Enzymes in organic synthesis. 44.[1] Stereoselective pig liver esterase-catalyzed hydrolyses of 3-substituted glutarate diesters of compactin-synthon interest[2]

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Pig liver esterase-catalyzed hydrolyses of dimethyl 3-hydroxy-, 3-methoxyethoxymethoxy-, and 3-benzyloxyglutarates occur with enantiotopic stereoselectivity to give the corresponding acid-ester products of 16–40% enantiomeric excess (ee). The results also provide another example of reversal of PLE-stereoselectivity.

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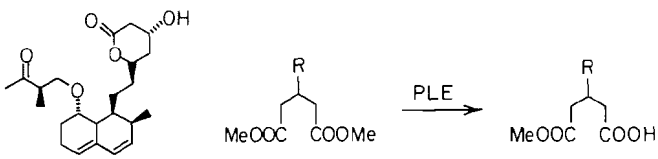
Les hydrolyses, catalysées par l'estérase du foie de porc, des hydroxy-3, méthoxyéthoxyméthoxy-3 et benzyloxy-3 glutarates de diméthyle se produisent avec une stéréosélectivité énantiotopique et elles fournissent les produits acide-esters correspondants avec un excès énantiomérique (eé) de 16–40%. Les résultats fournissent aussi un autre exemple de renversement de la stéréosélectivité de l'EFP.

[Traduit par la revue]

Compactin (**1**) and related compounds are potent inhibitors of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosyntheses (2*a*), and are consequently able to reduce plasma cholesterol levels in animals, including humans (2*b*). This property, combined with the very low toxicity (2), has elicited great interest in compactin and its analogues as hypocholesteremic drugs. As a result, the synthesis of compactins remains a very active field (3).

Chiral acid-esters such as **3***a* are potentially useful synthons for the therapeutically vital (4) lactone moiety of compactin. Our previous work on pig liver esterase (PLE)-catalyzed hydrolyses of 3-substituted glutarate diesters **2** had shown that enantiotopically selective hydrolyses could be effected to give acid-esters **3** of up to 97% enantiomeric excess (ee) (5). This encouraged us to evaluate the potential of PLE-catalyzed hydrolyses of the 3-oxygenated glutarate diesters **2***b–d* for the production of the potential compactin-lactone synthons **3***b–d*.



1 **2** **3**

a, R = Me
b, R = OH
c, R = OMEM
d, R = $OCH_2C_6H_5$

Results

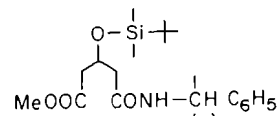
Diesters **2***b–d* were prepared in good yields by unexceptional methods. Each proved to be a reasonable substrate for PLE, having rates of hydrolyses (relative to that of the reference standard ethyl butyrate = 100) of 17 for **2***b*, 2 for **2***c*, and 4 for **2***d*. The preparative-scale hydrolyses of **2***b–d* proceeded smoothly to give high yields of the corresponding acid-esters **3***b–d*. The results are summarized in Table 1.

The absolute configurations of **3***b–d* were established by their correlations with (−)-(*R*)- and (+)-(*S*)-**3***b* of known (6) stereochemistry. The ee's of Table 1 were determined on the acid-esters **3***b* derived from **3***b–d* during the absolute configuration assignments, via their conversions to their (*S*)-1-phenethanamide derivatives **4** by the method of Heathcock and co-workers (7), followed by ^1H nmr examinations of the *tert*-butyl and methoxyl resonances.

TABLE 1. PLE-catalyzed hydrolyses of **2***b–d*[a]

Substrate	Product (% yield, % ee)
2*b*	(+)-(3*S*)-**3***b* (87, 16)
2*c*	(+)-(3*R*)-**3***c* (92, 39)
2*d*	(−)-(3*S*)-**3***d* (87, 40)

[a]At pH 7, 20°C.



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Discussion

The easily removable methoxyethoxymethyl (MEM) and benzyl functions were chosen as the hydroxyl-protecting groups for substrates **2***c,d* because of their stability during the acidic work-up involved in the enzyme-catalyzed reactions, and to avoid stereochemical complications introduced by the use of other common, but chiral, protecting groups. Substrates **2***b,c* were readily prepared by standard methods. However, due to the sensitivity of dimethyl 3-hydroxyglutarate (**2***b*) to base, benzylation attempts using sodium hydride – benzyl bromide conditions were unsuccessful in the preparation of **2***d*, and the acid-catalyzed procedure of Iversen and Bundle (8) was used instead. It was found that $BF_3 \cdot Et_2O$ was a more effective benzylation catalyst than the triflic acid used originally (8).

Although the relative rates of PLE-catalyzed hydrolyses of **2***b–d* were significantly slower than that of the ethyl butyrate

[1]For Part 43, see ref. 1.

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standard, each still remained a good candidate for preparative-scale reactions.[3]

The results of the preparative-scale experiments (Table 1) show that the ee levels are significantly increased by the conversion of the C3-OH group of **2***b* to the more bulky O-MEM and O-benzyl functions of **2***c* and **2***d* respectively. This ee increase on functionalization, and the reversal of stereoselectivity observed, parallel the observations recorded in the 3-amino- and 3-alkylglutarate series (5, 9). Rationalizing the many stereospecificity reversals within different series of substrates documented (5, 9, 10) for PLE now represents a formidable interpretative challenge.

There is interest (11, 12) in **3***b* as a chiral precursor of important target molecules other than compactin, with esterase-based methods complementing those involving reductions of β-ketoesters (13, 14).

While the current PLE-catalysis approach has not yet reached its goal of producing enantiomerically pure **3***b*,[4] further increases in ee levels should be attainable by control of reaction conditions (5, 15) and, by analogy with the 3-amino series (9), by different O-functionalization of **2***b*. Interestingly, when 3-methyl (16*a*) or 2,4-dimethyl (16*b*) substituents are present in **2***b* of 2,4-dimethyl (16*b*) substituents are present in **2***b*, PLE-catalyzed hydrolyses yield acid-esters of high ee's (16).

Experimental

The instrumentation and general purification and analytical methods used were as described previously (5). PLE (EC 3.1.1.1) was Sigma Chemical Company Type II (Lot 123F-0240). Unless noted otherwise, ir spectra were for films, and ^{1}H nmr and $[\alpha]_D^{25}$ measurements were taken on $CDCl_3$ and $CHCl_3$ solutions respectively.

Preparation of substrates

Dimethyl 3-hydroxyglutarate (**2***b*) was obtained in 80% yield by reduction (6) with $NaBH_4$ of dimethyl 3-ketoglutarate (Aldrich); **2***b* had bp 138–140°C (8 Torr; 1 Torr = 133.3 Pa); ir: 3430, 1740 cm^{-1}; ^{1}H nmr δ: 2.58 (4H, d, J = 6 Hz), 3.63 (1H, br s, OH), 3.75 (6H, s), and 4.48 (1H, quintet, J = 6.2 Hz) ppm.

*Dimethyl 3-methoxyethoxymethoxyglutarate (2*c*)*

Triethylamine (100 mL, 0.72 mol) was added dropwise with stirring at 20°C to a mixture of dimethyl 3-hydroxyglutarate (**2***b*, 5 g, 28.4 mmol) and methoxyethoxymethyl chloride (13.3 g, 107 mmol) at 25°C. The mixture was then refluxed for 1.5 days, filtered, and the solvent removed by rotoevaporation. The residual oil was taken up in diethyl ether (30 mL), and washed with saturated aqueous $NaHCO_3$ (10 mL) and then with brine (10 mL). The ether layer was dried ($MgSO_4$), filtered, and the solvent was removed by rotoevaporation. The crude oil was purified by flash chromatography (ether:hexanes (1:1) elution), followed by Kugelrohr distillation to afford dimethyl 3-methoxyethoxymethoxyglutarate (**2***c*, 6.9 g, 92% yield), bp 89–91°C (0.15 Torr); ir: 1736 cm^{-1}; ^{1}H nmr δ: 2.63 (4H, d, J = 6 Hz), 3.33 (3H, s), 3.37–3.77 (4H, m), 3.65 (6H, s), 4.38 (1H, quintet, J = 6 Hz), and 4.72 (2H, s) ppm. *Anal.* calcd. for $C_{11}H_{20}O_7$: C 49.99, H 7.63; found: C 49.97, H 7.61%.

*Dimethyl 3-benzyloxyglutarate (2*d*)*

Boron trifluoride – diethyl ether (catalytic amount, 0.05 mL) was added with stirring to a solution of dimethyl 3-hydroxyglutarate (**2***b*, 1.76 g, 10 mmol) and benzyl trichloroacetimidate (5.05 g, 20 mmol) in cyclohexane–CH_2Cl_2 (2:1; 100 mL). The reaction was kept for 3 h at 23°C prior to addition of more cyclohexane (100 mL). The organic layer was then filtered and washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and then with brine (50 mL). The dried ($MgSO_4$) solution was filtered, and the solvent was removed by rotoevaporation. The crude oil obtained was purified by flash chromatography (ethyl acetate:hexanes (1:10) elution) and then Kugelrohr-distilled to give dimethyl 3-benzyloxyglutarate (**2***d*, 1.87 g, 70% yield), bp 126–130°C (0.2 Torr); ir: 1739 cm^{-1}; ^{1}H nmr δ: 2.63 (4H, d, J = 6 Hz), 3.68 (6H, s), 4.32 (1H, quintet, J = 6 Hz), 4.58 (2H, s), and 7.32 (5H, s) ppm. *Anal.* calcd. for $C_{14}H_{18}O_5$: C 63.13, H 6.81; found: C 62.80, H 6.94%.

*PLE-catalyzed hydrolyses of 2*b–d

The relative rates of hydrolyses of **2***b–d* were determined as described previously (17).

The preparative-scale reactions were performed according to the standard procedure for symmetrical diester substrates (5). The results obtained (summarized in Table 1) were as follows:

Dimethyl 3-hydroxyglutarate (**2***b*, 1.0 g, 5.68 mmol) with PLE (400 units) for 80 min gave methyl hydrogen (3*S*)-3-hydroxyglutarate (**3***b*, 800 mg, 87% yield, 16% ee), bp 124–126°C (0.2 Torr); $[\alpha]_D^{25}$ +0.13° (*c* 15) (lit. (6) $[\alpha]_D^{22}$ −1.7° (*c* 12.5) for (3*R*)-**3***b*); ir: 3450–2610, 1734, 1715 cm^{-1}; ^{1}H nmr δ: 2.60 (4H, d, J = 6 Hz), 3.73 (3H, s), 4.45 (1H, quintet, J = 6 Hz), and 7.17 (2H, br s) ppm. *Anal.* calcd. for $C_6H_{10}O_5$: C 44.45, H 6.22; found: C 44.33, H 6.07%.

Dimethyl 3-methoxyethoxymethoxyglutarate (**2***c*, 750 mg, 2.84 mmol) with PLE (200 units) for 11 h yielded methyl hydrogen (3*R*)-3-methoxyethoxymethoxyglutarate (**3***c*, 655 mg, 92% yield, 39% ee), bp 140–145°C (0.25 Torr); $[\alpha]_D^{25}$ +1.0° (*c* 25); ir: 3430–2610, 1736, 1714 cm^{-1}; ^{1}H nmr δ: 2.68 (4H, d, J = 6 Hz), 3.37 (3H, s), 3.40–3.80 (4H, m), 3.68 (3H, s), 4.37 (1H, quintet, J = 6 Hz), 4.77 (2H, s), and 8.99 (1H, br s) ppm. *Anal.* calcd. for $C_{10}H_{18}O_7$: C 48.00, H 7.25; found: C 47.39, H 7.16%.

Dimethyl 3-benzyloxyglutarate (**2***d*, 500 mg, 1.88 mmol) with PLE (132 units) for 12 h afforded methyl hydrogen (3*S*)-3-benzyloxyglutarate (**3***d*, 410 mg, 87% yield, 40% ee), bp 145–147°C (0.2 Torr); $[\alpha]_D^{25}$ −0.3° (*c* 10); ir: 3440–2680, 1736, and 1714 cm^{-1}; ^{1}H nmr δ: 2.68 (2H, d, J = 6 Hz), 2.69 (2H, d, J = 6 Hz), 3.70 (3H, s), 4.32 (1H, quintet, J = 6 Hz), 4.60 (2H, s), 7.33 (5H, s), and 10.38 (1H, br s) ppm. *Anal.* calcd. for $C_{13}H_{16}O_5$: C 61.90, H 6.39; found: C 61.03, H 6.52%.

Absolute configuration determinations

Correlations were to the known (6) (−)-(3*R*)-**3***b*.

*Of (+)-3*c

The cleavage method of Boeckman and Potenza (18) was used. (+)-Methyl hydrogen methoxyethoxymethoxyglutarate (**3***c*, 635 mg, 2.54 mmol) in dry CH_2Cl_2 (10 mL) was treated dropwise with *o*-phenylene bromoboronate (19) in CH_2Cl_2 (0.2 M, 12.7 mL, 2.54 mmol) at 20°C. After stirring at 20°C for 30 min the mixture was treated with water (10 mL) and stirred for another 20 min. The reaction mixture was then extracted with ethyl acetate (4 × 50 mL), and the organic phase dried ($MgSO_4$), filtered, and rotoevaporated. The Kugelrohr distillation gave (−)-(3*R*)-**3***b* (339 mg, 82% yield), bp 123–128°C/0.2 Torr; $[\alpha]_D^{25}$ −0.43° (*c* 7.5, $CHCl_3$) (lit. (6) $[\alpha]_D^{22}$ −1.7° (*c* 12.5) for (3*R*)-**3***b*); ir and ^{1}H nmr identical to spectra obtained for (+)-(3*S*)-**3***b* above.

*Of (−)-3*d

A mixture of (−)-(3*S*)-monomethyl 3-benzyloxyglutarate ((−)-(3*S*)-**3***d*, 252 mg, 1.0 mmol) and pre-equilibrated 10% Pd/C (75 mg) in 95% ethanol (12 mL) was hydrogenated at 23°C and atmospheric pressure (20). Hydrogen uptake (1 equiv.) ceased after 12 h. The mixture was then filtered and rotoevaporated to give (+)-(3*S*)-**3***b* (146 mg, 90% yield), bp 122–125°C (0.2 Torr); $[\alpha]_D^{25}$ +0.78° (*c* 5.0); ir and ^{1}H nmr identical with those of (+)-(3*S*)-**3***b* above.

Enantiomeric excess determinations

The method of Rosen *et al.* (7) was used, using ^{1}H nmr measurements on the amides **4** obtained from (+)-(3*S*)- or (−)-(3*R*)-**3***c* above and (*S*)-1-phenylethanamine. The ee values obtained (accuracy: ±1%) are recorded in Table 1.

[3]Any ester with a rate of hydrolysis ≥ 0.02% of that of ethyl butyrate will work satisfactorily in synthetic-scale conversions.

[4]The 16% ee level of **2***b* attained in this study matches that reported by Tamm and co-workers (11). Under normal conditions, we do not observe higher ee levels (12) with any of our current PLE batches.

The properties of the reference amide diastereomers, separated by flash chromatography, are as follows:

(3*S*,1′*S*)-**4**: ^{1}H nmr (200 MHz) δ: 0.03 (3H, s), 0.07 (3H, s), 0.86[5] (9H, s, *t*-Bu), 1.47 (3H, d, J = 7.0 Hz), 2.39 (1H, d of d, J = 5.1, 14.8 Hz), 2.45 (1H, d, J = 1.6 Hz), 2.48 (1H, d, J = 0.8 Hz), 2.55 (1H, d of d, J = 5.1, 14.8 Hz), 3.65[5] (3H, s, -OMe), 4.50 (1H, m), 5.13 (1H, m), 6.60 (1H, br d, J = 7.6 Hz), and 7.32 (5H, m) ppm.

(3*R*,1′*S*)-**4**: ^{1}H nmr (200 MHz) δ: 0.03 (3H, s), 0.07 (3H, s), 0.80[5] (9H, s, *t*-Bu), 1.50 (3H, d, J = 7.0 Hz), 2.39 (1H, d of d, J = 5.3, 14.9 Hz), 2.59 (2H, d, J = 5.9 Hz), 3.68[5] (3H, s, -OMe), 4.52 (1H, m), 5.12 (1H, m), 6.60 (1H, br d, J = 7.6 Hz), and 7.31 (5H, m) ppm.

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[5]Used in ee determination.