Journal of Chromatography, 356 (1986) 433–437
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 446

Note

Separation of 1,2,4-triazole derivatives by high-performance liquid chromatography

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(First received October 29th, 1985; revised manuscript received December 30th, 1985)

Many 1,2,4-triazole derivatives have been reported to exhibit various biological activities and some have been used in agriculture as fungicides as well as plant growth regulators[1–3]. Gas chromatography was generally utilized for the separation and determination of triazoles in mixtures of pesticides[4,5] or in the environment[6–9]. The separation of triadimefon and some of its metabolites using a C_{18} reversed-phase column[10], of isomers of triadimenol on silica[11] and analogues of S-3308[12] by high-performance liquid chromatography (HPLC) have also been reported.

In this paper, we describe conditions for the separation of sixteen triazoles of two groups (based on whether the triazole nitrogen is bonded to an sp^3- or sp^2-hybridized carbon) by HPLC on a normal-phase silica gel column using mobile phases consisting of hexane and ethyl acetate. The relationships between structure and retention and the separation of geometric isomers and diastereomers are discussed.

EXPERIMENTAL

Materials

All 1,2,4-triazole derivatives studied were synthesized, purified and identified in this laboratory, except for triadimefon (Janhu Agrochemical Works, Jiangsu Province, China) which was recrystallized. The names or code numbers and chemical structures of these compounds are listed in Table I. Hexane and ethyl acetate, AR grade (Beijing Chemical Works, China) were employed.

Apparatus and methods

The HPLC measurements were carried out at ambient temperature on a Perkin-Elmer Series-3 liquid chromatograph equipped with an LC-15 ultraviolet detector. The wavelength selected for all measurements was 254 nm (0.064 a.u.f.s.). The stainless-steel column (250 mm × 4.6 mm I.D.) was packed with LiChrosorb Si 60, particle size 5 μm.

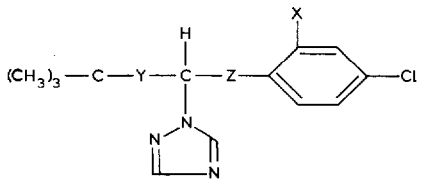
Individual compounds were dissolved in ethyl acetate at concentrations of approximately 0.1–2 mg/ml according to their detector response and mixtures were also prepared in ethyl acetate. The volumes of standard solution introduced onto the column were in the range of 3–8 μl.

Four mobile phases containing different volume ratios of hexane and ethyl

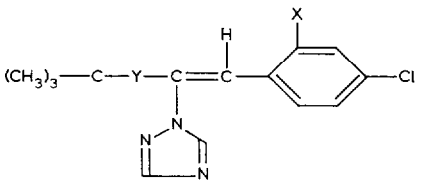
0021-9673/86/$03.50

TABLE I

COMPOUNDS STUDIED, THEIR NAMES OR CODE NUMBERS AND STRUCTURES



Group 1



Group 2

Compound	*Name or code number*	*Structure*				
		Group	*X*	*Y*	*Z*	*Configuration*
1	Triadimefon	1	H	CO	O	–
2	Triadimenol A	1	H	CHOH	O	*threo*
3	Triadimenol B	1	H	CHOH	O	*erythro*
4	Int. 1	1	H	CO	CH_2	–
5	Paclobutrazol	1	H	CHOH	CH_2	*threo*
6	Er-Pac	1	H	CHOH	CH_2	*erythro*
7	Int. 2	1	Cl	CO	CH_2	–
8	Diclobutrazol	1	Cl	CHOH	CH_2	*threo*
9	Int. 3	2	H	CO	–	*E*
10	Int. 3′	2	H	CO	–	*Z*
11	S-3307	2	H	CHOH	–	*E*
12	Z-SO7	2	H	CHOH	–	*Z*
13	Int. 4	2	Cl	CO	–	*E*
14	Int. 4′	2	Cl	CO	–	*Z*
15	S-3308	2	Cl	CHOH	–	*E*
16	Z-SO8	2	Cl	CHOH	–	*Z*

acetate were used with a constant flow-rate of 1.5 ml/min. The chart speed was kept at 5 mm/min.

RESULTS AND DISCUSSION

Working standards comprising individual 1,2,4-triazole derivatives were chromatographed to measure their retention time, t_R, under the conditions described. The capacity factor, k', for each compound was determined according to the equation $k' = (t_R - t_0)/t_0$, where t_0 (2.19 min) represents the solvent front. Table II gives the k' values obtained. The retention of the compounds increased with increasing percentage of hexane in the mobile phase. The dependences of k' on the polarity parameter[13], P', of the mobile phase for compounds 4, 5, 7, 9 and 13 are shown in Fig. 1. The retention order was consistent for most of the compounds tested with all the mobile phases used, while that for compounds 1, 11, 12 and 15 changed as the amount of hexane increased.

In liquid chromatography the retention order is chiefly based on the interaction of the solute with the stationary phase. As expected, the k' values were smaller for the ketone triazoles studied, 1, 4, 7, 9, 10 and 13 and 14, than for their alcohol

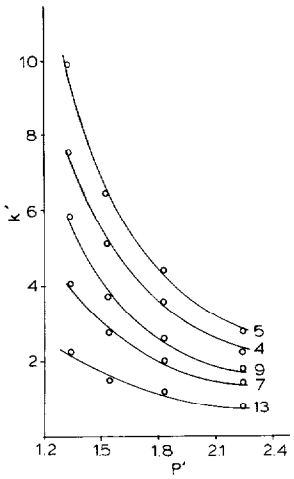


Fig. 1. Dependence of k' on the polarity of the mobile phase. P' = Polarity parameter[13] (hexane, 0.1; ethyl acetate, 4.4).

counterparts, 2 and 3, 5 and 6, 8, 11 and 12 and 15 and 16, which are more polar and more strongly attracted to the adsorbent surface. Compounds having two chlorine atoms on the phenyl ring have smaller k' values than their monochloro homologues, except that compound 12 (Z-4-Cl) was eluted a little earlier than compound 16 (Z-2,4-Cl_2). This may be due to the better match to the adsorbent surface for most

TABLE II

k' VALUES OF THE COMPOUNDS STUDIED

Compound	*Hexane–ethyl acetate volume ratio in mobile phase*			
	1:1	3:2	2:1	5:2
1	0.84	1.28	1.29	2.47
2	3.28	5.39	7.81	12.76
3	4.82	7.80	11.42	18.48
4	2.30	3.55	5.01	7.51
5	2.81	4.38	6.31	9.82
6	3.60	5.64	8.21	12.80
7	1.36	1.97	2.73	4.00
8	2.49	3.90	5.39	7.88
9	1.72	2.83	3.77	5.81
10	0.60	1.00	1.35	1.91
11	2.48	4.70	6.57	11.03
12	1.24	2.27	3.26	5.19
13	0.76	1.09	1.46	2.11
14	0.53	0.93	1.16	1.69
15	2.09	3.81	5.34	8.80
16	1.34	2.43	3.47	5.57

monochloro compounds than for their polychloro counterparts. For vinyltriazoles (group 2), the k' values are lower than those of the corresponding saturated ones (group 1), except for compound 15 which has a greater k' value than that of its saturated analogue 8 when eluted at higher percentages of hexane in the mobile phase. It is difficult to explain these observations; similar results were reported, *e.g.*, in the separation of aflatoxins[14]. All the vinyltriazoles studied have geometric isomers. Under the conditions proposed, each pair of E- and Z-isomers was separated adequately from each other. As a general rule, the k' value for the Z-isomer is lower than that of the E-isomer. The *trans* molecule is considered to provide a better match to the surface of the stationary phase. Funaki *et al.*[12] reported a similar phenomenon for S-3308 analogues.

Compounds 1, 4, 7, 11, 12, 15 and 16 each has an asymmetric carbon, while the reduction of C=O to CHOH in compound 1, 4 or 7 results in a further asymmetric carbon. Generally, enantiomers cannot be resolved by the usual chromatographic techniques, but under the conditions proposed, diastereoisomers were adequately separated. The *erythro* isomer 3 or 6 has a greater k' value than that of the *threo* isomer 2 or 5. The higher steric repulsion in the *erythro* isomers between the phenyl group and bulky *tert*-butyl group appears to cause different changes in lipophilicity.

Fig. 2 represents the separation of a mixture containing all sixteen 1, 2, 4-triazoles listed in Table I using hexane–ethyl acetate (3:2) as the mobile phase. Under these conditions, compounds 8 and 15 were co-eluted. Several early eluted compounds were not adequately separated, but most of the compounds were clearly separated from their isomeric counterparts. This finding should be of use in the identification of 1,2,4-triazole derivatives in the environment.

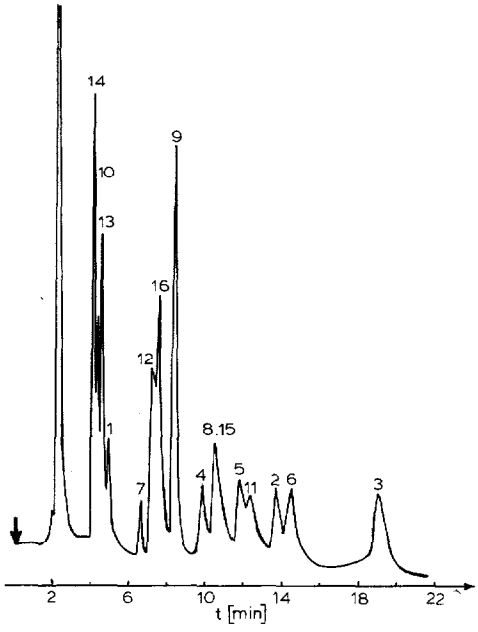


Fig. 2. Separation of 1,2,4-triazoles on silica gel as stationary phase. Mobile phase: hexane–ethyl acetate (3:2). Conditions are described in the text. Peak identification as in Table I.

ACKNOWLEDGEMENT

This work was supported in part by a grant from Jianhu Agrochemical Works, Jiangsu Province, China.

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