Tetrahedron: Asymmetry Vol. 4, No. 4, pp. 709-722, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00

Timolol Derivatives. I. X-ray, NMR and Theoretical Studies of the Crystallization of (*S*)-Timolol *O,O*-Diacetyl-L-tartaric Acid Monoester

Jussi Kivikoski,[a*] Jouko Vepsäläinen,[b] Reijo Suontamo,[a] Esko Pohjala[c] and Reino Laatikainen[b]

[a]Department of Chemistry, University of Jyväskylä, P.O. Box 35, SF-40351 Jyväskylä, Finland

[b]Department of Chemistry, University of Kuopio, P.O. Box 1627, SF-70211 Kuopio, Finland

[c]Leiras Oy, P.O. Box 33, SF-33721 Tampere, Finland

(*Received in UK 4 February* 1993)

Abstract: The absolute configurations of (*S*)-timolol hemihydrate and (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester were determined by single crystal X-ray diffraction. An NMR analysis based on the temperature dependence of vicinal coupling constants was carried out to characterize the conformational behaviour of the *S,R,R*- and *R,R,R*-forms in solution. The same conformation as in crystalline state was also found in solution, although with a rather low preference over some other conformations. Results of theoretical calculations using MNDO and AMBER force field methods are reported. An infinite chain of hydrogen bonds, along with other favourable inter- and intramolecular forces that are present in the crystal framework of the (*S*)-timolol-(*R,R*)-tartrate but not possible for (*R*)-timolol-(*R,R*)-tartrate, explain the astonishingly dominating crystallization of the *S,R,R*-form. Racemic timolol is accordingly easily resolved.

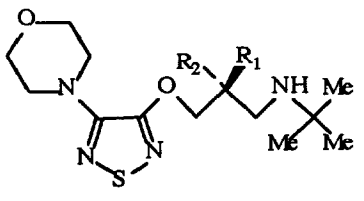
Introduction

Timolol, or (*S*)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (**1a**), is a non-selective β-adrenergic blocker, and its 1:1 maleate salt is currently the number one choice in the treatment of glaucoma and ocular hypertension.[1] The chiral aminoalcohol side-chain of **1a** can be constructed starting from suitably stereogenic three-carbon units such as D-glyceraldehyde derivatives[2] and glycidyl sulfonates.[3] Resolution of racemic timolol or its precursors[4] was of modest practical use until recently when a large-scale synthesis of (*S*)-timolol exploiting the resolution as *O,O*-diacetyl-(*R,R*)-tartaric [L-tartaric] acid monoester or (*S*)-[(1,1-dimethylethyl)amino]methyl-2-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]ethyl hydrogen (2*R*,3*R*)-2,3-bis(acetoxy)butanedioate (**2**) was introduced. In that process an optical purity of well over 90% for the *S,R,R*-form, and after hydrolysis the same amount for (*S*)-timolol, is achieved in a single crystallization step.[4] The expensive thiadiazole part of the remaining *R,R,R*-form **2'** can then easily be recycled for production of the racemic timolol. (*S*)-Timolol (and (*R*)-timolol obtained via the corresponding

R,S,S-tartrate) is then conveniently and efficiently purified up to 100% optical purity by crystallization as base hemihydrate **1b** (or **1b'**), irrespective of the origin of (*S*)- (or (*R*-)) timolol.[5]

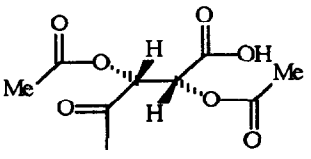
The crystal and molecular structures of (*S*)-timolol diacetyltartrate **2** and the hemihydrate **1b** have been determined earlier.[6] Here also their absolute configurations are determined, and they are shown not to invert in the hydrolysis of the *S,R,R*-ester.[7] ^{1}H-NMR spectra of esters **2** and **2'** were measured in an attempt to explain their extraordinarily facile resolution. Conformational analysis was concentrated on the C5-C6 and C6-C7 rotamers because the C5-C6-C7 system constitutes the active aminoalcohol part, similar in all β-blockers. Molecular modelling was done by AMBER force field and MNDO methods and the results was compared with the determined crystal structure of **2**.

Information about the inter- and intramolecular hydrogen bridges[8] and other interactions of β-blockers, as well as their divergent behaviour in different matrices, can assist the description of their three-dimensional mode of action at β-adrenoreceptors. The present study on the inter- and intramolecular forces of timolol and its tartaric acid esters is a contribution in this direction.[9]



	R_1	R_2	Config. of C6
1a	OH	H	*S*
1a'	H	OH	*R*
2	OR	H	*S*
2'	H	OR	*R*
1b	**1a** ·1/2 H_2O		*S*
1b'	**1a'**·1/2H_2O		*R*

R =



X-Ray Crystal Structure Analysis

(*S*)-Timolol hemihydrate **1b** and (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester **2** were recrystallized and remeasured to determine their absolute configurations. In an earlier study, not enough data were collected to allow the determination of absolute configuration. In addition it was found here that the monoester can crystallize in another polymorphic form (*mP*136).

Single crystals of both compounds (**1b** and **2**) were obtained at the interface of water and CH_2Cl_2 layers. For both compounds, cell dimensions and intensity data were measured on an Enraf-Nonius CAD-4 diffractometer up to θ = 75° (Cu Kα radiation). The data were corrected for absorption,[10] Lorentz and polarization effects. Atomic scattering factors were taken from International Tables.[11] The hydrogen atoms were placed geometrically (C-H distance 1.000 Å for **1b** and 1.025Å for **2**), except for the H atoms bonded to O or N atoms which were found from difference Fourier maps. The structures were refined by full-matrix least squares on F with merged Friedel pairs using the MolEN[12] program package. All non-H atoms were refined using anisotropic temperature factors, while the hydrogen atoms were treated as riding atoms with fixed temperature factor (5.0 $Å^2$). The crystal data given in Table 1 correspond to the crystal structures, which were refined to check the present against the earlier investigated structures.

Table 1: Crystal Data for	Hemihydrate **1b**	Monoester **2**
Molecular formula	$2C_{13}H_{24}N_4O_3S{\cdot}H_2O$	$C_{21}H_{32}N_4O_{10}S$ (*mP*136)
Formula weight	650.87	532.57
Crystal Data:		
Crystal system	monoclinic	monoclinic
Space group	C2	$P2_1$
a/Å	23.425(1)	9.770(1)
b/Å	6.381(2)	8.668(2)
c/Å	11.585(1)	16.319(1)
α/°	90	90
β/°	103.07(1)	103.60(1)
γ/°	90	90
V/Å^3	1686.9(5)	1343.1(4)
Z	2	2
D_c /g cm^{-3}	1.28	1.32
Linear absorption coeff./cm^{-1}	18.31	15.35
Crystal size/mm	0.15 x 0.20 x 0.25	0.20 x 0.20 x 0.25
Data collection:		
X-radiation	λ = 1.54178 Å Cu K_α	λ = 1.54178 Å Cu K_α
θ min., max./°	2, 75	2, 75
ω-scan parameters: A, B (°) (A + B tan θ)	A = 0.37 B = 0.14	A = 0.40 B = 0.14
Scan speed/° min^{-1}	0.6-5.5	0.6-5.5
Total data including Friedel opposities	3770	5707
Total unique data	1900	2958
Observed data, for [I > 3σ (I)]	1763	2116
Merging R	2.4%	4.0%
Absorption correction	0.822 (min.) 1.668 (max.)	0.788 (min.) 2.003 (max.)
Refinement:		
Solved by	Ref. 6	SHELXS-86[19]
Weighting scheme	$1/[(\sigma F_o)^2 + (S{\cdot}F_o)^2]$ (S = 0.04)	unit weights
Maximum residual electron density/eÅ^{-3}	0.27	0.17
Number of parameters	194	324
Final R	6.0%	4.7%
R_w	7.6%	4.4%
Flack's *x* parameter	0.001(1)	-0.01(2)

For hemihydrate **1b** the coordinates from the original paper were used for refinement. Relative to the earlier work there were no significant differences in the crystal structure. Within standard deviations the structure was exactly the same. The tables of bond angles, bond distances, torsional angles, anisotropic thermal parameters, atomic coordinates and list of structure factors have been deposited at the Cambridge Crystallographic Data Centre.[13]

In the earlier study the monoester **2** was crystallized with two molecules (conformers **A** and **B**) in the asymmetric unit (*mP*272). The main difference in the molecules was in two torsional angles. In the present case the monoester crystallized with one molecule (conformer **C**) in the asymmetric unit (*mP*136). Torsional angles as well as bond lengths and angles of the important part of the molecule are given in Table 2. The only significant differences relative to the earlier crystal structure are in the two torsional angles. The tables of bond angles, bond distances, torsional angles, anisotropic thermal parameters, atomic coordinates and list of structure factors have been deposited at the Cambridge Crystallographic Data Centre.[13] As in the previous structure there are two hydrogen bonds: one inter- and one intramolecular. The intermolecular bond is from N1 to O5 with N···O = 2.686(4) Å, HN···O = 1.785(3) Å and N-HN···O = 177.3(1)° and the intramolecular bond is from O6 to N1 with O···N = 2.804(6) Å, HO···N = 1.804(4) Å and O-HO···N = 179.7(2)°. The observed hydrogen bonding system is a typical example of a charge-assisted hydrogen bond. A view of the *S,R,R*-ester, its numbering scheme and hydrogen bonding system are shown in Figures 1 and 2.

Table 2: Values of the important torsional angles, bond angles and bond lengths for (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester.

	Present structure Conformer (**C**)	Earlier structure Conformer (**A**)	Conformer (**B**)
C8-O2-C7-C6/°	142.8(1)	155.2(1)	101.5(1)
C7-O2-C8-C9/°	-174.0(1)	179.0(1)	-158.8(1)
C6-C7-O2/°	107.3(3)	106.5(4)	108.9(4)
C7-O2-C8/°	117.9(3)	117.0(4)	115.8(4)
O2-C8-C9/°	119.5(4)	119.9(4)	121.5(5)
C6-C7/Å	1.505(7)	1.520(7)	1.521(7)
C7-O2/Å	1.439(5)	1.439(6)	1.453(6)
O2-C8/Å	1.343(6)	1.360(6)	1.365(6)
C8-C9/Å	1.440(7)	1.432(8)	1.452(8)

Determination of absolute configuration by X-ray diffraction is based on an anomalous scattering of X-rays. For both compounds, absorption (ψ-scan[14] with correction factors min. 0.852 and 0.914; max. 0.999 and 0.999 for **1b** and **2**, respectively), Lorentz and polarization effects were corrected and all but the Friedel related reflections were merged. Unit weights and 3384 reflections were used for the hemihydrate, and 5032 reflections for the monoester. Flack's *x* parameter[15-17] was refined using the *Xtal3.0*[18] program. For the hemihydrate the parameter obtained a value of 0.001(1); for the monoester it was -0.01(2). The anomalous effect of sulfur was clearly sufficient to give a very accurate result. The values and their standard deviations were reasonable, and the absolute configuration of the timolol part was shown to be *S* for both compounds, as anticipated.

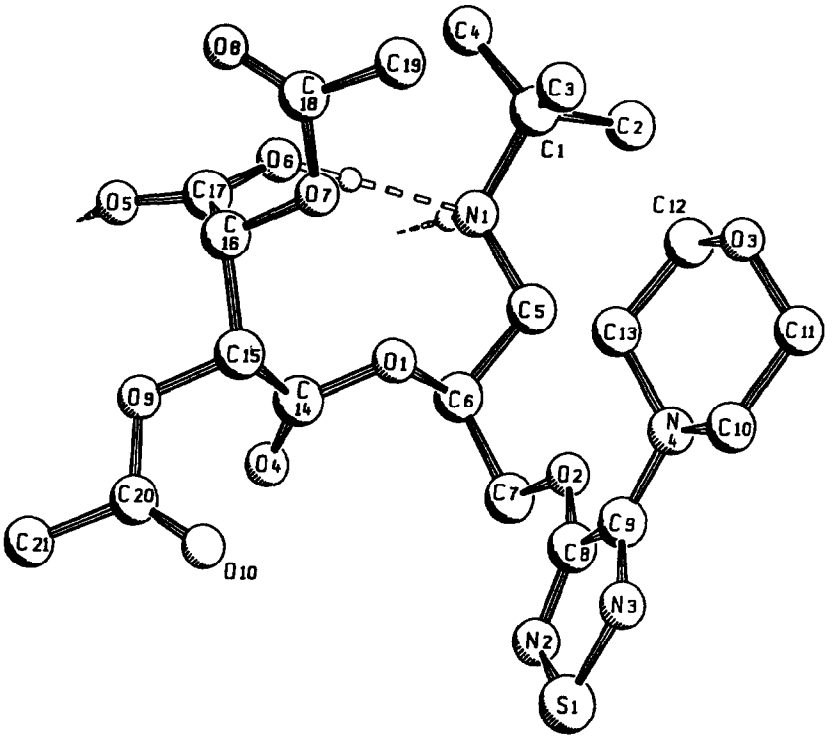


Figure 1: SCHAKAL[20] plot for (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester (**2**, conformer C).

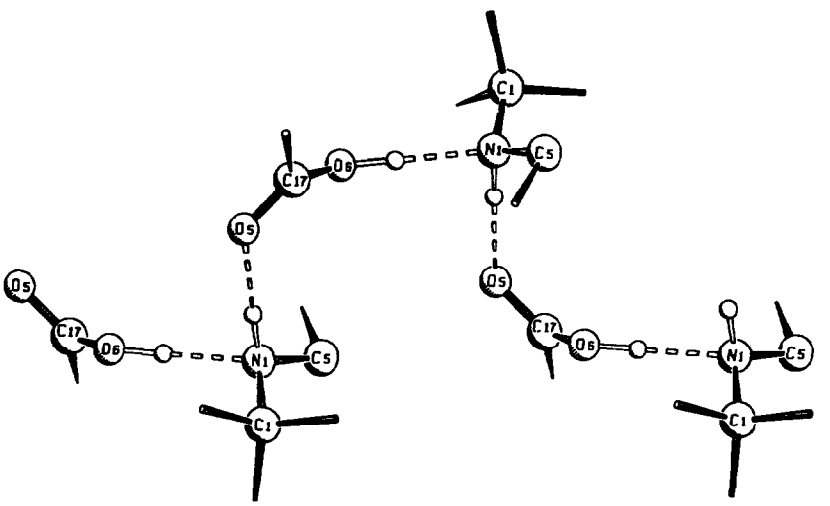


Figure 2: Hydrogen-bonding system for (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester (**2**, conformer C, fragments from three molecules).

Table 3: Fractional atomic coordinates and equivalent anisotropic temperature factors B_{eq} with standard deviations in parentheses for (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester (**2**, conformer C).

Atom	x	y	z	B_{eq}(Å^2)
S1	0.1808(2)	0.000	0.3932(1)	10.08(7)
O1	0.4438(3)	0.3425(4)	0.1804(2)	3.68(7)
O2	0.4236(3)	0.0606(4)	0.2547(2)	4.36(7)
O3	0.8580(5)	0.0812(8)	0.5087(3)	10.1(2)
O4	0.2329(3)	0.4254(5)	0.1076(2)	5.17(8)
O5	0.4542(4)	0.7806(4)	0.0741(2)	4.61(8)
O6	0.6040(4)	0.5832(4)	0.0981(2)	5.86(8)
O7	0.6196(3)	0.6009(4)	0.2684(2)	4.26(7)
O8	0.7441(4)	0.8172(5)	0.2653(2)	5.9(1)
O9	0.2580(3)	0.6928(4)	0.1908(2)	4.53(7)
O10	0.1326(4)	0.5486(6)	0.2603(2)	6.8(1)
N1	0.6487(3)	0.2660(5)	0.0935(2)	3.39(7)
N2	0.2171(4)	0.0336(6)	0.3013(3)	5.8(1)
N3	0.3419(6)	0.0074(9)	0.4515(3)	8.9(2)
N4	0.5723(5)	0.0292(7)	0.4299(3)	6.5(1)
C1	0.8099(5)	0.2493(7)	0.1083(3)	4.6(1)
C2	0.8402(6)	0.0868(8)	0.0811(4)	7.0(2)
C3	0.8727(6)	0.275(1)	0.2009(4)	7.7(2)
C4	0.8560(5)	0.3654(8)	0.0531(4)	6.3(2)
C5	0.5747(5)	0.1477(6)	0.1319(3)	4.0(1)
C6	0.4264(5)	0.2011(6)	0.1304(3)	3.55(9)
C7	0.3441(5)	0.0881(6)	0.1699(3)	4.0(1)
C8	0.3523(5)	0.0427(6)	0.3150(3)	4.6(1)
C9	0.4284(6)	0.0299(8)	0.4018(3)	5.8(1)
C10	0.6296(7)	-0.010(1)	0.5183(4)	9.4(2)
C11	0.7849(8)	-0.041(1)	0.5329(4)	10.4(3)
C12	0.8049(6)	0.111(1)	0.4209(4)	8.2(2)
C13	0.6533(6)	0.1521(9)	0.4026(4)	7.1(2)
C14	0.3381(4)	0.4458(6)	0.1600(3)	3.71(9)
C15	0.3723(4)	0.5880(6)	0.2168(3)	3.69(9)
C16	0.5061(4)	0.6758(6)	0.2097(3)	3.54(9)
C17	0.5253(5)	0.6789(6)	0.1190(3)	3.58(9)
C18	0.7360(5)	0.6914(8)	0.2946(3)	5.0(1)
C19	0.8441(6)	0.6080(9)	0.3597(3)	6.7(2)
C20	0.1377(5)	0.6530(7)	0.2138(3)	5.0(1)
C21	0.0193(5)	0.7560(8)	0.1709(4)	6.7(2)
HN1	0.6144	0.2728	0.0333	5.0
HO6	0.6192	0.4713	0.0956	5.0

Conformational NMR analysis

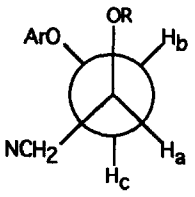
The ^1H and ^{13}C spectra were recorded on a Bruker AM 400 WB spectrometer operating at 400.133 MHz and 100.614 MHz, respectively. For ^{13}C measurements ca. 200 mmol and for ^1H ca. 50 mmol of sample were added to a solution containing 0.5 cm^3 of $CDCl_3$ and 0.1 cm^3 of C_6D_6. The sample solutions were prepared in 5-mm tubes using TMS as reference and shimming agent. Ordinary ^1H (sweep width, SW, 4000 Hz) and ^{13}C (SW 20000 Hz) spectra were acquired using 32 k data points. Temperature variable ^1H NMR spectra were recorded at 273, 278, 288, 306, 315, 325 and 330 K using 16 k (sweep width 1000 Hz) data points with resolution enhancement and zero filling to point resolution better than 0.01 Hz.

NMR chemical shifts for (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester **2**: δ_H 9.9 (1H, bs), 8.3 (1H, bs), 5.28 (1H, m), 5.10 (1H, d, $^3J_{HH}$ = 7.7 Hz), 4.94 (1H, d, $^3J_{HH}$ = 7.7 Hz), 4.74 (2H, m), 3.80 (4H, m), 3.52 (4H, m), 3.16 (2H, m), 2.17 (3H, s), 2.15 (3H, s), 1.45 (9H, s); δ_C 170.84 s, 170.52 s, 170.27 s, 167.63 s, 153.13 s, 150.04 s, 76.07 d, 74.12 d, 70.72 d, 69.21 t, 66.75 t, 57.68 s, 48.24 t, 42.81 t, 26.06 q, 21.17 q, 20.71 q.

NMR chemical shifts for (*R*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester **2'**: δ_H 5.65 (1H, m), 5.16 (1H, d, $^3J_{HH}$ = 7.8 Hz), 5.12 (1H, d, $^3J_{HH}$ = 7.8 Hz), 4.55 (2H, m), 3.74 (4H, m), 3.46 (4H, m), 3.44 (1H, m), 2.97 (1H, m), 2.07 (3H, s), 1.89 (3H, s), 1.30 (9H, s), NH and OH protons were not observed; δ_C 170.41 s, 170.38 s, 169.64 s, 166.46 s, 152.88 s, 149.64 s, 75.03 d, 75.02 d, 69.78 d, 68.98 t, 66.50 t, 57.40 s, 47.91 t, 41.88 t, 25.99 q, 20.80 q, 20.24 q.

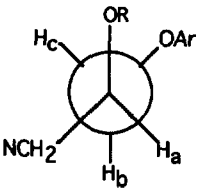
The populations of the C6-C5 and C6-C7 rotamers in solution can be estimated from the vicinal spin-spin coupling constants. In the present case there are totally nine stable conformers, designated I–IV, I–V, I–VI, II–IV etc. (see Scheme 1), where I, II and III refer to the C6-C7 bond rotamers and IV, V and VI to the C6-C5 rotamers. The C6-C7 couplings were determined at 7 temperatures, while the C6-C5 couplings were determined at only a few temperatures owing to the broad lines and a very tight spin system at lower temperatures (see Table 4).

As an assistance to NMR conformational analysis the geometries and energies of all nine conformers were computed by AMBER force field[21] and MNDO[22] methods (see theoretical calculations). Because of the numerous possible conformational minima, only the following procedure was applied: the AMBER calculation was allowed to bring the structure to the closest local minimum starting from the crystal structure, and this was then used to estimate the atomic charges by MNDO. The AMBER structure was refined using the calculated charges, and the final standard heats of formation were computed by MNDO. The search found three stable conformers for the (*S*)-derivative (I–VI, II–V, III–V, within 15.1 kJ/mol). For the (*R*)-derivative only conformers of II and III type were found. According to NMR analysis, conformer I-IV predominates in solution, just as it does in the crystal structure. The stability of this conformer is surprising because the two C6-C7 substituents have *gauche* interactions (see I in Scheme 1). A plausible explanation is association, in solution as well as in the crystal structure. A similar C6-C7 rotamer with two gauche interactions is also the most stable conformer for the *R,R,R*-form, although with clearly lower (1-4 kJ/mol) preference over the others, possibly for entropic reasons. The results of the theoretical calculations are at most in qualitative agreement with the NMR results; to obtain quantitative results, a more complete search would be needed through the conformational space. It is also rather difficult to lock the intramolecular N1-O6 hydrogen bond in the calculations. Evidently for these reasons the conformer I–IV is favourable and present in the crystal structure, even it does not have the lowest energy in the calculations. The calculations nevertheless show that all the essential dihedral angles are within 3-4° of 60° or 180°.



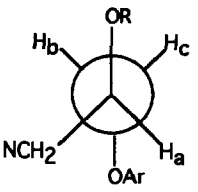
I

J_{ab} = 2.7 Hz
J_{ac} = 1.2 Hz



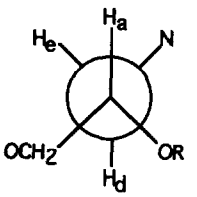
II

J_{ab} = 2.7 Hz
J_{ac} = 11.9 Hz



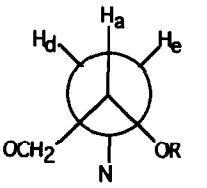
III

J_{ab} = 11.9 Hz
J_{ac} = 4.5 Hz



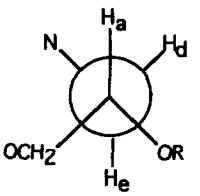
IV

J_{ad} = 12.3 Hz
J_{ae} = 2.5 Hz



V

J_{ad} = 1.7 Hz
J_{ae} = 3.3 Hz



VI

J_{ad} = 4.1 Hz
J_{ae} = 12.5 Hz

Scheme 1: The possible rotamers I-III for the C6-C7 bond, IV-VI for the C6-C5 bond and calculated $^3J_{HH}$ coupling constants. *Gauche* couplings were calculated by Haasnoot's equation[23] and *trans* couplings by that of Abraham[24] (Ar = [4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]).

The major difficulty in the NMR analysis was that the $C5H_2$ and $C7H_2$ proton signals could not be assigned on the basis of the spectra. However, the values of $^3J(CH_2, H)$ of the $C5H_2$ group (2.0 and 10.5 - 11.2 Hz, see Table 4) indicate that the major conformer, with population of ca. 90%, is the same as in the crystal structure (see Fig. 1 and rotamer IV in Scheme 1). The smallness of the $C7H_2$ couplings indicate that the H_a proton is *gauche* (g) to both of the $C7H_2$ protons, again supporting the same structure as in the crystal (rotamer I in Scheme 1). The values and their temperature dependence indicate the presence of considerable populations of the conformers of type II and III.

Table 4: Refined measured coupling constants for (*S*)- and (*R*)-timolol derivatives (**2** and **2'**).

	Temp.	Coupling constants [Hz]					
	[K]	J_{bc}	J_{ab}	J_{ac}	J_{de}	J_{ad}	J_{ae}
(*S*)-form	273	-12.106	3.010	3.595	-	-	-
	278	-12.130	3.027	3.645	-	-	-
	288	-12.131	3.078	3.761	-	-	-
	306	-12.122	3.164	3.904	-	-	-
	315	-12.166	3.215	3.963	-	-	-
	325	-12.110	3.267	4.022	-	-	-
	330	-12.107	3.299	4.055	-13.076	1.739	10.516
(*R*)-form	273	-12.034	5.740	3.407	-	-	-
	278	-11.959	5.565	3.513	-	-	-
	288	-11.918	5.492	3.570	-	-	-
	306	-11.904	5.384	3.713	-	-	-
	315	-11.909	5.351	3.754	-12.878	-	11.171
	325	-11.914	5.303	3.803	-12.925	-	11.136
	330	-11.912	5.277	3.830	-12.908	2.038	11.133

Thermodynamic analysis based on vicinal couplings:

If the values of the coupling constants, J_I and J_{II}, for two rotamers are known, the experimental determination of the coupling allows an estimation of their populations and free energy difference (ΔG).[25] If the observed value of the coupling is close to J_I, ΔG is sensitive to the value of J_I and insensitive to J_{II}. The accuracy of ΔG can be improved by using the temperature dependence of the coupling, as done here.[25] A determination of J at n temperatures leads to a group of n simultaneous nonlinear equations, the solution of which gives ΔG, J_I and J_{II}, usually at least two of them rather accurately. When the number of possible conformers and couplings are increased, as here, an unambiguous solution often becomes impossible.

The present ^3J vs. temperature data were analysed using the program EQUILA.[26] On the basis of the above argument we assume the following equilibrium for the C6-C7 rotamers:

$$\begin{aligned} I &= II \quad \Delta H_{II}, (\Delta S_{II}) \\ I &= III \quad \Delta H_{III}, (\Delta S_{III}) \end{aligned}$$

If we estimate the values of $^3J(H, CH_2)$ for the C6H-C7H_2 fragment of the conformers I–III using improved Karplus-type equations, the corresponding enthalpies, and sometimes the entropies, can be obtained using the program EQUILA. The coupling values in Scheme 1 for *gauche* protons are calculated using Haasnoot's[23] equation and the *trans* protons using that of Abraham.[24] The noteworthy point in the values is the very large and very small values for the J^g_{ac}'s, while the J^g_{ab}'s are of the same order. On the basis of our C5-C6 coupling data and the similar analysis reported by Kukarni & Coutinho,[27] we used a value of 11.5 Hz for $^3J^t$ instead of the much smaller value given by the Haasnoot equation. The same values are valid for both compounds.

(*S*)-timolol-(*R,R*)-tartaric acid monoester **2** Since the C7H_2 protons could not be assigned, there were two possible solutions to explore. The first assumes the assignment given in Table 4, in which case the program EQUILA gives an rms (the residual root mean square between the observed and computed couplings) of 31 mHz with ΔH_{II} = 2.7 and ΔH_{III} = 7.7 kJ/mol. These coupling data do not allow a complete optimization of the coupling constants, but if we adjust them so that the deviation of the optimized values from those given in Scheme 1 is one tenth as serious as the deviation between the observed and calculated couplings, the rms is reduced to 20 mHz and the values of the couplings stay within 0.1 - 0.4 Hz of the predicted values. A further reduction of rms to 10 mHz is possible, but ΔH_{II} and ΔH_{III} are not changed significantly from the values 2.9 and and 5.4 kJ/mol, respectively. The second solution assumes the opposite assignment, in which case the rms of the EQUILA fitting is 67 mHz, with ΔH_{II} = 3.9 and ΔH_{III} = 4.4 kJ/mol. The rms can be improved only to 41 mHz by optimizing the couplings with the same constraints as above. The result indicates that the characteristic difference between J^g_{ab} and J^g_{ac} is correct and can be used to assign the couplings.

It is also possible to improve the rms values by including the entropies. However, this does not significantly change the energy difference between the conformers (but replaces the enthalpies by free energies). It is also interesting that including of the entropies does not improve the rms of the second assignment as much as the rms of the first.

To conclude, the NMR analysis indicates that 20% and 10% of conformations II and III, respectively, are present in solution.

(*R*)-timolol-(*R,R*)-tartaric acid monoester **2'** In attempting a similar analysis for **2'**, we found the two alternative assignments of the CH_2 -signals to give the same rms, ca. 100 mHz, and this could not be improved greatly without the couplings of the conformers deviating significantly from the predicted values. However, a very good rms of 19 mHz is obtained simply by using the assignment given in Table 4 and allowing ΔS_{III} to fall to -10 J/K mol. The corresponding ΔH_{II} = 3.4 kJ/mol, ΔH_{III} = -1.4 kJ/mol and ΔG_{III} = 1.6 kJ/mol at 298 K.

The large negative entropy indicates that the III conformation is much more rigid than the I and II conformations. The entropy of 10 J/K mol corresponds to about 3.3-fold degeneracy for I and II. This offers an explanation, in addition to the 'H-bond model', for the poor crystallization ability of the (*R*)-timolol derivative, **2'**.

Theoretical calculations

Theoretical calculations and molecular modelling based on the crystal structure analysis of *S,R,R*-ester **2** were done as a support to the NMR analysis of both **2** and **2'**. Our purpose was to look for a possible hydrogen bonding system and non-bonding interactions in the (*R*)-timolol-(*R,R*)-ester, which so far has resisted crystallization. The two polymorphic forms of (*S*)-timolol-(*R,R*)-ester were modelled. In the first polymorphic form, two different conformations of *S,R,R* make a pair in the asymmetric unit (conformers **A** and **B**). In the second polymorph only one conformation is present (conformer **C**). Only torsion angles of the two substituents (H6 and C5 chain) on the stereogenic carbon were interchanged to make the corresponding models for (*R*)-timolol-(*R,R*)-ester. The rest of the molecule was kept as it is in the (*S*) configuration.

The semiempirical MNDO molecular orbital method,[22] as it is implemented in the GAUSSIAN 90 series of programs,[28] was chosen for the calculation of heats of formation for single molecules. No geometric optimization with MNDO was done because the large number (198) of internal coordinates to be optimized made this impractical.

Keeping the known crystal structures as a starting point, 18 additional configurations with non-*R,R* chirality on the tartaric acid part were constructed by a similar method to that described above. All 24 calculated forms are listed in Table 5, together with their MNDO heats of formation.

Table 5: MNDO heats of formation of the studied forms.

Molecule	E_{SCF}(H)	$\Delta H°_{298}$(kJ/mol)
A-R,R,R	-0.13443018	-357.39
A-R,R,S	-0.13393131	-351.86
A-R,S,R	0.07113912	186.90
A-R,S,S	0.02832507	74.40
A-S,R,R	-0.16130609	-423.79
A-S,R,S	-0.16296174	-428.14
A-S,S,R	0.03927702	103.20
A-S,S,S	-0.00593490	-15.57
B-R,R,R	-0.09919505	-260.63
B-R,R,S	-0.08993906	-236.30
B-R,S,R	0.43965131	1155.10
B-R,S,S	0.09340337	245.39
B-S,R,R	-0.12008130	-315.48
B-S,R,S	-0.12093800	-317.74
B-S,S,R	0.40758678	1070.86
B-S,S,S	0.05857478	153.91
C-R,R,R	-0.11778454	-309.45
C-R,R,S	-0.13039356	-342.56
C-R,S,R	0.20477481	538.00
C-R,S,S	0.05033198	132.22
C-S,R,R	-0.16064984	-422.07
C-S,R,S	-0.16446802	-432.12
C-S,S,R	0.16770248	440.62
C-S,S,S	0.01251108	32.87

All modelled *R,S,R*; *R,S,S*; *S,S,R* and *S,S,S* configurations of the polymorphic forms **A**, **B** and **C** are higher in energy than *S,R,R* configurations, implying that they are less likely candidates to form the kind of crystals that were found experimentally. For the conformations that were found in our crystals, molecule **A**-*S,R,R* has the lowest MNDO energy. Molecule **C**-*S,R,R* has virtually the same energy — only 1.72 kJ/mol higher. Molecule **B**-*S,R,R* is 108.31 kJ/mol higher in energy than **A**-*S,R,R*. Thus, if molecule **A**-*S,R,R* can crystallize, there should be no energetic reason why molecule **C**-*S,R,R* does not crystallize. All the *R,R,R* molecules are higher in MNDO energy than their *S,R,R* counterparts (the differences are 66.40, 54.85 and 112.62 kJ/mol for molecules **A**, **B** and **C**, respectively).

The second polymorph, with a pair of molecules **C**-*S,R,R* in the asymmetric unit, forms a more favourable crystal than the pair of molecules **A**-*S,R,R* and **B**-*S,R,R* in the first polymorph. The difference is 105.09 kJ/mol when packing effects are not included in the calculations (*i.e.*, when the pair is simply the sum of two single molecules). When similar pairs are constructed for the (*R*) molecules, the second polymorphic form is only 5.19 kJ/mol more favourable in energy than the first polymorph. To estimate the non-bonding (van der Waals, electrostatic and H-bonding) effects on the packing of the crystals, empirical force field calculations with the MOBY program[29] were carried out for the most favourable pairs of molecules in the two polymorphs. The program is based on the AMBER force field.[21] Standard parameters from the program were used for all

configurations. Partial atomic charges which were needed in the force field were taken from the MNDO calculations. Results are given in Table 6.

Table 6: Non-bonding AMBER force field energies (in kJ/mol) of the polymorphs.

Pair	van der Waals interaction	electrostatic interaction	H-bonding	total non-bonding energy
A-R,R,R+**B**-R,R,R	600.77	-179.51	-1.41	419.84
A-R,R,R+**B**-R,R,S	432.68	-183.92	-0.81	247.94
A-R,R,R+**B**-S,R,R	334.61	-191.35	-2.52	140.74
A-R,R,R+**B**-S,R,S	701.98	-186.56	-0.82	514.60
A-R,R,S+**B**-R,R,R	413.69	-195.11	-1.87	216.71
A-R,R,S+**B**-R,R,S	511.71	-187.30	-0.16	324.25
A-R,R,S+**B**-S,R,R	679.87	-182.36	-0.77	496.73
A-R,R,S+**B**-S,R,S	781.02	-186.17	-0.17	594.68
A-S,R,R+**B**-R,R,R	316.89	-192.91	-2.55	121.44
A-S,R,R+**B**-R,R,S	148.80	-197.14	-1.94	-50.29
A-S,R,R+**B**-S,R,R	51.50	-204.71	-3.65	-156.86
A-S,R,R+**B**-S,R,S	418.11	-195.90	-1.96	220.25
A-S,R,S+**B**-R,R,R	133.86	-192.33	-1.87	-60.34
A-S,R,S+**B**-R,R,S	231.86	-184.50	-0.16	47.21
A-S,R,S+**B**-S,R,R	400.02	-180.50	-0.76	218.76
A-S,R,S+**B**-S,R,S	501.18	-183.37	-0.17	317.64
C-R,R,R+**C**-R,R,R	688.88	-219.56	-4.53	464.79
C-R,R,R+**C**-R,R,S	777.03	-198.06	-2.55	576.43
C-R,R,R+**C**-S,R,R	367.34	-230.48	-2.43	134.43
C-R,R,R+**C**-S,R,S	422.99	-194.03	-2.28	226.67
C-R,R,S+**C**-R,R,S	865.08	-165.82	-0.56	698.70
C-R,R,S+**C**-S,R,R	455.47	-200.32	-0.44	254.70
C-R,R,S+**C**-S,R,S	511.11	-161.86	-0.29	348.96
C-S,R,R+**C**-S,R,R	134.98	-241.93	-0.33	-107.27
C-S,R,R+**C**-S,R,S	192.55	-203.62	-0.18	-11.26
C-S,R,S+**C**-S,R,S	248.15	-154.72	-0.03	93.40

The total non-bonding energies for both forms of the (*R*)-ester are high, mainly due to the unfavourable van der Waals interactions. Because of that, the (*R*)-timolol-(*R,R*)-ester does not crystallize under the same conditions as the known polymorphic forms of the (*S*)-timolol ester. The pair of molecules **A** and **B** have total non-bonding energy of -156.9 kJ/mol, and the same value for the pair of **C** molecules is -107.3 kJ/mol. Thus the two sets of molecules have equivalent packing effects when crystallization is modelled by starting with two molecules in known crystalline environment.

All the single molecules and pairs of molecules modelled in the fixed or "strained" crystalline state were also optimized to a nearest local minimum using the AMBER force field. With single molecules, MNDO energies were calculated for AMBER minima. All studied molecules, when relaxed from the strained crystal conformation in this way, gave closely similar MNDO energies, ranging from -582 to -611 kJ/mol for

A-N,X,Y, from -502 to -574 kJ/mol for **B**-N,X,Y and from -481 to -544 kJ/mol for **C**-N,X,Y, where N, X and Y are *S* or *R*.

Summary

The extraordinarily good crystallization property of the (*S*)-timolol *O,O*-diacetyl-(*R,R*)-tartaric acid monoester arises from the infinite hydrogen bonding system consisting of both inter- and intramolecular hydrogen bonds. According to NMR analysis and theoretical calculations, the *R,R,R*-form is unlikely to crystallize because the stabilizing intermolecular hydrogen bonds are lacking. NMR analysis of the *S,R,R*- and *R,R,R*-forms showed that the same C5-C6 rotamer (IV in Scheme 1) as in crystalline state predominates in solution. Furthermore, the most stable C6-C7 rotamer of the *S,R,R*-form is the same as in the crystal state (I in Scheme 1), but only with slight preference over the other two conformations. However, the molecules are very flexible in solution state and the smallest coupling constants corresponds to the most stable conformer. NMR analysis indicated the most stable conformer of the *R,R,R*-form: its C5-C6-C7 part proved to be the mirror image of that of the corresponding *S,R,R*-form. Theoretical calculations gave qualitatively reasonable results concerning the stability order of the different conformers of the *S,R,R*- and *R,R,R*-forms.

References

1. Kleeman, A.; Engel, J. *Pharmaceutische Wirkstoffe*, 2nd ed; Georg Thieme Verlag; Stuttgart, 1982; pp. 889-890 and references therein.
2. Weinstock, L. M.; Mulvey, D. M.; Tull, R. *J. Org. Chem.*, **1976**, 41, 3121-3124.
3. a) Baldwin, J. J.; McClure, D. E. US. Pat. 4,408,063 **1983**. b) Klunder, J. M.; Onami, T.; Sharpless, B. *J. Org. Chem.*, **1989**, 54, 1295-1304.
4. a) Wasson, B. K.; Gibson, W. K.; Stuart, R. S.; Williams, H. W. R.; Yates, C. H. *J. Med. Chem.*, **1972**, 15, 651-655. b) Nupponen, H.; Hietaniemi, L. (Leiras Oy); FI Pat. 71.933 **1989**.
5. Perälampi, M. (Leiras Oy); FI Pat. 82.240 **1991**.
6. Kivikoski, J.; Valkonen, J.; Pohjala, E. *Acta Crystallographica*, **1990**, C46, 2431-2435.
7. Hietaniemi, L.; Pohjala, E. *Finn. Chem. Lett.*, **1989**, 16, 61-66.
8. Lindner, W.; Leitner, C. *J. Chromatograph.*, **1984**, 316, 605-616.
9. a) Rouvinen, J.; Pohjala, E.; Vepsäläinen, J.; Mälkönen, P. *Drug Des. Deliv.*, **1990**, 5, 281-300. b) Lewell, X. Q. *Drug Des. Discov.*, **1992**, 9, 29-48.
10. Walker, N.; Stuart, D. *Acta Crystallographica*, **1983**, A39, 158-166.
11. International Tables for X-Ray Crystallography, Vol. IV, Kynoch Press; Birmingham, 1974.
12. MolEN, *An Interactive Structure Solution Procedure*, Enraf-Nonius, Delft, The Netherlands, 1990.
13. The tables of bond angles, bond distances, torsional angles, anisotropic thermal parameters, atomic coordinates and list of structure factors are available on request from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this paper.
14. North, A. C. T.; Phillips, D. C.; Mathews, F. S. *Acta Crystallographica*, **1968**, A24, 351-359.
15. Flack, H. D. *Acta Crystallographica*, **1983**, A39, 876-881.
16. Bernardinelli, G.; Flack, H. D. *Acta Crystallographica*, **1985**, A41, 500-511.
17. Bernardinelli, G.; Flack, H. D. *Acta Crystallographica*, **1987**, A43, 75-78.
18. Hall, S. R.; Stewart, J. M. *Xtal3.0* Reference Manual, Universities of Western Australia, Australia and Maryland, USA, 1990.
19. Sheldrick, G. M., *Crystallographic Computing 3*; Sheldrick, G. M.; Kruger, C.; Goddard, R. Eds.; Oxford University Press; Oxford, 1985.

20. Keller, E. SCHAKAL88, Kristallographishes Institut der Universität Freiburg, Germany, 1990.
21. a) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.*, **1984**, 106, 765-784. b) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.*, **1986**, 7, 230-252.
22. Dewar, M. J. S.; Thiel W. *J. Am. Chem. Soc.*, **1985**, 107, 3902-3909.
23. Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron*, **1980**, 36, 2783-2792.
24. Abraham, R. J.; Loftus, P.; Thomas, W. A. *Tetrahedron*, **1977**, 33, 1227-1234.
25. Garbish, E. W; Hawkins, B. L.; MacKey, K. D. *Conformational Analysis, Scope and Present Limitations*; Chiurdoglu, G. Ed.; Academic Press; New York, 1971; pp. 93-109.
26. a) Laatikainen, R.; Tuppurainen, K. *Magn. Reson. Chem.*, **1985**, 24, 588-594. b) Laatikainen, R.; Král, V. *J. Chem. Soc. Perkin II*, **1985**, 1091-1100.
27. Kulkarni, V. M.; Coutinho, E. C. *Ind. J. Chem.*, **1991**, 30B, 52-56.
28. GAUSSIAN 90, Frisch, M. J.; Head-Gordon, M.; Trucks, G. W.; Foresman, J. B.; Schlegel, H. B.; Raghavachari, K.; Robb, M. A.; Binkley, J. S.; Gonzales, C.; Defrees, D. J.; Fox, D. J.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Martin, R. L.; Kahn, L. R.; Stewart, J. J. P.; Topiol, S.; Pople, J. A. Gaussian Inc., Pittspurgh PA, USA, 1990.
29. Höweler, U.; MOBY, Molecular Modelling on the PC, Version 1.4, Springer-Verlag, Berlin, 1991.