Conformational analysis of A and B rings in 2-, 4-, and 6-bromosubstituted steroidal 4-en-3-ones by nuclear magnetic resonance

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*The conformational preference of A and B rings in four differently functionalized bromosubstituted 4-en-3-one steroids is studied by concerted application of high-resolution one- and two-dimensional nuclear magnetic resonance (NMR) techniques, such as homonuclear and heteronuclear correlated spectroscopy, transient and steady-state nOe spectroscopy, temperature-dependent chemical shift variation, and application of a modified Karplus equation. The steroids studied include 6β-bromocholest-4-en-3-one (**3**), 4,6β-dibromocholest-1,4-dien-3-one (**2**), 2α,4,6β-tribromocholest-4-en-3-one (**1**), and (25R)-2α,6β-dibromospirost-4-en-3-one (**4**). Steroids **1–4** were prepared by either acid-catalyzed or free-radical bromination from appropriate 4-en-3-one steroid. The study has yielded an insight into the factors responsible for conformational preferences of the A and B rings of these bromosubstituted steroids. Bromosubstitution at the 2α position is responsible for the inversion of the A ring to inverted 1β,2α-halfchair conformation. The electronic interaction between 4-bromine and carbonyl oxygen distorts the A-ring conformation further. Inversion of the A ring has a concomitant effect of distortion in the chair form of the B ring. Conformational preferences of A and B rings are not found to be influenced by transmission effect of a side chain or oxygenated ring system. Temperature-dependent NMR studies indicate the reduced conformational flexibility of the A ring for 2α-bromosubstituted steroids. Complete assignment of the ^{13}C and ^{1}H resonances of two of the steroids studied (**3** and **4**) is presented. (Steroids* **58:**170–177, 1993)

Keywords: 4-en-3-one steroids; two-dimensional nuclear magnetic resonance; bromosteroids; conformational analysis; total assignment; A and B rings

Introduction

Although understanding the relationship between molecular structure and biological reactivity has long been the goal of organic chemists, with steroids this extension of correlation has mainly involved the conformational preferences of the A and B rings. The endocrinological importance of a number of natural and synthetic 6-substituted hormones makes their global conformation an area of special interest.[1] The A ring in steroidal 4-en-3-ones has been a focal point of many x-ray studies.[2–4] A normal-1α,2β-halfchair conformation (Figure 1a) is reported for the A ring of a majority of 4-en-3-one steroids. The conformational preference of the A ring of 4-en-3-one steroids studied includes a wide spectrum of cases ranging from 2β-sofa through 1α,2β-halfchair to 1α-sofa, all of which have been considered as minor variations on fundamental normal-1α,2β-halfchair conformation. Wong and Clark[5] have studied the solution conformation by selective two-dimensional (2-D) indirect J spectroscopy for $^{2}J_{HH}$, whereas Schneider et al.[6] have obtained, by molecular mechanics 2 (MM2) calculations, 1α-sofa geometry as the energy minimum for the A ring of 6α-fluoro-11β-hydroxy-16α-methylprogesterone. Fluorine substitution at the 9α position has been found to distort but not invert the A ring in a number of cortisols.[7] Marat et al.[8] report a 1α,2β-halfchair A-ring conformation for a series of variously substituted 4-en-3-one steroids, except for 2β-acetoxy testosterone-17β-acetate, wherein the A ring is predominantly inverted to 1β,2α-halfchair (Figure 1c), mainly due to the flagpole interactions. Tori et al.[1,9] have studied the B-ring conformation by the deformation of ^{1}H nuclear magnetic resonance (NMR) signal

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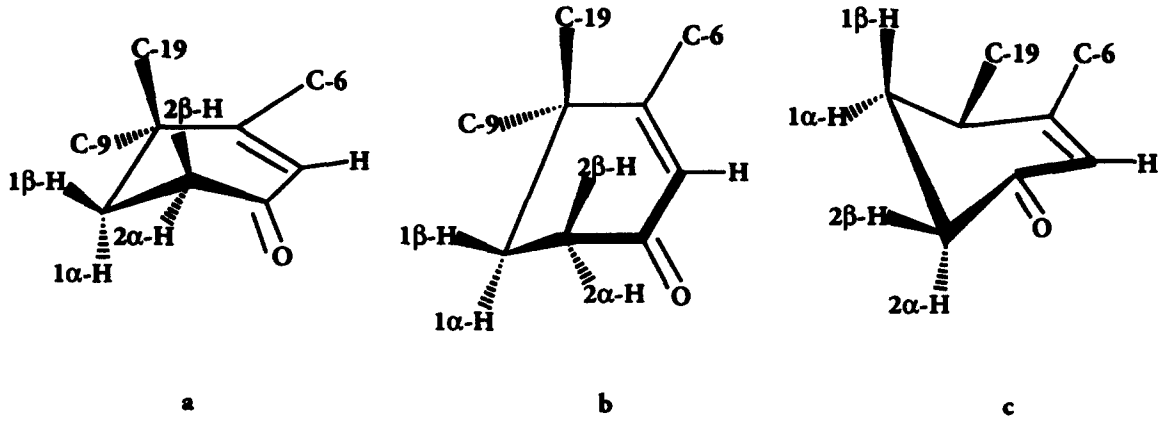


Figure 1 Three conformational preferences of A ring for 4-en-3-one steroids. (a) Normal 1α,2β-halfchair conformation; (b) 1α-sofa conformation; and (c) inverted 1β,2α-halfchair conformation. Notice the orientation of C-19 in each conformation. As the conformation changes from normal 1α,2β-halfchair to inverted 1β,2α-halfchair, the C-19 changes its orientation more toward the B ring.

pattern in 6β-chloro and 6β-bromosubstituted steroids and have attributed the observed long-range effects to distortion from the chair form of the B ring. A reservation about this conclusion was also expressed later by the same investigators.[10] Any further conclusion drawn from low-field NMR studies could be misleading. The known dependence of conformation on substitution pattern and the ambiguity in earlier literature led us to investigate the solution conformational preference of the A and B rings in some 4-en-3-one bromosubstituted steroids by the application of high-field NMR techniques.

Experimental

Melting points (mp) are uncorrected. Solvents were predried according to literature procedures. All compounds gave required elemental and mass analyses. Elemental analyses were performed on a CEST.MOD 011 analyzer, whereas electron impact (EI) mass spectra were obtained using a Shimadzu QP1000 spectrometer. The λ_{max} values were measured using a Shimadzu UV-160 spectrophotometer, and infrared (IR) spectra were obtained on a Perkin Elmer 168 spectrophotometer. Cholestenone and N-bromosuccinimide were purchased from Aldrich Chemical Co., USA. One-dimensional ^1H and ^{13}C NMR spectra of the compounds were recorded on a 500-MHz Bruker AM500 spectrometer equipped with an ASPECT 3000 computer, or a 300-MHz Varian VXR300S spectrometer with a digital resolution of 0.16 Hz at ambient temperature as solutions in $CDCl_3$. The ^1H NMR spectra were referenced with internal standard tetramethyl silane (TMS) as 0.0 ppm, whereas the central carbon line of $CDCl_3$ was set to 77.00 ppm for ^{13}C NMR. An amount of 10 mg in 0.50 ml each of **1–4** was used for ^1H, whereas 40 mg in 0.50 ml was used for ^{13}C NMR experiments. Variable-temperature ^1H NMR studies on **4** were performed on a 300-MHz Varian machine in the temperature range of −40 to 40 C by incrementing the temperature 10 C at a time. A $\pi/2$-t1-$\pi/2$-t2 pulse sequence[11] was used for generating proton-proton connectivities with 256 t1 increments and 512 W data size. The free induction decays (FIDs) were Fourier transformed onto a data matrix of 1K × 1K using sinebell window function. The absolute intensity correlation spectroscopy (COSY) experiments were symmetrized and plotted as contours. The 2-D transient nuclear Overhauser effects (nOes) for **4** were generated with a $\pi/2$-t1-$\pi/2$-τ-$\pi/2$-t2 pulse sequence[12] on the Varian machine with a mixing time of 1 second and a relaxation delay of 1.5 seconds. The FIDs were Fourier transformed onto a data matrix of 1K × 1K with phase-shifted sinebell window function. Negative nOes were observed for all protons in the 2-D experiments. The heteronuclear correlation experiments for **3** and **4** were performed using standard Varian software with 256 t1 increments and 512 data points. Zero filling to 1K × 1K data matrix was followed by Fourier transformation with sinebell window functions. Polarization transfer was optimized for $^1J_{CH}$ = 135 Hz. A relaxation delay of 1.5 seconds was used. SEFT (spin echo Fourier transform) experiment[13] for **4** was performed with the standard pulse sequence on a 270-MHz WM270 Bruker spectrometer. Nuclear Overhauser enhancement difference (NOED)[14] studies on **4** were performed on a Bruker AM500 spectrometer using standard Bruker software.

Preparation of bromocholestenones 1–3

To an ice-cooled (5–10 C) solution of cholest-4-en-3-one (3.09 g, 8 mmol) in anhydrous ether (175 ml) containing a few drops of aqueous HBr and acetic anhydride was added dropwise with vigorous stirring a solution of bromine (7.5 g, 47 mmol) in glacial acetic acid (75 ml) at a rate at which the solution decolorized. A slight excess of bromine was added and the mixture was further stirred for 30 minutes. The reaction mixture was concentrated in vacuo to half the original volume and filtered. The solid obtained was repeatedly washed with cooled ethanol and purified on silica gel (100–200 mesh) by eluting with mixtures of increasing polarity of benzene in petroleum ether [boiling point (bp) 60–80 C] to yield successively three brominated derivatives, 2α,4,6β-tribromocholest-4-en-3-one **(1)** (0.031 g, 0.62%), 4,6β-dibromocholest-1,4-dien-3-one **(2)** (0.62 g, 14.3%), and 6β-bromocholest-4-en-3-one **(3)** (0.90 g, 24.1%). These were recrystallized from methanol to obtain pure compounds.

2α,4,6β-Tribromocholest-4-en-3-one (1). mp 180–182 C; IR (KBr) $\bar{\nu}$ = 1,700 cm^{-1} (C=O), 1,605 (C=C); ultraviolet (UV) ($CHCl_3$) $\lambda_{max}(\log\varepsilon)$ = 279 nm (4.120); ^1H NMR ($CDCl_3$) δ = 5.66 (dd, J = 4.5, 2.1 Hz, 1H, 2β-H), 4.98 (dd, J = 3.9, 5.0 Hz, 1H, 6α-H), 1.63 (s, 3H, 19-H_3), 0.92 (d, J = 6.41, 3H, 21-H_3), 0.88 (d, J = 1.46 Hz, 3H, 26-H_3), 0.85 (d, J = 1.48 Hz, 3H, 27-H_3), 0.77 (s, 3H, 18-H_3).

4,6β-Dibromocholest-1,4-dien-3-one (2). mp 163–166 C; IR (KBr) $\bar{\nu}$ = 1,685 cm^{-1} (C=O), 1,606 (C=C); UV ($CHCl_3$)

λ_{max} (logε) = 279 nm (4.161); ^{1}H NMR ($CDCl_3$) δ = 6.63 (d, J = 2.0 Hz, 1H, 1-H), 6.54 (d, J = 2.0 Hz, 1H, 2-H), 4.94 (dd, J = 13.9, 5.3 Hz, 1 H, 6α-H), 1.22 (s, 3H, 19-H_3), 0.92 (d, J = 6.62 Hz, 21-H_3), 0.88 (d, J = 1.28 Hz, 26-H_3), 0.85 (d, J = 1.28 Hz, 27-H_3), 0.75 (s, 3H, 18-H_3).

6β-Bromocholest-4-en-3-one (3). mp 133–135 C (lit. 132 C[15]); IR (KBr) $\bar{\nu}$ = 1,675 cm^{-1} (C=O), 1,610 (C=C); UV ($CHCl_3$) λ_{max} = 249 nm; ^{1}H NMR ($CDCl_3$) δ = 5.88 (s, 1H, 4-H), 4.96 (dd, J = 3.8, 1.8 Hz, 1H, 6α-H), 2.48 (ddd, J = 17.4, 14.0, 5.0 Hz, 1H, 2β-H), 1.58 (s, 3H, 19-H_3), 0.92 (d, J = 6.5 Hz, 3H, 21-H_3), 0.87 (d, J = 2.1 Hz, 3H, 26-H_3), 0.86 (d, J = 2.1 Hz, 3H, 27-H_3), 0.77 (s, 3H, 18-H_3).

Preparation of (25R)-2α,6β-dibromospirost-4-en-3-one (4)

A mixture of (25R)-spirost-4-en-3-one (0.25 g, 0.61 mmol) and N-bromosuccinimide (NBS) (0.107 g, 0.51 mmol) was refluxed in freshly distilled anhydrous CCl_4 (25 ml) for 10 hours until all NBS at the bottom of the flask was gradually replaced by succinimide, which had risen to the surface of the mixture. Succinimide was filtered and the filtrates washed with anhydrous CCl_4. The solvent was removed in vacuo and the solid obtained repeatedly crystallized from methanol to yield pure (25R)-2α,6β-dibromospirost-4-en-3-one **(4)** (0.21 g, 60%, mp 182–184 C). IR (KBr) cm^{-1} = 1,685 (C=O), 1,610 (C=O); UV ($CHCl_3$) λ_{max} (logε) = 252 nm (4.012); ^{1}H NMR ($CDCl_3$) δ = 6.04 (s, 1H, 4-H), 4.99 (d, J = 2.9 Hz, 1H, 2β-H), 4.96 (dd, J = 14.4, 4.8 Hz, 1H, 6α-H), 1.67 (s, 3H, 19-H_3), 1.02 (d, J = 7.3 Hz, 3H, 21-H_3), 0.92 (s, 3H, 18-H_3), 0.83 (d, J = 6.1 Hz, 3H, 21-H_3).

Results and discussion

Bromination of Δ^4-3-ketosteroids has been a very well studied reaction, both synthetically and mechanistically.[15–17] Numerous products with bromine substitution and ene formation at the 2, 4, and 6 positions have been observed, with the precise reaction conditions governing the product's distribution. Bromination of Δ^4-3-ketosteroid **(5)** by acid catalysis[17,18] yielded three highly crystalline compounds after column chromatography on silica gel. The ^{1}H NMR spectra of the isolated steroids **1–3** indicated a deshielded 19-H_3 signal, implying a probable 6β-bromine substitution for all of them. The compound eluting first had no signal in the region of 5–7 ppm in its ^{1}H NMR spectrum (Figure 2a), indicating absence of 4-H. In addition, the NMR spectrum showed a signal (dd) at 5.65 δ assignable to 2α-bromine. In order to confirm the stereochemistry at the 2 position, a steady-state NOED experiment was performed by irradiating a 19-H_3 signal (data not shown). A one-dimensional (1-D) steady-state nOe experiment is normally more sensitive as compared to a 2-D transient nOe experiment for detection of less than 2% signal intensity enhancement. Weak positive enhancement (<5%) in the intensity of 2β-H was observed, confirming the correct assignment of substitution at the 2 position. The weak enhancement in intensity of the 2β-H signal was probably due to a number of magnetization relays occurring for the A-ring spin system and went undetected in the 2-D transient nOe experiment. The compound was hence tribromosteroid **1** (Scheme 1). The middle fraction provided a steroid that showed two doublets integrating for a proton each with J = 2.0 Hz in the olefinic region of the ^{1}H NMR spectrum; in addition, the EI mass spectrum (data not shown) showed a 1 : 2 : 1 intensity for the peaks assignable to molecular ions, indicating the structure to be dibromosteroid **2**. The final compound isolated showed only one olefinic proton at 5.85 δ in the deshielded region, implying 6β-bromosteroid **3** (Figure 2b). Free-radical bromination by the method of Calo et al.[19] of diosgenone **6** yielded a single compound as observed on thin-layer chromatography. The ^{1}H NMR spectrum of the compound showed signals at 6.1 δ (s, 4-H), 4.9 δ (dd, 6β-H), and 5.0 δ (d, 2α-H), indicating the structure to be dibromodiosgenone **4**. It is worthwhile to note here that the free-radical bromination method failed to provide any brominated product for steroid **5**, even after varying the reaction conditions. The inability of **5** to undergo this reaction is unexplainable. Bromosteroid **2** has not been reported to date to the best of our knowledge, although the corresponding 4,6-dibromosteroid without the 1-ene functionalization is well characterized.[17]

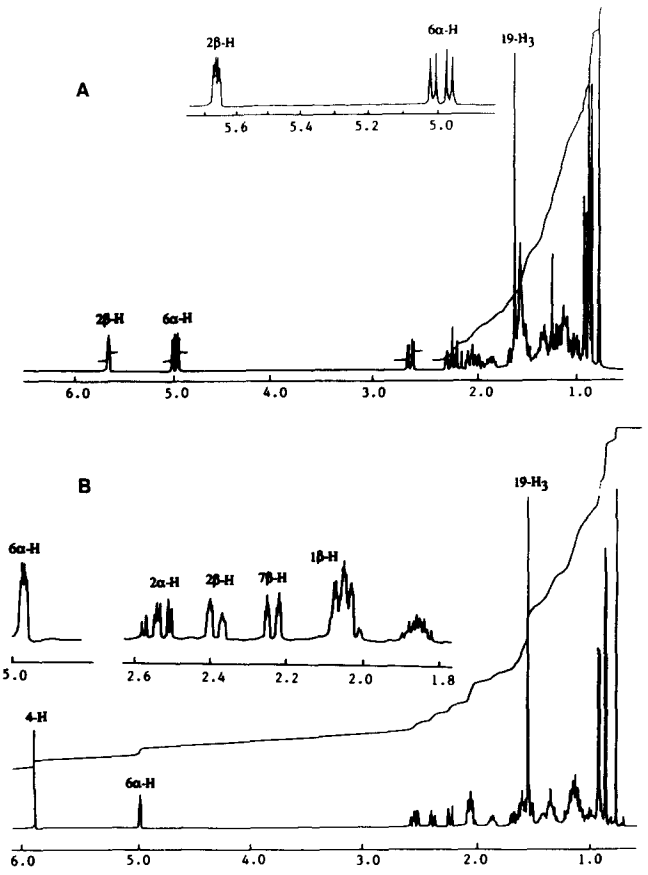
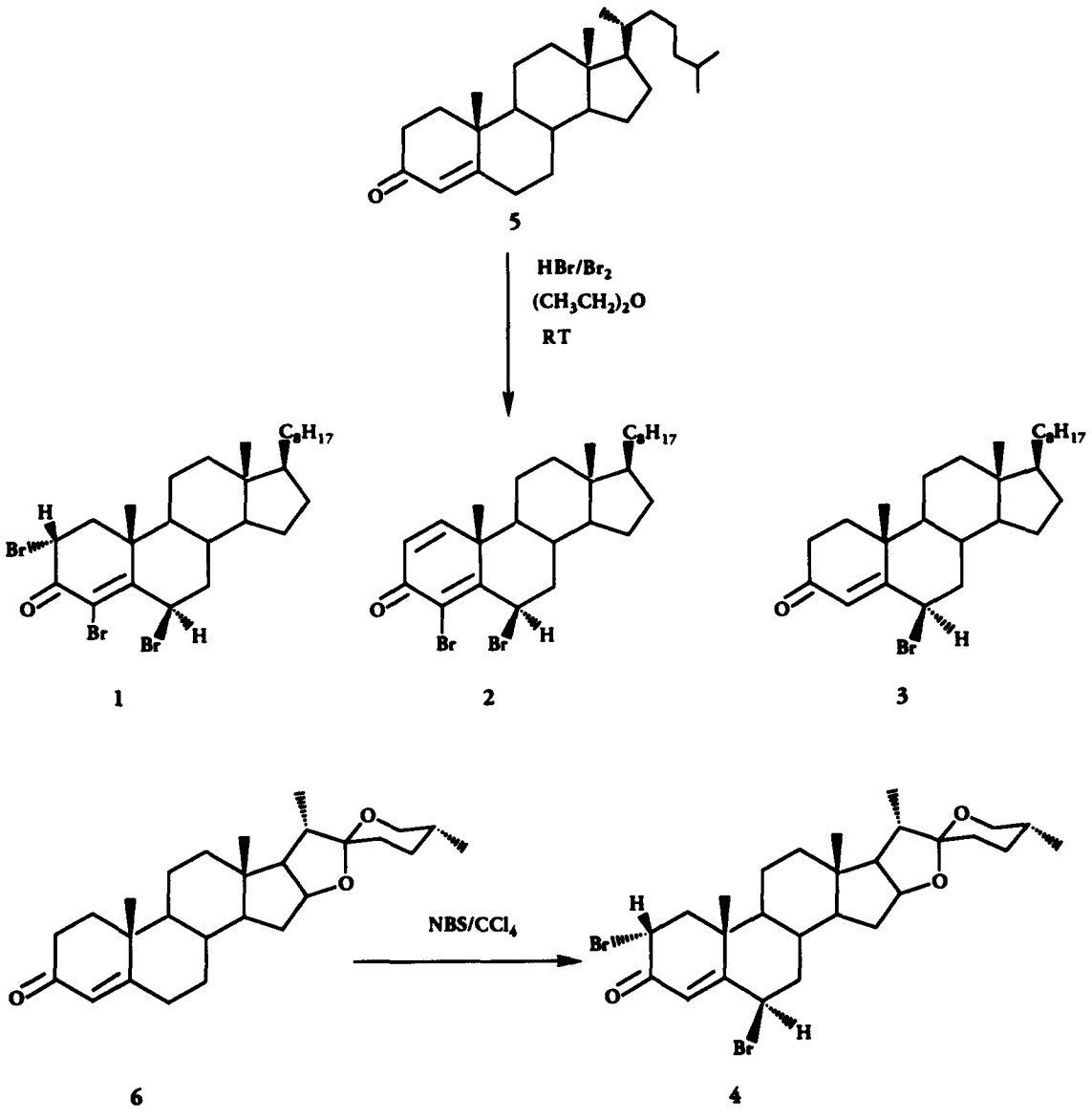


Figure 2 ^{1}H NMR spectra for steroids **1** (a) and **3** (b) at 500 MHz. Insets show the expanded regions of interest for calculation of dihedral angles of the protons of the A and B rings.

Because the ^{1}H NMR spectrum of a steroid is usually complicated by severe overlap of the multiplets, even at high fields, the conformational analysis of the four steroids was accomplished by the concerted applica-



Scheme 1 Preparation of bromosubstituted steroids **1–4.**

tion of 1-D and 2-D NMR techniques such as homonuclear COSY-90, ^{1}H ^{13}C COSY, NOESY, and steady-state NOED. The spin system of B-ring protons could be distinguished from A-ring protons by the connectivities of 2-H, which is expected to be deshielded more than 6-H. The assignment of ^{13}C resonances for steroids **3** and **4** were accomplished by using literature reports[20,21] and confirmed by heteronuclear COSY and SEFT experiments. The complete assignments of ^{1}H and ^{13}C resonances of **3** and **4** are given in Table 1. Multiplets corresponding to protons at C-2 and C-6 in all brominated derivatives were well resolved and separated from the rest of the methylene envelope, and the coupling constants could be calculated by first-order analysis.[22] As a result of the inherent shortcomings of the original and modified Karplus equations, the dihedral angles between the protons have been calculated by using the Karplus relationship given by Colucci et al. (Equation 1).[23] Though the empirical Karplus-type equation given by Imai and Osawa[24] gives the least standard deviation of error, it involves 11 mutually independent structural terms and 22 adjustable parameters. The relationship as given by Colucci et al.[23] (Equation 1), involving only three adjustable parameters, was therefore applied to obtain a relatively accurate description of the dihedral angles.

$$J = A + B\cos\Theta + C\cos 2\Theta + (S1 + S4)\cos(\Theta - 120) + (S2 + S3)\cos(\Theta + 120) \quad (1)$$

where A, B, and C are parameters optimized for this equation, the values for which are $A = 8.37$, $B = -2.83$, and $C = 7.44$. $S1$, $S2$, $S3$, and $S4$ are four substituent

Table 1 Assignment of ^{13}C and ^{1}H resonances in steroids **3** and **4**

	3				4			
		Proton				Proton		
	Carbon	α		β	Carbon	α		β
1	37.6	1.60		2.06	40.9	1.62		2.26
2	33.9	2.48		2.38	50.7	—		4.99
3	199.2	—		—	191.3	—		—
4	126.7		5.88		124.8		6.04	
5	165.5	—		—	165.7	—		—
6	52.2	4.96		—	49.8	4.96		—
7	40.7	1.51		2.23	50.0	2.16		2.62
8	30.5	—		1.96	30.3	—		1.62
9	52.7	0.96		—	52.7	1.00		—
10	38.1	—		—	41.8	—		—
11	20.8	1.60		1.60	20.8	1.55		1.55
12	39.3	2.01		1.09	39.3	1.82		1.20
13	42.3	—		—	40.5	—		—
14	55.0	1.26		—	54.9	1.18		—
15	23.9	1.01		1.55	31.6	1.38		1.99
16	27.9	1.82		1.29	80.4	4.45		—
17	55.9	1.12		—	62.2	1.80		—
18	11.8		0.77		16.4		0.92	
19	21.9		1.58		22.9		1.67	
20	35.9	—		1.30	41.8	—		1.91
21	18.5	0.92		—	14.5	1.02		—
22	35.5	1.13		1.05	109.2	—		—
23	23.6	1.30		1.17	31.5	1.63		1.63
24	39.3	2.04		1.13	28.8	1.48		1.62
25	27.8		1.51		30.0	—		2.22
26	22.4		0.87		66.9	3.40		3.50
27	22.7		0.86		17.1		0.81	

Chemical shifts are expressed in ppm.

constants of the ethane fragment of the ring, with *S*1 and *S*4 for the front carbon substituents, and *S*2 and *S*3 for the rear carbon substituents. The substituent constants used in the computation are 3.40 Hz (*S*1) for $COCHCH_2$, 3.28 Hz (*S*2) for Br, 2.44 Hz (*S*3) for t-butyl or equivalent framework, and 0.00 (*S*4) for H. Θ is the dihedral angle between protons under consideration.

Fitting the coupling constant into Equation 1 to obtain a corresponding dihedral angle was accomplished by using a program[25] in FORTRAN-77 on the CYBER-180/840 computer network. Corresponding to each coupling constant, Equation 1 was solved for all values of dihedral angle in the range 0–360° by incrementing it with 1/1,000th of a degree to double precision. This afforded four angles in four quadrants, because the equation is doubly Gaussian in the range. The error in the calculation was determined by back-substitution of the dihedral angles obtained by the computation. The angles with least error or the angles conforming to molecular modeling analysis were chosen for defining the conformations of the A and B rings. Equation 1 furnished dihedral angles (Table 2) that are different from those predicted by the extended and original Karplus relationships[26–30] by approximately 10° in a few cases, thus justifying its application.

The A-ring conformation of 6β-bromocholest-4-en-3-one **(3)** is deduced from the three-bond vicinal coupling between protons at C-1 and C-2, and geminal coupling of protons at C-2 (Table 2). For a 1α-sofa conformation (Figure 1b), the geminal coupling $^{2}J_{2\alpha,2\beta}$ is normally observed to be about 13 Hz. Because the value obtained is 17.4 Hz, 1α-sofa conformation is ruled out. An inverted-1β,2α-conformation would require $^{3}J_{1\alpha,2\beta}$ to be 3–4 Hz, which is not found to be the case. The vicinal coupling constants $^{3}J_{1\alpha,2\beta}$ and $^{3}J_{1\beta,2\beta}$ imply torsional angles of 178° and 46°; hence, the preferred conformation is normal-1α,2β-halfchair. An inverted-1β-2α conformation is not preferred, because it would increase the steric crowding between C-10 methyl and 6β-Br and deform the B ring. The 1,3-diaxial repulsions are minimized in the normal-1α,2β-halfchair conformation because the substituents at the 1 and 3 positions fall apart. According to a theoretical model of Barfield and Grant,[31] the C-3 carbonyl nearly bisects the H–C-2–H angle in a normal-1α,2β-halfchair conformation, which increases the geminal coupling $^{2}J_{2\alpha,2\beta}$ to approximately 17 Hz. This is indeed found to be the case. The observed vicinal couplings $^{3}J_{6\alpha,7\alpha}$ and $^{3}J_{6\alpha,7\beta}$ and the corresponding dihedral angles 42.6° and 60.3° show that the B ring nearly retains the chair conformation of a typical cyclohexane component of a steroid. The con-

Table 2 Chemical shifts and coupling constants of selected protons of rings A and B in steroids **1–4**

Compound	Chemical shifts[a]			Coupling constants[b]				
	2α	2β	6α	$^3J_{(1\alpha,2\beta)}$	$^3J_{(1\beta,2\beta)}$	$^2J_{(1\beta,2\beta)}$	$^3J_{(6\alpha,7\alpha)}$	$^3J_{(6\alpha,7\beta)}$
1	—	5.66	4.98	4.5 (59.0°)[c]	2.1 (76.4°)	—	5.0 (33.5°)	13.9 (173.4°)
2	—	—	4.94	—	—	—	5.3 (31.2°)	13.9 (173.4°)
3	2.34	2.48	4.96	14.0 (177.9°)	4.8 (45.7°)	17.4	3.8 (42.6°)	1.8 (60.3°)
4	—	4.99	4.96	—	2.9 (66.9°)	—	4.8 (35.0°)	14.4 (173.4°)

[a] Chemical shifts are expressed in ppm.
[b] Coupling constants are expressed in Hz.
[c] Angles correspond to molecular models.

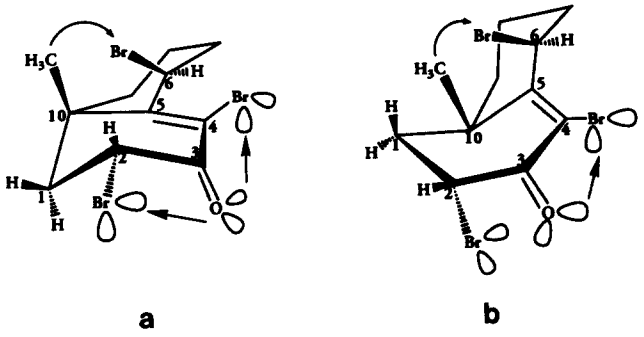


Figure 3 Stereoview of van der Waals and electrostatic interactions within A and B rings for steroids **1–4**. (a) Normal-1α,2β-halfchair conformation of the A ring with equatorial 2α-bromine and planar 4-bromine causes severe electrostatic interactions, indicated by straight arrows. Distorted B-ring conformation with 1,3-flagpole repulsion shown by curved arrow. (b) Inverted 1β,2α-halfchair conformation of the A ring with a twisted C-3–C-4 bond to relieve the electrostatic repulsion. This leads to increased distortion for the B ring.

sistency between the results obtained in the present NMR studies and those available from other studies such as ORD[32–35] speak in favor of our first-order approximation and proposed B-ring conformation of 6β-bromocholest-4-en-3-one **(3)**.

The chemical shift of 2β-H in (25R)-2α,6β-dibromospirost-4-en-3-one **(4)** and the calculated dihedral angles between 2β-H and protons at C-1 eliminate the possibility of a normal-1α,2β-conformation of the A ring. Molecular models suggest that in a 1α-sofa conformation, 2β-H lies perpendicular to 1α-H, with C-3, C-5, and C-1 coplanar. Qualitative NOED spectrum (data not shown) of bromosteroid **4** shows a weak enhancement of 19-H_3 signal on irradiation of the 2β-H signal, whereas the irradiation of the 19-H_3 signal shows a strong enhancement of 1β-H and a weak enhancement of 2β-H signals. These observations suggest an intermediate conformation of the A ring in **4** between an inverted-1β,2α-halfchair and a 1α-sofa (Figure 3a). The calculated dihedral angles (Table 2) corresponding to the couplings $^3J_{6\alpha,7\alpha}$ and $^3J_{6\alpha,7\beta}$ in the B ring of **4** are reasonable only if 6α-H assumes a pseudo-axial position, which suggests that the B ring is in a distorted form. The conformation of the A ring in **4** causes steric crowding between the C-10 methyl and 6β-Br, and this strain is minimized by the twisting of the B ring from a perfect chair conformation to a distorted chair. Temperature-dependent NMR studies (Figure 4) on 2α,6β-dibromo-(25R)-spirost-4-en-3-one **(4)** shed more light on these observations. Although the multiplets of 2β-H and 6α-H resolve at elevated temperatures, the chemical shifts of 6α-H, 2β-H, and 19-H_3 are found to be affected marginally by the variation of temperature from 40 to −40 C. This can be attributed to the fact that the conformation of the A ring is fully inverted with 2β-H, assuming complete equatorial position and coplanarity with C-3 carbonyl at lower temperatures. The inversion causes the flipping of C-10 methyl more toward the B ring, thereby increasing its 1,3-diaxial repulsive interactions with 6β-Br. The marginal deshielding of 6α-H at lower temperature may in part be attributed to the anisotropy of the C-4 π bond. At elevated temperatures, the thermal energy is partially sufficient to overcome the electronic interactions between 2α-Br and the carbonyl, which facilitates the adoption of an intermediate conformation between 1β,2α-halfchair and 1α-sofa by the A ring in **4**. Although the increase in temperature causes skeletal flipping of the A ring, it may not be quite conceivable to envisage the adoption of a normal 1α,2β-halfchair solution conformation at temperatures higher than 40 C because the electronic interactions between bromine and carbonyl are severe.

The calculated dihedral angles (Table 2) between A-ring protons in **1** eliminate the possibility of any normal-1α,2β-conformation. The only probable conformation adopted by the A ring is inverted 1β,2α-halfchair, which is attained by slight twisting of carbonyl, thereby disrupting conjugation in the A ring to relieve the electronic interactions between planar 4-bromine. This is significant in contrast to the 2β-acetoxy 4-en-3-one steroid reported by Marat et al.[8] where the A ring is predominantly inverted due to 1,3-diaxial steric interac-

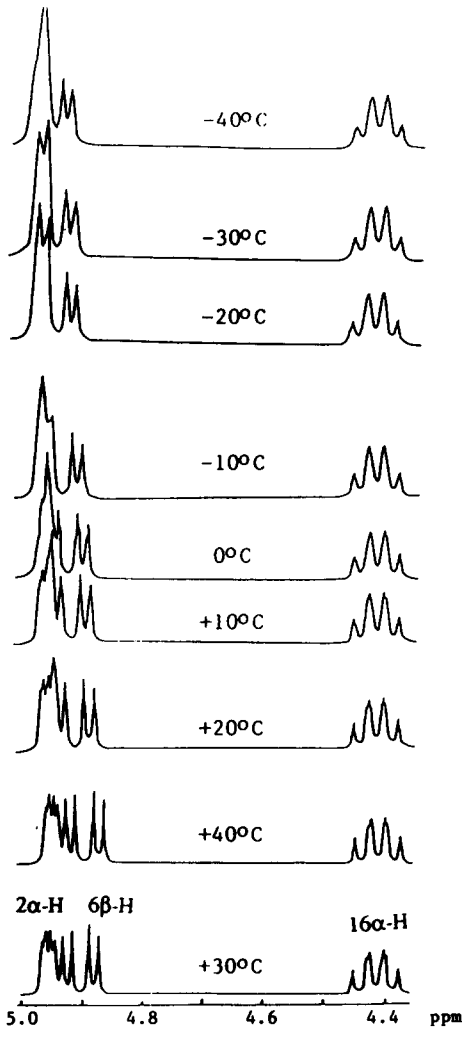


Figure 4 Temperature-dependent NMR studies for steroid **4** at 300 MHz. Only the region of interest is shown. All other signals remained unchanged with the variation in temperature. The gradual broadening of signals with decreasing temperature is probably due to decreasing molecular tumbling motion.

tions. The crucial role played by the planar 4-bromine in terms of electronic interactions with the carbonyl can be appreciated from the observed difference in A-ring inversion between steroids **1** and **4**. The B ring in 2α,4,6β-tribromocholestenone **(1)** is twisted, as can be seen from the calculated dihedral angles (Table 2). The A and B rings in steroid **1** are reported to adopt sofa and distorted chair conformations, respectively, in the crystalline state.[35] This is in contrast to our observations in the solution state. It is quite probable that crystal packing forces might slightly twist the A ring about C-2 (which is below the plane) to sofa conformation for proper stacking. In 4,6β-dibromocholestadienone **(2)** the planarity of the A ring causes strong 1,3-diaxial interactions between C-10 methyl and 6β-bromine. The dihedral angles (Table 2) between the protons at C-6 and C-7 show that the B ring is highly twisted.

In conclusion, any bulky halogen substitution at the 2 position in the A ring of Δ^4-3-keto steroids is expected to invert the ring irrespective of its configuration, and this inversion need not be explained only in terms of van der Waals repulsions, but other factors, such as electronic interactions, may also play a key role. The conformational preference of the A and B rings in these steroids is not at all influenced by the transmission effect, and it is essentially the conformation of the A ring that perturbs the B ring.

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