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# Benzoyl Group Participation in Epoxide Ring Opening of 26-Benzoxy-24,25-epoxycholesterol Derivatives; Reinvestigation of the Stereochemistry

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The benzoyl group participation in the epoxide ring opening reaction of 26-benzoxy-24,25-epoxycholesterol derivatives on treatment with perchloric acid in tetrahydrofuran was clearly demonstrated by the transformation of (carbonyl-$^{18}$O)-3$\beta$,26-dibenzoxy-24,25-epoxycholest-5-ene into (25-$^{18}$O)-24,25,26-trihydroxycholesterol derivatives.

**Keywords**——epoxide ring opening reaction; benzoyl group participation; 26-benzoxy-24,25-epoxycholesterol; carbonyl-$^{18}$O-label; mass spectrometry of $^{18}$O-sterol

Neighboring group participation by acyl groups has been widely reported in various chemical reactions.[2] Although many examples are known in steroid ring systems,[3] few reports have dealt with acyclic steroid side chains.[4] In the course of our synthetic work on 25,26-dihydroxyvitamin $D_3$,[5] we have undertaken acid catalyzed reactions of the 24,25-epoxides (**1a** and **2a**), which on reduction with lithium aluminum hydride gave the 25,26-diols (**3** and **4**), synthetic precursors of 25,26-dihydroxyvitamin $D_3$. The stereochemistry of the 24,25-epoxides (**1a** and **2a**) and hence the 25,26-diols (**3** and **4**) was deduced from the reaction pathway of the acid catalyzed ring-opening reaction of the epoxides (**1a** and **2a**), leading to the 24-benzoates (**5a** and **7a**) and the 26-benzoates (**6a** and **8a**). The results obtained from those previous experiments[5] favored path B (Chart 1), involving the initial attack of the benzoyl group at C-24 with inversion of configuration of this carbon, followed by equilibration of the acyloxonium intermediates which eventually collapsed into the 24-benzoates and the 26-benzoates.

However, the stereochemical assignments of the epoxides (**1a** and **2a**) made in the above study have been found to be incompatible with our recent results on Sharpless' asymmetric epoxidation of the (24*E*)-3$\beta$,26-dihydroxycholesta-5,24-diene derivative.[6] Therefore, we have reexamined the mechanism of the epoxide opening reaction with the use of the [carbonyl-$^{18}$O]epoxy-benzoates (**1b** and **2b**) or in medium containing [$^{18}$O]$H_2O$. The results indicated that the reaction proceeds not *via* path B but *via* path A.

Treatment of the more polar (**1a**) and the less polar (**2a**) epoxybenzoates[5] with a catalytic amount of perchloric acid in $H_2{}^{18}O$ and tetrahydrofuran (THF) afforded a mixture of the 24-benzoates and the 26-benzoates (**5b** and **6b**; **7b** and **8b**, respectively). Mass spectral analysis of four specimens of the tetrols (**9a** and **10a**), which were derived from **5b**, **6b**, **7b** and **8b** by saponification (NaOEt in EtOH) indicated that no $^{18}$O was incorporated in the tetrols. Also, the formation of [carbonyl-$^{18}$O]ethyl benzoate and ethyl benzoate (*ca.* 1 : 1) was observed. The results clearly preclude the possibility of the initial attack of a water molecule at either C-25 or C-24 of the epoxides (**1a** and **2a**) followed by benzoyl group migration.

The following experiments were undertaken in order to clarify which pathway (A or B) was operative. The more polar (**1b**) and less polar (**2b**) [carbonyl-$^{18}$O]epoxy-benzoates were prepared with [$^{18}$O]benzoyl chloride[7] (92% $^{18}$O enriched) as described in the previous paper.[5]

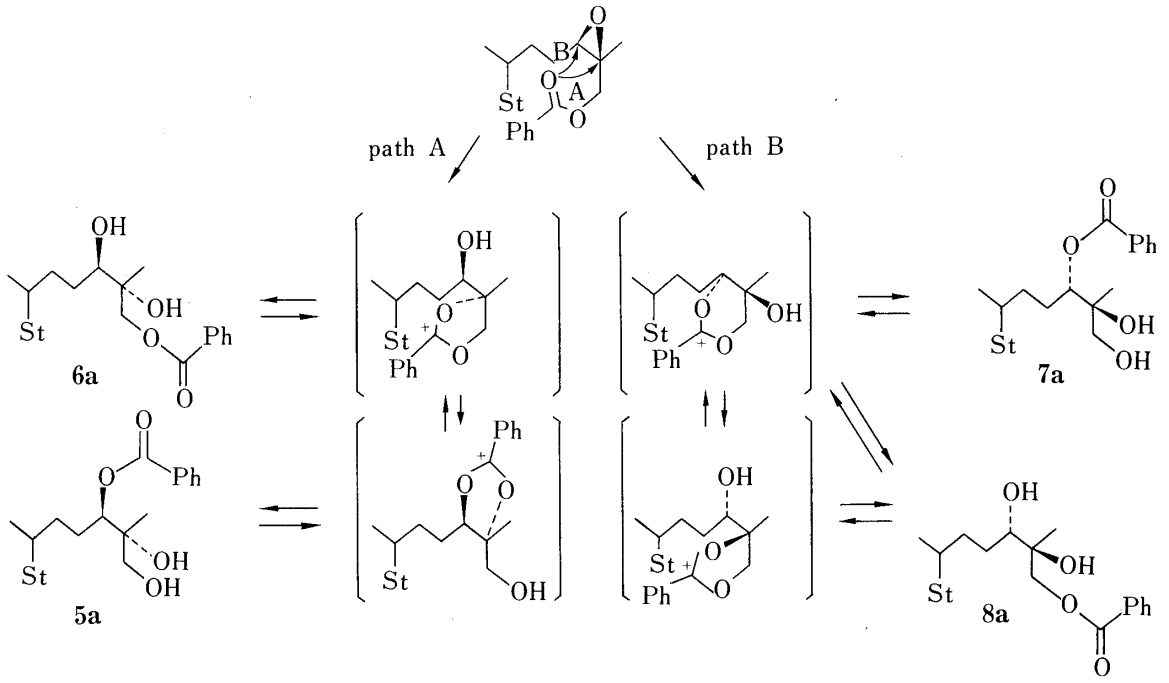
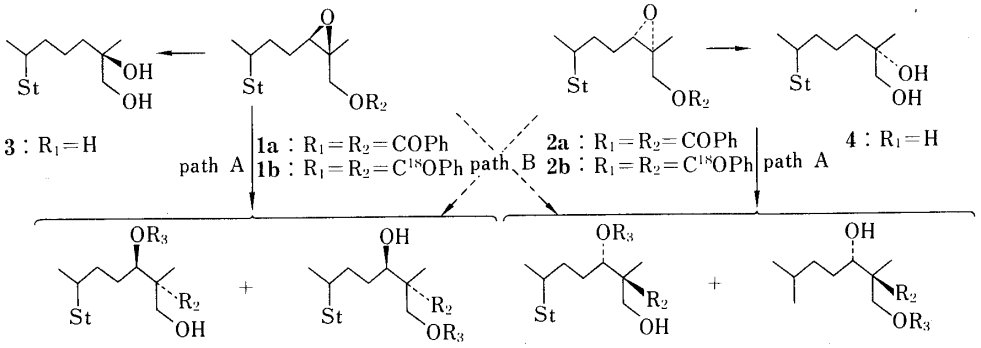
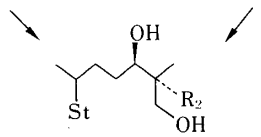


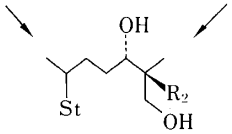

Chart 1



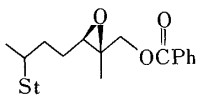

**5a** : $R_1=R_3=COPh$, $R_2=OH$
**5b** : $R_1=COPh$, $R_2=OH$, $R_3=C^{18}OPh$
**5c** : $R_1=C^{18}OPh$, $R_2={}^{18}OH$, $R_3=COPh$
**6a** : $R_1=R_3=COPh$, $R_2=OH$
**6b** : $R_1=COPh$, $R_2=OH$, $R_3=C^{18}OPh$
**6c** : $R_1=C^{18}OPh$, $R_2={}^{18}OH$, $R_3=COPh$
**7a** : $R_1=R_3=COPh$, $R_2=OH$
**7b** : $R_1=COPh$, $R_2=OH$, $R_3=C^{18}OPh$
**7c** : $R_1=C^{18}OPh$, $R_2={}^{18}OH$, $R_3=COPh$
**8a** : $R_1=R_3=COPh$, $R_2=OH$
**8b** : $R_1=COPh$, $R_2=OH$, $R_3=C^{18}OPh$
**8c** : $R_1=C^{18}OPh$, $R_2={}^{18}OH$, $R_3=COPh$



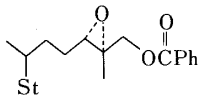
**9a** : $R_1=H$, $R_2=OH$
**9b** : $R_1=H$, $R_2={}^{18}OH$



**10a** : $R_1=H$, $R_2=OH$
**10b** : $R_1=H$, $R_2={}^{18}OH$



**11** : $R_1=THP$



**12** : $R_1=THP$

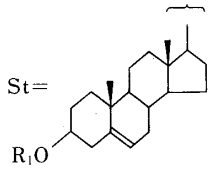



Chart 2

Treatment of **1b** with aqueous perchloric acid in THF afforded a mixture of 24-benzoate (**5c**) and 26-benzoate (**6c**). Saponification of **5c** and **6c** afforded the $^{18}O$-labelled 24,25,26-trihydroxycholesterol (**9b**). Its mass spectrum, together with that of the non-labelled material (**9a**) is shown in Fig. 1.

It can be seen that the mass fragments at *m/z* 248, 340, 325 and 77 indicate the incorporation of $^{18}O$-label into C-25 or C-26 and furthermore, the peaks at *m/z* 405 and 60 confirm the introduction of the label into C-25. Thus, the $^{18}O$-atom was located exclusively at C-25 of the tetrol **9b**, suggesting that **5c** and **6c** also have $^{18}O$ at the same position. The results were confirmed by mass spectral analysis of the 25-ketone (**13**) obtained from the 24-benzoate (**5c**) and of the 24-aldehyde (**14**) obtained from the 26-benzoate (**6c**) by oxidation with sodium periodate, because the fragments of **14**, at *m/z* 340 and 325, showed that the $^{18}O$-label was not

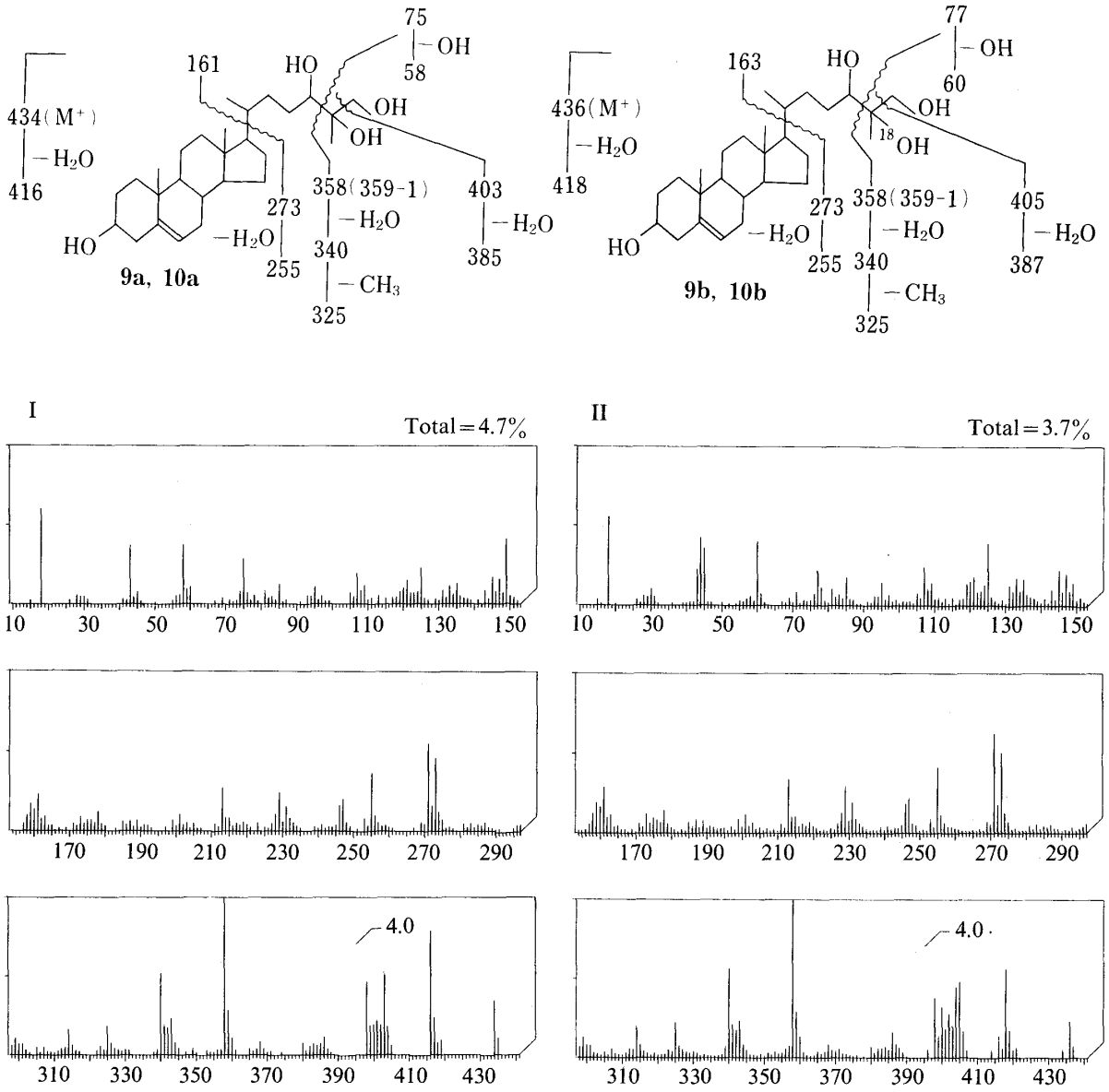
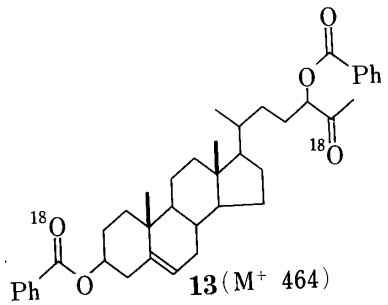



Fig. 1. I: Mass Spectrum of 24,25,26-Trihydroxycholesterol (**9a**) Derived from **5b**

Mass spectra of **9a** and **10a** obtained from **6b**, **7b** and **8b** were indistinguishable from this spectrum.

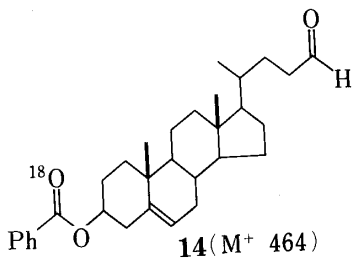
II: Mass Spectrum of [25-$^{18}O$]-24,25,26-Trihydroxycholesterol (**9b**) Derived from **5c**

Mass spectra of **9b** and **10b** derived from **6c**, **7c** and **8c** coincided with this spectrum.



**13** ($M^+$ 464)

fragments observed
490: $M^+ - PhC^{18}OOH$
475: $490 - CH_3$
385: $490 - PhCO$
368: $490 - PhCOOH$



**14** ($M^+$ 464)

fragments observed
340: $M^+ - PhC^{18}OOH$
325: $340 - CH_3$

Fig. 2. Main Mass Fragments of the 25-Ketone (**13**) and the 24-Aldehyde (**14**)

incorporated at C-24 at all, and the fragment of **13**, at *m/z* 368, showed that the label was present at C-25.

Thus, it is concluded that the carbonyl-$^{18}O$ atom of the 26-benzoxy group was selectively transferred to the C-25 position of **5c** and **6c**. Similarly, mass spectra (MS) of the tetrols (**10b**) obtained by hydrolysis of the 24-benzoate (**7c**) and 26-benzoate (**8c**), which were in turn prepared by treatment of the [carbonyl-$^{18}O$]epoxy-benzoate (**2b**) with aqueous perchloric acid, revealed that the carbonyl-$^{18}O$ label of the 26-benzoxy group was selectively introduced into the C-25 position of **7c** and **8c**.

Now it is clear that the initial attack by the carbonyl oxygen took place at C-25 and not C-24 in the epoxide ring opening reaction of **1a** and **2a**, and the oxygen introduced at C-25 remained there throughout the reaction. Taking into account the generally accepted stereochemical course of epoxide opening reaction,[2,3] it can reasonably be assumed that the reaction proceeded with inversion at C-25 and with retention at C-24. In conclusion, in this acyclic system, the reaction proceeded according to path A, involving a 5-membered ring mechanism as shown in Chart 1. Therefore, the following revision is required to the previous paper: the stereochemistry of the more polar epoxy-benzoate (**1a**) is 24*R*,25*S* and the 25,26-dihydroxycholesterol (and the corresponding vitamin $D_3$) obtained from the more polar epoxy-benzoate is 25*R*. The correct stereochemical relationships are illustrated in Chart 2.

We have also observed that the epoxide opening reaction of the other two epoxide isomers, (24*R*,25*R*)- and (24*S*,25*S*)-epoxybenzoates[6] (**11** and **12**) under the same conditions also proceeded *via* a 5-membered ring mechanism.[8] Hence, the 5-membered ring mechanism (path A) rather than the 6-membered one (path B) may be applicable to an expoxide opening reaction with acyl participation at least in an acyclic α-acyloxy-epoxide system. An analogous 5-membered ring mechanism was reported in the epoxide opening reaction of acyclic systems with phenylurethane participation.[9]

## Experimental

The proton nuclear magnetic resonance ($^1H$-NMR) spectra were recorded with a Hitachi R-24A or a JEOL PS-100 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. MS were run with a Shimadzu-LKB 9000S spectrometer (direct introduction, EI, 70 eV or 20 eV). Column chromatography was performed with silica gel (Wako gel C-200). Thin-layer chromatography (TLC) was carried out on pre-coated plates of silica gel (E. Merck). The usual work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying over magnesium sulfate, filtration, and evaporation *in vacuo*.

**Treatment of (24*R*, 25*S*)-3β,26-Dibenzoxy-24,25-epoxycholest-5-ene (1a) and (24*S*, 25*R*)-3β,26-Dibenzoxy-24,25-epoxycholest-5-ene (2a)**——A solution of the more polar epoxy-benzoate (**1a**) (20 mg) in THF (0.4 ml), 70% perchloric acid (3 μl) and $H_2{}^{18}O$ (0.2 ml) was stirred at room temperature for 48 h. The usual work-up (ethyl acetate extraction) and purification by preparative TLC [solvent, benzene–ethyl acetate (3 : 1)] gave (24*R*, 25*R*)-3β-benzoxy-24-([carbonyl-$^{18}$O]-benzoxy)-25,26-dihydroxycholest-5-ene (**5b**) (6 mg) and (24*R*, 25*R*)-3β-benzoxy-26-([carbonyl-$^{18}$O]-benzoxy)-24,25-dihydroxycholest-5-ene (**6b**) (10 mg). Each compound (2 mg) was refluxed in ethanol (1 ml) and tetrahydrofuran (1 ml) with sodium ethoxide (2 mg). The usual work-up (ethyl acetate extraction) afforded a mixture of 3β,24,25,26-tetrahydroxycholest-5-ene (**9a**) (1 mg): MS *m/z*: 434, 416, 403, 385, 358, 340, 325, 273, 255, 161, 75, 58; and ethyl benzoate containing [carbonyl-$^{18}$O]ethyl benzoate, which was extracted with dichloromethane and analyzed by GC-MS [column: 10% PEG-20M (1.5 m); temperature 145 °C; flow 30 ml/min; retention time 5.4 min]; MS *m/z* (relative intensity): 152 (14), 150 (16), 124 (22), 122 (25), 107 (72), 105 (100), 77 (89). Similar results were obtained by the same treatment of the less polar (24*S*, 25*R*)-3β,26-dibenzoxy-24,25-epoxycholest-5-ene (**2a**) with perchloric acid in $H_2{}^{18}O$.

**(24*R*, 25*S*)-[Carbonyl-$^{18}$O]-3β,26-dibenzoxy-24,25-epoxycholest-5-ene-(1b) and (24*S*, 25*R*)-[Carbonyl-$^{18}$O]-3β,26-dibenzoxy-24,25-epoxycholest-5-ene (2b)**——(24*E*)-3β,26-Dihydroxycholesta-5,24-diene[9] (44 mg) in pyridine (1 ml) was reacted with [$^{18}$O]benzoyl chloride (39 mg) at room temperature for 12 h. The usual work-up (ethyl acetate extraction) gave (24*E*)-[carbonyl-$^{18}$O]-3β,26-dibenzoxycholesta-5,24-diene (62 mg): NMR ($CDCl_3$): δ 0.70 (s, 3H, 13-Me), 0.96 (d, 3H, *J* = 6 Hz, 20-Me), 1.07 (s, 3H, 10-Me), 1.77 (s, 3H, 25-Me), 4.73 (br s, 2H, 26-H), 4.85 (m, 1H, 3α-H), 5.48 (m, 1H, 6-H), 5.61 (m, 1H, 24-H), 7.2—8.2 (m, 10H, phenyl). MS *m/z*: 488 ($M^+ - PhC^{18}OOH$), 364 ($M^+ - 2PhC^{18}OOH$).

The 3β,26-dibenzoate (60 mg) was stirred with osmium tetroxide (4 mg) and *N*-methylmorpholineoxide (100 mg) in tetrahydrofuran (5 ml), *tert*-butanol (8 ml) and water (0.8 ml) at room temperature for 12 h. The usual work-up (ethyl acetate extraction) afforded [carbonyl-$^{18}$O]-3β,26-dibenzoxy-24,25-dihydroxycholest-5-ene (61 mg): NMR ($CDCl_3$): δ 0.68 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me), 1.06 (s, 3H, 10-Me), 1.36 (s, 3H, 25-Me), 3.60 (m, 1H, 24-H), 4.22 (d, 1H, *J* = 12 Hz, 26-H), 4.48 (d, 1H, *J* = 12 Hz, 26-H), 4.85 (m, 1H, 3α-H), 5.47 (m, 1H, 6-H), 7.2—8.2 (m, 10H, phenyl).

The resulting 24,25-diol (60 mg) in pyridine (1.5 ml) was reacted with *p*-toluenesulfonyl chloride (60 mg) at room temperature for 12 h. The usual work-up yielded a crude 24-tosylate (62 mg): NMR ($CDCl_3$): δ 0.62 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me), 1.06 (s, 3H, 10-Me), 1.27 (s, 3H, 25-Me), 2.41 (s, 3H, $-SO_2-C_6H_4-\underline{CH_3}$), 4.30 (br s, 2H, 26-H), 4.85 (m, 2H, 3α- and 24-Hs), 5.47 (m, 1H, 6-H), 7.2—8.2 (m, 14H, aromatic).

The crude 24-tosylate (61 mg) was stirred with potassium carbonate (40 mg) in THF (1 ml), methanol (0.4 ml) and water (0.1 ml) at room temperature for 10 min. The usual work-up (ethyl acetate extraction) and preparative TLC (solvent, benzene) gave the epimeric pair of 24,25-epoxide (**1b** and **2b**). The more polar compound was (24*R*, 25*S*)-[carbonyl-$^{18}$O]-3β,26-dibenzoxy-24,25-epoxycholest-5-ene (**1b**) (15 mg): NMR ($CDCl_3$): δ 0.68 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me), 1.06 (s, 3H, 10-Me), 1.46 (s, 3H, 25-Me), 2.88 (m, 1H, 24-H), 4.31 (d, 1H, *J* = 12 Hz, 26-H), 4.48 (d, 1H, *J* = 12 Hz, 26-H), 4.85 (m, 1H, 3α-H), 5.47 (m, 1H, 6-H), 7.2—8.2 (m, 10H, phenyl); MS *m/z*: 504, 380, 365, 253, 124; high resolution MS *m/z*: 504.3479 ($M^+ - PhC^{18}OOH$). Calcd for $C_{34}H_{46}O_2{}^{18}O$, *m/z*: 504.3490. The less polar epoxide was (24*S*, 25*R*)-[carbonyl-$^{18}$O]-3β,26-dibenzoxy-24,25-epoxycholest-5-ene (**2b**) (16 mg): δ 0.68 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me), 1.06 (s, 3H, 10-Me), 1.46 (s, 3H, 25-Me), 2.88 (m, 1H, 24-H), 4.36 (br s, 2H, 26-H), 4.85 (m, 1H, 3α-H), 5.47 (m, 1H, 6-H), 7.2—8.2 (m, 10H, phenyl); MS *m/z*: 504, 380, 365, 253, 124; high resolution MS *m/z*: 504.3476 ($M^+ - PhC^{18}OOH$). Calcd for $C_{34}H_{46}O_2{}^{18}O$, *m/z*: 504.3490.

**(24*R*, 25*R*)-3β-([Carbonyl-$^{18}$O]benzoxy)-24-benzoxy-[25-$^{18}$O]-25,26-dihydroxycholest-5-ene (5c), (24*R*, 25*R*)-3β-([Carbonyl-$^{18}$O])benzoxy)-26-benzoxy-[25-$^{18}$O]-24,25-dihydroxycholest-5-ene (6c), (24*S*, 25*S*)-3β-([Carbonyl-$^{18}$O])-benzoxy)-24-benzoxy-[25-$^{18}$O]-25,26-dihydroxycholest-5-ene (7c) and (24*S*, 25*S*)-3β-([Carbonyl-$^{18}$O]benzoxy)-26-benzoxy-[25-$^{18}$O]-24,25-dihydroxycholest-5-ene (8c)**——Treatment of (24*R*, 25*S*)-[carbonyl-$^{18}$O]-epoxy benzoate (**1b**) (14 mg) with perchloric acid in THF–$H_2O$ in the manner described above afforded the more polar (**5c**) (5 mg) and the less polar diol (**6c**) (7 mg). Compounds **7c** (4 mg) and **8c** (7 mg) were obtained from (24*S*, 25*R*)-[carbonyl-$^{18}$O]epoxy-benzoate (**2b**) (13 mg) by the same procedure.

**9b**: NMR ($CDCl_3$): δ 0.68 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me). 1.05 (s, 3H, 10-Me), 1.24 (s, 3H, 25-Me), 3.52 (br s, 2H, 26-H), 4.85 (m, 1H, 3α-H), 5.24 (m, 1H, 24-H), 5.45 (m, 1H, 6-H), 7.2—8.2 (m, 10H, phenyl); MS *m/z*: 522, 504, 502, 400, 398, 382, 380, 370, 368, 340; high resolution MS *m/z*: 522.3586 ($M^+ - PhC^{18}OOH$). Calcd for $C_{34}H_{48}O_3{}^{18}O$, *m/z*: 522.3595.

**10b**: NMR ($CDCl_3$): δ 0.68 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me), 1.06 (s, 3H, 10-Me), 1.25 (s, 3H, 25-Me), 3.58 (m, 1H, 24-H), 4.24 (d, 1H, *J* = 12 Hz, 26-H), 4.44 (d, 1H, *J* = 12 Hz, 26-H), 4.85 (m, 1H, 3α-H), 5.45 (m, 1H, 6-H), 7.2—8.2 (m, 10H, phenyl); MS *m/z*: 522, 504, 502, 398, 380, 368, 340.

**11b**: NMR ($CDCl_3$): δ 0.63 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me), 1.05 (s, 3H, 10-Me), 1.24 (s, 3H, 25-Me), 3.52 (br s, 2H, 26-H), 4.85 (m, 1H, 3α-H), 5.24 (m, 1H, 24-H), 5.45 (m, 1H, 6-H), 7.2—8.2 (m, 10H, phenyl); MS *m/z*: 522, 504, 502, 400, 398, 382, 380, 370, 368, 340.

**12b**: NMR ($CDCl_3$): δ 0.68 (s, 3H, 13-Me), 0.94 (d, 3H, *J* = 6 Hz, 20-Me), 1.06 (s, 3H, 10-Me), 1.25 (s, 3H, 25-Me), 3.58 (m, 1H, 24-H), 4.24 (d, 1H, *J* = 12 Hz, 26-H), 4.44 (d, 1H, *J* = 12 Hz, 26-H), 4,85 (m, 1H, 3α-H), 5.45 (m, 1H,

6-H), 7.2—8.2 (m, 10H, phenyl); MS *m/z*: 522, 504, 502, 398, 380, 368, 340.

Each benzoate (2 mg) was hydrolyzed in ethanol (1 ml) and THF (1 ml) with sodium ethoxide (2 mg) under reflux for 1 h. The usual work-up (ethyl acetate extraction) gave [25-$^{18}$O]-3$\beta$,24,25,26-tetrahydroxycholest-5-ene (**9b** or **10b**) (1 mg): MS *m/z*: 436, 418, 405, 387, 358, 340, 325, 273, 255, 163, 77, 60; high resolution MS *m/z*: 436.3428 ($M^+$). Calcd for $C_{27}H_{46}O_3{}^{18}O$, *m/z*: 436.3439.

The 24-benzoate (**5c** or **7c**) (2 mg) was stirred in methanol (0.1 ml), THF (0.2 ml) and water (0.1 ml) with sodium periodate (10 mg) at room temperature for 4 h. The usual work-up (ethyl acetate extraction) provided compound **13** (1 mg): MS *m/z*: 490, 473, 385, 368; high resolution MS *m/z*: 490.3324 ($M^+ - PhC^{18}OOH$) Calcd for $C_{33}H_{44}O_2{}^{18}O$, *m/z*: 490.3333.

Similar treatment of the 26-benzoate (**6c** or **8c**) (2 mg) with sodium periodate afforded the aldehyde **14** (1 mg); MS *m/z*: 340, 325; high resolution MS *m/z*: 340.2759 ($M^+ - PhC^{18}OOH$). Calcd for $C_{24}H_{36}O$, *m/z*: 340.2677.

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