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Absolute configuration of 1β,10β-epoxydesacetoxymatricarin isolated from *Carthamus oxycantha* by means of TDDFT CD calculations

Zahid Hassan,[a] Hidayat Hussain,[b] Viqar Uddin Ahmad,[a] Shazia Anjum,[a] Gennaro Pescitelli,[c] Tibor Kurtán[d] and Karsten Krohn[b,*]

[a] *International Center for Chemical and Biological Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*
[b] *Department of Chemistry, University of Paderborn, Warburger Straße 100, 33098 Paderborn, Germany*
[c] *Università di Pisa, Dipartimento di Chimica e Chimica Industriale, via Risorgimento 35, 56126 Pisa, Italy*
[d] *Department of Organic Chemistry, University of Debrecen, PO Box 20, H-4010 Debrecen, Hungary*

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Abstract—The absolute configuration of 1β,10β-epoxydesacetoxymatricarin **1**, a sesquiterpenoid isolated from *Carthamus oxycantha*, was established by TDDFT CD calculations in combination with single crystal X-ray analysis. Since the solid-state CD spectrum shows contributions from intermolecular interactions in the crystal, **1** represents a test case for our solid-state CD/TDDFT approach.

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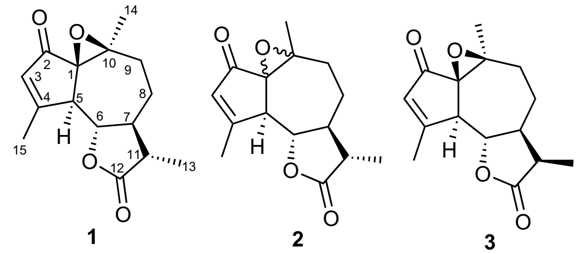
1. Introduction

Sesquiterpenoids are unique amongst the classes of natural products in terms of their great skeletal variety. Furthermore, the oxygenation and stereochemical patterns are very diverse.[1,2] Due to their biological properties and varied structural patterns, two groups of sesquiterpenoids, the guaianolides and pseudoguaianolides, have received particular attention with respect to their isolation and synthesis.[3] Guaianolides have a bicyclo[5.3.0]decane skeleton, characteristic of guaiane sesquiterpenes, with the attachment of a γ-lactone ring at C-6/C-7 or C-7/C-8, typically containing a methyl group or a vinylic methylene at C-11, plus methyl groups or vinylic methylenes attached at the C-4 and C-10 positions. As part of our program to characterize bioactive chemical constituents of terrestrial plants, we have investigated *Carthamus oxycantha* to isolate an interesting sesquiterpene, a 1,10-epoxyguaianolide **1**.

There are three recorded examples in the literature of 1,10-epoxyguaianolides with structural similarities to the compound we isolated (Fig. 1). The first example is 1β,10β-epoxydesacetoxymatricarin **1**, which was isolated from *Achillea abrotanoides* and *Achillea millefolium*.[4,5] The absolute stereochemistry of this compound was not established. Santachin **2** is another example; the compound was characterized only by ^{1}H NMR while the configuration of the epoxide ring was left undetermined.[6] The third example is 1,10-epoxyachillin **3**, a C-11 epimer of **1**, isolated by Gonzalez et al. from *Artemisia lanata*.[7] Later, the relative configuration of **3** was confirmed by X-ray studies.[8]



1 **2** **3**

Figure 1. Structure of 1β,10β-epoxydesacetoxymatricarin **1**, santachin **2**, and 1,10-epoxyachillin **3**.

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[*] Corresponding author. Tel.: +49 5251 602172; fax: +49 5251 603245; e-mail: k.krohn@uni-paderborn.de

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The stereochemical assignment of epoxide rings in complex natural structures is not an easy task, especially when the oxiranes are tetrasubstituted. ^{1}H NMR chemical shifts and coupling constants of proximal hydrogens are usually employed as predictive tools.[8–11] Nevertheless, the structural assignments based on group anisotropy effect of the epoxide ring are strongly conformation-dependent and caution is recommended in systems with some degree of conformational flexibility.[12] In the case of 1,10-epoxyguaianolides, the influence of the epoxide ring on the chemical shift of H-6 or H-8 has often been utilized for stereochemical assignments.[8,11] However, it has recently been shown that a secure assignment cannot be taken for granted using this criterion, even if both diastereoisomeric epoxides (1,10α and 1,10β) are available.[13] Additionally, ^{13}C NMR chemical shifts are not sufficient to determine the stereochemistry; and can occasionally lead to incorrect conclusions.[14] Having now reliably determined the relative configuration of **1** by X-ray analysis, we applied the solid-state CD/TDDFT methodology[15] for the determination of the absolute configuration on a molecule that may serve as a fixed point in this stereochemically diverse class of guaianolides.

2. Results and discussion

Compound **1** (Fig. 1) (colorless needles, mp 167–168 °C) was assigned the molecular formula $C_{15}H_{18}O_4$ by high resolution electron impact HREI-MS. The IR spectrum of **1** showed an absorption band due to a lactone carbonyl at 1757 cm^{-1}. The ^{1}H and ^{13}C NMR spectroscopic data of compound **1** were almost superimposable to those reported for1β,10β-epoxydesacetoxymatricarin, a 1,10-epoxyguaianolide previously isolated from *A. abrotanoides* and *A. millefolium*.[4,5] However, no specific rotation was reported for that epoxide. The relative structure of 1β,10β-epoxydesacetoxymatricarin **1** isolated by us from *C. oxycantha* was now determined by X-ray diffraction analysis (Fig. 2).

With the solid-state geometry of **1** available, we thought to apply our recently developed solid-state TDDFT/CD method[15] to determine the absolute configuration. In this method, the CD spectrum recorded in the solid-state as a KCl or KBr disc is compared with that calculated[16] with the TDDFT method,[17] employing the X-ray coordinates as the input structure. This approach benefits from two favorable attributes: (1) the conformational analysis of the compound in question, which may be time-consuming and a source of error, is removed; (2) since the very same geometry responsible for the observed solid-state CD is employed in the calculation, a good agreement can be expected.[15] On the other hand, a necessary prerequisite for the application of the solid-state TDDFT/CD method is that the origin of the solid-state CD is entirely molecular, that is, CD effects intrinsic to the solid-state (for example, arising from intermolecular couplings)[18] can be excluded, since they cannot be predicted by single-molecule TDDFT calculations. However, this is not entirely the case for compound **1**, as discussed in the following section.

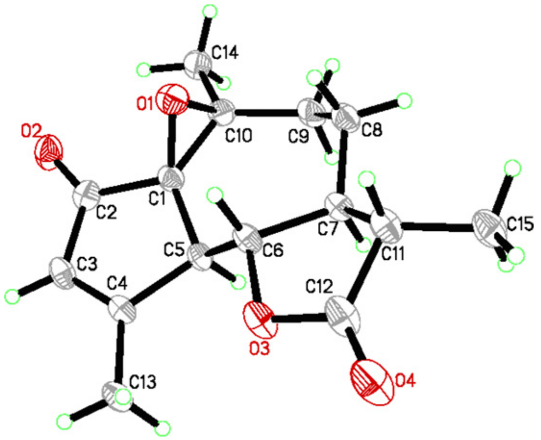

Figure 2. The asymmetric unit of compound **1**, showing 50% probability displacement ellipsoids.

In Figure 3, the solution (acetonitrile) and solid-state (KCl disc) CD spectra of (+)-**1** are compared. Although the main features observed in solution (positive Cotton effect, CE, at 345 nm, and negative stronger CE at 225 nm) are also found in the solid-state spectrum, new bands appear in the latter (positive CE at 255 and negative CE at 195 nm), and the first long-wavelength CE acquires a clear vibronic structure. While this latter effect may be due to the conformational stiffening typical of the solid-state, the appearance of the new bands might be caused by the presence of intermolecular couplings in the crystals.

Conformational searches and geometry optimizations run with MM and DFT methods confirmed that the differences between the solution and solid-state CD spectra could not simply be ascribed to a different conformation in the two states. Only two minima could be found, the most stable of which had a computed DFT geometry practically

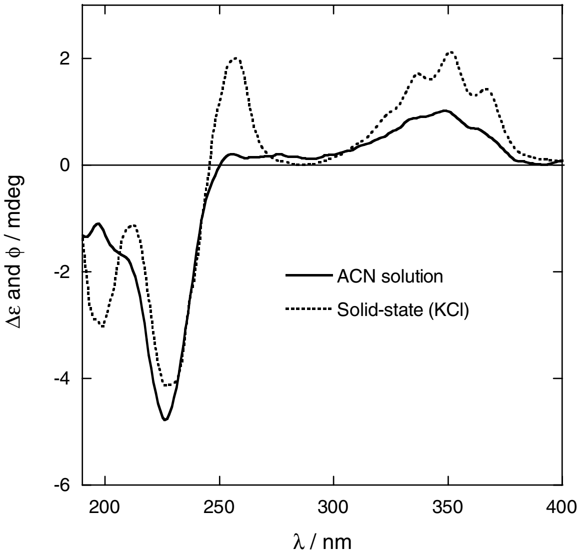

Figure 3. CD spectra of (+)-1β,10β-epoxydesacetoxymatricarin **1** in acetonitrile (ACN) solution and in the solid-state as KCl disc (ellipticity ϕ multiplied by 0.1 for better comparison).

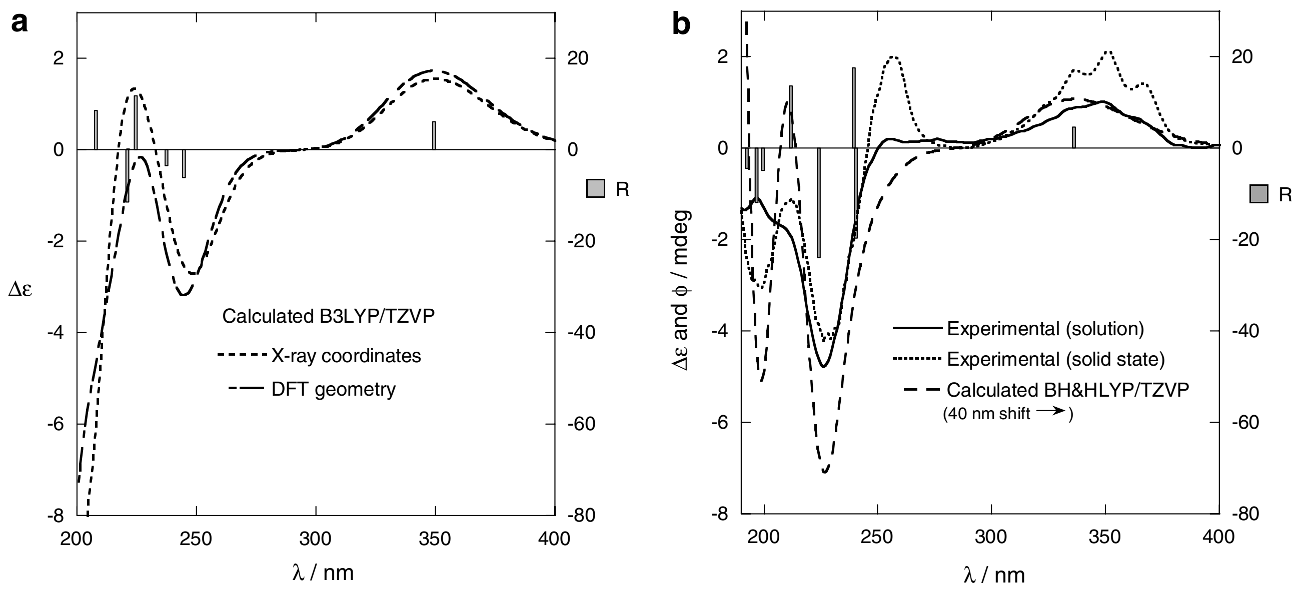

Figure 4. Experimental and calculated CD spectra of (1*R*,5*R*,6*S*,7*S*,10*S*,11*S*)-1β,10β-epoxydesacetoxymatricarin **1**; vertical bars represent rotational strengths R in 10^{-40} cgs. (a) Comparison between TDB3LYP/TZVP calculations using either X-ray coordinates or DFT geometry as input; (b) Comparison between experimental CD (solution and solid state) and TDBH & HLYP/TZVP calculation using X-ray coordinates.

coincident with the X-ray one (root-mean-square deviation for heavy atoms = 0.067 Å). The second minimum differed from the former due to a different conformation of the seven-membered ring (half-chair instead of chair); it had a much higher DFT energy (+12.4 kcal/mol) and was neglected.

The similarity between the solid-state and DFT-computed geometries is also reflected in the fact that practically coincident spectra were obtained (Fig. 4a) when they were employed as input structures for TDDFT calculations (B3LYP/TZVP). In the search for the best agreement with experimental CD, we ran several TDDFT calculations upon varying the functional (BP86, B3LYP, BH & HLYP) and the basis set (TZVP, aug-cc-pVDZ).[19] All of them consistently calculated, for (1*R*,5*R*,6*S*,7*S*,10*S*,11*S*)-**1**, a long-wavelength positive CE and a short-wavelength negative one separated by about 110–120 nm. The first band is due to the n–π* enone transition; the second band apparently results from a manifold of overlapping transitions including the enone π–π* one and various charge-transfer type transitions, also centered on ester and epoxide groups. In particular, the enone π–π* is responsible for the moderate absorption band at 217 nm (ε ≈ 11,000).

Calculated CD reproduce well the experimental spectra in terms of relative position and intensity of the first two bands, whereas higher-lying transitions are intrinsically less well predicted by TDDFT.[20] The agreement is especially good when employing B3LYP/TZVP (Fig. 4a) and BH & HLYP/TZVP (Fig. 4b) and if the experimental solution CD is considered. Thus, the absolute configuration of (+)-**1** can be assigned as (1*R*,5*R*,6*S*,7*S*,10*S*,11*S*). None of the calculations could reproduce the positive CE at 255 nm found in the solid-state spectrum (Fig. 4b). In this region, the enone π–π* may give rise to intermolecular couplings in the crystal, due to the proximity between closely packed molecules. For example, the shortest distance between enone and ester groups belonging to two different molecules is 3.7 Å and that between two enones 5.7 Å. We interpret the observed differences between solution and solid-state spectra for **1** (Fig. 3) as a combination of conformational stiffening and crystalline intermolecular interactions. In the majority of cases previously studied, no, or only marginal, crystalline intermolecular interactions were detected.[15] Thus, the analysis of the solid-state CD and the solution CD as outlined in the present case, may not only allow us to elucidate the absolute configuration of the compound in question, but at the same time also gives valuable experimental evidence about the possible existence of intermolecular couplings in the crystal, and may serve as a tool for the investigation of chirogenesis phenomena in the solid-state.[21]

Similar to other examples we previously reported,[15b] the application of our solid-state TDDFT CD approach to 1β,10β-epoxydesacetoxymatricarin **1** has to consider the presence of bands intrinsic to the solid-state in the CD spectrum. However, they are clearly recognizable upon inspection of the whole set of experimental and computed spectra, so that their impact on the configurational assignment procedure may be safely distinguished. Moreover, in the present situation, the difficulty is balanced by the structural rigidity observed for **1** and in fact, the X-ray structure may still serve as input for CD calculations, leading to a good agreement with the experimental solution spectrum.

3. Conclusions

In conclusion, the absolute configuration of (+)-1β,10β-epoxydesacetoxymatricarin **1** was established as (1*R*,5*R*,6*S*,7*S*,10*S*,11*S*) using TDDFT CD calculations in combination with single crystal X-ray analysis, in spite of intermolecular interactions in the crystal. Thus, **1** may serve as a fixed point in stereochemically diverse class of guaianolides. The comparison of solution and solid state CD spectra in combination with computational methods may give important experimental information about intermolecular interactions in the crystal.

4. Experimental

4.1. General procedures

^{1}H, 2D ^{1}H–^{1}H COSY, ^{13}C, 2D HMQC, and HMBC spectra were recorded with a Bruker Avance 500 MHz spectrometer. Chemical shifts are referenced to internal TMS ($\delta = 0$) and coupling constants J are reported in Hertz. Optical spectra were recorded with a NICOLET 510P FT-IR spectrometer, a UV-2101PC spectrometer, and Perkin–Elmer 241 polarimeter. The CD spectra were recorded on a J-810 spectropolarimeter. For the solid-state CD measurement of **1**, the disc was prepared by mixing about 249 mg KCl (optical grade, heated at 100 °C) and 98 μg **1** with a Perkin–Elmer vibrating mill for 5 min. The mixture was then pressed at 10 tons with a Perkin–Elmer press under vacuum to get a transparent disc for CD measurement. To check the microscopic anisotropy of the KCl disc, four spectra were recorded by rotating the disc with 90° intervals, which were slightly different. Solid-state CD spectra are shown as ϕ (mdeg).

4.2. Plant material

Fresh whole plants of *C. oxycantha* (2.4 kg) were collected from the northern areas of Pakistan (Swat) in June 2003 and identified by Professor M. Qaiser, at the Department of Botany, University of Karachi. A voucher specimen (KUGH, No. 68189) has been submitted to the herbarium of the same department.

4.3. Extraction and isolation

The air-dried aerial parts (2.4 kg) of *C. oxycantha* were soaked in methanol for a period of 25 days. The extract was evaporated under vacuum to get the crude extract. After fractionation a crude $CHCl_3$ extract (180 g) was chromatographed on a silica gel column chromatography using *n*-hexane with a gradient of $CHCl_3$ of up to 100% and followed by methanol, and yielded ten fractions. Fraction F_2 was eluted with a mixture of MeOH–$CHCl_3$ (0.5:9.5) yielding compound **1** (40.2 mg), R_f 0.61 MeOH–$CHCl_3$ (0.5:9.5).

1β,10β-Epoxydesacetoxymatricarin **1**: $[\alpha]_D^{20} = +26.0$ (c 0.16, CH_2Cl_2). White crystals, mp 167–168 °C (lit.[4]: 207 °C (uncorr.)). UV ($CHCl_3$): λ_{max} (log ε): 217 (4.04). CD (MeCN, λ (nm) ($\Delta\varepsilon$), $c = 5.41 \times 10^{-4}$): 362sh (0.65), 349 (0.98), 335sh (0.82), 253 (0.22), 226 (−4.83), 194 (−1.99). CD (KCl), λ (nm) ϕ (mdeg), 98 μg and 249 mg KCl: 366sh (7.34), 351 (11.15), 337sh (9.25), 323sh (5.28), 257 (11.91), 229 (−29.29), 197 (−20.18). IR ν_{max} ($CHCl_3$): =1757, 1700, 1605 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 6.20 (dq, $J = 2.2$, 1.3 Hz, 1H, H-3), 4.05 (t, $J = 9.5$ Hz, 1H, H-6), 3.03 (ddq, $J = 9.5$, 2.2, 1.3 Hz, H-5), 2.37 (t, $J = 1.3$ Hz, 3H, H-15), 2.24 (dq, $J = 12.2$, 7.6 Hz, H-11), 2.22 (ddd, $J = 13.8$, 5.6, 2.3 Hz, 1H, H-9β), 2.02 (ddd, $J = 13.8$, 11.9, 2.3 Hz, 1H, H-9α), 1.76 (s, 3H, H-14), 1.22 (d, $J = 7.6$ Hz, 3H, H-13), 1.67 (ddt, $J = 13.8$, 5.6, 2.3 Hz, H-8α), 1.54 (ddt, $J = 13.8$, 11.9, 11.9, 2.3 Hz, 1H, H-8β), 1.44 (dddd, $J = 12.2$, 9.6, 7.6, 2.7 Hz, H-7). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 201.0 (C-2), 178.1 (C-12), 176.4 (C-4), 133.1 (C-3), 80.2 (C-6), 67.0 (C-1), 65.2 (C-10), 56.4 (C-7), 49.3 (C-5), 40.8 (C-11), 34.6 (C-9), 22.1 (C-8), 21.2 (C-15), 18.9 (C-14), 12.3 (C-13). HREIMS: m/z 262.1197 (calcd 262.1204 for $C_{15}H_{18}O_4$). EIMS (rel. int.): m/z 262.1 $[M]^+$.

4.4. Crystal structure determination of 1β,10β-epoxydesacetoxymatricarin[22]

A plate shaped white crystal of compound **1** with dimension 0.38 × 0.03 × 0.15 mm was selected for X-ray diffraction studies. $C_{15}H_{18}O_4$: M_r 262.29; monoclinic; $a = 9.6492(5)$, $b = 5.7142(3)$, $c = 11.7981(6)$ Å; $\alpha = \gamma = 90°$, $\beta = 95.072(10)°$; $V = 647.97(6)$ Å^3, space group = $P2$ (1), $Z = 2$, $D_{calc} = 1.345$ g/cm^3, $F(000) = 280.0$, Mo Kα (λ 0.71073 Å). Intensity data of compound **1** were collected on a Bruker Smart Apex I, CCD 4-K area detector diffractometer; data reductions were performed using SAINT.[23] The structure was solved by direct methods[24] and refined by full-matrix least squares on F^2 using the SHELXTL-PC package. The intensity data within the θ range 1.73–25.0 were collected at 173 (2) K. A total of 6940 reflections were recorded, of which 2281 reflections were observed on the basis of $I > 2s$ (1). The final R and R_w were 0.0395 and 0.1054, respectively. The figure was plotted with the aid of ORTEP.[25] H atoms were placed in the calculated positions, with C–H distances in the range 0.93–0.98 Å. The U_{iso} values were constrained to be 1.5 U_{eq} of the carrier atom for methyl H atoms, and 1.2 U_{eq} for the remaining H atoms. In the absence of significant anomalous dispersion effects, Friedel pairs were merged before the final refinement.

4.5. Computational methods

Conformational searches were run employing a Molecular Merck force field (MMFF), with standard parameters and convergence criteria, implemented in SPARTAN'06, Wavefunction, Inc., Irvine CA. All minima thus found were optimized with DFT at B3LYP/6-31G(d) level.

DFT and TDDFT calculations were run with GAUSSIAN '03W, Revision D.01, Gaussian, Inc., Pittsburgh PA. A second input geometry for TDDFT calculations was obtained from the solid-state structure upon re-optimizing the hydrogen atoms only. TDDFT calculations were executed employing the GGA functional BP86 and the hybrid functionals B3LYP (with 20% HF exchange) and BH & HLYP (with 50% HF exchange); the basis sets employed were the triple-ζ split-valence TZVP and the augmented correlation-consistent double-ζ aug-cc-pVDZ.[19] All computed transitions responsible for the spectra shown in Figure 4 had energies below the estimated ionization potential (≈7 eV, B3LYP/TZVP).[20] CD spectra were generated using rotational strengths computed with dipole–length gauge formulation to which a Gaussian band-shape was applied with 4200 cm^{-1} half-height width, corresponding to 50 nm at 350 nm. Rotational strengths computed for all transitions with dipole–velocity gauge formulation differed from dipole–length values by less than 10% (TZVP basis set).

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