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Enantioselective Reduction of Some Aromatic Ketones by Baker's Yeast

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The reduction of aromatic ketones **1–6** by baker's yeast showed that acceptability of the substrates is not governed either by the number of aryl groups or by the distance of the carbonyl group from the aryl moiety. Relative rates of conversion of **3**, **4**, and **6** vs. ethyl acetoacetate as the "standard" substrate were reproducible in the order **3>6>4.** Strong inhibition of reduction of ethyl acetoacetate by benzene vanished at very low concentrations of both. Reduction of phenyl-pyrid-2'yl-methyl ketone (**1**) and its deazaanalogue (**6**) gave the chiral *sec.* alcohols of the opposite configuration. (−)−**7** and (+)−**12**, respectively. This could be ascribed to the prevalent binding of enol **1A** by the opposite enantioface, as compared to **6**. Absolute configuration of (−)−**7** is tentatively assigned as *R* by the empirical rule based on the ^{1}NMR spectra of Mosher's acid esters **13–15**. This assignment is confirmed by the single crystal X−ray structure determination of camphanic acid ester **16A**, which on hydrolysis affords (−)−**7.**

INTRODUCTION

The reduction of ketons by baker's yeast (*Saccharomyces cerevisiae*) has been known for a long time, and both the early and more recent work have been reviewed.[1a,b] The use of baker's yeast in preparation of optically active building blocks in the synthesis of natural products is increasing.[2a–2d] While the first synthetic transformation by yeast cells was included in "Organic Synthesis" as early as in 1943,[3] another transformation waited for 40 years to be included in the same monograph.[4]

The most explored reaction catalyzed by yeast is the reduction of the carbonyl group in substituted ketones,[5a–5c] β-or γ-diketones,[6a,b] α-keto acids,[7] β-keto acids,[8a–8d]

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γ- and β-keto acids.[9] The cited references should be regarded as exemplary, the exhaustive literature documentation can be found in ref. 1b. Reduction of some aromatic and heteroaromatic ketons is mentioned in the early papers of Mosher *et al.*[10a] and Červinka *et al.*[10b] Only sporadic examples can be found in the more recent literature.[11,12] Seebach explicitly stated[13a] that phenyl group in a substrate molecule might be the inhibitory subunit. Its effect seems to be compensated by any hydrophilic functionality in α, β, or γ position to the ketone carbonyl.[14,15] An important example of the baker's yeast mediated reduction of an aromatic ketone derivative represents the reduction of a pepstatin precursor in 30–50% chemical, and 60% optical yield.[16]

It was observed years ago that pyridine and N-methyl pyridinum salts exhibit strong inhibitory effect on the yeast alcohol dehydrogenase (YADH).[17a,b] Therefore, it could be expected that pyridyl ketons would not be good substrates for the NADH- dependent reductases of the yeast, either. However, pyridyl ketones are reduced by *Sporotrchum exile*,[18] *Cryptococcus macerans*,[18b,c] and when our work was in progress the reduction of 2- and 4-acetylpyridine by *Saccharomyces cerevisiae* was reported.[19,20]

As yet, there has been no report on a systematic study of the baker's yeast-catalyzed reduction of aromatic ketones. This study was undertaken in order to get a better insight into the intramolecular inhibitory effect of the aromatic ring in the reduction of **1–6**, as compared to the intermolecular effect of benzene on the reduction of ethyl acetoacetate as the standard substrate.[12,13b] These results were expected to aid determination of the conditions for reduction of phenyl-pyridilmethyl ketone (**1**), to the carbinol *R* **7**, which is the chiral intermediate in the envisaged enantioselective synthesis of sedamine, the alkaloid found in *Sedum acre*.

RESULTS AND DISCUSSION

Reduction of ketones **1–6** was performed in a series of experiments using fresh commercial baker's yeast. The reaction conditions were varied according to some of the most protocols.[4,10,12] Some results obtained with **2** are summarized in Figure. 1.

Figure 1(a) reveals that reduction of acetophenone was performed most effectively by the method of Bucciarelli *et al.*[12] (Method C), at a high yeast-to-substrate ratio. Slower reaction was noticed under Moshers' conditions (b–d).[10] A addition of thiamine and mineral salts (b,d) did not increase the limited yields even after prolonged reaction times.

The results of the reduction of ketones **1, 2, 5** and **6** are presented in Table I. The chemical and optical yields of (−)-**8** correspond to those reported in the literature,[10] whereas in the case of the reduction of propiophenone **5** the enantiomer of the opposite configuration (*R*) prevails. It was shown for **5** that keeping the cells in a freezer for some time does not change the reducing ability of yeast.

Attempted reduction of phenyl-pyridylmethyl ketone (**1**), in various experiments resulted in low-yields of carbinol **7**, formed with variable stereoselectivity (Table I).

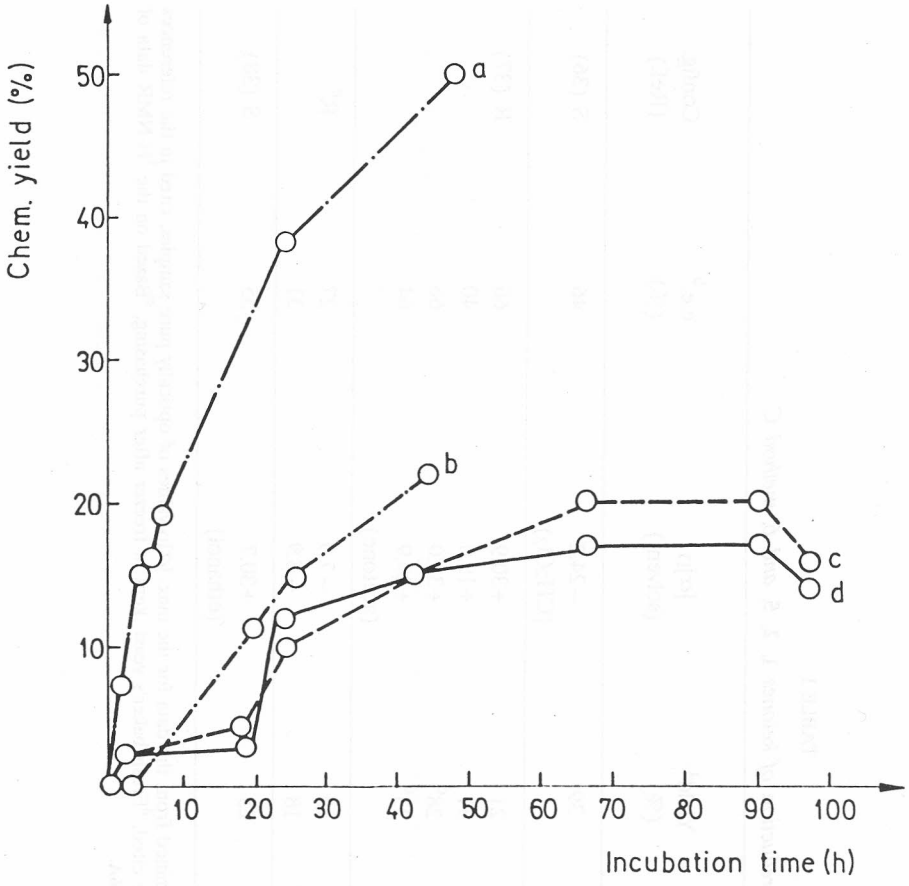


Figure 1. Reduction of acetophenone (**2**) with fresh commercial baker's yeast under various conditions; *a.* Method C, 8.5 mg substrate/g dry weight of yeast, *b.* Method B, 36 mg substrate/g dry weight of yeast, *c.* Modification of Method B in that the thiamine and mineral salts are excluded, 50 mg substrate/g dry weight of yeast, *d.* Method B, 50 mg substrate/g dry weight of yeast.

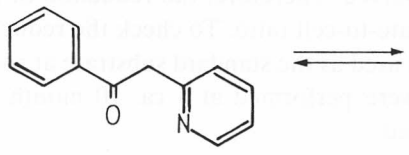
This indicates that some of several dehydrogenase enzymes present in the yeast could give rise to complementary enantioselectivity.[21] However, inversed enatnioselection in the reduction of **1** and **6** might also be related to their prefered form in solution. While the latter predominantes in the carbonylic form, we observed that the former is in equilibrium with its enolic form. In the UV spectrum of **1** (in EtOH), a strong maximum at 336 nm is present, wheras its ^{13}C NMR spectrum in $CDCl_3$ reveals ca. 1:1 mixture **1:1A.** A recent detailed study confirmed the unusual stability of enol **1A**, ascribed to the intramolecular hydrogen bonding.[22] Binding of the enolic form by the enantiotopic face to the active site of reducing enzyme(s) might explain the opposite enantioselectivity in the reduction of these two ketones. Related to pH dependent enolization, pH was followed in two reduction experiments with time, over 90 hrs; only a slow increase from 4.1 to 4.8 was observed.

TABLE I

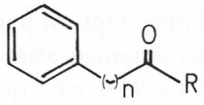
Baker's yeast reduction of ketones **1**, **2**, **5**, *and* **6**, *method C*

Substrate (mg substrate/g dry weight of yeast)	Incubation time (h)	Product	Yield[a] (%)	$[\alpha]_D$ (solvent)	e.e.[b] (%)	Config. (Ref.)
2 (9 mg/g)	48	(−)−**8**	39	−24.2 (CH_2Cl_2)	46	S (36)
5 (15 mg/g)[c]	25	(−)−**11**	21	+30.9	66	R (37)
			31	+19.0	40	
			28[d]	+31.0	66	
			33[d]	+29.9 (acetone)	64	
1 (11 mg/g)	72	(−)−**7**	12	−7.9	27	R[e]
			18	−8.9	31	
6 (9 mg/g)	144	(+)−**12**	25	+30.7 (ethanol)	55	S (38)

[a]Isolated yield by column chromatography (SiO_2). [b]Determined from the data for the max. $[\alpha]_D$ values of optically pure samples, cited in the references in the last column. [c]Two pairs of parallel experiments are cited, [d]Fresh baker's yeast, kept in freezer after purchasing, [e]Based on the ^{1}H-NMR data of its MTPA esters and X-ray structure determination of **16A**.

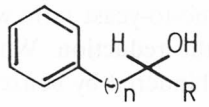


1 1A



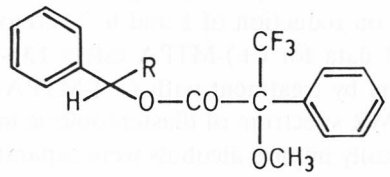
2 - 6

	2	3	4	5	6
n	0	1	2	0	0
R	CH_3	CH_3	CH_3	CH_2CH_3	CH_2Ph



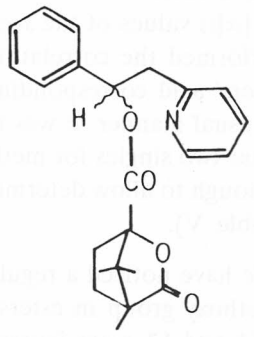
7–12

	7	8	9	10	11	12
n	0	0	1	2	0	0
R	CH_2Py	CH_3	CH_3	CH_3	CH_2CH_3	CH_2Ph

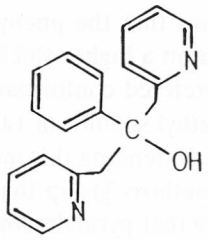


13–15

	R
13	CH_3
14	CH_2Ph
15	CH_2Py



16 A, 16B



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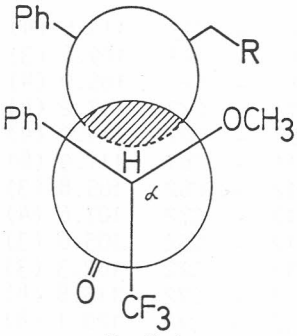
Complete inhibition of the reducing capacity of the yeast cell by high concentration of aromatic ketones was regularly observed. Therefore, the reduction of ketones **3, 4** and **6** was tried at a very low substrate-to-cell ratio. To check the reducing properties of the yeast, ethyl acetoacetate was used as the standard substrate at usual concentration.[4] Two series of experiments were performed at a ca. 10 month interval, and reproducible results were obtained.

Phenylacetone (**3**), with carbonyl group separated by one methylene group from the aromatic ring, was reduced with over 90% yield. Benzyl-phenyl ketone (**6**), in spite of two phenyl moieties in the molecule, was reduced with 60–65% yield, benzyl-acetone (**4**), quite surprisingly was reduced in 30–35% yield only. Optical purity of alcohol (+)-**9**, (no data reported for absolute configuration) obtained after 96 hrs of incubation of **3** was estimated according to lit. data[23] to ca. 95% e.e.; ($[\alpha]_D$ + 30.9), (c = 1.2 in dichloromethane), whereas for (+)-**10** obtained from **4** no data are reported for the optically pure compound. The effect of benzene on the reduction of ethyl acetoacetate was particularly informative. Its was added in a quantity equimolar to ethyl acetoacetate, imitating the ratio of aryl and alkyl subunits in aryl-alkyl ketones. In the experiment where the substrate and benzene-to-yeast ratio was high, (0.44 g/g of dry weight of the yeast) benzene inhibited the reduction. When their concentration was 10 times lower, the conversion was unhindered by benzene – ca. 100%, as determined by GLC.

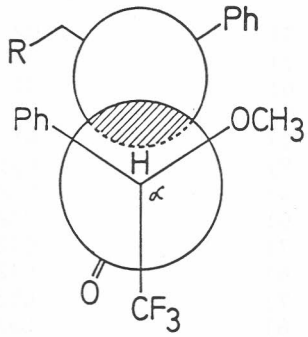
Determination of the Optical Purity and Absolute Configuration of (–)-7. – While the maximal rotation and absolute configuration were known for alcohols **8, 11** and **12**, neither value was reported for (–)-**7**. Particularly intriguing were the opposite $[\alpha]_D$ values of the *sec.*alcohols obtained on reduction of **1** and **6**. Therefore, we performed the correlation of the ^{1}H NMR data for (+)-MTPA esters **13–15** (Moshers' acid corresponding (+)-MTPA esters by treatment with (–)-MTPA-Cl in the usual manner. It was noted in the ^{1}H NMR spectrum of diastereomeric mixture that two singles for methoxy group of optically impure alcohols were separated well enough to allow determination of diastereomeric composition, (see Experimental, Table V).

We have noticed a regularity in the ^{1}H NMR spectra of **13–15**; the signals for the methoxy group in esters **13** and **14**, diastereomers obtained with (+)-enantiomers **11** and **12**, were inversed. This indicates that the phenyl group in compound **13** and the benzyl group in compound **14** exhibit a higher shielding effect. Inspection of Dreiding models of these esters in the prefered conformation[25] (Scheme 1.) reveals that the aromatic ring within phenylmethyl subunit in **14** can be brought closer to the methoxy group than the phenyl group. Extending this model to ester **15**, which showed the opposite pattern of signals for methoxy group than in **14**, tentative configuration assignment was allowed. Assuming that pyridylmethyl group in **15** exhibits a similar shielding effect to the phenylmethyl group in **14**, we concluded that configuration at C(1) in (–)-**7** is opposite to that in (+)- **12**, i.e. that it is *R*.



13 - 15 A

S . R - CONFIGURATION



13 - 15 B

S . S - CONFIGURATION

R
13 - CH_3
14 - Ph
15 - Py

Crystal and Molecular Structure of **16A**. – To confirm the tentatively assigned *R*-configuration to (–)-**7**., diastereomeric camphanic acid esters (**16A, 16B**) were prepared and **16A** was crystallized for X-ray structure determination. Good quality single crystals of **16A**– were obtained on slow crystallization from MeOH-H_2O (9:1). Its molecular geometry is described by interatomic distance and angles (Table II), while torsional angles are listed in Table III.

Perspective view of the **16A** molecule with atom numbering is given in Figure 2.

The absolute configuration at C(1) of **16A** has been determined as *R*. Camphanyl moiety of known absolute configuration, C(12) *S*, C(32) *R* [25, 26], was used as an internal standard. Bond distances correspond to the given atom type and hybridization. Geometry of camphanyl moiety is in agreement with literature.[13a, 26]

The ring puckering analysis[27] of camphanyl moiety shows boat conformation of the six-membered ring (Θ = 90.6(3), φ = 1.4(2), Q = 0.974(5)Å), and envelope for both five-membered rings with the C12 deviating from the four atom planes (for C12→C52 Θ = –146.8(5), Q = 0.589(4)Å; for O12→C62 φ = 105.9(4), Q = 0.577(4)Å).

Molecular packing is by van der Waals' interactions only. The shortest intermolecular contacts are between methylene (C42) and carbonyl (O62) [C42---O62, 3.427(6)Å, H421---O62, 2.5(2)Å, 174(2)° (x-1, y, z), C42---O62, 3.324(8)Å, H42---O62, 2.6 Å, 128(2)° (x-1/2, - y-1/2-1, -z+1), and methyl to carbonyl groups of camphanyl moieties C82-H821---O12, 3.467(6)Å, H821---O12, 2.5(1)Å. 146(2)°].

CONCLUSION

It can be concluded that no relation can be established between the number and distance of the phenyl groups from the carbonyl group and their "reducibility"

TABLE II

Interatomic distances and angles in **16A.**

C1	-	O1	1.457 (5)	C2	-	C1	-	C13	113.6 (4)
C1	-	C2	1.507 (7)	O1	-	C1	-	C13	109.6 (3)
C1	-	C13	1.503 (6)	O1	-	C1	-	C2	105.0 (4)
O1	-	C72	1.341 (6)	O1	-	O1	-	C72	117.2 (4)
C2	-	C21	1.513 (7)	O1	-	C2	-	C21	114.0 (4)
N11	-	C21	1.312 (8)	C21	-	N11	-	C61	117.9 (6)
N11	-	C61	1.311 (9)	C12	-	O12	-	C62	105.8 (3)
O12	-	C12	1.458 (5)	O12	-	C12	-	C72	107.6 (4)
O12	-	C62	1.371 (7)	O12	-	C12	-	C52	105.6 (3)
C12	-	C22	1.539 (6)	O12	-	C12	-	C22	102.3 (3)
C12	-	C52	1.534 (7)	C52	-	C12	-	C72	115.8 (4)
C12	-	C72	1.491 (6)	C22	-	C12	-	C72	120.1 (4)
C13	-	C23	1.369 (7)	C22	-	C12	-	C52	103.9 (4)
C13	-	C63	1.383 (8)	C1	-	C13	-	C63	119.8 (4)
C21	-	C31	1.369 (8)	C23	-	C13	-	C63	118.7 (5)
C22	-	C32	1.550 (6)	C2	-	C21	-	N11	116.4 (5)
C22	-	C82	1.542 (6)	N11	-	C21	-	C31	122.2 (5)
C22	-	C92	1.535 (7)	C2	-	C21	-	C31	121.4 (5)
C23	-	C33	1.382 (8)	C12	-	C22	-	C92	112.7 (4)
C31	-	C41	1.359 (9)	C12	-	C22	-	C82	115.5 (4)
C32	-	C42	1.546 (7)	C12	-	C22	-	C32	91.9 (3)
C32	-	C62	1.509 (7)	C82	-	C22	-	C92	108.6 (4)
C32	-	C102	1.500 (6)	C32	-	C22	-	C92	113.9 (4)
C33	-	C43	1.360 (9)	C32	-	C22	-	C82	113.6 (4)
C41	-	C51	1.350 (10)	C13	-	C23	-	C33	120.0 (5)
C42	-	C52	1.533 (8)	C21	-	C31	-	C41	119.7 (6)
C43	-	C53	1.367 (8)	C22	-	C32	-	C102	119.4 (4)
C51	-	C61	1.391 (11)	C22	-	C32	-	C62	98.6 (4)
C53	-	C63	1.381 (8)	C22	-	C32	-	C42	102.6 (4)
C62	-	O62	1.199 (7)	C62	-	C32	-	C102	114.3 (4)
C72	-	O72	1.200 (6)	C42	-	C32	-	C102	116.3 (4)
				C42	-	C32	-	C62	102.9 (4)
				C23	-	C33	-	C43	121.6 (6)
				C31	-	C41	-	C51	119.1 (6)
				C32	-	C42	-	C52	104.3 (4)
				C33	-	C43	-	C53	118.5 (6)
				C41	-	C51	-	C61	117.5 (6)
				C12	-	C52	-	C42	101.4 (4)
				C43	-	C53	-	C63	120.9 (6)
				N11	-	C61	-	C51	123.6 (6)
				O12	-	C62	-	C32	107.4 (4)
				C32	-	C62	-	O62	131.3 (5)
				O12	-	C62	-	O62	121.3 (5)
				C13	-	C63	-	C53	120.3 (5)
				O1	-	C72	-	C12	109.4 (4)
				C12	-	C72	-	O72	126.5 (4)
				O1	-	C72	-	O72	124.0 (4)

TABLE III

Torsion angles defining the absolute configuration and ring conformation in **16A** (°).

C23-C13-C1-O1	-124.3(5)
C21-C2-C1-O1	57.3(5)
C1-O1-C72-C12	-172.4(4)
O1-C72-C12-C22	-63.3(5)
O1-C72-C12-C52	62.8(5)
O1-C72-C12-O12	-179.5(3)
C12-C22-C32-C102	176.2(4)
C12-O12-C62-C32	1.9(5)
O12-C 62-C32-C42	68.8(5)
C62-C32-C42-C52	-68.9(5)
C32-C42-C52-C12	2.8(5)
C42-C52-C12-O12	68.8(4)
C52-C12-O12-C62	-74.4(4)
C12-C22-C32-C42	-53.4(4)
C22-C32-C42-C52	33.1(5)
C42-C52-C12-C22	-38.4(5)
C52-C12-C22-C32	56.6(4)
C12-C22-C32-C62	51.9(4)
C22-C32-C62-O12	-36.3(4)
C62-O12-C12-C22	34.0(4)
O12-C12-C22-C32	-53.1(4)

by the native yeast cells. Our results also leave open the question whether aromatic ketones are inherently bad substrates for all reducing enzymes present in the yeast cell,[22] or unfavourable interaction occurs at some other level. The possibility exists that selected yeast strains, grown in the presence of an aromatic ketone, might be more effective biocatalysts for preparation of optically active *sec.* alcohols. Our future efforts will be oriented to this aim.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Joel FX 90Q Fourier- transform spectrometer with tetramethylsilane as internal standard, chemical shifts are reported in δ values. IR spectra

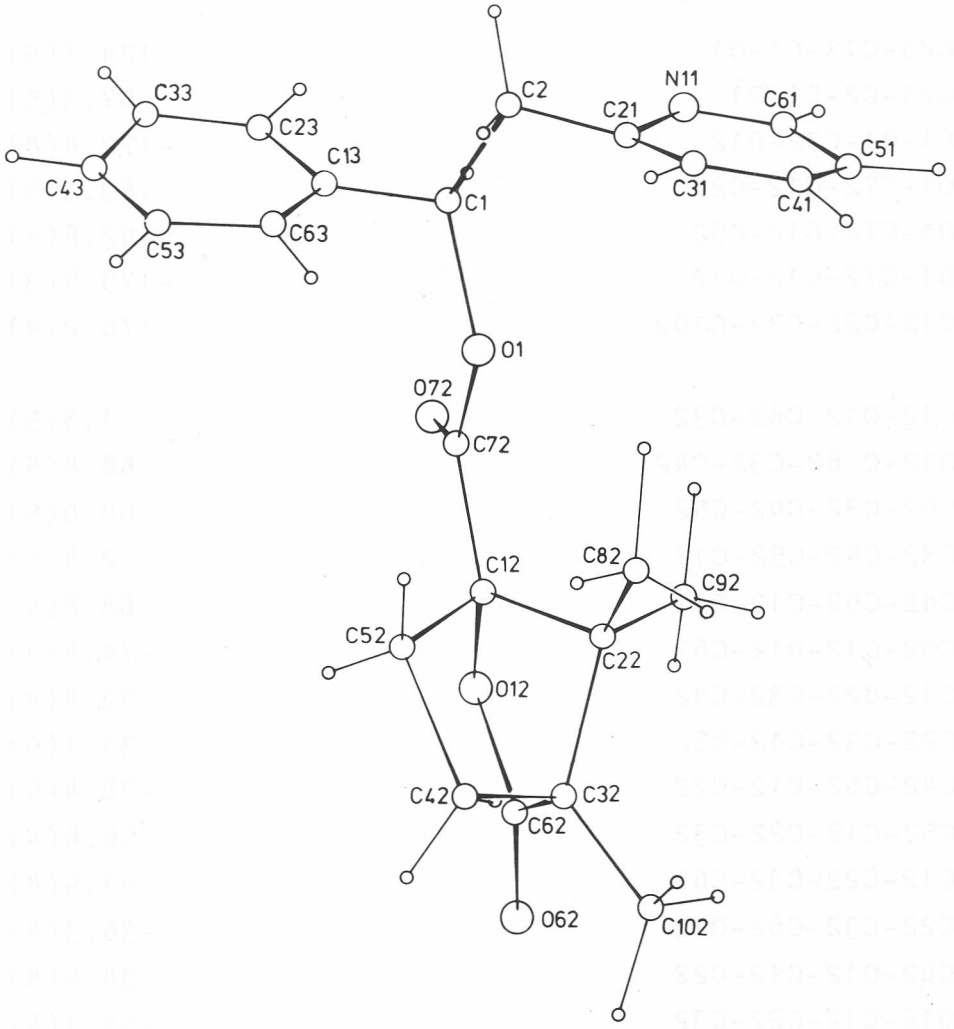


Figure 2. Perspective formula of **16A** with atom numbering.

were recorded with a Perkin-Elmer Model 137 spectrometer. Optical rotations were measured at ambient temperature with Perkin-Elmer 141 polarimeter, using 1 cm and 2 cm cells. TLC was performed on Merck's DC-alufolien with Kieselgel 60F-254, column chromatography was run over granular silica gel 0.063–0.2 mm or 0.004–0.063 mm (Merck). Organic extracts were regularly dried over Na_2SO_4 and evaporated *in vacuo*.

Compound **1** was prepared by modifying the method from ref. 28, compounds **2** and **5** were purchased from Aldrich and distilled before use. Compound **3** was prepared according to ref. 29, compound **4** according to ref. 30, compound **6** according to ref. 31. *S*–(–)–MTPA

(from Aldrich) was converted into *R*−(−)−MTPA-Cl according to ref. 24, camphanic acid chloride was prepared according to ref. 32.

Phenyl-pyrid-2'-yl-ketone (1). – To 300 ml of liq. ammonia, $FeNO_3$ $9H_2O$ (100 mg) was added and then metallic sodium over 5−10 min (4.6 g, 0.2 mol). After 10 min. of stirring at 30 °C, the deep-blue colour disappeared and a gray suspension of sodium amide was formed. Then, a solution of 2-methyl-pyridine (9.3 g, 0.1 mol) in diisopropylether (10 ml) was added dropwise over 10 min. After 15 min of stirring at 30 °C, ethylbenzoate (18.1 g, 0.12 mol), dissolved in diisopropylether (10 ml), was added over 4−5 min. Stirring was continued for 3.5 hrs, then 200 ml cf diisopropylether was added, stirred for 0.5 hrs, and water (250 ml) was added. Organic phase was separated, aqueous phase extracted with ether (2x100 ml), combined extracts were dried and evaporated leaving 16 g of crude **1.** Distillation afforded 9.9 g (52%) of pure **1**, b.p. 140−150 c/0.6 mm Hg, which solidified on standing. On crystallization from light petroleum, it had m.p. 57−59 C (lit [31] m.p. 59 °C).

When this reaction was performed, strictly following the literature method,[29] only 26% of **1** was obtained, along with 24% of

Phenyl-bis-pyrid-2'-yl-methyl-carbinol (17), which was separated by chromatography on silica gel column with dichloromethane- methanol (9.5:0.5) as eluent. On crystallization from cyclohexane m.p. 87−89 °C, IR: 3180 (broad), 1605, 1600, 1590, 1480, 1470, 1435, 1285, 1270, 1155, 1105, 1055, 1048, 1005, 790, 700 cm-1. ^{1}H NMR ($CDCl_3$) δ:3.33 (*q, J* = 16Hz, 4H), 7.0−7.5 (m, 11H), 8.34−8.42 (dq, 2H).[13] C NMR ($CDCl_3$) δ:49.10, 121.16, 125.06, 125.5, 126.08, 127.60, 135.95, 146.73, 148.08, 158.80.

Anal. $C_{19}H_{18}N_{20}$ (290.37) calc'd: C 78.59, H 6.24, N 9.60%.

found: C 78.73, H 5.94, N 9.71%.

General Method for Preparation of Rac. Carbonils 7−12. – Starting from 20 mmol of **1−6**, reduction with $NaBH_4$ (04 g, 10 mmol) was performed in methanol (60 ml). Products **8−11** were purified by distillation, crude product **12** by chromatography on a silica gel column with dichloromethane-light petroleum (8:2), as eluent, compound **7** by critallization from cyclohexane. Other data are listed in Table IV.

Preparation of MTPA Esters 13−15. – Enantiometrically enriched compounds **7, 11** and **12** (0.15−0.25 mmol) and a few drops of pyridine were added to the solution of MTPA-Cl (0.155−0.265 mmol) in 0.5 ml of $CDCl_3$, and deposited for 24 hrs. ^{1}H NMR control indicated practically complete conversion. The reaction mixture was purified on silica gel column (10 g) using chloroform as eluent.

Compound **13**: ^{1}H NMR ($CDCl_3$) δ: 0.75−1.0 (m, 2H), 3.45−3.55 (m, 3H OCH_3), 5.75−6.0 (m, 1H), 7.25−7.4 (m, 10H).

Compound **14**: ^{1}H NMR ($CDCl_3$) δ: 3.1−3.4 (m, 5H CH_2+OCH_3), 6.05−6.8 (m, CH_2OCH_3, 1H) 7.1−7.35 (m, 15H).

Compound **15:** ^{1}H NMR ($CDCl_3$) δ: 3.2−3.4 (m, 5H, CH_2+OCH_3), 6.4−7.0 (m, 1H), 7.1−7.36 (m, 13H), (m, 1H, N-CH).

Table V. shows data on enantiomeric purity of **7, 11** and **12**, as determined from their HMPTA-esters.

Camphanyl Esters of (+)-1-Phenyl-2-pyrid-2'yl-ethanol (16A, 16B). – To the ice-bath cooled solution of racemic **7** (1.75 g, 8.75 mmol) in chloroform (10 ml), camphanyl chloride (1.90 g, 8.75 mmol), dissolved in chloroform (10 ml) was added dropwise over 0.5 hrs. Stirring was continued for 72 hrs, the reaction mixture was first extracted with dil. aqueous bicarbonate, then with water, organic extracts were dried and evaporated. Crude product (2.79 g) was purified by column chromatography, etherchloroform (8:2) as eluent, affording 0.79 g of pure diastereomers **16A, 16B**, ratio ca. 1:1 according to ^{1}H NMR. ^{1}H NMR ($CDCl_3$) δ: 0.58, 0.73,

TABLE IV

Yields of the hydride reduction and characteristics physico-chemical data for (+)−7−12

Compd.	Yield (%)	B.p./mm Hg or m.p. (°C)	NMR ($CDCl_3$) ^1H-	^{13}C-	Lit. b.p./mm Hg or m.p. (°C) (ref.)
7	89[a]	111−112°	3.12(d, 2H), 5.16 (m, 1H), 5.22 (s, 1H 7.05−8.05(m, 9H)	45.9, 73.3, 121.6, 123.8, 125.8, 127.2 128.2, 136.7, 144.2, 148.5	−
8	71	70−76°/2−3	1.41(d, 3H), 2.59 (s, 1H), 4.72(q, J 6.4 Hz, 1H), 7.2−7.35 (m, 5H)	24.7, 69.4, 125.0 126.7, 127.8, 145.5	87°/10 (39)
9	63	65−70°/0.71°	1.22(d, 3H), 1.67 (s, 1H), 2.67−2.75 (m, 2H), 3.9−4.03 (m, 1H), 7.15−7.3(m, 5H)	22.8, 45.9, 68.9, 126.5, 128.6, 129.5 138.6	116−117°/23 (40)
10	90	90−100°/5	1.18(d, 3H), 1.6−1.85 (m, 2H), 2.58−2.78(m, 3H, CH_2+OH), 3.67−3.87 (m. 1H), 7.1−7.2−(m, 5H)	23.4, 32.1, 40.8, 67.0, 125.7, 128.3, 142.2	88−87°/4 (41)
11	88	70−80°/1	0.8−0.97 (m, 3H), 1.6−1.8 (m, 2H), 2.11(s, 1H) 4.55 (t, J 6.4 Hz. 1H) 7.2−7.36 (m, 5H)	9.7, 31.3, 75.2, 125.6, 126.7, 127.7, 144.3	77−82°/2 (37)
12	88	66−67°	2.0(d, 1H), 2.98(m, 2H), 4.78−4.95(m, 1H), 7.1−7.65(m, 10H)	46.0, 75.2, 125.9, 126.4, 127.4, 128.3, 129.5, 138.1 143.9	65−67° (38)

[a]*Anal.* $C_{13}H_{13}NO$ (199.25) calc'd: C 78.36, H 6.58, N 7.03.
found: C 78.54, H 6.70, N 7.33%.

TABLE V

Characteristic chemical shifts and enantiomeric purity of aryl-alkyl carbinols **7**, **11** *and* **12** *as determined by 1H-NMR spectra of the MTPA esters* **13–15.**

Enantiomer in excess	Diastereomeric mixture	^{1}H-NMR shift (ppm)		Ratio	d.e	e.e.	Config.
		OCH_3 (A)	OCH_3 (B)	OCH_3 (A) / OCH_3 (B)	(%, NMR)	(% polarimetr.)	(ref.)
(−)−7	15A,B	3.30	3.23	36.5/63.5	27	–	R[a]
(+)−12	14A,B	3.30	3.26	78.5/21.5	57	55	S(38)
(+)−11	13A,B	3.54	3.54	84.5/15.5	69	65	R(39)

[a]Assigned in this work.

0.84, 0.93, 1.05 (5xs, 18H for 6xCH_3), 1.74–2.27 (m, 4H, CH_2CH_2), 3.3–3.4 (m, 2H), 6.33–6.52 (m, 1H), 7.05–7.65 (m, 9H), 8.5 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 9.59, 16.25, 16.37, 16.48, 16.65, 28.95, 30.64, 45.04, 54.01, 54.74, 76.64, 76.92, 77.37, 90.97, 121.79, 124.16, 126.47, 128.27, 128.56, 136.79, 139.56, 149.44, 156.99, 166.37, 178.16.

Camphanyl Ester 16A. – Diastereomeric mixture **16A, 16B** (300 mg) was twice crystallized from hot ethylacetate. Slow cooling, each time overnight, afforded 86.3 mg of **16A,** over 95% pure. ^1H NMR ($CDCl_3$) δ: 0.73, 086, 1.05 (3s, 9H, 3xCH_3), 1.65–2.31 (m, 4H), 3.22–3.44 (m, 2H), 6.34–6.50 (m, 1H), 7.07–7.69 (m, 9H), 8.52–8.60 (m, 1H, NH).

Anal. $C_{23}H_{25}NO_3$ (331.44) calc'd: C 76.01, H 6.93, N 3.85%.

found: C 75.98, H 7.15, N 3.90.

(–)-1-Phenyl-2-pyrid-2'-yl-ethanol (7). Ester **16A** (74.2 mg, 0.19 mmol) was hydrolyzed with LiOH (16.8 mg, 0.4 mmol) in MeOH (5 ml). After 3 hrs of stirring at ambient temperature, 0.5 ml of the wet resin Dowex 50x2 (H+ form) were added. After brief stirring, the catalyst was filtered off, washed with MeOH, and the filtrate evaporated. Crude (–)-**7** was purified on silica gel column (10 g), elution with ether-chloroform (6.5:3.5) afforded 28.2 mg (83.6%) of chromatographically pure product, $[\alpha]_D$ –22.7 (c = 0.56, ethanol).

Asymmetric reductions with baker's yeast cells, GLC monitoring. – Samples from the reaction suspension, 1–10 ml- depending on the substrate concentration, were thoroughly extracted with ethylacetate, organic extracts dried, and 1–3 μl samples in 1% solution of the internal standard were injected. A Hewlett-Packard 5790A instrument with FI detector, and 3390A integrator was used. Separation was performed on column with 10% FFAP on Chromosorb W, (1.8 m x 1.8 inch). Column temperature was 80–120 °C (4°/min) for ethyl acetoacetate, 160 C for **2–5**, and 210 C for **6**. Nitrogen flow was 20 ml/min. Internal standards used to obtain calibration curves; n-butylacetate for reduction of ethyl acetoacetate, benzylcyanide for reduction of **2–5**, and phenyl-cyclohexyl ketone for reduction of **6**.

Method A. – Sucrose (37.5 g) was dissolved in 200 ml of lukewarm water and then 25 g of yeast ("Pliva", Zagreb) was suspended. After 1 hr of stirring, the substrate was added (50 mg of each **3, 4** or **6**, dissolved in 3 ml of ethanol, 2.5 g of ethyl acetoacetate). The reaction mixture was shaken at 28 °C, and after 25 hrs another portion of sucrose (25 g in 125 ml water) was added. At the same point, in experiment with ethyl acetoacetate, additional 2.5 g of substrate were added.[4] Samples of 10–15 ml of the reaction suspension were taken (only 2 ml samples in the case of ethyl acetoacetate) and extracted (3x25 ml of ethyl acetate). Dried extracts were evaporated and residual samples were dissolved in 1% solution of the standard for GLC analysis. When isolation of the products followed the above protocol, the crude product mixture was chromatographed on silica gel column, and the separated optically active alcohols were submitted to GLC, ^1H NMR, and optical rotation measurements. From these data, the % e.e.'s were calculated.

The same method was used for the reduction of ethyl acetoacetate in the presence of benzene. Ethyl acetoacetate (2.5 g or 0.25 g) and benzene (1.5 g of 0.15 g) were added at the beginning and after 24 hrs of incubation time.

Method B. – According to ref. 10a, the substrate was adsorbed on powdered cellulose or dissolved in a small amount of ethanol and added to the incubation mixture comprising glucose (60 g), traces of mineral salts, thiamine (0.01 g) fresh baker's yeast (60 g), and water (300 ml).

Method C. – Following the ref 12a, the substrate was added to a mixture of yeast (175 g) and water (200 ml). At 24 h intervals, the additional portions (85 g) of fresh yeast were added.

X-Ray Data. – Table VI. gives crystal data and details of structure determination of **16A**. Preliminary cell dimensions and space group ($P2_12_12_1$) were determined from oscillation and Weissenberg photographs recorded with CuKα radiation; final cell dimensions were refined from diffractometer measurements using 20 reflections.

The structure was solved by SHELX86.[33] Hydrogen atoms were introduced at calculated positions and refined with fixed geometry with respect to their carrier atoms. The H-atoms attached to C_1 and C_2 were determined from a difference Fourier map. A scale factor, the coordinates of non-H atoms and their anisotropic thermal parameters were refined. Scattering factors are those included in SHELX76.[34] Calculations were carried out on the IBM 4341 with SHELX76 and the program for analysis of molecular geometry.[35]

TABLE VI

Crystal data and details of structure determination of **16A**

Formula $C_{23}H_{25}NO_4$	
Formula wt 379.46	
Cryst. system orthorombic	
a / Å	6.271(2)
b / Å	14.452(6)
c / Å	22.423(5)
V / Å^3	2032.2
Z	4
D calc g cm^{-3}	1.24
Space group	$P2_12_12_1$
F(000)	808
T / K	293(1)
Radiation (graphite monochr) MoKα, λ = 0.71073Å	
Difractometer	Nonius-CAD4
μ (MoKα), cm^{-1}	0.79
Θ range, deg 2.5, 30	
Scan method	$\omega/2\ \Theta$
Scan interval, deg	1.1 + 35 tg Θ
No of reflections measured	3404
No of unique reflections with I $\geq$ 3 σ I	1618
Quantity minimized $\Sigma w \| \|F_o\| - \|F_c\| \|^2\ w^{-1} = \sigma^2 (F_o) + 0.001\ F^2$	
R, R_w, S	0.064, 0.068, 1.32
$(\Delta/\sigma)_{max}$	0.15 (C 23, Z)
$(\Delta\varphi)_{max}$; $(\Delta\varphi)_{min}$ (eÅ^{-3})	−0.24, 0.22

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SAŽETAK

Enantioselektivna redukcija nekih aromatskih ketona pekarskim kvascem

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Redukcija aromatskih ketona **1—6** pekarskim kvascem pokazala je da prihvatljivost supstrata nije određena ni brojem arilnih skupina niti udaljenošću karbonilne od arilne skupine. Relativne brzine konverzije **3, 4 i 6** prema etilacetoacetatu kao »standardnom« supstratu bile su reproducibilne u nizu **3>6>4.** Snažna inhibicija redukcije etilacetoacetata benzenom nestaje pri vrlo niskim koncentracijama obiju komponenata. Redukcija fenil-pirid-2'-il-metil ketona (**1**) i njegovog deaza- analoga (**6**) dala je *sec*-alkohole suprotnih konfiguracija, tj. (−)-**7** i (+)-**12**. Taj ishod može se pripisati pretežnom vezanju enola **1A** sa suprotne enantiostrane u odnosu na **6**. Apsolutna konfiguracija (−)-**7** tentativno je označena kao *R* na osnovi empirijskog pravila zasnovanoga na ^{1}H-NMR spektrima estera Mosherove kiseline sa **13—15**. Ta asignacija potvrđena je određivanjem strukture jediničnog kristala estera kamfan-kiseline **16A** X-zrakama, koji je nakon hidrolize dao enantiomer (−)-**7**.