2-Hexanoyl-1-tribromomethyl-1,2,3,4-tetrahydro-β-carboline: Crystal Structure Analysis of a Potent Inhibitor of Complex I of Mitochondrial Respiration* 

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The molecular structure of the title compound 2-hexanoyl-1-tribromomethyl-1,2,3,4-tetrahydro-β-carboline (3), a potent inhibitor of complex I of the mammalian mitochondrial respiratory chain, has been studied by single-crystal X-ray diffraction analysis. In the crystal, two heterochiral molecules of 3 (i.e., one R- and one S-configured molecule each) were found to be connected with one another in pairs via two intermolecular hydrogen bonds [O(215)...H(212)’ and O(215)’...H(212)] to form an overall achiral ‘dimeric’ subunit.

Introduction

The tetrahydro-β-carbolines comprise a group of tricyclic indole derivatives exhibiting distinct neuropharmacological properties. These compounds can be formed spontaneously, under quasi-physiological conditions from endogenous tryptamines reacting with aldehydes or α-keto acids by a Pictet-Spengler type condensation [1]. Our special interest is focused on highly halogenated compounds such as 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (‘TaClo’, 1), which is a potent dopaminergic neurotoxin [2–4] strongly affecting mammalian mitochondrial NADH-ubiquinone reductase (complex I) activity [5–7]. Remarkably, trace amounts of this toxic agent have been detected in blood samples of patients therapeutically treated with the soporific chloral hydrate [8].

Similar to 1, 1-tribromomethyl-1,2,3,4-tetrahydro-β-carboline (‘TaBro’, 2) has also been demonstrated to severely disturb the dopamine metabolism [9] and to be a potent complex I inhibitor [4, 6]. This bioactive heterocycle 2, which is formally derived from tribromoacetaldehyde, has not yet been found in man, it is a substance of exclusively synthetic origin. Since the extent of lipophilicity of those 1-trihalogenomethyl indol derivatives was found to distinctly favor their inhibitory capacity on complex I, we have synthesized a broad series of TaClo- and TaBro-related compounds for a comprehensive evaluation of their inhibitory potential on the enzymes of mammalian mitochondrial respiration [6, 10, 11]. Indeed, in the course of this work, the highly unpolar 2-hexanoyl-1,2,3,4-tetrahydro-β-carboline (3) turned out to be one of the most active agents displaying an even three times more effective inhibition (IC100 =

![Molecule 1](image1.png)

![Molecule 2](image2.png)

![Molecule 3](image3.png)

Fig. 1. The highly chlorinated tetrahydro-β-carboline ‘TaClo’ (1), endogenously formed from the biogenic amine tryptamine (‘Ta’) and the hypnotic chloral hydrate (‘Clo’), is a novel neurotoxic lead. The closely related bromal-derived heterocycle ‘TaBro’ (2) and its N-hexanoyl derivative 3 were both found to strongly block mitochondrial respiration.
200 μM) than its quite potent parent compound \( \text{IC}_{100} = 650 \mu M \) [6, 10]. In this paper, we report on the synthesis and X-ray diffraction analysis of 3 aiming at a more detailed knowledge concerning the three-dimensional structure of this tribromomethyl-substituted heterocycle.

**Results and Discussion**

TaBro (2) was converted into its 2-hexanoyl derivative 3 by a standard reaction procedure in dichloromethane in the presence of triethylamine, following a protocol previously elaborated for the synthesis of TaClo-related \( N \)-alkanoyl derivatives [11].

The molecular structure of 3 was determined by a single-crystal X-ray diffraction analysis. A molecule plot is shown in Fig. 2.

Besides confirming the constitution of this tricyclic indole compound, the X-ray investigations clearly revealed the huge 1-tribromomethyl group as well as the long-chained \( N \)-alkanoyl portion to be the most striking features about this molecule. The 1-CBr\(_3\) substituent is twisted out of the \( \beta \)-carboline ring plane, thus occupying a pseudo-axial position. The three voluminous bromine atoms at C(214) adopt a perfectly staggered orientation with respect to the C(21)–C(214) bond, thus minimizing their steric interactions with C(213) and the \( N \)-alkanoyl function. The \( N \)-hexanoyl part forms an ideal linear zigzag carbon chain showing transoid (i.e., antiperiplanar) arrays with the atoms C(215) up to C(219) being located in a joint plane. Only the terminal methyl group [C(220)] was found to be near-syn oriented, with respect to the alkyl chain formed by the carbon atoms C(217), C(218), and C(219). The dihedral angle of the C(217)–C(218)–C(219)–C(220) array was determined to be 62.3° corresponding to a gauche conformation of this aliphatic alkyl chain.

The X-ray structure analysis furthermore demonstrates the tetrahydropyrido part of 3 to adopt a partially planarized half-chair conformation, with only N(22) and, in particular, C(23) being located out of the \( \beta \)-carboline ring plane (see Fig. 2). The strong deviation of C(23) becomes manifest from the interplanar angle of 139.6° between the plane N(22)–C(23)–C(24) and the best plane C(21)–C(213)–C(25)–C(24), which shows a small dihedral angle of 0.7°. The enhanced sp\(^3\)-character of the acylated nitrogen atom of the tetrahydropyrido moiety is indicated by a distinct planarization of its environment with N(22) being located out of the plane C(21)–C(213)–C(215) by only 3.1 ppm, and by the C(21)–N(22)–C(23) angle, which is close to trigonal (115.8°).

![Fig. 2. SCHAKAL plot showing the molecular structure of 3 with a guide of the atomic numbering system adopted in the X-ray investigation. The compound was found to be racemic in the crystal; for presentation, only the enantiomer with the CBr\(_3\) group below the graphical plane, has been chosen, arbitrarily. The molecule is viewed horizontal to the tetrahydropyrido ring plane (hydrogen atoms have been omitted for reasons of clarity).](image-url)
The tetrahydro-β-carboline 3 was found to be racemic in the crystal. Two heterochiral molecules of 3 (i.e., one being R- and one being S-configured) are connected in pairs via intermolecular hydrogen bonds O(215)...H(212)' and O(215)'...H(212) (2.25 Å) to form an overall centrosymmetric and thus achiral ‘dimeric’ subunit (see Fig. 3). Obviously for steric reasons, due to the bulky bromine atoms at C(214), this meso-like ‘dimer’ of 3 is energetically favored, in comparison to a subunit of two stereochemically identical enantiomers.

Interestingly, there are also ‘solitaire’ molecules of 3 in the crystal. To distinguish between the two molecular ‘species’ of 3 (heterochirally associated vs. ‘solitaire’) in the crystal (see Table I), the prefix “2” was chosen for those of 3 forming a (heterochiral) dimeric subunit, while the prefix “1” was denoted to the ones that occur nearly solely, exhibiting only weak intermolecular bonding with distances > 3.6 Å. These two forms of molecular orientation of 3 in the crystal were observed to display an effect in particular on the half-chair conformation of the tetrahydropyrido part of 3. The acylated nitrogen atom N(12) is twisted out of the plane C(11)-C(13)-C(115) by 14.1 pm (vs. 3.1 pm for the ‘dimer’), and the interplanar angle between the plane N(12)-C(13)-C(14) and the best plane C(11)-C(113)-C(15)-C(14) was determined to be 139.6° (vs. 136.0° for the ‘dimer’).

From the diagram illustrated in Fig. 3, it became evident that two heterochiral molecules of 3 forming such a meso-like ‘dimer’ as described above, are accompanied by two solitaire molecules of 3, with their N-hexanoyl portions horizontally oriented to the tetrahydro-β-carboline ring systems of the dimer.

Remarkably, out of a series of various other N-acylated highly halogenated tetrahydro-β-carbolines investigated so far by X-ray crystallography [10, 11, 12-15], this TaBro derivative 3 was found to be the only compound showing such a packing principle as described above. All the other related heterocycles (e.g., 2-trifluoroacetyl-TaBro [10] or 2-propanoyl-TaClo [13]) were also found to be racemic in the crystal, but being connected to each other by a single intermolecular hydrogen bond H(12)...O(15), thus forming an approximately linear zigzag chain, e.g. for 2-trifluoroacetyl-TaBro parallel to [010].

Besides representing the most characteristic structural factors of 3, the 1-tribromomethyl group as well as the N-hexanoyl function have both been discussed to significantly contribute to the strong inhibitory capacity of this heterocycle towards mitochondrial respiration [10]. A presumable involvement of this trihalogenmethyl moiety of 3 in the inhibitory action on complex I has to be taken into account due to the finding that acetaldehyde-derived tetrahydro-β-carbolines affect mito-
chondrial enzymes only moderately [6, 7, 10]. Similar to other potent complex I inhibitors, among them capsaicin analogs [16] and phenylpyridinium-type compounds [17], enhancement of the lipophilic character of tetrahydro-β-carbolines, e.g., by introducing long-chain alkyl or alkoxy moieties (to be realized in compound 3) has also been claimed to play an important role for the inhibitory activities of those agents [10, 11]. Detailed structure-activity profiles (including comprehensive X-ray investigations), however, for the elucidation of structural factors required for an efficient inhibitory action on complex I turned out to be complicated and still ambiguous so far. Therefore, we are now aiming at a systematic modification of the β-carboline framework to hopefully gain new insight into structural properties necessary for an interaction with the binding environment of complex I.

**Experimental**

**General**

1H and 13C NMR spectra were recorded on a Bruker AC 250 spectrometer, and are referenced to internal acetone (1H, δ 2.01 ppm; 13C, δ 29.85 and 205.9 ppm). The melting point was determined on a Reichert-Jung Thermovar hot-stage apparatus and is uncorrected. The infrared spectrum was obtained on a Perkin-Elmer Model 1420 spectrophotometer. For mass spectrometry (electron ionization 70 eV), a Finnigan MAT 8200 instrument was used. The elemental analysis was performed by the Microanalysis Laboratory of the Institute of Inorganic Chemistry (University of Würzburg) on a Carlo Erba Elemental Analyzer M 1106 apparatus.

**Preparation of the tetrahydro-β-carboline 3**

To a suspension of 2-HCl [10] (200 mg, 0.44 mmol) in dry dichloromethane (15 ml), triethylamine (0.34 ml, 220 mg, 1.84 mmol) was added. The mixture was treated dropwise with n-hexanoyl chloride (190 µl, 186 mg, 1.38 mmol) at 0 °C, then stirred at room temperature for 2 h, and extracted with water (3×). The organic phases were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Purification of the residue by crystallization from methanol/petroleum ether provided 3 (170 mg, 0.32 mmol, 74% yield) as yellow crystals, m.p. 160 °C (dec). IR (KBr, cm⁻¹): ν 3280 (indole NH); 3030; 2940, 2920, 2850 (CH); 1660 (C=O). 1H NMR (CD3OD, 300 MHz): Table I. Selected bond lengths (Å) and angles (°) for 3.
250 MHz): \( \delta 0.98 \) (t, 3H, 6'-CH,); 1.35-1.40 (m, 2H, 2'-CH,); 2.49-2.76 (m, 2H, 2'-CH,); 2.89-2.95 (m, 2H, 4-H); 4.31-4.34 (m, 2H, 3-H); 6.89 (s, 1H, 1-H); 7.06 (dt, \( \delta 7.6 \) Hz, 1H, 5-H). 13 C NMR (C,D2O): \( \delta 106.9 \) (C-5'), 112.3 (C-4'); 33.9 (C-2'); 40.2 (C-3); 64.1 (C-1); 107.9; 112.3 (CBr3); 112.6; 119.2; 120.2; 123.4; 126.8; 128.8; 137.6; 173.4 (C=O). EI-MS (m/z) (rel. int.) [ion assignment] 442/440/438 (0.3/0.6/0.3) [M+-3Br], 264/262 (18/19) [M+-2Br-C6H10O], 281 (18) [M+-Br], 361/359 (92/94) [M+-HBr-Br], 21 [M+].

Analysis for C18H21Br3N2O (521.12):
- Calcd. C 41.49, H 4.06, N 5.38%.
- Found C 40.98, H 3.82, N 5.14%.

**Single-crystal X-ray diffraction analysis of 3**

The crystal chosen for X-ray investigations was a yellow lath with the approximate dimensions 0.10 \( \times \) 0.30 \( \times \) 1.20 mm. Data were collected on a Siemens P4 diffractometer using graphite-monochromated Mo-K\( \alpha \) radiation (\( \lambda = 0.71073 \) Å) in o-scan mode in the range of 1.75° < \( \theta < 27.5° \) [18].

C18H21Br3N2O (521.12 g mol\(^{-1}\)) crystallizes in the triclinic system, space group P1, with \( a = 11.120 \) (1), \( b = 13.407 \) (2), \( c = 15.577 \) (2) Å, \( \alpha = 96.98 (1)^\circ \), \( \beta = 108.39 \) (1)^\circ, \( \gamma = 114.15 \) (1)^\circ, \( V = 1924.7 \) (6) Å\(^3\), \( Z = 4 \), \( \mu (Mo-K\( \alpha \)) = 6.30 \) mm\(^{-1}\), and \( D_{\text{calc}} = 1.798 \) g cm\(^{-3}\). Unit cell parameters were determined by least-squares refinement using 64 centered reflections within 19.4° < \( \theta < 31.7° \). A total of 9344 reflections were collected to \( 26_{\text{max}} \) = 55° (\( h: -1 \rightarrow 14, k: -17 \rightarrow 16, l: -20 \rightarrow 20 \)) of which 8115 were unique. The structure was solved by direct phase determination and refined by full-matrix anisotropic least-squares with the aid of the program SHELXTL-PLUS [19]. In refinements, weights were used according to the scheme \( w = 1/\sigma^2(F_0) \). The refinement converged to the final agreement factors \( R = 0.065 \), and \( R_w = 0.059 \), for 442 parameters and 5575 observed reflections with \( F > 3\sigma(F) \); data-to-parameter ratio being 12.61. The electron density of the largest difference peak was found to be 0.85 eÅ\(^{-3}\), while that of the largest difference hole was 0.92 eÅ\(^{-3}\). All non-hydrogen atoms were refined anisotropically. The hydrogen positions were calculated using a riding model and were considered fixed with isotropic thermal parameters in all refinements.

Selected bond lengths and angles are listed in Table I according to the atom labels of the Figs. 2 and 3. Further crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2, 1EZ, UK [fax: (+ Int) 44-1223 336 033; e-mail: deposit@ccdc.com.ac.uk]. A complete listing of the atomic coordinates for 3 can be obtained free of charge, on request, on quoting the depository number CCDC-135682, the names of the authors, and the journal citation. Software used to prepare material for publication: SCHAKAL 88 [20].

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