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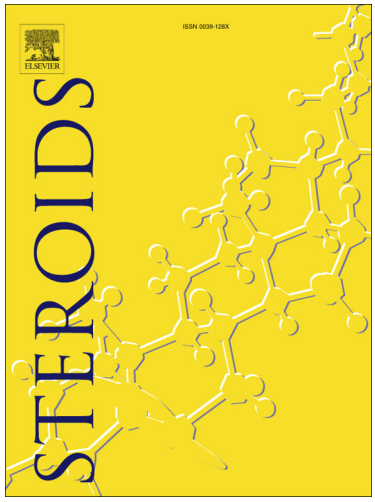

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Complete ^{1}H NMR assignment of cholesteryl benzoate

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ABSTRACT

The 750 MHz ^{1}H NMR spectrum of cholesteryl benzoate (**1b**) could be assigned completely, which means all chemical shifts and all coupling constants, including some long-range values, were established. This task was possible by extracting many approximate coupling constant values in the overlapped spectrum region from an HSQC experiment, and using these values in the ^{1}H iterative full spin analysis integrated in the PERCH NMR software. The task was facilitated using our published data for 3*β*-acetoxypregna-5,16-dien-20-one (**3**), the assignment data of the sesquiterpene benzoquinone dihydroperezone (**2**), also performed in the present study, which contains the same carbon atoms chain than cholesterol (**1a**), and an HSQC study of (25*R*)-27-deuteriocholesterol (**1c**) we prepared some 40 years ago. The HSQC values of **1c** in combination with the coupling constants of **1b** also allowed to completely assigning the spectrum of **1c**. The complete assignment of **1b** and **1c** further provided the opportunity to estimate the hydrogen shifts induced upon benzoylation of cholesterol. Comparison of the experimental vicinal coupling constants of **1b** with the values calculated using the Altona software provides an excellent correlation. In addition, a single crystal X-ray diffraction study of **1b** provided the molecular conformation in the solid state, which revealed the side chain adopts an extended conformation.

Keywords:

Cholesteryl benzoate; Iterative ^{1}H NMR analysis; X-ray structure; Dihydroperezone

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1. **Introduction**

Familiar and social conversations dealing with diseases, health, and blood levels of cholesterol (**1a**) are relatively frequent, suggesting this molecule, the sterol of the animal kingdom, seems nowadays to be very popular. This appears to be a natural consequence of almost three centuries in the study of **1a** since literature can be traced back to contributions published in 1733, 1775 and 1789, the first one when it was recognized that gallstones were soluble in alcohol or turpentine, as quoted in a superb book on cholesterol [1] published over half a century ago. It is extremely difficult to completely trace the scientific literature regarding cholesterol, since literally thousands of publications deal with almost any aspect of the still intriguing behavior of the molecule. Therefore, to this end it suffices here to say that despite many publications that continue to appear, at least four books published during the last 15 years testify the current interest on cholesterol [2-5].

The structure of cholesterol (**1a**) (Scheme 1) followed after extensive chemical studies that included degradation processes, as well as synthesis and preparation of derivatives of some degradation molecules. The information is described in some 25 publications that appeared during the last decade of the nineteenth century and the initial four decades of the twentieth century [1].

The complete biosynthesis of cholesterol (**1a**) has also been studied extensively [6] starting from its most basic precursor using [^{14}C]acetate. Although this required the tedious time-consuming carbon-by-carbon degradation processes, it allowed to completely mapping the two atoms of acetate in the sterol. The advent of continuous-wave ^{13}C NMR spectroscopy for studies of natural products and some of their derivatives in the late sixties of the last century provided us [7] the opportunity to study dihydroperezone (**2**), which

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possesses the same side chain than sterol **1a**. Since ^{13}C NMR spectroscopy suffered a very fast evolution by the introduction of the pulse Fourier transform methodologies, the ^{13}C NMR spectrum of cholesterol (**1a**) was almost completely assigned quite soon [8], excepting the distinction of the C-26 and C-27 signals. This spectroscopic technique changed dramatically the way biosynthetic studies were performed since years of efforts consumed by degradation processes of ^{14}C radioactive materials could be replaced by weeks of work [9] using ^{13}C NMR. The assignment of the spectrum of cholesterol (**1a**) was finally completed [10] when we prepared a sample of (25*S*)-27-deuteriocholesterol (**1c**) in a four steps sequence using an authentic sample of kryptogenin (cholest-5-ene-3*β*,26-diol-16,22-dione) isolated by Russel Marker around 1940.

As far as we know, the assignment of ^{1}H resonances of cholesterol (**1a**) has been attempted twice. In the first trial [11], the incomplete assignment of the hydrogen atoms of the A and B rings was accomplished by means of decoupling experiments and comparison of the ^{1}H spectrum of a partly deuterated sample. In the second case all chemical shifts of **1a** were described combining HSQC and HMBC correlations of the digitonin-cholesterol complex dissolved in pyridine [12]. Even though this study provided all ^{1}H chemical shifts of **1a**, most of them cannot be used as reference since some suffer strong solvent effects due to the aromatic solvent, as for instance the signals owing to the C-11 methylene. To complete the NMR characterization of cholesterol (**1a**) in a classic solvent like $CDCl_3$ it thus remains to have a detailed assignment of its ^{1}H spectrum, which must include the individual chemical shifts of all hydrogen atoms, as well as all homonuclear coupling constants. Although this is not an easy task due to the severe overlapping spin multiplets in the 1.54–1.90 ppm region, we took advantage of our recently published work [13] on the

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total assignment of 3β-acetoxypregna-5,16-dien-20-one (**3**) and of the availability of a sample of dihydroperezone (**2**) [7,10]. In addition, due to the difficulty to obtain highly purified samples of cholesterol (**1a**), which in this case is very important since minor impurities generating NMR signals in the observed spectra region can severely complicate spin-spin iteration processes, the molecule of choice was cholesteryl benzoate (**1b**) which eventually could be purified, after extensive recrystallizations, to afford a sample that was even suitable for a single crystal X-ray diffraction analysis.

2. Materials and methods

2.1 General

Cholesteryl benzoate (**1b**) was prepared by treatment of cholesterol (**1a**) with benzoyl chloride in pyridine. A sample of approximately 10 mg was placed in a 5 mm NMR sample tube, dissolved in 0.9 mL of $CDCl_3$ and the solution was degassed by slow bubbling of an argon stream under ultrasound for 5 min. This rendered a final volume of 0.5 mL to which a small amount of TMS in $CDCl_3$ was added. In turn, (25*R*)-27-deuteriocholesteol (**1c**) and dihydroperezone (**2**) were available from previous ^{13}C NMR studies [7,10].

2.2 NMR Spectra

All spectra were obtained on a Bruker Ascend 750 spectrometer equipped with a cryprobe. The ^{1}H NMR spectra were acquired with number of scans (NS) = 16, acquisition time (AQ) = 2.18 s, relaxation delay (RD) = 1.0 s, 90^{o} pulse width (P1) = 8.24 ms, spectral width (SW) = 15 000 Hz and FT size = 65536. The acquisition data for the 2D HSQC of **1b** were SW ^{1}H = 887.8 Hz with 2048 increments, and ^{13}C = 10352 Hz with 512 increments, AQ = 2.3 s, NS = 16 and RD = 2.0 s.

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2.3 ^{1}H NMR full spin analysis

Complete ^{1}H NMR spectra analysis of **1b**, **1c**, and **2** was achieved using the iterative full spin analysis (HiFSA) method [14] integrated in the PERCH v.2011.1 software (PERCH Solutions Ltd., Kuopio, Finland). The 750 MHz experimental data of **1b**, **1c**, and **2** were imported into the preparation unit (PAC) of the PERCH shell and subjected to phase and baseline correction, peak picking, and integration. Molecular models for **1b**, **1c**, and **2** were built using the molecular modeling module (MMS) and after geometry optimization a Monte Carlo conformers distribution was done. The most stable conformer for each molecule was used to obtain the initial calculated spectra.

In the case of dihydroperezone (**2**) the published [7,10] ^{13}C NMR chemical shifts in combination with a HSQC plot provided the ^{1}H chemical shifts and revealed which pairs of hydrogen atoms belong to the same methylene group. In addition, some approximate coupling constant values were taken from the published values for perezone (**4a**), *O*-methylperezone (**4b**), and 6-hydroxyperezone (**4c**) [15]. For the individual assignment of the *pro-R* and *pro-S* hydrogen atoms of the C-10, C-11, and C-12 methylene groups, several values were exchanged until the convergence was reached at the lowest total root-mean-square deviation (RMS) of 0.040.

In a similar but not identical approach, in the case of cholesteryl benzoate (**1b**) the initial chemical shift values were defined after extraction of the clean multiplets from a 2D HSQC experiment, while the initial coupling constant values were determined using the parameters of the two model compounds, 3β-acetoxypregna-5,16-dien-20-one (**3**) [13] and dihydroperezone (**2**) as is detailed in the Results and Discussion Section. The vicinal

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coupling constant values of **1b** were further tested using dihedral angle values obtained from the optimized conformer by means of the Altona software [16].

*2.4 Single crystal X-ray diffraction analysis of cholesteryl benzoate (**1b**)*

The data were collected on an Enraf-Nonius CAD4 diffractometer using Cu *Kα* radiation (λ = 1.54184 Å) at 293(2) K in the $\omega/2\theta$ scan mode. Crystal data were $C_{34}H_{50}O_2$, M = 490.74, tetragonal, space group $P4_12_12$, $a = b$ = 10.513(1) *Å*, c = 54.436(8) *Å*, V = 6016.8(1) $Å^3$, Z = 8, ρ = 1.083 mg/mm^3, μ = 0.492 mm^{-1}, total reflections = 4694, unique reflections 3987 (R_{int} 0.01%), observed reflections 2727. The structure was solved by direct methods using the SIR2002 program included in the WinGX v1.70.01 crystallographic software package. For the structural refinement, the non-hydrogen atoms were treated anisotropically, and the hydrogen atoms, included in the structure factor calculation, were refined isotropically. The final R indices were [I > 2σ(I)] R1 = 5.2% and wR2 = 13.4%. Largest difference peak and hole, 0.228 and -0.147 e.Å^3. The Olex2 v1.1.5 software[1] allowed to calculate the Flack parameter[2] x = 0.0(6) and Hooft parameter[3] y = -0.4(3). For the inverted structure these parameters were x = 1.0(6) and y = 1.4(3), respectively. A PLUTO plot of the X-ray structure is shown in Fig. 1. Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 1848799). Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. Fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

3. Results and discussion

In order to perform the detailed complete ^{1}H NMR assignment of cholesteryl benzoate (**1b**) through iterative full spin analysis (HiFSA) [14], the methodology used to determine the

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complete spectra parameters of several natural products [17-21], we relied on the previous knowdelge of the spectra parameters of two simpler related compounds: 3β-acetoxypregna-5,6-dien-20-one (**3**) we published a few years ago [13], which contains the cyclopentanoperhydrophenantrene core including the two angular methyl groups (Scheme 1), and dihydroperezone (**2**), which was assigned in the present work, containing the same saturated carbon atoms chain than cholesterol (**1a**). We used benzoate **1b** since it can be purified easierly than **1a**, by extensive recrystallizations, thus allowing to get a high quality ^{1}H NMR spectrum and a sample suitable for single crystal X-ray diffraction analysis (Fig. 1). Due to the iteration capability of the PERCH software, which compares the peaks of the experimental and calculated spectra, the high purity of a sample for HiFSA is mandatory, since experience dictates that small impurities that originate NMR signals in the studied spectra regions can severely complicate a good detailed assignment.

Thus, the 750 MHz free induction decay of **1b** was edited in the PAC module of the PERCH shell. Briefly, the atom coordinates obtained from the single crystal X-ray diffraction study were introduced in the molecular modeling module (MMS) and, after geometry optimization; the Monte Carlo conformer distribution analysis provided the most stable conformer which was used to obtain the initial calculated spectrum. The hydrogen atoms at positions C-3, C-4, C-6, C-18, C-19, and C-21 were easily recognized at this point. The positions of all remaining hydrogen atoms were determined using the HSQC correlations based on the reported ^{13}C assignments of **1a**. For this task, several sets of ^{13}C NMR data are available, although we selected those of the classic works that appeared in the seventies last century [8,9], which we know since then as correct. In a recent data set some discrepancies for the chemical shifts of C-16 and of the C-22 to C-25 atoms chain

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were suggested [22] but they should be ignored. In addition, to determine the heteronuclear correlations, we decided to disentangle the overlapped spin pattern by extraction of ^{1}H multiplicities (Fig. 2) from the HSQC experiment [23]. This method was very useful for the total assignment of 3β-acetoxypregna-5,16-dien-20-one (**3**) [13] and was originally developed by one of us for an early study of α-cedrene [24]. In the present study of **1b**, a SW of 887.8 Hz and AQ of 2.307 s gave a 4k data table which after zero filling to 64k and FT provided an ^{1}H NMR digital resolution around 0.1 Hz/point. Having the extraction of the traces from the HSQC plot at the carbon frequency, the precise recognition of the ^{1}H shifts of **1b** was possible, (Fig. 2). These ^{1}H chemical shifts were used to feed and update the initial values in the table editor of the PMS module. At this point it was also possible to define the α- and β- positions for the hydrogen atoms at C-1, C-2, C-7, C-11 and C-12 according to their signals shape, since for broader signals at least one additional large coupling constant, corresponding to the *axial* hydrogen atoms, were expected. Taking into account that the coupling constants of the A-, B-, and C-ring hydrogren atoms of **1b** keep similarity with those of 3β-acetoxypregna-5,6-dien-20-one (**3**) [13], since the conformational backbone should be similar in both compounds, we took advantage of the reported coupling constants for **3** and substituted them in the **1b** spectrum file. Using these parameters, the simulation of the less crowded spectrum region, at higher frequencies than 1.70 ppm, evidenced a high similarity with the experimental spectrum, and after a few iterations the simulation of this region was accomplished reaching a RMS of 0.052%.

For the most crowded window, in the 0.90–1.62 ppm region, in which the hydrogen atoms of the C-22 to C-25 side chain were spliced with many sterane hydrogen atoms, we took advantage of a sample of dihydroperezone (**2**) that was available from previous NMR

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studies [7,10]. The HSQC experiment of **2**, performed as in the case of **1b**, provided the plots depicted in Fig. 3, which for the C-10 to C-13 atoms, that are equivalent to the C-22 to C-25 atoms of **1b**, show that H-13 appears at 1.48 ppm, the two multiplets at C-10 provide signals near 1.52 and 1.71 ppm, those assigned to the two hydrogen atoms at C-11 appear at 1.24 and 1.14 ppm, the later being overlapped in the same multiplet than the two hydrogen atoms at C-12. Consequently, with this information and after several cycles of iteration, and the interchange of the hydrogen atoms of a given methylene group, a good agreement for the spectrum of **2**, providing a RMS value of 0.040%, could be obtained, as is shown in Fig. 3.

The coupling constant values obtained for dihydroperezone (**2**), which are summarized in Table 1, were used to complete the assignment of the spectrum of **1b**. After feeding the table editor of the PMS module and performing some additional manual adjustments for these values, a reduced number of iteration cycles finally provided an excellent concordance between the experimental and calculated spectra of **1b** reaching a RMS of 0.037%.

The complete data for cholesteryl benzoate (**1b**) are shown in Table 2, while the simulated spectrum is shown in Fig. 4. HiFSA calculations provide by default chemical shift and coupling constant values with six and four decimal places, respectively. Since the experimental 750 MHz spectra of **1b** and **2** were acquired with magnet homogeneities better than 0.8 Hz, chemical shift and coupling constant values in Tables 1 and 2 are presented with three and two digits, respectively, after the decimal point, as done for previous cases. [17-21]

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It is well known that in cyclohexanes the chemical shifts of *axial* hydrogen atoms appear at lower frequencies than the *equatorial* hydrogen atoms, except in the vicinity of substituents [25]. The *axial* hydrogen atoms at positions 1*α*, 2*β*, 7*α*, 11*β*, and 12*α* were not the exception for this trend, resonating in the 0.9–1.7 ppm range, in contrast to the *equatorial* hydrogen atoms which appeared in the 1.5–2.0 ppm range. The *axial*/*equatorial* distinction for the two methylene groups on ring D was opposite than that for the six member ring, the *quasi-axial* atoms being localized at higher frequencies than the *quasi-equatorial* atoms, rendering H-15*β* and H-16*α* at 1.078 and 1.840 ppm, respectively, and H-15*α* and H-16*β* at 1.589 and 1.266 ppm, respectively. This is in accordance with the assignments for some bile acids and some cholestane derivatives [26], although it is difficult to make a generalization due to the high conformational mobility of the five member ring and the nature of the substituent at C-17. In turn, the chemical shifts of the cholestane side chain C-20 to C-25 are in agreement with the values described for some cholestane steroids [26] having the same side chain. Finally, the methyl groups at C-18, C-19 and C-21 were observed at 0.692, 1.071 and 0.924 ppm, respectively, and it was possible for the first time to distinguish CH_3-26 and CH_3-27 (Fig. 5), at 0.871 and 0.867 ppm, respectively, taking advantage of our stereospecific C-27 deuterated sample prepared for the ^{13}C NMR assignment of cholesterol [10], in combination with an HSQC correlation measurement.

The main difficulty for the complete assignment of the ^{1}H NMR spectrum of **1b** resides on the precise knowledge of all homonuclear coupling constant values. Therefore, it is of relevance to evaluate these values in some detail, which means the *J* values of the rings and of the side chain require separate comments. Regarding the rings, the J_{gem} values

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of the methylene groups are consistent with strain-free systems with values between -12.40 and -13.73 Hz, although the geminal coupling constant of the C-7 methylene groups is much larger, -17.65 Hz, since it is influenced by the adjacent π-bond, as is well documented [27], and is also observed (Table 3) for the C-11 methylene in compounds **4a**–**c** [15]. The magnitude of vicinal coupling constants of rings A and C are in good agreement with chair conformations, while for ring B they agree with a half chair conformation. The *axial-axial* values for $J_{1\alpha,2\beta}$, $J_{2\beta,3}$, $J_{3,4\beta}$, $J_{7\alpha,8}$, $J_{8,9}$, $J_{8,14}$, $J_{9,11\beta}$ and $J_{11\beta,12\alpha}$, are in the 10.41–14.08 Hz range, the *axial-equatorial* values for $J_{1\alpha,2\alpha}$, $J_{1\beta,2\beta}$, $J_{2\alpha,3}$, $J_{3,4\alpha}$, $J_{7\beta,8}$, $J_{9,11\alpha}$, $J_{11\alpha,12\alpha}$, and $J_{11\beta,12\beta}$ are in the 3.72–5.37 Hz range, and the *equatorial-equatorial* values for $J_{1\beta,2\alpha}$ and $J_{11\alpha,12\beta}$ are 3.45 and 2.94 Hz, respectively. Regarding ring D, the $^3J_{HH}$ values are variable, since J_{cis} corresponding to $J_{14,15\beta}$, $J_{15\alpha,16\alpha}$, and $J_{15\beta,16\beta}$ show values of 12.80, 9.81, and 11.64 Hz, respectively, which are larger than the J_{trans} values corresponding to $J_{14,15\alpha}$, $J_{15\alpha,16\beta}$, and $J_{15\beta,16\alpha}$ of 7.38, 3.14, and 6.23 Hz, respectively, according to the tendency for an envelope conformation [28]. The remaining vicinal coupling constants, $J_{6,7\alpha}$ and $J_{6,7\beta}$ in ring B, and $J_{16\alpha,17}$ and $J_{16\beta,17}$ in ring D show values following a Karplus type relationship [29] for the first pair, and close to 9 Hz for the second pair.

In addition to the vicinal coupling constants, long range couplings can usually be observed in the ^{1}H NMR spectra of steroids. In this sense, the long-range $^4J_{HH}$ coupling constants observed in **1b** corresponding to the well-known W arrangements between H-1α and CH_3-19, of H-2α and H-4α, of H-9 and CH_3-19, and of H-6 and H-8 showed values smaller than 1 Hz [25], while the well-known $^4J_{HH}$ of H-12α and CH_3-18 shows a value of -0.63 Hz.

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Allylic coupling constants were observed for hydrogen atoms on either side of the C-5–C-6 double bond, as expected from some described steroids [25]. The magnitude of the allylic coupling between H-4α and H-6 (0.60 Hz) was smaller than those of H-4β and H-6 (2.07 Hz) in line with theoretical correlations of the torsion angles between the plane of the double bond and the adjacent methylene hydrogen atoms [25]. Homoallylic couplings were also seen between the methylene groups at C-4 and C-7, specifically for H-4β with H-7α and H-7β, of 3.51 and 2.62 Hz, respectively.

For the vicinal coupling constant values of the carbon atoms chain of **1b**, the values for $J_{22R,23R}$, $J_{22R,23S}$, $J_{22S,23R}$, $J_{22S,23S}$, $J_{23R,24R}$, $J_{23R,24S}$, $J_{23S,24R}$, and $J_{23S,24S}$ are in excellent agreement with those of dihydroperezone (**2**) and it is also interesting to note they provide similar values than those observed for $J_{2,3}$, $J_{2,3'}$, $J_{3,4}$ and $J_{3,4'}$, in *n*-hexane when measured in choloform [30]. The ${}^3J_{2,3}/{}^3J_{2,3'}$ and ${}^3J_{3,4}/{}^3J_{3,4}$ values in this *n*-alkane are 1.58 and 1.67 respectively, which are related to a *trans-gauche* equilibrium preference of C-2 with C-3 and of C-3 with C-4, being values close to 2 those which denote a major proportion of the *t* conformer [30]. In the case of **1b** the ratio ${}^3J_{22R,23S}/{}^3J_{22R,23S}$ is 2.2, while the ratio ${}^3J_{23R,24S}/{}^3J_{23R,24S}$ is 1.9, both values being comparable with those of *n*-hexane suggesting likewise a preference for a *t* conformation. The remaining vicinal coupling constants of the carbon atoms chain, $J_{24R,25}$ and $J_{24s,25}$, as well as those with the methyl groups $J_{20,21}$, $J_{25,26}$, and $J_{25,27}$ show traditional values in the 6-7 Hz range, also similar than those found in dihydroperezone (**2**).

The similarity of the vicinal coupling constant values of the side chains of cholesteryl benzoate (**1b**) and of dihydroperezone (**2**) suggests the spatial atom arrangement is similar in both molecules. Close inspection of the values shown in Table 3 reveals the

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only noticeable difference is observed between $J_{20,22S}$ = 2.58 Hz for **1b** as compared to $J_{8,10S}$ = 6.67 for **2**, which is attributed to the fact that in the former compound the side chain is appendage of a cyclopentanone moiety, while in the later it is appendage of a 2-hydroxy-1,4-benzoquinone. This is further evidenced from $J_{17,20}$ = 10.27 Hz in **1b**, which according to Altona [16] corresponds to an H(17)-C(17)-C(20)-H(20) dihedral angle of 157° in reasonable good agreement with the torsion angle of 176.9° derived from the X-ray study. In turn, the coupling constants of H-20 and the two hydrogen atoms at C-22 are 8.78 and 2.58 Hz, which according to Altona provide dihedral angle values of 146 and 63°, respectively, in reasonable agreement with the values (169.7 and 88.1°) provied by the torsion angles of the crystal structure. In the case of dihydroperezone (**2**) there is considerable less steric hindrance between the quinone ring and the sustituents at C-8, which results in an overall higher conformational mobility and therefore the coupling constants of H-8 and the two hydrogen atoms at C-10 are more similar one respect to the other one, providing values of 8.70 and 6.67 Hz. Furthermore, comparison of the vicinal coupling constant values of the C-8–C-10–C-11 fragment of **2** and those of **4a**–**c** (Table 3) reveals these values are quite similar, also suggesting the molecules share similar conformational space distributions in agreement with vibrational circular dichroism results obtained for **2** and **4a** [31].

In order to approximately validate the coupling constant values obtained by PERCH analysis of **1b**, the $^{3}J_{H–H}$ values of hydrogen atoms on the rings were compared with those calculated using the Altona software [16]. For this purpose, the molecular model of **1b** was constructed using the Spartan 04 (Wavefunction, Inc., Irvine, CA) software, and the conformational space was evaluated using the Monte Carlo protocol with molecular

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mechanics force field (MMFF94) in an energy window of 10 kcal/mol. This provided 20 conformers in the initial 3.14 kcal/mol energy gap which were subjected to single point calculations using DFT with the B3LYP functional and the 6-31G(d) basis set. The resulting 20 conformers in a 3.02 kcal/mol energy range, covering a Boltzmann population of 99.8% showed the D-ring in an envelope conformation. Comparison of the observed vicinal coupling constants with the values calculated by Altona show a good congruency as can be seen in Table 4. The R-squared (R^2) value derived from linear regression of experimental and calculated values was 0.9849, indicating an excellent correlation. This fact reveals the importance of a second methodology for verification of simulated ^{1}H NMR data.

The comparison of the ^{1}H NMR spectra of cholesteryl benzoate (**1b**) and (25*R*)-27-deuteriocholesterol (**1c**), shown in Fig. 5, in addition of allowing distinction of the methyl signals at positions 26 and 27, provides the opportunity to evaluate the H-3–H_2-4 spin system of both compounds. In the case of **1b** the pair of hydrogen atoms at C-4 have almost the same chemical shift and are coupled one with the other one while being differently coupled with H-3. The resulting ABX spin system shows a doublet (J = 7.62 Hz) at 2.467 ppm for the AB portion, which collapses to a singlet upon irradiation of H-3 at 4.863 ppm, revealing the existence of an uncommon virtual coupling spin system [32] for which it is quite difficult to accurate obtain coupling constant values beyond those estimated by the PERCH software. In contrast for **1c** the two H-4 signals have distinct chemical shifts as seen in Fig. 5.

Since the conformation of the rings system of **1b** and **1c** might essentially be the same, one would expect quite similar coupling constant values in both compounds.

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Therefore an HSQC experiment of **1c** provided the approximate chemical shifts of all hydrogen atoms, which in combination with the coupling constants of **1b** permitted to simulate the spectrum of **1c** to a nice RMS value of 0.042%. Comparison of the experimental and calculated spectra of **1c** is shown in Fig. 6, while the pertinent parameters are summarized in Table 5. In turn, comparison of the chemical shift values given in Table 5 for **1c** and those of **1b** shown in Table 2 reveals that benzoylation of cholesterol shifts the hydrogen atoms on the rings system in the magnitudes shown in Fig. 7. It can further be observed that the most influenced signals are those corresponding to the atoms on ring A, and as a general trend the magnitude of the induced shifts decrease for rings B, C, and D. In addition, all *axial* hydrogen atoms are more shifted than their *equatorial* counterparts on a given carbon atom, excepting the two signals owing to H_2-11 which are equally shifted.

4. Conclusions

All ^{1}H NMR chemical shifts and all homonuclear coupling constant values of the steroidal portion of cholesteryl benzoate (**1b**) were obtained by full spin analysis integrated in the PERCH NMR software in combination with a high resolution spectrum measured at 750 MHz under high magnet homogeneity conditions. For this goal it was necessary to resort to the ^{1}H NMR data of dihydroperezone (**2**), which possesses the same carbon atoms chain than cholesterol (**1a**), of 3*β*-acetoxypregna-5,16-dien-20-one (**3**), which possesses the sterol ring system including the two angular methyl groups, and of (25*S*)-27-deuteriocholesterol (**1c**) which allowed to ascribe the two terminal methyl groups which differ by only 0.004 ppm. The homonuclear hydrogen coupling constants of the C-8-C-10–

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C-11 fragment of dihydroperezone (**2**), are quite similar to those of perezone (**4a**), *O*-methylperezone (**4b**), and 6-hydroxyperezone (**4c**), suggesting they occupy similar conformational spaces. In turn, the complete side chains of cholesteryl benzoate (**1b**) and dihydroperezone (**2**) also show quite similar coupling constant values, suggesting extended chain conformers.

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SCHEME AND FIGURE LEGENDS

Scheme 1. Molecular formulas

Fig. 1. PLUTO plot of X-ray diffracted cholesteryl benzoate (**1b**)

Fig. 2. Partial ^{1}H NMR spectrum of **1b** showing the lower frequencies region. Multiplets extracted at the ^{13}C chemical shifts from HSQC are: C-9 at 50.1 ppm, C-14 at 56.8 ppm, C-17 at 56.2 ppm, C-24 at 39.5 ppm, C-23 at 23.9 ppm, C-22 at 36.2 ppm, C.-20 at 35.8 ppm, C-25 at 28.0 ppm, C-11 at 21.1 ppm, C-15 at 24.3 ppm, C-16 at 28.5 ppm, C-1 at 37.1 ppm, C-7 at 32.0 ppm, C-8 at 31.9 ppm, C-2 at 27.9 ppm and C-12 at 39.8 ppm

Fig. 3. Partial ^{1}H NMR spectrum of **2** showing the lower frequencies region. Multiplets extracted at the ^{13}C chemical shifts from HSQC are: C-11 at 25.9 ppm, C-13 at 27.9 ppm, C-10 at 34.3 ppm, and C-12 at 38.9 ppm, The labeled peak (*) is due to moisture

Fig. 4. Comparison of the PERCH calculated (top) and the experimental (bottom) ^{1}H NMR spectra of **1b** in $CDCl_3$ at 750 MHz

Fig. 5. Comparison of the experimental 750 MHz ^{1}H NMR spectra of cholesteryl benzoate (**1b**, bottom) and (25*R*)-27-deuteriocholesterol (**1c**, top). Signal expansions for **1b** are shown in Fig. 4. The labeled peak (*) is due to moisture

Fig. 6. Comparison of the PERCH calculated (top) and the experimental (bottom) ^{1}H NMR spectra of **1c** in $CDCl_3$ at 750 MHz

Fig. 7. Benzoylation induced ^{1}H NMR chemical shifts (in ppm) for cholesterol

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Table 1. Chemical shifts (in ppm), coupling orders, and *J*-values (in Hz) of dihydroperezone (**2**)

^{1}H	δ	multiplicity	*J*
6	6.481	d	$^4J_{6,7}$ = 1.73
7	2.059	d	$^4J_{6,7}$ = 1.73
8	3.035	ddd	$^3J_{8,9}$ = 7.08, $^3J_{8,10R}$ = 6.67, $^3J_{8,10S}$ = 8.70
9	1.197	d	$^3J_{8,9}$ = 7.08
10*R*	1.518	dddd	$^3J_{8,10R}$ = 6.67, $^2J_{10R,10S}$ = -13.37, $^3J_{10R,11R}$ = 5.72, $^3J_{10R,11S}$ = 10.39
10*S*	1.711	dddd	$^3J_{8,10S}$ = 8.70, $^2J_{10R,10S}$ = -13.37, $^3J_{10R,11S}$ = 10.42, $^3J_{10S,11S}$ = 4.80
11*R*	1.237	ddddd	$^3J_{10R,11R}$ = 5.72, $^3J_{10R,11S}$ = 10.42, $^2J_{11R,11S}$ = -12.91, $^3J_{11R,12R}$ = 5.26, $^3J_{11R,12S}$ = 10.27
11*S*	1.136	ddddd	$^3J_{10R,11S}$ = 10.39, $^3J_{10S,11S}$ = 4.80, $^2J_{11R,11S}$ = -12.91, $^3J_{11S,12R}$ = 10.49, $^3J_{11S,12S}$ = 5.43
12*R*	1.140	dddd	$^3J_{11R,12R}$ = 5.26, $^3J_{11S,12R}$ = 10.49, $^3J_{12R,12S}$ = -13.52, $^3J_{12R,13}$ = 7.00
12*S*	1.129	dddd	$^3J_{11R,12S}$ = 10.50, $^3J_{11S,12S}$ = 5.42, $^3J_{12R,12S}$ = -13.52, $^3J_{12S,13}$ = 6.80
13	1.480	ddqq	$^3J_{12R,13}$ = 7.00, $^3J_{12S,13}$ = 6.80, $^3J_{13,14}$ = 6.57, $^3J_{13,15}$ = 6.63
14	0.822	d	$^3J_{13,14}$ = 6.57
15	0.832	d	$^3J_{13,15}$ = 6.63

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Table 2. Chemical shifts (in ppm), coupling orders, and *J*-values (in Hz) of cholesteryl benzoate (**1b**)

^{1}H	δ	multiplicity	*J*
1α	1.217	dddq	$^2J_{1\alpha,1\beta}$ = -13.53, $^3J_{1\alpha,2\alpha}$ = 3.77, $^3J_{1\alpha,2\beta}$ = 14.08, $^4J_{1\alpha,19}$ = -0.62
1β	1.918	ddd	$^2J_{1\alpha,1\beta}$ = -13.53, $^3J_{1\beta,2\alpha}$ = 3.45, $^3J_{1\beta,2\beta}$ = 3.72
2α	2.000	ddddd	$^3J_{1\alpha,2\alpha}$ = 3.77, $^3J_{1\beta,2\alpha}$ = 3.45, $^2J_{2\alpha,2\beta}$ = -12.64, $^3J_{2\alpha,3}$ = 4.42, $^4J_{2\alpha,4\alpha}$ = -1.89
2β	1.739	dddd	$^3J_{1\alpha,2\beta}$ = 14.08, $^3J_{1\beta,2\beta}$ = 3.72, $^2J_{2\alpha,2\beta}$ = -12.64, $^3J_{2\beta,3}$ = 11.54
3	4.863	dddd	$^3J_{2\alpha,3}$ = 4.42, $^3J_{2\beta,3}$ = 11.54, $^3J_{3,4\alpha}$ = 5.18, $^3J_{3,4\beta}$ = 11.50
4α	2.468	ddd	$^4J_{2\alpha,4\alpha}$ = -1.89, $^3J_{3,4\alpha}$ = 5.18, $^2J_{4\alpha,4\beta}$ = -12.78, $^4J_{4\alpha,6}$ = 0.60
4β	2.466	ddddd	$^3J_{3,4\beta}$ = 11.50, $^2J_{4\alpha,4\beta}$ = -12.78, $^4J_{4\beta,6}$ = 2.07, $^4J_{4,7\alpha}$ = 3.51, $^4J_{4\beta,7\beta}$ = 2.62
6	5.421	dddd	$^4J_{4\alpha,6}$ = 0.60, $^4J_{4\beta,6}$ = 2.07, $^3J_{6,7\alpha}$ = 1.98, $^3J_{6,7\beta}$ = 5.11, $^4J_{6,8}$ = -0.49
7α	1.568	ddd	$^4J_{4\beta,7\alpha}$ = 3.51, $^3J_{6,7\alpha}$ = 1.98, $^2J_{7\alpha,7\beta}$ = -17.65, $^3J_{7\alpha,8}$ = 10.41
7β	1.992	ddd	$^4J_{4\beta,7\beta}$ = 2.62, $^3J_{6,7\beta}$ = 5.11, $^2J_{7\alpha,7\beta}$ = -17.65, $^3J_{7\beta,8}$ = 5.37
8	1.475	ddddd	$^4J_{6,8}$ = -0.49, $^3J_{7\alpha,8}$ = 10.41, $^3J_{7\beta,8}$ = 5.37, $^3J_{8,9}$ = 11.09, $^3J_{8,14}$ = 10.88
9	0.995	dddd	$^3J_{8,9}$ = 11.09, $^3J_{9,11\alpha}$ = 4.61, $^3J_{9,11\beta}$ = 12.56, $^4J_{9,19}$ = -0.37
11α	1.530	dddd	$^3J_{9,11\alpha}$ = 4.61, $^2J_{11\alpha,11\beta}$ = -13.73, $^3J_{11\alpha,12\alpha}$ = 3.97, $^3J_{11\alpha,12\beta}$ = 2.94
11β	1.479	dddd	$^3J_{9,11\beta}$ = 12.56, $^2J_{11\alpha,11\beta}$ = -13.73, $^3J_{11\beta,12\alpha}$ =13.44, $^3J_{11\beta,12\beta}$ = 4.06
12α	1.184	dddd	$^3J_{11\alpha,12\alpha}$ = 3.97, $^3J_{11\beta,12\alpha}$ = 13.44, $^2J_{12\alpha,12\beta}$ = -12.74, $^4J_{12\alpha,18}$ = -0.63
12β	2.025	ddd	$^3J_{11\alpha,12\beta}$ = 2.94, $^3J_{11\beta,12\beta}$ = 4.06, $^2J_{12\alpha,12\beta}$ = -12.74
14	1.018	dddd	$^3J_{8,14}$ = 10.88, $^3J_{14,15\alpha}$ = 7.38, $^3J_{14,15\beta}$ = 12.80
15α	1.589	dddd	$^3J_{14,15\alpha}$ = 7.38, $^2J_{15\alpha,15\beta}$ = -12.40, $^3J_{15\alpha,16\alpha}$ = 9.81, $^3J_{15\alpha,16\beta}$ = 3.14
15β	1.078	dddd	$^3J_{14,15\beta}$ = 12.80, $^2J_{15\alpha,15\beta}$ = -12.40, $^3J_{15\beta,16\alpha}$ = 6.23, $^3J_{15\beta,16\beta}$ = 11.64
16α	1.840	dddd	$^3J_{15\alpha,16\alpha}$ = 9.81, $^3J_{15\beta,16\alpha}$ = 6.23, $^2J_{16\alpha,16\beta}$ = -13.47, $^3J_{16\alpha,17}$ = 9.17
16β	1.266	dddd	$^3J_{15\alpha,16\beta}$ = 3.14, $^3J_{15\beta,16\beta}$ = 11.64, $^2J_{16\alpha,16\beta}$ = -13.47, $^3J_{16\beta,17}$ = 9.64
17	1.107	ddd	$^3J_{16\alpha,17}$ = 9.17, $^3J_{16\beta,17}$ = 9.64, $^3J_{17,20}$ = 10.27
18	0.692	d	$^4J_{12\alpha,18}$ = -0.63
19	1.071	dd	$^4J_{1\alpha,19}$ = -0.62, $^4J_{9,19}$ = -0.37
20	1.381	dddq	$^3J_{17,20}$ = 10.27, $^3J_{20,21}$ = 6.60, $^3J_{20,22R}$ = 8.74, $^3J_{20,22S}$ = 2.58

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Table 2. Continuation …

^{1}H	δ	multiplicity	J
21	0.924	d	$^3J_{20,21}$ = 6.60
22*R*	1.004	dddd	$^3J_{20,22R}$ = 8.74, $^2J_{22R,22S}$ = -13.34, $^3J_{22R,23R}$ = 4.77, $^3J_{22R,23S}$ = 10.51
22*S*	1.344	dddd	$^3J_{20,22S}$ = 2.58, $^2J_{22R,22S}$ = -13.34, $^3J_{22S,23R}$ = 10.78, $^3J_{22S,23S}$ = 5.43
23*R*	1.339	ddddd	$^3J_{22R,23R}$ = 4.77, $^3J_{22S,23R}$ = 10.78, $^2J_{23R,23S}$ = -12.65, $^3J_{23R,24R}$ = 5.25, $^3J_{23R,24S}$ = 10.11
23*S*	1.153	ddddd	$^3J_{22R,23S}$ = 10.51, $^3J_{22S,23S}$ = 5.43, $^2J_{23R,23S}$ = -12.65, $^3J_{23S,24R}$ = 10.47, $^3J_{23S,24S}$ = 5.28
24*R*	1.005	dddd	$^3J_{23R,24R}$ = 5.25, $^3J_{23S,24R}$ = 10.47, $^2J_{24R,24S}$ = -13.17, $^3J_{24R,25}$ = 6.60
24*S*	1.144	dddd	$^2J_{23R,24S}$ = 10.11, $^3J_{23S,24S}$ = 5.28, $^2J_{24R,24S}$ = -13.17, $^3J_{24S,25}$ = 7.12
25	1.521	ddqq	$^3J_{24R,25}$ = 6.60, $^3J_{24S,25}$ = 7.12, $^3J_{25,26}$ = 6.64, $^3J_{25,27}$ = 6.65
26 (*pro-R*)	0.871	d	$^3J_{25,26}$ = 6.64
27 (*pro-S*)	0.867	d	$^3J_{25,27}$ = 6.65

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Table 3. Side chain coupling constant values (in Hz) for cholesteryl benzoate (**1b**), dihydroperezone (**2**), perezone (**4a**), *O*-methylperezone (**4b**), and 6-hydroxyperezone (**4c**)

J	**4a**[a]	**4b**[a]	**4c**[a]	**2**	**1b**	*J*
8,10*R*	8.87	8.63	9.29	8.70	8.78	20,22*R*
8,10*S*	6.50	6.68	6.67	6.67	2.58	20,22*S*
10*R*,10*S*	-13.21	-13.25	-13.49	-13.37	-13.34	22*R*,22*S*
10*R*,11*R*	5.97	6.07	5.52	5.72	4.47	22*R*,23*R*
10*R*,11*S*	9.37	9.37	9.40	10.39	10.51	22*R*,23*S*
10*S*,11*R*	9.41	9.47	9.05	10.42	10.78	22*S*,23*R*
10*S*,11*S*	6.23	6.17	6.20	4.80	5.43	22*S*,23*S*
11*R*,11*S*	-14.09	-14.33	-14.72	-12.91	-12.66	23*R*,23*S*
11*R*,12*R*	-	-	-	5.26	5.25	23*R*,24*R*
11*R*,12*S*	-	-	-	10.27	10.11	23*R*,24*S*
11*S*,12*R*	-	-	-	10.49	10.47	23*S*,24*R*
11*S*,12*S*	-	-	-	5.43	5.28	23*S*,24*S*
12*R*,12*S*	-	-	-	-13.52	-13.17	24*R*,24*S*

[a] From reference [15].

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Table 4. Comparison of HiFSA determined coupling constants for cholesteryl benzoate (**1b)** with the vicinal coupling constants calculated using Altona.

J	**Exp**	**Cal**	**Δ**
1*α*,2*α*	3.77	3.94	-0.17
1*α*,2*β*	14.08	13.35	+0.73
1*β*,2*α*	3.45	3.10	+0.35
1*β*,2*β*	4.06	3.78	+0.28
2*α*,3	4.42	4.48	-0.06
2*β*,3	11.54	10.89	+0.65
3,4*α*	5.18	4.72	+0.46
3,4*β*	11.50	10.89	+0.61
7*α*,8	10.41	11.41	-1.00
7*β*,8	5.37	5.62	-0.25
8,9	11.09	12.06	-0.97
9,11*α*	4.61	4.09	+0.52
9,11*β*	12.56	12.14	+0.42
11*α*,12*α*	3.97	4.22	-0.25
11*α*,12*β*	2.94	2.74	+0.20
11*β*,12*α*	13.44	12.95	+0.49
11*β*,12*β*	4.06	4.26	-0.20
14,15*α*	7.38	6.07	-1.31
14,15*β*	12.80	11.50	+1.30
15*α*,16*α*	9.81	11.49	-1.68
15*α*,16*β*	3.14	1.75	+1.39
15*β*,16*α*	6.23	5.92	+0.31
15*β*,16*β*	11.64	11.39	+0.25
16*α*,17	9.17	8.51	+0.66
16*β*,17	9.64	9.11	+0.53

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Table 5. Chemical shifts (in ppm), coupling orders, and *J*-values (in Hz) of (25*R*)-27-deuteriocholesterol (**1c**).

^{1}H	δ	multiplicity	*J*
1α	1.079	dddq	$^2J_{1\alpha,1\beta}$ = -13.40, $^3J_{1\alpha,2\alpha}$ = 3.79, $^3J_{1\alpha,2\beta}$ = 13.90, $^4J_{1\alpha,19}$ = -0.64
1β	1.848	ddd	$^2J_{1\alpha,1\beta}$ = -13.93, $^3J_{1\beta,2\alpha}$ = 3.38, $^3J_{1\beta,2\beta}$ = 3.79
2α	1.835	ddddd	$^3J_{1\alpha,2\alpha}$ = 3.79, $^3J_{1\beta,2\alpha}$ = 3.38, $^2J_{2\alpha,2\beta}$ = -12.31, $^3J_{2\alpha,3}$ = 4.41, $^4J_{2\alpha,4\alpha}$ = -2.43
2β	1.501	dddd	$^3J_{1\alpha,2\beta}$ = 13.90, $^3J_{1\beta,2\beta}$ = 3.79, $^2J_{2\alpha,2\beta}$ = -12.31, $^3J_{2\beta,3}$ = 11.58
3	3.525	dddd	$^3J_{2\alpha,3}$ = 4.41, $^3J_{2\beta,3}$ = 11.58, $^3J_{3,4\alpha}$ = 4.84, $^3J_{3,4\beta}$ = 11.20
4α	2.294	ddd	$^4J_{2\alpha,4\alpha}$ = -2.43, $^3J_{3,4\alpha}$ = 4.84, $^2J_{4\alpha,4\beta}$ = -13.03, $^4J_{4\alpha,6}$ = 0.67
4β	2.236	ddddd	$^3J_{3,4\beta}$ = 11.20, $^2J_{4\alpha,4\beta}$ = -13.03, $^4J_{4\beta,6}$ = 2.00, $^4J_{4\beta,7\alpha}$ = 3.47, $^4J_{4\beta,7\beta}$ = 2.78
6	5.352	dddd	$^4J_{4\alpha,6}$ = 0.67, $^4J_{4\beta,6}$ = 2.00, $^3J_{6,7\alpha}$ = 2.11, $^3J_{6,7\beta}$ = 5.20, $^4J_{6,8}$ = -0.47
7α	1.528	ddd	$^4J_{4\beta,7\alpha}$ = 3.47, $^3J_{6,7\alpha}$ = 2.11, $^2J_{7\alpha,7\beta}$ = -17.78, $^3J_{7\alpha,8}$ = 10.52
7β	1.972	ddd	$^4J_{4\beta,7\beta}$ = 2.78, $^3J_{6,7\beta}$ = 5.20, $^2J_{7\alpha,7\beta}$ = -17.78, $^3J_{7\beta,8}$ = 5.38
8	1.455	ddddd	$^4J_{6,8}$ = -0.47, $^3J_{7\alpha,8}$ = 10.52, $^3J_{7\beta,8}$ = 5.38, $^3J_{8,9}$ = 11.19, $^3J_{8,14}$ = 10.45
9	0.928	dddd	$^3J_{8,9}$ = 11.19, $^3J_{9,11\alpha}$ = 4.70, $^3J_{9,11\beta}$ = 12.53, $^4J_{9,19}$ = -0.36
11α	1.509	dddd	$^3J_{9,11\alpha}$ = 4.70, $^2J_{11\alpha,11\beta}$ = -13.68, $^3J_{11\alpha,12\alpha}$ = 4.06, $^3J_{11\alpha,12\beta}$ = 3.10
11β	1.458	dddd	$^3J_{9,11\beta}$ = 12.53, $^2J_{11\alpha,11\beta}$ = -13.68, $^3J_{11\beta,12\alpha}$ =13.59, $^3J_{11\beta,12\beta}$ = 4.00
12α	1.156	dddd	$^3J_{11\alpha,12\alpha}$ = 4.06, $^3J_{11\beta,12\alpha}$ = 13.59, $^2J_{12\alpha,12\beta}$ = -12.73, $^4J_{12\alpha,18}$ = -0.54
12β	2.012	ddd	$^3J_{11\alpha,12\beta}$ = 3.10, $^3J_{11\beta,12\beta}$ = 4.00, $^2J_{12\alpha,12\beta}$ = -12.74
14	0.988	dddd	$^3J_{8,14}$ = 10.45, $^3J_{14,15\alpha}$ = 6.75, $^3J_{14,15\beta}$ = 12.72
15α	1.574	dddd	$^3J_{14,15\alpha}$ = 6.75, $^2J_{15\alpha,15\beta}$ = -12.30, $^3J_{15\alpha,16\alpha}$ = 9.95, $^3J_{15\alpha,16\beta}$ = 3.04
15β	1.069	dddd	$^3J_{14,15\beta}$ = 12.72, $^2J_{15\alpha,15\beta}$ = -12.30, $^3J_{15\beta,16\alpha}$ = 6.26, $^3J_{15\beta,16\beta}$ = 11.73
16α	1.828	dddd	$^3J_{15\alpha,16\alpha}$ = 9.95, $^3J_{15\beta,16\alpha}$ = 6.26, $^2J_{16\alpha,16\beta}$ = -13.47, $^3J_{16\alpha,17}$ = 9.17
16β	1.258	dddd	$^3J_{15\alpha,16\beta}$ = 3.04, $^3J_{15\beta,16\beta}$ = 11.73, $^2J_{16\alpha,16\beta}$ = -13.47, $^3J_{16\beta,17}$ = 9.54
17	1.089	ddd	$^3J_{16\alpha,17}$ = 9.17, $^3J_{16\beta,17}$ = 9.54, $^3J_{17,20}$ = 10.22
18	0.679	d	$^4J_{12\alpha,18}$ = -0.54
19	1.009	dd	$^4J_{1\alpha,19}$ = -0.64, $^4J_{9,19}$ = -0.36
20	1.373	dddq	$^3J_{17,20}$ = 10.22, $^3J_{20,21}$ = 6.58, $^3J_{20,22R}$ = 8.54, $^3J_{20,22S}$ = 2.76

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^{1}H	δ	multiplicity	J
21	0.915	d	$^3J_{20,21}$ = 6.58
22*R*	0.995	dddd	$^3J_{20,22R}$ = 8.54, $^2J_{22R,22S}$ = -13.50, $^3J_{22R,23R}$ = 4.82, $^3J_{22R,23S}$ = 9.88
22*S*	1.336	dddd	$^3J_{20,22S}$ = 2.76, $^2J_{22R,22S}$ = -13.50, $^3J_{22S,23R}$ = 10.60, $^3J_{22S,23S}$ = 5.06
23*R*	1.331	ddddd	$^3J_{22R,23R}$ = 4.82, $^3J_{22S,23R}$ = 10.60, $^2J_{23R,23S}$ = -12.47, $^3J_{23R,24R}$ = 5.25, $^3J_{23R,24S}$ = 10.53
23*S*	1.146	ddddd	$^3J_{22R,23S}$ = 9.88, $^3J_{22S,23S}$ = 5.06, $^2J_{23R,23S}$ = -12.47, $^3J_{23S,24R}$ = 10.98, $^3J_{23S,24S}$ = 5.05
24*R*	1.093	dddd	$^3J_{23R,24R}$ = 5.25, $^3J_{23S,24R}$ = 10.98, $^2J_{24R,24S}$ = -13.15, $^3J_{24R,25}$ = 5.99
24*S*	1.139	dddd	$^2J_{23R,24S}$ = 10.53, $^3J_{23S,24S}$ = 5.05, $^2J_{24R,24S}$ = -13.15, $^3J_{24S,25}$ = 6.46
25	1.510	ddqq	$^3J_{24R,25}$ = 5.99, $^3J_{24S,25}$ = 6.46, $^3J_{25,26}$ = 6.64, $^3J_{25,27}$ = 6.84
26 (*pro-R*)	0.861	d	$^3J_{25,26}$ = 6.64,
27 (*pro-S*)	0.847	dd	$^3J_{25,27}$ = 6.84, $^2J_{HD}$ = 1.32

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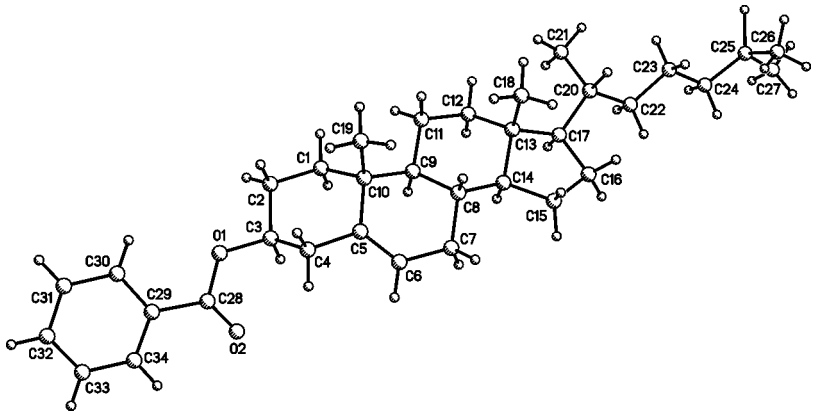

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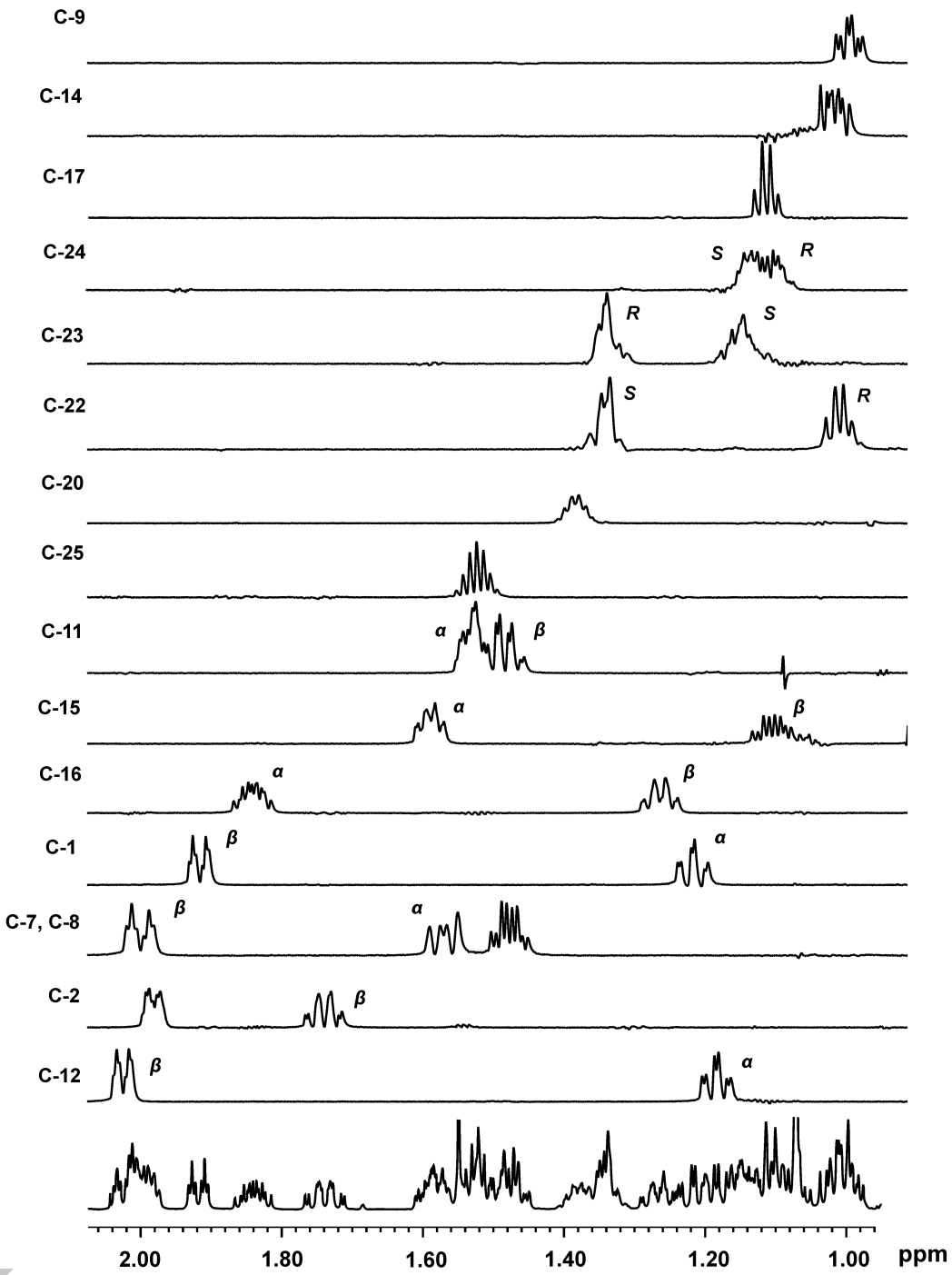

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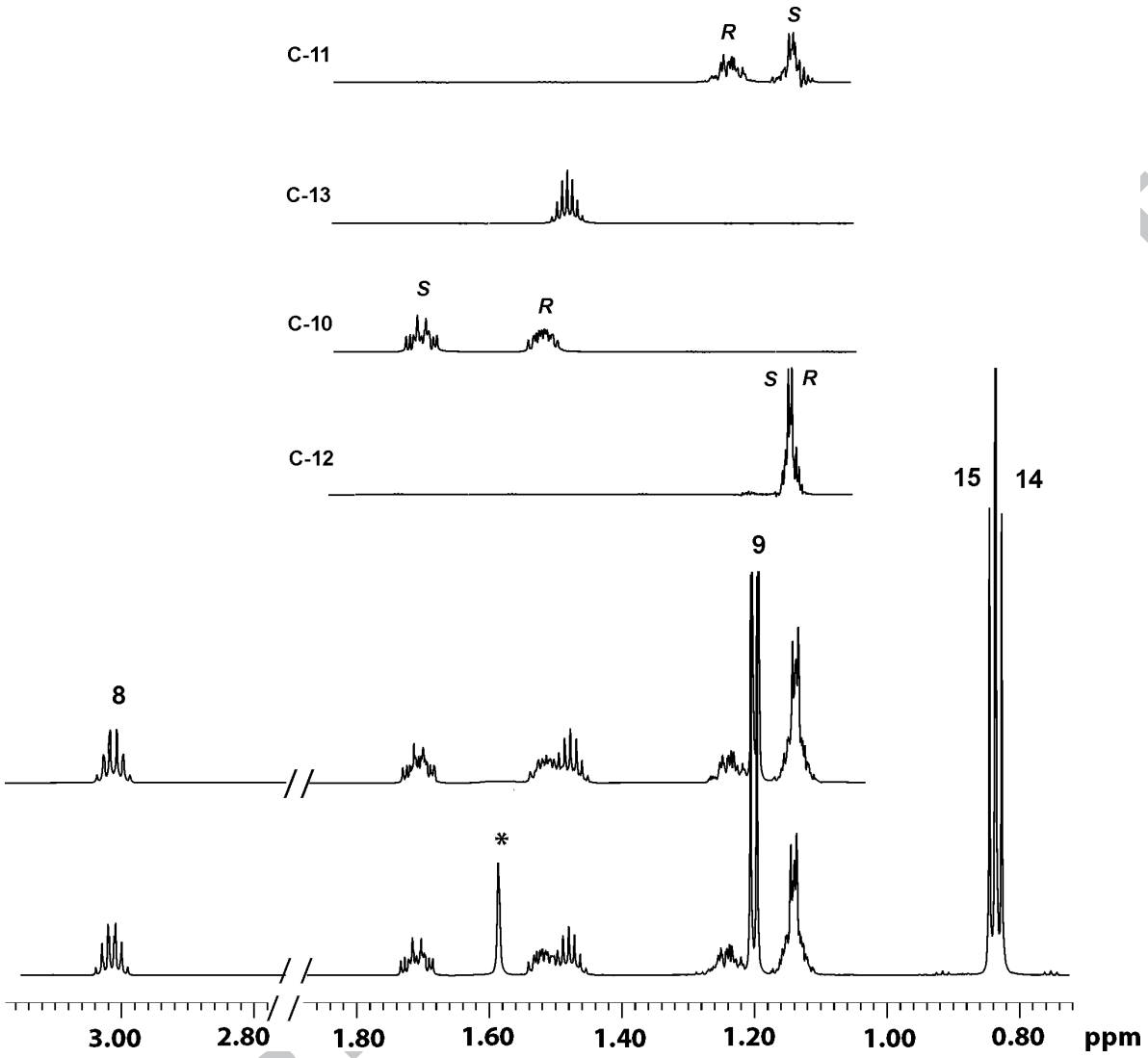

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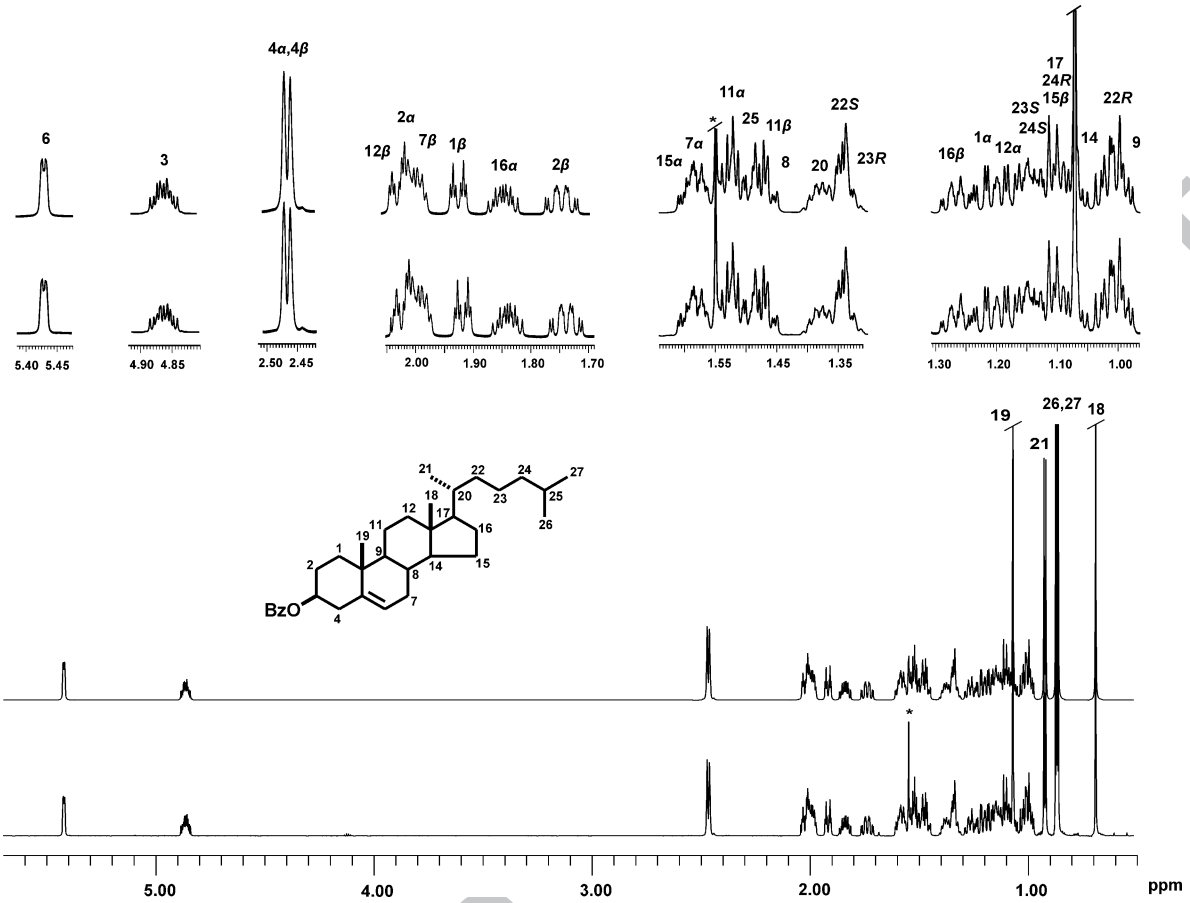

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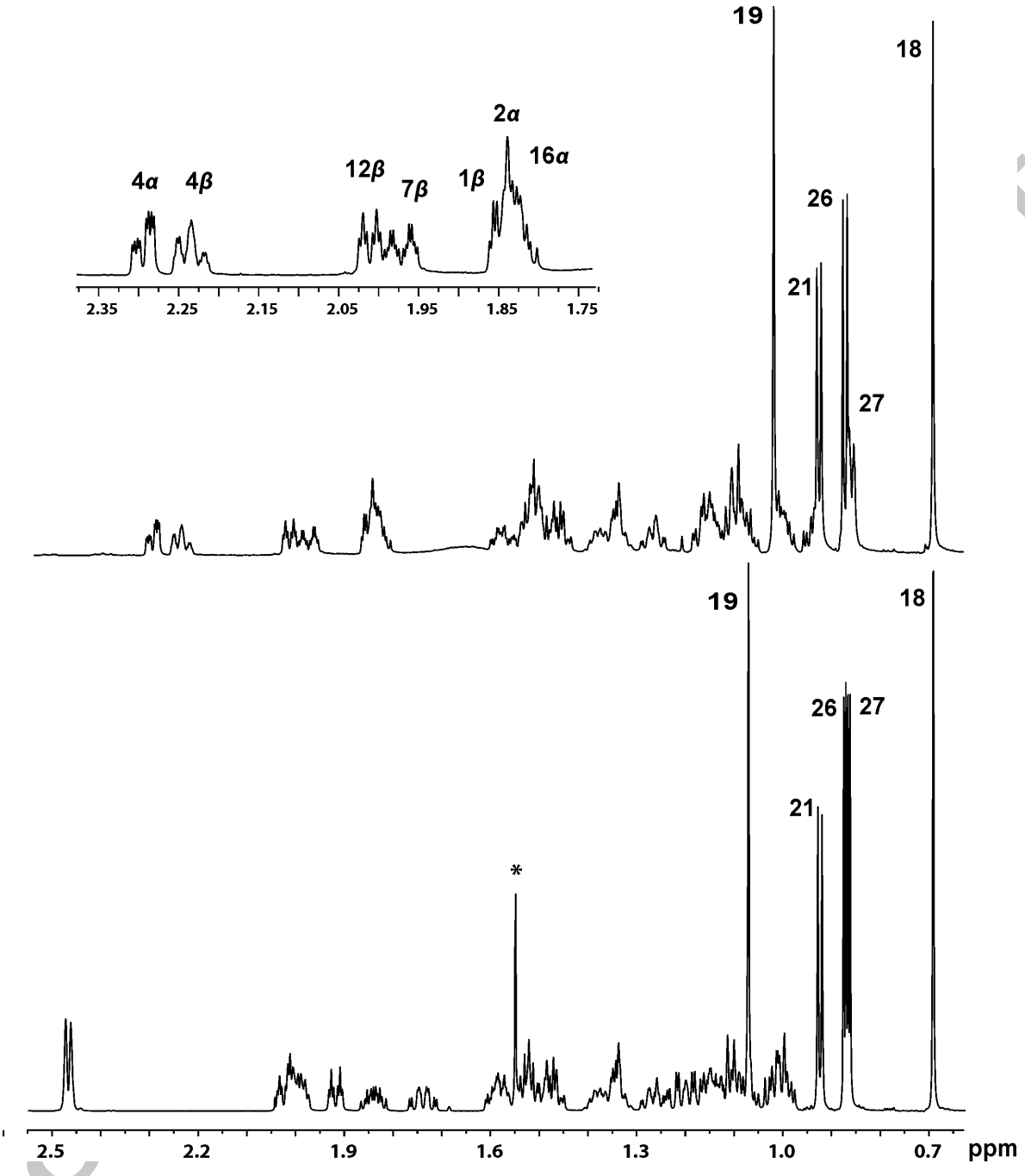

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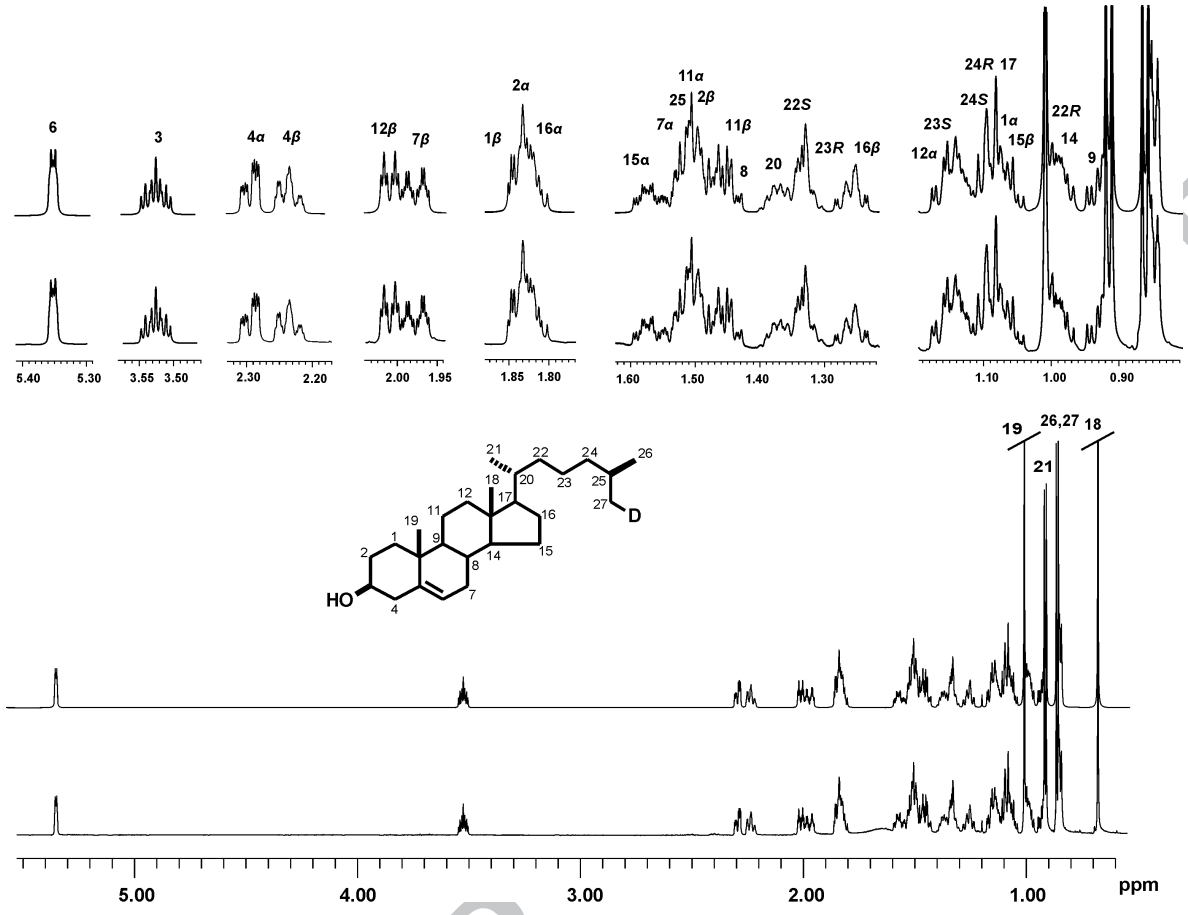

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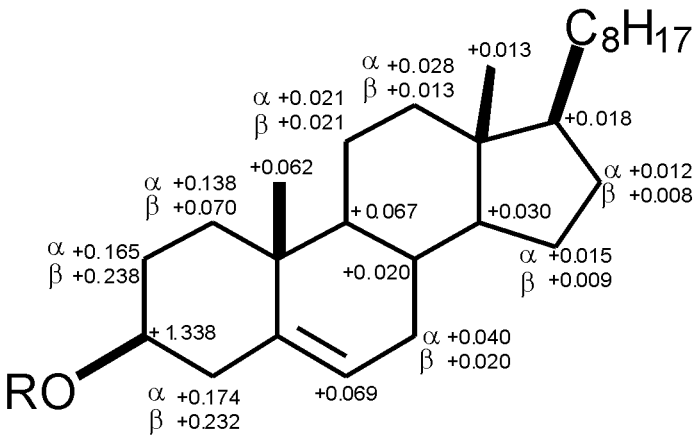

R = H or Bz

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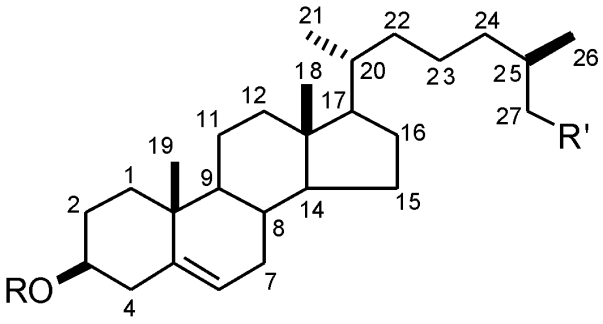
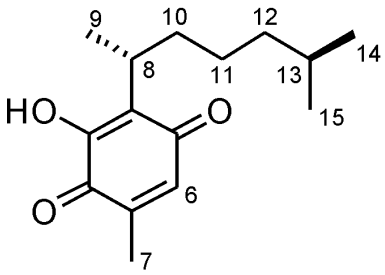


1a R = H, R' = H
1b R = Bz, R' = H
1c R = H, R' = D



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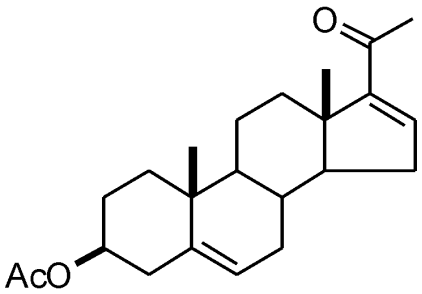

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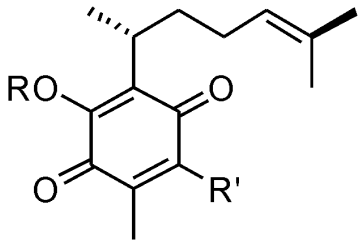

4a R = H, R' = H
4b R = Me, R' = H
4c R = H, R' = OH

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Highlights

- The total assignment of the ^{1}H NMR spectrum of cholesteryl benzoate is described.
- The data of dihydroperezone and of 3β-acetoxypregna-5,16-dien-20-one facilitated the task.
- Assignments were made extracting J_{HH} values from HSQC plots and using PERCH software.
- Experimental $^{3}J_{HH}$ and values calculated by ALTONA give excellent correlations.

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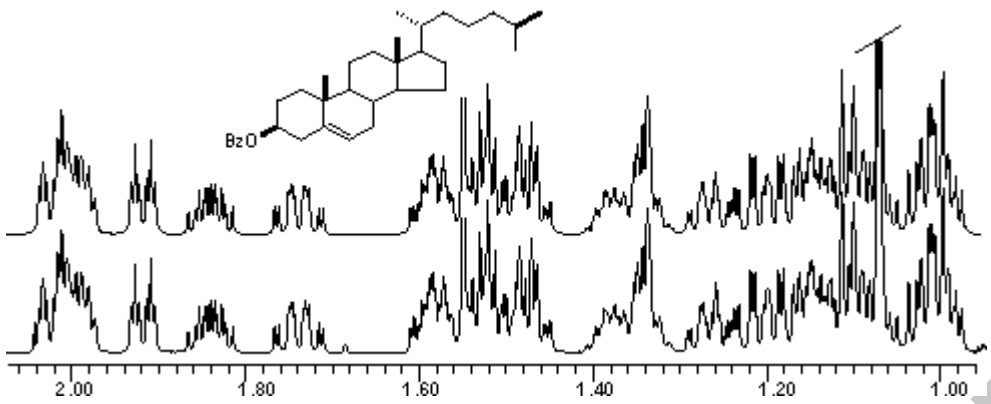

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