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# Evaluation of guanabana (*Annona muricata*) seed meal as a source of (*S*)-oxynitrilase

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**Abstract**—Guanabana seed meal is a source of (*S*)-oxynitrilase which biocatalyzes the enantioselective addition of HCN to aromatic, heteroaromatic and α,β-unsaturated aldehydes to produce cyanohydrins.
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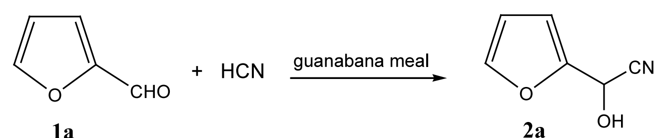
Nowadays the use of biocatalysts for the preparation of optically active compounds is increasing constantly, due to the advantages of biocatalysts over chemical chiral catalysts. It is well documented that enzymes accept a wide array of substrates, and catalyze enantio-, chemo- and regioselective reactions.[1,2] In the processes for the formation of carbon–carbon bonds, lyases are very attractive options and among them are the (*R*)- and (*S*)-oxynitrilases, that catalyze the addition of HCN to aldehydes or ketones to give (*R*)- or (*S*)-cyanohydrins respectively. Enzymatic procedures to obtain chiral cyanohydrins are very important, because these substances are very valuable starting materials for the synthesis of agrochemicals and drugs.[3–6]

We are currently engaged in the search for new and accessible sources of oxynitrilases,[7] and therefore we tested the capability of guanabana (*Annona muricata*) seed meal to biocatalyze the addition of HCN to 2-furfuraldehyde to produce the corresponding cyanohydrin.

It is well known that the enzymatic preparation of enantiopure cyanohydrins can be accompanied by the nonenzymatic formation of the unwanted racemic product. This disadvantage can be diminished using an aqueous–organic biphasic system, reducing the temperature and/or reducing the pH.[8,9] Since we were dealing with a new oxynitrilase source, we tested different reaction conditions for the biocatalyzed addition of HCN to 2-furfuraldehyde **1a** (Scheme 1). First, we worked at different pH values, the HCN generated in a KCN/citric acid buffer (pH 5.5, 5.0, 4.5, 4.0, 3.6) was extracted with isopropylether; second, the temperatures of the reaction were 4 and 25°C, the reaction mixture was stirred for 48 h, before the crude product was analyzed.

From the results in Table 1 it can be concluded that there is a relation between pH and the enantioselectiv-




**Scheme 1.**

**Table 1.** Enantiomeric excess conversion of the biocatalyzed addition of HCN to 2-furfuraldehyde

| Entry | pH | *T* (°C) | Conversion (%)[a] | Ee (%)[b] |
|---|---|---|---|---|
| 1 | 3.6 | 25 | 87 | 73 |
| 2 | 3.6 | 4 | 95 | 87 |
| 3 | 4.0 | 25 | 87 | 66 |
| 4 | 4.0 | 4 | 90 | 84 |
| 5 | 4.5 | 25 | 87 | 65 |
| 6 | 4.5 | 4 | 91 | 81 |
| 7 | 5.0 | 25 | 84 | 62 |
| 8 | 5.0 | 4 | 82 | 69 |
| 9 | 5.5 | 25 | 93 | 49 |
| 10 | 5.5 | 4 | 97 | 51 |

[a] Determined by GC as the naproxenate derivatives.[10]
[b] Determined by HPLC using Chiracel OD column.

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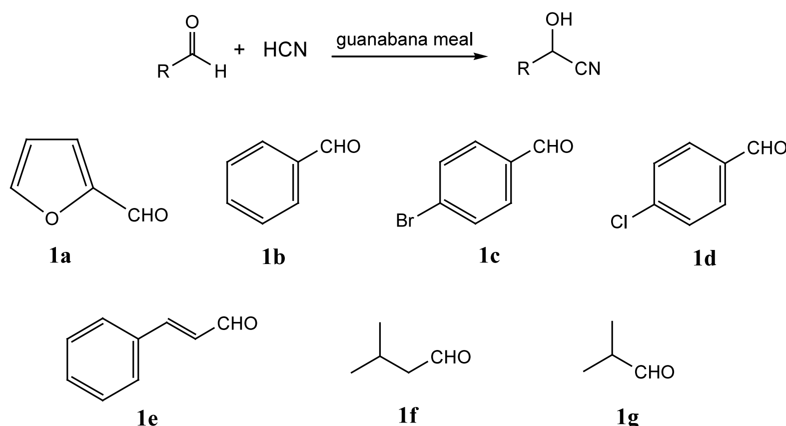


**Scheme 2.**

ity of the biocatalyzed reaction; at lower pH (3.6, 4.0, 4.5, entries 2, 4, 6) the enantiomeric excess is higher than 80%, whereas at pH 5.0 and 5.5 the enantiomeric excess was drastically reduced to 69 and 51% respectively (entries 8 and 10). The reaction temperature also played an important role over the enantioselectivity because at lower temperature the enantiomeric excess was higher, for example at pH 4.0 and 4°C (entry 4) the enantiomeric excess was 84%, in contrast to 66% at 25°C (entry 3).

Very interesting was the fact that the optical rotation of the cyanohydrin synthesized in this way was negative, meaning that the cyanohydrin of 2-furfuraldehyde prepared using defatted meal of guanabana has the (*R*)-configuration (assigned according Cahn–Ingold–Prelog) and the meal contains an (*S*)-oxynitrilase.[11]

Due to the fact that guanabana seeds are a new source of (*S*)-oxynitrilase, we decided to explore the scope and limitations of this biocatalytic material in the reaction of HCN addition to aromatic, heteroaromatic, α,β-unsaturated and aliphatic aldehydes (Scheme 2), at pH 4.0, 4°C.

From the results in Tables 1 and 2, it can be stated that the defatted meal of guanabana seeds biocatalyzed the addition of HCN to heteroaromatic **1a**, aromatic **1b–d** and α,β-unsaturated **1e** aldehydes, but did not work with aliphatic aldehydes **1f** and **1g**.

The best conversion and enantiomeric excess were obtained with the aldehyde **1a** (Table 1). The reaction with cinnamaldehyde **1e** proceeded in high enantiomeric excess (82%) but with low conversion; in the case of the reaction with aromatic aldehydes **1b–d**, the enantiomeric excess and conversions are not very high.

It is possible that the reaction conditions are adequate for the aldehyde **1a**, but not for aromatic or α,β-unsaturated aldehydes. All the cyanohydrins prepared in this way showed negative optical rotations, which corresponds to (*S*)-cyanohydrins, except for **2a** which configuration is '*R*'. In the reaction mixtures, only the corresponding cyanohydrin and the unreacted aldehyde were detected by $^{1}$H NMR.

From all these facts it can be stated that the defatted meal of guanabana seeds is a new source of (*S*)-oxynitrilase, a class of enzymes, which are much less common than (*R*)-oxynitrilases. The advantage of this source of (*S*)-oxynitrilase is that it is very cheap and accessible since guanabana can be purchased from local markets and the seeds are very abundant in the fruit. It is worth mentioning that the common sources of (*S*)-oxynitrilases, *Hevea brasiliens*[12] and *Manihot esculenta*,[13] have been overexpressed in several microorganisms in order to have sufficient amounts of the enzyme for synthetic applications.

## 1. Experimental

$^{1}$H NMR spectra were recorded on a Varian of 400 MHz instrument in $CDCl_3$ using tetramethylsilane as an internal reference. Optical rotations were measured using a Perkin Elmer 341 polarimeter and methylene chloride as solvent. Analysis of naproxenates was accomplished by gas chromatography on a Hewlett Packard 6890 series instrument using a HP-5 capillary column, and analysis of underivatized cyanohydrins by HPLC on a Hewlett Packard 1050 instrument, employing a Chiracel OD column with *n*-hexane/isopropanol mixtures.

**Table 2.** Cyanohydrins synthesized by the addition of HCN to aldehydes catalyzed by guanabana seed meal

| Product | Conversion (%)[a] | Ee (%)[b] |
|---|---|---|
| **2b** | 10 | 50 |
| **2c** | 24 | 46 |
| **2d** | 20 | 58 |
| **2e** | 11 | 82 |
| **2f** | nc | – |
| **2g** | nc | – |

[a] Determined by $^{1}$H NMR.
[b] Determined by HPLC using a Chiracel OD column.
nc, no conversion.

### 1.1. Guanabana seed meal

Guanabana fruit was purchased in local markets, the seeds from the fruit were peeled, then the meal was prepared by grinding the seeds and defatting three times with ethyl acetate, the meal was filtered, dried and stored at 4°C.

### 1.2. General procedure for the biocatalyzed addition of HCN to aldehydes

The HCN of an HCN/citric acid buffer solution (1N, 1.5 ml) was extracted with isopropyl ether (2×2.5 ml), then 0.02 ml of a citric acid/NaOH buffer (0.02 M) were added and the mixture was stirred for 10 min. Then the meal (200 mg) and the corresponding aldehyde (0.98 mmol) were added, the mixture was magnetically stirred at 4°C, 48 h for aldehyde **1a** and 144 h for the rest of the aldehydes. After that time, the reaction mixture was filtered, dried over sodium sulfate and the solvent evaporated under reduced pressure. The optical rotation of the mixture was determined as a methylene chloride solution, the enantiomeric excess was determined by HPLC using a Chiracel OD column, and the conversion percentage was determined by GC of the corresponding naproxenates or by $^{1}$H NMR.

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