Conformational Analysis of 10α-Cucurbitadienol[1]

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Evidence has been obtained on the solid state (determined by X-ray crystallography) and solution (determined by ^1H and ^{13}C NMR spectroscopy) properties of 10α-cucurbita-5,24-dien-3β-ol which indicate that the molecule orients into a bent rather than flat conformation, the 3β-OH group aligns into the unusual axial rather than the equatorial position, and the side chain orients into the right-handed conformation.

10α-Cucurbitadienol, a [19(10→9β)-*abeo*-10α-lanost-5-ene]-type migrated steroid (also referred to as the triterpenoid, anhydrolitsomentol), is the parent compound for the biologically active cucurbitacins. We became interested in the parent compound because, as a sterol-like molecule, it may possess some degree of architectural and substrate (*i.e.* binding to enzymes which act on the sterol substrate) parity with sterols.[1–3] In order to shed light on the physiological conformation of 10α-cucurbitadienol we undertook a study of the solid state and solution properties of this compound as the C-3 acetate and C-3 hydroxy. For thermodynamic reasons, we assumed that if a similarity existed in the solution and solid state geometries of 10α-cucurbitadienol then it would be unlikely to flip from one shape into the other after its initial formation by the squalene oxide cyclase[4] or secondarily through acid-induced formation from cycloartenol[5] (Scheme 1). A sample of 10α-cucurbitadienol isolated by us (W. D. N.) from pumpkin seeds possessed the same chromatographic and spectral properties as a sample obtained by chemical synthesis[5] and isolated from another plant source, gourd seed oil, by our visiting colleague (T. A.). The specimen derived by synthesis was converted into the C-3 acetate and subjected to an X-ray crystallographic analysis.‡

The perspective view of the crystal structure (Fig. 1) shows that the side chain orients into a 'right-handed' conformation (C-22 *trans*-oriented to C-13),[1a] similar to the solid state conformation observed for its structural isomers cycloartenol,[6] tirucallol[1a] and lanosterol[7] but not euphol,[1a] which maintains a 'left-handed' conformation due to the inversion of the configuration at C-20. Inversion of the configuration at C-10 results in conformational inversion in the A ring so that the C-3 hydroxy group becomes axial to ring A. In contrast to

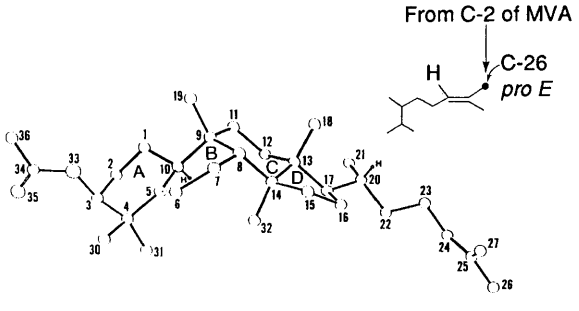

Fig. 1

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‡ *Crystal and refinement data* for $C_{32}H_{52}O_2$: M = 468.9, orthorhombic, space group $P2_12_12_1$ (D_2,[2] no. 19), a = 6.585(5), b = 13.796(16), c = 32.168(31) Å, β = 90.0°, U = 2922.6 Å^3, D_c = 1.07 g cm^{-3}, Z = 4, $F(000)$ = 1040, μ(Cu-Kα) = 4.54 cm^{-1}, R = 0.063, R_w = 0.066 for 2790 unique reflections with $|F_o| \geq 3\delta |F_o|$ in the range $3 \leq 2\theta \leq 114°$. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Date Centre. See Notice to Authors, Issue No. 1.

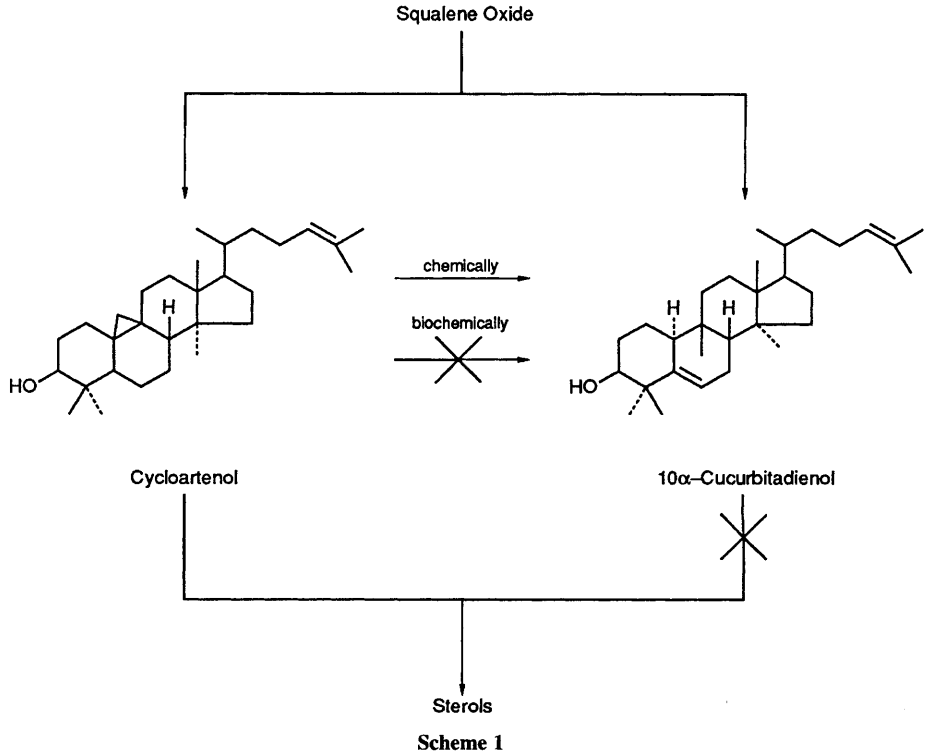
3-epicholesterol where the C-3 axial hydroxy group points toward the α-face, in 10α-curcurbitadienol, the axial hydroxy group points toward the β-face which should still allow for lipid–lipid or lipid–protein interactions in the membrane. However, we recognize this structural change may also contribute to the molecule's inability to act as a membrane insert. Torsion angles (*cf.* deposited materials) in 10α-cucurbitadienol indicate that rings B and C are locked rigidly in the sofa and chair conformations and ring B is nearly flat. By the *cis* relationship of the 8β-H and 9β-Me, the molecule assumes a bent conformation at the B–C ring junction. This conformation is maintained in solution as demonstrated by NMR studies, NOE experiments, on the 3-hydroxy compound.

The necessary ^1H shifts were obtained by carbon–proton shift correlations (CSMC),[8] long-range carbon–proton shift correlations[9] (LRCSMC), proton–proton shift correlations (COSY) and by relating differences in chemical shifts that result in derivatization of the C-3 OH group (**1**, free alcohol and **2**, C-3 acetate). The ^{13}C signals for the side chain carbons were based on cycloartenol[6] and lanosterol.[10] The methyls in **1** and **2** corresponding to H-21, H-26 and H-27 were previously assigned on the basis of shift reagent studies.[11] We confirmed the proton assignments by more modern NMR techniques. From 2D NOE experiments we observed that H-24 was correlated to the downfield vinyl methyl at δ 1.68 making that methyl *cis* to H-24 but *trans* to C-23. The downfield signal at δ 25.7 in the carbon spectra is correlated in the proton spectra to the downfield broad singlet at δ 1.687. Further confirmation by an NOE difference experiment that δ 1.68 was *cis* to H-24 was by irradiation of δ 1.68 and 1.60. An enhanced peak was observed at δ 5.09 only by irradiation of the downfield methyl. Because of much confusion in the literature, we prefer to use the following nomenclature for designating carbon positions: by the side chain rule introduced by Popják[12] and Nes,[13] the methyl group in squalene *trans* to C-23 which is derived from C-2-mevalonic acid becomes C-26 in the sterol[14,15] and cucurbitatane side chain, not the *cis* isopropylidene carbon, as inferred in the biosynthesis of cucurbitacin B[16] side chain or in the biosynthesis of cycloartenol[17] and related tetracycles[18,19] (Scheme 1). The location of the chemical shift for H-21 also suggested the 20*R*-stereochemistry[11,20] and that the 'right-handed' side chain conformation observed in the solid state is likely maintained in solution.[20] From the other methyl assignments of Akihisa[8] for methyls 19, 30 and 31 the corresponding carbon methyls for **1** and **2** were assigned (Table 1). With an NOE difference experiment, we confirmed the equatorial orientation for C-31. By irradiating in **1** Me-31 (the 4β methyl), a response was seen at δ 5.09 (H-6), suggesting that it is equatorial. That H-3 at δ 3.68 is a triplet with equal couplings to the two H-2 signals, indicates that H-3 is equatorial, thus making the 3-hydroxy axial. Of the peaks that shifted downfield upon hydrolysis of **2**, the peak in **1** at δ 28.9 is a CH_2, and must be C-2, and that δ 41.4 is a quaternary carbon and must be C-4. Additionally, on hydrolysis Me-31 shifts downfield in the proton spectrum to δ 1.134, which correlates to δ 25.4 methyl. In **2**, LRCSCM shows correlation from the C-4 δ 38.6 peak to Me-30 and Me-31. Of the remaining methine carbons in **2**, C-8, C-10 and C-17, the

Table 1 ^{1}H and ^{13}C NMR assignments for 10α-cucurbitadienol[a]

	Hydroxy **1**		Acetate **2**	
Carbon no.	$\delta_{^{13}C}$	$\delta_{^{1}H}$(*J*/Hz)	$\delta_{^{13}C}$	$\delta_{^{1}H}$(*J*/Hz)
1	21.1	1.55, 1.55	21.7	1.31, 1.61
2	28.9	1.70, 1.96	26.6	1.80, 1.80
3	76.6	3.68, br t (*J* 2.6)	78.8	4.691, t (*J* 2)
4	41.4	—	39.6	—
5	141.2	—	141.4	—
6	121.4	5.585, br d (*J* 6)	119.4	5.496, br d (*J* 6)
7	24.3	1.81, 2.39	24.2	1.81, 2.39
8	43.6	1.78	43.6	1.77
9	34.4	—	34.4	—
10	37.8	2.28	37.9	2.29
11	32.3	1.40, 1.70	32.2	1.43, 1.70
12	34.7	1.51, 1.77	34.8	1.47, 1.73
13	46.2	—	46.2	—
14	49.1	—	49.1	—
15	30.4	1.20, 1.20	30.4	1.18, 1.18
16	27.9	1.30, 1.83	27.9	1.33, 1.84
17	50.4	1.55	50.4	1.51
20	35.8	1.42	35.8	1.42
21	18.6	0.900, d (*J* 7)	18.6	0.883, d (*J* 6)
22	36.4	1.07, 1.42	36.4	1.07, 1.38
23	24.8	1.80, 2.04	24.8	1.83, 2.07
24	125.2	5.090, bt t (*J* 7)	125.2	5.075, t (*J* 7)
25	130.9	—	130.8	—
26	25.7	1.677	25.7	1.664
27	17.6	1.598	17.6	1.582
4α (C-30)	27.2	1.021	27.4	1.031
4β (C-31)	25.4	1.133	24.9	1.031
9β (C-19)	28.0	0.916	27.8	0.895
13β (C-18)	15.3	0.847	15.3	0.836
14α (C-32)	17.8	0.804	17.6	0.798
C=O	—	—	170.8	—
Ac	—	—	21.2	2.000

[a] NMR spectra were obtained at 200 MHz for proton and 50 MHz for carbon on a Nicolet NT-200. Assignments were facilitated for carbon by the attached proton test (S. C. Patt and J. N. Shoolery, *J. Magn. Reson.*, 1982, **46**, 535). Proton spectra were acquired with a 37° pulse angle at a 4.1 s repetition rate with a 4000 Hz spectral angle at a 2.0 s repetition rate with a 12500 Hz spectral width into 32K of memory, giving a digital resolution of 0.79 Hz. In the NOE difference experiments, the methyls were presaturated with a narrow irradiation band for a time greater than 10 × the longest T_1. Spectra were collected at ambient temperature with the decoupler off, alternately adding and subtracting on and off resonance irradiation. The chemical shifts (δ) in this table are given in ppm with tetramethylsilane (^{1}H NMR) or chloroform (^{13}C NMR) as internal standard. Compounds were dissolved in $CDCl_3$.



Scheme 1

δ 50.4 peaks shows LRCSCM correlation to Me-18 (see below), implying that this is C-17. This compares closely with the shift at δ 50.7 of C-17 in lanosterol.[10] The δ 37.8 peak correlates to a proton signal at δ 2.29 and the δ 43.6 peak correlates to a proton signal at δ 1.77. The downfield proton signal is undoubtedly H-10 adjacent to the 5,6-double bond. Of the quaternary signals for C-9, C-13 and C-14 the one in **2** at δ 34.4 shows LRCSCM correlation to the Me-19, indicating that this resonance corresponds to C-9. The signals at δ 46.2 and 49.1 are assigned as C-13 and C-14, respectively by comparison with the assignments reported for lanosterol.[10] Of the methylenes C-1, C-7, C-11, C-12, C-15 and C-16 the peak in **2** at δ 24.2 correlates to the H-7 signals at δ 2.39 and 1.81 (which are confirmed by COSY correlations to H-6). The δ 30.4 peak shows LRCSCM correlation to Me-18 (see below), making it C-12, the δ 32.2 peak shows LRCSCM correlation to the 19 methyl, making it C-11, and the δ 34.7 peak shows LRCSCM correlation to C-32, making it C-15. That leaves C-1 and C-16 for the δ 21.7 and 34.7 signals. The proton correlations of the δ 34.7 peak in **2** remains unchanged upon hydrolysis to **1**, suggesting that this peak may be C-16.

In an NOE difference experiment, irradiation of the methyl at δ 0.804 gave a response at δ 2.28 (H-10). No such response was seen when the δ 0.847 peak was irradiated. However, it is not possible to observe an NOE at H-10, which is on the α face, where the 13β methyl (Me-19) is irradiated. It is only possible to observe an NOE at H-10 if the molecule is bent at the C-ring so that the 14α methyl (Me-32) comes closer to H-10. Akihisa has assigned the methyl at δ 0.804 as Me-18, based on shift reagent studies. In Akihisa's shift reagent studies the δ 0.80 methyl has a larger relative shift than the δ 0.85 methyl. If, for purposes of ^{1}H NMR assignments, one assumes that 10α-cucurbitadienol maintains the same flat conformation as lanosterol, then Me-18 is somewhat closer to the 3β (axial) acetoxy than Me-32 and one assigns the methyls as reported. However, a bent conformation places Me-32 closer to the shift reagent so that the assignments, as shown in Table 1, are reversed from those previously reported.[11] Thus, cucurbitacin maintains a bent conformation in solution as well as the solid state and may therefore arise from the cyclization of squalene oxide as outlined previously,[21] and function in cellular biochemistry as the bent compound.

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