Pergamon

Tetrahedron, Vol. 53, No. 9, pp. 3101-3110, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00066-5

Biologically Active Metabolites from Fungi, 9.[1]

New Palmarumycins CP_{4a} and CP_5 from *Coniothyrium palmarum*: Structure Elucidation, Crystal Structure Analysis and Determination of the Absolute Configuration by CD Calculations

Karsten Krohn*[a], Karsten Beckmann[a], Ulrich Flörke[a], Hans-Jürgen Aust[b], Siegfried Draeger[b], Barbara Schulz[b], Stefan Busemann[c], and Gerhard Bringmann*[c]

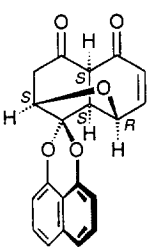
[a]Universität-GH-Paderborn, FB 13 Chemie und Chemietechnik,
Warburger Str. 100, D-33098 Paderborn, Germany
[b]Institut für Mikrobiologie, TU Braunschweig, Spielmannstr. 7, D-38106 Braunschweig, Germany
[c]Institut für Organische Chemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

Abstract: Two new palmarumycins CP_{4a} (**2**) and CP_5 (**3**) with an unusual bridged structure were isolated from *Coniothyrium palmarum*. The structure and relative configuration was elucidated by NMR and X-ray studies; the absolute configuration was determined by CD calculations. © 1997 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Recently we reported on the isolation and structure elucidation of palmarumycins C_1–C_{16} from *Coniothyrium* sp.[2] and CP_1–CP_4 from *Coniothyrium palmarum*.[3] Related compounds have been isolated from different species (see ref. in[2,3]); in more recent papers the DNA gyrase inhibition[4] and the absolute configuration of the diepoxin class of compounds[5] were published.

Palmarumycin CP_3 (**1**), isolated from *Coniothyrium palmarum*, showed a unique bridged ring system (Fig.1). The more polar fractions from the culture broth of *Coniothyrium palmarum* were screened for further compounds of this class and here we describe the isolation and determination of the absolute configuration of two new palmarumycins CP_{4a} and CP_5 (the index numbers of the CP compounds increase with polarity; for descriptions of the fungi and fermentation conditions see ref.[3]).



1

Fig. 1. Absolute configuration of palmarumycin CP_3 (**1**) [6]

RESULTS AND DISCUSSION

1. Isolation, Structure Elucidation and Crystal Structure Analysis of CP_{4a} and CP_5

The optically active compound CP_{4a} (**2**) (Fig. 2) of intermediate polarity between CP_3 and CP_4 showed a positive TLC-test with 2,4-dinitrophenylhydrazine. The presence of a carbonyl group was confirmed by a characteristic IR band at 1701 cm^{-1}. Typical NMR signals and also the characteristic fragment at m/z = 160 in the mass spectrum proved the presence of the 1,8-dioxygenated naphthalene fragment occurring in all palmarumycins[2]. In contrast to the spectrum of CP_3, a strong band at 3440 cm^{-1} indicated the presence of a hydroxy group.

The molecular formula of $C_{20}H_{18}O_5$ was deduced from the numbers of the protons and carbon atoms found in the ^1H and ^{13}C NMR spectra in combination with the mass spectrum. These spectra showed great similarity to those measured for palmarumycin CP_3 (**1**) and also (see below) to those for the second new metabolite CP_5 (**3**) (Fig. 2). However, the ^{13}C-signal for a carbonyl group at C-5 at δ = 189.9 seen in CP_3 (**1**)[3] was absent in the spectrum of CP_{4a} (**2**). Instead, signals at δ = 74.6 in the ^{13}C NMR and at 3.50 in the ^1H NMR spectrum appeared. Furthermore, the characteristic signals in the ^1H as well as in the ^{13}C NMR spectra for the $C_{6\text{-}7}$ double bond were absent in the spectra of both CP_{4a} (**2**) and CP_5 (**3**). A broad signal at δ = 4.3 was exchangeable with deuterated methanol. This indicated the reduction of one of the carbonyl groups in CP_3 to a hydroxyl group.

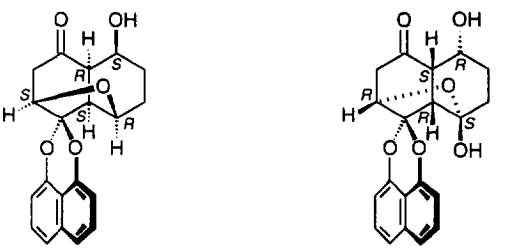
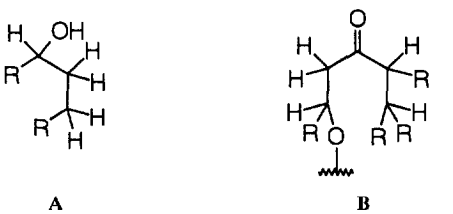


Fig. 2. Stereostructures of CP_{4a} (**2a**) and CP_5 (**3b**) (assignment of stereochemistry see text).

The remaining open question, i.e. which of the two carbonyls at C-4 or C-5 in CP_3 (**1**) was reduced, was solved by comparison with the spectral data of the more polar compound CP_5. This compound was also optically active and crystallized in colorless orthorhombic form. The presence of an additional hydroxyl group compared to CP_{4a} (**2**) was confirmed by the absence of the signals for one carbon proton at δ = 4.39, the occurrence of signals for one additional quarternary carbon and the presence of an additional exchangeable proton (2 exchangeable signals at δ = 4.1 and 4.7). One of these signals showed a large coupling of ^3J = 10 Hz indicating the connection with a tertiary carbon atom. This assumption was further confirmed by a HH-COSY experiment showing a correlation between the signal for the hydroxy proton at δ = 4.1 and the signal for the methine proton at δ = 3.7.

The two signals in the ^{13}C NMR spectrum at δ = 103.4 and 107.4 indicated the presence of *two* acetal carbon atoms. The low-field signal can be attributed to the typical spiro acetal fragment present in all palmarumycins. Consequently, the second hydroxy group must be located at C-8. The location of the remaining carbonyl and methylene groups was deduced from the coupling constants of the aliphatic protons using two-dimensional CH-COSY and HH-COSY experiments. Thus, the two protons of one methylene group at δ = 1.5 and 1.9 showed (in addition to a geminal coupling of 12.4 Hz) two ^3J couplings of 4.2 and 4.3 Hz with the

protons of a neighboring methylene group at δ = 1.9 and 2.2. The further cross-coupling with the methine proton at δ = 3.7 led to the fragment A (Scheme 1). The location of low-field signals for methylene groups at δ = 2.69 and 2.89 are typical for a neighboring carbonyl group and the ^3J-couplings of 2.40 and 2.41 Hz with the proton at δ = 4.60 prove the structure of fragment B (Scheme 1), in which the carbonyl group is located between the methylene and methine group at δ = 3.0 (4a-H).



Scheme 1. Fragment A and B of the 'upper' part of the molecule CP_5 (3).

A very similar picture is shown by the NMR spectra of the less polar compound CP_{4a} (2). The combination of the available structural information leads to structures **2** and **3** for CP_{4a} and CP_5, respectively, as shown in Fig. 2, but without stereochemical information.

The question of relative configuration of CP_{4a} and CP_5 can essentially be reduced to the orientation of the hydroxy group at C-5, the connection of the two hydroaromatic rings (*cis/trans*), and the relative position of the oxygen bridge. All these questions are difficult to answer by the analysis of ^1H NMR coupling constants alone. Fortunately, both compounds crystallized very nicely allowing crystal structure analysis. The results of the X-ray analysis showing the relative stereochemistry are presented in Fig. 3 and 4.

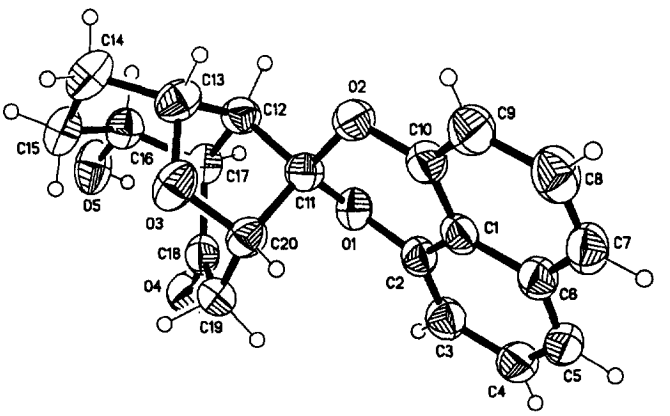


Fig. 3. The molecular structure of CP_{4a}; arbitrarily, the (correct) *2S,4aR,5S,8R,8aS*-enantiomer is drawn.

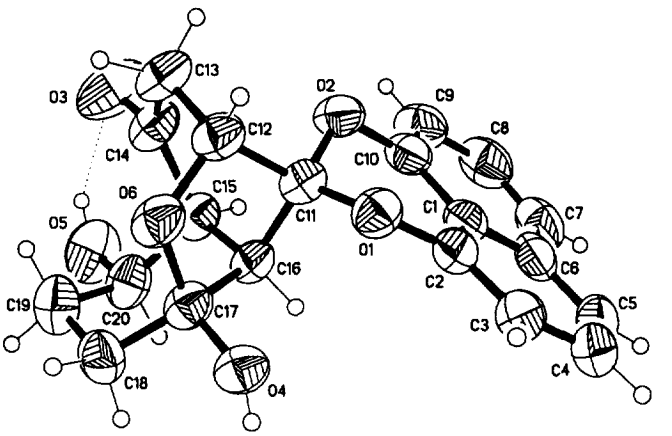


Fig. 4. The molecular structure of CP_5; arbitrarily, the (non-natural) *2R,4aS,5R,8S,8aR*-enantiomer is drawn.

2. Elucidation of the Absolute Configuration of CP_{4a} and CP_5 by Circular Dichroism Calculations

Given the known constitution and relative configuration of palmarumycin CP_5 (**3**), the elucidation of the absolute stereostructure of the compound, *i.e.* whether palmarumycin CP_5 has structure **3a** (2*S*,4a*R*,5*S*,8*R*,8a*S*) or **3b** (2*R*,4a*S*,5*R*,8*S*,8a*R*), see Fig. 5, was achieved using a method described recently,[6] the calculation of the circular dichroism (CD) spectrum of the compound and its comparison with the experimental one.

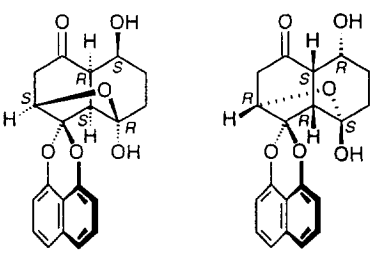


Fig. 5. Possible absolute stereostructures **3a** or **3b** for palmarumycin CP_5.

Following the procedure described in detail, recently,[6-8] the conformational behavior of **3** was calculated, arbitrarily starting with the 2*S*,4a*R*,5*S*,8*R*,8a*S*-enantiomer **3a**. The calculations gave rise to six minimum structures (**3a-A** to **3a-F**) (Fig. 6), which differ by three conformational parameters:

- the mutual orientation of the two molecular halves, by variation of the dihedral angle at the acetal oxygen functions as a 'hingejoint';[6]

- the structure of the conformationally flexible tricyclic 'upper' part of the molecule; and
- the rotation of the H atoms attached to the oxygen atoms at C-5 and C-8, about the adjacent C-O bonds

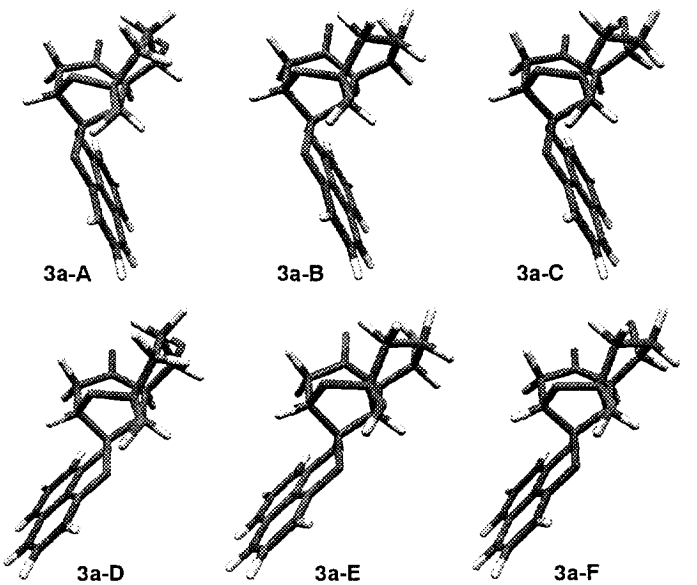


Fig. 6. Calculated minimum structures **3a-A** to **3a-F** of the **3a**-enantiomer of palmarumycin CP_5.

One of these conformers, **3a-A**, matches nicely (RMS value = 0.219) with the structure of palmarumycin CP_5 as found in the crystal (Fig. 7), underlining the reliability of the semiempirical calculation of the molecular structure.

Fig. 7. Matchplot of conformer **3a-A** with the structure of palmarumycin CP_5 experimentally found in the crystal. For the experimental structure, arbitrarily the same enantiomer is shown.

For these six conformational species **3a-A** to **3a-F**, the CD spectra were separately calculated and added up according to the Boltzmann statistic, to give the calculated spectra for **3a** and for its enantiomer, **3b**. Fig. 8 shows the good agreement of the experimental CD spectrum with the one calculated for the 2*R*,4a*S*,5*R*,8*S*,8a*R*-

enantiomer **3b**, whereas the one calculated for **3a** is virtually opposite to the experimental spectrum, showing the natural product to have 2*R*,4a*S*,5*R*,8*S*,8a*R*-configuration, *i.e.* stucture **3b**.

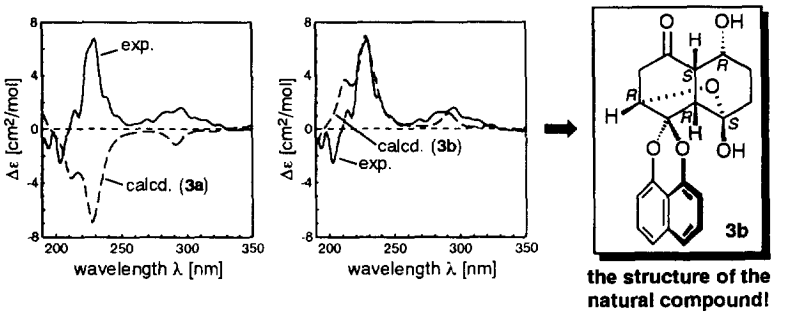


Fig. 8. Attribution of the absolute stereostructure of palmarumycin CP_5 as **3b**, by comparison of the theoretically predicted CD spectrum for the two possible enantiomers **3a** and **3b** with the experimental one for the natural product.

For the investigation of the absolute configuration of the second compound, palmarumycin CP_{4a} (**2a** or **2b**, see Fig. 9), the empirical comparison of its experimental CD spectrum with that of **3b** should not lead to a reliable attribution because of the pronounced differences of the two spectra (cf. Figs. 8 and 11). Therefore, as for **3b**, the CD spectrum of palmarumycin CP_{4a} was calculated, leading to an unambiguous assignment of the absolute configuration of this natural product.

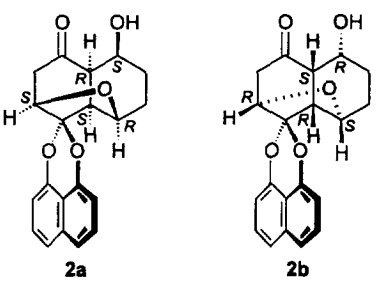


Fig. 9. Possible absolute stereostructures **2a** or **2b** for palmarumycin CP_{4a}.

Again, for the arbitrarily chosen enantiomer **2a** six conformers, **2a-A** to **2a-F** were found (Fig. 10), one of which (**2a-A**) again matches well with the structure experimentally found in the crystal (not shown, RMS value = 0.199).

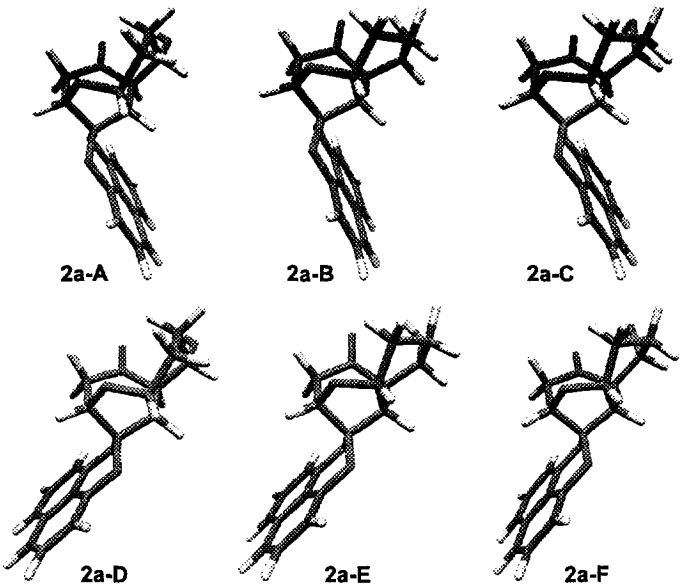


Fig. 10. Calculated minimum structures **2a-A** to **2a-F**, arbitrarily of the **2a**-enantiomer of palmarumycin CP_{4a}.

The calculation of the single CD spectra of these six conformational species, their Boltzmann-weighted addition and the comparison of the resulting overall CD spectrum with the experimental one, clearly showed palmarumycin CP_{4a} to be 2*S*,4a*R*,5*S*,8*R*,8a*S*-configured, *i.e.* to be represented by stereostructure **2a** (Fig. 11).

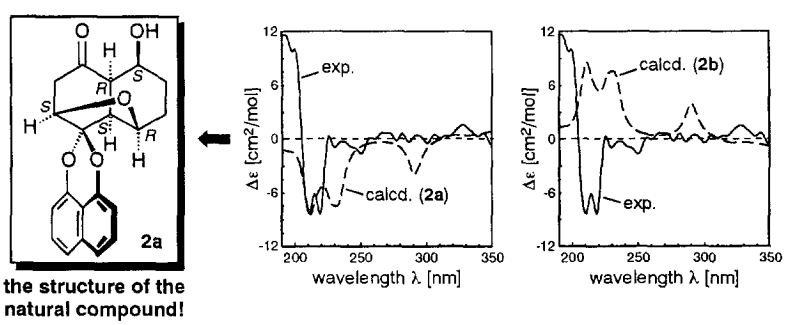
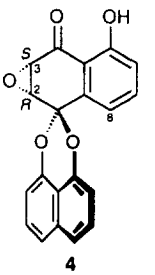


Fig. 11. Attribution of the absolute stereostructure of palmarumycin CP_{4a} as **2a**, by comparison of the theoretically predicted CD spectrum for the two possible enantiomers **2a** and **2b** with the experimental one for the natural product.

Unexpectedly, the oxygen-bridge from C-2 to C-8, which is a joint structural element of both palmarumycins CP_5 and CP_{4a}, is not located on the same side for both molecules, it is above the plain for CP_{4a}, but below the plain for CP_5. The opposite stereochemical array of the oxygen bridges might hint at a different biosynthetic origin of this bridge, possibly both from an 2,3-epoxide related to palmarumycin C_2 (**4**), the bridge being formed by attack of O-2 at C-8 in one case (**3b**), and by S_N2-type epoxide opening by an oxygen at C-8 in the other (**2a**).



4

Fig. 12. Palmarumycin C_2.

EXPERIMENTAL

For general methods and instrumentation see ref.[2] and for microbiological methods and culture conditions ref.[9]

Coniothyrium palmarum was incubated at 20 °C for 93 days in a biomalt softagar medium. The agar was homogenated using a Warring-Blender and the homogenate extracted three times with ethyl acetate (3 l). The combined organic phases were dried (Na_2SO_4), filtered, and the filtrate evaporated at reduced pressure to afford 7.0 g of a crude extract. The solution of the extract was chromatographed three times by column chromatography on silica gel with a gradient of CH_2Cl_2/MeOH (1 % - 10 % of MeOH) followed by layer chromatography on silica gel (CH_2Cl_2/5 % MeOH) and semipreparative HPLC on Si 60 (CH_2Cl_2/1% MeOH) to afford 29 mg of the metabolite CP_{4a} (**2**) from the fraction between CP_4[2] and CP_5. From the following more polar fraction 25 mg of CP_5 (**3**) were isolated.

(2S,4aR,5S,8R,8aS)-5-Hydroxy-2,3,5,6,7,8,8a-heptahydrospiro[2,8-epoxynaphthaline-1 (4H), 2´-naphtho-[1,8de][1,3-dioxin]-4(4aH)-one CP_{4a} (**2a**): Mp 213 °C; $[\alpha]_D^{20}$ = + 70.59 ° (c = 0.85 mg/ml; CH_2Cl_2); R_f 0.41 (CH_2Cl_2/2% MeOH); IR (KBr) 3440 (OH); 2936, 2929 (CH_2); 1701 (C=O); 1684, 1653, 1559, 1507, 1271 (ether); 1075, 1049 (ether, C-OH) cm^{-1}; UV (CH_2Cl_2): λ_{max} (lg ε) 333.6 (1.23), 319.2 (1.26), 305.4 (1.24) nm; ^{1}H-NMR ($CDCl_3$) δ 7.55 (dd, $J_{5',6'}$ = 8.39 Hz, $J_{5',7'}$ = 0.70 Hz, 1H, 5´-H), 7.54 (dd, $J_{4',3'}$ = 8.37 Hz, $J_{4',2'}$ = 0.94 Hz, 1H, 4´-H), 7.46 (dd, $J_{3',4'}$ = 8.39 Hz, $J_{3',2'}$ = 7.39 Hz, 1H, 3´-H), 7.43 (dd, $J_{6',5'}$ = 8.38 Hz, $J_{6',7'}$ = 7.51Hz, 1H, 6´-H), 7.03 (dd, $J_{2',3'}$ = 7.38 Hz, $J_{2',4'}$ = 0.96 Hz, 1H, 2´-H), 6.91 (dd, $J_{7',6'}$ = 7.50 Hz, $J_{7',5'}$ = 0.76, 1H, 7´-H), 4.61 (d, $J_{2,3}$ = 2.93 Hz, 1H, 2-H), 4.39 (pseudo-s, 1H, 8-H), 4.30 (d, $J_{OH,\ 5ax}$ = 10.0 Hz, 1H, 5-OH), 3.5 (m, $J_{5ax,\ 5\text{-}OH}$ = 9.89 Hz, $J_{5ax,\ 6e}$ = 4.78 Hz, 1H, 5 ax-H), 2.94 (dd, $J_{3e,3ax}$ = 19.23 Hz, $J_{3e,\ 2}$ = 2.16 Hz, $J_{3e,\ 4a}$ = 1.36 Hz, 1H, 3e-H), 2.73 (dd, $J_{3ax,\ 3e}$ = 19.30 Hz, $J_{3ax,\ 2}$ = 2.71 Hz, $J_{3ax,\ 4a}$ = 1.08 Hz, 1H, 3ax-H), 2.55 (dd, $J_{4a,\ 8a}$ = 5.6 Hz, $J_{4a,\ 3ax}$ = 1.03 Hz, 1H, 4a-H), 2.18 (m, $J_{8a,\ 4a}$ = 5.45 Hz, $J_{8a,\ 8}$ = 2.33 Hz, 1H, 8a-H), 1.65 (m, 4H, 6-H, 7-H); ^{13}C-NMR ($CDCl_3$) δ 214.7 (s, C-4), 146.5 (s, C-8´), 146.7 (s, C-1´), 134.3 (s, C-4a´), 127.4 (d, C-3´), 127.2 (d, C-6´), 121.5 (d, C-5´), 121.0 (d, C-4´), 113.9 (s, C-8a´), 109.7 (d, C-2´), 109.1 (d, C-7´), 106.9 (s, C-1), 74.6 (d, C-5), 74.0 (d, C-8), 71.0 (d, C-2), 49.0 (d, C-4a), 47.0 (t, C-3), 43.5 (d, C-8a), 27.1 (t, C-7), 26.6 (t, C-6); MS (100 °C): *m/z* (%) = 338 (100) [M^+], 252 (60), 197 (16), 160 (46), 115 (16), 105 (4), 79 (4), 55 (4). Found C, 69.71 %; H, 5.50 % Calcd. for $C_{20}H_{18}O_5$: C, 71.0 %; H 5.36 %.

(2R,4aS,5R,8S,8aR)-5,8-Dihydroxy-2,3,5,6,7,8a-hexahydrospiro-[2,8 epoxy-naphthaline-1 (4 H), 2´-naphtho[1,8 de][1,3]dioxin]-4(4aH)-one CP_5 (**3b**): Mp 168 °C; $[\alpha]_D^{20}$= + 45.45 ° (c = 1 mg/ml, CH_2Cl_2); R_f 0.29 (CH_2Cl_2/MeOH 2 %); IR (KBr) 3445 (OH); 2915, 2843 (CH_2); 1700 (C=O); 1635, 1410, 1381, 1271 (ether); 1096, 1051 (ether, C-OH) cm^{-1}; UV ($CHCl_3$): λ_{max} (lg ε) = 297 (3.41), 312 (3.57), 326 (3.60) nm; ^{1}H-NMR ($CDCl_3$) δ 7.60 (d, $J_{5',6'}$ = 8.3 Hz, 1 H, 5´-H), 7.59 (d, $J_{4',3'}$ = 8.2 Hz, 1H, 4´-H), 7.49 (dd, $J_{3',4'}$ = 8.3

Hz, $J_{3',2'}$ = 7.6 Hz, 1H, 3'-H), 7.45 ($J_{6',5'}$ = 8.3 Hz, $J_{6',7'}$ = 7.6 Hz, 1H, 6'-H), 7.10 (dd, $J_{2',3'}$ = 7.6 Hz, $J_{2',4'}$ = 0.6 Hz, 1H, 2'-H), 6.9 (dd, $J_{7',6'}$ = 7.5 Hz, $J_{7',5'}$ = 0.5 Hz, 1H, 7'-H), 4.7 (s, 1H, 8-OH), 4.6 (m, 1H, 2-H), 4.1 (d, $J_{OH,5}$ = 10 Hz, 1H, 5-OH), 3.7 (ddd, $J_{5ax,6ax}$ = 12.0 Hz, $J_{5ax,4a}$ = 4.7 Hz, $J_{5ax,6e}$ = 1.5 Hz, 1H, 5ax-H), 3.0 (m, $J_{4a,8a}$ = 8.7 Hz, $J_{4a,5ax}$ = 4.7 Hz, 1H, 4a-H), 2.9 (ddd, $J_{3ax,3e}$ = 19.3 Hz, $J_{3e,2}$ = 2.40 Hz, $J_{3e,4a}$ = 0.99 Hz, 1H, 3e-H), 2.7 (d, $J_{8a,4a}$ = 8.7 Hz, 1H, 8a-H), 2.68 (ddd, $J_{3ax,3e}$ = 19.3 Hz, $J_{3ax,2}$ = 2.41 Hz, $J_{3ax,4a}$ = 1.4 Hz, 1H, 3ax-H), 2.2 (ddd, $J_{7e,7ax}$ = 12.6 Hz, $J_{7e,6ax}$ = 4.3 Hz, $J_{7e,6e}$ = 4.2 Hz, 1H, 7e-H), 1.9 (m, 2H, 6ax-H, 7ax-H), 1.5 (ddd, $J_{6e,6ax}$ = 12.4 Hz, $J_{6e,7ax}$ = 4.3 Hz, $J_{6e,7e}$ = 4.2 Hz, 1H, 6e-H); ^{13}C-NMR ($CDCl_3$) δ 213.0 (s, C-4), 146.2 (s, C-8'), 145.7 (s, C-1'), 134.4 (s, C-4a'), 127.5 (d, C-3'), 127.4 (d, C-6'), 122.0 (d, C-5'), 121.9 (d, C-4'), 113.6 (s, C-8a'), 109.8 (d, C-2'), 109.5 (d, C-7'), 107.6 (s, C-1), 103.4 (s, C-8), 75.4 (d, C-2), 71.0 (d, C-5), 49.8 (d, C-4a), 47.7 (d, C-8a), 46.4 (t, C-3), 32.8 (t, C-7), 28.6 (t, C-6); MS (110 °C) *m/z* (%) = 354 (100) [M^+], 252 (84), 197 (16), 160 (70), 115 (14); HRMS found 354.110 ± 2 ppm; Calcd. 354.110340.

Crystal Structure Determination of CP_{4a} (**2a**):[10] $C_{20}H_{18}O_5$, M_r = 338.3, monoclinic, space group P 2_1, a = 679.7(3), b = 959.2(4), c = 1236.2(5) pm, β = 103.71(2)°, V = 783.0 10^6 pm^3, Z = 2, D_r = 1.435 g/cm^3, F(000) = 356, T = 298(1) K. Siemens R3m diffractometer, graphite monochromator, λ(MoKα) = 71.073 pm, μ = 0.10 mm^{-1}, colorless crystal, size 0.20 x 0.20 x 0.63 mm, ω-scan, 2798 intensities collected 3 < 2θ < 55°, -8 < h < 8, -12 < k < 12, 0 < l < 16, 3 standards every 400 reflections showed 13% decrease, intensities corrected accordingly, Lp correction, 2715 unique intensities (R_{int} = 0.021), 2261 with F > 4σ(F). Structure solved by direct methods[11], full-matrix least-squares refinement based on F^2 and 228 parameters[11], all but H atoms refined anisotropically, H atoms located from difference Fourier maps and refined with riding model on idealized positions, refinement converged at R1(F) = 0.043, wR2(F^2, all data) = 0.100, S = 1.04, max(Δ/σ) < 0.001, min/max height in final ΔF map -0.13/0.19 e/Å^3. Figure 3 shows the molecular structure.

Crystal Structure Determination of CP_5 (**3b**):[10] $C_{20}H_{18}O_6$, M_r = 354.3, orthorhombic, space group P $2_12_12_1$, a = 633.5(3), b = 1304.9(7), c = 1971.3(11) pm, V = 1630 10^6 pm^3, Z = 4, D_r = 1.444 g/cm^3, F(000) = 744, T = 298(1) K. Diffractometer and data collection as for **CP_{4a}**, μ = 0.11 mm^{-1}, colorless crystal, size 0.20 x 0.30 x 0.61 mm, 2176 intensities collected 3 < 2θ < 55°, 0 < h < 8, 0 < k < 16, 0 < l < 25, 3 standards every 400 reflections showed only random deviations, Lp correction, 1353 unique observed intensities with F > 4σ(F). Structure solution and refinement as for **CP_{4a}**, 238 parameters, refinement converged at R1(F) = 0.038, wR2(F^2, all data) = 0.110, S = 1.03, max(Δ/σ) < 0.001, min/max height in final ΔF map -0.16/0.18 e/Å^3. Figure 4 shows the molecular structure.

COMPUTATIONAL

The conformational analyses were carried out using the AM1 method[12] as implemented in the program VAMP 5.0.[13] The starting geometries were preoptimized by the TRIPOS[14] force field. For the calculation of the rotational strength R_{0a} of an electric transition from the groundstate ψ_0 to an excisted state ψ_a

$$R_{0a} = \Im\left\{\frac{e\hbar}{im(E_a - E_0)}\langle\psi_0|\hat{p}|\psi_a\rangle \cdot \langle\psi_a|\hat{m}|\psi_0\rangle\right\} \quad (1)$$

$$= \Im\left\{\frac{e\hbar}{im(E_a - E_0)}p_{0a} \cdot m_{a0}\right\}, \quad (2)$$

the wavefunctions of the excited states were obtained by a CNDO/S-CI calculation[15] using a CI expansion which includes 20 x 20 = 400 singly occupied configurations and the ground state determinant. The rotational strengths were then calculated by use of the BDZDO/MCDSPD program package,[16] following equation (2) to give origin-independent results even for approximated wavefunctions.

Corresponding to the Boltzmann statistic the CD spectra of all conformers were added up according to their heats of formation, leading to the theoretical overall CD spectrum. At last, for a better optical comparison with the experimental one, the rotational strenghts were transformed into $\Delta\varepsilon$ value according to

$$\Delta\varepsilon(\nu) = \frac{6.909hc_o\nu\sqrt{\varepsilon_0}}{8\pi^2 1000 N_A\sqrt{\mu_0}} R_{0a}, \tag{3}$$

and superimposed with an Gaussian band shape function

$$\sigma_{0a}(\lambda) = -\frac{1}{\Delta m\sqrt{\pi}} e^{-\left(\frac{\lambda-\lambda_a}{\Delta m}\right)^2} \tag{4}$$

with a halfband width of Δm = 5 nm, giving a $\Delta\varepsilon$ curve.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support by the BMBF (Bundesministerium für Bildung und Forschung, project no. 0310722) and the Fonds der Chemischen Industrie. Furthermore we wish to thank Prof. J. Fleischhauer (Universität Aachen) and J. W. Downing (University of Colorado) for providing the program package BDZDO/MCDSPD and K.-P. Gulden for porting it to LinuX.

REFERENCES AND NOTES

Dedicated to Prof. H. Paulsen on the occasion of his 75th birthday

1. Krohn, K.; Bahramsari, R.; Flörke, U.; L., K.; Kliche-Spory, C.; Michel, A.; Aust, H.-J.; Draeger, S.; Schulz, B.; Antus, S. *Phytochemistry* **1997**, in print.
2. Krohn, K.; Michel, A.; Flörke, U.; Aust, H.-J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1994**, 1093-1097.
3. Krohn, K.; Michel, A.; Flörke, U.; Aust, H.-J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1994**, 1099-1108.
4. Sakemi, S.; Inagaki, T.; Kaneda, K.; Hirai, H.; Iwata, E.; Sakakibara, T.; Yamauchi, Y.; Norcia, M.; Wondrack, L. M.; Sutcliffe, J. A.; Kojima, N. *J. Antibiot.* **1995**, 48, 134-142.
5. Schlingmann, G.; Matile, S.; Berova, N.; Nakanishi, K.; Carter, G. T. *Tetrahedron* **1996**, 52, 435-446.
6. Bringmann, G.; Busemann, S.; Krohn, K.; Beckmann, K. *Tetrahedron*, in print.
7. Bringmann, G.; Gulden, K.-P.; Busse, H.; Fleischhauer, J.; Kramer, B.; Zobel, E. *Tetrahedron* **1993**, 49, 3305-3312.
8. Bringmann, G.; Gulden, K.-P.; Hallock, Y. F.; Manfredi, K. P.; Cardellina II, J. H.; Boyd, M. R.; Kramer, B.; Fleischhauer, J. *Tetrahedron* **1994**, 50, 7807-7814.
9. Krohn, K.; Franke, C.; Jones, P. G.; Aust, H.-J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1992**, 789-798.
10. Further details of the crystal structure determinations are available on request from the Fachinformationszentrum Karlsruhe D-76344 Eggenstein-Leopoldshafen, Germany, on quoting the depository number CSD 406149 for CP_{4a} (**2a**), CSD 406150 for CP_5 (**3b**), the names of the authors, and the journal citation.
11. SHELXTL V5. Siemens Industrial automation, Inc. Analytical Instrumentation (1995).
12. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Steward, J. J. P. *J. Am. Chem. Soc.* **1985**, 107, 3902-3909.
13. Rauhut, G.; Chandrasekhar, J.; Alex, A.; Beck, B.; Sauer, W.; Clark, T.; VAMP 5.0, Oxford Molecular Limited. Oxford Science Park, Sandford on Thames, Oxford OX4 4GA, England.
14. SYBYL: Tripos Associates, 1699 St. Hanley Road, Suite 303, St. Louis, MO, 63144.
15. Del Bene, J.; Jaffé, H. H. *J. Chem. Phys.* **1968**, 48, 1807-1813.
16. Downing, J. W.; Programm package BDZDO/MCDSPD, Department of Chemistry and Biochemistry, University of Colorado, Boulder, USA; modified by Fleischhauer, J.; Schleker, W.; Kramer, B.; ported to LinuX by Gulden, K.-P.

(*Received in Germany* 6 *December* 1996; *accepted* 15 *January* 1997)