Biosynthesis of β-Sitosterol from [4-^{13}C]Mevalonic Acid and Sodium [1,2-^{13}C]Acetate in Tissue Cultures of *Isodon japonicus* Hara

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Summary The ^{13}C-labelling patterns in β-sitosterol, isolated from *Isodon japonicus* Hara tissue cultures fed with [4-^{13}C]mevalonic acid and [1,2-^{13}C]acetate, provide confirmatory evidence for the postulated backbone rearrangement during biosynthesis of β-sitosterol, and also suggest that biological alkylation at C-24 is stereospecific.

IT is well known that the biological conversion of squalene oxide into phytosterol *via* cycloartenol in higher plants

involves backbone rearrangement.[1] The distribution of mevalonate and acetate in the biosynthesis of phytosterol, however, has been determined only for a few positions by using radioisotopically labelled precursors.[2] We now report confirmatory experimental evidence for the postulated backbone rearrangement by ^{13}C n.m.r. studies of two ^{13}C-labelled specimens of β-sitosterol isolated from *Isodon japonicus* Hara tissue cultures† grown in two Linsmaier–Skoog liquid media, one containing [4-^{13}C]mevalonic acid[3a] and the other containing sodium [1,2-^{13}C]acetate.[3b] From the same tissue cultures, we obtained ^{13}C-labelled oleanene- and ursene-type triterpenes as reported previously.[3]

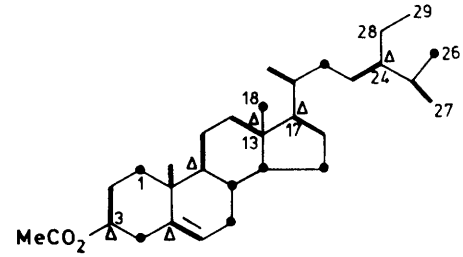
TABLE
Carbon-13 n.m.r. spectral data for β-sitosteryl acetate biosynthesized from sodium [1,2-^{13}C]acetate[a]

Atom	δc	Atom	δc
C-1	37·1s	C-16	28·3d[b]
C-2	27·8d[b]	C-17	56·1d[b]
C-3	74·0d (*J* 37 Hz)	C-18	11·9s
C-4	38·2s	C-19	19·3d (*J* 32 Hz)
C-5	139·7d (*J* 71 Hz)	C-20	36·2d (*J* 32 Hz)
C-6	122·7d (*J* 71 Hz)	C-21	18·8d (*J* 32 Hz)
C-7	31·9s	C-22	34·0s
C-8	31·9s	C-23	26·2d (*J* 36 Hz)
C-9	50·1d (*J* 35 Hz)	C-24	45·9d (*J* 35 Hz)[b]
C-10	36·6d (*J* 33 Hz)	C-25	29·3d[d]
C-11	21·1d (*J* 34 Hz)	C-26[d]	19·8s
C-12	39·8d[b]	C-27[d]	19·1d
C-13	42·4d[b]	C-28	23·1[c]
C-14	56·8s	C-29	11·9[c]
C-15	24·3s	CO*Me*	21·4[c]
		*C*OMe	170·4[c]

[a] ^{13}C Fourier transform n.m.r. spectra were recorded with a Varian NV-14 spectrometer operating at 15·087 MHz in $CDCl_3$ using 8-mm spinning tubes at room temperature (30 °C). Accuracies of chemical shifts δ and *J* values were *ca.* ±0·1 p.p.m. and ±1 Hz, respectively. [b] *J* Not exactly determinable owing to signal overlap. [c] Non-enriched ^{13}C signals. [d] Assignments of these signals were solely based on consideration of the steric effects of the epimeric ethyl groups at C-24 of this molecule and its C-24 epimer, clionasteryl acetate, upon δ(C-26) (22·7 p.p.m.) and δ(C-27) (22·9 p.p.m.) of cholesterol,[4] and are not unambiguous; δ values for C-23 to C-29 of clionasteryl acetate were 26·5, 46·2, 29·1, 19·0, 19·8, 23·1, and 12·4 p.p.m., respectively.

Proton-noise-decoupled ^{13}C Fourier transform n.m.r. spectra of β-sitosteryl acetate, for the ^{13}C-enriched and unenriched specimens, were compared. The ^{13}C signals of the natural-abundance compound were assigned by comparisons with the literature data on cholesterol[4] (Table). The spectrum of the sample from [4-^{13}C]mevalonic acid clearly shows that the six carbon atoms, C-3, C-5, C-9, C-13, C-17, and C-24, were enriched by *ca.* 12 times, while the samples of triterpenes obtained simultaneously from the same source were enriched by *ca.* 2—5 times as described previously.[3a] In addition to the enriched ^{13}C singlets due to C-13 and C-17, two satellite peaks split by ^{13}C–^{13}C spin coupling were observed for each signal as an AB-quartet (*J* 35 Hz), arising from incorporation of two labelled isoprene units into vicinal positions in the same molecule of β-sitosterol.

The spectrum of the sample from sodium [1,2-^{13}C]acetate, compared with the natural-abundance spectrum, clearly shows 13 singlets including those due to non-enriched C-28, C-29, and acetyl carbon atoms, and 18 doublets (Table). All the carbon atoms except C-28, C-29, and the acetyl carbon atoms were enriched by *ca.* 1·4 times, while the spectra of the triterpenes obtained simultaneously had shown *ca.* 2 times incorporations.[3b]‡ The ^{13}C-labelled patterns obtained here correspond with the well established biosynthetic pathway from acetate or mevalonate to cholesterol in mammals.[4] The appearance of singlet signals for C-14 and C-18 is attributable to 1 : 2 methyl migration from the C-14 to the C-13 position during biosynthesis of squalene oxide to β-sitosterol. This 1 : 2 methyl migration in cholesterol has been proved recently by feeding experiments with rats using mevalonate.[4]



Δ enriched ^{13}C atom from [4-^{13}C]MVA
— two coupled ^{13}C atoms from [1,2-^{13}C]$MeCO_2H$
• uncoupled ^{13}C atom from [1,2-^{13}C]$MeCO_2H$

In the biosynthesis of the alkyl group at C-24 of phytosterol in higher plants, a 24-ethylidene intermediate formed by double transmethylation from adenosyl methionine is converted into a $\Delta^{24(25)}$-compound, which is then reduced to give the saturated sterol side chain.[5] The enriched ^{13}C singlet at δ 19·8 p.p.m. and the doublet at 19·1 p.p.m. corresponding to C-26 and C-27 show that these two methyl groups have different origins, the former singlet being derived from C-2 of mevalonate and the latter doublet from C-6. This strongly suggests that the biological alkylation involving migration and reduction of the double bond may proceed stereospecifically, although these two signals could not yet be unambiguously assigned (Table).

(*Received*, 3*rd January* 1978; *Com.* 003.)

† G.l.c. examination showed that the phytosterol fraction isolated from the tissue cultures consists predominantly of β-sitosterol, a small amount of stigmasterol, and an almost negligible amount of campesterol. Thus, the labelled β-sitosteryl acetate was separated from stigmasteryl acetate by t.l.c. (30% w/w $AgNO_3$ impregnated silica-gel), and recrystallised.

‡ The ratio of incorporation of mevalonate into the sterol to that into the triterpenes (12 : 5) is apparently different from that for acetate incorporation (1·4 : 2).

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