Biosynthesis of Oleanene- and Ursene-type Triterpenes from [4-^{13}C]Mevalonic Acid in Tissue Cultures of *Isodon japonicus* Hara

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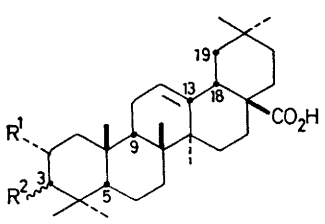
Summary On the basis of the ^{13}C-labelling patterns elucidated by ^{13}C n.m.r. spectroscopy in oleanene- and ursene-type triterpenes (**1**)—(**5**) isolated from *Isodon japonicus* tissue cultures fed with [4-^{13}C]mevalonic acid, Ruzicka's hypothesis for cyclisation of squalene 2,3-oxide to the pentacyclic triterpenes has been verified experimentally.

RUZICKA's group has proposed that pentacyclic triterpenes such as α- and β-amyrin arise by cyclisation of squalene† folded in a chair–chair–boat form with specific 1,2-hydride shifts.[1] Although an alternative mechanism involving a 1,3-hydride shift was considered later, the postulated 1,2-hydride shift has good experimental support.[2,3] However, the rearrangement of carbon atoms during formation of the olean-12-ene and the urs-12-ene skeleton from squalene 2,3-oxide has not yet been demonstrated owing to the difficulty of chemical degradation of the triterpenes biosynthetically labelled with ^{14}C.

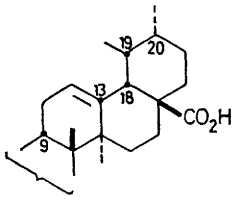
Recently, we have successfully assigned all ^{13}C n.m.r. signals for a number of olean-12-enes[4,5] and urs-12-enes.[5] Having elucidated ^{13}C-labelling patterns in some of these

†Recently, (*S*)-squalene 2,3-oxide was found to be the exclusive precursor of β-amyrin in plant systems; see D. H. R. Barton, T. R. Jarman, K. G. Watson, and D. A. Widdowson, *J.C.S. Chem. Comm.*, 1974, 861.

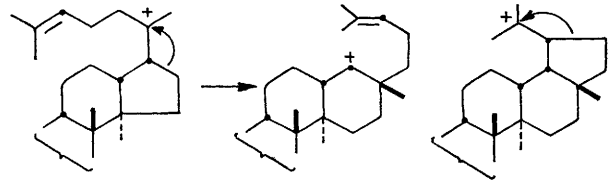
pentacyclic triterpenes synthesised from [4-^{13}C]mevalonic acid in tissue cultures of *Isodon japonicus* Hara by ^{13}C n.m.r. spectroscopy, we here show the occurence of the rearrangement in Ruzicka's hypothesis for the cyclisation of squalene to olean-12-ene- and urs-12-ene-type triterpenes.



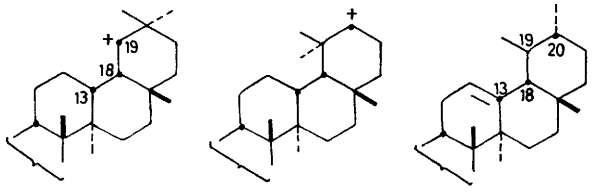
(1) R^1 = H, R^2 = β-OH
(2) R^1 = OH, R^2 = β-OH
(3) R^1 = OH, R^2 = α-OH
•: ^{13}C



(4) R^1 = H, R^2 = β-OH
(5) R^1 = OH, R^2 = β-OH



(6) (7) (8)



(9) (10) (11)

Tissue cultures of *Isodon japonicus* were grown in Linsmaier–Skoog liquid media; [4-^{13}C]mevalonic acid was prepared from [2-^{13}C]acetic acid by Cornforth's method.[8] A solution of [4-^{13}C]mevalonic acid (1 g; *ca.* 30% ^{13}C) in 50% ethanol (7·2 ml) was distributed among 18 bottles containing tissue cultures of *Isodon japonicus* (18 × 300 ml); cells were harvested after two weeks and extracted with methanol. Oleanolic acid (**1**), maslinic acid (**2**), 3-epimaslinic acid (**3**), ursolic acid (**4**), and 2α-hydroxyursolic acid (**5**) were obtained by repeated preparative t.l.c. as described previously.[5,6] ^{1}H-Noise-decoupled ^{13}C Fourier transform n.m.r. spectra of the methyl esters of these triterpenes were then compared for enriched and unenriched samples in $CDCl_3$ (see Table).

TABLE

Carbon-13 chemical shifts, δ(C), of enriched carbon atoms in $CDCl_3$[a]

Compound	δ(C) values[b] C-3	C-5	C-9	C-13	C-18	C-19
(1)	78·7	55·2	47·6	143·4	41·3	45·8
(2)	83·8	55·3	47·5	143·6	41·3	45·8
(3)	78·9	48·1	47·4	143·8	41·3	46·0
(4)	78·8	55·4	47·5	138·0	52·8	39·1 (or C-20)[c]
(5)	83·8	55·4	47·5	138·1	52·8	39·1 (or C-20)[c]

[a] ^{1}H-Noise-decoupled ^{13}C n.m.r. spectra were taken with a Varian NV-14 FT n.m.r. spectrometer operating at 15·09 MHz; precision *ca.* ±0·1. [b] Assignments and δ(C) values for the other carbon signals are reported in refs. 4 and 5. [c] We suggest that the enriched carbon is C-19 from Yb(fod)$_3$-induced ^{13}C shifts for the enriched and unenriched samples, the induced shift being apparently larger for C-19 than for C-20. However, unambiguous assignment must await further evidence.

The ^{13}C n.m.r. spectrum of methyl 3-epimaslinate clearly shows that the six carbons C-3, -5, -9, -13, -18, and -19, based on [4-^{13}C]mevalonic acid, were enriched by *ca.* 2 times. Similar ^{13}C-labelling patterns were observed in the spectra of the methyl esters of (**1**) and (**2**) with enrichments of *ca.* 5 and 2 times, respectively. The results are entirely in accord with Ruzicka's hypothesis for cyclisation from squalene to β-amyrin [(**6**) → (**7**) → (**8**) → (**9**) → β-amyrin].

In the biosynthesis of the ursene-type triterpenes (**4**) and (**5**), the D-ring is formed *via* process (**6**) → (**7**) as postulated for the biosynthesis of β-amyrin, since ^{13}C enrichment was clearly observed at C-18 together with C-3, C-5, C-9, and C-13 in (**4**) and (**5**) by *ca.* 5 and 2 times, respectively. Two possible mechanisms for E-ring formation have been considered so far.[1b,7,9] If the cation (**8**) or its equivalent is an intermediate, one of the enriched carbons originating from [4-^{13}C]mevalonic acid should be situated at C-19. On the other hand, if the E-ring is formed *via* process (**7**) → (**10**) → (**11**), the enriched carbon should be C-20. As indicated in the Table, although one signal corresponding to C-19 or C-20 of (**4**) and (**5**) was enriched by *ca.* 5 and 2 times, respectively, assignment of the enriched signal remains rather ambiguous because the C-19 and C-20 signals in these compounds are quite close to each other.[5] It is thus not possible at present to distinguish between these alternative mechanisms; further studies for differentiating between them are in progress.

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