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Note

The retention times of oximes in reversed-phase high-performance liquid chromatography*

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As with gas-liquid chromatography¹, the most significant uses of high-performance liquid chromatography (HPLC) include the study of the retention behaviour of specific classes of compounds^{2,3} and the separation of isomeric compounds^{4,5}. During our study of the use of shift reagents and ¹³C nuclear magnetic resonance for the assignment of stereochemistry to oximes⁶, HPLC, as well as nuclear magnetic resonance, was used to examine the purity of the reaction products, as well as the composition of the reaction products containing isomeric mixtures of the oximes. Subsequently, a retention behaviour study on these oximes of widely varying structure was carried out and the results are reported here. The structures of the oximes are shown in Fig. 1. An analysis of the results, shown in Table I, indicates that the *k'* values are determined by the number of carbons present in the oxime substituents, the orientation of the oxime hydroxyl, and the presence of internal constraints. The dependence of the retention time on the orientation of the hydroxyl group makes reversed-phase HPLC a practical technique for the preparative separation of oxime isomers.

EXPERIMENTAL

A Waters Assoc. ALC/GPC 201 liquid chromatograph, equipped with a U6K injection system, a 6000A solvent delivery system, and an R401 differential refractometer was used. The retention times were measured and recorded with a Hewlett-Packard 3390A reporting integrator. A μ Bondapak C₁₈ column, 30 cm \times 3.9 mm I.D., particle size 10 μ m (Waters Assoc.) was used to obtain the data in Table I. The column was thermostated at 25°C by means of a metal block which was connected in series with the housing of the refractive index detector to a Haake model FE constant-temperature circulating water bath.

Appropriate volumes of HPLC-grade water and methanol were mixed to give solvents of the desired composition. The flow-rate in all cases in the table was 0.5 ml/min. Cyclohexanone oxime (8) was used as the internal standard for all samples. Samples were prepared by dissolving 2 mg of the internal standard and an accurately weighed amount of 12-18 mg of the oxime, whose retention time was to be deter-

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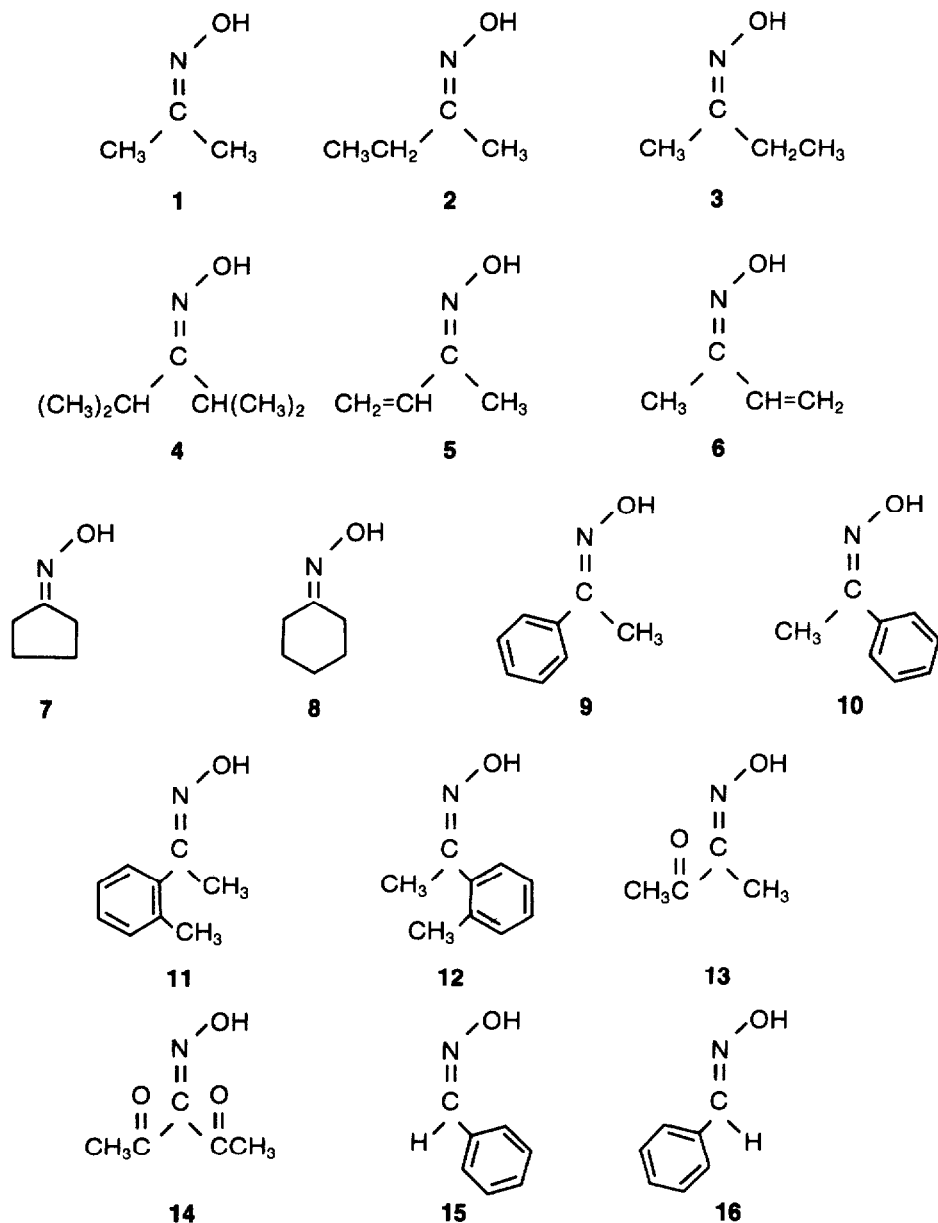


Fig. 1. Structures of the oximes.

mined, in one ml of the appropriate solvent. The injection volume was 15 μ l. The void volume was obtained by using injections of $^2\text{H}_2\text{O}$ ^{7,8}.

The oximes (1)–(10) were obtained as described previously⁶. The isomeric oxime pairs (2) and (3) and (5) and (6) were injected as mixtures (ratios of 76:24 and 85:15, respectively⁶). Oxime (13) was obtained commercially whereas (14) was synthesized from 2,4-pentanedione using the method of Wolff *et al.*⁹. The method of

Vogel¹⁰ was used to obtain (15) and (16). A mixture of (11) and (12) was obtained by the oximation of 2-methylacetophenone¹¹. The ratio of (11) to (12) was shown to be 67:33 as determined by a μ Bondapak C₁₈ column (30 cm \times 7.8 mm I.D.; solvent mixture: water-methanol, 50:50). Isomer (11) was obtained pure by recrystallization of the product mixture from light petroleum¹¹. A 50:50 mixture of (11) and (12) was obtained from the residues which could not be separated further by recrystallization, sublimation, or by column chromatography using silica gel (14-20 mesh). Oxime (12) (m.p. 78.3-78.8°C) was separated from its isomer using the μ Bondapak C₁₈ column (30 cm \times 7.8 mm I.D.; water-methanol, 50:50) and subsequent recrystallization from light petroleum (b.p. 35-60°C).

RESULTS AND DISCUSSION

There is no doubt that the factors affecting retention times in reverse-phase HPLC are complex^{12,13}. In this note, a qualitative approach is taken to explain the retention time behaviour. The retention times and k' values for the oximes, whose structure are shown in Fig. 1 are given in Table I for both 30:70 and 50:50 methanol-water.

Methanol-water (30:70)

The smallest k' value (0.39) is exhibited by (1) which has only two methyl groups attached to the oxime group. On the addition of a single methylene to one

TABLE I
THE CAPACITY FACTORS FOR THE OXIMES

Oxime	Methanol-water (30:70)*		Methanol-water (50:50)*		Ratio of k' values**
	t_R	k'	t_R	k'	
1	8.60	0.39	7.15	0.18	0.46
2	12.09	0.95	8.34	0.37	0.39
3	12.09	0.95	8.34	0.37	0.39
4	103.03	15.62	24.75	3.08	0.20
5	13.41	1.16	8.62	0.42	0.36
6	12.19	0.97	8.26	0.36	0.37
7	13.72	1.21	8.66	0.43	0.36
8	22.95	2.70	10.98	0.81	0.30
9	32.83	4.30	12.03	0.98	0.23
10	47.73	6.70	14.68	1.42	0.21
11	73.22	10.81	18.23	2.00	0.19
12	48.33	6.80	14.52	1.39	0.20
13	11.51	0.86	8.10	0.33	0.38
14	9.70	0.56	7.29	0.20	0.36
15	34.15	4.51	12.27	1.02	0.23
16	30.26	3.88	11.73	0.93	0.24

* t_0 Was determined from repeated injections of ²H₂O to be 6.07 and 6.20 for methanol-water 30:70 and 50:50, respectively. Values of k' were determined from $k' = (t_R - t_0)/t_0$.

** Ratios were obtained by dividing the k' values in methanol-water (50:50) by those in the methanol-water (30:70) composition.

substituent of (1), as in (2) and (3), the k' value increases in magnitude by 2.4 times to 0.95. This increase in k' with the addition of a saturated carbon is very similar to the 2.1–2.2-fold increase in k' found by Dufek¹⁴ for *n*-alkyl-phthalimides in methanol–water (40:60) for successive addition of saturated carbons. The effect of the orientation of the oxime hydroxyl in (2) and (3) is not large enough for separation to occur, although, when a larger column (30 cm × 7.8 mm I.D.) is used, partial separation is observed. On increasing the size of both substituents to isopropyl groups [as in (4)], the magnitude of k' increases to 15.62, the largest value in Table I. The fact that the retention times of these oximes behave predictably on the addition of saturated carbons, except when severe internal constraints are present (*vide infra*), is born out by a comparison of the k' value of (4) with that of (1). The increase in k' per saturated carbon going from (1) to (4) is 2.51, a value which is in the same range as that exhibited by other examples in this analysis.

It is interesting to note that the isomers (5) and (6) are cleanly separated in contrast to their saturated equivalents (2) and (3). The k' value of the *Z* isomer (6) ($k' = 0.97$) is only slightly larger than that of (2) and (3) ($k' = 0.95$) whereas that of the *E* isomer (5) is significantly higher ($k' = 1.16$).

Cyclopentanone oxime (7) has a k' value of 1.21, whereas the addition of a methylene group to the ring [cyclohexanone oxime (8)] gives a k' value (2.70) which is 2.2 times as large. In the aromatic isomeric oximes (9) and (10), the *E* isomer has a k' value of 4.30 whereas the *Z* isomer has a k' of 6.70. A comparison of the k' values of (9) and (10) with those of the isomeric pair (11) ($k' = 10.81$) and (12) ($k' = 6.80$), which have an additional methyl group in the *ortho* position of the benzene ring is informative. The *Z* isomers (10) and (12) have nearly identical k' values even though a saturated carbon has been added to (12), a fact which caused an increase of more than twice in the k' value between (1) and (2) and between (7) and (8). By contrast, the increase in k' from 4.30 for (9) to 10.81 for (11) is “normal” in the sense that k' increases by 2.5 times on the addition of a saturated carbon. Thus, the k' value of (12) is unusually low and the cause for this is undoubtedly a restriction on the orientation of the *o*-methylbenzene ring caused by the interaction of the *o*-methylbenzene and the oxime hydroxyl (models show a severe interaction) and the implications of this in terms of the interaction with the bonded phase and the solubility in the solvent. Fortier *et al.*¹⁵ have shown that steric factors in this type of oxime can cause large deviations in the dihedral angle between the plane of the aromatic ring and the plane of the oxime function.

It is also interesting to compare the k' values for (1), (2), (13), and (14) ($k' = 0.39, 0.95, 0.86,$ and 0.56 , respectively). Thus, the addition of a carbonyl group to (1) [to give (13)] causes the k' to increase to 0.86, a value which is not too different from when a saturated carbon is added to (1) [*i.e.* (2), $k' = 0.95$]. The addition of a carbonyl to (13) [*i.e.* (14), $k' = 0.56$] gives a significant decrease in k' instead of the expected increase. Again, models show severe constraints on the orientation of the groups attached to the oxime in (14).

The k' values for the isomeric pair (15) and (16) follow the trend set by the isomeric pair (9) and (10). Thus, the k' value for the *Z* isomer (15) decreases to 4.51 in comparison to that of the *Z* isomer (10), $k' = 6.70$. That of the *E*-isomer (16) goes to 3.88 from the value of 4.30 for *E* isomer (9). Thus, for these two pairs, the *E* isomers have the lower k' values. The decrease in retention time is considerably small-

er than would be expected on the removal of a saturated carbon and is undoubtedly due to the fact that the saturated carbon involved is attached directly to the oxime group.

The results for the four isomeric oxime pairs which exhibit different retention behaviour clearly indicate that HPLC retention times alone cannot be used to predict oxime stereochemistry since in two cases the *E* isomers elute first whereas the reverse is true for the other two. Except in the case of the isomers (2) and (3), however, the effect of the orientation of the oxime hydroxyl on retention time is sufficiently large to allow separation of the isomers by preparative techniques. In fact, as indicated in the experimental, the isomers (11) and (12) have been separated using a semi-preparative column.

Although, in general, the retention times of the oximes increase predictably with increasing molecular weight, there are significant exceptions as exemplified by the retention times of the oximes (12) and (14).

Methanol-water (50:50)

As expected¹⁶, the k' values in methanol-water (50:50) are only a fraction of the k' values at the 30:70 composition. Table I shows that the ratios of the k' values in the two solvents vary from 0.19 to 0.46 with the average for the sixteen oximes being 0.30. Similar values were found by Wang *et al.*¹⁶ for these two solvent compositions using different compounds. Interestingly, the ratios for the k' values between the two solvent compositions for isomeric oxime pairs are nearly identical in each case.

Methanol (100%)

In 100% methanol, the retention times of all of the oximes varied only slightly from the solvent front.

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