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Synthesis of α,β-unsaturated *(S)*-Cyanohydrins Using the Oxynitrilase from *Hevea brasiliensis*

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Abstract: α,β-unsaturated *(S)*-cyanohydrins derived from 2-propenal, 2-butenal, *(E)* and *(Z)*-2-hexenal and 2-hexynal are obtained in high enantiomeric purity (80-95% e.e.) by using an oxynitrilase isolated from the leaves of *Hevea brasiliensis*.

The preparation of enantiopure cyanohydrins has gained much attention in recent years, since they are valuable starting materials for various optically active compounds,[1] such as pharmaceuticals and agrochemicals.
Among the most efficient methods for the preparation of optically active cyanohydrins are the asymmetric chemical syntheses in the presence of chiral catalysts as well as by enzymes, using oxynitrilases, lipases and esterases. All these methods have been reviewed recently.[2,3]

A comparison of chemical and enzyme catalysed syntheses reveals that the latter is far more efficient in terms of enantiomeric purity of most of the products. Specifically, the enantioselective addition of HCN catalysed by oxynitrilases is finding greater importance.

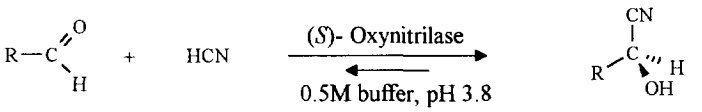
The literature on the resolution of cyanohydrin acetates by lipases and esterases reveals only one publication that deals with the preparation of enantiomerically enriched α,ß-unsaturated cyanohydrins. The enantiomeric excess reported was only moderate.[4] We have recently applied an oxynitrilase from *Hevea brasiliensis* for the synthesis of cyanohydrins.[5] This biocatalyst shows a broad applicability for the preparation of both aromatic and aliphatic (*S*)-cyanohydrins derived from aldehydes and ketones.[6]

In continuing our research on the application of this biocatalyst we wish to report on the synthesis of α,ß-unsaturated aliphatic cyanohydrins. These compounds are valuable synthetic intermediates. Some of them can be transformed into nonproteinogenic unsaturated ß,γ-D-amino acids (e.g. D-vinylglycine) which deserve great attention as part of compounds with biological activity.[7] They have been used as substrates for enzyme inhibition studies[8] and they are incorporated in semisynthetic antibacterials of the ß-lactam type.[9] Others have been used recently as starting materials for the synthesis of ß-blockers of the 1-(alkylamino)-3-(aryloxy)propan-2-ol type[10] and various 2,4-disubstituted 3-hydroxy butenolides (tetronic acids).[11] Furthermore, such α,ß-unsaturated cyanohydrins should serve as bifunctional building blocks due to the possession of both double and triple bonds. Transformations of the α-hydroxy nitrile functional moiety have been elaborated extensively.[1,2,12,13]

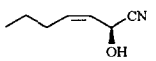
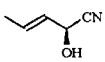
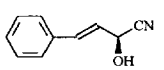
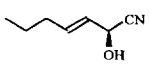
The enzyme catalysed synthesis of α,ß-unsaturated cyanohydrins has already been accomplished for the (*R*)-cyanohydrins derived from 2-propenal[14] and 2-butenal.[15] The preparation of the corresponding (*S*)-enantiomers was accomplished via conversion into the α-sulfonyloxy nitriles and subsequent nucleophilic substitution with various acetates occurs with inversion of configuration. In this case, extensive racemisation occurred with

benzylic substrates.[16] Another approach is the conversion of the chiral centre under Mitsunobu conditions.[17] Although this method is reported to proceed nearly without racemisation for benzylic and allylic substrates, this procedure affords several additional steps with concurrent loss of chemical yield.

The table lists cyanohydrins **1b - 6b** synthesised in high enantiomeric excess by application of the oxynitrilase from *Hevea brasiliensis.*[18]



1a - 6a **1b - 6b**

Compound No.	Yield(%)[19]	e.e.(%)[20]	Conf.[21]	$[\alpha]_D^{20}$ [22]	Compound No.	Yield(%)[19]	e.e.(%)[20]	Conf.[21]	$[\alpha]_D^{20}$ [22]
 **1b**	38/98	94	(*S*)[23]	+4.9 (0.64)	 **4b**	13/35	80	(*S*)[25]	+71.2 (0.8)
 **2b**	57/80	86	(*S*)[15]	+29.4 (0.56)	 **5b**	-/<5	-	-	-
 **3b**	17/46	95	(*S*)[24]	+20.3 (0.3)	 **6b**	43/88	80	(*S*)[25]	-20.4 (1.6)

Aldehydes **1a**, **2a**, **3a** and **5a** were commercially obtained. Aldehyde **4a**[26] and **6a**[27,28] as well as all racemic cyanohydrins for comparison purpose were prepared according to literature procedures.[29] The spectral data of known racemic compound **3b**[30] have not been reported and are therefore included in this paper.[31] Compounds **4b** and **6b** have not been reported in the literature up to now. Their spectral data are given in this paper.[32,33]

Procedure for the preparation of compounds (S) **-1b - 6b**

Crude enzyme preparation from *Hevea brasiliensis* (200 i.u. for compounds **1a** and **2a**, 400 i.u. for compounds **3a - 6a**) was suspended in 10 ml of 0.5 M aqueous sodium citrate buffer (pH 3.8). 2 mmoles of the distilled aldehyde was added and the mixture was chilled to 0° - 5° under stirring. 4 mmoles of KCN dissolved in 15 ml of cold 0.5M aqueous sodium citrate buffer, pH 3.8 was added within 20 minutes under continuous stirring. Cooling (0-5°C) was maintained throughout the reaction. The conversion to the products **1b - 6b** was monitored by GLC.[34] The aqueous phase was extracted three times with diethyl ether, the combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation. The crude products were purified by silica gel chromatography (eluent: petroleum ether/ethyl acetate 6:1 - 4:1). Evaporation of the solvent under reduced pressure afforded a colorless oil in all cases.

The high buffer concentration given above was necessary to keep pH 3.8 throughout the reaction, after all the potassium cyanide had been added. In addition to that, low temperature was applied to keep the concurrent

chemical reaction as low as possible. Using the above reaction conditions no conjugate addition of HCN to α,β -unsaturated aldehydes was observed.[35]

The highest reactivity was exhibited by aldehydes **1a** and **2a**. In case of **1a,** all the substrate had disappeared after a conversion time of 1 hour. Longer reaction time diminished the enantiomeric purity of the product, once the reaction came to equilibrium. When the chain length of the aldehyde was increased the lower rate of cyanohydrin formation had to be compensated by applying higher amount of enzyme (ca. 400 i.u./mmole aldehyde) as well as increased reaction time (3-4 hours). The transformation of the *(Z)*-olefinic aldehyde **4a** was accompanied by a 9% isomerisation to *(E)*-aldehyde **3a** during a reaction time of 4 hours. However, a slightly different ratio of the isomeric cyanohydrins was measured for **3b** and **4b** (5:95), resulting from the different rate of formation of the corresponding products. The isolated yields as given in the table are not optimised. The diminished yield of **1b** is mainly due to its high volatility,[36] resulting in loss of this product during the evaporation of the eluent after the purification on silica gel. In contrast to that, compounds **3b** and **4b** turned out to be unstable during purification on silica gel, resulting in reduced isolated yields (17% and 13% resp.) compared to conversion (46% and 35%) measured by GLC during the reaction. Interestingly, aldehyde **5a** was not sufficiently accepted by the enzyme and cyanohydrin **5b** could only be monitored in less than 5% yield by GLC. This aldehyde was recently reported not to be accepted by oxynitrilase from *Prunus amygdalis.*[15]

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18. For a procedure of the enzyme preparation see ref. 5.
19. Yield of isolated compound/yield determined by GLC before workup.
20. The enantiomeric purity of the corresponding cyanohydrin acetates was determined on a 30m x 0.25 mm permethyl-ß-cyclodextrin capillary column (Cyclodex B); nitrogen, carrier gas; 0.25 µm film thickness; For all compounds the elution order of enantiomers is (*R*) before (*S*).
21. The configuration of cyanohydrins **1b** and **2b** was assigned by comparison of their optical rotation values with literature reported data of the (*R*)-enantiomers (see ref. 15 and 23). The configuration of compounds **3b, 4b** and **6b** was not reported up to now. For the assignment see note 24 and 25.
22. Concentration in g/100ml chloroform.
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24. The configuration was established by comparison of the intensity of the ^{19}F NMR signals of the corresponding MPTA derivative with the MPTA ester of (*S*)-**2b**: ^{19}F NMR (282.4 MHz, $CDCl_3$): δ (ppm) = -71.98 (*S*)-enantiomer; -72.21 (*R*)-enantiomer; **3b**: -72.00 (*S*)-enantiomer; -72.22 (*R*)-enantiomer.
25. The configuration was assigned after catalytic hydrogenation to 2-hydroxyheptane nitrile (H_2/Pd-C, ethyl-acetate, 0.5 h); $[\alpha]_D^{20}$ of the (*S*)-enantiomer: -12.1 (c 1.4, chloroform; 84% e.e.); see note 5.
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31. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) = 6.04 (ddt, J = 15.4 Hz, 6.7 Hz, 1.1 Hz; 1H, olefinic H, C-atom 4). 5.58 (tdd, J = 15.4 Hz, 6.1 Hz, 1.4 Hz; 1H, olefinic H, C-atom 3); 4.92 (d, J = 6 Hz; 1H, CH-OH); 3.5 (br, s, 1H, OH); 2.1 (q, 2H, C-atom 5); 1.43 (m, 2H, C-atom 6); 0.91 (t, 3H, C-atom 7). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ (ppm) = 137.60 (C-3); 124.21 (C-4), 118.91 (CN); 61.77 (C-2); 34.03 (C-5); 21.85 (C-6); 13.65 (C-7).
32. ^1H NMR ($CDCl_3$): δ (ppm) = 0.93 (t, 3H, C-Atom 7); 1.45 (m, 2H, C-atom 6); 2.12 (m, 2H, C-atom 5); 2.65 (br, s, 1H, OH); 5.22 (d, J = 8.4 Hz; 1H, CH-OH); 5.60 (tdd, J = 10.6 Hz, 8.3 Hz, 1.5 Hz; 1H, olefinic H, C-atom 3); 5.81 (dt, J = 10.6 Hz, 7.4 Hz; 1H, olefinic H, C-atom 4). ^{13}C NMR ($CDCl_3$): δ (ppm) = 138.07 (C-3); 124.34 (C-4); 119.25 (CN); 57.42 (C-2); 30.04 (C-5); 22.45 (C-6); 13.82 (C-7).
33. ^1H NMR ($CDCl_3$): δ (ppm) = 5.19 (d, J = 1.6 Hz; 1H, CH-OH); 3.96 (br, s, 1H, OH); 2.24 (m, 2H, C-atom 5); 1.57 (m, 2H, C-atom 6); 1.0 (t, 3H, C-atom 7). ^{13}C NMR ($CDCl_3$): δ (ppm) = 116.81 (CN); 89.50 (C-3); 72.96 (C-4); 51.26 (C-2); 21.59 (C-4); 20.70 (C-5); 13.54 (C-6).
34. The cyanohydrins **1b - 6b** were derivatized to their corresponding acetates (Ac_2O, pyridine, DMAP cat.) prior to gaschromatographic analysis. The conditions were: 25m x 0.25µm DB-1701 fused silica capillary column, nitrogen, carrier gas.
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