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Short Communication

Reversed-phase high-performance liquid chromatography of ketoxime analogues of β-adrenergic blockers

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ABSTRACT

Reversed-phase high-performance liquid chromatography of aryloxyalkylaminoketone-oximes is optimized by implementing bonded-phase (C_8 and C_{18}) column packings deactivated for basic compounds, and competing base as mobile phase additive. The retention of *Z* isomers is prolonged because of intramolecular hydrogen bonding. The selectivity coefficient for the separation of *E* and *Z* compounds is influenced by internal constraints and intramolecular interaction of polar functional groups.

INTRODUCTION

Several compounds with hydroxyimino functional group show biological activity. A number of ketoximes (Fig. 1) to be used as potential antiglaucoma agents have recently been synthesized [1,2] based on the structure of some known β-adrenergic blockers. Most oximes are, however, known to exist in alternative *Z* (*syn*) or *E* (*anti*) configuration, and there may be differences in the activity of the stereoisomers [3]. The two isomers may even tend to form a equilibrium mixture, either by metabolism or by acid-catalyzed isomerization, in biological media [4]. A proper analytical method should, therefore, be able to distinguish the oxime isomers, and also to determine the composition of isomeric mixtures.

Based on retention data of selected model compounds [5], which have revealed the dependence of capacity factors (k') on the orientation of the oxime hydroxyl group, reversed-phase high-performance liquid chromatography (RP-HPLC) appears to be a suitable technique. However, predictions on compounds which have not been

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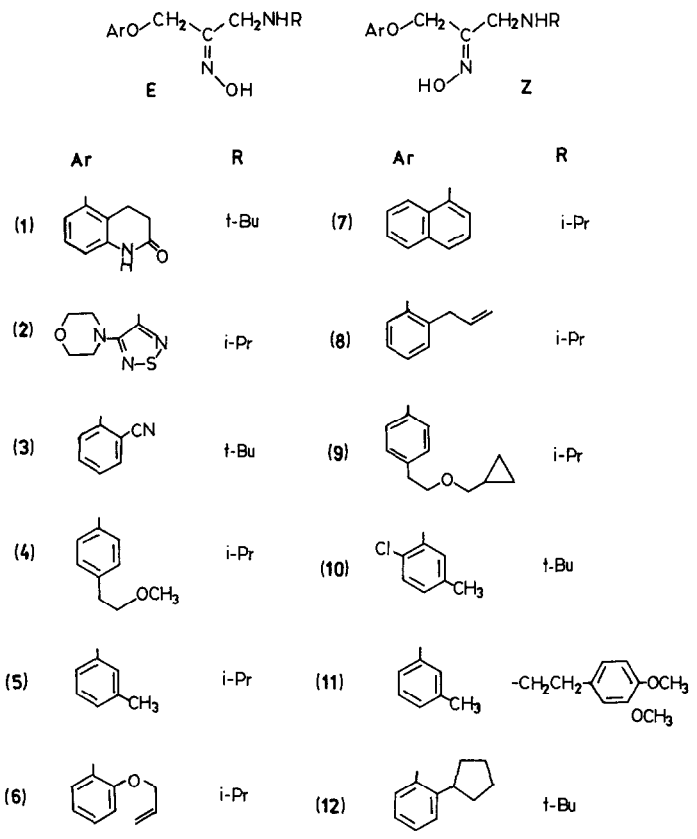

Fig. 1. Structure of the oximes. t-Bu = *tert.*-Butyl; i-Pr = isopropyl.

analyzed are rather difficult due to the existence of enumerable structural parameters that may affect the retention times. The elution order of *Z* and *E* isomers of oximes has not shown uniformity either [5], and there is no apparent explanation to this phenomenon. The evaluation of the retention behavior of aryloxyalkylamino-ketoximes involved in this study may also reveal the interaction of selected functional groups that can influence the separation of oximes in RP-HPLC.

EXPERIMENTAL

Chemicals and solvents

The preparation of oximes has been described previously [1,2]. HPLC-grade monobasic potassium phosphate, phosphoric acid (85%, w/w, aqueous solution), triethylamine and acetonitrile were supplied by Fisher Scientific (Fair Lawn, NJ, U.S.A.). Water was purified by reverse osmosis and ion exchange. Additional chemicals used throughout the studies were commercially available, analytical reagent-grade products.

High-performance liquid chromatography

The HPLC system consisted of Spectra-Physics (San Jose, CA, U.S.A.) SP 8810 precision isocratic pump, SP 8450 variable-wavelength UV–VIS detector and SP4290 computing integrator. Injections were made by a Rheodyne 7125 valve (Cotati, CA, U.S.A.) equipped with a 10-μl sample loop. A Spectroflow 430 (Kratos Analytical, Manchester, U.K.) low-pressure solvent mixer and gradient former was used to generate mobile phase compositions with variable organic modifier content.

We compared 5 cm × 4.6 mm I.D. Supelcosil LC-8-DB (octyldimethylsilyl bonded phase) and LC-18-DB (octadecyldimethylsilyl bonded phase) analytical columns with 5 μm particle-size packings in this study. The guard columns were 2-cm cartridges filled with 5 μm packing that contained the bonded phase identical to that of the analytical column. All columns and accessories were supplied by Supelco (Bellofonte, PA, U.S.A.).

The mobile phases applied for the separations were mixed from two components: 0.02 *M* monobasic phosphate buffer solution (pH was set to 3.0 with phoshoric acid) which also contained 100 μl/l triethylamine (final pH 3.15), and acetonitrile as organic modifier. The flow-rate was 1.0 or 1.5 ml/min.

RESULTS AND DISCUSSION

For selection of the analytical HPLC columns and appropriate separation conditions, one should consider certain inherent properties of the aryloxyalkylaminoketone-oximes (**1–12**) and their implications to reversed-phase chromatography. Upon attempting analyses on conventional bonded-phase (C_8 and C_{18}) columns, poor peak shapes (tailing) resulting in mostly inadequate, if any, separation of the oxime isomers were obtained [1]. This was due to the presence of the basic alkylamino functional groups, thus specifically deactivated (end-capped) column packings were applied. Additionally, a competing base (triethylamine) as mobile phase constituent was found to improve the peak shapes, although to a much lesser extent than the column deactivation. By taking these measures, even short (5 cm long, 5 μm packing) columns have provided efficient chromatographic performance. A value of 93000 theoretical plates/m has been obtained for the earlier eluting peak ($k' = 3.30$) of **7** under optimal conditions [6] (30% acetonitrile in the mobile phase), with resolution (R_s) reaching 2.33 and providing baseline separation of the oxime isomers.

Configurational analysis of isomeric aryloxyalkylaminoketone-oximes by ^1H and ^{13}C NMR spectrometry has been reported recently [2]. By using samples representing stereoisomerically pure samples (or significantly enriched in one of the oxime stereoisomers) characterized by the above techniques, the present studies have shown that the *E* isomers elute first in reversed-phase liquid chromatography. Intramolecular hydrogen-bonding between hydroximino group and the ethereal oxygen appears to provide an explanation to the prolonged retention of the *Z* isomer. This interaction probably represents a stronger bonding than the possible oxime-amine intramolecular association in the *E* isomer. The *Z* form also represents the thermodynamically more stable configuration of the compound [2].

Table I lists retention data and selectivity coefficients for C_8 and C_{18} bonded phases under identical chromatographic conditions. (Compound **12** represents an exception, since the elution of the isomers was not possible within reasonable time.)

TABLE I

CAPACITY FACTOR (k') AND SELECTIVITY COEFFICIENT (α) OF ARYLOXYALKYLAMINO-KETONE-OXIME ISOMERS

$k' = (t_R - t_0)/t_0$, where t_R = retention time and t_0 (determined by injection of sodium nitrate solution [7]) = dead time; $\alpha = k'(Z)/k'(E)$. Mobile phase: acetonitrile–aqueous buffer (20:80, v/v). See Experimental section for details.

Oxime	Octyldimethylsilyl (C_8) bonded phase			Octadecyldimethylsilyl (C_{18}) bonded phase		
	$k'(E)$	$k'(Z)$	α	$k'(E)$	$k'(Z)$	α
1	0.68	0.83	1.21	0.68	0.82	1.21
2	1.96	2.70	1.38	2.07	3.01	1.45
3	2.62	3.94	1.50	2.80	4.18	1.49
4	3.42	4.70	1.37	3.67	5.20	1.42
5	7.27	10.52	1.45	7.53	11.55	1.53
6	9.58	15.09	1.58	10.52	16.46	1.56
7	21.20	27.64	1.30	26.14	34.77	1.33
8	22.90	31.28	1.37	26.64	37.34	1.35
9	21.54	31.58	1.47	25.88	40.08	1.55
10	25.12	33.80	1.35	32.46	45.12	1.39
11	33.24	41.40	1.24	43.50	53.72	1.24
12[a]	(4.23)	(5.06)	(1.20)	(6.14)	(7.47)	(1.22)

[a] Eluted with acetonitrile–aqueous buffer (40:60, v/v).

This approach has allowed more relevant comparison of the retention characteristics than the one that applies different amounts of organic modifier to reach conditions preferred for real analyses [2]. Nevertheless, the order of lipophilicity from chromatographic data conforms to that predicted for the parent β-blockers [8] (aryloxyalkylaminoalcohols). The selectivity coefficient (α) varies only slightly, if at all, with the stationary phase for a given compound, but it is probably influenced by many structural features. Although one may observe a tendency that less polar aryloxyalkylaminoketone-oximes show less isomer separation, internal constraints [5] (steric factors) and intramolecular interaction (possible hydrogen bonding, dipole–dipole interactions, etc.) of polar functional groups with the oxime hydroxyl group should be responsible to the magnitude of α. Although the number of compounds involved in the study is limited, certain conclusions may be made. Bulky, non-polar substituents at *ortho* position of the aromatic moiety (**1**, **7**, **12**) will possibly decrease α. On the other hand, the separation of the oxime isomers is increased by the presence of polar functional groups (**3**, **6**) able to attract the oxime hydroxyl.

In conclusion, the retention behavior of aryloxyalkylaminoketone-oximes can be interpreted in terms of several intramolecular interactions, most notably hydrogen bonding between the functional groups, and steric effects. These interactions may affect the elution order of the *E* and *Z* isomers, and the selectivity coefficient. Continuing studies on a variety of other oximes are to reveal further structure–chromatographic retention relationships.

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