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# Analytical and preparative separation of the enantiomers of pyrazole phenyl ether herbicides on three chiral stationary phases

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ABSTRACT

Chiral pyrazole phenyl ethers (PPE) are highly active herbicides which are resolved by direct high-performance liquid chromatography on commercially available chiral stationary phases derived from N-3,5-dinitrobenzoyl derivatives of $\alpha$-amino acids or amines (Whelk-O 1). The chromatographic data for a series of PPEs is presented and the structure–enantioselectivity comparisions are made for three different chiral stationary phases. Chromatographic resolution obtained is suitable for determination of enantiomeric purities and, in some cases, for preparative resolution of the enantiomers. By collection of material from repetitive chromatographic runs using an automated preparative system, suitable quantities for whole plant biological evaluation of both enantiomers of 2-[5-[[4-chloro-1-methyl-5-(trifluoromethyl)-1H-pyrazol-3-yl]oxy]-2-nitrophenoxy]-N-methyl-butanamide were obtained in greater than 99% enantiomeric purity.

## INTRODUCTION

The pyrazole phenyl ether (PPE) herbicides, developed from unique, novel chemistry, are characterized by high unit activity and selectivity for a variety of crops [1,2]. A number of chiral candidates have advanced from greenhouse to field trials, including 2-[5-[[4-chloro-1-methyl-5-(trifluoromethyl)-1H-pyrazol-3-yl]oxy]-2-nitrophenoxy]-N-methyl-butanamide (PPE **2a**) which is highly effective for control of broadleaf weeds in cereal crops. This class of compounds, which cause rapid peroxidative desiccation of plant tissues, has been found to inhibit protoporphyrinogen oxidase (PROTOX) and the enantiomers of some of the candidates have been found to differ in herbicidal activity [3,4]. Differences in the activity of enantiomers of other PROTOX inhibitors have been reported in which the enantiomers were prepared by synthesis from chiral precursors [5]. Recently, the enantiomers of N-phenylimide herbicide S-23121, which also inhibit PROTOX, have been resolved chromatographically on a cellulose-based CSP (chiral stationary phase) to afford 2 mg of each enantiomer and the activity investigated by *in vitro* studies [6]. Although not PROTOX inhibitors, the 2-aryloxypropionate herbicides have common structural features with some of the PPEs (both can be prepared from lactic acid derivatives) and have been analytically resolved on a variety of CSPs [7–9].

The differences in herbicidal activity of enantiomers of 2-aryloxypropionic acid derivatives is well documented [10] and has recently led to the commercialization of enantiomerically pure or enriched products. Presumably, the advantages of improved unit activity led to ICI Americas' registration of the *R*- enantiomer of butyl 2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]-phenoxy]propanoate (Fusilade 2000) as an improved version of the original racemic product [11]. It is also recognized that the inactive or less active enantiomer can in some instances have

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undesirable effects and at the very least creates an unnecessary environmental burden on the treated system. Environmental pressures, as well as cost advantages, have led to the production and marketing of the single active *R*( + ) enantiomers of 2-(4-dichloro-2-methylphenoxy)-propanoic acid (CMPP) and 2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP) in Europe [7].

In this report, we have found that the enantiomers of lead PPE herbicide candidates and many of their analogs are separable by direct high-performance liquid chromatography on commercially available chiral stationary phases derived from either the N-3,5-dinitrobenzoyl derivatives of $\alpha$-amino acids [12] or the newly devised amine based Whelk-O 1 CSP [13]. These CSPs have allowed us to achieve preparative chromatographic resolution of greater than 400 mg of chiral herbicide **2a** using an automated preparative system for repetitive injection and collection of the enantiomers. The availability of pure quantites of each enantiomer allowed us to determine reliably the differences in whole plant activity as well as to provide materials for further biochemical studies.

## EXPERIMENTAL

### *General*

Analytical and preparative liquid chromatography was performed using Rainin (Woburn, MA, USA) HPX pumps, a Rheodyne 7125 injector equipped with a 20-$\mu$l sample loop for analytical injections or Rainin HPX pump for preparative injections, and either a Waters 481 or a Knauer (Bad Homburg, Germany) variable wavelength UV detector operated at 254 nm. Chromatography columns employed for analytical separations (4.6 mm I.D. × 250 mm) were obtained from Regis Chemical Co. (Morton Grove, IL, USA) and contained one of three CSPs. Two CSPs were derived from the N-3,5-dinitrobenzoyl derivatives of D-phenylglycine (CSP I, Regis #731021) and L-leucine (CSP II, Regis #731041), respectively, covalently bound to aminopropyl silanized silica. The third CSP was (*S*,*S*)-WHELK-O 1 (CSP III, Regis #786101). Mobile phases used were mixtures of HPLC grade 2-propanol and *n*-hexane. For all analytical runs, a flow rate of 2 ml/min was employed.

All melting points were recorded on a Thomas–Hoover or Mettler FP62 capillary melting point apparatus and are uncorrected. $^1$H NMR spectra were obtained on a Bruker 360 MHz, Varian EM-360, Varian XL-400, or IBM-300. Proton resonances are reported relative to the internal tetramethylsilane in $\delta$ (ppm). Elemental analysis were preformed by Atlantic Microlabs, Inc. (Norcross, GA, USA). Optical Rotations were obtained using a Rudolph (Flanders, NJ, USA) Autopol III polarimeter. Racemic PPEs, **2a–k**, and enantiomerically enriched samples of **2b**, **2e**, and **2g** (see Fig. 1) were obtained as previously described [2,4].

### *Chromatographic preparative resolution of ( ± )-2a*

The preparative chromatographic resolution of racemic ( ± )-**2a** was achieved using a semi-preparative (10 mm I.D. × 250 mm) column containing CSP II (Regis #731241). The mobile phase consisted of 2% 2-propanol–hexane and a 10 ml/min flow rate was employed. A Rainin HPX pump was used for injections and a Macintosh based controller afforded programmed or automated injections for multiple runs. Collection of the chromatographic fractions was achieved with a Gilson Model 201 fraction collector. The system was programmed using Rainin MACRABBIT software to allow automated injection of the racemate and collection of the enantiomerically pure fractions for multiple runs without operator intervention. Using this system, twenty consecutive runs consisting of an injection of 4 mg racemic **2a** per run were carried out in a 16-h time period. This process was repeated six times giving a total of 120 runs and

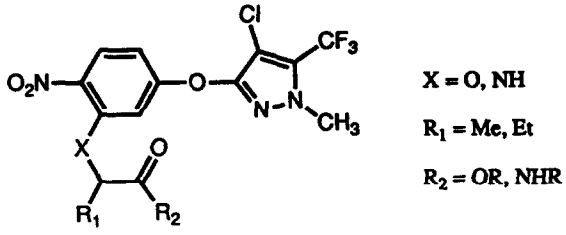


Fig. 1. PPE herbicides **2a–k**.

using over 48 l of mobile phase to afford, based on HPLC analysis, enantiomerically pure (*R*)-**2a** and (*S*)-**2a**. The R-(+) enantiomer of **2a** was eluted first (lower *k'*) on CSP II.

*(R)-2-[5-[[4-chloro-1-methyl-5-(trifluoromethyl)-1H-pyrazol-3-yl]oxy]-2-nitrophenoxy]-N-methyl-butanamide, (+)-2a.* Concentration of the combined collected chromatographic fractions of the first eluted peak (*k'*, 8.28) from 120 runs afforded 0.200 g (83%) of (*R*)-**2a** as a white solid: m.p. 140–141°C; $[\alpha]_D^{24}$ + 120° (*c* = 0.078, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 0.99 (t, 3H, *J* = 7.3 Hz), 2.08 (m, 2H), 2.88 (d, 3 H, *J* = 4.9 Hz), 3.96 (s, 3 H), 4.79 (t, 1 H, *J* = 5 Hz), 6.77 (dd, 1 H, *J* = 2.4, 9.2 Hz), 6.85 (d, 1H, *J* = 2.45 Hz), 7.20 (bs, 1H), 8.02 (d, 1H, *J* = 9.1 Hz); $^{19}F$ NMR ($CDCl_3$) $\delta$ − 61.1. Anal. Calc. for $C_{16}$ $H_{16}$ $N_4$ $O_5$ $F_3$ $Cl_1$: C, 44.0; H, 3.69; N, 12.83; found: C, 44.11; H, 3.70; N, 12.77.

*(S)-2-[5-[[4-chloro-1-methyl-5-(trifluoromethyl)-1H-pyrazol-3-yl]oxy]-2-nitrophenoxy]-N-methyl-butanamide, (−)-2a.* Concentration of the collected chromatographic fractions from the second eluted peak (*k'*, 9.43) afforded 0.195 g (81%) of (*S*)-**2a** as a white solid: m.p. 140–141°C; $[\alpha]_D^{24}$ − 130° (*c* = 0.15, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 1.00 (t, 3H, *J* = 7.4 Hz), 2.09 (m, 2H), 2.88 (d, 3 H, *J* = 4.9 Hz), 3.96 (s, 3 H), 4.79 (t, 1 H, *J* = 5 Hz), 6.79 (dd, 1 H, *J* = 2.4, 9.2 Hz), 6.85 (d, 1H, *J* = 2.50 Hz), 7.20 (bs, 1H), 8.02 (d, 1H, *J* = 9.2 Hz); $^{19}F$ NMR ($CDCl_3$) $\delta$ − 61.1. Anal. Calc. for $C_{16}$ $H_{16}$ $N_4$ $O_5$ $F_3$ $Cl_1$: C, 44.0; H, 3.69; N, 12.83; found: C, 44.10; H, 3.70; N, 12.84.

## RESULTS AND DISCUSSION

Two commercially available CSPs, derived from (*R*)-N-3,5-dinitrophenylglycine (CSP I) and (*S*)-N-3,5-dinitrophenylleucine (CSP II), have been shown to separate the enantiomers of compounds of diverse structural types [14]. Recent reports include resolutions of derivatives of α-hydroxycarbonyl compounds [15] and the herbicide Fluazifop-butyl [9] which are structurally similar to chiral PPEs. The third stationary phase investigated in these studies, CSP III, was developed specifically for the resolution of α-arylpropionic acid derivatives such as Naproxen [13]. We applied the HPLC-CSP technique to the direct separation of chiral PPEs **2a–k** using these three CSPs and found that for many of these compounds we could easily obtain baseline resolutions (Table 1). In a majority of the cases, the separations obtained allowed direct determination of enantiomeric purity. Analysis of an enantiomerically enriched sample of (*S*)-(−)-**2b**, which had been prepared by synthesis, afforded baseline separation and facile determination of an 88:12 mixture (76% e.e.) of the *S* and *R* enantiomers (Fig. 2).

All three of the CSPs were effective for the separation of most of the PPE herbicides **2a–k**, however, comparative enantioselectivities on the three phases can differ for individual compounds (Table I). The greatest differences between selectivity of the CSPs was observed for α-aryloxybutanamide, **2a**, and the α-aryloxypropionamides, **2b–d**. The primary amide **2c** was resolved on all three CSPs with the greatest enantioselectivity for CSP II (α = 1.40). Secondary amides **2a** and **2b**, however, gave the best separations on CSP III and afforded practically no separation on CSP I. Tertiary amide **2d**, on the other hand, was baseline resolved on CSP I and gave no separation on CSP III. In view of the excellent resolvability of the primary and secondary amides **2a–c** on the newer CSP III, the lack of separation of tertiary amide **2d** is surprising. However, since only one tertiary amide was investigated, we can not determine if this trend is general. The ester analogs of these compounds, **2e–i**, were resolvable on all three CSPs with only marginal differences in separability. Two N-aryl aminoacid derivatives, **2j–k**, were also investigated and found to give the best results on CSP II. The aminoester **2k** was not resolved on either CSP I or III, but was easily resolved on CSP II (α = 1.19). Four enantiomerically enriched samples were used to determine the elution order of the enantiomers. Interestingly, a reversal of elution order is observed for both CSP II and III between the alkanamides (**2a** and **2b**) and the alkanoate esters (**2e** and **2g**). On both CSPs the *S* enantiomers of the amides are preferentially retained, whereas the *R* enantiomers of the esters are retained. The elution order of the alkanoate esters **2e** and **2g** on CSP I (*S* retained) is

TABLE I

CHROMATOGRAPHIC RESOLUTION OF PPE HERBICIDES ON THREE CHIRAL STATIONARY PHASES

Chromatographic data were obtained with an analytical column (250 × 4.6 mm I.D.) containing CSP I, II or III. Detection, 254 nm; flow rate, 2 ml/min; mobile phase, 2-propanol in hexanes. (*S*) and (*R*) indicate the absolute configuration of the preferentially retained enantiomer.

| Compound | $R_1$ | $R_2$ | X | CSP I | | CSP II | | CSP III | | Mobile phase (% 2-propanol) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $k'$ | $\alpha$ | $k'$ | $\alpha$ | $k'$ | $\alpha$ | |
| **2a** | Et | $NHCH_3$ | O | 7.6 | 1.04 | 3.3 | 1.15(*S*) | 5.2 | 1.32(*S*) | 10 |
| | | | | | | 8.5 | 1.17 | | | 2 |
| **2b** | $CH_3$ | $NHCH_3$ | O | 10.0 | NS [a] | 4.8 | 1.10(*S*) | 7.5 | 1.29(*S*) | 10 |
| | | | | | | 13.2 | 1.13 | | | 2 |
| **2c** | $CH_3$ | $NH_2$ | O | 5.2 | 1.12 | 9.0 | 1.40 | 8.0 | 1.22 | 10 |
| **2d** | $CH_3$ | $NMe_2$ | O | 4.2 | 1.09 | 7.7 | 1.06 | 11.2 | NS | 10 |
| **2e** | $CH_3$ | $OCH_3$ | O | 14.1 | 1.08(*S*) | 6.7 | 1.06(*R*) | | | 2 |
| | | | | | | | | 3.9 | 1.11(*R*) | 10 |
| **2f** | Et | OEt | O | 3.0 | 1.07 | 3.8 | 1.07 | 2.4 | 1.12 | 2 |
| **2g** | $CH_3$ | OEt | O | 6.25 | 1.10(*S*) | | | | | 1.0 |
| | | | | | | 5.1 | 1.08(*R*) | | | 2 |
| | | | | | | | | 3.2 | 1.08(*R*) | 10 |
| **2h** | $CH_3$ | $OCH_2CH_2OMe$ | O | 4.8 | 1.04 | 9.7 | 1.07 | | | 2 |
| | | | | | | | | 6.1 | 1.08 | 10 |
| **2i** | $CH_3$ | $OCH_2CH_2OEt$ | O | 5.2 | 1.05 | 7.0 | 1.06 | | | 2 |
| | | | | | | | | 4.2 | 1.10 | 10 |
| **2j** | $CH_3$ | $NHCH_3$ | NH | 14.4 | 1.06 | 9.1 | 1.07 | 15.1 | 1.04 | 10 |
| **2k** | $CH_3$ | $OCH_3$ | NH | 6.9 | NS | 3.2 | 1.10 | 5.4 | NS | 10 |
| | | | | | | 8.9 | 1.19 | | | 0.5 |

[a] NS indicates an $\alpha$ value of less than 1.04.

consistent with that of CSP II (*R* retained) since the absolute configurations of these CSPs differ and are *R* (*d*-phenylglycine) and *S* (*l*-leucine), respectively.

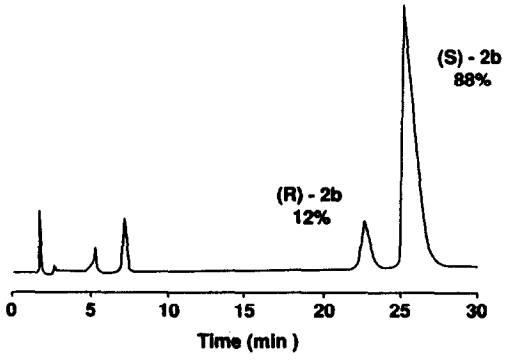


Fig. 2. Analytical separation and determination of the enantiomeric purity of an enriched sample of (*S*)-(−)-**2b** on CSP II. Chromatographic conditions are given in the Experimental section.

Enantiomerically enriched $\alpha$-aryloxypropionate herbicides, such as DPEs and pyridylphenyl ethers [16,17] have been prepared by synthesis from lactic acid precursors. Such synthetic strategies were employed for the preparation of PPE herbicides **2e** and **2g**, which, by derivitization to the secondary amide, also provided **2b**. While similar routes can be envisioned to synthetically prepare enantiomerically enriched samples of the $\alpha$-aryloxybutyrate PPEs **2a** and **2g**, lactic acid derivatives can not be employed as the "chiral pool" starting material and multistep synthetic sequences are required to obtain the desired enantiomerically pure butyrate precursors. By comparison, a direct chromatographic resolution of the available racemic (±)-**2a**, which was readily available, appeared more attractive. The primary drawback was the sample capacity of commercially available columns containing a suitable CSP. Although CSP III provided the

best analytical resloution of the racemate, a "semi-preparative" column (10 mm I.D. × 250 mm) containing an identical packing to the analytical column CSP II was chosen due to its availability within the timelines demanded for this project. The CSP II provided a nearly baseline separation of 4 mg of racemic (±)-**2a** (Fig. 3), while greater quantities in a single run led to loss of resolution and column overload. In order to meet the needs for biological testing, we required at least 100 mg of each enantiomer. Using an automation package for control of injection and collection of the eluent corresponding to the chromatographic peaks, 120 consecutive runs were carried out to resolve 480 mg of (±)-**2a** (Table II). Recovery of each enantiomer was in excess of 80% and the enantiomeric purities were greater than 99% (the HPLC detection limit for the minor isomers was approximately 0.5%). An advantage of the preparative chromatographic resolution of racemic mixtures is that one can obtain about equal quantities of both enantiomers in high purity which greatly facilitates comparisons of biological activity.

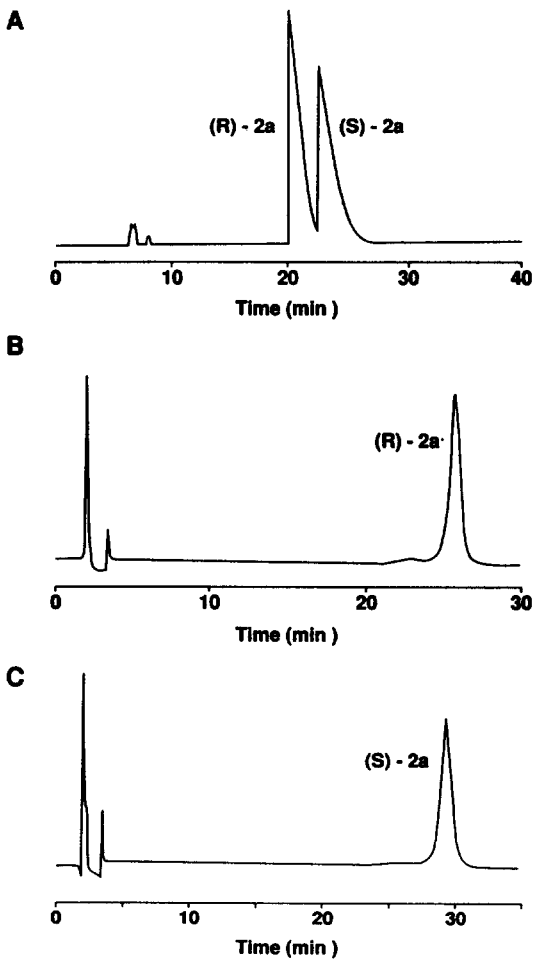


Fig. 3. Chromatographic resolution of the enantiomers of **2a** on CSP II. (A) preparative resolution of 4 mg of racemic **2a**; (B) and (C) analytical determination of enantiomeric purity of (*R*)- and (*S*)-**2a** obtained by collection of the first and second chromatographic peaks, respectively, from the preparative run. Chromatographic conditions are given in the Experimental section.

The absolute configuration of PPE (+)-**2b** has been previously assigned as *R* by synthesis from a lactic acid derivative of known configuration. For both **2a** and **2b**, we have found that the *R* enantiomer is eluted first on CSP II and III. The fact that both of these structurally unrelated CSPs exhibit the same elution orders for these pairs of analogs provides strong evidence of the absolute configuration of the enantiomers of **2a**. Thus, (+)-**2a**, which elutes first on CSP II and III, is assigned the *R* configuration by analogy with the enantiomers of the model compound **2b**. Such comparisons within sets of similar compounds have been made previously for 3,5-dinitrobenzoyl (DNB) derivatives of amines and alcohols on other CSPs [18]. The observed optical rotations of both (*R*)-**2a** and (*R*)-**2b** in chloroform exhibit positive specific rotations of + 120° and + 109°, respectively.

In summary, CSPs I–III afford baseline separations of a series of structurally related pyrazole phenyl ether herbicides. The separation factors

TABLE II

PREPARATIVE CHROMATOGRAPHIC RESOLUTION OF **2a**

| Compound [a] | Mass (mg) | %ee | [α] [b] |
|---|---|---|---|
| (*R*)-**2a** | 200 | > 98 | + 120 (*c* = 0.078, $CHCl_3$) |
| (*S*)-**2a** | 195 | > 98 | − 130 (*c* = 0.15, $CHCl_3$) |
| (*R*)-**2b** | - | > 95 | + 109 (*c* = 0.22, $CHCl_3$) |
| (*S*)-**2b** | - | > 95 | − 103 (*c* = 0.25, $CHCl_3$) |

[a] Compounds (*R*)- and (*S*)-**2b** were prepared by synthesis and recrystallized to obtain material of suitable enantiomeric purity.

[b] Dilute concentrations were employed for measurement of the optical rotation. Thus, the specific rotations are ± 10°.

and resolution of some of these PPEs can be large enough to allow for preparative resolutions. The power of this technique was demonstrated using an automated preparative system for resolution of **2a**, a chiral PPE which is not easily prepared by direct synthesis, in enantiomerically pure form. The availability of methods for obtaining enantiomerically pure compounds is important for complete evaluation of the biological activity of potential commercial candidates and can have impact on regulatory issues concerning stereoisomers, obtaining the most favorable patent positions and developing the best possible commercial products.

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