CONFORMATION OF RING A IN TRITERPENOID AND 4,4-DIMETHYLSTEROID 3-KETONES. CHEMICAL SHIFTS OF METHYL PROTONS AND LANTHANIDE AND BENZENE INDUCED SHIFTS*

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Chemical shifts of signals due to methyl groups in positions 10β (in $CDCl_3$) and 4α and 4β (in C_6D_6) in ^1H NMR spectra of pentacyclic triterpene 3-oxo derivatives (*V*, *VIII*, *IX*, and *XII*) are suitable for estimation of chair-boat equilibrium in the ring A. Benzene and lanthanide induced shifts of 4α and 4β-methyl protons were also used for this purpose. The results obtained with 2α-methyl-3-ketones (*III*, *X*) and 2β-methyl-3-ketones (*IV*, *XI*) as the respective chair and boat models agree well with those derived from other physical data (about 40% boat). The same methods were applied to 4,4-dimethylsteroid 3-ketones *XV*–*XVII*.

As shown recently by various physical data[1-6], 3-oxo derivatives of pentacyclic triterpenes (such as *V*, *VIII*, *IX*, and *XII*) and similar ketones containing 4α,4β,8β- and 10β-methyl groups exist as equilibrium mixtures of chair and boat conformations of the ring A. Both conformers are comparably populated: as estimated from vicinal coupling constants of the A-ring protons[1], ^{13}C chemical shifts of carbon atoms[2] in the rings A and B, dipole moments[3], CD spectra[3], and indirect methods (simulation of conformational equilibria by isomerization equilibria in model compounds[3]), the population of the boat form is about 40 ± 10%. On the other hand, in 4,4-dimethylsteroid 3-ketones without the 8β-methyl group (e.g. *XV* to *XVII*) the chair form highly predominates[1,5,7,8]. No conclusive proof of the boat form in these compounds has been presented although, according to the vicinal proton–proton coupling constants[1] and dipole moments[7], small quantities of this conformation cannot be excluded. Also molecular mechanics and combined molecular mechanics–quantum chemical calculations[8-11] indicate that the boat form may be significantly populated.

This communication concerns the use of ^1H NMR methyl signals for conformational study of ring A in the 3-oxo derivatives and for estimation of the chair–boat

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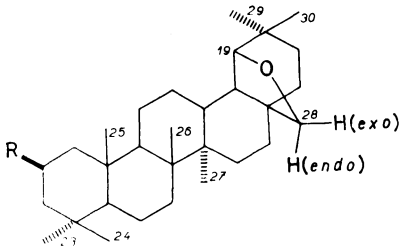
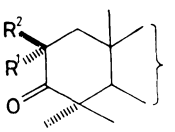
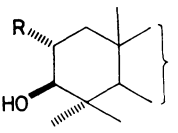
population. Since the spatial orientation of the 3-keto group relative to the skeletal methyl groups differs considerably in both conformers, the magnetic anisotropy of this group influences differently chemical shifts of methyl protons (particularly



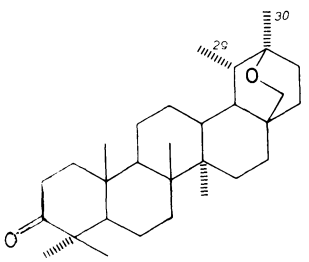
I, R = H
II, R = CH_3



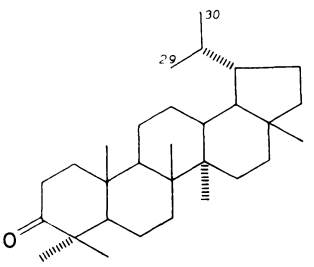
III, $R^1 = CH_3$; $R^2 = H$
IV, $R^1 = H$; $R^2 = CH_3$
V, $R^1 = R^2 = H$



VI, R = H
VII, R = CH_3



VIII

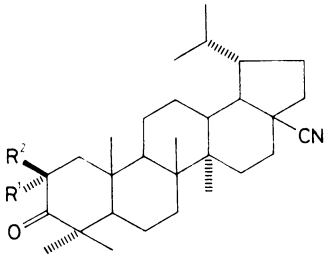


IX

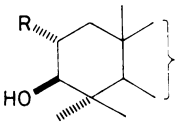
those of the 10β-methyl group; see e.g. refs[12,13]). First of all, it was necessary to ascribe unequivocally the observed signals to the individual methyl groups; this is often a difficult task, particularly in the case of methyl groups in positions 4, 8, 10, and 14. In the triterpenoid and steroid series this question is often solved using lanthanide shift reagents (LSR) (refs[14–16] and references therein). Moreover, the lanthanide induced shifts (LIS) well differentiate the 4α and 4β-methyl signals in the 3-oxo derivatives[8,16,17]; the same holds for the aromatic solvent induced shifts which sensitively indicate even small conformational changes in similar polycyclic ketones[6,13,16,18,19].

We have now measured the ^{1}H NMR spectra both in deuterochloroform and in hexadeuterobenzene, using tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium (Eu(fod)$_3$) in deuterochloroform. The measured set of compounds includes 19β,28-epoxy-18α-oleanane (*I*) and its derivatives *II*–*VII*, 20β,28-epoxy-18α,19β*H*-ursan-3-one (*VIII*), 3-lupanone (*IX*), lupane-28-nitriles *X*–*XIV* and 4,4-dimethyl-5α-cholestan-3-one (*XV*), all with the 5α-configuration. All the compounds have already been prepared (see refs[1,2]). As in our previous studies[1–3], the

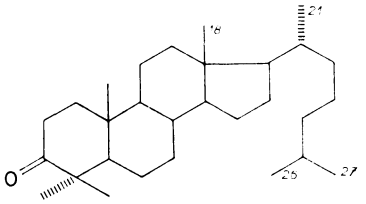
present conformational investigation of the 3-ketones is based on model compounds: 2α-methyl ketones *III* and *X* for the chair and 2β-isomers *IV* and *XI* for the boat form. Conformations of the model compounds have been derived previously[1] from the vicinal coupling constants. As concerns compounds without the keto group (measured for comparison and for an easier signal assignment), in compound *I* and in 3β-hydroxy derivatives *VI*, *VII*, *XIII*, and *XIV* the ring A adopts the chair form, whereas in the 2β-methyl derivative *II* it is in the boat conformation (see ref.[2] and references therein).



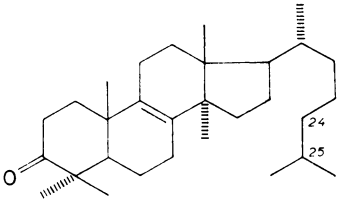
X, $R^1 = CH_3$; $R^2 = H$
XI, $R^1 = H$; $R^2 = CH_3$
XII, $R^1 = R^2 = H$



XIII, R = H
XIV, R = CH_3



XV



XVI
XVII, 24(25)-double bond

EXPERIMENTAL

^{1}H NMR spectra were measured on a Varian XL-200 FT-spectrometer (200 MHz) and on a Varian HA-100 (100 MHz) and a Tesla BS-487A (80 MHz) instruments (both CW-mode), concentration 15—30 mg/0·3—0·4 ml $CDCl_3$ or C_6D_6. The spectra were referenced to tetramethylsilane (TMS) as internal standard. In some cases, hexamethyldisiloxane (HMDS) was used as standard for solutions in C_6D_6 and the chemical shifts were referenced to TMS using the relationship $\delta(\text{TMS}) = \delta(\text{HMDS}) + 0{\cdot}113$. The typical acquisition parameters of the FT NMR spectra were as follows: spectral width 2 kHz, pulse width 4 μs (flip angle 45°), acquisition time and repetition time 5 s, data points 20 K (zero filling to 32 K), number of accumula-

tions about 100. In order to increase the resolution, exponential multiplication and gaussian apodization functions were used (with parameter RE = 0·3 and AF = 0·9). The chemical shifts in the FT NMR spectra were obtained by line-listing procedure and their accuracy, given by digital resolution (0·2 Hz) was ±0·001 ppm. For the CW spectra the shifts were taken from expanded spectra (1 Hz/cm) with comparable accuracy. In case of overlapping signals the accuracy was lower and the errors could achieve 0·005 ppm.

The spectra of all compounds in $CDCl_3$ and compounds *X*–*XII* and *XV* in C_6D_6 were measured at 200 MHz, spectra of compounds *I*, *III*–*V*, *VIII*, and *IX* in C_6D_6 at 100 MHz. Dilution studies using $CDCl_3$–C_6D_6 mixtures as solvents were performed at 80 MHz with HMDS as internal standard. Lanthanide induced shifts were measured in $CDCl_3$ at 100 MHz. To a solution of the triterpenoid (*c* 0·15–0·25 mol l^{-1}) were successively added three accurately weighed amounts of solid Eu(fod)$_3$ so as the final molar ratios LSR/substrate in the measured spectra were within the limits 0·05–0·08, 0·13–0·22 and 0·30–0·40.

RESULTS AND DISCUSSION

Assignment of Signals

Chemical shifts of methyl protons and protons adjacent to the ether bridge in the ring E in $CDCl_3$ solutions, and for most of the compounds also in C_6D_6, are summarized in Table I. The signals of the *endo*- and *exo*-protons in the C(28)H_2 group in ethers of the type *I* and *VIII* have been already assigned[20,21]. As concerns the methyl signals, the identification of the 10β-methyl (H-25) signals was facilitated by the fact that in most compounds these signals appeared as doublets (J = 0·5 to 1·0 Hz) or broad singlets caused by long-range coupling with H-1α. For most of the studied ketones we confirmed this coupling by spin-decoupling experiments[1]. Also the 14α-methyl signals (H-27) are broader or have a doublet character (especially in lupane compounds). Spectra of compounds *I*, *V*, *VI*, *VIII*, *IX* and similar derivatives without methyl group on C(2) were studied in deuterochloroform solutions already earlier[22–24] and therefore the identification of signals in compounds *I*–*IX* could be based on the assignments and general rules given in the cited studies. For lupane-28-nitrile derivatives *X*–*XIV* we assigned the signals analogously, taking into consideration the deshielding effect of the axial cyano group on the protons of the β-oriented (axial) methyl groups, particularly H-26 and H-25 (for analogies see ref.[20] and references therein).

The assignment of signals in ketones *III*–*V* is based on Eu(fod)$_3$ induced shifts. However, in these bifunctional derivatives the LSR coordinates with both the carbonyl oxygen in ring A and the ether oxygen in ring E. This complication is usually solved using monofunctional analogues[15,25]. As monofunctional derivatives we employed compounds *I*, *II*, and *IX*. Methyl signals in the individual spectra were identified in the usual manner from the dependence of chemical shifts on the molar ratio Eu(fod)$_3$/substrate. Within the concentration range (molar ratios 0–0·4) this dependence was not strictly linear, particularly for bifunctional derivatives (as

TABLE I

Proton chemical shifts of compounds *I*—*XV* in $CDCl_3$ and in C_6D_6 (given in parentheses). All signals are singlets unless stated otherwise

Compound	H-23	H-24	H-25	H-26	H-27	H-29[a]	H-30[a]	H-31[b]
I[c]	0·848	0·796	0·841[d]	0·975	0·922	0·929	0·796	—
	(0·901)	(≈0·852)	(≈0·852)	(0·924)	(0·887)[e]	(1·128)	(0·780)	—
II[c]	0·890	0·836	0·930	0·953	0·908	0·930	0·798	0·836
III[c]	1·064	1·042	1·135	1·034	0·901	0·933	0·796	1·021
	(1·176)	(≈0·908)	(0·879)	(≈0·908)	(0·808)	(1·123)	(0·787)	(1·101)
IV[c]	1·065	1·035	0·693[e]	≈0·957	≈0·957	0·934	0·805	0·987
	(0·920)	(≈1·129)	(0·623)[e]	(≈0·800)	(0·894)	(1·129)	(0·800)	(1·050)
V[c]	1·079	1·031	0·945[d]	1·011	≈0·927	0·933	0·799	—
	(1·082)	(0·989)	(0·754)[d]	(0·846)	(0·827)	(1·123)	(0·790)	—
VI[c]	0·974	0·765	0·846	0·974	0·913	0·928	0·795	—
VII[c]	0·974	0·774	0·873[e]	0·974	0·911	0·930	0·796	0·991
VIII[f]	1·076	≈1·031	0·955[d]	≈1·031	0·921	0·870[g]	≈1·031	—
	(1·086)	(0·988)	(0·746)[d]	(0·783)	(0·800)	(0·820)[g]	(1·153)	—

IX[h]	1·076	1·030	0·944[d]	1·076	0·936[d]	0·761[i]	0·841[i]	—
	(1·089)	(0·991)	(0·772)	(0·962)	(0·888)	(≈0·843)[i]	(≈0·926)[i]	—
X	1·066	1·048	≈1·149	≈1·149	0·918	0·758[i]	0·883[i]	1·014
	(1·186)	(0·884)	(0·860)[e]	(1·064)	(0·643)[d]	(0·641)[i]	(0·809)[i]	(1·118)
XI	1·066	1·039	0·702[d]	1·076	0·977[d]	0·769[i]	0·884[i]	0·981
	(0·910)	(1·115)	(0·591)[e]	(0·986)	(0·761)[e]	(0·665)[i]	(0·821)[i]	(1·048)
XII	1·079	1·036	0·966[d]	1·131	0·943[e]	0·764[i]	0·882[i]	—
	(1·097)	(0·965)	(0·720)[e]	(1·032)	(0·676)[e]	(0·631)[i]	(0·808)[i]	—
XIII	0·976	0·771	0·858[e]	1·090	0·929[d]	0·756[i]	0·876[i]	—
XIV	0·979	0·778	0·883[e]	1·087	0·928[e]	0·756[i]	0·877[i]	0·983
XV[j]	1·057	≈1·044	≈1·041[e]	0·860[i]	0·865[i]	—	—	—
	(1·116)	(0·933)	(0·791)	(0·931)[i]	(0·931)[i]	—	—	—

[a] Tentative assignment, the signals may be interchanged; [b] protons of C(2)–CH_3 group, doublet, $J \approx 6{\cdot}4$ Hz; [c] H-19: 3·53 s (3·63—3·64 s); H-28(*endo*): 3·78 dd (3·80—3·81 dd), $J = 7{\cdot}8$ and $\approx 1{\cdot}0$ Hz; H-28(*exo*): 3·43—3·45 d (3·42—3·43 d), $J = 7{\cdot}8$ Hz; [d] doublet, $J = 0{\cdot}5$—$1{\cdot}0$ Hz; [e] broad; [f] H-28(*endo*): 4·13 dd (4·20 dd), $J = 8{\cdot}5$ and $2{\cdot}5$ Hz; H-28(*exo*): 3·34 dd (3·38 dd), $J = 8{\cdot}5$ and $1{\cdot}2$ Hz; [g] doublet, $J = 7{\cdot}0$ Hz; [h] H-28: 0·763 s (0·798 s); [i] doublet $J = 6{\cdot}6$—$6{\cdot}9$ Hz; [j] H-23, H-24 and H-25 are protons of 4α-, 4β- and 10β-methyl group, resp.; H-18: 0·661 s (0·654 s); H-21: 0·898 d (1·007 d), $J = 6{\cdot}8$ Hz.

could have been expected[15,25]). To avoid errors introduced by the considerable dependence of the induced shifts on substrate concentration[15,25], weighing and measurement of volumes, we express the induced shifts as relative values (ΔEu)* given in % of the H-19 signal shift (i.e. normalized to ΔEu(H-19) = 100). In the case of 3-lupanone (*IX*) the values were normalized to H-23 (ΔEu(H-23) = 64) in order to obtain comparable values for compounds *IX* and *V*. The normalized shifts ΔEu were calculated for each concentration of LSR; in the range of the used concentrations no significant dependence of the thus-obtained values was observed for monofunctional as well as bifunctional derivatives (excepting ketone *V*; vide infra). This means that for bifunctional compounds *III* and *IV* the binding constants of complexes at both coordination sites are comparable. The mean ΔEu values, rounded to whole numbers, are given in Table II.

The found ΔEu values were compared with those calculated from the X-ray data. Of 19β,28-epoxy-18α-oleanane (*I*) derivatives only the ketone *V* was so far subjected to X-ray analysis[4]. The ring A in crystalline *V* exists in a boat conformation of an unusual geometry, one half of the ring corresponding to a classical boat and the other half resembling a twist-boat form. The geometry of this conformation (further denoted *BT*) is specific for the given crystalline state and does not correspond to the boat geometry in solution[4]. Therefore, we tested two other boat conformations that had been found[26] in two crystallographically nonidentical molecules of 2β--methyl ketone *XI*. Both of them correspond to a twist-boat form and differ somewhat in geometry: we denote them *T*(1) and *T*(2) (for the molecules 1 and 2, respectively, in ref.[26]). The geometry of the chair form (*C*) was taken from crystal data[27] for 2α-methyl ketone *X*. The endocyclic torsion angles, describing geometry of the A-ring in conformations used for calculation of the ΔEu values, are summarized n Table III. Atomic coordinates of skeletal carbon atoms (rings B–E) and carbon atoms of the A ring in conformation *BT* were taken from our previous paper[4]. For the conformations *C*, *T*(1) and *T*(2) the coordinates of the fragment C(1)···C(4) (including the methyl carbon atoms and the carbonyl oxygen) were taken from refs[26,27] and the fragment was attached to the C(10) and C(5) carbon atoms of the skeleton. The position of the hydrogen atoms in the methyl groups was approximated by an "average" hydrogen atom, situated 36 pm from the methyl carbon atom on the prolonged C—CH_3 bond. Atom H-19 and both atoms H-28 were placed in the theoretical positions. As usual[15,25], for the calculation of relative induced shifts ΔEu we considered only the pseudocontact shift which, according to the McConnell–Robertson equation[28], is proportional to the expression $(3\cos^2\Theta_i - 1)\, r_i^{-3}$, where r_i is the distance Eu—H_i and Θ_i is the angle H_i—Eu—O for each proton H_i.

* In this paper ΔEu denotes throughout the relative (normalized) shifts, induced by Eu(fod)$_3$. The positive values denote downfield, negative values upfield shifts.

As seen from Table II, in monofunctional compounds *I* and *II* the coordination of LSR to the ether oxygen results in an upfield shift of three skeletal methyl signals and a downfield shift also of three methyl signals, one methyl signal being practically unaffected; the H-28(*endo*) proton signal has a higher induced shift than the H-28(*exo*) one. These facts could not be explained if the europium atom were situated symmetrically to both protons on C(28), i.e. on the axis of the C(19)—O—C(28) angle. On the other hand, satisfactory results were obtained by shifting the Eu atom

TABLE II

Observed and calculated values (in parentheses) of chemical shifts ΔEu induced by $Eu(fod)_3$ in $CDCl_3$. The values are related to H-19 (ΔEu(H-19) = 100)

Protons	*I*		*II*		*III*		*IV*		*V*[a]		*IX*[b]	
H-23	0	(0)	0	(0)	42	(42)	30	(30)	64	(64)	64	(64)
H-24	−1	(−2)	−1	(−2)	28	(27)	38	(38)	59	(57)	60	(59)
H-25	−6	(−7)	−6	(−7)	12	(10)	20	(20)	27	(30)	32	(36)
H-26	−4	(−5)	−4	(−5)	3	(3)	3	(3)	8	(8)	12	(12)
H-27	13	(15)	14	(15)	16	(18)	20	(20)	20	(21)	8	(8)
H-28(*endo*)	101	(103)	102	(103)	100	(101)	101	(102)	100	(100)	5[c]	([d])
H-28(*exo*)	88	(97)	90	(97)	87	(87)	90	(90)	88	(88)	—	
H-29[e]	23	(25)	23	(25)	24	(24)	23	(24)	23	(24)	2	([d])
H-30[e]	21	(23)	21	(23)	22	(22)	23	(22)	22	(23)	2	([d])
H-31[f]	—	(−1[g])	−1	(−1)	39	(39)	39	(37)	—		—	

[a] Values given for molar ratio $Eu(fod)_3$/ketone $V = 0{\cdot}22$ and concentration of V 0·16 mol l^{-1}; [b] the values related to H-23 (ΔEu(H-23) = 64); [c] protons of $C(28)H_3$ group; [d] not calculated; [e] tentative assignment, values may be interchanged; [f] protons of $C(2)$–CH_3 group; [g] calculated for 2α-CH_3 in conformation *C*.

TABLE III

Endocyclic torsion angles (in °) in tested conformations of ring A

Conformation	C(1)–C(2)	C(2)–C(3)	C(3)–C(4)	C(4)–C(5)	C(5)–C(10)	C(10)–C(1)
C	−54	51	−49	51	−53	54
BT	0	−39	23	29	−64	47
T(1)	30	−62	28	35	−63	28
T(2)	30	−63	32	31	−61	28

from the mentioned axis toward the ring D (in a plane perpendicular to the plane C(19), O, C(28) and bisecting the angle C(19)—O—C(28)) so that the angle of the Eu—O connecting line and the C(19), O, C(28) plane was 10–15° and the Eu—O distance 280–300 pm. Values, calculated for the angle 12° and the Eu—O distance 290 pm, are given in Table II in parentheses. For compound *I* (chair conformation of the ring A) the calculations were performed for the conformation *C*, for the 2β-methyl derivative *II* (boat conformation) we calculated the values for the forms *BT*, *T*(1) and *T*(2). The values of ΔEu calculated for all these conformations differ only negligibly (within the experimental error), also the difference between the values found for *I* and *II* is only insignificant. Therefore, they cannot be used for distinguishing between the chair and boat forms of the ring A. The agreement between calculated and found values clearly confirms the assignment of the H-23 and H-27 signals in compounds *I* and *II*. However, the H-29 and H-30 protons cannot be unequivocally assigned because the observed ΔEu differ only little and, moreover, ΔEu values calculated for these protons change considerably even with a small change in position of the europium atom.

The experimental ΔEu values for compounds *I* and *II* were now employed for interpretation of data for the bifunctional derivatives *III* and *IV*, assuming that the shifts due to coordination of LSR to the ether and carbonyl oxygen atoms are additive. The ΔEu values for 2α-methyl ketone *III* (chair conformation) and 2β-methyl ketone *IV* (boat) were calculated as linear combinations of ΔEu values, calculated for the coordination LSR–carbonyl and ΔEu values, found for the coordination LSR–ether in *I* or *II*. The coefficients in the linear combination were chosen so as to get an agreement between the values calculated for H-19 and H-23 and those found for compound *III* or *IV* (i.e. the effect of the ketone was normalized to the found value of ΔEu(H-23)). It is worth notice that, whereas the complexation LSR–ether practically does not contribute to the ΔEu(H-23) value (see values for compounds *I* and *II* in Table II), in the case of H-19 the effect of the coordination LSR–carbonyl is not negligible and for all the tested conformations of the ring A contributes about 3% to the resulting value ΔEu(H-19) = 100 for both ketones *III* and *IV*. Under the simplest assumption that the europium atom lays on the C=O axis, the ΔEu values, calculated for Eu—O distances amounting to 270–360 pm, for all the conformations(*C*, *BT*, *T*(1), *T*(2)) agree approximately with the observed values, enabling thus an unequivocal assignment of methyl signals in ketones *III* and *IV*. Of the boat conformations tested, the best fit with the experimental data was obtained with the form *T*(2) whereas the worst with the conformation *BT*. Table II contains ΔEu values calculated for the conformation *C* in 2α-isomer *III* and conformation *T*(2) in 2β-isomer *IV* and for the Eu—O distance 340 pm. As seen, the greatest differences in the induced shifts between the chair (in *III*) and boat (in *IV*) conformations appear for the methyl groups in position 4 (H-23 and H-24).

In contrast to the mentioned compounds, in ketone *V* the relative induced shifts

of some protons depend significantly on the concentration of LSR. With increasing LSR/ketone molar ratio, the ΔEu values of protons in the neighbourhood of the carbonyl group decrease. The largest changes appear for H-23 and H-24, smaller for H-25, whereas other protons are practically unaffected. This shows that increasing concentration of LSR increases the proportion of LSR coordinated to the ether oxygen (the LSR–carbonyl binding constant is higher than the LSR–ether one[15]). Accordingly, for each $Eu(fod)_3$ concentration it was possible to obtain ΔEu values for ketone *V* as a linear combination of values for the monofunctional derivatives *I* (ether) and *IX* (ketone); the proportion of the values for *IX* decreased with increasing concentration of LSR. This at the same time confirmed that the shifts induced at both sites are additive. For sake of simplicity, Table II lists data for only one concentration of LSR in the middle of the measured concentration interval.

As already mentioned above, in ketones *V* and *IX* the ring A exists as an equilibrium mixture of chair and boat conformations (about 6 : 4). The ΔEu values in these compounds may be calculated from those found for the model compounds *I* and *III* (chair) and compounds *II* and *IV* (boat) under assumption that the coordination of LSR to the carbonyl oxygen has no effect on the conformational equilibrium, i.e. that the binding constants of the complexes are the same in both conformers. The validity of this assumption was tested on the methyl group in position 2 (H-31) whose orientation towards the carbonyl group is about the same in both the chair (2α-methyl in *III*) and in the boat (2β-methyl in *IV*). The ratio of induced H-31 shifts in *III* and *IV*, calculated for conformations *C*, *T*(1) and *T*(2), is close to 1; if we assume the same distance Eu—O in *III* and *IV* (250 – 360 ppm), the calculated ratio is 1·01 – 1·05. The experimental ratio 1·0 indicates that the differences between the binding constants in both conformers are minimal. The same conclusion has been reported[29] for conformational equilibrium in alkylcyclohexanones. The effect of coordination LSR–carbonyl in the chair form was separated by mere subtracting $\Delta Eu(I)$ from $\Delta Eu(III)$; a similar calculation was performed for the boat form ($\Delta Eu(IV) - \Delta Eu(II)$). We thus obtained characteristic values of ΔEu for the individual conformers which were weight-averaged in the ratio 6 : 4; this gave ΔEu values describing the effect of coordination LSR–carbonyl in the equilibrium mixture of both conformers. The values were further normalized to ΔEu(H-23) = 64 (found in ketones *V* and *IX*); in the case of ketone *V* we added the effect of LSR–ether coordination, approximated by the average ΔEu values of compounds *I* and *II*. The agreement of the thus-calculated and found values confirms the assignment of the H-23, H-24, H-25, H-26, and H-27 protons in these ketones (Table II). A similar agreement has also been obtained for the ΔEu values of ketone *V* at other two $Eu(fod)_3$ concentrations. It should be noted that the described procedure neglects the effect of coordination LSR–carbonyl on ΔEu(H-19); nevertheless, a more exact approach, including this effect (about 3%, vide supra) leads practically to the same results. The normalization to H-23 in ketone *V* is indispensable because the steric

effect caused by introduction of methyl into the position 2 evidently influences the binding constants in model compounds *III* and *IV* relatively to unsubstituted ketone *V*; we may, however, assume that this effect of methyl is the same in both conformers.

The assignment of methyl proton shifts in C_6D_6 solutions for compounds *I*, *III*–*V*, *VIII*, and *IX* (Table I) is based on correlation with the spectra measured in $CDCl_3$, using the method of stepwise dilution. For most signals, the dependence of chemical shifts on the $CDCl_3/C_6D_6$ ratio deviated considerably from linearity (see also refs[6,18]) and in the individual spectra usually some of the studied methyl signals overlapped. To get an unequivocal correlation, it was therefore necessary to measure the spectra in 8 to 15 different mixtures of both solvents. For the lupane--28-nitrile compounds *X*–*XII* the assignment is based on comparison with ketones *III*–*V*; the methyl signals for ketone *XV* were assigned according to refs[13,16].

The benzene induced shifts ($\Delta Bz = \delta(C_6D_6) - \delta(CDCl_3)$) of protons in the neighbourhood of carbonyl group in conformationally homogeneous ketones obey the known rules[13,16,19]: equatorial methyl groups on C(2) and C(4) are deshielded by benzene (ΔBz 0·06–0·12 ppm) whereas the axial ones are shielded (−0·13 to −0·16 ppm); the 10β-methyl groups are shielded too (−0·07 to −0·29 ppm). The data in Table I show further that the ether and cyano groups in the ring E are also specifically solvated by the aromatic solvent. In 19β,28-epoxy derivatives *I*, *III*–*V* one of the methyl groups on C(20) is strongly deshielded (0·19 ppm; H-29); the other one is influenced only negligibly (−0·01 ppm; H-30). In 20β,28-epoxy compound *VIII* the H-30 protons are deshielded (0·12 ppm) whereas the spatially close H-29 protons exhibit an opposite shift (−0·05 ppm). In lupanenitrile derivatives *X*–*XII* both methyls in the isopropyl group are shielded (about −0·07 and −0·13 ppm); in 3-lupanone *IX* they are deshielded (0·08 ppm for both groups). Strong shielding of H-27 protons in nitriles *X*–*XII* was observed (−0·2 to −0·3 ppm). The groups near the skeleton center (H-26, H-27 and partially also H-25) are undoubtedly influenced by solvation of both functional groups in the molecule.

Chemical Shifts of Methyl Protons and Conformation of Ring A

As follows from comparison of data for 2α-methyl-3-ketones *III* and *X* (chair) and their 2β-isomers *IV* and *XI* (boat) in Table I, conformation of the ring A affects chemical shifts of the H-23 to H-27 protons in both solvents used. Interestingly, H-23 and H-24 in $CDCl_3$ represent an exception: although these protons are the nearest to the 3-oxo group and their spatial relation to the carbonyl changes considerably with the chair–boat conversion, their chemical shifts for both conformers differ only negligibly. On the other hand, in perdeuterobenzene solution the difference is significant. However, the greatest differences between both conformers appear in $CDCl_3$ for the H-25 proton (see also Table IV). In ketones *V* and *XII* (without

methyl in position 2) the shifts of H-23 to H-27 lay between the values found in the model compounds (*III*, *IV*, *X*, and *XI*) as corresponds to the time-averaging of chemical shifts in a fast chair–boat interconversion. The population of the boat form was estimated only using the shifts of H-23, H-24, and H-25 for which the differences between the conformers were sufficiently large (greater than 0·2 pm).

The boat form population in ketones *V* and *XII* was estimated according to the relationship (*1*), where

$$\% \text{ boat} = 100\,(p - p_c)/(p_b - p_c) \qquad (1)$$

p is a time-averaged NMR parameter (chemical shift or induced shift) found for ketones *V* and *XII*, and p_c and p_b are parameters characteristic of the chair and boat form, respectively, as approximated by the values of model compounds (*III*, *X* and *IV*, *XI*) under assumption that a methyl in position 2 affects neither the parameters p_c and p_b nor the geometry of ring A in both conformers (see also refs[1–3]). If one neglects the effect of structural differences in the ring E between ketones *V*, *VIII*, and *IX* on chemical shifts of the H-23, H-24, and H-25 protons, the characteristic parameters p_c and p_b, obtained from 18α-oleanane model compounds *III* and *IV*, may also be used for estimation of the boat population in ketones *VIII* and *IX*. The

TABLE IV

Chemical shift differences ($\Delta\delta$) between the chair and the boat and estimated population of the boat form (% boat) in 3-oxotriterpenoids

Ketone	Solvent	Parameter	H-23	H-24	H-25
V	$CDCl_3$	$\Delta\delta^a$	0·001	−0·007	−0·442
		% boat[b]	—[c]	—[c]	43
	C_6D_6	$\Delta\delta^a$	−0·256	0·221	−0·256
		% boat[b]	37	37	49
VIII	$CDCl_3$	% boat[b]	—[c]	—[c]	41
	C_6D_6	% boat[b]	35	36	52
IX	$CDCl_3$	% boat[b]	—[c]	—[c]	43
	C_6D_6	% boat[b]	34	38	42
XII	$CDCl_3$	$\Delta\delta^d$	0·000	−0·009	−0·447
		% boat[e]	—[c]	—[c]	41
	C_6D_6	$\Delta\delta^d$	−0·276	0·231	−0·269
		% boat[e]	32	35	52

[a] $\Delta\delta = \delta(IV) - \delta(III)$; [b] calculated from the chemical shifts of *III* and *IV*; [c] the value could not be determined; [d] $\Delta\delta = \delta(XI) - \delta(X)$; [e] calculated from the chemical shifts of *X* and *XI*.

results are summarized in Table IV. In the same manner we estimated the boat population from the benzene induced shifts, ΔBz, under assumption that the chair–boat equilibrium is the same in both solvents. The values of ΔBz for the H-23, H-24, and H-25 protons, together with the populations calculated therefrom, are given in Table V. The mentioned assumption seems to be acceptable since – as calculated from the proton–proton coupling constants – the boat population in both solvents differs only by several per cent[1].

The boat populations in 3-oxotriterpenoid derivatives *V*, *VIII*, *IX* and *XII*, calculated from the H-23 and H-24 (in C_5D_6) and H-25 (in $CDCl_3$) data, range from 32% to 42%, being thus comparable with those obtained by other methods such as measurements of vicinal coupling constants of 1- and 2-protons[1] (30–40%), ^{13}C chemical shifts[2] (32–50%), dipole moments (39–45%), CD spectra (27–33%) or isomerization of model compounds[3] (40–51%). However, the results obtained from chemical shifts of the H-25 protons in C_6D_6, as well as from the ΔBz (H-25) values, are far more scattered and are not compatible with the other results. Possibly, the 2-methyl group in the model compounds affects solvation of the carbonyl group by the aromatic solvent and this influence manifests itself just in the behaviour of the H-25 signal.

The populations of the boat form, given in Table IV, suffer from a systematic error, caused by neglecting the effect of the 2-methyl group in the model compounds

TABLE V

Benzene induced shifts (ΔBz) and estimated population of the boat form (% boat, in parentheses) in 3-oxo derivatives

Ketone	Substituent at C(2)	ΔBz[a] (% boat)		
		H-23	H-24	H-25
III	α-CH_3	0·112	−0·134	−0·256
IV	β-CH_3	−0·145	0·094	−0·070
V[b]	—	0·003 (42)	−0·042 (40)	−0·191 (35)
VIII[b]	—	0·010 (40)	−0·043 (40)	−0·209 (25)
IX[b]	—	0·013 (39)	−0·039 (42)	−0·172 (45)
X	α-CH_3	0·120	−0·164	−0·289
XI	β-CH_3	−0·156	0·076	−0·111
XII[c]	—	0·018 (37)	−0·071 (39)	−0·246 (24)
XV[b,d]	—	0·059 (20)	−0·111 (10)	−0·250 (3)

[a] $\Delta Bz = \delta(\text{in } C_6D_6) - \delta(\text{in } CDCl_3)$; [b] % boat calculated from the data for *III* and *IV*; [c] % boat calculated from the data for *X* and *XI*; [d] H-23, H-24 and H-25 are protons of 4α-, 4β- and 10β-methyl groups, respectively.

on chemical shifts of the methyl groups. For the chair conformation in $CDCl_3$ solutions this effect can be estimated from the data for 3β-hydroxy derivatives with (*VII*, *XIV*) and without (*VI*, *XIII*) 2α-methyl group. Comparison of the chemical shifts in Table I shows that the effect of 2α-methyl group on the protons H-23 and H-24 is negligibly small (less than 0·01 ppm); the H-25 signal was shifted 0·026 ppm downfield. The effect of 2β-methyl group in the boat form could not be assessed because no suitable standard compounds were available. Considering that this effect is due predominantly to magnetic anisotropy of the C(2)—CH_3 bond, we can estimate from the X-ray diffraction data (conformations *C*, *T*(1) and *T*(2)), using McConnell–Robertson equation[28], that the 2β-methyl affects the chemical shift of H-25 only negligibly (+0·005 ppm). Correcting the shift of the H-25 signal in the chair form by 0·026 ppm and neglecting the effect of the 2β-methyl in the boat conformation, we obtained corrected values of the boat population which are 3–4% lower than given in Table IV (for solutions in $CDCl_3$). The final values are then within the limits 37–40% which is closer to the mean values, calculated from the vicinal coupling constants[1] (35–39%) for ketones *V*, *VIII*, *IX*, and *XII*.

The boat population in the equilibrium can also be calculated from the relative induced shifts, ΔEu, of the H-23 and H-24 protons which show the greatest differences between both conformers and in ketones *III*–*V* are in the least degree affected by the coordination LSR–ether. However, the characteristic values for the coordination LSR–carbonyl in the chair ($\Delta Eu_c = \Delta Eu(III) - \Delta Eu(I)$; 41·9 and 29·6 for H-23 and H-24, respectively) and in the boat form ($\Delta Eu_b = \Delta Eu(IV) - \Delta Eu(II)$; 29·3 and 39·8 for H-23 and H-24, respectively) cannot be directly used in Eq. (*1*) because the fundamental condition that the 2-methyl group in model compounds *III* and *IV* should not influence the ΔEu values is not fulfilled. As mentioned above, this methyl affects the binding constant of the complex LSR–carbonyl and thus also the relative shifts ΔEu. This fact can be expressed by equation (*2*)

$$\Delta Eu_c(1 - x)f_c + \Delta Eu_b x f_b = \Delta Eu_{obs} \qquad (2)$$

where x is the fraction of the boat in the equilibrium mixture, ΔEu_{obs} is the observed relative shift induced by coordination LSR–carbonyl in ketones without the 2-methyl group and f_c and f_b are unknown coefficients involving the effect of the 2-methyl in the chair and boat form, respectively. Under assumption of the same effect in both conformers ($f_c = f_b$), application of Eq. (*2*) to the induced shifts of H-23 and H-24 protons, using the above-mentioned values of ΔEu_c and ΔEu_b, leads to Eq. (*3*),

$$\% \text{ boat} = 100\,(41{\cdot}9 - 29{\cdot}6k)/(12{\cdot}6 + 10{\cdot}2k) \qquad (3)$$

where $k = \Delta Eu_{obs}$ (H-23)/ΔEu_{obs} (H-24). This means that for calculation of the boat

population it is sufficient to know only the ratio k for the unsubstituted ketones. The NMR spectral data of the ketone *V* at three different concentrations of $Eu(fod)_3$ give values $k = 1{\cdot}07-1{\cdot}09$ (after correction for coordination LSR–ether), corresponding to 41–43% of the boat; for ketone *IX* the values are $k = 1{\cdot}07$ and 43% of the boat.

According to the data published for 4,4-dimethyl-5α-cholestan-3-one (*XV*), the ratio of LSR–induced shifts of the 4α- and 4β-methyl protons, k, amounts to 1·27 (ref.[16]) or 1·25 (ref.[8]) which corresponds to 17% and 19% of the boat conformation, respectively. From the ΔBz values of these protons we calculated 20% and 10% of the boat (Table V). These values seem to be too high and do not correspond to vicinal coupling constants[1]. It is possible that our 2-methyl-3-oxotriterpenoids *III* and *IV* are not the ideal models for the 4,4-dimethylsteroid ketones, they are, nevertheless, certainly the best available ones. The recent molecular mechanics calculations[11] lead to 15% of the boat and a very similar value (17%) also follows from an entirely different method: as we have shown[3], in the isomerization of 2α and 2β--substituted 3-ketones (e.g. 2-bromoketones) the proportion of the 2β-isomer at equilibrium corresponds approximately to the population of boat form in conformational equilibrium in the unsubstituted ketone. Isomerization of 2α and 2β-bromo--4,4-dimethyl-5α-cholestan-3-ones gave[30] a mixture containing $17 \pm 5\%$ of the β-isomer. For 8-lanostene-3-one derivatives *XVI* and *XVII* the induced shift ratio $k = 1{\cdot}18-1{\cdot}14$ (refs[8,16]), i.e. 28–34% of the boat; isomerization of 2-bromo derivatives of 8-lanosten-3-one (*XVI*) gave $22 \pm 5\%$ of the β-isomer[30].

In conclusion, we may summarize that the chair–boat equilibrium in triterpenoid 3-ketones can apparently be best determined using shifts of the 10β-methyl protons in chloroform solutions: they exhibit largest differences for both conformers and their signal can easily be identified in the spectra because of its doublet character. Unfortunately, the "characteristic values" of the conformers, derived from our model compounds, cannot be used generally because the chemical shift of the 10β-methyl group may be considerably affected by structural changes in the other rings (B–E). This is true particularly if the molecule contains groups of high magnetic anisotropy. On the other hand, lanthanide or benzene induced shifts (ΔEu, ΔBz) of 4α- and 4β-methyl protons should be independent of structural changes at the sites sufficiently distant from the 3-oxo group and should be therefore generally applicable.

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