Tetrahedron Vol. 35, pp. 1409 to 1414
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0040-4020/79/0401-1409/$02.00/0

MICROBIOLOGICAL TRANSFORMATIONS—XII†

MICROBIOLOGICAL REDUCTION OF RACEMIC 1-(2',2',3'-TRIMETHYL-CYCLOPENT-3'-EN-1'-YL)PROPAN-2-ONE AND 1-(2',2',3'-TRIMETHYL-CYCLOPENT-3'-EN-1'-YL)BUTAN-2-ONE BY *RHODOTORULA MUCILAGINOSA*‡

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(*Received in UK 14 November 1978*)

Abstract—The microbiological reduction of (±)-1-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-propan-2-one (**4**) and (±)-1-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-butan-2-one (**5**) by *Rhodotorula mucilaginosa* was investigated. Both enantiomers of **4** are reduced stereospecifically to corresponding alcohols; (+)-(2*S*, 1'*R*)-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-propan-2-ol (**6**) and (−)-(2*S*,1'*S*)-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-propan-2-ol (**7**).

The substrate selectivity in the reduction of **5** was observed: *R* enantiomer of **5** yields stereospecifically (+)-(2*S*,1'*R*)-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-butan-2-ol (**8**) while *S*(−)**5** remains unchanged.

Racemic 1-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-propan-2-one (**4**) and 1-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-butan-2-one (**5**) are potential synthons of juvenile hormone analogues of the A or B type (Fig. 1). These racemic compounds, as we have found, exhibit a morphogenetic activity in tests on *Tenebrio molitor* and *Dysdercus cingulatus*.[1]

The starting material for their synthesis was synthetic (±) or natural (+)-camphor (**1**). The reactions proceeding via its oxime (**2**) and α-campholenonitrile (**3**) gave the desired ketones **4** and **5** in high purity and yields. The side chain in both ketones could be elongated using a sequence of selected reactions enabling the introduction of double bonds with E-geometry at the positions characteristic of the above mentioned juvenoids of the A and B type.[1]

Some examples of a difference in the biological activity of optically active juvenile hormones and juvenoids depending on the enantiomer used ase known.[2-6] Therefore, we expected some differences in the biological response between enantiomers of the presented compounds of the A and B type.

For that reason as well as for the determination of stabilities of certain structures in the natural environment, we attempted to resolve racemic ketones **4** and **5** by microbiological transformation by means of a strain of *Rhodotorula mucilaginosa* species. This species, selected from among 25 other ones by the screening method, seemed to be useful for the selective and stereospecific reduction of the CO group in the starting materials studied.

Previously we had demonstrated a microbiological reaction of this kind in the cases of synthetic and racemic steroid diketones and terpenic diketone,3-caren-2,5-dione.[7-10]

We carried out the microbiological reactions by the standard method using a concentration of 120–150 mg of the substrate in 1 dm^3 of *Rhodotorula mucilaginosa* culture grown on maltose nutrient. Products were isolated on an average after 10 days of transformation by means of column chromatography. The course of the transformation of (±) **4** was as follows (Fig. 2).

The reduction of the CO group only was observed and the optically active alcohols, (+)-(2*S*,1'*R*)-(2',2',3'-trimethyl-cyclopent-3'-en-1'-yl)-propan-2-ol (**6**) and (−)-(2*S*, 1'*S*)-(2',2',3'-trimethylcyclopent-3'-en-1'-yl)-butan-2-ol ((−)**7**), were formed. About 30% of the slightly optically active starting material with the predominance of (−)**4** remained unchanged. Some losses of products arose during isolation and chromatographic separation due to their volatility but from the amounts of products preparatively obtained it could be estimated that both alcohols are formed in almost the same ratio, (+)**6** slightly predominating. Both alcohols were oxidized with CrO_3 in pyridine to confirm the stereospecificity of reduction. Two optically active ketones, the enantiomers of starting (±)**4**, with specific rotations +19° and −18°, were obtained. Ketone (+)**4**, was subjected to repeated reduction by the use of *Rh.m.* giving again alcohol (+)**6**, chromatographically pure and identical by IR with that obtained from racemic **4**.

The structure of the products obtained was determined as follows (Table 1).

The absolute configuration on C-2 of alcohols (+)**6** and (−)**7** was designated as *S* by the method of Horeau.[11] Their IR spectra indicate the presence of an OH group at 3630 cm^{-1} and a double bond at 3040 and 800 cm^{-1}. The PMR spectra show protons of four Me groups an olefinic proton and a proton of an OH group. Their mass spectra have the same signal: $m/e M^+$ 168.

Both ketones, (+) and (−)**4**, have structures identical with that of starting (±)**4**. Their IR spectra indicate the presence of a CO group at 1720 cm^{-1} and a double bond at 3040 and 800 cm^{-1}. The PMR spectra contain the

†Part XI: *Arch. Immun. Ther. Exp.* **26**, 133 (1978).

‡The strain obtained from a collection of Prof. A. Nespiak, Department of Botany of Medical Academy, Wroclaw, Poland.

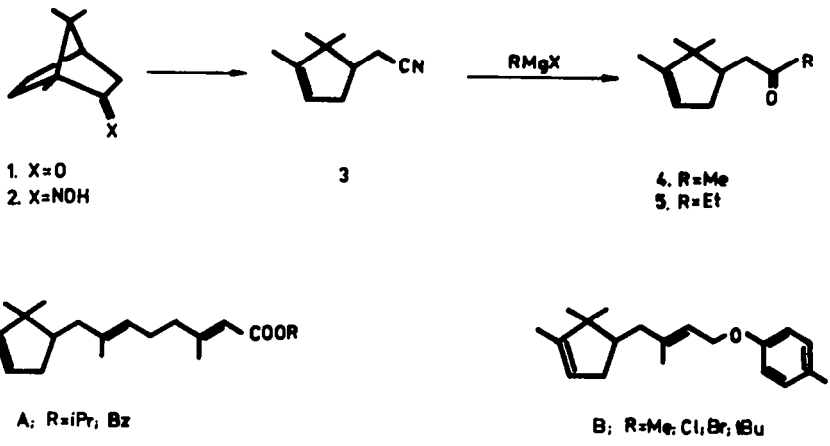

Fig. 1.

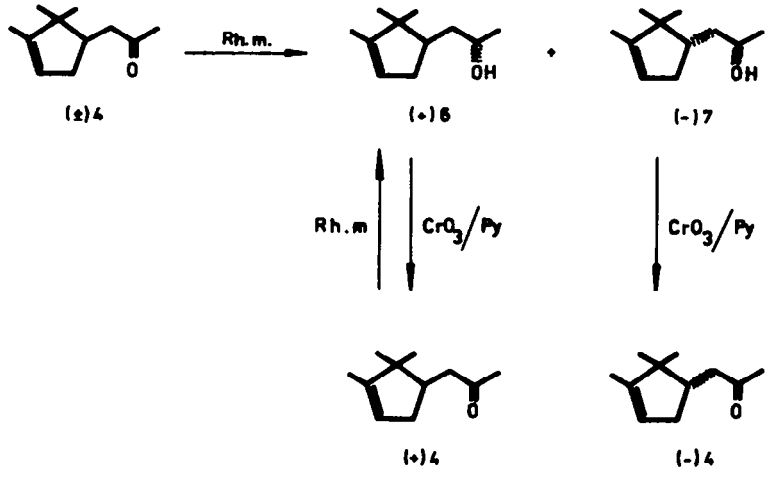

Fig. 2.

Table 1. Substrate (±)4 and its products

Compound	PMR					$[\alpha]_{546}^{20}$	IR cm^{-1}	MS
	C-2	C-3	C-4'	CH_3 CH_3	CH_3-/C-3'/			
(±)4		2.15/S/ 3H	5.2/S/ 1H	1.00/S/ 0.79/S/ 6H	1.6/S/ 3H		3042,1718 800 1054	
(+)6	3.82/m/ 1H	1.29/d/ 3H	5.25/S/ 1H	1.01/S/ 0.8 /S/ 6H	1.68/S/ 3H	+20°	3630 3040 800	m/e M^+ 168 CH_3+H_2O 135M
(-)7	3.85/m/ 1H	1.21/d/ 3H	5.2/S/ 1H	1.0/S/ 0.75/S/ 6H	1.62/S/ 3H	-3°	3630,3040 800	m/e M^+ 168
(+)4		2.16/S/ 3H	5.2/S/ 1H	1.00/S/ 0.79/S/ 6H	1.6/S/ 3H	+19°	1720,3040 800	
(-)4		2.15/S/ 3H	5.2/S/ 1H	1.00/S/ 0.79/S/ 6H	1.6 3H	-18°	1720,3040 800	

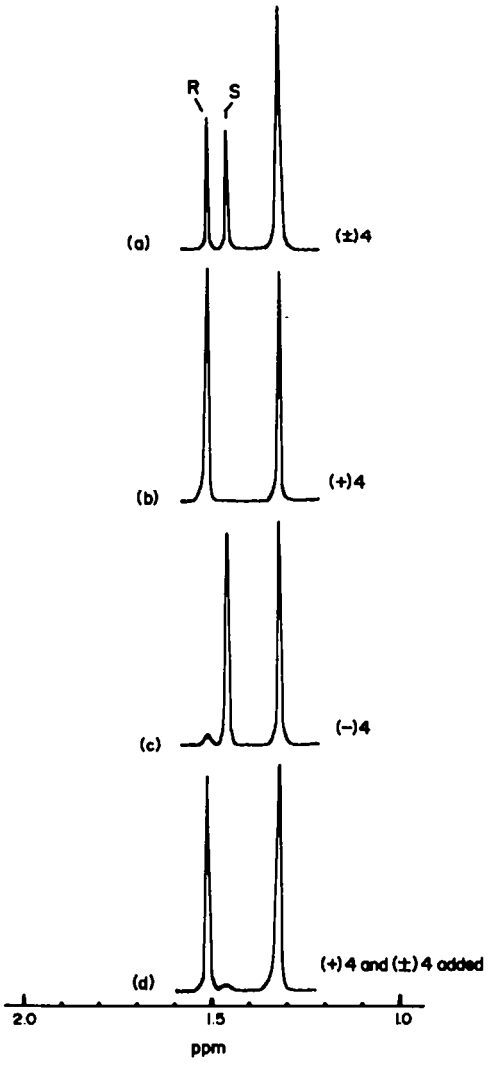

Fig. 3. Resolved Me groups resonances observed for the enantiomers of ketone 4 in the presence of Eu (tcf)$_3$: CCl_4 solution, the concentration of 4 *ca.* 0.6 M, Eu (tcf)$_3$: ketone 4 molar ratio-*ca.* 0.17, TMS as internal reference, 80 MHz, temperature 23°; (a) synthetic racemic 4, (b) and (c) optically active samples of 4 obtained via microbiological transformation, (d) optically pure (+)-(*R*) 4 obtained synthetically from (+)-camphor after addition of 3.0% of synthetic racemate (±)4; quantitative detection of 1.5% of *S* enantiomer in the mixture.

proton signals of four Me groups and an olefinic proton. The absolute configuration on C-1′ of all products was determined by comparison of the specific rotations of ketone (+)4 from microbiological transformations and that prepared from natural (+)-camphor. The enantiomeric purity of both (+)4 and (−)4 was determined directly using chiral shift reagent, tris-[3-trifluoroacetyl-d-camphorato]-europium (III). The signal of one of the two geminal Me groups of racemic 4 is split into two singlets in the presence of Eu(tcf)$_3$. The samples of (+)4 and (−)4 obtained via microbiological transformation were shown to be optically pure by this method which is able to detect qualitatively as low as 1.5% of enantiomer impurity in the optically active (+)4 (Fig. 3).

Substrate (±)5 was subjected to a similar microbiological reduction by *Rh.m.* Its course can be described as follows (Fig. 4).

Two chromatographically separable products, (+)-(2*S*,1′*R*)-(2′,2′,3′-trimethylcyclopent-3′-en-1′-yl) butan-2-ol ((+)8) and (−)-enantiomer of the substrate, (−)-(*S*)-(2′,2′,3′-trimethylcyclopent-3′-en-1′-yl)butan-2-one, were obtained. Alcohol (+)8 gave after oxidation with CrO_3 in pyridine(+)-enantiomer of the substrate (±)5. Ketone (+)5 obtained synthetically from natural (+)-camphor was subjected to the microbiological reduction in order to prove the selectivity of this process. Only alcohol (+)8 chromatographically identical with that obtained from the racemate, resulted. An additional microbiological reduction of ketone (−)5 ($(\alpha) - 15°$), obtained from the first transformation of racemic 5, was also carried out. The chromatographically and spectrally unchanged (−)5 was recovered but its specific rotation increased to −21°.

The structure of products was determined unequivocally (Table 2).

The *S*-configuration on C-2 of alcohol (+)8 was established as previously. The IR spectrum shows the presence of an OH group (3630 cm^{-1}) and a double bond (3045 and 800 cm^{-1}). In the PMR spectrum the proton signals of four Me groups, an olefinic proton and proton at the C atom bearing an OH group are present. Their multiplicity is in agreement with expectations. Both ketones (+)- and (−)5 have identical structures, enantiomeric each to other ($(\alpha) + 20°$ and −21°). Their IR spectra indicate the presence of the CO group (1715 cm^{-1}) and a double bond (3045 and 800 cm^{-1}). In the PMR spectra the proton signals of four Me groups and an olefinic proton are present.

The observations of the course of described trans-

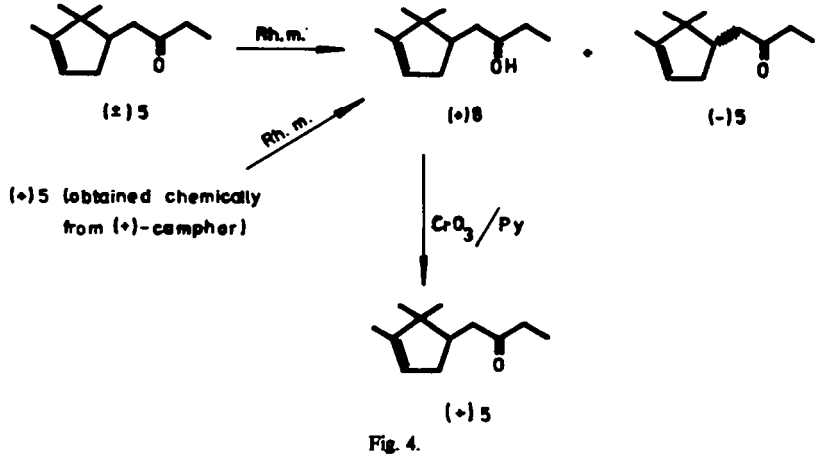

Fig. 4.

Table 2. Substrate (±)5 and its products

Compound	PMR					$[\alpha]_{546}^{20}$	IR/cm^{-1}/
	C_2	C_4	C'_4	H_3C CH_3	CH_3/C-3'/		
(±)5		1.08/t/ 3H	5.2/S/ 1H	1.0/S/ 0.8/S/ 6H	1.64/S/ 3H		3045,1715 800
(+)8	3.58/t/ 2H	0.98/t/ 3H	5.2/S/ 1H	1.0 /S/ 0.77/S/ 6H	1.62/S/ 3H	+17	3630,3045 800
(-)5		1.10/t/ 3H	5.23/S/ 1H	1.04/S/ 0.82/S/ 6H	1.65/S/ 3H	-21	3045,1715 800
(+)5		1.10/t/ 3H	5.22/S/ 1H	1.02/S/ 0.6/S/ 6H	1.64/S/ 3H	+20	3045,1715 800

formations suggest some general remarks:

It can be generally stated that the CO group in enantiomers (+)4 and (+)5 with the *R*-configuration on C-1' is more susceptible to the microbiological reduction with *Rh.m.* strain than in (−)4 and (−)5 with the *S*-configuration. This susceptibility becomes apparent in the different manner for each substrate. The transformation of ketone (±)4 (Fig. 2) proceeds with a small substrate selectivity because both enantiomers undergo reduction. The rate of this reduction is, however, slightly higher in the case of (+)4. The evidence is a small negative rotation of the unreacted starting material due to certain predominance of enantiomer (−)4 which reacts more slowly. At the same time this transformation is characterised by high stereoselectivity of reduction of the CO group giving alcohols (+)6 and (−)7 with the same *S*-configuration on C-2 from both enantiomeric ketones. The diastereomeric, chromatographically separable compounds with high optical purity are formed.

An additional indication of the high stereospecificity of microbiological reduction is the chromatographic homogeneity of alcohol (+)6, the only product of the microbiological reduction of optically pure ketone (+)4 obtained synthetically from (+) camphor.

In the microbiological transformation of ketone (±)5 (Fig. 4), the substrate selectivity and the stereospecificity of the reduction of the CO group is apparent. The transformation proceeds almost exclusively with the participation of one enantiomer (+)5 giving only one alcohol with the *S*-configuration on C-2. The second enantiomer almost does not react at all. The optical purity of two enantiomeric ketones (+)- and (−)5 obtained in this experiment is high, as evidenced by the almost equal absolute values of their specific rotations.

The analysis of these results prompted us to explain the relationship between the structure of the molecular fragment containing the CO group and the reaction course.

Conformational analysis based on Dreiding models and on the fundamental work of Prelog[12] showed that sizes of substituents in the proximity of the CO group are different in substrate 4 and 5. This difference occurs in the β-position (H in 4 an Me in 5) and is the reason for the observed stereospecificity and selectivity of reduction.

In our previous work[13] with the *Rh.m.* strain we found that in the case of identical substituents in α-positions to the CO group on C-3 in androstandione and dihydrotestosterone reduction proceeds non stereospecifically giving two alcohols, α and β. β-Substituents in the A/B *trans*-steroids do not cause, however, such great differences in steric hindrances as in the α-campholenic system. This fact is explained by the analysis of models of the compounds in question. The substrate specificity of microbiological reduction of α-campholenic ketone is different in comparison with the reduction of the corresponding CO group on C-17 in the steroid ketones studied by us earlier,[7,8] where the asymmetric C-13 atom and the C-16 methylene group occupied α-positions. The enantiomers C-13*S* react faster than C−13*R*. In the reductions of α-campholenic ketones reported here, the preferred substrate for the transformation is enantiomer C-1'*R* reacting more rapidly in the case of (±)4 or being the only reacting enantiomer in the case of (±)5.

Two explanations of this difference are possible. The first one is the different distances that substituents of differing sizes lie from the CO group. The second explanation could be based on the fact that the more reactive enantiomers, 13*S* and 1'*R*, have the reacting molecular fragment characteristic of the typical natural compounds. Similar observations have been reported for yeasts, to which *Rh.m.* strain belongs.[14]

The substituents (Me and Et group) in the ketones studied influence also decisively the reactivity of enantiomer C-1'*S*. Whereas the Me group in this enantiomer of (±)4 enables the reduction of ketone, though with a remarkable retardation of its rate in comparison with enantiomer C-1'*R*, the Et group in (−)5 makes this reaction practically impossible.

The presented results are the basis for the above suggestions but we are continuing investigations of consecutive derivatives of the α-campholenic system to confirm them.

EXPERIMENTAL

All cultures of microorganisms were grown on 2% nutrient "Malto" manufactured by "Warta"-Srem. A preliminary cultivation of cultures of 25 various microorganisms (screening) was performed in 200 ml flasks containing 100 ml of the sterile nutrient. After 2 days of growth at 27° with shaking, 10 mg of the substrate (±)4 or (±)5 dissolved in 1 ml of acetone were added to each flask and shaking was continued for 7 days. The chloroform extract of products was controlled by tlc.

The preparative cultivations were carried out in 2l. flasks containing 1 l. of *Rhodotorula mucilaginosa* cultures. The substrate was added in amounts 120–150 mg for 1 l. of the culture after 3 days of growth. The transformation was carried out for *ca* 10 days with shaking. Products were extracted with $CHCl_3$ and separated chromatographically on silica gel (Merck), eluent being mainly petroleum ether–acetone (10:1) mixture. The eluent for tlc was the petroleum ether–acetone (5:1) mixture.

The spectral measurements were carried out on following devices: IR: UR-20 (Zeiss) ($CHCl_3$); NMR: Varian 100 Mc; MS:209 GGMS (LKB Sweden); optical rotation: Polamat A Zeiss ($CHCl_3$).

(±)- and (+)-Campholenonitrile (3)

Nitrile 3 was obtained in 86% yield from (±)- and (+)-camphor according to Tiemann.[15] Glc showed the presence of the isomeric β-campholenonitrile (2,3,3-trimethylcyclopent-1-en-1-ylacetonitrile) (*ca.* 14%). Fractional distillation yielded 22% of homogeneous 3: b.p. 64–64.5°/(4 mm), n_D^{20} 1.4668, $[\alpha]_D^{20}$ + 6.68° (MeOH, *c* 15.3); lit.[15]: n_D^{20} 1.46653; $[\alpha]_D^{20}$ + 7.30° 1 dm. Fractions containing *ca.* 10% of β-campholenonitrile were used in ketones 4 and 5 preparations.

1-(2',2',3'-*Trimethylcyclopent-3'-en-1'-yl*)*propan-2-one* (4)

Nitrile 3 (29.8 g; 0.20 mole) was added dropwise to the boiling soln of MeMgI, prepared from 12.1 g (0.50 mole) Mg and 74.5 g (0.525 mole) MeI in 1500 ml ether during 4 hr. The mixture was refluxed for additional 4 hr and left overnight. After quenching with dil AcOH the mixture was stirred at 20° for 1 hr and the product extracted with ether. The extracts were washed with sat $NaHSO_3$aq, 10% NaOHaq and brine. Distillation of crude ketone through short Vigreux column yielded 28.5 g (85.5%) of ketone 4; b.p. 81–82.5/6 mm, n_D^{20} 1.4632. Glc revealed 5% of a contamination with shorter retention time (raising from isomeric β-campholenonitrile). Chromatographically homogeneous (glc, tlc) sample of 4 was obtained by preparative glc (20% Carbowax on Chromosorb W): n_D^{20} 1.4631, $[\alpha]_D^{20}$ + 25.4° (1 dm), lit.[16]: $[\alpha]_D^{20}$ + 18.48°.

1-(2',2',3'-*Trimethylcyclopent-3'-en-1'-yl*)*butan-2-one* (5)

Ketone 5 was obtained analogously to the above procedure using EtMgBr (0.40 mole) in 1200 ml of the ether and 0.20 mole of nitrile 3 yield: 26.0 g (72%), b.p. 83–85° (1 mm), n_D^{20} 1.4632. Glc purity: 97.5%. Tlc and glc pure sample was obtained by preparative glc and showed: n_D^{20} 1.4629, $[\alpha]_D^{20}$ + 24.14° (1 dm). Lit.[16]: $[\alpha]_D^{20}$ + 17.53°.

Transformation of (±) 4 *by* Rh.m.

The transformation of 300 mg of (±)4 was carried out as described above. 240 mg of product mixture, obtained after extraction, were chromatographed giving:

Starting material (±)4 and (−)4, $[\alpha]_D^{20}$ − 6° (*c* = 2)	70 mg
Alcohol (+)6 m.p. 54°, $[\alpha]_{546}^{20}$ − 20° (*c* = 6)	40 mg
Alcohol (−)7, $[\alpha]_{546}^{20}$ − 3° (*c* = 2)	35 mg

Oxidation of alcohols (+)6 *and* (−)7

50 mg of (+)6 or (−)7 were dissolved in 0.5 ml absolute pyridine, 50 mg CrO_3 in 0.5 ml pyridine were added and the mixture was allowed to stand at room temp for 24 hr. After extraction with ethyl ether and normal treatment, the extract was chromatographed on silica gel, the eluent being hexane-acetone (10:1). Alcohol (+)6 gave 35 mg of ketone (+)4, $[\alpha]_{546}^{20}$ + 19° (*c* = 10), alcohol (−)7 32 mg of ketone (−)4, $[\alpha]_{546}^{20}$ − 18° (*c* = 2.2).

Determination of absolute configuration of alcohols (+)6 *and* (−)7 *by the method of Horeau*[11]

To 42 mg (2.5×10^{-4} mol) of alcohol (+)6, 60.8 mg (18.6×10^{-4} mol) of (±)-α-phenylbutyric acid anhydride in pyridine were added and the mixture was allowed to stand overnight in a tightly closed flask. After this time the reaction was finished (tlc).

A drop of water was added and the mixture was refluxed for 30 min to hydrolyse an excess of anhydride. Then 5 ml of benzene were added and alkalysed with 0.1 N NaOH (phenolphthalein). The alkaline aqueous soln was successively extracted with benzene, chloroform and ether (10, 30 and 10 ml, resp.). The aqueous soln was acidified with a drop of conc. HCl and free α-phenylbutyric acid was extracted with benzene (3 × 15 ml). After drying and evaporating, 70 mg of the acid with rotation value of −0.35° (in benzene) were obtained. This means that alcohol (+)6 has the absolute configuration *S*.

The absolute configuration of alcohol (−)7 was determined similarly, the sample being 37 mg. After extraction, 68 mg of the acid were obtained, its rotation value being −0.2° (in benzene). Therefore, alcohol (−)7 has the *S*-configuration.

Microbiological transformation of ketone (+)4 *by means of* Rh.m.

This transformation was carried out similarly as above using 100 mg of (+)4 obtained by chemical oxidation of alcohol (+)6. Tlc showed the formation of only one alcohol. Column chromatography delivered pure (+)6 $[\alpha]_{546}^{20}$ + 18.5° (*c* = 2).

Microbiological transformation of ketone (±)5 *by means of* Rh.m.

The transformation of 450 mg of (±)5 was performed as above. 420 mg of a mixture obtained after extraction were chromatographed, the eluent being petroleum ether-acetone (20:1). 110 mg of ketone (−)5, $[\alpha]_{546}^{20}$ − 14° (*c* = 3) and 110 mg of alcohol (+)8, $[\alpha]_{546}^{20}$ + 17° (*c* = 3) were obtained. Tlc shows traces of a more polar substance near (+)8.

Ketone (−)5 was subjected to repeated microbiological transformation. The unchanged substrate, $[\alpha]_{546}^{20}$ − 21°, was recovered.

Determination of absolute configuration of alcohol (+)8

This determination was carried out as above using 37 mg of (+)8. 68 mg of the acid with rotation −0.2° (in benzene) were obtained after extraction. Therefore, alcohol (+)8 has the *S*-configuration.

Chemical oxidation of alcohol (+)8

50 mg of (+)8 were oxidized under conditions described above giving 40 mg of ketone (+)5, $[\alpha]_{546}^{20}$ + 17° (*c* = 3).

Transformation of ketone (+)5 *obtained synthetically*

The transformation of 100 mg of (+)5 obtained from (+)-camphor was carried out as previously giving 70 mg of alcohol (+)8, homogeneous by tlc, $[\alpha]_{546}^{20}$ + 17° (*c* = 3).

Acknowledgements—We express our gratitude to Mrs Urszula Walkowiak from the Institute of Organic and Physical Chemistry, Technical University, Wroclaw for PMR measurements. We thank also Mr. Andrzej Nosal and Mr Zygmunt Przepiórka from the same Institute for performing glc preparations.

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