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Stereo and chemical course of acid-catalyzed double bond migration of cholesta-5,7-dien-3β-ol to 5α-cholesta-8,14-dien-3β-ol

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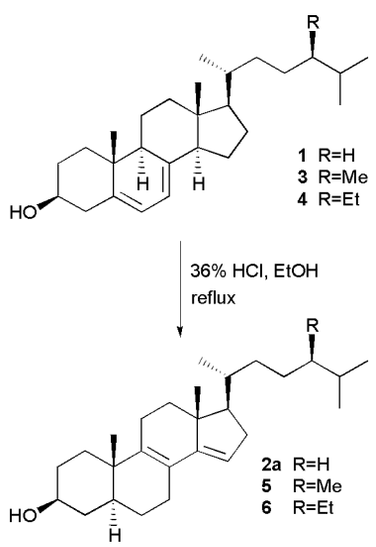
Acid-catalyzed double bond migration of steroid 5,7-dienes to the 5α-8,14-dienes, a well-known reaction in steroid chemistry, was reinvestigated by using cholesta-5,7-dien-3β-ol **1**, and the stereo and chemical course of this reaction was detailed. Treatment of **1** with 36% hydrochloric acid in refluxing ethanol for 3 h afforded a 6:11:65:16:2 mixture of dienols (93%): 5α- and 5β-cholesta-6,8(14)-dien-3β-ols **7a**,**b**, 5α- and 5β-cholesta-8,14-dien-3β-ols **2a**,**b**, and 5α-cholesta-14,16-dien-3β-ol **10a**, along with a mixture of enones (1.7%): 5β-cholest-8(14)-, 5β-cholest-14- and 14-*epi*-5β-cholest-8-en-3-ones **11**–**13**. The experiments using **7a**,**b** and **2a**,**b** suggested the reaction sequence: **1**→**7a**,**b**↔7,14-dienols **8a**,**b**↔**2a**,**b**↔8(14),15-dienols **9a**,**b**↔**10a**,**b**, in which **8a**,**b**↔**9a**,**b** should be also implicated. The initial step, **1** to **7a**,**b** proceeded irreversibly with the stereoselectivity, *ca.* 7:3 of 5α-H to 5β-H. Dienols **7a**, **8a**, **2a** and **10a** with 5α-H were identified, which were equilibrated at 6:0:92:2. Among dienols with 5β-H, only **7b** and **2b** were identified, which were equilibrated at 2:98, and this interconversion proceeded in competition with an intramolecular hydride shift from the C-3α to C-6α of **7b**, leading to the formation of a mixture of enones **11**–**13**. The considerable difference in activation energies between **1**→**7a**,**b** and **7a**,**b**→**8a**,**b**/**11**–**13** realized the predominant formation of **7a**,**b**: by treatment at 30 °C for 44 h, **1** gave a 53:27:5:15 mixture (94%) of **7a**, **7b**, **8a** and **2a**.

Introduction

Acid-catalyzed double bond migration of a 5,7-diene to the 8,14-diene with 5α-H in steroids, *e.g.*, cholesta-5,7-dien-3β-ol (7-dehydrocholesterol: **1**) to 5α-cholesta-8,14-dien-3β-ol **2a**, is a well-known reaction,[1,2] and the resulting 5α-8,14-dienes have been employed to prepare the biosynthetic intermediates and their labelled compounds useful for the investigation of steroid biosynthesis of mammals, plants, *etc.*[3–6] In recent years, we elucidated that *fackel-J79*, a dwarf mutant of *Arabidopsis thaliana* with unique phenotypes, had a defect of sterol C-14 reductase which reduces the 14-double bond in brassinosteroid biosynthesis and thus accumulated three abnormal sterols, **2a** and its (24*R*)-24-methyl and -ethyl congeners, *i.e.*, campesta-8,14-dienol **5** and stigmasta-8,14-dien-3β-ol **6** (sitosta-8,14-dienol) (Scheme 1).[7] This was clearly verified by GC-MS analysis using the synthetic 5α-8,14-dienols as the reference markers. However, in the synthesis, we could not attain the reported high yields under conventional reaction conditions, and found that these reactions were accompanied by the formation of several minor products, some of which were suspected to be the reaction intermediates and their C-5 epimers. Since there is no paper describing the actual features of this reaction including the chemical and stereo-selectivities, and the 8,14-diene functionality on the steroid should be a promising starting point for introducing a variety of substituents and functional groups to the C and D rings to assemble complex steroidal natural products, we undertook a study on the acid-catalyzed double bond migration of steroid 5,7-diene to the 5α-8,14-diene by using commercially available cholesta-5,7-dien-3β-ol **1** as a reaction substrate.

In this paper we detail the stereo and chemical course of this reaction on the basis of product analysis and equilibrium experiments, which enabled us to establish other acidic conditions to give steroid 6,8(14)-dienes predominantly. An intriguing reaction, an intramolecular hydride shift from the C-3α to C-6α observed on 5β-cholesta-6,8(14)-dien-3β-ol **7b** leading to the formation of 5β-cholest-8(14)-, 5β-cholest-14- and 14-*epi*-5β-cholest-8-en-3-ones **11**–**13**, is also presented in this context.



Scheme 1

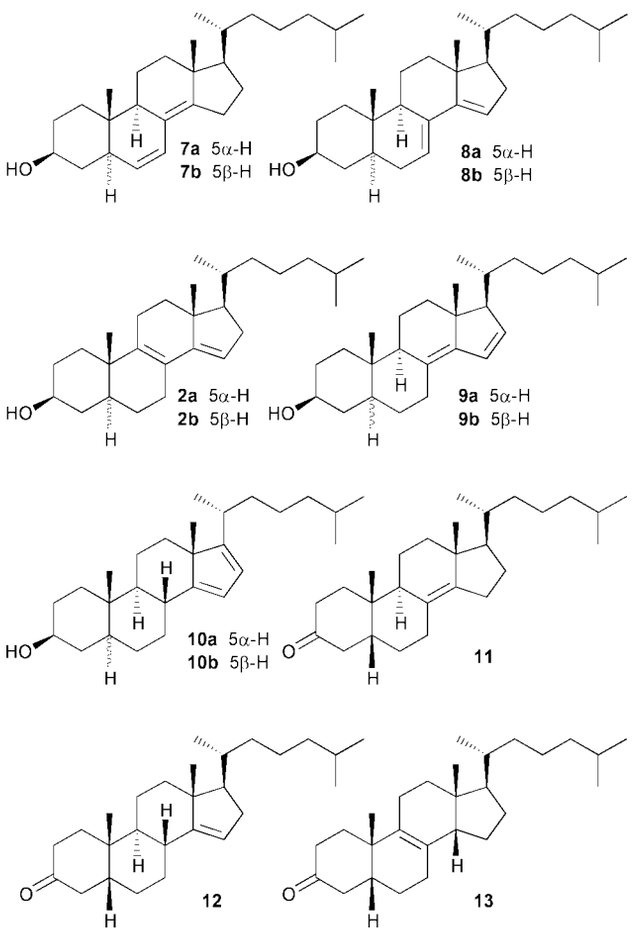
Results and discussion

When cholesta-5,7-dien-3β-ol **1**, campesta-5,7-dienol **3**[8] and stigmasta-5,7-dienol **4**[9] were subjected to acid-catalyzed diene migration from the 5,7-diene to the corresponding 8,14-diene by following the conventional procedure,[2] the yields of 5α-8,14-dienols, **2a**, **5** and **6**, were 55, 53 and 50%, respectively. Therefore, to clarify whether the unsatisfactory yields compared to those reported are inevitable or not, we first analyzed the

Table 1 Reaction of dienols **1**, **7a**,**b** and **2a**,**b**, with 36% hydrochloric acid in ethanol

Entry	Substrate	Reaction temperature	Reaction time/h	Product ratio (%)[a]	Combined yield (%)[b]
1	**1**	reflux	3	**7a** (5), **7b** (11), **2a** (64), **2b** (16), **10a** (2) a mixture of **11**, **12** and **13** (2)	95
2	**1**	30 °C	44	**7a** (53), **7b** (27), **8a** (5), **2a** (15)	94
3	**7a**	reflux	3	**7a** (7), **2a** (90), **10a** (3)	93
4	**2a**	reflux	3	**7a** (6), **2a** (92), **10a** (2)	93
5	**7b**	reflux	8	**7b** (4), **2b** (56), **11** (24), **12** (14), **13** (2)	96
6	**2b**	reflux	8	**7b** (1), **2b** (63), **11** (20), **12** (14), **13** (2)	85

[a] Ratios were calculated from ^{1}H NMR spectra in Entries 2–4, and by combination of the combined yields of dienol and enone mixtures, and their ^{1}H NMR spectra in Entries 1, 5 and 6. [b] After flash chromatography.



products formed from **1**. A solution of **1** (1.30 mmol) and 36% hydrochloric acid (1.0 cm^3) in ethanol (20 cm^3) was stirred at refluxing temperature for 3 h. After the usual aqueous work-up and flash chromatography on silica gel, a 6:11:65:16:2 mixture of dienols, 5α-cholesta-6,8(14)-dien-3β-ol **7a**, 5β-cholesta-6,8(14)-dien-3β-ol **7b**, 5α-cholesta-8,14-dien-3β-ol **2a**, 5β-cholesta-8,14-dien-3β-ol **2b** and 5α-cholesta-14,16-dien-3β-ol **10a**, was obtained in 93% combined yield along with 1.7% of a mixture of enones, 5β-cholest-8(14)-en-3-one **11**, 5β-cholest-14-en-3-one **12** and 14-*epi*-5β-cholest-8-en-3-one **13** (Entry 1 in Table 1). The ratio of **7a**, **7b**, **2a**, **2b**, **10a** was estimated from the ^{1}H NMR spectrum. The dienol mixture could be separated by high-performance liquid chromatography (HPLC), giving **7a**, **7b**, **2a**, **2b** and **10a** in 6.4, 9.0, 55, 14 and 2.2% isolated yields, respectively. Cholest-5-en-3β-ol was also isolated in 3.2% yield; this is a contaminant of commercial **1**. Fig. 1 shows a simplified HPLC spectrum of the dienol mixture, shown above, along with retention times of **1** and **8a**, the latter of which was obtained under other conditions (*vide infra*). Since the formation of enones **11**–**13** was assumed to be the result of an intramolecular hydride shift of **7b** as discussed later, the complete consumption of the starting material **1** indicated that the initial reaction of **1** leading to 6,8(14)-dienes **7a**,**b** was irreversible. Therefore, the stereoselectivity of this reaction was calculated as 69:31 of 5α-H to 5β-H, which is obviously determined kinetically at the initial protonation at C-5 of **1**.

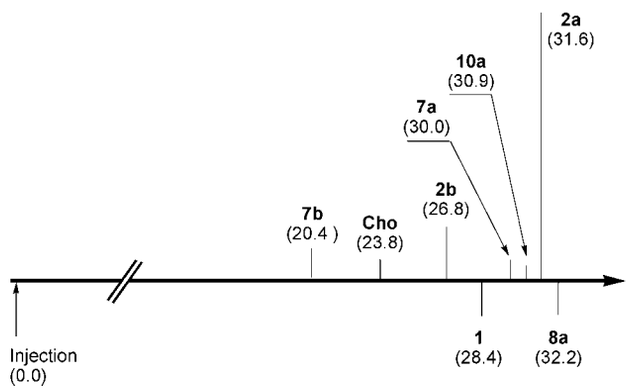
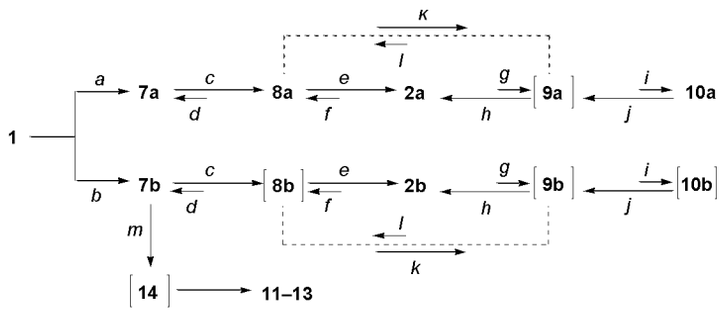


Fig. 1 Retention times/min of dienols identified in this work on HPLC. Simplified spectrum of a dienol mixture obtained by Entry 1 in Table 1 is shown above, where the length of each bar reflects the amount; others are shown below. Abbreviation **Cho** means cholest-5-en-3β-ol which is a contaminant in commercial **1**.

In order to increase the stereoselectivity biased to 5α-H by kinetic control, **1** was treated with 36% hydrochloric acid in a mixed solution of ethanol and benzene, 5:1, at 30 °C (a co-solvent, benzene, was necessary due to the low solubility of **1** in ethanol). After 44 h, the starting material was completely consumed, as monitored by ^{1}H NMR spectroscopy, and a 53:27:5:15 mixture of **7a**, **7b**, 5α-cholesta-7,14-dien-3β-ol **8a** and **2a** was obtained in 94% combined yield (Entry 2). Separation by HPLC gave **7a** and **7b** in 46 and 25% yields along with **8a** (4.8%) and **2a** (11%). The calculated stereoselectivity, 73:27 of 5α-H to 5β-H, was nearly the same as that attained under refluxing conditions, but the product distribution was completely different: *i.e.*, 6,8(14)-dienols **7a**,**b** predominantly formed. This indicates that the initial step, migration of the 5,7-diene in **1** to the 6,8(14)-diene in **7a**,**b**, proceeds much faster than further migrations or the intramolecular hydride shift of **7b**, and that **7b** is more stable than **7a**. Other attempts to prevent the formation of **8a** and **2a**, and to further enhance the 5α-H selectivity, resulted in near failure. Among them, a cationic resin, Dowex 50W, gave a similar result with some enhancement of the stereoselectivity. Treatment of **1** with Dowex 50W resin in ethanol at 70 °C for 51 h gave a 55:22:11:12 mixture of **7a**, **7b**, **8a** and **2a** in 96% combined yield, thus the stereoselectivity of 5α-H to 5β-H being 78:22. After separation, the isolated yields of **7a**, **7b**, **8a** and **2a** were 48, 21, 6.8 and 11%, respectively.

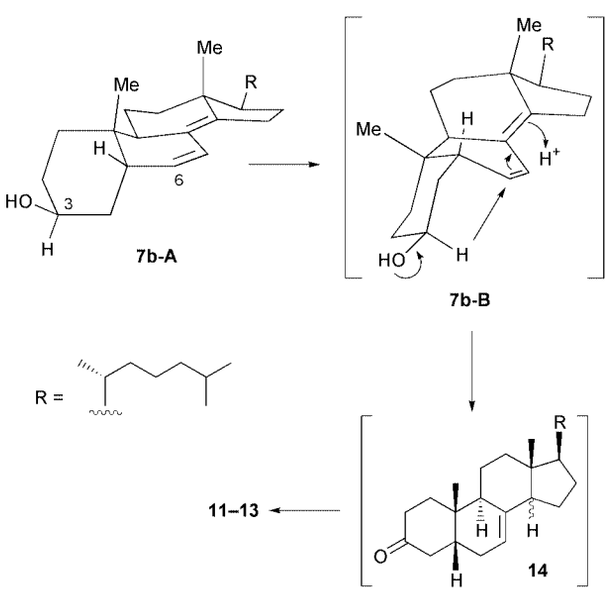
Further insight into the kinetic or thermodynamic correlation among dienols was obtained from reactions of the isolated **7a**, **2a**, **7b** and **2b** under the same acidic conditions at refluxing temperature. With 3 h treatment, both **7a** and **2a** gave a mixture of **7a**, **2a** and **10a** in nearly the same ratio (Entries



Scheme 2 Events taking place in treatment of cholesta-5,7-dien-3β-ol **1** with 36% hydrochloric acid in refluxing ethanol. Compounds in brackets are not identified. *Protonation and deprotonation positions (l)*: *a*: 5α-/14α-; *b*: 5β-/14α-; *c*: 6-/15-; *d*: 15-/6-; *e*: 7-/9α-; *f*: 9α-/7-; *g*: 9α-/16-; *h*: 16-/9α-; *i*: 8α-/17α-; *j*: 17α-/8α-; *k*: 7-/16-; *l*: 16-/7-; *m*: 14-/3β-OH, an intramolecular hydride shift of 3α-H to the C-6α position.

3 and 4), which indicated that dienols **7a**, **8a**, **2a**, **9a**, **10a** with 5α-H were equilibrated at a ratio of 6:0:92:0:2. On the other hand, the reaction of **7b** proceeded much more slowly than that of **7a**. After 3 h, *ca*. 45% of **7b** still remained, and after 8 h **7b** afforded a 4:56:24:14:2 mixture of **7b**, **2b** and **11**–**13** (Entry 5), while **2b** gave a 1:63:20:14:2 mixture of **7b**, **2b** and **11**–**13** after 8 h (Entry 6). Assuming that dienols **8b**, **9b** and **10b** should be implicated like **8a**, **9a** and **10a** with 5α-H, these results suggest that dienols **7b**, **8b**, **2b**, **9b**, **10b** with 5β-H are equilibrated at 2:0:98:0:0, although this process is impeded by an irreversible conversion of **7b** to enones **11**–**13**. On the basis of these data, all events taking place in treatment of **1** with 36% hydrochloric acid in refluxing ethanol can be eventually summarized as illustrated in Scheme 2.

With respect to the organic reaction mechanism, conversion of **7b** to enones **11**–**13**, is of special interest, which doubtless involves an intramolecular hydride shift, as depicted in Scheme 3. A 1,4-hydride-shift from the C-3α to C-6α occurs *via* a con-



Scheme 3 Acid-catalysed intramolecular hydride shift of 5β-cholesta-6,8(14)-dien-3β-ol **7b** *via* an unstable conformer **7b-B**.

former **7b-B**, which is one of the unstable conformers of **7b** satisfying the stereoelectric requirement for the reaction, to give intermediary **14**, 5β-cholest-7-en-3-one or its 14-epimer, which then isomerizes to the 8(14)-ene **11** and 14-ene **12**, and 14-*epi*-5β-cholest-8-en-3-one **13** by acid. The suitability of **7b-A** as a stable conformer of **7b** in a solution was deduced from the ^{1}H NMR spectrum: 3α-H resonates at δ 4.07 (br s, $\omega_{1/2}$ *ca*. 7 Hz) and has no large coupling constants due to an *anti*-vicinal coupling, indicating its equatorial orientation. It is well documented that secondary alcohols serve as hydride donors by their α-positioned hydrides as observed in the Meerwein–Ponndorf–Verley process, but no example has been reported so far of an intramolecular shift of α-H of a hydroxy function under acidic conditions.[10]

The structures of compounds, **7a**,**b**, **2b**, **10a**, **11**–**13**, **5** and **6** were verified by MS, and ^{1}H and ^{13}C NMR spectroscopy. NMR experiments on these compounds except for **5** and **6**, as well as the known **8a**[11] and **2a**[2] for reference, were carried out with 600 MHz by PFG-DQFCOSY, PFG-HMQC, PFG-HMBC and NOE difference experiments (400 MHz), by which all resonances were completely assigned (Experimental section). The stereochemistries of C-5 and C-9 of these compounds were respectively deduced from NOE difference experiments centered on 19-H_3, 5-H and 9-H. The NOE effects were observed between 19-H_3 and 5-H in **7b**, **2b** and **11**–**13** with 5β-H, but not observed in **7a**, **8a**, **2a** and **10a** with 5α-H. No effect was observed at 9-H of **7a**,**b**, **8a**, **2a**,**b**, **10a**, **11** and **12** with 9α-H when the 19-H_3 was irradiated. Similarly, NOE correlations between 8-H and both of 18-H_3 and 19-H_3 in **10a** and **12** demonstrated their 8β-configuration, and correlation between 14-H and 18-H_3 in **13** indicated the 14β-configuration. Some selected ^{1}H NMR data are shown in Table 2. The ^{1}H and ^{13}C NMR spectra of (24*R*)-24-methyl and -ethyl congeners of **2a** (**5** and **6**), were reasonably similar to those of **2a**.

In conclusion, a well-known reaction in steroid chemistry, acid-catalyzed double bond migration of steroid 5,7-dienes to the 5α-8,14-dienes was reinvestigated by using cholesta-5,7-dien-3β-ol **1**. The product analysis and equilibrium experiments suggested the reaction sequence: **1**→**7a**,**b**↔**8a**,**b**↔**2a**,**b**↔**9a**,**b**↔**10a**,**b**, in which **8a**,**b**↔**9a**,**b** should also be implicated. The initial step, **1** to 6,8(14)-dienol **7a**,**b**, proceeded irreversibly with stereoselectivity of *ca*. 7:3 of 5α-H to 5β-H. Dienols **7a**, **8a**, **2a** and **10a** with 5α-H were identified, which were equilibrated at 6:0:92:2. On the other hand, among dienols with 5β-H, only **7b** and **2b** were identified, which were equilibrated at 2:98. The conversion of **7b** to **8b** competed with an intramolecular hydride shift from the C-3α to C-6α, resulting in the formation of enones **11**–**13**. These results proved that moderate but not high yields of 5α-8,14-dienols, **2a**, **5** and **6**, from 5,7-dienols, **1**, **3** and **4**, were reasonable for this acidic migration reaction. The considerable difference in activation energies between **1**→**7a**,**b** and **7a**,**b**→**8a**,**b** or **11**–**13** realized the reaction conditions for the predominant formation of 6,8(14)-dienols **7a**,**b** from **1**.

Experimental

General

Melting points (mp) were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. NMR measurements were performed on a Bruker AC-300, JEOL JNM-A400 or JEOL JNM-A600 spectrometer. All spectra were recorded using standard pulse sequences. Chemical shifts were recorded as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0 ppm) for ^{1}H or to the solvent ($CDCl_3$) (δ 77.0 ppm) for ^{13}C as an internal reference. All *J*-values are given in Hz.

Table 2 Selected ^{1}H-NMR Data (δ: multiplicity, J in Hz) of dienols, **7a**,**b**, **8a**, **2a**,**b** and **10a**, and enones **11**–**13**

Compound	Atom	3-H or 4α-H	18-H_3	19-H_3	Olefinic protons	
7a	3-H	3.68: tt *J* 10.7, 4.9	0.89: s	0.64: s	5.26: dd *J* 9.8, 1.5	6.13: dd *J* 9.8, 2.9
7b	3-H	4.09: br s $\omega_{1/2}$ *ca.* 7	0.89: s	0.76: s	5.52: dd *J* 9.8, 5.4	6.09: d *J* 9.8
8a	3-H	3.60: tt *J* 11.2, 4.4	0.83: s	0.79: s	5.50: dd *J* 3.4, 2.0	5.75: m
2a	3-H	3.63: tt *J* 10.7, 4.9	0.82: s	0.99: s	5.36: dd *J* 2.5, 2.0	
2b	3-H	3.89: m $\omega_{1/2}$ *ca.* 16	0.83: s	1.12: s	5.39: br s	
10a	3-H	3.59: tt *J* 11.2, 4.9	0.97: s	0.92: s	5.81: dd *J* 2.0, 2.0	5.96: d *J* 2.0
11	4α-H	2.82: dd *J* 14.7, 14.2	0.87: s	0.90: s	—	
12	4α-H	2.68: dd *J* 14.7, 14.2	0.93: s	1.03: s	5.20: m	
13	4α-H	2.33: ddd *J* 14.2, 9.8, 1.0	0.90: s	1.14: s	—	

Mass spectra, EI-MS and HR-EI-MS, were obtained with a JEOL-SX102 mass spectrometer. Analytical thin-layer chromatography (TLC) was conducted on micro-slides coated with Merck Kieselgel KG60F-254; the developed plates were stained with 10% (w/v) vanillin in concentrated sulfuric acid at 180 °C. All reactions were carried out under a nitrogen atmosphere. Flash chromatography was conducted using silica gel FL-60D [Fuji Silysia Chemical Ltd.] as the adsorbent. High-performance liquid chromatography (HPLC) was conducted with Senshu Pak PG-S60-5251 (20 mm i.d. × 25 cm; Senshu Scientific Co.) at a flow rate of 9.0 cm^3 min^{-1}; the peaks were detected by a refractive index detector. The ratios of mixed solvents were v/v. Commercial cholesta-5,7-dien-3β-ol **1** (7-dehydrocholesterol: Sigma) was purified by flash chromatography on silica gel before use, and still contained *ca.* 3% of cholest-5-en-3β-ol. The ratios of compound mixtures were estimated from the ^{1}H NMR spectra.

Acid treatment of cholesta-5,7-dien-3β-ol 1

With 36% hydrochloric acid (HCl) at reflux temperature. A solution of cholesta-5,7-dien-3β-ol **1** (500 mg, 1.30 mmol) and 36% HCl (1.0 cm^3) in EtOH (20 cm^3) was refluxed for 3 h. After removal of EtOH under reduced pressure, the residue was diluted with water and extracted with Et_2O. The extracts were successively washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated. The residue was subjected to flash chromatography using hexane–AcOEt (4:1) as eluent. The fraction eluted at R_f 0.61 gave compounds (8.5 mg, 1.7%) which had no ultra-violet absoption by 254 nm irradiation on TLC, and which were found to be a mixture of 5β-cholest-8(14)-en-3-one **11**, 5β-cholest-14-en-3-one **12** and 14-*epi*-5β-cholest-8-en-3-one **13** from the ^{1}H NMR spectrum. The isolation and structural assignment of **11**–**13** were performed from the experiment on treatment of 5β-cholesta-6,8(14)-dien-3β-ol **7b** with 36% HCl, as mentioned below.

The fraction eluted at R_f 0.18–0.27 gave a dienol mixture (465 mg, 93%) consisting of 5α-cholesta-6,8(14)-dien-3β-ol **7a**, 5β-cholesta-6,8(14)-dien-3β-ol **7b**, 5α-cholesta-8,14-dien-3β-ol **2a**, 5β-cholesta-8,14-dien-3β-ol **2b** and 5α-cholesta-14,16-dien-3β-ol **10a** in a ratio of 6:11:65:16:2. This mixture was subjected to HPLC using hexane–AcOEt (4:1) as a mobile phase. Elutions at different R_ts as shown in Fig. 1 gave **7b** (45 mg, 9.0%), 5α-cholest-5-en-3β-ol (16 mg, 3.2%), **2b** (71 mg, 14%), **7a** (32 mg, 6.4%), **10a** (11 mg, 2.2%) and **2a** (273 mg, 55%), successively.

5α-Cholesta-6,8(14)-dien-3β-ol ***7a***. Colorless needles, mp 90–98 °C (MeOH); δ_H (600 MHz) 0.64 (3H, s, 19-H_3), 0.864 (3H, d, *J* 6.8 Hz, 26-H_3), 0.867 (3H, d, *J* 6.8 Hz, 27-H_3), 0.89 (3H, s, 18-H_3), 0.94 (3H, d, *J* 6.8 Hz, 21-H_3), 1.07 and 1.38 (each 1H, each m, 22-H_2), 1.10 and 1.17 (each 1H, each m, 24-H_2), 1.14 (1H, m, 1α-H), 1.14 and 1.36 (each 1H, each m, 23-H_2), 1.19 (1H, m, 17-H), 1.26 (1H, ddd, *J* 12.7, 12.7 and 3.4 Hz, 12α-H), 1.38 (1H, m, 4β-H), 1.44 and 1.90 (each 1H, each m, 16-H_2), 1.47 (1H, m, 11β-H), 1.47 (1H, m, 20-H), 1.48 (1H, m, 2β-H), 1.52 (1H, m, 25-H), 1.62 (1H, m, 11α-H), 1.69 (1H, ddd, *J* 13.2, 3.4 and 3.4 Hz, 1β-H), 1.78 (1H, m, 4α-H), 1.88 (1H, m, 2α-H), 1.92 (1H, m, 9-H), 2.01 (1H, ddd, *J* 12.7, 3.4 and 3.4 Hz, 12β-H), 2.06 (1H, br dm, *J* 13.2 Hz, 5-H), 2.30 (1H, m, 15β-H), 2.39 (1H, m, 15α-H), 3.68 (1H, tt, *J* 10.7 and 4.9 Hz, 3-H), 5.26 (1H, dd, *J* 9.8 and 1.5 Hz, 6-H), 6.13 (1H, dd, *J* 9.8 and 2.9 Hz, 7-H); δ_C (150 MHz) 11.31 (C-19), 18.88 (C-21), 19.24 (C-18), 19.73 (C-11), 22.56 (C-26), 22.79 (C-27), 23.68 (C-23), 24.93 (C-15), 27.35 (C-16), 28.02 (C-25), 31.48 (C-2), 34.77 (C-20), 35.15 (C-1), 35.68 (C-10), 35.88 (C-22), 36.59 (C-4), 36.76 (C-12), 39.52 (C-24), 43.65 (C-13), 44.75 (C-5), 48.15 (C-9), 55.92 (C-17), 71.44 (C-3), 125.23 (C-8), 125.75 (C-7), 129.38 (C-6), 147.54 (C-14); EI-MS *m/z* 384 (M^+, 11%), 366 ($M^+ - H_2O$, 48), 351 ($M^+ - H_2O - CH_3$, 38), 271 ($M^+ - C_8H_{17}$, 28), 253 ($M^+ - C_8H_{17} - H_2O$, 100), 199 (36); HR-EI-MS *m/z* M^+: Found, 384.3389. Calc. for $C_{27}H_{44}O$, 384.3392.

5β-Cholesta-6,8(14)-dien-3β-ol ***7b***. Colorless needles, mp 100 °C (MeOH); δ_H (600 MHz) 0.76 (3H, s, 19-H_3), 0.866 (3H, d, *J* 6.8 Hz, 26-H_3), 0.869 (3H, d, *J* 6.8 Hz, 27-H_3), 0.89 (3H, s, 18-H_3), 0.95 (3H, d, *J* 6.4 Hz, 21-H_3), 1.08 and 1.38 (each 1H, each m, 22-H_2), 1.13 (2H, 24-H_2), 1.17 and 1.37 (each 1H, each m, 23-H_2), 1.21 (1H, m, 17-H), 1.34 (1H, m, 12α-H), 1.44 and 1.89 (each 1H, each m, 16-H_2), 1.45–1.55 (2H, 11-H_2), 1.48 (1H, m, 4α-H), 1.48 (1H, m, 20-H), 1.53 (1H, m, 25-H), 1.58 (2H, 1-H_2), 1.58 (2H, 2-H_2), 1.76 (1H, m, 4β-H), 2.04 (1H, ddd, *J* 12.2, 3.4 and 3.4 Hz, 12β-H), 2.14 (1H, ddd, *J* 12.7, 5.4 and 4.9 Hz, 5-H), 2.28 and 2.38 (each 1H, each m, 15-H_2), 2.43 (1H, m, 9-H), 4.09 (1H, m, $\omega_{1/2}$ *ca.* 7 Hz, 3-H), 5.52 (1H, dd, *J* 9.8 and 5.4 Hz, 6-H), 6.09 (1H, d, *J* 9.8 Hz, 7-H); δ_C (150 MHz) 18.92 (C-21), 19.05 (C-18), 19.46 (C-11), 22.55 (C-26), 22.78 (C-27), 23.06 (C-19), 23.67 (C-23), 24.92 (C-15), 27.38 (C-16), 28.01 (C-25), 28.07 (C-2), 28.17 (C-1), 34.21 (C-9), 34.73 (C-20), 34.53 (C-10), 35.90 (C-22), 36.59 (C-4), 37.43 (C-12), 39.09 (C-5), 39.52 (C-24), 43.59 (C-13), 55.99 (C-17), 66.31 (C-3), 124.46 (C-7), 124.86 (C-8), 129.82 (C-6), 147.04 (C-14); EI-MS *m/z* 384 (M^+, 19%), 366 ($M^+ - H_2O$, 76), 351 ($M^+ - H_2O - CH_3$, 40), 271 ($M^+ - C_8H_{17}$, 41), 253 ($M^+ - C_8H_{17} - H_2O$,

100), 199 (50); HR-EI-MS *m/z* M^+: Found, 384.3389. Calc. for $C_{27}H_{44}O$, 384.3392.

5α-Cholesta-8,14-dien-3β-ol ***2a***. Colorless scales, mp 111–113 °C (MeOH) [lit.,[2] mp 116–117 °C (MeOH)]; δ_H (600 MHz) 0.82 (3H, s, 18-H_3), 0.868 (3H, d, *J* 6.8 Hz, 26-H_3), 0.871 (3H, d, *J* 6.8 Hz, 27-H_3), 0.94 (3H, d, *J* 6.4 Hz, 21-H_3), 0.99 (3H, s, 19-H_3), 1.05 and 1.38 (each 1H, each m, 22-H_2), 1.08 and 1.20 (each 1H, each m, 24-H_2), 1.18 and 1.37 (each 1H, each m, 23-H_2), 1.26 (1H, ddd, *J* 13.2, 13.2 and 3.9 Hz, 1α-H), 1.38 (1H, m, 4β-H), 1.40 (1H, m, 12α-H), 1.44 and 1.58 (each 1H, each m, 6-H_2), 1.48 (1H, m, 2β-H), 1.50 (1H, m, 5-H), 1.52 (1H, m, 17-H), 1.53 (1H, m, 25-H), 1.61 (1H, m, 20-H), 1.68 (1H, m, 4α-H), 1.85 (1H, ddd, *J* 13.2, 3.9 and 3.0 Hz, 1β-H), 1.88 (1H, m, 2α-H), 2.03 (1H, m, 12β-H), 2.07 and 2.35 (each 1H, each m, 16-H_2), 2.10 and 2.35 (each 1H, each m, 7-H_2), 2.18 (1H, m, 11β-H), 2.22 (1H, m, 11α-H), 3.63 (1H, tt, *J* 10.7 and 4.9 Hz, 3-H), 5.36 (1H, dd, *J* 2.5 and 2.0 Hz, 15-H); δ_C (150 MHz) 15.66 (C-18), 18.34 (C-19), 18.87 (C-21), 21.84 (C-11), 22.53 (C-26), 22.78 (C-27), 23.73 (C-23), 25.28 (C-6), 27.99 (C-7), 27.99 (C-25), 31.69 (C-2), 34.04 (C-20), 35.31 (C-1), 35.90 (C-16), 36.10 (C-22), 36.52 (C-10), 36.93 (C-12), 38.30 (C-4), 39.50 (C-24), 45.02 (C-13), 40.93 (C-5), 57.22 (C-17), 71.00 (C-3), 117.41 (C-15), 123.07 (C-8), 140.84 (C-9), 151.08 (C-14); EI-MS *m/z* 384 (M^+, 100%), 369 ($M^+ - CH_3$, 15), 351 ($M^+ - CH_3 - H_2O$, 30), 271 ($M^+ - C_8H_{17}$, 20); HR-EI-MS *m/z* M^+: Found, 384.3395. Calc. for $C_{27}H_{44}O$, 384.3392.

5β-Cholesta-8,14-dien-3β-ol ***2b***. Colorless needles, mp 50–53 °C (MeOH); δ_H (600 MHz) 0.83 (3H, s, 18-H_3), 0.869 (3H, d, *J* 6.8 Hz, 26-H_3), 0.871 (3H, d, *J* 6.8 Hz, 27-H_3), 0.94 (3H, d, *J* 6.3 Hz, 21-H_3), 1.06 and 1.38 (each 1H, each m, 22-H_2), 1.10 and 1.18 (each 1H, each m, 24-H_2), 1.12 (3H, s, 19-H_3), 1.17 and 1.38 (each 1H, each m, 23-H_2), 1.37 (1H, m, 12α-H), 1.46 and 1.58 (each 1H, each m, 2-H_2), 1.53 (1H, m, 17-H), 1.53 (1H, m, 25-H), 1.56 and 1.70 (each 1H, each m, 6-H_2), 1.58 and 1.67 (each 1H, each m, 1-H_2), 1.63 (1H, m, 20-H), 1.68 (1H, m, 5-H), 1.70 (2H, 4-H_2), 1.98 and 2.33 (each 1H, each m, 7-H_2), 2.03 (1H, m, 12β-H), 2.07 (1H, m, 11α-H), 2.08 (1H, m, 16β-H), 2.24 (1H, m, 11β-H), 2.37 (1H, m, 16α-H), 3.89 (1H, m, $\omega_{1/2}$ *ca.* 16 Hz, 3-H), 5.39 (1H, br s, 15-H); δ_C (150 MHz) 15.33 (C-18), 18.84 (C-21), 22.54 (C-26), 22.77 (C-11), 22.77 (C-27), 23.69 (C-23), 24.87 (C-6), 24.87 (C-7), 25.09 (C-19, br), 27.99 (C-25), 30.96 (C-1), 31.16 (C-2), 33.97 (C-20), 35.85 (C-16), 36.09 (C-22), 36.92 (C-4), 36.92 (C-12), 36.98 (C-10, br), 39.29 (C-5, br), 39.50 (C-24), 45.07 (C-13), 57.20 (C-17), 67.33 (C-3), 117.57 (C-15), 123.54 (C-8), 138.92 (C-9, br), 150.77 (C-14); EI-MS *m/z* 384 (M^+, 100%), 369 ($M^+ - CH_3$, 44), 351 ($M^+ - CH_3 - H_2O$, 35), 271 ($M^+ - C_8H_{17}$, 9); HR-EI-MS *m/z* M^+: Found, 384.3398. Calc. for $C_{27}H_{44}O$, 384.3392.

5α-Cholesta-14,16-dien-3β-ol ***10a***. Colorless wax; δ_H (600 MHz) 0.60 (1H, ddd, *J* 12.2, 11.2 and 3.9 Hz, 9-H), 0.84 (3H, d, *J* 6.8 Hz, 26-H_3), 0.85 (3H, d, *J* 6.8 Hz, 27-H_3), 0.86 (1H, m, 12α-H), 0.92 (3H, s, 19-H_3), 0.97 (3H, s, 18-H_3), 0.97 (1H, ddd, *J* 13.2, 13.2 and 3.4 Hz, 1α-H), 1.05 (3H, d, *J* 6.3 Hz, 21-H_3), 1.14 (1H, m, 5-H), 1.15 (2H, 24-H_2), 1.26 (2H, 23-H_2), 1.33 and 1.59 (each 1H, each m, 4-H_2), 1.38 and 1.50 (each 1H, each m, 22-H_2), 1.39 (1H, m, 11β-H), 1.40 and 1.48 (each 1H, each m, 6-H_2), 1.45 and 1.80 (each 1H, each m, 2-H_2), 1.48 and 1.94 (each 1H, each m, 7-H_2), 1.50 (1H, m, 25-H), 1.60 (1H, m, 11α-H), 1.75 (1H, ddd, *J* 13.2, 3.4 and 3.4 Hz, 1β-H), 1.96 (1H, m, 12β-H), 2.21 (1H, m, 8-H), 2.29 (1H, m, 20-H), 3.59 (1H, tt, *J* 11.2 and 4.9 Hz, 3-H), 5.81 (1H, dd, *J* 2.0 and 2.0 Hz, 15-H), 5.96 (1H, d, *J* 2.0 Hz, 16-H); δ_C (150 MHz) 12.42 (C-19), 18.72 (C-18), 21.32 (C-11), 22.66 (C-26), 22.66 (C-27), 22.99 (C-21), 25.56 (C-23), 27.92 (C-25), 28.47 (C-6), 29.72 (C-7), 31.46 (C-2), 31.46 (C-20), 35.55 (C-8), 35.96 (C-10), 36.16 (C-12), 37.38 (C-1), 38.13 (C-4), 38.25 (C-22), 39.20 (C-24), 44.71 (C-5), 53.57 (C-13), 57.47 (C-9), 71.25 (C-3), 117.62 (C-15), 120.98 (C-16), 158.79 (C-14), 164.10 (C-17); EI-MS *m/z* 384 (M^+, 13%), 366 ($M^+ - H_2O$, 30), 299 ($M^+ - C_6H_{13}$, 45), 281 ($M^+ - C_6H_{13} - H_2O$, 100); HR-EI-MS *m/z* M^+: Found, 384.3400. Calc. for $C_{27}H_{44}O$, 384.3392.

With 36% HCl at 30 °C. A solution of **1** (250 mg, 0.65 mmol) and 36% HCl (0.5 cm^3) in EtOH (10 cm^3) and benzene (2 cm^3) was stirred at 30 °C for 44 h. The same work-up and flash chromatography as described above gave a 53 : 27 : 5 : 15 dienol mixture (235 mg, 94%) of **7a**, **7b**, 5α-cholesta-7,14-dien-3β-ol **8a** and **2a**. Separation by HPLC using hexane–AcOEt (4 : 1) as a mobile phase gave **7b** (63 mg, 25%), **7a** (115 mg, 46%), **2a** (28 mg, 11%) and **8a** (R_t 32.2 min: 12 mg, 4.8%), successively.

5α-Cholesta-7,14-dien-3β-ol ***8a***. Colorless plates, mp 78–80 °C (MeOH) [lit.,[11] mp 103–105 °C (MeOH)]; δ_H (600 MHz) 0.79 (3H, s, 19-H_3), 0.83 (3H, s, 18-H_3), 0.868 (3H, d, *J* 6.8 Hz, 26-H_3), 0.871 (3H, d, *J* 6.8 Hz, 27-H_3), 0.92 (3H, d, *J* 6.0 Hz, 21-H_3), 1.05 and 1.36 (each 1H, each m, 22-H_2), 1.07 (1H, m, 1α-H), 1.14 and 1.36 (each 1H, each m, 23-H_2), 1.15 (2H, 24-H_2), 1.25 (1H, m, 4β-H), 1.31 (1H, m, 12α-H), 1.41 (1H, m, 2β-H), 1.42 (1H, m, 5-H), 1.49 (1H, m, 11β-H), 1.53 (1H, m, 25-H), 1.57 (1H, m, 17-H), 1.58 (1H, m, 20-H), 1.60 (1H, m, 11α-H), 1.72 (1H, m, 4α-H), 1.72 (1H, m, 9-H), 1.78–1.90 (2H, 6-H_2), 1.81 (1H, m, 2α-H), 1.85 (1H, m, 1β-H), 1.90 (1H, m, 16-H), 2.03 (1H, ddd, *J* 12.2, 3.4 and 3.4 Hz, 12β-H), 2.31 (1H, ddd, *J* 15.6, 6.8 and 3.4 Hz, 16-H), 3.60 (1H, tt, *J* 11.2 and 4.4 Hz, 3-H), 5.50 (1H, dd, *J* 3.4 and 2.0 Hz, 15-H), 5.75 (1H, m, 7-H); δ_C (150 MHz) 12.37 (C-19), 16.50 (C-18), 18.92 (C-21), 20.97 (C-11), 22.55 (C-26), 22.81 (C-27), 23.75 (C-23), 28.01 (C-25), 30.28 (C-6), 31.53 (C-2), 33.88 (C-10), 34.12 (C-20), 35.16 (C-16), 36.06 (C-22), 36.80 (C-1), 37.92 (C-4), 39.50 (C-24), 39.68 (C-5), 40.16 (C-12), 46.47 (C-13), 49.76 (C-9), 58.70 (C-17), 70.95 (C-3), 119.45 (C-15), 120.17 (C-7), 134.51 (C-8), 152.07 (C-14); EI-MS *m/z* 384 (M^+, 39%), 366 ($M^+ - H_2O$, 74), 351 ($M^+ - H_2O - CH_3$, 53), 271 ($M^+ - C_8H_{17}$, 46), 253 ($M^+ - C_8H_{17} - H_2O$, 100); HR-EI-MS *m/z* M^+: Found, 384.3406. Calc. for $C_{27}H_{44}O$, 384.3392.

With Dowex resin. Purchased resin Dowex-50W-X2 (H^+ form), was used after being successively washed with dist. H_2O, 2 M NaOH, dist. H_2O and 2 M HCl, then dist. H_2O until the filtrate was neutral, and finally washed with ethanol. A heterogeneous mixture of **1** (250 mg, 0.65 mmol) and Dowex resin (500 mg) in EtOH (10 cm^3) was stirred at 70 °C for 51 h. The resin was filtered off through a glass filter and washed with Et_2O. The filtrate was concentrated *in vacuo*, and the residue was subjected to the purification and separation procedure as described above. Flash chromatography gave a 55 : 22 : 11 : 12 dienol mixture (239 mg, 96%) of **7a**, **7b**, **8a** and **2a**. HPLC gave **7b** (52 mg, 21%), **7a** (119 mg, 48%), **2a** (27 mg, 11%) and **8a** (17 mg, 6.8%), successively.

Campesta-8,14-dienol 5

Campesta-5,7-dienol **3** was prepared from campesterol supplied by Tama Biochemical Co., Ltd. according to a method reported by Kircher and Rosenstein.[8] A solution of **3** (7.2 mg, 0.018 mmol) and 36% HCl (0.025 cm^3) in EtOH (0.5 cm^3) was refluxed for 3 h. After the same work-up as that for treatment of **1** with 36% HCl and flash chromatography using hexane–AcOEt (4 : 1), the product mixture was subjected to HPLC using hexane–AcOEt (4 : 1) as a mobile phase. The elution at R_t 32.0 min gave campesta-8,14-dienol **5** (3.8 mg, 53%): colorless scales, mp 111–113 °C (MeOH); δ_H (300 MHz) 0.78 (3H, d, *J* 6.9 Hz, 26-H_3), 0.81 (3H, d, *J* 7.0 Hz, 27-H_3), 0.82 (3H, s, 18-H_3), 0.86 (3H, d, *J* 6.8 Hz, 28-H_3), 0.93 (3H, d, *J* 6.2 Hz, 21-H_3), 0.99 (3H, s, 19-H_3), 3.63 (1H, tt, *J* 10.9 and 4.7 Hz, 3-H), 5.36 (1H, br s, 15-H); δ_C (75 MHz) 15.38, 15.70, 18.26, 18.37, 18.89 and 20.19 (C-18, -19, -21, -26, -27 and -28), 21.88, 25.32, 26.61, 30.21, 31.74, 33.67, 35.35, 35.92, 36.98 and 38.35 (C-1, -2, -4, -6, -7, -11, -12, -16, -22 and -23), 32.46, 34.17, 38.90, 40.98 and 57.22 (C-5, -17, -20, -24 and -25), 36.57 and 45.08

(C-10 and -13), 71.03 (C-3), 117.42 (C-15), 123.12 (C-8), 140.86 (C-9), 151.14 (C-14); EI-MS *m/z* 398 (M^+, 100%), 380 ($M^+ - H_2O$, 73), 365 ($M^+ - H_2O - CH_3$, 68), 271 ($M^+ - C_9H_{19}$, 17), 253 ($M^+ - C_9H_{19} - H_2O$, 20); HR-EI-MS *m/z* (M^+): Found, 398.3551. Calc. for $C_{28}H_{46}O$, 398.3549.

Stigmasta-8,14-dien-3β-ol 6

Stigmasta-5,7-dien-3β-ol **4** was prepared from stigmast-5-en-3β-ol supplied by Tama Biochemical Co., Ltd. according to a method reported by Kircher.[9] A solution of **4** (13.4 mg, 0.032 mmol) and 36% HCl (0.05 cm^3) in EtOH (1 cm^3) was refluxed for 3 h. The same work-up and purification procedure as described above gave stigmasta-8,14-dien-3β-ol **6** (6.7 mg, 50%): R_t 30.0 min; colorless scales, mp 105–106 °C (MeOH); δ_H (300 MHz) 0.82 (3H, s, 18-H_3), 0.82 and 0.84 (each 3H, each d, each *J* 7.2 Hz, 26- and 27-H_3), 0.95 (3H, d, *J* 6.1 Hz, 21-H_3), 0.99 (3H, s, 19-H_3), 0.85 (3H, t, *J* 6.8 Hz, 29-H_3), 3.63 (1H, tt, *J* 10.8 and 4.8 Hz, 3-H), 5.36 (1H, br s, 15-H); δ_C (75 MHz) 11.98, 15.70, 18.37, 18.96, 19.03 and 19.78 (C-18, -19, -21, -26, -27 and -29), 21.87, 23.04, 25.31, 26.00, 26.61, 31.73, 33.90, 35.35, 35.94, 36.97 and 38.35 (C-1, -2, -4, -6, -7, -11, -12, -16, -22, -23 and -28), 29.15, 34.40, 40.98, 45.89 and 57.15 (C-5, -17, -20, -24 and -25), 36.56 and 45.07 (C-10 and -13), 71.03 (C-3), 117.42 (C-15), 123.11 (C-8), 140.87 (C-9), 151.14 (C-14); EI-MS *m/z* 412 (M^+, 100%), 394 ($M^+ - H_2O$, 72), 379 ($M^+ - H_2O - CH_3$, 64), 271 ($M^+ - C_{10}H_{21}$, 18), 253 ($M^+ - C_{10}H_{21} - H_2O$, 19); HR-EI-MS *m/z* ($M^+$): Found, 412.3714. Calc. for $C_{29}H_{48}O$, 412.3705.

Treatment of dienols, 7a,b and 2a,b, with 36% HCl at reflux temperature

Treatment of 7a. A solution of **7a** (15 mg, 0.039 mmol) and 36% HCl (0.05 cm^3) in EtOH (1.0 cm^3) was refluxed for 3 h. After the same work-up as that for treatment of **1** with 36% HCl, flash chromatography using hexane–AcOEt (4 : 1) as an eluent gave a 7 : 90 : 3 mixture of **7a**, **2a** and **10a** (14 mg, 93%).

Treatment of 7b. A solution of **7b** (55 mg, 0.14 mmol) and 36% HCl (0.11 cm^3) in EtOH (2.2 cm^3) was refluxed. After 3 h, the ^{1}H NMR spectrum showed that *ca.* 45% of the starting material **7b** still remained; thus, the reaction was further continued for 8 h. After the same work-up as that for treatment of **1** with 36% HCl at refluxing temperature, flash chromatography using hexane–AcOEt (4 : 1) as an eluent gave an enone mixture consisting of **11**–**13** (21 mg, 38%) and a 6 : 94 mixture of **7b** and **2b** (32 mg, 58%). The enone mixture was subjected to HPLC using hexane–AcOEt (100 : 1) as a mobile phase, affording a 88 : 12 mixture (R_t 13.4 min, 7.8 mg, 14%) of 5β-cholest-14-en-3-one **12** and 14-*epi*-5β-cholest-8-en-3-one **13**, and 5β-cholest-8(14)-en-3-one **11** (R_t 14.0 min, 12 mg, 22%). This mixture was separated by HPLC using hexane–^{i}PrOH (100 : 1) to give **12** (R_t 8.6 min, 5.0 mg), **13** (R_t 9.2 min, 1.1 mg) and their mixture (4.2 mg).

5β-Cholest-8(14)-en-3-one ***11***. Colorless foam; δ_H (600 MHz) 0.869 (3H, d, *J* 6.8 Hz, 26-H_3), 0.872 (3H, d, *J* 6.8 Hz, 27-H_3), 0.87 (3H, s, 18-H_3), 0.90 (3H, s, 19-H_3), 0.95 (3H, d, *J* 6.4 Hz, 21-H_3), 1.08 and 1.39 (each 1H, each m, 22-H_2), 1.14 (2H, 24-H_2), 1.15 and 1.36 (each 1H, each m, 23-H_2), 1.16 (1H, m, 17-H), 1.21 (1H, m, 12α-H), 1.28 and 1.84 (each 1H, each m, 6-H_2), 1.40 and 1.85 (each 1H, each m, 16-H_2), 1.47 (1H, m, 20-H), 1.49 (1H, ddd, *J* 14.2, 14.2 and 4.9 Hz, 1β-H), 1.53 (1H, m, 25-H), 1.58 (2H, 11-H_2), 1.83 (1H, m, 5-H), 1.93 (1H, m, 7α-H), 2.01 (1H, m, 12β-H), 2.02 (1H, m, 1α-H), 2.08 (1H, ddd, *J* 14.7, 3.9 and 2.4 Hz, 4β-H), 2.21 and 2.27 (each 1H, each m, 15-H_2), 2.24 (1H, m, 2β-H), 2.26 (1H, m, 7β-H), 2.42 (1H, ddd, *J* 14.7, 14.2 and 5.4 Hz, 2α-H), 2.52 (1H, m, 9-H), 2.82 (1H, dd, *J* 14.7 and 14.2 Hz, 4α-H); δ_C (150 MHz) 18.14 (C-18), 19.11 (C-21), 19.84 (C-11), 22.57 (C-26), 22.80 (C-27), 22.99 (C-19), 23.75 (C-23), 23.91 (C-7), 25.95 (C-15), 26.77 (C-6), 27.10 (C-16), 28.02 (C-25), 34.44 (C-20), 35.87 (C-9), 35.96 (C-22), 36.21 (C-1), 36.42 (C-10), 37.15 (C-12), 37.72 (C-2), 39.55 (C-24), 42.13 (C-4), 42.80 (C-13), 44.51 (C-5), 56.99 (C-17), 125.40 (C-8), 143.35 (C-14), 213.18 (C-3); EI-MS *m/z* 384 (M^+, 100%), 369 ($M^+ - CH_3$, 26), 351 (65), 271 ($M^+ - C_8H_{17}$, 30); HR-EI-MS *m/z* (M^+): Found, 384.3391. Calc. for $C_{27}H_{44}O$, 384.3392.

5β-Cholest-14-en-3-one ***12***. Colorless amorphous powder; δ_H (600 MHz) 0.870 (3H, d, *J* 6.8 Hz, 26-H_3), 0.872 (3H, d, *J* 6.8 Hz, 27-H_3), 0.92 (3H, d, *J* 6.0 Hz, 21-H_3), 0.93 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.05 and 1.38 (each 1H, each m, 22-H_2), 1.08–1.22 (2H, 24-H_2), 1.17 and 1.37 (each 1H, each m, 23-H_2), 1.32 (1H, ddd, *J* 13.2, 13.2 and 3.4 Hz, 12α-H), 1.33 and 1.94 (each 1H, each m, 6-H_2), 1.42 (1H, m, 1β-H), 1.42 and 1.50 (each 1H, each m, 11-H_2), 1.47 (1H, m, 7α-H), 1.53 (1H, m, 9-H), 1.53 (1H, m, 25-H), 1.55 (1H, m, 17-H), 1.60 (1H, m, 20-H), 1.73 (1H, m, 7β-H), 1.82 (1H, m, 5-H), 1.93 and 2.31 (each 1H, each m, 16-H_2), 2.02 (1H, ddd, *J* 14.7, 4.9 and 2.4 Hz, 4β-H), 2.06 (1H, ddd, *J* 13.2, 2.4 and 2.4 Hz, 12β-H), 2.08 (1H, m, 1α-H), 2.12 (1H, m, 8-H), 2.16 (1H, m, 2β-H), 2.34 (1H, ddd, *J* 14.7, 14.7 and 5.4 Hz, 2α-H), 2.68 (1H, dd, *J* 14.7 and 14.2 Hz, 4α-H), 5.20 (1H, m, 15-H); δ_C (150 MHz) 16.88 (C-18), 18.95 (C-21), 22.04 (C-11), 22.40 (C-19), 22.57 (C-26), 22.80 (C-27), 23.57 (C-7), 23.68 (C-23), 26.30 (C-6), 28.04 (C-25), 33.89 (C-20), 34.83 (C-8), 35.08 (C-10), 35.65 (C-16), 36.06 (C-22), 36.85 (C-1), 37.28 (C-2), 39.53 (C-24), 40.35 (C-9), 42.26 (C-4), 42.43 (C-12), 44.27 (C-5), 47.21 (C-13), 58.82 (C-17), 117.61 (C-15), 154.90 (C-14), 213.19 (C-3); EI-MS *m/z* 384 (M^+, 9%), 271 ($M^+ - C_8H_{17}$, 100), 253 (13); HR-EI-MS *m/z* (M^+): Found, 384.3386. Calc. for $C_{27}H_{44}O$, 384.3392.

14-epi-5β-Cholest-8-en-3-one ***13***. Colorless amorphous powder; δ_H (600 MHz) 0.864 (3H, d, *J* 6.4 Hz, 26-H_3), 0.867 (3H, d, *J* 6.8 Hz, 27-H_3), 0.90 (3H, s, 18-H_3), 0.93 (3H, d, *J* 6.4 Hz, 21-H_3), 1.01 and 1.37 (each 1H, each m, 22-H_2), 1.07 and 1.98 (each 1H, each m, 15-H_2), 1.14 (3H, s, 19-H_3), 1.14 (2H, 24-H_2), 1.15 and 1.37 (each 1H, each m, 23-H_2), 1.27 and 1.75 (each 1H, each m, 16-H_2), 1.37 (1H, m, 12β-H), 1.37 (1H, m, 17-H), 1.40 and 1.97 (each 1H, each m, 6-H_2), 1.49 (1H, m, 20-H), 1.52 (1H, m, 25-H), 1.58 (1H, m, 1β-H), 1.62 (1H, m, 12α-H), 1.73 and 2.15 (each 1H, each m, 7-H_2), 1.79 (1H, dd, *J* 8.3 and 8.3 Hz, 14-H), 1.85 and 2.00 (each 1H, each m, 11-H_2), 1.92 (1H, m, 5-H), 2.09 (1H, m, 1α-H), 2.11 (1H, m, 2α-H), 2.22 (1H, m, 2β-H), 2.25 (1H, ddd, *J* 14.2, 5.4 and 2.0 Hz, 4β-H), 2.33 (1H, ddd, *J* 14.2, 9.8 and 1.0 Hz, 4α-H); δ_C (150 MHz) 19.84 (C-21), 21.17 (C-11), 22.57 (C-26), 22.80 (C-27), 23.11 (C-18), 24.16 (C-6), 24.42 (C-23), 26.10 (C-7), 26.68 (C-19), 28.01 (C-25), 28.50 (C-16), 30.67 (C-15), 33.55 (C-20), 35.64 (C-22), 35.68 (C-1), 35.68 (C-12), 36.69 (C-10), 38.48 (C-2), 39.50 (C-24), 41.21 (C-13), 42.72 (C-5), 42.89 (C-4), 52.22 (C-17), 54.11 (C-14), 130.15 (C-9), 132.63 (C-8), 213.44 (C-3); EI-MS *m/z* 384 (M^+, 100%), 369 ($M^+ - CH_3$, 44), 351 (48), 314 (95), 271 ($M^+ - C_8H_{17}$, 46); HR-EI-MS *m/z* (M^+): Found, 384.3396. Calc. for $C_{27}H_{44}O$, 384.3392.

Treatment of 2a. A solution of **2a** (15 mg, 0.039 mmol) and 36% HCl (0.05 cm^3) in EtOH (1.0 cm^3) was refluxed for 3 h. After the same work-up as that for treatment of **1** with 36% HCl, flash chromatography using hexane–AcOEt (4 : 1) as eluent gave a 6 : 92 : 2 mixture of **7a**, **2a** and **10a** (14 mg, 93%).

Treatment of 2b. A solution of **2b** (35 mg, 0.091 mmol) and 36% HCl (0.07 cm^3) in EtOH (1.4 cm^3) was refluxed for 8 h. The same work-up and column chromatography as those for treatment **7b** gave an enone mixture consisting of **11**–**13** (11 mg, 31%) and a 2 : 98 mixture of **7b** and **2b** (19 mg, 54%). The HPLC of the enone mixture using hexane–AcOEt (100 : 1) as eluent gave a 86 : 14 mixture of **12** and **13** (4.7 mg, 13%) and **11** (5.6 mg, 16%).

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