Journal of Molecular Structure 892 (2008) 246–253

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Molecular association via halogen bonding and other weak interactions in the crystal structures of 11-bromo-12-oxo-5β-cholan derivatives

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ARTICLE INFO

Article history:
Received 3 May 2008
Received in revised form 26 May 2008
Accepted 27 May 2008
Available online 12 June 2008

Keywords:
11-Bromosteroids
Halogen bonding
Crystal structure
Cholic acid

ABSTRACT

Methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **2**, methyl 11α-bromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **3**, methyl 11β-bromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **4** and methyl 11,11-dibromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **5** were synthesized. The crystal structures of these molecules were resolved to study the effect of bulky bromine atom in the steroid skeleton of cholic acid with different stereo-chemical orientations at C-11 on the two-dimensional arrangement of molecules and solid-state properties. All the molecules associate only via weak intermolecular interactions in their crystal structures, notable one being the Halogen Bonded assembly (C–Br. . .O) in **5**.

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1. Introduction

Stereoselective C-11 functionalization in steroids is one of the challenging targets for synthetic organic chemists as it involves severe steric interactions caused due to C-18 and C-19 angular methyl groups. Introduction of C-11 α-hydroxyl functionality via microbial hydroxylation by Syntex group [1] and via long-range chemical functionalization by Breslow [2] are well documented. Steroids with C-11 functionality are well known for biological activity and are present in a number of naturally occurring molecules such as cortisone, hydrocortisone and corticosterone [3,4]. Much more potent synthetic corticosteroids such as dexamethasone, triamcinolone and fluticasone also possess C-11 hydroxy functionality [5]. We planned to study the effect of bulky halogen atom (bromine) at C-11 in the steroid skeleton of cholic acid **1** with different stereo-chemical orientations on the crystal structure and two-dimensional arrangement of molecules. Cholic acid **1**, a main bile acid, is a biosurfactant involved in the digestion of dietary lipids [6]. It has unique distribution of three hydrophilic hydroxyl groups in the α-face, and the β-face is hydrophobic consisting of only hydrocarbons. Because of this cholic acid is often considered to be a facial amphiphile. Due to its interesting structural features and commercial availability, cholic acid is a popular building block in supramolecular chemistry [7] and to construct environmentally responsive molecules [8,9]. Herein, we would like to report the synthesis and supramolecular as well as spectral properties of methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **2**, methyl 11α-bromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **3**, methyl 11β-bromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **4** and methyl 11α,11β-dibromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate **5**, respectively. The synthesis of compound **5** and the unique halogen bonding of this novel molecule in steroid have been reported herein for the first time.

2. Experimental

2.1. Synthesis

All chemicals were commercially available and used as received, except that the solvents were dried and purified by distillation. Flash column chromatography was carried out using 230–400 mesh silica gel. For TLC analysis, precoated plates of silica gel 60 F_{254} (Merck) were used. Spots were visualized with UV light and/or with dipping in a phosphomolybdic acid solution and charring on a hot plate.

2.1.1. Methyl 3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate (2)

Compound **2** was synthesized in an overall 88% yield starting from cholic acid **1** as reported by us earlier [10,11] (Scheme 1). White solid, mp 175–176 °C (EtOAc); IR and NMR spectroscopic data was consistent with that reported in the literature.

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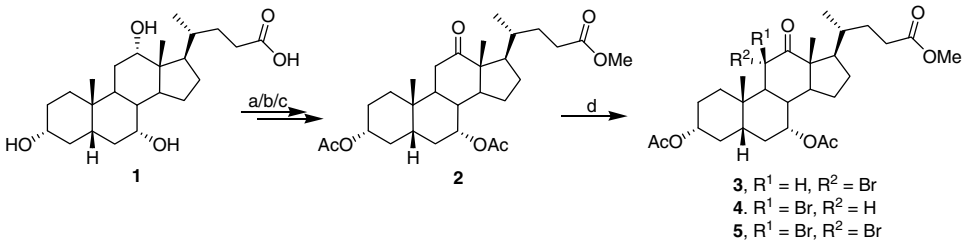
doi:10.1016/j.molstruc.2008.05.051



Scheme 1. Reagents and conditions. (a) CH_3OH, TsOH, 28 °C, 24 h, 98%; (b) Ac_2O, DMAP, Et_3N, DCM, 28 °C, 4–5 h, 92%; (c) CrO_3, H_2SO_4, Acetone, 10 °C, 5 min, 98%; (d) Br_2, Benzene, 28 °C, 6 days, 88%.

*2.1.2. Methyl 11α-bromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate (**3**), Methyl 11β-bromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate (**4**), Methyl 11,11-dibromo-3α,7α-diacetoxy-12-oxo-5β-cholan-24-oate (**5**)*

To a solution of **2** (1.008 g, 2 mmol) in benzene (10 mL), a bromine solution (1 mL, 2 M in benzene) was slowly added with stirring, at 30 °C in dark. After 6 days, TLC analysis showed the total consumption of the starting material. The reaction mixture was diluted with 150 mL of ethyl acetate and poured in a 250 mL separatory funnel. The organic layer was washed with 10% $Na_2S_2O_5$ (2 × 10 mL), cold H_2O (2 × 10 mL), brine (2 × 10 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude product. The residue (1.35 g) was chromatographed on silica gel (EtOAc/Pet Ether, 1:4) to yield the dibromo compound **5** (0.05 g, 4%), mp 181–182 °C (EtOAc); $[\alpha]_D^{25}$ +40.00 (*c* 1.1, $CHCl_3$); IR (KBr) 2955, 1737, 1716 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.02 (d, *J* = 6.3 Hz, 3H), 1.37 (s, 3H), 1.54 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 3.04 (bd, *J* = 15.3 Hz, 3 Hz), 3.38 (d, *J* = 11.4 Hz, 1H), 3.67 (s, 3H), 4.67 (m, 1H), 4.97 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.6, 18.4, 21.4, 21.4, 24.0, 26.6, 27.0, 29.7, 30.4, 31.1, 31.3, 34.5, 35.5, 36.2, 39.6, 40.3, 46.3, 49.0, 49.5, 51.5, 54.1, 54.7, 71.4, 73.6, 74.8, 169.9, 170.7, 174.5, 194.4; MS (LCMS) *m/z*: 685.1 $[M+Na]^+$; HRESIMS m/z 685.1152 $[M+Na]^+$ ($C_{29}H_{42}Br_2NaO_7$; calcd. 685.1174). Anal. Calcd. for $C_{29}H_{42}Br_2O_7$: C, 52.58; H, 6.39. Found: C, 52.60; H, 6.04. On further elution with the same solvent system furnished β-bromo compound **4** (0.22 g, 19%), mp 183–184 °C (EtOAc); and followed by α-bromo compound **3** (0.76 g, 65%), mp 202–203 °C (EtOAc). IR and NMR spectroscopic data for compounds **3** and **4** are consistent with that reported in literature [12].

2.2. Methods

Melting points were obtained with Buchi Melting Point apparatus B-540. Optical rotations were obtained on Bellingham & Stanley ADP-220 Polarimeter. Specific rotations ($[\alpha]_D$) are reported in deg/dm, and the concentration (*c*) is given in g/100 mL in the specific solvent. Ultraviolet spectroscopy was performed using Perkin–Elemer instrument, Lambda 35 UV/VIS Spectrometer. CD spectra were taken on spectropolarimeter, Jasco J-715 at 25 °C using solutions of the products in methanol exhibiting absorbance values in the range 0.1–0.2 at 220 nm. Fourier transform infrared (FTIR) spectra were recorded on Schimadzu 8400 series FTIR instrument. For the IR spectrum 1 mg of sample was dispersed in 300 mg of KBr and the pellets were prepared with a mini-press at 10 tonnes after grinding manually in a mortar. IR spectra were acquired accumulating 40 scans at 8 cm^{-1} resolution. Only diagnostic bands are reported on cm^{-1} scale. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer at 500.00 and 125.78 MHz, respectively. The chemical shifts are given in ppm relative to tetramethylsilane. Mass spectra were recorded on LC-MS/MS-TOF API QSTAR PULSAR spectrometer, samples introduced by infusion method using Electrospray Ionisation Technique. EI and CI mass spectra were recorded on an AEI MS-50 and AEI MS-9 spectrometer, respectively. High resolution mass spectra were obtained on a Kratos MS-80 spectrometer. Elemental analyses were performed by CHNS-O EA 1108-Elemental analyser, Carloerba Instrument (Italy) and were within ±0.4% of calculated values.

2.3. Single-crystal structure determination: X-ray measurements

Single crystals of **2, 3, 4** and **5** were grown from a hot saturated filtered solution of these compounds in ethyl acetate. Suitable crystals were obtained by slow evaporation of the solvent at room temperature (RT). Compound **2** was crystallized as colorless long needles where as crystals of compounds **3**, **4** and **5** were obtained as thin plates. The best amongst them were selected using Leica polarizing microscope. X-ray diffraction data of all the compounds were collected on a Bruker SMART APEX CCD diffractometer with omega and phi scan mode, $\lambda_{Mo\,K\alpha}$ = 0.71073 Å at *T* = 297(2) K. All the data were corrected for Lorentzian, polarization, and absorption effects using Bruker's SAINT and SADABS programs. The crystal structures were solved by direct methods using SHELXS-97 and the refinement was performed by full matrix least squares on F^2 using SHELXL-97 [13]. Hydrogen atoms were included in the refinement as per the riding model. Molecular graphics were from Mercury (http://www.ccdc.cam.ac.uk/prods/mercury). Selected details about the crystal structure, experiment and structure solution and refinement are given in Table 2. Geometrical parameters for intermolecular interactions are included in Table 3. The crystallographic-information-files (CIFs) have been deposited at the Cambridge Crystallographic Database Centre as a supplementary publication. The respective CCDC numbers are given in Table 2.

3. Results and discussion

*3.1. Synthesis of compounds (**2**), (**3**), (**4**) and (**5**)*

In the course of our studies on the synthesis of various bile acid conjugates [12] we have recently synthesized [10] C-11 azido/amino functionalized novel cholic acid derivatives. These C-11 functionalized cholic acid derivatives induced host cell fusion during the progression of HIV-1 infection and produced multinucleated giant cells [11]. We were interested in the investigation of the effect of bulky bromine atom in steroid skeleton of cholic acid with different stereo-chemical orientations at C-11 on the two-dimensional arrangement of molecules and solid-state properties. Bromination of 12-oxo steroids has been widely explored [14]. However, there is no report on the study of crystal structure properties of these 11-bromo compounds. A stereoselective high-yield bromination of compound **2** to compound **3** was demonstrated [14b] by Yanuka et al. and reproduced by us [11]. Recently we have elaborated cholic acid **1** to its 11α-bromo and 11β-bromo derivatives **3** and **4** via 12-oxo methyl cholate **2** using bromine in benzene [10]. Detailed investigation of the bromination reaction on compound **2** with excess bromine and longer reaction period led to the isolation of hitherto unknown

Table 1
Some representative ^{1}H, ^{13}C NMR chemical shifts and IR spectroscopy data

Compound	^{1}H NMR, δ ppm and coupling constants J in Hz						C-12 carbonyl	
	Methyl group			Proton (H) at			^{13}C NMR δ ppm	IR ν_{max} cm^{-1}
	C-18	C-19	C-21	C-9	C-11	C-1$_e$		
2	1.03	1.03	0.85 d, J = 5.8	–	–	–	213.3	1701
3	1.03	1.21	0.86 d, J = 5.8	2.79 dd, J = 10.7	5.01 d, J = 10.7	2.90 dt, J = 15.4 and 3.0	202.3	1718
4	1.36	1.38	0.93 d, J = 6.6	2.64 dd, J = 11.7 and 5.9	4.42 d, J = 5.9	–	203.4	1697
5	1.36	1.52	1.00 d, J = 5.9	3.35 d, J = 11.4	–	3.02 dt, J = 15.2 and 3.1	194.3	1716

Table 2
Crystal data for compounds **2**, **3**, **4** and **5**

Compound No.	2	3	4	5
Empirical formula	$C_{29}H_{44}O_7$	$C_{29}H_{43}BrO_7$	$C_{29}H_{43}BrO_7$	$C_{29}H_{42}Br_2O_7$
Formula weight	504.64	583.54	583.54	662.45
Temperature (K)	297(2)	297(2)	297(2)	297(2)
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Orthorhombic	Monoclinic	Monoclinic
Space group	*C*2	$P2_12_12_1$	$P2_1$	$P2_1$
a (Å)	27.845(15)	10.059(2)	10.575(11)	8.825(5)
b (Å)	6.284(4)	11.392(3)	6.541(7)	11.778(6)
c (Å)	18.143(10)	25.629(6)	20.83(2	15.053(8)
α (°)	90	90	90	90
β (°)	122.219(8)	90	97.966(18)	101.755(8)
γ (°)	90	90	90	90
Volume (Å^3)	2686(3)	2936.9(12)	1427(3)	1531.8(13)
Z, Calculated density (g/cm^3)	4, 1.248	4, 1.320	2, 1.358	2, 1.436
Absorption coefficient (mm^{-1})	0.088	1.442	1.484	2.688
F(000)	1096	1232	616	684
Crystal size (mm^3)	0.45 × 0.05 × 0.03	0.58 × 0.36 × 0.17	0.99 × 0.24 × 0.04	0.87 × 0.45 × 0.12
Theta range (data collection) (°)	2.27–25.00	2.39–24.99	1.97–25.00	2.21–25.00
Index ranges	−32 <= *h* <= 32	−5 <= *h* <= 11	−12 <= *h* <= 12	−10 <= *h* <= 10
	−7 <= *k* <= 7	−12 <= *k* <= 13	−7 <= *k* <= 7	−13 <= *k* <= 13
	−21 <= *l* <= 21	−30< = *l* <= 30	−24 <= *l* <= 24	−17 <= *l* <= 17
Reflections collected/unique	12,738/4690 [*R*(int) = 0.1103]	14,791/5145 [*R*(int) = 0.0346]	10,137/4796 [*R*(int) = 0.0466]	10,530/5087 [*R*(int) = 0.0296]
Completeness to θ = 25.00 (%)	99.7	99.7	99.9	99.8
Max. and min. transmission	0.9972 and 0.9616	0.7916 and 0.4884	0.9430 and 0.3212	0.7351 and 0.2038
Data/restraints/parameters	4690/1/332	5145/0/340	4796/1/340	5087/1/349
Goodness-of-fit on F^2	1.003	1.017	0.994	0.990
Final R indices [I > 2σ(I)]				
R_1	0.0768	0.0540	0.0630	0.0383
wR_2	0.1703	0.1429	0.1623	0.0898
R indices (all data)				
R_1	0.1248	0.0698	0.1012	0.0484
wR_2	0.1968	0.1531	0.2031	0.0965
Absolute structure parameter	3(2)	0.039(12)	−0.010(18)	−0.005(8)
Extinction coefficient	0.0054(10)	None	None	None
Largest diff. peak and hole (Å^{-3})	0.276 and −0.218	0.690 and −0.369	0.531 and −0.346	0.363 and −0.235
CCDC No.	676282	676283	676284	676285

C-11 dibrominated product, namely methyl 3α,7α-diacetoxy-11α,11β-dibromo-12-oxo-5β-cholan-24-oate **5** in 4% yield (Scheme 1). The high resolution mass spectra of **5** showed the expected molecular ion peak and in the ^{13}C NMR spectra a quaternary C-11 carbon appeared at δ 74.76 ppm. The absolute structure of 12-oxo compound **2** and bromo ketones **3, 4, 5** has been established by single-crystal X-ray analysis (Fig. 1).

3.2. Spectroscopic discussion

Some interesting observations can be made on the ^{1}H, ^{13}C NMR and IR spectra of the bromo ketones (**3, 4** and **5**) with respect to the starting 12-oxo compound **2** (Table 1, Fig. 2). Deshielding effect of ∼0.18 δ ppm was observed on C-19 methyl group in 11α-bromo compound **3** in comparison with 12-oxo compound **2**, while deshielding effect of ∼0.33 to 0.35 δ ppm on both C-18 and C-19 methyl's was observed in 11β-bromo compound **4** in comparison with 12-oxo compound **2**. Similarly, deshielding effect of ∼0.14 δ ppm was observed on C-19 methyl group in 11-dibromo compound **5** in comparison with 11β-bromo compound **4**, while deshielding effect of about 0.31–0.32 δ ppm on both C-19 and C-18 methyl's were observed in 11-dibromo compound **5** in comparison with 11α-bromo compound **3**.

Thus, C-11α-bromine contributes for the deshielding (∼0.14–0.18 δ ppm of only C-19 methyl protons where as C-11β-bromine contributes for the deshielding (∼0.31–0.35 δ ppm of both C-18 and C-19 methyl protons. The C-11α-bromine also

Table 3
Geometrical parameters for intermolecular interactions

Compound	D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	⟨(D–H···A)
2	C(3)–H(3B)···O(2)[a]	0.98	2.61	3.522(7)	155
	C(15)–H(15A)···O(4)[b]	0.97	2.73	3.558(6)	144
	C(29)–H(29B)···O(4)[c]	0.96	2.55	3.412(8)	149
	C(29)–H(29A)···O(1)[d]	0.96	2.82	3.732(8)	159
	C(18)–H(18C)···O(5)[e]	0.96	2.63	3.398(6)	138
	C(19)–H(19B)···O(5)[e]	0.96	2.52	3.439(6)	159
	C(8)–H(8B)···O(5)[e]	0.98	2.46	3.415(6)	165
3	C(26)–H(26A)···Br(1)[f]	0.96	3.15	3.856(6)	132
	C(28)–H(28B)···O(5)[g]	0.96	2.74	3.477(6)	134
	C(29)–H(29B)···O(2)[g]	0.96	2.81	3.748(12)	165
	C(26)–H(26C)···O(6)[g]	0.96	2.66	3.483(15)	145
	C(19)–H(19A)···O(6)[h]	0.96	2.77	3.628(14)	149
	C(5)–H(5)···O(6)[h]	0.98	2.79	3.734(12)	161
	C(2)–H(2A)···O(7)[i]	0.97	2.82	3.601(8)	139
	C(21)–H(21A)···O(1)[i]	0.96	2.70	3.651(7)	172
4	C(26)–H(26C)···O(2)[j]	0.96	2.68	3.503(10)	144
	C(18)–H(18C)···O(5)[k]	0.96	2.92	3.720(10)	141
	C(28)–H(28A)···O(6)[l]	0.96	2.71	3.22(2)	114
	C(15)–H(15A)···O(6)[l]	0.97	2.56	3.393(19)	144
	C(23)–H(23A)···O(4)[m]	0.97	2.60	3.476(17)	151
	C(3)–H(3B)···O(2)[n]	0.98	2.44	3.366(9)	159
	C(19)–H(19B)···O(5)[k]	0.96	2.74	3.698(10)	173
	C(8)–H(8B)···O(5)[k]	0.98	2.47	3.382(9)	154
5	C(11)–Br(2)···O(5)[o]	1.967(3)	3.256(3)	–	139.3(4)
	C(14)–H(14A)···Br(2)[p]	0.98	3.11	4.032(4)	158
	C(28)–H(28A)···O(5)[p]	0.96	2.46	3.198(6)	134
	C(15)–H(15B)···Br(1)[q]	0.97	2.99	3.920(5)	161
	C(26)–H(26B)···O(4)[r]	0.96	2.51	3.461(8)	171

Symmetry codes: [a] $-x+1/2, y-1/2, -z$; [b] $-x+1/2, y+1/2, -z+1$; [c] $x-1/2, y+1/2, z$; [d] $x-1/2, y-1/2, z$; [e] $x, y-1, z$; [f] $-x+1, y+1/2, -z+3/2$; [g] $x-1/2, -y+1/2, -z+2$; [h] $-x+3/2, -y+1, z-1/2$; [i] $x+1/2, -y+1/2, -z+2$; [j] $-x+2, y+1/2, -z$; [k] $x, y-1, z$; [l] $-x+1, y-1/2, -z+1$; [m] $x-1, y+1, z$; [n] $-x+2, y-1/2, -z$; [o] $y+1/2, -z$, [p] $-x+1, y-1/2, -z$; [q] $x+1, y, z$; [r] $x-1, y, z$.

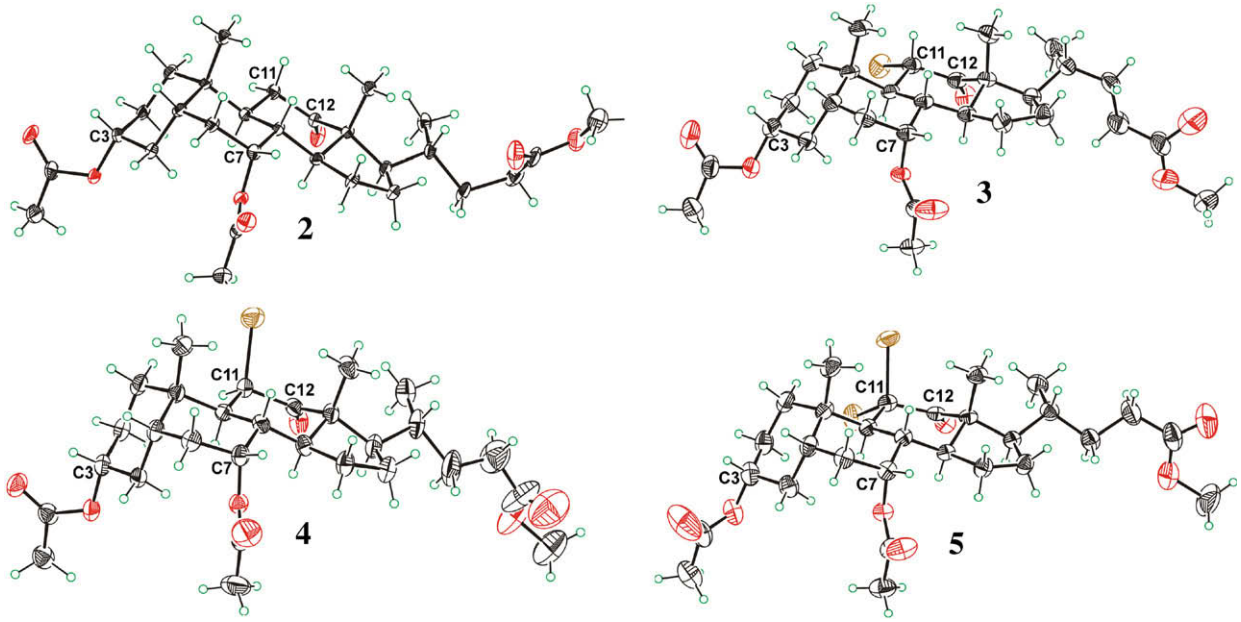


Fig. 1. ORTEP views of compounds **2**, **3**, **4** and **5**.

contributes for the deshielding of C-1 equatorial (C-1_e) proton through space [15] this can be observed in compounds **3** and **5**, in which there is a presence of C-11α-bromine. These observations demonstrate a well-defined effect of bulky bromine atom through space on the chemical shifts of C-18, C-19 angular methyl protons and C-1_e proton. Earlier we have demonstrated such kind of anisotropy by C-11 azido and amino functionalities [10].

Carbonyl resonance of C-12 in compound **2** appeared at 213.3 ppm (Table 1). As expected [16], marked shielded shift (shielding) of about 10 ppm was observed for carbonyl resonances in 11-bromo-12-oxo compounds **3** (202.3) and **4** (203.4). This clearly suggests that irrespective of the stereochemistry at C-11 position the bromo substitution accounts for shielding of about 10 ppm, accordingly carbonyl resonance of C-12 in C-11-*gem*-dibromo compound **5** appeared at 194.3 ppm. In this compound

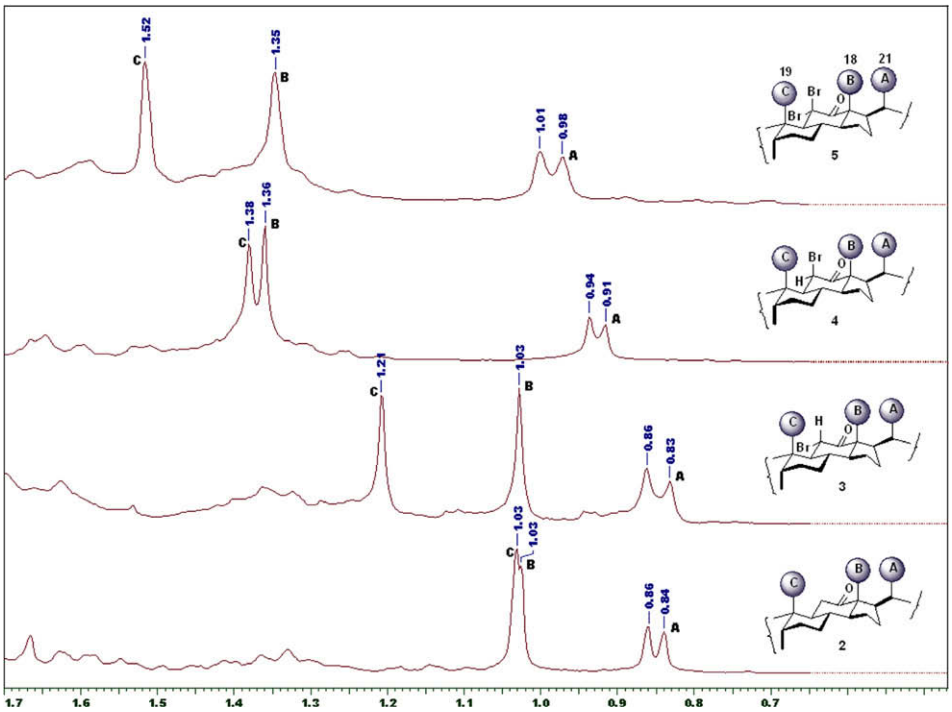


Fig. 2. Partial ^{1}H NMR spectra of **2**, **3**, **4** and **5** showing the effect of C-11 substitution on C-18, C-19 and C-21 methyl groups.

two bromine atoms showed an additive effect of about 19 ppm on C-12 carbonyl resonance.

An equatorial α-bromo substituent produced a marked shift in the infrared carbonyl stretching frequency (about 17 cm^{-1} in case of 11α-bromo-12-oxo compound **3** and 15 cm^{-1} in case of C-11-*gem*-dibromo-12-oxo compound **5**) relative to that of the parent ketone **2**. An axial halogen has a negligible effect on the carbonyl stretching frequency and this can be observed in case of compounds **4** and **5**. This is in accordance [17] with equatorial α-bromo ketone.

The circular dichroism (CD) curves for the three bromoketones **3**, **4** and **5** are compared with that of the parent 12-oxo compound **2** (Fig. 3). The axial nature of the bromine atom in compounds **4** and **5** was clearly evident from its strong negative Cotton effects, indicating a very high degree of asymmetry in these compounds. The similarity of the circular dichroism absorption (positive sign) of the 11α-bromo ketone **3** and the parent compound **2** is characteristic of the equatorial bromine substituent. These findings are consistent with the octant rule [18].

In conclusion ^{1}H NMR, IR and CD spectroscopy showed a constant and pronounced effect of bulky bromine atom through space which depends on the axial or equatorial orientation of bulky bromine atom.

3.3. Single crystal X-ray diffraction studies

The effects of sequential replacement of methylene-H atoms at the C11 position of **2** by the bromine atom on molecular organization in crystals were studied. The effect of classical H-bonding interaction on the molecular packing of bile acid skeleton was recently reported by Zhao [19] and Tato et al. [20]. In order to study the interplay of weak intermolecular interactions on the molecular aggregation of C11 functionalized bile acid skeleton in the absence of conventional H-bonding, the C3 and C7 hydroxyl groups as well

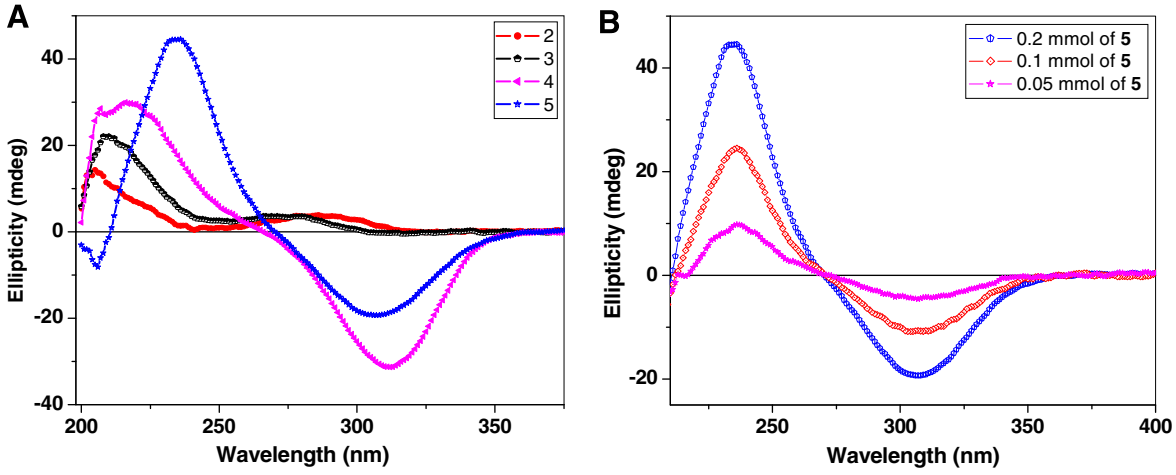


Fig. 3. (A) CD of compounds **2**, **3**, **4** and **5**; (B) CD of **5** at variable concentrations.

as C24 carboxylic acid was masked with acetate and methyl ester functionalities, respectively. As expected, the molecular packing in all the four structures was dominated by C–H...O interactions (Table 3).

In **2**, molecules make trifurcated C–H...O interactions with unit-translated molecules along *b*-axis forming a molecular chain involving carbonyl oxygen O5 and the methyl hydrogen from C18, C19 and C8 atoms (Fig. 4A). These chains are zipped by 2_1-screw axis via C3B–H3B...O2 interactions bringing the head groups in close proximity. These layers make cohesions through C29–H29B...O4 and C29–H29A...O1 interactions along the *a*-axis and via C15–H15A...O4 contacts along the *c*-axis (Table 3, Fig. 4B). The molecular organization does not exhibit typical 'head-to-tail' organization generally observed in steroids [19].

In **3**, bromine at the C-11 alpha position breaks the trifurcated C–H...O linked molecular assembly seen in **2**. Molecules along *a*-axis are linked by C28–H28B...O5 contacts across crystallographic 2_1-screw axis forming helical assembly (Fig. 5A). In addition, four other C–H...O interactions (C2–H2A...O7, C29–H29B...O2, C21–H21A...O1 and C26–H26C...O6, Table 3) are made between the 2_1 related molecules along the helix that brings the C3-acatate group and the side chain in close proximity. These helices are stitched together by C5–H5...O6, C19–H19A...O6 and C26–H26A...Br1 interactions (Fig. 5B). The helices along the *a*-axis are discretely packed by creating well-guided tunnel.

In **4** with Br atom at β position of C11, the organization of the molecule is very similar to **2** along *b*-axis (Fig. 6A). Molecules made trifurcated C–H...O assembly with longer C18–H18C...O5, C19–H19B...O5 (Table 3) interactions but similar C8–H8B...O5 interaction as compared to **2** (Fig. 4). Again here, these molecular chains are glued through C3–H3B...O2 interactions along crystallographic twofold screw axis by bringing the heads together. In addition, one more C–H...O interaction (C26–H26C...O2) is involved in bridging these chains as compare to **2** giving more stability to the association. These layers are zipped along the 2_1-screw axis via bifurcated weak C–H...O interactions (C28–H28A...O6 and C15–H15A...O6) between acetate group at C7 and side chain at C17. Molecular packing viewed down the *b*-axis shows the dimeric assemblies of layers, which are connected via C23–H23A...O4 contact (Fig. 6B). It is interesting to note that Br atom in this molecule does not make any short contact.

In **5**, replacement of both the H-atoms at C-11 by Br atoms causes an interesting effect on molecular organization. Molecules in **5** are helically assembled around the crystallographic 2_1-screw axis via halogen bonding (Br...O) and C–H...O contacts (Fig. 7A). Beta bromine atom at C-11 interacts with the carbonyl oxygen (O5) of the 2_1-screw axis molecule via C11–Br2...O5 contact. The same carbonyl oxygen (O5) accepts H atom from C28-H28A of C7 acetate group of the next 2_1-screw axis molecule to make C–H...O interaction (Table 3). In continuing this pattern, each successive molecule gets a twist of 180° to generate helicity along the *b*-axis, which coincides with the crystallographic twofold screw axis. The Br2...O5 distance (3.256 Å) is much less than the sum of their van der Waals radii (3.35 Å) although the angle of approach deviates from linearity (⟨C11–Br2...O5 = 139.33°). The same is the case with C28–H28A...O5 contact (H28A...O5 = 2.455 Å and ⟨C28–H28A...O5 = 133.93°). These successive helices are held together along the *a*-axis through C26–H26C...O4 and C15–H15B...Br1 (Fig. 7B).

The molecular assembly via 'Halogen bonding', a non covalent interaction between the halogen atoms (as acceptors of electron density) and lone-pair possessing atoms (mostly O and N) was vastly studied since last decade due to its application in the field of crystal engineering, molecular recognition, solid-state synthesis [21]. To the best of our knowledge, structure of **5** is the first example of halogen bonding recognized in steroid structures. Since we

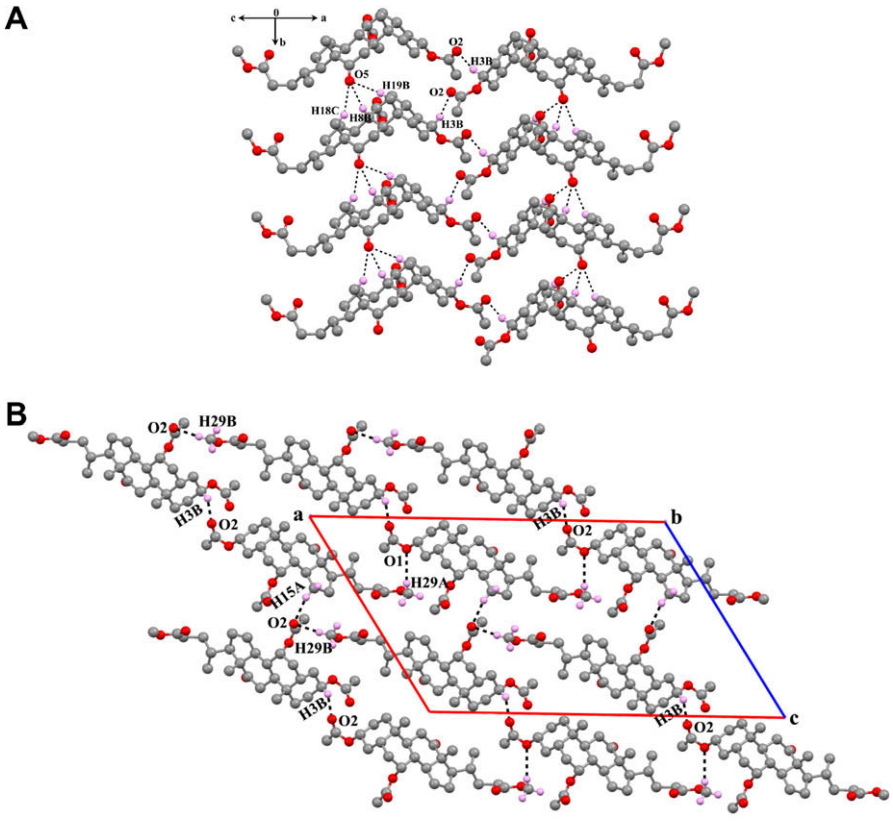


Fig. 4. Association of molecules in **2** showing (A) formation of molecular chain via trifurcated C–H...O interactions and (B) bridging of the layers viewed down *b*-axis.

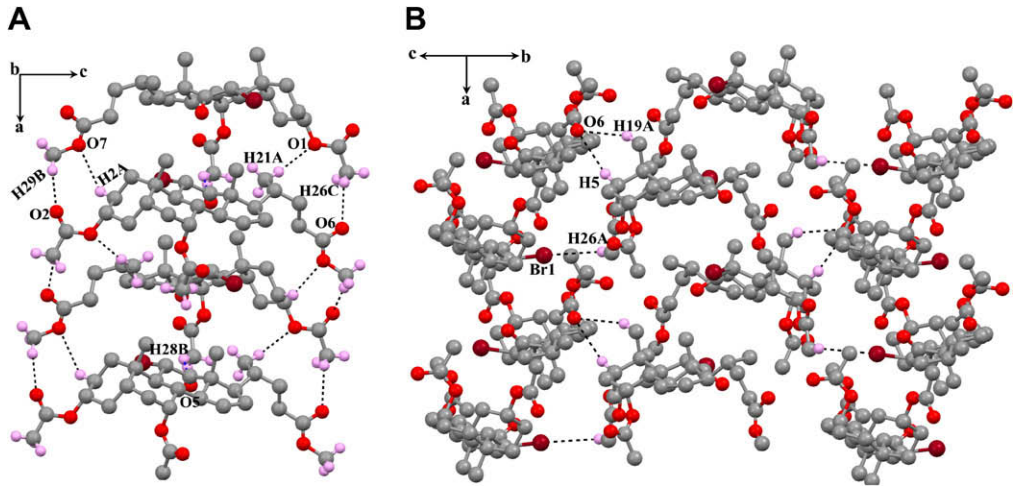


Fig. 5. View of molecular packing in **3** showing (A) helical assembly along the *a*-axis and (B) interlinking of the helices via C–H...O interactions.

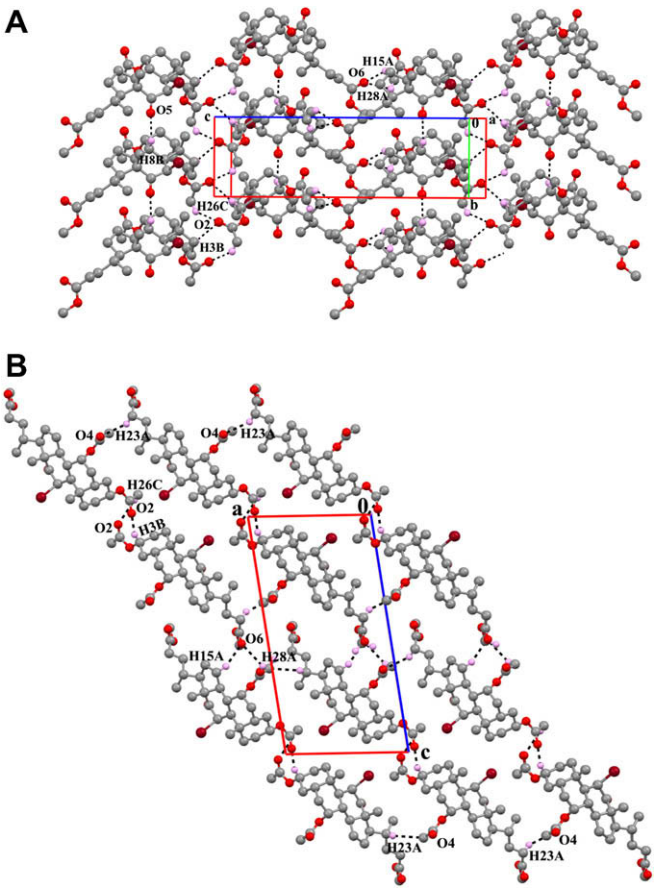


Fig. 6. Molecular packing viewed down *a*-axis in **4** (A) viewed down b-axis (B) association of the molecules via C–H...O interactions.

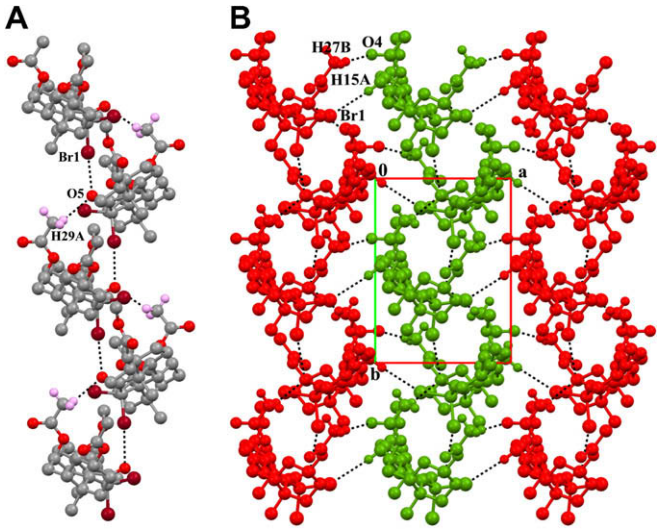


Fig. 7. (A) Helical self-assembly through C–Br...O and C–H...O interactions in **5** along the 2_1-axis and (B) interlinking of helices along *a*-axis via C–H...O and C–H...Br contacts.

observed halogen bonding contact for the first time in steroids, we carried out a CSD search to examine its occurrence and preferred geometries in other steroid structures [22]. The constrains applied were $R < 0.10$, distances $\leqslant$ sum of van der Waals radii and angles in the range 130–180°. All searches were carried out with error-free coordinates and restricted entries of disordered, ionic, polymeric and powdered structures. The CSD search included halogens (X = F, Cl, Br, I) and differently hybridized oxygen atoms (carbonyl and ether). Out of 104 structures containing halogen atoms and CO groups in steroid structures, 7 structures showed C–X...O interaction. In all the seven structures, only carbonyl oxygen was involved in the short contact with Cl, Br and I, but no hits were found for F. This weak interaction was considered to have a role in binding of halo-steroids to the carbonyl of the peptide chain of the receptor protein that offers optimum desired binding affinity, as observed in the crystal structure of an inhibitor-protein complex between the inhibitor 4,5,6,7-tetra-bromobenzotriazole and phosphor-CDK2-cyclin A [23].

The best fit of the four steroids **2**, **3**, **4** and **5** (Fig. 8) reveals the large conformational differences, maximum seen in the side chains at C-3 and C-17. It is note worthy to see some amount of flexibility upon bulky substitution at C11 in the steroid skeleton itself, which is normally taken as a rigid framework.

4. Conclusion

Steroids with C-11 functionality are well known for their biological activity. Keeping this in mind the effect of bulky bromine atom at C-11 in steroid skeleton of cholic acid with different stereo-chemical orientations on the molecular organization in solid-

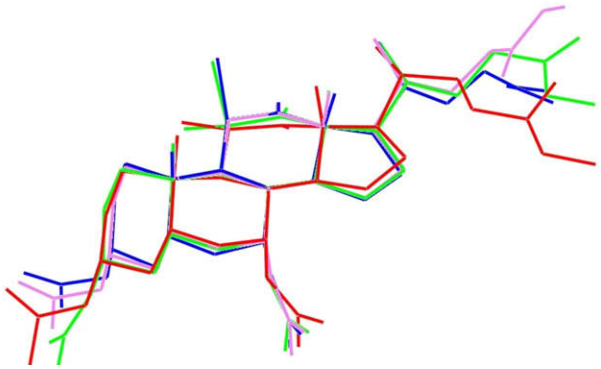


Fig. 8. The overlap of molecules **2** (pink), **3** (red), **4** (blue) and **5** (green). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

state has been investigated. In the present study synthesis of hitherto unknown C-11 dibrominated product, namely methyl 3α,7α-diacetoxy-11α,11β-dibromo-12-oxo-5β-cholan-24-oate **5** has been achieved. All the structures **2**, **3**, **4** and **5** devoid of conventional H-bonding, exhibit patterns of molecular agglomeration via C–H...O and C–H...Br interactions which can provide understanding in steroid–protein interactions. Compound **5** exhibits molecular association via C–Br...O short contact. Halogen bonding recognized for the first time in steroid structure, which is thought to have implications in achieving higher binding affinities of these structures to the receptor proteins.

5. Supplementary material

Crystallographic data for the structural analysis of **2**, **3**, **4** and **5** have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 676282, 676283, 676284 and 676285, respectively. Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union road, Cambridge CB2 1EZ, UK (fax: C44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk) or via www.ccdc.cam.ac.uk/conts/retrieving.html.

Acknowledgements

DBS thanks Council of Scientific and Industrial Research (CSIR), New Delhi for the Senior Research Fellowship. Emeritus Scientist Scheme awarded to BGH by the CSIR, New Delhi is gratefully acknowledged.

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