Tetrahedron Vol 41, No. 7, pp. 1347 to 1352, 1985
Printed in Great Britain.

0040-4020/85 $3.00 + 00

ENZYME CATALYSED HYDROLYSIS OF DIALKYLATED PROPANEDIOIC ACID DIESTERS, CHAIN LENGTH DEPENDENT REVERSAL OF ENANTIOSELECTIVITY

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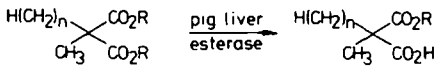
(*Received in UK* 2 *January* 1985)

ABSTRACT: Enzyme catalysed hydrolyses of dialkylated propanedioic acid diesters have been studied. A novel change of enantioselectivity from pro-S to pro-R in the hydrolysis of ester groups was observed, depending on the chain length of the alkyl substituents of the substrate. Optically pure (S)-(-)-α-methylphenylalanine was prepared by α-chymotrypsin catalysed hydrolysis of benzylmethylpropanedioic acid dimethyl ester.

Enzymes are becoming increasingly important as valuable catalysts for asymmetric synthesis. Particularly for the hydrolytic enzymes there are many examples of their synthetic utility. Their ability to catalyse hydrolysis of prochiral diesters of unnatural substrates to provide optically active monoesters has been studied by several research groups.[1] For the preparation of chiral synthons we have studied the selectivity of enzymatic hydrolysis of dialkylated propanedioic acid diesters 1-13 (Schemes 1, 2). The enzymes used were the commercially available pig liver esterase and chymotrypsin. Our investigation was performed in order to study structural effects on the kinetics and enantioselectivity of the hydrolysis and to provide data for studies on structure/activity relationships in enzyme catalysed reactions. Furthermore, the monoesters 14-26 obtained after hydrolysis may serve as chiral building blocks in the synthesis of more complex compounds of biological or pharmacological interest, e.g. α-methyl aminoacids or barbiturates.[2] Attempts have also been made to decarboxylate the monoesters into optically active α-alkylated acids or esters.

Scheme 1



R=Me	R=Et	R=Me	R=Et
1 n=2	8 n=2	14 n=2	21 n=2
2 n=3	9 n=3	15 n=3	22 n=3
3 n=4	10 n=4	16 n=4	23 n=4
4 n=5	11 n=5	17 n=5	24 n=5
5 n=6	12 n=8	18 n=6	25 n=8
6 n=7		19 n=7	

RESULTS

A remarkable reversal of the enantioselectivity was observed for pig liver esterase catalysed hydrolysis of dialkylated propanedioic acid diesters. Substrates with a methyl substituent and a short alkyl chain 1-3 and 8-10 gave up to 73 % e.e. of the S-enantiomer whereas the corresponding homologues 4-6 and 11 yielded the R-enantiomer. Almost 90 % e.e. was obtained for the hexyl 5 and heptyl 6 derivatives (Figure 1). A great difference in enantioselectivity was found for the methyl as compared with the ethyl diesters (Figure 1).

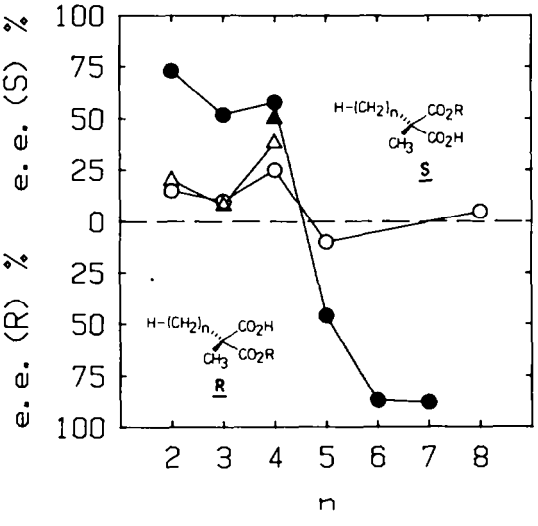
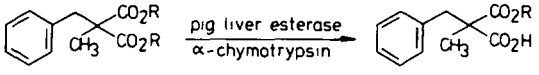


Figure 1. Enantiomeric excess (e.e.) of the monoesters of dialkylated propanedioic acids obtained by hydrolysis of the corresponding diesters catalysed by pig liver esterase (●:R=Me, ○:R=Et). The triangles denote results obtained by Schneider *et al.*[2]

Optically pure benzylmethylpropanedioic acid monoester was obtained when either the corresponding dimethyl ester 7 or diethyl ester 13 was hydrolysed by chymotrypsin (Scheme 2). Chymotrypsin did not catalyse the hydrolysis of any of the other substrates (1-6, 8-12).

Scheme 2



7 R=Me
13 R=Et

20 R=Me
26 R=Et

The determinations of the enantiomeric excess were made by NMR studies on the mono esters in the presence of optically pure 1-phenylethylamine.[3] The absolute configuration was established by a transformation of the optically pure (+)-benzylmethylpropanedioic acid monomethyl ester (20) (from chymotrypsin hydrolysis) to the optically pure (S)-(-)-α-methylphenylalanine (27) via acyl azide formation followed by a Curtius rearrangement[4] (Scheme 3). The Curtius rearrangement is known to proceed with retention of configuration.[4] Since the absolute configuration of (S)-(-)- α-methylphenylalanine is known this chemical transformation proved that the chymotrypsin catalysed hydrolysis of benzylmethylpropanedioic acid dimethyl ester (7) gave the R-(+)-monoester 20.

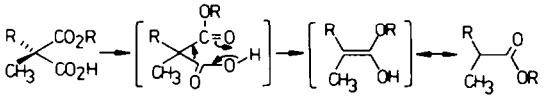
Scheme 3

R-20 S-27

The pig liver esterase catalysed hydrolysis of the substrate 7 provided the S-enantiomer of 16 % e.e. as shown by NMR studies in a comparison with the optically pure R-(+)-monoester of benzylmethylpropanedioic acid (20). The shift differences of the diastereotopic protons in the 1-phenylethylamine salts of the monoesters derived from pig liver esterase hydrolysis showed that the enantiomer in excess is the same (S) for all short chain compounds (14-16, 21-23) and that the opposite enantiomer (R) is in excess for the homologues (17-19, 24). The optical rotation measurements were also consistent with this change of enantiomeric preference of the enzyme. The short chain products (14-16, 21-23) exhibited (-)-rotation whereas the long chain products (17-19, 24) showed (+)-rotation.

Thermal decarboxylation of the monoesters gave the racemic α-alkylated esters. This result confirms the cyclic mechanism proposed for this decarboxylation[5] (Scheme 4). Radical decarboxylation[6] also gave racemic compounds.

Scheme 4



DISCUSSION

Detailed knowledge of the active site of pig liver esterase is limited. The active site is known to have an active serine and the enzyme thus belongs to the serine hydrolases, but the topology of the active site is unknown.[7]

Recently several authors have reported on the stereoselective hydrolysis of monocyclic meso-1,2-diesters using pig liver esterase.[8,9,10] A ring-size induced enantiospecificity is observed. The pro-S ester group is hydrolysed in small rings whereas the cyclopentyl analogue afforded an almost racemic product and the pro-R ester group is hydrolysed in the cyclohexyl derivative. The stereochemical requirements for high enantiomeric excess upon hydrolysis of diesters by pig liver esterase were discussed in more detail by Mohr et al.[10] Their model for structural/ activity relationship is not easily compared with ours since most of their substrates were β-diesters and ours are all α-diesters. In our case we observed a change in enantioselectivity enforced by the length of one of the alkylgroups. Between butyl and pentyl substituents there was a change from a preferential hydrolysis of the pro-R to the pro-S ester group of the dimethyl ester of the dialkylated propanedioic acids. It is very likely that the short side chains interact with a hydrophobic site in the enzyme but that this site is too small for the longer alkyl groups. The substrates with a longer side chain have therefore to interact with their methyl group in the hydrophobic site resulting in an inversion of the enantioselectivity. This model provides an explanation for the enantioselectivity in the hydrolysis of both the α- and β-diesters so far studied. The great difference in enantiomeric excess between the dimethyl esters and the diethyl esters may be explained by a greater ability of the ethyl ester group for binding to the hydrophobic site. Such a binding of the ester groups may result in a lower enantiomeric excess as observed. Schneider et al.[2] have also investigated the hydrolysis of dialkylated propanedioic acid diesters. They studied only short chain alkyl groups and mainly the diethyl esters and thus did not observe high enantiomeric excess (Figure 1). The best result was obtained with the dimethyl ester of methylphenylpropanedioic acid .

We have determined the initial specific activity of the pig liver esterase when applied to the hydrolysis of our substrates. The activity ranges from a few up to ten percent of its maximal activity with good substrates (Figure 2). This demonstrates that it is possible to use these reactions for preparative purposes. There is a significant and interesting decrease of the enzymatic activity in the hydrolysis of the propyl derivatives 2 and 9 (Figure 2), which parallels the observed decrease in the enantiomeric excess (Figure 1). Further studies on the structure/activity relationships may provide a rationale for this observation.

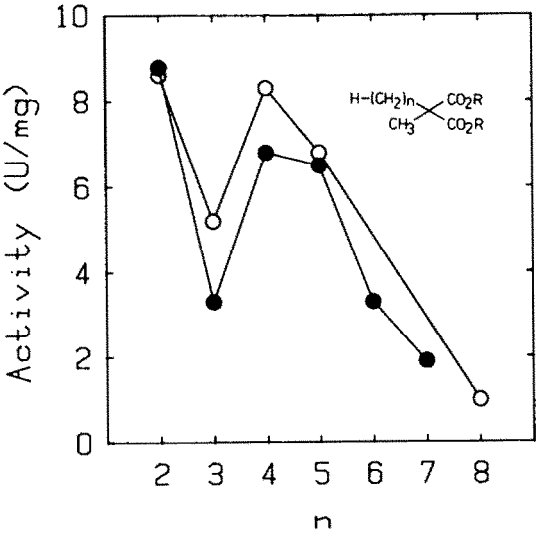


Figure 2. Initial rate of hydrolysis of dialkylated propanedioic acid diesters (●:R=Me, ○:R=Et) with pig liver esterase as determined in a pH-stat.

Monoalkylated propanedioic acid diesters were hydrolysed ten times faster, but resulted in racemic monoesters after isolation and purification. We have also tested subtilisin and chymotrypsin which were active against the monoalkylated diesters up to the pentyl and hexyl derivatives respectively but totally inactive with the dialkylated diesters. The only dialkylated derivatives that were hydrolysed by chymotrypsin were the benzylmethylpropanedioic acid diesters. The activity was very low (10^{-3} of the activity towards natural substrates).

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker WP 200 spectrometer with TMS as internal standard. Analytical GC was performed on a PYE 204 instrument with a flame ionisation detector using fused silica capillary columns. IR spectra were recorded on a PYE Unicam SP 1000 spectrophotometer. Optical rotations were determined on a Perkin Elmer 141 polarimeter. Due to the low optical rotations of some of the products enantiomeric excess are determined by NMR methods. In some of these cases the optical rotation could not be accurately recorded and therefore the $[\alpha]_D$ values are not reported. Liquid chromatography was performed with Merck silica gel (0.040-0.063 mm) using gradient elution with hexane with increasing amounts of ethyl acetate.[11] All reactions of air- and water-sensitive materials were performed under inert conditions (N_2 or Ar). Pig liver esterase (EC 3.1.1.1) ,α-chymotrypsin (EC 3.4.21.1) and subtilisin carlsberg (EC 3.4.21.14) were purchased from Sigma.

DIALKYLATED PROPANEDIOIC ACID DIESTERS

Typical procedure: Clean cut sodium (1.75 g, 76 mmol) was added in pieces to dry methanol (450 ml). When the reaction had ceased freshly destilled propanedioic acid dimethyl ester (10 g, 76 mmol) was added and the mixture was stirred at room temperature for 0.5-1 h. Benzyl bromide (13 g, 76 mmol) was added and the mixture was refluxed for 0.5 h then most of the solvent was removed under reduced pressure and aqueous HCl (0.5 M, 100 ml) was added. Extraction with diethyl ether (3x100 ml), drying ($MgSO_4$) and then destillation yielded 11.8 g (70 %) benzylpropanedioic acid dimethyl ester. This product was treated with sodium methoxide (1.1 eq) followed by addition of destilled methyl iodide (1.1 eq) in the same manner as above and yielded after work up and destillation 11.4 g (90 %, >99 % pure) of 7. In a few cases, e.g. methylpropylpropanedioic acid dimethyl ester, further purification by chromatography was necessary.

Ethylmethylpropanedioic acid dimethyl ester (1) : ^1H NMR δ: 3.72 (s,6H), 1.98-1.85 (q,2H), 1.40 (s,3H), 0.87 (t,3H). ^{13}C NMR δ: 172.7, 54.1, 52.3, 28.6, 19.4, 8.7.

Methylpropylpropanedioic acid dimethyl ester (2) : ^1H NMR δ: 3.72 (s,6H), 1.88-1.79 (m,2H), 1.41 (s,3H), 1.27-1.21 (m,2H), 0.93 (t,3H). ^{13}C NMR δ:172.9, 53.8, 52.3, 37.9, 20.0, 17.7, 14.4.

Butylmethylpropanedioic acid dimethyl ester (3) : ^{1}H NMR δ: 3.72 (s,6H), 1.90-1.80 (m,2H), 1.41 (s,3H), 1.35-1.1 (m,4H), 0.90 (t,3H). ^{13}C NMR δ: 172.9, 53.7, 52.3, 35.4, 26.5, 22.9, 20.0, 13.8.

Methylpentylpropanedioic acid dimethyl ester (4) : ^{1}H NMR δ: 3.72 (s,6H), 1.89-1.81 (m,2H), 1.41 (s,3H), 1.31-1.21 (unresolved m,6H), 0.88 (s,3H). ^{13}C NMR δ: 172.9, 53.8, 52.3, 35.7, 32.0, 23.9, 22.4, 20.0, 13.9.

Hexylmethylpropanedioic acid dimethyl ester (5) : ^{1}H NMR δ: 3.72 (s,6H), 1.89-1.81 (mm,2H), 1.41 (s,3H), 1.27-1.18 (unres. m,8H), 0.88 (t,3H). ^{13}C NMR δ: 172.7, 53.6, 52.1, 35.6, 31.4, 29.4, 24.1, 22.4, 19.8, 13.8.

Heptylmethylpropanedioic acid dimethyl ester (6) : ^{1}H NMR δ: 3.72 (s,6H), 1.89-1.81 (mm,2H), 1.41 (s,3H), 1.26-1.18 (unres. m,10H), 0.88 (t,3H). ^{13}C NMR δ: 172.7, 53.7, 52.2, 35.6, 31.6, 29.7, 28.9, 24.2, 22.5, 19.9, 13.9.

Benzylmethylpropanedioic acid dimethyl ester (7) : ^{1}H NMR δ: 7.28-7.10 (unres. m,5H), 3.74 (s,6H), 3.23 (s,2H), 1.35 (s,3H). ^{13}C NMR δ: 172.3, 136.0, 130.1, 128.2, 127.0, 54.9, 52.5, 41.3, 19.8.

Ethylmethylpropanedioic acid diethyl ester (8) : ^{1}H NMR δ: 4.18 (q,4H), 1.91 (q,2H), 1.39 (s,3H), 1.25 (t,6H), 0.87 (t,3H).

Methylpropylpropanedioic acid diethyl ester (9) : ^{1}H NMR δ: 4.18 (q,4H), 1.88-1.78 (m,2H), 1.39 (s,3H), 1.24 (t,6H), 0.90 (t,3H).

Butylmethylpropanedioic acid diethyl ester (10) : ^{1}H NMR δ: 4.18 (q,4H), 1.90-1.75 (m,2H), 1.39 (s,3H), 1.30-1.20 (m,10H), 0.90 (t,3H).

Methylpentylpropanedioic acid diethyl ester (11) : ^{1}H NMR δ: 4.15 (q,4H), 1.85-1.77 (m,2H), 1.36 (s,3H) 1.3-1.18 (m,12H), 0.84 (t,3H).

Methyloctylpropanedioic acid diethyl ester (12) : ^{1}H NMR δ: 4.15 (q,4H), 1.85-1.77 (m,2H), 1.38 (s,3H), 1.33-1.11 (m,20H), 0.84 (t,3H).

Benzylmethylpropanedioic acid diethyl ester (13) : ^{1}H NMR δ: 7.26-7.13 (m,5H), 4.20 (q,4H), 3.23 (s,2H), 1.34 (s,3H), 1.25 (t,6H). ^{13}C NMR δ: 171.9, 136.2, 130.2, 128.1, 126.8, 61.3, 54.8, 41.1, 19.7, 14.0.

DIALKYLATED PROPANEDIOIC ACID MONOESTERS

Incubations: Typical procedure : Reactions were carried out in 20 ml buffered batches containing DMSO (25%) with tris(hydroxymethyl)aminomethane (Tris-HCl, 0.375 M, pH 7.5), substrate (100-150 mg, 25 mM) and pig liver esterase (1-2 mg) or α-chymotrypsin (100-350 mg). Reaction temperature was kept at 22-25°C. The reactions were monitored by GC analyses of diethyl ether extracts of the reaction mixture in the presence of an internal standard (n-alkanes). After completion of the reaction (chymotrypsin 24-48 h, pig liver esterase 4-12 h) the mixture was acidified (HCl, 2 M) and the product was extracted with diethyl ether. Purification by back extraction ($NaHCO_3$), acidification (HCl, 2 M) and extraction yielded 90-98 % of the desired product.

Rate determination : The initial reaction velocities were determined in a Radiometer pH-stat under inert conditions (N_2) at 30 °C and maintained at pH 7.5 or pH 8.0 with NaOH (0.010 M). The reaction mixture contained dialkyl diester substrate (50 mM), DMSO (25%), $CaCl_2$ (0.075 M) and pig liver esterase (0.028 mg) or chymotrypsin (1.7 mg) in a total volume of 2.0 ml. One unit of enzyme activity was defined as the amount of enzyme catalysing the hydrolysis of 1 μmol of substrate per minute under the conditions described.

Ethylmethylpropanedioic acid monomethyl ester (14) : 73 % e.e. $[\alpha]_D$=-0.1° $[\alpha]_{365}$=-1.3° (c=1, $CHCl_3$). ^{1}H NMR δ: 9.62 (bs,1H), 3.76 (s,3H), 2.02-1.87 (q,2H), 1.44 (s,3H), 0.91 (t,3H). ^{13}C NMR δ: 177.6, 172.9, 52.6 (2C), 29.0, 19.6, 8.9.

Methylpropylpropanedioic acid monomethyl ester (15) : 52 % e.e. ^{1}H NMR δ: 3.76 (s,3H), 1.92-1.80 (m,2H), 1.46 (s,3H), 1.35-1.20 (m,2H), 0.94 (t,3H). ^{13}C NMR δ: 176.7, 173.5, 53.5, 52.7, 38.4, 20.4, 17.9, 14.2.

Butylmethylpropanedioic acid monomethyl ester (16) : 58 % e.e. ^{1}H NMR δ: 3.7 (s,3H), 1.95-1.85 (m,2H), 1.45 (s,3H), 1.4-1.2 (m,4H), 0.9 (t,3H). ^{13}C NMR δ: 177.7, 173.1, 53.7, 52.7, 35.7, 26.6, 22.9, 20.2, 13.8.

Methylpentylpropanedioic acid monomethyl ester (17) : 46 % e.e. ^{1}H NMR δ: 3.72 (s,3H), 1.82 (m,2H), 1.42 (s,3H), 1.3-1.2 (m,6H), 0.84 (t,3H). ^{13}C NMR δ: 177.8, 172.8, 53.4, 52.4, 35.6, 31.7, 23.8, 22.2, 19.9, 13.7.

Hexylmethylpropanedioic acid monomethyl ester (18) : 87 % e.e. $[\alpha]_D$=1.1° $[\alpha]_{365}$=3.5° (c=1, $CHCl_3$). ^{1}H NMR δ: 3.76 (s,3H), 1.8-1.95 (unres. m,2H) 1.45 (s,3H), 1.3-1,1 (unres. m,8H), 0.88 (t,3H). ^{13}C NMR δ: 177.4, 173.2, 53.7, 52.6, 36.0, 31.5, 29.4, 24.4, 22.5, 20.2, 14.0.

Heptylmethylpropanedioic acid monomethyl ester (19) : 88 % e.e. $[\alpha]_D$=1.3° $[\alpha]_{365}$=4° (c=1.2, $CHCl_3$). ^{1}H NMR δ: 3.76 (s,3H), 1.8-1.9 (unres. m,2H), 1.45 (s,3H), 1.3-1.1 (unres. m,10H), 0.88 (t,3H). ^{13}C NMR δ: 177.5, 173.1, 53.7, 52.6, 36.0, 31.7, 29.7, 29.0, 24.4, 22.6, 20.2, 14.0.

Benzylmethylpropanedioic acid monomethyl ester (20) : Pig liver esterase 16 % e.e.. Chymotrypsin >98 % e.e. $[\alpha]_D$=4.8° (c=1.86, $CHCl_3$) ^{1}H NMR δ: 7.3-7.1 (unres. m,5H), 3.77 (s,3H), 3.31 (d,J=13.6 Hz,1H), 3.21 (d,J=13.6 Hz,1H), 1.42 (s,3H). ^{13}C NMR δ: 177.3, 172.4, 135.7, 130.1, 128.3, 127.1, 54.9, 52.7, 41.5, 20.0.

Ethylmethylpropanedioic acid monoethyl ester (21) : 15 % e.e. ^{1}H NMR δ: 8.95 (bs,1H), 4.22 (q,2H), 1.93 (q,2H), 1.43 (s,3H), 1.24 (t,3H), 0.91 (t,3H).

Methylpropylpropanedioic acid monoethyl ester (22) : 10 % e.e. ^{1}H NMR δ: 10.7 (bs,1H), 4.22 (q,2H), 1.9-1.8 (m,2H), 1.45 (s,3H), 1.25-1.35 (unres. m,5H), 0.94 (t,3H). ^{13}C NMR δ: 175.7, 172.8, 61.6, 53.7, 38.2, 20.2, 17.8, 14.3, 14.0.

Butylmethylpropanedioic acid monoethyl ester (23) : 25 % e.e. ^{1}H NMR δ: 4.2 (q,2H), 1.95-1.8 (unres. m,2H), 1.4 (s,3H) 1.35-1.15 (unres. m,7H), 0.90 (t,3H).

Methylpentylpropanedioic acid monoethyl ester (24) : 10 % e.e. ^{1}H NMR δ: 4.2 (q,2H), 1.95-1.8 (unres. m,2H), 1.4 (s,3H) 1.35-1.15 (unres. m,9H), 0.90 (t,3H).

Methyloctylpropanedioic acid monoethyl ester (25) : 5 % e.e. ^{1}H NMR δ: 4.16 (q,2H), 1.8 (bs,2H), 1.38 (s,3H), 1.3-1.15 (unres. m,15H), 0.81 (t,3H).

Benzylmethylpropanedioic acid monoethyl ester (26) : Pig liver eterase 23 % e.e.. Chymotrypsin >98 % e.e. $[\alpha]_D$=5.4° (c=1.56, $CHCl_3$) ^{1}H NMR δ: 7.3-7.1 (unres. m,5H), 4.23 (q,2H), 3.31 (d,J=13.6 Hz,1H), 3.18 (d,J=13.6 Hz,1H), 1.45 (s,3H), 1.28 (t,3H). ^{13}C NMR δ: 177.1, 172.2, 135.8, 130.1, 128.3, 127.1, 61.9, 54.8, 41.5, 20.1, 13.9.

Determination of absolute configuration, (S)-α-methylphenylalanine (S-27)[12]: (R)-Benzylmethylpropanedioic acid monomethyl ester (R-20) (0.25 g, 1.12 mmol) was dissolved in water (0.25 ml) and acetone (0.5 ml) at 0°C. Triethylamine (0.14 g, 1.34 mmol) in acetone (2.5 ml) and then ethyl chloroformate (165 mg, 1.74 mmol) in acetone (1 ml) were added slowly. After 30 min at 0 °C sodium azide (165 mg, 1.74 mmol) in water (0.5 ml) was added dropwise. One hour later the mixture was poured into an excess of ice water and extracted with (Et_2O), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was dissolved in toluene (2 ml) and heated to 100°C until no more nitrogen was evolved. Removal of toluene under reduced pressure gave almost pure isocyanate by its IR spectra (2270 cm^{-1}). The isocyanate was suspended in aqueous HCl (20%) and heated under reflux for 3 h. The crude amino acid thus formed was purified by ion exchange chromatography (Dowex 50 WX 8) eluated with NH_4OH (1 M). This procedure yielded 170 mg (86 %) of S-27. Analytical sample was obtained by crystallisation from methanol. $[\alpha]_D$=-22° (c=1,H_2O) m.p. 308-309°C (dec.) ^{1}H NMR (D_2O) δ: 7.4-7.2 (unres m,5H), 3.26 (d,J=14.2 Hz,1H), 2.94 (d,J=14.2 Hz,1H), 1.52 (s,3H). ^{13}C NMR (D_2O) δ: 178.6, 136.8, 132.6, 131.5, 130.3, 64.7, 45.2, 24.9.

ACKNOWLEDGEMENTS

We thank the National Swedish Board for Technical Development (STU). "Axel och Margaret Ax:son Johnsons Stiftelse" is greatfully acknowledged for financial support to the chromatographic equipment.

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