Eur Biophys J (1989) 17:159–166

European Biophysics Journal

Comparative conformational analysis of cholesterol and ergosterol by molecular mechanics

M. Bagiński [1*], A. Tempczyk [2], and E. Borowski [1]

[1] Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, PL-80-952 Gdańsk, Poland
[2] Institute of Chemistry, University of Gdańsk, PL-80-952 Gdańsk, Poland

Received February 4, 1988/Accepted in revised form May 15, 1989

Abstract. A comparative conformational analysis of cholesterol and ergosterol has been carried out using molecular mechanics methods. These studies are aimed at giving a better understanding of the molecular nature of the interaction of these sterols with polyene macrolide antibiotics. Structures of cholesterol and ergosterol determined by X-ray methods have been used as initial geometries of these molecules for force field calculations. The calculation of steric energy has also been made for conformations which do not appear in the crystal. The latter conformers have different conformations of the side chain as well as different conformations of rings A and D. The rotational barriers around bonds C17–C20 and C20–C22 have also been calculated. The results obtained on differences and similarities in the conformations of cholesterol and ergosterol allow us to postulate a mechanism for differential interaction with the antibiotics. The relatively rigid side chain of ergosterol (stretched molecule) in comparison with the flexible side chain of cholesterol (bent molecule), allows better intermolecular contact of the first sterol molecule with a polyene macrolide and in consequence facilitates complex formation involving Van der Waal's forces.

Key words: Conformational analysis, sterol conformation, cholesterol, ergosterol, molecular mechanics calculations

Introduction

Cholesterol and ergosterol are well-known steroids which play an essential role in the cell membrane of eucaryotic organisms (Oldfield and Chapman 1972; Demel and De Kruyff, 1976). Ergosterol is the principal fungal sterol while cholesterol is the sterol found in animal cell membranes. Both sterols are targets for polyene macrolide antifungal antibiotics such as amphotericin B (AmB). The anticellular action of AmB and other polyene macrolides is due to an interaction with the sterol in the cell membrane (for recent reviews see: Bolard 1986; Medoff et al. 1983; Cybulska 1981). It has been suggested that the relative specificity of AmB for fungi is based on a difference in the affinity of the drug for ergosterol and cholesterol (Kotler-Brajtburg et al. 1974; Teerlink et al. 1980). The molecular basis for the preferential binding of AmB to ergosterol, as compared to cholesterol, is unknown. Ergosterol differs from cholesterol in having an additional methyl group at C24 in the side chain and two additional double bonds, one at C7 in ring B of the nucleus and the other one at C22 in the side chain (Fig. 1a and b). It was proposed that the significant structural feature in ergosterol which determines its greater ability to interact with AmB is the double bond at C7 in ring B (Clejan and Bittman 1985). It has been also postulated that the amphotericin B-sterol interac-

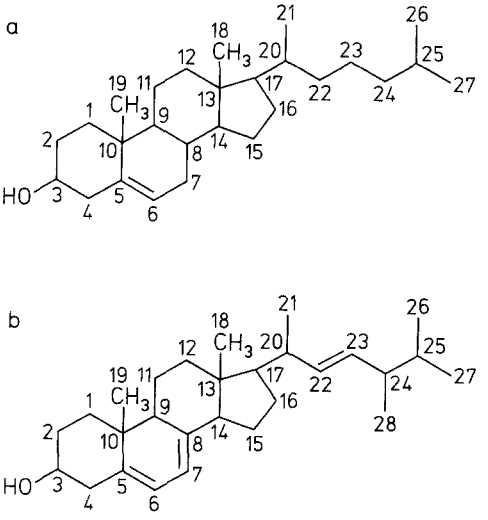

Fig. 1a. Cholesterol – cholest-5-en-3β-ol. **b.** Ergosterol – ergosta-5,7-dien-3β-ol

* To whom offprint requests should be sent

tion depends on the structure of the side chain of the sterol (Nakamura et al. 1980; Hervé et al. 1989; Debouzy et al. 1988). However, the question still remains whether the differential effect on ergosterol as compared to cholesterol containing membranes originates solely from a preferential binding of AmB to ergosterol (direct effect) or from different consequences of the binding (indirect effect), due to the different influence of ergosterol and cholesterol on the overall properties of the membrane.

In the present paper, molecular mechanics calculations were used to carry out a comparative conformational analysis for the above sterols. This is a theoretical approach to the problem which allows us to answer some questions at the molecular level. The primary structural differences between cholesterol and ergosterol are rather small. Therefore it is of importance to look for other molecular differences, including conformation, which might throw some light on the molecular nature of the differential interaction of polyene macrolides with these sterols. This in turn, could facilitate the rational modification of these antibiotics aimed at the development of antifungal drugs with improved selective toxicity.

There are few physical methods (Kirk 1982, 1983, 1986) for determining the structure of steroids but force field calculations present an economical way to obtain realistic geometries and to perform a conformational analysis for both sterols. Generally, this is a useful method for all steroids (Romers et al. 1974; Schneider et al. 1982; Duax and Fronckowiak 1982; Duax and Griffin 1985), for which molecular mechanics calculations are in good agreement with X-ray solid state and NMR studies.

Each of the substituents in the steroidal nucleus changes the molecular geometry a little, but in spite of this fact the carbocyclic nucleus is rather rigid and only the side chain is flexible (Stockton and Smith 1976; Dufourc et al. 1984). In the present calculations the rings A, B and D were taken into consideration. The conformation of the side chain and rotational barriers around bonds C17–C20 and C20–C22 have also been considered.

Theory and methods

All calculations were carried out by means of the MM2 (Allinger and Yuh 1980) and MM2P (Allinger and Yuh 1982) programs for cholesterol and ergosterol respectively (program MM2P is MM2 with MMP1 PI subroutines). The total strain energy was formed by a stretching, a bending, a stretch-bend, a torsional and a non-bonded term using the standard MM2 force field including lone pairs on the hydroxyl oxygen. For the delocalized electronic system (Π-system) in ergosterol, a standard VESCF calculation was undertaken in the program MM2P (Allinger and Sprague 1973). Initial geometry (only heavy atoms) for both compounds was taken from the crystallographic data (Shieh et al. 1981; Hull and Woolfson 1976). The hydrogen atoms were allocated using standard internal coordinates for hydrocarbons. To calculate the energy for an envelope conformation in ring D the torsion angle C13–C17–C16–C15 was constrained. For geometries, the energy value was obtained by full relaxation of all internal coordinates except the torsion angles C16–C17–C20–C22 and

Table 1. Calculated torsion angles (decimal degrees) of cholesterol side chain in the eight independent molecules and relative steric energy (kcal/mol)

Molecule	ω_0	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	E
A	−54	−177	−179	172	171	62	−175	0.15
B	−55	−178	−170	173	174	63	−174	0.00
C	−58	176	60	176	−64	−179	− 56	0.54
D	−55	−178	−176	173	172	63	−174	0.02
E	−54	−177	−178	173	171	63	−175	0.16
F	−56	−179	−168	172	175	63	−174	0.01
G	−58	176	60	177	−64	−179	− 56	0.54
H	−54	−177	−174	174	173	64	−173	0.01

Table 2. Calculated torsion angles (decimal degrees) of ergosterol side chain in the two independent molecules and relative steric energy (kcal/mol)

Molecule	ω_0	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	ω_7	E
A	−65	173	−112	179	128	180	−58	−108	0.00
B	−60	180	−123	−179	103	−63	62	−130	0.41

C17–C20–C22–C23 during rotational barrier calculations. Torsional angle increments of 15° were used in the computation of the strain energy for rotations around the C17–C20 and C20–C22 bonds. The dihedral angles are defined:

ω_0 = C13–C17–C20–C21
ω_1 = C13–C17–C20–C22
ω_2 = C17–C20–C22–C23
ω_3 = C20–C22–C23–C24
ω_4 = C22–C23–C24–C25
ω_5 = C23–C24–C25–C26
ω_6 = C23–C24–C25–C27
ω_7 = C22–C23–C24–C28 for ergosterol

All calculations were performed on EMC R-32 or EMIX 86XT computers.

Results

The first step was a calculation of the energy for all independent molecules from the crystallographic unit cell (Shieh et al. 1981; Hull and Woolfson 1976). Calculated torsion angles for cholesterol and ergosterol side chains are presented in Tables 1 and 2, respectively. The corresponding energies recorded in these tables are relative to the most stable conformation B for cholesterol and A for ergosterol. Six of the eight chains in the cholesterol crystal are in the typical extended, all-*trans* conformation with (−)*syn*clinal conformation for C24–C25 bond[1]. The other two molecules (C and G) have their side chain in the (+)*syn*clinal conformation for the C20–C22 bond, (−)*syn*clinal conformation for C23–C24 bond and (+)*syn*clinal conformation for C24–C25.

The second step was a comparison between calculated data from molecular mechanics and structural features from X-ray studies. It was only made for the carbocyclic rings. Bond distances, valence angles and torsion angles for cholesterol, averaged over the eight independent molecules, are given in Tables 3a, b, c respectively. Table 4a, b, c gives the same values for ergosterol, averaged over the two independent molecules. In view of the results from Table 3 the agreement for bonds, valency and torsion angles is surprisingly good. For ergosterol the agreement is not so good because the X-ray data are of poor quality. Owing to its high sensitivity to X-rays, the ergosterol crystal suffered severe radiation damage during data collection (R is 0.242 and estimated standard deviations are high) (Hull and Woolfson 1976).

In order to compare conformations of the cholesterol and ergosterol nucleus, the parameters of asymmetry were calculated. In Table 5 the parameters of asymmetry are listed for rings A and B for both calcu-

1 According to the IUPAC nomenclature convention (Pure and Applied Chemistry (1976) 45:11–13) when two substituents are chemically indistinguishable a third one, hydrogen atom at C25 in this case, determines the conformation

Table 3a. Comparision of experimental and calculated bond distances (Å), averaged over the eight independent molecules in cholesterol carbocyclic rings

Bond	Exp.	Calc.	Bond	Exp.	Calc.
1–10	1.557	1.551	9–11	1.547	1.545
1– 2	1.536	1.535	11–12	1.532	1.540
2– 3	1.504	1.534	12–13	1.533	1.542
3– 4	1.527	1.533	13–14	1.542	1.546
4– 5	1.512	1.515	8–14	1.519	1.536
5–10	1.522	1.526	14–15	1.544	1.530
5– 6	1.325	1.343	15–16	1.555	1.541
6– 7	1.499	1.500	16–17	1.567	1.553
7– 8	1.533	1.535	13–17	1.552	1.555
8– 9	1.542	1.544	10–19	1.549	1.546
9–10	1.548	1.562	13–18	1.537	1.543

Table 3b. Comparision of experimental and calculated valency angles (decimal degrees), averaged over the eight independent molecules in cholesterol carbocyclic rings

Angle	Exp.	Calc.	Angle	Exp.	Calc.
2– 1–10	114.3	114.7	8– 9–11	111.5	111.5
3– 2– 1	110.4	110.1	9–11–12	114.6	114.2
4– 3– 2	110.3	110.0	11–12–13	111.2	111.8
5– 4– 3	111.2	110.8	12–13–14	106.5	105.0
10– 5– 4	116.6	117.4	13–14– 8	115.4	115.0
1–10– 5	108.3	108.5	14– 8– 9	109.7	110.0
10– 5– 6	122.6	122.8	13–14–15	104.5	104.2
5– 6– 7	125.3	124.9	14–15–16	103.4	103.0
6– 7– 8	112.7	112.7	15–16–17	106.8	107.8
7– 8– 9	108.8	109.8	16–17–13	103.4	103.1
8– 9–10	112.5	112.3	17–13–14	100.2	99.9
9–10– 5	110.0	110.5			

Table 3c. Comparision of experimental and calculated torsion angles (decimal degrees), averaged over the eight independent molecules in cholesterol carbocyclic rings

Angle	Exp.	Calc.	Angle	Exp.	Calc.
10– 1– 2– 3	−56	−56	11– 9– 8–14	−49	−48
1– 2– 3– 4	58	58	9– 8–14–13	57	58
2– 3– 4– 5	−56	−56	8–14–13–12	−61	−61
3– 4– 5–10	53	53	14–13–12–11	56	58
4– 5–10– 1	−48	−47	13–12–11– 9	−55	−56
5–10– 1– 2	49	49	12–11– 9– 8	50	49
10– 5– 6– 7	1	−1	17–13–14–15	47	47
5– 6– 7– 8	13	16	13–14–15–16	−34	−36
6– 7– 8– 9	−43	−44	14–15–16–17	8	10
7– 8– 9–10	62	60	15–16–17–13	21	19
8– 9–10– 5	−47	−45	16–17–13–14	−41	−39
9–10– 5– 6	16	16			

Table 4a. Comparision of experimental and calculated bond distances (Å), averaged over the two independent molecules in ergosterol carbocyclic rings

Bond	Exp.	Calc.	Bond	Exp.	Calc.
1–10	1.577	1.549	9–11	1.571	1.546
1– 2	1.537	1.535	11–12	1.570	1.539
2– 3	1.666	1.534	12–13	1.620	1.542
3– 4	1.551	1.532	13–14	1.660	1.543
4– 5	1.459	1.513	14– 8	1.405	1.508
5–10	1.669	1.525	14–15	1.500	1.530
5– 6	1.390	1.351	15–16	1.554	1.541
6– 7	1.432	1.470	16–17	1.719	1.552
7– 8	1.369	1.350	17–13	1.579	1.551
8– 9	1.657	1.518	10–19	1.478	1.545
9–10	1.545	1.563	13–18	1.421	1.543

Table 4b. Comparision of experimental and calculated valency angles (decimal degrees), averaged over the two independent molecules in ergosterol carbocyclic rings

Angle	Exp.	Calc.	Angle	Exp.	Calc.
2– 1–10	111.0	114.6	8– 9–11	108.4	112.3
3– 2– 1	105.8	109.8	9–11–12	111.4	114.6
4– 3– 2	104.1	109.9	11–12–13	106.3	111.5
5– 4– 3	108.4	111.7	12–13–14	101.6	105.4
10– 5– 4	117.5	118.5	13–14– 8	112.8	112.7
1–10– 5	105.5	109.8	14– 8– 9	116.9	115.6
10– 5– 6	113.8	120.0	13–14–15	103.8	104.1
5– 6– 7	127.2	121.3	14–15–16	106.4	103.3
6– 7– 8	124.0	120.3	15–16–17	105.9	107.6
7– 8– 9	113.3	119.8	16–17–13	98.8	103.8
8– 9–10	113.7	112.4	17–13–14	98.7	100.6
9–10– 5	109.6	109.1			

Table 4c. Comparision of experimental and calculated torsion angles (decimal degrees), averaged over the two independent molecules in ergosterol carbocyclic rings

Angle	Exp.	Calc.	Angle	Exp.	Calc.
10– 1– 2– 3	−67	−57	11– 9– 8–14	−39	−41
1– 2– 3– 4	71	60	9– 8–14–13	54	53
2– 3– 4– 5	−65	−55	8–14–13–12	−67	−61
3– 4– 5–10	59	48	14–13–12–11	67	61
4– 5–10– 1	−50	−42	13–12–11– 9	−62	−53
5–10– 1– 2	54	46	12–11– 9– 8	43	41
10– 5– 6– 7	0	−6	17–13–14–15	51	46
5– 6– 7– 8	−17	−15	13–14–15–16	−34	−36
6– 7– 8– 9	−2	−1	14–15–16–17	5	11
7– 8– 9–10	35	31	15–16–17–13	27	16
8– 9–10– 5	−48	−46	16–17–13–14	−44	−37
9–10– 5– 6	32	36			

lated compounds – conformation A in ergosterol and conformation B in cholesterol (see Tables 2 and 1). Using standard parameters of asymmetry ΔC_s and ΔC_2 (Duax et al. 1976) it is possible to measure the degree of departure from ideal symmetry. Related torsion angles are compared in a way that will result in a

Table 5. Conformations of rings A, B and D of cholesterol and ergosterol (molecule B in Table 1 and A in Table 2) as described by deviation from ideal forms (parameters of asymmetry for rings A and B, parameters of pseudosymmetry for ring D)

Angle (decimal degrees)	Parameters of asymmetry or pseudosymmetry
Cholesterol	
1– 2– 3– 4 = 58.7	Ring A
2– 3– 4– 5 = −56.5	
3– 4– 5–10 = 52.6	$\Delta C_s(3) = 2.5$
4– 5–10– 1 = −46.6	Chair conformation
5–10– 1– 2 = 47.9	
10– 1– 2– 3 = −56.1	
5– 6– 7– 8 = 14.8	Ring B
6– 7– 8– 9 = −42.6	
7– 8– 9–10 = 59.9	$\Delta C_2(5–6) = 2.5$
8– 9–10– 5 = −45.6	Half-chair conformation
9–10– 5– 6 = 16.6	
10– 5– 6– 7 = − 1.4	
ϕ_0 = 47.5	Ring D
ϕ_1 = − 39.2	
ϕ_2 = 18.1	Δ = 8.1 ϕ_{max} = 48.2 (for $j = 2$)
ϕ_3 = 10.8	13β, 14α-half-chair conformation
ϕ_4 = − 36.2	
Ergosterol	
1– 2– 3– 4 = 59.7	Ring A
2– 3– 4– 5 = −54.6	
3– 4– 5–10 = 47.8	$\Delta C_s(3) = 6.61$
4– 5–10– 1 = −42.2	Chair conformation
5–10– 1– 2 = 46.5	
5– 6– 7– 8 = −15.0	Ring B
6– 7– 8– 9 = − 0.2	
7– 8– 9–10 = 32.0	$\Delta C_2(6–7) = 4.4$
8– 9–10– 5 = −47.3	5,7-diplanar conformation
9–10– 5– 6 = − 5.2	
ϕ_0 = 45.7	Ring D
ϕ_1 = − 37.2	
ϕ_2 = 16.2	Δ = 5.0 ϕ_{max} = 46.3 (for $j = 2$)
ϕ_3 = 11.7	13β, 14α-half-chair conformation
ϕ_4 = − 35.6	

value of zero if the symmetry in question is present. Mirror related torsion angles are inversely related (same magnitude, opposite sign) and such torsion angles are compared by addition. The two-fold related torsion angles are directly related (same magnitude and sign) and are compared by substraction. Equation (1) is used to calculate the mirror plane asymmetry parameters (ΔC_s), and Eq. (2) is used to calculate the two-fold asymmetry parameters (ΔC_2).

$$\Delta C_s = \sqrt{\frac{\sum_{i=1}^{m} (\phi_i + \phi_i')^2}{m}} \tag{1}$$

$$\Delta C_2 = \sqrt{\frac{\sum_{i=1}^{m} (\phi_i - \phi_i')^2}{m}}. \tag{2}$$

Here m is the number of individual comparisons and ϕ_i and ϕ_i' are the symmetry related torsion angles. For ring D another two parameters, ϕ_m and Δ (Altona et al. 1968), are presented in Table 5. The ϕ_m is the maximum angle of puckering and Δ is the phase angle of pseudorotation which are related to the endocyclic torsion angle ϕ_j by Eqs. (3) and (4):

$$\phi_j = \phi_m \cos(\Delta/2 + j\delta), \tag{3}$$

where $j = 0, 1, 2, 3$ or 4 and $\delta = 144°$ and

$$\tan(\Delta/2) = [(\phi_2 + \phi_4) - (\phi_1 + \phi_3)]/(3.0777\,\phi_0). \tag{4}$$

The numbering of torsion angles used in ring D is indicated in Fig. 2. Ring A in cholesterol is in the chair conformation. Ring B is in the half-chair conformation and ring D is in the 13β, 14α-half-chair conformation. Ring A in ergosterol is in the chair conformation. Ring B is in 1,3-diplanar chair conformation and ring D is in 13β, 14α-half-chair conformation. These are typical conformations for the rings in steroids.

In the next step the conformation was changed independently for rings A and D in the cholesterol molecule. The calculation was performed for the new initial geometry (conformation B – see Table 1). Table 6 presents the results of these computations together with the relative energy compared to the conformation listed in Table 5. Ring A is in the twist conformation. Ring D is in the 14α-envelope conformation or the 13β-envelope conformation. Each change of conformation of the D or A ring caused increasing steric energy. New conformations are not energetically preferable. Rings A and D in ergosterol have the same conformation as in the cholesterol molecule; for this reason independent calculations were not carried out.

The fourth stage was a conformational analysis of the alkyl side chain. For both compounds the most energetically stable conformations obtained during the first step calculation (see Tables 1 and 2) were taken as the models for generation of new conformations. Conformer B was chosen for cholesterol, and conformer A for ergosterol. Several new conformations of the side chain were generated for cholesterol and ergosterol. It was not possible to generate all the side chain conformers to find the global minimum as there are several hundred possible conformers. Only sectional analysis was carried out to find differences between cholesterol and ergosterol. The side chain torsion angles for every conformation are listed in Table 7a and b for cholesterol and ergosterol respectively. Next to the angles is recorded the relative energy compared to the most stable conformation listed in Tables 1 and 2. Some of the conformations of the side chain presented in Table 7a exist in the solid state in compounds having the same alkyl side chain (Duax et al. 1980).

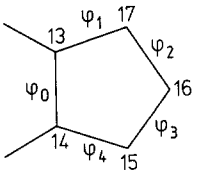

Fig. 2. Convention for the numbering of endocyclic torsion angles in ring D

Table 6. Conformations of rings A and D of cholesterol after change as described by deviation from ideal forms (parameters of asymmetry for ring A, parameters of pseudosymmetry for ring D). Relative energy (kcal/mol) (compared to molecule B in Table 1) is listed together with parameters

Angle (decimal degrees)	Parameters of asymmetry or pseudosymmetry and relative energy
Cholesterol	
1– 2– 3– 4 = – 27.8	Ring A $E = 4.69$
2– 3– 4– 5 = – 32.6	
3– 4– 5–10 = 68.2	$\Delta C_s(3) = 3.4$
4– 5–10– 1 = – 32.4	Twist conformation
5–10– 1– 2 = – 32.6	
10– 1– 2– 3 = 64.9	
$\phi_0 =$ 45.7	Ring D $E = 0.78$
$\phi_1 =$ – 43.2	
$\phi_2 =$ 27.5	$\Delta = 32.2$ $\phi_{max} = 49.2$ (for $j = 2$)
$\phi_3 =$ 0.8	13β-envelope conformation
$\phi_4 =$ – 29.3	
$\phi_0 =$ 46.8	Ring D $E = 2.49$
$\phi_1 =$ – 28.4	
$\phi_2 =$ 0.1	$\Delta = -35.6$ $\phi_{max} = 49.2$ (for $j = 0$)
$\phi_3 =$ 28.6	14α-envelope conformation
$\phi_4 =$ – 46.2	

The last step was a calculation of rotational barriers. The most important positions in the side chain are bonds C20–C22 and C17–C20. Because of steric hindrance it can be assumed that rotational barriers for these bonds are high in comparision with the other bonds of the side chain. For these two bonds rotational barriers were found. Figure 3a presents curves of the potential energy as a function of rotational angle for cholesterol. The curves for ergosterol are given in Fig. 3b. Cholesterol has a three-fold rotational barrier for both bonds. Ergosterol has a three-fold rotational barrier for the bond C17–C20 and a two-fold barrier for the bond C20–C22.

The energetically prefered conformation (–)*anti*clinal or (+)*syn*clinal for the bond C20–C22 with other combinations for the bond C23–C24 effect the overall shape so that most of the ergosterol conformers are flat. The cholesterol side chain is more flexible and its flat shape is only one of several possible conformations.

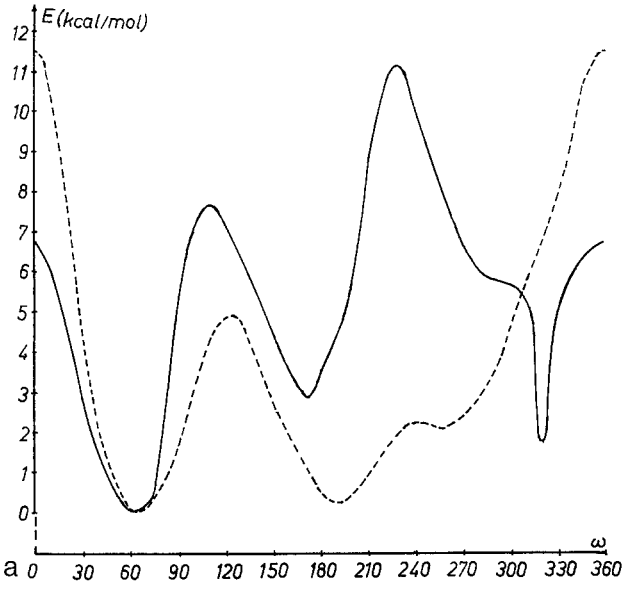

Fig. 3a. Rotational barriers around bond C17–C20 (torsion angle C16–C17–C20–C22 is changed – solid curve) and bond C20–C22 (torsion angle C17–C20–C22–C23 is changed – dashed curve) in cholesterol

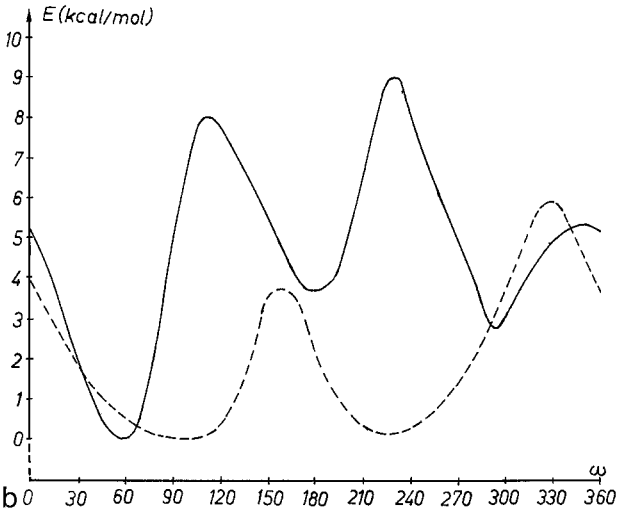

Fig. 3b. Rotational barriers around bond C17–C20 (torsion angle C16–C17–C20–C22 is changed – solid curve) and bond C20–C22 (torsion angle C17–C20–C22–C23 is changed – dashed curve) in ergosterol

Table 7a. Calculated torsion angles (decimal degrees) of cholesterol side chain in other generated structures and relative steric energy (kcal/mol) compared to conformation B in Table 1

Conform.	ω_0	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	E
B1	−56	179	64	176	176	64	−173	−0.16
B2	−56	179	63	178	−175	174	−63	−0.18
B3	−55	−178	−173	175	−177	174	−63	0.09
B4	−53	−175	−89	−180	179	64	−173	1.84
B5	−51	−172	−74	−168	−170	176	−61	2.00
B6	−56	−179	−170	173	−62	−177	−55	0.78
B7	71	−58	−165	176	171	62	−175	2.91
B8	71	−58	−165	177	−178	172	−65	2.91
B9	−147	88	−160	174	−177	171	−67	1.81
B10	−147	88	−163	173	173	61	−176	1.74
B11	−56	178	62	173	61	58	−179	0.25
B12	−57	−180	−173	59	177	172	−66	0.71
B13	−55	−177	−172	170	58	58	−179	0.49

Table 7b. Calculated torsion angles (decimal degrees) of ergosterol side chain in other generated structures and relative steric energy (kcal/mol) compared to conformation A in Table 2

Conform.	ω_0	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	ω_7	E
A1	−63	176	−105	177	144	180	−58	−91	0.29
A2	−64	175	−105	177	161	−59	66	−73	1.24
A3	−64	174	−107	178	169	72	−163	−65	1.64
A4	−61	178	−130	−179	123	−179	−57	−112	0.02
A5	−60	175	78	180	131	−178	−55	−104	0.22
A6	−65	175	−125	178	−76	180	−58	51	0.18
A7	−61	178	−118	−178	−62	179	−58	64	0.14
A8	−61	178	−141	−179	−66	−177	−54	60	0.26
A9	−65	171	56	180	−69	−177	−54	57	0.70
A10	−63	173	66	−180	−63	−177	−55	64	0.56
A11	−65	173	−113	179	123	−180	−57	−113	0.08

Discussion

Conformational analysis allows us to draw some conclusions about the structure of the cholesterol and ergosterol molecules. The calculated geometry for the carbocyclic skeleton is almost the same in all independent cholesterol and ergosterol molecules. These calculated data are in a good agreement with the experimental structure in the solid state (defined by X-ray study). It means that all independent molecules (eight for cholesterol and two for ergosterol) in the solid state are near the energy minimum. Crystal packing forces only slightly deform the carbocyclic rings. The cholesterol nucleus with the chair conformation in ring A, the half-chair conformation in ring B and the 13β, 14α-half-chair conformation in ring D is the most stable one. Similarly, the ergosterol nucleus with the chair conformation in ring A, the 1,3-diplanar chair conformation in ring B and the 13β, 14α-half-chair conformation in ring D is also the most stable one.

The fact that the sterol nucleus is rigid is rather well-known. Present calculations confirm this and give some more information on the rigidity of alternative structures. As can be seen from Tables 5 and 6 differences between two levels corresponding to energy-minimum conformations amount to 4.69 kcal/mol for ring A and 0.78 or 2.49 kcal/mol for ring D. The cholesterol molecule needs more energy to change the conformation of the A or D rings. The barrier for inversion of the cyclohexane ring lies at about 10.5 kcal/mol above the chair form (Allinger 1977). Hence only small deformations from the optimal structure of the cholesterol nucleus are available and probably the most flexible places are rings D (the energy level of the 13β-envelope is near the level of the 13β, 14α-half-chair) and B (Duax et al. 1980). Of course such deformations in ring B do not change the overall conformation and are similar to those occuring in the solid state. The fact that only the rigid carbocyclic nucleus can be taken into account is also important for interaction with flat polyenes (Ganis et al. 1971).

Selective conformational analysis of the side chain confirms that conformers appearing in the solid state (B2, B3, B11, B12, B13 – see Table 7a)[2] (Duax et al. 1980) are energetically most favoured. This explains why all of them are so typical in the crystal state. Other conformations have only a slightly higher potential energy from 0.78 to 2.91 kcal/mol. This means that most of them can also exist. In this case only rotational barriers limit a change of conformation.

The calculations allow us to define some similarities and differences between the structures of cholesterol and ergosterol with regard to their interaction with polyenes:

Similarities

a) In spite of the different conformations in ring B the overall nucleus shape of both sterols is similar and relatively flat.
b) The position of the hydroxyl group in relation to the carbocyclic ring in cholesterol and ergosterol is the same.
c) Both cholesterol and ergosterol nuclei are rigid and only small deformations are possible.
d) The side chain is flexible in the part comprising carbon atoms C23–C27 (or C23–C28 for ergosterol). Owing to the steric hindrance of the methyl group at C13 and C20, and the hydrogen atoms at C16 the rotational barriers for bond C17–C20 are high in both cases.

Differences

a) The conformation of ring B in cholesterol differs from the conformation of this ring in ergosterol. Ergosterol has an additional double bond in ring B. This means that ring B in ergosterol can be deformed in a different way.
b) The sterols have different geometries of the side chain. The existance of the double bond in the ergosterol side chain constrains this chain and limits the number of conformers (the bond C22–C23 does not rotate, bonds C20–C22 and C23–C24 have only a two-fold barrier for rotation).

The results of our studies show some differences in the conformation of cholesterol and ergosterol. Because the source of antibiotic receptor binding energies are probably Van der Waal's interactions – i.e., the dispersion energy – with sterols (De Kruyff et al. 1974 and papers cited there), the data obtained about these differences can be useful for understanding the molecular nature of different affinity of the polyene macrolide molecule for both sterols. This problem requires further study. However it may be expected that interaction between flat sterol and flat polyene would be the strongest, as in such a case the contact between both molecules is optimal, as judged from inspection of space-filling models (De Kruyff and Demel 1974). The hydrophobic interaction of the rigid double bond system of amphotericin B with the rigid steroid nucleus and relatively less flexible side chain of ergosterol must be more favourable than the interaction between the same part of amphotericin B with the rigid steroid nucleus and more flexible alkyl chain of cholesterol. This suggests that the conformational possibilities of the hydrocarbon side chain of both sterols molecules may determine the selectivity of their interaction with

2 Conformers in Duax paper are D_2, A, D_3, C, and B respectively

the polyene backbone. Further experiments as well as calculations are required to substantiate this proposition.

Acknowledgements: The authors acknowledge financial support for these studies from Project RPBP-R.P.II.10., Ministry of National Education, Poland. We are also grateful to A. Konitz for help with computer programs.

References

Allinger NL (1977) Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V_1 and V_2 torsional terms. J Am Chem Soc 99:8127–8134

Allinger NL, Sprague JT (1973) Calculation of the structures of hydrocarbons containing delocalized electronic systems by the molecular mechanics method. J Am Chem Soc 95:3893–3907

Allinger NL, Yuh YH (1980) Computer program MM2, University of Georgia, Athens (USA)

Allinger NL, Yuh YH (1982) Computer program MM2P, University of Georgia, Athens (USA)

Altona C, Geise HJ, Romers C (1968) Conformation of non-aromatic ring compounds – XXV. Geometry and conformation of ring D in some steroids from X-ray structure determinations. Tetrahedron 24:13–32

Bolard J (1986) How do the polyene macrolide antibiotics affect the cellular membrane properties? Biochim Biophys Acta 864:257–304

Clejan S, Bittman R (1985) Rates of Amphotericin B and filipin association with sterols. J Biol Chem 260:2884–2889

Cybulska B (1981) The role of structural factors in the modification of membrane permeability by polyene macrolide antibiotics. In: Proceedings of Sixth School on Biophysics of Membrane Transport (School Proceedngs, Poland), pp 124–149

Debouzy JC, Hervé M, Borowski E, Cybulska B, Neumann JM, Gary-Bobo CM (1988) Study by ^{31}P, ^{13}C NMR of the interaction of polyene antibiotics with sterols: antifungal selectivity. In: Proceedings of 2nd International Symposium on Molecular Aspects of Chemotherapy (Symposium Abstracts, Poland), pp 84

De Kruyff B, Demel RA (1974) Polyene antibiotic-sterol interactions in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes. III. Molecular structure of the polyene antibiotic-cholesterol complexes. Biochim Biophys Acta 339:57–70

De Kruyff B, Gerritsen WJ, Oerlemans A, Demel RA, Van Deenen LLM (1974) Polyene antibiotic-sterol interactions in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes. I. Specifity of the membrane pereability changes induced by the polyene antibiotics. Biochim Biophys Acta 339:30–40

Demel RA, De Kruyff B (1976) The function of sterols in membranes. Biochim Biophys Acta 457:109–132

Duax WL, Fronckowiak MD (1982) A comparison between crystallografic data and molecular mechanics calculations on the side chain and backbone conformations of steroids. In: Intramolecular Dynamics (Jerusalem Symp Quantum Chem Biochem). D Reidel, Dordrecht, pp 505–524

Duax WL, Griffin JF (1985) A comparison of crystallographic observations and molecular mechanics calculations of the conformations of steroids and steroids analogs. Stud Org Chem 20 (Nat Prod Chem): 385–396

Duax WL, Weeks CM, Rohrer DC (1976) Crystal structure of steroids. Top Stereochem 9:271–283

Duax WL, Griffin JF, Rohrer DC, Weeks CM (1980) Conformational analysis of sterols: comparison of X-ray crystallographic observations with data from other sources. Lipids 15:783–792

Dufourc EJ, Parish EJ, Chitrakorn S, Smith ICP (1984) Structural and dynamical details of cholesterol-lipid interaction as reveled by deuterium NMR. Biochemistry 23:6062–6071

Ganis P, Avitable G, Mechlinski W, Schaffner CP (1971) Polyene macrolide antibiotic amphotericin B. Crystal structure of the N-iodoacetyl derivative. J Am Chem Soc 93:4560–4564

Hervé M, Debouzy JC, Borowski E, Cybulska B, Gary-Bobo CM (1989) The role of the carboxyl and amino groups of polyene macrolides in their interactions with sterols and their selective toxicity. A ^{31}P-NMR study. Biochim Biophys Acta 980:261–272

Hull SE, Woolfson MM (1976) The crystal structure of erosterol monohydrate. Acta Crystallogr B32:2370–2373

Kirk DN (1982) Physical methods. Terpenoids Steroids 11: 165–186

Kirk DN (1983) Physical methods. Terpenoids Steroids 12: 269–287

Kirk DN (1986) Steroids: Physical methods. Nat Prod Rep 3:505–513

Kotler-Brajtburg J, Price HD, Medoff G, Schlessinger D, Kobayashi GS (1974) Molecular basis for the selective toxicity of amphotericin B for yest and filipin for animal cells. Antimicrob Agents Chemother 5:377–382

Medoff G, Bolard J, Brajtburg J, Kobayashi GS (1983) Antifungal agents useful in therapy of systemic fungal infections. Ann Rev Pharmacol Toxicol 23:303–330

Nakamura T, Nishikawa M, Inoue K, Nojima S, Akiyama T, Sankawa U (1980) Phosphatidylcholine liposomes containing cholesterol analogues with side chains of various lengths. Chem Phys Lipids 26:101–110

Oldfield E, Chapman D (1972) Dynamics of lipids in membranes: heterogenity and the role of cholesterol. FEBS Lett 23:285–297

Romers C, Altona C, Jacobs HJC, De Graaff RAG (1974) Steroid conformations from X-ray analysis data. Terpenoids Steroids 4:531–583

Shieh HS, Hoard LG, Nordman CE (1981) The structure of cholesterol. Acta Crystallogr B37:1538–1543

Schneider HJ, Buchheit U, Gschwendtner W, Lonsdorfer M (1982) Steric distrotions and polar effects in steroids: molecular mechanics calculations and ^{13}C-NMR-investigations. In: Mol Struct Biol Activity Proc. Meet. Elsevier, Amsterdam, pp 165–179

Stockton GW, Smith CP (1976) A deuterium nuclear magnetic resonance study of the condensing effect of cholesterol on egg phosphatidylcholine bilayer membranes. I. Perdeuterated fatty acid probes. Chem Phys Lipids 17:251–263

Teerlink T, De Kruyff B, Demel RA (1980) The action of pimaricin, etruscomycin and amphotericin B on liposomes with varying sterol content. Biochim Biophys Acta 599:484–492