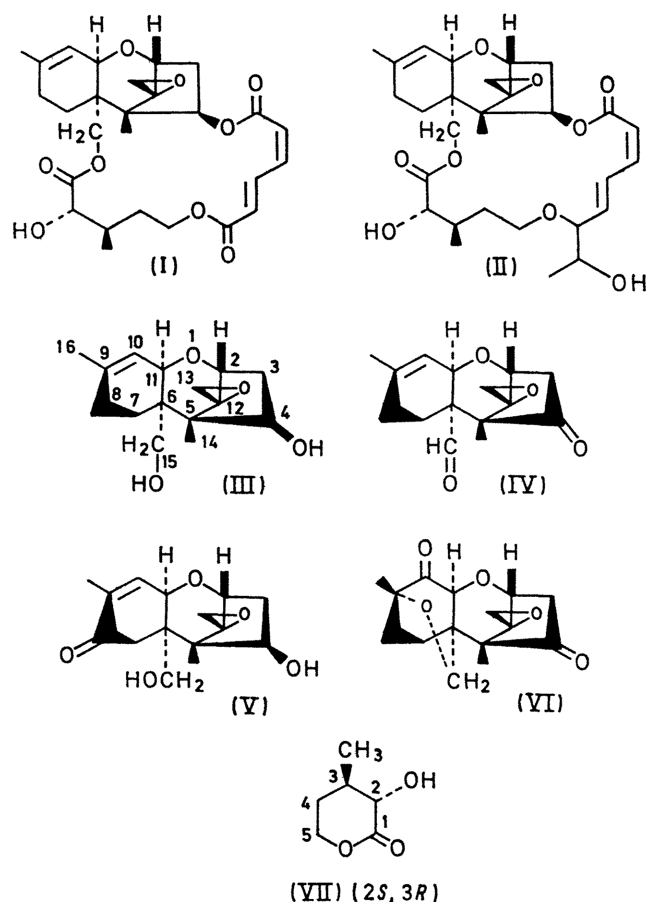
Biosynthesis of Verrucarol, the Sesquiterpene Moiety of the Verrucarins and Roridins

By R. ACHINI, B. MÜLLER, and CH. TAMM*
(*Institut für Organische Chemie der Universität Basel* CH-4056 *Basel, Switzerland*)

Summary Incorporation experiments with (3*R*)-[5-^{14}C]-mevalonate and both enantiomers of [2-^{3}H]mevalonate into verrucarin A and roridin A indicate that hydroxylation at C-4 of verrucarol proceeds with retention of configuration; they confirm that C-8 and not C-10 is derived specifically from C-2 of mevalonate, and that the *pro*-2-*S* hydrogen of mevalonate is lost during the biosynthesis of verrucarinolactone.

VERRUCAROL (III)[1] is the sesquiterpene moiety of the verrucarins and roridins, a class of macrocyclic di- and tri-ester antibiotics isolated from cultures of *Myrothecium verrucaria* and *M. roridum*[2] It is obtained by base-catalysed hydrolysis of the metabolites by which verrucarin A (I)[3] yields verrucarinolactone (VII) and *cis,trans*-muconic acid as further products and roridin A (II)[4] gives roridinic acid.

Earlier work[5] confirmed the sesquiterpene nature of the trichothecane[6] skeleton (three molecules of mevalonic acid were incorporated) and also indicated that various rearrangements occurred subsequently. The (3*R*) enantiomer of mevalonic acid was first incorporated into verrucarol (III) and verrucarinolactone (VII) by feeding (3*R*)-[5-^{14}C]mevalonate to the mould followed by degradation of the metabolites. To study the mechanism of later stages, specifically tritiated mevalonates were incorporated into verrucarol (III). Administration of [2-$^{3}H_2$,2-^{14}C]-sodium mevalonate (^{3}H : ^{14}C, 9·6 : 1) to growing cultures of *Myrothecium* and isolation of the metabolites verrucarin A (I) and roridin A (II) followed by hydrolysis of (II) gave verrucarol (III) (^{3}H : ^{14}C, 7·37 : 1) corresponding to 4·61 tritium atoms The departure from the expected value (5·0 tritium atoms) may reflect the reversible mode of action of prenyl isomerase.[7] Oxidation of the verrucarol to the keto-aldehyde (IV) resulted in the loss of a fifth of the tritium activity consistent with the attachment of one tritium atom to C-4. The loss of one tritium atom and not a fractional amount indicates that the hydroxylation at C-4 is stereospecific, and is inconsistent with the presence of tritium at C-15. Further oxidative transformation of verrucarol (III) using selenium dioxide gave the 8-oxo-compound (V); the ratio ^{3}H to ^{14}C of this was three fifths that of verrucarol indicating that two tritium atoms had been eliminated from C-8. To confirm that tritium was



absent from C-10, verrucarol (III) was epoxidised and converted by known steps[8] into the diketo-ether (VI) with the loss of only the C-4 tritium atom. Location of the

radioactivity at C-8 and not C-10 is in agreement with the formation of verrucarol by the proposed route *via* bisabolene.[8]

To determine the stereochemistry of the hydroxylation step at C-4 (3*R*)-[(2S)-2-^{3}H]/(3*S*)-[(2*R*)-2-^{3}H] and (3*S*)-[(2*S*)-2-^{3}H]/(3*R*)-[(2*R*)-2-^{3}H] sodium mevalonates were fed to the moulds in separate experiments (Table). It would be expected that only the (3*R*)-mevalonate would be incorporated into the sesquiterpene moiety of verrucarin A assuming that the compounds are derived from farnesol which had been built up normally.[9] Oxidation of verrucarol derived from the (3*R*)-[(2*R*)-2-^{3}H]/(3*S*)-[(2*S*)-2-^{3}H]-mevalonate experiment to the keto-aldehyde (IV) produced no change in the specific activity indicating that C-4 carried no tritium. The conversion of the verrucarol derived from the (3*R*)-[(2*S*)-2-^{3}H]/(3*S*)-[(2*R*)-2-^{3}H]-mevalonate into the keto-aldehyde (IV) resulted in a 35—40%† loss of tritium activity consistent with incorporation of three atoms of tritium into verrucarol, one at C-4. Hence the orientation of the C-4 hydroxy-group corresponds to the "*pro*-2-*R*" hydrogen atom of mevalonate and, on the basis of current biogenetic proposals of a bisabolene intermediate,[10] indicates that hydroxylation occurs with retention of configuration. These conclusions agree with those for tricothecin and trichodermol (roridin C).[11]

TABLE

Tracer experiments of Myrothecium verrucaria

Mevalonate precursor	Compound	Specific activity (d.p.m.) ^{3}H/mmol	^{14}C/mmol	^{3}H : ^{14}C Activity ratio	^{3}H : ^{14}C atomic ratio	% Incorporation
[2-^{3}H,2-^{14}C](^{3}H : ^{14}C, 9·6 : 1)	Verrucarin A (I)	110·2 × 10^5	15·78 × 10^5	7·0	—	0·45
	Roridin A (II)	73·0 × 10^5	12·3 × 10^5	5·94	—	3·0
	Verrucarol (III)	48·2 × 10^5	65·4 × 10^4	7·37	4·61(5·0)*	
	Keto-aldehyde (IV)	38·9 × 10^5	65·5 × 10^4	5·95	3·72(4·0)*	
	8-Ketone (V)	29·0 × 10^5	63·5 × 10^4	4·56	2·78(3·0)*	
	Diketo-ether (VI)	39·6 × 10^5	64·7 × 10^4	6·12	3·79(4·1)*	
(3*R*)-[(2*S*)-2-^{3}H]/3*S*-[(2*R*)-2-^{3}H]	Verrucarin A (I)	34·5 × 10^4				0·33
	Verrucarol (III)	37·0 × 10^4				
	Keto-aldehyde (IV)	22·0 × 10^4				
	Verrucarinolactone (VII)	0·3 × 10^4				
(3*R*)-[(2*R*)-2-^{3}H]/3*S*-[(2*S*)-2-^{3}H]	Verrucarin A (I)	14·4 × 10^4				0·02
	Verrucarol (III)	9·8 × 10^4				
	Keto-aldehyde (VI)	10·3 × 10^4				
	Verrucarinolactone (VII)	6·2 × 10^4				
(3*R*)-[5-^{14}C]	Verrucarin A		30·1 × 10^3			1·5
	Roridin A (II)		63·8 × 10^3			2·2
	Verrucarol (III)		21·6 × 10^3			
	Verrucarinolactone (VII)		8·1 × 10^3			

* Values corrected for verrucarol = 5 tritium atoms.

It is of interest that only (3*R*)-[(2*R*)-2-^{3}H]/(3*S*)-[(2*S*)-2-^{3}H]-mevalonate is incorporated into the macrocyclic ring of verrucarin A (Table). The feeding experiment with (3*R*)-[5-^{14}C]-mevalonate indicates that the "*pro*-2*S*" hydrogen of mevalonate is lost in the formation of verrucarinolactone. These results together with full details of the degradation of the macrocyclic ring of verrucarin A and roridin A will be reported elsewhere.

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† Reduced accuracy owing to low activity.

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