Synthesis and crystal structure of [26,27-$^2H_6$] 24-*epi*-cathasterone

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The first synthesis of [26,27-$^2H_6$]24-*epi*-cathasterone **8** *via* (20*S*)-3β-acetoxy-6,6-(ethylenedioxy)-20-formyl-5α-pregnane **5** starting from stigmasterol is described. The aldehyde **5** was alkylated with lithium butyldimethyl-(*E*)-2,3-dimethyl[3,3,3,4,4,4-$^2H_6$]butenylaluminate **6** prepared from 3-[$^2H_3$]methyl[4,4,4-$^2H_3$]but-1-yne. The structure was determined using spectral data and X-ray crystallographic analysis.

Introduction

The brassinosteroids are steroidal phytohormones ubiquitously occurring in the plant kingdom, exhibiting high growth promoting and antistress activity.[1] Cathasterone ((22*S*,24*R*)-3β,22-dihydroxy-5α-ergostan-6-one) was shown to be a biosynthetic precursor of brassinolide in cultured cells of *Catharanthus roseus*[2] by feeding experiments with deuterium-labelled cathasterone. We synthesised deuterium-labelled 24-*epi*-cathasterone ([26,27-$^2H_6$](22*S*,24*S*)-3β,22-dihydroxy-5α-ergostan-6-one) as the corresponding precursor in the analogously naturally occurring 24-*epi*-brassinosteroid series.[3,4] Here we report the first synthesis of [26,27-$^2H_6$]*epi*-cathasterone **8** *via* (20*S*)-3β-acetoxy-6,6-(ethylenedioxy)-20-formyl-5α-pregnane **5** starting from stigmasterol. The intermediate aldehyde **5** was alkylated with lithium butyldimethyl-(*E*)-2,3-dimethyl[3,3,3,4,4,4-$^2H_6$]-butenylaluminate **6**. [26,27-$^2H_6$]24-*epi*-cathasterone was needed for biosynthetic studies and also for the determining of the endogenous level of 24-*epi*-cathasterone in plants.

Results and discussion

For the synthesis of [26,27-$^2H_6$]24-*epi*-cathasterone **8** stigmasterol was used as the starting material. Our synthetic route is shown in Scheme 1. At first (22*E*,24*S*)-3β,5-cyclo-24-ethylcholest-22-en-6-one **1** was prepared from stigmasterol as described by Lichtblau.[5] Then the cyclopropane ring was opened with AcOH and H_2SO_4 to give (22*E*,24*S*)-3β-hydroxy-24-ethyl-5α-cholest-22-en-6-one in a similar manner as Aburatani *et al.*[6] had described for the preparation of (22*R*,23*R*,24*S*)-3β,22,23-triacetoxy-5α-ergostan-6-one. In the next steps the hydroxy and the keto groups were protected as acetoxy and ethylenedioxy, respectively.[7] Then the double bond in the side chain was oxidised with OsO_4. The obtained 22,23-diole **4** was cleaved with H_5IO_6 in presence of a small amount of pyridine to prevent the extensive loss of the protecting group at the keto group.[8] The desired aldehyde **5** was obtained in 84.2% yield of the diol. The alkylation of the aldehyde with lithium butyldimethyl-(*E*)-2,3-dimethyl[3,3,3,4,4,4-$^2H_6$]butenylaluminate **6** gave after purification by flash chromatography on SiO_2 and by preparative HPLC 21% of the desired (22*R*)-allylic alcohol **7** ([26,27-$^2H_6$]3β-acetoxy-6,6-(ethylenedioxy)-22*R*-hydroxy-24-methyl-5α-cholest-23-ene). This (22*R*)-allylic alcohol was catalytically hydrogenated using 10% Pt on carbon as a catalyst. In the next two steps the protecting groups were removed. The dioxolane-type acetal was cleaved at first by transacetalisation[9] and then the acetyl-group from the 3β-position was removed with NaOMe. In the next step the obtained crude [26,27-$^2H_6$]24-*epi*-cathasterone was purified by flash chromatography (SiO_2, EtOAc–*n*-hexane 1 : 1 to 7 : 3 v/v) and preparative HPLC (octadecylsilane (ODS), MeCN–H_2O 95 : 5). 3.5 mg purified [26,27-$^2H_6$]24-*epi*-cathasterone **8** was obtained, 14.9% from the [26,27-$^2H_6$]3β-acetoxy-6,6-(ethylenedioxy)-22*R*-hydroxy-24-methyl-5α-cholest-23-ene **7**.

Table 1 Selected torsion angles τ/° for [26,27-$^2H_6$]24-*epi*-cathasterone

Atoms	Molecule A	Molecule B
C(13)–C(17)–C(20)–C(21)	−54.9(11)	−50.7(12)
C(13)–C(17)–C(20)–C(22)	−179.0(8)	−177.9(9)
C(17)–C(20)–C(22)–C(23)	178.5(8)	−163.9(8)
C(17)–C(20)–C(22)–O(3)	55.3(9)	71.4(10)
C(20)–C(22)–C(23)–C(24)	169.5(8)	−173.8(9)
C(22)–C(23)–C(24)–C(25)	151.9(9)	−177.1(9)
C(22)–C(23)–C(24)–C(28)	−81.6(12)	−51.4(13)
C(23)–C(24)–C(25)–C(26)	−69.9(13)	−53.6(12)
C(23)–C(24)–C(25)–C(27)	56.6(14)	69.0(13)

The deuteriation rate of the labelled 24-*epi*-cathasterone amounts to 86%. In the NMR-spectra of the deuteriated compounds we therefore found small signals for the positions 26 and 27 due to non-labelled compounds.

The deuterium labelled 24-*epi*-cathasterone is suitable for biosynthetic studies and for the determining of the endogenous levels of 24-*epi*-cathasterone in plants because a sufficient amount of 6 H-atoms is exchanged.

The structure of the [26,27-$^2H_6$]24-*epi*-cathasterone was confirmed by X-ray crystallographic analysis (Fig. 1 and 2, Table 1 and 2) and NMR (Table 3). The spectral data (MS, ^1H NMR and ^{13}C NMR) of the intermediate compounds and of the [26,27-$^2H_6$]24-*epi*-cathasterone are in agreement with the given structures.

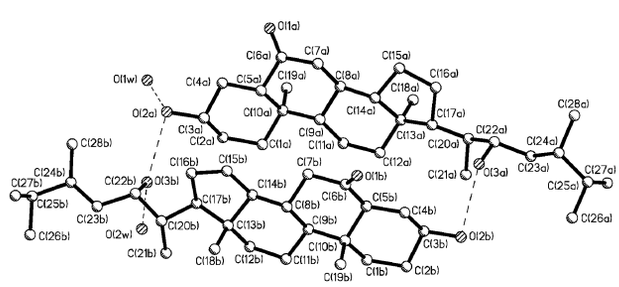

Fig. 1 Structure of the two molecules in the unit cell of [26,27-$^2H_6$]24-*epi*-cathasterone with atom labelling scheme (H atoms have been omitted for clarity, O–H ··· O bridges shown as broken lines connecting the donor and acceptor O atoms).[16]

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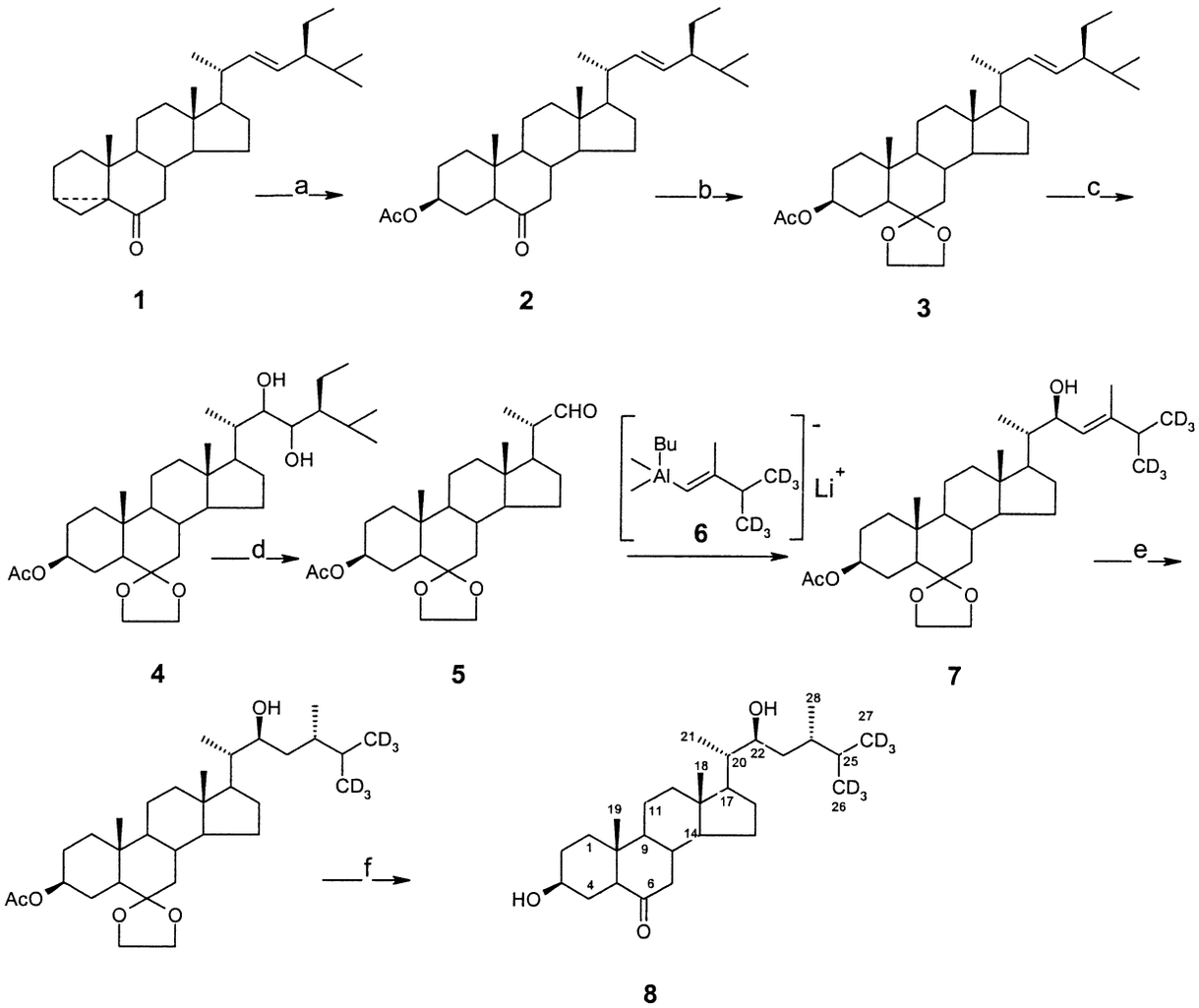

Scheme 1 a: AcOH, H_2SO_4, Py–$(Ac)_2O$; b: 2,2-dimethyl-1,3-dioxolane, *p*TsOH; c: OsO_4; d: H_5IO_6, $NaHCO_3$; e: 10% Pt/C; f: *p*-Ts-pyridine.

X-Ray †

[26,27-$^2H_6$]24-*epi*-Cathasterone crystallizes in the triclinic space group *P*1 with two steroid and two crystal water molecules per unit cell, *i.e.* the two molecules are symmetrically independent (Fig. 1). Both of them show the expected bond lengths and angles, which are in good agreement in the range of the standard uncertainties with one another and also with that of similar steroid molecules like 3-acetyl-22,24-di-*epi*-cathasterone.[10] ‡ Besides effects caused by the different constitution (torsion angles including O(2) and absolute configuration of C(22) of the reference molecule) and by disorder (O(1) in molecule A, which could not be resolved because of the poor quality of data), considerable conformational variations are restricted to the side chain of the steroid molecule (see Table 1). While these chains (C(20) to C(25)) are essential in the *all-trans* form for both molecules A and B of the title compound (all torsion angles are in the anti-periplanar range of conformation, *cf.* Fig. 1), the reference molecule shows different partial conformations, readily explainable by packing effects. The conformational differences between the chains of molecules A and B are also due to their arrangement within the crystal and the corresponding intermolecular interactions.

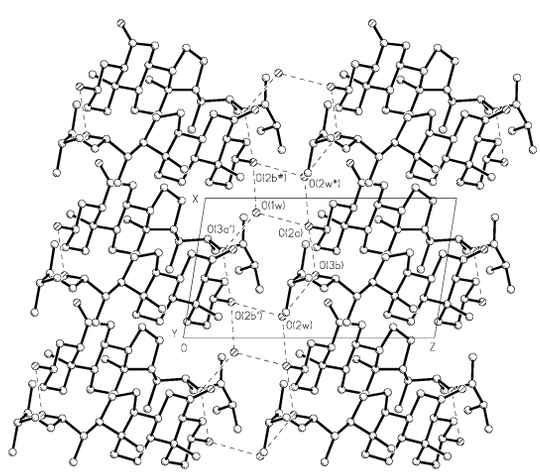

Fig. 2 Crystal packing of [26,27-$^2H_6$]24-*epi*-cathasterone in a projection along the crystallographic *y*-axis showing the O–H ⋯ O bridging system as broken lines connecting the donor and acceptor O atoms (symmetry relationships for atoms labelled a, b, w ⟶ *x*, *y*, *z*; a′, b′ ⟶ *x*, 1 + *y*, −1 + *z*; w* ⟶ 1 + *x*, *y*, *z*; b# ⟶ 1 + *x*, 1 + *y*, −1 + *z*).[16]

The head-to-tail packing of a pair of neighbouring molecules A and B is fixed by two H bonds between the hydroxylic groups at C(3) of one molecule and C(22) of the other molecule, respectively (*cf.* Fig. 1), including two of the four H-donor functions of the pair of molecules. Such pairs are stacked by translation along the crystallographic *x*-axis. To make full use of their H-bonding potential in the packing would probably lead to steric problems in the sense described for the case of

† CCDC reference number(s) 183401. See http://www.rsc.org/suppdata/p1/b2/b203323b/ for crystallographic files in .cif or other electronic format.

‡ The molecular structure presented in[10] and the corresponding data in the Cambridge Structural Database[11] show the compound to be (22*R*,24*S*)-3β-acetoxy-22-hydroxy-5α-ergostan-6-one (3-acetyl-22,24-di-*epi*-cathasterone), not 3-acetyl-24-*epi*-cathasterone as given in Ref. 10.

Table 2 Geometrical H-bond parameters for [26,27-$^2H_6$]24-*epi*-cathasterone. (Labels and the appropriate symmetry operators correspond to Fig. 1 and 2)

Atoms D–H ··· A	Distance/Å D–H	H ··· A	D ··· A	Angle/° D–H ··· A
O(2a)–H(2ao) ··· O(3b)	0.820	1.990	2.773(8)	159.4
O(3a′)–H(3ao′) ··· O(1w)	0.820	1.965	2.778(9)	171.0
O(2b′)–H(2bo′) ··· O(3a′)	0.820	2.079	2.854(9)	157.7
O(3b)–H(3bo) ··· O(2w)	0.820	2.045	2.824(9)	158.6
O(1w)–H(1w1) ··· O(2a)	0.960	1.943	2.871(9)	161.9
O(1w)–H(2w1) ··· O(2b$^{\#}$)	0.960	2.058	3.015(8)	174.1
O(2w)–H(1w2) ··· O(2b)	0.960	1.993	2.877(10)	152.2
O(2w*)–H(2w2*) ··· O(2a)	0.960	2.160	3.088(9)	162.2

Table 3 ^{1}H NMR data (δ, multiplicity, coupling constants/Hz) for protons and ^{13}C chemical shifts for carbons of [26,27-$^2H_6$]24-*epi*-cathasterone

Position	δ_{13C}	$\delta_{1H}\alpha/\beta$ [a]
1	36.6	1.25/1.78
2	30.7	1.85/1.40
3	70.7	3.581 dddd(11.3/11.3/4.6/4.6)
4	30.0	1.90/1.48
5	56.7	2.217 dd (12.5/2.6)
6	210.9	–
7	46.7	1.966 dd (13.2/13.2) α 2.324 dd (13.2/4.6) β
8	37.9	1.79
9	53.8	1.24
10	40.9	–
11	21.5	1.62/1.35
12	39.4	1.26/2.029 ddd (12.6/3.0/3.0)
13	42.8	–
14	56.6	1.28
15	23.9	1.53/1.102 dddd (12.0/12.0/12.0/6.2)
16	27.6	1.95/1.33
17	52.7	1.50
18	11.9	0.677 s
19	13.1	0.759 s
20	41.4	1.36
21	11.8	0.917 d (6.8)
22	71.1	3.745 ddd (9.4/3.2/1.5)
23	40.3	1.55 A 1.010 ddd (13.7/9.6/3.3) B
24	34.9	1.48
25	32.1	1.52
26	20.0 [b]	–; 0.872 d (6.8) [b]
27	17.8 [b]	–; 0.823 d (6.8) [b]
28	15.1	0.816 d (6.7)

[a] Values without multiplicity are chemical shifts of the GHSQC. [b] Signal of the residual non deuteriated compound

monoalcohols by Brock and Duncan.[12] Thus, the stacks are held together by hydrogen bonds to the crystal water molecules (*cf.* Fig. 2) stabilizing the packing as a "gluing factor" as reported by Krygowski *et al.*[13] Neighbouring stacks in [01–1] direction as shown in Fig. 2 are connected by additional H bonds to the water molecules forming hydrogen-bonded sheets parallel to the crystallographic (011) plane.

The hydrogen-bond system itself may be described as ribbon-like, consisting of alternating fused 4- and 6-O atom rings including all of the six O and the eight H atoms of the hydroxylic groups and the water molecules within the structure. For an exact determination of the hydrogen-bonding pattern, the actual H-atom positions should be known. The current structure analysis allows only a (not uniquely definable) localization by geometrical considerations, but the topology of the H-bond system requires, besides their H-donor function, the O(2) atoms of both molecules to act as double acceptors, but the O(3) atoms as well as the water-O atoms as acceptors for one H bond only. Judging from the donor–acceptor distances (Table 2), two groups of moderate H bonds may be distinguished, one with the six smaller distances within the 6-O atom ring and the other one containing the two O(2) ··· O(w) bridges between two adjacent 6-O atom rings with significantly longer O ··· O distances, thus forming the 4-O atom ring of the ribbon.

NMR

Structure elucidation and unambiguous assignment of ^{1}H and ^{13}C NMR signals of **8** were done by means of one- and two-dimensional NMR experiments including one-bond (GHSQC) and multiple-bond (GHMBC) ^{1}H, ^{13}C shift correlation. The position of the CD_3 groups was verified by HMBC correlation of the residual proton signal of Me-26 and Me-27 (δ 0.872 d (6.8 Hz); δ 0.823 d (6.8 Hz), relative intensity each *ca.* 0.45 H). Both ^{1}H doublets show correlations with C-24 and C-25. The ^{13}C chemical shift of the C-25 HMBC correlation peak belonging to the residual proton signals of Me-26/Me-27 is downfield shifted by 0.4 ppm in comparison to the C-25 HMBC correlation peak belonging to the proton signals H-23A/H-23B/Me-28 of the deuteriated compound. This can be explained by the isotope effect of the six ^{2}H atoms on C-25 in **8**.

The ^{1}H and ^{13}C NMR data of **8** are summarized in Table 3.

Infrared and Raman spectra

Although both techniques do not exhibit the potential to elucidate the structure of the molecules independently of other procedures, they may give further characterisation, particularly on molecular interactions. Therefore we have incorporated these techniques into our study and the results are given below.

The IR spectrum shows some dominant bands as given in the Experimental section. On cooling additional features become significant. Thus the OH region shows 3 strong overlapping bands, namely (after using the fitting procedure) at ν/cm^{-1} 3333, 3233, and 3146, whereas the band near 3407 cm^{-1} disappears in the background and may be assigned as traces of water in KBr. The band near 3333 cm^{-1} shows a large half width of *ca.* 200 cm^{-1}, double of each of both other bands. Moreover a broad band appears with decreasing temperature at 850 cm^{-1} ($\nu_{1/2}$ = *ca.* 200 cm^{-1}).

As it was to be expected, the Raman spectrum is extremely rich in sharp lines. All in all, the Raman spectrum appears very well-resolved, whereas the IR spectrum does not exhibit similar sharp bands probably due to the influence of hydrogen bonding.

Because of the manifoldness of similar C–C groups their vibration frequencies are nearly the same. An analogous situation is likely to happen for the CH groups. Therefore we could not expect to find sufficiently characteristic features in the IR spectrum to deduce the given structure of the compound. Nevertheless, the well-performed deuteriation and the existence of the C=O group are as obvious as the band near 1058 cm^{-1}, probably representing the C–OH bond. The large difference between the CO vibration of Raman spectra and IR band of 7 cm^{-1} should be mentioned. But there should exist some information on hydrogen bonding. The three bands, presented

in the OH region at liquid nitrogen temperature, which surpass on cooling all other bands in intensity, may be taken as an indication of different species of hydrogen bonding. As we learn from the X-ray data the donor acceptor distances (oxygen–oxygen) are different in the case of water, and additionally, both water molecules differ in A–D distances up to 0.3 Å. Similar relationships are valid for the C–OH groups, which also form long distance (3.015 Å) and short distance (*ca.* 2.8 Å) hydrogen bonds. The large distances are to be found in the four membered ring. In the six membered ring the donor–acceptor distances between 2.77 and 2.87 Å are similar. Taking the distances as the deciding factor for the similarity of bond strength, then a remarkable coupling should exist between the vibrations of the six membered ring but the longer oxygen–oxygen distance in the four membered ring should lead to a higher frequency of the OH-vibration. Therefore we assign the high frequency OH-band to the vibration of the long distance interactions in the four membered ring (O(2w*)–O(2a) and O(2b#)–O(1w)). The difference in these bond lengths (3.088 Å and 3.015 Å) may lead to two bands overlapping each other and forming the unresolved broad band near 3333 cm^{-1}. The coupling between the other bonds belonging to the COH group as well as to a water molecule should lead to the rather narrow bands near ν/cm^{-1} 3233 and 3146, exhibiting rather similar half widths of *ca.* 100 cm^{-1}. The band near 850 cm^{-1} represents an overlap of the different deformation frequencies of the hydrogen bonds. The sharpness and the position of the C=O band and its existence in the Raman spectrum denies it taking part in the hydrogen bond mechanism. All in all, the steric relations determine this rather special structure.

In spite of its excellent quality in the Raman spectrum there are too many well-resolved lines between 1400 cm^{-1} and 500 cm^{-1}, namely 42 (17 of these with similar intensities in the order of magnitude of that of the CO line) for us to discuss. Only the region 2050 cm^{-1} to 2230 cm^{-1} gives a well understandable combination of lines, proving the well-performed deuteriation.

Experimental

Methods and materials

All commercial reagents were used without further purification.

For flash column chromatography Merck silica gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM) was used. Melting points (uncorrected) were determined on a Boetius heating table.

High resolution EI-MS was obtained from a MasSpec of the firm Micromass. EIMS (DIS): 70 eV, AMD 402 (AMD Intectra.), positive ion ESI mass spectra.

[26,27-$^{2}H_6$](22*S*,24*S*)-3β,22-Dihydroxy-5α-ergostan-6-one was recrystallized from MeOH–water mixture to give comparatively small single crystals suitable for X-ray crystal structure analysis. A crystal with dimensions 0.27 × 0.18 × 0.07 mm was mounted on a Stoe Stadi4 diffractometer to determine lattice parameters and intensity data of X-ray reflections at ambient conditions.[14]

Because crystallisation only takes place in the presence of water and crystals are a prerequisite for structure elucidation by X-ray analysis, IR and Raman spectra were also performed using these crystals. Infrared spectra are gained using KBr method (0.37 mg in 500 mg KBr) on the Bruker instrument IFS 25. For hydrogen bond characterisation the sample was cooled by liquid nitrogen. The Raman spectrum was obtained from one crystal of about 0.8 mg on the Bruker IFS 66. 100 runs were added to give the final spectrum.

NMR: 1D: VARIAN GEMINI spectrometer 300, 300.24 MHz and 75.5 MHz, solvents CD_3OD and $CDCl_3$, 2D: (GHMBC, GHSQC and ^{1}H–^{1}H-COSY) NMR VARIAN UNITY 500 spectrometer, 499.83 MHz, solvent CD_3OD and $CDCl_3$, TMS as internal standard.

The preparative HPLC was carried out on a Knauer instrument with a YMC-column on ODS, 5 µm, 20 × 150 mm, MeCN–H_2O as eluent and UV detection at 210 nm. YMC-column on ODS-A, 5 µm, 4,6 × 250 mm, MeCN–H_2O as eluent was used for analytical HPLC.

(22*E*, 24*S*)-3β-Acetoxy-24-ethyl-5α-cholest-22-en-6-one 2

(22*E*,24*S*)-3β,5-Cyclo-24-ethylcholest-22-en-6-one **1** (4.05 g) was dissolved in glacial acetic acid (90 ml). H_2SO_4 (22.5 ml, 2.5 M) was added to this solution. The mixture was stirred and heated under reflux for 2 h. The solution was cooled and diluted with crushed ice and water. The precipitate was collected, washed with water and then dissolved in ether. The ether phase was washed with aq. $NaHCO_3$ and water, dried over Na_2SO_4 and concentrated *in vacuo*. The residue (4.45 g) was dissolved in pyridine (7 ml) and acetic anhydride (7 ml). The mixture was stirred at room temperature for 16 h and then heated under reflux for 1 h. The solution was cooled and diluted with crushed ice and water. After addition of HCl the desired product was extracted with $CHCl_3$. The chloroform extract was washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from acetone to give 3.5 g (75.4%) compound **2**. TLC (silufol 2 × *n*-hexane–EtOAc 9 : 1 v/v) R_f 0.38 mp 146–148 °C (from hexane); EIMS: *m*/*z* 470 (M^+, 30.7), 427 ($M^+ - CH_3CO$, 5.7), 367 (41.4), 358 ($M^+ - C_8H_{16}$(fission C-20/C-22 + 1H), 52.1), 349 (24.3), 329 ($M^+ - C_{10}H_{21}$(fission C-17/C-20 + 2H), 100), 316 (359 − CH_3CO, 57.1), 303 (50), 299 (35.7), 271 (30.7), 245 (22.8), 149 (58.6), 123 (33.6), 95 (52.1), 83 (63.6%); ^{1}H NMR: δ_H 0.681 (3H, s, H-18), 0.772 (3H, s, H-19), 0.792 (3H, d, (6.7) H-27*), 0.803 (3H, t, (7.3) H-29), 0.843 (3H, d, (6.4) H-26*), 1.021 (3H, d, (6.6) H-21), 2.029 (3H, s, CH_3COO–), 4.670 (1H, m, H-3), 5.018 (1H, dd, (15.2/8.5)) and 5.146 (1H, dd, (15.2/8.5)) (H-22 and H-23); * exchangeable;^{13}C NMR: δ_C 12.3 (2C), 13.1, 19.0, 21.2 (2C), 21.4, 21.6, 24.1, 25.4, 26.2, 26.9, 28.8, 31.9, 36.4, 38.0, 39.4, 40.4, 41.0, 42.9, 46.7, 51.2, 53.9, 55.9, 56.5, 56.8, 72.8 (C-3), 129.5 and 137.8 (C-22 and C-23), 170.4 (COO–), 210.1 (C-6).

(22*E*,24*S*)-3β-Acetoxy-6,6-(ethylenedioxy)-24-ethyl-5α-cholest-22-ene 3

2,2-Dimethyl-1,3-dioxolane (60 ml) and *p*TsOH (200 mg) were added to compound **2** (5.879 g). The mixture was stirred and heated to 110 °C under reflux for 16 h. The formed acetone was removed by distillation. The reaction was stopped by addition of K_2CO_3 (355 mg). The excess of 2,2-dimethyl-1,3-dioxolane was removed *in vacuo* and the residue was dissolved in ether. The ether phase was washed with brine and dried with Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography on SiO_2. The product was eluated with *n*-hexane–EtOAc (92 : 8 v/v). Amorphous **3** (5 g, 77.8%) was yielded.

TLC (silufol 2 × *n*-hexane–EtOAc 9 : 1 v/v); R_f 0.43; EIMS: *m*/*z* 514 (M^+, 19.3), 375 ($M^+ - C_{10}H_{19}$ (fission C-17/C-20), 1.4), 317 ($C_{21}H_{33}O_2$, 100), 178 (5.7), 99 (7.1), 83 (5.0%);^{1}H NMR: δ_H 0.687 (3H, s, H-18), 0.794 (3H, d, (6.5) H-27*), 0.801 (3H, t, (7.3) H-29), 0.844 (3H, d, (6.5) H-26*), 0.956 (3H, s, H-19), 1.009 (3H, d, (6.6) H-21), 2.029 (3H, s, CH_3COO–), 3.740 m and 3.910 m (2 × OCH_2), 4.683 (1H, m, H-3), 5.006 (1H, dd, 15.1/8.5) and 5.145 (1H, dd, 15.1/8.3) (H-22 and H-23), *exchangeable; ^{13}C NMR: δ_C 12.3 (2C), 14.2, 19.1, 21.1 (2C), 21.3, 21.5, 24.3, 25.3, 25.4, 27.3, 28.9, 31.9, 33.4, 36.9, 38.0, 39.7, 40.5, 41.3, 42.5, 50.5, 51.2, 53.5, 56.0 (2C), 64.2 and 65.4 (2 × CH_2O), 73.9 (C-3), 109.4 (C-6), 129.2 and 138.1 (C-22 and C-23), 170.3 (COO–).

(24*S*)-3β-Acetoxy-6,6-(ethylenedioxy)-22,23-dihydroxy-24-ethyl-5α-cholestane 4

A mixture consisting of amorphous **3** (2.93 g), *N*-methylmorpholine *N*-oxide (3.34 g), $NaHCO_3$ (241 mg),

$MeSO_2NH_2$ (545 mg), THF (50 ml), water (12.5 ml) and OsO_4 (5 ml, 2.5% in tBuOH) was stirred for 4 days at 40 °C. The starting compound disappeared as shown by TLC (silufol *n*-hexane–EtOAc 7 : 3 v/v). The spot at R_f 0.50 for the starting compound was absent and two new spots appeared at R_f 0.28 and R_f 0.09. Solid Na_2SO_3 (6.5 g) and water (45 ml) were added and the mixture was stirred for 1 h. The solvent was removed and the residue was extracted with EtOAc. The EtOAc phase was washed with brine and dried with Na_2SO_4. The solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2 *n*-hexane–EtOAc 8 : 2 to 6 : 4 v/v). Both compounds (87.7%) were isolated in 11 : 1 proportion (R_f 0.28 : 0.09). 2.52 g mp 97–100 °C (from *n*-hexane) and 215 mg mp 128–130 °C (from *n*-hexane).

The spectroscopic data given below are for the substance with mp 97–100 °C.

EIMS: *m*/*z* 548 (M^+, 20.7), 434 ($M^+ - C_7H_{14}$ (fission C-22/C-23 + 1H), 54.3), 404 ($M^+ - C_8H_{16}O_2$ (fission C-22/C-23 + 1H), 22.8), 373 ($M^+ - C_{10}H_{23}O_2$ (fission C-17/C-20–2H), 34.3), 351 ($C_{21}H_{35}O_4$, 100), 237 ($C_{14}H_{21}O_3$, 69.3), 178 (20), 99 ($C_5H_7O_2$, 72.8%); ^{1}H NMR: δ_H 0.710 (3H, s, H-18), 0.955 (3H, s, H-19), 2.031 (3H, s, CH_3COO–), 3.617 (2H, br s, H-22, H-23), 3.753 m and 3.902 m (2 × CH_2O), 4.693 (1H, m, H-3); ^{13}C NMR: δ_C 65.4 and 69.5 (2 × CH_2O), 72.2 and 73.9 (C-22 and C-23), 76.6 (C-3), 109.4 (C-6), 170.6 (COO–).

(20*S*)-3β-Acetoxy-6,6-(ethylenedioxy)-20-formyl-5α-pregnane 5

Compound **4** (1.59 g) was dissolved in THF (67 ml). To this stirred solution dry pyridine (333 µl), solid $NaHCO_3$ (243 mg) and finally periodic acid (660 mg) were added under argon. The mixture was stirred for 24 h at room temperature under argon. The precipitated HIO_3 was removed by filtration. The filtrate was concentrated *in vacuo*. To the residue 5% aq. $NaHSO_3$ (33.3 ml) was added and the mixture was stirred for 30 min. Then 10% aq. Na_2CO_3 was added and the mixture was stirred for another 30 min. The desired product was extracted with CH_2Cl_2 (3 × 35 ml). The CH_2Cl_2 phase was washed successively with 5% saturated aq. $NaHCO_3$ (35 ml) and with brine (35 ml) and then dried with Na_2SO_4. The solvent was evaporated under reduced pressure to give 1.055 g (84.2%) amorphous aldehyde **5** which we did not purify.

3-[$^2H_3$]Methyl[4,4,4-$^2H_3$]but-1-yn-3-ol

Into stirred liquid ammonia (700 ml) under argon containing hydrated ferric nitrate (244 mg) as a catalyst sodium (15,73 g) in small pieces was added during 30 min. Then triphenylmethane as an indicator for the conversion of sodamide to sodium acetylide was added to the solution. In the next step acetylene purified by bubbling through sulfuric acid was added until the solution lost its red colour. Then to this intensively stirred solution dry acetone[$^2H_6$] (50 ml in 30 min) was added dropwise, with continued addition of acetylene. This mixture was stirred for 14 h and the ammonia was allowed to evaporate. To the residue diethyl ether (100 ml) and a solution of aqueous ammonium chloride (68 g in 186 ml water) were added. The layers were separated and the aqueous phase was extracted three times with diethyl ether (300 ml). All ether layers were added to each other and were dried with sodium sulfate. The product was isolated by fractional distillation over succinic acid. Yield was 33 g (55%) 3-[$^2H_3$]methyl[4,4,4-$^2H_3$]but-1-yn-3-ol bp 98–104 °C. The deuteriation rate was found to be 86.6%, because the acetylene in the steel flask is dissolved in acetone, sodium acetylide reacted at a low rate with swept away acetone, although the acetylene was added through sulfuric acid in order to remove the acetone.

1-Bromo-3-[$^2H_3$]methyl[4,4,4-$^2H_3$]buta-1,2-diene

3-[$^2H_3$]Methyl[4,4,4-$^2H_3$]but-1-yn-3-ol (18 g) was added to a stirred mixture of powdered cuprous bromide (10 g), powdered ammonium bromide (8 g), copper powder (0.5 g) and hydrobromic acid (48% w/w; 48 ml). The mixture was stirred and warmed up to 30 °C for 1.5 h. Then in the infrared spectrum of the upper layer the OH band (3400 cm^{-1}) disappeared. The mixture was cooled, filtered and the residue was washed with *n*-hexane. The hexane phase was washed with 48% hydrobromic acid until the lower layer showed no violet coloration. The hexane layer was dried (Na_2SO_4) and fractionated *in vacuo*. Yield was 15.2 g (49.7%) 1-bromo-3-[$^2H_3$]methyl[4,4,4-$^2H_3$]buta-1,2-diene bp 55–60 °C/70 mmHg. ν_{max} (CCl_4/cm^{-1} 644 (vs νCBr), 1166 (s δCH), 1959 (s νC=C=C).

3-[$^2H_3$]Methyl[4,4,4-$^2H_3$]but-1-yne

A slurry of lithium aluminium hydride (1.88 g) in diethylcarbitol§ (60 ml) purified by distillation from lithium aluminium hydride was stirred under argon atmosphere and cooled in an ice–salt bath. To this slurry 15.2 g 1-bromo-3-[$^2H_3$]-methyl[4,4,4-$^2H_3$]buta-1,2-diene were added dropwise. The reaction mixture was stirred for 20 h, during this time the mixture was allowed to warm up to room temperature. 8 ml water were added and 3-[$^2H_3$]methyl[4,4,4-$^2H_3$]but-1-yne (bp 30–35 °C) was distilled from the crude mixture. 4.77 g (64.9%). ν_{max} (neat)/cm^{-1}: 2226 (m νC≡C), 3313 (vs νCH of C≡CH).

Preparation of the organoaluminium reagent 6

4 ml of a solution of trimethylaluminium in *n*-heptane (2 M) were added to a stirred suspension of bis(cyclopentadienyl)-zirconium dichloride (Cp_2ZrCl_2) (1 g) in dry CH_2Cl_2 (10 ml) under argon. The mixture was stirred for 30 min at room temperature to give a homogeneous yellow solution. To this stirred mixture 3-[$^2H_3$] methyl[4,4,4-$^2H_3$]but-1-yne (609 mg = 0.92 ml) was dropped under argon and the stirring was continued for 105 min at room temperature. Then the excess of Me_3Al and of the solvent were removed under reduced pressure at 30 °C. To the residue dry *n*-hexane (5 ml) was added to precipitate Cp_2ZrCl_2. The supernatant was transferred to another flask under argon. This *n*-hexane-extraction of the organoaluminate was repeated two more times. 2.6 ml of a solution of *n*-BuLi in *n*-hexane (1.6 M) was added dropwise under argon to the stirred and cooled (−10 °C) *n*-hexane solution of the organoaluminate **6**. After 30 min the bath temperature was raised to 2–5 °C and the mixture was stirred for 1 h at that temperature.

[26,27-$^2H_6$]3β-Acetoxy-6,6-(ethylenedioxy)-22*R*-hydroxy-24-methyl-5α-cholest-23-ene 7

703 mg of the aldehyde **5** was dissolved in dry THF (5 ml) and then added dropwise to the stirred and cooled (−10 to −30 °C) solution of the organoaluminate **6** in *n*-hexane under argon. After 5 min the bath temperature was raised to 2–5 °C. The mixture was stirred overnight at room temperature. The next day the mixture was diluted with THF (5 ml) and quenched with aq. NH_4Cl at ice cooling. Then the mixture was extracted with ether and EtOAc. The extract was washed with water, dried with Na_2SO_4 and then the solvent was removed under reduced pressure. The residue was purified by flash chromatography with *n*-hexane–EtOAc (7 : 3 v/v) as a eluent to give 238 mg (28.0%) raw product. This raw product was purified by preparative HPLC (ODS MeCN–H_2O 95 : 5).

EIMS: *m*/*z* 522 (M^+, 0.2), 504 ($M^+ - H_2O$, 8.6), 404 ($M^+ - C_7H_6O^2H_6$ (fission C-20/C-22–1H), 100), 373 ($M^+ - C_9H_{13}O^2H_6$ (fission C-17/C-20 + 2H), 42.8), 178 (17.8), 119

§ The IUPAC name for diethylcarbitol is di(ethylene glycol) diethyl ether.

($C_7H_7O^2H_6$, 39.3), 99 (40.7%); ^{1}H NMR: δ_H 0.683 (3H, s, H-18), 0.729 (1H, ddd (12.1/10.7/4.1) H-9), 0.950 (3H, d (6.7) H-21), 0.957 (3H, s, H-19), 1.611 (3H, d, (1.2) H-28), 2.029 (3H, s, CH_3COO–), 2.185 (1H, s, H-25), 3.751 m and 3.918 m (2 × CH_2O), 4.461 (1H, dd (7.9/1.6) H-22), 4.696 (1H, m, H-3), 5.318 (1H, dq (7.9/1.2) H-23).

[26,27-$^2H_6$]24-*epi*-Cathasterone 8

A small amount of the catalyst (10% Pt/C) was suspended in benzene (15 ml) and with H_2 hydrogenated for 5 h. Then 28 mg of compound **7** dissolved in benzene (1 ml) were added and the mixture was hydrogenated for 12 h. The catalyst and the solvent were removed. Then the residue was dissolved in aq. acetone (1 ml) to cleave the dioxolane-type acetal by transacetalization with pyridinium toluene-*p*-sulfonate as a catalyst. After addition of pyridinium toluene-*p*-sulfonate (7.5 mg) the mixture was refluxed for 3 h. The solvent was then removed *in vacuo* and the residue dissolved in EtOAc. After the washing with water and aq. $NaHCO_3$ and drying with Na_2SO_4 the solvent was removed under reduced pressure. The residue was dissolved in dry methanol and 0. 5 M NaOMe (100 µl). After 3 h the reaction was stopped with acetic acid and the mixture was purified by flash chromatography (SiO_2 EtOAc–*n*-hexane 1 : 1 to 7 : 3 v/v) and after them by preparative HPLC (ODS MeCN–H_2O 95 : 5). 3.5 mg (14.9%) [26,27-$^2H_6$]24-*epi*-cathasterone were obtained.

Mp 184–189 °C (MeOH–H_2O); EIMS: *m*/*z* 438 (M^+, 0.7), 420 (1.0), 347 (2.8), 318 ($M^+ - C_7H_8{}^2H_6$ (fission C-20/C-22–1H), 100), 300 (5.0), 248 (10.0), 139 (17.8%); *m*/*z* (EI) 438.398514 ($M^+ \cdot C_{28}H_{42}D_6O_3$ requires 438.398006), 420.387871 ($M^+ - H_2O \cdot C_{28}H_{40}D_6O_2$ requires 420.387442). X-Ray: Crystal data, $C_{28}H_{42}D_6O_3 \cdot H_2O$, M = 456.72, triclinic, space group $P\bar{1}$ (no. 1), a = 7.4742(10), b = 14.290(3), c = 14.776(3) Å, α = 62.376(11), β = 77.377(19), γ = 79.801(12)°, V = 1359.2(4) Å^3, Z = 2, T = 293(2) K, μ(Mo-K_{ga}) = 0.71 mm^{-1}, 10882 reflections measured, 5359 unique reflections (R_{int} = 0.179) used in the calculations,[15] final $wR(F^2)$ = 0.152 for all data, final R = 0.072 for 1912 reflections with $I > 2\sigma(I)$. IR: ν/cm^{-1} 3407, 2934, 2209, 1713, 1422, 1383, 1252, 1175, 1059, and 963. RAMAN: Only the most dominant lines are given: ν/cm^{-1} 2218, 2207, 2123, 2071, 1720, 1440, 1344, 1267, 1175, 1126, 1057, 995, and 838. ^{1}H NMR and ^{13}C NMR in Table 3

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