Biosynthetic origin of C-26 and C-27 of the phytoecdysteroids cyasterone and 29-norcyasterone in *Ajuga* hairy roots

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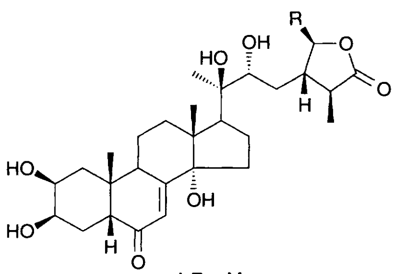
Feeding of [$^{13}C_2$] acetate to the hairy root culture of *Ajuga reptans* var. *atropurpurea* and ^{13}C NMR analysis of the biosynthesized cyasterone and 29-norcyasterone reveal that the lactone carbonyl carbon of these phytoecdysteroids is derived from C-2 of mevalonate, whereas the methyl group on C-25 comes from C-6.

A number of ecdysteroids have been isolated from plants.[1] Approximately one third of these phytoecdysteroids have one- or two-carbon substituents at their C-24 positions. It is, therefore, reasonable to assume that 24-alkylated phytoecdysteroids are biosynthesized from plant sterols or their biosynthetic precursors which have one- or two-carbon units at C-24. This idea is supported by our previous studies, in which ^{13}C-labelled cholesterol was incorporated into 20-hydroxyecdysone, but not into cyasterone **1** and 29-norcyasterone **2** in the hairy roots of *Ajuga reptans* var. *atropurpurea*.[2,3] However, little is known about the mechanism of 24-alkylated phytoecdysteroid biosynthesis.[4] We have initiated studies on this problem using the *Ajuga* culture hairy roots. In this communication we report the metabolic origin of C-26 and C-27 in **1** and **2** and their correlation with the (*E*)- and (*Z*)-methyl groups of a putative Δ^{24}-sterol precursor, *e.g.* desmosterol or cycloartenol, and with C-2 and C-6 of mevalonate. Isolation of cyasterone has been previously reported from *Cyathula capitata*[5] and several *Ajuga* species,[6] and 29-norcyasterone from *Ajuga reptans*.[7] The sterol fraction of the *Ajuga* hairy roots is composed of 22,23-didehydroclerosterol, clerosterol **3** and cholesterol.[8]

The studies were based on the feeding of ^{13}C-labelled substrates and ^{13}C NMR analysis of the purified products.[2] Thus, [$^{13}C_2$] acetate (33% labelled acetate,† 30 mg in 250 ml liquid medium per flask, 8 flasks) was fed to the hairy roots in the same manner as described previously.[2] The isolated cyasterone (2.8 mg) and 29-norcyasterone (3.4 mg) were analysed by ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum of **1** is given in Fig. 1. The signals for C-25 (δ 43.5[2]) and the methyl group on C-25 (δ 16.0) clearly show flanking doublets (36.6 Hz) resulting from the incorporation of a ^{13}C-labelled acetate unit. In contrast, the signal of the carbonyl carbon (δ 181.8) is not accompanied by any such satellite peaks. Similarly, the ^{13}C NMR spectrum of **2** also showed the flanking doublets (36.6 Hz) for C-25 and the methyl group on C-25 (δ 44.4[2]), and a singlet for the carbonyl carbon (δ 181.9).



1 R = Me
2 R = H

It is inferred from these results that C-2 of mevalonate is incorporated as the lactone carbonyl carbon in **1** and **2**, *via* the (*E*)-methyl group of a Δ^{24}-sterol precursor, and that C-6 of mevalonate gives rise to the methyl group on C-25 *via* the (*Z*)-methyl group of a Δ^{24}-sterol precursor (Scheme 1).[9]

We recently reported on the mechanism of clerosterol biosynthesis in *Ajuga* hairy roots, in which the (*E*)- and (*Z*)-methyl groups of desmosterol become the methyl on C-25 and the *exo*-methylene carbon, respectively.[8] Furthermore, we have also shown that clerosterol is converted into cyasterone.[10] These new results imply that the methyl group on C-25 of clerosterol **3** is oxidatively converted into the lactone carbonyl carbon, whereas the *exo*-methylene group is reduced stereospecifically into the methyl group on C-25 of **1**.

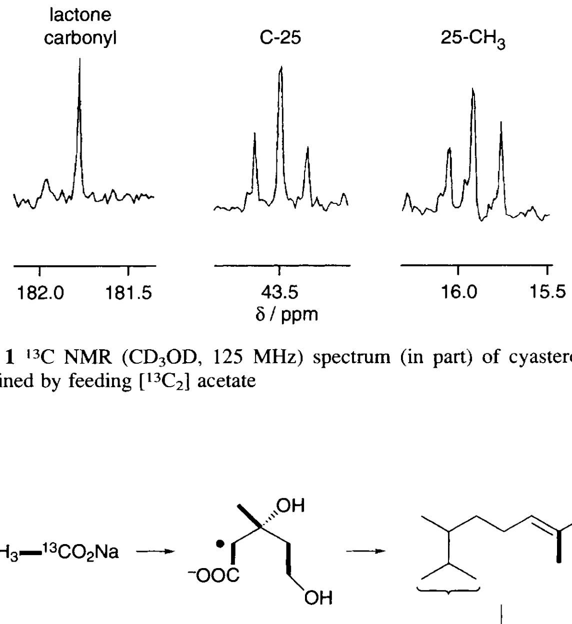
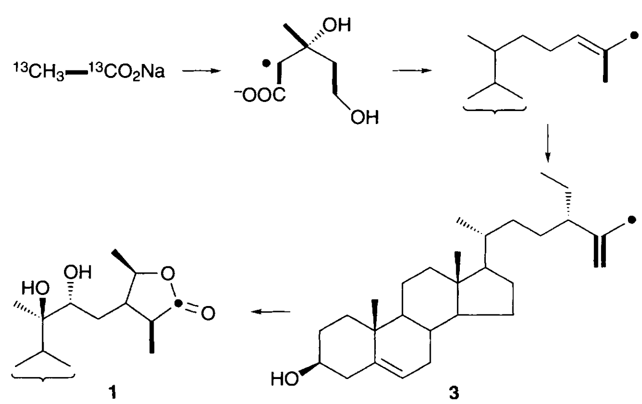


Fig. 1 ^{13}C NMR (CD_3OD, 125 MHz) spectrum (in part) of cyasterone obtained by feeding [$^{13}C_2$] acetate



Scheme 1 The metabolic correlation of C-26 and C-27 carbons during biosynthesis of cyasterone. Bold lines indicate acetate units and dots refer the carbon originated from C-2 of mevalonate.

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Footnote

† [$^{13}C_2$]acetate was diluted with an appropriate amount of non-labelled acetate to avoid the possibility that the ^{13}C-acetate molecules are incorporated at the adjacent positions of the products which might disturb NMR analysis due to the presence of additional couplings.

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