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Molecular and crystal structure of an amphiphile: 4′-propoxybiphenyl-4-methyl-*N*,*N*-dimethylamineoxide dihydrate

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Abstract

A novel amphiphile, 4′-propoxybiphenyl-4-methyl-*N*,*N*-dimethylamineoxide, has been synthesized, crystallized ($P2_1/a$, $a = 9.084$ Å, $b = 8.911$ Å, $c = 22.460$ Å, $\beta = 96.224°$) and its crystal structure was determined. The amphiphile forms a bilayer in which the amineoxide oxygen of each molecule binds two water molecules. In the hydrophobic part of the bilayer the biphenyls form edge-to-face contacts, in the polar layer there is a hydrogen bonding network. The potential use of the compound as a detergent for membrane proteins has been demonstrated and the relevance of the amineoxide hydrate for other detergents discussed.

Keywords: Amphiphile; Membrane protein; X-ray crystallography

1. Introduction

Mixed protein-detergent micelles with functionally intact proteins are a prerequisite for chromatographic purification procedures and for crystallization experiments of integral membrane proteins. Of these, only two classes have been analysed with X-ray crystallography at high resolution: reaction centres [1,2] and porins [3–5]. Lauryldimethylamineoxide has been used successfully for the purification and crystallization of reaction centres, light harvesting complexes and porins of purple bacteria [6–9].

Amphiphiles with a novel hydrophobic part consisting of a short alkyl chain and a rigid biphenyl group have been used as mild detergents to solubilise integral membrane proteins [10]. Several amphiphilic compounds with alkylbiphenyl groups coupled to polyethyleneoxide-, amineoxide- and maltoside polar groups have been synthesized [11].

Here we report the crystal structure of one of these, 4′-propoxybiphenyl-4-methyl-*N*,*N*-dimethylamineoxide (PBIPAO) which is useful as a detergent for solubilising membrane proteins.

2. Materials and methods

2.1. Synthesis of 4′-propoxybiphenyl-4-methyl-N,N-dimethylamineoxide (PBIPAO)

4′-Propoxybiphenyl-4-*N*,*N*-dimethylmethanamide was synthesized using 4′-hydroxybiphenyl-4-carbonic acid (kindly provided to us by Dr. E. Poetsch, Merck, Darmstadt, Germany), propyl bromide and dimethylamine [12,13]. A further intermediate was 4′-propoxybiphenyl-4-methyl-*N*,*N*-dimethylamine, which was converted to the amineoxide.

The final compound was checked for purity by thin-layer chromatography and proton NMR spectroscopy. The melting point was 162° C, the critical micellar concentration was found to be 0.42 ± 0.02 mM by surface tension measurement.

2.2. Test for detergent (solubilization) activity

The solubilization capacity for membrane proteins was tested with two proteins, the B800–850 complex and the reaction centre-B875 complex from *Rhodopseudomonas palustris*. After solubilising and purifying the protein in a conventional detergent, it is bound to a small anion exchanger column. The column is washed free of detergent and then equilibrated with a buffer of low ionic strength, containing the amphiphile at 5-fold of the critical micellar

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concentration. An amphiphile is a solubilising detergent, if it elutes the protein from the column immediately after increasing the ionic strength in the buffer to about 0.5.

2.3. Crystallization

A saturated solution of PBIPAO was prepared in an equimolar mixture of water and methanol at room temperature. Due to evaporation of methanol small crystals or precipitates formed. Methanol then was added so that the solution just became clear. After further slow evaporation overnight, large crystals (up to $50 \times 2000 \times 4000$ μm) grew as flat sheets.

Crystal data $C_{18}H_{23}NO_2 \cdot 2H_2O$; mol wt. 321.4; monoclinic; $a = 9.084 \pm 0.001$ Å, $b = 8.911 \pm 0.001$ Å, $c = 22.460 \pm 0.002$ Å, $\beta = 96.224 \pm 0.002°$; $V = 1807.36$ Å^3;

Table 1
Fractional coordinates ($\times 10^4$) and anisotropic temperature factor coefficients T ($\times 10^3$)

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	4461 (3)	804 (4)	9343 (1)	45(1)	69(1)	51(1)	6(1)	0(1)	9(1)
C2	3503 (3)	2988 (3)	8785 (2)	46(1)	48(1)	67(2)	3(1)	8(1)	−3(1)
C3	3118 (3)	465 (3)	8337 (1)	50(1)	50(1)	58(1)	−2(1)	−1(1)	−8(1)
C4	4462 (2)	384 (3)	8009 (1)	49(1)	44(1)	46(1)	−4(1)	0(1)	−3(1)
C5	5470 (3)	−772 (3)	8108 (1)	74(1)	36(1)	51(1)	1(1)	9(1)	−3(1)
C6	6682 (3)	−873 (3)	7791 (1)	69(1)	37(1)	52(1)	1(1)	7(1)	11(1)
C7	6936 (3)	165 (2)	7349 (1)	57(1)	35(1)	40(1)	−4(1)	−1(1)	0(1)
C8	5910 (3)	1322 (3)	7253 (1)	57(1)	48(1)	46(1)	9(1)	0(1)	3(1)
C9	4710 (3)	1416 (3)	7574 (1)	54(1)	52(1)	53(1)	5(1)	0(1)	11(1)
C10	8235 (3)	49 (2)	7011 (1)	60(1)	38(1)	44(1)	−4(1)	1(1)	2(1)
C11	9545 (3)	−629 (3)	7250 (1)	71(1)	53(1)	45(1)	1(1)	0(1)	11(1)
C12	10761 (3)	−760 (3)	6940 (1)	56(1)	66(1)	57(2)	0(1)	0(1)	14(1)
C13	10714 (3)	−181 (3)	6372 (1)	62(1)	53(1)	57(2)	0(1)	8(1)	5(1)
C14	9438 (3)	521 (3)	6123 (1)	73(2)	74(1)	48(1)	15(1)	11(1)	8(1)
C15	8230 (3)	633 (3)	6438 (1)	62(1)	60(1)	51(1)	6(1)	2(1)	11(1)
C16	13149 (3)	−1085 (4)	6233 (2)	58(1)	98(2)	77(2)	−7(1)	3(1)	4(1)
C17	14171 (5)	−1053 (5)	5764 (2)	75(2)	126(3)	116(3)	19(2)	32(2)	17(2)
C18	13601 (6)	−1881 (6)	5218 (2)	121(3)	186(5)	86(3)	14(3)	35(3)	30(3)
N1	3257 (2)	1382 (2)	8908 (1)	33(1)	46(1)	46(1)	1(0)	0(0)	−2(0)
O1	1914 (2)	1254 (2)	9151 (1)	36(0)	62(1)	68(1)	0(0)	12(0)	−5(0)
O2	11846 (2)	−256 (2)	6020 (1)	70(1)	90(1)	79(1)	15(1)	23(1)	14(1)
O3	686 (3)	−1539 (2)	9137 (1)	73(1)	53(1)	123(2)	−20(1)	44(1)	−9(1)
O4	1938 (3)	1646 (3)	10378 (1)	73(1)	143(2)	75(1)	−16(1)	31(1)	36(1)
H11	5381(29)	851(25)	9201(11)	54					
H12	4230(24)	−234(29)	9419(11)	54					
H13	4411(24)	1432(25)	9707(12)	54					
H21	2624(26)	3281(23)	8494(12)	52					
H22	4406(27)	3101(24)	8644(11)	52					
H23	3450(25)	3482(25)	9168(13)	52					
H31	2868(24)	−511(27)	8481(11)	51					
H32	2317(26)	936(25)	8088(11)	51					
H51	5247(23)	−1567(24)	8403(11)	53					
H61	7373(25)	−1632(24)	7882(11)	49					
H81	6082(21)	2057(24)	6949(11)	43					
H91	4068(25)	2211(26)	7503(10)	51					
H111	9574(25)	−981(25)	7650(13)	54					
H121	11700(29)	−1221(24)	7119(11)	56					
H141	9449(27)	966(27)	5755(13)	63					
H151	7397(27)	1083(26)	6272(12)	57					
H161	12731(29)	−2168(32)	6318(12)	74					
H162	−8512(31)	4338(30)	3421(15)	74					
H171	15136(44)	−1481(36)	5877(17)	106					
H172	14141(34)	119(41)	5611(15)	106					
H181	12831(44)	−2676(46)	5245(20)	123					
H182	14371(42)	−1856(40)	4948(20)	123					
H183	12634(49)	−1346(43)	5091(18)	123					
HO31	1036(33)	−712(35)	9191(13)	75					
HO32	−136(37)	−1556(27)	9327(13)	75					
HO41	2785(36)	2159(32)	10520(13)	86					
HO42	1957(35)	1459(32)	10001(17)	86					

For hydrogens, the single isotropic temperature factor is given which was held fixed during the refinement. The expression for T is $T = \exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}k^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$.

$\rho = 1.177$ g/cm^3; linear absorption coefficient μ (Mo K_α) = 0.8/cm; systematic extinctions: h_{0l} for h odd; space group $P2_1/a$. X-ray intensity data were collected on a crystal of dimensions $0.05 \times 1.0 \times 2.0$ mm^3 with graphite-monochromatized Mo K_α radiation from a rotating anode source (50 kV, 30 mA) on an image plate detector (STOE & Cie, Darmstadt, Germany) at a crystal-detector distance of 70 mm. 139 5-min frames of 1° rotation angle were taken and subsequently reduced with the XDS program [14]. 2127 unique reflections ($R = 0.042$, $2\theta < 45°$ corresponding to 0.93 Å resolution) with $I > 4\sigma(I)$ were obtained after symmetry averaging of 6213 reflections.

2.4. Structure determination and refinement

The structure was solved by direct methods using SHELXS-86 [15]. The solution with the best figure of merit ($c_{fom} = 0.118$) showed the characteristic atomic arrangement of the biphenyl moiety and gave $R = 0.323$. Isotropic refinement of the heavy atoms with SHELX-76 converged at $R = 0.164$. All hydrogen atoms were found in difference maps and included in the model, they were assigned the isotropic temperature factors of the heavy atoms to which they were bonded. On further refinement of positional parameters of all atoms and anisotropic temperature factors of the heavy atoms, the R-factor reduced to 0.069. Positions and temperature factors of all atoms are given in Table 1.

3. Results and discussion

PBIPAO solubilised the B800–850 complex and the reaction centre-B875 complex of *Rhodopseudomonas palustris*, two integral membrane protein complexes [16]. It is thus useful as a detergent for membrane proteins.

Fig. 1 shows the conformation of PBIPAO in the crystal. The N-O bond is pointing away from the hydrophobic part of the molecule towards the two hydrogen-bonded water molecules. The torsion angle O2-C16-C17-C18 of the propyl chain amounts to 69°. The two phenyl rings are twisted against each other by 27.5°.

In the crystal (Fig. 2), the amphiphilic PBIPAO molecules form a bilayer with a thickness of 22.4 Å (corresponding to the unit cell c-axis) which segregates the polar ends of the molecules into a 7 Å wide polar and a 15.4 Å wide hydrophobic layer. Each amineoxide oxygen accepts two hydrogen bonds, one each from the two water molecules (see Table 2). One of these two hydrogen bonds binds to a water molecule of the opposite PBIPAO layer, thus creating the crystal contact to this layer (unit cell c-direction). The other hydrogen bond forms an angle of 95.0° with the former and connects to a water molecule in the same layer to which the oxygen atom belongs (b-direction). Each of the two water molecules donates bonds to and accepts bonds from symmetry related waters, so that the 4 water molecules interdigitated between each pair of amineoxide oxygens (see Fig. 2) are part of an infinite zig-zag chain of waters along the a-direction of the crystal (not shown).

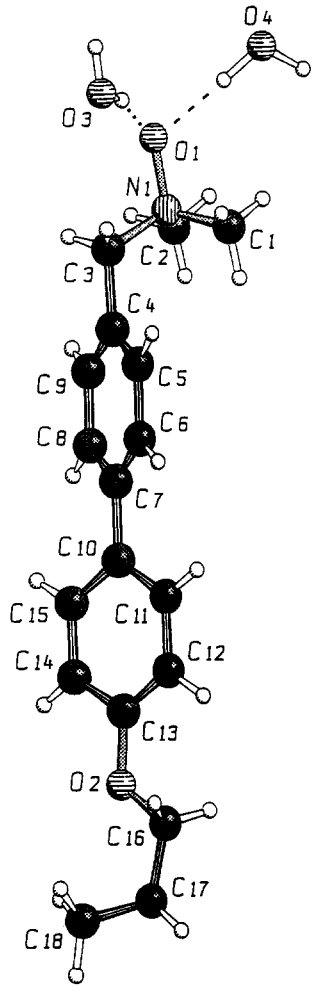

Fig. 1. Ball-and-stick model [17] of PBIPAO with two hydrogen-bonded water molecules.

The biphenyl groups are not stacked parallel in van der Waals contact according to the optimal packing requirement. Instead, phenyl rings of adjacent groups are roughly at right angles to each other and show edge-to-face contacts as often found in proteins and oligopeptides [18]. This arrangement is favoured due to the orientational dependence of the quadrupole–quadrupole interaction of both rings. The strong tendency of PBIPAO to form lamellar liquid crystalline phases [19] may be due to this interaction.

The two phenyl rings are linked by a single bond so that they are free to rotate against each other [20]. However in the coplanar conformation there is sterical strain due to the hydrogens at C6, C8, C11 and C15. While in the vapor phase and in the liquid phase the two phenyl rings in biphenyl are twisted by 44° [21] and 35° [22] against each

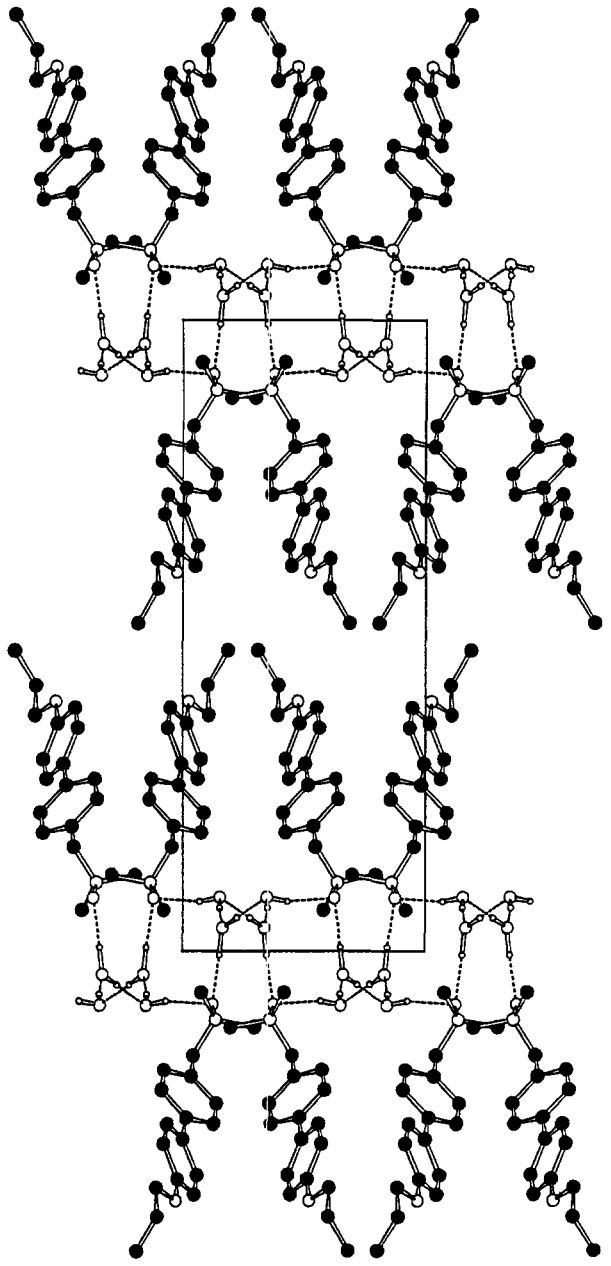

Fig. 2. Crystal arrangement of PBIPAO molecules and water molecules. Hydrogen bonds are drawn by broken lines. The unit cell edges shown are *c* (vertical) and *b* (horizontal).

other, respectively, in crystals the twist angle depends upon packing requirements which can be modified by substituents at positions adjacent to the C7-C10 bond. Biphenyl was found planar [20,23–25] in spite of the steric strain, while substituted biphenyls showed twist angles between 21° and 54° [26–32].

In case of a loose packing due to a clathrate structure [33], biphenyl showed a ring twist of 33°, close to the value in the present work and in the liquid state. The space available in the clathrate cage and in the rigid hydrogen bonding network of our crystal possibly does not require tight packing of the biphenyl groups. A twist of both rings is thus possible, which relieves the strain of the coplanar conformation.

The free space available to the propoxybiphenyl group in presence of the strongly hydrated *N*-linked oxygen is so large, that the former group is tilted with respect to the surface of the polar phase by 45°. The tighter packing of the former group which is thus possible is used to form the energetically favourable edge-to-face arrangement. A tilt of the hydrophobic group is also found in the $L_{\beta'}$-phase of phospholipids [34]. The tilt indicates an overall conical shape of the hydrated molecule, so that in a different arrangement PBIPAO could also from micelles. Indeed we found a critical micellar concentration of 0.42 mM and solubilization capacity for membrane proteins. Amineoxide groups in micelles of lauryldimethylamineoxide are hydrated to a similar degree (approx. 2.5 water molecules per group) [35]. In single-chain lipids [36] and carbohydrates [37] close packing of interdigitated all-*trans* alkyl chains frequently contributes to the crystal contacts. The interacting biphenyls seem to interfere with this arrangement, as the propyl chain at the apolar end assumes a twisted conformation with increasing temperature factor coefficients towards the terminal methyl group (see Table 1).

The poor solubilising capacity and mildness of some other biphenyl detergents for membrane proteins [11] may to be due to the energy of the quadrupole interaction of the biphenyl group. In conventional detergents the hydrophobic parts of the molecules interact only by hydrophobic interactions by which they also bind to the hydrophobic surface of the protein. In contrast, proteins do not possess suitably arranged biphenyl groups at their surface so that the biphenyl detergents in contact with the protein possess an unfavourable surface energy as compared to those in the bulk micellar phase.

In contrast to amineoxides, carbohydrates have both hydrogen bonding donors and acceptors. Crystals of carbohydrate amphiphiles thus tend to exclude water molecules

Table 2
Hydrogen bond geometry in crystals of PBIPAO

	Symm. operat. applied to A	D · · · A distance	H · · · A distance	D-H · · · A angle
O3-HO31..O1	x, y, z	2.72	1.93	168°
O4-HO42..O1	x, y, z	2.78	1.92	173°
O4-HO41..O3	$\bar{x}-1/2, y+1/2, \bar{z}$	2.82	1.91	170°
O3-HO32..O4	$\bar{x}-1, \bar{y}-1, \bar{z}+1$	2.73	1.83	172°

Distances in Å, angles in degrees. D, donor atom, A, acceptor atom.

[38–47]. The lack of suitable hydrogen bonding donors in amineoxide compounds explains the requirement for the inclusion of water molecules into their crystalline lattice.

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