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Asymmetric syntheses and lanthanide-induced CD studies of (24R and 24S) 5β-cholestane-3α,7α,12α,24,25-pentols

B. Dayal[1]*, Keshava Rao[1], G. Salen[1]

W. M. Seong[1], B. N. Pramanik[2],E. C. Huang[2], and V. Toome[3]

[1]Dept. of Medicine, UMDNJ-New Jersey Medical School, Newark, N.J. 07103*,
[2]Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033,
and [3]Roche Research Center, Hoffmann-La Roche Inc. Nutley, NJ 07110

ABSTRACT: 5β-cholest-24-ene-3α,7α,12α-triol was efficiently converted to the corresponding (24R and 24S) isomers of 5β-cholestane-3α,7α,12α,24,25-pentols with the Os-catalyzed Sharpless asymmetric dihydroxylation process. Utilizing a new phthalazine class of cinchona alkaloid based ligands, namely $(DHQD)_2PHAL$ and $(DHQ)_2PHAL$ and the use of methylsulfonamide for the acceleration of the reaction rate, the enantiomeric excess achieved ranged from 95-98%. The absolute configurations and enantiomeric excess of the (24R,24S)-5β-cholestane-3α,7α,12α,24,25-pentols achieved were confirmed by the lanthanide-induced CD Cotton effect Measurements.

INTRODUCTION

Cerebrotendinous xanthomatosis (CTX) patients transform cholesterol into bile acids predominantly via the 25-hydroxylation pathway (1-6). This pathway involves the 25-hydroxylation of 5β-cholestane-3α,7α,12α-triol to give 5β-cholestane-3α,7α,12α,25-tetrol followed by stereospecific 24S-hydroxylation to yield 5β-cholestane-3α,7α,12α,24S,25-pentol (5-8). More recent results have indicated that hepatic mitochondrial 27-hydroxylation is also abnormal in CTX subjects and several point mutations in the sterol 27-hydroxylation gene have been described (9-12). In order to investigate further the sequence of side chain hydroxylations and the enzymatic block in bile acid synthesis in (CTX) we required an efficient and large scale syntheses of (24R and 24S) 5β-cholestane-3α,7α,12α,24,25-pentols in stereochemically defined pure forms. Utilizing a new phthalazine class of cinchona alkaloid based chiral ligands which were recently disclosed by Sharpless (13-15) we have synthesized (24R and 24S) 5β-cholestanepentols in extremely high levels of enantioselectivity. This synthetic approach provided the most powerful method for controlling relative and absolute configuration of the two 5β-cholestanepentols required for biosynthetic studies (Fig.1).

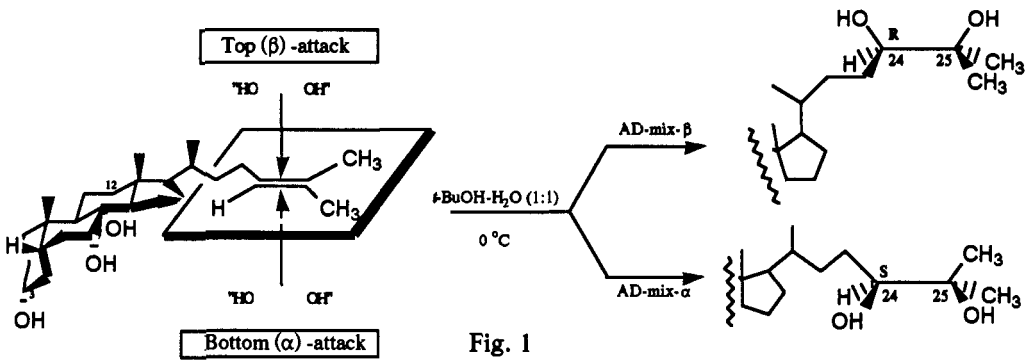

Fig. 1

General Methods: The general procedure, including the measurements of melting points, flash chromatography, thin-layer chromatography (TLC) , capillary GLC analysis and mass spectra (MS) is same as described previously (16). The nuclear magnetic resonance spectra (^1H and ^{13}C) were recorded on a Varian XL-400 (400 MHz) spectrometer in $CDCl_3$ solution. Proton and carbon chemical shifts are reported relative to tetramethylsilane (TMS). In addition, Distortionless Enhancement by Polarization Transfer (DEPT) spectra were also recorded on XL-400 spectrometer at 100 MHz for ^{13}C nuclei (17). Mass spectra (MS) of the bile alcohols were obtained with a JEOL JMS-HX 110A high resoultion mass spectrometer (HR-MS). Fast atom bombardment mass spectrometric (FAB-MS) studies of these bile alcohols were conducted using NBA (nitrobenzyl alcohol) as a matrix and MeOH as a solvent (18,19). Lanthanide-induced CD studies of the bile alcohols were accomplished as described in our previous publication.(6,16)

Experimental and results: *Asymmetric dihydroxylation of 5β-cholestane-3α, 7α, 12α-Δ^{24}- triol (Fig. 1, I). Representative procedure for 24S, (β)-pentol:* To a well-stirred mixture of (AD-mix-α, 1.8 g) in 20 ml of 1 : 1 tert-butanol-water (10 ml each), MeSO2NH2 (0.19 g, 2 mmol) at 0 °C was added 5β-cholestane-3α, 7α, 12α-Δ^{24}- triol (0.509 g, 1.22 mmol) (16). The mixture was stirred at 0 °C overnight, then 6g of solid Na_2SO_3 was added and left stirring at room temp. for 1 h. The tert-butanol layer was separated and the aqueous layer was extracted with EtOAc (3 x 25 ml). The combined organic layers were washed with water and brine, dried over Na_2SO_4, concentrated, and the residue flash chromatographed (ethyl acetate : chloroform (90 : 10) to give pure 24 β-pentol (455 mg, 89 %). ^1H, NMR, 24S, 25-pentol (400 MHz, $CDCl_3$ + CD_3OD) δ 0.70 (s, 18-CH_3), 0.90 (s,19-CH_3), 1.0 (d, C-21-CH_3, 6Hz), 1.2/1.17 (26 CH_3 + 27 CH_3), 3.32 (d,d,10 Hz, 24H), 3.45 (m, H-3), 3.86 (br, s, H-7) 4.0 (hr, s, H-12). The CD spectrum of the 24S,25-pentol in the presence of Eu(fod)3 showed $\Delta\varepsilon_{307}$ = + 9.4 degree x cm^2 x $dmol^{-1}$ (first Cotton effect), and $\Delta\varepsilon_{284}$ = -5.9 degree x cm^2 x $dmol^{-1}$ (second Cotton effect) (6,16). These positive Cotton effects measured at its maximum value, around 310 nm, were found to correlate with the chirality of the two hydroxyl groups (6,16) having 1,2 glycol system in the side chain and thus the chirality at C-24 hydroxyl group in this pentol was assigned as S. Using a similar protocol 24R(α)-pentol was synthesized

from AD-mix-β, ^{1}H-NMR for 24R, 25-pentol (400 MHz, CDCl3 + CD3OD) δ 0.70 (s, 18-CH_3), 0.90 (s,19-CH_3), 1.0 (d, C-21CH_3, 6 Hz), 1.2/1.17 (26 CH_3 + 27 CH_3), 3.25 (t, 24H), 3.41(m, H-3), 3.83 (s, H-7) 4.0 (br, s, H-12). The CD spectrum of the 24R,25-pentol in the presence of Eu(fod)3 showed $\Delta\varepsilon_{307}$ = -5.83 degree x cm^2 x dmol^{-1} (first Cotton effect), and $\Delta\varepsilon_{284}$ = + 409 degree x cm^2 x dmol^{-1} (second Cotton effect) (6). These positive Cotton effects measured at its maximum value, around 310 nm, were found to correlate with the chirality of the two hydroxyl groups having 1,2 glycol system in the side chain and thus the chirality at C-24 hydroxyl group in this pentol was assigned as R.

The FAB mass spectra of both R and S isomers provided protonated and sodiated molecular ions at m/z 453 and 475, respectively, and a weak signal at m/z 905 which corresponded to $[2M + H]^+$. In addition, the mass spectra displayed a number of fragment ions representing successive loss of H_2O molecules from the protonated molecular ions at m/z 435 $[M + H - 4H_2O]^+$, 417 $[M + H - 2H_2O]^+$, 399 $[M + H - 3H_2O]^+$, 381 $[M + H - 4H_2O]^+$, and 363 $[M + H - 5H_2O]^+$.

[Note: The receipe for the preparation of AD-mix-α or AD-mix-β has adequately been described by Sharpless and his associates (13,14).

Discussion: The configuration at C-24 of (24R)-pentol and (24S)-pentol was further established by NMR experiments and verified by CD analysis (16) (see experimental). The results of ^{1}H- and ^{13}C- NMR studies are summarized in Table 1. The C-24H in (24R) pentol shows triplet at δ 3.4 and in (24S) shows doublet at 3.2 in ^{1}H NMR. The ^{13}C spectrum in $CDCl_3$ exhibits 30 signals between 140 and 10 ppm (data not shown) and analyzed by DEPT-135 spectrum in $CDCl_3$ which allowed the identification of 7 quaternary carbons, 5 CH's, 10 CH_2's, 8 CH_3's. However, these ^{13}C data could not differntiate 24S-pentol from 24R-pentol. FAB-MS data of (24R)-and (24S)-pentols in the presence of NaCl (18,19) provided very intense molecular ions 475=$[M + Na]^+$ as base peaks in the spectra. Since the fragmentation pattern was similar for both samples as discussed above therefore, this data could not differentiate 24S-pentol from the 24R-pentol.

Table. 1 ^{1}H- and ^{13}C-NMR data for (24R) and (24S) pentols (in $CDCl_3$ solution).

Types	^{1}H	^{13}C
5β-cholestane-3α, 7α, 12α, 24R,25-pentol.	3.4 (t) C-24H	78.6 (C-24)
5β-cholestane-3α, 7α, 12α, 24S,25-pentol.	3.2 (d) C-24H	79.6 (C-24)

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