STRUCTURAL EVALUATION OF THE ENZYMATIC RESOLUTION OF TRICYCLO[5.2.1.0^{2,6}]DECENE-2-CARBOXYLATES USING PIG'S LIVER ESTERASE

M. Mamaghani[1*], A. J. H. Klunder[2] and B. Zwanenburg[2]

[1]*Department of Chemistry, Faculty of Sciences, Gilan University, P. O. Box 1914, Rasht, Islamic Republic of Iran*
[2]*Department of Organic Chemistry, NSR Center for Molecular Structure, Design and Synthesis, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*

Abstract

Moderate to excellent entantio- and stereoselectivities (ee's 54-100%) were observed in PLE catalyzed hydrolysis of (±)-ethyl 5-oxo-endo-tricyclo[5.2.1.0^{2,6}] deca-8-ene-2-carboxylate **5** and the structurally related open chain bicyclic structures (±)-ethyl 3-acetylbicyclo[2.2.1]hept-5-ene-2-carboxylates **7**, **9** and (±)-ethyl 3-propanoylbicyclo[2.2.1]hept-5-ene-2-carboxylates **8**, **10**. A pronounced preference for hydrolysis of the *exo- vs.* the *endo*-ester function was observed.

Introduction

The use of enzymes in organic synthesis is now well documented [1]. In particular, hydrolases are effective catalysts in the chemo-, regio- and stereoselective hydrolysis of carboxylic esters. In addition, these enzymes have been successfully employed for the synthesis of enantiopure compounds either by kinetic resolution of chiral compounds or in asymmetric synthesis. In connection with our studies on the utilization of polycyclic structures as chirons for biologically interesting cyclopentenoids [2]we report the enzymatic kinetic resolution of tricyclo [5.2.1.0^{2,6}]decadienone carboxylate **1** applying pig's liver esterase (PLE) [3]. Using PLE in a buffered water solution at pH 8 with acetonitril (0.2 M) as a co-solvent at 20°C leads to a nearly complete resolution to give both the carboxylic acid (-)-**2** and the remaining ester (+)-**1** in high optical and chemical yields (Scheme 1). The effectiveness of this resolution is quite surprising as the tricyclodecadienone skeleton is sterically quite demanding and the rigidity of the carbon skeleton dose not allow any adaptation to the requirements of the active site of the enzyme. Moreover, the ester function in **1** is positioned at a bridgehead carbon. To our knowledge no other examples have been reported that involves such an effective PLE-hydrolysis of polycyclic esters in which the ester function is attached to a tertiary bridgehead carbon atom. The uniqueness of the PLE-catalyzed hydrolysis of **1** and the high synthetic potential of these chiral polycyclic esters prompted us to investigate the geometrical and electronic constraints required for an effective ester hydrolysis in such systems. In a first approach norbornene type 2,3-di-esters **3** were shown to undergo PLE-mediated hydrolysis with appreciable rate only for the

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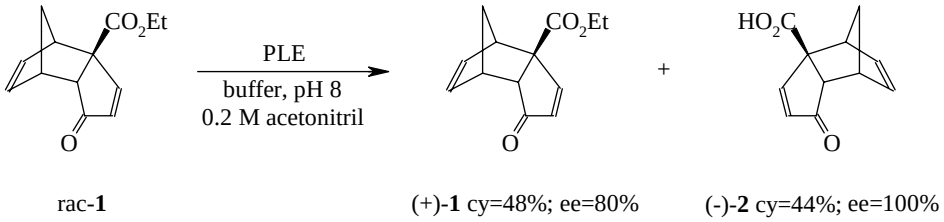
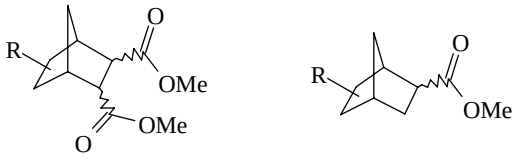
* *E-mail: m-chem41@cd.gu.ac.ir*

Scheme 1

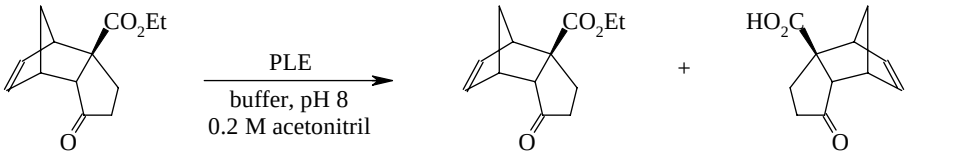


rac-**1** (+)-**1** cy=48%; ee=80% (-)-**2** cy=44%; ee=100%



3 **4**

Scheme 2



rac-**5** (-)-**5** cy=49%; ee=85% (+)-**6** cy=50%; ee=94%

trans-diesters and with almost complete stereoselective conversion of the *exo*-carboxylate ester function [4]. Pronounced stereoselectivity for *exo*-hydrolysis was also observed for the corresponding bicyclo[2.2.1] heptene mono-carboxylates **4**. These results conform to the substrate model proposed by Tamm *et al.* [5] for PLE mediated ester hydrolyses. In this model a polar substituent positioned *trans* to the carboxylate ester function is a prerequisite for effective hydrolysis. In *endo*-tricyclodecadienone **1** the cyclopentenone carbonyl function can be considered such a polar *trans*-substituent and therefore this structure also fits into the Tamm's substrate model. As the result of cyclopentenone ring-annulation with the norbornene moiety the position of the C_5-ketone function in **1** is conformationally completely fixed. In addition this ketone function forms part of a planar enone system. Both structural features may play an essential role in determining the effectiveness of the PLE-mediated hydrolysis of **1**. In this paper these structural effects are evaluated.

Results

In order to establish a structural effect of the enone moiety in **1** on the PLE-catalyzed hydrolysis, the enone alkene function in *rac*-**1** was regioselectively reduced by lithium aluminum hydride in tetrahydrofuran at -78°C to give tricyclodecenone **5** [6]. This tricyclic ester *rac*-**5** was subjected to PLE hydrolysis under identical conditions as used for the enzymatic resolution of **1** (pH 8.0, 0.1 M phosphate buffer solution with 1% acetonitril as the co-solvent, room temperature) [3]. The reaction was stopped at 50% conversion which needed about 7 h work-up and purification by column chromatography afforded unreacted ester (-)-**5** and acid (+)-**6** in both 50% yield (Scheme 2). The analytically pure carboxylic acid (+)-**6** (m.p. 109-110°C) had $[\alpha]_D^{20}$=+106° (c=0.32, MeOH) which corresponds with ee=94% based on the optical rotation of the optically pure acid (-) **6** $[\alpha]_D^{20}$=+114° (c=1.12, MeOH) which is available from enantiopure (+)-**1** using the same reductive procedure as used for the reduction of *rac*-**5** followed by chemical hydrolysis [6]. The optical yield of the remaining ester (-)-**5** $[\alpha]_D^{20}$=-78°(c=0.53, MeOH) was calculated to be 85%. The (+) methyl ester of (+)-**6** (ee=94%) was obtained by treatment of (+) **6** with diazomethane.

Comparing the results of the PLE-mediated hydrolysis of ethyl ester **5** with those of **1** shows that

both the hydrolysis rate and the enantioselectivity do not significantly change. Therefore, it may be concluded that the enone moiety dose not interact electronically or sterically with the active site of the enzyme but is simply accommodated in the available space. An interesting phenomenon is the dramatic change in direction of optical rotations observed for the carboxylic acid (+)-**6** and ester (-)-**5**, both lacking the C_4-C_5 olefinic bond, as compared with enone acid (-)-**2** and ester (+)-**1**. The possibility that the PLE-mediated hydrolysis of *rac*-**5** could have led to opposite absolute stereochemistry as found for the hydrolysis of **1** is excluded as reduction of optically pure (+)-ester **1** with established absolute configuration also gives (-)-ester **5**. Although the enone function evidently does not effect the chemical transformation involved here, its absence dramatically changes the chiroptical properties of the tricyclodecenone system.

To investigate the effect of cyclopentanone annulation we synthesized and studied the open chain norbornenyl ketones **7** and **9**. Both compounds closely resemble tricyclic ketone **6**; however, now the ketone moiety is not conformationally restricted.

Bicycloheptenyl keto-esters **7** and **9** were synthesized applying Diels-Alder methodology. Utilizing (E)-ethyl 4-oxo-2-pentanoate [7] as the dienophile a mixture of the desired ethyl 3-*endo*-acetylbicyclo[2.2.1]heptene-2-*exo*-carboxylate **7** and its *exo*-acetyl isomer **9** was obtained in a 1.2:1 ratio and in quantitative yield (Scheme 3). The synthesis of bicyclic ethylketone **8** required (E)-ethyl 4-oxo-2-hexenoate [7] as the dienophile. This compound was prepared in 50% yield by condensation of 2-oxo-triphenylphosphoranylidene-butane with ethyl glyoxalate. With cyclopentadiene a mixture of ethyl 3-*endo*-propanoylbicyclo[2.2.1]hept-5-ene-2-exocarboxylate **8** and ethyl 3-*exo*-propanoylbicyclo[2.2.1]hept-5-ene-2-endo-carboxylate **10** was obtained in a 55:45 ratio and in quantitative yield.

Conventional separation of these *endo/exo*-adducts e.g. by column chromatography appeared impossible. Based on the anticipation that the *endo*-ester functions in these adducts are much less apt to undergo PLE-mediated hydrolysis than the *exo*-ester functions [4], we subjected these mixtures of carboxylate esters to hydrolysis with PLE (0.1 M phosphate buffer solution, pH=7.8, room temperature) in order to accomplish their separation. The reactions were carefully monitored by gas chromatography.

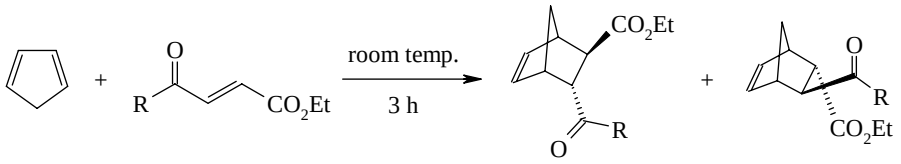
Incubation of the acetyl carboxylate ester mixture of **7** and **9** with PLE immediately initiated the hydrolysis process. However, after about 1 ½ h the rate of the reaction considerably decreased. GLC-analysis showed that after this period the reaction had proceeded up to 27% conversion. Workup led to the isolation of a mixture of unreacted ester adducts and a single carboxylic acid in 69% and 27% yield, respectively. The carboxylic acid could be identified as ethyl *endo*-3-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate **11** (Scheme 4). It showed an optical rotation $[\alpha]_D^{20}$=+79.8° (c=0.56, MeOH) which corresponds with ee=71% as was established with ^{1}HNMR-spectroscopy (400 MHz) using Eu(hfc)$_3$ as chiral shift reagent. A sample of this (+)-carboxylic acid **11** was transformed into its methyl ester by treatment with diazomethane. No trace of the *endo*-methyl ester corresponding to **9** was detected showing that this enzymatic hydrolysis had occurred with complete *exo/endo*-stereoselectivity and appreciable enantioselectivity. Purification by column chromatography (hexane/ethyl acetate) gave an analytically pure sample of **11**, $[\alpha]_D^{20}$=+80° (c=0.73, MeOH) which again calculated for ee=71%.

The addition of acetonitril (1%) as co-solvent in the PLE-mediated hydrolysis of the ester mixture of **7** and **9** was found to have, just as for tricyclodecadienone ester **1**, a profitable effect on the enantioselectivity. Again the reaction slowed down at a conversion of 27% which was reached after 70 min. Workup yielded optically pure carboxylic acid **11**, $[\alpha]_D^{20}$=+113° (c=0.81, MeOH) in 22% yield after recrystallization from di-isopropylether (m.p. 146-146.5°C). The remaining esters **13** were recovered in 72% yield.

Incubation of the ethylketone carboxylates *exo*-**8** and *endo*-**10** with PLE under standard conditions led initially to a relatively fast hydrolysis reaction which slowed down considerably again at 26% conversion which being reached after 1¼ h. Upon acidic work up, *exo*-carboxylic acid **12** was isolated as a single compound in 25% yield. Purification by column chromatography gave analytically pure acid (+)-**12**, m.p. 64-65°, which exhibited an optical rotation $[\alpha]_D^{20}$=+85.1° (MeOH) corresponding with ee=74%. Treatment of this (+)-acid **12** with diazomethane gave the corresponding (+)-methyl ester with the same optical purity as the original acid. Interestingly, the addition of acetonitril as a co-solvent had only minor effect on the hydrolysis of *exo*-ester **8** but, in contrast to the hydrolysis of acetyl ester **7**, both the rate of hydrolysis and its enantioselectivity decreased. A conversion of 26% was reached only after 2 ¼ h to give (+)-acid **12**, $[\alpha]_D^{20}$=+79.1° (MeOH) in 25% yield with ee=69%.

After separation of the acid fractions, the remaining ester mixtures **13** and **14** were again subjected to PLE hydrolysis (Scheme 5). In a rather sluggish reaction a conversion of ~70% was reached after 30 h. GLC-analysis showed that all *exo*-carboxylate had now been hydrolyzed. Removal of the acidic fraction obtained from the hydrolysis of **13** provided ethyl 3-*exo*-acetylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate **15** in 23% yield after purification by column chromatography

Scheme 3

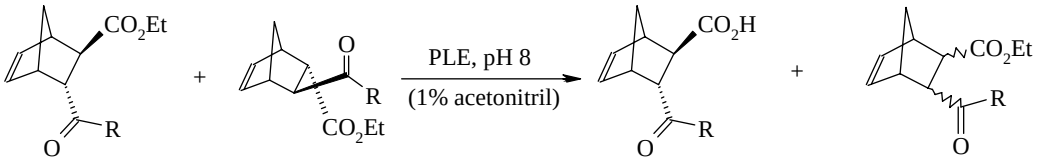


R=CH_3, C_2H_5

7 R=CH_3 **9** R=CH_3

8 R=C_2H_5 **10** R=C_2H_5

Scheme 4



R=CH_3 **7** **9**

R=C_2H_5 **8** **10**

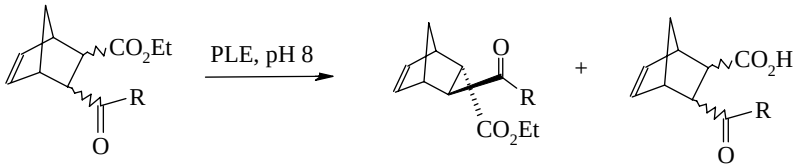
(+)-**11** cy=27%; ee=71% **13**

(+)-**11** cy=22%; ee=100% (acetonitril) **14**

(+)-**12** cy=24%; ee=74%

(+)-**12** cy=25%; ee=69% (acetonitril)

Scheme 5



R=CH_3 **13**

R=C_2H_5 **14**

(+)-**15** cy=23%; ee=54% **17**

(+)-**16** cy=29%; ee=56% **18**

(hexane/ethyl acetate). ^{1}HNMR spectroscopic analysis (400 MHz) using $Eu(hfc)_3$ showed this ester **15**, $[\alpha]_D^{20}$=+49.5° (c=0.79, MeOH) to have an optical purity of 54%. In a similar way the mixture of unreacted esters **14**, which was recovered from the hydrolysis of **8** and **10** in 72% yield, gave a single ester which was isolated in 29% yield and which was identified as ethyl 3-*exo*-propanoylbicyclo[2.2.1]heptene-2-*endo*-carboxylate **16**. Its optical rotation, $[\alpha]_D^{20}$=+61.3° was shown by ^{1}HNMR-spectroscopy to correspond with an ee=56%.

The above results show that longer reaction times not only lead to complete hydrolysis of the *exo*-ester function in both enantiomers of **7** and **8** but also results in partial hydrolysis of the *endo*-carboxylate function in *rac*-**9** and **10** with appreciable enantioselectivity.

Discussion

The tricyclo[5.2.1.$0^{2,6}$]decadienone 2-carboxylate **1** is unique in its behaviour towards PLE. Both the esterically demanding tricyclic skeleton and the bridge head position of the ester moiety do not prevent this chiral ester to be an excellent substrate for PLE-mediated hydrolysis. The conformational rigidity of this system is suspected to be a crucial geometrical factor for the almost complete enantioselectivity observed when *rac*-**1** is subjected to enzymatic hydrolysis. A similar high degree of rigidity is maintained in ester **5**

which lacks the enone double hand as is present in **1**. If the enone double bond in **1** does not play a significant role, either esterically or electronically, in the PLE-mediated hydrolysis high enantioselectivity is again expected for **5** when subjected to PLE-hydrolysis. Indeed, the observed rate of hydrolysis and the optical yields obtained for both the isolated acid (+)-**6** (ee=94%) and the remaining –ester (-) **5** (ee=84%) at about 50% conversion do not significantly differ from those of **1**. In order to further substantiate the effect of rigidity on the efficiency of PLE-hydrolysis two closely related bicyclo[2.2.1]heptene ketones **7** and **8** were investigated. The synthesis of these ketones using Diels-Alder methodology lead unavoidable to about 1:1-mixtures of *exo*- and *endo*-carboxylates. Based on our earlier studies with norbornene *endo*- and *exo*-carboxylate esters, it was assumed that the *exo*-esters would be hydrolyzed much faster than the corresponding *endo*-carboxylates and that depending on the degree of enantioselectivity of this hydrolysis separation of the *endo/exo*-carboxylate mixture would be possible. This turned out to be true. In a relatively fast and complete stereoselective reaction about half of the *exo*-esters **7** and **8** was hydrolyzed to the corresponding *exo*-carboxylic acids **7** and **8**. The considerable drop in hydrolysis rate at 27% conversion and the relatively high enantioselectivity observed here (~70% ee) show that both open chain ketone carboxylates **7** and **8** can nicely be resolved by PLE, although with somewhat lower optical efficiency as for the tricyclodecenone system. Interestingly, the nature of the ketone alkyl group R does not seem to have much effect on both the rate and enantioselectivity of hydrolysis when the enzyme is applied in the absence of acetonitril as a co-solvent. However, a rather impressive difference is observed in the presence of 1% of acetonitril. For the acetyl ester **7** complete enantioselectivity is now obtained at a somewhat higher rate of hydrolysis whereas a negligible effect on both the hydrolysis rate and the enantioselectivity is observed for the propanoyl ester **8**. Although an unambiguous explanation for this difference in behaviour can not be given, the more lipophylic nature of **8** as compared with **7** may play a role here.

This leads to the conclusion that cyclopentanone annulation is not a prerequisite for efficient optical resolution of tricyclodecenone 2-carboxylates but that restriction of the conformational freedom around the ketone moiety somewhat adds to its efficiency. With respect to stereoselectivity of PLE-mediated hydrolysis of *exo*- and *endo*-carboxylates **7** and **9**, and **8** and **10**, it was again found that in these bicyclic esters, the *exo*-carboxylate function is hydrolyzed considerably faster than the *endo*-ester function. In this respect these results entirely conform to the results obtained for the corresponding norbornane esters and again nicely fit in Tamm's PLE-substrate model.

Experimental Section

General Remarks

Melting points were measured with a Reichert Thermopan microscope and are uncorrected. IR spectra were taken on a Perkin Elmer 298 infrared spectrophotometer. ^{1}HNMR spectra were recorded on a Bruker AM-400, using TMS as an internal standard. For mass spectra a Bruker double focusing VG 7070E mass spectrometer was used. Flash chromatography was carried out at a pressure of ca. 1.5 bar, a column length of 15-25 cm and a column diameter of 1-4 cm, using Merck Kieselgel 60H, unless stated otherwise. All solvents used were dried and distilled according to standard procedures.

PLE catalyzed hydrolysis of (±)-ethyl 5-oxo-*endo*-tricyclo[5.2.1.0^{2,6}]deca-8-ene-2-carboxylate (5)

To a stirred 0.1 M phosphate buffer solution (17.20 ml., pH=8, 1% acetonitril) was successively added (±)-**5** (0.3 gr., 1.36 mmol.) and PLE (32.5 µl) at room temperature. The pH was kept constant at 8 by continuous addition of aqueous Na_2CO_3 solution until pH=10 was reached. The reaction mixture was extracted with ether (3×60 ml.) the combined ether extracts were dried ($MgSO_4$) and evaporated to give unreacted (-)-ethyl 5-oxo-*endo*-tricyclo[5.2.1.0^{2,6}]deca-8-ene-2-carboxylate (-)-**5** (0.145 gr., 0.66 mmol.) in 49% yield, $[\alpha]^D_{20}$=-78° (c=0.53, MeOH), ee=85%. ^{1}HNMR ($CDCl_3$): δ 6.28 (dd, 1H); 6.25 (dd, 1H); 4.21 (q, 2H); 3.35 (d, 1H); 3.24 (s,br., 1H); 2.37 (m, 1H); 2.11 (m, 2H); 1.81 (m, 1H); 1.62 (m, 1H); 1.29 (t, 3H) ppm. IR (neat):3060; 1720; 1230; 1175 cm^{-1}. Mass (EI): m/e M$^+$-C_2H_4O (1%); 149 (15%); 141 (5%); 140 (0.2%); 66(12%). EI/HRMS m/e: 221.1168 [calc. for $C_{13}H_{16}O_3$: 221.1178].

The remaining aqueous layer was acidified by dilute H_2SO_4 aq. to pH=3 and extracted with ether (3×60 ml.). The combined ether extracts were dried ($MgSO_4$) and evaporated to give (+)-5-oxo-*endo*-tricyclo[5.2.1.0^{2,6}] deca-8-ene-2-carboxylic acid (+)-**6** (0.130 gr., 0.67 mmol.; 50% yield) which was purified by column chromatography (hexane/EtOAc 1:1), $[\alpha]^D_{20}$=+106° (c=32, MeOH), ee=94%, m.p.=109-110°C. ^{1}HNMR($CDCl_3$): δ 6.30 (dd, 1H); 3.39 (s, 1H): 3.35 (d, 1H); 3.27 (s, 1H); 2.41-2.36 (m, 1H); 2.29-2.22 (m, 1H); 2.15-2.07 (m, 1H); 1.88-1.81 (m, 1H); 1.65 (s, 2H) ppm. IR (CH_2Cl_2): 3500-2500; 1730; 1690 cm^{-1}. EI/HRMS m/e: 193.08628±0.00058 [calc. for $C_{11}H_{12}O_3$ (M$^+$+1): 193.086471].

A sample of carboxylic acid (+)-**6** was methylated applying an ethereal solution of diazomethane to

provide (+)-methyl 5-oxo-*endo*-tricyclo[5.2.1.$0^{2,6}$]deca-8-ene-2-carboxylate which was purified by column chromatography (hexane/EtOAc 5:10), $[\alpha]^{D}_{20}$=+104° (c=0.4, MeOH), ee=94%. ^{1}HNMR($CDCl_3$): δ 6.28 (dd, 1H); 6.25 (dd, 1H); 3.76 (s, 3H); 3.35 (t, 2H); 3.24 (s, 1H); 2.37-2.32 (m, 1H); 2.18-2.03 (m, 2H); 1.84-1.78 (m, 1H); 1.62-1.55 (m, 2H) ppm. IR (neat): 1715, 1230, 1170 cm^{-1}.

(E)-Ethyl 4-oxo-2-pentanoate

Crude ethyl glyoxalate (15 gr. 0.147 mol) was added to a suspension of triphenylphosphoranylidene acetone (35.2 gr., 0.111 mol.) in dry benzene (450 ml.). The mixture was refluxed for 44 h and any water was removed by the use of a Dean-Stark separator. The solvent was first distilled at atmospheric pressure in order to remove most of the benzene and then under vacue to remove all of the benzene. The residue was extracted with several portion of hexane (500 ml.). The combined solvent extracts were evaporated and the crude product was purified by column chromatography (hexane/ether 4:1) to give (E)-ethyl 4-oxo-2-pentenoate (11.06 gr., 0.078 mol.) in 70% yield. ^{1}HNMR(in $CDCl_3$): δ 7.19 (d, 1H), J=16 Hz); 6.63 (d, 1H, J=16 Hz); 4.27 (q, 2H); 2.38 (s, 3H); 1.33 (s, 3H); 1.33 (t, 3H) ppm. IR (neat): 2980; 1720; 1680; 1420; 1360; 1280; 1260; 1180; 1180; 980 cm^{-1}.

Ethyl 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate (7) and ethyl 3-*exo*-acetylbicyclo [2.2.1]hept-5-ene-2-*endo*-carboxylate (11)

Freshly distilled cyclopentadiene (4.35 gr., 65.9 mmol.) in benzene (10 ml.) was added slowly to a stirred solution of (E)-ethyl 4-oxo-2-pentanoate (9 gr., 63.38 mmol.) in benzene (20 ml.) at room temperature. An exothermic reaction occurred. The mixture was stirred at room temperature for another 3 h. The solvent was removed under vacuo to provide a mixture of **7** and **11** (13 gr., 62.5 mmol.; *exo/endo* ratio 56.44 according to GLC and ^{1}HNMR analysis) in 99% yield.

PLE-catalyzed hydrolysis of ethyl 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate (7) and ethyl 3-*exo*-acetylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate (9)

The mixture of **7** and **9** (1 gr., 4.8 mmol.; *exo/endo* ratio 56:44) as obtained from the Diels-Alder reaction of (E)-ethyl 4-oxo-2-pentanoate and cyclopentadiene, was subjected to PLE hydrolysis using the standard procedure (0.1 M phosphate buffer solution, pH=8). After 1 ½ h and 28% conversion a dramatic change in the rate of hydrolysis is observed. When the reaction was stopped here unreacted esters **13** (0.691 gr. 3.32, mmol.) were recovered in 69% yield (*exo/endo* ratio 38:62). The corresponding 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylic acid (+)-**11** (0.24 gr., 1.32 mmol.) was isolated in 27% yield, m.p.=121-4°C; $[\alpha]^{D}_{20}$=+76.8° (c=0.56, MeOH); ee=71%. ^{1}HNMR($CDCl_3$): δ 6.26 (dd, 1H); 5.98 (dd, 1H); 3.41 (t, 1H); 3.35 (s, br., 1H); 2.83 (dd, 1H); 2.18 (s, 3H); 1.7 (d, 1H); 1.5 (m, 1H) ppm. IR (CH_2Cl_2): 3500-2500; 1700; 1410; 1355; 1330; 1305; 1178; 910; 870 cm^{-1}. Mass (EI); m/e (M^+, 0.93%, M^++1, 0.17%); 137 (22%); 119 (16%); 115 (18%); 91 (16%); 66(100%); 65 (12%); 43 (41%); 28 (11%). EI/HRMS m/e: 180.07871±0.00084 [calc. for $C_{10}H_{12}O_3$: 180.07864].

A sample of *exo* acid (+)-**11** was transformed into its methyl ester by treatment with an ethereal solution of diazomethane. The methyl 3-*endo*-acetylbicyclo [2.2.1]hept-5-ene-2-*exo*-carboxylate was purified (99% pure by G.C) by column chromatography (eluent hexane/EtOAc 7:1), m.p.=35-38°C; $[\alpha]^{D}_{20}$=+80° (c=0.73, MeOH), ee=71%. ^{1}HNMR($CDCl_3$): δ 6.25 (dd, 1H); 5.99 (dd, 1H); 3.70 (s, 3H); 3.42 (t, 1H); 3.32 (s, br., 1H); 3.13 (s, br., 1H); 2.17 (s, 3H); 1.70 (d, 1H); 1.48 (m, 1H) ppm. IR (CH_2Cl_2): 1705; 1430; 1305; 1330; 1305; 1240; 1175; 1110; 910; 870 cm^{-1}. Mass (EI); m/e (M^+, 0.1%); 151 (8%); 150 (4%); 149 (66%); 135 (6%); 119 (11%); 97 (20%); 91 (11%); 69 (11%); 66 (62%). EI/HRMS m/e: 194.09465±0.00077 [calc. for $C_{11}H_{14}O_3$: 194.09429].

A sample of unreacted esters **13** (0.651 gr., 3.12 mmol., *endo/exo* ratio 63:37) was subjected to PLE hydrolysis using the standard conditions. The reaction was stopped after 30 h at 69% conversion. At this stage of the reaction no trace of the *exo*-carboxylate **7** could be detected by GLC. Acidic work up gave a mixture of *endo/exo* acids **17** (0.400 gr., 2.22 mmol.) in 71% total yield. Pure *endo*-ester (+)-**15** (0.150 gr., 0.72 mmol.) was obtained in 23% yield, $[\alpha]^{D}_{20}$=+49.5 (c=0.97, MeOH), ee=54%. ^{1}HNMR(in $CDCl_3$): δ 6.30 (dd, 1H); 6.10 (dd, 1H); 4.09 (q, 2H); 3.36 (t, 1H); 3.24 (s, br., 1H); 3.04 (s, br., 1H); 2.82 (d, 1H); 2.27 (s, 3H); 1.43 (m, 2H); 1.23 (t, 3H) ppm. IR (neat): 3050; 1700; 1350; 1175; 1110; 1030 cm^{-1}. Mass(EI): m/e (M^+, 2%); 193 (0.5%); 179 (0.5%); 163 (10%); 149 (100%); 135 (26%); 119 (19%); 97 (33%); 91 (23%); 66 (86%). EI/HRMS m/e: 208.10998±0.00061 [calc. for $C_{12}H_{16}O_3$: 208.10994].

PLE catalyzed hydrolysis of (7) and (9) in the presence of acetonitril as co-solvent

A mixture of *exo/endo* adducts **7** and **9** (1.04 gr., 5 mmol.) was subjected to PLE hydrolysis at pH=8 (0.1 M phosphate buffer solution) in the presence of acetonitril (1%). The reaction was stopped at 28% conversion after 70 min reaction time. Alkaline workup gave a mixture of unreacted esters **13** (0.75 g, 3.60 mmol) in 72% yield. After acidification and extraction with ether 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-

carbocyclic acid **11** (0.19 gr., 1.05 mmol.) was obtained in 22% yield. Recrystallization from di-isopropylether gave optically pure **11**, m.p.=146-146.5°C, $[\alpha]^{D}_{20}$=+113° (c=0.81, MeOH), ee=100%.

The recovered ester mixture **13** (0.6 gr., 2.9 mmol.) was also subjected to PLE-hydrolysis using identical conditions (pH=8, phosphate buffer with acetonitril (1%) as co-solvent). The reaction was now stopped at 65% conversion which was reached after 14 days. Ethyl 3-*exo*-acetylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate (+)-**15** (0.126 gr.) was isolated from the neutral fraction in 21% yield, $[\alpha]^{D}_{20}$=+22.5° (c=0.96, MeOH), ee=25%. Acidic workup yielded a mixture of *exo*- and *endo*-carboxylic acids **17** (0.230 gr.).

(E)-Ethyl 4-oxo-2-hexenoate and ethyl 2-methyl-4-oxo-2-hexenoate

A mixture of 1-methyl-2-oxo-triphenylphosphoranylidenebutane and 2-oxo-triphenylphosphoranylidenebutane (15 gr.) and ethyl glyoxylate (9.6 g., 93.7 mmol) in benzene (250 ml.) was heated under reflux for 16 h [7]. Water was removed using a Dean-Stark separator. The solvent was distilled off at atmospheric pressure and the residue extracted with several portions of hexane/EtOAc mixture (4:1; 400ml.). The combined solvent extracts were evaporated in vacuo and the mixture of esters separated by column chromatography (hexane/ether 7:1). (E)-Ethyl 4-oxo-2-hexenoate (2 gr., 12.8 mmol.) was obtained as a colorless liquid. ^{1}HNMR(in $CDCl_3$): δ 7.08 (d, 1H, J=16 Hz); 6.66 (d, 1H, J=16 Hz); 4.26 (q, 2H, J=7.1 Hz); 2.67 (q, 2H, J=7.2 Hz); 1.32 (t, -3H, J=7.1 Hz); 1.13 (t, 3H, J=7.2 Hz) ppm. IR(neat: 3060; 2980; 1717; 1700; 1680; 1636; 1455; 1363; 1275; 1180; 1114; 1025; 980 cm^{-1}. Mass (EI): m/e (M^+, 3%, M^++1, 1%); 127 (63%); 111 (20%); 29 (77%). EI/HRMS m/e 156.07864±0.00077 [calc. for $C_8H_{12}O_3$: 156.07864]. Ethyl 2-methyl-4-oxo-2-hexenoate (2.4 gr., 14.11 mmol.) was isolated as a white crystalline solid, m.p.= 32-32.5°C. ^{1}HNMR(in $CDCl_3$): 6.56 (q, 1H, J=1.4 Hz); 4.24 (q, 2H, J=7.1 Hz); 2.73 (q, 2H, J=7.2 Hz); 2.24 (d, 3H, J=1.4 Hz); 1.32 (t, 3H, J=7.1 Hz); 1.12 (t, 3H, J=7.2 Hz) ppm. IR(CH_2Cl_2): 2965; 2925; 1710; 1680; 1635; 1440; 1362; 1212; 1182; 1090; 1050; 980; 880 cm^{-1}. Mass (EI): m/e (M^+, 4.3%, M^++1, 2%); 141 (30%); 125 (21%); 124 (37%); 85 (20%); 57 (35%); 29 (100%). EI/HRMS m/e 170.094341±0.00084 [calc. for $C_9H_{14}O_3$: 170.09429].

Ethyl 3-*endo*-propanoylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate (8) and ethyl 3-*exo*-propanoylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate (10)

To an ice cooled magnetically stirred solution of (E)-ethyl 4-oxo-2-hexenoate (1.92 gr., 12.3 mmol.) in benzene (5 ml.), freshly distilled cyclopentadiene (0.87 gr., 13.18 mmol.) in benzene (4 ml.) solution was added dropwise over a period of 15 min. The cooling bath was removed and stirring continued for 6 h at room temperature. The solvent was evaporated under vacuo to provide a mixture of desired cycloadducts (2.7 gr., 12.2 mmol.) in 99% yield (*exo/endo* ratio 1.2:1).

PLE catalyzed hydrolysis of ethyl 3-*endo*-propanoylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate (8) and ethyl 3-*exo*-propanoylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate (10)

A mixture of *endo/exo*-adducts **8** and **10** (6 g, 2.7 mmol., *exo/endo* ratio 1.2:1) was subjected to PLE hydrolysis using the standard procedure. The reaction was stopped at 26% conversion after 12 h of reaction time to provide 3-*endo*-propanoylbicyclo[2.2.1]hept-5-ene-2-exo-carboxylic acid **12** (0.127 gr., 0.654 mmol.) in 25% yield and with ee=74% after purification by column chromatography (hexane/EtOAc 2:1; 99% pure by G.C.). m.p.=64.5°C, $[\alpha]^{D}_{20}$=+85.1° (c=0.57, MeOH). ^{1}HNMR($CDCl_3$): δ 6.30 (dd, 1H); 5.95 (dd, 1H); 3.40 (t, 1H); 3.30 (s, 1H); 3.22 (br. s, 1H); 2.86 (dd, 1H); 2.49 (q, 2H, J=7.2 Hz); 1.70 (d, 1H); 1.50 (m, 1H); 1.04 (t, 3H, J=7.2 Hz) ppm. IR (CH_2Cl_2): 3500-2500; 2964; 1695; 1450; 1410; 1330; 1215; 1135; 1105; cm^{-1}. Mass (EI): m/e (M^+, 2%; M^++1, 0.1%); 176 (10%); 150 (1%); 149 (11%); 137 (33%); 129 (25%); 119 (21%); 111 (15%); 99 (23%); 66 (100%).

A mixture of esters **14** (0.435 gr., 1.96 mmol., *exo/endo* ratio 44:56) was recovered in 72%. This mixture of esters (0.400 gr., 1.80 mmol.) was again subjected to PLE hydrolysis, using the standard procedure. The reaction was now stopped at 69% conversion after 12 days of reaction time (fresh PLE (86 µl) was added after 18 h) to give ethyl 3-*exo*-propanoylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate **16** (0.117 gr., 0.527 mmol.). After purification by column chromatography (hexane/EtOAc: 5:1) **16** was obtained in 29% yield and with ee=56%, $[\alpha]^{D}_{20}$=+61.26° (c=0.63, MeOH). ^{1}HNMR($CDCl_3$): δ 6.28 (dd, 1H); 6.08 (dd, 1H); 4.08 (q, 2H, J=7.1 Hz); 3.36 (t, 1H); 3.25 (s, br., 1H); 2.99 (s, br., 1H); 2.81 (dd, 1H); 2.59 (d of q, 2H, J=7.2, 2.8 Hz); 1.57 (d, 1H); 1.38 (m, 1H); 1.23 (t, 3H, J=7.1 Hz); 1.08 (t, 3H, J=7.2 Hz) ppm. IR(neat): 3055; 2970; 1715; 1700; 1450; 1360; 1330; 1300; 1260; 1190; 1110; 1024; 910; 860; 715 cm^{-1}. Mass (EI): m/e (M^+, 6%, M^++1, 1%); 177 912.6%); 176 (19.3%); 165 (20.6%); 1557 (27.7%); 149 (37.6%); 127(11.2%); 119 (24.1%); 66 (100%). Besides **16** a mixture of acids **18** (0.234 gr., 1.21 mmol., *endo/exo* ratio 45:55) was isolated in 67% yield.

A sample of 3-*endo*-propanoylcyclo[2.2.1]hept-5-ene-2-*exo*-carboxylic acid **12** was transformed into its methyl ester by reacting with ethereal diazomethane.

The ester was purified by column chromatography (hexane/EtOAc 5:1) to give pure methyl ester as colorless oil (>99% pure by G.C.) $[\alpha]^{D}_{20}$=+85.7° (c=0.7, MeOH) with ee=74%. ^{1}HNMR(in $CDCl_3$): δ 6.26 (dd, 1H); 5.94 (dd, 1H); 3.69 (s, 3H); 3.41 (t, 1H); 3.27 (s, br., 1H); 3.13 (s, br., 1H); 2.81 (dd, 1H); 2.48 (q, 2H, J=7.2 Hz); 1.7 (d, 1H); 1.48 (m, 1H); 1.03 (t, 3H, J=7.2 Hz) ppm. IR (neat): 3055; 2940; 1700; 1430; 1330; 1240; 1175; 1105; 1020; 960; 898; 730 cm^{-1}. Mass (EI): m/e (M^+, 7%, M^++1, 0.8%); 177 (6%); 151 (24%); 143 (22%); 119 (25%); 113 (23%); 111 (33%); 91 (15%); 66 (100%).

PLE catalyzed hydrolysis of (8) and (10) in the presence of acetonitril as co-solvent

A mixture of *exo/endo* adducts **8** and **10** (0.92 gr., 4.1 mmol.) was hydrolyzed by PLE (198 µl) at pH=8 (0.1 M phosphate buffer solution) in the presence of acetonitril (1%). The reaction was stopped at 26% conversion after 22 h reaction time to give a mixture of unreacted esters **14** (0.62 gr., 2.8 mmol.) and 3-*endo*-propanoylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylic acid **12** (0.2 gr., 1 mmol.) in 25% yield and with ee=69% $[\alpha]^{D}_{20}$=+79% (c=0.67, MeOH).

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