62. Quantitative Analysis of Enzyme-Mediated Hydrolytic Processes and Their Products Using HPLC and NMR Techniques

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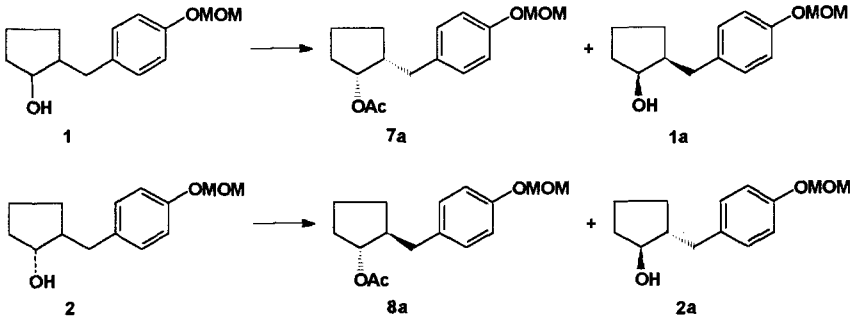
Second derivatives of the UV spectra of 2-(4-alkoxybenzyl)cyclopentan-1-ols and their acetates were used for the quantitative analysis of reaction kinetics of enzyme-mediated hydrolytic processes. To determine the enantiomeric purity of the products using non-chiral HPLC columns, their diastereoisomeric esters of 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (MTPA) were prepared. The absolute configuration of the products was established using a combination of the results of a HPLC analysis and $^1H/^{19}F$-NMR measurements of the diastereoisomeric MTP esters. The analytical method described consists of an easy routine HPLC analysis which can sometimes be used as a quick on-line analysis of the reaction kinetics and as a quick stepwise analysis of the optical purity and the absolute configuration of the products.

Introduction. – During our research, we often have to analyze reaction mixtures in which chiral compounds are products of enzyme-mediated resolution reactions involving racemic parent substrates. It is obvious that the UV spectra of the racemic parent substrates and the chiral products should be very similar or even identical. In this research, we focused our attention on the derivatives of 2-(4-alkoxybenzyl)cyclopentan-1-ols **1**–**6** (*Scheme 1*), the enantiomers of which are useful chiral intermediates in the synthesis of insect juvenile hormone analogs. Enzymic transesterifications studied with the isomers of 2-(4-methoxybenzyl)cyclopentanol (*i.e.*, with the structures analogous to **1** and **2**) has been published recently [1] [2]. The main products **1a**–**12a** (*i.e.*, the main chiral products **7a**–**12a** and the deracemized substrates **1a**–**6a**) of the enzymic process involving **1**–**6** as substrates are shown in *Scheme 1*, together with the corresponding minor products **1b**–**12b** of which were determined by the analytical method described below and are considered to be optical impurities of **1a**–**12a**.

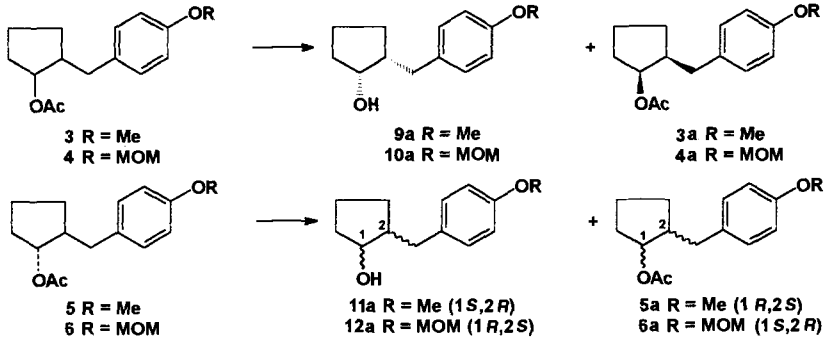
Due to the presence of an aromatic ring in the structure, the compounds **1**–**12** display characteristic UV spectra showing two maxima, at λ *ca.* 220 and 280 nm (*Fig. 1*). The latter of these two maxima is the lower one, but its second-order derivative shows important features, which may be used in the on-line HPLC analysis of reaction kinetics. The second-order derivative of the UV maximum at λ *ca.* 280 nm displays several thin minima, the intensity of which is dependent on the concentration of the compound studies in the reaction mixture. Such a concentration dependence was reported by *Piot* and coworkers to be linear and used to develop a quantitative HPLC analysis of aromatic

Scheme 1. *Processes Mediated by Selected Biochemical Systems*

ENZYMIC RESOLUTION: TRANSESTERIFICATION

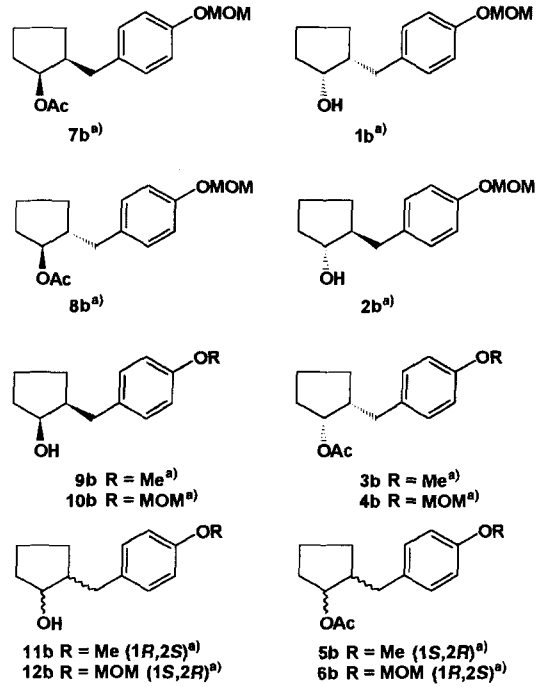


ENZYMIC RESOLUTION: HYDROLYSIS



MOM = $MeOCH_2$

[a]) Minor chiral products.



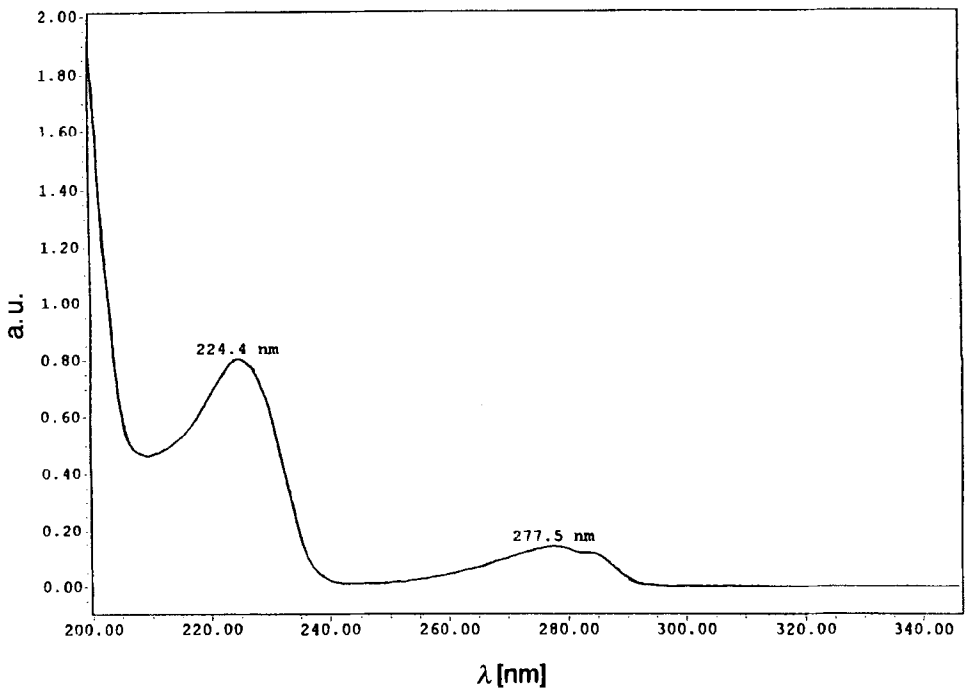


Fig. 1. *A typical zero-order UV spectrum of the compounds studied*

amino acid containing peptides in biological fluids [3]. We now applied this method to the quantitative analysis of low-molecular-weight organic compounds.

The method of *Piot* and coworkers is based on the dependence of the $\partial^2 A/\partial \lambda^2$ value (*i.e.*, the second derivative of the appropriate maximum of the UV spectra) on the concentration of the compound studied. The graphic line of this dependence, based on reference measurements, should be established for a sufficiently wide concentration range to cover the expected concentrations of the samples to be studied. Generally, the most characteristic feature of the second-order derivative spectrum is its 'very' minimum displayed at the same wavelength λ at which the zero-order UV spectrum displays its maximum [4]. On both sides (left and right) of this minimum in the second-order derivative spectrum, two 'very' maxima occur. To find the $\partial^2 A/\partial \lambda^2$ value, a line parallel to the y-axis (A, absorbency) should be drawn at the 'very' minimum of the second-order derivative spectrum (the x-axis value, λ). Another line should connect both neighboring maxima. The y-axis value of the point of intersection of both lines shows the $\partial^2 A/\partial \lambda^2$ value (*Fig. 2*).

Results and Discussion. – *General.* The samples of the reaction mixtures were taken periodically (*cf. Exper. Part* for details) and analyzed by reversed-phase HPLC. To prove the linearity of the dependence of the second-order derivative of the UV maximum found at λ *ca.* 280 nm (the $\partial^2 A/\partial \lambda^2$ values) on the concentration, each reference sample **1**–**6** was prepared in several concentrations and submitted to analysis. Results are shown in

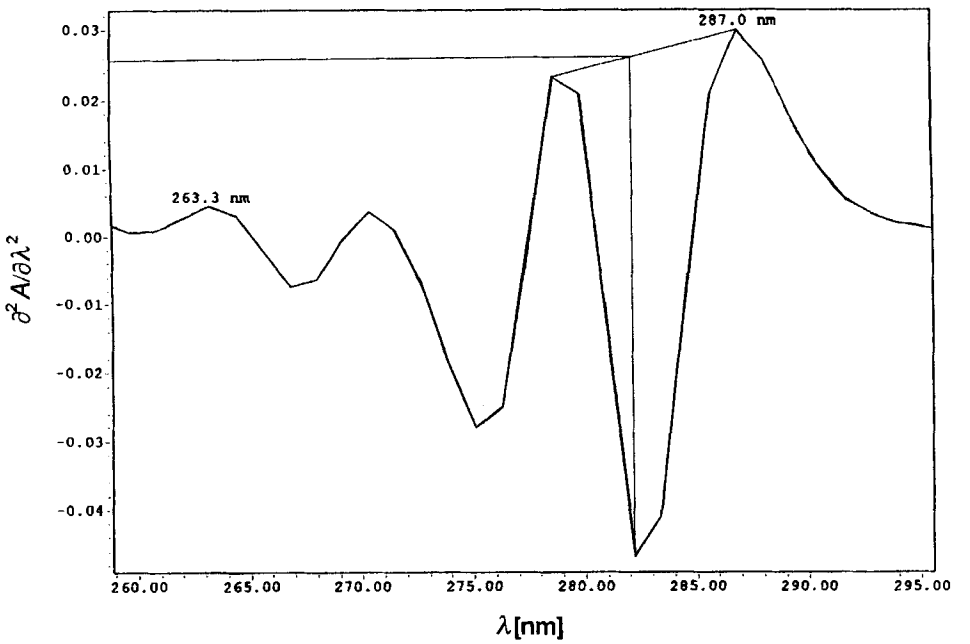


Fig. 2. *Second-order derivative of the maximum of the UV spectrum at λ 280 nm: a way for a graphic calculation of* $\partial^2 A/\partial\lambda^2$ (see text)

Fig. 3. This graphic dependence allows the determination of the concentration of each compound analyzed on the basis of the calculated value of the second derivative $\partial^2 A/\partial\lambda^2$ of the appropriate UV maximum. When the reaction kinetics is studied, during which the concentration of a substrate decreases and the concentration of a product increases simultaneously, the ratio of both compounds may be determined by an on-line analysis of the reaction mixture. This is illustrated by the results of such calculations for the reaction course of selected enzymic processes (*Figs. 4–6*). The method was very efficient for the determination of the moment of maximum conversion (*Fig. 6*) when a single enantiomer is expected to be produced by the enzymic process. Exceeding the maximum conversion (*i.e.*, 50% yield) indicates lower enantioselectivity of the process, *i.e.*, the enzyme employed is able to accept both enantiomers for the biotransformation, although with different reaction kinetics. The application of such a biochemical system to the production of chiral compounds of the type studied is inconvenient. *Figs. 4–6* show a variety in the course of the enzymic process studied, provided that different substrates were employed.

Enzyme-Mediated Transesterification. The behavior of the lipases employed for the enzyme-mediated transesterification of **1** depended on the absence or presence of molecular sieves in the reaction mixture. While the process mediated by porcine pancreatic lipase (PPL) was forwarded in the presence of molecular sieves (*i.e.* removal of as much H_2O as possible, even during the entire reaction period of 7 days), both the lipase from *Candida cylindracea* (CCL) and the lipase from *Geotrichum candidum* (GCL) preferred the absence of the molecular sieves (*i.e.* a certain quantity of H_2O in a reaction mixture)

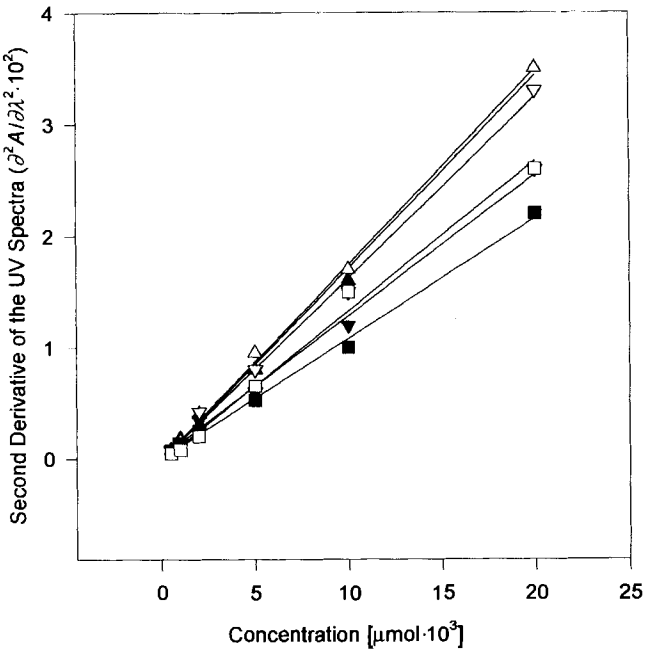


Fig. 3 *Calibration lines.* ▼ **1**; ▽ **2**; ▲ **3**; ■ **4**; △ **5**; □ **6**.

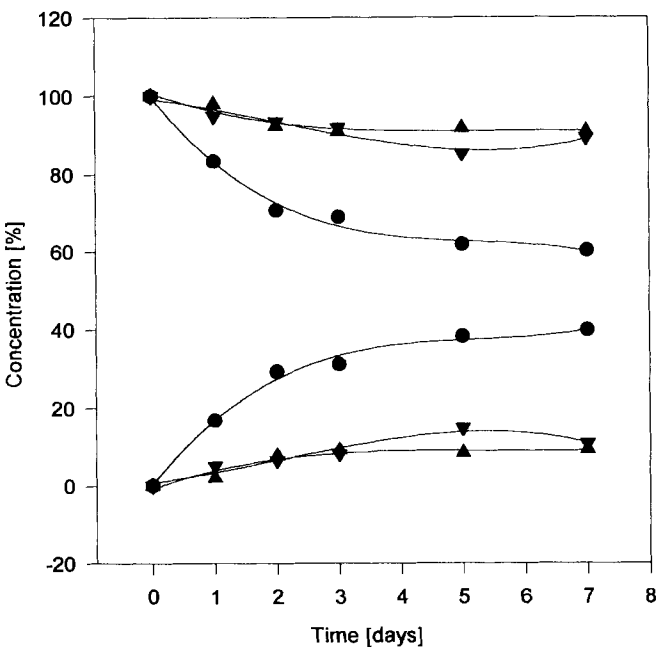


Fig. 4. *Enzyme-mediated transesterification of* **1**: *absence of molecular sieves.* ● PPL; ▼ GCL; ▲ CCL.

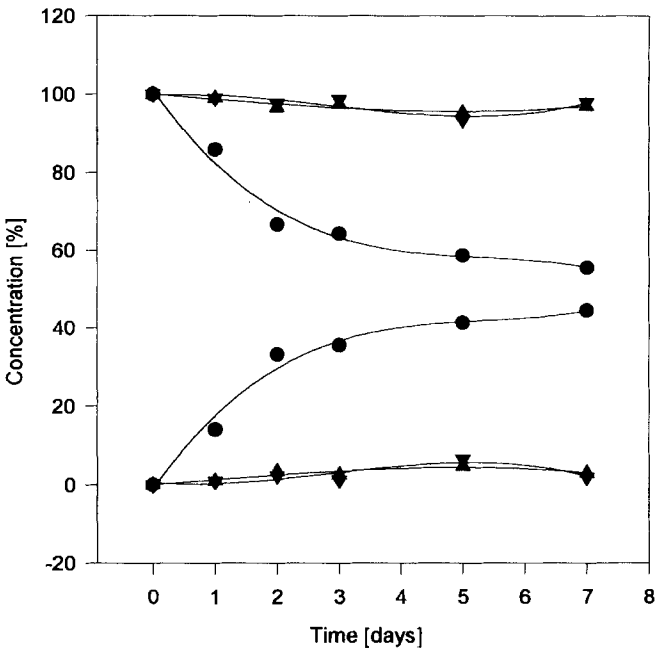


Fig. 5. *Enzyme-mediated transesterification of* **1**: *presence of molecular sieves.* ● PPL; ▼ GCL; ▲ CCL.

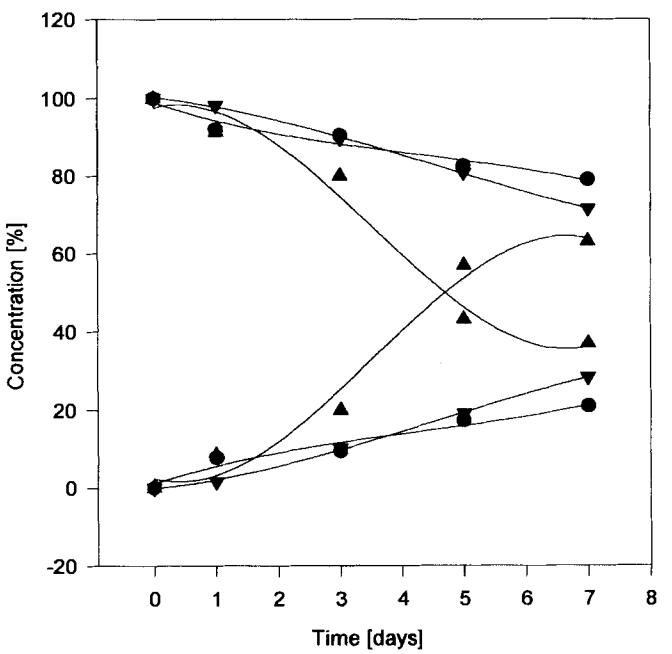


Fig. 6. *Enzyme-mediated hydrolysis of* **6**: *an example of an advantageous on-line HPLC analysis.* ● PPL; ▼ GCL; ▲ CCL.

to mediate the process towards improving the reaction yields. Among the three lipases studied, PPL was the best enzyme for mediating the transesterification of the substrates **1** and **2**.

Enzyme-Mediated Hydrolysis. Both *cis*-configurated 2-(4-alkoxybenzyl)cyclopentyl acetates **3** or **4** underwent enzymic hydrolysis with lower chemical yields than the *trans*-isomers **5** or **6**. The best results in this series of experiments were obtained with the GCL-mediated hydrolysis when substrates **3** and **4** were employed, and with the CCL-mediated reactions in the case of substrates **5** and **6**.

Determination of Enantiomeric Purity of the Products. Tables 1 and *2* summarize – among others – the enantiomeric purity of the main chiral products (ee_p) and that of the deracemized substrate (ee_s) resulting from successful enzymic reactions. Acetates **7a** or **8a** are considered to be the chiral products of the enzymic transesterification of **1** and **2**, respectively, while the untreated but chiral alcohols **1a** and **2a** are the deracemized substrates (see *Scheme 1*). Analogously, alcohols **9a**, **10a**, **11a**, and **12a** are the chiral products of the enzymic hydrolysis of **3**–**6**, whereas the acetates **3a**, **4a**, **5a**, and **6a** are the corresponding deracemized substrates. To calculate the enantiomeric-excess values (ee_p and ee_s, resp.), the ratio of the corresponding enantiomers **1a**–**12a**/**1b**–**12b** was determined by an analysis of diastereoisomeric derivatives of the respective chiral compounds.

To evaluate quantitatively the selected enzymic reactions, an earlier described method [5] was used. Accordingly, three key parameters allow an efficient evaluation of an enzyme-mediated resolution process: the optical purity of the product, expressed as enantiomeric excess of the chiral product (ee_p) or that of the deracemized substrate (ee_s), the extent of conversion of the racemic substrate c (*Eqn. 1*), and the enantiomeric ratio E (*Eqn. 2*). The great advantage of the E value consists in its independence of substrate concentration [5]. Thus, in general experiments with different substrate concentrations can be compared. In principle, the product of an enzyme-mediated resolution reaction can be recycled an infinite number of times to achieve absolute optical purity ($ee_p > 0.9999$). In reality, however, it would be more convenient to select an enzymic system with the enantiomeric ratio $E \geq 10$ and subject the product not more than twice

Table 1. *Enzyme-Mediated Transesterification: Selected Results*

Entry	Substrate	Enzyme	Main chiral product[a])	Chemical yield [%]	ee_p	Deracemized substrate[b])	ee_s	c[c])	E[d])
1	**1**	PPL	**7a(14a)**	37	0.98	**1a(14c)**	0.26	0.21	116.4
2	**1**	PPL[e])	**7a(14a)**	38	0.98	**1a(14c)**	0.58	0.37	288.3
3	**2**	PPL	**8a(16a)**	42	0.99	**2a(16c)**	0.47	0.32	2550
4	**2**	PPL[e])	**8a(16a)**	47	> 0.99	**2a(16c)**	> 0.99	0.50	1057
5	**2**	CCL	**8a(16a)**	42	0.25	**2a(16c)**	0.57	0.69	2.8
6	**2**	GCL	**8a(16a)**	20	0.99	**2a(16c)**	0.53	0.35	215.0

[a]) Analyzed *Mosher* ester in parentheses.
[b]) Deracemized substrate in chiral form; analyzed *Mosher* ester in parentheses.
[c]) c = extent of conversion of the racemic substrate.
[d]) e = enantiomeric ratio.
[e]) Experiment carried out in the presence of molecular sieves.

Table 2. *Enzyme-Mediated Hydrolysis: Selected Results*

Entry	Substrate	Enzyme	Main chiral product[a])	Chemical yield [%]	ee_p	Deracemized substrate[b])	ee_s	c[c])	E[d])
7	**3**	GCL	**9a(13a)**	22	0.95	**3a(13c)**	0.28	0.24	21.9
8	**5**	PPL	**11a(15c)**	38	> 0.99	**5a(15a)**	0.33	0.25	274.9
9	**5**	CCL	**11a(15c)**	55	> 0.99	**5a(15a)**	0.92	0.48	1986
10	**5**	GCL	**11a(15c)**	40	> 0.99	**5a(15a)**	0.24	0.20	61.9
11	**4**	GCL	**10a(14a)**	24	0.94	**4a(14c)**	0.29	0.24	31.2
12	**6**	CCL	**12a(16a)**	55	0.47	**6a(16c)**	0.02	0.04	2.9
13	**6**	GCL	**12a(16a)**	33	0.98	**6a(16c)**	0.60	0.38	174.2

[a]) Analyzed *Mosher* ester in parentheses.
[b]) Deracemized substrate in chiral form; analyzed *Mosher* ester in parentheses.
[c]) c = extent of conversion of the racemic substrate.
[d]) e = enantiomeric ratio.

to recycling to prepare enantiomers with $ee_p > 0.98$. The importance of the E value is based on this finding. The higher is the E value, the higher is the value of the corresponding enzyme-mediated process in a selection procedure aiming to use the optimum enzymic system for the production of a chiral compound with requested absolute configuration.

$$c = ee_s/(ee_s + ee_p) \quad (1)$$

$$E = \ln[(1 - c)\,(1 - ee_s)]/\ln[(1 - c)\,(1 + ee_s)] \quad (2)$$

Of the biochemical transformations described in *Tables 1* and *2*, only two showed an enantiomeric ratio $E < 10$ (*cf. Entries 5* and *12*), resulting in products with modest optical purity (ee_p 0.25 and 0.47, resp.). To the contrary, biochemical (enzymic) systems displaying an enantiomeric ratio $E > 10$ were able to produce the desired structures with optical purities $ee_p > 0.98$. Generally, PPL was the most successful biochemical system capable of producing enantiomers of 2-(4-alkoxybenzyl)cyclopentanol with a high optical purity. Nevertheless, a relationship between the substrate structure and the enzyme exists, which shows that even the less successful enzymic system (such as that using CCL) may accept selected structures with high stereospecificity (*cf. Entry 9 vs. Entries 5* and *12*).

Absolute Configuration of the Products. The assignment of the absolute configuration of the chiral compounds **1a**–**12a** and **1b**–**12b** was based on the evaluation of the ^{1}H- and ^{19}F-NMR spectra of appropriate diastereoisomeric derivatives. The acetates **7a** (= **4b**), **7b** (= **4a**), **8a** (= **6b**), **8b** (= **6a**), **3a**, **3b**, **5a** and **5b** were first transformed by alkaline hydrolysis to the corresponding alcohols **1b** (= **10a**), **1a** (= **10b**), **2b** (= **12a**), **2a** (= **12b**), **9b**, **9a**, **11b**, and **11a** which were esterified using 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (= α-methoxy-α-(trifluoromethyl)phenylacetic acid; MTPA, *Mosher*'s acid) [6–9] (*Scheme 2*). The *Mosher* esters **13a,c**, **14a,c**, **15a,c**, and **16a,c** were derived from (*R*)-MTPA, while **13b,d**, **14b,d**, **15b,d**, and **16b,d** were obtained with (*S*)-MTPA. Analysis was only made in the case of the experiments described in *Tables 1* and *2*. *Table 3* shows ^{1}H- and ^{19}F-NMR chemical-shift values of selected signals

of **13a,c**, **14a,c**, **15a,c**, and **16a,c**, (derived from **1a–12a** and **1b–12b**) which are essential for assigning the absolute configuration. The principle of evaluation of the NMR spectra of diastereoisomeric compounds was based on a general paper of *Rinaldi* [10], on a recent paper dealing with a high-field-NMR modification *Mosher*'s method [11], and on our most recent paper [2] in which the assignment of the absolute configuration of 1,2-disubstituted cyclopentane systems is described in detail.

The NMR analysis of the *Mosher* esters **13a–d**, **14a,b**, **15a–d**, and **16a–d** (*Scheme 2*) was complicated due to the conformational flexibility of the five-membered ring. Considering steric interactions, the energy difference between axial and equatorial methylcyclopentane is likely to be substantially lower than between the corresponding methylcyclohexane conformers (0.54 kcal mol^{-1} *vs.* 1.70 kcal mol^{-1}) [12] [13]. It is evident from the ^{1}H-NMR spectra of the 2-substituted cyclopentanols described above or of their *Mosher* esters that they do not exist as single discrete conformers; rather they undergo pseudorotation through several potential minima which are separated by low energy barriers. Nevertheless, the differences in the chemical shift of H–C(1) and in the sum of the coupling constants of the corresponding signal (*m*) allowed to confirm the relative configuration at C(1) and C(2) (*cis*-isomers: 5.27 ppm ($\Sigma J \approx 11$ Hz, H–C(1)); *trans*-isomers: 5.11 ppm ($\Sigma J > 17$ Hz, H–C(1))). These values suggest a pseudosequatorial arrangement of both H–C(1) (deshielded) and the $ArCH_2$ substituent in the *cis*-isomers, whereas in the *trans*-derivatives, H–C(1) rather adopts a pseudoaxial position when the bulkier $ArCH_2$ substituent is assumed to be stabilized in pseudoequatorial position. The data presented here for 2-substituted cyclopentanols are very similar to those published by *Guyon et al.* [14].

The absolute configuration of the chiral products and of the deracemized substrates **1a–12a** and **1b–12b** (*Scheme 1*) was assigned on the basis of the chemical-shift difference between the H-atoms of the $ArCH_2$ moiety in the ^{1}H-NMR spectra of the diastereoisomer *Mosher* esters among **13a–d**, **14a–d**, **15a–d**, and **16a–d**. The chemical-shift values of these CH_2 protons were of fundamental importance in the determination of the absolute configuration of the diastereoisomeric *Moshers* esters [10]. Considering the observed findings, the CF_3 group and the carbonyl O-atom are eclipsed so that the preferred conformation of the system displays the carbonyl H-atom (H–C(1)) eclipsed with the carbonyl group. An extended *trans* ester conformation places one of the groups close to the phenyl ring. This group is, therefore, more shielded and thus shifted to higher field [2]. The theoretical background for the explanation of the changes in the ^{19}F-NMR spectra is based on the differenes in shielding/deshielding of the CF_3 moiety by the carbonyl group. The coexistence of a bulky group and the phenyl moiety on the same side of the CF_3–C–CO–CH plane produces a deviation of the CF_3 group from this plane, and results in an increase of the shielding of the appropriate signal. Due to this mechanism, the final effect results in the different signs of the chemical shifts in the ^{1}H- and ^{19}F-NMR spectra. Based both on the known absolute configuration at the chiral C(1) center, and on the relative configuration at C(1) and C(2), the absolute configuration at C(2) can be unambiguously established [2] [10].

HPLC Analysis. Finally, a relationship between the absolute configuration of the *Mosher* esters and their retention times on HPLC analysis was studied. *Table 4* summarizes the results obtained with the *Mosher* esters derived from (+)-(*R*)-MTPA and shows that the retention time t_R of the esters with (2*R*)-configuration at the cyclopentane unit

Table 3. *Selected NMR Data of* **1a**–**12a** *and* **1b**–**12b** (*Scheme 1*) *and of* Mosher *Esters* (*Scheme 3*)[a])

Products (corresp. *Mosher* Esters)		Abs. Configuration of the product		^{19}F-NMR (*Mosher* esters)		^{1}H-NMR					
						ArCH_2 (*Mosher* esters)		MeO (*Mosher* esters)		arom. H	
major	minor	major	minor	major	minor	major	minor	major	minor	major	minor
7a(14a)	**7b(14c)**	(1*R*,2*R*)	(1*S*,2*S*)	− 67.36	− 67.22	2.51, 2.70	2.41, 2.62	3.531	3.595	6.92, 6.98	6.89
1a(14c)	**1b(14a)**	(1*S*,2*S*)	(1*R*,2*R*)	− 67.22	− 67.36	2.41, 2.62	2.51, 2.70	3.595	3.531	6.89	6.92, 6.98
8a(16a)	**8b(16c)**	(1*R*,2*S*)	(1*S*,2*R*)	− 67.75	− 67.84	2.49, 2.74	2.42, 2.72	3.463	3.507	6.95, 7.07	6.93, 7.02
2a(16c)	**2b(16a)**	(1*S*,2*R*)	(1*R*,2*R*)	− 67.84	− 67.75	2.42, 2.72	2.49, 2.74	3.508	3.463	6.93, 7.02	6.95, 7.07
9a(13a)	**9b(13c)**	(1*R*,2*R*)	(1*S*,2*S*)	− 67.39	[b])	2.50, 2.71	[b])	3.534	[b])	6.79, 6.99	[b])
10a(14a)	**10b(14c)**	(1*R*,2*R*)	(1*S*,2*S*)	− 67.36	[b])	2.51, 2.70	[b])	3.531	[b])	6.92, 6.98	[b])
3a(13c)	**3b(13a)**	(1*S*,2*S*)	(1*R*,2*R*)	− 67.22	− 67.39	2.41, 2.62	2.50, 2.71	3.596	3.534	6.75, 6.91	6.79, 6.99
4a(14c)	**4b(14a)**	(1*S*,2*S*)	(1*R*,2*R*)	− 67.22	− 67.36	2.41, 2.62	2.51, 2.70	3.596	3.531	6.89	6.92, 6.98
11a(15c)	**11b(15a)**	(1*S*,2*R*)	(1*R*,2*S*)	− 67.85	− 67.76	2.42, 2.71	2.49, 2.74	3.505	3.466	6.79, 7.02	6.82, 7.07
12a(16a)	**12b(16c)**	(1*R*,2*R*)	(1*S*,2*R*)	− 67.75	[b])	2.49, 2.74	[b])	3.463	[b])	6.95, 7.07	[b])
5a(15a)	**5b(15c)**	(1*R*,2*S*)	(1*S*,2*R*)	− 67.76	− 67.84	2.49, 2.74	2.42, 2.71	3.467	3.505	6.82, 7.07	6.79, 7.02
6a(16c)	**6b(16a)**	(1*S*,2*R*)	(1*R*,2*S*)	− 67.84	− 67.75	2.42, 2.72	2.49, 2.74	3.507	3.463	6.93, 7.02	6.95, 7.07

[a]) *Cf. Experimental* for the conditions of the NMR measurements.
[b]) Minor product not interpretable by NMR.

are longer than those of the corresponding esters with (2*S*)-configuration. Referring to *Scheme 1* which shows the major chiral products, the major deracemized substrates, and their minor opposite enantiomers resulting from the enzymic reactions, then the $\Delta(t_R(\text{maj.})\text{-}t_R(\text{min.}))$ value (see *Table 4*) corresponds with the absolute configuration of the alcohol or acetate studied. If this Δ value is positive, the major compounds studied posses the (2*R*)-configuration at the cyclopentane ring, and if Δ is negative, the major compounds studied posses the (2*S*)-configuration. These results were obtained after hydrolysis of the chiral acetates **7a** (= **4b**), **7b** (= **4a**), **8a** (= **6b**), **8b** (= **6a**), **3a**, **3b**, **5a** and **5b** to the corresponding chiral alcohols **1b** (= **10a**), **1a** (= **10b**), **2b** (= **12a**), **2a** (**12b**), **9b**, **9a**, **11b**, and **11a** (*Scheme 2*). In several cases, an enzymic formation or removal of the acetic moiety according to *Scheme 1*, and a subsequent alkaline removal of the acetic moiety from the structures for analytical purposes (*Scheme 2*) result in identical chiral structures, but not in identical ee values, which can be demonstrated by *Scheme 1*: the formation of the chiral acetate couple **7a**/**7b** by transesterification of **1** *vs.* the formation of a chiral alcohol couple **10a**/**10b** by enzymic hydrolysis of **4** *etc.* *Scheme 2* illustrates the chemical transformation of the chiral acetates **7a** and **7b** into the alcohols identical with **10a** and **10b** by their absolute configuration but not by their enantiomeric purity. Several other examples may be traced by comparing *Schemes 1* and *2*. No evidence is available so far for a general validity of the above identified rule connecting the $\Delta(t_R)$ and the absolute configuration at C(2) of the cyclopentane ring; however, studies will also focus on this topic during a future investigation.

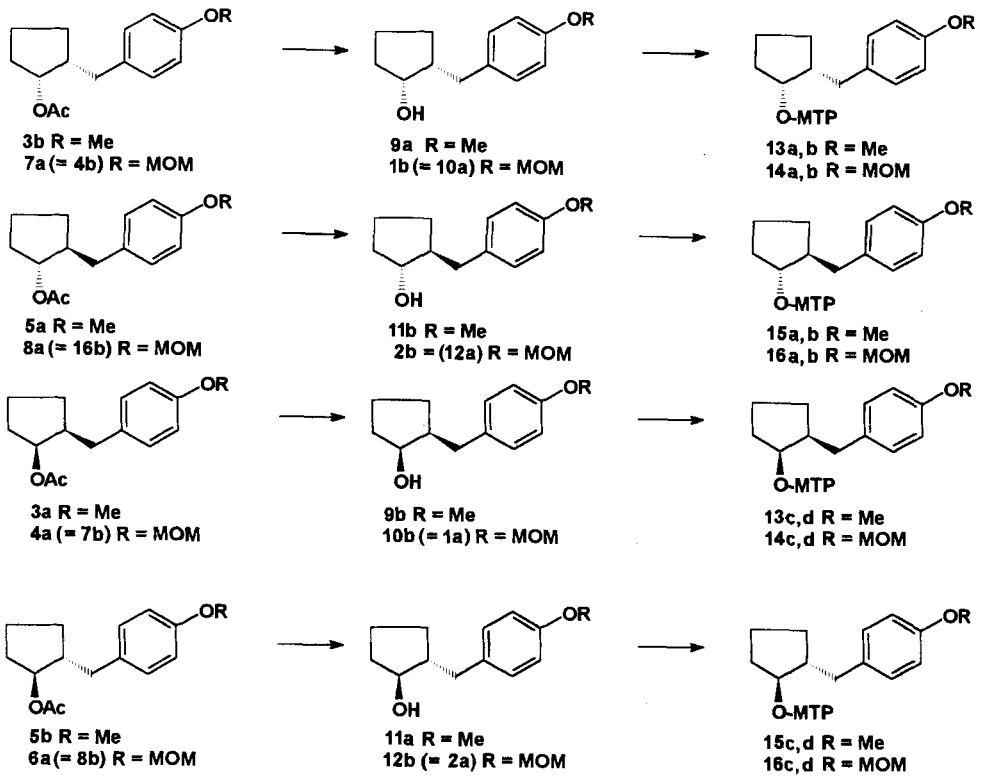
Table 4. *HPLC Analysis of the* (R)*-MTP Ester: Retention Times* t_R [min]

Acetate ((*R*)-MTP ester)	t_r(ester)[a]	$\Delta(t_R(\text{maj.}) - t_R(\text{min.}))$[b]	Alcohol ((*R*)-MTPA ester)	t_R(ester)[a]	$\Delta(t_R(\text{maj.}) - t_R(\text{min.}))$[b]
7a(**14a**)	22.70	+ 1.18	**1a**(**14c**)	21.61	– 1.12
7b(**14c**)	21.52		**1b**(**14a**)	22.73	
8a(**16a**)	19.53	– 0.62	**2a**(**16c**)	20.12	+ 0.37
8b(**16c**)	20.15		**2b**(**16a**)	19.75	
3a(**13c**)	23.80	– 1.24	**9a**(**13a**)	24.98	+ 1.20
3b(**13a**)	25.04		**9b**(**13c**)	23.78	
5a(**15a**)	19.90	– 0.66	**11a**(**15c**)	20.74	+ 0.66
5b(**15c**)	20.56		**11b**(**15a**)	20.08	

[a]) t_R of the appropriate (*R*)-MTP ester.
[b]) t_R of the *Mosher* ester of the major compound minus t_R of that of the minor compound.

The explanations given in the preceding paragraph on the absolute configuration of the chiral products were also supported by a difference in the chromatographic behavior of the *Mosher* esters. The diastereoisomer couples **13a**/**13c** and **14a**/**14c** derived from the chiral *trans*-alcohols. This finding can also be explained by an easier conformational flexibility occurring in the respective diastereoisomeric esters **15a**/**15c** and **16a**/**16c** in comparison with **13a**/**13c** and **14a**/**14c** (*cf. Table 4*).

Scheme 2. *Chemical Transformations of the Chiral Products and the Deracemized Substrates of the* Mosher *(MTP) Esters*



MOM = $MeOCH_2$, MTP = (*R*)- or (*S*)-PhC(MeO)(CF_3)C(=O)

Experimental. – *General.* Separations of the reaction mixtures were performed by column chromatography on silica gel (*Herrmann*, Köln-Ehrenfeld, Germany). TLC: precoated silica-gel plates. HPLC: Reaction kinetics on a *Waters* instrument consisting of a *Waters 600* controller, a *Waters 717 plus* autosampler, and a *Waters 996* photodiode array detector, *Sepharon Si-C18* (250 × 3.9 (i.d.) mm) reversed-phase column, $MeCN/H_2O$ 3:2 as mobile phase, flow rate 1.0 ml min^{-1}; analyses of the diastereoisomeric *Mosher* esters on a *TSP (Thermoseparation Products)* instrument equipped with a *ConstaMetric 4100 Bio* pump, a *SpectroMonitor 5000 UV DAD*, and a series of 3 columns (250 × 4(i.d.) mm each) filled with a *Biosphere Si-100* (5 μm) silica-gel phase, light petroleum ether/Et_2O 9:1 as mobile phase, flow rate 1.0 ml min^{-1}. Spectra: *Varian Unity 500* spectrometer (FT mode) at 499.8 (^1H) or 470.27 MHz (^{19}F); in $CDCl_3$ rel. to. $SiMe_4$ as internal reference (^1H) or rel. to hexafluorobenzene as external reference (δ −162.9 ppm; ^{19}F).

Enzymes. Porcine pancreatic lipase (PPL; 135 U mg^{-1}; *Sigma*), lipase from *Candida cylindracea* (CCL; 1010 U mg^{-1}; *Sigma*), and lipase from *Geotrichum candidum* (GGL; activity not given; *Amano*) were used to mediate the enzymic reactions.

Enzyme-Mediated Transesterification. A lipase (PPL, CCL, or GCL; 20 mg) was added to a soln. of the substrate **1** or **2** (0.23 mmol) in vinyl acetate (2 ml), in some cases (see *Table 1*) in the presence of molecular sieves (20 mg). The reaction was performed in a glass vial under stirring at r.t. for 7 days. Samples were taken after 24, 48, 72, and 120 h. Final workup consisted in filtering the enzyme off, evaporation of the solvent, and chromatographic separation of the residue. Chemical yields of the products of selected successful reactions are shown in

Table 1, and the analysis of the reaction kinetics of the process is demonstrated in *Figs. 4* and *5*, which focus on the difference observed in the reaction kinetics of the same enzymic process in the absence or in the presence of molecular sieves.

Enzyme Mediated Hydrolysis. A lipase (PPL, CCL, or GCL; 20 mg) was added to a suspension of the substrates (**3** and **5**, 0.20 mmol; **4** and **6**, 0.18 mmol) in a phosphate buffer (2 ml, pH 6.5). The reaction was performed in glass vials under stirring at r.t. for 7 days. Samples were taken after 24, 72, and 120 h. Final workup consisted in extracting the org. compounds into AcOEt, drying the extract (Na_2SO_4), evaporation of the solvent, and chromatographic separation of the residue. Chemical yields of the products of selected successful reactions are shown in *Table 2*, and the analysis of the reaction kinetics of the process is demonstrated in *Fig. 6*, which focus on the advantage of the on-line analysis for a precise determination of the time of 50% conversion (observed after the fifth day).

Alkaline Hydrolysis of Chiral Acetates. In a typical experiment, a 10% soln. of KOH in MeOH (1 ml) was added to a soln. of acetates **7a,b**, **8a,b**, **3a,b**, **4a,b**, **5a,b**, or **6a,b** (0.05 mmol) in MeOH (0.2 ml). The mixture was allowed to stand overnight at r.t. After evaporation of an excess of MeOH, the residue was purified by column chromatography (silica gel), affording the chiral alcohols **1b,a**, **2b,a**, **9b,a**, **10b,a**, **11b,a**, or **12b,a**, resp., in 80–90% yield.

Synthesis of the Mosher *Esters.* In a typical experiment, a soln. of either of the enantiomers of 3,3,3-trifluoro-2-methyl-2-phenylpropanoyl chloride (0.07 mmol) in benzene (500 µl) and a soln. of 4-(dimethylamino)pyridine (0.005 mmol) in pyridine (30 µl) were added to a soln. of the chiral alcohol **1b,a**, **2b,a**, **9b,a**, **10b,a**, **11b,a** or **12b,a** (0.05 mmol) in benzene (200 µl). The mixture was either stirred or allowed to stand at r.t. for 3–5 h. Thereafter, benzene was evaporated and the residue dissolved in light petroleum ether and purified by column chromatography (silica gel), affording the respective esters **13a–d**, **14a–d**, **15a–d**, and **16a–d** in 85–95% yields.

Conclusion. – Comparing the results shown in *Tables 1–3* and those shown in *Figs. 4–6*, several optimum enzymic processes may be traced out. The biochemical system using PPL was found to mediate successfully the enzymic transesterification of the racemic substrates **1** and **2** producing **7a** and **8a**, respectively, with high enantioselectivity. Moreover, a modification of the procedure allowed the preparation of **8a** and **2a** with enantiomeric purities of ee > 0.99 in a one-step reaction (*Table 1*, *Entry 4*). Multiple recycling could be required to prepare **1a** with the same enantiomeric purity. Concerning the influence of molecular sieves on the enzymic transesterification, it should be pointed out that the presence or absence of molecular sieves was the only modification of the reaction conditions. Therefore, the results showing the effect of removal of H_2O from the system by molecular sieves are of great importance. *Figs. 4* and *5* demonstrate that the enzymic transesterification may be forwarded or impeded by the presence of molecular sieves. The lipases employed reacted in different ways to the presence of molecular sieves, a phenomenon which is in coincidence with the different affinity of these lipases to H_2O. However, the proper cause of this effect was not studied.

All three biochemical systems hydrolyzed the substrate **5** with high enantioselectivity to result in chiral alcohol **11a**. Among them, only the system using CCL was able to produce the by-product **5a** with an ee of 0.92 in a one-step procedure. Enantioselective enzyme-mediated hydrolysis of the substrates **3**, **4**, and **6** was observed using a GCL-based biochemical system (*Table 2*, *Entries 7*, *11*, and *13*), but the deracemized substrates **3a**, **4a**, and **6a**, respectively, were obtained only with modest enantiomeric purity.

Second-order derivatives of the UV spectra of the reaction products in an on-line HPLC analysis of the crude reaction mixtures were found to be a convenient way to rapidly analyze the reaction kinetics, thus allowing to determine the appropriate time for stopping enzymic reactions. The method can also assist in optimizing conditions.

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