307. The Mode of Incorporation of Farnesyl Pyrophosphate into Verrucarol

Preliminary Communication[1])

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Summary. In the course of the biosynthesis of verrucarol (**3**) from farnesyl pyrophosphate in *Myrothecium roridum*, strain S 1135, a hydride shift occurs from the central double bond of the precursor to C(2) of the product.

Verrucarol (**3**) [2] is the neutral sesquiterpene moiety of the verrucarins and roridins, a class of macrocyclic di- and triester antibiotics isolated from cultures of *Myrothecium verrucaria* and *M. roridum* [3]. It is obtained by base catalysed hydrolysis from verrucarin A (**1**) [4] together with *cis,trans*-muconic acid and verrucarinic acid, isolated as lactone, and from roridin A (**2**) [5] next to roridinic acid (**6**).

The sesquiterpene nature of the basic trichothecane skeleton [6] of **3** was established by the incorporation of various labelled mevalonic acids into trichothecolone (**4**) [7], trichodermol (roridin C) (**5**) [8] and verrucarol (**3**) [9]. In addition *all-trans* farnesyl pyrophosphate [10] and trichodiene (**8**) [11] have been recognized as precursors of trichothecolone (**4**) in *Trichothecium roseum.*

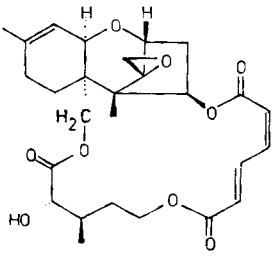
To cast additional light on the mechanism responsible for the transformation of farnesyl pyrophosphate into trichothecane derivatives, we have now investigated the incorporation of [6-^{3}H[-*trans*-farnesyl pyrophosphate into verrucarol (**3**). The required labelled material was prepared as follows:

The trisnor-aldehyde available from the selective ozonolysis [12] of geranyl tetrahydropyranyl ether was labelled with tritium as shown in **9** through reduction with [^{3}H]-$NaBH_4$ followed by subsequent oxidation of the resulting alcohol with CrO_3 in pyridine methylene chloride [13]. The labelled compound **9** was then converted to [6-^{3}H]-*all-trans* farnesol (**10**) by the methode of *Corey* & *Yamamoto* [14]. A [^{14}C]-farnesol standard, labelled at C(12) and C(13) as indicated in **11**, was obtained by the *Wittig* reaction of the trisnor-aldehyde from farnesyl acetate with [1-^{14}C)-isopropylidenediphenylphosphorane, prepared by the alkylation of ethylidenphosphorane with [^{14}C]-methyl iodide.

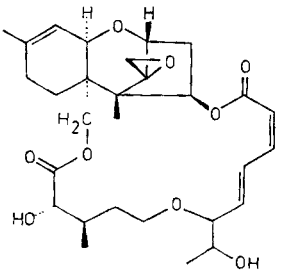
The two labelled farnesols **10** and **11** were admixed to give a ^{3}H:^{14}C ratio of 4.50. After conversion to the pyrophosphates a total [^{14}C]-activity of $4.0 \cdot 10^6$ dpm was fed to a stirred culture of *Myrothecium roridum*, strain S 1135. The usual work-up [3] gave as the main product radioactive roridin A (**2**) showing a total incorporation of 0.22% and no change in the ^{3}H:^{14}C ratio (*cf.* Table). Base catalysed hydrolysis of the labelled metabolite **2** gave radioactive verrucarol (**3**) (Prep. A) (100% of total activity;

[1]) Verrucarins and Roridins, 26th Commun.; 25th Commun. see [1].

Scheme 1



1



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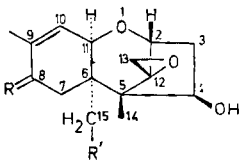
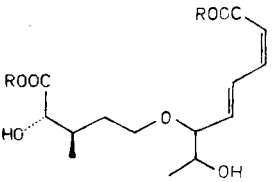
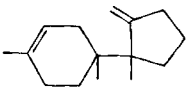


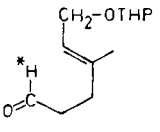
3 R = H_2 R′ = OH
4 R = O R′ = H
5 R = H_2 R′ = H



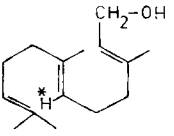
6 R = H **7** R = CH_3



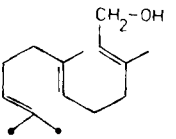
8



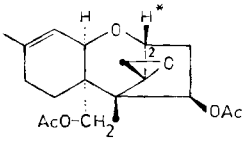
9



10

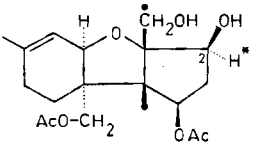


11



12

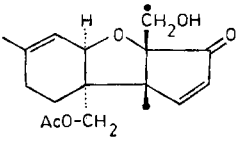
H_2SO_4
dioxane



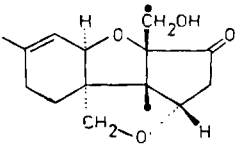
13

MnO_2
acetonitril

KOH
MeOH



14



15

^3H:^{14}C = 4.40). The roridinic acid (**6**), isolated as dimethyl ester **7** contained less than 5% of the activity, thus showing that incorporation had been specific. The mixture of the minor metabolites was hydrolysed as well and yielded a further amount of verrucarol (**3**) (Prep. B) with the same ratio of radioactivities.

For the localization of the ^3H-label, the two samples of verrucarol were pooled and converted into the di-O-acetyl derivative **12**. Acid catalysed rearrangement of **12** [2] yielded the apotrichothecane derivative **13** with only a slight change of the isotopic ratio. Subsequent selective oxidation of **13** with MnO_2 in acetonitril gave, with concurrent elimination of acetic acid, the oily α,β-unsaturated ketone **14**. Hydrolysis of **14** with methanolic KOH was accompanied by internal 1,4-addition to the conjugated double bond and resulted in the formation of **15**.

The almost complete loss of tritium radioactivity associated with the conversion of **13** into **15** (*cf.* Table) serves to locate the tritium label specifically at C(2) or, less likely at C(3), in verrucarol (**3**). This result, which confirms and extends previous findings on the biosynthesis of trichothecolone (**4**) from [1,1-^3H)-geranyl pyrophosphate [15], requires an intramolecular hydride shift in the formation of the trichothecane skeleton. Since the terminus of a similar hydride migration has been detected unambiguously for the formation of the biogenetically related cuparane system [16] the available evidence for the biosynthesis of verrucarol and its congeners [17] can now be summarized as in *scheme 2*.

Whereas more work is needed to elucidate all the details of the conversion (**a**→**b**) [10] [18] it is clear that the double addition to the central double bond of the immediate

Scheme 2

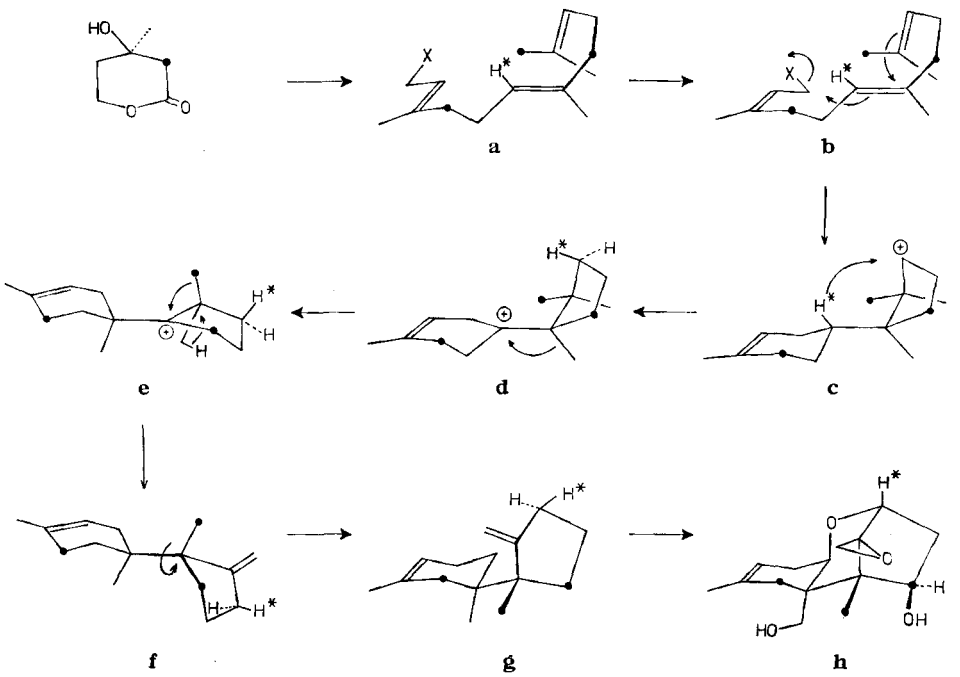


Table. *Distribution of Radioactivity*

Compound	Specific Activity dpm/mmol		^3H:^{14}C-Activity Ratio	Percent of total ^3H
	^3H	^{14}C		
[6-^{3}H-12,13-$^{14}C_2$]-farnesyl pyrophosphate			4.50	
Roridin A (**2**)	$54.8 \cdot 10^4$	$12.1 \cdot 10^4$	4.52	100
Dimethyl roridinate (**7**)	$2.2 \cdot 10^4$	$0.57 \cdot 10^4$	3.96	4.2
Verrucarol (**3**) Prep. A	$53.2 \cdot 10^4$	$12.1 \cdot 10^4$	4.40	97
Verrucarol (**3**) Prep. B	$40.4 \cdot 10^4$	$9.05 \cdot 10^4$	4.46	–
Diol **13**	$42.9 \cdot 10^4$	$10.0 \cdot 10^4$	4.29	100
Ketoether **15**	$1.05 \cdot 10^4$	$9.75 \cdot 10^4$	0.11	2.4

aliphatic precursor (**b** → **c**) occurs in an overall *cis*-fashion and that the insertion of the oxygen function at C(2) (*cf.* **g** → **h**) takes place with retention of configuration.

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REFERENCES

[1] *Ch. Tamm, B. Böhner & W. Zürcher*, Helv. *55*, 510 (1972).
[2] *J. Gutzwiller, R. Mauli, H. P. Sigg & Ch. Tamm*, Helv. *47*, 2234 (1964).
[3] *E. Härri, W. Löffler, H. P. Sigg, H. Stähelin, Ch. Stoll, Ch. Tamm & D. Wiesinger*, Helv. *45*, 839 (1962); *B. Böhner, E. Fetz, E. Härri, H. P. Sigg, Ch. Stoll & Ch. Tamm*, ibid., *48*, 1079 (1965); *W. Zürcher & Ch. Tamm*, Helv. *49*, 2594 (1966).
[4] *J. Gutzwiller & Ch. Tamm*, Helv. *48*, 157 (1965).
[5] *B. Böhner & Ch. Tamm*, Helv. *49*, 2527 (1966).
[6] *W. O. Godtfredsen, J. F. Grove & Ch. Tamm*, Helv. *50*, 1666 (1967).
[7] *E. R. H. Jones & G. Lowe*, J. chem. Soc. *1960*, 3959.
[8] *B. Achilladelis, P. M. Adams & J. R. Hanson*, Chem. Commun. *1970*, 511.
[9] *R. Achini, B. Müller & Ch. Tamm*, Chem. Commun. *1971*, 404.
[10] *R. E. Evans, A. M. Holtom & J. R. Hanson*, Chem. Commun. *1973*, 465.
[11] *Y. Machida & S. Nozoe*, Tetrahedron Letters *1972*, 1969.
[12] *S. Escher, P. Loewe & D. Arigoni*, Chem. Commun. *1970*, 823; *E. Bertele & P. Schudel*, Helv. *50*, 2445 (1967); *E. J. Corey, K. Achiwa & J. A. Katzenellenbogen*, J. Amer. chem. Soc. *91*, 4318 (1969); *G. Stork, M. Gregson & P. A. Grieco*, Tetrahedron Letters *1969*, 1391.
[13] *J. C. Collins*, Tetrahedron Letters *1968*, 3363.
[14] *E. J. Corey & H. Yamamoto*, J. Amer. chem. Soc. *92*, 6637 (1970).
[15] *P. M. Adams & J. R. Hanson*, Chem. Commun. *1971*, 1414.
[16] *D. E. Cane, S. Godtfredsen & D. Arigoni*, Helv. *56* (1973), in press.
[17] Cf. *K. H. Overton*, Senior Reporter: A Specialist Periodical Report: Terpenoids and Steroids, Vol. 2, pg. 204, The Chemical Society, London, W1V OBN, 1972.
[18] *K. H. Overton & F. M. Roberts*, Chem. Commun. *1973*, 378.