Journal of Chromatography, 295 (1984) 299–303
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,767

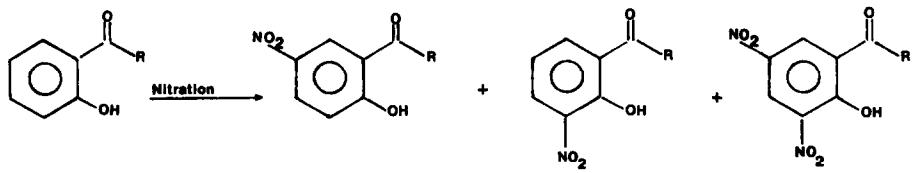
Note

Thin-layer and high-performance liquid chromatographic identification of some nitrohydroxyacylphenones

ELENA GAGLIARDO*, GIOVANNI MANTEGAZZA and PAOLO ORIGGI
Research and Development Reagents, Analytical Division, Farmitalia Carlo Erba, Via Imbonati 24, 20159 Milan (Italy)
and
EMILIO LAZZARI
Institut of Pharmaceutical and Toxicologycal Chemistry, University of Bologna, Bologna (Italy)
(First received September 20th, 1983; revised manuscript received March 20th, 1984)

The nitro derivatives of *o*-hydroxyacylphenones[1–3] are valuable intermediates in organic synthesis, *e.g.*, for the preparation of nitrochromones and flavones[2] and more recently as starting materials for new types of alkylating agents[4–6]. These compounds raise some interesting analytical problems in their high-performance liquid chromatographic (HPLC) identification as possible contaminants in the final products. The nitration of *o*-hydroxyacylphenones gives mixtures of 3-nitro, 5-nitro and 3,5-dinitro derivatives, the relative amounts of which can be evaluated by HPLC.

Some examples of the separation of phenolic compounds by HPLC have been reported[7–11], but, to our knowledge, there are no examples of chromatographic separations of the nitro derivatives of *o*-hydroxyaceto-, propio- and -butyrophenone. The nitration reaction is shown in Scheme I.



Scheme I. Scheme of nitration of the hydroxyacylphenones.

The purpose of this work was to study the conditions for the separation of this type of phenolic compound by HPLC, after prior investigation by thin-layer chromatography (TLC).

0021-9673/84/$03.00

EXPERIMENTAL

Apparatus

An SP 8000 B liquid chromatograph equipped with a data system, an automatic injector (10-μl loop) and an SP 8400 variable-wavelength UV–visible detector was employed.

Chemicals

Reagents and solvents were obtained from Carlo Erba. The methanol, chloroform *n*-hexane and water were HPLC-grade solvents.

Stratocrom SIF $_{254}C_{18}W$ TLC plates were used. The standards for chromatography were of reagent-grade purity. TLC of the nitro derivatives was carried out using 20 × 20 glass plates with a 0.25 mm layer of silanized silica C_{18}.

The concentration of solutions of the standards was 10^{-3} *M* in methanol–water (6:4). The concentration of the nitration mixtures was 50 mg per 100 ml in the same solvent. The mixture and the standards were applied to the plates using microsampling pipettes: 5 μl for the solutions of standards, 10 μl for the nitration mixture of aceto- and propiophenones and 30 μl for the nitration mixture of butyrophenones. In the HPLC investigations the same solutions of standards were used and 10 μl were injected.

HPLC column and support

The support was 10-μm silica ODS. The bare silica was home-made[12]. The characteristics of bare silica are as follows: specific surface area, 500 m^2/g; pore volume, 0.8 ml/g; and mean pore diameter, 60 Å. The specific surface area and the porosity were determined by using Sorptomatic 1800 and Porosimeter 200 instruments (Carlo Erba). The mean particle size was determined with a Coulter Counter. The 10-μm silica was derivatized with octadecylchlorosilane and trimethylchlorosilane as described previously[13,14]. The stationary phase was further characterized by its carbon content, determined by elemental analysis to be 22%.

TLC investigation prior to HPLC separation

The behaviour of the nitro derivatives on thin-layer plates of octadecylsilica was investigated using different eluent mixtures. The best results were obtained with chloroform–*n*-hexane (1:9) and good results were also obtained with methanol–water (65:35) (see Table I).

The chromatograms were developed to a distance of 15 cm and the nitro derivatives were revealed under UV light (250 nm).

HPLC investigation

After the trial TLC study, HPLC of the nitro derivatives using similar solvents was studied on a 250 × 4.6 mm I.D. Column of 10-μm C_{18} silanized silica. Chloroform–*n*-hexane (1:9) was not suitable, because the peaks were eluted with the solvent front. Even when the proportion of chloroform was reduced in order to increase the retention of the sample compounds the peaks were tailed and the resolution was poor.

The best results were obtained with methanol–water, as follows: for nitrohy-

TABLE I

R_F VALUES OBTAINED USING DIFFERENT ELUENTS

Compound	*Eluent*	
	Methanol–water (65:35)	*n-Hexane–chloroform (90:10)*
o-Hydroxyacetophenone	0.35	0.83
3-Nitro-*o*-hydroxyacetophenone	0.69	0.69
5-Nitro-*o*-hydroxyacetophenone	0.73	0.40
3,5-Dinitro-*o*-hydroxyacetophenone	0.76	0.07*
o-Hydroxypropiophenone	0.27	0.79
3-Nitro-*o*-hydroxypropiophenone	0.61	0.67
5-Nitro-*o*-Hydroxypropiophenone	0.66	0.41
3,5-Dinitro-*o*-hydroxypropiophenone	0.75	0.00*
o-Hydroxybutyrophenone	0.19	0.80
3-Nitro-*o*-hydroxybutyrophenone	0.48	0.65
5-Nitro-*o*-hydroxybutyrophenone	0.55	0.38
3,5-Dinitro-*o*-hydroxybutyrophenone	0.74	0.00*

* The 3,5-dinitro derivatives can be moved from the starting point using as the eluent *n*-hexane–chloroform (80:20); however, the other compounds cannot be separated by this means.

droxyacetophenones, methanol–water (8:2), flow-rate 0.7 ml/min; for nitrohydroxypropio- and -butyrophenone, methanol–water (7:3), flow-rate 0.9 ml/min.

Water (10 μl) was injected under the same chromatographic conditions in order to determine the dead time (t_0). The results are given in Table II and Figs. 1–3 show the chromatograms obtained.

TABLE II

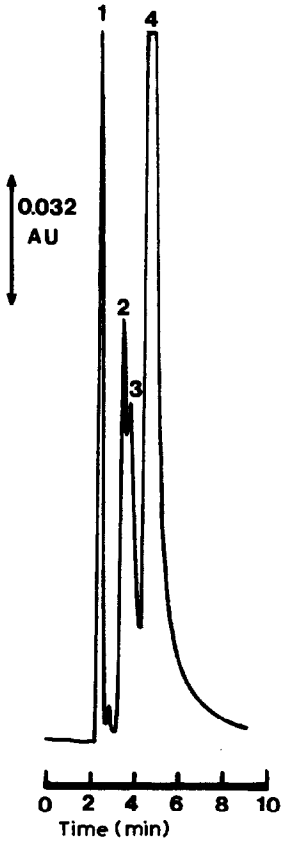
RETENTION TIMES AND RESOLUTION DATA FOR SEPARATION OF NITROHYDROXYACYLPHENONES ON A 10-μm C_{18} COLUMN

For nitro-*o*-hydroxyacetophenones, flow-rate = 0.7 ml/min and dead time (t_0) = 1.39 min. For nitro-*o*-hydroxypropio- and butyrophenones, flow-rate = 0.9 ml/min and dead time (t_0) = 1.40 min.

Compound	k'	α	R_s
3,5-Dinitro-*o*-hydroxyacetophenone	0.31		
3-Nitro-*o*-hydroxyacetophenone	0.92	2.9	5.5
5-Nitro-*o*-hydroxyacetophenone	1.08	1.2	1.5
o-Hydroxyacetophenone	1.56	1.4	3.1
3,5-Dinitro-*o*-hydroxypropiophenone	0.36		
3-Nitro-*o*-hydroxypropiophenone	2.35	6.52	10.5
5-Nitro-*o*-hydroxypropiophenone	3.56	1.21	2.4
o-Hydroxypropiophenone	4.25	1.19	2.3
3,5-Dinitro-*o*-hydroxybutyrophenone	0.49		
3-Nitro-*o*-hydroxybutyrophenone	4.62	9.4	13.0
5-Nitro-*o*-hydroxybutyrophenone	8.00	1.73	6.6
o-Hydroxybutyrophenone	10.76	1.34	4.1

RESULTS AND DISCUSSION

Comparison of the k' values of nitro derivatives shows that the retention increases from the 3-nitro derivatives to the un-nitrated starting compounds. This is due to the mesomeric effect ($-M$) of the nitro group in *ortho* and *para* positions to the hydroxy group. The introduction of a second nitro group into a *para* or *ortho* position further weakens the retention owing to the increase in molecular polarity, which increases the affinity to the mobile phase. The 3,5-dinitro derivatives are the least retained compounds. The weaker retention of 3-nitro (*ortho*) derivatives in comparison with 5-nitro (*para*) derivatives is presumably due to the formation of an intramolecular hydrogen bond; 3-nitro derivatives are slightly more hydrophobic and are more retained.



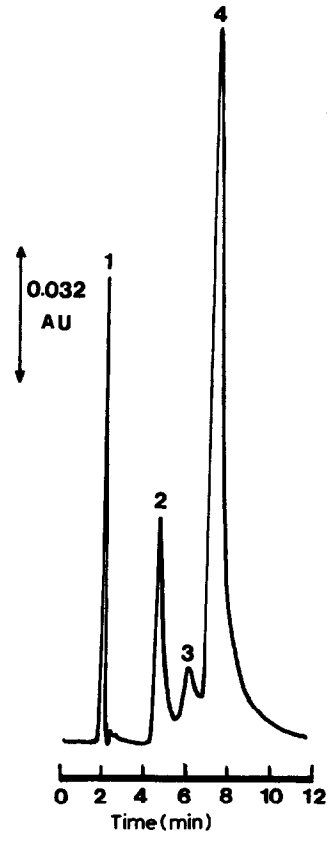


Fig. 1. Chromatogram of separation of nitro derivatives of acetophenones on a C_{18} column at a flow-rate of 0.7 ml/min of methanol–water (8:2) with UV detection at 254 nm. Peaks: (1) 3,5-dinitro-; (2) 3-nitro-; (3) 5-nitro-*o*-hydroxyacetophenone; (4) *o*-hydroxyacetophenone.

Fig. 2. Chromatogram of separation of nitro derivatives of propiophenones on a C_{18} column at a flow-rate of 0.9 ml/min of methanol–water (7:3) with UV detection at 254 nm. Peaks: (1) 3,5-dinitro-; (2) 3-nitro-; (3) 5-nitro-*o*-hydroxypropiophenone; (4) *o*-hydroxypropiophenone.

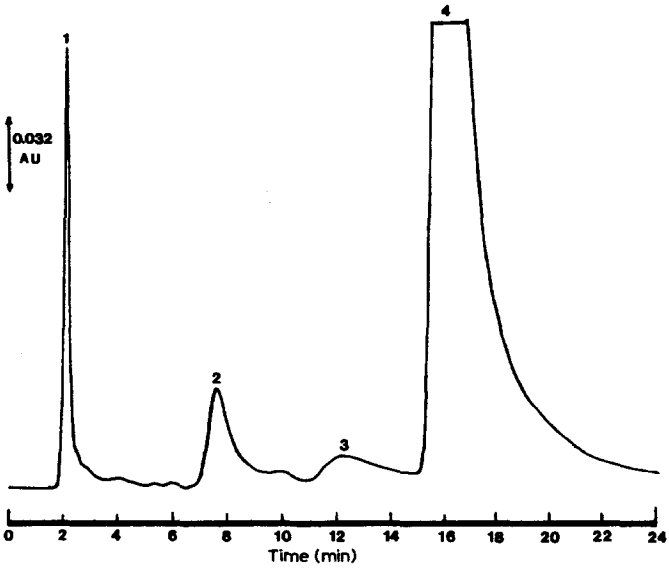


Fig. 3. Chromatogram of separation of nitro derivatives of butyrophenones on a C_{18} column at a flow-rate of 0.9 ml/min methanol–water (7:3) with UV detection at 254 nm. Peaks: (1) 3,5-dinitro-; (2) 3-nitro-; (3) 5-nitro-*o*-hydroxybutyrophenone; (4) *o*-hydroxybutyrophenone.

The greater retention of butyrophenone derivatives compared with the corresponding propio- and acetophenones is due the effect of the alkyl chain (methyl, ethyl, propyl) bonded to the carbonyl group.

REFERENCES

1 S. S. Joshi and H. Singh, *J. Amer. Chem. Soc.*, 76 (1954) 4993.
2 P. Da Re, P. Valenti, G. Ceccarelli and G. Caccia, *Ann. Chim. (Rome)*, 60 (1970) 215.
3 P. Da Re, L. Verlicchi and I. Setnikar, *J. Med. Chem.*, 10 (1967) 266.
4 A. Andreani, M. Ramboldi, D. Bonazzi, G. Fabbri, R. Bossa and I. Galatulos, *Eur. J. Med. Chem.*, 18 (1983) 351.
5 A. Andreani, M. Ramboldi, D. Bonazzi, G. Fabbri, R. Bossa and I. Galatulos, *J. Pharm. Sci.*, 72 (7) (1983) 814.
6 A. Andreani, M. Ramboldi, D. Bonazzi, G. Fabbri, R. Bossa and I. Galatulos, *Arch. Pharm. (Weinheim)*, 316 (2) (1983) 141.
7 J. B. Murphy and C. A. Stutte, *Anal. Biochem.*, 86 (1978) 220.
8 J. M. Hardin and C. A. Stutte, *Anal. Biochem.*, 102 (1980) 124.
9 L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, 116 (1976) 271.
10 M. Vanhaelen and R. Vanhaelen-Fastré, *J. Chromatogr.*, 187 (1980) 255.
11 M. W. Wilkinson, G. Sweeney and G. A. Iacobuca, *J. Chromatogr.*, 132 (1977) 349.
12 R. K. Iler and E. Matisevic, *Surface and Colloid Science*, Vol. 6, Wiley, London, 1973.
13 I. J. Kirkland and J. J. De Stefano, *J. Chromatogr. Sci.*, 8 (1970) 309.
14 P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 149 (1978) 211.