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The Absolute Configuration of Dibromopalau'amine

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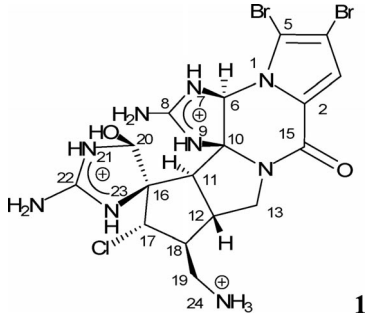
We determined the ensemble of conformations and the relative configuration of dibromopalau'amine by using NMR parameters such as ^3J couplings, ROEs and residual dipolar couplings. Based on the ensemble electronic circular dichroism (ECD) and optical rotatory dispersion (ORD) spectra were calculated using DFT and compared to experiment. By this method the absolute configuration of natural (–)-dibromopalau'amine was determined as 6*S*,10*R*,11*S*,12*S*,16*R*,17*S*,18*S*,20*S*.

Introduction

The molecular formula of palau'amine, the most prominent member of a whole family of marine natural products (the pyrrole-imidazole alkaloids) comprising the axinellamines and massadine, was first published in 1993.[1] The compound soon started to be a challenge for synthetic chemists because of its structural complexity and the corresponding analytical problems. Since the molecule could not be crystallized so far, NMR spectroscopy is left as the analytical method of choice for determination of the configuration. The relative configuration proposed in 1993[1] was soon questioned and revised in 1998 at C20 using NMR spectroscopy.[2] Almost 10 years later, a further revision was done in 2007: The configuration at C20 had to be set back to the 1993 state, C12 and C17 had to be inverted. The inversion of C12 was derived from the relative configuration of C11 and C12 at the junction between the two five membered rings of the tetracyclic core structure. While this junction was assigned to be *cis*,[1,2] the $^3J_{H11,H12}$ coupling of 14.5 Hz was incompatible with a *cis* configuration and suggested *trans*[3] (Scheme 1). The relative configuration was recently corroborated by the synthesis of racemic palau'amine.[4] The synthetic product with a *trans* configuration at C11/C12 exhibited identical NMR spectra compared to the isolated natural product. However, to date the absolute configuration of none of the members of the palau'amine family has been established. Palau'amine is not the only molecule with unknown absolute, but known relative configuration.[5]



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Scheme 1. Molecular formula of natural (–)-(6*S*,10*R*,11*S*,12*S*,16*R*,17*S*,18*S*,20*S*)-dibromopalau'amine **1**.

Here, we determine the ensemble of conformations of dibromopalau'amine (**1**) as accurately as possible by NMR parameters such as ^3J couplings, ROEs and residual dipolar couplings (RDCs).[5,6] With this ensemble the relative configuration[3a] with its 8 stereogenic centers is validated. Then, we calculate from the experimentally determined conformational ensemble electronic circular dichroism (ECD) and optical rotatory dispersion (ORD) spectra and compare them to the experimental ones. Since ORD and ECD depend on the absolute configuration, we can determine the absolute configuration of dibromopalau'amine (Scheme 1).

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Results and Discussion

Experimental and calculated chiroptical properties only agree if the underlying structural model is accurate with respect to all levels of the structure: constitution, configuration and conformation.[7] For the constitution, the protonation level of the two guanidines and the amino group was determined by careful integration of the NH signals in the 1D proton spectrum (see Supporting Information). Four protons for each guanidine in the imidazole rings and 3 protons for the amino group N-24 were found. Thus dibromopalau'amine in DMSO is a triple cation (Scheme 1) without any sign of tautomerism. This is not surprising, since the molecule was purified by HPLC in the presence of TFA in the last step according to the literature.[3a]

With respect to relative configuration, residual dipolar couplings were induced by orienting ca. 6 mg of dibrompalau'amine in PAN gel,[8] (Supporting Information for details). Six RDCs could be reliably extracted from internuclear vectors that are rigid in the molecule. Figure 1 shows the fits of the RDCs to the H11–H12 *trans* configuration (Q = 0.246, Figure 1, a)[3] and the previously proposed H11–H12 *cis* configuration (Q = 0.558, Figure 1, b).[2] This once more confirms the assignment of the relative configuration as shown in Scheme 1. Since dipolar couplings are not only sensitive to configuration but also to conformation, this excellent fit also indicates that the conformation of the hexacyclic ring system is well reproduced by the conformation that we used to back calculate the RDCs. In the next paragraph, we describe, how the conformational ensemble of dibromopalau'amine was obtained.

Despite the hexacyclic nature of dibromopalau'amine conformational heterogeneity can occur due to the rotation about the C18–C19 and the C20–O20 bond. The conformation about the C18–C19 bond could be determined, and the prochiral protons at C19 could be assigned. $^3J_{HH}$ couplings of the diastereotopic protons at C19 to the proton at C18 ($J_{H19proR,H18}$ around 12, $J_{H19proS,H18}$ around 2 Hz), $^3J_{HC}$ couplings ($J_{H19proR,C12}$ absent, $J_{H19proS,C12}$ present) as well as ROEs between the three protons ($ROE_{H19proR,H18}$ absent, $ROE_{H19proS,H18}$ present: integral compatible with *gauche* conformation) allowed the assignment of the C19 protons and the determination of the dihedral angle (N–C19–C18–C17) to be *trans*. Minor conformations – if present at all – have a cumulative population of less than 20%. With this dihedral angle fixed, only the C20-O20 bond was left undetermined. DFT optimizations on the B3LYP/6-31G(d) level of theory resulted in two energy minima with OH dihedrals (H–O–C20–C16) of –80.5° (conformer 1) and +157° (conformer 2), respectively. This fits to the experimental value of $^3J_{OH,H20}$ = 5 Hz[3a] indicating equally populated conformers assuming 0 Hz for conformer 1 and 10 Hz for conformer 2.[9]

With the two conformers, we applied ECD spectroscopy to determine the absolute configuration of natural (–)-dibromopalau'amine. This chiroptical method has been successfully applied to a number of organic molecules in combination with time-dependent density functional theory (TD-DFT).[10] The experimental and calculated ECD spectra are shown in Figure 2. The experimental spectrum (red curve) shows a negative band with a maximum at 288 nm, a zero crossing at 272 nm and a positive band at 263 nm. ECD values at wavelengths smaller than 260 nm have to be discarded because of the strong absorption of DMSO and will not be taken for comparison with the calculated values. Calculations for **1** were carried out using a solvent model (PCM) at the B3LYP/6-311G(d,p) level of theory.

The two characteristic bands around 290 nm and 260 nm, and the zero crossing at 272 nm are well reproduced with respect to wavelength and intensity. It is inter-

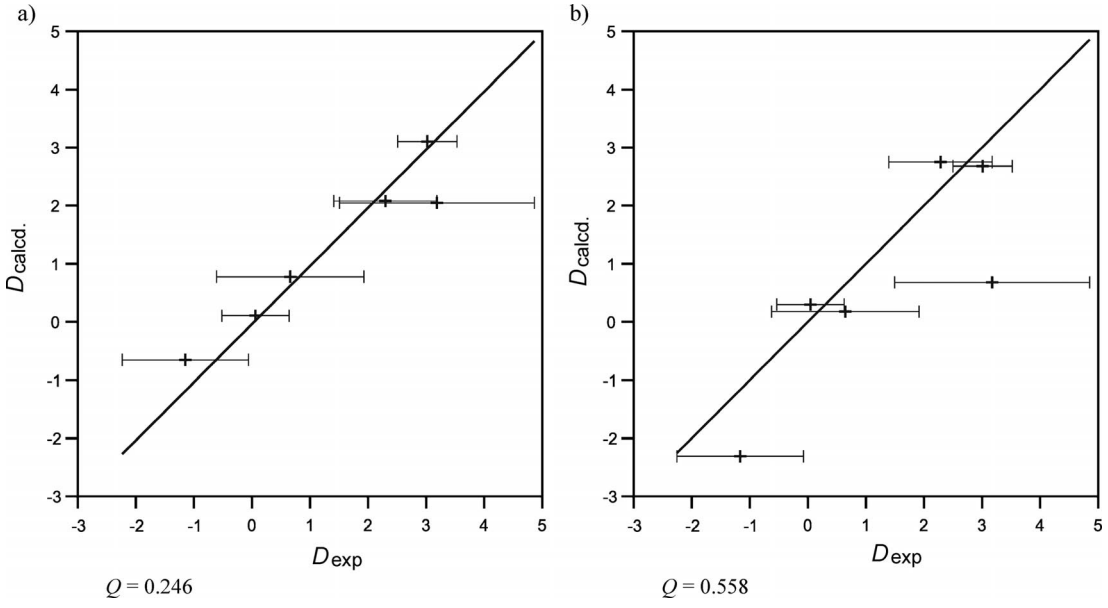

Figure 1. Fit of RDCs to **1**,[3a] b) to 12-*epi*-**1**.

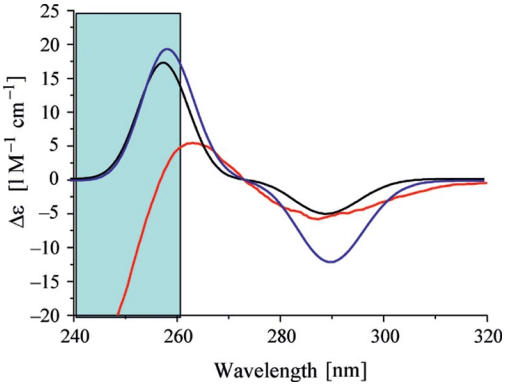

Figure 2. Experimental and calculated ECD spectra (Lorentz-shape) with a bandwidth of 0.11 eV according to the estimated width of the experimental spectrum; calculations were done using DMSO as solvent (PCM) at the B3LYP-SCRF/6-311G(d,p)//B3LYP/6-31G(d) level of theory; in red: experimental; in black: conformer 1; in blue: conformer 2. The blue shaded area indicates the part of the spectrum where a strong UV absorption of DMSO prevents reliable measurement of the ECD.

esting to note that the agreement between theoretical and experimental spectrum could only be achieved when the correct conformations together with a solvent model were used. The in vacuo calculated UV spectra showed no agreement to the experimental spectrum (see Supporting Information). Such a finding is not new. In a study of a biflavonoid[11] it was shown that the incorporation of a solvent model improves the match with the experiment.

To verify the ECD assignment, experimental ORD data in DMSO and methanol at 589, 578 and 546 nm were compared with calculated ones (Table 1). ORD at 436 nm and 365 nm could not be obtained accurately because the absorption was too high.

Table 1. ORD of dibromopalau'amine.

λ	$[\alpha]$[a]	$[\alpha]$ NMR-derived[b]
589 nm	–87.4	–182.8
	(–67.3)	(–183.3)
578 nm	–91.5	–191.6
	(–70.7)	(–189.7)
546 nm	–105.0	–220.2
	(–82.2)	(–219.9)
436 nm	–110	–282.5
		(–285.8)
365 nm	–115	–358.1
		(–355.4)

[a] Experimental optical rotation of 6.25 mg of dibromopalau'amine in 1 mL of methanol, in parentheses of 5.5 mg of dibromopalau'amine in 1 mL of DMSO. [b] Optical rotation calculated from the NMR-derived ensemble with methanol as continuum solvent, in parentheses with DMSO as continuum solvent; calculations were done using methanol or DMSO as solvent (PCM) at the B3LYP-SCRF/6-31G(d)//B3LYP/6-31G(d) level of theory.

The calculated and experimental ORD values have the same sign for all five wavelengths and thus show a monosignate behaviour for **1**. We can make this statement despite the fact that in the low wavelength range, 436 and 365 nm, the experimental data can only be taken qualitatively. For monosignate spectra the reliability of the ORD derived configuration is considered high especially when the ORD is measured at several wavelengths[12] and exceeds a certain threshold[13,14] as discussed below. This is in contrast to ORD curves that change sign (bisignate). With bisignate ORD curves the absolute configuration could not have been derived reliably from a comparison between calculated and experimental $[\alpha]$ values at a single wavelength even if coupled cluster (CC) calculations had been used.[14] Thus we have a reliable answer here, because fortunately dibromopalau'amine's ORD is monosignate.

As mentioned, despite considerable deviations of the absolute value, monosignate ORD curves can be interpreted reliably,[14] if the measured value exceeds 80 [degrees·(dm·g/cm^3)$^{-1}$]. This is indeed the case for **1** (Table 1). As an example of such a large difference between experimental and calculated ORD values (4*S*,5*S*)-*cis*-cytoxazone has been described by Giorgio et al.[15] Its absolute configuration was known but it showed an experimental $[\alpha]_D$ of +67 as average in three solvents, roughly half of the value for the calculated Boltzmann-weighted average of +139. This difference increased when going to 546 nm with an $[\alpha]_{exp}$ average of +75.3 and an $[\alpha]_{calcd.}$ average of +165. Similar to the case of dibromopalau'amine, the sign was sufficient for the correct assignment of the configuration in this example despite the large difference in the absolute value of the ORD at multiple wavelengths. To judge the reliability of ab initio calculations of chiroptical properties of molecules of the complexity of **1** (54 atoms) a case study of Giorgio et al.[16] is relevant. These authors revealed that the same basis set that we used [6-31G(d)] is able to compute chiroptical data of a complex (52 atoms) and flexible (6 conformers) molecule [(–)-chimonanthine] reliably.

Conclusions

In summary, we base our assignment of naturally occurring (–)-dibromopalau'amine to be 6*S*,10*R*,11*S*,12*S*,16*R*,17*S*,18*S*,20*S* on the following points:

1. A state of the art level of theory (DFT/B3LYP for chiroptical data) and state of the art geometry optimization [B3LYP/6-31G(d)] for complex molecules such as dibromopalau'amine were applied. The latter was cross validated with NMR spectroscopic data (^3J couplings, NOEs and RDCs).
2. The absolute, experimental $[\alpha]$ values are relatively high {above 80 [degrees·(dm·g/cm^3)$^{-1}$]}, were measured at five wavelenghts and were monosignate enabling reliable determination of the absolute configuration.
4. $[\alpha]$ calculations with and without inclusion of a solvent model were used, together with test calculations with the larger basis set 6-311G(2d,p) (see Supporting Information).
5. The two diagnostic bands around 290 nm and 260 nm in the experimental ECD spectrum are well reproduced by the calculated spectra of the two conformers.

In summary, we have presented a combined experimental and theoretical approach starting with the determination of NMR-derived conformational ensembles which enables the

reliable calculation of chiroptical spectra. By comparison with experimental spectra the determination of the absolute configuration of even more complex molecules that cannot be crystallized becomes possible. We are also glad to report that this configuration agrees with biosynthetic arguments relating **1** with the "pre-axinellamines".[17]

Supporting Information (see also the footnote on the first page of this article): Computational details, NMR spectroscopy (proton spectrum, RDC data, ^{19}F spectrum) and chiroptical data (ORD and ECD/UV).

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