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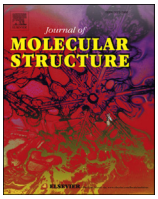

Structural and chiroptical analysis of naturally occurring (−)-strychnine

F. Reinscheid [a], M. Schmidt [a], H. Abromeit [b], S. Liening [b], G.K.E. Scriba [b], U.M. Reinscheid [a, *]

[a] NMR-based Structural Biology, Max-Planck-Institute of Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
[b] Pharmaceutical Chemistry, Friedrich-Schiller Universität, Philosophenweg 14, 07743 Jena, Germany

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ABSTRACT

Structural aspects such as chemical exchange, dimerization, solvent association, nitrogen inversion and protonation status of strychnine were investigated using experimental and calculated data. The information was mainly interpreted in view of a successful determination of the absolute configuration (AC) with strychnine (base and salt) as test molecule due to its importance in chemistry. By geometry optimization a stable isomer of protonated strychnine was found with an inverted nitrogen, however, 25 kcal/mol higher in energy. It is shown that solvent association can be assumed in protic solvents such as methanol and dimerization to a small extent in polar/protic solvents. However, the monomeric structural model neglecting explicit solvent molecules still allows the correct prediction of the AC of base and hydrochloride using optical rotation and ECD data.

1. Introduction

Recently, it was stated that “today the focus of chemical research is much more on function than on structure.” [1] Although it is still more difficult to give answers to functional questions on the same scientific level as has been achieved for structural research, it is important to push the limits in structural descriptions even further. Since a number of stereochemical misassignments of natural products can be found in the literature (for reviews) [2–5], studying the structure of known molecules may give surprising results. Two recent examples are the correct determination of the absolute configuration (AC) of the antimalarial drug mefloquine [6], and the discovery of the yet hidden structural flexibility of strychnine [7]. Even this extremely well studied (first isolation in 1818 by Pelletier and Caventou) [8] prototypic molecule of rigidity turned out to exist as two conformers in solution. Consequently, all functional effects due to the presence of a minor conformer will be neglected if only the crystal structure of strychnine [9,10] is considered.

The structure of strychnine (Scheme 1) has been established by Robinson [11]. Soon after it was confirmed by total synthesis [12] and x-ray crystallography [13]. The absolute configuration of naturally occurring (−)-strychnine was determined by Peerdeman in 1956 using x-ray crystallography [14]. Apart from the general chemical importance of strychnine, there is an astonishing funnel effect in all synthetic schemes published so far: either the Wieland-Gumlich aldehyde is prepared, or isostrychnine [15,16]. From these intermediates known conversions lead to strychnine, but are quite often not performed. In addition, comparing the IR spectra presented as proof for the successful synthesis of strychnine [17] leads to the conclusion that two identical spectra are shown. To the best of our knowledge, the only further structural information concerning the conversion of isostrychnine into strychnine [18] was given by Magnus et al. [19] in their introduction to the second total synthesis of strychnine, more than 30 years after Woodward's first total synthesis. Magnus et al. [19] mentioned without giving experimental details, that by using different reaction conditions as Prelog et al. [18], i.e. ethanolic potassium hydroxide at 80 °C, almost no conversion into strychnine was observed, and in case of cesium carbonate in *tert*-butyl alcohol as solvent 13-*epi*-isostrychnine was formed. In conclusion, the strychnine chemistry is far from being well understood. This motivated us to further analyse experimentally and computationally the structural model of strychnine,

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* Corresponding author.
E-mail address: urei@nmr.mpibpc.mpg.de (U.M. Reinscheid).

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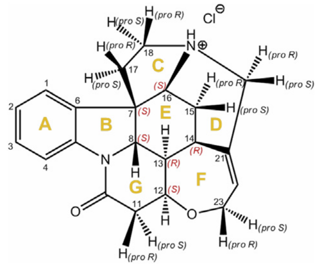

Scheme 1. Stereochemical formula of (−)-strychnine HCl.

followed by a chiroptical analysis. Insofar serves strychnine as a test molecule to explore the success and limitations of the purely chiroptical approach to determine the AC of a chiral compound.

2. Results and discussion

2.1. Structural model

In our first publication about the structure of strychnine, we showed the first experimental and quantitative evidence of a minor conformer in solution using low-temperature NMR (Fig. 1) [7a]. Structure calculation of this low-populated conformer is successful on the mpw1pw91/cc-pvdz level of theory, but not on the often used B3LYP/6-31G(d) level of theory [7a]. Earlier work by Butts et al. (2011) [7b] presented calculated quantitative information. In addition, a third low-populated conformer was predicted by computation [52]. To date, there is no experimental evidence for its existence so it was not further investigated.

In the present study we investigate structural aspects in order to explore the success and limitations of the determination of the absolute configuration by comparing experimental and calculated chiroptical data. Strychnine represents an excellent test molecule for the structural work since it is still a challenging synthetic target and a typically complex natural product. From our analysis we want to derive limits that can be further tested and maybe pushed even further by using smaller natural products such as limonene [20].

2.2. Chemical exchange in ring G

The protons at C11 of strychnine are acidic. It is known that under alkaline conditions these protons can be exchanged by tritiated water [21]. At a hydroxide concentration of 0.1 M, the exchange rate amounts to $4.32 \times 10^4\ s^{-1}$. However, without using a large excess of hydroxide anions the rate rapidly drops to very small values so that under the conditions of our NMR measurements (measurement times of hours, strychnine at low concentrations of <20 mM, 25 °C (pH of 9.5 (base in water) and pH of 5.5 (strychnine sulphate in water) as saturated solutions) [22] almost no exchange will take place so that the experimental, structural data are not contaminated by fast exchange of the H11 protons. However, this reaction is important for the isostrychnine-strychnine conversion [18]. Likewise, a lactamization reaction is much too slow to produce ring-opened derivatives during the measurements [23], but could be relevant for the isostrychnine-strychnine conversion.

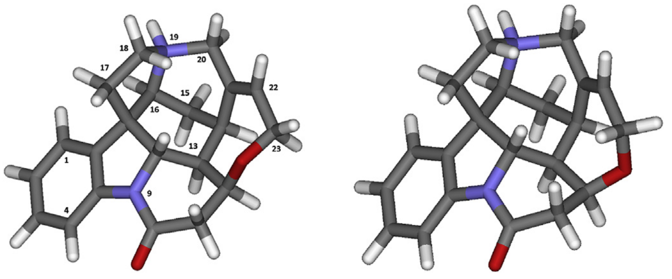

Fig. 1. Geometry-optimized (mpw1pw91/cc-pvdz, IEFPCM: methanol) structure of protonated strychnine (major conformer on the left, minor conformer on the right with a population of 5.9% of strychnine HCl in methanol at 298 K) [7].

2.3. Dimerization, ion-pairing

Mostad [9] analyzed strychnine base by x-ray crystallography. He concluded that the amide oxygen is mostly involved in the intermolecular interactions in the crystal. In order to test the dimerization of (−)-strychnine base, two samples with different concentrations were compared: 2 mg/mL (6 mM) and 100 mg/mL (300 mM) in $CDCl_3$ at 298 K. The proton spectra showed concentration dependent resonances affecting the protons near the aliphatic amine with an upfield shift of more than 0.1 ppm for the high concentration compared to the low concentration, i.e. H16, H18 pro*S*, H20 pro*R* and pro*S*, and H22, located on one side of the 3D-model (Fig. 1; see Supporting information). These data indicate aggregation. In contrast with our results, Metaxas and Cort [24] did not observe concentration dependent effects for the base in chloroform. However, the concentration range studied might be too small so that weak associations were not detected. Assuming a low association constant of 1 $[M]^{-1}$, 6% of the strychnine base molecules would exist as dimer in our high concentration sample (100 mg/mL, 300 mM). For comparison, with an association constant of 100 M^{-1}, 12% of the strychnine base molecules would exist as dimers in our low concentration sample.

For salts of strychnine a concentration dependent change of proton chemical shifts of protons near the protonated tertiary amine were observed (maximum of −8.7 ppb/mM for one of the H20 protons of (−)-strychnine nitrate in $CDCl_3$) [24]. Interestingly, in the same solvent counter-ion dependent proton chemical shifts near the aliphatic nitrogen varied up to 0.3 ppm which can be explained by different ion-pairs formed. Since we have measured strychnine HCl in much more polar and H-bond accepting solvents (DMSO-*d6* and methanol-*d3*), ion-pair formation should be decreased compared to $CDCl_3$, and concomitantly the tendency to form ionic aggregates would be reduced. In agreement, Moreno et al. [25] observed ion-pairing for brucine BF_4 in chloroform which decreased in acetone-*d6* at a 2 mM concentration. With larger anions, strong ion-pairing was observed even in acetone as polar solvent so that we can assume that for strychnine HCl a reduced ion-pairing is reasonable due to the smaller size of the chloride anion compared to $BF4^-$. In agreement, by comparing two samples 2 mg/mL (5 mM) and 65 mg/mL (163 mM) in methanol-*d3* at 298 K, we observed that the protons surrounding the aliphatic nitrogen are mostly affected but to a lower extent compared to the base in chloroform, i.e. H15 pro*R*, H16, H17 pro*R*, H18 pro*S* by more than 0.03 ppm (see Supporting information). In this case, up- and downfield shifts were observed. Interestingly, H1 at the aromatic ring is shifted by 0.04 ppm downfield at the higher concentration. These concentration dependent differences in chemical shifts are smaller than the values observed by Metaxas and Cort [24] due to the different solvent effect on the aggregation/ion-pairing process. The latter is much more pronounced in apolar solvents such as chloroform used by Metaxas and Cort [24] compared to the polar solvent methanol-*d3* in our study. In conclusion, there are clear indications of aggregation of strychnine base (in $CDCl_3$) and strychnine HCl (in methanol-*d3*) at very high concentrations. It is therefore reasonable to use a monomer as structural model if the chiroptical analysis is performed at sufficiently low concentrations, i.e. <20 mM. In polar solvents and at low concentrations of