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GAS CHROMATOGRAPHIC DETERMINATION OF PHENOLS AS 2,4-DINITROPHENYL ETHERS USING GLASS CAPILLARY COLUMNS AND AN ELECTRON-CAPTURE DETECTOR

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SUMMARY

An improved gas chromatographic method is described for the determination of alkyl- and alkoxy-substituted phenols as their 2,4-dinitrophenyl ethers. The gas chromatographic analysis is performed on OV-210 glass capillary or SP-2100 fused silica capillary columns using electron-capture detection. The separation of *o*-, *m*- and *p*-substituted phenols and the isomers of dimethylphenols has been achieved. The sensitivity limit of electron-capture detection for various 2,4-dinitrophenyl ethers is 0.01–0.09 ng injected and the coefficient of variation is about 5%.

The reaction conditions for ether formation are described. The optimum pH, temperature and reaction time are 11.5, 50°C and 40 min, respectively.

INTRODUCTION

Gas chromatography (GC) has been used for several years for the determination of phenols in a variety of samples. Although free phenols can be determined as such using a flame-ionization detector (FID)[1–5], their concentration is frequently so low that the sensitivity of the FID is insufficient. When the phenol content is at the nanogram level it becomes necessary to produce phenol derivatives so as to exploit the extremely sensitive specific detectors, such as the electron-capture detector (ECD) and nitrogen and flame photometric detectors.

The ECD has proved to be one of the most useful for this purpose, as many phenol derivatives can be formed that contain groups towards which the detector is sensitive. For instance, esters of halogenated acids such as trifluoroacetates[6] and chloroacetates[7] have been used, although the former cause difficulties because of their ready hydrolysis in the presence of water[6]. In contrast, α-bromo-2,3,4,5,6-pentafluorotoluene[8–10], 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene[10], heptafluorobutyrylimidazole[11] and 1-fluoro-2,4-dinitrobenzene[10,12,13] form derivatives with phenols that not only give a good ECD response but also are stable in aqueous solution.

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The derivatives chosen for this work were 2,4-dinitrophenol ethers, which, in addition to satisfying the need for water stability and ECD sensitivity, could also be readily synthesized.

EXPERIMENTAL

Apparatus

The ether derivatives were analysed on Hewlett-Packard 7620A and 5730A gas chromatographs fitted with ^{63}Ni ECDs. The 7620A model was modified so as to be suitable for glass capillary columns by constructing a glass splitter, while the 5730A was fitted with a Hewlett-Packard 18740B splitter. Argon–methane (95:5) make-up gas was connected between the end of the column and the detector. Peak areas on the chromatograms and quantitative results were measured using a Hewlett-Packard 3352B laboratory data system. The glass capillary was drawn on a Hewlett-Packard Hupe + Busch 1045B.

Columns

The capillary columns used for the analyses were a glass column, 50 m × 0.3 mm I.D., coated with OV-210, prepared in our laboratories, and a Hewlett-Packard fused silica column, 50 m × 0.2 mm I.D., coated with SP-2100. We produced the glass capillary from soda-glass (O.D. 8 mm, I.D. 4 mm; Glaswerk Wertheim, Wertheim, G.F.R.). The internal surface of the capillary was first etched with concentrated hydrochloric acid[14] and then deactivated with benzyltriphenylphosphonium chloride[15]. Finally, the column was coated using the dynamic method described by Schomburg and Husmann[16].

Gas chromatographic conditions

The flow-rate of the helium carrier gas was a steady 1.2 ml/min for the OV-210 column and 0.35 ml/min for the SP-2100 column, and that of the argon–methane (95:5) make-up gas was 45 ml/min. The temperatures of the injection block and detector were 250°C and 300°C, respectively. Injections were made at 60°C with a splitless technique. The column temperature was kept constant at 60°C for 4 min and then raised rapidly at 31°C/min to 220°C, and maintained there until the end of the run.

Reagents

1-Fluoro-2,4-dinitrobenzene and triethylamine were obtained from Fluka (Buchs, Switzerland) and *n*-hexane from J. T. Baker (Deventer, The Netherlands). The phenols examined were obtained from the suppliers listed in Table I, and were purified before use by vacuum distillation or recrystallization from light petroleum (b.p. 40–60°C; May and Baker, Dagenham, Great Britain). The following were used to prepare the buffer solution of Teorell and Stenhagen[17]: citric acid, boric acid, phosphoric acid, hydrochloric acid (all from E. Merck, Darmstadt, G.F.R.), and sodium hydroxide (EKA, Bohus, Sweden).

Synthesis of 2,4-dinitrophenyl ethers of phenols

The phenols were converted into their 2,4-dinitrophenyl ethers using the method of Reinheimer *et al.*[18]. The products were recrystallized from water–ethanol.

TABLE I

PHENOLS USED AND SUPPLIERS

Mixture No.	*Phenol*	*Supplier*
1	Phenol	Merck
	o-Cresol	Fluka
	2,6-Dimethylphenol	K& K Labs., Plainview, NY, U.S.A.
	m-Cresol	Union Chimique, Brussels, Belgium
	p-Cresol	Fluka
	2,4-Dimethylphenol	Fluka
	3,5-Dimethylphenol	Fluka
	2,3-Dimethylphenol	Fluka
	p-Ethylphenol	Fluka
	3,4-Dimethylphenol	Fluka
	p-Isopropylphenol	Pfalz& Bauer, New York, U.S.A.
	p-*n*-Propylphenol	K& K Labs.
	2,3,5,6-Tetramethylphenol	EGA-Chemie, Steinheim, G.F.R.
	p-*tert*.-Butylphenol	Fluka
	p-Ethylguaiacol	K& K Labs.
2	*o*-Ethylphenol	Fluka
	2,5-Dimethylphenol	Fluka
	o-*n*-Propylphenol	K& K Labs.
	o-Allylphenol	Pfalz& Bauer
	m-Ethylphenol	Fluka
	Guaiacol	Polak's Frutal Works, Amersfoort, The Netherlands
	2,3,5-Trimethylphenol	Fluka
	p-Methylguaiacol	Merck
	p-*sec*.-Butylphenol	Aldrich-Europe, Beerse, Belgium
	Eugenol	Fluka

Preparation of 2,4-dinitrophenyl ether derivatives on the micro-scale

Because all of the phenols studied cannot be completely separated by OV-210 or SP-2100 capillary columns, they were divided into two groups (Table I) and analysed separately.

The preparation procedure was developed from the method of Cohen *et al.*[12]. A phenol mixture (15 ml of a 45% aqueous ethanolic solution containing 0.08–0.95 mg/l of each phenol in the group) was pipetted into a pear-shaped flask and 1-fluoro-2,4-dinitrobenzene (1 ml of a 2% ethanolic solution) and buffer (10 ml, pH 11.5) were added. The mixture was shaken briefly and left to stand at 50°C for 40 min. The ethers formed were extracted with *n*-hexane, the separated *n*-hexane layer was dried on a sodium sulphate column and 1 μl of the extract was taken for GC analysis.

RESULTS AND DISCUSSION

Reaction conditions

The pH and temperature of the reaction mixture were found to have substantial effects on the rate of formation of the 2,4-dinitrophenyl ethers. It can be seen from Table II that the optimal pH for the reaction is about 11.5, which agrees well with the results of Cook *et al.*[13].

TABLE II

INFLUENCE OF pH ON THE FORMATION OF THE 2,4-DINITROPHENYL ETHERS OF PHENOLS

Mixture No.	*Phenol*	*Area of phenol peak/area of internal standard* peak*						
		pH 8.6	*pH 9.6*	*pH 10.1*	*pH 11.0*	*pH 11.6*	*pH 12.1*	*pH 12.8*
1	Phenol	0.15	0.66	0.83	1.09	1.10	0.63	0.14
	o-Cresol	0.05	0.27	0.48	0.77	0.81	0.44	0.09
	2,6-Dimethylphenol	0.03	0.13	0.20	0.29	0.39	0.21	0.03
	m-Cresol	0.17	0.70	0.91	1.09	1.07	0.70	0.19
	p-Cresol	0.14	0.77	1.12	1.19	1.23	0.95	0.25
	2,4-Dimethylphenol	0.13	0.27	0.47	0.78	0.75	0.51	0.12
	3,5-Dimethylphenol	0.26	0.83	1.10	1.62	1.48	1.02	0.25
	2,3-Dimethylphenol	0.15	0.34	0.56	1.01	1.17	0.71	0.15
	p-Ethylphenol	0.29	0.83	1.03	1.25	1.21	0.99	0.31
	p-Isopropylphenol	0.17	0.60	0.72	0.88	0.85	0.73	0.23
	p-*n*-Propylphenol	0.34	0.83	0.99	1.14	1.13	1.03	0.33
	2,3,5,6-Tetramethylphenol	0.06	0.08	0.12	0.41	0.73	0.53	0.11
	p-tert.-Butylphenol	0.31	0.87	1.09	1.28	1.23	1.12	0.35
	p-Ethylguaiacol	0.44	1.24	1.57	1.63	1.69	1.54	0.47
2	*o*-Ethylphenol	0.08	0.41	0.94	1.42	1.60	0.89	0.17
	2,5-Dimethylphenol	0.07	0.24	0.58	0.86	0.92	0.57	0.12
	o-*n*-Propylphenol	0.15	0.64	1.51	2.41	2.68	1.59	0.28
	o-Allylphenol	0.11	0.40	0.80	1.04	1.09	0.65	0.12
	m-Ethylphenol	0.32	1.16	1.61	1.69	1.65	1.34	0.31
	Guaiacol	0.57	2.27	3.22	3.54	3.40	2.34	0.48
	2,3,5-Trimethylphenol	0.13	0.47	1.00	1.69	1.89	1.34	0.36
	p-Methylguaiacol	0.22	0.57	0.62	0.68	0.68	0.67	0.23
	p-sec.-Butylphenol	0.67	1.13	1.37	1.35	1.42	1.37	0.78
	Eugenol	0.69	1.66	1.90	1.90	2.06	1.90	0.58

* The 2,4-dinitrophenyl ether internal standard used in mixture 1 was of 3,4-dimethylphenol and that in mixture 2 of *o*-cresol.

Table III shows that the reaction time is influenced to some extent by the position of the alkyl and alkoxy substituents in the phenolic ring, with the *o*-substituted phenols reacting considerably more slowly. This is well illustrated by 2,6-dimethylphenol and 2,3,5,6-tetramethylphenol, which have two *ortho* substituents; it appears that steric hindrance contributes to the reduced reaction rate. In general, the reaction rate for a substituted phenol seems to increase in the order $o < m < p$.

It can be seen from Table III that suitable reaction conditions are 40 min at 50°C, using which all of the phenols examined had reacted to completion.

Gas chromatography

The effect of flow-rate of the make-up gas on the response of the ECD is shown in Fig. 1. The optimal flow-rate is 45 ml/min.

Figs. 2 and 3 show chromatograms of the mixture of 2,4-dinitrophenyl ethers on the OV-210 and SP-2100 columns respectively. The peaks are not as sharp as those often obtained on capillary columns, and there is some peak tailing. This has been shown by Fitzpatrick *et al.*[19] to be a consequence of the detector dimensions.

TABLE III

DEPENDENCE OF THE TIME REQUIRED TO REACH MAXIMAL CONVERSION ON REACTION TEMPERATURE

Mixture No.	*Phenol*	*Time required (min)*						
		20°C	*25°C*	*30°C*	*40°C*	*50°C*	*60°C*	*70°C*
1	Phenol	50	40	40	30	10	10	5
	o-Cresol	120	120	30	30	20	10	5
	2,6-Dimethylphenol	>120	>120	>120	>120	40	30	10
	m-Cresol	50	40	30	10	10	10	5
	p-Cresol	40	10	10	10	10	5	5
	2,4-Dimethylphenol	60	50	40	20	10	10	5
	3,5-Dimethylphenol	60	40	40	10	10	10	5
	2,3-Dimethylphenol	>120	120	50	30	30	10	10
	p-Ethylphenol	20	10	10	10	5	5	5
	3,4-Dimethylphenol	10	10	10	5	5	5	5
	p-Isopropylphenol	10	10	5	5	5	5	5
	p-*n*-Propylphenol	20	10	5	5	5	5	5
	2,3,5,6-Tetramethylphenol	>120	120	50	30	30	30	10
	p-*tert*.-Butylphenol	20	10	10	10	5	5	5
	p-Ethylguaiacol	10	10	5	5	5	5	5
2	*o*-Ethylphenol	120	50	40	30	10	10	5
	2,5-Dimethylphenol	120	50	40	30	10	10	5
	o-*n*-Propylphenol	120	50	40	30	10	10	5
	o-Allylphenol	120	40	40	30	10	10	5
	m-Ethylphenol	20	20	20	5	5	5	5
	Guaiacol	20	20	20	10	10	5	5
	2,3,5-Trimethylphenol	50	40	20	10	10	5	5
	p-Methylguaiacol	5	5	5	5	5	5	5
	p-*sec*.-Butylphenol	10	10	5	5	5	5	5
	Eugenol	10	10	5	5	5	5	5

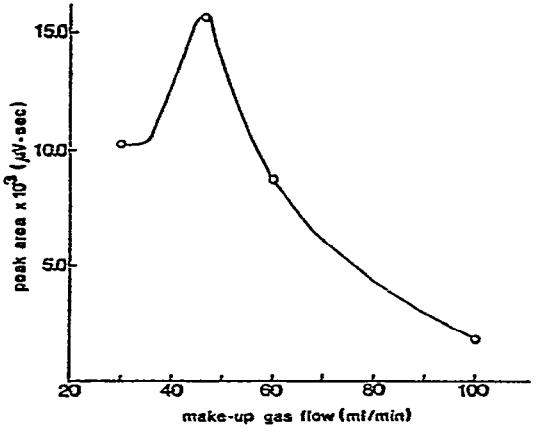

Fig. 1. Dependence of ECD sensitivity on the flow-rate of make-up gas.

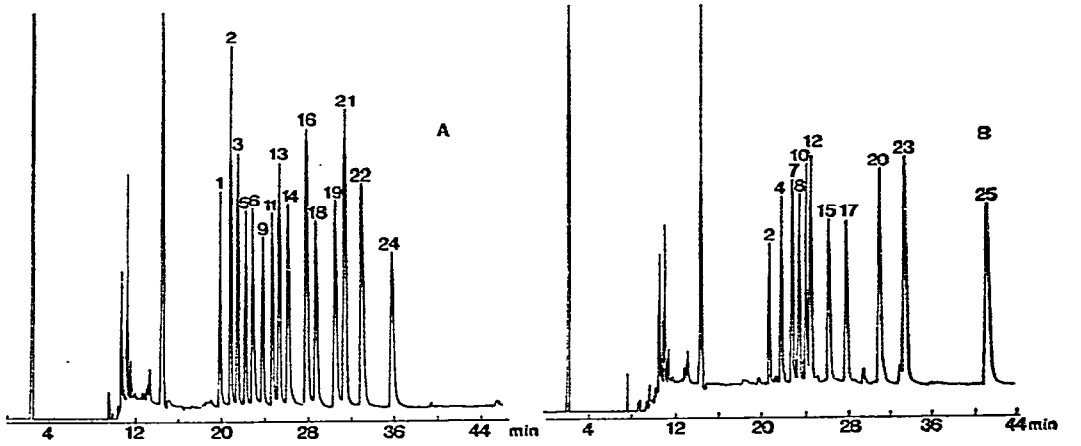

Fig. 2. Gas chromatograms of (A) mixture 1 and (B) mixture 2 of 2,4-dinitrophenyl ethers of phenols using an OV-210 glass capillary column. For peak identifications see Table IV.

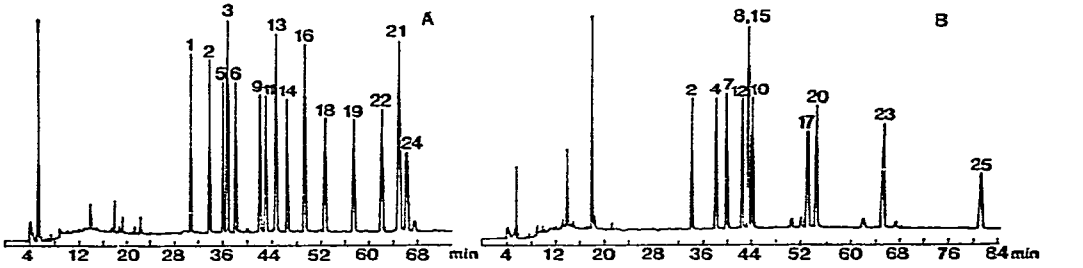

Fig. 3. Gas chromatograms of (A) mixture 1 and (B) mixture 2 of 2,4-dinitrophenyl ethers of phenols using an SP-2100 fused silica capillary column. For peak identifications see Table IV.

Further, it is apparent that retention times on the SP-2100 column are substantially longer than on the OV-210 column.

Table IV gives the retention times of the derivatives and the response factors, both with reference to the 2,4-dinitrophenyl ether of *o*-cresol, and the detector sensitivity. With each column the nature and position of the substituents in the parent phenol markedly affect the retention times of the derivatives, increasing in the order $o < m < p$. A corresponding *ortho* effect has also been reported as normal for free phenols[12]. Considering the nature of the substituents, the retention time increases with carbon number, is slightly lower for branched compared than straight chains, and unsaturated substituents result in longer retention times than the corresponding saturated groups. The retention times of derivatives containing more than one unit of the same substituent depend on their combined effect. For dimethyl-substituted phenol ethers, for example, it increases in the order 2,6- < 2,5- < 2,4- < 3,5- < 2,3- < 3,4-. As typified by the methyl-substituted phenyl ethers, the retention time increases with increasing number of substituents, as expected.

Both columns successfully resolve *o*-, *m*- and *p*-substituted phenyl ethers, which is often not possible with the parent phenols. Moreover, the dimethyl isomers

TABLE IV

RELATIVE RETENTION TIMES (RRT) AND GC RESPONSE FACTORS (RF) RELATIVE TO *o*-CRESOL 2,4-DINITROPHENYL ETHER, AND ECD SENSITIVITY (ES) OF PHENOL 2,4-DINITROPHENYL ETHERS

Mixture No.	*Parent phenol*	*Peak No.**	*RRT*		*RF*	*ES***
			OV-210	*SP-2100*		*($g \cdot 10^{-9}$)*
1	Phenol	1	0.954	0.908	1.294	0.01
	o-Cresol	2	1.000	1.000	1.000	0.01
	2,6-Dimethylphenol	3	1.031	1.081	0.916	0.01
	m-Cresol	5	1.064	1.066	1.124	0.01
	p-Cresol	6	1.096	1.120	1.282	0.01
	2,4-Dimethylphenol	9	1.139	1.237	1.115	0.01
	3,5-Dimethylphenol	11	1.183	1.265	1.455	0.02
	2,3-Dimethylphenol	13	1.207	1.314	1.161	0.02
	p-Ethylphenol	14	1.250	1.366	1.537	0.02
	3,4-Dimethylphenol	16	1.329	1.452	1.546	0.03
	p-Isopropylphenol	18	1.369	1.549	1.524	0.02
	p-n-Propylphenol	19	1.455	1.690	1.919	0.02
	2,3,5,6-Tetramethylphenol	21	1.497	1.907	1.379	0.04
	p-tert.-Butylphenol	22	1.569	1.825	1.772	0.03
	p-Ethylguaiacol	24	1.703	1.944	2.316	0.03
2	*o*-Ethylphenol	4	1.054	1.122	0.921	0.01
	2,5-Dimethylphenol	7	1.102	1.174	0.989	0.01
	o-n-Propylphenol	8	1.131	1.279	0.928	0.01
	o-Allylphenol	10	1.163	1.298	0.962	0.02
	m-Ethylphenol	12	1.182	1.251	0.892	0.02
	Guaiacol	15	1.261	1.279	1.447	0.02
	2,3,5-Trimethylphenol	17	1.337	1.561	1.794	0.03
	p-Methylguaiacol	20	1.477	1.599	1.699	0.05
	p-sec.-Butylphenol	23	1.586	1.915	1.727	0.06
	Eugenol	25	1.956	2.389	2.444	0.09

* See Figs. 2 and 3.

** Electron-capture sensitivity expressed as the amount of derivative injected to produce a peak with a height 5% of full scale at a baseline noise level of 1%.

can be separated from each other, although, as revealed by Table IV, neither column completely resolves all of the phenyl ethers investigated.

The effect of the nature and position of substituents on the response factors varies according to the retention times.

Table IV also gives the sensitivity of the Hewlett-Packard ^{63}Ni ECD, which is higher than that reported by Cohen *et al.*[12]. The reduced sensitivity at longer retention times is a consequence of peak broadening.

Reproducibility of the determination

The reproducibility of the method is shown in Table V, which summarizes the results of four independent determinations. The coefficient of variation in the concentration range 0.08–0.95 mg/l is about 5%, which means that the method is suitable for the quantitative determination of phenols.

TABLE V

ACCURACY AND REPRODUCIBILITY OF THE METHOD

Average of four independent determinations.

Mixture No.	*Parent phenol*	*Amount added (mg/l)*	*Amount found (mg/l)*	*Standard deviation (mg/l)*
1	Phenol	0.08	0.09	0.004
	2.6-Dimethylphenol	0.33	0.38	0.013
	m-Cresol	0.09	0.09	0.002
	p-Cresol	0.11	0.12	0.005
	2,4-Dimethylphenol	0.10	0.11	0.019
	3,5-Dimethylphenol	0.15	0.17	0.018
	2,3-Dimethylphenol	0.16	0.16	0.006
	p-Ethylphenol	0.17	0.18	0.006
	3,4-Dimethylphenol	0.26	0.29	0.026
	p-Isopropylphenol	0.19	0.20	0.004
	p-n-Propylphenol	0.19	0.19	0.002
	2,3,5,6-Tetramethylphenol	0.95	0.95	0.034
	p-tert.-Butylphenol	0.36	0.41	0.035
	p-Ethylguaiacol	0.26	0.25	0.002
2	*o*-Ethylphenol	0.08	0.08	0.001
	2,5-Dimethylphenol	0.15	0.15	0.001
	o-n-Propylphenol	0.15	0.15	0.003
	o-Allylphenol	0.16	0.16	0.003
	m-Ethylphenol	0.11	0.11	0.003
	Guaiacol	0.12	0.12	0.006
	2,3,5-Trimethylphenol	0.21	0.21	0.004
	p-Methylguaiacol	0.27	0.26	0.014
	p-sec.-Butylphenol	0.25	0.25	0.014
	Eugenol	0.26	0.25	0.022

CONCLUSION

The method described can be applied successfully to the quantitative determination of samples containing very low levels of phenols. The resolution of derivatives containing several isomers is readily accomplished and, because a selective detector is used, the phenyl derivatives can be easily identified in a complex mixture containing components towards which the ECD is insensitive. The method is to be used to analyse alcoholic beverages.

REFERENCES

1 J. Hrivnak and J. Macák, *Anal. Chem.*, 43 (1971) 1039.
2 A. Di Corcia, *J. Chromatogr.*, 80 (1973) 69.
3 R. W. Souter and R. H. Bishara, *J. Chromatogr.*, 140 (1977) 245.
4 J. Buryan, J. Macák and V. M. Nabivach, *J. Chromatogr.*, 148 (1978) 203.
5 Y. Hoshika and G. Muto, *J. Chromatogr.*, 157 (1978) 277.
6 A. T. Shulgin, *Anal. Chem.*, 36 (1964) 920.
7 R. J. Argauer, *Anal. Chem.*, 40 (1968) 122.
8 F. K. Kawahara, *Anal. Chem.*, 40 (1968) 1009.
9 L. G. Johnson, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1503.
10 J. N. Seiber, D. G. Crosby, H. Fouda and C. J. Soderquist, *J. Chromatogr.*, 73 (1972) 89.

11 L. L. Lamparski and T. J. Nestrick, *J. Chromatogr.*, 156 (1978) 143.
12 I. C. Cohen, J. Norcup, J. H. A. Ruzicka and B. B. Wheals, *J. Chromatogr.*, 44 (1969) 251.
13 R. F. Cook, J. E. Jackson, J. M. Shuttleworth, O. H. Fullmer and G. H. Fujie, *J. Agr. Food Chem.*, 25 (1977) 1013.
14 G. Alexander, G. Garzó and G. Pályi, *J. Chromatogr.*, 91 (1974) 25.
15 H. T. Badings, J. J. van der Pol and J. G. Wassink, *Chromatographia*, 8 (1975) 440.
16 G. Schomburg and H. Husmann, *Chromatographia*, 8 (1975) 517.
17 T. Teorell and E. Stenhagen, *Biochem. Z.*, 299 (1939) 417.
18 J. D. Reinheimer, J. P. Douglass, H. Leister and M. B. Voelkel, *J. Org. Chem.*, 22 (1957) 1743.
19 F. A. Fitzpatrick, D. A. Stringfellow, J. Maclouf and M. Rigaud, *J. Chromatogr.*, 177 (1979) 51.