Tetrahedron Vol. 36, pp. 91 to 96
© Pergamon Press Ltd., 1980. Printed in Great Britain

0040-4020/80/0101-0091/$02.00/0

SYNTHESIS OF OPTICALLY ACTIVE ALKYNYL ALCOHOLS AND α-HYDROXY ESTERS BY MICROBIAL ASYMMETRIC HYDROLYSIS OF THE CORRESPONDING ACETATES[a]

KENJI MORI* and HIROKO AKAO

Department of Agricultural Chemistry, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

(*Received in Japan 27 April 1979*)

Abstract—Asymmetric hydrolysis of the acetates of racemic alkynyl alcohols and α-hydroxy esters by *Bacillus subtilis* var. *Niger* afforded optically active acetates and alcohols in 7–90% optical purities. The both enantiomers of optically pure mandelic acid were prepared by this microbial method.

The capacity of microorganisms to synthesise various organic compounds is indeed fantastic which is now intensively investigated by microbial chemists. From synthetic chemists' point of view, however, a microorganism or an enzyme can be regarded as a kind of reagent which executes a particular transformation of organic compounds. Although this concept is gradually prevailing among organic chemists,[1] there are still vast possibilities of employing these bio-catalysts in preparative organic transformations. Doubtlessly the enantio-selectivity is the most noteworthy feature of a microbial reaction. We therefore began our studies to define scope and limitation of bio-catalytic reactions on a variety of organic substrates. Here we report in detail our findings on the asymmetric hydrolysis of acetates of racemic alkyl alkynyl carbinols and α-hydroxy esters by *Bacillus subtilis* var. *Niger*.[2] This bacterium is known to effect asymmetric hydrolysis of acetates of some terpene alcohols.[3-5]

Preparation of optically active alkynyl alcohols

Optically active alkynyl alcohols are useful intermediates in natural products syntheses. For example, optically active forms of vitamin E,[6] steroids,[7] and dried bean beetle pheromone[8] were prepared from alkynyl alcohols. They can be prepared either by optical resolution of the racemates or by asymmetric reduction of the corresponding acetylenic ketones in the presence of (+)-(2*S*, 3*R*) - 4 - dimethylamino - 3 - methyl - 1,2 - diphenyl - 2 - butanol (Darvon alcohol).[7] However, the resolution is tedious and the reduction requires rather inaccessible "Darvon alcohol".

When acetates of racemic alkyl alkynyl carbinols (±)-**1** were shaken for 3 days at 30° with *Bacillus subtilis* var. *Niger* grown in 2% nutrient broth, optically active (*R*)-alkynyl acetates (*R*)-**1** and (*S*)-alkynyl alcohols (*S*)-**2** were obtained as shown in Table 1.

The absolute configuration of the hydrolysis products **2** was assigned to be *S* by correlation with the reported data in the cases of **2a**,[9] **2b**,[10b] and **2e**.[6] The *S* absolute stereochemistry of (−)-**2d** was deduced by the conversion of 90% optically pure (+)-**2d** to (*S*)-(+)-decan-3-ol.[11] The (+)-enyne alcohol **2f** also belonged to the (*S*)-series, since (−)-**2f** yielded the natural enantiomer of the Japanese beetle pheromone [(*R*, *Z*) - 5 - (1 - decenyl)oxacyclopentan - 2 - one].[12] In any of the above cases, the NMR signal due to the O*Me* protons of the (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester **3**[13] of an (*S*)-alcohol **2** appeared at a higher field than that of the enantiomeric (*R*)-alcohol in the presence of the Sievers' reagent, Eu(fod)$_3$. By this correlation, it was possible to deduce the absolute configuration of **2c** to be *S*.

The optical purities of the products were determined by the NMR analysis of the (*S*)-(−)-MTPA esters **3** of the alcohols **2** in the presence of Eu(fod)$_3$ (Experimental).[13] Alternatively, the MTPA esters **3** were analyzed by glc. The retention times of the MTPA esters of (*S*)-alcohols were always shorter than those of the (*R*)-alcohols. The results obtained by these two methods were in good accord. An acetate (*R*)-**1** with unknown optical purity was hydrolyzed to compare the $[\alpha]_D$ value of the corresponding alcohol with that of the enantiomeric alcohol whose optical purity was determined by the MTPA ester method. In this manner the optical purities of all of the products were determined as shown in Table 1.

It is now clear that the bacterium always preferentially hydrolyses (*S*)-acetates to give a mixture of (*S*)-alcohols and unchanged (*R*)-acetates, although in varying optical yields. The acetates with smaller alkyl groups, (±)-**1a**, **1b** and **1e**, yielded products with higher optical purities, while the acetates with larger alkyl groups, (±)-**1c**, **1d** and **1f**, gave unsatisfactory results. No hydrolysis took place in the case of (±)-**1g** where R and R' were both bulky alkynyl and alkyl groups.

In view of the simplicity of the experimental procedure, this microbial method is an excellent preparative method for chiral alkyl alkynyl carbinols of known absolute configuration. Notably it is possible to obtain the both enantiomers by a single fermentation, employing chromatographic separation of the products, (*R*)-**1** and (*S*)-**2**.

Preparation of optically active α-hydroxy esters

Acetophenone is widely employed in studying the

[a]Preparative Bioorganic Chemistry—I. The experimental part of this work was mainly taken from the B.Sc. Thesis of H.A. (1979).

[b](*S*)-(−)-Hex-1-yn-3-ol was prepared in 3.5% optical purity by reduction of the corresponding acetylenic ketone with 3 - O - benzyl - 1,2 - O - cyclohexylidene - α - D - glucofuranose - LAH complex.

Table 1. Asymmetric hydrolysis of some acetates of racemic alkyl alkynyl carbinols with *Bacillus subtilis* var. *Niger*

Substrate	Product ratio[a] (R)-1 : (S)-2	Isolated yield (R)-1 : (S)-2	obsd. $[\alpha]_D$ (optical purities)[b] of Acetate (R)-1	and Alcohol (S)-2
1a	1 : 2.8	9% : 20%	+91.6°[c] (72%)	-22.5°[c] (54%)
1b	1 : 1.6	22% : 36%	+88.1°[c] (90%)	-24.0°[c] (68%)
1c	1 : 1.3	28% : 28%	+11.1°[c] (16%)	-3.2°[c] (14%)
1d	1 : 0.4	53% : 25%	+3.2°[c] (7%)	-2.1°[c] (16%)
1e	1 : 1.8	25% : 36%	+78.9°[c] (74%)	-7.6°[d] (54%)
1f	1 : 0.7	28% : 14%	+17.4°[e] (34%)	+3.2°[e] (40%)
1g	1 : 0	— —	———	———

a As determined by GLC or NMR analyses of the crude products before chromatographic purification. The area ratio of the signals due to CHOH (δ4.2) and CHOAc (δ5.2) protons was compared.

b As determined by the NMR measurement of MTPA esters.

c Measured as ether soln.

d Measured as $CHCl_3$ soln.

e Measured as n-hexane soln.

asymmetric reduction of prochiral ketones to optically active alcohols with chiral metal hydrides.[14] Recently reduction of acetophenone with LAH and (*S*)-2-(2,6-xylidinomethyl) pyrrolidine was reported to give (*S*)-α-methylbenzyl alcohol in 95% enantiomeric excess.[15] In order to compare the enantioselectivity of the microbial asymmetric hydrolysis with that of the chemical reduction, we fed the racemic acetate (±)-**4** of α-methylbenzyl alcohol to *Bacillus subtilis* var. *Niger*. The resulting products were (*R*)-acetate **4**, $[\alpha]_D^{22}$ +20.0° (cyclopentane), and (*S*)-alcohol **5**, $[\alpha]_D^{22}$ -17.7° (cyclopentane). They were obtained in 54% [(*R*)-**4**] and 24% [(*S*)-**5**] yield, respectively, and their optical purities were 21% [(*R*)-**4**] and 41% [(*S*)-**5**] as based on the optical rotation, $[\alpha]_D^{21}$-43.1° (cyclopentane), of pure (*S*)-(−)-α-methylbenzyl alcohol **5**.[16] Thus, in this particular case, the microbial enantioselectivity was far less satisfactory than the chemical enantioselectivity with chiral metal hydrides.

It occurred to us, however, that the selection of a crystalline chiral molecule as a target might enable us to obtain optically pure final products by recrystallising partially resolved materials available by microbial hydrolysis. We therefore chose mandelic acid **8** and treated the racemic acetate **6** of ethyl mandelate with *Bacillus subtilis* var. *Niger* for 3 days. To our pleasure the acetate group was preferentially hydrolysed, leaving the ethoxycarbonyl group intact. Partially resolved (*R*)-acetoxy ester **6** and (*S*)-hydroxy ester **7** were obtained in 31 and 27% isolated yield, respectively. These were separately heated under reflux with dil. HCl to effect hydrolysis. Starting from 15.9 g of (±)-**6**, 2.1 g of (*R*)-(−)-mandelic acid **8** was obtained. Its optical purity was 73% as determined by comparing its optical rotation, $[\alpha]_D^{20}$-115° (water), with the authentic value, $[\alpha]_D$-158° (water).[17] This was recrystallized three times from water to give 985 mg of (*R*)-(−)-mandelic acid, $[\alpha]_D^{19.5}$-154° (water). Similarly 3.3 g of (+)-mandelic acid (*S*)-**8** was obtained in 47% optical purity. This was recrystallised four times from water to give 300 mg of (*S*)-(+)-mandelic acid, $[\alpha]_D^{20.5}$ + 153° (water). These mandelic acid enantiomers were 97–97.5% optically pure and comparable in its purity with highly purified commercial materials (cf. Aldrich Gold Label materials, $[\alpha]_D^{20}$ + 154° and −153°). We were thus able to obtain both (+)- and (−)-forms of highly optically pure mandelic acid by microbial resolution without employing expensive alkaloids as resolving agents. It should be added that the phenyl group of (±)-**4** corresponds to the alkynyl (-C≡C-R') group of (±)-**1**, while that of (±)-**6** to the alkyl group (R) of (±)-**1** in the stereochemical recognition by the esterase of *Bacillus subtilis* var. *Niger*.

The success described above encouraged us to attempt the microbial hydrolysis of the racemic acetate (±)-**9** of ethyl leucinate. In this case the rate of hydrolysis was exceedingly slow and the shaking culture with *Bacillus subtilis* var. *Niger* was continued for 12 days to give 4.3 g of an oil from 12.9 g of (±)-**9**. The oil was proved to be a mixture of an acetoxy ester **9** and a hydroxy ester **10** in 2:1 ratio. The both were obtained pure after chromatographic separation. To our surprise, both the acetate **9** (816 mg), $[\alpha]_D^{20.5}$ + 37.1° (ether), and the hydroxy ester **10** (404 mg), $[\alpha]_D^{20}$ + 14.2° (ether), were shown to be (*R*)-enantiomers by comparing their optical rotations with those of authentic samples. Their optical purities were 90% for (*R*)-**9** and 78.5% for (*R*)-**10**. This result may be explained by the preferential hydrolysis of the (*S*)-acetate **9** to give (*S*)-hydroxy ester **10**, which was further metabolized by the microorganism. The derivatives of

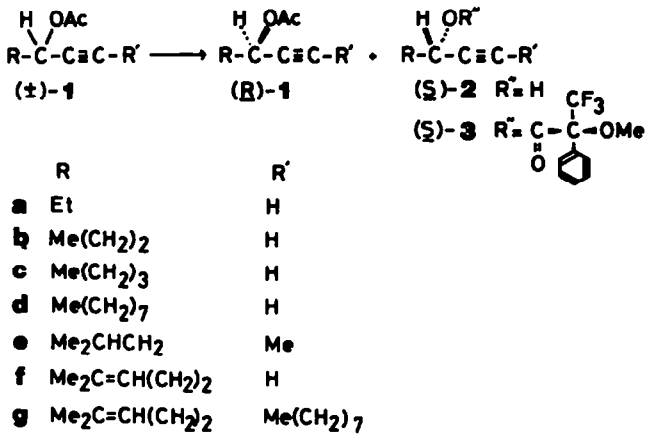

Fig. 1.

unnatural (*R*)-form of leucinic acid were less readily attacked by the bacterium.

In conclusion quite an interesting enantioselectivity was observed in the course of microbial hydrolysis of various racemic acetates of alkyl alkynyl carbinols and α-hydroxy esters. This will be useful as preparative means for chiral molecules.

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra refer to films unless otherwise specified and were determined on a Jasco IRA-1 spectrometer. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer. Optical rotations were measured on a Jasco DIP-4 polarimeter. glc analyses were performed on a Yanaco G 80 or G-550F gas chromatographs.

General fermentation procedure

Basillus subtilis var. *Niger* was cultured for 2 days at 30° in a nutrient broth (100 ml containing beef extract (0.33 g), peptone (1.11 g) and NaCl (0.56 g)). Then *ca.* 2 g of a substrate (±)-1, etc. was added and the culture medium was shaken for 3 days at 30°. The broth was extracted with ether. The ether extract was washed with brine, dried ($MgSO_4$) and concentrated. The residual oil was chromatographed over neutral alumina (Woelm, grade II). Elution with n-pentane (or hexane) gave an acetate (*R*)-1, etc. Further elution with n-pentane (or hexane)-ether (1:1) gave an alcohol (*S*)-2, etc. Their structures and purities were fully checked as described below.

Hydrolysis of (±)-*pent*-1-*yn*-3-*yl acetate* (±)-1a

(a) *Preparation of* (±)-1a. Commercially available (±)-2a, n_D^{25} 1.4297, ν_{max} ~3400 (s), 3300 (s), 2120 (w), 1100 (m), 1050 (m), 1020 (s), 970 (s) cm^{-1}, was treated with Ac_2O–C_5H_5N to give (±)-1a, b.p. 68–69°/40 mm, n_D^{23} 1.4175; ν_{max} 3300 (m), 2140 (w), 1745 (s), 1380 (s), 1240 (s), 1030 (s) cm^{-1}; δ 0.99 (3H, t, J = 7Hz), 1.65 (2H, q, J = 7Hz), 2.00 (3H, s), 2.31 (1H, d, J = 2Hz), 5.20 (1H, dt, J_1 = 2, J_2 = 7Hz).

(b) *Microbial hydrolysis.* The acetate (±)-1a (1.91 g) gave an oil (*ca* 1.5 g) upon microbial hydrolysis. This was separated by chromatography to give 184 mg of (*R*)-1a, b.p. 60–61°/30 mm, n_D^{22} 1.4178; $[\alpha]_D^{22}$ +91.6° (*c* = 0.92, ether) [lit.[9] $[\alpha]_{546}^{20}$ +125° (ether)]. The IR spectrum of (*R*)-1a was identical with that of (±)-1a. Later fractions gave 254 mg of (*S*)-2a, b.p. 53°/30 mm, n_D^{22} 1.4290; $[\alpha]_D^{22}$ −22.5° (*c* = 0.914, ether) [lit.[9] $[\alpha]_{546}^{20}$ +36.8° (*c* = 2.5, ether) for 93% optically pure (*R*)-2a]. The IR spectrum of (*S*)-2a was identical with that of (±)-2a. The MTPA ester (*S*)-3a was prepared in the usual manner from 30 mg of (*S*)-2a: ν_{max} 3300 (m), 2140 (w), 1760 (s), 1250 (s), 1180 (s), 1130 (s), 1020 (s), 1000 (s), 720 (s) cm^{-1}; δ 0.90 (3H, t, J = 7Hz), 1.75 (2H, q, J = 7Hz), 2.43 (1H, d, J = 2Hz), 3.52 (3H, br. s), 5.41 (1H, dt, J_1 = 2, J_2 = 7Hz), ~7.3 (5H, m); δ (100 MHz, CCl_4, in the presence of 30 mg of Eu $(fod)_3$) 0*Me* 4.46 (77%), 4.72 (23%). The optical purity of (*S*)-2a was therefore 54%. (*S*)-2a (150 mg) was acetylated (Ac_2O/C_5H_5N) to give 118 mg of (*S*)-1a, b.p. 60°/33 mm, n_D^{22}

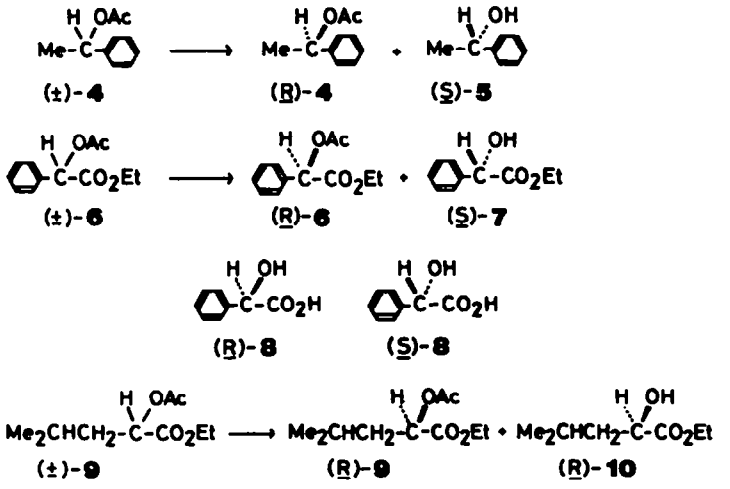

Fig. 2.

1.4169; $[\alpha]_D^{22}$ −68.9° (c = 0.601, ether). Since this was 54% optically pure, our (*R*)-1a was (91.6/68.9) × 54 = 72% optically pure.

Hydrolysis of (±)-*hex*-1-*yn*-3-*yl acetate* (±)-1b

(a) *Preparation of* (±)-1b. Commercially available (±)-2b, n_D^{22} 1.4309, ν_{max} ~3400 (s), 3300 (s), 2120 (w), 1120 (m), 1065 (m), 1030 (s), 1010 (s), 985 (m) cm^{-1}, was treated with Ac_2O–C_5H_5N to give (±)-1b, b.p. 68–70°/20 mm, n_D^{22} 1.4216; ν_{max} 3300 (m), 2140 (w), 1750 (s), 1380 (s), 1250 (s), 1240 (s), 1070 (m), 1025 (s), 985 (m) cm^{-1}; δ 0.94 (3H, m), ~1.1 to ~1.8 (4H, m), 2.00 (3H, s), 2.30 (1H, d, J = 2Hz), 5.30 (1H, dt, J_1 = 2, J_2 = 7Hz).

(b) *Microbial hydrolysis.* The acetate (±)-1b (1.87 g) gave an oil (*ca.* 1.5 g) upon microbial hydrolysis. This was separated by chromatography to give 407 mg of (*R*)-1b, b.p. 74–75°/30 mm, n_D^{22} 1.4223; $[\alpha]_D^{22}$ + 88.1° (c = 2.531, ether). The IR spectrum of (*R*)-1b was identical with that of (±)-1b. Later fractions gave 469 mg of (*S*)-2b, b.p. 62°/22 mm, n_D^{22} 1.4311; $[\alpha]_D^{22}$ −24.0° (c = 2.363, ether) [lit.[10] $[\alpha]_D^{20}$ −1.12° (neat) for 3.5% optically pure (*S*)-2b)]. The IR spectrum of (*S*)-2b was identical with that of (±)-2b. (*R*)-1b (250 mg) was hydrolysed (KOH/MeOH–H_2O) to give 150 mg of (*R*)-2b, b.p. 63 ~ 64°/25 mm, n_D^{22} 1.4320; $[\alpha]_D^{22}$ + 31.7° (c = 1.046, ether). The MTPA ester of (*R*)-2b was prepared in the usual manner from 30 mg of (*R*)-2b; ν_{max} 3300 (m), 2980 (m), 2130 (w), 1760 (s), 1460 (m), 1280 (s), 1260 (s), 1200 (s), 1180 (s), 1130 (s), 1020 (s), 1000 (m), 725 (m) cm^{-1}; δ 0.98 (3H, m), ~1.2 to ~2.1 (4H, m), 2.38 (1H, d, J = 2Hz), 3.48 (3H, s), 5.46 (1H, dt, J_1 = 2, J_2 = 7Hz), ~7.3 (5H, m); δ (in the presence of 30 mg of Eu (fod)$_3$) O*Me* 4.28 (5%), 4.47 (95%). The optical purity of (*R*)-2b and (*R*)-1b was therefore 90%. The optical purity of (*S*)-2b was (24.0/31.7) × 90 = 68%.

Hydrolysis of (±)-*hept*-1-*yn*-3-*yl acetate* (±)-1c

(a) Preparation of (±)-1c. Commercially available (±)-2c, n_D^{22} 1.4351, ν_{max} ~3400 (s), 3300 (s), 2960 (s), 2880 (s), 2120 (w), 1475 (m), 1050 (s), 1020 (s) cm^{-1}, was treated with Ac_2O–C_5H_5N to give (±)-1c, b.p. 83–84°/20 mm, $n_D^{22.5}$ 1.4249, ν_{max} 3300 (m), 2130 (w), 1750 (s), 1380 (m), 1240 (s), 1020 (m) cm^{-1}; δ 0.92 (3H, m), ~1.1 to ~1.9 (6H, m), 2.00 (3H, s), 2.30 (1H, d, J = 2.5Hz), 5.23 (1H, dt, J_1 = 2.5, J_2 = 7Hz). (Found: C, 69.67; H, 9.09. $C_9H_{14}O_2$ requires: C, 70.10; H, 9.15%).

(b) *Microbial hydrolysis.* The acetate (±)-1c (2.06 g), upon microbial hydrolysis, gave an oil (*ca.* 1.5 g). This was chromatographed to give 562 mg of (*R*)-1c as earlier fractions, b.p. 82°/19 mm, n_D^{22} 1.4254, $[\alpha]_D^{22}$ + 11.1° (c = 2.971, ether). Its IR spectrum was identical with that of (±)-1c. Later fractions gave 402 mg of (*S*)-2c, b.p. 73°/20 mm, n_D^{22} 1.4346, $[\alpha]_D^{22}$ −3.2° (c = 2.29, ether). The MTPA ester (*S*)-3c was prepared in the usual manner from 30 mg of (*S*)-2c: ν_{max} 3320 (m), 2240 (w), 1760 (s), 1290 (s), 1270 (s), 1250 (s), 1200 (s), 1180 (s), 1130 (m), 1025 (m), 1000 (m), 730 (m) cm^{-1}; δ 0.86 (3H, m), ~1.0 to ~2.0 (6H, m), 2.35 (1H, q), 3.50 (3H, br. s), ~7.30 (5H, m); δ (in the presence of 30 mg of Eu (fod)$_3$) O*Me* 4.30 (57%), 4.60 (43%). The optical purity of (*S*)-2c was therefore 14%. (*R*)-1c (470 mg) was saponified (KOH/MeOH–H_2O) to give 254 mg of (*R*)-2c, b.p. 72–73°/20 mm, n_D^{22} 1.4353; $[\alpha]_D^{22}$ + 3.7° (c = 2.331, ether). The optical purity of (*R*)-1c was therefore (3.7/3.2) × 14 = 16%.

Hydrolysis of (±)-*undec*-1-*yn*-3-*yl acetate* (±)-1d

(a) *Preparation of* (±)-1d. (±)-Undec-1-yn-3-ol (±)-2d, n_D^{22} 1.4442, ν_{max} ~3400 (m), 3310 (m), 2940 (s), 2870 (m), 2120 (w), 1470 (m), 1030 (m) cm^{-1}, was treated with Ac_2O–C_5H_5N to give (±)-1d, b.p. 105–108°/3 mm, n_D^{22} 1.4356, ν_{max} 3300 (m), 2940 (s), 2830 (m), 2120 (w), 1750 (s), 1470 (m), 1380 (m), 1240 (s), 1120 (w), 1020 (m), 980 (w) cm^{-1}; δ 0.88 (3H, m), ~1.0 to ~1.9 (14H, m), 2.00 (3H, s), 2.30 (1H, d, J = 2.5Hz), 5.25 (1H, dt, J_1 = 2.5, J_2 = 7 Hz). (Found: C, 73.87; H, 10.51. $C_{13}H_{22}O_2$ requires: C, 74.24; H, 10.54%).

(b) *Microbial hydrolysis.* The acetate (±)-1d (1.80 g) gave *ca* 1.7 g of an oil upon microbial hydrolysis. This was chromatographed to give 958 mg of (*R*)-1d, b.p. 97–99°/2 mm, n_D^{22} 1.4363; $[\alpha]_D^{22}$ + 3.2° (c = 3.332, ether). Its IR spectrum was identical with that of (±)-1d. Later fractions gave 334 mg of (*S*)-2d, b.p. 103–104°/3 mm, n_D^{22} 1.4459; $[\alpha]_D^{22}$ −2.1° (c = 2.935, ether). The MTPA ester (*S*)-3d was prepared in the usual manner from 30 mg of (*S*)-2d: ν_{max} 3300 (m), 2920 (s), 2840 (m), 1750 (s), 1270 (s), 1250 (s), 1180 (s), 1170 (s), 1115 (m), 1005 (m), 990 (m) cm^{-1}; δ 0.85 (3H, m), ~1.0 to ~2.0 (14H, m), 2.32 (1H, d, J = 2.5 Hz), 3.45 (3H, s), 5.42 (1H, dt, J_1 = 2.5, J_2 = 7 Hz), ~7.35 (5H, m); δ (in the presence of 30 mg of Eu (fod)$_3$) O*Me* 4.85 (58%), 5.35 (42%). The optical purity of (*S*)-2d was therefore 16%. (*R*)-1d (900 mg) was saponified (KOH/MeOH–H_2O) to give 583 mg of (*R*)-2d, b.p. 92°/1 mm, n_D^{22} 1.4459; $[\alpha]_D^{22}$ + 0.91° (c = 5.271, ether). The optical purity of (*R*)-2d and (*R*)-1d was (0.91/2.1) × 16 = 7%. Another sample of (*R*)-2d was obtained by resolving the phthalic half ester of (±)-2d[11] and showed $[\alpha]_D^{22}$ + 14.0° (c = 1.128, ether). The MTPA ester of this (*R*)-2d was prepared in the usual manner and its NMR spectrum was measured in the presence of Eu(fod)$_3$: δ O*Me* 4.65 (5%), 5.08 (95%). This sample was therefore of 90% optical purity.

Hydrolysis of (±)-6-*methylhept*-2-*yn*-4-*yl acetate* (±)-1e

(a) *Preparation of* (±)-1e. (±) - 6 - Methylhept - 5 - yn - 4 - ol 2e was synthesised as described:[6] b.p. 61–64°/4 mm, n_D^{22} 1.4466, ν_{max} ~3360 (m), 2250 (w), 1480 (m), 1400 (m), 1380 (m), 1160 (m) 1060 (m), 1040 (m), 990 (m) cm^{-1}; δ 0.95 (6H, d, J = 7 Hz), ~1.2 to ~1.7 (3H, m), 1.80 (3H, d, J = 2.5 Hz), 2.95 (1H, s, –OH), 4.25 (1H, dt, J_1 = 2.5, J_2 = 6 Hz). This was acetylated (Ac_2O/C_5H_5N) to give (±)-1e, b.p. 72–73°/5 mm, n_D^{22} 1.4352; ν_{max} 2970 (m), 2260 (w), 1750 (s), 1470 (m), 1380 (m), 1250 (s), 1180 (m), 1050 (m), 1020 (m), 960 (m), 950 (m) cm^{-1}; δ 0.90 (6H, d, J = 7 Hz), ~1.2–~1.7 (3H, m), 1.78 (3H, d, J = 2 Hz), 1.95 (3H, s), 5.25 (1H, dt, J_1 = 2, J_2 = 6 Hz). (Found: C, 70.97; H, 9.62. $C_{10}H_{16}O_2$ requires: C, 71.39; H, 9.59%).

(b) *Microbial hydrolysis.* The acetate (±)-1e (1.82 g) gave *ca* 1.5 g of an oil upon microbial hydrolysis. This was chromatographed to give 453 mg of (*R*)-1e, b.p. 63–64°/2 mm, n_D^{22} 1.4367; $[\alpha]_D^{22}$ + 78.9° (c = 2.204, ether). Later fractions gave (*S*)-2e (489 mg), b.p. 82°/22 mm, n_D^{22} 1.4460, $[\alpha]_D^{22}$ −16.8° (c = 2.583, ether); $[\alpha]_D^{22}$ −7.56° (c = 2.038, $CHCl_3$). The (*R*)-acetate (*R*)-acetate (*R*)-1e (400 mg) was saponified to give (*R*)-2e, b.p. 98–99°/33 mm, n_D^{22} 1.4465; $[\alpha]_D^{22}$ + 10.44° (c = 2.078, $CHCl_3$) [lit.[6] $[\alpha]_D^{25}$ −13.02° (c = 5.05, $CHCl_3$) for 95.6% optically pure (*S*)-2e]. The optical purity of (*R*)-2e was therefore 77% on the basis of optical rotation. The MTPA ester of (*R*)-2e was prepared in the usual manner: ν_{max} 2980 (m), 2260 (w), 1760 (s), 1285 (s), 1260 (s), 1195 (s), 1180 (s), 1130 (m), 1020 (m), 995 (m) cm^{-1}; δ 0.94 (6H, d, J = 6 Hz), ~1.5 to ~1.8 (3H, m), 1.80 (3H, d, J = 2 Hz), 3.48 (3H, s), 5.42 (1H, dt, J_1 = 2, J_2 = 6 Hz), ~7.32 (5H, m); δ (in the presence of 30 mg of Eu (fod)$_3$) O*Me* 4.55 (13%), 4.80 (87%). The optical purity of (*R*)-2e was therefore 74%. The optical purity of (*S*)-2e was (7.56/10.44) × 74 = 54%.

Hydrolysis of (±) - 7 - *methyloct* - 6 - *en* - 1 - *yn* - 3 - *yl acetate* (±) - 1f

(a) *Preparation of* (±)-1f. (±) - 7 - Methyloct - 6 - en - 1 - yn - 3 - ol (±)-2f[12] was acetylated (Ac_2O/C_5H_5N) to give (±)-1f, b.p. 93–95°/19 mm, n_D^{22} 1.4509; ν_{max} 3460 (w), 3300 (m), 2980 (m), 2940 (m), 2870 (m), 2130 (w), 1750 (s), 1675 (w), 1450 (m), 1380 (s), 1300 (w), 1240 (s), 1175 (m), 1115 (m), 1090 (m), 1025 (s), 980 (m), 960 (m), 925 (w), 670 (m) cm^{-1}; δ 1.60 (3H, s), 1.68 (3H, s), 1.77 ~ 2.23 (4H, m), 1.99 (3H, s), 2.33 (1H, d), 4.82–5.38 (2H, m).

(b) *Microbial hydrolysis.* The acetate (±)-1f (2.03 g) gave 1.63 g of an oil upon microbial hydrolysis. This was chromatographed to give 568 mg of (*R*)-1f, b.p. 110–112°/21 mm, n_D^{22} 1.4496; $[\alpha]_D^{22}$ + 17.4° (c = 2.52, n-hexane). (Found: C, 73.37; H, 9.03. $C_{11}H_{16}O_2$ requires: C, 73.30; H, 8.95%). Later fractions gave 210 mg of (*S*)-2f, b.p. 90°/8 mm, n_D^{22} 1.4660; $[\alpha]_D^{22.5}$ + 3.2° (c = 1.7, n-hexane); ν_{max} 3380 (m, br), 3300 (s), 2980 (m), 2940 (s), 2870 (m), 2125 (w), 1680 (w), 1450 (m), 1385 (m), 1350 (m), 1260 (m), 1170 (w), 1120 (m), 1070 (s), 1020 (m), 940 (w), 890 (w), 840 (w), 665 (m) cm^{-1}; δ 1.61 (3H, *s*), 1.68 (3H, s), 1.46 ~ 2.20 (4H, m), 2.09 (1H, s), 2.30 (1H, d, J = 2Hz) 4.24 (1H, t, J = 5.5 Hz), 5.08 (1H, t, J = 7 Hz). This was converted to the MTPA ester (*S*)-3f in the usual manner: ν_{max} 3310 (m), 2960 (m), 2140 (w), 1760 (s), 1460 (m), 1290 (s), 1260 (s), 1200 (s), 1180 (s), 1130 (m), 1020 (s), 1000 (m), 795 (m), 770 (m), 720 (m) cm^{-1}; δ 1.56 (3H, s), 1.66 (3H, s), ~1.7 to ~2.3 (4H, m), 2.36 (1H, d, J = 2Hz), 3.47 (3H, s), 5.00 (1H, br. t), 5.40 (1H, dt, J_1 = 2, J_2 = 6Hz), ~7.30 (5H, m); δ (in

the presence of 30 mg of Eu(fod)$_3$) *OMe* 4.21 (70%), 4.38 (30%). The optical purity of (*S*)-**2f** was therefore 40%. The acetate (*R*)-**1f** was saponified (KOH/MeOH–H_2O) to give (*R*)-**2f**, b.p. 102°/21 mm, n_D^{22} 1.4641; $[\alpha]_D^{22}$ −2.7° (*c* = 2.594, n-hexane). The optical purity of (*R*)-**2f** and (*R*)-**1f** was (2.7/3.2) × 40 = 34%.

Attempted hydrolysis of (±) - 2 - *methylhexadec* - 2 - *en* - 7 - *yn* - 6 - *yl acetate* (±) - **1g**. The known alcohol (±)-**2g** was acetylated and the product (1.78 g) was submitted to the microbial hydrolysis for 4 days. No hydrolysis product (*S*)-**2g** was detectable.

Hydrolysis of (±)-*α-methylbenzyl acetate* (±)-**4**

(a) *Preparation of* (±)-**4**. Commercially available (±)-**5**, n_D^{22} 1.5253, ν_{max} 3320 (s), 3080 (m), 3040 (m), 2980 (s), 2930 (m), 2880 (m), 1600 (w), 1490 (m), 1450 (s), 1370 (m), 1310 (m), 1205 (m), 1100 (s), 1080 (s), 1030 (m), 1010 (m), 990 (m), 900 (m), 760 (s), 700 (s) cm^{-1}; δ 1.25 (3H, d, J = 6Hz), 3.52 (1H, br. s), 4.60 (1H, q, J = 6 Hz), 7.15 (5H, s), was acetylated (Ac_2O/C_5H_5N) to give (±)-**4**, b.p. 108 ~ 109°/24 mm, n_D^{22} 1.4921; ν_{max} 3080 (m), 3050 (m), 2980 (s), 2940 (m), 1750 (s), 1605 (w), 1585 (w), 1500 (m), 1450 (m), 1380 (s), 1250 (s), 1215 (s), 1065 (s), 1030 (s), 940 (m), 850 (w), 760 (s) cm^{-1}; δ 1.45 (3H, d, J = 6 Hz), 1.90 (3H, s), 5.78 (1H, q, J = 6 Hz), 7.20 (5H, s).

Microbial hydrolysis. The acetate (±)-**4** (2.07 g) gave 1.8 g of an oil upon microbial hydrolysis. This was chromatographed to give 1.127 g of (*R*)-**4**, b.p. 110°/24 mm, n_D^{22} 1.4915; $[\alpha]_D^{22}$ + 20.0° (*c* = 3.551, cyclopentane). Its IR spectrum was identical with that of (±)-**4**. Later fractions gave 372 mg of (*S*)-**5**, b.p. 103°/24 mm, n_D^{22} 1.5227; $[\alpha]_D^{22}$ −17.7° (*c* = 2.077, cyclopentane). Its IR spectrum was identical with that of (±)-**5**. The optically pure (*S*)-**5** was reported to show $[\alpha]_D^{21}$ −43.1° (*c* = 7.19, cyclopentane).[16] The optical purity of our (*S*)-**5** was therefore 41%. The MTPA ester of (*S*)-**5** was prepared in the usual manner: ν_{max} 3090 (w), 3060 (w), 3000 (m), 2960 (m), 2860 (w), 1760 (s), 1610 (w), 1590 (w), 1500 (m), 1460 (m), 1390 (w), 1330 (w), 1280 (s), 1270 (s), 1180 (s), 1130 (s), 1080 (m), 1060 (m), 1020 (s), 1000 (m), 765 (s), 720 (s), 705 (s) cm^{-1}; δ 1.50 (2H, d, J = 6 Hz), 1.58 (1H, d, J = 6 Hz), 3.40 (2H, s), 3.48 (1H, s), 6.0 (1H, m), 7.15–7.25 (10H); δ (in the presence of 30 mg of Eu(fod)$_3$) *OMe* 4.28 (34%), 4.58 (66%). The optical purity of (*S*)-**5** was therefore 32%. The acetate (*R*)-**4** was saponified to give (*R*)-**5**, b.p. 103–105°/25 mm, n_D^{22} 1.5240; $[\alpha]_D^{22}$ + 9.1° (*c* = 2.471, cyclopentane). The optical purity of (*R*)-**4** and (*R*)-**5** is therefore (9.1/17.7) × 32 = 16.5%. On the basis of optical rotation, however, the optical purity was (9.1/43.1) × 100 = 21%.

Determination of optical purity by GLC analysis of MTPA esters

The above described MTPA esters 3 were analyzed by glc (Column: 5% LAC 2R 446 on Diasolid L, 1.5 m × 3 mm at 184°; Carrier gas: N_2, 1.0 ~ 1.2 kg/cm^2). (*S*)-**3a**: Rt 4.5 min (73.3%), 5.1 min (26.7%)—46.6% optical purity. (*R*)-**3b**: Rt 5.8 min (3.8%), 6.6 min (96.2%)—92.4% optical purity. (*S*)-**3c**: Rt 12.4 min (60.0%), 14.3 min (40.0%)—20.0% optical purity. (*S*)-**3d**: Rt 25.5 min (59.4%), 29.5 min (40.6%)—18.8% optical purity. (*R*)-**3e**: Rt 7.7 min (10.6%), 8.7 min (89.4%)—78.8% optical purity. (*S*)-**3f**: Rt 15.5 min (73.5%), 17.7 min (26.5%)—47.0% optical purity. (*S*)-(−)-MTPA ester of (*S*)-**5**: Rt 19.3 min (66.5%), 20.7 min (33.5%)–33.0% optical purity. These data were in good accord with those obtained by the NMR measurement.

Preparation of the both enantiomers of mandelic acid **8**

(a) *Preparation of* (±)-**6**. Ethyl (±)-mandelate (±)-**7** (24 g) was acetylated with Ac_2O (30 ml) and C_5H_5N (30 ml). Conventional work-up yielded 24.9 g (84%) of (±)-**6**, b.p. 127–129.5°/5.5 mm, $n_D^{25.5}$ 1.4908; ν_{max} 3060 (w), 3030 (w), 2980 (m), 2920 (w), 1750 (s), 1600 (w), 1585 (w), 1500 (m), 1455 (m), 1380 (s), 1340 (m), 1305 (m), 1275 (m), 1240 (s), 1215 (s), 1190 (s), 1085 (m), 1055 (s), 1030 (m), 960 (m), 925 (w), 855 (w), 795 (w), 740 (m), 700 (m) cm^{-1}; δ 1.14 (3H, t, J = 7 Hz), 2.05 (3H, s), 4.02 (2H, q, J = 7 Hz), 5.37 (1H, s, –O*H*), 7.06 ~ 7.45 (5H, m); glc (Column, 3% SE30, 1.5 m × 2 mm at 140°; Carrier gas, N_2 1.2 kg/cm^2): Rt 1.9 min. (Found: C, 64.07; H, 6.36. Calc. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35%).

(b) *Microbial hydrolysis.* The acetate (±)-**6** (15.9 g) was shaken with *B. subtilis* culture (4 flasks with 150 ml each of 2% nutrient broth; precultured for 4 days) for 3 days at 30°. The turbid culture medium was extracted with ether. The ether extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 9.146 g of an oil. This was a mixture of **6** and **7** as revealed by IR and glc. This was chromatographed over 110 g of Merck Kieselgel 60. Elution with n-hexane-ether (9:1) gave (*R*)-**6** (4.85 g). Further elution with n-hexane–ether (9:1 ~ 4:1) gave (*S*)-**7** (3.5 g). The IR spectra of these products were identical with those of the racemates.

(c) *Preparation of pure* (R)-(−)-*mandelic acid* (*R*)-**8**. The acetoxy ester (*R*)-**6** (4.85 g) was mixed with 2.4 N HCl (180 ml) and the mixture was stirred and heated under reflux for 4 ~ 5 hr. The resulting soln was concentrated *in vacuo* to give crystalline (*R*)-**8**. This was dissolved in water, decolorized with charcoal and concentrated *in vacuo*. The residue was recrystallised from water. The first recrystallisation yielded 2.092 g of impure (*R*)-**8**, $[\alpha]_D^{20}$ −115° (*c* = 0.175, H_2O), with optical purity of 73%. The second recrystallisation yielded 1.418 g of the acid, m.p. 121–124°. The third recrystallisation yielded 1.18 g of (*R*)-**8**, m.p. 124–125°, $[\alpha]_D^{20}$ −146° (*c* = 0.164, H_2O), with optical purity of 93%. The fourth recrystallisation gave 985 mg of (*R*)-**8**, m.p. 126 ~ 126.5°, $[\alpha]_D^{20}$ −154° (*c* = 0.267, H_2O), with optical purity of 97.5%. The IR spectrum was identical with that of an authentic material, ν_{max} (Nujol) 3420 (m), ~2600 (m), 1715 (s), 1250 (s), 1200 (s), 1100 (m), 1065 (s), 935 (m), 860 (m), 775 (m), 730 (m), 700 (m) cm^{-1}.

(d) *Preparation of pure* (S)-(+)-*mandelic acid* (*S*)-**8**. The hydroxy ester (*S*)-**7** (3.5 g) was mixed with 2.4N HCl (130 ml) and the mixture was stirred and heated under reflux for 5–6 hr. The resulting soln was concentrated *in vacuo* to give crystalline (*S*)-**8**. This was recrystallised from water. The first recrystallisation yielded 3.271 g of impure (*S*)-**8**, $[\alpha]_D^{20}$ + 73.7° (*c* = 0.19, H_2O), with optical purity of 47%. The second recrystallisation gave 1.344 g of the acid, m.p. 113 ~ 116°. The third recrystallisation yielded 1.077 g of (*S*)-**8**, $[\alpha]_D^{20}$ + 107° (*c* = 0.122, H_2O), with optical purity of 68%. The fourth recrystallisation gave 543 mg of (*S*)-**8**, $[\alpha]_D^{20}$ + 145° (*c* = 0.137, H_2O), with optical purity of 93%. The fifth recrystallisation gave 300 mg of pure (*S*)-**8**, m.p. 124–125°, $[\alpha]_D^{21}$ + 153° (*c* = 0.153, H_2O). The IR spectrum was identical with that of (*R*)-**8**.

Hydrolysis of the acetate (±)-**9** *of ethyl* (±)-*leucinate*

(a) *Preparation of* (±)-**9** Ethyl (±)-**10** (25.2 g, 87%) was prepared from (±)-leucinic acid (24 g) in the conventional manner,[18] b.p. 81.5–82°/14 mm, n_D^{20} 1.4233; ν_{max} 3450 (m), 2875 (s), 2830 (m), 1730 (s), 1470 (m), 1390 (m), 1370 (m), 1275 (s), 1210 (s), 1145 (s), 1090 (s), 1015 (m), 930 (w), 860 (w), 740 (w); δ 0.93 (6H, d, J = 6 Hz), 1.28 (3H, t, J = 7 Hz), 1.46 (2H, t, J = 6 Hz), 1.81 (1H, m, J = 6 Hz), 2.59 (1H, s, –O*H*), 4.00 (1H, t, J = 6 Hz), 4.18 (2H, q, J = 7 Hz); glc (3% SE 30, 1.5 m × 2 mm at 88°; Carrier gas, N_2, 1.05 kg/cm^2): Rt 1.1 min. (Found: C, 59.72; H, 10.00. Calc. for $C_8H_{16}O_3$: δ, 59.98; H, 10.07%). This was acetylated with Ac_2O/C_5H_5N to give (±)-**9** b.p. 92–94°/9.5 mm, n_D^{20} 1.4178; ν_{max} 2950 (m), 2860 (m), 1750 (s), 1470 (m), 1445 (w), 1435 (w), 1380 (m), 1350 (w), 1320 (w), 1285 (m), 1250 (s), 1235 (s), 1200 (s), 1175 (m), 1150 (m), 1130 (m), 1080 (m), 1030 (m), 960 (w), 940 (w), 920 (w), 855 (w), 820 (w), 755 (w), 700 (br. w) cm^{-1}; δ 0.95 (6H, d, J = 6 Hz), 1.27 (3H, t, J = 7 Hz), 1.50–1.99 (3H, m), 2.06 (3H, s), 4.15 (2H, q, J = 7 Hz), 4.78–5.01 (1H, m); glc (Column: 3% SE 30, 1.5 m × 2 mm at 88°; Carrier gas, N_2, 1.05 kg/cm^2): Rt 3.2 min. (Found: C, 59.20; H, 8.94. $C_{10}H_{18}O_4$ requires: C, 59.38; H, 8.97%).

(b) *Microbial hydrolysis.* The acetoxy ester (±)-**9** (12.94 g) was shaken with *B. subtilis*, precultured for 4 days in 5 flasks containing 150 ml each of 2% nutrient broth, for 12 days at 30°. Then the turbid culture medium was extracted with ether. The ether extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue (4.23 g) was a mixture of **9** and **10** (2:1) as revealed by glc. This was chromatographed over Merck Kieselgel 60 (52 g). Elution with n-hexane–ether (19:1) gave (*R*)-**9** (816.2 mg), b.p. 118–125°/23 mm, n_D^{20} 1.4160, $[\alpha]_D^{20.5}$ + 37.1° (*c* = 0.447, ether). A pure sample of (*S*)-**9** was prepared by acetylating pure

(*S*)-**10**. Its optical rotation value was: $[\alpha]_D^{20}$ −41.2° (c = 1.888, ether). The optical purity of (*R*)-**9** was therefore 90%. Its IR and NMR spectra were identical with those of (±)-**9**. Further elution with n-hexane-ether (19: 1~9:1) gave 543.6 mg of (*R*)-**9** containing 5% of (*R*)-**10**. Later fractions gave 403.6 mg of (*R*)-**10**, b.p. 112–114°/22.5 mm, n_D^{20} 1.4217; $[\alpha]_D^{20}$ + 14.2° (c = 0.827, ether). An authentic sample of (*S*)-**10** was prepared as described.[18] Its optical rotation value was: $[\alpha]_D^{20}$ − 18.06° (c = 1.229%, ether). The optical purity of (*R*)-**10** was therefore 78.5%. Its IR and NMR spectra were identical with those of (±)-**10**.

Acknowledgements—We thank Dr. T. Oritani, Tohoku University, for his kind supply of *Bacillus subtilis* var. *Niger*. Thanks are due to Prof. T. Beppu and Dr. H. Shoun, this Department, for their advices and helps in carrying out the fermentation. We are grateful to Messrs T. Nukada and K. Sato for their supply of **3d**, **3f** and **3g**.

REFERENCES

1. *Applications of Biochemical Systems in Organic Chemistry*, Part I (Edited by J. B. Jones, C. J. Sih and D. Perlman), Wiley, New York (1976).
2. Preliminary communication: K. Mori and H. Akao, *Tetrahedron Letters* 4127 (1978).
3. T. Oritani and K. Yamashita, *Agric. Biol. Chem.* **37**, 1687, 1691, 1695 (1973).
4. T. Oritani and K. Yamashita, *Ibid.* **38**, 1961, 1965 (1974).
5. T. Oritani and K. Yamashita, *Ibid.* **39**, 89 (1975).
6. K. K. Chan, N. C. Cohen, J. P. DeNoble, A. C. Specian, Jr. and G. Saucy, *J. Org. Chem.* **41**, 3497 (1976).
7. R. S. Brinkmeyer and V. M. Kapoor, *J. Am. Chem. Soc.* **99**, 8339 (1977).
8. W. H. Pirkle and C. W. Boeder, *J. Org. Chem.* **43**, 2091 (1978).
9. F. Bohlmann and G. Grau, *Chem. Ber.* **98**, 2608 (1965).
10. R. S. Landor, B. J. Miller and A. R. Tatchell, *J. Chem. Soc.* (C) 2339 (1971).
11. K. Mori and T. Nukada, unpublished results.
12. K. Sato, T. Nakayama and K. Mori, *Agric. Biol. Chem.* **43**, 1571 (1979).
13. J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.* **95**, 512 (1973).
14. D. Valentine, Jr. and J. W. Scott, *Synthesis* 329 (1978).
15. M. Asami and T. Mukaiyama, *Heterocycles* **12**, 499 (1979).
16. S. Yamaguchi and H. S. Mosher, *J. Org. Chem.* **38**, 1970 (1973).
17. *Dictionary of Organic Compounds* (Edited by I. Heilbron), Vol. III, p. 212. Oxford University Press, New York (1953).
18. K. Mori, *Tetrahedron* **32**, 1101 (1976).