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Note

High-performance liquid chromatographic behaviour of phytoactive derivatives of diazines

M. T. LUGARI* and C. A. MAGGIALI

Istituto di Chimica Farmaceutica e Tossicologica dell'Università, Via M. D'Azeglio n. 85, 43100 Parma (Italy)

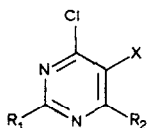
and

A. MANGIA

Istituto di Chimica Generale ed Inorganica dell'Università, Via M. D'Azeglio n. 85, 43100 Parma (Italy)

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A new class of herbicides derived from pyrimidine has been recently synthesized¹⁻³. These compounds, which have steric analogies with the phytocidal derivatives of 1,3,5-triazine, are generally phytotoxic, with different biological activity, depending on the nature and position of the substituents on the pyrimidine ring. In some cases the activity is comparable with that of atrazine^{1,2}.



X = H, Cl, SCH₃, SOCH₃, SO₂CH₃

R₁, R₂ = Cl, NHCH₃, NHC₂H₅, NH-i-C₃H₇, NH-i-C₄H₉

In this paper the chromatographic behaviour of homologous series of these compounds was studied to assess the conditions for a preparative separation of mixtures obtained in the synthetic process and to check the possibility of determination via high-performance liquid chromatography (HPLC) of these products in view of their potential as herbicides. Chromatographic methods, using both gas and liquid chromatography, have been proposed for the triazine herbicides⁴⁻¹⁰. The influence on the chromatographic behaviour of the nature and position of the substituents was investigated using reversed-phase and adsorption systems.

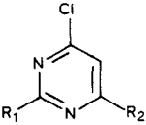
EXPERIMENTAL

The compounds were synthesized from 2,4,6-trichloropyrimidine and selected amines³. Mono- or disubstituted derivatives were obtained depending on the reactivity of the chlorine atoms; this reactivity was influenced by the nature of the substituent group in 5-position. Isomeric mixtures were separated by column chromatography; purified products were characterized by elemental analysis, m.p., IR and NMR spectra.

A Perkin-Elmer Series 3B chromatograph, equipped with a variable-wavelength spectrophotometric detector, LC75, was used. The columns were LiChrosorb

TABLE I

RETENTION VOLUMES OF 6-CHLOROPYRIMIDINE DERIVATIVES WITH X = H ON THE RP-18 COLUMN

|  | R_1 | | V' (ml) | R_2 | | V' (ml) |
|--|-------|------------------------------------|-----------|------------------------------------|-------|-----------|
| | R_1 | R_2 | V' (ml) | R_1 | R_2 | V' (ml) |
| | Cl | NHC ₂ H ₅ | 1.3 | NHC ₂ H ₅ | Cl | 3.5 |
| | Cl | NH-i-C ₃ H ₇ | 1.8 | NH-i-C ₃ H ₇ | Cl | 4.8 |
| | Cl | NH-i-C ₄ H ₉ | 2.6 | NH-i-C ₄ H ₉ | Cl | 6.3 |

Si 60 and LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.). The mobile phases were tetrahydrofuran (THF)-*n*-hexane for adsorption chromatography and methanol-water on the reversed-phase column. Samples were injected as acetonitrile solutions using a Rheodyne injection valve. All solvents were chromatographic grade (Carlo Erba, Milan, Italy). Isocratic elutions were carried out.

RESULTS AND DISCUSSION

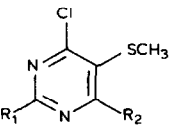
Among the synthesized compounds, some series of derivatives were chosen, related by the nature of the substituents in positions 2, 4 and 5, to correlate the chromatographic behaviour with the steric and electronic features of the substituents on the pyrimidine ring.

Table I gives the corrected retention volumes (V'), obtained on an RP-18 column, using methanol-water (80:20) as mobile phase, for a series of isomers having X = H and a Cl atom and an NH-alkyl group in the 2- and 4-positions. The compounds with $R_1 = \text{Cl}$ and $R_2 = \text{NH-alkyl}$ have lower retention volumes than 2-amino isomers. The compound with $R_1 = \text{Cl}$ and $R_2 = \text{NH-i-C}_4\text{H}_9$ (the longest aminoalkyl chain here examined) is eluted before the compound with $R_1 = \text{NHC}_2\text{H}_5$ and $R_2 = \text{Cl}$, which has the shortest chain in the other isomeric series. As expected, the retention volumes increase with increasing length of the chain on the amino group, in the same series.

The 2,4-disubstituted diamino derivatives with X = H show too high an affinity for the stationary phase and cannot be eluted using a reversed-phase system. The separation of the products with $R_1 = R_2 = \text{NHC}_2\text{H}_5, \text{NH-n-C}_3\text{H}_7, \text{NH-i-C}_3\text{H}_7, \text{NH-i-C}_4\text{H}_9$ was achieved on a silica column, using *n*-hexane-THF (90:10) as mobile phase. The retention volume (ml) are 16.8, 8.0, 6.7, and 6.2, respectively, with an elution order opposite to the length of the alkyl chain.

TABLE II

RETENTION VOLUMES OF 6-CHLOROPYRIMIDINE DERIVATIVES WITH X = SCH₃ ON THE RP-18 COLUMN

|  | R_1 | | V' (ml) | R_2 | | V' (ml) |
|--|-------|------------------------------------|-----------|------------------------------------|-------|-----------|
| | R_1 | R_2 | V' (ml) | R_1 | R_2 | V' (ml) |
| | Cl | NHCH ₃ | 2.0 | NHCH ₃ | Cl | 3.7 |
| | Cl | NHC ₂ H ₅ | 2.7 | NHC ₂ H ₅ | Cl | 5.1 |
| | Cl | NH-i-C ₃ H ₇ | 4.0 | NH-i-C ₃ H ₇ | Cl | 6.9 |
| | Cl | NH-i-C ₄ H ₉ | 5.2 | NH-i-C ₄ H ₉ | Cl | 9.4 |

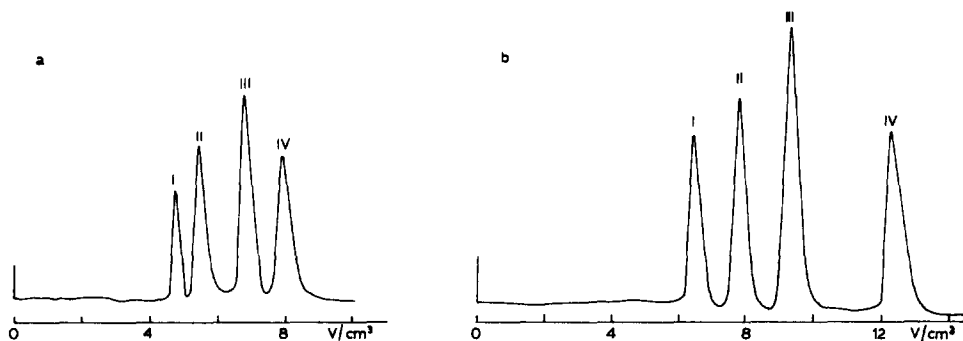


Fig. 1. Separation of 6-chloropyrimidine derivatives with $X = \text{SCH}_3$ on RP-18 column. Mobile phase, methanol-water (80:20); flow-rate, 1 ml min^{-1} ; $\lambda = 275 \text{ nm}$. (a) $R_1 = \text{Cl}$, $R_2 = \text{NHCH}_3$ (I), NHC_2H_5 (II), $\text{NH-i-C}_3\text{H}_7$ (III), $\text{NH-i-C}_4\text{H}_9$ (IV). (b) $R_2 = \text{Cl}$, $R_1 = \text{NHCH}_3$ (I), NHC_2H_5 (II), $\text{NH-i-C}_3\text{H}_7$ (III), $\text{NH-i-C}_4\text{H}_9$ (IV).

In the series with $X = \text{SCH}_3$, (this function seems to induce some selectivity in the phytocidal action) the isomers having $R_1 = \text{Cl}$ and $R_2 = \text{NH-alkyl}$ have lower retention times (Table II) than the corresponding positional isomers on an RP-18 column. In this case the compound with $R_1 = \text{NHC}_2\text{H}_5$ and $R_2 = \text{Cl}$ has a retention volume (5.1 ml) slightly lower than that of the derivative with $R_1 = \text{Cl}$ and $R_2 = \text{NH-i-C}_4\text{H}_9$ (5.2 ml). Within the same series of positional isomers, the retention volumes increase as the length of the alkyl chain increases. Fig. 1 shows the separation of the series of isomers using a methanol-water (80:20) mobile phase. The corresponding dependence of $\log V'$ on the number of carbon atoms in the aminoalkyl group is shown in Fig. 2.

The separation of 2,4-diisopropylamino derivatives with different groups in position 5 was obtained on silica using *n*-hexane-THF (90:10) as mobile phase (Fig.

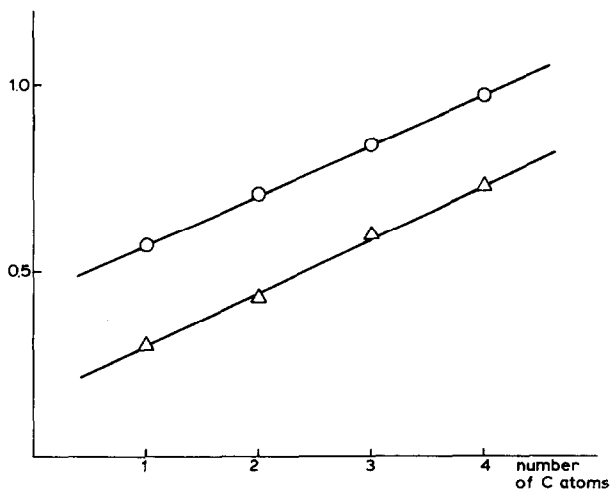


Fig. 2. Dependence of $\log V'$ on the number of carbon atoms in the aminoalkyl group in the $X = \text{SCH}_3$ series. $R_1 = \text{Cl}$, $R_2 = \text{NH-alkyl}$; $R_1 = \text{NH-alkyl}$, $R_2 = \text{Cl}$.

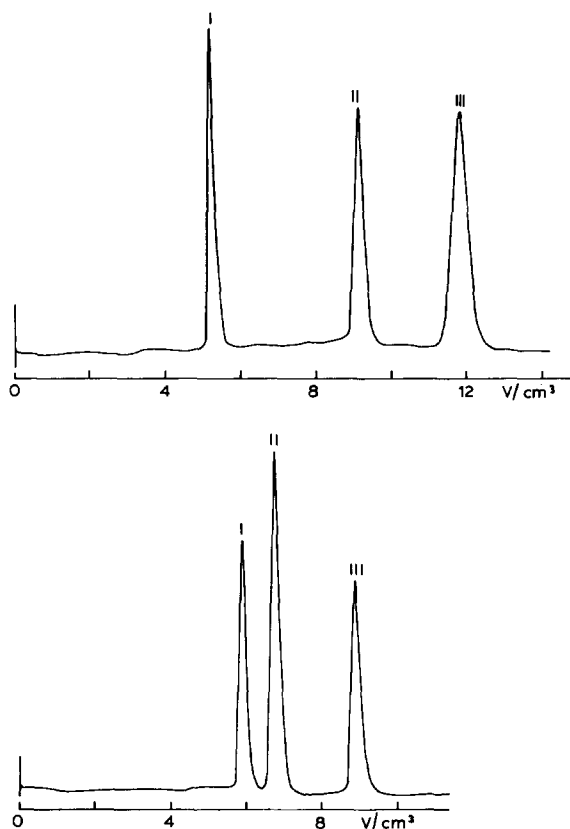


Fig. 3. Separations of NH-*i*-C₃H₇ 2,4-disubstituted derivatives on silica column. Mobile phase, *n*-hexane-THF (90:10); flow-rate, 1 ml min⁻¹, $\lambda = 275$ nm. (a) R₁ = R₂ = NH-*i*-C₃H₇; X = SCH₃ (I), SOCH₃ (II), SO₂CH₃ (III). (b) R₁ = R₂ = NH-*i*-C₃H₇; X = C₂H₅ (I), CH₃ (II), H (III).

3). In this series are compounds of some biological interest. The elution order of sulphur containing compounds is SCH₃, SOCH₃, SO₂CH₃, with $V' = 2.4, 6.3,$ and 9.2 ml, respectively. For compounds with X = H, CH₃, C₂H₅ the retention volumes are 6.3, 4.1, and 3.4 ml, respectively.

To evaluate the concentration range in which these herbicides could be determined via HPLC and UV detection, the dependence of the detector response at $\lambda = 275$ nm on the concentration of the derivative was tested for the compound with X = SCH₃, R₁ = Cl and R₂ = NH-*i*-C₃H₇. Acetonitrile solutions (concentration range 1–10) were injected (sample volumes 10 μ l); thus the amount of product injected ranged between 10 and 100 ng. The injection of each sample was repeated five times. The relative standard deviation of the peak height was 6% at lowest and 2% at highest concentration level. The absorbance data *versus* amount of compound injected fit the linear equation $y = 17.83x + 0.31$, where y is the area of the peak in cm² (recorder sensitivity $2 \cdot 10^{-3} \mu\text{A cm}^{-1}$) and x is the amount of compound injected, in nanograms. The relative standard deviation of the slope is 3% for $n = 9$. The approximate detection limit (signal-to-noise ratio *ca.* 3) is *ca.* 5 ng.

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