Synthesis of 24-Methyl Sterols Stereospecifically labelled with ^2H in the Isopropyl Methyl Groups. ^{13}C NMR Spectral Assignment of C-26 and C-27 Resonances

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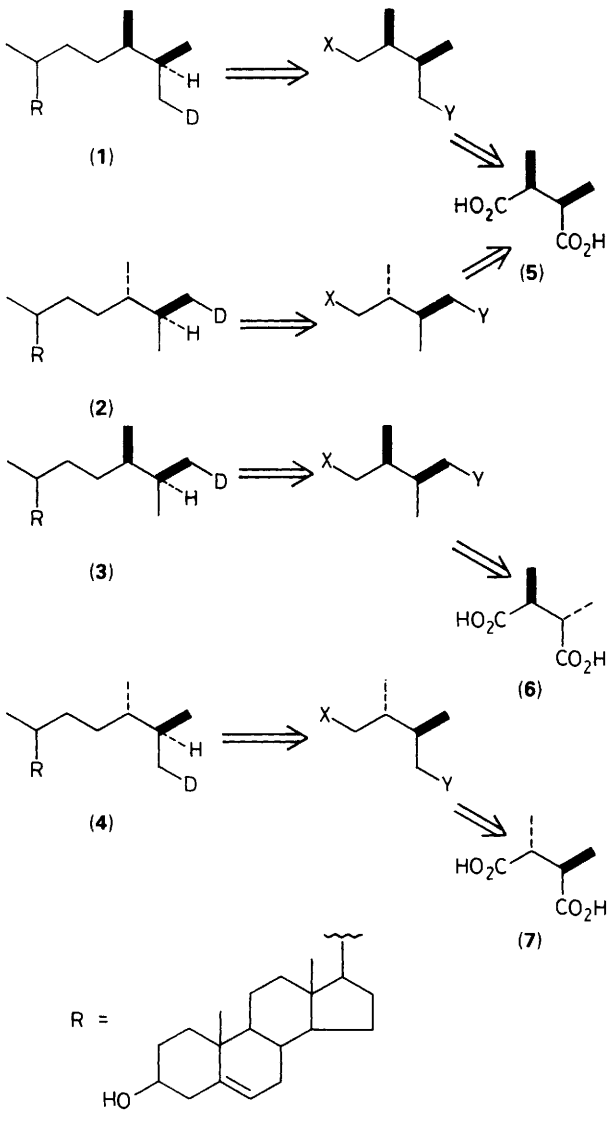
Through analysis of the ^{13}C NMR spectra of (25*S*)-[27-^2H]campesterol (**1**) and (25*R*)-[26-^2H]dihydrobrassicasterol (**2**), the C-26 and C-27 resonances have been unambiguously assigned; the biosynthetic applications are discussed.

The stereochemistry of the hydride migration from C-24 to C-25, which occurs during the alkylation of the 24-position operated by *S*-adenosylmethionine on a $\Delta^{24(25)}$-precursor, is among the stereochemical problems related to the construction of the 24-alkyl side chain during the biosynthesis of phytosterols which have been studied and discussed.[1—2] The stereochemical events at C-25, a consequence of this migration, can be determined through a biosynthetic experiment, provided that the following three conditions are satisfied: (i) the two diastereotopic C-26 (*pro-R*) and C-27 (*pro-S*) methyl groups are distinguishable by spectroscopic methods; (ii) they are obtained differently labelled at the end of the biosynthetic experiment in order to give rise to differently shaped signals in the spectrum of the biosynthetic phytosterol; (iii) their signals are unequivocally assigned.



Scheme 1

The first point deserves no further attention, as the ^{13}C NMR resonances of C-26 and C-27 of phytosterols are well distinguished signals.[3] The second requirement may be easily accomplished if suitably labelled precursors are used (*e.g.*, use of $C^2H_3CO_2Na$ in order to obtain phytosterol $^2H_2$ and $^2H_3$ labels on the isopropyl methyl groups, or use of $^{13}CH_3{}^{13}CO_2Na$ to get a product in which one of the methyl groups resonates as a singlet in the proton decoupled ^{13}C NMR spectrum, while the other resonates as a doublet). The third requirement deserves further discussion as the assignment of the signals should rely on the analysis of products obtained stereospecifically labelled.

Although some reports concerning the biosynthesis of 24-methyl sterols have been published,[2] in campesterol and in dihydrobrassicasterol, the C-26 and C-27 signals have been assigned only by comparison with analogous[2b] or model[3a] compounds.

We report here a stereospecific synthesis of (25*S*)-[27-^2H]campesterol (**1**)† and (25*R*)-[26-^2H]dihydrobrassicasterol (**2**), which allows a definitive assignment of the resonances of the C-26 and C-27 atoms in the ^{13}C NMR spectra of the two isomeric compounds.

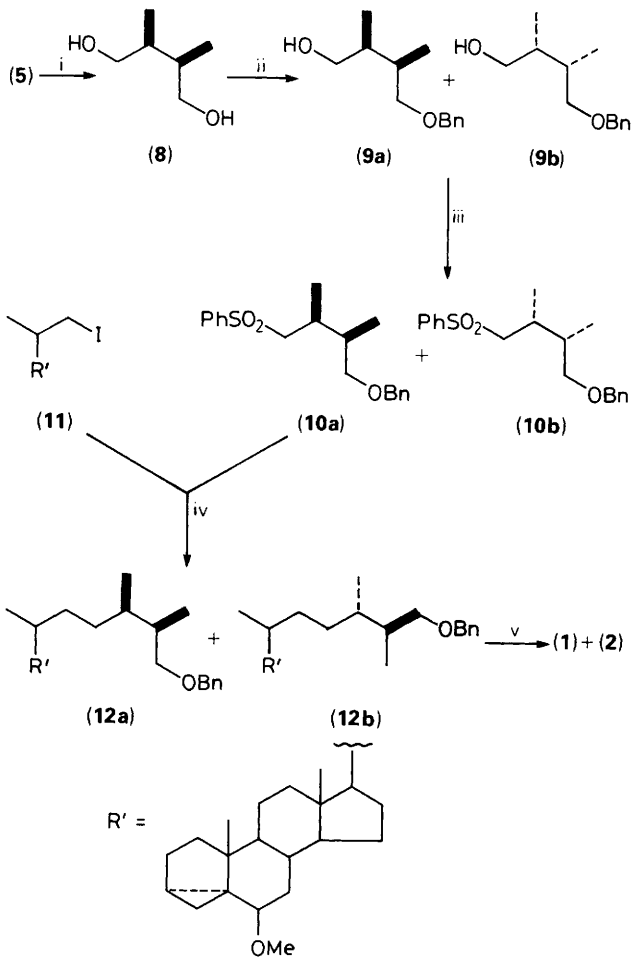
Our approach is illustrated in Scheme 1, which shows that 2,3-dimethyl succinic acid can furnish synthons with the correct configuration at the two stereocentres, which will become the C-24 and C-25 stereocentres in the target 24-methyl sterols. It can be easily observed that, among the

Table 1. ^{13}C NMR resonances[a] of the side-chain carbon atoms of compounds (**1**) and (**2**) and of their unlabelled analogues.

C	(1)	(2)	Campesterol[c]	Dihydro-brassicasterol[c]
20	35.95	36.25	35.96	36.26
21	18.77	18.96	18.77	18.95
22	33.80	33.80	33.80	33.80
23	30.39	30.68	30.37	30.67
24	38.92	39.16	38.92	39.17
25	32.41[b]	31.49[b]	32.49	31.54
26	20.24	17.39[b]	20.26	17.68
27	18.03[b]	20.54	18.32	20.56
28	15.46	15.52	15.44	15.51

[a] Relative to C^2HCl_3 (δ 77.00). [b] Shifted to high fields owing to α or β isotopic shifts. [c] Ref. 3a.

† The numbering of C-26 and C-27 should be exchanged in compounds (**1**) and (**4**); however, for convenience, we designated the (*pro-R*) methyl group as C-26 throughout this paper.



Scheme 2. *Reagents and conditions*: i, ref. 4a; ii, NaH–BnBr (Bn = benzyl) (1 equiv.), THF, room temp., 4 h; iii, (a), $MeSO_2Cl$, NEt_3, CH_2Cl_2, 0 °C, 3.5 h; (b) NaH–PhSH, THF, room temp., 4 h; (c) *m*-chloroperbenzoic acid, CH_2Cl_2, room temp., 3 h; iv, (a) LDA, THF, hexamethylphosphoramide, −70 to 0 °C, 5 h; (b) Na–Hg, EtOH, room temp., 24 h; v, (a) H_2, Pd/C, AcOEt, 20 h; (b) $MeSO_2Cl$, pyridine, 0 °C, 4 h; (c) $LiAl^2H_4$, THF, room temp., 18 h; (d) toluene-*p*-sulphonic acid (*p*-TSA), dioxane–H_2O, 70 °C, 3.5 h.

three stereoisomers of this dicarboxylic acid, the (*SS*)-isomer (**6**) will give rise to (25*R*)-[26-^{2}H]campesterol (**3**) and the (*RR*)-isomer (**7**) to (25*S*)-[27-^{2}H]dihydrobrassicasterol (**4**), whereas the *meso*-2,3-dimethylsuccinic acid (**5**) will give rise to a mixture of (25*S*)-[27-^{2}H]campesterol (**1**) and (25*R*)-[26-^{2}H]dihydrobrassicasterol (**2**). As a first approach to this synthetic problem, we have chosen the *meso*-acid (**5**) as starting material for several reasons. Firstly, it is commercially available. Secondly, it can give, in a single synthetic sequence, both the two target 24-methyl phytosterols, and so, thirdly, as the signals of the methyl groups in the side chain of the two compounds are easily distinguishable in the ^{13}C NMR spectra,[3] a single experiment can solve the problem of the assignment of the resonances of the isopropyl methyl groups for both the 24-isomeric compounds.

To this aim, *meso*-2,3-dimethylsuccinic acid (**5**) was transformed (Scheme 2) into the known *meso*-2,3-dimethylbutane-1,4-diol[4] (**8**). Monobenzylation of diol (**8**) afforded (±)-4-benzyloxy-2,3-dimethylbutan-1-ol‡ (**9a,b**), which was transformed[5] into the (±)-sulphone (**10a,b**), through mesylation, displacement with phenyl sulphide, and oxidation of the obtained sulphide with *m*-chloroperbenzoic acid. The coupling reaction with the steroidal counterpart was accomplished[5] by reacting the sulphone (**10a,b**), after treatment with lithium di-isopropylamide (LDA) in tetrahydrofuran (THF), with the known[6] 6β-methoxy-3α,5-cyclo-5α-23,24-dinorcholan-22-iodide (**11**) to afford, after removal of the phenylsulphone moiety with sodium amalgam, the 26-benzyloxy derivatives (**12a,b**). Hydrogenolysis of the 26-*O*-benzyl group with Pd/C, mesylation, reduction of the 26-mesylate with lithium aluminium deuteride, and cleavage of the *i*-methyl ether afforded the desired deuteriated compounds (25*S*)-[27-^{2}H]campesterol (**1**) and (25*R*)-[26-^{2}H]dihydrobrassicasterol (**2**).

The ^{1}H-decoupled ^{13}C NMR spectrum of the mixture of (**1**) and (**2**) showed the two expected series of singlets. However, in both series a signal was detected as a triplet, owing to the ^{2}H–^{13}C coupling, at δ 17.39 (*J* 19 Hz) and at δ 18.03 (*J* 19 Hz) (Table 1). As the resonances in the deuteriated samples are shifted to high fields owing to the α isotopic shift, the signal at δ 17.39 corresponds to that at δ 17.68 of the unlabelled dihydrobrassicasterol, and the signal at δ 18.03 corresponds to that at δ 18.32 of the unlabelled campesterol.

The relative configuration of C-24 and C-25 in the deuteriated 24-methylsterols reflects the configuration of the starting *meso*-dimethylsuccinic acid. Therefore, as in the deuteriated campesterol (**1**), the deuterium atom is located on the *pro*-(*S*)-isopropyl methyl group. In the ^{13}C NMR spectrum of campesterol, the signal at δ 18.32 must therefore be attributed to C-27 (and the signal at δ 20.26 to C-26). Analogously, as in the deuteriated dihydrobrassicasterol (**2**), the deuterium atom is located on the *pro*-(*R*)-methyl group, for dihydrobrassicasterol, the signal at δ 17.68 must be attributed to C-26 (and the signal at δ 20.56 to C-27).§ These assignments provide unequivocal proof of the conclusions previously drawn by Seo *et al.*[2b] on the stereochemistry at C-25 in the biosynthesis of campesterol and dihydrobrassicasterol.

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‡ All the new compounds gave satisfactory elemental analyses and were characterized using NMR spectroscopy at 200 MHz.

§ These results confirm the assignments reported in refs. 2b and 3a.