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CHROMATOGRAPHIC INVESTIGATION OF HERBICIDE ANTIDOTES WITH SPIRO-OXAZOLIDINE STRUCTURES

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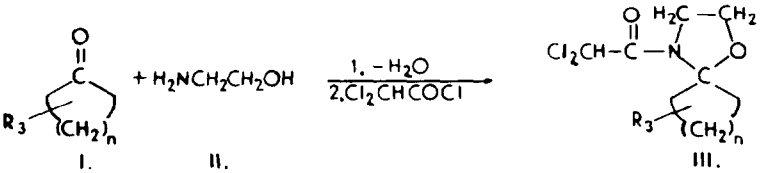
SUMMARY

A high-performance liquid chromatographic (HPLC) method is reported for the investigation and determination of herbicide antidotes with spiro-oxazolidine structures. The syntheses of these compounds were followed by thin-layer chromatography, and HPLC. The components of the end products were separated by preparative column chromatography and their structures were identified by IR, NMR, HPLC and synthetic investigations. The isolated products then served as standards for quantitative HPLC of acylated spiro-oxazolidines. From the HPLC results it is concluded that during acylation of oxazolidines both esters and amides are formed as a consequence of a tautomeric equilibrium between the intermediates.

INTRODUCTION

By condensation of cyclic ketones (I) and ethanolamine (II) followed by dichloroacetylation, spiro-oxazolidines (III) were synthesized which possessed the properties of herbicide antidotes[1] (see scheme A). The first, condensation, step has been investigated by a number of workers[2–6]. It has been known for a long time that 1,2-amino-alcohols react with ketones or aldehydes to produce a tautomeric mixture consisting of an oxazolidine (V), as a ring tautomer, and a Schiff's base (IV). The components are in equilibrium and their rates of interconversion depend on the structures of the starting materials[2–6] (see scheme B).



I. II. III.

n = 4, 5
R = H, CH_3

Scheme A.

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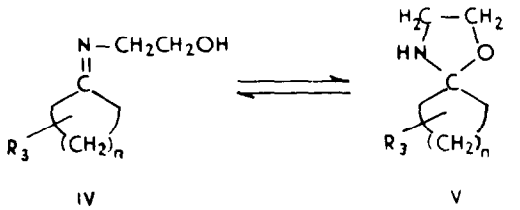
TABLE I

PHYSICAL CONSTANTS OF CONDENSATION PRODUCTS OF CYCLIC KETONES WITH ETHANOLAMINE

B.p. = boiling point.

Starting ketone	*B.p. (°C/mbar)*	R_M			*IR (cm^{-1})*		^1H *NMR (ppm)*		*Schiff's base content (%) from*		
		Found	*Calc. for*		*Open C=N OH*	*Ring NH O-C-N*	*Open OH*	*Ring NH*	R_M	*IR*	^1H *NMR*
			Schiff's base	*Oxazolidine*							
Cyclopentanone	94–96/26*	36.13	36.86	35.36	1670	3250 1080–1175	2.95	1.78	54	52	50
2,2,4-Trimethyl-cyclopentanone	103/4§§§		50.70	49.20	1670 3350		3.0	–	–	95	96
2,4,4-Trimethyl-cyclopentanone	109/4	50.17	50.70	49.20	1675 3350	1050–1160	3.15	1.35	65	74	75
Cyclohexanone	92–93/33**	39.74***	41.44	39.90	–	3200 1025–1105	–	1.88	0	0	0
3,3,5-Trimethyl-cyclohexanone	105–110/40§	53.95	55.30	53.70	1660	3200 1045–1110	3.10	1.98	16§§	10	15

* 66–66.5/2.6[4].
** 89–90/21[4].
*** 39.66[4].
§ 132/30[2].
§§ 23%[2].
§§§ Crystal, m.p. 41–43°C.



IV V

Scheme B.

As a consequence of this ring–chain tautomerism, during the second, acylation, step of the synthesis both esters (by O-acylation) and amides (by N-acylation) can be formed[2].

In this study the end products of these reactions were investigated by high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and preparative LC.

EXPERIMENTAL

Materials

All compounds to be acylated were prepared by azeotropic condensation of the corresponding cyclic ketones and ethanolamine in boiling benzene, according to the convenient procedure described by Bergmann *et al.*[3]. The resulting solutions were fractionated in a Todd column *in vacuo*. The fractions were collected and investigated. Acylations were carried out with dichloroacetyl chloride in the presence of triethylamine in the same solvent[1]. The solid acylated products were crystallized from ethanol–water, the others being used as brown oils. The compounds and their physical constants are given in Table I. All melting and boiling points were uncorrected.

Compounds synthesized by the general procedure were as follows: spirocyclohexane-1,2′-oxazolidine[1], *i.e.*, 1-oxa-4-azaspiro[4.5]decane*; spirocyclohexane-1,2′-(N-dichloroacetyl)oxazolidine[1]; spirocyclopentane-1,2′-oxazolidine[4]; spirocyclopentane-1,2′-(N-dichloroacetyl)oxazolidine[1]; (±)spiro-(3,3,5-trimethyl)cyclohexane-1,2′-oxazolidine[2]; (±)spiro-(3,3,5-trimethyl)cyclohexane-1,2′-(N-dichloroacetyl)oxazolidine[1]; (±)N-(2,2,4-trimethyl)cyclopentylideneethanolamine; (±)N-(2,2,4-trimethyl)cyclopentylideneethanolamine dichloroacetate; (±)N-(2,4,4-trimethyl)cyclopentylideneethanolamine; (±)N-(2,4,4-trimethyl)cyclopentylideneethanolamine dichloroacetate; N-(2-dichloroacetoxyethyl)dichloroacetamide[1]; N-(2-hydroxyethyl)-dichloroacetamide[8]; 2-aminoethyl dichloroacetate[9].

Spectroscopic measurements

For IR spectra a Zeiss Specord 75 IR spectrometer was used. The quantitative IR data based on the azomethine absorption (ε = 145.6 l mol^{-1} cm^{-1})[10] were measured in carbon tetrachloride solution (5 mg/ml) using a Zeiss UR 10 spectrometer. For ^{1}H NMR spectra a Varian A 60D instrument was used. The samples were dissolved in deuterochloroform.

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* Nomenclature used for the investigated compounds follows the rule recommended by Daash[7].

Determination of molecular refraction (R_M)

The refractive indices, n, were determined with an Abbe refractometer; densities, d, were measured by a picnometric method. The data necessary for the calculation of molecular refractions were determined at the standard temperature of 25°C. Results are given in Table I.

High-performance liquid chromatography

Chromatographic separations were performed on a laboratory assembled instrument, of which the principal components were a reciprocating piston pump Type 1515 (Orlita, Giessen, F.R.G.) and a variable-wavelength photometer fitted to a 10-μl flow-cell (Model 212; Cecil, Cambridge, U.K.). The column effluents were monitored at 215 nm. Columns (125 × 4 mm I.D.) were of internally polished stainless steel. Injection by a 5-μl microsyringe was made centrally through a septum into a bed of glass beads placed on top of the column packing. The packing material was reversed-phase SAS-Hypersil[11] with a particle size of around 6 μm (Shandon Southern, Runcorn, U.K.). The columns were packed conventionally by the slurry method. All solvents used were p.a. quality and the mobile phases were methanol–water mixtures. The chromatograph was operated isocratically at ambient temperature and the mobile phase flow-rate was 1.2 ml/min. A 5-μl volume of a solution of the products (1–25 μg) was injected. Peaks were recorded on a Type OH-814/1 chart recorder (Radelkis, Hungary), and characterized by their retention times, t_R, and capacity factors, k'.

Determination of the Schiff's base content in "oxazolidines"

In order to distinguish the oxazolidine and Schiff's base structures which are possible for the condensation products, the molecular refraction, IR and NMR spectra have been used. The experimental data showed that the products consisted of two isomeric forms. The presence of the oxazolidine ring and azomethine structures is evidenced by the R_M data (Table I). The (non-conjugated) C=N double bond absorbs in the IR at about 1670 cm^{-1}, whilst the oxazolidine system is characterized by three bands (1149–1185, 1116–1139 and 1086–1118 cm^{-1}) in the 1100–1200 cm^{-1} region (Table I). The Schiff's base products also exhibited OH absorptions. The ^1H NMR signals of the OH proton in the Schiff's bases and NH proton in the oxazolidine rings were characteristic (see Table I).

These methods also enabled a quantitation of the Schiff's base content in the mixture (see Table I). Thus, the theoretical molecular refraction values for the Schiff's base and oxazolidine structures were calculated. The comparison of calculated and found values gave the compositions of the condensation products. Quantitative IR measurements were made at 1670 cm^{-1} (where C=N bond absorbs) using a molar extinction coefficient, ε, of 145.6 (ε = 150[10]). Integration of the NH and OH proton NMR signals yield the composition directly (see Table I).

Thin-layer chromatography

TLC was carried out on Merck silica plates (Kieselgel 60, Art. 5553, 80 × 40 mm and 20 × 8 cm) with dichloromethane–methanol (96:4) as developing solvent. The spots were detected by treatment with iodine vapour or, after chlorination, by use of KI–*o*-toluidine spray reagent.

Preparative column liquid chromatography

Crude products (1–2 g) in 3–5 ml methanol was introduced into a 80 × 4 cm glass column packed with Merck silica (70–230 mesh, 0.063–0.200 mm, Art. 7734), and eluted with dichloromethane containing 4% (v/v) methanol. 3-ml Fractions were collected (flow-rate 60 ml/h) by an OR 606 Labor MIM fraction collector (Esztergom, Hungary). They were checked for identity and homogeneity by TLC or analytical HPLC, followed by pooling and evaporation. The structure of the residues were inferred by IR, NMR, HPLC and synthetic investigations.

RESULTS

For investigation of the end products and of acylation reaction mixtures, a reversed-phase HPLC technique was developed with SAS-Hypersil packing material[11]. In general, 1–25 μg of the pure or crude derivatives were chromatographed. Typical chromatograms are shown in Figs. 1 and 2. Chromatography required about

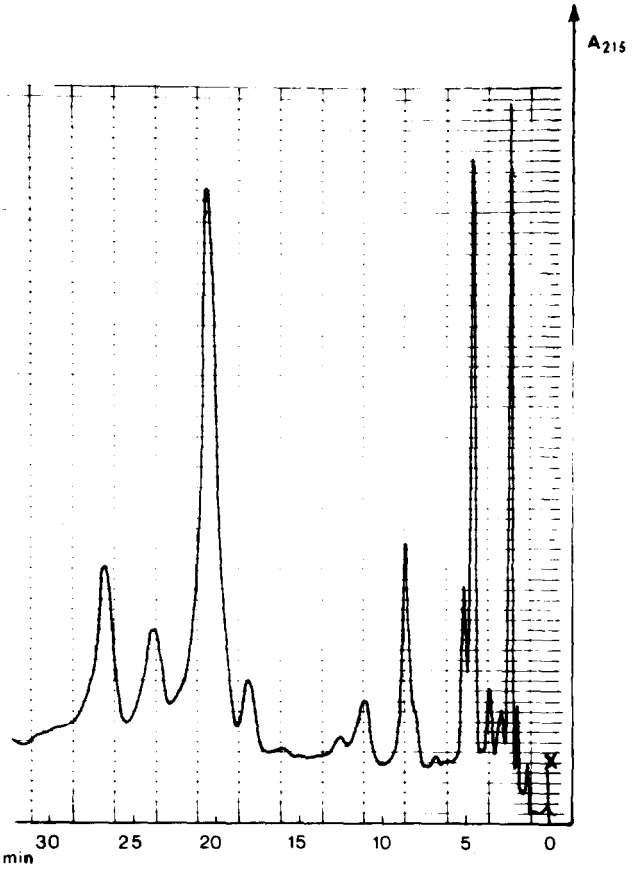


Fig. 1. Chromatogram of crude product obtained in the reaction of 2,2,4-trimethylcyclopentanone and ethanolamine followed by dichloroacetylation. Packing: SAS-Hypersil. Eluent: methanol–water (40:60, v/v). Chromatographic conditions as in Experimental.

TABLE II

PHYSICAL CONSTANTS AND CHROMATOGRAPHIC PARAMETERS OF DICHLOROACETYLATED "OXAZOLIDINES"

Chromatographic conditions as in Experimental. M.p. = melting point.

Starting ketone	*M.p. (°C), n*	*TLC R_F*	*HPLC k'*				*IR (cm^{-1}) C=O (amide I) -O-C-N-*	*^1H NMR (ppm) in $CHCl_2$*
Acetone	116–118*	0.34	2.5				1650, 1050–1160	6.05 (amide)
Cyclopentanone	Crude: oil	Multicomponent:		1.5	3.8	14.6	1670, 1660, 1080–1170	6.3 6.6
	after cryst. 79–80*	0.78	14.6				1650, 1080–1170	6.6
2,2,4-Trimethylcyclopentanone	Crude: oil 1.4909	Multicomponent:		1.5 32.0	3.7	19.5	1750–1770 (C=O ester) 1655–1665 (C=O amide) 1670 (C=N)	6.4 6.7 5.9 6.3 (esters)
2,4,4-Trimethylcyclopentanone	Crude: oil 1.4859	Multicomponent:		1.4 35.4	3.6	24.1	1750–1770 1655–1665 1670	6.4 6.7 5.9 6.3
Cyclohexanone	105–108**	0.67	8.7				1667, 1050–1165	6.10
3,3,5-Trimethylcyclohexanone	121–123	0.92	27.5				1665, 1065–1160	6.15

* Ref. 18.
** Ref. 19.

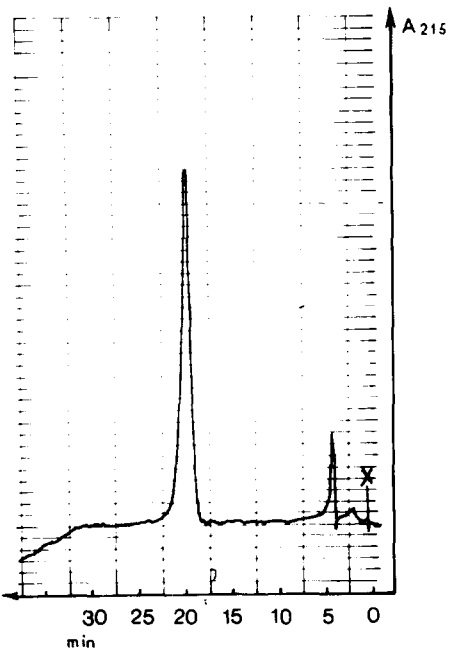


Fig. 2. Chromatogram of spiro-(3,3,5-trimethyl)cyclohexane-1,2'-(N-dichloroacetyl)oxazolidine. Details as in Fig. 1.

30 min. The mobile phases were methanol–water, 40:60, 50:50 and 60:40 (v/v) chosen by optimization. The pressure was varied between 80 and 90 bars, and the flow-rate was about 1–1.2 ml/min. The column effluents were monitored at 215 nm, based on the absorbance of the amide chromophore. Baseline separations were achieved isocratically with methanol–water (40:60) as eluent.

From the HPLC data summarized in Table II it is seen, that the cyclohexanone derivatives were separated as a single peak, analytically pure (see also the IR, NMR data). They could be isolated in homogeneous form and easily obtained as crystalline compounds.

The behaviour of the cyclopentanone derivatives is quite different from that of the cyclohexanones, more peaks being eluted (Fig. 1). Surprisingly, the predicted retention time (23 min) of the expected N-acyl-spiro-oxazolidine was very different from that of the substance present in the reaction mixture. This type of compound was not found in the crude end product, nor in the reaction mixture. According to our experience[1], it is not easy to isolate end products from these mixtures, as mixed oils may result. Their IR and NMR spectra indicate the presence of both esters and amides: $\nu_{C=O}$ (ester) and $\nu_{C=O}$ (amide) frequencies appeared at 1750–1770 cm^{-1} and 1650 cm^{-1}, respectively. The proportions of the dichloroacetates and dichloroacetamide could be determined from the NMR signals, since the chemical shift of the $\geq CHCl_2$ protons is dependent on the ester or amide structure according to the Schoolery rule[12].

The separation of components from the mixed end products was achieved by preparative chromatography on a silica column with dichloromethane–methanol as

TABLE III

PHYSICAL CONSTANTS OF COMPONENTS OBTAINED BY PREPARATIVE LC

Chromatographic conditions as in Experimental. For nomenclature of the components see the text.

Starting ketone	*Compt.*	*M.p. (°C), n*	*TLC R_f*	*HPLC k'*	*IR (cm^{-1}) C=O (amide, ester) C=N*	*^1H NMR (ppm) in $CHCl_2$ (amide, ester)*	*Quantity (%)*
2,2,4-Trimethyl-cyclopentanone	I.	1.4999	0.89	32.0	1765, 1670	6.00	30–35
	II.	1.5079	0.77	3.7	1680, 1765, 3300 (amide)	6.47, 6.78	16–20
	III.	1.5022	0.50	19.5	3400 (OH), 1665 (amide)	6.35 5.78 (>C=CH)	10–15
	IV.	85–86	0.17	1.5	3280, 3140, 1650	6.47	7–25
	V.	99–102	0.08	1.1	1765	6.75	–
2,4,4-Trimethyl-cyclopentanone	I.	1.4869	0.85	35.4	1765, 1680	6.00	35–45
	II.	1.5071	0.74	3.6	See above (II)		12–20
	III.	1.5004	0.48	24.1	3450, 1670	6.37 5.76	10–15
	IV.	85–86	0.19	1.4	See above (IV)		12–15
	V.	99–102	0.07	1.1	See above (V)		–

eluent. The substances isolated from the pure fractions were identified by IR, NMR, HPLC and synthetic investigations. In the case of 2,2,4-trimethylcyclopentanone the following components of the crude end product were isolated and identified in the order of the elution: (±)N-(2,2,4-trimethyl)cyclopentylideneethanolamine dichloroacetate; N-(2-dichloroacetoxyethyl)dichloroacetamide; (±)N-(2,2,4-trimethyl-1-cyclopentenyl)-N-(2-hydroxyethyl)dichloroacetamide; N-(2-hydroxyethyl)dichloroacetamide[8]; 2-aminoethyldichloroacetate hydrochloride[9]. The characterization of the components is found in Table III. They served as standards for the quantitative HPLC and qualitative TLC (see Fig. 3). In the case of the 2,4,4-trimethylcyclopentanone the same types of components were obtained. The average compositions of the synthetic series were determined by quantitative HPLC (see Table III).

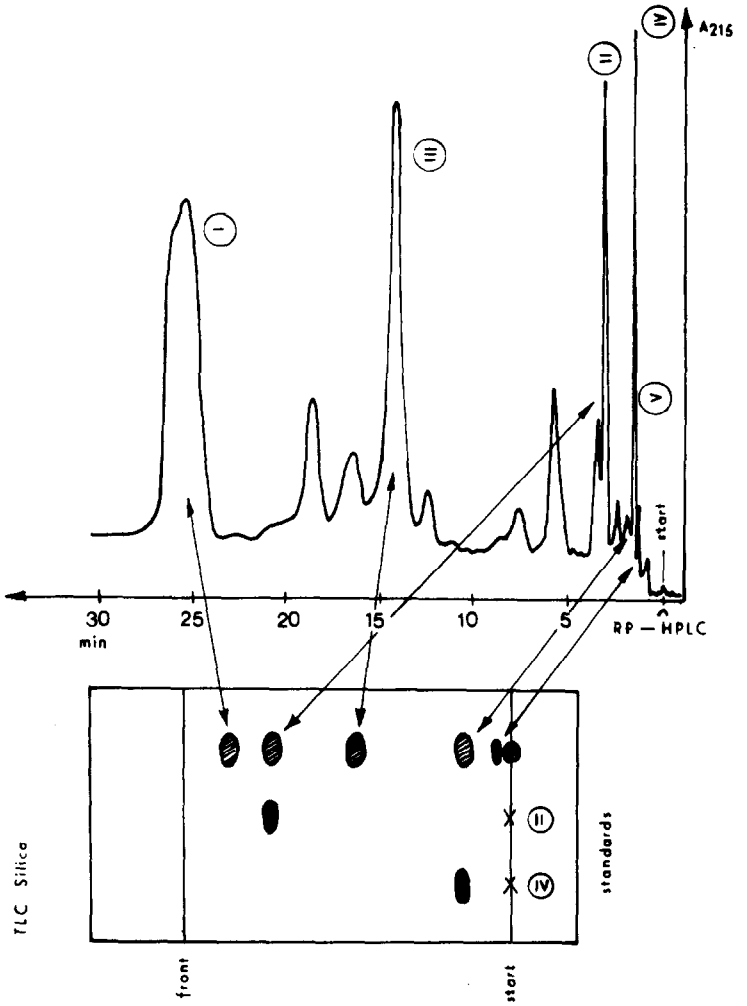
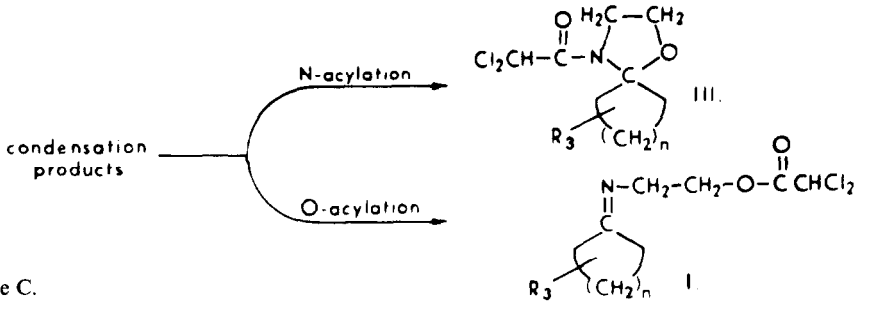


Fig. 3. Comparison of HPLC peaks to TLC spots. HPLC eluent: methanol–water (40:60); TLC eluent: dichloromethane–methanol (96:4).

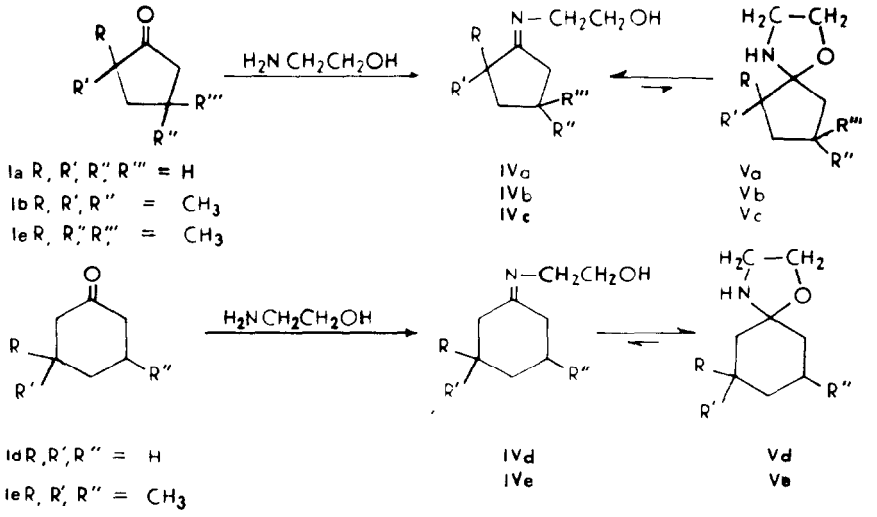
The HPLC monitoring of the dichloroacetylation of condensation products shows that the O- and N-acyl derivatives of ethanolamine, being more polar than esters and amides containing on alicyclic group, elute before the acyloxazolidines. The substitution of the cycloalkyl residue with alkyl groups results in faster elution of a compound under the same chromatographic conditions (*cf.*, N-dichloroacetyl-2,2-dimethyloxazolidine, $k' = 2.5$). The presence of methyl groups on the ring increases the retention times on reversed-phase columns because of the higher hydrophobocity of the compound (*cf.*, Id and Ie). The values of k' decreased with increasing concentration of methanol in the mobile phase. The obtained HPLC resolution suggested the possibility to study the side reactions and secondary transformations during the acylation of oxazolidines.

DISCUSSION

The chromatographic investigations provided further structural evidence for the existence of the ring–chain tautomerism of 1,3-oxazolidines. The dichloroacetylation step led to mixed products containing amide, because of N-acylation of the oxazolidine ring, and ester, because of O-acylation of the appropriate Schiff's base[2,13,14] (see scheme C).

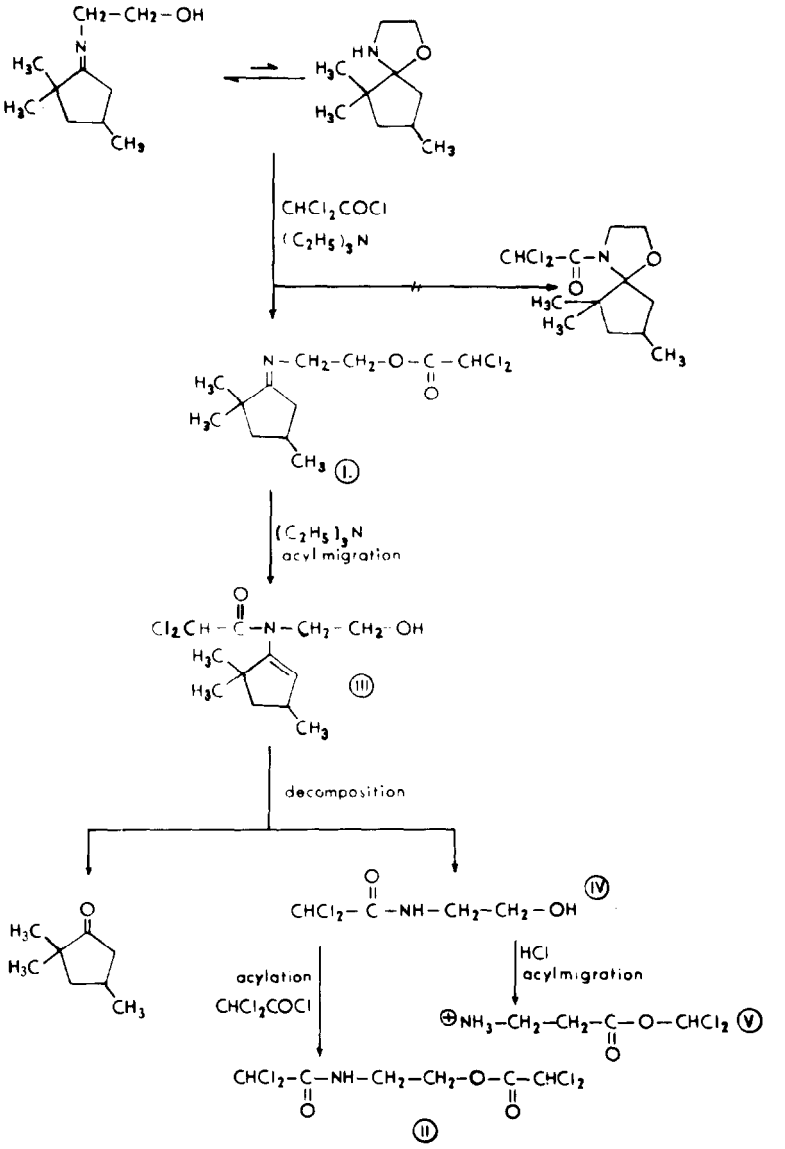


Scheme C.



Scheme D.

In principle any chemical reaction becomes useless as a source of structural evidence if these are two forms which tend to establish an equilibrium. As here the fast acylation proceeded with both tautomers, the data obtained are in good agreement with the results of the investigation of the intermediates (Table I). Since the N- and O-acylation proceeded simultaneously, the composition of the products is determined mostly by the rate of equilibration of the ring and open-chain forms (IV and V). According to data of Pihlaja and Aaljoki[5] the equilibrium constants of oxazolidines are influenced by inter- and intramolecular hydrogen bonding. In general,



Scheme E.

the presence of methyl groups weakens the ability of the ring tautomer to form intermolecular hydrogen bonds stabilizing this tautomer.

From the results summarized in Table I it is seen that the behaviour of cyclopentanones (I, $n = 4$) towards ethanolamine is very interesting, especially if it is compared with that of cyclohexanones (I, $n = 5$). Thus, a six-membered ring at spiro position-2 apparently stabilizes the ring tautomer, whereas the presence of a five-membered ring enhances the proportion of the open-chain form. On the other hand, the conformation of cyclohexane ring seems to be more favourable for spiro-oxazolidines (see scheme D).

Thus, in the case of cyclohexanones, chiefly N-acylated oxazolidines are produced. In the case of cyclopentanone, N- and O-acylation proceeded simultaneously. On increasing the number of methyl substituents nearly 100% of the Schiff's base was achieved (2,2,4- and 2,4,4-derivatives). Here, O-acylation predominated over N-acylation. Unfortunately, the ester formed was only a primary product. It suffered secondary transformations because of possible N → O and O → N acyl migrations characteristic of any acyl derivative of ethanolamine[13,15,16]. As a consequence of these side reactions, decompositions also took place. So the acylation finally led to a mixture. On the basis of HPLC, preparative LC, spectroscopic and synthetic evidence, the following pathway is suggested for the 2,2,4-trimethyl derivative (Ib) (see scheme E).

CONCLUSIONS

It is concluded that after acylation of oxazolidines both esters and amides are formed, but in different quantities depending on the structure of the starting 1,3-amino-alcohol and ketone. Therefore the compositions of herbicide antidotes with oxazolidine structures must be revised in many cases. Using combined HPLC, preparative LC and spectroscopic analysis for the study of the acylation step and of the intermediary condensates, a precise method could be developed for controlling the reactions. From the summarized results it seems that in the reaction with 1,2-amino-alcohols, cyclohexylketones have a greater tendency to give oxazolidines than have cyclopentylketones. The latter favour the formation of Schiff's bases[13,17]. So, according to the chromatographic studies, their acylation yields quite different results.

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