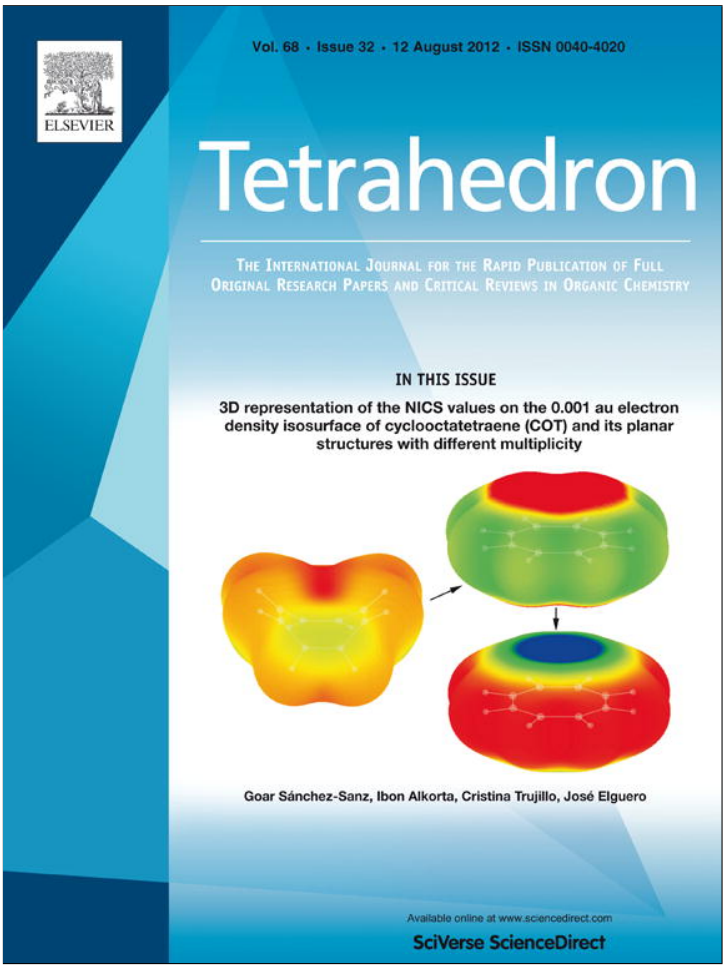

Tetrahedron 68 (2012) 6485–6491

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

Oxidation with selenium dioxide: the first report of solvent-selective steroidal aromatization, efficient access to 4β,7α-dihydroxy steroids, and syntheses of natural diaromatic ergosterols

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ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form 20 May 2012
Accepted 27 May 2012
Available online 1 June 2012

Keywords:
Selenium dioxide
Aromatization
4β,7α-Dihydroxy steroids
Dehydrogenation
Natural products

ABSTRACT

Selenium dioxide oxidation of cholesterol reveals a solvent-dependent product selectivity and facile one-pot synthesis of three derivatives, including aromatic analogues of naturally occurring ergosterol. Efficient access to 4β,7α-dihydroxy cholesterol is described. Analogous chemistry of β-sitosterol and diosgenin is also reported. The protocol is found effective to synthesize two diaromatic ergosterol natural products. A brief description of the molecular structures of the representative diaromatic cholesterol derivative and the triacetylated 4β,7α-dihydroxy cholesterol derivative are proven by X-ray crystallography.

1. Introduction

Selenium dioxide-mediated oxidation is regarded as one of the most reliable and predictable methods for allylic hydroxylation, especially in the steroid field.[1–5] Despite the proven biological importance of oxygen bearing functionalities in steroidal systems[4,6–9] no systematic approach has been adopted to explore the oxidizing ability of selenium dioxide (SeO_2) in steroidal systems. The present report demonstrates there is ample opportunity to elaborate the chemistry of steroidal systems based on the oxidizing ability of SeO_2.

The reaction of SeO_2 with cholesterol was reported to produce 4β-hydroxy cholesterol as the only product[2,3] whereas with the same reagent, cholesteryl acetate and benzoate yielded 4β-hydroxylated and 6α-hydroxylated products, respectively.[2] The observation was supported by Marker et al.[5] who studied the action of SeO_2 on stigmasterol, stigmasteryl acetate and sitosteryl acetate. Among the oxysterols in human circulation, the major one, 4β-hydroxy cholesterol, is, along with other oxysterols, degraded generally to bile acids via 7α-hydroxylation as the rate-limiting step.[7,8] In addition, other hydroxy derivatives of cholesterol are drawing attention due to their profound importance in human metabolism. This motivates the investigation of their structure–activity relationship in order to correlate disease with drug treatment.[4,8,9] Clearly, there exists an enormous demand to synthesize 4β,7α-dihydroxy steroids, preferably in more direct and easier routes than existing synthetic methodologies.[10] Motivated by the above, we were encouraged to synthesize oxysterols and to explore the oxidation behaviour of SeO_2 on steroids. For the present study, cholesterol was chosen as the representative molecule. The results thus obtained were subsequently extended to ergosterol, β-sitosterol and diosgenin.

2. Results and discussion

In particular, we wish to report the results of a solvent-dependent investigation of SeO_2 oxidation on cholesterol, which resulted in the formation of naphthalene analogue (**2a**) via selective aromatization of rings A and B of cholesterol, together with 4β-hydroxy (**3a**) and 4β,7α-dihydroxy (**4a**) derivatives (Scheme 1). Cholesteryl acetate and benzoate also yielded the naphthalene analogues along with the other two products as reported[2] (Scheme 2).

Steroidal dehydrogenation of a maximum two hydrogen atoms using selenium dioxide[3,11] as well as steroidal aromatization (using other reagents) and their biological implications have been reported.[12] But, achieving dehydrogenation using SeO_2 by removing as

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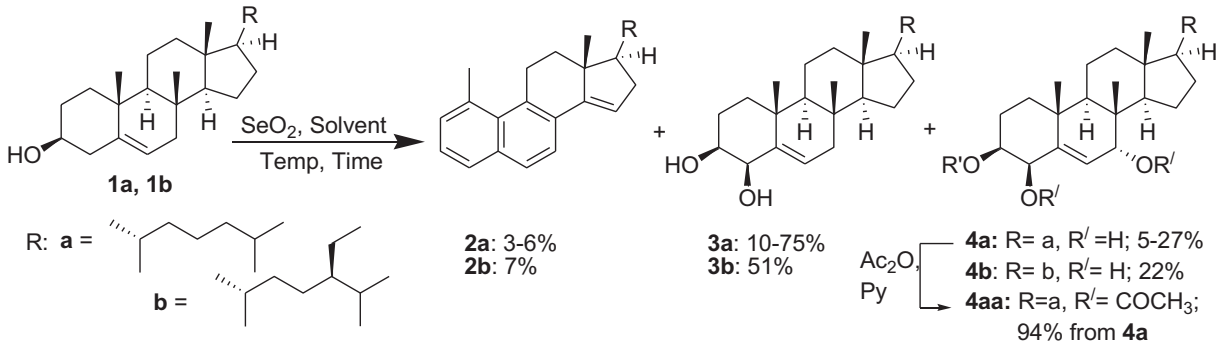
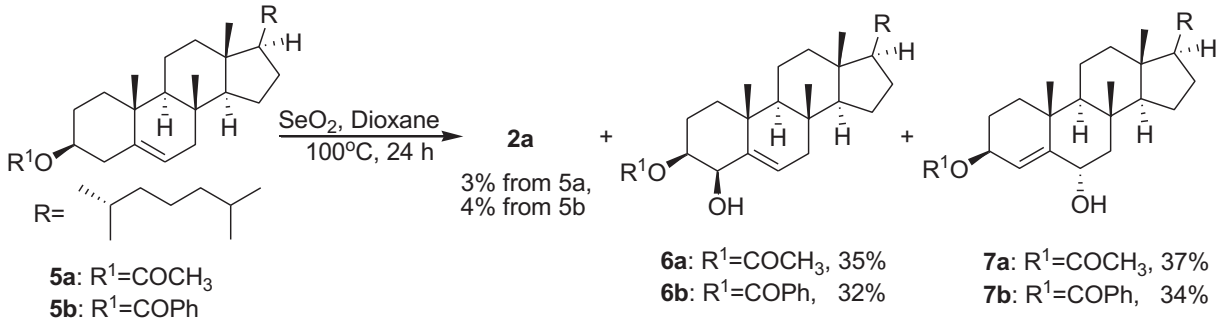
http://dx.doi.org/10.1016/j.tet.2012.05.110



Scheme 1. SeO_2 oxidation of cholesterol (**1a**) and β-sitosterol (**1b**).



Scheme 2. SeO_2 oxidation of cholesteryl acetate (**5a**) and benzoate (**5b**).

many as nine hydrogen atoms from a cholesterol molecule to induce aromatization (e.g., formation of naphthalene analogue, **2a**) in a solvent-specific reaction is unprecedented. Of note, only ethereal solvents with two oxygens such as 1,4-dioxane, 1,3-dioxalane, 1,2-dimethoxy ethane and 1,2-diethoxy ethane are able to yield **2a**. To the best of our knowledge, this is the first report of the solvent-selective aromatization using selenium dioxide.

The products have been fully characterized (see Experimental section and Supplementary data) by elemental analysis and spectroscopy (IR, ^1H, ^{13}C, DEPT-135 NMR, mass), and in the cases of **2a** (to confirm the structure) and **4aa** (to confirm the α-configuration of the 7-OH group in **4a** and, by analogy, that in **4b** and **13**), by single crystal X-ray crystallography (Figs. 1 and 2). The molecular structures of **2a** and **4aa** are described in Supplementary data.[13]

aprotic solvents (entries 17–19) were used. To obtain the diol, **3a**, the use of ether as solvent, particularly THF (entry 8), was found to be best, whereas for triol, **4a**, 1,4-dioxane (moist, entry 2) gave maximum yield. Lower alcohols appeared to give better yield than their long chain analogues (entries 9–12). Dihydroxy alcohol, e.g., ethylene glycol, was also examined but was found to be ineffective (entry 13). Basic solvent, e.g., pyridine (entry 14) was found effective in converting cholesterol into the diol and triol whereas triethylamine and morpholine (entries 15 and 16) were found to be ineffective. This is presumably due to the higher basicity of morpholine (pK_b=8.33) and triethylamine (pK_b=11.01) compared to pyridine (pK_b=5.21). The reaction was also attempted in strong basic medium with the use of K_2CO_3 and NaOH in aqueous ethanol, 1,4-dioxane and acetonitrile. However, the extent of

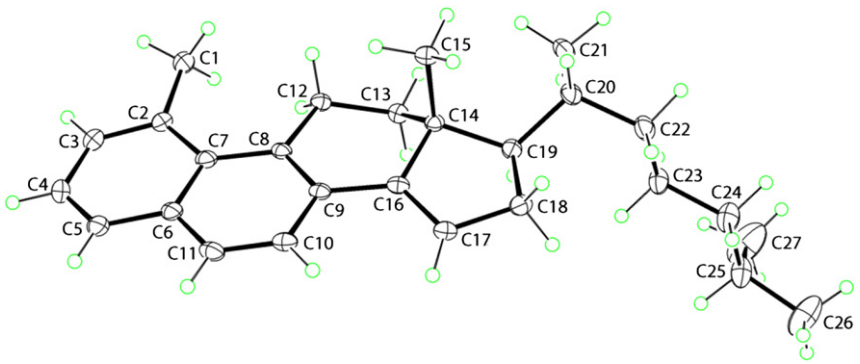


Fig. 1. Molecular structure of **2a** and atom labelling.

The results of the systematic study have revealed that the composition of the products is largely solvent dependent (Table 1). Four different classes of solvents viz. (i) ethers (entries 1–8), (ii) alcohols (entries 9–13), (iii) basic (entries 14–16) and (iv) polar transformation was negligible in each case. Among the polar aprotic solvents used, DMSO resulted in good overall transformation (entry 19), whereas acetonitrile (entry 17) and DMF (entry 18) gave poor yields.

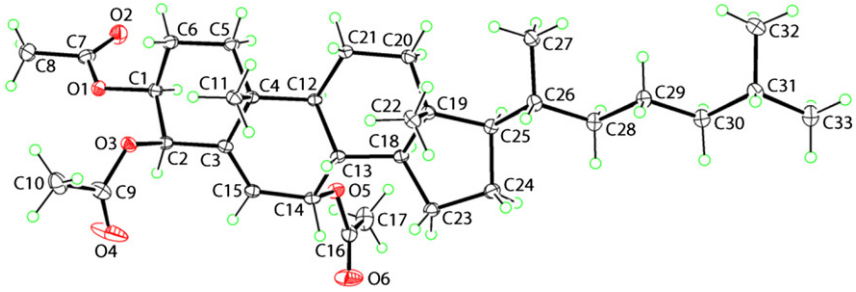


Fig. 2. Molecular structure of **4aa** and atom labelling.

Table 1
Product composition for the reaction of cholesterol with selenium dioxide[a] in different solvents

Entry	Solvent system	Reaction condition	Yield[b,c] (%)		
			2a	**3a**	**4a**
1	1,4-Dioxane	100 °C, 24 h	3	35	6
2	1,4-Dioxane/water (50:1)	100 °C, 24 h	5	50	26
		Reflux, 1 h	6	34	27
		Reflux, 3 h	Trace	46	8
3	1,4-Dioxane/water (5:1)	100 °C, 24 h	Trace	64	5
4	1,3-Dioxalane	Reflux, 24 h	6	32	5
		Reflux, 6 h	Trace	42	8
5	2-Methoxy ethanol	100 °C, 24 h	NF	48	Trace
6	1,2-Dimethoxy ethane	Reflux, 24 h	6	54	6
7	1,2-Diethoxy ethane	Reflux, 24 h	5	51	11
8	Tetrahydrofuran	Reflux, 24 h	NF	75	17
9	Ethanol	Reflux, 24 h	NF	35	11
10	*tert*-Butanol	Reflux, 24 h	NR	57	15
11	Octanol	Reflux, 40 h	NR	NR	NR
12	Decanol	Reflux, 40 h	NR	NR	NR
13	Ethylene glycol	100 °C, 24 h	NR	NR	NR
		Reflux, 5 h	NF	10	Trace
14	Pyridine	Reflux, 24 h	NF	62	13
15	Triethylamine	Reflux, 40 h	NR	NR	NR
16	Morpholine	100 °C, 40 h	NR	NR	NR
17	Acetonitrile	Reflux, 24 h	NF	21	5
18	DMF	100 °C, 24 h	NF	28	11
19	DMSO	100 °C, 24 h	NF	60	5
20	On solid support[d]	MW,[e] min	NF	30	Trace

[a] All the reactions were performed on 580 mg, 1.5 mmol of cholesterol.
[b] Yield refers to isolated pure compounds.
[c] NF=not found, NR=no reaction.
[d] On preactivated silica gel 60–120, after making dust.
[e] Domestic microwave, at 600 W.

It was observed that the aromatized product **2a** was formed when the oxidation was carried out in 1,4-dioxane (entries 1–3), 1,3-dioxalane (entry 4), 1,2-dimethoxy ethane (entry 6) and in 1,2-diethoxy ethane (entry 7). All other solvents used (Table 1) failed to furnish **2a** indicating the likely participation of both ethereal oxygens situated at 1,4-positions in the aromatization process. In a separate experiment, carried out under solvent-free microwave induced conditions, the reaction produced diol (**3a**) but not **2a** (entry 20).

By contrast, the synthesis of **4a**[10] (Scheme 1) is a simple and convenient one-step reaction. From the solvent-dependant study (Table 1), a maximum of 27% yield of the triol **4a** was obtained using 1,4-dioxane (moist, entry 2) as the solvent. It was clear that SeO_2 induced allylic hydroxylation in **3a** to form **4a** and hence, we tried reacting **3a** with SeO_2 in the identical reaction conditions, which produced **4a** in 72% yield.[14] As the formation of **4a** is one of the important findings, we changed the reaction conditions little bit to raise the yield of **4a** by carrying out the reaction directly with cholesterol (Table 2).[15] Firstly, we conducted the reactions with longer period, viz., 48 h, 72 h and 96 h. Among these conditions, maximum of 46% yield was obtained at 72 h (entry 2). The composition of the reactants along with reaction time was then also varied taking different mole ratios of cholesterol to selenium dioxide. Interestingly, it was seen that 1:7 mole ratio of the reactants furnished better result (Table 2, entry 5) than the 1:3.5 mole ratio (Table 1, entry 2) towards the formation of **4a**. However, increased reaction time or, the other mole compositions taking even excess selenium dioxide could not result satisfactory yield of the product **4a** (entries 6–8). Besides, we could not isolate diaromatic cholesterol **2a** from the reactions in these conditions and the diol **3a** was isolated at poor yields. Thus it may be concluded that, though these reaction conditions furnished better results towards the yield of **4a**, the conditions rendered unsuitable for the formation of both the aromatized product as well as the diol. Hence it seemed to be advantageous to have **4a** via **3a**.

Table 2
Optimization of the yield of **4a** directly from cholesterol

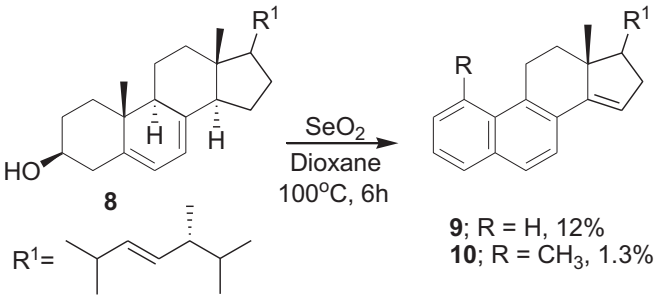
Entry	Reactant composition[a]	Reaction condition[b]	Yield[c] (%)
1	1:3.5	100 °C, 48 h	32[d]
2	1:3.5	100 °C, 72 h	46
3	1:3.5	100 °C, 96 h	43
4	1:3.5	Reflux, 1 h	20[d]
5	1:7	100 °C, 24 h	44
6	1:7	100 °C, 48 h	37
7	1:10.5	100 °C, 24 h	38
8	1:14	100 °C, 48 h	28

[a] Mole ratio of cholesterol to selenium dioxide; on 580 mg, 1.5 mmol of cholesterol.
[b] All the reactions were carried out in 1,4-dioxane/water=50:1.
[c] Aromatized product **2a** was not found, diol **3a** was isolated at 6% yield except entries 1 and 4.
[d] Compound **3a** was isolated at 10% and 28% yields, respectively, from entries 1 and 4.

In 2004, Qin and Liu,[16] isolated two aromatized ergosterol derivatives (**9** and **10**) from the ascomycete *Daldinia concentrica*, and described the long-sought after biological precursor steroids for organic matter in Earth's subsurface. Of the two compounds isolated, the latter, **10**, bears an unusual methyl group at position 1. According to the authors, the aromatized products arise due to microbial action on the precursor molecule ergosterol.

Having the natural diaromatic ergosterol analogue of cholesterol, we obviously were interested to apply the reaction protocol on ergosterol (**8**). As anticipated, the reaction furnished both the natural compounds (Scheme 3; **9** and **10**) in relatively better yields compared to cholesterol.[17] Surprisingly, the fact that the major product was **9** (12%) rather than **10** (1.3%) gives plausible mechanistic hints of the involvement of the 7-dehydro skeleton in the formation of 1-hydro derivative (**9**) rather than 1-methyl product (**10**). However, we could not isolate **10** as a single compound rather it was in a mixture with **9** (9:1, by NMR).[18] In order to optimize the yield, different reaction conditions were explored using selective solvents (Table 3). Ethanol and 2-methoxy ethanol failed to furnish

9 and/or **10**. However, the synthesis of these naturally occurring compounds using our methodology ruled out the possibility of incidental formation of the aromatized cholesterol analogue in our previous experiments.



Scheme 3. Synthesis of natural diaromatic ergosterol derivatives **9** and **10**.

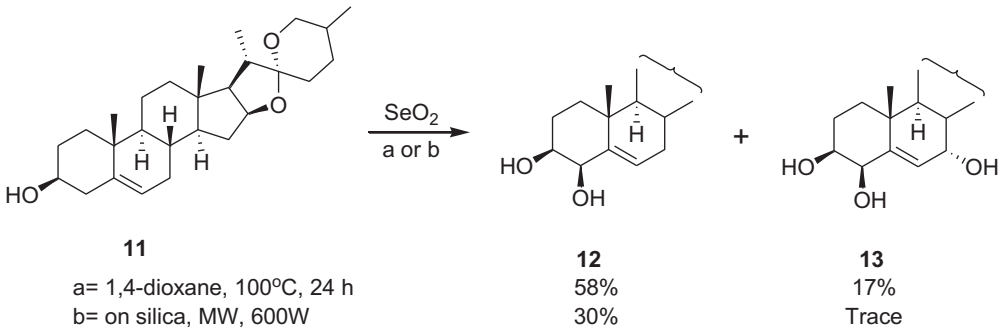
Table 3
Optimization[a] of the yield of the natural product **9**

Entry	Solvent system	Reaction condition	Yield (%)	
			9	**10**
1	1,4-Dioxane/water (50:1)	(a) 100 °C, 2 h	5.5	<1
		(b) 100 °C, 6 h	12	1.3
		(c) 100 °C, 10 h	7	<1
2	Ethanol	Reflux, 24 h	NF[b]	NF[b]
3	2-Methoxy ethanol	100 °C, 24 h	NF[b]	NF[b]

[a] Thorough solvent study of the reaction of cholesterol with SeO_2 (Table 1 above) has prompted us to choose selective solvents and reaction conditions.
[b] NF=not found.

4β-Hydroxy cholesterol (**2a**) did not furnish the corresponding diaromatic derivative whereas ergosterol (**8**, possessing C5=C6 as well as C7=C8 double bond in ring-B) yielded the diaromatic ergosterol derivative. So there exists a possibility of the involvement of A-ring and more specifically the 4β-hydrogen towards the formation of the aromatized products. Under identical reaction conditions, neither the diol **3a** nor the triol **4a** could be converted to the aromatized product **2a**. Rather **3a** produced, as mentioned previously, **4a** in a very good yield whereas **4a** did not react at all. The mechanism for the conversion of **1a** to **2a** is unclear at this stage, although it may be concluded that hydroxylation (as in **3a** and **4a**) is, apparently, not a precursor to form **2a**. Moreover, since the yield of **2a** does not vary substantially when prepared from **1a** or its acetate/benzoate derivatives under identical conditions (Scheme 2), the groups at the 3β position are not likely acting as leaving groups in these reactions. However, a detail experimental investigation with a number of differently substituted starting materials may lead to a clear mechanistic approach for the aromatization process.

To extend the scope of the protocol, we carried out analogous reactions on β-sitosterol (**1b**, Scheme 1) and diosgenin (**11**, Scheme 4) under identical conditions. On reaction, β-sitosterol, like cholesterol, produced (in 1,4-dioxane at 100 °C) the aromatic analogue (**2b**, 7%), 4β-hydroxy β-sitosterol (**3b**, 51%) and 4β,7α-dihydroxy β-sitosterol (**4b**, 22%). By contrast, diosgenin (both at elevated temperature and microwave induced solvent-free conditions, Scheme 4) yielded 4β-hydroxy diosgenin (**12**, 30–58%) and 4β,7α-dihydroxy diosgenin (**13**, trace-17%) but no aromatic analogue. Conversions of the corresponding diols (**3b** and **12**) to the triols were also achieved in high yields (**4b**, 72%; **13**, 69%).[14]



Scheme 4. Action of SeO_2 on diosgenin (**11**).

Again, it is noted that the yield of **2a** is rather poor (maximum reproducible yield 6%) compared to those of the other two products (**3a** and **4a**). As has been pointed out earlier, the formation of **2a** is very significant because the reaction (of SeO_2 and cholesterol) involves a selective aromatization of rings A and B of the tetracyclic skeleton with simultaneous regioselective formation of a double bond between C-14 and C-15.

On the basis of the previous literature,[19] it may be anticipated that the aromatization is accompanied with the methyl migration (from **1a**, **1b** and **8**) as well as demethylation (from **8**) of the methyl situated at the ring juncture (C-19) in the starting materials. The solvent selectivity suggests a possible explanation towards the regioselective dehydrogenation. It was assumed that a transient dioxonium intermediate of the ethers may be formed with selenium moiety offering the furnished regioselectivity.

3. Conclusion

In summary, we have accomplished the syntheses of natural diaromatic ergosterol derivatives and other steroidal analogues in an unprecedented simple, one-pot and convenient synthetic route. In the process, we have established the key factor as the selectivity of the solvents (having 1,4-ethereal oxygens) towards the formation of the aromatized products. Thorough solvent-dependant study of the model reaction reveals valuable product composition, which may be exploited, specially, for the synthesis of biologically important steroid molecules. By using the established solvent-selective steroidal methodology, the yield of the natural product, diaromatic ergosterol (**9**) was optimized at 12%. The same reaction is also found to be an easy and efficient access to the human metabolism research demanded 4β,7α-dihydroxy steroids. Furthermore, single crystal X-ray crystallography has resolved the

molecular structures, for the first time in their class, of similar diaromatic cholesterol derivative (**2a**) and triacetylated 4β,7α-dihydroxy cholesterol derivative (**4aa**).

4. Experimental section

4.1. General

Melting points were measured in open capillary methods and were uncorrected. The FAB mass spectra were recorded on a Jeol SX 102/Da-600 mass spectrometer/Data System using Argon/Xenon as the FAB gas. The DART-MS was recorded on a JEOL-AccuTOF JMS-T100LC mass spectrometer having a DART (Direct Analysis in Real Time) source. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avance 300 MHz FT-NMR spectrometer using 5 mm BBO probe. Either $CDCl_3$ or DMSO-d_6 or CD_3COCD_3 was used as solvent and TMS as reference material. Data are presented as follows: Chemical shift—in parts per million on the scale relative to δ_{TMS}=0; coupling constant—*J*/Hz. Elemental analysis was performed using a Vario EL-III elementary analyser. Infrared spectra were recorded on Shimadzu FT-IR 8300 Spectrometer as neat or thin films (KBr or Nujol) as indicated in the experimental procedures, and at room temperature. Frequencies are given in wave numbers (cm^{-1}). For column chromatography silica gel G, 60–120 mesh was used with petroleum ether/ethyl acetate mixture as the eluent. For thin layer chromatography (TLC), freshly made silica gel plates (using silica gel for TLC+petroleum ether) were used and visualization was achieved by staining with iodine.

4.2. X-ray crystallography[20]

Intensity data for **2a** and **4aa** were measured at 100 K on a Bruker SMART APEX diffractometer with Mo Kα radiation. Data processing (APEX2 and SAINT)[1x] and absorption correction for **2a** (SADABS)[2x] were accomplished by standard methods. The structures were solved by direct-methods with SHELXS-97[3x] and refinement (anisotropic displacement parameters, hydrogen atoms in the riding model approximation and a weighting scheme of the form $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$ for $P = (F_o^2 + 2F_c^2)/3$) was on F^2 by means of SHELXL-97.[3x] The absolute structures of both **2a** and **4aa** could not be determined experimentally and so for each, it was assigned based on that of cholesterol. As such, 2082 (**2a**) and 3934 (**4aa**) Friedel pairs were averaged in the final refinements. For the refinement of **2a**, high thermal motion was displayed by the C27 atom. However, multiple sites were not resolved for this atom. For the refinement of **4aa**, the O4 atom displayed elongated anisotropic displacement parameters and hence, this atom was refined with the ISOR command in SHELXL-97.[3x] Figs. 1 and 2 were drawn with ORTEP[4x] at the 50% probability level. Data manipulation and interpretation were with WinGX[5x] and PLATON.[6x]

4.3. Representative procedure for the oxidation reactions

To a solution of **1a** (770 mg, 2 mmol) in dioxane (15 mL) was added selenium dioxide (777 mg, 7 mmol), the mixture was heated at 100 °C for 24 h. The reaction mixture was then cooled and the black selenium deposited was filtered off. To the filtrate, ether (50 mL) was poured and was washed successively with water and then with saturated brine solution, dried over Na_2SO_4 and concentrated in vacuo to give a reddish gummy residue. The compounds presented therein, were then separated by column chromatography eluted successively by petroleum ether, petroleum ether/ethyl acetate=17:3 and petroleum ether/ethyl acetate=3:2 to afford **2a** (44 mg, 6%), **3a** (403 mg, 50%) and **4a** (217 mg, 26%), respectively.

The proportion of reactants to selenium dioxide, for the other oxidation reactions, was kept constant throughout the various experiments.

4.4. Product characterization

*4.4.1. 1-Methyl-19-norcholesta-1,3,5(10),6,8(9),14(15)-hexaene (**2a**).* Needle shaped white crystals ($CHCl_3$/MeOH), mp 96–97 °C; 3–6% yield; R_f (petroleum ether) 0.95; ^{1}H NMR (300 MHz, CD_3COCD_3): 0.89 (d, *J*=6.6 Hz, 6H), 1.05 (s, 3H), 1.08 (d, *J*=6.0 Hz, 3H), 2.50–2.59 (m, 1H), 2.95 (s, 3H), 3.55–3.59 (m, 2H), 6.23 (s, 1H), 7.25 (m, 2H), 7.59–7.64 (m, 2H), 7.72 (d, *J*=9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 14.8 (CH_3), 19.0 (CH_3), 22.6 (CH_3), 22.8 (CH_3), 23.8 (CH_2), 27.3 (CH_3), 28.0 (CH), 28.7 (CH_2), 34.1 (CH), 35.9 (CH_2), 36.1 (CH_2), 37.2 (CH_2), 39.6 (CH_2), 44.4 (C), 57.5 (CH), 120.4 (CH), 123.2 (CH), 124.8 (CH), 127.5 (CH), 127.8 (CH), 129.2 (C), 130.7 (CH), 132.5 (C), 133.7 (C), 134.6 (C), 135.5 (C), 148.8 (C); IR (Nujol, cm^{-1}): 3057, 1380, 1365, 1278, 1257, 1103, 1060, 987, 935, 894, 821, 788, 754. DART-MS (ESI^+), *m*/*z*: 362 ($[M+2H]^+$, 27%), 361 ($[M+H]^+$, 100), 360 ($[M]^+$, 30), 359 (23), 347 (5). Elemental analyses: found: C, 89.88; H, 10.15. $C_{27}H_{36}$ requires C, 89.93; H, 10.07%.

*4.4.2. 4β-Hydroxy cholesterol (3β,4β-dihydroxy-5-cholestene, **3a**).* Needle shaped white crystals ($CHCl_3$/MeOH), mp 168–170 °C; 10–75% yield; R_f (30% ethyl acetate/petroleum ether) 0.45; ^{1}H NMR (300 MHz, $CDCl_3$): 0.68 (s, 3H), 0.86 (d, *J*=6.0 Hz, 6H), 0.90 (d, *J*=6.3 Hz), 1.18 (s, 3H), 1.25–1.66 (m, 13H), 1.81–2.10 (m, 8H), 3.54–3.56 (m, 1H), 4.13 (s, 1H), 5.67 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.9 (CH_3), 18.7 (CH_3), 20.5 (CH_2), 21.1 (CH_3), 22.6 (CH_3), 22.8 (CH_3), 23.8 (CH_2), 24.3 (CH_2), 25.4 (CH_2), 28.0 (CH), 28.2 (CH_2), 31.9 (CH), 32.1 (CH_2), 35.8 (CH), 36.0 (C), 36.2 (CH_2), 36.9 (CH_2), 39.5 (CH_2), 39.7 (CH_2), 42.3 (C), 50.2 (CH), 56.1 (CH), 56.9 (CH), 72.5 (CH), 77.3 (CH), 128.8 (CH), 142.8 (C); IR (KBr, cm^{-1}): 3382 (br), 1168, 978; FABMS (ESI^+), *m*/*z*: 402 (12%), 401 (11), 399 (13), 386 (29), 385 (100), 384 (50), 383 (48), 368 (28), 367 (62). Elemental analyses: found: C, 80.44; H, 11.59. $C_{27}H_{46}O_2$ requires C, 80.53; H, 11.52%.

*4.4.3. 4β,7α-Dihydroxy cholesterol (3β,4β,7α-trihydroxy-5-cholestene, **4a**).* White amorphous solid, mp 193–194 °C; 5–78% yield; R_f (70% ethyl acetate/petroleum ether) 0.55; ^{1}H NMR (300 MHz, $CDCl_3$): 0.69 (s, 3H), 0.86 (d, *J*=6.6 Hz, 6H), 0.92 (d, *J*=6.6 Hz, 3H), 1.18 (s, 3H), 3.57–3.65 (m, 1H), 3.94 (t, *J*=3.0 Hz, 1H), 4.18 (d, *J*=3.0 Hz, 1 Hz), 5.86 (d, *J*=3.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.6 (CH_3), 18.7 (CH_3), 19.4 (CH_3), 20.1 (CH_2), 22.6 (CH_3), 22.8 (CH_3), 23.8 (CH_2), 24.4 (CH_2), 25.1 (CH_2), 28.0 (CH), 28.3 (CH_2), 35.8 (CH), 36.2 (CH_2), 36.7 (CH_2), 37.0 (C), 37.6 (CH), 39.1 (CH_2), 39.5 (CH_2), 42.1 (C), 42.6 (CH), 49.4 (CH), 55.9 (CH), 65.3 (CH), 72.1 (CH), 77.0 (CH), 129.7 (CH), 147.0 (C); IR (KBr, cm^{-1}): 3349, 1153, 1066, 965. DART-MS (ESI^+), *m*/*z*: 402 (6%), 401 (26), 385 (5), 384 (28), 383 (100), 366 (4), 365 (10). Elemental analyses: found: C, 77.37; H, 11.17. $C_{27}H_{46}O_3$ requires C, 77.45; H, 11.08%.

*4.4.4. 1-Methyl-19-norβ-sitosta-1,3,5(10),6,8(9),14(15)-hexaene (**2b**).* Needle shaped white crystals (CH_2Cl_2/MeOH), mp 87–88 °C, 7% yield; R_f (petroleum ether) 0.95; ^{1}H NMR (300 MHz, $CDCl_3$): 0.87 (d, *J*=7.2 Hz, 6H), 0.94 (d, *J*=7.2 Hz, 3H), 1.07 (s, 3H), 1.10 (s, 3H), 1.15–1.35 (m, 6H), 1.39–1.60 (m, 4H), 1.68–1.74 (m, 4H), 2.19–2.35 (m, 2H), 2.94 (s, 3H), 3.51–3.55 (m, 2H), 6.15 (s, 1H), 7.20–7.25 (m, 2H), 7.55–7.60 (m, 2H), 7.66 (d, *J*=8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 12.0, 14.8, 19.0, 19.9, 23.1, 26.0, 27.3, 28.7, 29.2, 29.4, 33.9, 34.5, 36.0, 37.2, 44.5, 45.9, 57.4, 120.4, 123.2, 124.8, 127.6, 127.8, 129.2, 130.7, 132.5, 133.7, 134.6, 135.6, 148.9; IR (Nujol, cm^{-1}): 3049, 1367, 1278, 1112, 987, 896, 821, 844, 789, 755. Elemental analyses: found: C, 89.69; H, 10.31. $C_{29}H_{40}$ requires C, 89.62; H, 10.38%.

*4.4.5. 4β-Hydroxy β-sitosterol (3β,4β-dihydroxy-5-β-sitostene, **3b**).* Needle shaped white crystals ($CHCl_3$/MeOH), mp 162–164 °C,

51% yield; R_f (30% ethyl acetate/petroleum ether) 0.45; ^{1}H NMR (300 MHz, $CDCl_3$): 0.68 (s, 1H), 0.81 (d, J=6.9 Hz, 3H), 0.86 (d, J=6.9 Hz, 6H), 0.92 (d, J=6.6 Hz, 3H), 1.18 (s, 3H), 3.51–3.61 (m, 1H), 4.15 (s, 1H), 5.68 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.9, 12.0, 18.8, 19.0, 19.8, 20.5, 21.1, 23.1, 24.3, 25.4, 26.0, 28.2, 29.1, 31.8, 32.1, 33.9, 36.0, 36.1, 36.9, 39.7, 42.3, 45.8, 50.2, 56.0, 56.9, 72.5, 77.3, 128.8, 142.8; IR (Nujol, cm^{-1}): 3392, 1172, 1069, 977. Elemental analyses: found: C, 80.89; H,11.66. $C_{29}H_{50}O_2$ requires C, 80.86; H, 11.71%.

4.4.6. 4β,7α-Dihydroxy β-sitosterol (3β,4β,7α-trihydroxy-5-β-sitostene, ***4b****).* White solid, mp 192–193 °C; 22–72% yield; R_f (70% ethyl acetate/petroleum ether) 0.55; ^{1}H NMR (300 MHz, DMSO-d_6): 0.63 (s, 3H), 0.79 (d, J=6.3 Hz, 3H), 0.83 (d, J=6.0 Hz, 6H), 0.91 (d, J=6.3 Hz, 3H), 1.07 (s, 3H), 3.26–3.38 (m, 1H), 3.64 (t, J=3.0 Hz, 1H), 3.89 (d, J=3 Hz, 1H), 5.58 (d, J=4.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): 11.9, 12.2, 12.3, 19.1, 19.4, 19.6, 20.2, 23.0, 24.2, 25.4, 25.8, 28.4, 29.1, 33.8, 36.0, 36.9, 37.4, 37.9, 41.9, 42.3, 45.6, 49.4, 55.8, 63.8, 72.1, 77.1, 129.2, 145.7; IR (Nujol, cm^{-1}): 3392, 1165, 1078, 967. Elemental analyses: found: C, 77.88; H, 11.32. $C_{29}H_{50}O_3$ requires C, 77.96; H, 11.29%.

*4.4.7. 3β,4β,7α-Triacetoxy-5-cholestene (****4aa****).* Colourless cubic crystals ($CHCl_3$/MeOH), mp 170–171 °C; 94% yield from **4a**; R_f (5% ethyl acetate/petroleum ether) 0.75; ^{1}H NMR (300 MHz, $CDCl_3$): 0.66 (s, 3H), 0.86 (d, J=6.3 Hz, 6H), 0.92 (d, J=6.6 Hz, 3H), 1.14 (s, 3H), 2.01 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 4.77–4.82 (m, 1H), 5.06 (t, J=4.5 Hz, 1H), 5.52 (s, 1H), 5.93 (d, J=6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.4 (CH_3), 18.8 (CH_3), 20.2 (CH_2), 21.2 (CH_3), 21.3 (CH_3), 21.6 (CH_3), 22.3 (CH_2), 22.6 (CH_3), 22.8 (CH_3), 23.9 (CH_2), 24.1 (CH_2), 28.0 (CH), 28.1 (CH_2), 35.7 (CH), 35.8 (CH), 36.2 (CH_2), 36.3 (CH_2), 37.0 (C), 38.9 (CH_2), 39.5 (CH_2), 42.2 (CH), 43.5 (CH), 49.0 (CH), 55.9 (CH), 67.6 (CH), 72.2 (CH), 75.0 (CH), 128.4 (CH), 143.9 (C), 169.7 (C), 170.2 (C), 170.4 (C); IR (Nujol, cm^{-1}): 1734, 1365, 1246, 1044, 1012, 976, 941, 889. DART-MS (ESI$^+$), m/z: 485 (6%), 426 (29), 425 (100), 384 (5), 383 (17), 366 (5), 365 (16). Elemental analyses: found: C, 79.69; H, 10.62. $C_{33}H_{52}O_3$ requires C, 79.77; H, 10.56%.

*4.4.8. 3β-Acetoxy-4β-hydroxy-5-cholestene (****6a****).* White solid, mp 175–176 °C, 35% yield; R_f (15% ethyl acetate/petroleum ether) 0.45; ^{1}H NMR (300 MHz, $CDCl_3$): 0.68 (s, 3H), 0.86 (d, J=6.6 Hz, 6H), 0.91 (d, J=6.6 Hz, 3H), 1.22 (s, 3H), 2.11 (s, 3H), 4.25 (s, 1H), 4.69–4.76 (m, 1H), 5.71 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.9 (CH_3), 18.7 (CH_3), 20.5 (CH_2), 21.1 (CH_3), 21.4 (CH_3), 21.7 (CH_2), 22.6 (CH_3), 22.8 (CH_3), 23.8 (CH_2), 24.2 (CH_2), 28.0 (CH), 28.2 (CH_2), 31.7 (CH), 32.1 (CH_2), 35.8 (CH), 36.2 (CH_2), 36.6 (C), 36.9 (CH_2), 39.5 (CH_2), 39.6 (CH_2), 42.3 (C), 50.2 (CH), 56.1 (CH), 56.8 (CH), 75.5 (CH), 75.6 (CH), 129.5 (CH), 141.5 (C), 170.2 (C); IR (Nujol, cm^{-1}): 3412, 1737, 1279, 1046. DART-MS (ESI$^+$), m/z: 429 (11%), 428 (61). 427 (100%), 385 (16), 368 (18), 367 (58). Elemental analyses: found: C, 78.39; H, 10.79. $C_{29}H_{48}O_3$ requires C, 78.31; H, 10.89%.

*4.4.9. 3β-Acetoxy-6α-hydroxy-4-cholestene (****7a****).* White solid, mp 139–140 °C, 32% yield; R_f (20% ethyl acetate/petroleum ether) 0.45; ^{1}H NMR (300 MHz, $CDCl_3$): 0.68 (s, 3H), 0.87 (d, J=6.6 Hz, 6H), 0.91 (d, J=6.6 Hz, 3H), 1.18 (s, 3H), 2.08 (s, 1H), 3.60–3.69 (m, 1H), 5.38 (d, J=2.7 Hz, 1H), 5.85 (d, J=3.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.8 (CH_3), 18.7 (CH_3), 20.4 (CH_3), 20.6 (CH_2), 21.6 (CH_3), 22.6 (CH_3), 22.8 (CH_3), 23.8 (CH_2), 24.2 (CH_2), 25.8 (CH_2), 28.0 (CH), 28.2 (CH_2), 31.6 (CH), 32.1 (CH_2), 35.8 (CH), 36.0 (C), 36.2 (CH_2), 36.8 (CH_2), 39.5 (CH_2), 39.6 (CH_2), 42.3 (C), 50.2 (CH), 56.1 (CH), 56.8 (CH), 71.7 (CH), 79.3 (CH), 128.8 (CH), 138.8 (C), 171.2 (C); IR (Nujol, cm^{-1}): 3398, 1738, 1260, 1237, 1074. DART-MS (ESI$^+$), m/z: 428 (14%), 427 (43), 385 (77), 368 (29). Elemental analyses: found: C, 78.38; H, 10.96. $C_{29}H_{48}O_3$ requires C, 78.31; H, 10.89%.

*4.4.10. 3β-Benzoxy-4β-hydroxy-5-cholestene (****6b****).* White feather like crystals ($CHCl_3$/MeOH), mp 202–204 °C, 37% yield; R_f (10% ethyl acetate/petroleum ether) 0.55; ^{1}H NMR (300 MHz, $CDCl_3$): 0.74 (s, 3H), 0.92 (d, J=6.3 Hz, 6H), 0.97 (d, J=6.3 Hz, 3H), 1.31 (s, 3H), 2.21 (s, 1H), 4.45 (s, 1H), 5.01–5.06 (m, 1H), 5.79 (s, 1H), 7.49 (t, J=7.5 Hz, 2H), 7.61 (t, J=6.6 Hz, 1H), 8.11 (d, J=7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.3, 18.1, 20.0, 20.5, 21.3, 22.0, 22.3, 23.3, 23.7, 27.5, 27.7, 31.2, 31.6, 35.2, 35.6, 35.7, 36.5, 39.0, 39.1, 41.8, 49.7, 55.5, 56.3, 75.1, 75.5, 127.8 (2), 129.1 (3), 129.7, 132.5, 140.9, 165.1; IR (Nujol, cm^{-1}): 3533 (sharp), 1694, 1682, 1282, 1127, 1068, 1026, 967, 920, 845, 709. DART-MS (ESI$^+$), m/z: 491 (7%), 490 (37), 489 (100), 368 (3), 367 (10). Elemental analyses: found: C, 80.65; H, 9.84. $C_{34}H_{50}O_3$ requires C, 80.57; H, 9.95%.

*4.4.11. 3β-Benzoxy-6α-hydroxy-4-cholestene (****7b****).* Needle shaped white crystals ($CHCl_3$/MeOH), mp 142–144 °C, 34% yield; R_f (12% ethyl acetate/petroleum ether) 0.55; ^{1}H NMR (300 MHz, $CDCl_3$): 0.66 (s, 3H), 0.86 (d, J=6.6 Hz, 6H), 0.91 (d, J=6.3 Hz, 3H), 1.20 (s, 3H), 3.75 (t, J=6 Hz, 1H), 5.67 (s, 1H), 5.93 (d, J=3 Hz, 1H), 7.44 (t, J=7.5, 2H), 7.55 (t, J=7.2 Hz, 1H), 8.04 (d, J=8.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.8, 18.7, 20.7, 22.6, 22.8, 23.8, 24.2, 26.0, 28.0, 28.2, 31.7, 32.1, 35.8, 36.0, 36.2, 36.8, 39.5, 39.6, 42.3, 50.2, 56.1, 56.8, 72.0, 80.0, 128.4, 128.4, 129.6, 129.6, 130.5, 132.0, 133.0, 138.9, 166.8; IR (Nujol, cm^{-1}): 3442, 3392, 1686, 1674, 1273, 1170, 1110, 977, 899, 759, 711. DART-MS (ESI$^+$), m/z: 491 (4%), 490 (36), 489 (100), 386 (26), 385 (89), 368 (24), 367 (83). Elemental analyses: found: C, 80.48; H, 9.86. $C_{34}H_{50}O_3$ requires C, 80.57; H, 9.95%.

4.4.12. 4β-Hydroxy diosgenin (3β,4β-dihydroxy-5-spirostene, ***12****).* White solid, mp 171–173 °C, 30–58% yield; R_f (30% ethyl acetate/petroleum ether) 0.45; ^{1}H NMR (300 MHz, $CDCl_3$): 0.79 (d, J=3.0 Hz, 3H), 0.80 (s, 3H), 0.97 (d, J=6.8 Hz, 3H), 1.21 (s, 3H), 2.32 (s, 1H), 2.43 (d, J=6 Hz, 1H), 3.37 (t, J=10.8 Hz, 1H), 3.46 (d, J=3.0 Hz, 1H), 3.48–3.55 (m, 1H), 4.13 (d, J=3 Hz, 1H), 4.40 (dd, J=15.0 and 7.5 Hz, 1H), 5.66 (d, J=2.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 14.5 (CH_3), 16.3 (CH_3), 17.1 (CH_3), 20.3 (CH_2), 21.0 (CH_3), 25.3 (CH_2), 28.8 (CH_2), 30.3 (CH), 31.4 (CH_2), 31.8 (CH_2), 32.2 (CH), 36.2 (C), 36.9 (CH_2), 39.7 (CH_2), 40.3 (C), 41.6 (CH), 50.1 (CH), 56.6 (CH_2), 62.0 (CH), 66.9 (CH_2), 72.4 (CH), 77.2 (CH), 80.8 (CH), 109.3 (C), 128.3 (CH), 142.9 (C); IR (Nujol, cm^{-1}): 3392, 1169, 1047, 976. FABMS, m/z: 432 (37%), 431 (90), 430 (39), 429 (77), 414 (49), 413 (100), 412 (27), 411 (28), 395 (25). Elemental analyses: found: C, 75.21; H, 9.79. $C_{27}H_{42}O_4$ requires C, 75.29; H, 9.84%.

4.4.13. 4β,7α-Dihydroxy diosgenin (3β,4β,7α-trihydroxy-5-spirostene, ***13****).* White solid, mp 201–202 °C, 17–69% yield; R_f (70% ethyl acetate/petroleum ether) 0.50; ^{1}H NMR (300 MHz, $CDCl_3$): 0.79 (d, J=3.0 Hz, 3H), 0.80 (s, 3H), 0.98 (d, J=6.9 Hz, 3H), 1.20 (s, 3H), 3.37 (t, J=10.8 Hz, 1H), 3.45–3.49 (m, 1H), 3.52–3.64 (m, 1H), 3.94 (t, J=4.5 Hz, 1H), 4.09–4.19 (m, 1H), 4.48 (dd, J=15.0 and 7.5 Hz, 1H), 5.86 (d, J=4.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6 (CH_3), 16.1 (CH_3), 17.2 (CH_3), 19.4 (CH_3), 20.0 (CH_2), 25.1 (CH_2), 28.8 (CH_2), 30.3 (CH), 31.4 (CH_2), 31.9 (CH_2), 36.6 (CH_2), 37.2 (CH), 39.1 (CH_2), 40.4 (C), 41.7 (CH), 42.5 (CH), 49.0 (CH), 62.0 (CH), 65.2 (CH), 66.9 (CH_2), 72.1 (CH), 76.9 (CH), 80.8 (CH), 109.3 (C), 129.6 (CH), 146.9 (C); IR (Nujol, cm^{-1}): 3395, 1172, 1054, 978. Elemental analyses: found: C, 72.49; H, 9.41. $C_{27}H_{42}O_5$ requires C, 72.59; H, 9.48%.

*4.4.14. 19-Norergosta-1,3,5,7,9,14,22-heptaene (****9****).* Pale yellow needles, mp 125–126 °C (Chloroform/methanol), 5.5–12% yield; R_f (petroleum ether) 0.95; ^{1}H NMR (300 MHz, $CDCl_3$): 0.84 (d, J=4.2 Hz, 3H), 0.88 (d, J=4.2 Hz, 3H), 0.95 (s, 3H), 0.98 (d, J=5.1 Hz, 3H), 1.15 (d, J=6.6 Hz, 3H), 1.52 (m, 1H), 1.76 (m, 1H), 1.90 (m, 1H), 1.61 and 2.33 (m, 2H), 2.33 (m, 1H), 2.21 and 2.36 (m, 2H), 3.14 and 3.29 (m, 2H), 5.24 (m, 1H), 5.30 (m, 1H), 6.12 (t, J=3 Hz, 1H), 7.42 (m, 1H), 7.45 (m, 1H), 7.50 (m, 1H), 7.64 (m, 1H), 7.77 (d, J=7.8 Hz, 1H), 8.01 (d, J=9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 15.4 (CH_3), 17.7

(CH_3)19.7 (CH_3), 20.0 (CH_3), 21.2 (CH_3), 23.7 (CH_2), 33.1 (CH), 36.5 (CH_2), 36.9 (CH_2), 39.0 (CH), 42.9 (CH), 45.2 (C), 57.2 (CH), 120.7 (CH), 123.6 (CH), 123.7 (CH), 125.2 (CH), 126.1 (CH), 126.3 (CH), 128.5 (CH),128.5 (C), 130.2 (C), 132.5 (CH), 132.8 (2C), 135.3 (CH) , 148.1 (C). These spectral data match that originally reported by Qin and Liu,[16] a summary of which is found in Table S1 in Supplementary data.

Acknowledgements

The authors are thankful to CDRI, Lucknow, India, for mass spectrometry and elemental analysis. A.S. and J.D. thank UGC (New Delhi) and CSIR (New Delhi), respectively, for the award of Junior Research Fellowships. The Ministry of Higher Education (Malaysia) is also thanked for support of the crystallographic facility and for research funding through the High-Impact Research scheme (UM.C/HIR/MOHE/SC/12).

Supplementary data

Copies of ^{1}H NMR, ^{13}C NMR and DEPT-135 NMR spectra, NMR comparison sheet for the natural and synthetic diaromatic ergosterol **9**, description of the molecular structures of **2a** and **4aa** (with crystallographic data). Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.05.110.

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13. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 828345 (for **2a**) and CCDC 828346 (for **4aa**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
14. The reactions of 4β-hydroxy steroids (i.e., the diols) with SeO_2 were conducted for 48 h. After usual work up a deep red gummy residue was obtained, which seemed apparently, not to contain any solid product. The corresponding 4β,7α-dihydroxy steroids (i.e., the triols) were finally purified from it after repeated column chromatography
15. The authors are thankful to one of the honorary reviewers for the valuable suggestion of improving the yield of **4a** starting directly from cholesterol.
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17. When we formed 9 from ergosterol, attention was directed to the reaction of cholesterol. The reaction was conducted again at 2 h, 6 h and 12 h. Although ^{1}H NMR indicated the formation of the corresponding analogue, the amount was negligible (see Supplementary data).
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