56. Biosynthesis of the Verrucarins and Roridins. Part 4. The Mode of Incorporation of (3*R*)-[(5*R*)-5-^{3}H]-Mevalonate into Verrucarol. Evidence for the Identity of the C(11)-Hydrogen Atom of the Trichothecane Skeleton with the (5*R*)-Hydrogen Atom of (3*R*)-Mevalonic Acid

Verrucarins and Roridins, 30th Communication [1]

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(19. XII. 74)

Summary. Incorporation of (3*R*)-[(5*R*)-5-^{3}H]-mevalonate into verrucarin followed by hydrolyses to verrucarol (**2**) and transformation of the latter to the spirolactol **5** and the spirolactone **6** successively, demonstrate the identity of the C(11)-hydrogen of the trichothecane skeleton with the 5-'pro-*R*' hydrogen atom of (3*R*)-mevalonate.

It has been demonstrated by us [2], and independently by other authors [3], that it is not the *cis,cis* form of farnesol as anticipated by earlier workers [4], but the *cis,trans* isomer **12** which undergoes cyclization to form the trichothecane skeleton. The evidence of a hydrogen 1,5-shift has ruled out bisabolene as an intermediate and has shown that farnesol cyclizes directly [5] [6]. The isomerization of *trans,trans*-farnesol (**8**) to *cis,trans*-farnesol (**12**) is an oxydation-reduction reaction with *trans, trans*-farnesal (**10**) and *cis,trans*-farnesal (**11**) as intermediates [3b] [7], whereby one hydrogen atom of the skeleton is lost in the first step. Very recently *Overton & Roberts* [8] have demonstrated that the 'pro-*S*' hydrogen atom is eliminated in the *trans-cis* reaction when a tissue culture of *Andrographis paniculata* is used; conversely *Imai & Marumo* [9] have found that the 'pro-*R*' hydrogen atom is abstracted in the *trans-cis* isomerization in the fungal system of *Helminthosporium sativum*. In both cases the hydrogen exchange was shown to be stereospecific. If farnesol which is not labelled stereospecifically at C(1) (C(1) of farnesol corresponds to C(5) of mevalonic acid) is used as precursor, this loss of a specific hydrogen atom cannot be recognized in the trichothecane skeleton because the corresponding 11-position is trisubstituted. The discovery of the new metabolites **17** and **18** as biogenetic intermediates of the cyclization of farnesol has provided further information on the mechanism of trichothecane biosynthesis [10]. Thus the transformation of trichodiene (**14**) into trichodiol (**17**) must be accompanied again by the loss of a hydrogen atom.

To cast additional light on the origin of the C(11) hydrogen atom of the trichothecane skeleton, *i.e.* the mechanism of the cyclization of trichodiene (**14**), we have investigated the incorporation of the stereospecifically tritiated precursor, (3*R*)-[(5*R*)-5-^{3}H]-mevalonate (**1**)[1]), into verrucarol (**2**) *via* verrucarin A and B. 3(*R,S*)-

[1]) We are most grateful to Prof. Dr. *D. Arigoni*, ETH, Zürich, for the generous gift of a specimen of this material.

[2-^{14}C]-mevalonate was used as internal standard. The two labelled mevalonates were admixed to give a ^{3}H:^{14}C ratio of 6.2. They were administered to a growing culture of *Myrothecium verrucaria*, strain S 118/F, when the production of the desired metabolites had just started. A semiquantitative method for the determination of the metabolites, using thin layer chromatography (TLC.), was applied. Extraction of the culture broth with ethyl acetate, and chromatography of the crude extract on a silica gel column yielded radioactive verrucarin A [11] as main product, with a total incorporation of 1%, together with verrucarin B [11]. The ^{3}H:^{14}C ratio was 11.6 in both metabolites (*cf.* Table). Base catalysed hydrolysis of the labelled verrucarin A gave radioactive verrucarol (**2**), radioactive verrucarinolactone and radioinactive *cis,trans*-muconic acid (isolated as dimethyl ester). The active hydrolysis products showed no change in the ^{3}H:^{14}C ratio. The distribution is in agreement with the earlier findings, demonstrating that 3 molecules of mevalonate are incorporated into verrucarol and one into verrucarinic acid. Accordingly the tritium labels in verrucarol should be found at C(3), C(7) and C(11). The unchanged ^{3}H:^{14}C ratio indicates that no tritium had been eliminated during the biosynthesis. It is interesting to note that the rate of incorporation of [(5*R*)-5-^{3}H]-mevalonate was about twice as high as that of [2-^{14}C]-mevalonate[2]. Whereas the former possesses the natural 3*R*-configuration, the latter is the racemic 3(*R*,*S*)-form.

Table. *Distribution of Radioactivity*[3])

Compound	Specific Activity dpm/mmol		^{3}H:^{14}C Activity	% of Total ^{3}H	Abs. Rate of Incorporation
	^{3}H	^{14}C	Ratio		% (^{14}C)
Sodium (3*R*)-[(5*R*)-5-^{3}H]-+ (3*R*,*S*)-[2-^{14}C]-mevalonates			6.2		
Verrucarin A	$98.89 \cdot 10^4$	$8.54 \cdot 10^4$	11.6	100	0.97
Verrucarin B	$95.70 \cdot 10^4$	$8.25 \cdot 10^4$	11.6		0.13
Verrucarol (**2**)	$71.02 \cdot 10^4$	$6.48 \cdot 10^4$	11.0	76	
Verrucarinolactone	$29.76 \cdot 10^4$	$2.64 \cdot 10^4$	11.3	31	
Dimethyl *cis,trans*-muconate	0	0	–	0	
Hydroxy-keto aldehyde **4**	$69.03 \cdot 10^4$	$6.25 \cdot 10^4$	11.0	100[a])	
Spirolactol **5**	$47.25 \cdot 10^4$	$6.02 \cdot 10^4$	7.8	66.5[a])	
Spirolactone **6**	$23.34 \cdot 10^4$	$5.90 \cdot 10^4$	3.9	32.8[a])	

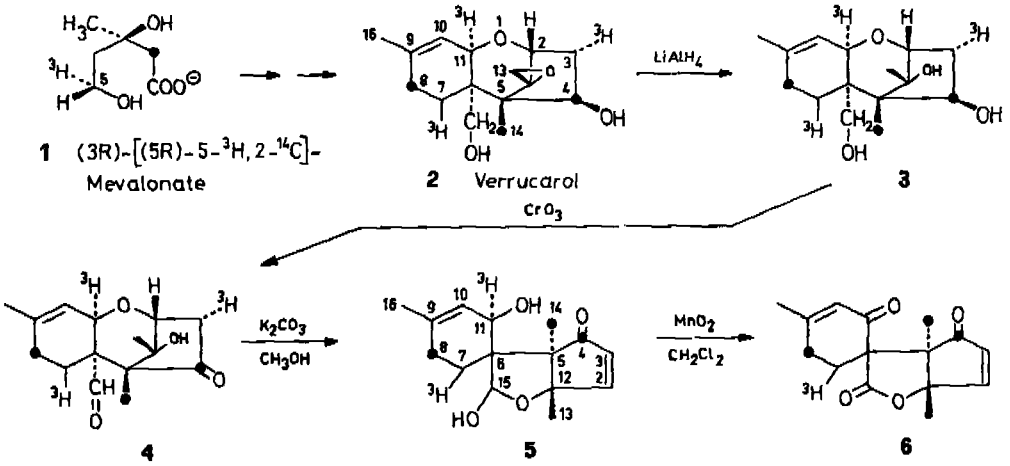
[a]) Related to verrucarol as 100%.

[2]) The slight deviation of the values of the ^{3}H:^{14}C ratio, found for verrucarin A and B, and for verrucarol and verrucarinolactone, from the theoretical ratio of 12.4 might be due to some exchange of tritium with the culture fluid and to the fact that the preparation of (3*R*)-mevalonate was not absolutely optically pure.

[3]) We thank *H. Galliker*, Isotopenlaboratorium *Sandoz* A.G., Basel, for the radioactivity determinations. These were carried out by dissolving the samples in toluene scintillation fluid and measuring directly on a Tri-Carb *Packard* Model 3375 scintillation spectrometer. Precision of measurement is ± 5% for the doubly labelled samples.

This result confirms the previous findings that it is the natural enantiomer of mevalonic acid which is used as precursor in the biosynthesis of the trichothecanes [12]. In order to preclude wrong conclusions due to unexpected migrations of tritium, we have determined the tritium labelling at C(11) as well as at C(3) in the following manner (*cf. Scheme 1*).

Scheme 1

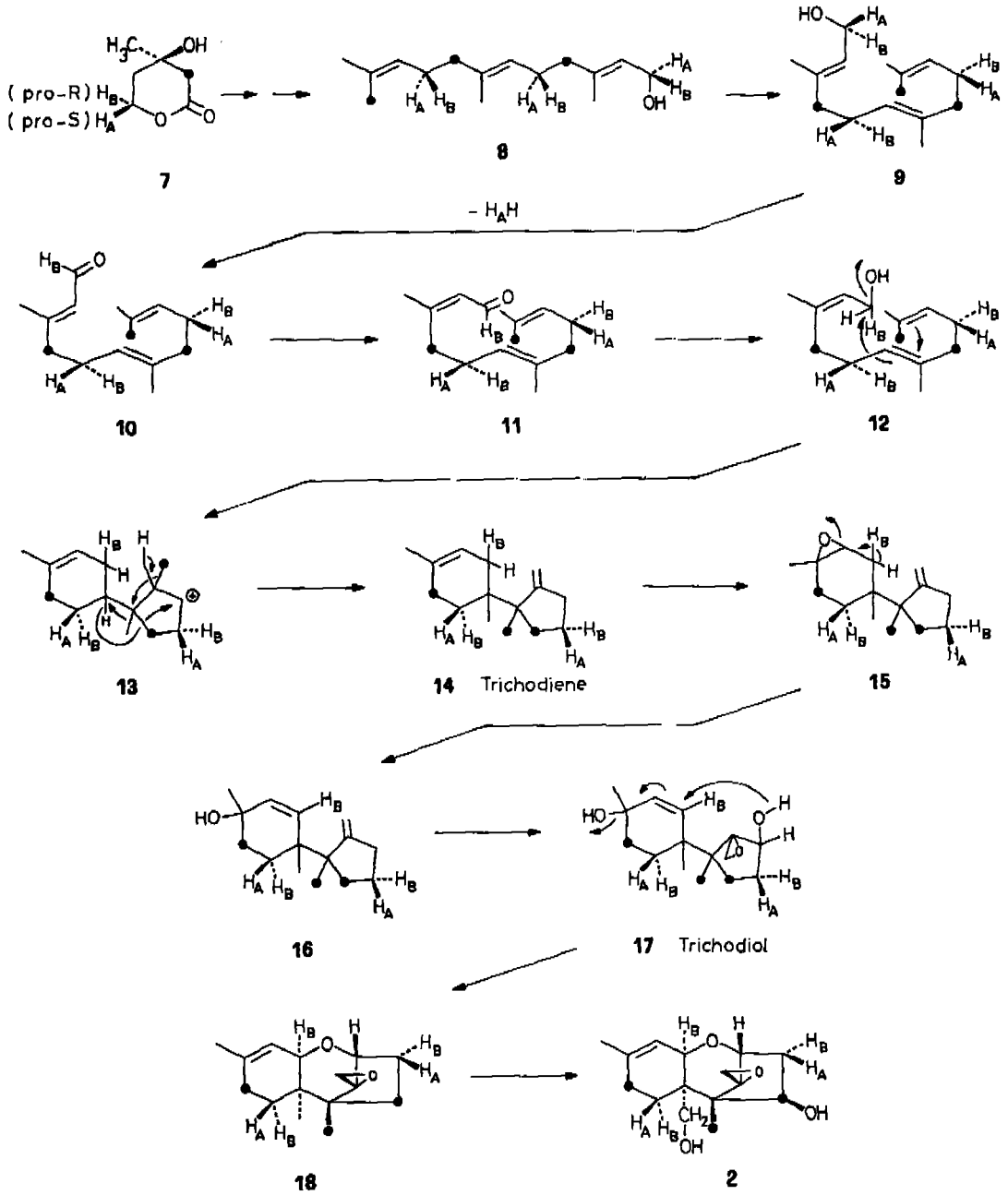


1 (3R)-[(5R)-5-^{3}H, 2-^{14}C]-Mevalonate **2** Verrucarol **3** **4** **5** **6**

Reductive cleavage of the epoxy group of verrucarol (**2**) by treatment with $LiAlH_4$ in tetrahydrofuran yielded the triol **3**. It was oxidized by CrO_3/H_2SO_4 in acetone to the hydroxy keto aldehyde **4**. The $^3H:^{14}C$ ratio remained unchanged by these transformations. Treatment of the hydroxy keto aldehyde **4** with potassium carbonate in aqueous methanol led to the spirolactol **5**. The mechanism of this rearrangement reaction and proof of structure of this product will be discussed in a later communication. Simultaneously tritium attached to C(3), *i.e.* in α-position to the carbonyl group, is exchanged (*cf.* [11]). Therefore the mole specific tritium activity in the spirolactol **5** is reduced by one third as compared to its predecessor **4**, thus proving the presence of one tritium atom at C(3) in verrucarol (**2**). Subsequent oxidation of **5** with MnO_2 in methylene chloride gave the spirolactone **6**. The loss of a further third of the original tritium activity on conversion of **5** to **6** serves to locate the tritium label at C(11). The presence of the third tritium label at C(7) follows from results obtained by exchange reactions carried out with trichothecolone [11].

The results demonstrate that the isomerization of *all-trans*-farnesol (**8**) to *cis,trans*-farnesol (**12**) *via* the aldehydes **10** and **11** is a stereospecific reaction (*cf. Scheme 2*). The hydrogen atom H_A, which corresponds to the 5-'pro-*S*' hydrogen atom of mevalonolactone (**7**) is eliminated whereas the 5-'pro-*R*' hydrogen atom H_B is retained. In addition, in the course of the transformation of trichodiene (**14**) (whose formation *via* the proposed intermediate **13** would require an intramolecular hydride shift) to trichodiol (**17**), it is again the 11-hydrogen atom H_B which is retained. The

Scheme 2



same hydrogen atom, which was added in the course of the oxidation-reduction process (**8** → **12**), is removed in this later stage of the biosynthetic pathway.

We thank Prof. *D. Arigoni*, ETH, Zürich, for very stimulating discussions. Support of this work by the «*Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung*» (Projects no. 2.675.72 and 2.0550.73) and by *Sandoz* A.G., Basel, is gratefully acknowledged.

Experimental Part

1. Culture experiment. – *Medium:* 2.67 g NH_4Cl, 2.0 g KH_2PO_4, 2.0 g $MgSO_4 \cdot 7H_2O$, 20.0 g Glucose, 2.0 g malt extract, 2.0 g peptone and 2.0 g yeast extract per l of distilled water. 1.5 l of sterile culture medium were placed in a 2 l fermenter and inoculated under sterile conditions with 100 ml of a spore suspension of *Myrothecium verrucaria*, strain S 118/F. The fermentation took place at 27° with stirring (450 rpm) and air circulation (1.5 l per min). 2.8 μCi sodium (3*R*,*S*)-[2-^{14}C]-mevalonate and 17 μCi sodium (3*R*)-[(5*R*)-5-^{3}H]-mevalonate dissolved in 10 ml H_2O were added with a milipore sterile filter syringe 28 h after inoculation. The culture solution was extracted with ethyl acetate 93 h after addition of the precursors, the organic solution washed with H_2O, dried with $MgSO_4$ and evaporated to dryness *in vacuo*. The crude extract was chromatographed on 125 g of silica gel (0.05–0.2 mm from *E. Merck* A.G., Darmstadt). The elution was accomplished with methylene chloride containing 1% of methanol. In this manner a crystalline crude mixture of verrucarine A and B was separated from more polar material. Recrystallization from ether yielded 400 mg of crystalline mixture. It was separated by preparative TLC. Verrucarin A and B were obtained in a ratio of 7.6:1. They were identical with authentic material in all respects. The samples were dried 8 h at 0.02 Torr and 40°.

Verrucarin A: Crystallization 1: 2000 dpm/mg ^{3}H; 173 dpm/mg ^{14}C. Crystallization 2: 1950 dpm/mg ^{3}H; 166 dpm/mg ^{14}C. Crystallization 3: 1960 dpm/mg ^{3}H; 172 dpm/mg ^{14}C.

Verrucarin B: Crystallization 1: 1900 dpm/mg ^{3}H; 167 dpm/mg ^{14}C. Crystallization 2: 1930 dpm/mg ^{3}H; 164 dpm/mg ^{14}C.

2. Hydrolysis of verrucarin A and B. Verrucarin A and a mixture of verrucarin A and B were hydrolyzed separately with K_2CO_3 in aqueous methanol according to the procedure described by *Gutzwiller & Tamm* [13]. The hydrolysis products from the verrucarin A sample gave the following radioactivities:

Verrucarol (**2**), recrystallized from ether/methylene chloride: Crystallization 1 (m.p. 157–158°): 2660 dpm/mg ^{3}H; 242 dpm/mg ^{14}C. Crystallization 2 (m.p. 157–158°): 2690 dpm/mg ^{3}H; 258 dpm/mg ^{14}C. Crystallization 3 (m.p. 158–159°): 2660 dpm/mg ^{3}H; 233 dpm/mg ^{14}C.

Verrucarinolactone, recrystallized from ether after sublimation at 60° and 15 Torr: Crystallization 1 (m.p. 97–98°): 2280 dpm/mg ^{3}H; 205 dpm/mg ^{14}C. Crystallization 2 (m.p. 97–98°): 2240 dpm/mg ^{3}H; 212 dpm/mg ^{14}C. Crystallization 3 (m.p. 99–100°): 2310 dpm/mg ^{3}H; 200 dpm/mg ^{14}C. Crystallization 4 (m.p. 98–101°): 2340 dpm/mg ^{3}H; 195 dpm/mg ^{14}C.

Dimethyl cis,trans-*muconate*, prepared from isolated *cis,trans*-muconic acid by treatment with diazomethane, and recrystallized from ether/petroleum ether: 0 dpm/mg ^{3}H; 0 dpm/mg ^{14}C.

3. Reactions of verrucarol (2). – 3.1. *Reduction with $LiAlH_4$ to the triol* **3**. 158 mg of **2** were reduced with $LiAlH_4$ in tetrahydrofuran according to the procedure described by *Gutzwiller & Tamm* [13]. After purification of the crude product by preparative TLC. with methylene chloride containing 10% of methanol, 151 mg of triol **3** with m.p. 144–145° was obtained. It was identical with authentic material in all respects.

3.2. *Oxidation of triol* **3** *to the hydroxy keto aldehyde* **4**. A solution of 110 mg of triol **3** in 12 ml of acetone was treated with 0,36 ml of CrO_3-H_2SO_4 solution (2,67 g CrO_3, 2.3 ml of conc. H_2SO_4 and H_2O *ad* 10 ml) at 50° for 5 min. After addition of 20 ml of H_2O the acetone was removed *in vacuo*. After usual work up, 74 mg of hydroxy keto aldehyde **4**, m.p. 185–191°, were obtained by crystallization from ether. Crystallization 1 (m.p. 185–191°): 2590 dpm/mg ^{3}H; 230 dpm/mg ^{14}C. Crystallization 2 (m.p. 190–192°): 2650 dpm/mg ^{3}H; 235 dpm/mg ^{14}C. Crystallization 3 (m.p. 190–192°): 2610 dpm/mg ^{3}H; 240 dpm/mg ^{14}C.

3.3. *Rearrangement of the hydroxy keto aldehyde* **4** *to the spirolactol* **5**. A solution of 64 mg of **4** in 6.4 ml of methanol was treated with a solution of 220 mg K_2CO_3 in 3.2 ml H_2O for 1 h at 60°. After extraction with methylene chloride and evaporation, 62 mg of crude product were obtained. Purification by preparative TLC. with benzene/ethyl acetate 2:1 yielded, after crystallization from ether, 29 mg of pure spirolactol **5** of m.p. 128–131°. Crystallization 1 (m.p. 128–131°): 1770 dpm/mg ^{3}H; 222 dpm/mg ^{14}C. Crystallization 2 (m.p. 130–132°): 1786 dpm/mg ^{3}H; 223 dpm/mg ^{14}C. Crystallization 3 (m.p. 130–132°): 1820 dpm/mg ^{3}H; 239 dpm/mg ^{14}C.

3.4. *Oxidation of the spirolactol* **5** *to the spirolactone* **6**. A solution of 20 mg of spirolactol **5** in 2 ml of methylene chloride was refluxed with 300 mg of MnO_2 with stirring. After filtration through 0.5 g of silica gel and purification with active carbon, the crude product was crystallized from ether/petroleum ether to give 13 mg of pure spirolactone **6** of m.p. 184–186°. Crystallization 1 (m.p. 184–186°): 888 dpm/mg ^3H; 228 dpm/mg ^{14}C. Crystallization 2 (m.p. 183–185°): 860 dpm/mg ^3H; 233 dpm/mg ^{14}C. Crystallization 3 (m.p. 185–186°): 943 dpm/mg ^3H; 220 dpm/mg ^{14}C.

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57. Azidiniumsalze[1])

13. Mitteilung [1]

Polarographie der Azidiniumsalze substituierter Chinoline

von **Heinz Balli** und **Dieter Schelz**[2])

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(28. XI. 74)

Zusammenfassung. 1-Äthyl-2-azido-6-X-chinolinium-tetrafluoroborate (X = NH_2, OCH_3, CH_3, H, Cl, Br, C_6H_5, NO_2, $CO_2C_2H_5$) werden an der Quecksilbertropfelektrode in Wasser ($\mu = 0{,}05$, 25°) irreversibel durch einen Zweielektronenübergang reduziert. Die Halbstufenpotentiale werden bestimmt und zeigen eine lineare Korrelation mit σ_X.

Polarographische Untersuchungen an organischen Aziden sind erst in letzter Zeit bekannt geworden [3] [4]. Wir berichten hier über die polarographische Reduk-

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[1]) *Anmerkung der Redaktion*: Dieser Name ist nicht in den IUPAC-Regeln der organischen Nomenklatur zu finden. Seine Ableitung wird in der 1. Mitt. [Liebigs Ann. Chem. *647*, 1 (1961)] dargelegt. Um den Zusammenhang mit früheren Abhandlungen der gleichen Reihe zu wahren, wird auf Wunsch von Prof. *Balli* der Titel «Azidiniumsalze» beibehalten.

[2]) Aus der Dissertation *D. Schelz* [2].