Biosynthesis of the Terpenes Maslinic Acid and 3-Epimaslinic Acid in Tissue Cultures of *Isodon japonicus* Hara

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

Summary 3-Epimaslinic acid, an oleanane-type triterpene, was biosynthesized from maslinic acid *via* the 3-oxo-derivative; the mechanism involving cyclization of (3*R*)-2,3-oxidosqualene can be excluded in the biosynthesis of 3α-hydroxy-triterpenoids.

THE callus derived from *Isodon japonicus* Hara retains the ability to synthesize oleanolic acid (I), maslinic acid (II), and 3-epimaslinic acid (III), though diterpene derivatives formed in the intact plant are not produced in the callus.[1]

Ruzicka *et al.*[2] have proposed that oleanane type triterpenoids arise by cyclization of squalene folded in a chair–chair–chair–boat–boat form; the labelling patterns of the 3β-hydroxyoleanane-type triterpenoid from [2-^{14}C]mevalonic acid and 3*R*-[(4*R*)-4-$^{3}H_1$]mevalonic acid have been established[3] and the hypothetical mechanism conclusively confirmed. On the other hand, Halsall *et al.*[4] and Moss *et al.*[5] proposed that the 3α-hydroxytriterpenoid could arise from the (3*R*)-2,3-oxidosqualene folded in a boat–chair–chair form. Halsall *et al.* also referred to the possibility of a ketonic intermediate.

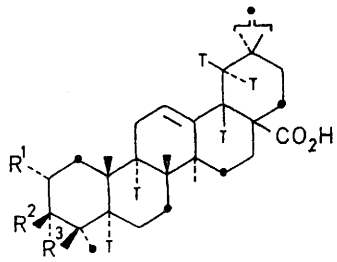
We here report the biosynthetic relationship between maslinic acid and 3-epimaslinic acid in *Isodon japonicus* tissue cultures. Cells of *Isodon japonicus* tissue cultures[1] grown on Linsmaier–Skoog agar medium containing 3*R*-[2-^{14}C; (4*R*)-4-$^{3}H_1$]mevalonic acid [^{14}C 25 μCi; ^{3}H/^{14}C 3·89] were extracted with boiling methanol. Compounds (I)—(III) were isolated from the extract by preparative t.l.c. on silica gel plates, and their methyl esters (IV)—(VI) were recrystallized from methanol to constant specific radioactivity after addition of carrier triterpenes. As shown in the Table the esters (IV) and (V) contained six tritium atoms as expected while the 3α-epimer (VI) had only five. Treatment of the esters (V) and (VI) with *p*-nitrobenzoyl chloride in pyridine gave the 2-*p*-nitrobenzoates (VII) and (VIII),† respectively, which were oxidized with Jones' reagent to give the corresponding 3-oxo-derivatives (IX) and (X). Reduction of (IX) and (X) with an excess of $NaBH_4$ in ethanol gave methyl maslinate (XI) and (XII) and *p*-nitrobenzyl alcohol, (XI) and (XII) both containing five tritium atoms. This result indicates that one of the tritium atoms was located at C-3 in biosynthesized maslinic acid (II)

TABLE

^{3}H : ^{14}C ratios of oleanane derivatives formed from 3*R*-[2-^{14}C; (4*R*)-4-$^{3}H_1$]mevalonic acid (^{3}H/^{14}C = 3·89) in tissue cultures of *Isodon japonicus*

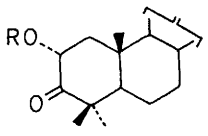
Compound	Incorporation %	^{3}H : ^{14}C ratio	^{3}H : ^{14}C atomic ratio (based upon IV)		Theoretical atomic ratio
Methyl oleanolate (IV)	1·34	3·82	5·89 : 6	6 : 6	6 : 6
Methyl maslinate (V)	5·33	3·75	5·80 : 6	5·90 : 6	6 : 6
Methyl 3-epimaslinate (VI) ..	0·82	3·08	4·75 : 6	4·84 : 6	5 : 6
(VII) : 2-nitrobenzoate of (V) ..		3·82	5·89 : 6	6 : 6	6 : 6
(VIII) : 2-nitrobenzoate of (VI) ..		3·16	4·87 : 6	4·96 : 6	5 : 6
3-Oxo-derivative (IX)		3·37	5·19 : 6	5·29 : 6	5 : 6
3-Oxo-derivative (X)		3·15	4·86 : 6	4·95 : 6	5 : 6
Methyl maslinate (XI)		3·25	5·01 : 6	5·10 : 6	5 : 6
Methyl maslinate (XII)		3·21	4·95 : 6	5·04 : 6	5 : 6

† The position of the *p*-nitrobenzoyl group in (VII) was established by its n.m.r. spectrum [δ 3·33 (d, *J* 10 Hz, 3-H) and 5·20 (m, 2-H)] and that of (IX) [δ 5·87 (q, *J* 14 and 16 Hz, 2-H)]. (IX) was identical with (X) in its i.r. and t.l.c. properties.



•= ^{14}C, T= ^{3}H

(I) R^1= R^3= H, R^2= OH
(II) R^1= R^2= OH, R^3= H
(III) R^1= R^3= OH, R^2= H



(IX) and (X) *p*-NO_2·C_6H_4·CO
(XIV) R = H

while no tritium was present at C-3 in biosynthesized 3-epimaslinic acid (III) since it was eliminated during biosynthesis. [$^{14}C_6$]Maslinic acid ($1{\cdot}36 \times 10^6$ d.p.m.) biosynthesized from [2-^{14}C]mevalonic acid was mixed with water containing 0·3% of Tween 80 and the mixture was added to cells of *Isodon japonicus* tissue cultures under sterile conditions. After six days 3-epimaslinic acid (XIII) was extracted and isolated as described above, and the methyl ester of (XIII) was recrystallized to constant specific radioactivity after addition of carrier compound. The methyl 3-epimaslinate obtained was radioactive ($1{\cdot}49 \times 10^5$ d.p.m./mmol; incorporation 0·35%). Therefore, 3-epimaslinic acid is biosynthesized from maslinic acid *via* the 3-oxo-derivative (XIV), and a mechanism involving cyclization of (3*R*)-2,3-oxidosqualene can be excluded in 3α-hydroxytriterpenoid biosynthesis.

Moreover, the apparent biological reduction of the 3-oxo-group of (XIV) must give exclusively the α-hydroxy-compound (III) since the $^{3}H/^{14}C$ ratio for maslinic acid biosynthesized from 3*R*-[2-^{14}C; (4*R*)-4-$^{3}H_1$]mevalonic acid is exactly identical with the atomic ratio 6 : 6.

(*Received, 6th July* 1973; *Com.* 966.)

[1] Y. Tomita, S. Seo, and E. Sakurai, in preparation.
[2] L. Ruzicka, *Experientia*, 1953, **9**, 357; A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, 1955, **38**, 1890.
[3] D. Arigoni, *Experientia*, 1958, **14**, 153; H. H. Rees, G. Britton, and T. W. Goodwin, *Biochem. J.*, 1968, **106**, 659.
[4] G. P. Cotterell, T. G. Halsall, and M. J. Wriglesworth, *J. Chem. Soc.* (*C*), 1970, 739.
[5] G. P. Moss and S. A. Nicelaids, *Chem. Comm.*, 1969, 1072.