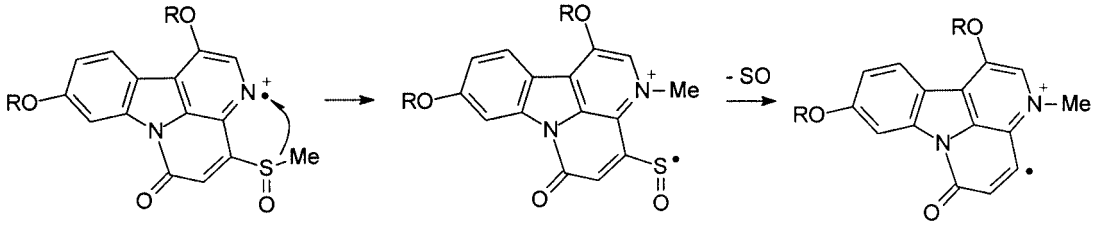

Figure 2. Formation of the prominent $M^+ - SO$ ion in the EI-MS of curtisin derivatives

used in the calculation of the CD and the UV spectrum, we performed geometry optimizations employing density functional theory using the B3LYP[12] functional and a TZVP (triple ζ for valence orbitals plus polarization functions) basis set.[13] Four energetically low-lying local minima have been obtained at the B3LYP/TZVP level of DF theory by changing the orientation of the hydroxy groups at C1 and C9. In all four structures, whose relative energies lie within a range of less than 2.6 kcal/mol, the hydroxylic hydrogen atoms are located in the molecular plane and no stationary points with out-of-plane hydrogen atoms could be found. Other conformers differing by the orientation of the −S(=O)−Me group have also been obtained, however, their energies are so high that they will not contribute to the final averaged CD and UV spectra. The corresponding spectra for each structure have been calculated with the TDDFT/B3LYP/TZVP method. The rotational strengths have been obtained using the origin-independent dipole-velocity formalism.[14] The calculated CD and UV curves for each conformer have been generated as a sum of Gaussians, centered at the wavelengths of the corresponding transitions, multiplied with the rotational and the oscillator strength, respectively. Boltzmann weights (T = 298 K) for each of the four structures have been calculated using the corresponding total energies. Addition of the Boltzmann-weighted spectra of the single conformers gives the resulting CD and UV curve shown in Figure 3.[15,16]

Compared with the experimental one, the calculated CD spectrum is blue-shifted by an average value of 25 nm. As far as the signs of the bands are concerned, the major difference between the calculated and the measured CD curves is the broad and weakly positive band calculated at 346 nm, which has no counterpart in the measured spectrum. Such weakly positive bands also occur in the single CD spectra of all four conformers. These bands all share the common feature that they have no underlying transitions close to their maxima but only very small rotational strengths near to the intersections of the curve with the abscissa. The calculated CD and UV spectra of the four most stable isomers are very similar. In Figure 4 we give the calculated CD and UV spectrum of the most stable conformer of **6a**. We, therefore, consider these small positive rotational strengths as belonging to the CD bands flanking the broad positive band, and conclude that the latter one does not correspond to a Cotton effect. The Cotton effect calculated at 387 nm is negative and its main contribution is due to an $n_{O=S} \rightarrow \pi^*$ transition from an orbital below the HOMO to the LUMO, and we correlate it with the one observed at 420 nm. The next Cotton effect calculated at 312 nm is also negative. It is also due to an $n \rightarrow \pi^*$ transition where the nonbonding orbital has its largest coefficients at the carbonyl oxygen and the S=O group. This band corresponds to the negative one observed at 333 nm. The first positive Cotton effect has been calculated at 276 nm. We assign it to

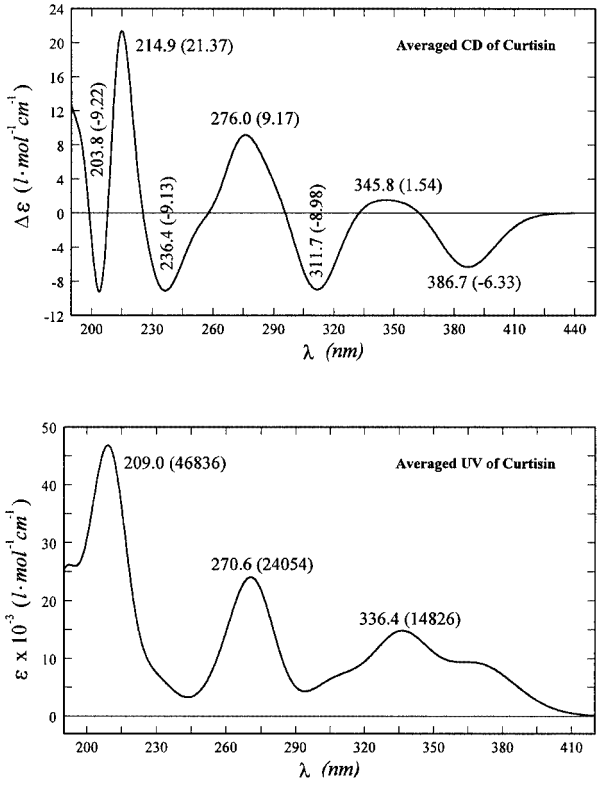

Figure 3. CD and UV spectrum of curtisin (**6a**) calculated at the TDDFT/B3LYP/TZVP level (averaged). The numbers in parentheses are the corresponding Δε and ε values

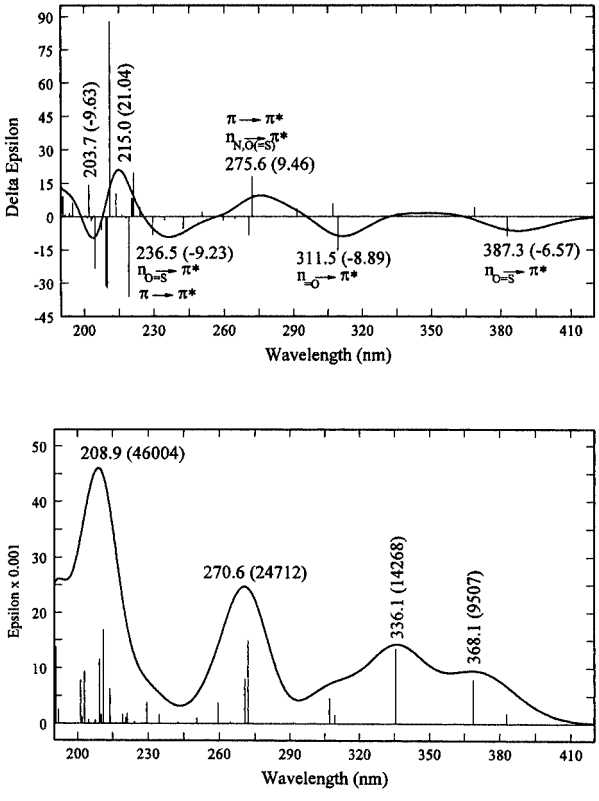

Figure 4. CD (above) and UV (below) spectrum of the most stable conformer of curtisin (**6a**) calculated at the TDDFT/B3LYP/TZVP level (averaged). The numbers in parentheses are the corresponding Δε and ε values

the one observed at 295 nm. According to our calculation it is dominated by a $\pi \rightarrow \pi^*$ transition but contains also significant contributions from an $n \rightarrow \pi^*$ excitation where the nonbonding orbital has its largest coefficients at the oxygen atom of the S=O group and at the N atom of the pyridine segment. The CD band calculated at 237 nm with a negative $\Delta\varepsilon$ is correlated with the one observed at 263 nm. This Cotton effect is dominated by an almost pure $n \rightarrow \pi^*$ transition and a combination of three $\pi \rightarrow \pi^*$ excitations calculated at 243 and 229 nm for the most stable isomer. The strongest positive band of the CD spectrum has been observed at 243 and calculated at 215 nm, and its sign is governed by a transition with a strongly positive rotational strength calculated between 210 and 215 nm. Finally the negative Cotton effect calculated at 204 nm has its experimental counterpart at 227 nm. Thus, the measured and the calculated CD spectra agree as far as the signs of all Cotton effects are concerned. Since the spectrum has been calculated for the (*S*)-isomer, we conclude that the configuration of the stereogenic sulfur atom in curtisin is also (*S*).

Conclusions

The co-occurrence of canthin-6-one (**1**) with several of its methylthio derivatives indicates a unique biosynthetic relationship. The mushroom is obviously able to introduce methylthio residues specifically at the electron deficient C-4, C-5, C-9, and C-11 centers. β-Carboline-1-propanoic acid (**9**) is the biosynthetic precursor of canthin-6-one (**1**) in plants,[17] and the occurrence of both compounds in *B. curtisii* points to the same biosynthetic relationship.

Canthin-6-one alkaloids have not been isolated from fungi before. Due to its bright color and other characteristics, *Boletus curtisii* has been transferred by Singer[18] to *Pulveroboletus* Murr. This taxonomically rather controversial genus is characterized by the occurrence of pulvinic acid pigments,[19] often in halogenated or *O*-methylated form.[19,20] Our present investigation demonstrates that the pigments of *B. curtisii* belong to a new class of chromoalkaloids, differing profoundly from the pulvinic acids responsible for the bright color of other *Pulveroboletus* and many *Boletus* species. From these results it appears necessary to revise the taxonomic position of *B. curtisii* within the Boletineae.

Experimental Section

General: Evaporation of the solvents was performed under reduced pressure using a rotary evaporator. Melting points (uncorrected): Reichert Thermovar. Column chromatography: Silica gel 60 (Merck), Sephadex LH-20 (Pharmacia). Analytical TLC: silica gel 60 F_{254} aluminum foils (Merck); solvent system A: toluene/HCO_2Et/HCO_2H (10:5:3); B: $CHCl_3$/acetone (10:1), C CH_2Cl_2/acetone/MeOH (10:1:1). Preparative TLC: TLC glass plates with silica gel 60 F_{254} 2 mm, (Merck). Analytical HPLC: Waters/Millipore with photodiode array detector, Nucleosil C18 column, 250 × 4.6 mm, 5 μm (Macherey−Nagel), flow: 1 mL/min; solvent A: 10% MeOH/90% H_2O; solvent B: 90% MeOH/10% H_2O; gradient I: start 60% A/40% B, 40 min 10% A/90% B, 43 min 100% B; gradient II: start 90% A/10% B, 50 min 50% A/50% B, 60 min 100% B. Preparative HPLC: Waters/Millipore with Knauer Variable Wavelength Monitor (detection at 215 nm), Nucleosil C18 column, 250 × 20 mm, 7 μm (Macherey−Nagel), with precolumn 30 × 20 mm, flow: 6.75 mL/min; same solvent system as in analytical HPLC; start 70% A/30% B, 60 min to 10% A/90% B, 65 min 10% A/90% B, 70 min to 100% B, 100 min 100% B. UV: Varian Cary 17 spectrophotometer. CD: Circulardichrograph III NRS-Jouan-Roussel. IR: Perkin−Elmer 1420. NMR: Bruker WM 400 (^{1}H at 400, ^{13}C at 100.6 MHz), Bruker AC 200 (^{1}H at 200, ^{13}C at 50.3 MHz), chemical shifts in δ relative to [D_6]DMSO as internal standard. Multiplets due to $^1J_{C,H}$ couplings are indicated by capital letters. ^{15}N NMR: Bruker AMX 600 (80.8 MHz) with DMF as external standard. EIMS: A.E.I. MS 30 and MS 50 with data system DS 50, direct inlet (DI) at 70 eV and 180 °C if not mentioned otherwise. MS [(+)-FAB]: Kratos Concept-H-System, matrix thioglycerol. All calculations have been performed with the TURBOMOLE[21] package of quantum-chemical programs running on the facilities of the computing center of the RWTH Aachen.

Mushrooms: Fruit bodies of *Boletus curtisii* (det. W. Steglich) were collected in August 1991 near Chapel Hill, North Carolina (USA), and lyophilized after collection. A voucher specimen is kept in the herbarium of the Ludwig-Maximilians-Universität, Department of Chemistry, München.

Isolation Procedure: The lyophilized, powdered fruit bodies (40 g) were exhaustively extracted with MeOH (4 × 250 mL). The combined extracts were concentrated in vacuo, filtered through a RP-18 cartouche and analyzed by analytical HPLC. The 6 main fractions were separated by preparative HPLC and the individual fractions further purified as indicated in Scheme 1. Fraction 1 yielded on preparative HPLC (solvent A: 10% MeOH/90% H_2O; solvent B: 90% MeOH/10% H_2O; start 90% A and 10% B, 50 min 50% A and 50% B, 60 min 100% B) 2-methyl-β-carbolinium-1-propanoate (**10**, 8 mg) and β-carbolin-1-propanoic acid (**9**, 18 mg). Fraction 2 was purified by chromatography on Sephadex LH-20 (MeOH) and subsequently silica gel (CH_2Cl_2/acetone/MeOH, 10:1:1) to yield the two yellow pigments curtisin (**6a**, 32 mg) and 9-deoxycurtisin (**8**, 5.4 mg). Fractions 3 and 4 afforded canthin-6-one (**1**, 24 mg) and 4-(methylthio)canthin-6-one (**2**, 27 mg), respectively. Purification was achieved by silica gel chromatography with $CHCl_3$/acetone (10:1) or $CHCl_3$/acetone (20:1), respectively. Purification of fraction 5 on a silica gel column ($CHCl_3$/acetone, 10:1) yielded 9-(methylthio)-canthin-6-one (**4**, 1.2 mg). Finally, fraction 6 could be separated by preparative TLC (toluene/HCO_2Et/HCO_2H/AcOH, 12:6:2:1) to yield (11-methylthio)canthin-6-one (**5**, 6 mg) and 5-(methylthio)-canthin-6-one (**3**, 14 mg).

Canthin-6-one (1): Pale yellow solid, m.p. 161−163 °C (m.p. 162.5−163.5 °C[2]); R_f (TLC) = 0.27 (system A), 0.38 (system B), 0.71 (system C); t_R (HPLC) = 30.1 min (gradient I). UV (MeOH): λ_{max} (ε_{rel}) = 226 (1.0), 250 (0.63), 260 (0.64), 268 (0.60), 300 (0.40), 330 (sh, 0.25), 346 (0.40), 362 (0.63), 380 (0.56) nm. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.75 (d, J = 5.0 Hz, 1 H, 2-H), 8.57 (dd, J = 7.8, 1.2 Hz, 1 H, 8-H), 8.01 (dd, J = 7.8, 1.2 Hz, 1 H, 11-H), 7.94 (d, J = 9.8 Hz, 1 H, 4-H), 7.86 (d, J = 5.0 Hz, 1 H, 1-H), 7.62 (ddd, J = 7.8, 7.8, 1.2 Hz, 1 H, 9-H), 7.45 (ddd, J = 7.8, 7.8, 1.2 Hz, 1 H, 10-H), 6.91 (d, J = 9.8 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 159.6 (dd, J = 11, 2 Hz, C-6), 145.9 (Dd, J = 180, 3 Hz, C-2), 139.6 (D, J = 166 Hz, C-4), 139.5 (dd, J = 9, 9 Hz, C-7a), 136.2 (dd, J = 12, 12 Hz, C-3a), 132.1 (dd, J = 8, 6 Hz, C-11c), 130.9 (Dd, J = 163, 8 Hz, C-9), 130.3 (dd, J = 7,

3 Hz, C-11b), 129.0 (D, J = 169 Hz, C-5), 125.7 (Dd, J = 164, 8 Hz, C-10), 124.4 (dd, J = 7, 7 Hz, C-11a), 122.7 (Dd, J = 162, 9 Hz, C-11), 117.3 (Dd, J = 170, 8 Hz, C-8), 116.4 (Dd, J = 166, 9 Hz, C-1) ppm. MS (EI): m/z (%) = 222 (11), 221 (16), 220 (100) [M^+], 194 (6), 193 (17), 192 (63, $C_{13}H_8N_2$), 191 (7, $C_{13}H_7N_2$), 165 (9), 164 (9, $C_{12}H_6N$), 139 (8, $C_{10}H_5N$). HRMS (EI): m/z = 220.0637 [M^+] (calcd. for $C_{14}H_8N_2O$: 220.0637).

4-(Methylthio)canthin-6-one (2): Colorless solid, m.p. 257−258 °C (m.p. 252.5−253.5 °C[2]); R_f (TLC) = 0.54 (system A), 0.77 (system B), 0.95 (system C); t_R (HPLC) = 37.3 min (gradient I). UV (MeOH): λ_{max} (log ε) = 212 (4.40), 236 (4.29), 254 (4.16), 296 (4.06), 310 (sh, 3.94), 344 (sh, 3.88), 360 (4.04), 376 (3.99) nm. IR (KBr): $\tilde{\nu}$ = 3040 (w), 1660 (ss), 1630 (m), 1595 (m), 1580 (m), 1525 (m), 1450 (m), 1430 (m), 1360 (m), 1325 (s), 1310 (m), 1290 (m), 1270 (m), 1245 (m), 1220 (w), 1170 (w), 1155 (w), 1125 (w), 1100 (w), 1060 (w), 1010 (w), 975 (m), 910 (w), 870 (w), 840 (m), 820 (m), 785 (w), 750 (m), 665 (w) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.72 (d, J = 5.0 Hz, 1 H, 2-H), 8.55 (dd, J = 7.6, 1.2 Hz, 1 H, 8-H), 8.01 (dd, J = 7.6, 1.2 Hz, 1 H, 11-H), 7.91 (d, J = 5.0 Hz, 1 H, 1-H), 7.64 (ddd, J = 7.6, 7.6, 1.2 Hz, 1 H, 9-H), 7.45 (ddd, J = 7.6, 7.6, 1.2 Hz, 1 H, 10-H), 6.46 (s, 1 H, 5-H), 2.56 (s, 3 H, SCH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 158.3 (s, C-6), 155.1 (qd, J = 4, 3 Hz, C-4), 144.8 (Dd, J = 182, 3 Hz, C-2), 139.4 (dd, J = 10, 9 Hz, C-7a), 135.0 (dd, J = 12, 10 Hz, C-3a), 130.9 (Dd, J = 163, 8 Hz, C-9), 130.8 (d, J = 8 Hz, C-11c), 130.7 (dd, J = 8, 3 Hz, C-11b), 125.3 (Dd, J = 164, 7 Hz, C-10), 124.2 (m, C-11a), 122.6 (Dd, J = 162, 9 Hz, C-11), 117.4 (D, J = 168 Hz, C-5), 117.0 (Dd, J = 171, 8 Hz, C-8), 116.9 (Dd, J = 166, 9 Hz, C-1), 13.9 (Q, J = 141 Hz, SCH_3) ppm. MS (EI): m/z (%) = 267 (12), 266 (100) [M^+], 265 (9, $C_{15}H_9N_2OS$), 238 (16, $C_{14}H_{10}N_2S$), 237 (15, $C_{14}H_9N_2S$), 221 (14, $C_{14}H_9N_2O$), 220 (12, $C_{14}H_8N_2O$), 206 (12), 205 (25, $C_{14}H_9N_2$), 195 (9, $C_{13}H_{11}N_2$), 193 (28), 192 (42, $C_{13}H_8N_2$), 57 (17). HRMS (EI): m/z = 266.0509 [M^+] (calcd. for $C_{15}H_{10}N_2OS$: 266.0514).

5-(Methylthio)canthin-6-one (3): Yellow solid, m.p. 198−200 °C; R_f (TLC) = 0.37 (system A), 0.47 (system B), 0.93 (system C); t_R (HPLC) = 42.1 min (gradient I). UV ($CHCl_3$): λ_{max} (log ε) = 252 (4.27), 258 (4.28), 306 (3.90), 344 (sh, 3.77), 360 (4.07), 378 (4.23), 396 (4.05) nm. IR (KBr): $\tilde{\nu}$ = 3050 (w), 3020 (w), 2910 (w), 1650 (ss), 1625 (s), 1595 (m), 1580 (m), 1525 (w), 1450 (m), 1430 (s), 1355 (s), 1330 (s), 1310 (m), 1290 (m), 1270 (m), 1245 (w), 1220 (m), 1210 (m), 1150 (w), 1090 (m), 1055 (w), 1020 (m), 915 (m), 885 (m), 820 (w), 780 (m), 745 (s), 730 (m), 655 (w), 620 (w) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.72 (d, J = 5.0 Hz, 1 H, 2-H), 8.59 (br. d, J = 8.0 Hz, 1 H, 8-H), 8.03 (br. d, J = 8 Hz, 1 H, 11-H), 7.81 (d, J = 5.0 Hz, 1 H, 1-H), 7.65 (ddd, J = 8, 8, 1 Hz, 1 H, 9-H), 7.52 (s, 1 H, 4-H), 7.48 (ddd, J = 8, 8, 1 Hz, 1 H, 10-H), 2.53 (s, 3 H, SCH_3) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 157.1, 145.9, 143.3, 139.3, 136.3, 130.6, 129.9, 129.3, 127.2, 125.8, 125.0, 122.7, 117.4, 114.7, 14.7 ppm. MS (EI): m/z (%) = 266 (37) [M^+], 234 (12), 233 (79, $C_{15}H_9N_2O$), 205 (23, $C_{14}H_9N_2$), 192 (10, $C_{13}H_8N_2$), 179 (11, $C_{12}H_7N_2$). HRMS (EI): m/z = 266.0517 [M^+] (calcd. for $C_{15}H_{10}N_2OS$: 266.0514).

9-(Methylthio)canthin-6-one (4): Yellow solid; R_f (TLC) = 0.34 (system A), 0.35 (system B); 0.78 (system C); t_R (HPLC) = 40.7 min (gradient I). UV ($CHCl_3$): λ_{max} (ε_{rel}) = 208 (1.0), 284 (0.47), 318 (0.33), 362 (0.40) nm. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.79 (d, J = 5.0 Hz, 1 H, 2-H), 8.54 (d, J = 1.5 Hz, 1 H, 8-H), 8.02 (d, J = 9.8 Hz, 1 H, 4-H), 7.96 (d, J = 8.0 Hz, 1 H, 11-H), 7.89 (d, J = 5.0 Hz, 1 H, 1-H), 7.37 (dd, J = 8.0, 1.5 Hz, 1 H, 10-H), 6.96 (d, J = 9.8 Hz, 1 H, 5-H), 2.63 (s, 3 H, SCH_3) ppm. MS (EI): m/z (%) = 266 (100) [M^+], 265 (5), 251 (16), 233 (23), 223 (8), 221 (11), 220 (12), 207 (8), 205 (7), 192 (17), 179 (17), 69 (9). HRMS (EI): m/z = 266.0521 [M^+] (calcd. for $C_{15}H_{10}N_2OS$: 266.0514).

11-(Methylthio)canthin-6-one (5): Lemon yellow solid, m.p. 211−214 °C; R_f (TLC) = 0.42 (system A), 0.41 (system B), 0.87 (system C); t_R (HPLC) = 41.7 min (gradient I). UV ($CHCl_3$): λ_{max} (log ε) = 244 (4.04), 268 (4.05), 328 (sh, 3.72), 340 (3.75), 368 (3.75), 386 (3.87), 400 (sh, 3.71) nm. IR (KBr): $\tilde{\nu}$ = 2950 (m), 2910 (m), 2820 (w), 1690 (s), 1670 (s), 1620 (m), 1580 (w), 1560 (w), 1480 (w), 1450 (w), 1425 (s), 1405 (m), 1385 (w), 1335 (m, sh), 1320 (m), 1295 (m), 1255 (s), 1220 (w), 1175 (w), 1145 (w), 1120 (m, sh), 1100 (s), 1085 (s, sh), 1050 (s), 1010 (s), 840 (m), 795 (s), 785 (s), 775 (m), 740 (w), 625 (w) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.84 (d, J = 5.0 Hz, 1 H, 2-H), 8.49 (dd, J = 8.0, 0.8 Hz, 1 H, 8-H), 8.26 (d, J = 5.0 Hz, 1 H, 1-H), 8.04 (d, J = 9.8 Hz, 1 H, 4-H), 7.65 (dd, J = 8.0, 8.0 Hz, 1 H, 9-H), 7.32 (br. d, J = 8.0 Hz, 1 H, 10-H), 6.97 (d, J = 9.8 Hz, 1 H, 5-H), 2.71 (s, 3 H, SCH_3) ppm. MS (EI): m/z (%) = 266 (100) [M^+], 251 (16, $C_{14}H_7N_2OS$), 238 (7), 233 (30, $C_{15}H_9N_2O$), 223 (8), 205 (9), 196 (6), 179 (12, $C_{12}H_7N_2$). HRMS (EI): m/z = 266.0506 [M^+] (calcd. for $C_{15}H_{10}N_2OS$: 266.0514).

Curtisin (6a): Pale yellow solid; in acetone yellow-green, in DMSO green fluorescence; m.p. >340 °C; R_f (TLC) = 0.36 (system A), 0.42 (system C); t_R (HPLC) = 19.5 min (gradient I). $[\alpha]_D^{21}$ = −149 (c = 0.08, MeOH). UV/Vis (MeOH): λ_{max} (log ε) = 239 (sh, 4.14), 257 (sh, 4.16), 303 (4.16), 416 (4.09) nm. CD (MeOH): $[\Theta]_{227}$ −3130, $[\Theta]_{230}$ 0, $[\Theta]_{243}$ +24540, $[\Theta]_{253}$ 0, $[\Theta]_{263}$ −36570, $[\Theta]_{282}$ 0, $[\Theta]_{295}$ +10830, $[\Theta]_{316}$ 0, $[\Theta]_{333}$ −5440, $[\Theta]_{420}$ −9140. IR (KBr): $\tilde{\nu}$ = 3400−3000 (s), 1650 (ss), 1600 (ss), 1485 (w), 1460 (m), 1430 (s), 1410 (w), 1360 (m), 1320 (ss), 1275 (s), 1240 (s), 1190 (w), 1140 (w), 1105 (w), 1035 (m), 1010 (s), 990 (w), 955 (w) cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO): δ = 8.14 (s, 1-H, 2-H), 8.01 (d, J = 2.2 Hz, 1 H, 8-H), 7.98 (d, J = 8.5 Hz, 1-H, 11-H), 6.96 (dd, J = 8.5, 2.2 Hz, 1 H, 10-H), 6.67 (s, 1 H, 5-H), 3.05 (s, 3 H, $SOCH_3$) ppm. ^{13}C NMR (100.6, [D_6]DMSO): δ = 158.7 (d, J = 2 Hz, C-6), 158.0 (dm, J = 8 Hz, C-9), 156.9 (m, C-1), 156.5 ("quint", J = 4 Hz, C-4), 139.0 (Dm, J = 178 Hz, C-2), 138.2 (dd, J = 9, 2 Hz, C-7a), 134.3 (s, C-11c), 124.1 (D, J = 164 Hz, C-11), 120.3 (m, C-3a), 116.7 (m, C-11a), 114.2 (m, C-11b), 114.0 (Dd, J = 160, 4 Hz, C-10), 113.6 (Dm, J = 169 Hz, C-5), 103.0 (Dd, J = 167, 4 Hz, C-8), 41.8 (Q, J = 140, $SOCH_3$) ppm. MS (EI, DI 300 °C): m/z (%) = 314 (2) [M^+], 298 (37) [M − O]$^+$, 297 (7) [M − OH]$^+$, 284 (13), 270 (7), 269 (8), 267 (15), 266 (98) [M − SO]$^+$, 237 (18), 225 (23), 224 (100). HRMS (EI): m/z = 314.0352 [M^+] (calcd. for $C_{15}H_{10}N_2O_4S$: 314.0361).

***O,O*-Dimethylcurtisin (6b):** To a stirred solution of **6**a (3 mg) in dry acetone (30 mL) was added anhydrous K_2CO_3 (50 mg). The mixture was treated dropwise with dimethyl sulfate (1 mL), and the stirring was continued for 1 h at room temperature until the reaction was complete (TLC control). After partitioning between water and EtOAc, the organic layers were dried (Na_2SO_4) and concentrated in vacuo. Chromatography of the residue on Sephadex LH-20 with MeOH followed by flash chromatography on silica gel (EtOAc/hexanes, 5:2) yielded pure dimethyl ether **6b** (2.8 mg). R_f (TLC) = 0.29 (system 1, light green fluorescence). $[\alpha]_D^{21}$ = −86 (c = 0.16, $CHCl_3$). UV/Vis ($CHCl_3$): λ_{max} (ε_{rel}) = 238 (0.6), 253 (0.4), 258 (0.47), 279 (0.7), 287 (1.0), 310 (0.3), 353 (0.7), 377.5 (0.6) nm. CD ($CHCl_3$): λ_{max} ($\Delta\varepsilon_{rel}$) = 250.5 (0.25), 253.5 (0), 266 (−0.3), 282 (0), 285 (0.25), 288 (0), 317 (0.75), 327 (0.7), 388.5 (0), 360.1 (−1.0), 376 (−0.85), 405 (0), 480 (0.1), 585 (0) nm. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.38 (s, 1 H, 2-H), 8.18 (d, J = 2.2 Hz, 1 H, 8-H),

8.05 (d, J = 9 Hz, 1-H, 11-H), 7.34 (s, 1 H, 5-H), 7.08 (dd, J = 9.0, 2.2 Hz, 1 H, 10-H), 4.23 (s, 3 H, 1-OCH_3), 3.97 (s, 3 H, 9-OCH_3), 3.16 (s, 3 H, $SOCH_3$) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 162.1 (dm, J = 11 Hz, C-9), 158.7 (d, J = 1 Hz, C-6), 156.8 (m, C-4), 152.1 (qd, J = 4, 1 Hz, C-1), 140.1 (dd, J = 10, 2 Hz, C-7a), 133.4 (s, C-11c), 129.5 (D, J = 181 Hz, C-2), 126.0 (m, C-3a), 125.4 (D, J = 166 Hz, C-11), 121.9 (D, J = 173 Hz, C-5), 117.8 (m, C-11b), 116.6 (dd, J = 8, 8 Hz, C-11a), 114.9 (Dd, J = 162, 4 Hz, C-10), 101.0 (Dd, J = 169, 5 Hz, C-8), 56.9, 56.0 (each OCH_3), 42.2 ($SOCH_3$) ppm. ^{15}N NMR (60.8 MHz, $CDCl_3$): δ = 152 (N-7), 79 (N-3) ppm. MS (EI, DI 180 °C): m/z (%) = 342 (23.5) [M^+], 326 (13) [$M - O]^+$, 294 (100) [$M - SO]^+$, 280 (16), 279 (16), 252 (55), 251 (11). HRMS (EI): m/z = 342.0680 [M^+] (calcd. for $C_{15}H_{10}N_2O_4S$: 342.0674).

***O*,*O*-Bis(4-bromobenzoyl)curtisin (6c):** To a solution of **6a** (4 mg) and a catalytic amount of DMAP in pyridine (2 mL) at 0 °C was added 4-bromobenzoyl chloride (25 mg) in CCl_4 (0.5 mL). The mixture was stirred for 24 h at room temperature, quenched with saturated aqueous NH_4Cl and equilibrated between $CHCl_3$ and water. The organic phases were washed with brine (2 ×), dried (Na_2SO_4) and concentrated in vacuo. Column chromatography on silica gel with toluene/acetone (7:1) afforded **6c** as yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.68 (s, 1 H, 2-H), 8.57 (d, J = 2.1 Hz, 1-H, 8-H), 8.21, 8.07 (each d, J = 8.7 Hz, 2 H), 7.85 (d, J = 8.4 Hz, 1 H, 11-H), 7.79, 7.68 (each d, J = 8.7 Hz, 2 H), 7.49 (s, 1 H, 5-H), 7.35 (dd, J = 8.4, 2.1 Hz, 1 H, 10-H), 3.20 (s, 3 H, $SOCH_3$).

***O*,*O*-Diacetyl-*S*-deoxycurtisin (7):** To **6a** (6 mg) in acetic anhydride (1.5 mL) were added at 0 °C with stirring 0.2 mL of a solution prepared from acetic anhydride (1 mL) and conc. H_2SO_4 (3 drops). The suspension yielded within 4 min a clear solution exhibiting a yellow-green fluorescence. After stirring for 3 h at room temperature, the mixture was worked-up as described for **6c**. Column chromatography on silica gel with CH_2Cl_2/acetone (30:1) yielded **7** (3 mg) as a colorless solid. M. p. 266−267 °C. UV (MeOH): λ_{max} (log ε) = 238 (4.14), 256 (3.99), 294 (3.94), 312 (sh, 3.83), 342 (sh, 3.76), 358 (3.94), 374 (3.91) nm. IR (KBr): $\tilde{\nu}$ = 1750 (s), 1655 (s), 1625 (w), 1595 (w), 1525 (w), 1455 (w), 1430 (m), 1410 (m), 1360 (m), 1315 (m), 1260 (m), 1245 (m), 1195 (ss, br), 1130 (w), 1080 (w), 1000 (w), 960 (w), 930 (w), 895 (w), 865 (w), 830 (w), 640 (w), 630 (w) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.59 (s, 1 H, 2-H), 8.39 (dd, J = 2.2, 0.4 Hz, 1 H, 8-H), 7.88 (dd, J = 8.5, 0.4 Hz, 1 H, 11-H), 7.24 (dd, J = 8.5, 2.2 Hz, 1 H, 10-H), 6.49 (s, 1 H, 5-H), 2.59, 2.56, 2.38 (each 3 H) ppm. MS (EI): m/z (%) = 382 (35) [M^+], 340 (26), 299 (13), 298 (100), 280 (35), 270 (9), 252 (15), 250 (8), 237 (8), 224 (9). HRMS (EI): m/z = 382.0642 [M^+] (calcd. for $C_{19}H_{14}N_2O_5S$: 382.0623).

9-Deoxycurtisin (8): Pale yellow solid, in acetone light green fluorescence; R_f (TLC) = 0.43 (system A), 0.46 (system C); t_R (HPLC) = 22.3 min (gradient I). $[\alpha]_D^{20.5}$ = ca. −70 (c = 0.05, MeOH). UV/Vis (MeOH): λ_{max} (ε_{rel}) = 210 (1.0), 242 (sh, 0.30), 276 (sh, 0.21), 286 (0.26), 308 (0.19), 352 (0.18), 366 (0.30), 384 (0.35), 414 (0.20) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon_{rel}$) = 230 (0), 240 (+0.62), 249 (0), 263 (−1.0), 278 (0), 285 (+0.38), 295 (+0.23), 310 (0), 343 (−0.49), 353 (−0.46), 363 (−0.57), 374 (−0.39), 382 (−0.52), 408 (−0.18), 460 (0) nm. IR (KBr): $\tilde{\nu}$ = 3430 (m, br), 3020 (w), 2960 (w), 2925 (m), 2860 (w), 1675 (ss), 1615 (m), 1605 (s), 1580 (w), 1560 (w), 1470 (w), 1440 (m), 1415 (w), 1390 (ss), 1345 (ss), 1315 (m), 1280 (w), 1255 (m), 1215 (w), 1190 (w), 1160 (w), 1105 (w), 1095 (w), 1040 (ss), 980 (w), 880 (w), 795 (w), 760 (m), 750 (m), 695 (w), 650 (w) cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.67 (br. d, J = 7.5 Hz, 1 H, 8-H), 8.38 (br. d, J = 7.5 Hz, 1-H, 11-H), 8.23 (br. s, 1 H, 2-H), 7.65 (br. dd, J = 7.5, 7.5 Hz, 1 H, 9-H*), 7.59 (br. dd, J = 7.5, 7.5 Hz, 1 H, 10-H*), 6.97 (br. s, 1 H, 5-H), 3.20 (s, 3 H, $SOCH_3$) ppm, *assignments interchangeable. ^{13}C NMR (100.6 MHz, [D_6]DMSO): δ = 158.6, 156.7, 156.2, 138.0, 136.8, 134.0, 128.3, 125.8, 124.6, 123.5, 121.4, 116.1, 115.0, 113.8, 41.7 ppm. MS (EI, DI 180 °C): m/z (%) = 298 (19) [M^+], 267 (2), 266 (19) [$M - SO]^+$, 234 (3), 233 (15), 220 (8), 219 (12). HRMS (EI): m/z = 298.0416 [M^+] (calcd. for $C_{15}H_{10}N_2O_3S$: 298.0412).

β-Carboline-1-propanoic Acid (9): Pale yellow solid, m.p. 203−208 °C (m.p. 215−216 °C[9]); R_f (TLC) = 0.15 (system A); t_R (HPLC) = 36.3 min (gradient II). UV (MeOH): λ_{max} (lg ε) = 212 (4.24), 236 (4.47), 250 (sh, 4.34), 282 (sh, 3.92), 288 (4.10), 302 (sh, 3.64), 338 (3.57), 350 (3.60) nm. ^{1}H NMR (200 MHz, CD_3OD): δ = 8.19 (m, 2 H), 8.09 (d, J = 6.2 Hz, 1 H), 7.61 (m, 2 H), 7.29 (m, 1 H), 3.49 (br. t, J = 7.2 Hz, 2 H, CH_2_1′), 2.84 (br. t, J = 7.2 Hz, 2 H, CH_2-2′) ppm. MS (EI): m/z (%) = 241 (16), 240 (27) [M^+], 222 (11, $C_{14}H_{10}N_2O$), 221 (10, $C_{14}H_9N_2O$), 196 (47), 195 (100, $C_{13}H_{11}N_2$), 194 (25), 193 (31, $C_{13}H_9N_2$), 168 (12, $C_{11}H_8N_2$), 140 (10, $C_{10}H_6N$). HRMS (EI): m/z = 240.0894 [M^+] (calcd. for $C_{14}H_{12}N_2O_2$: 240.0899).

2-Methyl-β-carbolinium-1-propanoate (10): Straw-yellow solid, m.p. >340 °C; R_f (TLC) = 0.11 (system A); t_R (HPLC) = 31.7 min (gradient II). UV (MeOH): λ_{max} (lg ε) = 254 (4.46), 308 (4.29), 374 (3.67) nm. IR (KBr): $\tilde{\nu}$ = 3400 (s, br), 3040 (w), 2925 (w, sh), 2900 (m), 2825 (w), 1625 (s, sh), 1620 (s), 1565 (ss, br), 1515 (m), 1490 (m), 1440 (w), 1380 (s), 1325 (s), 1310 (m), 1295 (w, sh), 925 (w), 745 (m) cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.48 (d, J = 6.0 Hz, 1 H, 3-H*), 8.45 (d, J = 6.0 Hz, 1 H, 4-H*), 8.38 (br. d, J = 8.0 Hz, 1 H, 8-H), 7.77−7.85 (m, 5-H, 2 H, 6-H**), 7.48 (ddd, J = 8.0, 8.0, 1.5 Hz, 1 H, 7-H**), 4.53 (s, 3 H, CH_3), 3.78 (br. t, J = 7.5 Hz, 2 H, CH_2_1′), 2.74 (br. t, J = 7.5 Hz, 2 H, CH_2_2′) ppm. ^{13}C NMR (100.6 MHz, CD_3OD): δ = 178.8, 145.6, 144.9, 136.5, 135.6, 133.7, 133.0, 124.1, 123.0, 121.4, 116.7, 113.9, 45.3, 35.9, 27.3 ppm. MS (EI): m/z (%) = 237 (14), 236 (100, $C_{15}H_{12}N_2O$) [$M - H_2O]^+$, 235 (68, $C_{15}H_{11}N_2O$), 220 (5, $C_{14}H_8N_2O$), 208 (22), 207 (100, $C_{14}H_{11}N_2$), 206 (17), 205 (8, $C_{14}H_9N_2$), 192 (8), 182 (12), 103 (15). MS [(+)-FAB]: m/z = 255.1 [$M + H]^+$ (100%).

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[15]
$$\Delta\varepsilon = \sum_{i=1}^{N} w_i \cdot \Delta\varepsilon_i ,$$
$$w_i = exp(-E_i/R\cdot T)/[\sum_{j=1}^{N} exp(-E_j/R\cdot T)]$$
N is the number of located stationary points and $\Delta\varepsilon$ the superposition of Gaussians. w_i and E_i are the Boltzmann factor and the energy of the i-th local minimum, respectively. The Gaussians were generated using the empirical formula $\Gamma = k\cdot\lambda^{1.5}$ for the half bandwidth Γ at $\Delta\varepsilon/e$. The parameter k was set to 0.00375, which yields half bandwidths from 14.8 to 69.5 between 250 and 700 nm.
[16] Further details of the calculations, like the spectra of the single conformers and their geometries, are available from the authors upon request.
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