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Biocatalytic Preparation of (R)-(-)-1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene, a Synthon for Potent Agricultural Pyrethroids[1]

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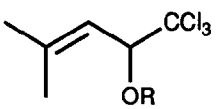
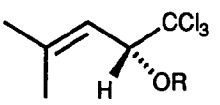
Abstract: Asymmetric hydrolysis of acetate (±)-**2** mediated by *B.subtilis* provided a very simple and cheap method for obtaining alcohol (R)-(-)-**1a** in high chemical yield and optical purity (ee ≥ 98%).

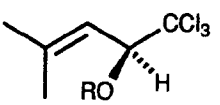
In the context of our program[2] on synthetic pyrethroids[3], we required optically active (R)-(-)-1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene **1a**[4], the starting material for the syntheses of NRDC 182 (A) and the optically active form of commercial cypermethrin (B), both potent agricultural pyrethroids. While the racemic alcohol **1** is readily available by a modification of the reported procedure[4], its chemical resolution is particularly difficult, due to the low reactivity of the alcohol functionality.



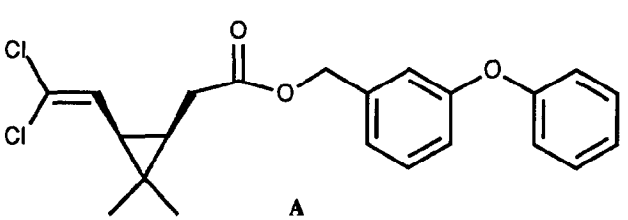
(±)-**1**, R = H
(±)-**2**, R = $COCH_3$



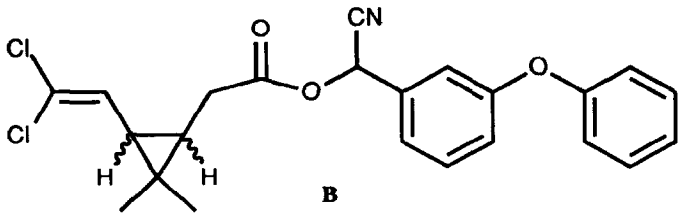
(R)-(-)-**1a**, R = H
(R)-(+)-**2a**, R = $COCH_3$



(S)-(+)-**1b**, R = H
(S)-(-)-**2b**, R = $COCH_3$



A



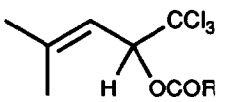
B

We therefore resorted to a biocatalytic[5] resolution procedure. On subjecting racemic esters **2-8**[6] to whole cell biocatalysis we found (Table 1) that the organism *Bacillus subtilis* 2010 from NCIM, effected the enantiospecific hydrolysis of acetate (±)-**2**[7] to furnish the desired alcohol (R)-(-)-**1a** (mp 109°C)[8] in high chemical yield (40%, theoretical 50%) and optical purity (ee ≥ 98%). Esters **3-6** were also hydrolysed, although at reduced rates, with a marked preference for the R isomer. As may be expected, the formate ester **8** was hydrolysed very rapidly, however, the nitrobenzoate **7**, the only solid compound in the series, was not hydrolysed at all. The experiments were carried out by incubating the substrate (0.5% v/v) in 100 ml of a 24 hr culture broth of *B.subtilis* on a shaker at 30° for 48 hr. Ether extraction followed by chromatography separated the alcohol **1a** and unhydrolysed ester.

For a preparative method the acetate (±)-**2** was hydrolysed in 3 batches of 1.5 ml each under the conditions described (incubation time 24 hr). After extraction with ether, the material was combined and crystallised directly (petroleum ether) avoiding chromatography, to yield **1a** (1.7 g, 38%), with ee ≥ 98% (Table I, footnote d,e). Chromatography of the residue gave the unhydrolysed acetate (S)-(-)-**2b** in good chemical yield (2.18 g, 40%). The optical purity of this material was enhanced by resubjecting it to the action of *B.subtilis*. Acetate **2b** so obtained $[\alpha]_D^{23}$ -0.82 (c 7.5, $CHCl_3$), ee ≥ 98% (by nmr, Table I, footnote d,e) was used for the studies described below. Chemical hydrolysis (KOH, EtOH, 90%) provided the antipodal alcohol (S)-(+)-**1b** $[\alpha]_D^{25}$ +12.0 (c 2, $CHCl_3$), mp 109°C.

The high degree of enantiospecificity observed in the above work was confirmed by subjecting the enantiomers (R)-(+)-**2a** and (S)-(-)-**2b** separately to the action of *B.subtilis* and monitoring the rates of hydrolysis (Table II). After 2 hr the R-isomer was almost completely hydrolysed, while the S-isomer showed negligible conversion even with extended reaction times.

With a facile method in hand for preparing the desired **1a**, we sought to improve the overall efficiency of the process. It was observed that good hydrolytic conversions (~38%) could be obtained in experiments with up to 1% acetate concentration beyond which the conversions decreased drastically. Several strategies were successfully tried to overcome this limitation. Some of the promising results, with scope for further improvement are recorded in Table III. Working with a substrate concentration of 2% (4 times the original concentration), satisfactory results were realised by the incorporation of surfactant TX-100 (entry 1) and by the use of increased biomass, (cell : substrate ratio 1:1), (entry 2). With optimisation, the formate ester (entry 3) can be used to advantage allowing increased substrate concentration along with considerable reduction in reaction time.



2-8

Table I : Asymmetric hydrolysis of racemates 2-8 to produce 1a

Substrate, R		Conversion[a],% GC	$[\alpha]_D^{25}$ b,c	%, ee
2	gCH_3-	42	-12.0	98[d,e]
3	nC_2H_5-	40	-12.0	98[f]
4	nC_4H_9-	30	-11.6	96.6[f]
5	nC_7H_{15}-	12	-11.5	95.8[f]
6	C_6H_5-	15	-11.9	99[f]
7	pNO_2-C_6H_4-	0	---	---
8	H-	100	---	---

[a] Isolated yields are of the same order. [b] (c 2, $CHCl_3$), [c] Lit.[4] $[\alpha]_D$ 12.1 (c 1, $CHCl_3$). [d] Determined by 300 MHz ^1H nmr of the acetate using $Eu(tfc)_3$ [e] The value indicates that no trace of the other enantiomer was detected by nmr; an artificially prepared mixture of R and S enantiomers (95:5) under the conditions shows well separated peaks with a clear base line for the methyl ester group of the two enantiomers. [f] Determined by comparison with $[\alpha]_D$ 12.0;[g] Incubation time 24 hrs.

Table II : Hydrolysis[a] of (R)-(+)-2a[b] and (S)-(-)-2b

Time (mins)	Conversion %	
	(R)-(+)-**2a**	(S)-(-)-**2b**
40	13.3	0
40	25.2	0
80	38	0
120	97	0.2
240	100	0.6

[a] Experimental conditions : 0.1 ml of substrate was added to cells from a 50 ml culture broth of *B.subtilis*, suspended in tris-HCl buffer (50 ml, pH 8.0) and incubated at 30°. Flasks were removed at intervals and the ether extract monitored by GC. [b] $[\alpha]_D^{23}$ +0.93 (c, 7.5, $CHCl_3$), ee ≥ 98% (Table I, footnote d,e)

Table III : Asymmetric Hydrolysis at 2% substrate concentration

Entry	Substrate	Time hr.	Reaction conditions	Conversion%	$[\alpha]_D^{25d}$	ee,%[e]
1	(±)-**2**	48	a	45	-11.8	97.5
2	(±)-**2**	48	b	40	-11.2	92.5
3	(±)-**8**	6	c	33	-11.0	90.0

[a] Substrate dispersed in 10 ml buffer (Tris HCl, pH 8.0) along with 0.1 ml Triton X-100 added to the reaction medium. [b] Cells harvested from one culture flask were resuspended in another culture broth to obtain medium with increased biomass. [c] Normal [d] (c 2, $CHCl_3$) [e] Calculated by comparison with $[\alpha]_D^{25}$ -12.0.

In conclusion, the biocatalytic method described here is distinctly superior to the reported chemical resolution, in terms of cost, experimental ease and consequently for scale-up work.

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References and notes:

1.a) Presented in part at the 17th IUPAC International Symposium on Chemistry of Natural Products, New Delhi, India, Feb 4-9, 1990.

b) Z. Muljiani, S.R. Gadre, S. Modak and R.B. Mitra, Indian Patent Appl. No. 651/DEL/87.

2. Z. Muljiani, A.R.A.S. Deshmukh, S.R. Gadre and V.S. Joshi, *Synth. Commun.* 1987, **17**, 25 and references therein.

3. M. Elliot, Synthetic Pyrethroids, ACS Symposium Series 42, ACS, Washington, D.C. 1977.

4. C.E. Hatch III, J.S. Baum, T. Takashima and K. Kondo, *J. Org. Chem.*, 1980, **45**, 3281.

5. For reviews on biocatalysis in organic synthesis see

a) J.B. Jones, *Tetrahedron*, 1986, **42**, 3351.

b) S. Butt and S.M. Roberts, *Natural Product Reports*, 1986, 489.

6. Prepared from **1**: **2**, acetic anhydride pyridine, 92%; **3-7**, corresponding acid chloride, triethyamine in CH_2Cl_2; **8**, acetic formic anhydride.

7. Preliminary experiments with PPL (Sigma) were not encouraging. Whole cell hydrolysis with *Pseudomonas aeruginosa* and *P. lemoneri* gave 25-30% conversion.

8. The reported mp 79-81°C is incorrect. We found alcohol (±)-**1** exists as a conglomerate, the racemic compound exhibiting minimum melting point 79-80°C.