Biosynthesis of Ursene-type Triterpenes from Sodium [1,2-^{13}C]Acetate in Tissue Cultures of *Isodon japonicus* Hara and Re-assignments of ^{13}C N.m.r. Signals in Urs-12-enes

By SHUJIRO SEO, YUTAKA TOMITA,* and KAZUO TORI
(*Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan*)

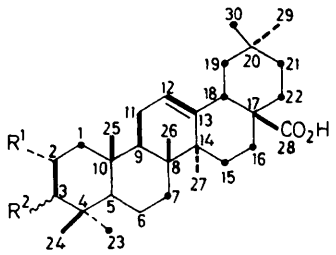
Summary The mechanism of biosynthesis of the E-ring in ursene-type triterpenes has been elucidated by ^{13}C n.m.r. studies of the ^{13}C-doubly labelled ursolic acids (**4**) and (**5**) isolated from *Isodon japonicus* tissue cultures fed with sodium [1,2-^{13}C]acetate.

IN a previous paper,[1] we presented experimental verification for Ruzicka's hypothesis[2] for cyclisation of squalene 2,3-oxide to pentacyclic triterpenes on the basis of ^{13}C-labelling patterns elucidated by ^{13}C n.m.r. spectroscopy[3] in the oleanene-type triterpenes, oleanolic (**1**), maslinic (**2**), and 3-epimaslinic acids (**3**), isolated from *Isodon japonicus* tissue cultures fed with [4-^{13}C]mevalonic acid. However, for the ursene-type triterpenes, ursolic (**4**) and 2α-hydroxyursolic acids (**5**), obtained simultaneously from the callus, the mechanism of E-ring biosynthesis remains ambiguous.

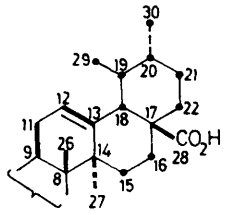
We now report a further ^{13}C n.m.r. study of the five triterpenes (**1**)—(**5**)† isolated from tissue cultures grown in Linsmaier-Skoog liquid media containing sodium [1,2-^{13}C]-acetate (0·15 mg cm^{-3} of a 2 : 1 mixture of unlabelled and [1,2-^{13}C]acetate) in a manner similar to that described previously.[1,4] This study elucidates an unambiguous biosynthetic route to the ursene-type triterpenes.

Prior to examinations of ^{13}C-doubly-labelled products, we re-examined the ^{13}C FT n.m.r. spectra of the methyl esters (**1a**)—(**5a**) of the triterpenes (**1**)—(**5**), because the ^{13}C signal assignments previously reported,[5] particularly for the methyl groups, involve a few ambiguities which may lead to incorrect results, and because Knight[6] has published different signal assignments for α- and β-amyrins. Several ^{1}H single-frequency off-resonance decoupling experiments[7] were carried out on the ^{13}C spectra of (**1a**) and (**4a**) in [^{2}H]-chloroform containing various amounts of Eu(fod)$_3$.[8] Since signals due to the methyl and some other protons in the triterpene–Eu(fod)$_3$ mixtures were assignable as reported recently,[9] the ^{13}C methyl signals were confirmed on the basis of the signal multiplicities and the magnitudes of residual couplings. The ^{1}H and ^{13}C signals were followed by plotting signal shift *vs.* Eu(fod)$_3$ concentration. As a result, the previous assignments[5] for C-11, C-27, and C-29 of (**4a**), its acetate and ketone, and (**5a**) were found to be interconverted as shown in the Table.‡

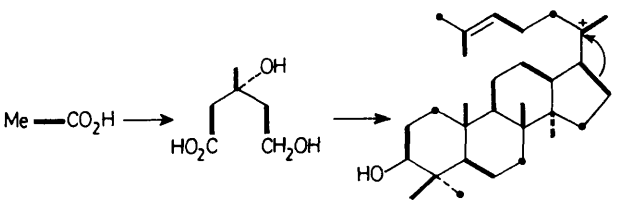
The ^{1}H-noise-decoupled spectra of (**4a**) and (**5a**) biosynthetically prepared from sodium [1,2-^{13}C]acetate, overlapping with the natural-abundance spectra, clearly showed eighteen doublets and twelve singlets. These ^{13}C double-labelling patterns, in particular the appearance of singlet signals for C-19, C-20, C-21, C-29, and C-30, provide confirmatory evidence that the biosynthesis of ursene-type triterpenes proceeds along a route indicated in the scheme

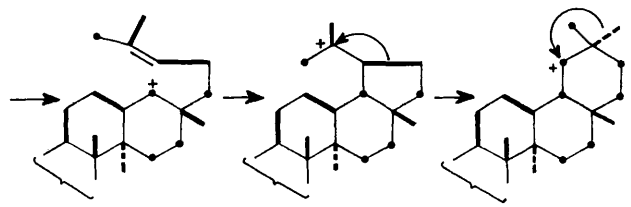


(1) R^1 = H, R^2 = β-OH
(2) R^1 = OH, R^2 = β-OH
(3) R^1 = OH, R^2 = α-OH

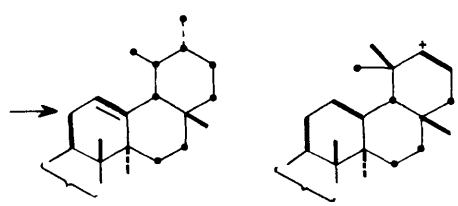


(4) R^1 = H, R^2 = β-OH
(5) R^1 = OH, R^2 = β-OH





(6)



(7)

▬ ^{13}C labelled, formed from complete Me▬CO_2H unit
• ^{13}C labelled, formed from isolated Me or CO_2H unit

† The compounds (**1a**), (**2a**), (**3a**), (**4a**) and (**5a**) were enriched by *ca.* 2·0, 2·3, 2·5, 2·0 and 2·2 times, respectively.

‡ We have also confirmed that Knight's assignments[6] for α- and β-amyrins are correct on the basis of similar experiments.

TABLE

Carbon-13 n.m.r. spectral data on methyl 3-epimaslinate (**3a**), ursolate (**4a**), and 2α-hydroxyursolate (**5a**) biosynthetically synthesized from sodium [1,2-^{13}C]acetate[a]

	(**3a**)			(**4a**)			(**5a**)		
atom	δC	Multiplicity	*J*/Hz	δC	Multiplicity	*J*/Hz	δC	Multiplicity	*J*/Hz
C-1	41·7	s		38·8	s		46·8	s	
C-2	66·5	d	38	27·3	d	38[e]	68·9	d	38
C-3	78·9	d	37	78·8	d	36	83·8	d	38
C-4	38·5[c]	d	35[e]	38·8	d	38[e]	39·1	d	36[e]
C-5	48·1	d	34	55·4	d	35	55·4	d	36
C-6	18·1	d	35[e]	18·4	d	36	18·4	d	36[e]
C-7	32·5	s		33·0	s		32·9	s	
C-8	39·7	d	36[e]	39·6	d	38[e]	39·6	d	38[e]
C-9	47·4	d	34[e]	47·5	d	36[e]	47·5	d	36[e]
C-10	38·3[c]	d	35[e]	37·0	d	38[e]	38·3	d	37[e]
C-11	23·4[b]	d	35	23·3[b]	d	36[e]	23·4[b]	d	36[e]
C-12	122·1	d	73	125·5	d	71	125·3	d	73
C-13	143·8	d	72	138·0	d	72	138·1	d	73
C-14	41·9	d	36[e]	42·0	d	37[e]	42·1	d	36[e]
C-15	27·7	s		28·2	s		28·0	s	
C-16	23·2[b]	s		24·3	s		24·3	s	
C-17	46·8	d	55[e]	48·1	d	56[e]	48·1	d	56
C-18	41·3	s		52·8	s		52·8	s	
C-19	46·0	s		39·1[c]	s		39·1[c]	s	
C-20	30·7	d	36	38·8[c]	s		38·9[c]	s	
C-21	34·0	s		30·7	s		30·7	s	
C-22	32·5	s		36·7	s		36·7	s	
C-23	28·5	s		28·2	s		28·7	s	
C-24	21·9	d	36[e]	15·5*d*	d	38[e]	17·0	d	36[e]
C-25	16·4	d	36	15·7*d*	d	38[e]	17·0	d	36[e]
C-26	17·0	d	36[e]	16·9	d	37[e]	17·0	d	36[e]
C-27	26·2	d	35	23·6[b]	d	36	23·7[b]	d	36[e]
C-28	178·1	d	55	177·7	d	56	177·9	d	56
C-29	33·2	d	36	16·9[b]	s		17·0[b]	s	
C-30	23·6	s		21·2	s		21·2	s	

[a] ^{13}C FT n.m.r. spectra were taken with a Varian NV-14 spectrometer operating at 15·09 MHz in [^{2}H]-chloroform using 8 mm tubes at room temperature (30 °C). Accuracies of chemical shifts δ_C and *J*-values were about ±0·1 and ±1 Hz, respectively.[b] The assignments of these signals were revised; *cf.* ref. 5.[c,d] The assignments of these signals were only based on experiments using the shift reagent and may be reversed. [e] These *J*-values may be accurate to ±2 Hz because of overlapping of one peak of the doublet signals with other signals.

postulated earlier,[2] and that an alternative mechanism[1] involving an intermediate (**7**) can be excluded.

The ^{13}C n.m.r. spectra of (**1a**)—(**3a**) simultaneously obtained from the callus gave twenty doublets and ten singlets. These ^{13}C labelling patterns also strongly support the biosynthetic mechanism of oleanene-type triterpenes verified previously,[1] and that oleanene- and ursene-type triterpenes are formed *via* a process involving the same intermediate (**6**). Furthermore, the C-23 and C-30 methyl groups in (**1a**)—(**3a**), each appearing as a singlet, were clearly demonstrated to be derived from C-2 of mevalonic acid, confirming the results of earlier studies.[10] It should be noted that the C-20 and C-29 signals in (**3a**) appear as an AB-type quartet at δ_C 30·7 and 33·2, respectively (*J* 36 Hz); this fact also supports the above result.§

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§ In the ^{13}C signal assignments for (**3a**) previously reported,[5] those for C-11 and C-16 should be interchanged; the other assignments were confirmed as a result of the present study; δ(C-1) 47·7 in ref. 5 should read 41·7.

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