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An efficient enzymatic preparation of (+)- and (−)-conduritol E, a cyclitol with C_2 symmetry

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Abstract: Lypozyme® IM (immobilised lipase from *Mucor miehei*) catalyses the enantiomeric alcoholysis of tetraacetylconduritol E (±)-**2** to give enantiopure (1*R*,2*R*,3*R*,4*R*)-tetrahydroxycyclohex-5-ene, (−)-**1**, and the unreacted ester (1*S*,2*S*,3*S*,4*S*)-tetraacetyloxycyclohex-5-ene, (+)-**2**. The latter was transformed by basic hydrolysis into (+)-**1** in high yield and 95% ee. Selective amination of partial ester (−)-**3**, obtained by short alcoholysis of (±)-**2**, furnished the previously unreported conduramine F-4, (−)-**4**.
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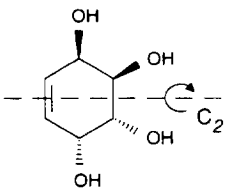
Introduction

Conduritols, 5-cyclohexen-1,2,3,4-tetrols, are a class of polyols valuable as starting material for the synthesis of biologically active compounds.[1] They have been used in the preparation of epoxyconduritols possessing glucosidase-inhibition activity and inositols acting as cell mediators,[2] as well as for the synthesis of aminoconduritols that are moieties in bioactive compounds of complex structure, for instance lycoricidine,[3] and pancratistatin.[4]

The ten possible stereoisomers, two *meso*-forms (conduritols A and D) and four couples of enantiomers (conduritols B, C, E and F), are obtained with difficulty in stereochemically pure form, due to the presence of four stereogenic centres in the cyclohexene system.



(±)-**1**

As part of a programme directed to the synthesis of cyclitols,[5] we considered the preparation of enantiopure conduritols E, for which only few, multi-step stereocontrolled syntheses are available,[2c,6] all but one giving unsatisfactory yields. Although no enzymatic resolution of racemic conduritols has been reported, we decided to try the biocatalysed resolution of (±)-**1**, since reactions promoted by lipases have been effectively utilised to transform *meso*-conduritols or their derivatives into optically active compounds. For instance, 1,4-dibutyryl conduritol A was converted into the corresponding 2,3-ketal, which by enantiotoposelective hydrolysis in the presence of a recombinant *Fusarium solani pisi* cutinase gave an optically active hydroxyester.[6b] From the same ketal the enantiomer was obtained exploiting the opposite reaction, esterification in organic medium with lipase from *Pseudomonas cepacia* as catalyst.[7] Recently in our laboratory the tetraacetate of conduritol D has been desymmetrised by alcoholysis in the presence of lipases.[5b] With these results in mind we envisaged

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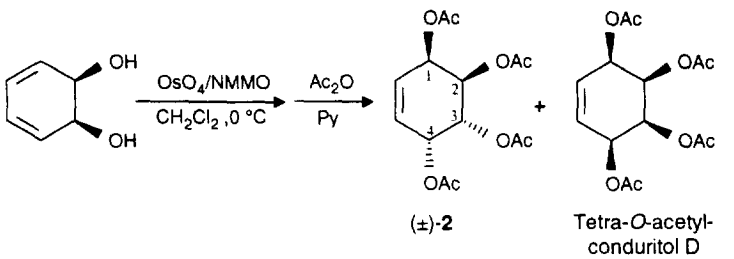
that the biocatalysed resolution of conduritol E, particularly intriguing in view of the presence of four stereogenic centres and the C_2 symmetry of the substrate, could be carried out successfully.

Here we report the results concerning the biocatalysed resolution of (±)-**1**, together with the synthesis of the previously unreported (−)-conduramine F-4.

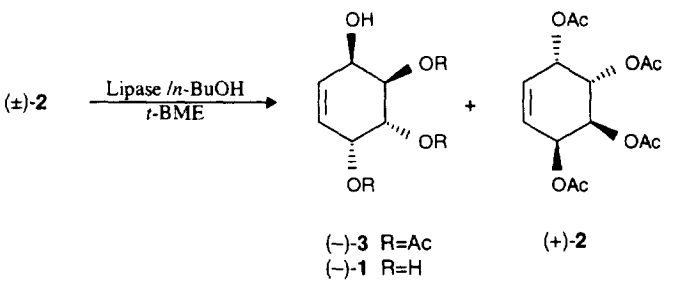
Results and discussion

Due to the sparing solubility of (±)-**1** in common organic solvents, we decided to subject its tetraacetate (±)-**2** to alcoholysis catalysed by an appropriate enzyme. The desired substrate was prepared in 63% yield, according to the Carless' procedure,[8] from 1,2-dihydroxycyclohexa-3,5-diene by dihydroxylation with OsO_4 in the presence of *N*-methylmorpholine oxide followed by conventional acetylation. In addition, minor amounts of tetra-*O*-acetylconduritol D were also isolated (Scheme 1).



Scheme 1.

Ester (±)-**2** was subjected to alcoholysis with *n*-butanol in *tert*-butylmethyl ether for 48 h, using as catalyst three different enzymes previously found to catalyse efficiently the asymmetrisation of conduritol D with high enantioselectivity and *R*-stereopreference,[5b] namely lipase from porcine pancreas (PPL), lipase from *Candida cylindracea* (CCL) and lipase from *Mucor miehei* (Lipozyme® IM). In the conditions adopted, CCL gave poor conversion (5%) and PPL was completely inactive. Conversely, the reaction rate was high in the presence of Lipozyme® IM and after 5 h conversion reached 22%. GC analysis of the reaction mixture showed the presence of unreacted ester (+)-**2** and a single product, (−)-**3**, whose ^{1}H NMR spectrum contained three resonances for acetyl groups. The presence of a dd-signal at δ 4.53 correlated with one of the olefinic protons and assignable to a hydroxymethine proton indicated the allylic nature of the free OH. On the basis of this and the result of its chemical hydrolysis, which afforded (−)-conduritol E, (−)-**1**, compound (−)-**3** was assigned the structure of (1*R*)-hydroxy-(2*R*,3*R*,4*R*)-triacetoxycyclohex-5-ene (Scheme 2). Its enantiomeric purity, determined by GC on chiral column after conventional acetylation, was consistent with an *E* value >100 for the alcoholysis.



Scheme 2.

When the alcoholysis of (±)-**2** was protracted the concentration of (−)-**3** did not increase substantially and at the same time products of further deacylation began to form. After 48 h incubation,

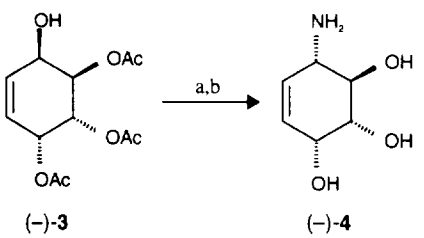
conversion reached ca. 50% and column chromatography of the reaction mixture gave unreacted tetraester (+)-**2** (48% yield, ee >95% determined by GC on chiral column), whose chemical hydrolysis furnished (+)-conduritol E, (+)-**1**.

A more polar chromatographic fraction contained a complex mixture of partially acylated compounds that was not investigated further, but was hydrolysed (dil. NH_4OH) to afford (−)-**1** in 45% yield (ee >95%).

Further prolonging of the incubation time toward completeness of the alcoholysis and direct production of (−)-**1** is not advisable since the product tends to precipitate on the catalyst.

The high regioselectivity observed in the first alcoholysis step of (1*R*,2*R*,3*R*,4*R*)-tetraacetoxycyclohex-5-ene (−)-**2** can find an explanation in the less steric hindrance to the lipase action experienced by the allylic acetoxyls in comparison with those located at C-2 and C-3. However, the first reaction product, (−)-**3**, can undergo further alcoholysis either at positions C-4, due to the C_2 symmetry of the molecule, or at position C-2, now accessible owing to the reduced hindrance. The diols so formed still possess ester functions with the correct *R*-stereochemistry for recognition by Lipozyme® IM and consequently suffer alcoholysis until the formation of the final product, (−)-**1**.

Ester (−)-**3**, due to the presence of a free hydroxyl, is suitable for the synthesis of cyclitols with controlled chirality. Amination of (−)-**3** under the Mitsunobu conditions[9] yielded the corresponding phthalimido-derivative, that by treatment with aqueous CH_3NH_2 gave in high yield an aminoalcohol, whose ^1H NMR spectrum showed a resonance at δ 3.44, assigned by heteronuclear correlation to the aminomethine proton. Coupling of this proton with those located on the olefinic system confirmed the amination at C-1 and the formation of the desired conduramine F-4, (−)-**4**. (Scheme 3).



(−)-**3** (−)-**4**

a) DEAD, Phthalimide, Ph_3P
b) aq. CH_3NH_2

Scheme 3.

Conclusion

The results obtained in the present work evidence the potentiality of lipase in the enantiomeric separation of chiral substrates of complex chirality. Resolution of (±)-conduritol E has been successfully carried out via alcoholysis catalysed by lipase from *Mucor miehei* obtaining both enantiomers with high chemical and optical yields. Taking advantage of the faster alcoholysis rate of ester group located at the allylic position it is possible to obtain in reasonable yield partial ester (−)-**3**, suitable for transformation into the unreported conduramine F-4, (−)-**4**.

Experimental section

General

Lipases from *Candida cylindracea* and porcine pancreas were obtained from Sigma; Lipozyme® IM (immobilised lipase from *Mucor miehei*) is a registered mark from Novo Nordisk. Optical rotations were measured with a DIP 135 JASCO polarimeter. Preparative chromatography was performed on silica-gel 60-F_{254} (Merck), Lichropep Si Diol 40–63 μm (Merck) or Dowex 50 W X 8 resin (Fluka). ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ at 250.13 and 62.9 MHz on a Bruker AC-250

spectrometer with tetramethylsilane as internal standard. Chemical shifts are reported in ppm (δ) and coupling constants (*J*) are given in Hz. 2D NMR (COSY and C–H correlation) experiments were performed using standard Bruker microprograms. GC analysis were performed on a HP-5, 5% phenylmethylsilicone or Chiraldex G–DA (dialkyl γ-cyclodextrin) capillary columns, N_2 as carrier gas, flame ionization detector.

Oxidation of cis-*1,2-dihydroxycyclohexa-3,5-diene*

N-Methylmorpholine-*N*-oxide (550 mg, 4.70 mmol) and OsO_4 (12 mg, 0.05 mmol) were added to a solution of *cis*-1,2-dihydroxycyclohexa-3,5-diene (500 mg, 4.46 mmol) in CH_2Cl_2 (50 mL). The resulting mixture was stirred at 4°C for 24 h, then quenched by evaporation of the solvent. The residue was subjected to standard acetylation (Ac_2O in pyridine) followed by column chromatography (acetone/CH_2Cl_2 1:9 vol/vol as the eluent) affording, after crystallization from ethyl acetate/hexane, 380 mg of 1,2,3,4-tetra-*O*-acetylconduritol D (27% yield) and 880 mg of (±)-1,2,3,4-tetra-*O*-acetylconduritol E (63% yield) (±)-**2**.

General procedure for small-scale enzymatic alcoholysis of (±)-2

Enzyme (20 mg/mL) and *n*-BuOH (23 μL/mL, 2 equiv) were added to a solution of (±)-**2** (10 mg/mL) in *tert*-butylmethyl ether (*t*-BME). The suspension was stirred at 300 rpm and 45°C and the progress of the reaction monitored by GC analysis of aliquots, injected after acylation with propionic anhydride/pyridine. The reaction was stopped by filtering off the enzyme and the solution was taken to dryness. The residue was subjected to chromatography on Si Diol eluting with ethyl acetate/hexane (4:6 vol/vol). Enantiomeric excesses (ee) of (+)-**2** were determined by GC analysis. The stereochemistry of (+)-**2** was assigned by chemical correlation with (+)-conduritol E.

Preparative enzymatic alcoholysis of (±)-1,2,3,4-tetracetyloxy-cyclohex-5-ene, (±)-2

Lipozyme® IM (500 mg) and *n*-BuOH (0.44 mL, 4.83 mmol) were added to a solution of (±)-**2** (250 mg, 0.79 mmol) in *t*-BME (25 mL). The reaction mixture was stirred at 45°C and 300 rpm for 5 h (conv. 22%), the suspension was filtered and the filtrate taken to dryness. The residue was chromatographed on Si Diol (ethyl acetate/hexane 4:6 vol/vol) to give (1*R*)-hydroxy-(2*R*,3*R*,4*R*)-triacetyloxycyclohex-5-ene, (−)-**3** (43 mg, 20% yield), $[\alpha]_D$ −200 (*c* 0.8, $CHCl_3$). ^{1}H NMR: δ 2.02 (3H, s), 2.06 (3H, s), 2.13 (3H, s), 4.53 (1H, dd, *J*=4.2 and 4.0), 5.33 (1H, dd, *J*=9.8 and 4.0), 5.47 (1H, dd, *J*=9.8 and 4.0), 5.62 (1H, dd, *J*=4.5 and 4.0), 5.81 (1H, dd, *J*=9.9 and 4.5), 6.00 (1H, *J*=9.9 and 4.2), ^{13}C NMR: δ 20.67, 20.82, 20.87, 65.11, 66.49, 66.69, 69.30, 126.15, 131.33, 169.97, 170.20, 170.26. Conventional acetylation of (−)-**3** afforded (−)-**2**, ee >95%, $[\alpha]_D$ −245 (*c* 0.8, $CHCl_3$).

In an analogous experiment the incubation time was protracted to a total of 48 h. The reaction mixture was chromatographed on Si Diol (ethyl acetate/hexane 2:3 vol/vol) to give (+)-**2** (240 mg, 48% yield); ee >95% $[\alpha]_D$ +244 (*c* 1.6, $CHCl_3$). This ester by treatment with dil. NH_4OH gave (+)-conduritol E (+)-**1** (115 mg, overall yield 49%). The more polar fractions, containing partially acylated compounds, were pooled and taken to dryness. The residue was hydrolysed as above to give (−)-conduritol E (−)-**1** (110 mg, 47% yield); ee >95%, $[\alpha]_D$ −280 (*c* 0.3, H_2O), (lit.[6b] $[\alpha]_D$ −294 (*c* 1, H_2O)).

Preparation of (−)-(1S,2R,3R,4R)-1-amino-2,3,4-trihydroxy-cyclohex-5-ene (Conduramine F-4), (−)-4

A toluene solution (4 mL) containing triphenylphosphine (115 mg, 0.44 mmol), phthalimide (65 mg, 0.44 mmol) and diethyl azodicarboxylate (0.070 mL, 0.44 mmol) was added to a toluene (4 mL) solution of (−)-**3** (100 mg, 0.37 mmol). The reaction mixture was shaken at rt and monitored by TLC analysis. After 3 h the suspension was filtered and methylamine (40% aqueous solution, 4 mL) was added to the filtrate. Stirring at rt was maintained for 15 min. The aqueous layer was then separated and after elimination of methylamine by evaporation under reduced pressure applied to a Dowex-50 W (H^+ form) column. The product (47 mg, yield 88%) was recovered by elution with 2N NH_4OH:

$[\alpha]_D$ −35 (*c* 0.3, CH_3OH), ^1H NMR (D_2O): δ 3.44 (1H, m), 3.60 (2H, m), 4.26 (1H, dd, *J*=3.2 and 4.9), 5.71 (1H, dd, *J*=9.9 and 1.9), 5.91 (1H, ddd, J=9.9, 5.0 and 2.4); ^{13}C NMR (D_2O): δ 55.33, 67.61, 72.29, 72.42, 129.62, 130.39.

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