

MICROBIOLOGICAL TRANSFORMATIONS—XV†

MICROBIOLOGICAL TRANSFORMATION OF 1-(2,2,3-TRIMETHYLCYCLOPENT-3-EN-YL)-PROPAN-2-ONE AND THEIR HOMOLOGUES BY *ACREMONIUM ROSEUM* s.s. GAMS 1971

A. SIEWIŃSKI, J. DMOCHOWSKA-GŁADYSZ and T. KOŹEK
Academy of Agriculture, ul. Norwida 25, 50-375 Wrocław, Poland

A. ZABŹA and K. DERDZIŃSKI
Institute of Organic and Physical Chemistry, Technical University, 50-370 Wrocław, Poland

and

A. NESPIAK

Medical Academy, 51-601 Wrocław, Poland

(Received in UK 3 August 1982)

Abstract—The microbiological transformations of 1-(2,2,3-trimethylcyclopent-3-en-yl)-propan-2-one (**2a**) and its four (**2b–e**) homologues by *Acromonium roseum* were investigated. For **2a, b** and **e** ketones Baeyer–Villiger oxydation was predominant reaction, but ketone **2c** and **d** were mainly hydroxylated.

In support of our studies on the biotransformations of isoprenoid compounds by means of fungi,^{1,2} *Acromonium roseum* is characterized by its ability to grow on a variety of organic substrates, and the strain we used in our biotransformations was isolated from wheaten straw and cultivated on maltose nutrient.

As model compounds for these investigations we selected a series of alkyl (2,2,3-trimethyl-3-cyclopentenyl) methyl ketones **2a–e** with the chirality centre in the cyclopentenyl ring in β -position to the CO group. Thus, using racemic and/or optically active ketones as the substrates, we were able to study not only the general type of reactions but also the enantiomeric selectivity of the investigated biotransformations.

The starting ketones **2a–e** were synthesized from both racemic and natural (+)-camphor which were converted (via oxime) into racemic and (+)-(R)- α -campholenitrile,¹ and these compounds were used in Grignard reactions with appropriate alkylmagnesium halides under optimized conditions to give pure products in high yields³ (Fig. 1).

The microbiological transformations were carried out

in submerged cultures of *Acromonium roseum* containing about 250 mg of the substrate per 1l. of the culture. After ten or two days of biotransformation at 27° the products were isolated by extraction with chloroform and the crude mixture of products was analyzed by TLC, GC, GC-MS and/or chromatographed to separate the compounds formed.

The products of the biotransformations (Fig. 2) indicate the oxidative properties of *Acromonium roseum*. Depending on the structure of the substrate, both Baeyer–Villiger and/or the allylic oxidation were observed as reported for other microorganisms.

The type of reaction and enantiomeric selectivity of the biotransformations by *Acromonium roseum* depend on the structure of starting ketone. In the case of methyl ketone **2a** only the Baeyer–Villiger oxidation was observed. In the products mixture, apart from the starting ketone **2a**, we found alcohol **3** (2,2,3-trimethyl-3-cyclopentenyl-methanol) and acid **4** which was probably the product of subsequent oxidation of alcohol **3**. If the biotransformation was carried out for 10 days, only traces of substrate remained. However, carrying out the biotransformation for 2 days only, we found that the enantiomer (–)-(S)-**2a** was more reactive. This was evidenced by the sign of optical rotation of the regenerated

†Part XIV In *Polish J. Chem.* in press.

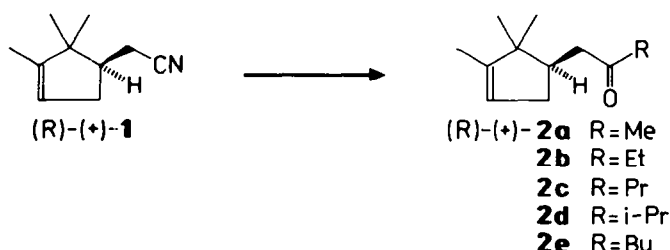
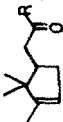

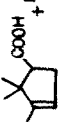
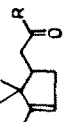
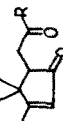



Fig. 1.

Fig. 2. The products of the biotransformations of investigated ketones.

starting material	products					
 2	 3	 4	 5	 6	 7	unreacted starting material 2
2a R = CH ₃ ^{**} [α] _D ²⁰ ₅₄₆ +1° ^{***} [α] _D ²⁰ ₅₄₆ 30% ^{**} [α] _D ²⁰ ₅₄₆ 0°	50%	32%	0°	-	-	20%
2b R = C ₂ H ₅ ^{**} [α] _D ²⁰ ₅₄₆ +4,5° ^{***} [α] _D ²⁰ ₅₄₆ 32% ^{**} [α] _D ²⁰ ₅₄₆ 0°	40%	35%	0°	traces	-	25%
2c R = n-C ₃ H ₇ ^{**} [α] _D ²⁰ ₅₄₆ traces ^{**} [α] _D ²⁰ ₅₄₆ -	traces	traces	traces	22%	-	45%
2d R = i-C ₃ H ₇ ^{**} [α] _D ²⁰ ₅₄₆ -	-	-	10%	0°	-	64%
2e R = n-C ₄ H ₉ ^{**} [α] _D ²⁰ ₅₄₆ 20% ^{**} [α] _D ²⁰ ₅₄₆ -4,5°	20%	5%	5%	5%	traces	60%

* 2 days transformation

*** 10 days transformation

ketone: (+)-2a, as well as of the resulting alcohol (+)-3. In a separate experiment we established that from the (+)-(R) enantiomer of ketone 2a, the alcohol (-)(S) 3 was formed. The change of chirality sign is not connected with the inversion at the chiral C atom, but with the change of formal sequence of substituents at this chiral center.

The Bayer-Villiger oxidation also predominated in the biotransformation of ethyl ketone 2b—the closest homologue of 2a. Thus, we isolated from the products mixture alcohol 3 and acid 4, and after shorter time of biotransformation (2 days), the unreacted ketone (+)-2b. Precise GC-MS analysis revealed the presence of traces of products of allylic oxidation: hydroxyketone 5b and diketone 6b (which were identified taking advantage of the chromatographic and spectroscopic data obtained for their homologues which were isolated in substantial amounts). The Bayer-Villiger oxidation was also regioselective and the sign of the optical rotation of the isolated alcohol (+)-3 suggested that the enantiomeric preference of this reaction was identical to that observed for methyl ketone 2a.

The biotransformations of the two next homologues: propyl and isopropyl ketones 2c and 2d followed completely different courses. In both these cases the allylic oxidation predominated yielding hydroxyketones 5c,d and traces of diketones 6c respectively. In the biotransformation of n-propyl ketone 2c only small amounts of Bayer-Villiger products resulted: alcohol 3 and acid 4 were detected by GC-MS. The rate of the biotransformation of ketones 2c and 2d was much lower in comparison to methyl and ethyl ketones 2a and 2b. Regenerated ketones 2c and 2d were completely devoid of optical activity (ketone 2d) or exhibited small optical activity (ketone 2c), which may suggest the lack of enantiomeric selectivity in these biotransformations.

The most complex reaction was observed for n-butyl ketone 2e. In addition to the regioselective Bayer-Villiger and the allylic oxidations (hydroxyketone 5e and diketone 6e), diol 7 was found, which seemed to be a product of both types of oxidation. In spite of the complex character of the reaction, we observed an exceptionally high enantiomeric selectivity. Alcohol (-)(S)-3 was formed in almost 100% enantiomeric purity.

Analysis of the spectroscopic data of the compounds mentioned is simplified by the presence of characteristic signals connected with the 2,2,3-trimethyl-3-cyclopentenyl moiety in their IR and ¹H-NMR spectra. These are, for example, two singlets for geminal Me groups at about $\delta = 0.85$ and 1.0 ppm in their ¹H-NMR spectrum, and the sharp strong band at 800 cm⁻¹ for trisubstituted double bond in the IR spectra.

In all cases investigated the Bayer-Villiger oxidation was regioselective. Crude mixtures of products were treated with diazomethane prior to GC-MS in order to convert free acids into volatile methyl esters. Mass spectra were recorded for low electron energy (15 eV) in order to avoid deeper fragmentation and to allow molecular ions of the labile compounds to appear in the spectrum. Since the second possible product of the Bayer-Villiger oxidation should be α -campholenic acid (8), we synthesized this from nitrile 1 (Experimental). However, no trace of this compound could be detected, thus proving the regioselectivity of the reaction.

The GC-MS analyses were also applied to the detection of nonisolable compounds in the product mixtures, e.g. alcohol 3 and acid 4 (products from ketone 2c), and

the allylic oxidation products: hydroxyketones 5b and diketones 6b-e. The assignment of the structure of 5b to a peak eluted as the last one of products from ketone 2b was based on the comparison of chromatographic data (GC) and the MS of 5b with those of hydroxyketones; 5c-e (which were isolated in pure state from corresponding mixtures and their structures were unequivocally assigned by IR and ¹H-NMR spectra). The MS spectra of 5b and 5c-e were very similar; the differences being well justified by their homologous character (Experimental). Thus, intensive ions at *m/e* 106, 107, 109, 149, 95, 124, 121 appeared in all the MS spectra in similar relative intensities, while the other intensive ions as (M-18)⁺, (M-33)⁺, (RCO)⁺ and (R)⁺ appeared at *m/e* values depending on the nature of R attached to CO group (Experimental).

The structures of other products of allylic oxidation: diketones 6b-e were proposed on the ground of comparison of their MS spectra with that of ketone 6a. We had obtained this compound in larger amounts in other biotransformation by means of *Botrytis cinerea*¹ and its structure was proved by the IR, UV and ¹H-NMR spectra. In all these MS spectra we found the presence of ions typical of diketone 6a, as (C₉H₁₃O)⁺ *m/e* 137, (C₈H₁₁O)⁺ *m/e* 123, (C₁₀H₁₃O₂)⁺ *m/e* 165, (C₈H₁₃)⁺ *m/e* 109 (Experimental), which could be explained only when one additional CO group is included to the 5-membered ring. The discussion of the pathways of these MS fragmentations will be presented in a separate paper.

We have evidenced the enantiomeric selectivity of the bio-transformation only for the Bayer-Villiger oxidation. In a series of the NMR measurements of racemic alcohol 3 with chiral shift reagent [tris - (3 - trifluoroacetyl - d - camphorato) - europium (III)], we found that the signals of one of the two geminal Me groups was split into two singlets. Searching for the correlation between the chemical shifts of these two singlets, the change of position of other signals in the ¹H-NMR spectrum with different amounts of Eu(tfc)₃, we observed that the best results were obtained when the molar ratio of Eu(tfc)₃ to alcohol 3 was 0.075 (Fig. 3 and Diagram 1). At a lower temperature (-25°) it was possible to integrate two separated signals of the Me groups of both enantiomers and to observe the splitting of signal of other gem-Me groups (Fig. 3). In a separate experiment using this method we demonstrated the possibility of qualitative detection of enantiomeric impurity as low as 2%, in the optically active form of (-)(S)-alcohol 3 (Fig. 4). We have also observed interesting differences in the signals of methylene protons, deshielded by OH in the -CH₂OH group in racemic and pure (-)(S)-enantiomer of alcohol 3. These protons are diastereotopic and in pure (-)(S) enantiomer reveals a doublet in the ¹H-NMR spectrum, which is rather the AB part of the more complicated of ABX system. In the case of racemic alcohol 3 we observed a multiplet, which probably was the result of overlapping of two AB parts derived from both enantiomers (Fig. 5).

EXPERIMENTAL

Transformations were carried out in 2 dm³ flasks containing 1 dm³ *Acremonium roseum* cultures on maltose nutrient at 27° with shaking. After 3 days of growth the substrates were added in amounts 250 mg for 1 dm³ of cultures. Products were extracted with CHCl₃ after 10 or 2 days of transformation. Crude mixtures were separated chromatographically on silicagel, using hexane-ethyl ether mixtures (10:1 or 3:1) as eluent.

The spectral measurements were carried out on the following

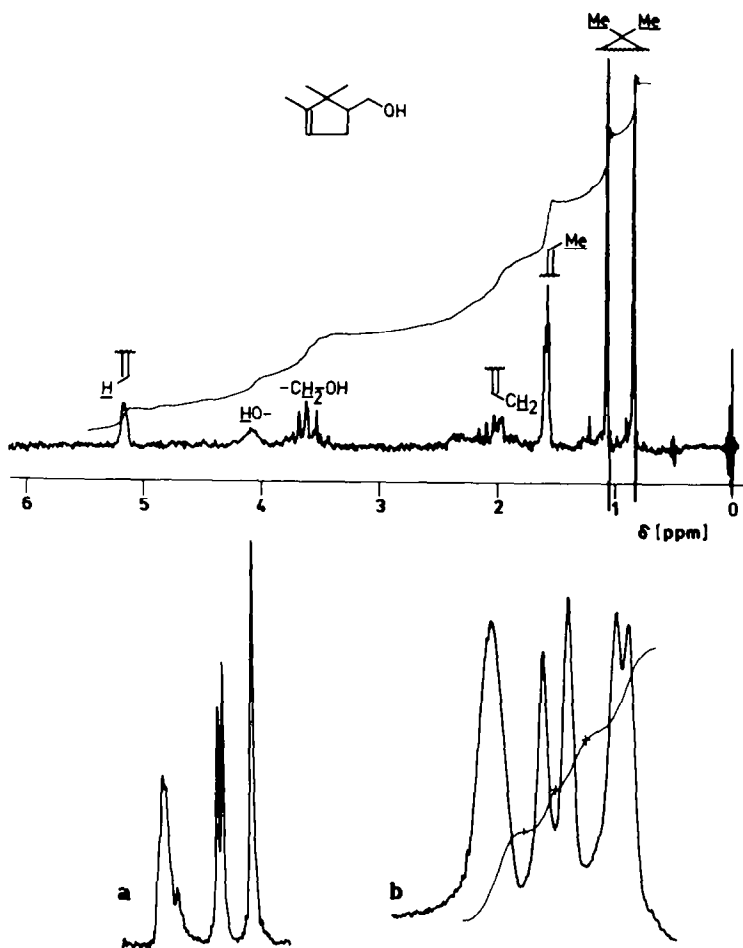


Fig. 3. NMR spectrum of alcohol (-)(S)-3. (a) Me groups of NMR spectrum of alcohol (-)(S)-3 with Eu(tfc), at 0°. (b) Me groups of NMR spectrum of alcohol (-)(S)-3 with Eu(tfc), at -25°.

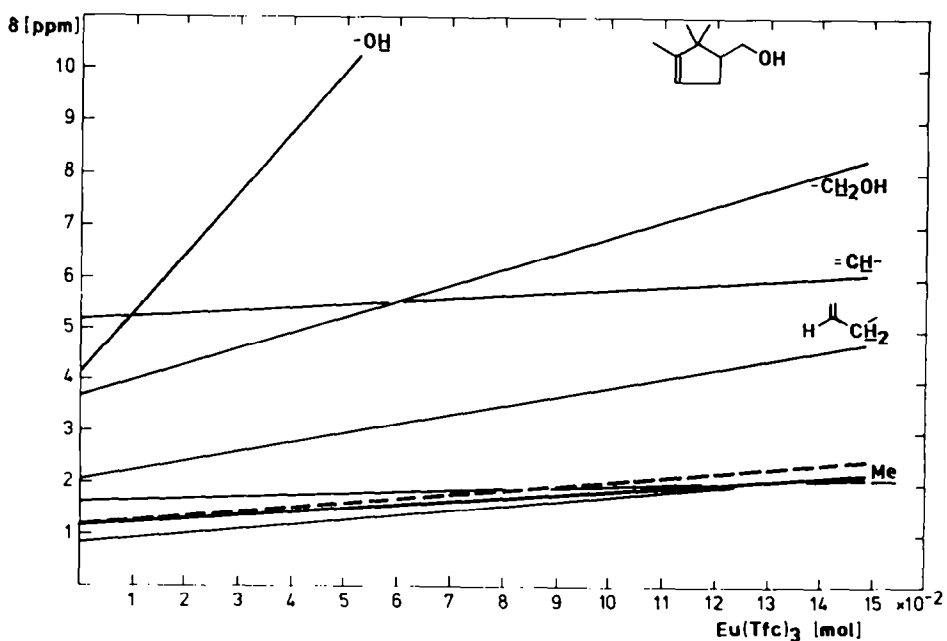


Diagram 1. The correlation between the chemical shifts of the NMR signals of alcohol 3 with different amounts of Eu(tfc)₃.

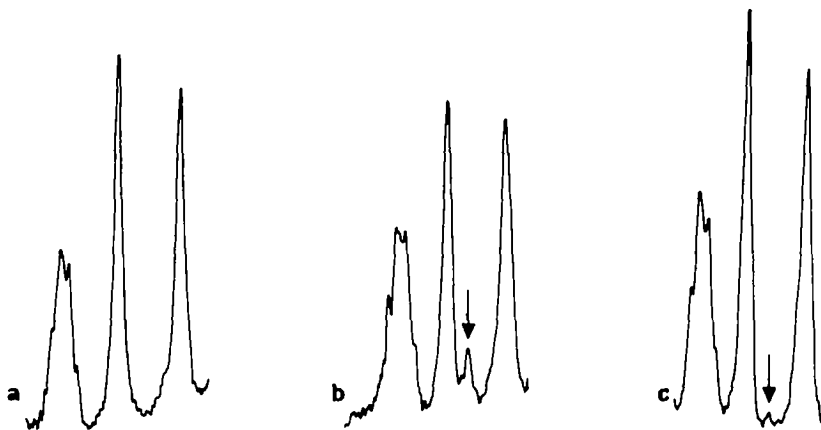


Fig. 4. Resolved Me groups resonances observed for the enantiomer $(-)(S)$ -3 in presence of $\text{Eu}(\text{tfc})_3$ in CCl_4 solution at 0° . (a) optically pure $(-)(S)$ -3. (b) $(-)(S)$ -3 with 10% of racemate (\pm) -3. (c) quantitative detection of 2% (R) -3 in the mixture.

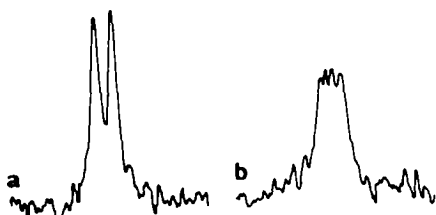


Fig. 5. $-\text{CH}_2\text{OH}$ proton signals of (a) $(-)(S)$ -3. (b) (\pm) -3.

devices: IR: UR-20/Zeiss (film), $^1\text{H-NMR}$: Varian 100 Mc or Tesla 100 Mc with TMS as internal standard, MS: 209 GCMS (LKB Sweden) 15 eV, optical rotation Polamat A (Zeiss) in CHCl_3 ($c \approx 3$).

(\pm) and $(+)$ -Campholenitrile 1. This was obtained in 86% yield from (\pm) and $(+)$ -camphor according to Tiemann.⁵ GLC showed the presence of the isomeric β -campholenitrile (2,3,3-trimethylcyclopent-1-en-ylacetone nitrile) (ca 14%). Fractional distillation yielded 22% of homogeneous 1: b.p. $64\text{--}64.5^\circ$ (4 mm, n_D^{20} 1.4668, $(\alpha)_D^{20} + 6.68^\circ$) MeOH, c 15.3; fractions containing ca 10% of β -campholenitrile were used in **2a-d** preparations.

1-(2,2,3-Trimethylcyclopent-3-en-yl)propan-2-one (**2a**). Nitrile 1 (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 an 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% NaOH aq and brine. Distillation of the crude ketone through short Vigreux column yielded 28.5 g (85.5%) of **2a** b.p. $81\text{--}82.5/6$ mm, n_D^{20} 1.4632. GLC revealed 5% of a contamination with shorter retention time (raising from isomeric β -campholenitrile). Chromatographically homogeneous (GLC, TLC) sample of **2a** was obtained by preparative GLC (20% Carbowax on Chromosorb W): n_D^{20} 1.4631, $(\alpha)_D^{20} + 25.4^\circ$ (1 dm). IR: $\nu = 3042, 1718, 800\text{ cm}^{-1}$. $^1\text{H-NMR}$: $\delta = 5.2$ (b.s. 1H), 2.15 (s 3H) 1.6 (s 3H), 1.0 (s 3H); 0.79 (s 3H). MS: m/e 166 (M^+ ; 2); 109 (15); 108 (100); 93 (22).

1-(2,2,3-Trimethylcyclopent-3-en-yl)butan-2-one (**2b**). This was obtained as above using EtMgBr (0.40 mole) in 1200 ml ethyl ether and 0.20 mole of **1**, yield: 26.0 g (72%), b.p. $83\text{--}85^\circ$ (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, $(\alpha)_D^{20} + 24.14^\circ$ (1 dm). IR: $\nu = 3045, 1715, 800\text{ cm}^{-1}$; $^1\text{H-NMR}$: $\delta = 5.2$ (b.s. 1H); 1.64 (s 3H); 1.08 (t $J = 5$ Hz, 3H); 1.0 (s 3H); 0.80 (s 3H); MS: $m/e = 180$ (M^+ ; 2); 109 (10); 108 (100); 93 (10).

1-(2,2,3-Trimethylcyclopent-3-en-yl)pentan-2-one (**2c**). This was obtained as previously using PrMgBr 0.2 mole in 600 ml ethyl ether and 0.1 mole of **1**, yield: 12.7 g (**2c**)—65.4%. IR: 3045, 1720, 800 cm^{-1} ; $^1\text{H-NMR}$: $\delta = 5.2$ (bs 1H); 1.62 (bs 3H) 0.98 (s 3H); 0.91 (t—3H $J = 5$ Hz); 0.77 (s 3H). MS: $m/e = 194$ (M^+ 18), 109 (10), 108 (100); 93 (8).

1-(2,2,3-Trimethylcyclopent-3-enyl)-3-methylbutan-2-one (**2d**). This was obtained using $i\text{-PrMgBr}$ 0.2 mole in 600 ml ethyl ether and 0.1 mole of **1**, yield: 9.4 g 48.4% **2d**. IR: 3045, 1715, 800 cm^{-1} ; $^1\text{H-NMR}$: $\delta = 5.25$ (bs 1H); 1.56 (bs 3H); 1.07 (s 3H); 0.96 (d 6H $J = 4$ Hz); 0.73 (s 3H). MS: m/e 184 (M^+ 3); 109 (12); 108 (100); 93 (9).

1-(2,2,3-Trimethylcyclopent-3-en-yl)hexan-2-one (**2e**). This was obtained using BuMgBr 0.2 mole in 600 ml ethyl ether and 0.1 mole of **1**, yield: 13.3 g (63.8%) **2e**. IR: $\nu = 3045, 1715, 800\text{ cm}^{-1}$; $^1\text{H-NMR}$: $\delta = 5.20$ (bs 1H); 1.62 (bs 3H); 0.98 (s 3H); 0.9 (t 3H $J = 5$ Hz); 0.78 (s 3H); MS: m/e 208 (M^+ 2); 109 (9); 108 (100); 93 (6).

Transformations of (\pm) -**2a** ketone

A. After 10 days the following products were obtained after chromatography:

(i) 75 mg (30%) alcohol (\pm) -3: IR: $\nu = 3640, 3040, 800\text{ cm}^{-1}$; $^1\text{H-NMR}$: $\delta = 5.12$ (bs 1H); 3.66 (m 2H); 1.68 (bs 3H); 1.07 (s 3H); 0.85 (s 3H); MS: m/e (rel intensity) = 140 (M^+ 49); 125 (74); 122 (30); 109 (13); 108 (9); 107 (100); $(\alpha)_{546}^{20} = 0$.

(ii) 80 mg (32%) acid (\pm) -4 IR: $\nu = 3600\text{--}2400$; 1715, 800 cm^{-1} . Crude mixture after transformation was esterified with ether soln of CH_2N_2 and the following products were obtained after chromatography:

(iii) 70 mg alcohol (\pm) -3 (spectral data as above).

(iv) 75 mg methyl ester of acid (\pm) -4: IR: $\nu = 3020, 1750, 800\text{ cm}^{-1}$; $^1\text{H-NMR}$: $\delta = 5.12$ (bs 1H); 3.68 (s 3H); 1.6 (bs 3H); 1.2 (s 3H); 0.85 (s 3H); MS: $m/e = 169$ (11); 168 (M^+ 100); 153 (80); 137 (19); 125 (78); 124 (47); 103 (50); 108 (90); 93 (60);

(v) Traces of the starting **2a** ketone have been also found (by GC-MS): MS: $m/e = 166$ (M^+ 1); 110 (3); 109 (11); 108 (100); 93 (14).

B. After 2 days the following products were obtained:

(i) 50 mg (20%) ketone $(+)$ -**2a** (spectral data as above) $(\alpha)_{546}^{20} = +6$;

(ii) 125 mg (50%) alcohol $(+)$ -3 (spectral data as above) $(\alpha)_{546}^{20} = +1$.

Transformation of $(+)$ -**2a** ketone

After 3 days 70 mg alcohol $(-)$ -3 (spectral data as above) $(\alpha)_{546}^{20} = -4.5$ was obtained.

Transformations of (\pm) -**2b** ketone

A. After 10 days the following products were obtained:

(i) 80 mg (32%) alcohol (\pm) -3 (spectral data as above); $(\alpha)_{546}^{20} = 0$;

(ii) 85 mg (35%) acid (\pm)-4 IR as above; $^1\text{H-NMR}$ $\delta = 5.2$ (s 1H); 2.9 (t J = 8.5 Hz 1H); 1.62 (s 3H); 1.25 (s 3H); 0.96 (s 3H).

In GC-MS spectrum of estrified (CH_2N_2) mixture after transformation there were found:

(iii) traces of **5b** MS: $m/e = 178$ ($\text{M}^+ - \text{H}_2\text{O}$ 12); 124 (9); 121 (8); 109 (12); 107 (16); 106 (100); 95 (14); 57 (57);

(iv) traces of **6b** MS: $m/e = 194$ ($\text{M}^+ - 3$); 165 (31); 139 (12); 138 (100); 137 (78); 123 (48); 109 (7); 83 (5); 29 (5).

B. After 2 days the following products were obtained:

(i) 120 mg ketone (+)-**2b** (IR, $^1\text{H-NMR}$ identical to those of starting ketone); $(\alpha)_{346}^{20} = +4.5$;

(ii) 60 mg alcohol (+)-**3** (IR, $^1\text{H-NMR}$ as above); $(\alpha)_{346}^{20} = +1$.

Transformation of (\pm)-**2c** ketone

After 10 days the following products were obtained:

(i) 120 mg (45%) ketone ($-$)-**2c**; IR, $^1\text{H-NMR}$ identical as those of starting ketone; $(\alpha)_{346}^{20} = -2$;

(ii) 56 mg (22%) hydroxyketone ($-$)-**5c**; IR: $\nu = 3620, 3050, 1718, 800 \text{ cm}^{-1}$; $^1\text{H-NMR}$: $\delta = 5.48$ (s 1H); 4.12 (s 2H); 1.03 (s 3H); 0.9 (t J = 3 Hz 3H); 0.83 (s 3H); MS: $m/e = 192$ ($\text{M}^+ - \text{H}_2\text{O}$ 13); 149 (14); 124 (12); 121 (13); 109 (33); 107 (25); 106 (100); 95 (30); 71 (38); 43 (21); $(\alpha)_{346}^{20} = -2$. In GC-MS spectrum of estrified (CH_2N_2) mixture after transformation of (\pm)-**2c** there were also found:

(i) traces of **6c** MS: $m/e = 208$ ($\text{M}^+ - 1$); 165 (25); 139 (16); 138 (100); 137 (71); 124 (16); 123 (64); 109 (7); 71 (8); 43 (5);

(ii) traces of **3** MS: $m/e = 140$ ($\text{M}^+ - 42$); 126 (8); 125 (82); 122 (35); 109 (13); 108 (9); 95 (16); 81 (6); 43 (15);

(iii) methyl ester of **4** MS: $m/e = 169$ (11); 168 ($\text{M}^+ - 100$); 153 (75); 137 (23); 125 (63); 121 (48); 109 (56); 108 (98); 94 (8); 93 (65).

Transformation of (\pm)-**2d** ketone

After 10 days the following products were obtained:

(i) 160 mg (64%) ketone (\pm)-**2d**; IR, $^1\text{H-NMR}$ identical as those of starting ketone; $(\alpha)_{346}^{20} = 0$;

(ii) 25 mg (10%) hydroxyketone **5d**; IR: $\nu = 3640, 3050, 1720, 800 \text{ cm}^{-1}$; $^1\text{H-NMR}$: $\delta = 5.5$ (s 1H); 4.12 (s 2H); 1.6 (s 3H); 1.1 (d J = 5 Hz 6H); 0.88 (s 3H); MS: $m/e = 192$ ($\text{M}^+ - \text{H}_2\text{O}$ 18); 167 (16); 149 (63); 139 (21); 109 (16); 107 (18); 106 (100); 97 (15); 71 (35); $(\alpha)_{346}^{20} = 0$. Compounds **3** and **4** were not found (by GC-MS) in the mixture after transformation.

Transformation of (\pm)-**2e** ketone

After 10 days the following products were obtained:

(i) 150 mg (60%) ketone ($-$)-**2e**; IR, $^1\text{H-NMR}$ identical as those of starting ketone; $(\alpha)_{346}^{20} = -4$;

(ii) 50 mg (20%) alcohol ($-$)-**3**; IR, $^1\text{H-NMR}$, MS identical with **3** (obtained from **2a** ketone); $(\alpha)_{346}^{20} = -4.5$.

(iii) 15 mg (6%) acid **4**; IR, $^1\text{H-NMR}$ identical with those obtained from **2a**;

(iv) 10 mg (4%) hydroxyketone **5e**; $^1\text{H-NMR}$ (HMDS as external standard); 5.78 (bs 1H); 4.35 (bs 2H); 1.34 (s 3H); 1.14 (s 1H); MS: $m/e = 206$ ($\text{M}^+ - \text{H}_2\text{O}$ 10); 149 (14); 125 (9); 124 (15); 123 (6); 121 (6); 106 (100); 85 (28);

(v) 12 mg (5%) diol **7e**; IR: $\nu = 3650, 3040, 800 \text{ cm}^{-1}$; $^1\text{H-NMR}$ (HMDS as external standard); $\delta = 5.94$ (s 1H); 4.55 (s 2H); 4.1 (m 2H); 1.53 (s 3H); 1.35 (s 3H); MS: $m/e = 138$ ($\text{M}^+ - \text{H}_2\text{O}$ 28) 125 (11); 123 (30); 109 (19); 108 (14); 197 (64); 105 (24); 93 (100); 81 (10). In GC-MS spectrum of estrified (CH_2N_2) mixture after transformation there were found:

(i) traces of diketone: MS: $m/e = 222$ ($\text{M}^+ - 8$); 180 (62); 165 (26); 138 (68); 137 (100); 124 (16); 123 (63); 109 (13); 96 (15); 82 (13).

Methyl ester of *a*-campholenic acid **8**. 4.47 g (0.03 m) of **1** and 3.36 (0.06 m) KOH in 75 ml MeOH + H_2O (4:1) was refluxed during 10 hr. The neutral fraction was separated with petrol ether after MeOH evaporation and addition of 30 ml H_2O . The residue was acidified with H_2SO_4 and extracted with ethyl ether. The crude acid was estrified (CH_2N_2) and after chromatography the methyl ester was obtained: IR: $\nu = 3040, 1740, 800 \text{ cm}^{-1}$; $^1\text{H-NMR}$: $\delta = 5.15$ (b.s. 1H); 3.6 (s 3H); 1.62 (b.s. 3H); 1.02 (s. 3H); 0.82 (s. 3H). MS: $m/e = 182$ ($\text{M}^+ - 25$); 168 (4); 167 (34); 150 (8); 135 (7); 109 (17); 108 (100); 107 (22); 93 (12).

Acknowledgements—We thank the Polish Academy of Sciences (Grant MR.I.12) and the Polish Ministry of Science, Higher Education and Technology (Grant R.I.09) for financial support.

REFERENCES

1. Siewiński, J. Dmochowska-Gładysz, T. Kofek, A. Zabża and K. Derdziński, *Tetrahedron* **35**, 1409 (1979).
2. A. Siewiński, W. Peczyńska-Czoch, A. Zabża and A. Szewczuk, *Ibid.* **33**, 1139 (1977).
3. K. Derdziński, M. Wawrzeńczyk and A. Zabża, Submitted for publication.
4. J. Dmochowska-Gładysz, T. Kofek, A. Siewiński, K. Derdziński, A. Zabża and A. Nespiak, Submitted for publication.
5. F. Tiemann, *Ber. Dtsch. Chem. Ges.* **29**, 3006 (1896).