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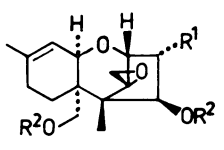
Structural studies on bioactive microbial metabolites

Christoph Tamm

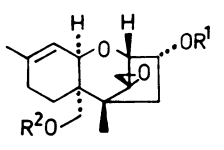
Institute of Organic Chemistry, University of Basel
CH-4056 Basel, Switzerland

Abstract – The trichothecenes are a family of toxic mould metabolites. The structures of new minor metabolites of the producing microorganisms have been elucidated. Some are C_{15}-compounds which possess the apotrichothecane skeleton. Macrocyclic trichothecenes have been synthesized using diacetoxyscirpenol (anguidine) as as starting material. Methods were developed for the stereoselective synthesis of the macrocyclic building blocks. By the use of the enzymes pig liver esterase and α-chymotrypsin and of $MoO_5 \cdot Py \cdot HMPT$ complex highly functionalized chiral synthons were prepared which are suitable for the construction of a variety of bioactive natural products.

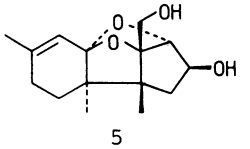
Many moulds, especially various species of Fungi imperfecti such as Fusarium or Myrothecium, produce trichothecenes (ref. 1). Numerous members of this growing class of secondary metabolites exhibit a wide range of interesting biological effects, such as cytostatic (antileukemic) activity, but they are also highly toxic. They belong to the class of the sesquiterpenes and can be divided into three groups (ref. 2): 1) the sesquiterpene alcohols, simple esters and glycosides (ref. 3), 2) the macrocyclic di- and triesters, most often derived from verrucarol, and 3) the trichoverroids, which possess only a portion of the macrocyclic moiety. Owing to their unusual chemical and biological properties, both the simple sesquiterpenes and the macrocyclic esters have been the goal of a variety of synthetic efforts. For our own synthetic work larger quantities of diacetoxyscirpenol (anguidine) (2) were required. It is the major metabolite of Fusarium sambucinum (ref. 4). We have isolated seven minor metabolites. Whereas four of these substances are nitrogeneous compounds, the other three are sesquiterpenes. Structures 5 and 7 were assigned to sambucinol ($C_{15}H_{22}O_4$) and sambucoin ($C_{15}H_{22}O_3$) respectively. The structures were confirmed by X-ray analysis (ref. 5) but the absolute configurations are still unknown. However, it is reasonable to assume that they correspond to the absolute configuration of diacetoxyscirpenol (2). The structure of the third C_{15}-substance ($C_{15}H_{22}O_3$) (ref. 6) has not been established yet. The isolation of a fourth sesquiterpene ($C_{15}H_{24}O_3$), seco-deoxysambucinol (6) has been reported by a Canadian group (ref. 7). It is interesting to note that both, sambucinol (5) and seco-deoxysambucinol (6) possess the apotrichothecane skeleton. The biogenetic relationship of the new minor metabolites with the trichothecenes appears to be obvious. Two pathways are proposed, both starting with trichodiene (8), the well established precursor of the trichothecenes (ref. 8).



1 $R^1=R^2=H$
2 $R^1=OH$; $R^2=Ac$



3 $R^1=R^2=Ac$
4 $R^1=H$; $R^2=Ac$



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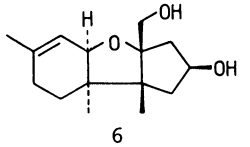

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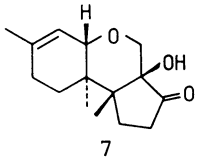

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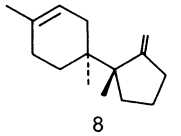

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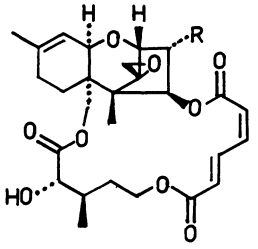
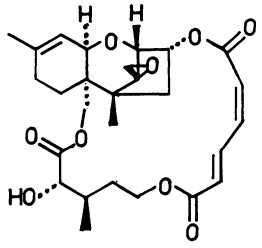
After completion of the synthesis of the macrocyclic trichothecene triesters verrucarin A (9) from verrucarol (1) and 3α-hydroxyverrucarin A (10) from anguidine (2) respectively (ref. 9) – compound 10 has not been found in nature yet – the macrocyclic isomer 11 of verrucarin A (9) was synthesized from calonectrin (3). The comparison of the biological properties of the unnatural analogues 10 and 11 with those of the natural macrocyclic metabolites will be of special interest. For this purpose



9 R=H
10 R=OH



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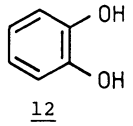
an efficient procedure for the conversion of diacetoxyscirpenol (2) into calonectrin (3) and deacetylcalonectrin (4) using the Barton deoxygenation method as key reaction has been developed (ref. 10).

The building blocks for the macrocyclic moiety, (E,Z)-muconic acid and verrucarinic acid, which contain suitable protecting groups, were synthesized in the following manner.
Treatment of catechol (12) with O_2/CuCl in the presence of methylmercaptoethanol gave the (Z,Z)-half ester 13, which was isomerized to the (E,Z)-half ester 14 by heating with water. The trans-protected and the cis-protected half esters 15 and 16 respectively were also obtained from catechol (12). Ionisation shifts in the ^{1}H-NMR spectra served for the determination of the esterification sites (ref. 6,9).



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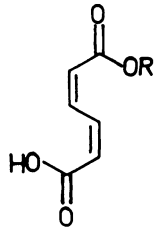
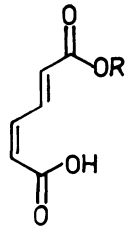
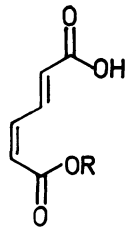


13 R=CH_2CH_2SMe

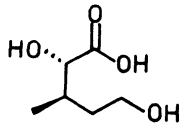


14 R=CH_2CH_2SMe
15 R=$CH_2CH_2SiMe_3$



16 R=CH_2CH_2SMe

Optically active verrucarinic acid (2S,3R)-2,5-dihydroxy-3-methylpentanoic acid)(17) and its lactone 18 which are suitably protected for the subsequent condensation reaction with the trichothecene and muconic acid derivatives, have been synthesized by five routes. The first synthesis



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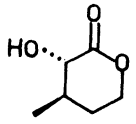

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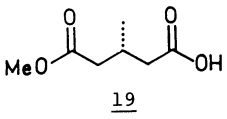
uses an asymmetric hydroboration of an olefin, the second a Sharpless epoxidation and the third the enantioselective hydrolysis of dimethyl-3-methylglutarate by pig liver esterase (PLE) or α-chymotrypsin for introducing chirality (ref. 11). The key steps of the fourth approach involve a diastereoselective alkylation of a (-)-(S)-malic acid ester and the regioselective reduction of one carboxyl function to a methyl group (ref. 12). The fifth synthesis uses a stereoselective addition of an allylsilane to a chiral glyoxylate (ref. 12). 8-Phenylmenthol served as the chiral directing group.

In the third synthesis of verrucarinic acid chirality was introduced by the application of PLE and α-chymotrypsin. By the use of enzymes which possess low substrate selectivity but which at the same time catalyse reactions with a high degree of stereoselectivity, new chiral synthons for the construction of optically active natural products can be produced (ref. 13). We have therefore studied the PLE-catalyzed hydrolysis of dimethyl esters of symmetrical dicarboxylic acids, including meso-diacids, cis-1,2-cycloalkanedicarboxylic acids and diacids with a prochiral centre (ref. 14, 15). The products of these stereoselective hydrolyses are chiral monoesters of dicarboxylic acids, with an enantiomeric excess (e.e.) from 10% to 100%. The following conclusions were drawn: 1) To achieve high stereoselectivity the distance of the prochiral centre from the ester group has to be restricted to the α- or β-position. 2) Approximate additivity of structural parameters on enzyme stereoselectivity is observed. 3) Rigid conformation of a substrate, imposed by a cyclic structure, affects higher stereoselectivity as compared to an acyclic analogue. In six-membered ring substrates the ester group must be in an equatorial position. 4) Substituents of different polarity and different size show opposite effects on the selectivity of enzyme hydrolysis.
The (R)-half ester 19 of 3-methylglutaric acid which was obtained from 3-methylglutarate with PLE, served for the synthesis of the chiral bromoester 20, a synthon for the construction of the macrocyclic moieties of some cytochalasins (ref. 16). (S)-Glutamic acid provided the second centre of chirality.



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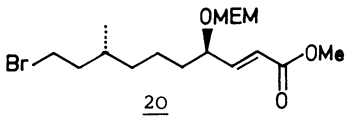

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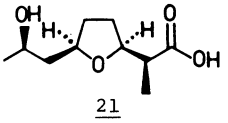

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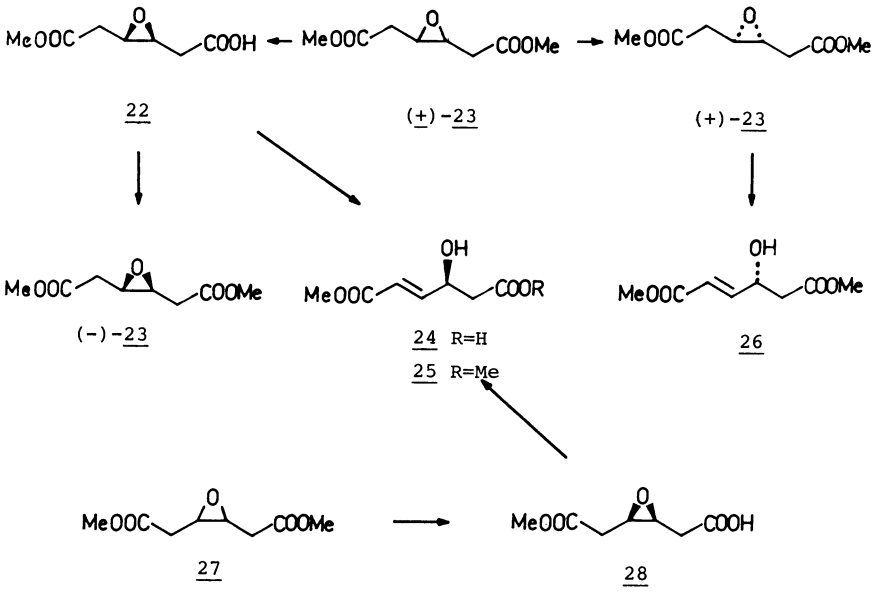
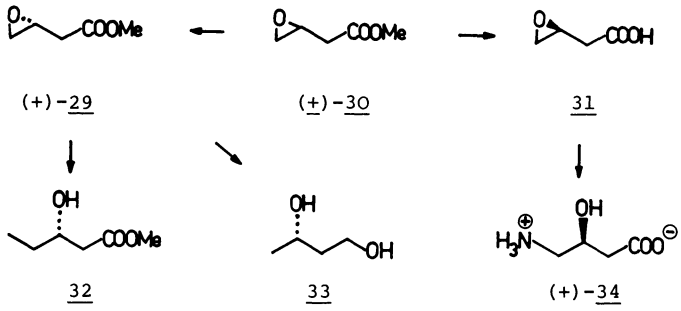
The hydrolysis of dimethyl-3-hydroxyglutarate with PLE was reinvestigated in view of a synthesis of optically active nonactic acid (21) (ref. 6).

As anticipated from our enzyme model, the diester was found to be a bad substrate. The e.e. was only 22% ((S)-configuration). With α-chymotrypsin an e.e. of 68% ((R)-configuration) was observed. The e.e. was determined by HPLC-analysis of diastereoisomeric camphanoic acid derivatives.

Interesting results were obtained with the dimethyl-3,4-epoxyadipates (ref. 6). By their kinetic resolution by PLE chiral β-hydroxyesters and acids are accessible. Treatment of the racemic diester 23 which has a

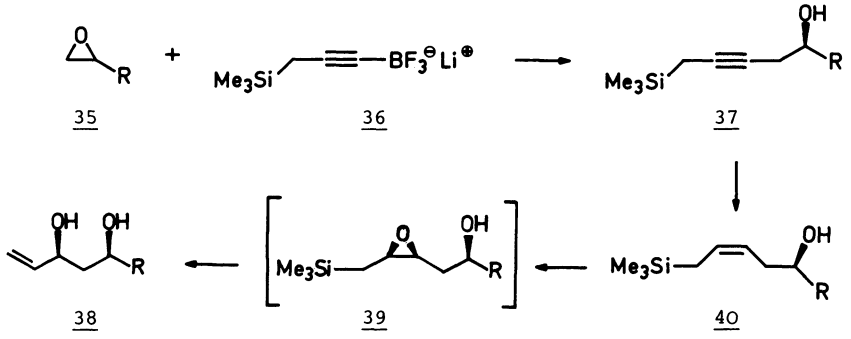


C_2-axis, with PLE gave the (+)-diester 23 and the monoester 22 in high optical yields. Esterification of 22 led to the (-)-diester 23. The epoxymonoester 22 was transformed to the unsaturated β-hydroxy acid 24 which is a C_6-building block with four different functionalities, and to the diester 25. In analogous manner (+)-23 yielded the enantiomeric diester 26. On the other hand, the meso-epoxydiester 27 was rapidly hydrolysed by PLE with almost 100% selectivity to give the optically pure half ester 28. The latter was transformed to the unsaturated hydroxy-diester 25.
According to our enzyme model methyl-(±)-3,4-epoxybutanoate should be a good substrate for PLE as well. The enzymic hydrolysis of (±)-30 gave the (+)-ester 29 and the acid 31 (ref. 6).

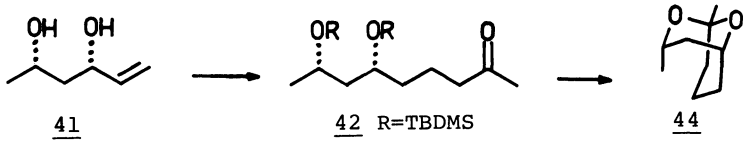


The latter was converted to (+)-4-amino-3-hydroxybutanoic acid (34), the enantiomer of the hypotensive agent GABOB (e.e. 97%). The optically active β-hydroxyester 32 and the 1,3-diol 33 were prepared from the epoxyester (+)-29.

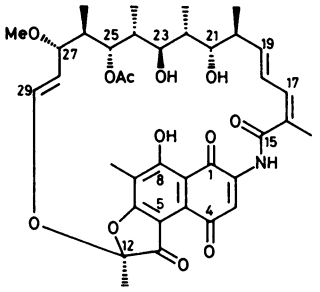
Many natural products contain a 1,3-diol substructure. Therefore we have studied the stereoselective synthesis of functionalized 1,3-diols (ref. 6). It was observed that V^{5+}-catalyzed t.-butylhydroperoxide epoxidation of (Z)-5-hydroxy-2-alkenylsilanes 40 exhibits excellent erythro selectivity. The resulting epoxides 39 undergo fragmentation to yield 1,3-diols of type 38. The required allylsilanes are prepared from the epoxide 35. Reaction of the latter with the lithium complex 36 led to the intermediate 37.



It was interesting to note that vinylsilanes do not react with V^{5+}-catalyzed t.-butylhydroperoxide under the same conditions. The erythro-1,3-diol 41 prepared by this method was used for the stereoselective synthesis of endo-1,3-dimethyl-2,9-dioxabicyclo[3.3.1]nonane (44) via the protected ketone 42 (ref. 6). The latter underwent, after deprotection to 43, cyclization to the desired bicyclic ketal 44. The latter was isolated from Norway spruce which is attacked by the ambrosia beetle Trypodendron lineatum Oliv..



The high enantioselectivity of the hydrolysis of dimethyl xylo-3-hydroxy-2,4-dimethylglutarate (47) to the monoester 48 by PLE offers the opportunity to use this chiron for the stereocontrolled synthesis of the epimer 55 of the diester 45 (ref. 6). The latter corresponds to the C-19 to C-27 segment of the antibiotic rifamycin S (46). The half ester 48 was reduced with borane dimethylsulfide complex and transformed to the acetonide 49. Reduction of 49 with DIBAH afforded the aldehyde 50, which was reacted with the enolate of 2,4-dimethylphenylpropionate. The resulting ester 51 was converted to the alcohol 53 by four steps via the derivative 52. Swern oxidation of 53 led to the aldehyde 54. After



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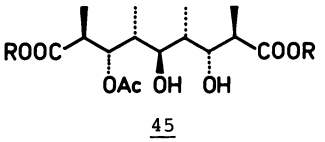

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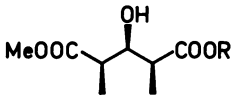
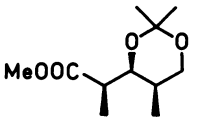


47 R=Me

48 R=H



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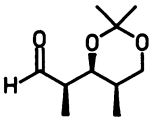

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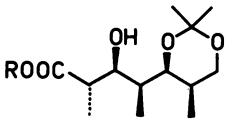
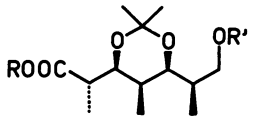


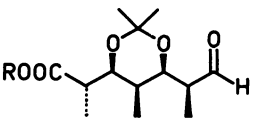
51 R=2,6-Dimethylphenyl



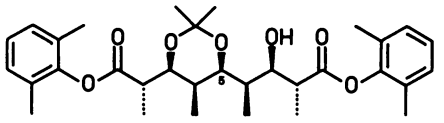
R=2,6-Dimethylphenyl

52 R'=TBDMS

53 R'=H



54 R=2,6-Dimethylphenyl



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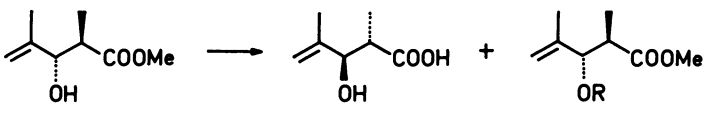
the second aldol condensation with 2,4-dimethylphenylpropionate the diester 55 was obtained. It contains 7 centres of chirality. For the preparation of 45 C-5 has to be inverted. In an alternative approach to the desired target molecule 45 the hydrolysis of the racemic ester 56 with PLE was reinvestigated (ref. 6, 17). It yielded the acid (-)-57 and the ester (+)-56. After hydroboration of the protected derivative 58 and Swern oxidation, the aldehyde 59 was obtained. Chain elongation by treatment of 59 with crotylbromide/$CrCl_2$ led to the intermediate 60.

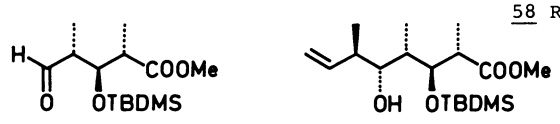


(±)-56

(-)-57

(+)-56 R=H

58 R=TBDMS



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The studies of the synthesis of verrucarinic acid have prompted us to explore the possibility of the diastereoselective hydroxylation of chiral ester enolates with $MoO_5 \cdot Py \cdot HMPT$ (ref. 18) but also with 2-(phenylsulfonyl)-3-phenyloxaziridine (ref. 19). It is known that both the (Z)- and (E)-isomers of the enolates are accessible by changing the conditions of deprotonation, i.e. by the use of LICA and LICA/HMPT complex as base in THF respectively (Fig. 1). Esters of 3-phenylpropionic

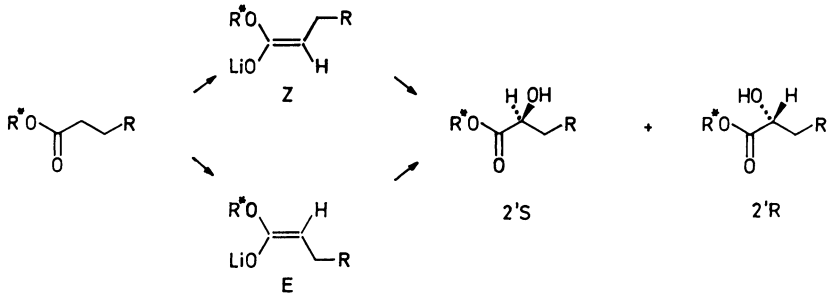


Fig. 1

acid served as substrates in the preliminary experiments (ref. 20). They were prepared from chiral alcohols (R*-OH) derived from (+)-camphor. For steric reasons the reagent attacks from the less hindered side of the enolate i.e. the (Z)-enolate from the Re-face leading to the (2'R)-hydroxy ester, and the (E)-enolate from the Si-face forming the (2'S)-hydroxy ester respectively (Fig. 2).

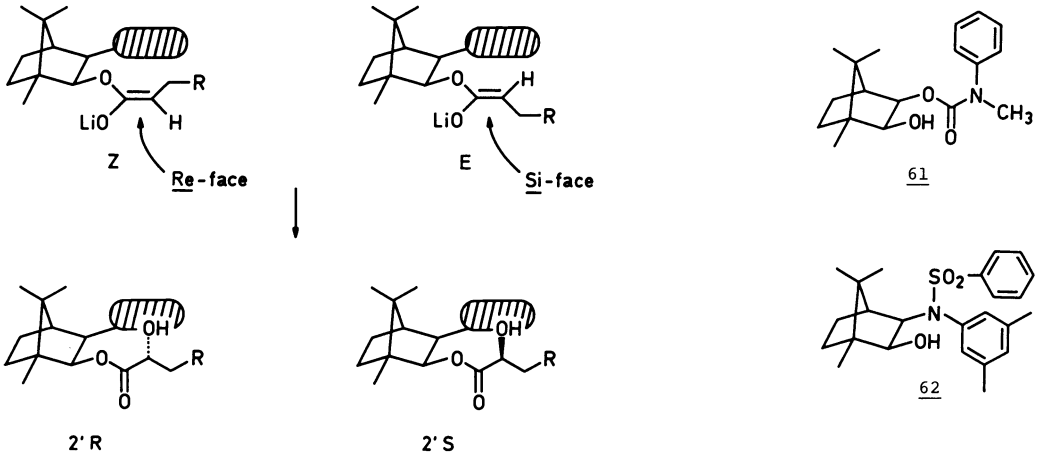


Fig. 2

Best results were obtained with the alcohols 61 and 62 respectively. Remarkable dependence of the R',S'-ratio from the base added, was noted for the esters of the alcohols 61 and 62 with phenylpropionic acid as shown by Table 1.

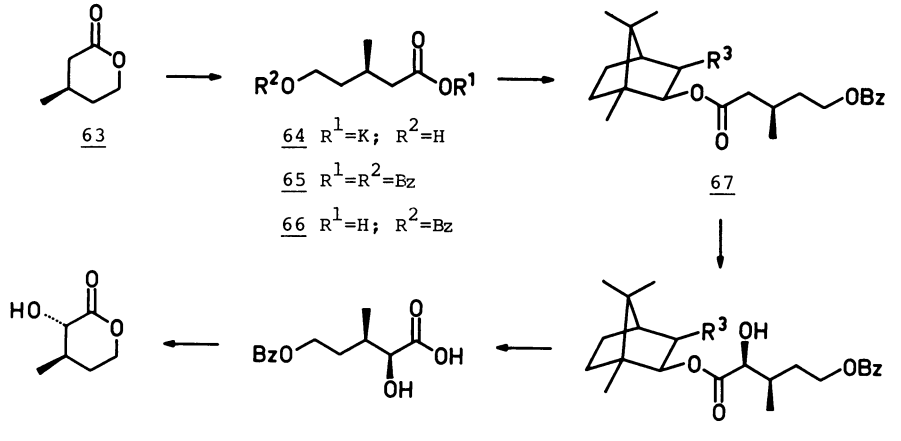
Table 1 Hydroxylation of Esters of Phenylpropionic Acid

Base	Alcohol	R'S'Ratio	Yield (isol.)
2 eq LICA/HMPT	61	14:86	50%
	62	7:93	50%
2 eq LICA+HMPT, after deprotonation	61	57:43	57%
	62	82:18	60%
2 eq LDA + DMPU, after deprotonation	62	28:72	40%
2 eq LHMDS	62	9:91	30%
2 eq KHMDS	62	1:99	73%

In order to enhance the diastereoselectivity of the hydroxylation and to avoid the toxic HMPT, base and hydroxylating conditions were varied using the ester of phenylpropionic acid with alcohol 62 (ref. 6). The best R',S'-ratio of 28:72 was obtained with 2-equivalents of LDA and addition of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) after deprotonation. However, replacing the base by lithium hexamethyldisilazide (LHMDS) the ratio was found to be 9:91 (yield 30%). Quite unexpectedly

a remarkable increase of isolated yield (73%) and R',S'-ratio to 1:99 was achieved by the addition of 8 eq of potassium sec-butoxide during depronation using 2 eq of KHMDS as base. Other alkoxides showed the same effect. Replacement of MoOPH by the oxaziridine decreased the diastereoselectivity.

The same excellent result was also obtained with the ester 62 with propionic acid and (3R)-5-benzyloxy-3-methylpentanoic acid (66). However the yield was lower in the latter case.
Finally we made use of the high diastereoselectivity of the hydroxylation reaction for an improved stereoselective synthesis of verrucarinolactone (18). The δ-lactone 63, an intermediate of one of our earlier syntheses (ref. 11) served as starting material. It was transformed to the potassium salt 64. Treatment with benzylbromide and tetrabutylammonium



iodide led to the benzyl ester 65. The latter was hydrolysed to the acid 66. Condensation of 66 with the chiral alcohol 62 in the presence of carbonyldiimidazole and sodium yielded the ester 67. By subsequent hydroxylation as described above, and hydrolysis with KOH in aqueous methanol the hydroxyacid 68 was obtained. It was converted to verrucarinolactone (18) by catalytic hydrogenation with palladium in acetic acid/THF.

After completion of these investigations results on the α-acetoxylation of O-silylated camphorsulfonamide esters with lead tetraacetate (ref. 21) and the hydroxylation of imide enolates with the oxaziridine (ref. 22) were reported. Also in these cases high diastereoselectivities were observed.

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REFERENCES

1. J.R. Bamburg & F.M. Strong, in "Microbial Toxins", Vol. 3, ed. S. Kadis, Academic Press, New York, 1973, p. 207; Ch. Tamm, Progr. Chem. Org. Nat. Prod. 31, 63 (1974); J.R. Bamburg, in "Mycotoxins and Other Fungal Related Food Problems", ed. J.V. Rodricks, American Chemical Society, Washington D.C., 1976; Ch. Tamm, in"Mycotoxins in Human and Animal Health", eds. V. Rodricks, C.W. Hesseltine and M.A. Mehlman, Pathotox Publishers, Park Forest South, Illinois, 1977, p. 209; B.B. Jarvis & E.P. Mazzola, Acc. Chem. Res. 15, 388 (1982).
2. B.B. Jarvis, J.O. Midivo, G.P. Stahly, G. Pavanasasivam & E.P. Mazzola, Tetrahedron Lett. 21, 787 (1980); B.B. Jarvis, V.M. Vrudhula & J.O. Midivo, J. Org. Chem. 48, 2576 (1983).

3. C.P. Gorst-Allman, P.S. Steyn, R. Vleggar & C.J. Rabie, J. Chem. Soc. Perkin Trans. I, 1985, 1553.

4. E. Flury, R. Mauli & H.P. Sigg, Chem. Commun. 1965, 26; H.P. Sigg, R. Mauli, E. Flury & D. Hauser, Helv. Chim. Acta 48, 962 (1965): A.W. Dawkins, J.F. Grove & B.K. Tidd, Chem. Commun. 1965, 27; A.W. Dawkins, J. Chem. Soc. (C) 1966, 116.

5. P. Mohr, Ch. Tamm, W. Zürcher & M. Zehnder, Helv. Chim. Acta 67, 406 (1984).

6. Ch. Tamm et al. Unpublished results.

7. Ch. Tamm, Sixth International Symposium on Mycotoxins and Phycotoxins, 22-25 July 1985, Pretoria, RSA.

8. Ch. Tamm & W. Breitenstein, in "The Biosynthesis of Mycotoxins" ed. P.S. Steyn, Academic Press, New York, 1980, p. 69; G.A. Cordell, Chem. Rev. 76, 425 (1976); D.E. Cane, S. Swanson & P.P. Murthy, J. Amer. Chem. Soc. 103, 2136 (1981).

9. P. Mohr, M. Tori, P. Grossen, P. Herold & Ch. Tamm, Helv. Chim. Acta, 65, 1412 (1982).

10. N. Jeker, P. Mohr & Ch. Tamm, Tetrahedron Lett. 25, 5637 (1984).

11. P. Herold, P. Mohr & Ch. Tamm, Helv. Chim. Acta 66, 744 (1983).

12. P. Grossen, P. Herold, P. Mohr & Ch. Tamm, Helv. Chim. Acta 67, 1625 (1984).

13. F.C. Huang, L.F.H. Lee, R.S.D. Mittal, P.R. Ravikumar, J.A. Chan, C.J. Sih, E. Caspi & C.R. Eck, J. Amer. Chem. Soc. 97, 4144 (1975). J.B. Jones, C.J. Sih & D. Perlman, "Application of Biochemical Systems in Organic Chemistry", J. Wiley & Sons, New York, 1976; J.B. Jones, M.A.W. Finck & I.J. Jakovac, Can. J. Chem.60, 2007 (1982); G.M. Whitesides & C.-H. Wong, Alrichim. Acta 16, 27 (1983); D.W. Brooks & J.T. Palmer, Tetrahedron Lett. 24, 3059 (1983).

14. P. Mohr, N. Waespe-Sarcevic, Ch. Tamm, K. Gawronska & J.K. Gawronski, Helv. Chim. Acta 66, 2501 (1983).

15. M. Ohno, S. Kobayashi, T. Iimori, Y.-F. Wang & T. Izawa, J. Amer. Chem. Soc. 103, 2405 (1981). H. Kotani, Y. Kuze, S. Uchida, T. Miyabe, T. Iimori, K. Okano, S. Kobayashi & M. Ohno, Agr. Biol. Chem. 47, 1363 (1983). C.J. Francis & J.B. Jones, J. Chem. Soc. Commun. 1984, 579; G. Salbioni, M.L. Shea & J.B. Jones, ibid. 1984, 236. M. Schneider, N. Engel & H. Boensmann, Angew. Chem. 96, 54 (1984); H.-J. Gais, K.L. Lukas, ibid. 96, 140 (1984); H.-J. Gais & T. Lied, ibid. 96, 143 (1984); Y.-F. Wang & Ch.J. Sih, Tetrahedron Lett. 25, 4999 (1984); Y.-F. Wang, C.-S. Chen, G. Girdaukas & Ch.J. Sih, J. Amer. Chem. Soc. 106, 3695 (1984); K. Laumen, E.H. Reimerdes & M. Schneider, Tetrahedron Lett. 26, 407 (1985).

16. J. Ackermann, N. Waespe-Šarčevič & Ch. Tamm, Helv. Chim. Acta, 67, 254 (1984).

17. C. Chen, Y. Fujimoto, G. Girdaukas & C.J. Sih, J. Amer. Chem. Soc. 104, 7294 (1982).

18. E. Vedeijs, J. Amer. Chem. Soc. 96, 5944 (1974); E. Vedeijis, D.A. Engler & J.E. Telschow, J. Org. Chem. 43, 188 (1978).

19. F.A. Davis, L.C. Vishwakarma, C. Billmers & J.M. Finn, J. Org. Chem. 49, 3241 (1984).

20. R. Gamboni, P. Mohr, N. Waespe-Šarčevič & Ch. Tamm, Tetrahedron Lett. 26, 203 (1985).

21. W. Oppolzer, R. Dudfield, Helv. Chim. Acta 68, 216 (1985).

22. D.A. Evans, M.M. Morrissey & R.L. Dorow, J. Amer. Chem. Soc. 107, 4346 (1985); F.A. Davis & L.C. Vishwakarma, Tetrahedron Lett. 26, 3539 (1985).