BIOTRANSFORMATION OF 2-(4-METHOXYBENZYL)-1-CYCLOHEXANONE BY MEANS OF *Saccharomyces cerevisiae*

Zdeněk WIMMER, Miloš BUDĚŠÍNSKÝ, Tomáš MACEK, Aleš SVATOŠ, David ŠAMAN, Soňa VAŠÍČKOVÁ and Miroslav ROMAŇUK

Institute of Organic Chemitry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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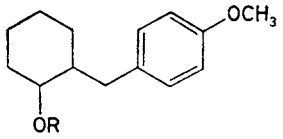
Biotransformation reduction of 2-(4-methoxybenzyl)-1-cyclohexanone was investigated. A method was elaborated for the preparation of some diastereoisomeric alcohols derived from the racemic title ketone and for the determination of the products obtained. The study resulted in the synthesis of *cis*-(1*S*, 2*S*)-(+)-2-(4-methoxybenzyl)-1-cyclohexanol and *trans*-(1*S*, 2*R*)-(+)-2-(4-methoxybenzyl)-1-cyclohexanol in optical purity of at least 91·6 ± 1·0% and 97·5 ± 1·0%, respectively.

In our previous paper[1] we reported on the determination of conformations of isomeric *cis*- or *trans*-1-acyloxy-2-(4-methoxybenzyl)-cyclohexanes. Both isomeric series of compounds were obtained on reduction of 2-(4-methoxybenzyl)-1-cyclohexanone with lithium aluminum hydride and subsequent esterification of the chromatographically separated isomeric 2-(4-methoxybenzyl)-1-cyclohexanols with corresponding acyl chloride. The *cis*- or *trans*-esters *III* and *IV* obtained were racemic. On the basis of the width of the multiplets of H-1 signals in the ^{1}H NMR spectra of compounds *I*–*IV* we could demonstrate that in the *trans*-isomers both substituents assume equatorial positions, while in the *cis*-isomers the *p*-methoxybenzyl group is equatorial and the hydroxy or the acyloxy group is axial.

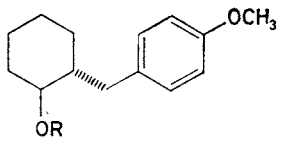
In this study we used the yeast *Saccharomyces cerevisiae* for the reduction of 2-(4-methoxybenzyl)-1-cyclohexanone. We found that the reduction proceeds stereospecifically with a high enantiomeric excess of one of the diastereoisomers with *cis*- or *trans*-configuration. We used either NMR spectroscopy or a few step chemical correlation with the described optically active *cis*- or *trans*-hexahydro-2(3*H*)benzofuranones, respectively, for the determination of absolute configurations on the atoms C(1) and C(2) in the alcohols *I* and *II* obtained.

The principle of the NMR determination of absolute configuration requires the preparation of a pair of diastereoisomeric compounds, or at least, of diastereoisomeric dynamic complexes with the compound of a known absolute configuration and of interpretable shielding effects in NMR spectra[2]. For the purposes of the synthesis of diastereoisomeric esters of the isomers of 2-(4-methoxybenzyl)-1-cyclohexanols obtained, with 2-methoxy-2-phenyl-3,3,3-trifluoropropanoic acid (MTPA),

we elaborated a method for microscale preparation[3], which we found better than the classical method of preparation of esters by means of the chloride of this chiral acid[4-7]. The absolute configuration at the carbon atom carrying the esterified



I, R = H
III, R = COX
V, R = (*R*)-MTPA
VI, R = (*S*)-MTPA

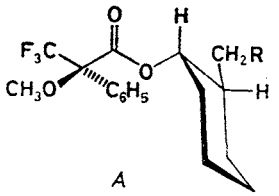


II, R = H
IV, R = COX
VII, R = (*R*)-MTPA
VIII, R = (*S*)-MTPA

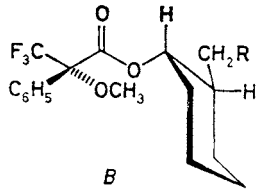
X = CH_3, $CH(CH_3)_2$, $C(CH_3)_3$

hydroxyl can be determined from the differences of the chemical shifts of the hydrogen signals of the substituents of the investigated carbon atoms in the pair of the diastereoisomeric esters with (*R*) or (*S*) configuration of MTPA. Another method, especially suitable in cases where the required chemical shifts of the hydrogen signals of substituents are not available, is based on differing ^{19}F NMR shifts of the CF_3 group signals in diastereoisomeric esters with known absolute configurations of the MTPA moiety. In this paper we also tested the possibility of using ^{13}C NMR spectra of diastereoisomeric MTPA ester, which has been neglected so far.

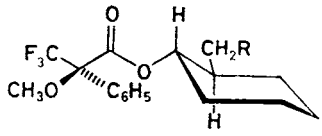
Two pairs of diastereoisomeric MTPA esters *V*, *VI* and *VII*, *VIII* were prepared from the respective alcohols *I* and *II* by means of (*R*)- and (*S*)-MTPA. Their ^1H NMR spectra (Table I) proved identical conformational behaviour of the substituents as shown[1] for alcohols *I*, *II* and acyloxy derivatives *III*, *IV*. Since the signals of hydrogens in positions 2 and 6 in the spectra are components of a complex multiplet, together with the signals of further hydrogens of the molecule, only the signals of hydrogens H-7 and H-7′ were available for the determination of the absolute configuration at C(1). A detailed comparison of the ^1H NMR spectra of MTPA esters of the *cis*-alcohol *I* indicated that the signals of hydrogens H-7 and H-7′ in (*R*)-MTPA-ester *V* are shifted upfield in comparison with the (*S*)-MTPA-ester *VI* (δ 2·46 and 2·25 in comparison with δ 2·52 and 2·33). An upfield shift is observed for the known preferred conformation of the MTPA esters[2] (formulae *A*–*D*), caused by the shielding effect of the phenyl group, explicable only for the (*S*)-configuration at C(1). Partial conformational formulae *A* and *B* belong to the MTPA esters *V* and *VI* prepared, and from them it is evident that in the (*R*)-MTPA-ester *A* the phenyl group is closer to the $C(7)H_2$ hydrogens, causing an upfield shift of their signals in comparison with the (*S*)-MTPA-ester *B*. In an analogous manner the absolute



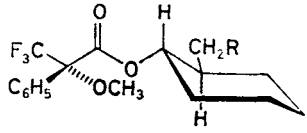
A



B



C



D

R = $C_6H_4OCH_3$

configuration at C(1) in the *trans*-alcohol *II* was also determined. The upfield shifts of the signals H-7 and H-7′ in (*R*)-MTPA-ester *VII* in comparison with the (*S*)--MTPA-ester *VIII* (δ 2·71 and 2·08 *versus* δ 2·89 and 2·18) again lead to the (*S*)--configuration at C(1) and the situation indicated by partial conformational formulae *C* and *D*.

The ^{19}F NMR spectra lead to the same conclusion on the (*S*)-configuration at C(1). The upfield shift of the signal of CF_3 in ester *V*, with respect to *VI* (δ −8·05 *versus* −7·90) and an analogous difference in the pair of esters *VII* and *VIII* (δ −7·73 *versus* −7·62) is in agreement with the expected protruding of the CF_3 group from the eclipsed arrangement with the carbonyl, in consequence of steric interaction of the bulkier groups[2] (in our case phenyl and the C(2)-substituent), which is operative in the (*R*)-MTPA-esters *V* and *VII* (formulae *A* and *C*).

The ^{13}C NMR chemical shifts of starting alcohols *I* and *II* and the MTPA esters *V*–*VIII* are listed in Table I. The assignment of the signals was done on the basis of the chemical shift values, of the number of directly bound hydrogen atoms and of the known acylation effects. In principle, for the determination of the absolute configuration at C(1), the signals of close carbon atoms on both sides from C(1) should be utilisable, *i.e.* C(6) on one side, and C(2) and C(7) on the other. Using the same model as in the interpretation of the ^1H NMR spectra with the dominant shielding effect of the phenyl, the observed upfield shift of the signal C(7) in (*R*)--MTPA-esters in the pairs *V*, *VI* and *VII*, *VIII* (see Tables I and II) may be again interpreted in the sense of (*S*)-configuration at C(1) in alcohols *I* and *II*. For C(2) and C(6), however, the observed difference of the shifts in both pairs of esters has

the same sign, which is in contradiction with the expectation (+ for C(6), but − for C(2)). Hence, it may be assumed that in addition to the orientation of the phenyl group, further effects (for example certain conformational differences in the pairs of diastereoisomeric esters) also contribute to the observed values of the shifts of C(2) and C(6) and thus prevent their utilisation for the given purpose. From an

TABLE I

^1H, ^{13}C, and ^{19}F NMR parameters of alcohols *I*, *II* and corresponding MTPA-esters *V*–*VIII* in deuteriochloroform

Parameter	*I*	*V*	*VI*	*II*	*VII*	*VIII*	*E* + *F*[a]
δ(H-1)	3·80	5·19	5·16	3·28	4·80	4·79	—
W(H-1)[b]	9·0	9·4	11·0	24·0	24·4	23·5	—
δ(H-7)	2·66	2·46	2·52	3·07	2·71	2·89	3·15
δ(H-7′)	2·48	2·25	2·33	2·33	2·08	2·18	2·40[c]
J(7, 2)	7·5	6·8	6·7	4·0	3·0	3·2	4·2
J(7′, 2)	7·5	8·0	8·0	8·9	9·8	9·9	—
J(7, 7′)	−13·6	−13·8	−13·6	−13·5	−13·4	−13·5	−13·3
δ(H-10, 12)	6·82	6·79	6·80	6·82	6·77	6·81	6·80
δ(H-9, 13)	7·10	6·93	6·95	7·09	6·89	6·99	7·06
δ(H-14)	3·78	3·78	3·78	3·78	3·77	3·78	3·77
				^{13}C NMR			
δ(C-1)	68·47	75·45	75·78	74·35	79·57	79·79	212·84
δ(C-2)	43·62	42·79	42·67	47·00	43·84	43·52	52·61
δ(C-3)	26·30	26·56	26·61	29·92	29·55	29·41	34·39[d]
δ(C-4)	25·23	25·00	25·04	25·38	24·75	24·61	24·96
δ(C-5)	20·32	20·76	20·35	24·85	24·41	24·25	28·00
δ(C-6)	33·19	29·98	29·85	35·70	31·52	30·96	42·08
δ(C-7)	37·65	37·34	37·88	37·96	37·19	37·49	33·30[d]
δ(C-8)	132·97	132·06	131·98	132·64	131·73	131·63	132·22
δ(C-9, 13)	129·91	129·83	129·83	130·19	130·15	130·11	129·96
δ(C-10, 12)	113·59	113·72	113·76	113·52	113·59	113·67	113·65
δ(C-11)	157·66	157·88	157·90	157·65	157·86	157·91	157·81
δ(C-14)	55·16	55·22	55·23	55·13	55·22	55·21	55·16
				^{19}F NMR			
δ(CF_3)	—	−8·05	−7·90	—	−7·73	−7·62	—

[a] Racemic 2-(4-methoxybenzyl)-1-cyclohexanone, [b] width of the multiplet of H-1 (the sum of couplings *J*(1, 2), *J*(1, 6), and *J*(1, 6′)); [c] multiplet of 4 H (H-2, H-6, H-6′, H-7′); [d] interchange possible.

analysis of models (structures *A* – *D*), it is evident that C(7) has the best precondition (distance, angle) for indicating the orientation toward phenyl by its chemical shift in the pairs of the MTPA esters and for affording the necessary data for the determination of absolute configuration at C(1). Hence, it is evident that, when using ^{13}C NMR data of MTPA esters for the determination of absolute configuration, great care must be taken and those carbon atoms selected the shifts of which should be used for the deduction of the configuration.

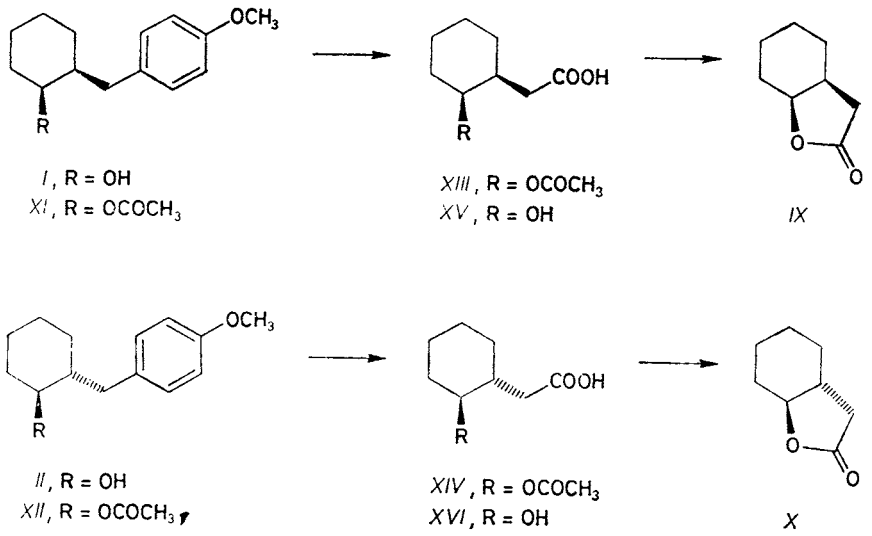
From the determined (*S*) absolute configuration at C(1) and the known relative configurations of substituents in positions 1 and 2 the absolute configuration at C(2) also follows. The prepared alcohols can thus be structurally defined as *cis*-(1*S*, 2*S*)- or *trans*-(1*S*, 2*R*)-2-(4-methoxybenzyl)-1-cyclohexanol, and the formulae *I* and *II*, repectively, show their absolute configurations.

The checking of this result was made by chemical correlation of both respective alcohols *I* and *II* with the known respective enantiomers of *cis*- and *trans*-hexahydro-2(3*H*)benzofuranone. The alcohols *I* and *II* were first converted to acetoxy derivatives *XI* and *XII*, which were oxidized[8] by Ru^{4+}–$NaIO_4$ to the respective *cis*- or *trans*-2-acetoxycyclohexaneacetic acids *XIII* and *XIV*. After the elimination of the protecting group, *cis*- or *trans*-2-hydroxycyclohexaneacetic acids *XV* and *XVI* were obtained, which were submitted to cyclization[9] under formation of corresponding optically active *cis*- or *trans*-hexahydro-2(3*H*)benzofuranones *IX* and *X*,

TABLE II
^{1}H, ^{13}C, and ^{19}F NMR chemical shift differences for diastereoisomeric pairs of MTPA-esters *V*, *VI* and *VII*, *VIII*

Atom	$\Delta\delta$(*V* – *VI*)	$\Delta\delta$(*VII* – *VIII*)
	^{1}H NMR	
H-1	0·03	0·01
H-7	−0·06	−0·18
H-7′	−0·08	−0·10
	^{13}C NMR	
C-1	−0·33	−0·22
C-2	0·12	0·32
C-6	0·13	0·56
C-7	−0·54	−0·30
	^{19}F NMR	
CF_3	−0·15	−0·11

respectively. All the reaction steps took place with the retention of configuration at C(1) and C(2) of the starting alcohols *I* and *II*. From Scheme 1 it is evident that alcohol *I* gives rise to lactone *IX*, while alcohol *II* affords lactone *X* by the same reaction path.

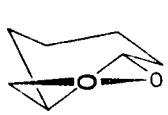


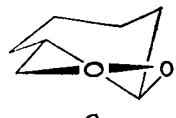
SCHEME 1

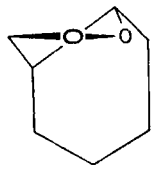
For the determination of absolute configuration of lactones *IX* and *X* a combination of CD, IR, and ^{1}H NMR spectra was made use of. A proof that compounds *IX* and *X* are always one of the isomers of *cis*- or *trans*-lactone was achieved by evaluating both IR and ^{1}H NMR spectra. The absolute configuration *IX* and *X* was then determined on the basis of a combination of the ^{1}H NMR and CD data. According to Japanese authors[10,11] the sign of the Cotton effect of γ-lactones is determined as the sum of the contributions of two factors. One of them is the contribution determined by the chirality of the five-membered ring, and the second one is the contribution following from the orientation of the substituents at C(3). If taking into consideration that C(3) in lactones *IX* and *X* always carries only two hydrogen atoms, the decisive contribution will be the value following from the chirality of the ring.

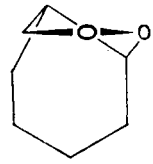
When comparing both possible enantiomeric structures Q_1 and Q_2 of *trans*-lactone *X* with the measured value $\Delta\varepsilon = -0{\cdot}6$, it is evident that the experimentally determined $\Delta\varepsilon$ value corresponds to configuration Q_1, *i.e.* *trans*-(8*S*, 9*R*)-hexahydro-2(3*H*)benzofuranone. While the molecule of *trans*-lactone *X* is relatively rigid, the

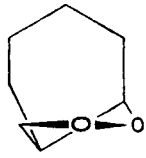
cis-lactone *IX* displays higher flexibility of both rings. Either enantiomer of the *cis*-lactone *IX* can assume, theoretically, two possible conformations (structures $Q_3 - Q_6$). For the stereostructural interpretation of the measured value $\Delta\varepsilon = +0{\cdot}2$


Q_1


Q_2


Q_3


Q_4


Q_5


Q_6

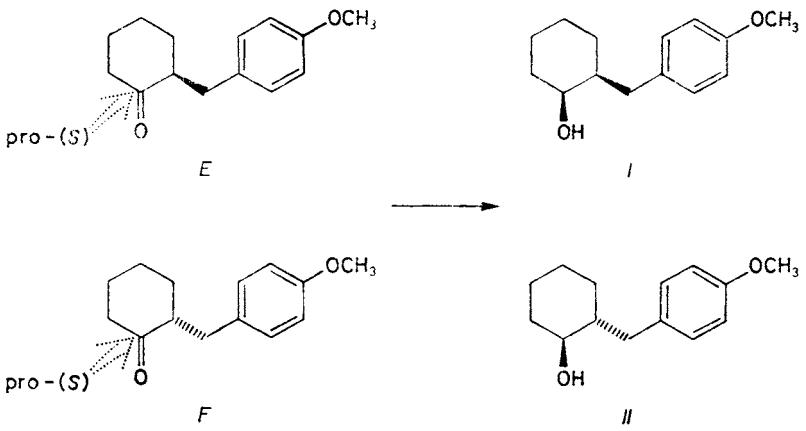
it was necessary to determine the preferred conformation of *cis*-lactone *IX*. It can be inferred from the vicinal coupling constants in the ^{1}H NMR spectrum that the configurations Q_3 or Q_6 are distinctly preferred. Measuring the CD spectra at various temperatures (in the +40°C to −80°C range) it was shown that under these conditions this conformation remains unchanged. However, the measured value, $\Delta\varepsilon = +0{\cdot}2$, only agrees with the structure Q_3 (taking into consideration the contribution introduced into the total value of $\Delta\varepsilon$ by the steric orientation of the six-membered ring), and therefore it is possible to define the *cis*-lactone *IX* as *cis*-(8*S*, 9*S*)-hexahydro-2(3*H*)benzofuranone on the basis of both these facts.

The absolute configuration of alcohols *I* and *II* could be reinferred from the determined absolute configurations of lactones *IX* and *X* (Scheme 1). The chemical correlation employed, leading from compounds *I* or *II* to lactones *IX* or *X*, respectively, takes place with the retention of configuration on both atoms involved, C(1) and C(2), in alcohols *I* and *II*. Using CD spectra the absolute configuration of alcohols

I and *II* could be confirmed indirectly, proposed on the basis of measurements of the NMR spectra of their diastereoisomeric derivatives *V*–*VIII*.

As a further possibility of determining absolute configurations of lactones *IX* and *X* and alcohols *I* and *II* we measured also the sign and the value of their optical rotation. On the basis of the data obtained and by their comparison with the published values of optical rotation[9] it was also possible to determine unambiguously the absolute configurations of lactones *IX* and *X*. Hence, lactone *IX* is defined as (8*S*, 9*S*)-(−)- and lactone *X* as (8*S*, 9*R*)-(−)-hexahydro-2(3*H*)benzofuranone. The absolute configurations of alcohols *I* and *II* were completed on the basis of the optical rotation values to *cis*-(1*S*, 2*S*)-(+)- or *trans*-(1*S*, 2*R*)-(+)-2-(4-methoxybenzyl)-1-cyclohexanol, respectively.

The optical purity achieved in the biotransformation of enantiomers *E* and *F* of the racemic ketone (Scheme 2) was determined from the ratio of diastereoisomeric MTPA esters of alcohols *I* and *II* by means of HPLC, under the conditions mentioned



SCHEME 2

in Experimental. During chromatography, a major peak appeared in the region of the retention times corresponding to MTPA esters in the case of both alcohols, accompanied by several minor peaks, representing 2–4% of the total. In order to make a rigorous assignment we found it favourable that we had prepared MTPA esters derived both from (*R*)-(+)-MTPA and from (*S*)-(−)-MTPA. Thus we had a set of enantiomeric and diastereoisomeric pairs of esters at our disposal, the chromatographic behaviour of which would coincide with the aforementioned phenomenon. While the retention times of enantiomers will be always identical (within the range of experimental deviations), those of diastereoisomers will always differ (Table III). When passing from (*R*)-MTPA-ester to the (*S*)-MTPA-ester, a change in retention

times takes place, owing to the fact that the chromatographic behaviour of the major (*R*)-MTPA-ester corresponds to that of the minor (*S*)-MTPA-ester, because they have become enantiomers. This procedure may be used for accurate determination of chromatographic peaks corresponding to the minor diastereoisomers in MTPA esters of the alcohols determined. It is generally utilisable mainly if one of the diastereoisomers prevails distinctly and when it is possible to meet the situation discussed. The optical purity determined, and the retention times for corresponding diastereoisomers are given in Table III.

From the relative representation of diastereoisomeric alcohols *I* and *II*, formed during biotransformation (1 : 1 according to HPLC analysis), it follows that in enzymatic reduction of the racemic mixture of the starting ketone *E* and *F* a preferred reduction of any of the two enantiomers does not take place and the observed ratio *I*/*II* suggests that the reduction of both enantiomers takes place at the same rate and that a kinetic resolution does not take place (Scheme 2). The slightly lower degree of enantioselectivity for the formation of *cis*-alcohol *I*, minimally 91·6 ± 1·0% e.e., as opposed to the determined value, 97·5 ± 1·0% e.e., in *trans*-alcohol *II* indicates that the enantiomer *F* is more advantageous for the enzymatic reduction, apparently for steric reasons. The reduction of the prochiral keto group takes place from the pro-(*S*) side, which is in agreement with earlier data, even though different microorganisms had been used[12].

TABLE III

HPLC analysis and Δε values (CD spectra) of MTPA-esters *V*— *VIII*

Compound	Retention time, min	Content %	Enantiomeric excess, %	$\Delta\varepsilon^a$ (λ)
V	36·5[b]	4·18	91·6	+3·0
	38·1	95·20		(229)
VI	36·5	94·10	95·1	−1·6
	38·2[c]	2·40		(235)
VII	29·9	98·30	98·2	+2·5
	32·0[d]	0·58		(229)
VIII	30·1[e]	1·24	97·5	−1·2
	32·2	98·80		(235)

[a] For the major compound; [b] (*R*)-MTPA-ester of *cis*-(1*R*, 2*R*)-*I*; [c] (*S*)-MTPA-ester of *cis*-(1*R*, 2*R*)-*I*; [d] (*R*)-MTPA-ester of *trans*-(1*R*, 2*S*)-*II*; [e] (*S*)-MTPA-ester of *trans*-(1*R*, 2*S*)-*II*.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-200 spectrometer at 200 or 50·31 MHz frequencies, respectively, in deuteriochloroform, using tetramethylsilane as internal reference. The chemical shifts and coupling constants of hydrogen atoms were obtained by first order analysis. The ^{19}F NMR spectra were recorded on a Tesla BS-497 spectrometer at 94·1 MHz frequency, in deuteriochloroform, with a capillary containing trifluoroacetic acid as external reference. The IR spectra were recorded on a Perkin–Elmer 580 instrument in tetrachloromethane. The CD spectra were obtained from a Roussel–Jouan II instrument in methanol and the UV spectrum was recorded on a Cary 219 spectrophotometer in methanol, 1 cm. HPLC analyses were carried out on a Hewlett–Packard HP 1090 instrument, coupled with a HP-85B microcomputer. Detection was carried out at 220 nm wavelenght by means of an ultraviolet detector DAD; integration was carried out at 220 nm using a DPU multichannel integrator. A set of 4 columns connected in series was used for analysis, each 150 × 3·2 (i.d.) mm, with Separon Six of particle size 5 μm as stationary phase. Light petroleum with 2·5% ether and 0·05% ethanol was used as mobile phase, flow rate 0·5 ml/min. Optical rotations were measured on an Opton (Carl Zeiss, Jena) polarimeter, with $\pm 0{\cdot}01°$ accuracy. Column chromatographies were carried out on silica gel (Gebr. Herrman, Koeln-Ehrenfeld). Silica gel G, type 60 according to Stahl (Merck, Darmstadt) for TLC analyses was used.

Biotransformation with *Saccharomyces cerevisiae*

The yeast *Saccharomyces cerevisiae*, strain 22 (origin: Dr. C. Williamson, National Institute of Medical Research, London, U.K.) was cultivated in liquid malt medium diluted to 8° Bg, at 27 ± 1°C, for 48 h. The yeast obtained was centrifuged (10 min at 2 000 rpm), washed repeatedly with 100 ml of phosphate buffer (pH 6·5, 0·06 mol l^{-1}), transferred into flasks containing the substrate, *i.e.* 2-(4-methoxybenzyl)-1-cyclohexanone (totally 300 mg), in a $2{\cdot}3 \cdot 10^{-3}$ mol l^{-1} concentration (0·05 g of substrate in 100 ml of the same phosphate buffer). The dependence of

TABLE IV

Course of reduction of 2-(4-methoxybenzyl)-1-cyclohexanone with *Saccharomyces cerevisiae*, strain 22

Time[a] days	Content, %[b]	
	ketone *E* + *F*	alcohols *I* + *II*
0	100	0
2	65	35
3	61	39
7	56	44
8[c]	48	52

[a] At the indicated time the content of one biotransformation flask was worked up; [b] the content of all compounds by GLC analysis; [c] the content of three remaining flasks was worked up together.

the biotransformation course on time was followed over 2 to 8 days from the application (Table IV). The biotransformation products obtained were separated by column chromatography on silica gel and both respective pure alcohols *I* (69 mg) and *II* (74·5 mg) were characterized as *cis*-(1*S*, 2*S*)-(+)-2-(4-methoxybenzyl)-1-cyclohexanol, $[\alpha]_D^{20}$ +21° (*c* 0·37, CH_3OH), or *trans*-(1*S*, 2*R*)-(+)-2-(4-methoxybenzyl)-1-cyclohexanol, $[\alpha]_D^{20}$ +38° (*c* 0·18, CH_3OH). For spectral characterization see Table I. Further, 140 mg of the starting racemic ketone were obtained. IR spectrum (cm^{-1}): 1 710, 1 615, 1 515. UV spectrum (1 mol^{-1} cm^{-1}): ε_{284} = 1 600, ε_{278} = 1 780, ε_{224} = 9 000.

MTPA esters *V*—*VIII*

A general procedure used for the preparation of microamounts of MTPA esters was described recently[3]. Compounds *V*—*VIII* were obtained in 50—70% yields and their characterizations by spectral data are summarized in Tables I and III.

cis-(8*S*, 9*S*)- and *trans*-(8*S*, 9*R*)-Hexahydro-2(3*H*)benzofuranones (*IX* and *X*)

a) Alcohol *I* or *II* (0·13 or 0·16 mmol, respectively) was submitted to acetylation with acetic anhydride (0·2 ml) in pyridine (1 ml). Both diastereoisomers *XI* and *XII* were obtained in about 95% yields.

b) Both respective acetoxy derivatives *XI* and *XII* (0·125 or 0·15 mmol, respectively) were dissolved in a mixture of tetrachloromethane and freshly distilled acetonitrile 1 : 1 (1·2 ml). A solution of sodium periodate (0·25 g) in water (0·9 ml) and ruthenium oxide (10 mg) were added under vigorous stirring to the mixture. The vigorous stirring continued for 48—72 h. The mixture was diluted with dichloromethane (2 ml) and the product was extracted and dried. Column chromatography on silica gel afforded the products *XIII* or *XIV* in 60 or 70% yield, respectively.

c) The pure respective products *XIII* or *XIV* (0·072 or 0·14 mmol, respectively) were reacted with lithium hydroxide (1 or 2 mmol, respectively) in 75% aqueous tetrahydrofuran (1·5 ml) to give hydroxy acids *XV* or *XVI*, respectively, in about 90% yield.

d) Hydroxy acid *XV* or *XVI* (0·063 or 0·165 mmol, respectively) was heated in benzene (10 ml) under azeotropic distillation of water, for 3—4 h. Column chromatography on silica gel gave pure *IX* (5·5 mg, 62%) or *X* (12 mg, 68%), respectively. Lactone *IX*: ^{1}H NMR spectrum (C^2HCl_3): 4·51 q (H-8, J = 4·2 Hz), 2·61 dd (H-3, J(3, 3′) = 16·4; J(3, 9) = 6·7 Hz), 2·38 m (H-9, W = 31 Hz), 2·24 ddd (H-3′, J(3′, 3) = 16·4; J(3′, 9) = 2·7; J(3′, 4) = 0·6 Hz), 2·08 bdq (H-7, J(7, 7′) = 14·4; J(7, 6) = J(7, 6′) = J(7, 8) = 4·2 Hz), 1·21—1·79 m (7 H). IR spectrum (cm^{-1}): 1 785, 1 172. CD spectrum: $\Delta\varepsilon_{213}$ = +0·2 (CH_3OH, 0·1 cm); $[\alpha]_D^{24}$ −42° (*c* 1·6, CH_3OH). Lactone *X*: ^{1}H NMR spectrum (C^2HCl_3): 3·78 ddd (H-8, J = 11·1, 10·4, and 3·8 Hz), 2·51 dd (H-3, J(3, 3′) = 16·0; J(3, 9) = 6·3 Hz), 2·23 m (H-7), 2·22 dd (H-3′, J(3′, 3) = 16·0; J(3′, 9) = 13·0 Hz), 1·08—2·15 m (8 H). IR spectrum (cm^{-1}): 1 792, 1 187. CD spectrum: $\Delta\varepsilon_{217}$ = −0·6 (CH_3OH, 0·1 cm); $[\alpha]_D^{24}$ −86° (*c* 0·3, CH_3OH).

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