158. Biosynthesis of the Verrucarins and Roridins. Part 1. The Transformation of Mevalonic Acid into Verrucarinic Acid. Evidence for a Hydrogen 1,2-Shift[1])

Verrucarins and Roridins, 27th Communication [2]

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Summary. Incorporation experiments using sodium [2-^{14}C]-, [2-^{3}H]-, (3*R*)-[5-^{14}C]- and [2-^{3}H, 2-^{14}C]-mevalonates and with mevalonates stereospecifically tritiated at C(2) demonstrate the transformation of mevalonic acid (**8**) into verrucarinic acid (**5**). Degradation experiments showed that this transformation occurs with a hydrogen 1,2-shift of the 'pro-2*R*' hydrogen atom of mevalonate to C(3) of verrucarinate. A possible mechanistic pathway is discussed.

1. Introduction. – The major components of the antibiotic complex of the verrucarins and roridins, which have been isolated from cultures of *Myrothecium verrucaria* (*Albertini et Schweinitz*) *Ditmar ex Fries* and *Myrothecium roridum Tode ex Fries* [3]–[5] and investigated during the past years, are verrucarin A and roridin A. They are macrocyclic esters of the sesquiterpene alcohol verrucarol (**3**) [6] which possesses the tricyclic trichothecane skeleton [7], and they differ from each other by the nature of the acidic hydrolysis products. Whereas verrucarin A yields *cis,trans*-muconic acid (**4**) and (2*S*,3*R*)-verrucarinic acid (**5**) (isolated as verrucarinolactone (**6**)), roridin A gives the dicarboxylic acid roridinic acid (**7**) as the single acidic product. Interestingly roridinic acid contains verrucarinic acid and partially reduced *cis,trans*-muconic acid as structural elements. Verrucarin A has been shown to be the cyclic triester **1** [8]–[10] of verrucarol, and roridin A the corresponding cyclic diester **2** [11].

The complicated structures of the verrucarins and roridins presuppose correspondingly complex biogenetic pathways. For their elucidation, a series of feeding experiments were carried out by addition of likely ^{14}C- and ^{3}H-labelled precursors to growing cultures of *Myrothecium*. Earlier work suggested the sesquiterpene nature of the trichothecane skeleton [12] (three molecules of mevalonic acid were incorporated) and indicated that various rearrangements occurred subsequently. The results obtained with verrucarol (**3**)[2]) and their implications for the mechanism of the later biosynthetic stages will be reported in a following paper [14]. The isomeric relationship between verrucarinic acid (**5**) and mevalonic acid (**8**) suggested that the latter might not only be a precursor of verrucarol, but also of verrucarinic acid. In this paper experimental evidence is presented showing this assumption to be correct. In addition, the results obtained allow the proposal of possible mechanisms for the biogenetic transformation of mevalonic acid into verrucarinic acid.

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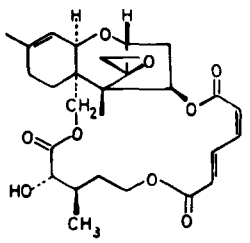
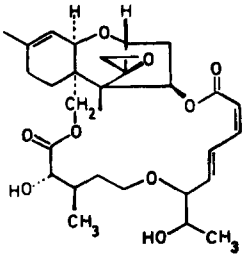
[1]) Presented in part at the XXIIIrd International Congress of Pure and Applied Chemistry, Boston, Mass., USA, 26–30th July 1971 [1].

[2]) For preliminary communications of some results, see [13], [2].

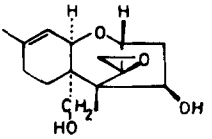
2. Incorporations and Degradations. – In order to achieve an optimal incorporation of the precursors, the rate of biological formation of verrucarin A (**1**) and roridin A (**2**) was determined by semiquantitative evaluation of their UV. absorption on thin layer plates. The resulting curve shows that intense production of the metabolites begins about 56 h after inoculation of the culture medium, and so the ^{14}C-labelled and specifically tritiated mevalonates were administered at that time to growing cultures of *Myrothecium roridum,* strain S 1135. Verrucarin A (**1**) was isolated



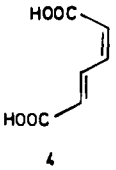
1 Verrucarin A



2 Roridin A



3 Verrucarol



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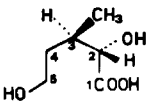
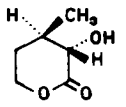
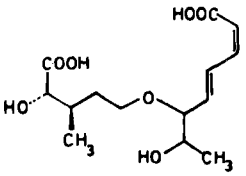


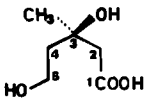
5 (2S,3R)-Verrucarinic Acid



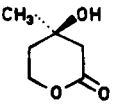
6 (2S,3R)-Verrucarinolactone



7 Roridinic Acid



8 (3R)-Mevalonic Acid



9 (3R)-Mevalonolactone

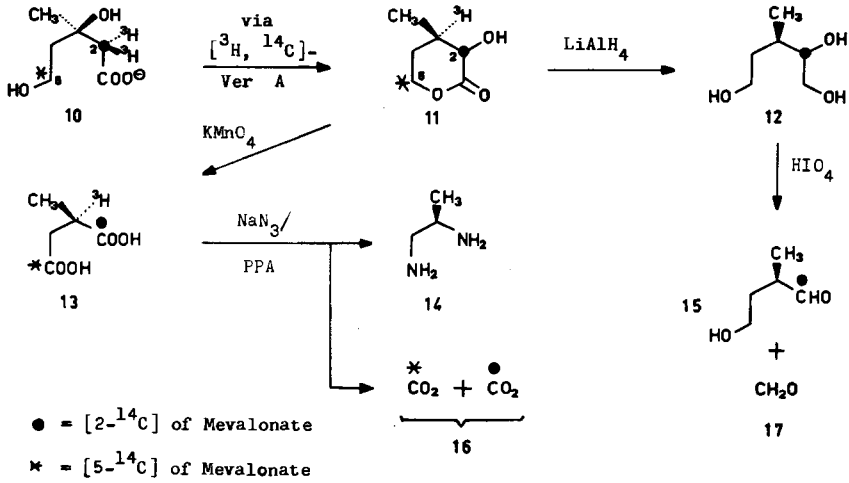
and purified by extraction with ethyl acetate and chromatography on a silicagel column. The administered precursors and the radioactivity of the isolated samples of **1** are listed in Table 1.

The radioactive verrucarin A preparations were hydrolysed with potassium carbonate in aqueous methanol. The neutral verrucarol (**3**) was separated readily from the acidic hydrolysis products *cis,trans*-muconic acid (**4**) and verrucarinic acid (**5**). The latter was separated from **4** by sublimation of the crystalline lactone **6**. *Cis,trans*-muconic acid was then transformed into dimethyl muconate with diazomethane. The radioactivities of the verrucarin A hydrolysis products and their derivatives are summarized in Table 1. These results clearly demonstrate that mevalonate is incorporated into verrucarin A (**1**), and that the radioactivity is distributed between verrucarol and verrucarinolactone (**6**), *cis,trans*-muconic acid being radioinactive. Three molecules of mevalonic acid (**8**) are incorporated into verrucarol thereby, confirming the sesquiterpene nature of the trichothecane skeleton [12], and one molecule into verrucarinolactone. Although the mole-specific ratio of the ^{14}C activity of these two components is approximately 3:1, these conclusions should be accepted with caution since verrucarol and verrucarinic acid are built up by two specific pathways which are probably linked together at a late stage. Thus the observed activity distribution in various sections may just reflect the relative efficiencies of conversion of the precursor for each of the pathways rather than the number of precursor units involved. However, since there was no pool of intermediates present prior to the addition of the precursor, and since the compound studied appears to be the major metabolite (*i.e.* practically all the verrucarol and verrucarinic acid and related intermediates produced by the mould are converted to the final metabolite), the radioactivity distribution will approximate to the ratio of units required to construct each moiety.

The incorporation of (3*R*)-[5-^{14}C]-mevalonate constitutes the basis of all further experiments which were carried out with racemic (3*R,S*)-mevalonate, since this demonstrates that it is in fact the natural enantiomer which is used as precursor for both sections of the verrucarin A molecule.

For the localization of the radioactivities, verrucarinolactone (**11**), obtained after administration of [2-^{14}C]-mevalonate (**10**), was reduced to the triol **12** with $LiAlH_4$. Cleavage of the triol **12** with HIO_4 gave the hydroxyaldehyde **15** and practically radio-inactive formaldehyde (**17**) which was isolated as its dimedone derivative. Therefore the radioactivity is assumed to be located at C(2) of verrucarinolactone (**11**). To confirm this assumption, **11** was oxidized to (*R*)-(+)-methylsuccinic acid (**13**) [8]. *Schmidt* degradation of the acid **13** with NaN_3 and polyphosphoric acid yielded radioinactive 1,2-diaminopropane (**14**) (isolated as picrate) and two equivalents of CO_2, corresponding to C(2) and C(5) of verrucarinic acid (**5**). The isolated $BaCO_3$ contained about 50% of the original radioactivity as would be expected due to the formation of two equivalents of CO_2. The radio-inactivity of the 1,2-diaminopropane demonstrates that the carbon skeleton of mevalonate had not undergone a rearrangement or an exchange of the state of oxidation of C(1) and C(5). Additional evidence for the biogenetic identity of C(2) of verrucarinate with C(2) of the isomeric mevalonate was provided by the oxidative degradation of the verrucarinolactone **11** isolated after feeding (3*R*)-[5-^{14}C]-mevalonate (**10**). The resulting methylsuccinic acid **13** contained the total amount of the original radioactivity. Subsequent *Schmidt* degradation gave 2 equivalents of CO_2, which again contained about half of the original radioactivity.

Incorporation of (3*R*, *S*)-[2-^{14}C]-, [2-^{3}H]- and of [5-^{14}C]-Mevalonate into Verrucarinate

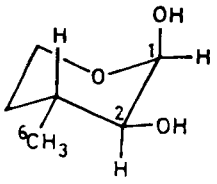


Ver A = Verrucarin A
PPA = Polyphosphoric acid.

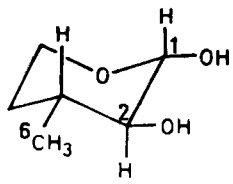
A preliminary experiment with the verrucarinolactone **11** obtained after addition of [2-^{3}H]-mevalonate (**10**) to the mould indicated a hydrogen shift from C(2) of mevalonate to C(3) of verrucarinate since, surprisingly, tritium activity still appeared in the *R*-(+)-methylsuccinic acid **13**.

In order to test this assumption and to elucidate the mechanism of the transformation of mevalonic acid into verrucarinic acid, the incorporation of doubly labelled [2-^{3}H, 2-^{14}C]-mevalonate (**18**) and of stereospecifically tritiated mevalonates was studied.

Verrucarinolactone (**19**) resulting from the administration of [2-^{3}H, 2-^{14}C]-mevalonate (**18**) to the mould, was treated with LiAlH$_4$ in ether. In addition to the triol **23**, indicated above as **12**, the lactol **20** was obtained as a second reaction product. Further treatment with LiAlH$_4$ in tetrahydrofuran under more vigorous conditions transformed the lactol **20** in to the triol **23**. Compound **20** reacted slowly with 3,5-dinitrophenylhydrazine in acidic solution, indicating the presence of a masked aldehyde group. The lactol was reoxidized readily to the original verrucarinolactone by treatment with bromine in water. Since the reduction of the carbonyl group of verrucarinolactone creates a new centre of chirality at C(1), two diastereoisomers



20a



20b

can be formed. According to the NMR. spectrum, the lactol **20** is in fact such a mixture of **20a** and **20b**. The 6-methyl group appears not as doublet but as double doublet at 0.95–1.1 ppm.

The axial C(1)-proton of **20b** is a doublet at 4.38 ppm (J = 7 Hz), the equatorial in **20a**, however, is a doublet at 5.05 ppm (J = 4 Hz). The proton at C(2) is a double

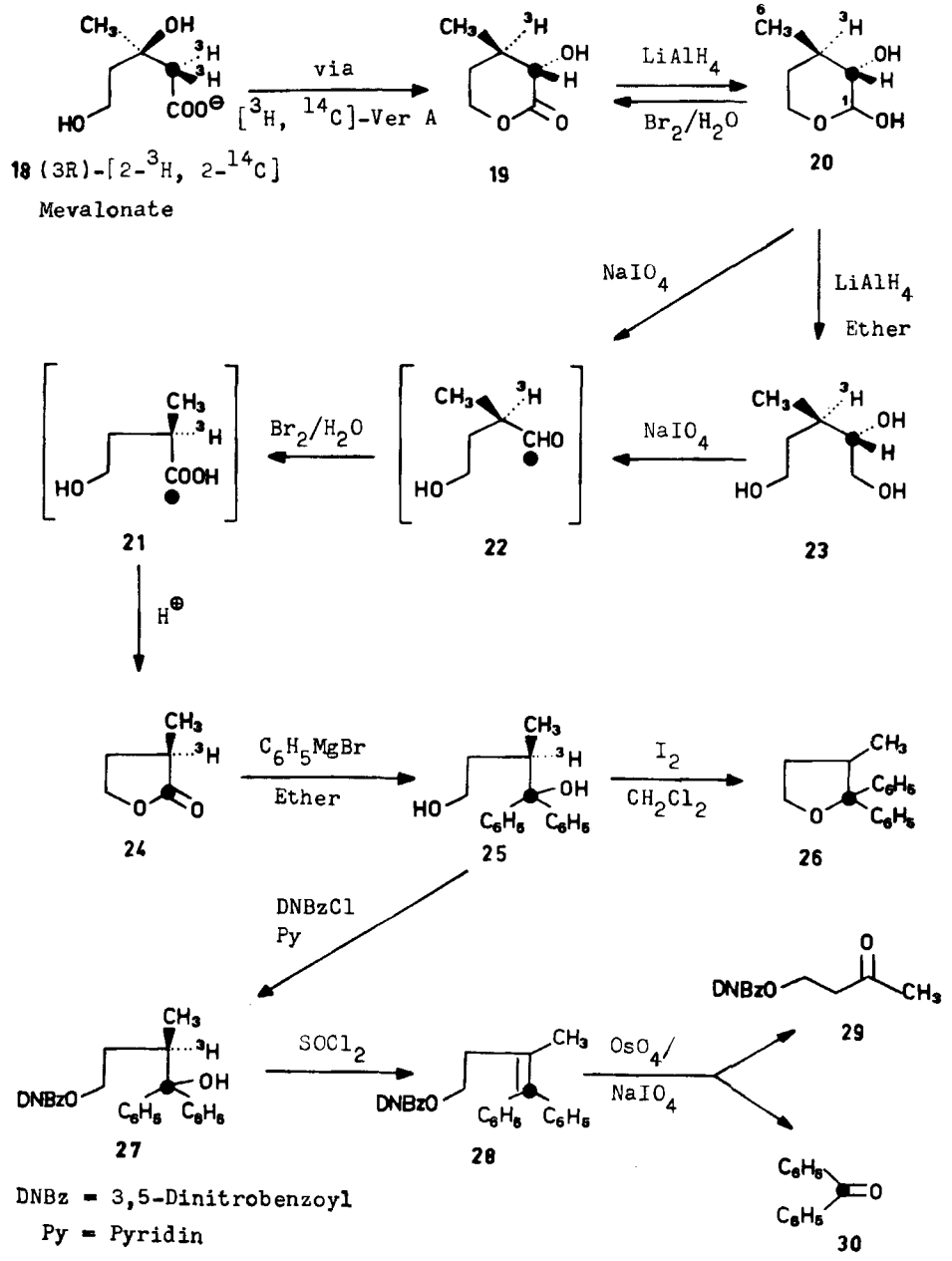


Table 1. *Radioactivity of verrucarin A* (**1**) *and its transformation products*

Mevalonate precursor[3]) (sodium salt)	Activ. of precursor mCi	Culture fluid liter	Compound	Specific activity dpm/mmol ^3H	Specific activity dpm/mmol ^{14}C	Abs. rate of incorp. %	% of total
[2-^{14}C]	0.4	20	*Verrucarin A* (**1**)		$97.0 \cdot 10^4$	0.06	100
			verrucarol (**3**)		$60.3 \cdot 10^4$	–	62
			verrucarinolactone (**6**)		$29.4 \cdot 10^4$	–	30.2 100
			methylsuccinic acid (**13**)		$27.3 \cdot 10^4$	–	93
			methylsuccinic acid diluted		$17.5 \cdot 10^4$	–	100
			$BaCO_3$		$9.1 \cdot 10^4$	–	52
			1,2-diaminopropane picrate		–	–	–
			verrucarinolactone (**6**) diluted		$5.6 \cdot 10^4$	–	100
			formal dimedone		$0.2 \cdot 10^4$	–	3.6
(3*R*)-[5-^{14}C]	0.01	5	*Verrucarin A* (**1**)		$30.1 \cdot 10^3$	0.6	100
			verrucarol (**3**)		$21.6 \cdot 10^3$		70
			dimethyl *cis,trans*-muconate		0		0
			verrucarinolactone (**6**)		$7.9 \cdot 10^3$		26 100
			methylsuccinic acid (**13**)		$7.5 \cdot 10^3$		95
			$BaCO_3$		$0.27 \cdot 10^3$		35
[2-^{3}H]	1	20	*Verrucarin A* (**1**)	$167.4 \cdot 10^4$		0.28	100
			verrucarol (**3**)	$140.4 \cdot 10^4$			83.6
			verrucarinolactone (**6**)	$24.4 \cdot 10^4$			14.6
			verrucarinolactone (**6**) after dilution	$13.6 \cdot 10^4$			100
			methylsuccinic acid (**13**)	$15.0 \cdot 10^4$			113
[2-^{14}C]	0.5	20	*Verrucarin A* (**1**)	$110.4 \cdot 10^5$	$15.7 \cdot 10^5$	0.45	100
[2-^{3}H]	5.0		verrucarol (**3**)	$97.5 \cdot 10^5$	$12.7 \cdot 10^5$		81
			dimethyl *cis,trans*-muconate	0	0		0
			verrucarinolactone (**6**)	$18.6 \cdot 10^5$	$4.2 \cdot 10^5$		27
(3*R*)-[(2*S*-2-^{3}H)] (3*S*)-[(2*R*-2-^{3}H)]	0.25	20	*Verrucarin A* (**1**)	$34.3 \cdot 10^4$		0.07	
			verrucarol (**3**)	$37.0 \cdot 10^4$			100
			verrucarinolactone (**6**)	$0.35 \cdot 10^4$			<1
(3*R*)-[(2*R*-2-^{3}H)] (3*S*)-[(2*S*-2-^{3}H)]	0.25	20	*Verrucarin A* (**1**)	$14.4 \cdot 10^4$		0.04	100
			verrucarol (**3**)	$9.7 \cdot 10^4$			67
			verrucarinolactone (**6**)	$6.2 \cdot 10^4$			43
			methylsuccinic acid **35**	$5.0 \cdot 10^4$			35

[3]) The radioactive precursors were purchased from the *Radiochemical Centre*, Amersham, Buckinghamshire/England, with the exception of sodium (3*R*)-[5-^{14}C]-mevalonate, which was kindly provided by Prof. Dr. *Arigoni*, ETH, Zürich.

doublet appearing at 3.12 ppm (J = 4 and 10 Hz) in the case of **20a**, and at 2.88 ppm (J = 7 and 10 Hz) in the diastereoisomer **20b**. These assignments were confirmed by spin spin decoupling experiments. The hydroxylic hydrogens are exchanged by D_2O.

Upon further degradation with aqueous $NaIO_4$, both the lactol **20** and the triol **23** yielded the same aldehyde **22**. In order to avoid an exchange of tritium by enolization, the reaction was carried out under neutral conditions. The aldehyde **22** was not isolated but oxidized directly with Br_2 in water to the carboxylic acid **21**. The protons formed during both reactions were neutralized by the addition of the calculated amount of $NaHCO_3$ solution. As shown later by the unchanged ^3H:^{14}C ratio, neither exchange of tritium nor racemization had taken place. The final product isolated from these reactions was not the carboxylic acid **21**, but the butyrolactone **24**. Since the lactonization requires acidic conditions, $NaIO_3$ formed in the course of cleavage of the triol **23** was reduced *in situ* to sodium iodide by Na_2SO_3, since by addition of acid to the reaction mixture before the reduction of the iodate, bromine and iodine would be liberated from bromide and iodide present. After saturation of the aqueous reaction mixture with NaCl, it was possible to extract the butyrolactone **24** almost quantitatively with ether in a *Kutscher-Steudel* apparatus. In this manner the lactone **24** was obtained from the triol **23** in 93% yield.

Further degradation reactions were first elaborated using a synthetic radioinactive γ-butyrolactone. Diethyl methylmalonate was transformed into diethyl methyl-(2-hydroxyethyl)-malonate with ethylene oxide in ethanol (*cf.* [15]). Hydrolysis and decarboxylation gave the desired (±)-2-methyl-γ-butyrolactone (**24**). The lactone **24** was treated with phenylmagnesium bromide in abs. ether. Whereas the resulting (±)-diphenylcarbinol was crystalline, the radioactive (2*R*)-2-methyl-1,1-diphenyl-butane-1,4-diol (**25**) was amorphous. Treatment of the carbinol **25** with I_2 in dichloromethane did not lead to the desired elimination product, but yielded instead the tetrahydrofuran derivative **26**. In order to avoid this cyclization, the primary hydroxyl group of the diol **25** was esterified selectively to derivative **27** using 3,5-dinitrobenzoyl chloride in pyridine. It was crystalline only in the racemic series. Treatment of either racemic or natural (2*R*)-**27** with $SOCl_2$ in pyridine at 22° yielded the same optically inactive, crystalline olefin **28**. Oxidative cleavage of the double bond with $NaIO_4$ and catalytic amounts of OsO_4 in aqueous dioxane occurred readily to give the 3,5-dinitrobenzoyl derivative **29** of 4-hydroxy-butan-2-one and benzophenone (**30**), which were separated by silicagel chromatography.

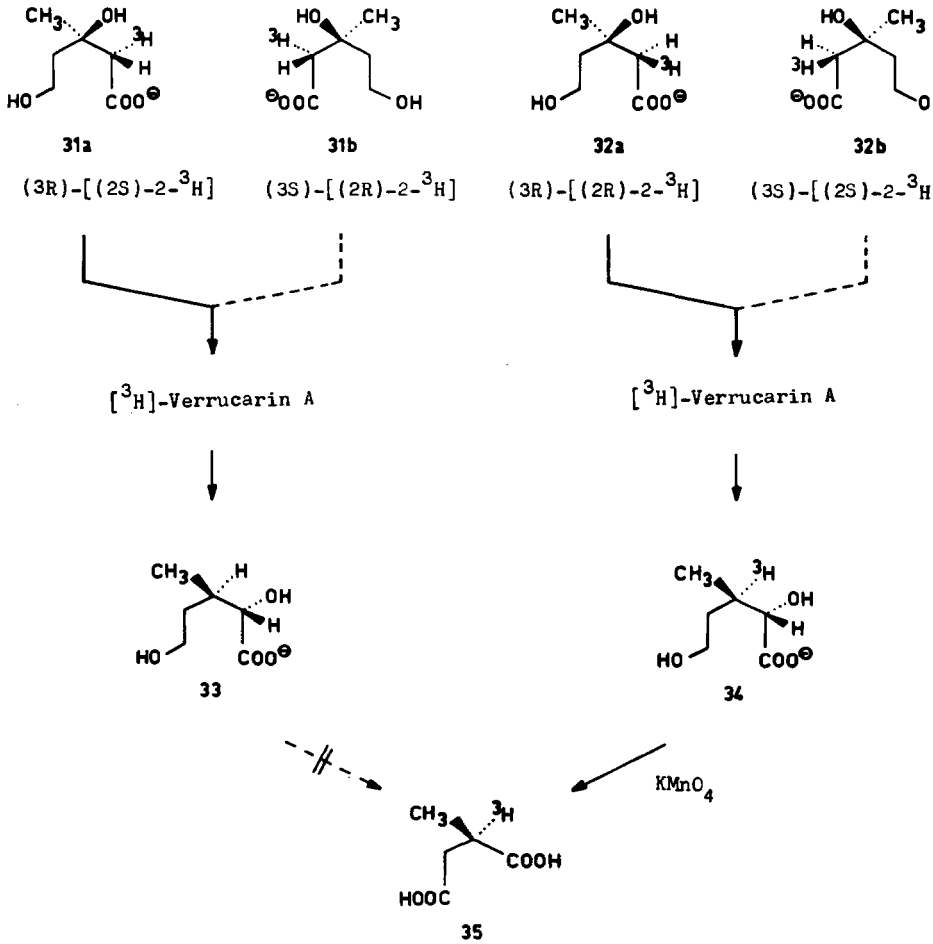
The radioactivities of the main degradation products described are listed in Table 2.

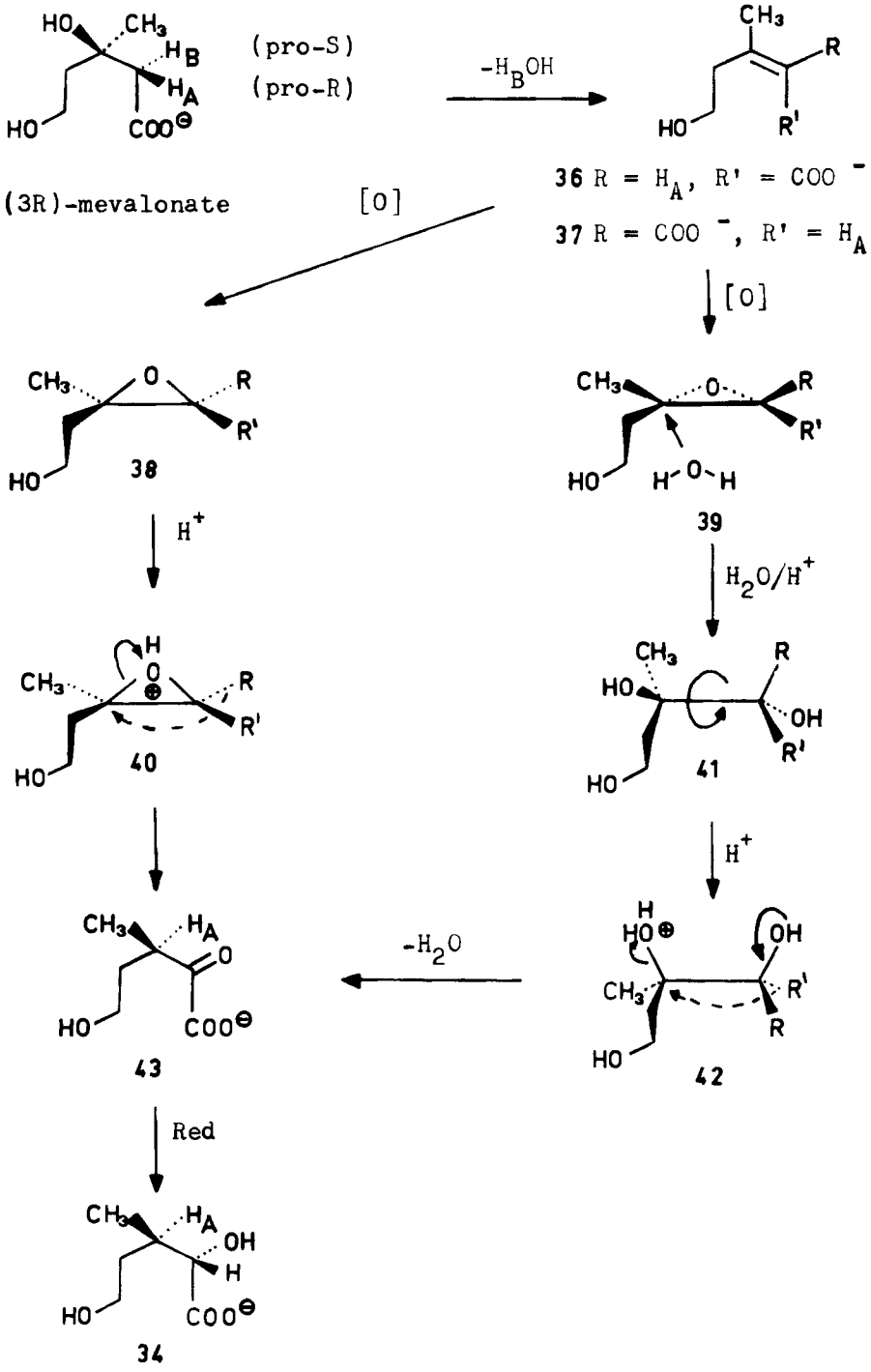
The unchanged ^3H:^{14}C ratio of the carbinol **27** as compared with verrucarinolactone (**19**) shows that no tritium has been lost during the reaction sequence. Consequently the complete loss of tritium activity with the retention of the full ^{14}C-activity in the olefin **28** is a significant result. Since after oxidative cleavage of compound **28** 99.5% of the original ^{14}C-activity is found to be in benzophenone **30** and none in the methyl ketone **29**, C(2) of verrucarinic acid (**5**) is biogenetically identical with C(2) of mevalonic acid (**8**). The absence of tritium activity in the olefin **28** provides strong evidence for the anticipated hydrogen transfer from C(2) of mevalonic acid to C(3) of verrucarinic acid.

Table 2. *Radioactivity of the degradation products of verrucarinolactone with incorporated[2-^3H, 2-^{14}C]-mevalonate*

Compound	Radioactivity (dpm/mmol)		
	^3H	^{14}C	^3H:^{14}C
Verrucarinolactone **19**	17.04×10^5	4.16×10^5	4.09
Carbinol **27**	16.60×10^5	4.02×10^5	4.12
Olefin **28**	0.19×10^5	4.15×10^5	–
Methyl ketone **29**	0.10×10^5	0.00×10^5	–
Benzophenone (**30**)	0.03×10^5	4.13×10^5	–

To study the mechanism of the hydrogen transfer, the stereospecifically tritiated mevalonates, namely sodium (3*R*)-[(2*S*)-2-^3H]/(3*S*)-[(2*R*)-2-^{3}H]-mevalonate (**31**) and sodium (3*R*)-[(2*R*)-2-^{3}H]/(3*S*)-[(2*S*)-2-^{3}H]-mevalonate (**32**) were administered to cultures of *Myrothecium* in separate experiments. After feeding the mevalonate **31** and subsequent hydrolysis of [^{3}H]-verrucarin A, radio-inactive verrucarinate (**33**)




(pro-S)
(pro-R)
$-H_BOH$
32a (3R)-mevalonate
[O]
36 R = H_A, R' = COO^-
37 R = COO^-, R' = H_A
38
39
40
41
42
43
H^+
H_2O/H^+
$-H_2O$
Red
34

was isolated, the total radioactivity being located in verrucarol. The experiment with the mevalonate **32** gave radioactive verrucarinate (**34**) containing ca. 40% of the activity of verrucarin A and radioactive verrucarol (60% of original tritium). Since the natural (3*R*)-enantiomer of mevalonate is incorporated in verrucarinate, it is the (3*R*)-[(2*R*)-2-^{3}H]-mevalonate **31a** which is transformed to verrucarinate. Consequently it is the 'pro-2*S*' hydrogen atom of mevalonate that is lost in the formation of verrucarinate. The 'pro-2*R*' hydrogen is retained and transferred to C(3) of verrucarinate (**34**). In consequence, oxidation of the radioactive **34** with $KMnO_4$ gave radioactive methylsuccinic acid (**35**).

A possible mechanism for the biogenetic formation of verrucarinate from mevalonate is outlined in the following scheme. The loss of the 'pro-2*S*' hydrogen H_B in mevalonate **32a** agrees with the *trans*-elimination of H_2O leading to *cis*-anhydromevalonate **36**. However, a formal *cis*-elimination yields *trans*-anhydromevalonate (**37**). Epoxidation of the double bond gives rise to two pairs of enantiomeric glycidic acids, **38** and **39**. By protonation of the 'β-epoxide' **38** the oxonium ion **40** is formed. Subsequent cleavage of the C(3)-oxygen bond and hydride 1,2-shift of the 'pro-2*R*' hydrogen atom H_A of mevalonate **32a** leads to the ketone **43** with concurrent inversion of the chiral centre C(3) by analogy with a pinacol rearrangement[4]). Both the *cis*- and *trans*-anhydromevalonates yield the same product because of the disappearance of chirality at C(2). The last step in the formation of (2*S*,3*R*)-verrucarinate (**34**) constitutes the stereospecific reduction of 2-dehydro-verrucarinate (**43**). – Since the configuration of the epoxy group in the epoxy-anhydromevalonate obtained by hydrolysis of the metabolite verrucarin B [17] is still unknown, the 'α-epoxide' **39** cannot be excluded *a priori* as an intermediate. However, the steric position of the methyl group at C(3) of verrucarinate (**34**) requires a rearrangement with retention of the configuration, which is possible by a double inversion at C(3). Hydrolytic cleavage of the C(3)-oxygen bond of the oxirane ring forms the diol **41** with inversion at C(3). Subsequent pinacol rearrangement with a hydride 1,2-shift involving H_A requires a torsion of the C(2)–C(3) bond to form the diol **42**, and leads to the ketone **43** as well. – The cleavage of the 'α-epoxide' at C(2) which is favoured under basic conditions, yields the enantiomer of diol **41**. Due to the transfer of H_A, the following pinacol rearrangement leads to inversion at C(3) and consequently to a final product possessing the wrong configuration.

The loss of the 'pro-2*S*' hydrogen in mevalonate appears to preclude a mechanism involving vitamin B_{12} coenzyme [18].

On the basis of these mechanistic considerations, we believe the 'β-epoxide' **38** to be the real intermediate.

The isolation of metabolites from *Myrothecium* species containing anhydromevalonic acid (verrucarin J [19]), 2,3-epoxy-anhydro-mevalonic acid (verrucarin B [17]) and 2-dehydro-verrucarinic acid (2'-dehydroverrucarin A [5]) provides strong support for the proposed sequence of reactions. An analogous sequence is also found in the roridin series.

Support of this work by the «*Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung*» and by *Sandoz A.G.*, Basel, is gratefully acknowledged.

[4]) For analogous rearrangements of glycidic esters catalyzed by BF_3-ether *cf.* [16].

Experimental Part

1. General Methods. – The melting points were determined on a *Kofler* block and are uncorrected. Elemental analyses were carried out in the micro-analytical laboratories of the Institute (*E. Thommen*). The infrared (IR.) spectra were recorded with a *Perkin-Elmer* Model 125 IR grating spectrophotometer, and the ultraviolet (UV.) spectra were recorded with a *Beckmann* Model DK2 spectrophotometer in the spectral laboratories of the Institute (*K. Aegerter*).

The 100 MHz nuclear magnetic resonance (NMR.) spectra were determined by *H. Huber, E. Jutzi,* and *E. Wullschleger* in the Physikalisch-Chemisches Institut der Universität Basel on a *Varian* HA-100 D spectrometer. The spectral laboratory of our Institute (*K. Aegerter*) recorded the 60 MHz NMR. spectra on a *Varian* A-60 instrument. Abbreviations used are: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br. = broad.

The optical rotations were measured with a *Perkin-Elmer* Model 141 polarimeter. We thank *H. Galliker*, Isotopenlabor der Firma *Sandoz A.G.*, Basel, for the radioactivity determinations. The samples were dissolved in toluene scintillation fluid, and the activity was determined on a Tri-Carb (*Packard*) Model 3375 liquid scintillation spectrometer by direct measurement. The precision of measurement for doubly marked samples is ± 5%, and for singly marked samples is ± 1% unless specified otherwise. Gas chromatography (GC.) was carried out on a *Perkin-Elmer* Model F 11 instrument, using silanized columns containing 10% SE 30 on Chromosorb W. For column chromatography, silica gel 0.05–0.2 mm from *E. Merck A.G.*, Darmstadt, was employed. For column chromatography of UV.-absorbing substances a Uvicord Type LKB 4701 A (254 nm) was used. Preparative thin-layer chromatography (TLC.) was carried out on silica gel PF254 (*Merck*), and ordinary TLC. was carried out on silica gel G (*Merck*). Usual work-up means: extraction with the solvent stated; washing with 2N H_2SO_4, 2N Na_2CO_3, and water; drying with $MgSO_4$; concentration *in vacuo*. Before determination of radioactivity, the samples were dried 8 h at 0.02 Torr and 40°, unless otherwise stated.

2. Determination of production rates of roridin A and verrucarin A. – The culture experiments were carried out by *Sandoz A.G.* Medium: 7 g casamino acids, 2 g KH_2PO_4, 2 g $MgSO_4 \cdot 7H_2O$, 20 g glucose, 2 g malt, 2 g peptone, 2 g yeast, and 1 liter of distilled water, pH 5.28 (not adjusted). The sterile culture medium (10 l) was placed in a fermenter and inoculated under sterile conditions with 50 ml of a spore suspension of *Myrothecium roridum* Strain S 1135. The fermentation took place at 27° with stirring (450 rpm) and air circulation (0.4 l per liter of culture medium per min.)

Every 6 h 100 ml samples were removed under sterile conditions and immediately extracted with ethyl acetate. The extracts were washed three times with 100 ml of water, dried with $MgSO_4$, and concentrated. Chromatography TLC. of the samples was effected with methylene chloride/methanol (98:2) against standards of roridin A (0.001 mg) and verrucarin A (0.004 mg). By comparison of the UV. absorption (254 nm) of the samples, the amounts of roridin A and verrucarin A were calculated.

3. Culture experiments with addition of sodium [(2-^{3}H, 2-^{14}C)]-mevalonate. – Equal amounts of sodium 0.5 mCi [2-^{14}C]- and 5.0 mCi [2-^{3}H]-mevalonate were added to each of two 10 l fermenters with a millipore sterile filter syringe 50 h after inoculation. The culture solutions were extracted with ethyl acetate 41 h after addition of the precursors, and the crude extract (34 g) was chromatographed on 2 kg of silica gel. The elution was accomplished with methylene chloride: for fractions no. 1–32: 0.5% methanol was added; for no. 33–53: 0.75% methanol; for no. 54–57: 1%; for no. 58–63: 1.5%; for no. 64–79: 2%; for no. 80–83: 3%; for no. 84–91: 4%; for no. 92–95: 5%; for no. 96–100: 10%. The substances were identified by TLC. comparison and IR. spectroscopy.

Fractions no. 32–38 (602 mg) yielded 411 mg verrucarin B after chromatography on 12 g of silica gel. For radioactivity measurements, *verrucarin B* was recrystallized five times from methylene chloride/ether:

Crystallization 3: 32600 dpm/mg ^{3}H 4610 dpm/mg ^{14}C
Crystallization 4: 33800 dpm/mg ^{3}H 4880 dpm/mg ^{14}C
Crystallization 5: 34600 dpm/mg ^{3}H 5000 dpm/mg ^{14}C

Repeated chromatography of fractions no. 40–53 (3.112 g) on 320 g silica gel yielded 2.325 g of pure *verrucarin A* (**1**). For radioactivity determination, the substance was recrystallized from methylene chloride/ether:

Crystallization 2: 22000 dpm/mg ^{3}H 3170 dpm/mg ^{14}C
Crystallization 3: 21900 dpm/mg ^{3}H 3110 dpm/mg ^{14}C
Crystallization 4: 22000 dpm/mg ^{3}H 3140 dpm/mg ^{14}C
Crystallization 5: 21600 dpm/mg ^{3}H 3130 dpm/mg ^{14}C

Fractions 58–61 (528 mg) were chromatographed on 5 g silica gel to produce 290 mg of *roridin E*.

Fractions 62–67 gave primarily roridin D. Fraction 66 (524 mg) yielded pure *roridin D*. For radioactivity determination it was recrystallized from methylene chloride/ether:

Crystallization 3: 14800 dpm/mg ^{3}H 2020 dpm/mg ^{14}C
Crystallization 4: 15300 dpm/mg ^{3}H 2120 dpm/mg ^{14}C
Crystallization 5: 14600 dpm/mg ^{3}H 1990 dpm/mg ^{14}C

Fractions 68–79 contained primarily roridin A (**2**). Fraction 72 (3.1 g) was recrystallized to produce pure *roridin A*:

Crystallization 3: 15600 dpm/mg ^{3}H 2170 dpm/mg ^{14}C
Crystallization 4: 15200 dpm/mg ^{3}H 2180 dpm/mg ^{14}C
Crystallization 5: 15100 dpm/mg ^{3}H 2180 dpm/mg ^{14}C

Culture experiments with other precursors were carried out in the same fashion. The results of radioactivity determination of the various samples of verrucarin A are listed in Table 1.

4. Hydrolysis of verrucarin A (1). – The method used was that of *Gutzwiller & Tamm* [8] with K_2CO_3 in aqueous methanol; the results are given below.

a) **1** *with incorporated [2-^{3}H, 2-^{14}C]-mevalonate*: average activity of 22000 dpm/mg ^{3}H and 3140 dpm/mg ^{14}C.

Verrucarol (**3**), recrystallized from ether, m.p. 163–164°:

Crystallization 4: 36300 dpm/mg ^{3}H 4800 dpm/mg ^{14}C
Crystallization 5: 36600 dpm/mg ^{3}H 4790 dpm/mg ^{14}C
Crystallization 6: 36500 dpm/mg ^{3}H 4820 dpm/mg ^{14}C

Verrucarinolactone (**6**), recrystallized from ether and dried at room temp., m.p. 103–104°:

Crystallization 1: 14500 dpm/mg ^{3}H 3310 dpm/mg ^{14}C
Crystallization 2: 14200 dpm/mg ^{3}H 3290 dpm/mg ^{14}C
Crystallization 3: 14300 dpm/mg ^{3}H 3300 dpm/mg ^{14}C

Dimethylmuconate, recrystallized from ether, m.p. 75–76°, showed no ^{3}H or ^{14}C activity.

b) **1** *with incorporated* (3R)-[(2S)-*2-^{3}H*]/(3S)-[(2R)-*2-^{3}H*]-*mevalonate* (**31**): average activity of 683 dpm/mg ^{3}H. Verrucarol (**3**), recrystallized from acetone/ether:

Crystallization 2: 1400 dpm/mg ^{3}H
Crystallization 3: 1400 dpm/mg ^{3}H
Crystallization 4: 1370 dpm/mg ^{3}H

Verrucarinolactone (**6**), recrystallized from ether and sublimated, m.p. 103–104°:

1st. sublimation: 40 dpm/mg ^{3}H
2nd. sublimation: 15 dpm/mg ^{3}H

Muconic acid (**4**) was not measured[5]).

c) **1** *with incorporated* (3R)-[(2R)-*2-^{3}H*]/(3S)-[(2S)-*2-^{3}H*]-*mevalonate* (**32**): average activity of 287 dpm/mg ^{3}H. Verrucarol (**3**), recrystallized from acetone/ether, m.p. 157–160°:

Crystallization 4: 370 dpm/mg ^{3}H
Crystallization 5: 330 dpm/mg ^{3}H
Crystallization 6: 360 dpm/mg ^{3}H

[5]) It was shown in 4a that no mevalonate was incorporated in muconic acid.

Verrucarinolactone (**6**), m.p. 103–104°:

1st. sublimation: 498 dpm/mg ^3H
2nd. sublimation: 458 dpm/mg ^3H

Muconic acid (**4**) was not measured[5]).

d) **1** *with incorporated* (*3*R)-[*5*-^{14}C]-*mevalonate*: average activity of 62 dpm/mg ^{14}C. Verrucarol (**3**), recrystallized from acetone/ether, m.p. 162–163°:

Crystallization 2: 81 dpm/mg ^{14}C
Crystallization 3: 80 dpm/mg ^{14}C
Crystallization 4: 81 dpm/mg ^{14}C

Verrucarinolactone (**6**), recrystallized from ether and dried at room temp., m.p. 103–104°:

Crystallization 1: 61 dpm/mg ^{14}C
Crystallization 2: 62 dpm/mg ^{14}C
Crystallization 3: 62 dpm/mg ^{14}C

Dimethylmuconate, m.p. 75–76°, showed no radioactivity.

e) **1** *with incorporated* [*2*-^{14}C]-*mevalonate:* average activity of 1220 dpm/mg ^{14}C. Verrucarol (**3**), recrystallized from acetone/ether:

Crystallization 5: 1490 dpm/mg ^{14}C
Crystallization 6: 1370 dpm/mg ^{14}C

Verrucarinolactone (**6**), crystallized and sublimed, m.p. 103–104°, 1430 dpm/mg.

f) **1** *with incorporated* [*2*-^3H]-*mevalonate*: average activity of 3330 dpm/mg ^3H. Verrucarol (**3**), recrystallized from acetone/ether, m.p. 158–163°:

Crystallization 2: 5190 dpm/mg ^3H
Crystallization 3: 5470 dpm/mg ^3H
Crystallization 4: 5120 dpm/mg ^3H

Verrucarinolactone (**6**) m.p. 102–104°:

1st. sublimation: 1930 dpm/mg ^3H
2nd. sublimation: 1830 dpm/mg ^3H

5. Degradation of verrucarinolactones 11 with incorporated [2-^{14}C] and/or [2-^3H]- and/or [5-^{14}C]-mevalonate. – 5.1. *Isolation of C(1) as formaldehyde from* **11** *with incorporated* [*2*-^{14}C]-*mevalonate.* The verrucarinolactone **11** (19 mg; ^{14}C activity, 2260 dpm/mg) was diluted with 81 mg of inactive lactone and dissolved in 30 ml of absolute ether. After cooling to 0°, 54 mg $LiAlH_4$ were added [8], and the mixture was refluxed 3.5 h. The mixture was cooled to 0°, 2N H_2SO_4 was added carefully until both phases were clear, the phases separated and the ether phase was evaporated.

The aqueous layer was neutralized with Na_2CO_3 and adjusted to pH 6 with 294 mg of $HIO_4 \cdot 2H_2O$. After being stirred overnight, the resulting *formaldehyde* (**17**) solution was steam-distilled during 30 min into 200 ml of dimedone solution (2 g dimedone in 300 ml water) [20]. Piperidine (4 drops) was added to the solution and the mixture was refluxed 5 min. *Formaldimedone*, which crystallized from this mixture, was filtered and recrystallized 7 times from methanol: m.p. 194–194.5°, identical with authentic material by mixed m.p. and TLC. The substance from the fourth crystallization was dried for analysis.

$C_{17}H_{24}O_4$ (292.36) Calc. C 69.83 H 8.27% Found C 69.19 H 8.06%

Activities: Crystallization 3: 53 dpm/mg; 4: 33 dpm/mg; 7: 7 dpm/mg.

5.2. *Oxidation of* **11** *to methylsuccinic acid* (**13**) [8]. – 5.2.a. *Using* **11** *with incorporated* [*2*-^{14}C]-*mevalonate.* The lactone **11** (63 mg, activity 2260 dpm/mg) was shaken with a solution of 200 mg $KMnO_4$ in 35 ml 2N H_2SO_4 at 22°. After 40 min, an additional 50 mg $KMnO_4$ were added, and 2 h later the violet mixture was treated with solid $NaHSO_3$ until the solution was colourless and clear. The mixture was saturated with NaCl and extracted 16 h in a *Kutscher-Steudel* apparatus. The crude extract (38 mg) produced 19.1 mg (*R*)-(+)-methylsuccinic acid (**13**) as needles, after two recrystallizations from ether/petroleum ether 1:2, m.p. 110.5–112.5°; identical with authentic

material by mixed m.p. The major portion was diluted with commercial inactive racemic methylsuccinic acid (*purum*, Fa. *Fluka A.G.*, Chemische Fabrik, Buchs SG).
Activities: Crystallization 2: 2070 dpm/mg, diluted sample: 1030 dpm/mg.

5.2.b. *Using* **11** *with incorporated* (*3*R)-[*5*-^{14}*C*]-*mevalonate* (49 mg, 62 dpm/mg) gave methyl succinic acid (**13**) after treatment as described in 5.2.a. Purification with activated charcoal and crystallization gave 11 mg **13**: crystallization 2: m.p. 109–111°, 57 dpm/mg.

5.2.c. *Using* **11** *with incorporated*[*2*-^{3}*H*]-*mevalonate.* The lactone (54 mg, 1875 dpm/mg) was diluted with 54 mg of inactive material to a radioactivity of 1050 dpm/mg. A degradation similar to that in 5.2.a gave, after two recrystallizations, 23 mg **13**, m.p. 114–117°; m.p. identical mixed with authentic material; radioactivity: 1140 dpm/mg.

5.2.d. As in 5.2.a, 95 mg of the radioactive verrucarinolactone **11** (average activity 470 dpm/mg) *with incorporated* (*3*R)-[(*2*R)-*2*-^{3}*H*]/(*3*S)-[(*2*S)-*2*-^{3}*H*]-mevalonate (**32**) were degraded. The resulting 16 mg of crystalline methylsuccinic acid (**13**) were recrystallized four times:

Crystallization 2: m.p. 104–106°, 360 dpm/mg
Crystallization 3: m.p. 109–111°, 390 dpm/mg
Crystallization 4: m.p. 107–110°, 390 dpm/mg

5.3. Schmidt-*degradation of methylsuccinic acid* (**13**). 5.3.a. Treatment of 16.7 mg of diluted radioactive methylsuccinic acid (**13**) (from [2-^{14}C]-mevalonate, 1330 dpm/mg from 5.2.a) with 90 mg NaN_3 and 1.4 g polyphosphoric acid[6]) was carried out in the *Britt* apparatus [22] for 1.5 h at 80–90°. (The $Ba(OH)_2$ solution for CO_2-absorption had been prepared as follows: an excess of $Ba(OH)_2$ was dissolved in H_2O, which had been distilled twice in a quartz-apparatus; 900 ml of this saturated solution were mixed with 180 ml 10.75% $BaCl_2$ solution and filtered under N_2.) The $BaCO_3$ produced by the *Schmidt* degradation was centrifuged, washed three times with twice distilled H_2O, twice with methanol, and then dried under a quartz lamp to constant weight. (3 control experiments without carbonic acid gave less then 0.5 mg $BaCO_3$). – Radioactivity: 465 dpm/mg.

Aqueous 30% NaOH (6 ml) was added to the residual mixture in the *Britt* apparatus, the solution was distilled and the distillate treated with 40 mg picric acid in 3 ml water. After warming on a waterbath to complete solution, and then cooling, *1,2-diaminopropane* (**14**) *dipicrate* crystallized. The crystals were washed twice with cold water and recrystallized twice from ethanol. Yield: 7.5 mg picrate as yellow needles, m.p. 250–252° (dec.). – Radioactivity after drying at 100°: 0 dpm/mg.

Inactive standard material (1,2-diaminopropane dipicrate from inactive *rac.* methylsuccinic acid by *Schmidt* degradation): m.p. 236,5–238° (dec.).

$C_{15}H_{16}N_8O_{14}$ (532,34) Calc. C 33.84 H 3.02% Found C 34.08 H 3.11%

Methylsuccinic acid (**13**) (9 mg with incorporated (3*R*)-[5-^{14}C]-mevalonate) (from 5.2.b) with the average activity of 57 dpm/mg was degraded in the same manner. 14 mg of $BaCO_3$ were obtained; 1,2-diaminopropanedipicrate could not be isolated.

Radioactivity of $BaCO_3$: sample a) 14 dpm/mg; sample b) 14 dpm/mg

6. Verrucarinolactone 19 with incorporated (3*R*)-[2-^{3}H, 2-^{14}C]-mevalonate and its degradation products. – 6.1. *Reduction with* $LiAlH_4$. A solution of 91 mg (0.70 mmol) verrucarinolactone (**19**) in 50 ml absolute ether was added slowly to a suspension of 200 mg $LiAlH_4$ in 50 ml abs. ether at 0° during 30 min, and the mixture kept for 2 h at 40°. After cooling to room temp., 200 ml methylene chloride were added and the excess $LiAlH_4$ was destroyed with ice. The mixture was acidified with the minimum amount of 2N sulfuric acid, with vigorous stirring, then neutralized with solid $NaHCO_3$, and the water phase removed by addition of $MgSO_4$. The latter (partly hydrated) was filtered, ground and washed twice with ether. The combined organic extracts were evaporated to produce 104 mg crude product which showed two components in TLC. (methylene chloride/methanol 4:1). Preparative TLC. gave 14 mg (0.104 mmol = 14.8%) of pure *3-methylpentane-1,2,5-triol* (**23**) as a colourless oil. The TLC.-, IR.-, NMR.-data and degradation reactions were identical with those of authentic material. $[\alpha]_D^{25} = +8.9° \pm 1°$ ($c = 0.74$ in acetone).

[6]) For its preparation see [21].

In addition to triol **23** it was possible to isolate 78 mg (0.582 mmol = 83%) of the less polar *2,5-dihydroxy-3-methyl-pentanol δ-hemiacetal* (**20**), which distilled at 60° and 0.15 Torr. $[\alpha]_D^{25} = -69° \pm 1°$ ($c = 0.947$ in acetone). IR. spectrum (film) bands at: 3450 (OH, very br.) 1460, 1380, 1260, 1070, 1010, 960, 910 cm^{-1}. NMR. spectrum: see discussion.

6.2. *Reduction of 2,5-dihydroxy-3-methyl-pentanal δ-hemiacetal* (**20**). A solution of 85 mg (0.63 mmol) **20** in 12 ml abs. tetrahydrofurane (THF) was added at 0° during 30 min to a suspension of 200 mg $LiAlH_4$ in 40 ml THF. After being refluxed 20 h, nearly all of the starting material was consumed. Working up as in experiment 6.1., gave 84 mg (0.82 mmol = 99%) *3-methyl-pentane-1,2,5-triol* (**23**), which contained traces of starting material and side products.

6.3. *Verrucarinolactone* (**19**) *from 2,5-dihydroxy-3-methyl-pentanal δ-hemiacetal* (**20**). Lactol **20** (25 mg, 0.189 mmol) was treated with 0.86 ml bromine-water (0.189 mmol bromine) and five drops of 2N H_2SO_4 and stirred at room temperature in a closed apparatus. After 30 min, the bromine solution was decolourized to the extent of approximately 90%, and in the next 2 h no further decolourization was observed. Treatment of the aqueous solution with solid $NaHSO_3$ was followed by addition of 100 ml of ether and 100 ml of methylene chloride. The acid was neutralized with solid $NaHCO_3$ under vigorous stirring. The mixture was dried with $MgSO_4$ and the solvent evaporated to give 13 mg (0.10 mmol = 55%) of crystalline product. Sublimation at 60°/15 Torr and crystallization from ether produced the pure verricarunolactone (**19**), which was identical with authentic material by TLC., mixed m.p., and optical rotation.

6.4. *Cleavage of (±)-3-methylpentane-1,2,5-triol* (**23**) *with $NaIO_4$*. Triol **23**[7]) (28.6 mg, 0.123 mmol) was added to a solution of 95 mg of $NaIO_4$ in 4 ml of water at room temperature. The slightly acid solution was neutralized with solid $NaHCO_3$. After 30 min the resulting aldehyde **22** and formaldehyde were titrated with bromine-water (11.2 g bromine+13.4 g $KHCO_3$+10 g KBr at 100 ml H_2O). At the beginning, the bromine-water was decolourized rapidly, but after 2.5 h (total 1 ml bromine-solution added) no further decolourization was observed. After addition of ice, the solution was acidified with conc. HCl, and 2.2 ml of a solution of 16 g $NaHSO_3$+15,6 g $KHCO_3$ in 200 ml water were added immediately. The excess of $NaHSO_3$ was backtitrated with bromine-water. When the solution had stood at pH 1 for 15 min (to effect the lactonization), the mixture was neutralized and re-acidified with NaH_2PO_4. The solution was saturated with NaCl and extracted with ether for three hours in a *Kutscher-Steudel* apparatus. GC. analysis (oven 160°, injector 220°, carrier gas N_2) showed that the solution contained 19.8 mg (0.198 mmol = 93%) of 2-methyl-γ-butyrolactone (**24**), which was identical by GC. and IR. with synthetic material (cf. exp. 7.1.).

6.5. *Cleavage of 2,5-dihydroxy-3-methyl-pentanal δ-hemiacetal* (**20**). Hemiacetal **20** (77 mg, 0.58 mmol) was treated with $NaIO_4$ and bromine-water as in experiment 6.4. After GC.-analysis, the ether extract contained 13 mg (0.13 mmol = 22%) of lactone **24**.

6.6. *Reaction of 2-methyl-δ-butyrolactone* (**24**) *with phenylmagnesium bromide*. The ether solution of **24** (from exp. 6.4.) was concentrated to two ml on a waterbath and dried with $MgSO_4$. Magnesium turnings (150 mg) and 0.1 ml bromobenzene in 0.35 ml abs. ether were mixed in a 10 ml vessel. When the reaction had started, 0.5 ml bromobenzene in 2 ml abs. ether were added over 30 min. The ether solution of **24** was added to the *Grignard* solution and heated for 30 min, when there was still an excess of *Grignard* reagent present. After addition of ice and NH_4Cl, the mixture was worked up in the usual manner with methylene chloride. The crude product was chromatographed on 10 g of SiO_2 with methylene chloride/methanol 2.5%. The resulting 19 mg (0.074 mmol = 37%) of *1,1-diphenyl-2-methyl-butane-1,4-diol* (**25**), m.p. 108–110°, were identical with synthetic material (*cf.* exp. 7.2.) by TLC., m.p. and mixed m.p.

7. Synthesis of compounds for use as reference standards. – 7.1. *Synthetic (±)-2-methyl-γ-butyrolactone* (**24**). 2.67 g of clean sodium and 20 g (0.115 mol) of methylmalonic acid ethyl ester were added to 200 ml of abs. ether. After cooling to −15°, 4 g (0.091 mol) of freshly distilled ethylene oxide[8]) were added. The solution was kept at −15° over night, refluxed

[7]) (±)-3-Methylpentane-1,2,5-triol (**23**) was synthetized according to [23].

[8]) Produced by dropwise addition of 20 ml of chloroethanol to a mixture of 30 g sand and 30 g solid KOH. After drying with KOH, the gas was condensed.

for 2 h, and the ethanol distilled off. The mixture was stirred over night after addition of 4.5 g NaOH in 100 ml of water. Acidification with 45% H_2SO_4 and warming resulted in CO_2-evolution. The acidic reaction product was extracted four times with methylene chloride, washed twice with a $NaHCO_3$ solution and dried with $MgSO_4$. The resulting 5.72 g of crude product were fractionally distilled at 82–85°/14 Torr to give 3.56 g (35.6 mmol = 39%) of GC.-pure (±)-2-methyl-γ-butyrolactone (**24**). IR. spectrum (film): bands at 3510 (weak), 1765 (C=O, br.) 1465, 1380, 1175, 1020, 920 cm^{-1}. NMR. spectrum (in CCl_4): signals at 1,26 ppm (*d*, CH_3); 4.05–4.35 ppm (*d* of *t*, CH_2—O); 1.5–2.7 ppm (*m*, 3 H).

7.2. *Synthetic 1,1-diphenyl-2-methyl-butane-1,4-diol* (**25**). A solution of 2-methyl-γ-butyrolactone (**24**) (108 mg, 1.08 mmol) in 2 ml ether was added to a *Grignard* solution of 100 mg magnesium turnings and 650 mg bromobenzene in 2 ml ether during 15 min. After 15 min of reflux, the mixture was hydrolysed with ice, $(NH_4)_2SO_4$ was added and the mixture worked up in the usual manner with ether. Chromatography of the crude product on 10 g SiO_2 with methylene chloride produced 30 mg of biphenyl, 15 mg of an unknown by-product, and 247 mg (0.965 mmol = 88%) of 1,1-diphenyl-2-methyl-butane-1,4-diol (**25**), m.p. 108–110°. IR. spectrum (in methylene chloride): bands at 3600 (OH, associated); 3350 (OH); 1600 (weak); 1490, 1450 (weak); 1040 cm^{-1}. UV. spectrum (in ethanol): maxima at 195 nm (log ε = 4.741), 253 nm (2,67), 259 nm (2.71), 268 nm (2.48). NMR. spectrum (in $CDCl_3$): signals at 1.91 ppm (*d*, CH_3); 2.95 ppm (*m*, 1H); 3.65 ppm (*d* of *t*, CH_2—OH); 2.40 ppm (OH, 2H), *ca.* 1.6 ppm (*m*, 2H); *ca.* 7.4 ppm (aromatic, 10H).

$C_{17}H_{20}O_2$ (256.3) Calc. C 79.65 H 7.86% Found C 79.39 H 7.88%

7.3. *Synthetic 4-O-(3,5-dinitrobenzoyl)-1,1-diphenyl-2-methyl-butane-1,4-diol* (**27**). A solution of 93 mg (0.363 mmol) **25** in 12 ml pyridine was stirred over night at room temperature with 450 mg 3.5-dinitrobenzoyl chloride. The reaction mixture was poured over ice and worked up in the usual manner with ether and the resulting 186 mg of crude product were chromatographed on 30 g SiO_2 with methylene chloride to give 138 mg (0.306 mmol = 84%) of pure **27**, crystallized from methylene chloride/petroleum ether, m.p. 171–172.5°. IR. spectrum (in methylene chloride): bands at 3600 (OH; 1730 (C=O); 1630, 1550 (NO_2); 1350 (NO_2); 1270, 1170, 1080 cm^{-1}. UV. spectrum (in ethanol): maxima at 195 nm (log ε = 4.839); shoulder at 216 nm (4.548).NMR. spectrum (in $CDCl_3$): signals at 1.02 ppm (*d*, CH_3); 1.3–2.1 ppm (*m*, 1H); 4.35–4.65 ppm (*q*, 2 H); 7.1–7.7 ppm (aromatic, 10 H); 8.6–8.8 ppm (aromatic; 3 H). Mass spectrum: base peak at *m/e* 432 ($M^+ - H_2O$).

7.4. *Synthetic 1-O-(3,5-dinitrobenzoyl)-3-methyl-4,4-diphenyl-3-buten-1-ol* (**28**). 27 mg (0.6 mmol) **27** were cooled to −80° and 5 ml $SOCl_2$ added. The $SOCl_2$ was removed in vacuum during 15 min after warming to room temp. Two additions of methylene chloride and evaporation in vacuum resulted in an odorless crude product which was chromatographed on 5 g SiO_2 and crystallized from ether to produce 23 mg (0.532 mmol = 88%) **28**. Recrystallization from ether gave light yellow needles, m.p. 149–151°. – IR. spectrum (in methylene chloride): bands at 1730 (C=O); 1630, 1540 (NO_2); 1340 (NO_2); 1160, 1070, 960, 920 cm^{-1}. UV. spectrum (in ethanol): maxima at 199 nm (log ε = 4.778); shoulder at 227 nm (4.520). NMR. spectrum (in $CDCl_3$): signals at 1.90 ppm (*s*, CH_3); [2.72 ppm (*t*, 2 H), 4.60 ppm (*t*, A_2X_2-system)], 7.1–7.4 ppm (aromatic; 10 H), 8.7–8.8 ppm (aromatic, 3 H). Mass spectrum: molecular ion at *m/e* 432.

7.5. *Cleavage of synthetic 1-O-(3,5-dinitrobenzoyl)-4,4-diphenyl-3-methyl-3-buten-1-ol* (**28**) *with* $NaIO_4$. 25 mg of OsO_4, 70 mg of $NaIO_4$ in 0.3 ml water and 3 drops of pyridine were added to a solution of 48 mg (0.105 mmol) **28** in 1.4 ml abs. dioxane. The reaction mixture was stirred vigorously at 50° and fine needles crystallized out. All the starting material was consumed after 9 h and two new products were observed by TLC. Usual work-up and washing of the ether layer with $NaHSO_3$ solution produced 48 mg crude product. Chromatography on 5 g SiO_2 gave 13 mg (0.071 mmol = 67%) of *benzophenone* (**30**), which were recrystallized from petroleum ether; identical m.p. and mixed m.p. with authentic material.

Further elution with methylene chloride/methanol 1.3% gave 20 mg (0.071 mmol = 67%) of *4-(3,5-dinitrobenzoyloxy)-butan-2-one* (**29**), which were crystallized in fine needles from ether, m.p. 102–103°. – IR. spectrum (on methylene chloride): bands at 1730 (C=O, broad), 1630, 1545 (NO_2), 1350 (NO_2), 1160, 1080 (weak), 920 (weak) cm^{-1}. NMR. spectrum (in $CDCl_3$): signals at 2.26 ppm (*s*, CH_2), [3.00 ppm (*t*, 2 H), 4,72 ppm (*t*, 2 H) A_2X_2-system], 8.7–9.0 ppm (aromatic, 3 H). Mass spectrum: molecular ion at *m/e* 282.

7.6. *Synthetic 1,1-diphenyl-2-methyl-tetrahydrofuran* (**26**). A solution of 53 mg (0.207 mmol) **25** in 3 ml of methylene chloride was treated with 5 mg of iodine. After refluxing for 15 min, the solution was decoloured with a $NaHSO_3$ solution and dried with $MgSO_4$. The 46 mg of crude product which resulted contained traces of non-polar impuritics. Chromatography on 8 g of SiO_2 with methylene chloride produced 43 mg (0.18 mmol = 87%) of pure 1,1-diphenyl-2-methyl-tetrahydrofuran (**26**), m.p. 37–40°. – IR. spectrum (film): bands at 2980, 2890, 1490, 1450, 1070, 1030, 760, 710 cm^{-1}. NMR. spectrum (in $CDCl_3$): signals at 0.81 ppm (*d*, CH_3), 1.2–2.2 ppm (*m*, 2 H), 2.9–3.2 ppm (*q*, 1 H), 3.6–4.4 ppm (*m*, 2 H), 7.1–7.6 ppm (aromatic, 10 H).

$C_{17}H_{18}O$ (238.3) Calc. C 85.67 H 7.61% Found C 85.50 H 7.52%

8. Degradation of the verrucarinolactone 19 with incorporated [2-^{3}H, 2-^{14}C]-mevalonate. A portion of 99.5 mg (0.765 mmol) **19** (average activity of 13000 dpm/mg [^{3}H] and 3200 dpm/mg [^{14}C]) was reduced with $LiAlH_4$ in THF as in experiment 6.2. The resulting *triol* **23** (96 mg = 0.716 mmol = 93%) was oxidized with bromide-water and $NaIO_4$ in the same manner as in exp. 6.4. According to GC. analysis, 54 mg (0.54 mmol = 75%) of *radioactive* (2R)-*2-methyl-γ-butyrolactone* (**24**) were produced. Treatment with phenylmagnesium bromide gave 93 mg (0.363 mmol = 67%) of *pure diol* **25**, which did not crystallize. $[\alpha]_D^{22} = -26° \pm 1°$ ($c = 1.99$ in methylene chloride).

Reaction of **25** with 3,5-dinitrobenzoyl chloride in pyridine as in 7.3. resulted in 138 mg (0.306 mmol = 84%) of *ester* **27**, which also did not crystallize. The sample **27** was identical with synthetic material by TLC. and NMR. $[\alpha]_D^{22} = -11° \pm 1°$ ($c = 1.22$ in methylene chloride). – Radioactivity: sample 1: 3620 dpm/mg ^{3}H, 911 dpm/mg ^{14}C; sample 2: 3690 dpm/mg ^{3}H, 894 dpm/mg ^{14}C.

110 mg (0.224 mmol) of **27** was treated with $SOCl_2$ as in 7.4. Chromatographic purification produced 90 mg (0.208 mmol = 85%) of olefin **28**, which crystallized from ether as yellow needles; identical with synthetic material by TLC., m.p. and mixed melting point. Radioactivity: Crystallization 3: 49 dpm/mg ^{3}H, 932 dpm/mg ^{14}C; Crystallization 4: 48 dpm/mg ^{3}H, 970 dpm/mg ^{14}C, Crystallization 5: 45 dpm/mg ^{3}H, 962 dpm/mg ^{14}C.

The *olefin* **28** (83 mg = 0.192 mmol) was oxidized with $NaIO_4$ and OsO_4 in dioxane-water analogously to exp. 7.5. The isolated *benzophenone* (**30**) (15.5 mg = 0.085 mmol = 44%) and *methyl ketone* **29** (7.5 mg = 0.026 mmol = 14%) were identical with authentic material by TLC., m.p. and mixed m.p. Radioactivity: a) *Benzophenone* (**30**): Crystallization 2: 35 dpm/mg ^{3}H 2240 dpm/mg ^{14}C; Crystallization 3: 21 dpm/mg ^{3}H 2270 dpm/mg ^{14}C; Crystallization 4: 96 dpm/mg ^{3}H 2150 dpm/mg ^{14}C.

b) *Methyl ketone* **29**: Crystallization 2: 62 dpm/mg ^{3}H, 11 dpm/mg ^{14}C; Crystallization 3: 37 dpm/mg ^{3}H, 2 dpm/mg ^{14}C.

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159. Structure cristalline et moléculaire d'un dérivé méthyl-hexopyranoside à C(4) hybridé sp^2

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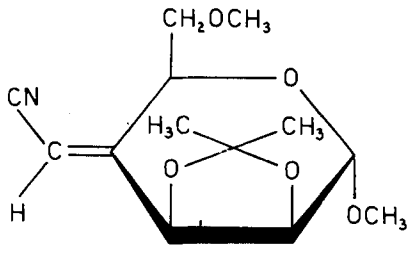
(13. III. 74)

Summary. The derivative $C_{13}H_{19}NO_5$ crystallizes in space group $P2_12_12_1$ with $a = 9.371$, $b = 11.815$, $c = 13.207$ Å and $Z = 4$. The structure was solved by direct methods and refined by full-matrix least-squares to $R = 0.058$. The pyranose ring exists in the $^3S_1$ conformation (or in the equivalent, but here structurally less consistent, $^0S_4$ conformation). The dioxolane ring has an envelope conformation. Strong intramolecular interactions between the bulky substituents suggest that the ensuing strain energy is assumed, for a significant part, by the twisted-boat conformation of the pyranose ring.

Une série de sucres ramifiés à cycle pyrannique hybridé sp^2 en C(4) a été récemment l'objet d'une étude conformationnelle par RMN. [1]. La présente détermination structurale aux rayons X du méthyl-désoxy-4-O-isopropylidène-2,3-O-méthyl-6-*cis*-(H–C(4')–C(4)–C(3))-cyanomethylène-4-α-D-*lyxo*-hexopyranoside (**1**) confirme la conformation moyenne déduite des observations spectroscopiques et montre l'incidence des substituants volumineux dans l'adoption d'une conformation croisée par le cycle pyrannique.



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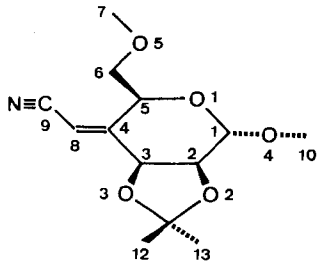

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