Tetrahedron: Asymmetry Vol. 3, No. 3, pp. 431-436, 1992
Printed in Great Britain

0957-4166/92 $3.00+.00
Pergamon Press plc

Enzymatic Asymmetric Synthesis of *Cis*-4-cyclopentene-1,3-dimethanol monoacetate.

Mounia MEKRAMI and Sames SICSIC*

CERCOA.CNRS, 2 rue Henry Dunant, F-94320 THIAIS.

(*Received* 20 *January* 1992)

Abstract: Asymmetric synthesis of *cis*-4-cyclopentene-1,3-dimethanol monoacetate was performed via enzymatic hydrolysis of the diacetate or enzymatic acetylation of the diol precursor. Esterases and lipases were studied, and *Candida cylindracea* lipase was shown to afford the (-)- enantiomer with ee>97%.

In the synthesis of carbocyclic analogues of ribofuranose containing compounds[1-7], *cis*-4-cyclopentene-1,3-dimethanol monoacetate **1** is a useful intermediate as the hydroxymethyl group can be easily converted into the carboxylic acid and further into the amine.

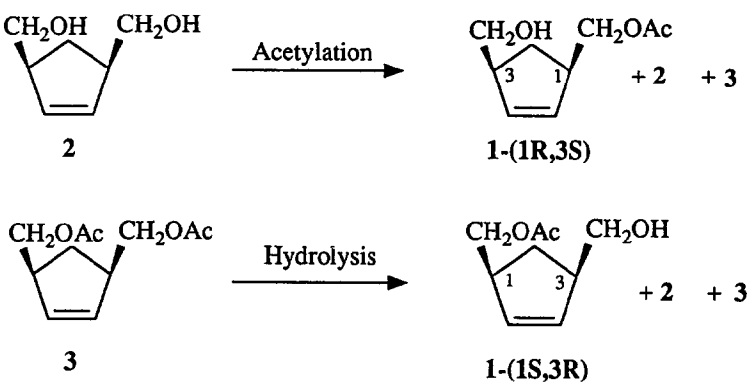

Fig.1

As the availability of **1** which contains two stereogenic carbons, in an enantiomerically pure form is essential to mimic the natural ribofuranose containing compounds, we planned to study different ways using the enantioselective potential of enzymes.

The principle followed was to differentiate between the two enantiotopic groups of a *meso* molecule leading to an asymmetric one (fig 1). Esterases and lipases are known for their broad substrate

spectrum, for their efficiency in racemic resolution procedures [8], and for their ability to differentiate enantiotopic groups in *meso* substrates. Furthermore this ability is expressed both in hydrolysis of esters and in their synthesis by acyl transfer (transesterification).

In cycloalkane series many papers have described works following this principle[9-16]. More specially, Schneider using lipases from porcine pancreas (PPL) and from *Pseudomonas sp.* (SAM II)[9,16] and Guanti using pig liver esterase (PLE)[10] have reported results on the differentiation of hydroxymethyl or acetoxymethyl groups separated by two carbon atoms. Our work concerns the differentiation of the same groups separated by three carbon atoms in cyclopentene series.

The results from the literature clearly showed that the hydrolysis as well as the synthesis operates on the acetoxymethyl or the hydroxymethyl attached to the cycloalkyl carbon which has S configuration respectively. It was interesting to examine whether the enantioselectivity of the enzymatic reactions was unchanged for our molecule having the acetoxymethyl groups (molecule **3**) or hydroxymethyl groups (molecule **2**) in 1,3-positions and also a double bond in the β position relative to each of these groups. For this study we screened different commercial lipases and esterases and two industrial lipases, Esterase 30000 (E.GB) from Gist Brocades, France and the genetically modified lipase (L.PGS) from Plant Genetic System, Belgium.

The hydrolysis reactions of the diacetate **3** were run in water maintaining the pH at 7 with 1N NaOH, and were stopped when one equivalent of acetic acid has been liberated.

For the synthesis reactions of the monoacetate **1** from the diol **2**, different conditions were tested, modifying the acetylating agent, the solvent and the stoicheiometry of the reagents. Our best conditions were for reactions run in toluene using the well known vinyl acetate as acetylating agent[17], with a twofold excess relative to the diol. The reactions gave mono and diacetates, the formation of which was followed by GPC and were stopped when the best ratio monoacetate/diacetate was reached.

ENZYME* (entry)	TIME (h)	DIOL (%)	MONOAc (%)	$[\alpha]_D^{25}$**	ee (%)	(conf)
PPL (1)	6		98	10.9	55	(1S,3R)
CC (2)	24	13	45	6.5	30	(1S,3R)
PF (3)	23	11	43	-6.0	39	(1R,3S)
PLE (4)	24	20	55	5.2	30	(1S,3R)
WG (5)	24	32	34	2.1	10	(1S,3R)
ALHP (6)	4	20	55	5.2	38	(1S,3R)
ALPP (7)	23	22	45	6.4	35	(1S,3R)
E.GB (8)	24	20	62	4.9	28	(1S,3R)
ALBP (9)	24	20	46	13.0	70	(1S,3R)
L.PGS (10)	0.5	11	59	9.1	62	(1S,3R)

(*)CC: *candida cylindracea*; PF: *pseudomonas fluorescens*; WG: wheat germ; ALHP: acetone liver horse powder; ALPP: acetone liver porcine powder; ALBP: acetone liver beef powder. (**)Specific optical rotation of monoacetate **1**.

TABLE 1- Enzymatic hydrolysis.

The enantiomeric excess of monoacetate **1** was determined by GPC using a chiral column coated with cyclodextrin. The results of the enzymatic hydrolysis are indicated in table 1. Only PPL gives exclusively monohydrolysis, but the enantiomeric excess of the monoacetate produced is not satisfactory. The best enantiomeric excesses are obtained with ALBP and L.PGS, but the chemical yields of the monoacetate are low. It is remarkable that the preferentially hydrolyzed acetate is attached in all cases but one to the carbon which has the configuration R.

The results of the enzymatic synthesis of the monoacetate **1** are summarized in table 2. Only the synthesis catalyzed by CC (entry 11) has both a good chemical yield and an excellent enantiomeric excess of 97%. As for the enzymatic hydrolysis, the hydroxymethyl preferentially acetylated is attached to the carbon which has the configuration R. This experiment using CC was scaled up to a 0.1 mol level. After 25h the reaction gave 73% of the monoacetate and after liquid chromatography purification, **1**-(1R,3S) was obtained with a yield of 55% relatively to the diol **2** and an ee>97%.

ENZYME* (entry)	TIME (h)	DIAc (%)	MONOAc (%)	$[\alpha]_D^{25}$**	ee (%)	(conf)
CC (11)	20	12	68	-20.0	97	(1R,3S)
ALHP (12)	48	2	46	-9.9	57	(1R,3S)
PF (13)	48	26	43	6.2	32	(1S,3R)
ALPP (14)	48		30	-4.2	26	(1R,3S)
PPL (15)	48	7	57	-3.6	18	(1R,3S)
ALBP (16)	48	2	32	-3.0	14	(1R,3S)
WG (17)	24	3	69	-2.0	13	(1R,3S)
Lipo (18)	48	21	42	-1.8	13	(1R,3S)
E.GB (19)	24	18	60	-8.6	46	(1R,3S)
L.PGS (20)	4	26	50	-7.2	42	(1R,3S)

(*) Lipo: *mucor miehei* immobilized (from Novo). (**) Specific optical rotation of monoacetate **1**.

TABLE 2- Enzymatic acetylation.

The absolute configuration of the monoacetates was unambiguously assigned by chemical correlation with (-)-(1S,4R)-4-(acetylamino)-2-cyclopentene-1-carboxylic acid **4** (fig. 2) which was correlated to a natural product[18,19]. This product **4** was obtained by enzymatic resolution of the corresponding methyl ester using PLE in a toluene-water mixture. In this solvent system, and after 40% hydrolysis, the acid **4** isolated has an $[\alpha]_D^{25}$=-72 (c=1, CCl_4) higher than the value we have previously reported for the same hydrolysis in water, $[\alpha]_D^{25}$=-42. The transformation of **4** led to (1S,4R)-4-(benzyloxycarbonylamino)-2-cyclopentene-1-methanol **6** which has an $[\alpha]_D^{25}$=+52 (c=1, CCl_4). The correlation was done with the product **1** (entry 11, table 2) obtained by the acetylation catalyzed by CC. This product was transformed to 4-(benzyloxycarbonylamino)-2-cyclopentene-1-methanol **8** which has an $[\alpha]_D^{25}$= -48 (c=1, CCl_4). Conclusively the product **8** has the configuration (1R,4S) and consequently the product **1** (entry 11) has the configuration (1R,3S).

Our results clearly show that in all cases but one (PF, entries 3 and 13) the enzymatic reactions take place on the hydroxymethyl or the acetoxymethyl attached to the carbon which has the

configuration **R**, in opposition to the results of Schneider where these groups are always attached to the carbon which has the configuration **S**. This opposition could be only formally due to the Cahn-Ingold-Prelog rules where the priority order for the determination of the absolute configuration is determined irrespective of the size of the substituents[20]. It also could be due to the fact that the function where the reaction takes place is not directly attached to the stereogenic carbon atom. In these conditions, other structural factors of the alcohol moiety controlling the specificity of the enzymes must be understood.

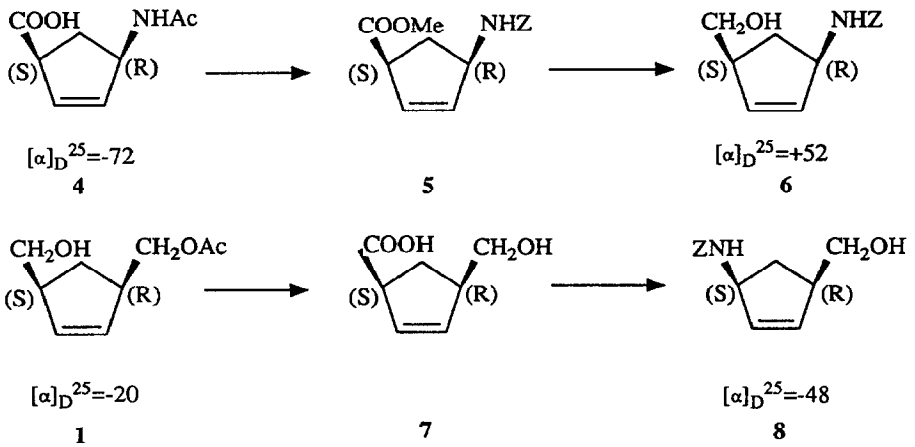

Fig.2

EXPERIMENTAL PART

The enantiomeric excesses were determined by GPC using a chiral WCOT fused silica (25m, 0.25 m) column from Chrompack with the following conditions: N_2, 1bar; temperature program, 100°C for 18mn, then progression of 30°C/mn up to 200°C. Retention times: (+)-1, 24.14mn, (-)-1, 25.13mn. The optical rotations were measured in CCl_4, c=1.

Cis-4-cyclopentene-1,3-dimethanol 2.[21]
^{1}Hnmr ($CDCl_3$): δ 5.65 (s, 2H), 4.22 (s, 2H), 3.46 (d, 4H, J=6.2Hz), 2.9 (m, 2H), 2.1 (dt, 1H, J_1=13.7Hz, J_2=9.1Hz), 1.3 (dt, 1H, J_1=13.6Hz, J_2=5.6Hz)ppm, ^{13}Cnmr ($CDCl_3$): δ 132 (C4, C5), 67 (CH_2OH), 48 (C1, C3), 28 (C2)ppm.

Cis-4-cyclopentene-1,3-dimethanol diacetate 3.
A solution of pyridine (10ml) containing 1g (7.8 mmol) of the diol 2, and 5ml of acetic anhydride was left at room temperature for 12h. Pyridine and excess acetic anhydride were evaporated, the oily residue was dissolved in ether (15ml),the solution was washed with water and brine, and dried ($MgSO_4$). Evaporation of ether led to an oily residue which gave after distillation ($Eb_{0.2}$:125°C) 1.52g of product 3 (92% yield). ^{1}Hnmr ($CDCl_3$): δ 5.7 (s, 2H), 4 (ddd, 4H, J_1=13.8Hz, J_2=7.6Hz, J_3=6.3Hz), 3 (m, 2H), 2.2 (dt, 1H, J_1=13.8Hz, J_2=6.9Hz), 2 (s, 6H), 1.1 (dt, 1H, J_1=13.9Hz, J_2=4.9Hz)ppm. ^{13}Cnmr ($CDCl_3$): δ 170 (CO), 134 (vinylic), 68 (CH_2O), 45 (allylic), 28 (C2), 20 (methyl)ppm. Mass: 212 (M), 197 (M-CH_3), 182 (M-2CH_3), 154 (M-CH_3-acetyl), 94 (M-2 acetyl).

Cis-4-cyclopentene-1,3-dimethanol monoacetate 1 (chemical).
A solution of pyridine (10ml) containing 1g (7.8 mmol) of the diol 2 and 0.8g (7.8mmol) of acetic anhydride was left at room temperature for 8h. Pyridine and unreacted anhydride were evaporated and the residual oil was dissolved in ethyl acetate and this solution was washed with water and brine and dried ($MgSO_4$). Evaporation of ethyl acetate led to an oily residue which was analyzed by GPC (CP Sil 19CB, 10m). The three products 1 (62%), 2 (20%) and 3 (18%) were detected, and separated with liquid column

chromatography (silica gel, ethyl acetate-petroleum ether, 20-80). 0.77g of monoacetate **1** (58% yield) was obtained. ^{1}Hnmr ($CDCl_3$): δ 5.76 (ddd, 2H, J_1=5.7Hz, J_2=1.8Hz, J_3=1.8Hz), 3.95 (ddd, 2H, J_1=10.6Hz, J_2=6.7Hz, J_3=6.7Hz), 3.5 (d, 2H, J=5.9Hz), 2.9 (m, 2H), 2.18 (ddd, 1H, J_1=13.7Hz, J_2=8.9Hz, J_3=6.6Hz), 1.99 (s, 3H), 1.1 (ddd, 1H, J_1=13.7Hz, J_2=6.4Hz, J_3=2.7Hz)ppm. ^{13}Cnmr ($CDCl_3$): δ 170 (CO), 134 (C5), 132 (C4), 68 (C6), 66 (C7), 48 (C1), 45 (C3), 28 (C2), 18 (CH_3)ppm. Mass: 170 (M), 169 (M-1), 155 (M-CH_3), 111 (M-OAc).

Enzymatic hydrolysis:
To 7ml of a stirred mixture of enzyme [22] in distilled water (pH 7.0, 35°C), the substrate **3** (0.5g) was added. The pH was maintained at 7.0 by addition of 1M NaOH with a pHstat. The reaction was stopped when one equivalent of base has been consumed, by addition of acetone. The mixture was then evaporated and treated as indicated before.

Enzymatic acetylation:
A flask containing 0.5g of substrate **2**, 0.724ml of vinyl acetate, 7ml of toluene and enzyme [23] was vigorously shaken at room temperature. The reaction was stopped by filtration of the mixture, and the filtrate was evaporated. The residual product was separated as indicated before.

(1R,3S)-4-cyclopentene-1,3-dimethanol monoacetate **1** (enzymatic).
A flask containing 120ml of toluene, 12.8g of the diol **2**, 8.6g of vinyl acetate and 2.6g of CC lipase was vigorously shaken (120u/mn) at room temperature. After 25h the reaction was stopped by filtration, and the filtrate was evaporated to dryness. The resulted oily residue was fractionated by liquid chromatography, giving rise to 9.35g of (1R,3S)-**1**, $[\alpha]_D^{25}$=-20 (c=1, CCl_4).

(1S,4R)-4-(hydroxymethyl)-2-cyclopentene-1-carboxylic acid **7**.
To a solution of acetone (15ml) containing 0.66g (3.88mmol) of monoacetate (1R,3S)-**1** obtained from enzymatic acetylation using *Candida cylindracea* lipase and maintained at 0°C, 3.5ml of Jones reagent[24] was added dropwise during 40mn. The mixture was stirred for 3h at room temperature, filtered and evaporated. The oily residue was dissolved in ether, and this solution treated with 1N NaOH and water. The aqueous phases were gathered, acidified with 1N HCl and extracted with ether. The ethereal phases were gathered, dried ($MgSO_4$) and evaporated leading to 0.26g (47% yield) of the crude product **7**. ^{1}Hnmr (D_2O): δ 5.75 (ddd, 2H, J_1=10.4Hz, J_2=10.2Hz, J_3=6.69Hz), 3.9 (d, 2H, J=6.5Hz), 3.6 (m, 1H), 3 (m, 1H), 2.6 (m, 1H), 1.8 (m, 1H)ppm. ^{13}Cnmr (D_2O): δ 185 (CO), 140 (C2), 134 (C3), 67 (C6), 58 (C1), 48 (C4), 32 (C5)ppm.

(1R,4S)-4-(benzyloxycarbonylamino)-2-cyclopentene-1-methanol **8**:
To a solution of dry THF (10ml) containing 0.464g (3.26mmol) of the acid **7** and 0.46ml of triethylamine, was added 0.315ml (3.26mmol) of ethyl chloroformate at 0°C under nitrogen. The solution was stirred for 30mn, and a solution of 0.64g (9.78mmol) of sodium azide in 5ml of water was added. The solution was stirred for 30mn at 0°C and for 2h at room temperature. Then the solution was extracted with ethyl acetate (3x20ml), the organic phase washed with brine, aqueous sodium bicarbonate 10% and water, and dried (Na_2SO_4). Evaporation of the solvent led to 0.35g of an oily residue which was dissolved in toluene (5ml). This solution was heated at 80°C for two hours, and evaporated under vacuum giving rise to a residue which was dissolved in benzyl alcohol (10ml). This solution was heated at 90°C for 15h and the alcohol evaporated under vacuum leading to 0.57g of an oily residue which was purified by column chromatography (silica, CH_2Cl_2-CH_3OH, 98-2). 0.52g of a cristalline product was obtained (65% yield), F=121-2°C, $[\alpha]_D^{25}$=-48 (c=1, CCl_4). ^{1}Hnmr ($CDCl_3$): δ 7.8 (broad s, 1H), 7.25 (m, 5H), 5.97 (dd, 1H, J_1=9.6Hz, J_2=6.3Hz), 5.76 (dd, 1H, J_1=9.8Hz, J_2=6.7Hz), 5.28 (s, 2H), 4.12 (d, 1H, J=6.3Hz), 3.9 (broad s, 1H), 3.74 (dt, 1H, J_1=7.4Hz, J_2=6.7Hz), 3.6 (d, 2H, J=7.3Hz), 2.48 (m, 1H), 1.8 (m, 1H)ppm. Mass: 247 (M), 246, 216 (M-CH_2OH), 170 (M-C_6H_5), 156 (M-$C_6H_5CH_2$).

(1S,4R)-4-(acetylamino)-2-cyclopentene-1-carboxylic acid **4**.
In 25ml of water containing 5mg of PLE (0.65ml), was added a solution of 3.2g (17.5mmol) of cis-N-acetyl-4-amino-cyclopent-2-ene carboxylic acid methyl ester in toluene (7ml)[18]. The reaction was carried out at 35°C, maintaining the pH=7 with 1N NaOH. It was stopped at 40% consumption of the substrate. Work up of the acid **4** was done as described[18]. 1.01g (86% yield) was obtained. $[\alpha]_D^{25}$=-72 (c=1, MeOH).

(1S,4R)-4-(benzyloxycarbonylamino)-2-cyclopentene-1-carboxylic acid methyl ester **5**.
A solution of 6N HCl (10ml) containing 0.9g (7.25mmol) of the acid **4** was refluxed for 4h. The solution was then evaporated to dryness and the residue dissolved in 2N NaOH (15ml). This solution was cooled (0°C) before the addition dropwise of 1.6g of benzyl chloroformate (9.45mmol), and 2N NaOH (7ml) under vigorous stirring, maintaining the pH at 9. Then the mixture was left at room temperature (2h), and washed with ether and acidified with 1N HCl (pH=1). An oil separated which was extracted with ether (4x20ml), the organic phase was dried ($MgSO_4$) and evaporated leading to an oily residue (1.23g). This residue was suspended in 2,2-dimethoxy propane, then concentrated HCl (4.5ml) was added and the resulted solution left at room temperature for 15h. The solution was then evaporated to dryness and the residue extracted with ether. The organic phase was washed with water, brine and dried ($MgSO_4$), and evaporated leading to 1.02g of a viscous product (50% yield). ^{1}Hnmr ($CDCl_3$): δ 7.6 (broad s, 1H), 7.25 (m, 5H), 6.2

(d, 1H, J=10.1Hz), 6 (d, 1H, J=9.8Hz), 5.28 (s, 2H), 3.6 (t, 1H, J=7.22Hz), 3.4 (s, 3H), 3.22 (t, 1H, J=6.63Hz), 2.5 (m, 1H), 2 (m, 1H)ppm.

(1S,4R)-4-(benzyloxycarbonylamino)-2-cyclopentene-1-methanol **6**.
In a solution of dry THF (40ml) containing 0.7g (2.54mmol) of **5** maintained at 0°C, was added dropwise 2.54ml of a solution of 1N lithium triethyl borohydride. The mixture was stirred for 30mn, then the solvent was evaporated, the residue dissolved in methanol and the solution treated with 0.1N HCl at 0°C. The resulting solution was evaporated and the residue was washed with acetone and purified by liquid column chromatography (silica gel, CH_2Cl_2-MeOH, 98-2). 0.42g of the cristalline product **6** was obtained (67% yield). F=121-2°C. This product has the same chromatographic properties and nmr data than **8**. $[\alpha]_D^{25}$=+52 (c=1, CCl_4).

REFERENCES

1. Marquez, V.E.; Lim, M.I.; *Med. Res. Rev.* **1986**, 6, 1-40.
2. Cermak, R.C.; Vince, R.; *Tetrahedron Lett.* **1981**, 22, 2331-2332.
3. Kam, B.L.; Oppenheimer, N.J.; *J. Org. Chem.* **1981**, 46, 3268-3272.
4. Deardorff, D.R.; Shulman, M.J.; Sheppeck II, J.E.; *Tetrahedron Lett.* **1989**, 30, 6625-6628.
5. Chen, J.; Grim, M.; Rock, C.; Chan, K.; *Tetrahedron Lett.* **1989**, 30, 5543-5546.
6. Vince, R.; Bromwell, J.; *Biochem. Biophys. Res. Comm.* **1990**, 168, 912-916.
7. Vince, R.; Hua, M.; *J. Med. Chem.* **1990**, 33, 17-21.
8. Sih, C.J.; Wu, S.-H.; in Eliel, E.L. and Wilen, S.H. Eds. *Topics in Stereochemistry* vol.19, Wiley, New-York, 1989, pp.63-125 and references cited herein.
9. Laumen, K.; Schneider, M.; *Tetrahedron Lett.* **1985**, 26, 2073-2076.
10. Guanti, G.; Banfi, L.; Narisano, E.; Riva, R; Thea, S.; *Tetrahedron Lett.* **1986**, 27, 4639-4642.
11. Deardorff, D.R.; Matthews, A.J.; Scott Mc Meekin D.; Craney, C.L.; *Tetrahedron Lett.* **1986**, 27,1255-1256.
12. Breitgoff, D.; Laumen, K.; Schneider, M.P.; *J. Chem. Soc., Chem. Commun.* **1986**, 1523-1524.
13. Kerscher, V.; Kreiser, W.; *Tetrahedron Lett.* **1987**, 28, 531-534.
14. Sugai, T.; Mori, K.; *Synthesis* **1988**, 19-22.
15. Theil, F.; Ballschuh, S.; Schick, H.; Haupt, M.; Häfner, B.; Schwarz, S.; *Synthesis* **1988**, 540-541.
16. Ader, V.; Breitgoff, D.; Klein, P.; Laumen, K.E.; Schneider, M.P.; *Tetrahedron Lett.* **1989**, 30, 1793-1796.
17. Degueil-Casting, H.; De Jeso, B.; Drouillard, S.; Maillard, B.; *Tetrahedron Lett.* **1987**, 28, 953-954.
18. Sicsic, S.; Ikbal, M.; Le Goffic, F.; *Tetrahedron Lett.* **1987**, 28, 1887-1888.
19. Arita, M.; Adachi, K.; Ito, Y.; Sawai, H.; Ohno, M.; *J. Amer. Chem. Soc.* **1983**, 105, 4049-4055.
20. Cahn, R.S.; Ingold, C.; Prelog, V.; *Angew. Chem. Int. Ed. Eng.* **1966**, 5, 385-415.
21. Grob, G.A.; Pfaendler, H.R.; *Helv. Chim. Acta* **1970**, 53, 2156-2159.
22. PPL (type II), CC (type VII), ALHP,ALPP, ALBP, from Sigma, 100mg; WG (type I) from Sigma, 150mg; PF from Fluka, 10mg; PLE from Boehringer, 0.1ml; E.GB, 50mg; L.PGS, 10mg.
23. 100mg of enzyme, except for PF and PGS, 10mg and E.GB, 50mg.
24. Bowers, A.; Halsall, T.G.; Jones, E.R.H.; Lemin, A.J.; *J. Chem. Soc.* **1953**, 2548-2560.