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Note

High-performance liquid chromatographic analysis of the *endo–exo* isomer ratio in the 5-substituted 2-norbornene series

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In recent years the Diels–Alder reaction has attracted considerable interest owing to its synthetic usefulness in producing products with particular stereochemical features[1–6]. The *endo–exo* isomer ratio of the Diels–Alder adducts of cyclopentadiene with methyl vinyl ketone and with derivatives of acrylic and methacrylic acid have been determined by gas chromatography[7–12] and ^{1}H and ^{13}C NMR spectroscopy[13–21]. Gel-permeation chromatographic analyses have generally been carried out using packed columns containing as the stationary phase SE-30[8,10], 3% OV-17[4], Carbowax 20M[8], 400[9] or 1500[3,9,22], EGSSX[8], 15% UCON[11], 5% PEG[7,10], PEGS[10] or 20% TCPE[10,11], and Chromosorb W[3,9,11,22] or Chromosorb W AW DMCS[7] as the support, at temperatures below 110–120°C. The restrictions concerning the temperature of the injector and the column were determined by the fact that a retro-Diels–Alder reaction could take place at high temperatures.

NMR spectroscopy has been used in most instances for the elucidation or attribution of the stereochemical structures of the pure products isolated from mixtures of isomers and only occasionally for the determination of the *endo–exo* ratio[7,10,22]. The direct ^{1}H NMR analysis of samples taken from the reaction mixtures was not possible because the presence of water or other solvents containing hydrogen atoms.

As part of current investigations on the Diels–Alder reaction in our laboratory[23,24], we needed to develop another simple and satisfactory method for the determination of the *endo–exo* isomer ratio in the final reaction mixture. This paper reports the results on the application of high-performance liquid chromatography (HPLC) to the determination of the *endo–exo* isomer ratio of some 5-substituted 2-norbornenes, prepared from cyclopentadiene and methyl vinyl ketone or derivatives of acrylic and methacrylic acid.

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EXPERIMENTAL

The HPLC system consisted of a Millipore–Waters unit (Model 510) with an automatic gradient controller, Lambda Max (Model 481) UV detector with variable wavelength and a Model 745B integrator were used. The analyses were carried out on a Nova-Pak C_{18} (5 μm) reversed-phase column at 210 nm. Methanol and acetonitrile solvents were of HPLC grade (Aldrich). Water was doubly distilled.

The samples were prepared by reported methods[6,25] from cyclopentadiene (obtained from decomposition of commercial dicyclopentadiene at 170°C and stored at −10°C) and the corresponding commercially available dienophiles: methyl vinyl ketone (Fluka), acrylonitrile (Janssen), methyl and *tert.*-butyl acrylate and methyl methacrylate (Fluka). The *endo–exo* ratio was determined after extraction of the final product and evaporation of the solvent without purification or after directly sampling from the reaction mixture.

RESULTS AND DISCUSSION

HPLC possesses all the advantages necessary for the purpose of our investigations, permitting a simple and effective analysis under mild analytical conditions. Because of the relatively non-polar structure of the compounds and their good solubility in methanol, we tried to carry out the analyses on a C_{18} reversed-phase column with methanol or methanol–water mixtures as the mobile phase, but our first attempts to separate the two stereoisomers failed. We were obliged to replace the methanol with acetonitrile, possessing a lower elution strength[26] (ε_0 is 0.95 for methanol and 0.65 for acetonitrile).

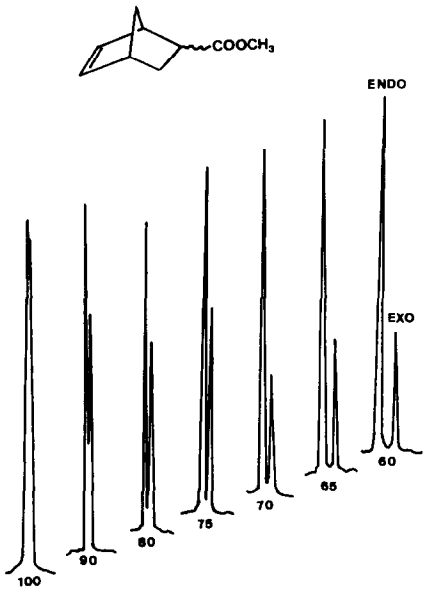
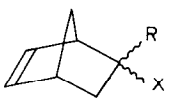


Fig. 1. Separation of the *endo–exo* isomers with different compositions of the acetonitrile–water mobile phase (the percentage of acetonitrile is given under the peaks).

TABLE I

ANALYTICAL CONDITIONS FOR THE DETERMINATION OF THE *ENDO-EXO* ISOMERS ON A C_{18} REVERSED-PHASE COLUMN

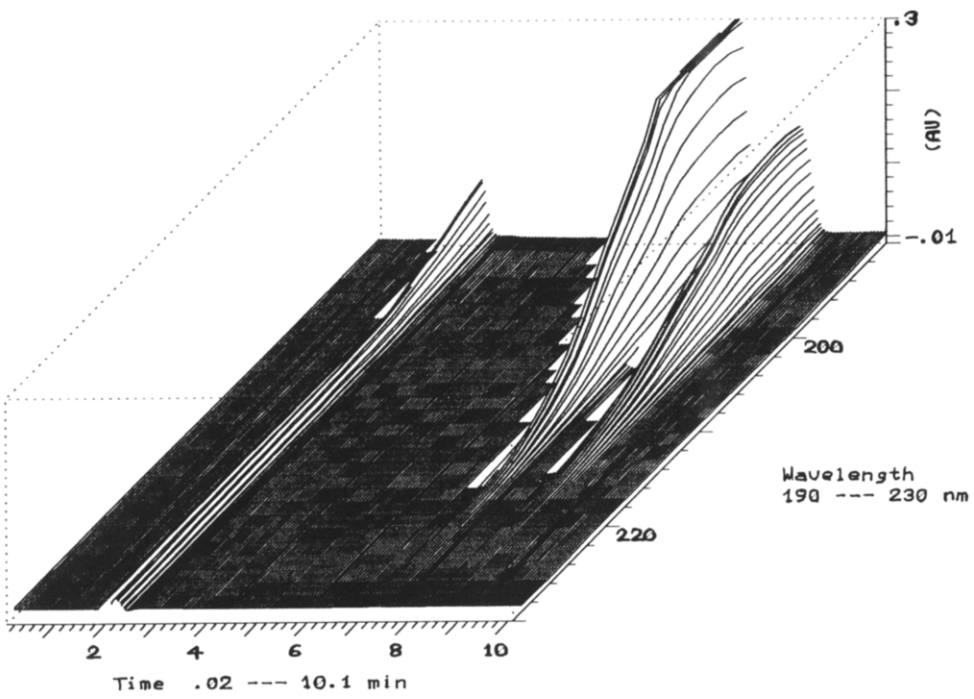
Flow-rate of mobile phase, 9.5 ml/min at room temperature.



R	*X*	*Acetonitrile–water mobile phase*	*Retention time (min)*		*Endo–exo ratio*		
			*Observed**	*Average***	*Observed**	*Average*	*Precision (%)*
H	$COCH_3$	50:50	5.38, 5.40, 5.41	5.39 ± 0.12	91.34, 91.97, 92.03	91.78 ± 0.35	0.380
			6.56, 6.57, 6.57	6.57 ± 0.16	8.66, 8.03, 7.97	8.22 ± 0.35	
H	CN	40:60	7.21, 7.18, 7.11	7.16 ± 0.37	67.66, 67.13, 67.41	67.40 ± 0.26	0.386
			8.49, 8.44, 8.39	8.44 ± 0.46	32.34, 32.87, 32.59	32.60 ± 0.26	
H	$COOCH_3$	60:40	5.24, 5.32, 5.29	5.30 ± 0.11	88.06, 87.39, 87.80	87.75 ± 0.36	0.410
			6.12, 6.18, 6.16	6.18 ± 0.24	11.94, 12.61, 12.20	12.25 ± 0.36	
H	$COOC(CH_3)_3$	70:30	8.82, 8.80, 8.82	8.75 ± 0.16	82.82, 82.64, 82.45	87.64 ± 0.19	0.230
			10.43, 10.42, 10.45	10.36 ± 0.21	17.18, 17.36, 17.55	17.36 ± 0.19	
CH_3	$COOCH_3$	60:40	7.11, 6.64, 6.68	6.86 ± 0.26	29.93, 29.45, 29.24	29.51 ± 0.34	0.482
			8.55, 7.90, 7.92	8.18 ± 0.39	70.07, 70.55, 70.76	70.48 ± 0.34	

* For three consecutive analyses under the same analytical conditions.

** For more than 40 analyses.



Waters 990	Spectrum index	plot (peak & slope)	correct		
11-27-1987	10:47:52	Time range	0 --- 10 min	Sample name	DOBREV.acn7
Sampling time	25 msec *4	Interval	1.01 sec		
Y-scale	.065 AU/FS	Baseline	OFF		
Sense	high 10	Slope	.01 AU/min		
Paper speed	9 mm/min				
Resolution	1.4 nm	Wavelength	190 --- 230 nm		

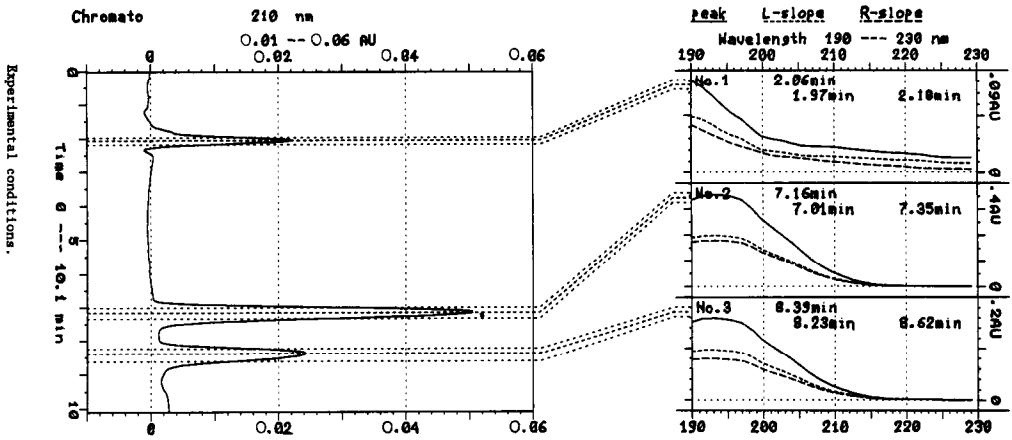


Fig. 2. Separation of the *endo–exo* isomers of 5-cyano-2-norbornene. Mobile phase: acetonitrile–water (40:60); Waters 990 photodiode array detector.

Immediately we observed some splitting of the isomer peak (Fig. 1). Using acetonitrile–water mixtures with different compositions, we obtained a very good chromatographic resolution of the peaks of the two *endo–exo* isomers in all instances, which permitted their good integration and precise dosage (Fig. 2). The peaks were identified from the ^{1}H NMR spectra or by comparison with samples having a known isomer ratio. It should be noted that in these instances, because of the reversed-phase character of the column, the first peak was always that of the *endo* isomer and not that of the *exo* isomer as in gas chromatographic analysis.

Typical conditions of the analyses, the observed retention times and the calculated isomer ratios are given in Table I. In each instance we obtained good reproducibility of the retention times and of the calculated isomer ratios. This method has also been used successfully for the determination of the isomer ratio after direct sampling from the reaction mixture.

CONCLUSION

These studies have shown that by varying the composition of the acetonitrile–water mobile phase, very good HPLC resolution of the mixture of *endo–exo* stereoisomers can be achieved. The optimal chromatographic conditions for each of the examples studied were determined and the isomer ratio was calculated accurately.

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