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On the Preparation of Methyl and Ethyl (R)-(-)-3-Hydroxy-valerate by Depolymerization of a Mixed PHB/PHV Biopolymer

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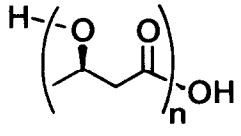
Abstract: *The microbial polyester containing 70-80% 3-hydroxy-butanoate and 20-30% 3-hydroxy-pentanoate (PHB/PHV, 2) is depolymerized to give monomeric esters of (R)-configuration. These are separated by fractional distillation. (R)-3-Hydroxy-pentanoate (4) is thus made readily available as enantiomerically pure starting material for syntheses.*

Besides glycogen, the polyester derived from (R)-3-hydroxybutyrate, PHB (1) is the second most ubiquitous microbial energy reserve material[2)]. If an essential nutrient, such as a nitrogen source is missing from the medium, while an excess of a carbon source such as fructose or glucose is supplied, many microorganisms will start polymerizing (R)-3-hydroxy-butyryl-CoA to PHB by a polymerase bound to the PHB *granulae*[3-5)]. There is a microorganism, *Alcaligenes eutrophus H 16*, which will store under appropriate conditions[6)] up to 80% of its dry



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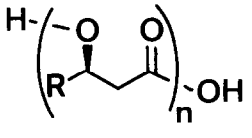
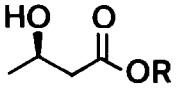
(PHB)



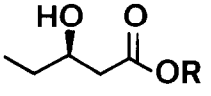
2: R = CH_3, with up to 30% R = C_2H_5

(PHB/PHV-copolymer)



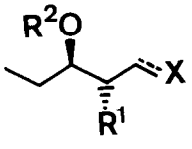
3a: R = CH_3

3b: R = C_2H_5



4a: R = CH_3

4b: R = C_2H_5



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weight as PHB. A laboratory procedure exists, by which PHB can be prepared on a 50 g scale in a 6 l flask[7]. Industrial scale biotechnological production is also possible, so that PHB is now commercially available[8]. Reductive and alcoholytic depolymerizations have been reported[7,9] which supply the valuable monomeric chiral building blocks (R)-butane-1,3-diol and (R)-3-hydroxy-butanoates (3) for syntheses[10]. The (S)-enantiomer of 3 is available by yeast reduction of acetoacetate under anaerobic or aerobic conditions[11].

In 1972, *L.L. Wallen* discovered a biopolymer which consisted of 3-hydroxy-butyrate and -valerate units[12]. In the meantime, it has become possible to produce the copolymer 2 using *Alcaligenes eutrophus NC IB* [13]. The microorganism is "forced" to incorporate the valerate into the polymer by feeding a mixture of propionic acid and glucose as carbon source while inducing the storage phase by omitting another essential nutrient. The mixed polymer 2 is also commercially available[8], so that it appeared feasible to prepare enantiomerically pure 3-hydroxy-valerates from it. We used the conditions[7] for depolymerization which we had previously applied to PHB, and obtained mixtures of 3-hydroxy-butyrate and -valerate which could be cleanly separated by fractional distillation through a *Fischer Spaltrohrkolonne HMS 500*[14] of 50 cm length and of *ca.* 90 plates. A spinning band column can also be used. At 12 Torr, the boiling point difference of the ethyl esters 3b and 4b is 10°C. The samples of 4 thus obtained have the highest specific rotations reported for this compound. It has (R)-configuration, by comparison with literature data[15].

With the following procedures, chiral non-racemic building blocks 5 with an ethyl-carbinol center of chirality become readily available[16]. Natural products containing this kind of center are: serricornine[17a], anhydro-serricornine[17a], cirramycin A_1[17b], narbomycin[17c], erythromycin B [17d], tetranactin [17e] and a pheromone of the smaller european elm bark beetle[17f].

Procedures

We had two samples of the copolymer 2 at our disposal[8], containing 22-23 mole % hydroxy-valerate units; one was a white powder, the other one a pale, glassy, rubberlike material.

Methyl (3R)-3-hydroxy-pentanoate (4a): A 4 L round-bottomed one neck flask containing a 7 cm magnetic stirring bar is charged with 134 g of dry, pure PHB/PHV biopolymer (white powder), corresponding to 1.17 mole PHB and 0.33 mole PHV. After addition of 1.1 L absolute

1.2 dichloro-ethane and of 1.1 L of a carefully prepared solution of 32 mL of concentrated H_2SO_4 in absolute methanol, with swirling, the flask is equipped with a reflux condenser with drying tube, the stirrer is started, and the mixture is heated to gentle reflux for 48 hours. A colorless solution is formed from the very beginning of heating. Three 5 L separatory funnels are charged with 440 mL half-saturated aq. NaCl, 320 mL sat. aq. $NaHCO_3$, and 320 ml sat. aq. NaCl. The cooled depo lymerization solution is added to the first funnel, and the organic layer formed after shaking is transferred to the second separatory funnel, from there into the third one, and finally into a flask containing dry magnesium sulfate. Three 1.1 L portions of dichloromethane are passed through the three funnels as described above for the original solution. The dried four solutions are combined and concentrated in a rotatory evaporator (water aspirator vacuum; 35°C). The residue is distilled (62-68°/11 Torr) to give 164.0 g (90%) of a mixture of the two esters. This mixture is subjected to fractional distillation through the above mentioned *Spaltrohrkolonne*[14] (reflux ratio 5:1, external dropping rate 1/sec.). At a constant pressure of 20 Torr, the following fractions were collected: 1.5 g (forerun) 62-73°C; 119.6 g (87% 3a) 73-74°C; 6.9 g (mixed fraction) 74-84°C; 24.0 g (55% 4a) 84°C; there was a residue of 3.8 g which gave another 3.2 g of 4a upon *Kugelrohr*distillation. Total yield of 4a: 27.2 g (62%), n_D^{20} = 1.4211, d_4^{23} = 1.025 g/cm^3, $[\alpha]_D^{23}$ = -18.8° (neat), -35.7° (c = 1, $CHCl_3$) [Lit. (*Hasegawa* et al.[15]): -16.3° (neat)], IR spectrum and elemental analysis as expected for 4a; ^{1}H-NMR ($CDCl_3$): 0.97 (*t*, 3 H, CH_3-CH_2), 1.37-1.67 (*m*, 2 H, CH_3-CH_2), 2.40-2.53 (*m*, 2 H, CH_2CO), 3.00 (*d*, *J* = 4.5, 1 H, OH, exchangeable with D_2O), 3.72 (*s*, 3 H, OCH_3), 3.82-4.05 (*m*, 1 H, O-CH). - The butanoate 3a had $[\alpha]_D^{23}$ = -47.3° (c = 1, $CHCl_3$).

Ethyl (3R)-3-hydroxy-pentanoate (4b): From 120 g 2 (rubber-like material, 1.03 mole PHB, 0.31 mole PHV content), in 1 l dichloroethane and 1 l CH_3OH/H_2SO_4, with 400 ml half-saturated NaCl, 300 ml sat. $NaHCO_3$, 300 ml sat. NaCl, 1 x 1 l and 2 x 0.5 l CH_2Cl_2 for the workup procedure, the yield of the distilled mixture of 3b and 4b was 151.6 g (85%), b.p. 65-80°/ 11 Torr. - Fractional distillation at 12 Torr, as above, gave 1.8 g (forerun) 66-69°C; 113.7 g (84% 3b) 69-70°C; 5.2 g (mixed fraction) 71-79°C; 24.1 g (of 4b) 80°C; from the residue of 4.0 g another 2.6 g of 4b were isolated as above. Total yield of 4b: 26.7 g (59%), n_D^{20} = 1.4259, d_4^{23} = 0.992 g/cm^3, $[\alpha]_D^{23}$ = -17.6° (neat), -34.6° (c = 5, $CHCl_3$) [Lit. (*Serck-Hanssen*[15]): -31.4°], IR spectrum and elemental analysis as expected for 4b; ^{1}H-NMR ($CDCl_3$): 0.97 (*t*, *J* = 7, 3 H, CH_3-CH_2-C), 1.27 (*t*, *J* = 7, 3 H, CH_3CH_2O), 1.43-1.72 (*m*, 2 H, CH_3-CH_2-C), 2.37-2.50 (*m*, 2 H, CH_2-CO), 3.03 (*d*, 1 H, OH), 3.77-4.10 (*m*, 1 H, O-CH), 4.15 (*q*, *J* = 7, 2 H, OCH_2-CH_3). - The butanoate 3b had $[\alpha]_D^{20}$ = -43.2° (c = 1, $CHCl_3$).

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