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Recent studies on mould metabolites

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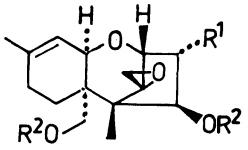
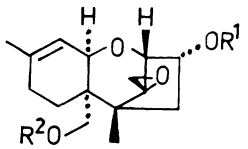
Abstract - Anguidine (diacetoxyscirpenol) is the major metabolite of Fusarium sambucinum (Fungi imperfecti). A series of minor metabolites has been isolated from cultures of this microorganism. Some of their structures have been elucidated. Their biogenetic relationship to the trichothecenes is discussed. Macrocyclic trichothecenes have been synthesized using anguidine as the starting material. Special attention is paid to the stereoselective synthesis of the building blocks of the macrocyclic segment. Methods for the asymmetric synthesis of α-hydroxy-esters have been developed. The enantioselectivity of the enzymes, pig liver esterase and α-chymotrypsin, has also been studied extensively in order to prepare chiral synthons from symmetrical starting materials. The former are suitable for the construction of a variety of optically active mycotoxins and other natural products.

1 MINOR METABOLITES OF *FUSARIUM SAMBUCINUM*

The trichothecenes are a growing class of closely related sesquiterpenoid secondary metabolites, produced by moulds, especially by various species of Fungi imperfecti (ref. 1). Many members of this family display a wide range of biological effects, such as cytostatic (antileukemic) activity, but they are also highly toxic. They can be divided into three groups: 1) the simple sesquiterpenes, 2) the macrocyclic di- and triesters, most often derived from verrucarol, and 3) the trichoverroids, which possess only a portion of the macrolidic moiety (ref. 2). Owing to their extraordinary properties, many trichothecenes, both the simple sesquiterpenes and their macrocyclic esters have been the target of synthetic efforts. For our own synthetic work larger quantities of anguidine (diacetoxyscirpenol) (2) were required. It is the major metabolite of Fusarium sambucinum (ref. 3). After working up a large scale fermentation and careful chromatographic separations we isolated seven minor metabolites. Whereas three of these substances are sesquiterpenes, the other four are nitrogenous compounds. On the basis of the analytical and spectroscopic data structures 8 and 10 were assigned to sambucoin ($C_{15}H_{22}O_3$) and sambucinol ($C_{15}H_{22}O_4$) respectively. The structures were confirmed by X-ray analysis (ref. 4) but the absolute configurations are still unknown. However, it is reasonable to assume that they correspond to the absolute configuration of anguidine (2).

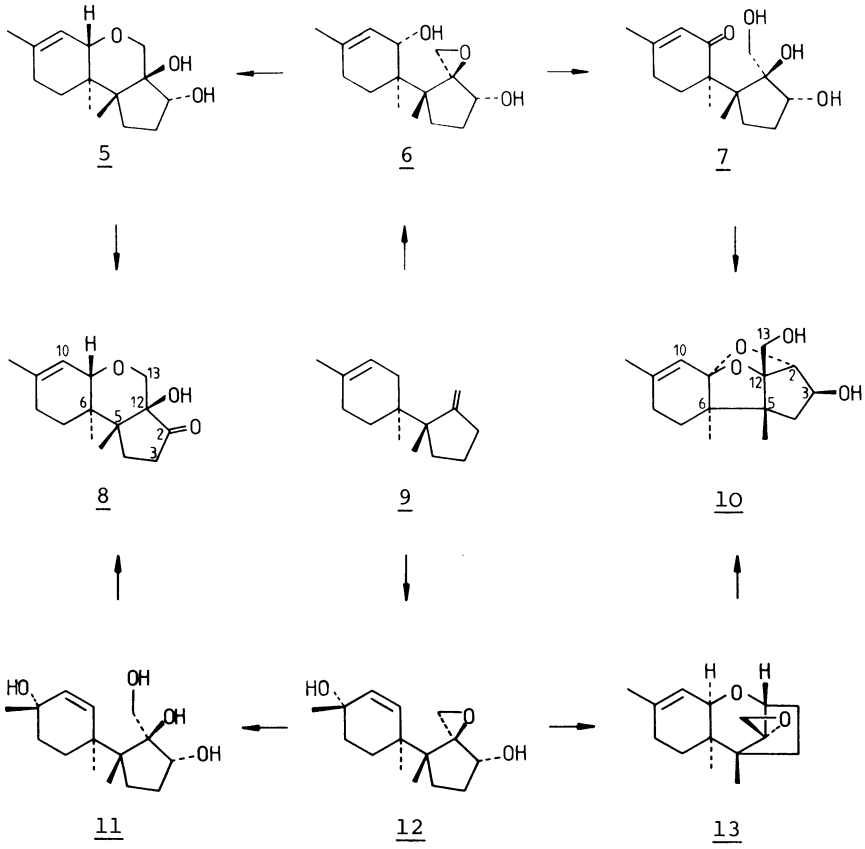


1 $R^1=R^2=H$
2 $R^1=OH$; $R^2=Ac$



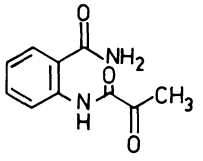
3 $R^1=R^2=Ac$
4 $R^1=H$; $R^2=Ac$

The structure of the third C_{15}-compound ($C_{15}H_{22}O_3$) remains to be elucidated. The structure formulae of 8 and 10 exhibit remarkable features: the absence of the 12,13-epoxy groups, the hitherto unknown acetal bridging in 10 and the unusual attachment of the cyclopentane ring in 8. Although incorporation studies have not yet been carried out, a biogenetic relationship with the trichothecene seems to be obvious. Two possibilities are proposed,both starting with trichodiene (9), the well established precursor of the trichothecenes (ref. 5). In the first, 9 is transformed to trichodiol (12). Pyran ring formation by attack of the 2-hydroxy group leads to 12,13-epoxy-trichothec-9-ene (13), which is also a naturally occurring metabolite. Subsequent oxidation at C(11) and C(3), epoxide opening, and acetalization would finally complete the biosynthesis of 10. On the other hand, hydrolysis of 12 would yield the tetrol 11, which, by attack of the primary hydroxyl group, can directly cyclize to form the sambucoin skeleton. The alternative pathway would involve two allylic hydroxylations leading after epoxidation to the key intermediate 6. Nucleophilic attack at C(13) produces 5, the immediate precursors of sambucoin (8), whereas oxidation to the α,β-unsaturated ketone, epoxide opening, acetalization and oxygenation at C(3) complete, via 7, the formation of sambucinol (10). In both pathways leading to 10 inversion at C(12) must take place.



Three of the four nitrogen containing minor metabolites of Fusarium sambucinum were identified as 2-pyruvylamino-benzamide ($C_{10}H_{10}N_2O_3$) (14), 2-acetyl-4(3H)-quinazolinone ($C_{10}H_8N_2O_2$) (15), and 2-(1-hydroxyethyl)-4(3H)-quinazolinone (chrysogine) ($C_{10}H_{10}N_2O_2$) (16) (ref. 6). Compound 14 is an anti-auxin first isolated from Colletotrichum lagenarium (ref. 7). Cyclization by dehydration leads to 15, which had been found in Fusarium culmorum together with trichothec-9-ene-8-ones (ref. 8). The third metabolite, chrysogine (16) has been isolated earlier from cultures of Penicillium chrysogenum (ref. 9). So far, it has not been found as a metabolite of Fusarium. The chirality is

unknown. The structure of the fourth nitrogenous metabolite of Fusarium sambucinum, $C_{17}H_{19}NO_3$, appears to be more complex. Its elucidation is in progress.



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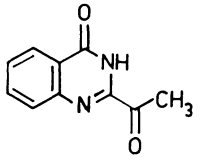

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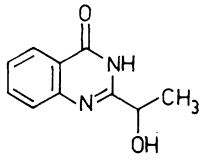

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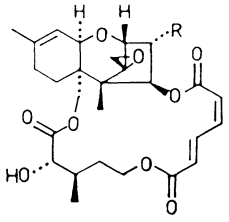
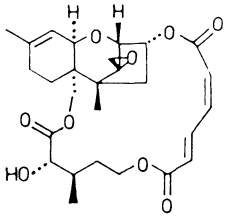
2 SYNTHESIS OF MACROCYCLIC ANALOGUES OF VERRUCARIN A

The synthesis of the macrocyclic isomer 19 of verrucarin A (17) from calonectrin (3) has been the next goal of our synthetic work having synthesized the macrocyclic trichothecene triesters verrucarin A (17) from verrucarol (1) and 3α-hydroxyverrucarin A (18) from anguidine (2) (ref. 10), the latter being an unnatural analogue. For this purpose an efficient procedure for the conversion of anguidine (2) into calonectrin (3) and deacetylcalonetrin (4) in seven steps using the Barton deoxygenation via the thiocarbonylimidazole derivative as key reaction has been utilised (ref. 11).



17 R=H

18 R=OH



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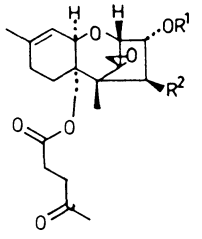
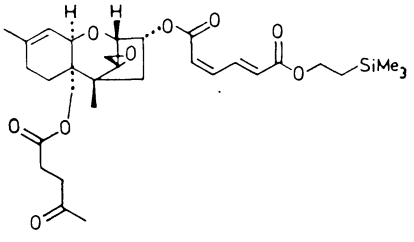


20 R^1=THP; R^2=OH

21 R^1=R^2=H



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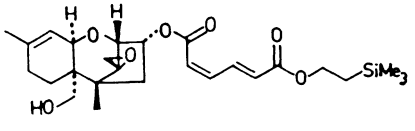

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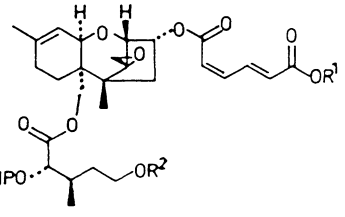


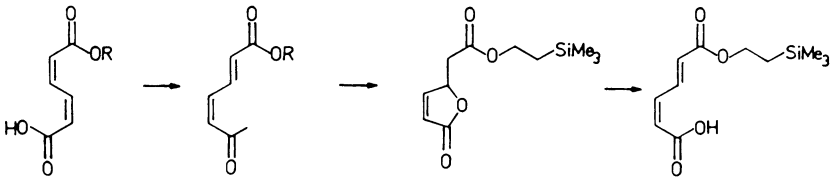
24 R^1=$C_2H_4SiMe_3$; R^2=TBDMS

25 R^1=R^2=H

For the synthesis of 19, the deoxygenation reaction of the derivative 20 of anguidine (2) leads to 21 after removal of the THP-group. The latter was reacted with the trans-protected (E,Z)-muconic acid to yield 22. Deprotection of the 15-hydroxy group to 23 was achieved with sodium borohydride. It is planned to transform 23 by the condensation with a suitable verrucarinic acid (ref. 10) and deprotection via 24 into 25. By final macrocyclization the desired triester 19 should be obtained.

3 SYNTHESIS OF CHIRAL BUILDING BLOCKS

The suitable functionalized derivatives of monoprotected (E,Z)-muconic acid and verrucarinic acid, which were needed as building blocks for the macrocyclic moiety, were synthesized in the following manner. Treatment of catechol with O_2/CuCl in the presence of methylmercaptoethanol gave the (Z,Z)-half ester 27, which on heating with water isomerized to the (E,Z)-half ester 29. Ionisation shifts in the ^{1}H-NMR-spectra were used for the determination

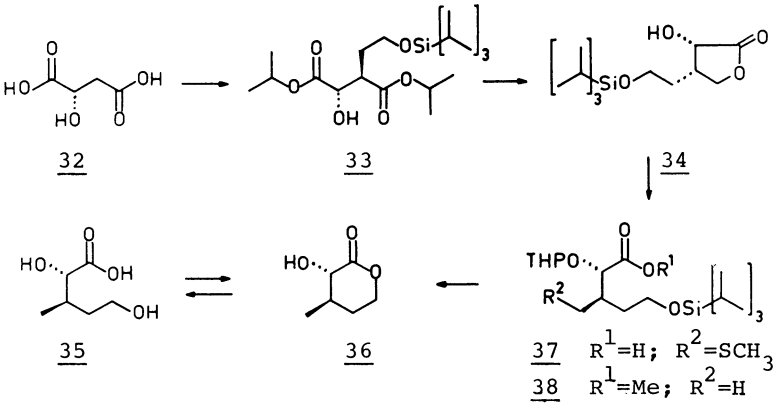


26 R=H 28 R=H 30 31

27 R=CH_2CH_2SMe 29 R=CH_2CH_2SMe

of the esterification site in monoesters of muconiç acids (ref. 6, 10). The site of this isomerization is in accord with a mechanism involving anchimeric assistance of the free carboxy group. A second trans-protected half ester 31 was obtained from (Z,Z)-muconic acid (26) by condensation with β-trimethylsilylethanol via the lactone 30.

Five approaches have been developed for the synthesis of optically active verrucarinic acid ((2S,3R)-2,5-dihydroxy-3-methylpentanoic acid) (35) which is suitably protected for the subsequent condensation with the trichothecene derivatives. The first route 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) for introducing the chirality (ref. 12). These two very recent syntheses are discussed in more detail (ref. 13). 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 . (S)-Malic acid (32) was esterified with 2-propanol and then alkylated with 1-iodo-2-(triisopropylsilyloxy)ethane. The electrophile entered the molecule with 90%



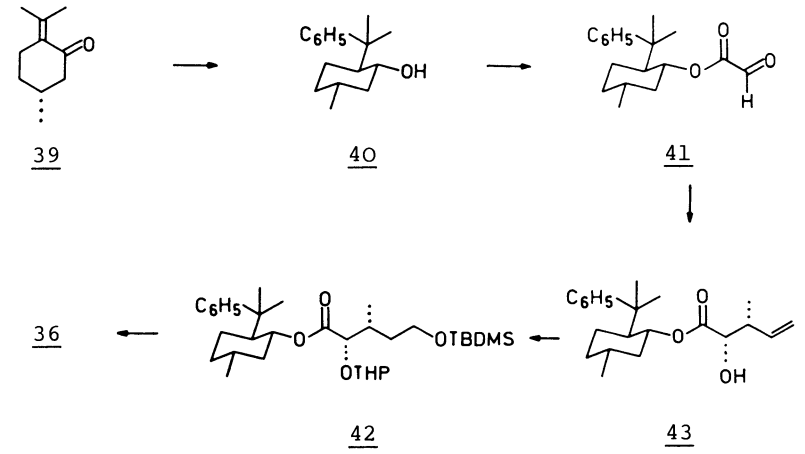
32 33 34

35 36 37 R^1=H; R^2=SCH_3

38 R^1=Me; R^2=H

diastereoselectivity yielding predominantly the desired isomer 33. Subsequent careful alkaline hydrolysis and reduction of the resulting half ester led directly to the lactone 34. After protection and alkyl-O-fission with NaSMe in HMPH the carbocyclic acid 37 was obtained. After esterification the synthesis was completed by desulfuration with Raney-Ni yielding the protected verrucarinic acid methyl ester 38. It was converted to verrucarinolactone 36.

The fifth synthesis involved a stereoselective addition of an allylsilane to a chiral glyoxylate.



39 40 41 36 42 43

8-Phenylmenthol (40), which is accessible from (+)-(R)-pulegone (39) served as the chiral directing group. It was transformed into the key intermediate 40 by esterification with bromoacetic acid followed by a Kornblum oxidation. When 41 was treated with [(E)-2-butenyl]trimethylsilane and $BF_3 \cdot Et_2O$ as catalyst (with $TiCl_4$ as catalyst a complex mixture was obtained), the desired erythro-alcohol 42 was the predominant product. It was easily converted to verrucarinolactone (35).

The studies on the synthesis of verrucarinic acid have prompted us to explore the possibility of diastereoselective hydroxylation of chiral ester enolates with $MoO_5 \cdot Py \cdot HMPT$ (ref. 14). Esters of 3-phenylpropionic acid served as substrates (ref. 15). They were prepared from chiral alcohols (R*-OH) derived from (+)-camphor. The deprotonation was performed using LICA and LICA/HMPT complex as base in THF respectively, because under these conditions both the (Z)- and (E)-isomers of the enolates are accessible. Depending on the nature of the chiral alcohol, high diastereoselection was observed either via the (Z)- or (E)-enolates.

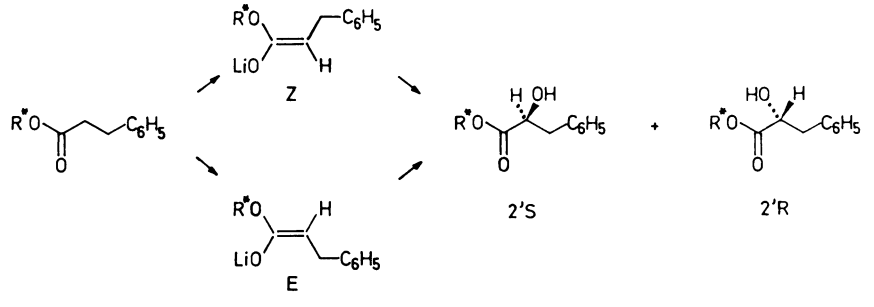


Fig. 1

Best results were obtained with the alcohols 44 and 45 respectively.

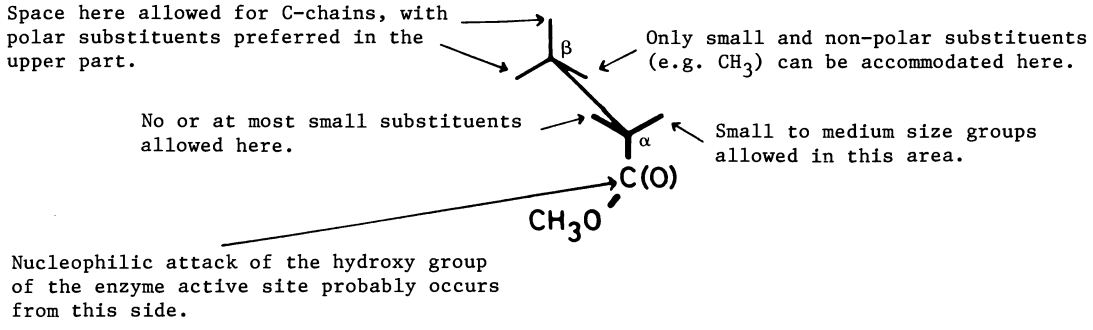
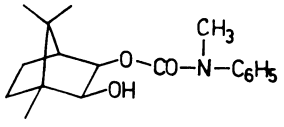


Fig. 2

As mentioned earlier, the chirality in one of our verrucarinic acid syntheses was introduced by the stereoselective hydrolysis of a symmetrical diester with pig liver esterase (PLE). By the use of enzymes which possess low substrate selectivity but which at the same time catalyse reactions with a high degree of stereoselectivity, a large number of new chiral synthons for the construction of optically active natural products can be produced (ref. 16). 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. 17, 18). 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 (cf. Fig. 2):(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 acylic 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 of 3-methylglutaric acid 46, which was obtained from 3-methylglutarate with PLE, was used for the synthesis of the chiral bromoester 51, a synthon for the construction of the macrocyclic moieties of some cytochalasins such as the cytochalasins B (phomin) (52) and D (53) (ref. 19). (S)-glutamic acid (47) served for the introduction of the second centre of chirality. 46 was transformed to the C_5-bromide 48, and 47 to the C_5-epoxide 49. Coupling of 48 and 49 gave decanol 50, which was converted to the synthon 51.



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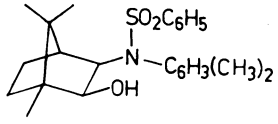

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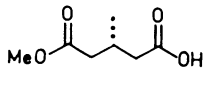

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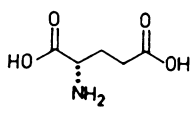

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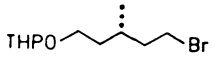

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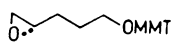

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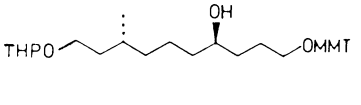

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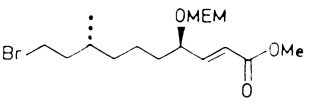

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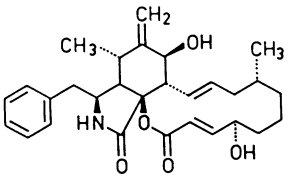

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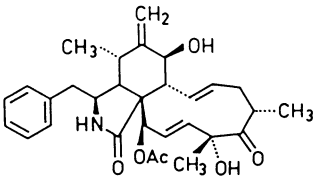

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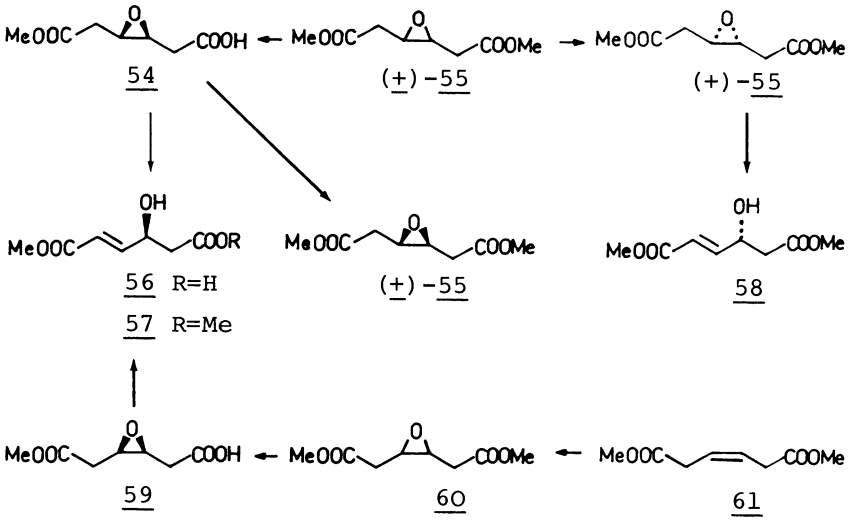
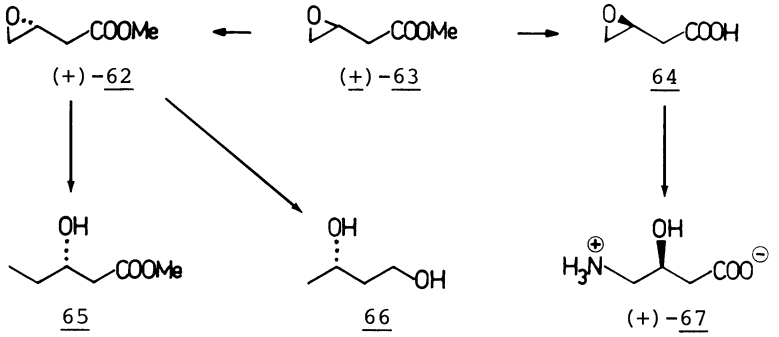
In view of a synthesis of optically active nonactic acid, the hydrolysis of dimethyl 3-hydroxyglutarate with PLE was reinvestigated(ref. 6). As anticipated from our enzyme model, the diester was found to be a bad substrate. The e.e. was only 22% (S-configuration). With chymotrypsin an e.e. of 68% (R-configuration) was observed. The e.e. was determined by HPLC-analysis of diastereoisomeric camphanoic acid derivatives. Microbial esterases are superior.

Interesting results were obtained with the dimethyl 3,4-epoxyadipates (ref.6). Their kinetic resolution by PLE provides an access to chiral α-hydroxyesters and acids.



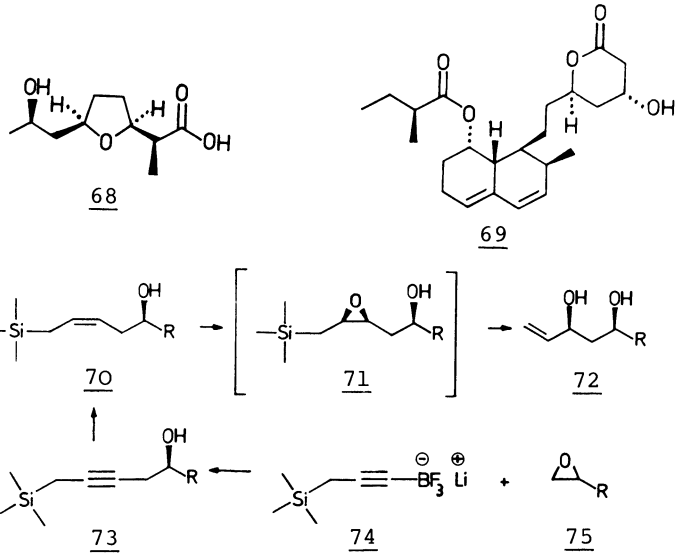
Treatment of the racemic diester 55 which has a C_2-axis, with PLE led to the (+)-diester 55 and the monoester 54 in high optical yields. Esterification of 54 led to the (-)-diester 55. 54 was converted to the unsaturated acid 56, a C_6-building block with four different functionalities by base catalysed elimination. Similarly (+)-55 gave the unsaturated β-hydroxydiester 58. On the other hand, the meso-epoxydiester 60, which was prepared from the (Z)-olefin 56, was rapidly hydrolysed by PLE with almost 100% selectivity to give the optically pure half ester 59. The latter was converted to 57. Thus, the enzyme cleaves the same ester group as in 3-hydroxyglutarate, but with much higher selectivity in the case of the epoxide 60.

According to our enzyme model methyl (+)-3,4-epoxybutanoates would be a good substrate for PLE as well (ref. 6). The enzymic hydrolysis yielded the (+)-ester 62 and the acid 64. The latter afforded (+)-γ-amino-2-hydroxy-butyric acid (67), the enantiomer of the hypotensive agent GABOB (e.e. 97%) upon treatment with ammonia. (+)-62 was transformed to the β-hydroxy ester 65 and the 1,3-diol 66.



(+)-62 (±)-63 64 65 66 (+)-67

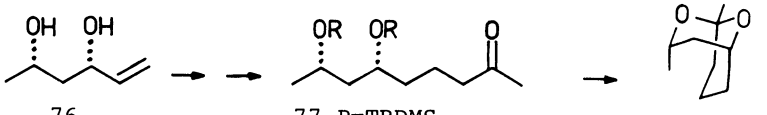
Since many natural products contain a 1,3-diol substructure (e.g. nonactic acid (68) or compactin (69), the stereoselective synthesis of functionalized 1,3-diols was studied (ref. 6). It was found that V^{5+}-catalyzed t-butylhydroperoxide epoxidation of (Z)-5-hydroxy-2-alkenylsilanes 70 exhibit excellent erythro selectivity. The resulting epoxides 71 undergo fragmentation to afford 1,3-diols of type 72. The allylsilanes are prepared from the terminal



68 69 70 71 72 73 74 75

epoxide 75. Reaction of the latter with the Li-acetylid $BF_3 \cdot Et_2O$ complex 74 yielded the intermediate 73. It is interesting to note that vinylsilanes are unreactive towards V^5-catalysed t-butylhydroperoxide reaction.

The new methodology for the construction of erythro-1,3-diols was used for a short stereoselective synthesis of endo-1,3-dimethyl-2,9-dioxabicyclo [3.3.1]nonane (79) (ref. 6). The compound was isolated from Norway spruce which is attacked by the ambrosia beetle (Trypodendron lineatum Oliv.). The diol 76 was converted to the protected ketone 77, which after deprotection to 78 underwent cyclization to the desired bicyclic ketal 79.



76 77 R=TBDMS 78 R=H 79

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