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Application of crude preparations of leaves from food plants for the formation of cyanohydrins with high enantiomeric excesses

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Abstract—Crude preparations obtained from mamey (*Pouteria sapota*), capulin (*Prunus serotina* var. *capulli*) and peach (*Prunus persica*) leaves were used to catalyze the enantioselective addition of HCN to a variety of aldehydes. The corresponding cyanohydrins were obtained with high levels of enantioselectivity, comparable with those obtained with other catalysts used for the same purpose.

1. Introduction

The enzymes α-hydroxynitrile lyases (HNLs, also known as oxynitrilases) are involved in the cyanogenesis process of many plants. The release of HCN during cyanogenesis is a two-step process, which starts with cleavage of the sugar moiety from the cyanogenic glycoside triggered by the action of one or more β-glycosidases. In the second step, the resulting cyanohydrin, which is relatively unstable, undergoes further degradation either spontaneously or by the action of an α-hydroxynitrile lyase to produce HCN and the corresponding carbonyl compound.[1] As with many other catalysts, the enzymes are able to catalyze chemical reactions in both directions and therefore, α-hydroxynitrile lyases can be used either in the preparation of cyanohydrins from aldehydes and a suitable source of HCN[2] or for the reverse process. One potential practical inconvenience of this approach is the competition between the enzymatic reaction and the non-biocatalyzed addition of HCN to the carbonyl compound. It was only after Effenberger et al.[3] reported that the non-biocatalyzed competing reaction could be suppressed by the use of water–immiscible organic solvents, such as ethyl acetate, and after the cloning of the gene for the HNL of *Hevea brasiliensis* enzyme,[4] along with the seminal work by Griengl et al.[5] which describes the use of (*S*)-hydroxynitrile lyases to catalyze the formation of cyanohydrins from aliphatic, aromatic and heterocyclic aldehydes, that this biocatalytic approach became a practical synthetic methodology, which can provide cyanohydrins with high enantiomeric excess. Nowadays, the use of organic solvents as the reaction media for the addition of HCN to carbonyl compounds catalyzed by HNLs has become a fundamental tool for many investigations related to the preparation of enantiopure cyanohydrins.[6]

In recent years, several modifications to the original procedure introduced by Effennberger for the preparation of (*R*)- and (*S*)-cyanohydrins[3] have been developed. Among them, the possibility of using crude preparations obtained for example from almond,[7] apple[8] and mamey seeds[9] in the case of (*R*)-HNLs, and sorghum shoots[10] or crude preparation of guanabana seeds[11] for (*S*)-HNLs, instead of isolated enzymes is worth noting. Regarding the reaction medium, a biphasic system[12] can be used instead of pure organic solvents,[3] and the use of ionic liquids to conduct the reactions has been recently reported.[13] The use of several alternative sources of cyanide has been reported and in some cases, HCN can be prepared in situ from sodium cyanide and either acetic[7] or citric[14] acid buffers. In other cases, HCN was obtained from acetone cyanohydrin[15] or other racemic cyanohydrins[16] via a transcyanation reaction. Recently, Asano et al.[17] reported the screening of 163 species of plants as potential sources of HNLs and established that homogenates from *Baliosperman montanum* showed (*S*)-HNL activity, whereas leaves and seeds of *Passiflora edulis* and seeds from *Eriobotrya japanica*,

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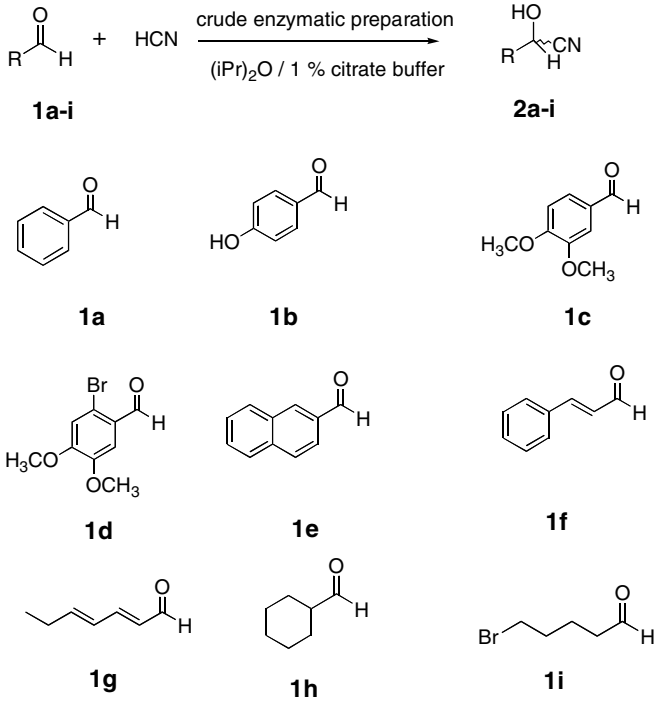
Chaenomles sinensis, *Sorbus aucuparia*, *Prunus mume* and *Prunus persica* displayed (*R*)-HNL activity. Several reviews dealing with the isolation and applications of HNLs in stereoselective organic synthesis have also been published in recent years.[2,18–20]

In a previous report from our group, we have reported some new sources of HNLs;[21] hereafter we report the results of testing the capability of crude enzymatic preparations of leaves from capulin, peach and mamey to catalyze the stereoselective addition of HCN to a broad range of aldehydes **1a**–**i** in order to prepare cyanohydrins **2a**–**i** (Scheme 1). Basically, our approach consisted of the extraction, with diisopropyl ether, of HCN generated in a solution of KCN and a citric acid buffer (pH 4.5). This solution was then added to the crude enzymatic preparation, followed by the addition of the corresponding aldehyde. Under these reaction conditions, the non-biocatalyzed addition of HCN to the aldehyde is almost completely suppressed in most cases, and the corresponding cyanohydrins are obtained in high enantiomeric excess.

2. Results and discussion

Crude enzymatic preparations of capulin (*Prunus serotina*), peach (*P. persica*) and mamey (*Pouteria sapota*) leaves were used as biocatalysts for the enantioselective addition of HCN to several aldehydes **1a**–**i**, to produce the corresponding (*R*)-cyanohydrins with high enantiomeric excesses (Scheme 1). Remarkably, this methodology is suitable for both aliphatic and aromatic substrates. According to the results shown in Table 1, all the crude enzymatic preparations biocatalyzed the transformation of aldehydes into



Scheme 1.

Table 1. Enantioselective cyanohydrin formation using crude preparations of leaves as biocatalysts

Aldehyde	Capulin		Peach		Mamey	
	%ee[a]	%Conv.[b]	%ee[a]	%Conv.[b]	%ee[a]	%Conv.[b]
1a	>99	95	90	90	90	92
1b	92	60	92	70	88	60
1c	82	34	82	36	80	29
1d	4	39	4	34	4	41
1e	98	71	>99	62	94	16
1f	96	40	94	41	96	40
1g	>99	62	>99	73	80	50
1h	38[c]	>99	32[c]	>99	40[c]	>99
1i	93	>99	94	>99	90	>99

[a] Determined by Chiral HPLC on a Chiracel OD column.
[b] Determined by ^{1}H NMR spectra.
[c] Determined by GC on a HP-5 capillary column using the naproxenate derivative.

cyanohydrins, under the conditions employed, although they were not optimized for each substrate.

For aldehyde **1a**, the best results came from capulin leaves (>99% ee and 95% of conversion), whereas peach and mamey leaves displayed similar results between them (90% ee, 90% and 92% of conversion, respectively). If we compare the results from Table 1 with those previously reported from our group for mandelonitrile **2a** (capulin and mamey seeds meal, 89% and 70% ee, and 92% and 76% conversions, respectively),[22] we can conclude that both are sources of HNLs with good catalytic capacity. It is important to emphasize that capulin fruits are seasonal, whereas the leaves are almost perennial, and consequently the use of leaves guarantees access to the biocatalysts material almost all year long.

In the case of the biotransformation of aldehyde **1b** into cyanohydrin **2b**, moderate conversions were achieved using the three biocatalysts (60–70%), with ee's ranging from 88% to 92%. These results are similar to those reported by Kanerva[8,10] using crude preparations of almond, apple and cherry seeds (57–66% conversion and 89–96% ee, respectively) in diisopropyl ether and a 0.1 M tartrate buffer (2% v/v), pH 4.5, after 66–71 h of reaction. The results suggest the existence of an electronic effect from the *p*-hydroxyl group in **1b**, with respect to the unsubstituted benzaldehyde **1a**, resulting in a lower enantioselectivity.

The reaction of **1c**, a more substituted and bulkier substrate, resulted in a big decrease in conversion (29–36%), although the enantioselectivity was still good (80–82% ee). These results are in sharp contrast with those reported by Kanerva,[8,10] who used almond, apple and cherry seeds meal (82–86% conversion and 93–94% ee) with reaction times between 66 and 71 h under the conditions mentioned earlier.

The introduction of a bulkier substituent (bromine) *ortho* to the reactive center, as in aldehyde **1d**, had a severe impact on the enantioselectivity of this process, resulting in an almost racemic product **2d**, even though the conversion was moderate (34–41%) for the three biocatalysts

(Table 1). These results might be optimized by using the reaction conditions reported by Sheldon[23] for the chemoenzymatic preparation of the cyanohydrin of 2-chlorobenzaldehyde.

According to Riva,[24] the use of purified HNL from *Prunus amygdalus* in diisopropyl ether and a citrate buffer (pH 5.5) gave 99% conversion and 95% ee of **2e**. The use of our three biocatalyst sources afforded similar results for enantioselectivity (98%, 100% and 94% ee for capulin, peach and mamey, respectively), but the extent of the reaction was lower using mamey preparation (only 16% conversion).

On the other hand, the reactivity of the α,β-unsaturated aldehyde **1f** towards HCN addition did not show significant differences among the three biocatalysts, with respect to conversion (40–41%) and enantioselectivity (96–94%). We previously reported 99% ee for **2f** using defatted mamey seed meal in diisopropyl ether and a citrate buffer, and found that the conversion was dependent on the concentration of the citrate buffer solution.[9] It might be possible to improve the conversion by modifying the concentration of the citrate buffer.

The addition of HCN to compound **1g**, another α,β-unsaturated aldehyde, showed excellent enantioselectivity (>99% ee) and moderate conversion (63% and 73%, respectively) with capulin and peach crude preparations, although for mamey the results were slightly lower (80% ee and 50% conversion). Previously, we reported a 79% ee and 51% conversion for cyanohydrin **2g** using defatted mamey seed meal in diisopropyl ether and citrate buffer (pH 5.0).[9]

For the bulkier aliphatic cyclohexanecarboxyaldehyde, **1h**, enantioselectivity for the reaction was low in all cases (38%, 32% and 40% ee), although the conversion to **2h** was excellent (>99%).

In the case of cyanohydrin from **1i**, the enantioselectivity for the three biocatalysts was similar (93%, 94% and 90% ee) with the same conversion (>99%). This result is important, because Gotor[16] reported 91% ee and 81% yield for the preparation of cyanohydrin **2i** using an almond crude extract as the biocatalyst in diisopropyl ether and (±)2-hydroxy-2-methylhexanonitrile as cyanide source in a transcyanation reaction, whereas our procedure consisted of a direct hydrocyanation process.

3. Conclusion

We have demonstrated that crude preparations from capulin, peach and mamey leaves are excellent and efficient sources of HNLs to prepare cyanohydrins with high levels of enantioselectivities. This methodology represents an inexpensive and reliable alternative, since the leaves are available almost all year round. Remarkably, this procedure can be used for preparative purposes with both aliphatic and aromatic substrates. In addition to the broad substrate scope, an additional advantage is the simplicity of manipulation and storage of the biocatalytic material.

4. Experimental

Reagents and solvents were purchased from either Baker or Aldrich and used without any further purification. ^{1}H NMR spectra were recorded on a 400 MHz Varian instrument in $CDCl_3$, using tetramethylsilane (TMS) as an internal reference. Enantiomeric excesses were determined using a Chiracel OD column (eluent: *n*-hexane/isopropanol mixtures) in a Hewlett–Packard 1050 instrument, equipped with a diode array detector by the analysis of underivatized cyanohydrins **2a**, **2c**–**g**; the diacetylated derivative of cyanohydrin **2b** and the naproxenate[25] of cyanohydrin **2i**. Cyanohydrin **2h** was derivatized as the corresponding naproxenate and the enantiomeric excess was determined by gas chromatography in a Hewlett–Packard 6890 instrument using a HP-5 capillary column.

4.1. Preparation of the crude enzymatic materials

Young leaves of mamey, capulin and peach were collected from the corresponding garden tree. In order to remove water, greasy material and some pigments that could interfere with the biotransformation, the leaves were blended sequentially three times with enough acetone to completely cover the material. After filtering and discarding the solvent each time, the resulting solid was air-dried in a fumehood and stored in tight closed jars at 5 °C until use.

4.2. General procedure for the biotransformation of aldehydes 1a–i into cyanohydrins 2a–i

In a typical experiment, 1.5 mL of a 1.0 M buffer solution of KCN/citric acid (pH 4.5) was extracted three times with diisopropyl ether (1.5 mL each time). The organic extracts containing HCN were combined and added to a vessel containing the crude enzymatic preparation (150 mg). Then 45 μL of citrate buffer (0.1 M, pH 4.5) was added, followed by the addition of aldehyde **1a**–**i** (0.1–0.18 mmol). The resulting mixture was magnetically stirred at room temperature for 24 h, followed by the addition of anhydrous Na_2SO_4 to the reaction and filtration; the filtrate was then evaporated under reduced pressure. The conversion percentage was measured by the ^{1}H NMR of the crude product and the enantiomeric excess of the underivatized cyanohydrins **2a** and **2c**–**g**, the diacetylated cyanohydrin **2b** and naproxenate derivative[25] of cyanohydrin **2i** were measured by HPLC. The cyanohydrin obtained from aldehyde **1h** was derivatized with (*S*)-naproxen chloride[25] and further analyzed by GC. Racemic cyanohydrins were prepared from the corresponding aldehydes according to a reported procedure, all of which showed spectral data according to literature reports, and were used as references during measurements.[26,27]

Naproxenate of **2i**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (m, 3H), 7.35 (m, 1H), 7.13 (m, 2H), 5.33 (t, 1H), 3.91 (m, 3H), 3.41 (m, 2H), 1.75 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 18.0, 18.2, 31.3, 31.5, 32.3, 32.6, 44.9, 45.0, 60.6, 60.9, 119.0, 119.1, 125.5, 125.8, 126.0, 127.2, 129.0, 133.5, 134.1.

Cyanohydrin **2i**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.26 (d, 1H, H-2), 1.79 (m, 3H), 1.19 (m, 2H).

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