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Separation and interconversion of 3-amino-2-cyanoacrylates by high-performance liquid chromatography

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SUMMARY

High-performance liquid chromatography (HPLC) is the preferred method for the resolution of *Z*/*E* isomers. The partly known interconversion of the *Z*/*E* isomers of methyl and ethyl 3-substituted amino-2-cyanoacrylates and amides was observed and quantitatively followed by HPLC analysis. In a solution of alkaline pH and containing methanol instead of acetonitrile, the interconversion is faster. The use of gas chromatography for the analysis of the isomers for unsubstituted aminocyanoacrylates was impracticable owing to the easy interconversion in the sample chamber.

INTRODUCTION

Alkyl 3-amino-2-cyanoacrylates are important intermediates in the synthesis of pyrimidines used in the pharmaceutical industry. Knippel *et al.*[1] measured the *Z*/*E* interconversion of 3-mono- and -disubstituted-amino-2-cyanoacrylates by ^{1}H NMR spectroscopy. Bellanato *et al.*[2] used IR spectroscopy for characterizing these compounds and observed qualitative evidence for the rotation around the C=C double bond in solutions of the N-monosubstituted compounds.

Jacobson *et al.*[3] studied the *cis–trans* isomerization of peptides containing proline by high-performance liquid chromatography (HPLC). They calculated the rate constants of this reaction from the distortion of the peak shapes. Jansen and Both-Miedema[4]. reported the instability of phenylthiohydantoin amino acids in the HPLC mobile phase during automatic HPLC analysis.

Our aim here was to investigate the applicability of the *Z*/*E* isomeric amino-2-cyanoacrylates in the synthesis of some pharmaceutical products. It is known from the work of Knippel *et al.*[1] and Bellanato *et al.*[2] that the interconversion between the isomers is easy. No method has been reported for the quantitative measurement of unsubstituted aminocyanoacrylates. In this paper, the use of HPLC for the analysis of unsubstituted and substituted 3-amino-2-cyanoacrylates is demonstrated.

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EXPERIMENTAL

Apparatus

A Varian (Palo Alto, CA, U.S.A.) Model 5000 liquid chromatograph with a Pye Unicam (Cambridge, U.K.) LC-3 UV detector was used at 272 nm. A 25 cm × 4.6 mm I.D. reversed-phase LiChrosorb RP-8 column (particle size 10 μm) (Merck, Darmstadt, F.R.G.) was used. The mobile phase was acetonitrile–distilled water (40:60, v/v) at a flow-rate of 1 ml/min. Injection was carried out with a Valco 10-μl loop injector.

For gas chromatographic analysis use was made of a Hewlett-Packard (Avondale, PA, U.S.A) Model 5890A gas chromatograph with a flame ionization detector, an HP 3394A reporting integrator and an HP-5 column (5% phenylmethylsilicone, cross-linked; fused-silica capillary column, 25 m × 0.31 mm I.D., 0.52-μm film thickness). The flow-rate of the carrier gas (hydrogen) was 5 ml/min with a splitting ratio of 200.

For structure elucidation of the unknown compounds, NMR, UV and IR spectra were measured using Varian FT80A, Pye Unicam SP 1800 and Perkin-Elmer 783 instruments, respectively.

A Mettler Titriprocessor was used for measuring the pH of the eluents.

Chemicals and materials

Acetonitrile, toluene and chloroform (chromatographic grade) and perdeuterated dimethyl sulphoxide (DMSO-d_6) (spectroscopic grade) were obtained from Merck and methanol and KH_2PO_4 (analytical-reagent grade) from Reanal (Budapest, Hungary). Distilled water was used throughout. Eluent of pH 4 was prepared by dissolving 0.05% KH_2PO_4 in distilled water and adjusting the pH to 4 with phosphoric acid while monitoring the pH with the Mettler titrimeter. Mixing this buffer with acetonitrile in a volumetric ratio of 40:60 gave a mobile phase of pH 4.

UV and IR spectra were measured in acetonitrile solution and as potassium bromide pellets, respectively. 3-Amino-2-cyanoacrylates were prepared by a method similar to that described by Bredereck[5]. The compounds studied are listed in Table I. The isomers were separated by crystallization of the isomer mixture from toluene. The solubility of the *E* isomer is lower. The *Z* isomer can be crystallized from the filtrate. So far only compounds **2** and **3** have been separated.

TABLE I

AMINOCYANOACRYLATES PREPARED AND INVESTIGATED

$R_2NCH=C(CN)COX$

No.	*R*	*X*	*Isomer*
1	H	OCH_3	Mixture
2	H	OC_2H_5	*Z*
3	H	OC_2H_5	*E*
4	H	NH_2	*E*
5	CH_3	NH_2	*E*
6	CH_3	OC_2H_5	*E*

Procedures

For HPLC measurements 4–5 mg of material were weighed on an analytical microbalance and dissolved in 10 ml acetonitrile. A 10-μl volume of the sample was first injected immediately after dissolution. The injection was repeated after given periods of time. The column temperature was 25°C, the same as that for the sample solutions.

For gas chromatography, 1 μl of 1% ethanol solutions was injected into the sample chamber maintained at 220°C. The temperatures of the detector and column were 240 and 140°C (isothermal), respectively.

RESULTS AND DISCUSSION

The IR spectra and the assignments of the prepared and investigate cyanoacrylates are summarized in Table II. The results were found to correspond with those of Bellanato *et al.*[2].

TABLE II

IR ABSORPTIONS (cm^{-1}) AND ASSIGNMENTS (SYMBOLS AS PROPOSED BY SOHÁR *et al.*[6]) OF THE PREPARED COMPOUNDS $R_2NCH=C(CN)COX$

R	*X*	ν_{as} *(NH₂)*	ν_s *(NH₂)*	*ν (CN)*	*ν (C=O)*	*ν (C=C)*	β_s *(NH₂)*	*ν (C–N)*	*γ (NH₂)*	*Isomer*
H	C_2H_5O	3330	3180	2220	1675	1610	–	1160	730	*E*
H	C_2H_5O	3380	3250	2210	1680	1610	1560	1230	730	*Z*
H	CH_3O	3320	3200	2210	1680	1620	1550	1220	780	
H	NH_2	3350	3150	2210	1670[a]	1620	1560	1180	770	
CH_3	NH_2	3380	3200	2200	1665[a]	1640	–	1140	–	
CH_3	C_2H_5O	–	–	2210	1700	1625	–	1220	–	

[a] Amide I band.

In DMSO-d_6 at room temperature simple ^1H NMR spectra were obtained *e.g.*, for ethyl 3-amino-2-cyanoacrylate; ^{13}C NMR, because of the longer time needed, resulted in duplication of the bands, in the same way as at 80°C with ^1H NMR. We can assign the bands to the isomers, separated by crystallization (Table III).

On injecting either **2** or **3** into the gas chromatograph the same chromatograms were obtained, showing that isomerization takes place in the sample chamber. According to the quantitative evaluation, in the thermodynamic equilibrium the ratio of the *Z*/*E* isomers is 30:70 (w/w). The chromatogram is shown in Fig. 1.

The N,N-disubstituted compounds, *e.g.*, ethyl 3-dimethylamino-2-cyanoacrylate (**6**), showed a single peak under the same chromatographic conditions, owing to the small tendency for rotation around the double bond.

When methyl or ethyl 3-amino-2-cyanoacrylate in methanol or ethanol solution was injected repeatedly into the HPLC column, two peaks appeared and their ratio as a function of time changed as shown in Fig. 2. Thermodynamic equilibrium was attained in neutral solution in 2 h, whereas above pH 7 this happened within a few seconds. In acidic solutions the aminocyanoacrylates are not isomerized; for this

TABLE III

^1H AND ^{13}C NMR BANDS 2 AND 3

The CH band of isomer **2** could not be assigned because of the near NH_2 bands.

Method	*Band*	*δ(ppm)*	
		E	*Z*
^1H NMR	CH_3	1.20	1.23
	CH_2	4.11	4.14
	CH	8.06	–
^{13}C NMR	C-1	165.21	166.48
	C-2	71.13	69.91
	C-3	158.49	159.25
	CN	116.45	119.21
	CH_2	59.67	59.58
	CH_3	14.36	14.30
	Coupling constant (Hz)		
	^3J(CO,H)	3.36	10.07
	^3J(CN,H)	9.99	4.86

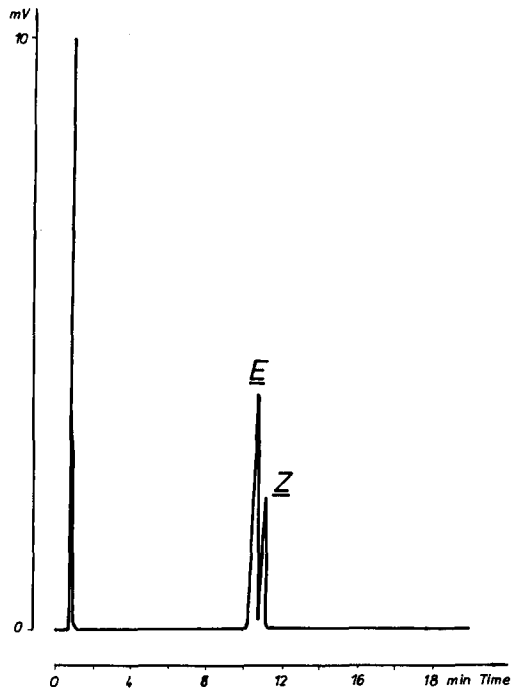

Fig. 1. GC of (*E*)-ethyl 3-amino-2-cyanoacrylate. HP-5 column. Temperature: injector, 220°C; detector, 240°C; oven, 140°C, isothermal.

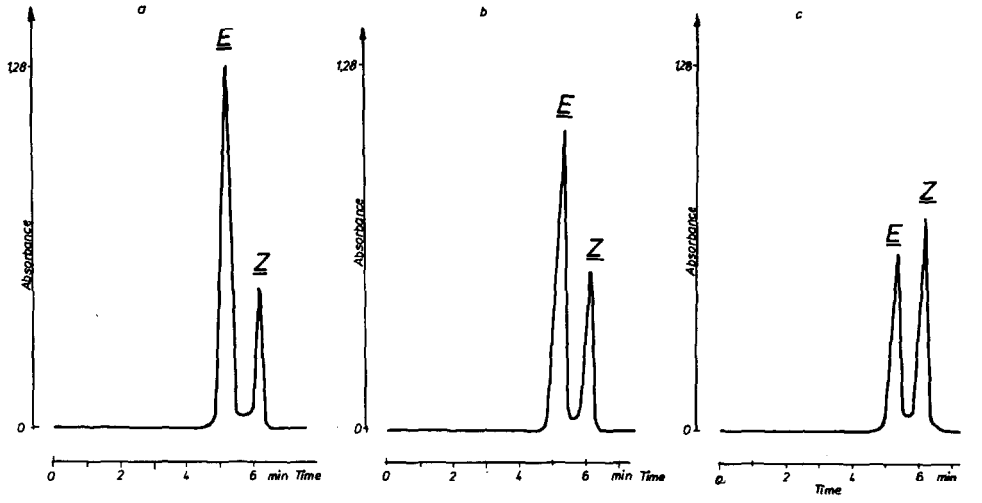

Fig. 2. HPLC of pure *E* isomer of ethyl 3-amino-2-cyanoacrylate. Injection at (a) 20, (b) 40 and (c) 120 min after dissolution in ethanol (5 mg in 10 ml). RP-8 column; mobile phase, acetonitrile–phosphate buffer (40:60, v/v); wavelength, 272 nm.

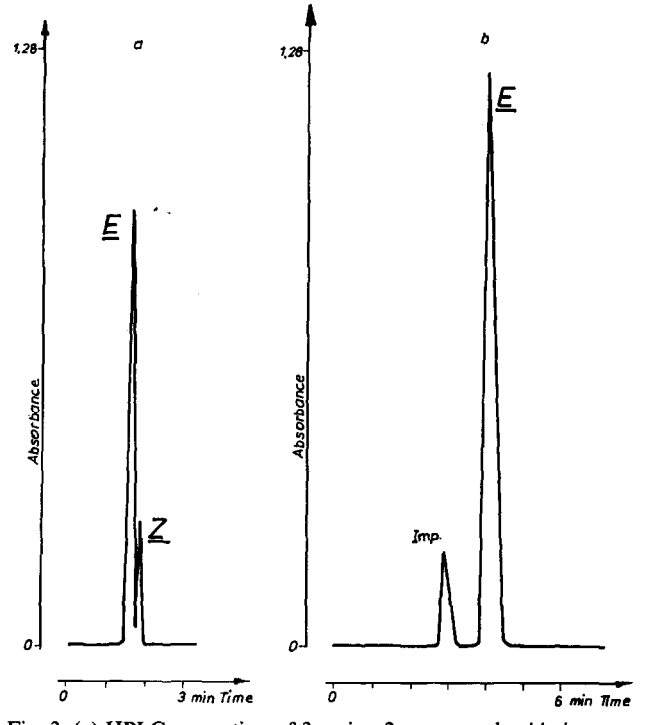

Fig. 3. (a) HPLC separation of 3-amino-2-cyanoacrylamide isomers and (b) HPLC of ethyl 3-dimethylamino-2-cyanoacrylate (*E* isomer). Conditions as in Fig. 2.

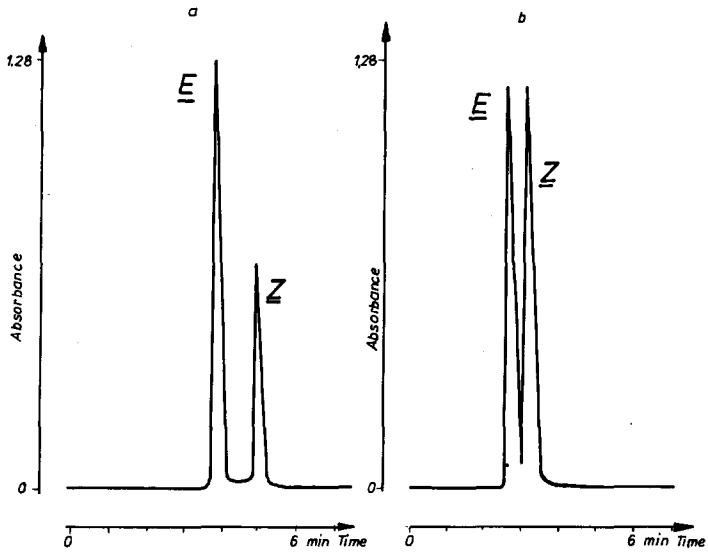

Fig. 4. HPLC separation of (a) ethyl and (b) methyl 3-amino-2-cyanoacrylate isomers. Conditions as in Fig. 2.

reason, we used a mobile phase of pH 4. The retention times of these compounds change very little with the pH of the mobile phase.

It is important to emphasize that an eluent of alkaline pH must not be used in the analysis of these compounds. In an alkaline mobile phase the just separated isomers are immediately reconverted to each other during the elution, resulting in a broad peak. A similar phenomenon was described by Jacobson *et al.*[3] and Jansen and Both-Miedema[4]

HPLC showed that in acetonitrile solution, unlike in alcohols, the unsubstituted compounds do not undergo isomerization for a few hours because of the absence of hydrogen.

Using an RP-18 column the separation was the same as that on the RP-8 column, but the retention times were longer.

TABLE IV

UV ABSORBANCES OF AMINOCYANOACRYLATES IN ACETONITRILE

Compound	*Absorbance maximum (nm)*	$A^{1\%}_{1\,cm}$
1	270	Isomer mixture
2	274	1040
3	276	1260
4	273	1467
5	286	1360
6	285	1285

We also succeeded in resolving the *Z*/*E* isomers of 3-amino-2-cyanoacrylamide and methyl 3-amino-2-cyanoacrylate (Figs. 3 and 4).

In Table IV the UV absorbances of aminocyamoacrylates measured in acetonitrile are summarized.

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