A short and efficient synthesis of heptadeuterated 2,2,4,4,5,7,7-d7-cholestane (1) from cholesterol (3) is described. The deuterated material will be useful for the analysis of different sources of petroleum in analytical geochemistry laboratories as internal standard for quantification of steranes via gas chromatography–mass spectrometry (GC–MS).

Keywords: total synthesis; steroids; deuterated standard.
Straightforward synthesis of 2,2,4,4,5,7,7-d7-cholestane

up in the usual way to produce the new chemical biomarker 2,2,4,4,5,7,7-d7-cholestane (1) in yield of 73% after purification by flash chromatography. This is the first synthesis of (1), which we believe will find wide use as a biomarker due to the ease of preparation and high degree of deuteration.

The same sequence of reactions was used to prepare the expensive, commercially available tetradeuterated cholestane (2) also in good yields (Scheme 3).

It is worth noting that the synthesis of deuterated cholestane structures 1 and 2 from cheap and easily available cholesterol (3) described herein can be used in environmental analysis laboratories to determine the source of spills, which sometimes occur during petroleum extraction and transportation.

CONCLUSIONS

Compound 1, a novel structure, presented on mass spectral analysis three specific peaks representing deuterated fragments from ring B (Scheme 4). This structural characteristic represents an additional advantage by eliminating the presence of spectral interferences which could be a problem with commercially available cholestane-d4 (2) currently used in quantitative studies of steranes from petroleum sources.

For the next step in our analytical work related to samples of petroleum, we will investigate the use of 2,2,4,4,5,7,7-d7-cholestane (1), in quantification studies of sterane compounds in matrices from several areas of petroleum production in Brazil.

Scheme 2. i) PCC (7.0 eq.), CH3Cl2, 83% (ref. 5); ii) H2, 70 psi, ethyl acetate, 100% (ref. 2); iii) NaOD/D2O, dioxane, 91%; iv) Tosyl-NHNH2, MeOH/NaBH4, MeOH, 86%

Scheme 3. i) PCC (1.0 eq.), CH3Cl2, 90% (ref. 2); ii) H2, 70 psi, ethyl acetate, 100% (ref. 5); iii) NaOD/D2O, dioxane, 93%; iv) Tosyl-NHNH2, MeOH/NaBH4, MeOH, 87%

Scheme 4. Masse of the main fragment ions from 2,2,4,4,5,7,7-d7-cholestane
EXPERIMENTAL

General experimental procedures

Melting points were determined in a Melt-Temp apparatus. $^1$H and $^{13}$C NMR spectra were obtained in GEMINI-200MHZ spectrometer with Me$_2$Si ($\delta = 0.00$) as internal standard using as solvents CDCl$_3$ or MeOD. The abbreviations used to describe multiplicity of the signals are s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet). Chemical shifts were expressed as $\delta$ values (parts per million) utilizing tetramethylsilane as reference. Low resolution mass spectra were obtained in an Auto Specq, in the EI mode at 70 eV and high resolution spectra using a Varian MAT-CH7 instrument at 70 eV. High-resolution mass spectra were taken on an ATLAS MS-12, Consolidated 12-110 B, and FINNEGAN 400 mass spectrometers at 70 eV. All chemicals and solvents commercially available were purchased from Aldrich Co. (USA) or TEDIA-Brazil.

4-cholesten-3,6-diene (4)

A continuously stirred suspension of PCC (0.75 g, 3.50 mmol) in DCM (10 mL) was added to cholesterol (3) (0.193 g, 0.50 mmol) in DCM (10 mL). This reaction mixture was stirred under nitrogen at room temperature for 24 h after which it was filtered through a layer of silica gel and washed with diethyl ether (5 × 20 mL). The solvent was evaporated to give a crude product which was further purified by flash chromatography on silica gel column using as eluent a mixture of hexanes and ethyl acetate (4:1) affording the 4-cholesten-3,6-diene (4) in a yield of 0.1865 g, (83%) as a pale yellow solid; mp: 116 °C.

IR (KBr) 1715, 1691, cm$^{-1}$

HRMS (EI) m/z calcd. for C$_{31}$H$_{40}$O: 390.3800; found: 390.3786.

Anal. Calcd. for C$_{31}$H$_{39}$O; C, 81.85; H, 10.62. Found C, 81.25; H, 10.59.

3,6-cholestan-4-ol (5)

For the hydrogenation reaction, 4-cholesten-3,6-dione (4, 0.208 g) or 3-cholestanone (2, 0.025 g, 6.46 X 10$^{-2}$ mmol) was added to anhydrous sodium carbonate (0.20 g) in DCM (10 mL). The reaction mixture was stirred under nitrogen at 70 °C for 24 h after which it was filtered through a layer of silica gel and washed with diethyl ether (5 × 20 mL). The solvent was evaporated to give a crude product which was further purified by flash chromatography on silica gel column using as eluent a mixture of hexanes and ethyl acetate (4:1) affording the 3,6-cholestan-4-ol (5) in quantitative yield (0.024 g) or 3-cholestanone (2, 0.025 g) as a white solid; mp: 169 and 129 °C respectively.

3,6-cholestan-2,2,4,4,5,7,7-d$_7$ (6) and 3-cholestanone-2,2,4,4-d$_6$ (9)

A 25 mL round-bottomed flask equipped with a reflux condenser was charged with 3,6-cholestanone (5, 0.0259 g, 6.46 X 10$^{-2}$ mmol) or (8, 0.025 g, 6.46 X 10$^{-2}$ mmol) in 5 mL of dioxane. To this solution, 0.5 mL of 10% NaOD in D$_2$O was added. The reaction mixture was refluxed for 36 hours and cooled to room temperature after which the solvent was removed under reduced pressure. The residue was dissolved in diethyl ether and washed successively with water. The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography using as eluent ethyl acetate and hexanes (1:9) giving 3,6-cholestan-2,2,4,4,5,7,7-d$_7$ (6) in a 91% yield (0.024 g) or 3-cholestanone-2,2,4,4-d$_6$ (9), in a 93% yield, (0.023 g) both as white solids; mp: 169 and 129 °C respectively.

IR (KBr) 2183 $\nu_{\text{C=O}}$ 1715, 1691, cm$^{-1}$

HRMS (EI) m/z calcd. for C$_{31}$H$_{39}$D$_{21}$O: 390.3800; found: 390.3786.

Anal. Calcd. for C$_{31}$H$_{39}$D$_{21}$O; C, 79.54; H, 11.07. Found: C, 80.81; H, 11.03.

3,6-cholestan-2,2,4,4,5,7,7-d$_7$-cholestanone (1)

A 25 mL round-bottomed flask was charged with 3,6-cholestan-2,2,4,4,5,7,7-d$_7$ (6, 0.0701 g, 0.17 mmol) or (9, 3-cholestanone-2,2,4,4-d$_6$, 0.0663 g, 0.17 mmol) or tosylhydrazide (0.090 g, 209.2 (C-3), 211.4 (C-6).

MS (EI, 70 eV): m/z (%) = 137 (20), 245 (100), 262 (15), 287 (60), 386 (25), 400 (100 [M$^+$.])

HRMS (EI) m/z calcd. for C$_{31}$H$_{40}$O$_2$: 400.3341; found: 400.3337.

Anal. Calcd. for C$_{31}$H$_{39}$O$_2$; C, 80.94; H, 11.07. Found: C, 80.81; H, 11.03.

IR (KBr) 2183 $\nu_{\text{C=O}}$ 1715, 1691, cm$^{-1}$

HRMS (EI) m/z calcd. for C$_{31}$H$_{39}$D$_{21}$O$_2$: 407.3781; found: 407.3786.

Anal. Calcd. for C$_{31}$H$_{39}$D$_{21}$O$_2$; C, 79.54; H, 12.61. Found C, 79.29; H, 12.59.

3,6-cholestan-2,2,4,4-d$_6$ (9)

IR (KBr) 2201 $\nu_{\text{C=O}}$ 1715 cm$^{-1}$

HRMS (EI) m/z calcd. for C$_{31}$H$_{39}$D$_{21}$O$_2$: 390.3800; found: 390.3803.

2,2,4,4,5,7,7-d$_7$-cholestanone (1)
Straightforward synthesis of 2,2,4,4,5,7,7-d7-cholestane

Comptes Rendus de l’Academie des Sciences, Serie IIc: Chimie

IR (KBr) 2183 ν(C–D), 1670 cm⁻¹.

1H NMR (200 MHz, CDCl₃): δ = 0.81-2.37 (m, 35H), 0.72 (s, 6H).

13C NMR (50 MHz, CDCl₃): δ = 12.3 (C-18), 12.4 (C-19), 18.9 (C-21), 21.0 (C-11), 22.2 (C-2), 22.7 (C-26 e C27), 23.0 (C-23), 24.0 (C-15), 24.4 (C-3), 28.2 (C-25), 28.4 (C-16) 29.3 (C-6 e C-4), 32.4 (C-7), 35.7 (C-8) 36.0 (C-20), 36.4 (C-10 e C-22), 38.8 (C-1), 39.7 (C-24), 40.3 (C-12), 42.8 (C-13), 47.0 (C-5), 55.0 (C-9), 56.5 (C-17), 56.8 (C-14).

MS (EI, 70 eV): m/z (%) = 156 (30), 224 (100), 262, (50), 362 (8).

HRMS: m/z calcd. for C₅₃H₄₁D₁₇: 376.4007; found: 376.4009.

Acknowledgment

The authors gratefully acknowledge financial support from FAPERJ, CNPq and CENPES-PETROBRAS for the Master Science fellowship to M. G. de Miranda.

Supplementary Material

The 1H-NMR, 13C-NMR and mass spectra for compounds 1, 4, 5 and 6 are available at http://quimicanova.sbq.org.br, in PDF file, with free access.

References