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Note

High-performance liquid chromatography of phytotoxic substances

II. Behaviour of imidazopyrimidines

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During research on the synthesis, biological properties and analytical characterization of phytotoxic substances, a new series of derivatives of imidazopyrimidines has been obtained[1–5]. Most of the synthesized compounds show some phytocidal activity (*e.g.*, towards *Sorghum vulgare, Hordeum hexasticum* and *Pisum sativum*).

Starting from the 5,7-dichloroimidazo[1,2-*a*]pyrimidine, by reaction with selected amines a series of derivatives with an alkylamino group in the 5-position (series I) was obtained[2]. The 7-alkylamino-5-chloroimidazopyrimidines (series II) were synthesized indirectly[3], because nucleophilic substitution of the chlorine atom in the 7-position was not possible. The corresponding members of the two series show different biological activities, with respect to both selectivity and potency. Different activities are also observed within each series depending on the length of the alkyl chain on the amino group.

The differences in the biological activities of these compounds mean that effective separation methods, both on an analytical and preparative scale, are required. The high-performance liquid chromatographic (HPLC) behaviour of some of these compounds has been tested, in a study on the chromatographic properties of phytocide derivatives[6]. In the present study the differences in the chromatographic behaviour of compounds of the two series has been compared with their chemical and biological properties. Table I lists the compounds examined.

EXPERIMENTAL

A Perkin-Elmer Series 3B chromatograph, equipped with a variable-wavelength spectrophotometric detector, LC 75, was used. The column (25 cm × 4 mm I.D.) contained LiChrosorb-NH_2 (Merck, Darmstadt, F.R.G.), and the mobile phase was methanol–dichloromethane. Samples were injected as acetonitrile solutions using a Rheodyne injection valve. All solvents were chromatographic grade (Carlo Erba, Milan, Italy). When possible, isocratic elutions were carried out; in some cases gradient elution was required, with the methanol–dichloromethane ratio varying between 3:100 and 0.1:100.

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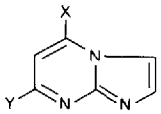
TABLE I

5,7-DISUBSTITUTED IMIDAZO[1,2-*a*]PYRIMIDINES



Series I		*Series II*	
X	*Y*	*X*	*Y*
NH_2	Cl	Cl	NH_2
$NHCH_3$	Cl	Cl	$NHCH_3$
NHC_2H_5	Cl	Cl	NHC_2H_5
NHC_3H_7-*n*	Cl	Cl	NHC_3H_7-*n*
NHC_3H_7-*iso*	Cl	Cl	NHC_3H_7-*iso*
NHC_4H_9-*iso*	Cl	Cl	NHC_4H_9-*iso*
NHC_6H_{11}-*cyclo*	Cl	Cl	NHC_5H_{11}-*n*
$N(C_2H_5)_2$	Cl	Cl	$N(C_2H_5)_2$
$N(CH_2)_4O$	Cl	Cl	$N(CH_2)_4O$
$NHCH_2C_6H_5$	Cl		

RESULTS AND DISCUSSION

Different kinds of column were tested for the separation of the imidazopyrimidine derivatives. The best results were obtained using the basic stationary phase LiChrosorb-NH_2 and methanol–dichloromethane mixtures as mobile phase.

Comparing the retention volumes of the corresponding positional isomers (Table II), it appears that the compounds of series I show higher retention volumes than those of series II with the same percentage of methanol in the mobile phase. This behaviour is in accordance with the difference systematically found in some physical properties between the two isomeric series[2], *e.g.*, solubility, melting point, R_F values and ^1H NMR spectral behaviour with respect to the chemical shifts of the

TABLE II

RETENTION VOLUMES (ml) OF 5,7-DISUBSTITUTED IMIDAZO[1,2-*a*]PYRIMIDINES ON THE LICHROSORB-NH_2 COLUMN

Group	*Series I methanol–dichloromethane (5:95)*	*Series II methanol–dichloromethane*		
		5:95	*1.2:98.8*	*0.5:99.5*
$N(C_2H_5)_2 \equiv N(CH_2)_4O$	1.0	0.7	0.8	
$NHC_6H_{11}(NHC_5H_{11})$	5.5			6.4
NHC_4H_9-*iso*	7.2		1.5	6.9
NHC_3H_7 ($n \equiv iso$)	8.0		2.1	9.6
$NHCH_2C_6H_5$	11.4			
NHC_2H_5	12.7	1.5	3.1	15.8
$NHCH_3$	23.7	2.6		
NH_2	–	9.6		

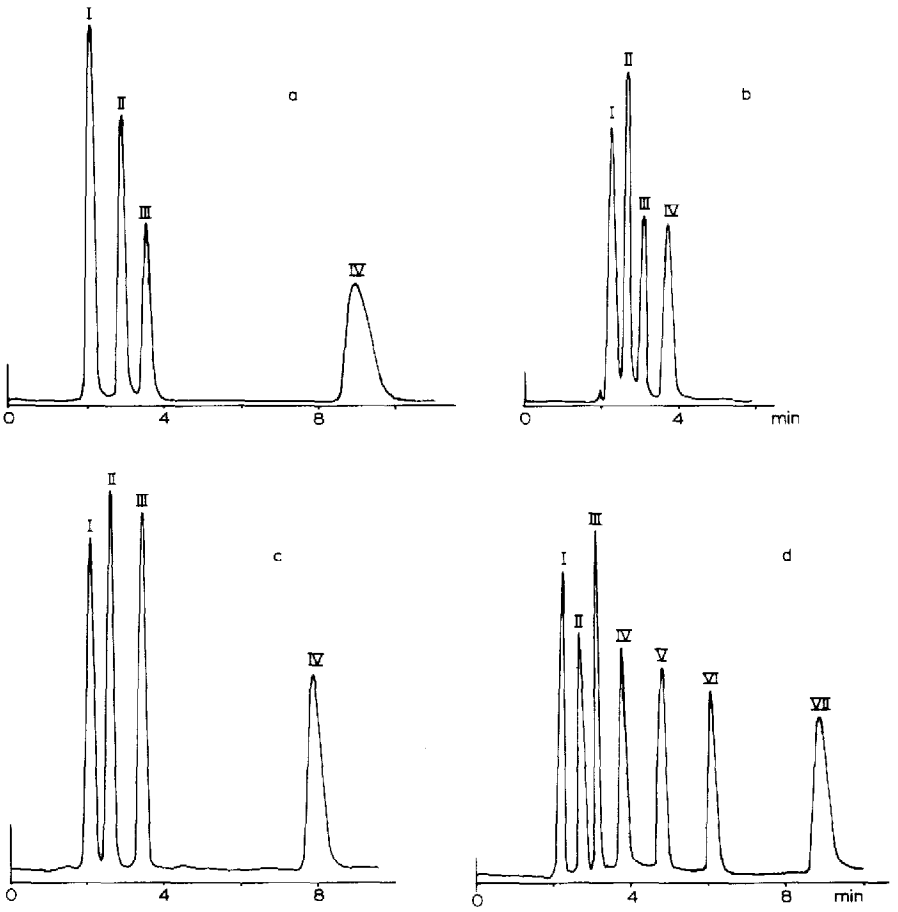


Fig. 1. Separation of imidazopyrimidines on a LiChrosorb-NH_2 column. Flow-rate: 1.6 ml min^{-1}. UV detection at 275 nm. a, Mobile phase: methanol–dichloromethane (15:85). Peaks: Y = Cl, X = NHC_3H_7(I); NHC_2H_5(II); $NHCH_3$(III); NH_2(IV). b, Mobile phase: methanol–dichloromethane (1.5:98.5). Peaks: X = Cl, Y = $N(C_2H_5)_2$(I); NHC_4H_9(II); NHC_3H_7(III); NHC_2H_5(IV). c, Mobile phase: methanol–dichloromethane (5:95). Peaks: X = Cl, Y = $N(C_2H_5)_2$(I); NHC_2H_5(II); $NHCH_3$(III); NH_2(IV). d, Gradient elution, 0.1–3% methanol. Peaks: X = Cl, Y = $N(C_2H_5)_2$(I); NHC_5H_{11}(II); NHC_4H_9(III); NHC_3H_7(IV); NHC_2H_5(V); $NHCH_3$(VI); NH_2(VII).

protons in the 2- and 3-positions. The chemical shift values are systematically higher for compounds of series I (Table III); on the contrary, the δ values of the proton in the 6-position are systematically higher in series II.

Fig. 1a shows the separation of series I derivatives using methanol–dichloromethane (15:85) as mobile phase and Fig. 1b, c show the separation of sets of imidazopyrimidines of series II using different mobile phases. Owing to the considerable and different effect of the percentage of methanol on the retention volumes of these compounds, it is not possible to separate all compounds by isocratic elution; Fig. 1d shows the separation of all compounds of this series by gradient elution, with the methanol–dichloromethane ratio varying between 3:100 and 0.1:100.

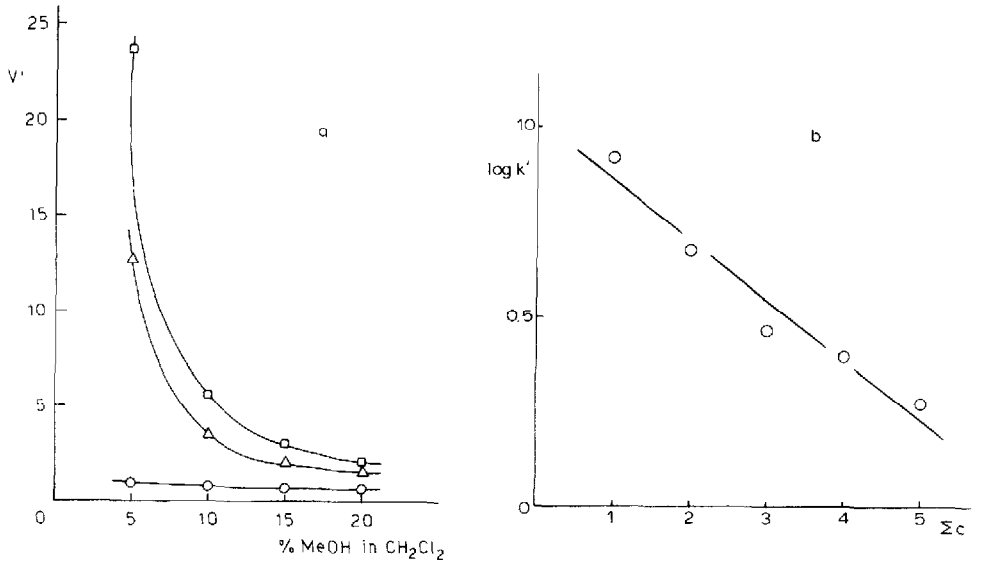


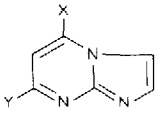
Fig. 2. a, Dependence of the retention volumes (V', in ml) on the percentage of methanol (MeOH) in the mobile phase for some 5-alkylamino derivatives: ○, 5-diethylamino; △, 5-ethylamino; □, 5-methylamino. b, Dependence of log k' on the number of carbon atoms (Σc) of the 5-alkylamino group.

In both series of imidazopyrimidines a similar dependence of the corrected retention volumes on the percentage of methanol is observed, although over a different range of percentages. For example, Fig. 2a shows the dependence observed for some 5-alkylamino derivatives. A similar relationship is observed, in both series, between the retention volumes and the number of carbon atoms in the alkylamino

TABLE III

CHEMICAL SHIFT VALUES FOR 5,7-DISUBSTITUTED IMIDAZO[1,2-*a*]PYRIMIDINES IN DEUTERATED DIMETHYL SULPHOXIDE

TMS = tetramethylsilane.



		δ (ppm relative to TMS)		
X	*Y*	*2-H*	*3-H*	*6-H*
Cl	Cl	7.65	7.55	6.90
NHC_3H_7-*n*	Cl	7.97	7.50	6.15
Cl	NHC_3H_7-*n*	7.45	7.25	6.63
NHC_4H_9-*iso*	Cl	7.95	7.50	6.20
Cl	NHC_4H_9-*iso*	7.40	7.25	6.60
$N(C_2H_5)_2$	Cl	7.60	7.55	6.45
Cl	$N(C_2H_5)_2$	7.40	7.30	6.90
$N(CH_2)_4O$	Cl	7.80	7.65	6.58
Cl	$N(CH_2)_4O$	7.47	7.32	7.12

chain. The retention volumes decrease with increasing length of the chain, and consequentely the hydrophobicity. The corresponding dependence of log capacity factor (k') on the number of carbon atoms is depicted in Fig. 2b for the 5-alkylamino derivatives and methanol–dichloromethane (5:95) as mobile phase.

From a comparison of the chromatographic behaviour and phytocidal potency[1,2], the compounds having higher activity are those with larger retention volumes in series I; the opposite trend is observed in the second series, where the derivatives with smaller retention times show higher activities.

In order to examine the possibility of determination of these compounds via HPLC, the dependence of the response of the UV detector on the quantity of injected compound was tested at 275 nm for 5-isobutylamino-7-chloroimidazo[1,2-*a*]-pyrimidine, which is one of the most significant members of this class of compounds. Volumes of 10 μl of acetonitrile solutions (1–10 ppm) were injected. The experimental data (corresponding to five injections at each level of concentration with a relative standard deviation ranging between 7 and 3%) can be expressed in terms of the linear equation $y = 0.26 + 32.09x$, where y is the peak area in cm^2 (recorder sensitivity $2 \cdot 10^{-3}$ a.u.) and x is the amount in nanograms of the compound injected. The relative standard deviation of the slope is 3% for $n = 7$. The detection limit (signal-to-noise ratio = 3) corresponds to about 2 ng.

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