1. The Conformation of Cycloartenol Investigated by NMR and Molecular Mechanics

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Cycloartenol (**4**), a natural plant sterol, was shown to be an effective membrane reinforcer; this was attributed to its conformation. We now present a conformational analysis of **4** by molecular modeling and NMR. Molecular modeling suggests that two conformations **I** and **II** coexist, differing mainly at the level of ring C, and of nearly equal energy, **I** and **II** each having ring A and B in a chair and half-chair conformation, respectively, with ring C 1,3-diplanar in **I** (solid-state structure as determined by X-ray crystallography) and in chair conformation in **II**. A complete assignment of the ^{1}H- and ^{13}C-NMR spectra of **4** and the entire coupling network in rings A and B is determined by various modern NMR techniques. The conformation of rings A and B thus determined is in agreement with conformations **I** and **II**. Low-temperature NMR experiments show a fast equilibrium between two conformations, presumably **I** and **II**. It is concluded, therefore, that the cyclopropane ring of **4** produces a flexibility at the level of ring C which may be important for the membrane properties.

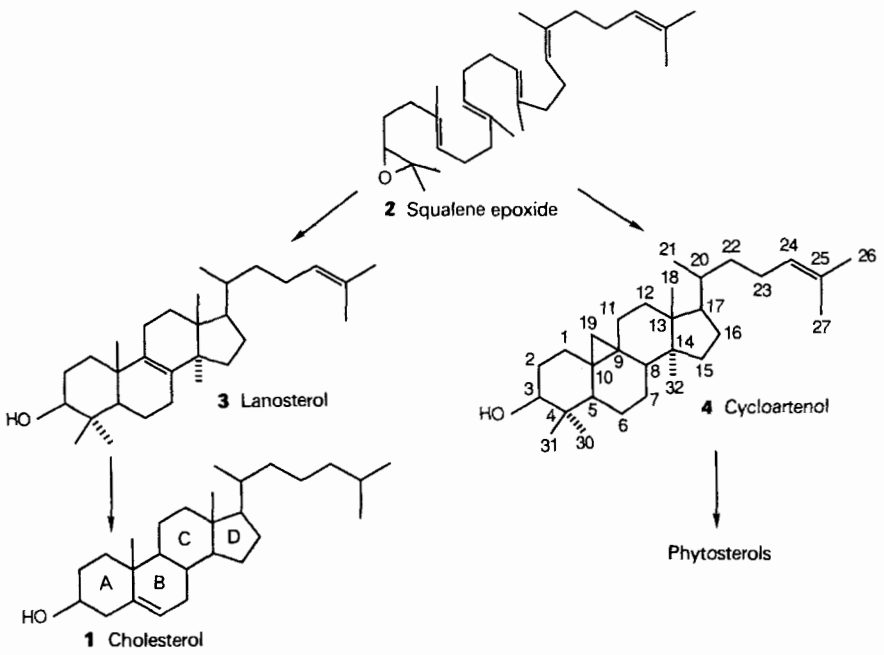
1. Introduction. – Cholesterol (**1**) is a universal reinforcer of eucaryotic phospholipidic membranes [1]. Its biosynthesis *(Scheme)* from squalene epoxide (**2**) proceeds *via* lanosterol (**3**) in animals and fungi, while cycloartenol (**4**) is the precursor in green plants [2] and at least one protozoon, *Acanthamoeba polyphaga* [3].

Lanosterol (**3**) and cycloartenol (**4**) are precursors of the final sterols and are structurally identical, except that in **4**, a cyclopropane ring between C(9), C(10), and C(19)[1]), replaces the CH_3(19) group and the C(8)=C(9) bond of **3**. However, they differ markedly in their interactions with membranes: *Bloch* and coworkers have shown that **4** is, in certain cases, *in vitro* and *in vivo*, nearly as effective as cholesterol (**1**) in stabilizing the membranes, whereas **3** does not play such a role [5–7]. This has been confirmed by the finding of *Benveniste* and coworkers [8] that higher plants, treated with an inhibitor of the cyclopropane-opening enzyme, can survive and then contain 9,10-dihydro-19-norcyclopropa[9,10]sterols as their major membrane sterols.

The remarkable difference of behaviour of the isomers cycloartenol (**4**) and lanosterol (**3**) is obviously linked to their different capacities to induce cooperative intermolecular *van der Waals* interactions with the phospholipidic membrane; a similar 'explanation' lies at the base of the unrelated observation that, while long-chain fatty esters of **3** show no

[1]) Steroid numbering system, according to the IUPAC-IUB rules [4]. We use the following abbreviations for the conformations of the 6-membered rings A–C: chair (*C*), boat (*B*), and half-chair (*H*).

Scheme. *Biosynthetic Pathway of Sterols from Squalene Epoxide* (**2**) via *Lanosterol* (**3**) *or Cycloartenol* (**4**)



second-order phase transition, those of cholesterol (**1**) and **4** form the well known cholesteric liquid-crystal phases [9] [10].

Bloch suggested that a flat α-face of sterol derivatives is essential for a good interaction with phospholipids in membranes and for the stabilizing effect [7]. The axial α-CH_3–C(14) (= C(32)) of **3** would be the most perturbing, followed by α-CH_3–C(4) (= C(30)) and β-CH_3–C(4) (= C(31)), and for that reason, they would have to be removed in the biosynthesis of **1** . Moreover, **4** would have a conformation (rings A–C: *C*/*B*/*C*^{1})) in which α-CH_3–C(14) would not protrude, but rather be embedded in a pocket of axial H-atoms *(Fig. 1)*. In this interesting interpretation of the function of **4**, the

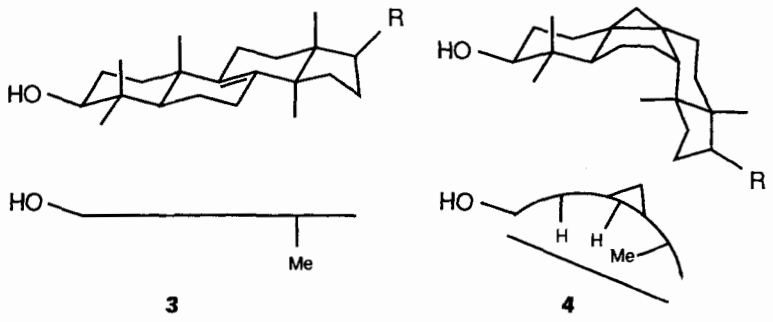


Fig. 1. *Conformation of lanosterol* (**3**) *and cycloartenol* (**4**) *proposed by* Bloch [7]. The bent structure of **4** would give it a flat α-face.

hypothesis of a particular conformation plays a crucial role; it was supported, however, by no other evidence than ball-and-stick models, and highly schematic drawings, and ran against the evidence from X-ray structural studies, which shows that *Bloch*'s hypothetical conformation was not that observed in the crystal (see below).

Modern NMR techniques give now the possibility to analyse completely the conformation of molecules as complex as cycloartenol (**4**), and molecular mechanics makes it possible to compare the energies of various conformers. The study presented here shows that three conformations of **4** are possible, two of which are in fast equilibrium, which leads to the hypothesis that the conformation present in the membranes can be different from that observed in solution at room temperature, and gives a physicochemical basis to *Bloch*'s hypothesis.

2. Results and Discussion. – 2.1. *Comparison of the Conformations in the Solid State.* Crystalline structures of several sterol derivatives are available for comparison, those of cholesteryl octanoate [11], of lanosteryl iodoacetate [12], and of the cycloartenol derivative (23*R*)-3α-methoxy-9,19-cyclo-9β-lanost-24-en-27,23-diol bis(*p*-bromobenzoate) [13].

Using the SYBYL software[2]), the crystallographic data have been extracted from the *Cambridge Crystallographic Database* and analyzed. Simple transformations of these molecules allow the construction of cholesterol (**1**), lanosterol (**3**), and cycloartenol (**4**) models, without modification of the cyclic systems. Of special interest for conformational analysis are the dihedral angles in the polycyclic systems represented in *Fig. 2.* These angles correspond to the following descriptions (following [14]): rings A–C in **1**, *C*/*H*/*C*; rings A–C in **3**, *C*/1,2 diplanar/1,2 diplanar; rings A–C in **4**, *C*/*H*/1,3 diplanar[1]). Comparison of the overall shapes of **3** and **4** in the solid state does not reveal any major difference, and the conformation of **4** revealed by X-ray crystallography is not the bent

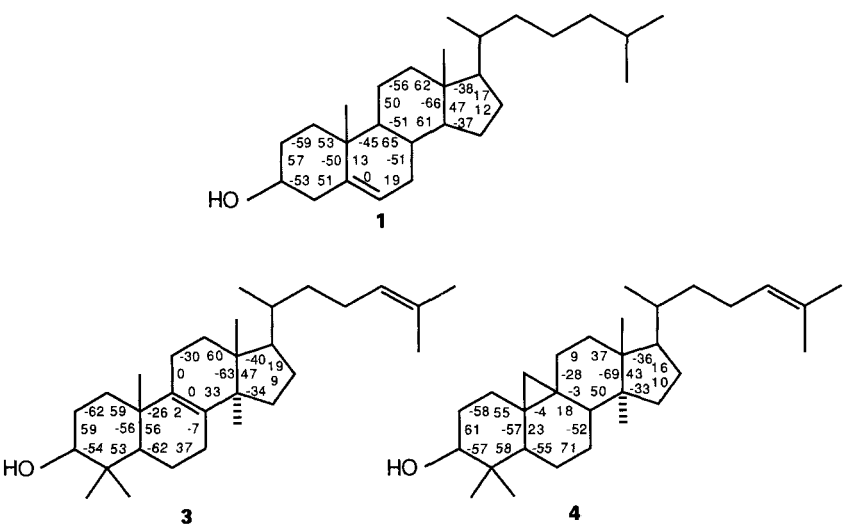


Fig. 2. *Dihedral angles in the cyclic system of cholesterol* (**1**), *lanosterol* (**3**), *and cycloartenol* (**4**) *in the crystalline state*

[2]) SYBYL Molecular Modeling System, Vers. 5,1, *Tripos Associates Inc.*, St. Louis, Missouri, 1987.

one assumed by *Bloch* [7]. However, on the basis of simple *Dreiding* models, the formation of a chair in ring C and the inversion of ring B looks reasonable. Therefore, we have started a detailed conformational analysis using molecular mechanics.

2.2. *Molecular Mechanics of Cycloartenol* (**4**). The use of molecular-mechanics programmes should enable one to investigate conformations of the isolated molecules. Using the SYBYL software, we have constructed several starting conformations for rings A–C among which **I** with a *C/H/*1,3 diplanar (crystal structure), **II** with a *C/H/C*, and **III** with a *C/B/C* conformation (similar to *Bloch*'s assumption; see *Fig. 3*). Energy minimization does not give the global energy minimum but rather the relative minimum closest to the starting conformation; to describe the conformational space of a molecule, it is, therefore, necessary to start from several possible conformations and to perform energy minimization on each of them. Such a procedure, when performed on cyclohexane, cyclohexene, *cis*- and *trans*-decalins and hydrindanes gave results in agreement with experimental data [14] [15].

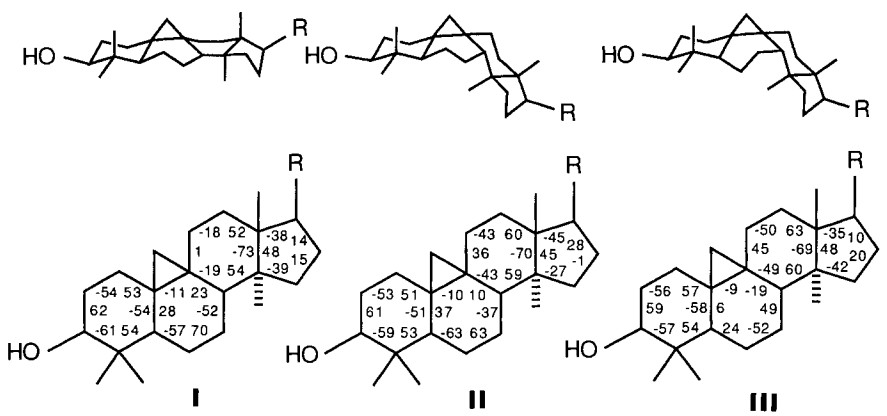


Fig. 3. *Dihedral angles obtained after energy minimization of the structures* **I** (*C/H/H* for rings A–C; crystalline structure), **II** (*C/H/C* for rings A–C), *and* **III** (*C/B/C* for rings A–C; from *Bloch*). In structures **II** and **III**, ring B became 1,2 diplanar.

Energy minimization for the three most stable conformers **I–III** gives the dihedral angles reported in *Fig. 3*. The major geometrical transformations occurring during energy minimization are the following. (*i*) Chain and ring A are not affected. (*ii*) Ring C of **I** is slightly modified and adopts an *H* conformation. (*iii*) In structure **III**, the initial *B* conformation of ring B is transformed into a 1,2-diplanar cyclohexene (in any case, ring B can be described as a cyclohexene because the dihedral angle C(5)–C(10)–C(9)–C(8) is close to zero). (*iv*) The structures **II** and **III** are similar, except for ring B which takes up conformations roughly symmetrical through the plane of the molecule (C(6) up and C(7) down in **II**, C(6) down and C(7) up in **III**), ring C having a *C* conformation in both molecules.

The calculated energies of these conformers are given in *Table 1*. It appears that structure **I** derived by minimal changes from the crystallographic structure is not the most stable one for the isolated molecule: conformer **II**, in which ring C is in a *C* instead of *H* conformation for **I**, is more stable by 6.3 kJ/mol. A conformational change of ring B

Table 1. *Energy* (kJ/mol) *of Minimum-Energy Conformers* **I–III** *of Cycloartenol* (**4**)[a])

Conformation[b])	I	II	III
Bond stretching	2.1	2.9	2.1
Angle bending	437.2	446.8	451.9
Torsional energy	129.6	106.2	127.9
1,4 *van der Waals*	–43.9	–45.6	–44.7
Van der Waals	–58.1	–49.7	–56.0
Total energy	466.9	460.6	481.2
Energy differences	6.3	0	20.6

[a]) The option Maximin2 and the standard force-field parameters of the SYBYL software were used.
[b]) Energy minimization was also performed on six other starting conformations which gave much higher energies than those of **I–III**.

leads, however, to a conformer **III** of slightly higher energy (20.6 kJ/mol). The relatively small differences obtained by these calculations suggest, but do not prove, the possibility of conformational equilibria, and cannot exclude completely any of the three conformations studied. Therefore, we have initiated a detailed NMR study of the conformations prevailing in solution.

2.3. *NMR Analysis.* After assignment of all NMR signals, the conformation of a molecule can, in principle, be deduced from the coupling constants between protons using a *Karplus*-type relationship and from NOE experiments. Experiments at lower temperatures have to be performed to check for conformational equilibria. We have run these studies on cycloartenol (**4**).

2.3.1. *Resonance Assignments.* After preliminary experiments in $CDCl_3$, (D_6)benzene is selected as a solvent because it gives more splitting of the ^{1}H-NMR signals *(Fig. 4)*.

An unambiguous and complete assignment of the ^{13}C-NMR spectrum of **4** in $CDCl_3$ has been obtained by *Kamisako et al.* using ^{13}C labelled cycloartenol biosynthesized from (^{13}C)acetate [16] and has been confirmed by a 2D-INADEQUATE experiment [17]. A ^{1}H,^{13}C correlation (2D-COSY) gives now the ^{1}H-NMR chemical shifts of **4** from the

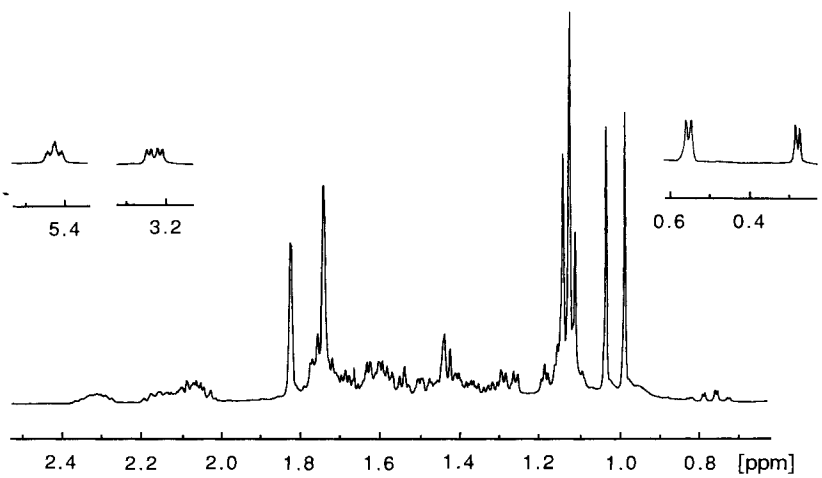


Fig. 4. *400-MHz ^{1}H-NMR ((D_6)benzene) of cycloartenol* (**4**)

Table 2. *Assignment of the ^{13}C- and ^{1}H-NMR Chemical Shifts of Cycloartenol* (**4**)

Atom number	δ(C)		δ(H)[c]	Comments
	$CDCl_3$[a]	C_6D_6[b]		
1	32.06	32.27	1.50 (α), 1.18 (β)	decoupling
2	30.48	30.91	1.72 (α), 1.62 (β)	NOE Me(31)
3	78.90	78.43	3.22 (α)	
4	40.56	40.71	–	
5	47.22	47.34	1.27 (α)	
6	21.17	21.38	1.62 (α), 0.76 (β)	NOE Me(31), $H_{'endo'}$–C(19)
7	26.07	26.36	1.13 (α), 1.38 (β)	from 1D-COSY
8	48.00	48.29	1.56 (β)	
9	20.12	20.05	–	
10	26.25	26.36	–	
11	26.60	26.75	2.09 (α), 1.12 (β)	NOE ME(32), $H_{'endo'}$–C(19)
12	33.02	33.34	1.75 (α, β)	
13	45.40	45.62	–	
14	48.91	49.10	–	
15	35.65	35.94	1.43 (α, β)	
16	28.18	28.56	1.48, 2.08	
17	52.38	52.78	1.76	
18	18.05	18.38	1.13	
19	29.90	30.02	0.55 (*'endo'*), 0.28 (*'exo'*)	NOE H_β–C(6)
20	35.94	36.30	1.62	
21	18.31	18.56	1.12	*d*
22	36.44	36.85	1.30, 1.73	
23	25.02	25.48	2.18, 2.30	
24	125.35	125.77	5.42	
25	130.84	130.82	–	
26	17.64	17.74	1.75	
27	25.70	25.90	1.83	
30	25.49	25.75	1.14	
31	14.04	14.32	0.99	
32	19.36	19.60	1.03	

[a]) From [16].
[b]) At 100.6 MHz, δ relative to TMS (tetramethylsilane), reference C_6D_6 (= 128.01 ppm).
[c]) At 400.1 MHz, δ relative to TMS, reference C_6HD_5 (= 7.27 ppm); δ (±0.02 ppm) from the 2D spectra.

known ^{13}C chemical shifts *(Table 2)*. The assignments of *Table 2* are in complete agreement with the performed $^{1}H,^{13}C$ chemical shift correlation, $^{1}H,^{1}H$ chemical shift correlation, 1D-COSY, *J*-resolved spectroscopy, and NOE.

Our ^{13}C-NMR data ((D_6)benzene) show slight differences (up to 0.5 ppm) when compared with [16] (data for $CDCl_3$). Therefore, there is some ambiguity for C-atoms with very close chemical shifts. This is the case for the pairs $CH_2(7)/CH_2(11)$ and $CH_3(18)/CH_3(21)$. As Me(21) is coupled with H–C(20), it gives a *d* readily recognized in the ^{1}H-NMR spectrum. In the case of $CH_2(7)/CH_2(11)$, the assignment is made by the 1D-COSY experiment described below.

In the case of CH_2 groups with two non-equivalent protons, the distinction between α and β protons is based upon NOE's in the ^{1}H-NMR: irradiation of Me(32) gives NOE's on H_α–C(7), H_α–C(15), H_α–C(12), and H_α–C(11), irradiation of Me(31) on H_β–C(6) and H_β–C(2), and irradiation of H_β–C(6) on $H_{'endo'}$–C(19).

The *qd* of H_β–C(6) at 0.76 ppm is isolated from the other protons. This strong shielding indicates that H_β–C(6) lies in the vicinity of the C_3 axis of the cyclopropane ring. H_β–C(7) is not so strongly influenced by the cyclopropane ring (1.15 ppm), and this is a first indication disfavouring a conformation like **III**.

2.3.2. *Coupling Constants and Dihedral Angles.* A 1D-COSY experiment [18] [19] allows the selective detection of the protons coupled to H_β–C(6) and, therefore, the analysis of the spin system H–C(5), H_α–C(6), H_β–C(6), H_α–C(7), H_β–C(7) *(Fig. 5)*.

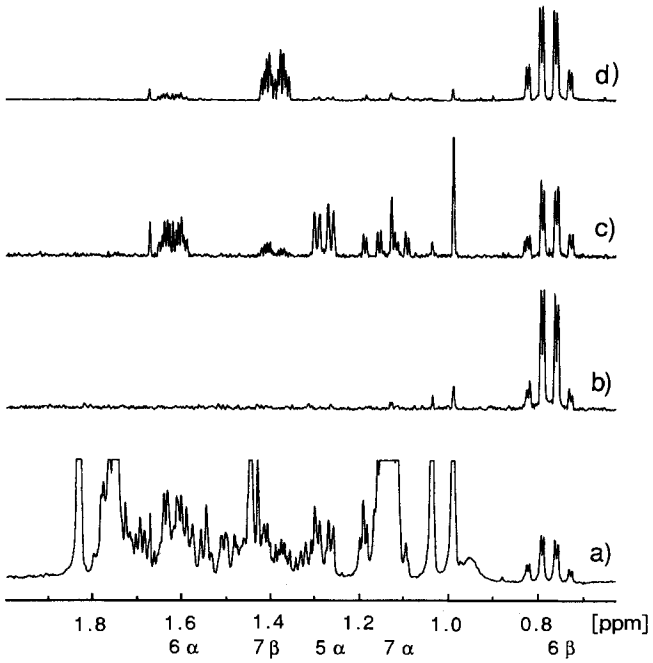


Fig. 5. *1D-COSY spectrum ((D$_6$)benzene) of cycloartenol* (**4**). *a)* Normal spectrum. *b)* Selective excitation of H_β–C(6) with a 60-ms *Gauss*ian pulse (absolute-value spectrum). *c)* 1D-COSY obtained with an additional delay $\tau = 10$ ms, making τ_{eff} equal to 40 ms ($\tau_{eff} = \tau$ + half the duration of the *Gauss*ian pulse); H_α–C(6), H_α–C(5), and H_α–C(7) are observed, with J to H_β–C(6) of *ca.* 12 Hz. *d)* Same as *c)*, with $\tau = 50$ ms, making τ_{eff} equal to 80 ms; H_β–C(7) is then detected ($J = 2.5$ Hz).

After a *Gauss*ian pulse selective for H_β–C(6), there is polarization transfer to the protons coupled to H_β–C(6). The amplitude of transfer depends on the function $\sin(\pi J \tau_{eff})$, where J is the coupling constant and τ_{eff} the time of transfer (τ_{eff} = half the *Gauss*ian pulse duration + delay τ). For $J = 12.5$ Hz, the optimum value of τ_{eff} is 40 ms. This condition detects the protons H_α–C(7), H_α–C(5), and H_α–C(6) which present a J of 12.5 Hz with H_β–C(6) *(Fig. 5c)*. For $\tau_{eff} = 80$ ms, these protons are not detected anymore but instead H_β–C(7) appears *(Fig. 5d)*.

This experiment allows the unambiguous signal assignment and determination of all coupling constants of this spin system by a first-order analysis.

From the *J*-resolved experiment, the determination of the coupling constants in the system H_α–C(1), H_β–C(1), H_α–C(2), H_β–C(2), H_α–C(3) was straightforward.

The ^{1}H,^{1}H-coupling constants of ring A and B of **4** are shown in *Table 3* together with the approximate dihedral angles H–C–C–H calculated by a *Karplus*-type relationship [20]. From these data, approximate values are calculated for the C–C–C–C dihedral angles (using standard values for the angles H–C–H). They correspond to $60 \pm 15°$ and $-60 \pm 15°$, alternatively, in ring A (chair conformation). For ring B, we obtain the angles C(10)–C(5)–C(6)–C(7) = $-60 \pm 15°$, C(5)–C(6)–C(7)–C(8) = $60 \pm 15°$, and C(6)–C(7)–C(8)–C(9) = $-50 \pm 15°$. These values are in agreement with structures **I**

Table 3. *$^1H,^1H$-Coupling Constants* (Hz) *in Rings A and B of Cycloartenol* (**4**), *Approximate Dihedral Angles H–C–C–H in Parentheses*[a])

	Ring A				
	1α	2α	3α	1β	2β
1α		2.5 (74°)		12.5	12.5 (170°)
2α	2.5 (74°)		4.0 (60°)	3.5 (64°)	13.0
3α		4.0 (60°)			12.0 (165°)
1β	12.5	3.5 (64°)			3.5 (64°)
2β	12.5 (170°)	13.0	12.0 (165°)	3.5 (64°)	

	Ring B					
	5α	6α	7α	6β	7β	8β
5α		4.5 (56°)		13.0 (180°)		
6α	4.5 (56°)		3.0 (68°)	13.0	5.0 (53°)	
7α		3.0 (68°)		13.0 (180°)	12.0	12.0 (165°)
6β	13.0 (180°)	13.0	13.0 (180°)		2.5 (74°)	
7β		5.0 (53°)	12.0	2.5 (74°)		4.5 (56°)
8β			12.0 (165°)		4.5 (56°)	

[a]) The absolute values are calculated using $J = 7 - \cos\Phi + 5\cos 2\Phi$ [20]. Due to the uncertainty on the coupling constants and the *Karplus*-type relationship, the angles are given with an error of ±15°.

and **II** and, despite their inaccuracy, exclude structure **III**, that is to say the inversion of ring B or the existence of a *B* conformation for ring B. They are not accurate enough, however, to distinguish undoubtedly between **I** and **II**, although the agreement is better with structure **I**. The same indication comes from the dihedral angle H_β–C(8)–C(7)–H_β which is −53° in **I** and −39° in **II**; the experimental value $J = 4.5$ Hz corresponds to *ca.* ±56°.

A clear distinction between **I** and **II** should have come from the analysis of the spin system H–C(11)/H–C(12). Unfortunately, H_α–C(12) and H_β–C(12) have the same chemical shift so that coupling constants with H_α–C(11) and H_β–C(11) cannot be read out easily, even not in other solvents than benzene ($CDCl_3$, CD_2Cl_2, 6% CD_3OD in CD_2Cl_2).

2.3.3. *Nuclear* Overhauser *Effects*. Giving access to inter-proton distances, NOE's can, in principle, give informations on the solution conformation of molecules. This approach has recently been used by *Nes et al.* in the case of several 9β,19-cyclosterols [21]. They have observed that there is a stronger NOE between Me(18) and H_{endo}–C(19) than between Me(32) and H_{endo}–C(19) which shows the existence of a 'flat' conformation corresponding to our conformation **I**. Their results are unambiguous for dehydropollinasterol in which Me groups are absent at C(4) (which could influence the conformation). They are not so clear with the 4,4-dimethyl compound as the effects observed could also due to interaction with α-CH_3–C(4) (= C(30)). In the case of **4**, there is severe overlap of the resonances of Me(30) and Me(18), and we consider that no convincing conclusion can be obtained.

In our hands, the irradiation of Me(31) gives a clear NOE on Me(30), H_β–C(2), and H_β–C(6) as expected from the structure of **4**. More interesting is the irradiation of Me(32) *(Fig. 6)*: NOE's are observed at the signals of H–C(16)/H_α–C(11) (2.07 ppm), H–C(12)/H–C(17) (1.75 ppm), H–C(15)/H–C(16) (1.46 ppm), and H_α–C(7) (1.15 ppm). For

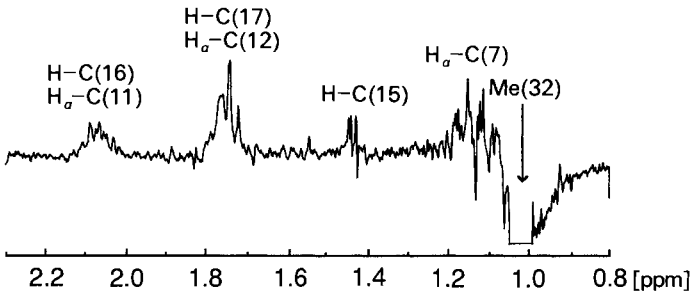


Fig. 6. *NOE difference spectrum ((D$_6$)benzene) of cycloartenol* (**4**) *after irradiation of Me(32) at 1.03 ppm.* Decoupler power 55 L, presaturation during 10 s, 704 scans. NOE's at signals of H–C(16)/H_α–C(11), H–C(17)/H_α–C(12), H–C(15), and H_α–C(7), but not at 1.62 ppm for H_α–C(6).

conformation **III**, the protons closest to Me(32) are H_α–C(6) (1.74 Å between H_α–C(6) and the closest Me(32) proton), H_α–C(12) (1.91 Å), H_α–C(17) (2.23 Å), and H_α–C(15) (2.42 Å), and for conformation **I**, those are H_α–C(7) (1.94 Å), H_α–C(11) (2.08 Å), H_α–C(17) (2.18 Å), H_α–C(12) (2.31 Å), and H_α–C(15) (2.41 Å). This clearly confirms that conformation **III** does not exist in solution (no NOE at 1.60 ppm for H_α–C(6)). The distinction between **I** and **II** is again more difficult. In conformation **II** unlike in conformation **I**, the distance between H_α–C(11) and Me(32) (3.22 Å) is such that no NOE should be observed. However, the overlap between H_α–C(11) and H_α–C(16) (d(H_α–C(16),Me(32)) = 2.66 Å) makes the situation rather complex.

In conclusion, for a molecule such as **4** quantitative analysis of the NOE being very difficult, one should be very careful in drawing conclusions. Still, conformation **III** can be excluded, and there are indications for the existence of conformation **I** in solution at room temperature.

2.3.4. *Temperature Dependence.* It has so far been implicitly assumed that polycyclic terpenoids like **4** are rigid molecules which take up only one conformation: however, the above results make it imperative to study temperature dependence of the NMR spectra in order to detect an eventual equilibrium between several conformations in solution.

We have analyzed the NMR spectrum of **4** at temperatures between 303 and 198 K, in CD_2Cl_2/CD_3OD 15:1. In this temperature range, the Me resonances do not show any specific line broadening, indicating that the equilibria, if any, lead to fast exchange, even at the lowest temperature. All the protons in the molecule experience a high field shift at lower temperature, which is specific: δ(303 K) – δ(243 K) is 0.07 ppm for protons far from ring C (H_α–C(1), H_β–C(1), H_β–C(2), H_α–C(3), H_α–C(6), H–C(18), H–C(21), H–C(25), H–C(26), H–C(30), H–C(31), H–C(32)), it is higher for protons H_α–C(7) (0.11 ppm), H_β–C(7) (0.11 ppm), H_{endo}–C(19) (0.12 ppm), H_β–C(6) (0.10 ppm), H_β–C(8) (0.13 ppm), and H_β–C(11) (0.11 ppm), and lower for protons H_{exo}–C(19) (0.03 ppm), H_α–C(11) (0.02 ppm). Thus, the chemical shifts of protons situated around the B ring/C ring junction experience abnormal temperature dependence. This may be taken as the first indication that conformational equilibrium is occuring.

In order to get more information, in particular on the coupling constants, we have performed phase-sensitive DQF(double quantum filter)-COSY experiments [22] at 303 and 223 K in $CDCl_3/CD_3OD$ 15:1. In this new solvent, the assignment is obtained straightforwardly from a $^{13}C,^1H$ correlation and from the COSY itself. Most of the 2D

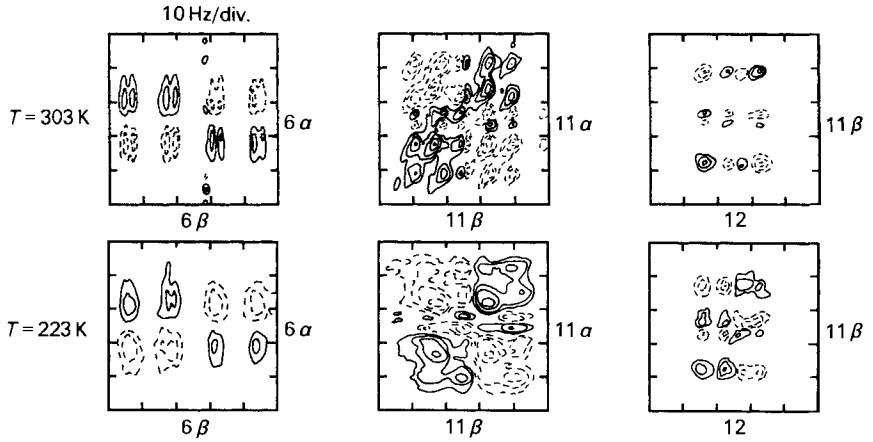


Fig. 7. *Portions of the phase-sensitive DQF-COSY spectrum* (contour plot) *of cycloartenol* (**4**) *in* CD_2Cl_2/CD_3OH *15:1 at 303 and 223 K.* Positive peaks are plotted with solid lines, negative peaks with broken lines. Sweep width 850 Hz, digital resolution 0.83 Hz/point in both directions. Zero filling in dimension 2 does not change the peak shape. The same sample and identical acquisition and processing parameters have been used at 303 and 223 K. Temp. calibration on a sample of 4% CD_3OH/CH_3OH.

pattern is identical at both temperatures although the chemical shifts are slightly changed. We have represented in *Fig. 7* the correlation signals for the pairs H_α–C(6)/H_β–C(6) (ring B), H_α–C(11)/H_β–C(11) (ring C), and H–C(12)/H_β–C(11) (ring C). Vertical and horizontal cross-sections of these figures give the *m* structures of the protons involved. It is clear that no change is observable in the *m* structure of H_α–C(6)/H_β–C(6), and the same is true for the other protons of rings A and B. Thus, rings A and B have only one conformation in this temperature range. However, clear differences appear for the signals of H_α–C(11), H_β–C(11), and H–C(12). At 303 K, H_β–C(11) presents the structure of 2 *t*, which degenerate into 2 *d* at 223 K. The active coupling in the cross-section H–C(12)/H_β–C(11) (causing antiphase signals) is also very different at 303 and 223 K. This shows that we are in the presence of a mixture of conformations of ring C: the relative populations of the conformers change with the temperature, thus affecting the apparent *m* structure of H_α–C(11), H_β–C(11), and H–C(12)[3]). We have not been able to lower the temperature enough to obtain only one conformation and characterize it. This experiment demonstrates that in cycloartenol (**4**) ring C is not rigidly fixed in only one conformation. Considering the results of the molecular modeling, it is highly probable that the structures in equilibrium are structures **I** and **II**.

In the previous paragraphs, we have discussed results concerning dihedral angles and NOE. Having observed well defined coupling constants in rings A and B, the conclusions have not to be modified as these rings are indeed rigid. However, we have not been able to determine dihedral angles in ring C, observing that H_α–C(12) and H_β–C(12) give superimposed signals in all examined solvents. This, of course, may be fortuitous, but it can also be due to the dynamics of ring C, if these protons are exchanging rapidly their position in space. NOE data are even more complex to analyze if there is exchange

[3]) However, at the present time, we cannot exclude that some of these differences might be due to a slight temperature effect on the chemical-shift difference of the 2 H–C(12) resulting in different higher-order cross-peaks.

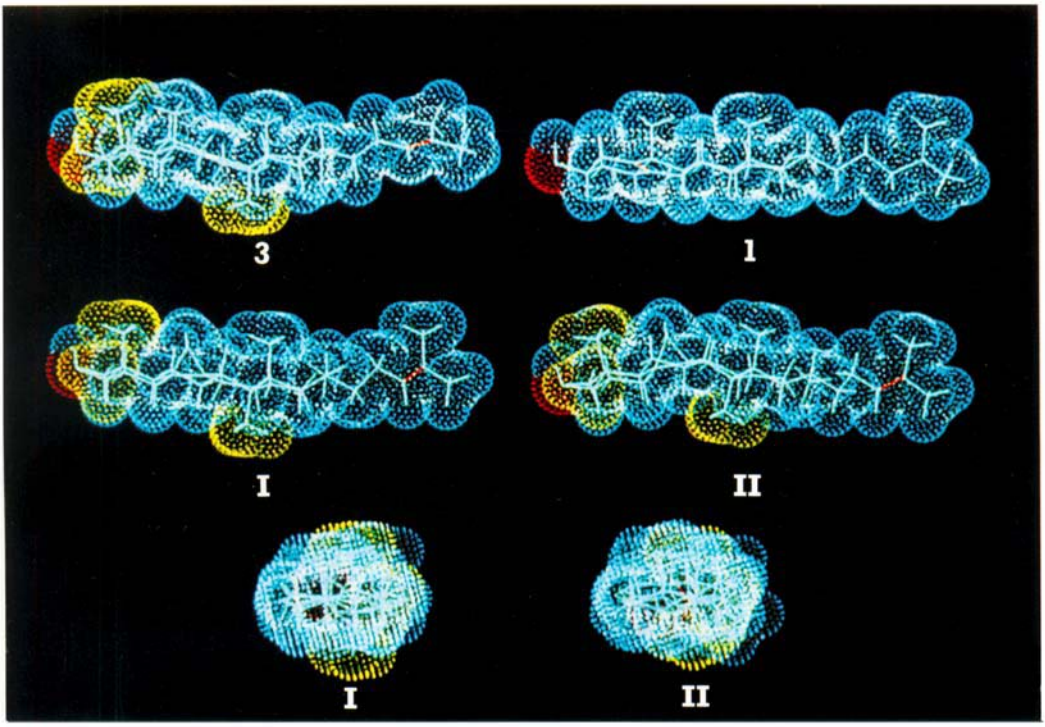


Fig. 8. *Comparison of the* van der Waals *volumes of cholesterol* (**1**; upper right), *lanosterol* (**3**; upper left), *cycloartenol* (**4**; conformation **I**; middle left and lower left), *cycloartenol* (**4**, conformation **II**; middle right and lower right). $CH_3(30)$, $CH_3(31)$, and $CH_3(32)$ are shown in yellow.

between several conformations. The NOE's observed which are compatible with conformation **I** (see [19] and our data) can be observed even if conformation **II** is present in large proportions, as this kind of transfered NOE is well known to be efficient [23].

In conclusion, among the three conformations of lowest energy considered, one, *i.e.* **III** which has the highest energy, has been discarded after the NMR analysis. The other two having the same conformations for ring A (*C*) and B (*H*) differ mainly by the shape of ring C. The change in this ring occurs in particular by modification of the dihedral angle C(9)–C(11)–C(12)–C(13) which is equal to +9° in the crystalline state and would be equal to −60° in a pure *C* conformation. Optimized structures for cycloartenol (**4**) have been found with angles equal to −18 (**I**) and −43° (**II**). The NMR data, namely the coupling constants between protons at C(11) and C(12), have shown that some rotation occurs at this level. This may give the molecule a global shape resembling either cholesterol (**1**) for **II**, or lanosterol (**3**) for **I**, in particular concerning the CH_3–C(14) (= C(32)) which is masked for **II** but not for **I** *(Fig. 8)*. Also, when included in a membrane, **4** could adapt its conformation in order to optimize the cooperative *van der Waals* interactions with the other constituents, in particular the phospholipids.

3. Experimental Part. – The purity of cycloartenol (**4**) was checked by TLC. Molecular modeling: SYBYL software[2]) on a *DEC MicroVax II/PS 390*. NMR experiments: *Bruker AM 400* spectrometer (software DISNMR); when not otherwise specified, at r.t.

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G. O. wishes to dedicate this paper to the memory of his friend and predecessor, Prof. *E. Lederer*.

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