Communications to the Editor

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SYNTHESIS OF USEFUL CHIRAL SYNTHONS (2R, 3R)-2-METHYLMALATE AND ITS CONGENERS VIA MICROBIAL ASYMMETRIC REDUCTION

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Asymmetric reduction of the commercially available diethyl 2-methyl-3-oxosuccinate 4 with Candida albicans gave a mixture of (3R)-methylmalates 5a and 5b. These were subjected to simple chemical processes and subsequent column chromatographic separation to provide the optically pure, useful chiral synthon 6a or 7a in good yield.

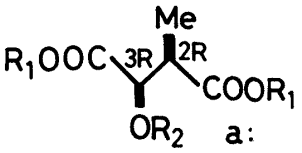
KEYWORDS———microbiologically asymmetric reduction; yeast; 2-methylmalate; α-methyl β-keto ester

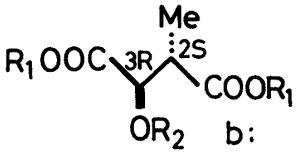
To obtain a functionalized 2-methyl-3-hydroxy propionic acid, a useful building block for the synthesis of polyoxygenated natural products, we previously examined a microbiological reduction of dimethyl 2-methyl-3-oxosuccinate (1). We found that although the desired dimethyl (2R, 3R)-2-methylmalate 2a was obtained in high optical purity (95% e.e.) along with (2S, 3R)-isomer 2b (58% e.e.) by the use of Candida albicans, the total yield of the products was only 22% and a chromatographic separation of the isomer 2b was quite difficult.[1] These difficulties were partially overcome by replacing one of the methoxycarbonyl group in 1 with a furyl group which could be regarded as a carboxylic acid equivalent.[2] However, a large scale conversion of a furyl group into carboxylic acid by ozonolysis is difficult in practice. Therefore, a still better substrate was sought.

Meanwhile, in microbiological reduction of 3, a great advantage of ethyl ester over methyl ester was observed.[3] Thus, expecting the same trends in the present case, we reduced diethyl 2-methyl-3-oxosuccinate 4 with a variety of yeasts. The selected results are shown in Table I. In all cases, the products were found to be mixtures of (2R, 3R)-syn-5a[4] and (2S, 3R)-anti-5b.[4] Determination of the absolute structure, product ratio and optical purity of each isomer was achieved in the same way as described for the reduction of dimethyl ester 1.[1] The desired (2R, 3R)-isomer 5a was obtained with high optical purity (>99% e.e.). Moreover, a great increase in total yield of the mixture of 5a and 5b (more than 80% yield) was observed, particularly when Candida albicans was used. To examine the effect of the concentration of substrates, a series of the experiments were carried out using Candida albicans. The results are shown in Table II. The total yield and optical purity of 5a were quite satisfactory in every case but the ratio of the desired 5a decreased appreciably with the increase in substrate concentration, which shows that a 0.1% solution of 4 is optimal.

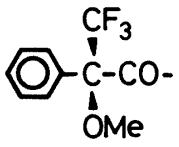
The remaining serious problem is the separation of anti-isomer 5b, which was always produced. Presumably when configurational isomers are subjected to separation by column chromatography, the

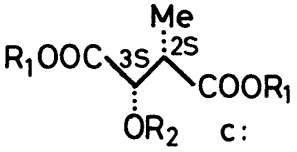
effect of a configurational difference of substituents would appear more subtly in cyclic compounds than the corresponding acyclic compounds. Thus, a mixture of microbiological reduction products (Table II, entry 4: 5a, >99% e.e.; 5b, 33% e.e.) was treated with BH_3-Me_2S in the presence of a trace amount of $NaBH_4$[5] and then lactonized with CF_3COOH, producing a mixture of the hydroxy lactones (6a,b and 7a,b). These could be separated as expected into a mixture (15%) of 7a and 7b, 6a[7] (41%) and 6b[7] (28%) by the usual column chromatography. A mixture of 7a and 7b could also be separated into 7a[7] and 7b[7] by using lobar column chromatography. The structure of the isomeric lactones thus obtained were confirmed by converting the authentic syn-2a[8] and anti-2b[8] into (6a, 7a) and (6b, 7b), repectively, in the same way as above.

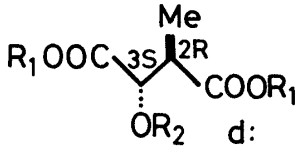




(+)-MTPA:





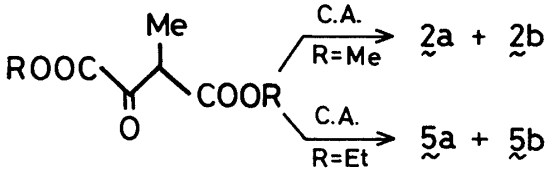


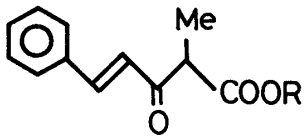
a: (2R, 3R)
b: (2S, 3R)
c: (2S, 3S)
d: (2R, 3S)

2 R_1=Me R_2=H

5 R_1=Et R_2=H

8 R_1=Et R_2=(+)-MTPA



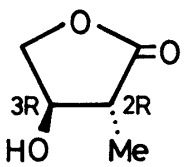


1 R=Me

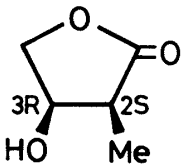
4 R=Et

3 R=Me or Et

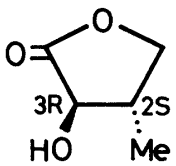
C.A.: Candida albicans



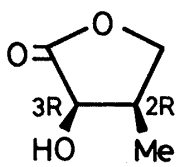
6a



6b



7a



7b

Table I

Entry	Microorganisms	Yield (%)	syn/anti	Optical purity (% e.e.)
1[a)]	Saccharomyces cerevisiae	85[d)]	43/57	syn-5a; 79 anti-5b; 31
2[b)]	Saccharomyces fermentati	33[e)]	44/56	syn-5a; 82 anti-5b; 44
3[b)]	Endomycopsis fibligera	51[e)]	68/32	syn-5a; 90 anti-5b; 46
4[b)]	Hansenula anomala	50[e)]	51/49	syn-5a; 95 anti-5b; 42
5[b)]	Hasenula anomala NI-7572	47[e)]	58/42	syn-5a; 92 anti-5b; 25
6[b)]	Saccharomyces acidifaciens	42[e)]	56/44	syn-5a; 93 anti-5b; 32
7[c)]	Candida albicans	84[d)]	55/45	syn-5a;>99 anti-5b; 77

a) A mixture of substrate 4 (2 g), sucrose (20 g), Baker's yeast (Saccharomyces cerevisiae, 20 g) in water (120 ml) was shaken for 24 h at 30°C.

b) Yeasts were incubated in 100 ml of liquid medium[1)] and the mixtures were shaken for 3 d at 30°C. Then ca. 50 mg of substrate 4 was added and the whole was again shaken for 3 d at 30°C.

c) A test tube containing 10 ml of the reported liquid medium[1)] was inoculated with Candida albicans and cultured at 30°C for 2 d with shaking. Then 1 ml of the seed culture was transfered to 450 ml of the same medium as mentioned above. After 2 d cultivation, 500 mg of 4 was added to the 450 ml of seed culture, then the whole was again shaken for 2 d at 30°C.

d) Isolated yield.

e) Yield as (+)-MTPA ester.[6)]

Table II

Entry	Concentration of substrate 4	Yield (%)	syn/anti	Optical purity (% e.e.)
1	500 mg/ 450 ml	84	55/45	syn-5a; >99 anti-5b; 77
2	1.03 g/ 800 ml	83	52/48	syn-5a; >99 anti-5b; 25
3	1.27 g/ 800 ml	82	44/56	syn-5a; >99 anti-5b; 31
4	1.38 g/ 800 ml	90	47/53	syn-5a; >99 anti-5b; 33
5	1.563 g/ 800 ml	90	39/61	syn-5a; >99 anti-5b; 25
6	1.765 g/ 800 ml	90	32/68	syn-5a; >99 anti-5b; 24
7	2.196 g/ 800 ml	89	38/62	syn-5a; >99 anti-5b; 25

Thus, it become possible to obtain optically pure (2R, 3R)-6a and (2S, 3R)-7a corresponding to the (2R, 3R)-methylmalate in quantities from commercially available diethyl ester 4 by asymmetric reduction with Candida albicans, followed by simple chemical processes and column chromatographic separation.

Synthesis of the biologically active natural product is now being undertaken in this laboratory using the optically pure 6a or 7a obtained by the present method.

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REFERENCES AND NOTES

1) H. Akita, A. Furuichi, H. Koshiji, K. Horikoshi and T. Oishi, Chem. Pharm. Bull., 31, 4384 (1983).

2) a) Idem, Tetrahedron Lett., 23, 4051 (1982); b) Idem, Chem. Pharm. Bull., 32, 1333 (1984).

3) H. Akita, H. Koshiji, A. Furuichi, K. Horikoshi and T. Oishi, Tetrahedron Lett., 24, 2009 (1983).

4) The asymmetric reduction products 5a and 5b of 4 with Saccharomyces cerevisiae were separated by means of high pressure liquid chromatography to the less polar fraction anti-5b and the more polar fraction syn-5a, eluted with n-hexane-AcOEt (5:1). Among these, anti-5b was identical with an authentic sample in respect to $[\alpha]_D$[9] and ^{13}C-NMR.[10] syn-5a; $[\alpha]_D^{27}$ +1.78° (c=5, ether), IR (CCl_4): 1735, 3525 cm^{-1}, MS (as 3,5-dinitrobenzoate): Calcd for $C_{16}H_{18}N_2O_{10}$; 398.095. Found; 398.094, ^{1}H-NMR ($CDCl_3$) δ: 1.170 (d, J=7.2 Hz, 3H; C_2-Me), 4.604 (dd, J=4.6, 3.7 Hz, 1H; C_3-H), ^{13}C-NMR ($CDCl_3$) δ: 10.781 (C_2-Me), 43.290 (C_2), 71.627 (C_3-OH), anti-5b; $[\alpha]_D^{27}$ +4.54° (c=5, ether), IR (CCl_4): 1735, 3525 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ: 1.298 (d, J=7.2 Hz, 3H; C_2-Me), 4.267 (dd, J=3.5, 6.4 Hz, 1H; C_3-H), ^{13}C-NMR ($CDCl_3$) δ: 12.840 (C_2-Me), 43.453 (C_2), 72.654 (C_3-OH). To determine optical purity, the relationship between the absolute structure of each isomer 5a-d and chemical shift due to C_3-H of the corresponding (+)-MTPA esters 8a-d was determined. 8a; δ 5.770 (d, J=3.4 Hz), 8b; δ 5.401 (d, J=5.1 Hz), 8c; δ 5.795 (d, J=3.4 Hz), 8d; δ 5.418 (d, J=5.1 Hz)

5) S. Saito, T. Hasegawa, M. Inaba, R. Nishida, T. Fujii, S. Nomizu, and T. Moriwake, Chemistry Lett., 1984, 1389.

6) a) J. A. Dale, D. L. Dull, and H. S. Mosher, J. Org. Chem., 34, 2543 (1969).
b) J. A. Dale and H. S. Mosher, J. Am. Chem. Soc., 95, 512 (1973).

7) 6a; $[\alpha]_D^{20}$ +55.1° (c=2, $CHCl_3$), IR (CCl_4): 1790, 3440 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ: 1.311 (d, J=7.3 Hz, 3H; C_2-Me), 2.530-2.600 (m, 1H; C_2-H), 6b; $[\alpha]_D^{20}$ +6.82° (c=2.2, $CHCl_3$), IR (CCl_4): 1790, 3430 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ: 1.281 (d, J=7.2 Hz, 3H; C_2-Me), 2.617-2.685 (m, 1H; C_2-H), 7a; $[\alpha]_D^{20}$ +70.33° (c=2.73, $CHCl_3$), IR (CCl_4): 1790, 3430 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ: 1.252 (d, J=6.6 Hz, 3H; C_2-Me), 2.485-2.606 (m, 1H; C_2-H), 4.047 (d, J=10.5 Hz, 1H; C_3-H), 7b; $[\alpha]_D^{20}$ +2.31° (c=1.95, $CHCl_3$), IR (CCl_4): 1790, 3440 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ: 1.118 (d, J=7.1 Hz, 3H; C_2-Me), 2.724-2.813 (m. 1H; C_2-H), 4.503 (d, J=7.3 Hz, 1H; C_3-H)

8) The authentic syn-2a and anti-2b were synthesized by Izumi's method.[11]

9) K. Mori and H. Iwasawa, Tetrahedron, 36, 87 (1980).

10) D. Seebach and D. Wasmuth, Helv. Chim. Acta, 63, 197 (1980).

11) S. Tatsumi, M. Imaida, and Y. Izumi, Bull. Chem. Soc. Japan, 39, 1818 (1969).

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