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# Structural Effects on the Enantioselective Acetylation of 4-Hydroxychromans Catalyzed by Microbial Lipases

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**Abstract**: Kinetic resolutions of a series of racemic 4-hydroxychromans by the *Candida cylindracea* lipase catalysed acetylation are described. Correlation between structure (conformation) and enantioselectivity is discussed.

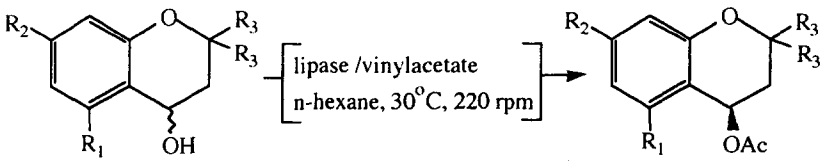
## INTRODUCTION

4-Hydroxychromans represent a class of chiral compounds used for the synthesis of various benzopyran derivatives[1,2]. Their preparation in optically pure form has not yet been described. Prompted by the recently achieved stereoselective hydrolysis and acylation of some acyclic[3,4] and cyclic *sec* alcohols[5,6], we have investigated kinetic resolutions of racemic 4-hydroxychromans **1-5** by microbial lipases. The efficacy of the biocatalytic transformation was particularly intriguing in view of their different conformational properties and chemical stability, depending on the groups $R_1$ and $R_2$.

## RESULTS AND DISCUSSION

Preliminary screening of 14 lipases was performed with racemic **1** and **4**, and revealed *Candida cylindracea* lipase (CCL) as the only one active and highly enantioselective; *Pseudomonas fluorescens* (PFL) lipase was also active with **1**, not however with **3** and **4**, representatives of 2,2-dimethylderivatives, which possess bulky group in the β-position to the reacting stereogenic center. The observed difference in reactivity between PFL and CCL with **1** and 2,2-dimethyl derivatives **2-5** is not surprising in view of the well known preference of the latter for bulky substrates. The former can be extremely selective on structurally "slim" counterparts[7]; our recent study shows how effective PFL is in the stereoselective hydrolysis and acetylation of stereoisomeric macrocyclic lactones that differ in the absolute conformation of the macrocyclic ring[5,6]. CCL was therefore used in all subsequent experiments with **1-5**. Acylation was performed with vinylacetate in n-hexane, an apolar solvent that usually gives the optimal results in this reaction[8,9], Scheme 1. The yields and enantioselectivities reported in the Table 1. have regularly been determined by achiral and chiral HPLC, respectively. For the preparative-scale experiments the yields are given, for some less reactive lipases the conversions after 24 or 48 hrs are indicated.

Scheme 1




| | $R_1$ | $R_2$ | $R_3$ | |
|---|---|---|---|---|
| (±) **1** | H | H | H | (+) **6** |
| (±) **2** | H | H | $CH_3$ | (+) **7** |
| (±) **3** | H | $OCH_3$ | $CH_3$ | (+) **8** |
| (±) **4** | $CH_3$ | H | $CH_3$ | (+) **9** |
| (±) **5** | $CH_3$ | $OCH_3$ | $CH_3$ | (+) **10** |

Difference in the reactivity between 5-methyl derivatives **4,5** as compared to **1-3**, was particularly significant. Progress curves for two pairs of 5-methyl and 5-demethyl derivatives, **2,3** and **4,5**, are presented in the Figures 1 and 2.

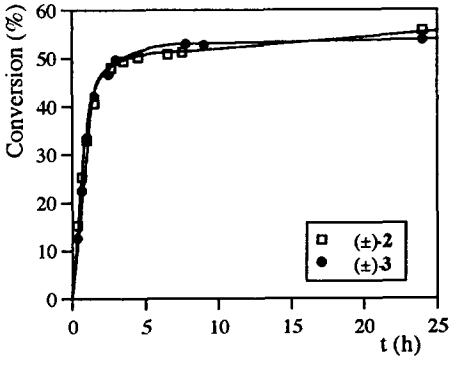



**Figure 1.** Acetylation of *rac.*4-hydroxy-5-demethyl chromans **2** and **3** with CCL in n-hexane

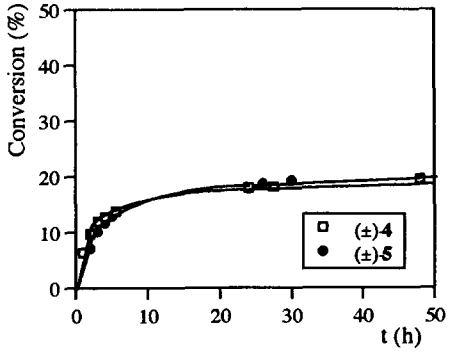



**Figure 2.** Acetylation of *rac* 4-hydroxy-5-methyl chromans **4** and **5** with CCL in n-hexane

Whereas lipase catalyzed acetylation of **2** and **3** approaches *ca.* 50% conversion after 5-10 hrs, affording 4-O-acetyl derivatives (+)-**7** and (+)-**8** with nearly 100% enantiomeric excess, 5-methyl congeners **4** and **5** react much slower, reaching only 20% conversion after 48 hrs.

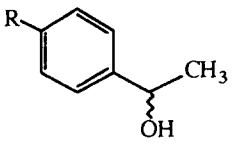
Naemura *et al*[12] have recently proposed a predictive active site model for PFL (lipase YS from *Pseudomonas fluorescens*, Amano) for the series of primary and secondary alcohols, mainly derivatives of cyclohexanol, e.g. **13,14**, or its open-chain or polycondensed congeners (**11,12**; **14,15**). Since chroman derivative **1** differ from 1-hydroxybenzocyclohexane **13** only by the oxygen present in the saturated ring, comparison of some of our data with previous work has provided additional informations about the relative sterical requirements at the active sites of these two microbial lipases.

**Table 1:** Tansesterification of **1-5** with lipases in n-hexane.

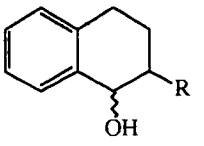
| Substrate | Entry | Lipase | t (h) | Conversion (%) | Acetate e.e. (%) | Alcohol e.e.(%) | E |
|---|---|---|---|---|---|---|---|
| (±)-**1** | 1 | *Candida cylindracea* | 1.25 | 44.9 | 74.9 | 54.8 | |
| | 2 | (Amano) | 2.3 | 51.1 | 71.0 | 65.2 | 13 |
| | 3 | *Pseudomonas species* | 1.25 | 38.0 | 100 | 60.9 | |
| | 4 | | 1.3 | 46.7 | 100 | 81.5 | >250 |
| | 5 | | 1.3* | 48.5 | 99.2 | 93.4 | >250 |
| | 6 | Lipozyme | 24 | 46.3 | 96.7 | 94.0 | 157 |
| | 7 | *Humicola lanuginosa* | 24 | 21.7 | | | |
| | 8 | *Penicillium camemberti* | 24 | 15.6 | | | |
| | 9 | *Geotrichum candidum* | 24 | 14.8 | | | |
| (±)-**2** | 10 | *Candida cylindracea* (Sigma) | 24 | 51.8 | 95.5 | 100 | >250 |
| | 11 | *Candida cylindracea* | 24 | 53.8 | 85.6 | 100 | 81 |
| | 12 | (Amano) | 2* | 48.4 | 100 | 94.4 | >250 |
| | 12 | | 24* | 53.5 | 81.3 | 100 | 33 |
| | 13 | | 0.5* | 23.4 | 100 | 31.1 | >250 |
| | 14 | *Penicillium camemberti* | 24 | 11.2 | | | |
| (±)-**3** | 15 | *Candida cylindracea* | 2 | 28.5 | 100 | 38.4 | >250 |
| | 15 | (Sigma) | 4 | 41.3 | 100 | 69.3 | >250 |
| | 15 | | 6 | 47.0 | 98.2 | 86.0 | |
| | 15 | | 24 | 53.1 | 90.6 | 100 | >250 |
| | 16 | | 24 | 52.3 | | | |
| | 17 | *Candida cylindracea* | 2 | 52.0 | 93.8 | 99.0 | >250 |
| | 17 | (Amano) | 3 | 54.3 | 89.7 | 100 | >250 |
| | 17 | | 6 | 54.9 | 87.2 | 100 | >250 |
| | 17 | | 24 | 57.1 | 77.9 | 100 | >250 |
| | 18 | | 24 | 53.8 | | | |
| | 19 | | 24 | 52.5 | | | |
| | 20 | | 24* | 56.9 | 82.3 | 100 | >250 |
| | 21 | *Penicillium camemberti* | 24 | 14.2 | | | |
| (±)-**4** | 22 | *Candida cylindracea* | 48 | 14.5 | | | |
| | 23 | (Sigma) | 48 | 14.3 | 100 | 14.3 | >250 |
| | 24 | *Candida cylindracea* | 48 | 19.5 | 100 | 25.4 | >250 |
| | 25 | (Amano) | 48* | 22.5 | 100 | 32.0 | >250 |
| (±)-**5** | 26 | *Candida cylindracea* | 48 | 14.2 | | | |
| | 27 | (Sigma) | 48 | 14.7 | | | |
| | 28 | *Candida cylindracea* | 48 | 19.4 | | | |
| | 29 | (Amano) | 48 | 19.3 | | | |
| | 30 | | 48* | 19.0 | 100 | 22.4 | >250 |

* Preparative scale of transesterification

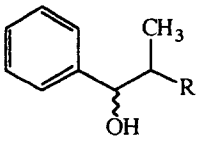
In parallel we examined the kinetic resolution of **1** by PFL and CCL in n-hexane and diisopropylether, since this latter solvent was regularly used by Naemura *et al.* The results are presented in the Table 2. They revealed that PFL is more selective than CCL; the results for **1** in diisopropylether are close to those obtained by Naemura for **13**. Since PFL was catalytically inactive with the representatives of 2,2-dimethyl derivatives **2** and **3** this indicate that the active site of CCL can much better accommodate the remote dimethyl group.



| | R |
|---|---|
| **11** | H |
| **12** | $CH_3O$ |



| | R |
|---|---|
| **13** | H |
| **14** | $CH_3$ |



| | R |
|---|---|
| **15** | H |
| **16** | $CH_3$ |

**Table 2:** Comparison of acetylation of (±)-**1** and (±)-**13** with lipases in n-hexane and diisopropylether

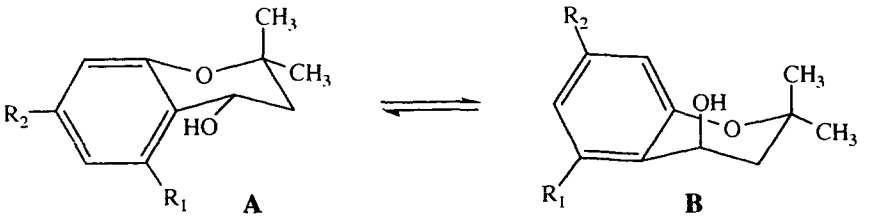
| Substrate | Lipase | Organic solvent | t (h) | Conversion (%) | Acetate e.e. (%) | Alcohol e.e. (%) | E | Ref. |
|---|---|---|---|---|---|---|---|---|
| (±)-**1** | CCL | n-hexane | 24 | 66.3 | 51.1 | 91.5 | | this work |
| | CCL | n-hexane | 3* | 44.8 | 84.5 | 65.1 | 24 | this work |
| | PFL | n-hexane | 24 | 50.1 | 96.7 | 100 | >250 | this work |
| | PFL | diisopropylether | 24 | 50.0 | 100 | 100 | >250 | this work |
| (±)-**13** | PFL | diisopropylether | 7 | 49.7 | 93 | 92 | 94 | 7 |

*Preparative scale of transesterification.

Slower acetylation rate for **4,5** as compared to **1-3**, can be ascribed to the steric hindrance of 5-methyl group, effecting the conformational equilibrium in the Scheme 2. $^1H$ NMR data for **4,5** indicate the conformer B as the prevaling in the equilibrium. Exact calculations, based on the coupling constants for 7-tosyl congeners of **5**, has shown that conformer B could amount to over 70%, whereas for 5-demethyl congeners the dominance of the conformer A was confirmed[1]. The assumption that conformers with pseudoaxialy exposed hydroxy group are more reactive is supported by the reports of Schneider[10] and Triantaphylides[11], who observed that α-*trans* substituted cyclohexanols are more reactive than their *cis*-isomers in lipase catalyzed acylations.

There is no observable effect of 2,2-dimethyl group in **2-5**, as compared to **1** on the rate of acetylation with CCL; the enantioselectivity at *ca.* 50% conversion is notably lower, however. There is also no effect of the 7-methoxy group in **3,5** as compared to **2,4**. Since no effect of the *para*-methoxy group was also observed for PFL with the couple **11,12**, there is accumulated evidence that both lipases easily accomodate a large group within $HL_T$ back-side sector of the active site, but only CCL can accomodate a bulky group in the $HL_1$ back-side sector, Fig. 3.

Scheme 2




The effect of the 5-methyl group in **4** and **5** is particularly interesting. The (+)-enantiomers of **4** and **5** exhibited notably lower reactivity than their demethyl counterparts **2** and **3**, though both pairs of the racemic substrates afforded (+)-acetates and (+)-alcohols with 100% enantiomeric excess. These results are complementary to those reported[12] for **14** and **16**. This active-site model presented in Fig. 3 suggests that reactive enantiomers of **2,3** undergo acetylation in their less stable conformation, with the hydroxy group in the *pseudo-axial* position. The steric hindrance by 5-methyl group makes **4,5** less reactive in spite of the much higher ratio of the conformer with *pseudo-axial* hydroxy group in the equilibrium in solution.

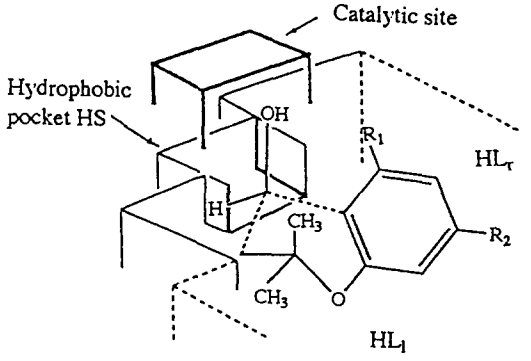



**Figure 3.** Reactive conformation of 4-hydroxy-2,2-dimethylchromans superimposed on the PFL active site (according to Naemura *et al.*[12]).

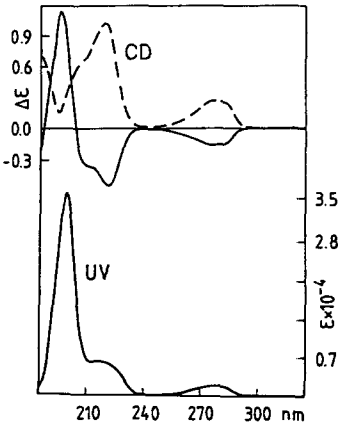
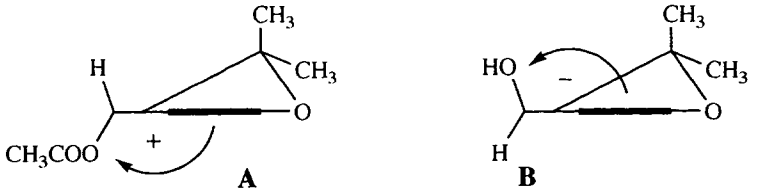



**Figure 4.** CD spectra of **1** (——) and **6** (----), and UV spectrum of **6**, in MeCN

For $HL_1$ space was shown, by the inhibiting effect of the α-methyl groups in **14** and **16**, that it is highly sensitive on the steric requirements; in fact, both enantiomers of **14** and **16** were completely inert with PFL, whereas (+)-enantiomers of their α-demethyl counterparts **13** and **15** have been acylated with high rate and over 90% enantioselectivity. Our results with **2,3** *vs.* **4,5** indicate that the PFL back-side space of the hydrophobic pocket $HL_1$ is "narrower" than in CCL.

The instability of 4-acetoxy-7-methoxy derivatives (+)-**8** and (+)-**10** precluded their isolation; both compounds eliminate AcOH even during chromatography on silica gel or neutral alumina. Since 7-demethoxy derivatives (+)-**6**, (+)-**7** and (+)-**9** proved quite stable under these conditions, the obvious driving force for the elimination in the former represents stabilization of benzylic carbocation by the *para*-positioned 7-methoxy group. For some 4-hydroxychromans related to **2-5**, the elimination of water under harsh conditions in the presence of the acid catalyst, forming 2,2-dimethyl-2*H*-chromenes, was already observed[1]; this elimination is obviously facilitated for acetates of 7-methoxy derivatives **3,4**.

As to the chiroptical properties of the alcohols **1-5** and acetates **6-10**, it is interesting to note that alcohols have regularly changed the sign of $[\alpha]_D$ on acylation, since the same sign was observed for the unacylated (remaining) alcohols (+)-**2-5** and the acetates of their (-)-enantiomers, *i.e.* for (+)-**7** and (+)-**9**. The acetate of the (+)-enantiomer of **1** inverts the sign of $[\alpha]_D$ to (-). On the bases of the known specificity of *Candida cylindracea* lipase for *R*-alcohols[10,11,13] we have assumed *R* configuration for acetates **6-10**. CD spectra confirmed this assumption; the acetyl derivatives **6-10** exhibited in the region of $^1L_b$ ($^1B_{2u}$) and $^1L_a$ ($^1B_{1u}$) band two positive Cotton effects, alcohols instead showed up two negative bands in the same region, as exemplified for **6** and **1** in Figure 4. Since the CD of 4-substituted chromans is governed by the chirality of the ring and chiral contribution from the substituent at C(4)[14], the envelope ("sofa") conformation of P-helicity (**A**) can be assigned for *R*-acetates, and the opposite one (**B**) for *S*-alcohols.




In conclusion, we have shown that kinetic resolution catalyzed by CCL can be used in preparation of the optically pure 2,2-dimethyl-4-hydroxychromans. A number of microbial enzymes proved inactive, in particular PFL, presumably because CCL is the only one that could accomodate bulky 2,2-dimethyl group within its active site. The results are therefore also of interest for ulterior probing of the hydrophobic binding region of the active site of CCL and PFL.

**Acknowledgements**. The authors sre indebted to Prof. M. Hollòsi, Eötvös Lorand University, Budapest, for helpfull discussion of the CD spectra. This work was supported by the Europian Communities (ERBCIPECT 926003 8385), by the Croatian Ministry of Science and Technology (Project No. 1-07-255), by the Hungarian Ministry of Culture and Education (Grant No. 14/94), and by Alkaloida Chem. Co., Ltd. Thanks are due to Amano Co. for generous gift of the lipases, and to Novo Norisk for Lipozyme used in this work.

## EXPERIMENTAL SECTION

Melting points are determined on Electrothermal 9100 apparatus, and are not corrected. Ir spectra were obtained for KBr pallets, on a Perkin Elmer M 137 spectrometer. 1H- and 13C-NMR spectra were recorded on a Varian XL-GEM 300 spectrometer; shifts are given in ppm downfield from TMS. Optical rotations were measured on Optical Activity LTD automatic polarimeter AA-10.

HPLC was performed on a Hewlett Packard instrument Series 1050 with UV detector ($\lambda$=254 nm) and HP integrator 3396B. Reactions were monitored using Econosphere RP C8 5$\mu$ column (250 x 4.6 mm) under following conditions: methanol-water 5:2 as eluent, flow rate 0.5 mL/min, pressure 106 bar for substrate (±)-**4** and (±)-**5**, and methanol-water 5:2 as eluent, flow rate 0.70 mL/min, pressure 115 bar for substrate (±)-**1**, (±)-**2** and (±)-**3** The retention times were: **1**: 4.9 min, **6**: 5.7 min, **2**: 5.2 min, **7**: 6.6 min, **3**: 5.3 min, **8**: 7.58 min, **4**: 8.1 min, **9**: 10.8 min, **5**: 8.18 min, **10**: 10.6 min. Determination of the enantiomeric excess was performed using Chiralcel OJ column (250 x 4.6 mm) with Chiralcel OJ precolumn (50 x 4.6 mm) in n-hexane-isopropanol 90:10 as eluent, flow rate 0.5 mL/min, pressure 15-16 bar and column temperature 30°C for compounds (±)-**2** (±)-**3** and (±)-**4**, n-hexane-isopropanol 92:8 as eluent, flow rate 0.6 mL/min, pressure 20 bar at room temperature for (±)-**1** and Chiralcel OD-R column (250 x 4.6 mm) with RP precolumn for (±)-**5** in methanol-water 6:4 as eluent, flow rate 0.5 ml/min, preasure 91 bar, and column temperature 40°C. Chiralcel OJ column does not separate enantiomers of acetate **7**. The retention times were: 25.2 and 36.7

min. for the enantiomers of (±)-**1**; 13.7 and 18.9 min. for the enantiomers of (±)-**2**; 22.7 and 29.2 min. for the enantiomers of (±)-**3**;11.8 and 12.9 min. for the enantiomers of (±)-**4**; 22.4 and 23.9 min. for the enantiomers of (±)-**5**.

Racemic 4-hydroxychromans (±)-**1-5** were prepared according to known method[15,16].

*Screening of microbial lipases for kinetic resolution of (±)-1-5 in n-hexane*

Activity of 15 microbial lipases was investigated, *Pseudomonas species, Humicola lanuginosa, Aspergillus niger, Geotrichum candidum, Rhizopus oryzae, Mucor javanicus, Candida cylindracea, Candida lipolytica, Penicillium camembertii, Rhizopus niveus, Rhizopus delemar,* all supplied by Amano, *Candida cylindracea* (Sigma, Activity 665 U/mg), *Mucor miehei* i *Pseudomonas fluorescens* lipases (Fluka, activity 24.2 and 31.5 U/mg) and Lipozyme (immobilyzed *Mucor miehei* lipase from Novo Nordisk, activity 49 BIU/g). All transesterification were performed in n-hexane.

The progress curves were determined with *ca.* 10 mg of substrate, and CCL lipase (*ca.* 10 mg, 1.67 mg/ml) suspended in n-hexane (5 ml), at 30°C in the thermostated shaker. Reaction was initiated by addition of vinylacetate (1 ml, 10 mmol). Samples (30 µl) were taken at regular time intervals, filtered through millipore filter, evaporated, desolved in the mobile phase (100 µl) and analyzed by HPLC on RP C8 column. The enantiomeric purity was determined on Chiralcel OJ column for substrate (±)-**1**, (±)-**2**, (±)-**3**, (±)-**4** and on Chiralcel OD-R column for (±)-**5**.

*General procedure for preparative acetylation of* (±)-**1-5**.

Substrate (*ca.* 100 mg) was dissolved in n-hexane (20 ml). To this solution was added *Candida cylindracea* lipase (*ca.* 100 mg, 4.5 mg/ml) and suspension was thermostated at 30°C. After few minutes vinylacetate (2 ml) was added and reaction mixture was shaken at 220 rpm and 30°C. After indicated reaction periods the reaction mixture was elaborated by the standard work-up procedure.

**(-)-4-Hydroxychroman**, (-)-**1**.-Reaction was stopped after 3 h at 44.8% of conversion, the reaction mixture was filtered, evaporated and purified by chromatography on $SiO_2$ with $CH_2Cl_2$/cyclohexane (6:4) as eluant. Chemically pure and enantiomerically enriched alcohol (-)-**1** (e.e. 65.1%; $[\alpha]_D$ -41.5, c 2.62 in $CHCl_3$) was isolated. $^1H$ NMR.($CDCl_3$) δ 7.24 (1H, d), 7.16 (1H, t), 6.88 (1H, t), 6.79 (1H, d), 4.65 (1H, s), 4.17 (2H, t), 2.68 (1H, s), 2.08.1.87 (2H, m). $^{13}C$ NMR.($CDCl_3$) δ 154.4, 129.6, 129.5, 124.1, 120.3, 116.8, 62.8, 61.8, 30.4.

**(+)-2,2-Dimethyl-4-hydroxychroman**, (+)-**2**.-Reaction was stopped after 30 minutes at 23.4% of conversion, the work-up of the reaction mixture was performed as described for (+)-**1**, and crude product was purified on $SiO_2$ with $CH_2Cl_2$/cyclohexane (8:2) as eluant. Chemically pure and enantiomerically enriched alcohol (+)-**2** (e.e. 31.7%; $[\alpha]_D$ +16.2, c 2.62 in $CHCl_3$) was isolated.

When the reaction was stopped after 24 h at 53.5% of conversion, chemically and enantiomerically pure alcohol (+)-**2** (e.e. 100%; $[\alpha]_D$ +51.5, c 1.12 in $CHCl_3$) was isolated. $^1H$ NMR.($CDCl_3$) δ 7.45 (1H, d), 7.18 (1H, t), 6.93 (1H, t), 6.79 (1H, d), 4.86 (1H, t), 2.18 (1H, dd), 1.87 (1H, dd), 1.66 (1H, s), 1.45 (3H, s), 1.32 (3H, s). $^{13}C$ NMR.($CDCl_3$) δ 153.1, 129.2, 127.6, 124.3, 120.2, 117.1, 75.2, 65.5, 42.6, 28.9, 25.7.-

**(+)-2,2-Dimethyl-4-hydroxy-7-methoxychroman**, (+)-**3**.-Reaction was stopped after 24 h at 56.9% of conversion, usual work-up and chromatography on $SiO_2$ with $CH_2Cl_2$/cyclohexane (1:1) afforded chemically and enantiomerically pure alcohol (+)-**3** (e.e. 100%; $[\alpha]_D$ +22.1, c 0.724 in $CHCl_3$). $^1H$ NMR.($CDCl_3$) δ 7.33 (1H, d), 6.50 (1H, dd), 6.32 (1H, d), 4.76 (1H, t), 3.74 (3H, s), 2.10 (1H, dd), 2.00 (1H, s), 1.80 (1H, dd), 1.42 (3H, s), 1.29 (3H, s). $^{13}C$ NMR.($CDCl_3$) δ 160.4, 154.2, 128.5, 116.6, 107.4, 101.2, 75.3, 63.1, 54.9, 42.5, 28.4, 25.7.

**(+)-2,2-Dimethyl-4-hydroxy-5-methylchroman**, (+)-**4**.-Reaction was stoped after 48 h at 22.5% of conversion; usual work-up and chromatography on $SiO_2$ with $CH_2Cl_2$ afforded chemically and enantiomerically enriched alcohol (+)-**4** (e.e. 32.0%; $[\alpha]_D$ +1.0, c 1.12 in $CHCl_3$). $^1H$ NMR.($CDCl_3$) δ 7.10 (1H,t), 6.68 (1H, d), 6.76 (1H, d), 4.91 (1H, t), 2.43 (3H, s), 2.09 (2H, d), 1.60 (1H, s), 1.44 (3H, s), 1.39 (3H, s). 300 MHz-$^{13}C$ NMR.($CDCl_3$) δ 153.4, 138.6, 129.0, 122.2, 121.2, 115.3, 73.2, 62.0, 42.3, 28.9, 26.0, 18.9.

**(+)-2,2-Dimethyl-4-hydroxy-7-methoxy-5-methylchroman**, (+)-**5**. Reaction was stoped after 48 h at 19.0% of conversion, usual work-up and chromatography on $SiO_2$ with $CH_2Cl_2$ afforded enantiomerically

enriched alcohol (+)-**5** (e.e. 22.4%; $[\alpha]_D$ +1.1, c 1.69 in $CHCl_3$). $^1H$ NMR.($CDCl_3$) δ 6.34 (1H, d), 6.21 (1H, d), 4.81 (1H, t), 3.72 (3H, s), 2.37 (3H, s), 2.02 (2H, d), 1.42 (3H, s), 1.37 (3H, s). $^{13}C$ NMR.($CDCl_3$) δ 160.0, 154.5, 139.7, 113.9, 109.6, 99.2, 73.6, 61.7, 54.9, 42.3, 29.0, 26.0, 19.0.

**(+)-4-Acetoxychroman**, (+)-**6**.-Reaction was stopped after 3 h at 44.8% of conversion, usual work-up and chromatography on $SiO_2$ with $CH_2Cl_2$/cyclohexane (6:4) afforded chemically and optically pure acetate (+)-**6** (e.e. 84.5%; $[\alpha]_D$ +158.0, c 0.23 in $CHCl_3$. $^1H$ NMR.($CDCl_3$) δ 7.25 (2H, tt), 6.91 (1H, d), 6.86 (1H, d), 5.94 (1H, t), 4.32-4.18 (2H, m), 2.29-2.02 (2H, m), 2.01 (3H, s). $^{13}C$ NMR.($CDCl_3$) δ 170.5, 155.2, 130.6, 130.1, 120.4, 120.1, 116.9, 65.0, 61.8, 28.0, 21.1.

**(+)-4-Acetoxy-2,2-dimethylchroman**, (+)-**7**.-Reaction was stopped after 30 minutes at 23.4% of conversion, usual work-up and chromatography on $SiO_2$ with $CH_2Cl_2$/cyclohexane (8:2) afforded chemically and optically pure acetate (+)-**7** (e.e. 100%; $[\alpha]_D$ +54.8, c 0.228 in $CHCl_3$).

When the reaction was stoped after 24 h at 53.5% of conversion, chemically pure acetate (+)-**7** (e.e. 81.3%; $[\alpha]_D$ +44.6, c 0.628 in $CHCl_3$) was isolated. $^1H$ NMR.($CDCl_3$) δ 7.22 (1H, d), 6.90 (1H, t), 6.88 (1H, t), 6.00 (1H, t), 2.23 (1H, dd), 2.12 (3H, s), 1.98 (1H, dd), 1.42 (3H, s), 1.38 (3H, s).

**(+)-4-Acetoxy-2,2-dimethyl-5-methylchroman**, (+)-**9**.-Reaction was stopped after 48 h at 22.5% of conversion, usual work-up and chromatography on $SiO_2$ with $CH_2Cl_2$ afforded chemically and optically pure acetate (+)-**9** (e.e. 100%; $[\alpha]_D$ +31.6, c 1.93 in $CHCl_3$). $^1H$ NMR.($CDCl_3$) δ 7.14 (1H,t), 6.76 (1H, d), 6.71 (1H, d), 5.97 (1H, t), 2.20 (3H, s), 2.15 (2H, d), 2.08 (3H, s), 1.40 (3H, s), 1.38 (3H, s).

## REFERENCES AND NOTES


1. Lévai, A.; Toth, G.; Szòllosy, A.; Timàr, T., *Monatsh. Chem.* **1990**, *121*, 403-411.
2. G. P. Ellis, Ed., *Chromenes, Chromanones, and Chromones*, John Wiley & Sons, Ltd., New York, 1977.
3. Knezović, S.; Lévai, A.; Šunjić, V. *Tetrahedron: Asymmetry*, **1993**, *4*, 313.
4. Ružić, S. K.; Avdagić, A.; Cotarca, L.; Gelo-Pujić, M.; Šunjić, V. *Biocatalysis*, **1994**, *9*, 49-60.
5. Gelo, M.; Šunjić, V. *Tetrahedron*, **1992**, *48*, 6511.
6. Gelo, M.; Antolić, S.; Kojić-Prodić, B.; Šunjić, V. *Tetrahedron*, **1994**, in press.
7. Faber, K. *Biotransformations in Organic Chemistry*, Springer Verlag, 1992, pp. 86-90.
8. Parida, S.; Dordick, J. S. *J. Am. Chem. Soc.* **1991**, *113*, 2253-2259.
9. Nordin, O.; Hedenstrom, E.; Hogeberg, H.-E. *Tetrahedron: Asymmetry*, **1994**, *5*, 785-788.
10. Laumen, K.; Seemayer, R.; Schneider, M., *J. Chem. Soc. Chem. Commun.* **1990**, 49-51.
11. Langrand, G.; Secchi, M.; Buono, G.; Baratti, J.; Triantaphylides, C., *Tetrahedron Lett.* **1985**, *26*, 1857-1860.
12. Naemura, K.; Fukuda R.; Konishi, M.; Hirose, K.; Tobe, Y., *J. Chem. Soc. Perkin Trans. 1*, **1994**, 1253-1256.
13. Ref. 7, pp. 82-86.
14. Snatzke, G.; Snatzke, F.; Tökes, A. L.; Ràkosi, M.; Bògnar, R. *Tetrahedron*, **1973**, *29*, 909-912.
15. Timàr, T.; Jaszberenyi, J. Cs.; Kover, K. F.; Batta, Gy. *Acta Chim. Hung.* **1988**, *125*, 457-463.
16. Sebök, P.; Timàr, T.; Eszenyi, T.; Patonay, T.;*J. Org. Chem.* **1994**, *59*, 6318-6321.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