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Spectral Assignments and Reference Data

^{13}C NMR spectral assignment of five epimeric 3α- *versus* 3β-functionalized cholestane pairs

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^{13}C NMR chemical shift assignments of five α- and β-epimeric pairs of cholestanes functionalized at C-3 are presented. Empirical increment estimations proved to be a valuable tool for the unequivocal structural elucidation when compared with the chemical shift values of cholestanes derivatized by introduction of *N*- and *S*-containing groups at C-3 in equatorial and axial positions. Moreover, the possibility is demonstrated to anticipate the effect of –OC(S)R substituents at neighboring carbon atoms of the ring A backbone. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: ^{1}H NMR; ^{13}C NMR; DEPT; COSY; HMQC; cholestane derivatives; substituent increments

INTRODUCTION

Hemisynthetic derivatives of cholestane, **1**–**10** (Scheme 1), have been our subject of interest as model precursors in an ongoing study seeking novel strategies for the functionalization of inactivated carbons in terpenes in the presence of ferric catalysts through Patin chemistry.[1] For this purpose, groups containing *N*- and *S*-heteroatoms had to be introduced at C-3 position in cholestanol.[2] These hemisynthetic cholestane derivatives could potentially be useful since the insertion of these functional groups not introduced by biosynthetic processes awards new biological activities. Following this idea, a structure-activity relationship study of the influence of α- and β- configuration of substituents at C-3 in relation to antimicrobial activities[2] was performed based on the series of C-3 epimer pairs synthesized.

The ^{13}C NMR spectral assignment of the five functionalized cholestanes 3α-/3β- epimer pairs **1**–**10** based on the common cholestane skeleton are presented here. It is our belief that the present study can contribute in further assignments of all carbons present in ring A backbone of related compounds possessing equatorial and axial groups in C-3 position.

Moreover, although cholestan-3α-ol (**1**),[3] cholestan-3β-ol (**2**)[3] have already been described with ^{13}C NMR data fully assigned, and the 3α-benzoate (**3**)[4] has been characterized although with interchange between C-12 and C-16 carbon chemical shifts, we could not find the full assignment of β-derivatives **4**, **6**, **8**, and **10** in the literature and, to the best of our knowledge, the α-derivatives **7** and **9** have never been reported previously. The synthesis of compound **5** has been reported by Barton *et al.*[5] without any NMR assignment.

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RESULTS AND DISCUSSION

Cholestan-3β-ol (**2**) was synthesized following previously described procedures.[6] The epimerization to cholestan-3α-ol (**1**) with the hydroxyl group in the axial position[7] was accomplished[8] starting from tosylation of **2** followed by reaction with KNO_2 in dry DMF. All derivatives **3**–**10** were prepared following typical procedures described in the literature (Scheme 1).

The benzoylated derivatives **3** and **4** were prepared by reaction of the cholestan-3-ols **1** and **2**, respectively, with benzoyl chloride in dry pyridine.[9] The preparation of xanthates **5** and **6** was carried out by reaction of the cholestan-3-ol **1** and **2**, respectively, with sodium hydride in dry THF in the presence of a catalytic amount of imidazole followed by treatment with carbon disulfide and methyl iodide.[5,10] Lawesson's reagent[11] was used to prepare the thiobenzoates **7** and **8** from benzoylated **3** and **4**, respectively. Thiocarbamates **9** and **10** were synthesized from xanthates **5** and **6**, respectively, by reaction with diethylamine in petroleum ether based on methods already described.[12]

The structural analysis and ^{13}C NMR spectral assignment (Table 1) for all cholestanes presented here were, in general, based on the data previously reported for cholestane backbone,[13] together with calculated increment values for cyclohexane substituted with hydroxyl and benzoyloxy groups in axial and equatorial position,[13] after ^{13}C NMR assignment for cholestane derivative **8** performed by a complete analysis of the ^{1}H NMR, ^{13}C NMR, DEPT135, COSY and HMQC spectral data.

^{13}C and DEPT135 studies afforded the identification of all quaternary carbons along with all signals in B, C and D rings as well as in the side chain. The C-3 assignment for all cholestane derivatives **1**–**10** was easily determined, through evidence of the ^{13}C-chemical shifts expected to a sp^3-hybridized carbon substituted by oxygen containing groups, namely, hydroxyl and benzoyloxy.[13] The assignment of the remaining carbons in ring A for the compounds **1**–**4** was accomplished by analogy of the experimental values with the estimated increment values using calculated values for cyclohexane substituted in axial and equatorial position.[13]

The experimental chemical shifts [δ_{Ci}(exp.)], thus obtained for compounds **1**–**4**, were compared with the calculated values [δ_{Ci}(calc.)], which were estimated by adding increments outlined for hydroxy [$\Delta_{\delta-i}$(OH)] and benzoyloxy substituents [$\Delta_{\delta-i}$OBz)] in axial and equatorial position of a cyclohexane to the respective chemical shifts values of the unsubstituted cholestane.[13] In general, the experimental and the calculated values correlate fairly well (within ±1 ppm) although some noticeable differences up to |3.3| ppm exist. These discrepancies may be explained by the influence of two additional alkyl groups present in the ring A of the cholestanes, which have been not considered in the model cyclohexane.

The overall ring A signal assignments of **5**–**10** compounds were deduced on the basis of the previous assignment of **1**–**4** taking the axial *versus* equatorial position of the 3-substituted groups as reference.

Table 2 summarizes all increments $\Delta_{\delta-i}$(group) [= δ_{Ci}(cholestane) – δ_{Ci}(exp.)] calculated for the carbons in the ring A of cholestane derivatives **1**–**10** due to the presence of an axial and equatorial 3-substituent by different groups. They are presented relative to the corresponding values of the reference compound cholestane.

From Table 2 it can be concluded that the deshielding effect of any *O*-substituted functional group in an equatorial position is generally stronger in relation to the axial position with differences of increment values, for about 3–4 ppm. The thiocarbonyl α-increment values are always higher than those of the carbonyl group, irrespective of axial or equatorial position. Despite both C-2 and C-4 being C$_\beta$ carbons, the increment value of the first is lower whenever a C-3 substituent is in axial or equatorial position. The influence of the C-3 substituent on the increment value of carbon C$_\delta$ (C-10) is quite negligible due to its distant position.

In conclusion, the increment values presented in Table 2 allow the estimation of the chemical-shift value of all the carbons present in ring A of any cholestane analogue with a –OC(S)R substituents in C-3 position.

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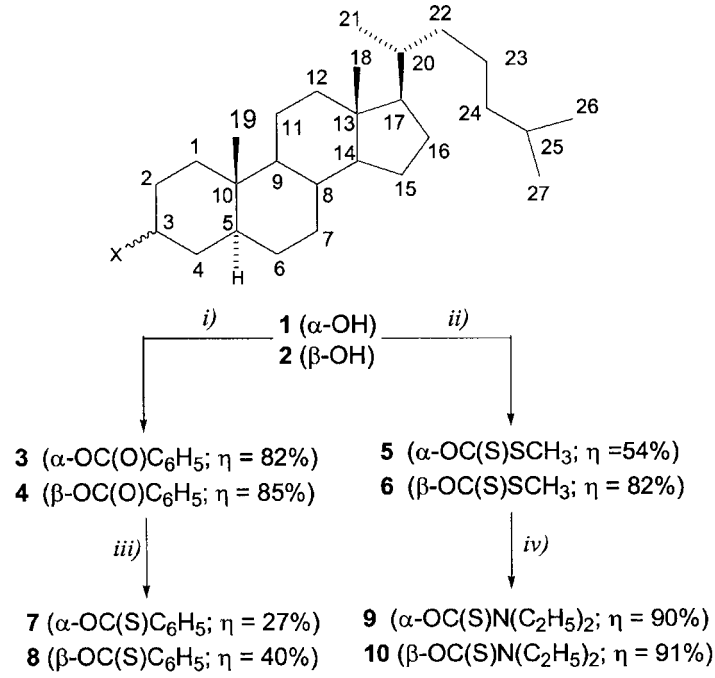


Scheme 1. Synthesis of cholestanes functionalized at C-3 (**1**–**10**).

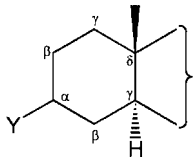
Table 1. ^{13}C NMR chemical shifts, δ (ppm), for cholestane (first column)[a] and its derivatives **1**–**10**[b]

		Substituents in axial position					Substituents in equatorial position				
C	[a]	**1**	**3**	**5**	**7**	**9**	**2**	**4**	**6**	**8**	**10**
1	39.2	32.2	35.8	33.3	35.8	32.9	37.0	36.2	35.8	36.2	34.0
2	22.7	29.1	26.3	28.4	25.5	28.1	31.5	27.6	26.9	26.7	28.2
3	27.3	66.6	70.8	80.6	77.9	76.8	71.4	74.4	83.6	81.5	80.1
4	29.2	36.0	33.3	36.2	33.3	33.6	38.2	35.5	35.5	35.6	35.3
5	47.6	39.2	42.6	40.6	42.5	40.9	44.8	44.7	44.6	44.6	42.5
6	29.6	28.6	28.4	28.2	28.4	28.3	28.7	28.7	28.6	28.7	28.6
7	32.6	32.0	32.0	31.9	31.9	32.1	32.1	32.0	32.0	32.0	31.9
8	36.0	35.5	33.0	35.5	34.5	35.4	35.5	34.2	33.3	35.5	35.4
9	55.3	54.4	54.4	54.3	54.1	54.7	54.3	54.3	54.2	54.2	54.1
10	36.7	36.1	35.9	35.8	35.8	35.7	35.4	36.8	36.2	36.7	35.7
11	21.3	20.8	20.9	20.9	20.9	20.8	21.2	21.2	21.3	21.2	21.1
19	12.5	11.2	11.4	11.4	13.1	11.4	12.3	12.3	12.3	12.3	13.2
3′/5′	–	–	128.3	–	128.0	–	–	128.2	–	127.9	–
2′/6′	–	–	129.6	–	128.7	–	–	129.5	–	128.8	–
4″	–	–	131.2	–	132.7	–	–	131.0	–	132.5	–
1′	–	–	132.6	–	139.0	–	–	132.6	–	139.0	–
C═O	–	–	165.9	–	–	–	–	166.1	–	–	–
C═S	–	–	–	214.7	210.0	186.3	–	–	215.3	210.7	186.4
S–CH_3	–	–	–	18.7	–	–	–	–	18.7	–	–
N$\underline{C}H_2CH_3$	–	–	–	–	–	43.3	–	–	–	–	44.4
N$CH_2\underline{C}H_3$	–	–	–	–	–	47.3	–	–	–	–	47.4

[a] Reported data for cholestane.[13a]

[b] The position of the additional aromatic carbons present in the benzoate **3**–**4** and thiobenzoate **7**–**8** derivatives are indicated by a prime.

Table 2. Increment values ($\Delta_{\delta-i}$-C_i) calculated for all carbons in ring A of the cholestane derivatives **1**–**10** with equatorial or axial substituents (in ppm)



	$\Delta_{\delta-i}$-C_α		$\Delta_{\delta-i}$-C_β				$\Delta_{\delta-i}$-C_γ				$\Delta_{\delta-i}$-C_δ	
	eq.	ax.	eq.		ax.		eq.		ax.		eq.	ax.
Y	C-3		C-2	C-4	C-2	C-4	C-1	C-5	C-1	C-5	C-10	
OH	44.1	39.3	8.8	9.0	6.4	6.8	−2.2	−2.8	−7.0	−8.4	−1.3	−0.6
$OC(O)C_6H_5$	47.1	43.5	4.9	6.3	3.6	4.1	−3.0	−2.9	−3.4	−5.0	0.1	−0.8
$OC(S)SCH_3$	56.3	53.3	4.2	6.3	5.7	7.0	−3.4	−3.0	−5.9	−7.0	−0.5	−0.9
$OC(S)N(C_2H_5)_2$	52.8	49.5	5.5	6.1	5.4	4.4	−5.2	−5.1	−6.5	−6.7	−1.0	−1.0
$OC(S)C_6H_5$	54.2	50.6	4.0	6.4	2.8	4.1	−3.0	−3.0	−3.4	−5.1	−0.0	−0.9

EXPERIMENTAL

The synthesis of all cholestane derivatives **1**–**10** was carried out by methods based on literature[6–13] and previously described in detail.[2]

NMR spectra were recorded on a Bruker AMX 300 spectrometer operating at 300 (^{1}H) and 75 MHz (^{13}C) at room temperature (300 K) in $CDCl_3$ with TMS as internal standard, equipped with a 5-mm QNP probe (^{1}H: 90° pulse width 7.8 μs; ^{13}C: 90° pulse width 7.5 μs).

^{1}H NMR spectra were obtained using a 4237 Hz spectral window with 32 768 data points using LB = 0.3 Hz and GB = 0.0 in processing. ^{13}C NMR spectra were obtained using a 19 230-Hz spectral window with 131 072 data points using LB = 1.0 Hz and GB = 0.0 in processing.

HMQC spectra were recorded as 2048 (t_2) × 128 (t_1) and 2048 (F_2) × 1024 (F_1) with eight scans and 2 s relaxation delay. COSY spectra were recorded as 1024 (t_2) × 256 (t_1) and 1024 (F_2) × 256 (F_1) with four scans. Standard Bruker software has been used.

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REFERENCES

1. Patin H, Mignani G. *J. Chem. Soc., Chem. Commun.* 1979; **16**: 685.
2. Ramos SS. *Funcionalização de Carbonos Inactivados em Terpenos e Estudos Preliminares da Relação Estrutura-Actividade*, Ph.D. Dissertation, Universidade da Beira Interior, 2005.
3. Eggert H, VanAntwerp CL, Bhacca NS, Djerassi C. *J. Org. Chem.* 1976; **41**: 71.
4. Camp D, Jenkins ID. *Aust. J. Chem.* 1988; **41**: 1835.
5. Barton DHR, Choi S-Y. *Tetrahedron Lett.* 1996; **37**: 2695.
6. Augustine RL. *Org. React. Steroid Chem.* 1972; **18**: 111.
7. Radüchel B. *Synthesis* 1980; **4**: 292.
8. King LC, Bigelow MJ. *J. Am. Chem. Soc.* 1952; **74**: 3338.
9. Gawronski JK, Reddy SM, Walborsky HM. *J. Am. Chem. Soc.* 1987; **109**: 6726.
10. Dembele A, Deshayes H, Pete J-P. *Bull. Soc. Chim. Fr.* 1988; **4**: 671.
11. Jang DO, Song SH, Cho DH. *Tetrahedron* 1999; **55**: 3479.
12. Barrett AGM, Prokopiou PA, Barton DHR. *J. Chem. Soc., Perkin Trans. 1* 1981; 1510.
13. (a) Kalinowski HO, Berger S, Braun S. *Carbon-13 NMR Spectroscopy*. John Wiley & Sons: Great Britain, 1988; (b) Blunt JW, Stothers JB. *Org. Magn. Reson.* 1977; **9**: 439.