CHROM. 5265

A CRITICAL EVALUATION OF SOME FACTORS INFLUENCING THE QUANTITATIVE ISOLATION AND CHARACTERISATION OF CARBONYL COMPOUNDS FROM OXIDISING FATS

J. D. CRASKE
Unilever Australia Pty. Ltd., Balmain, 2041 (Australia)
AND
R. A. EDWARDS
The University of New South Wales, Kensington (Australia)

(Received January 18th, 1971)

SUMMARY

The dinitrophenylhydrazine–phosphoric acid–Celite column first proposed by SCHWARTZ for the conversion of carbonyl compounds in oxidising fats to their dinitrophenylhydrazones (DNPs) has been studied and a number of factors which can influence the stoichiometry of its performance are critically evaluated. It is concluded that the column should be considered to be both an equilibration and a reaction column and that the high yields of monocarbonyl hydrazones obtained in practice arise because of the mass action effect of the high concentration of dinitrophenylhydrazine and low concentration of free water. Since equilibrium in a reversible reaction can be achieved from either direction, it is shown that, to ascertain the degree of conversion of carbonyl compounds in a SCHWARTZ column, the simplest method is to apply a known amount of the dinitrophenylhydrazone itself and to determine the yield of hydrazone eluted from the column under the selected experimental conditions. By considering the DNP to be a form of stabilised aldehyde, the problems inherent in accurately applying to a column a known amount of a labile compound can be circumvented.

Whilst the importance of careful pretreatment of solvents to render them carbonyl-free has previously been stressed, it is now shown that it is of equal importance to employ stable solvents that will remain carbonyl-free in use. Cyclohexane and benzene are relatively stable solvents and methods for their purification are discussed.

Alumina has been found to be an unreliable adsorbent for the isolation of DNPs, giving recoveries both higher and lower than the theoretical value. Unreacted dinitrophenylhydrazine, a small amount of which is eluted from the SCHWARTZ column together with the DNPs, and which is strongly adsorbed to alumina, can act with alumina as a reaction column and generate extra DNPs from traces of spurious carbonyls present in the solvents used for elution. The ion-exchange method of SZONYI has been found to be a more reliable technique for purifying DNPs from unreacted dinitrophenylhydrazine and from fat.

Observance of the above factors and a number of minor modifications has given rise to a carbonyl determination method of improved quantitative reliability.

INTRODUCTION

It is now well established that the first reaction to take place in autoxidising fats is a free radical chain reaction involving attack at the methylene groups adjacent to the double bonds and leading to the formation of hydroperoxides. A detailed discussion of autoxidation is beyond the scope of this paper, being extensively treated by a number of authors[1–3]. Whilst the hydroperoxides are odourless, they are labile and undergo a variety of secondary reactions to form high-molecular-weight products by further oxidation and/or polymerisation, and lower-molecular-weight products by reactions such as scission or dehydration. From the standpoint of the onset of reversion flavour in fats, one of the most important secondary reactions is that of hydroperoxide scission, which can lead to the generation, *inter alia*, of carbonyl compounds of intense flavour.

Of the many methods published for the isolation of carbonyl compounds from, or their determination in autoxidising fats, three approaches are the most common, *viz.* distillation[4–9], conversion to water-soluble Girard hydrazones[10,11] and conversion to 2,4-dinitrophenylhydrazones (DNPs)[12–15].

Distillation offers the advantage that volatile components other than carbonyl compounds can be isolated. On the other hand, non-volatile carbonyl compounds cannot be determined and, whilst they probably do not contribute to flavour, their characterisation may be of importance in a study of the oxidation process. Furthermore, oxidising fats may generate a wide variety of carbonyl compounds ranging from very volatile to non-volatile and, by distillation techniques, it is difficult to guarantee quantitative recovery of the highly volatile components and also complete vaporisation of those compounds of high boiling point. Furthermore, distillation may give rise to artifacts by scission of hydroperoxides.

The Girard hydrazone technique has not found favour, due to doubts about whether quantitative conversion of micro quantities of carbonyl compounds can be achieved and whether different classes of carbonyl compounds convert to the same degree. The DNP has become the derivative of most popular choice as it is readily formed in high yield, individual derivatives can now be separated on the basis of both class and chainlength by a variety of chromatographic techniques, and characterisation is readily accomplished both by IR and UV spectrophotometric techniques.

Early methods for the conversion of micro amounts of carbonyl compounds to their DNPs were adaptations of the classical technique for preparation of the derivative on a macro scale[16], *viz.* acid-catalysed reaction in solution. Quantitative estimation was effected spectrophotometrically. LAPPIN AND CLARK[17] measured the absorbance of the wine-red colour formed from the DNP in alkaline medium and HENICK *et al.*[13] adapted this method to lipids by employing a reaction medium comprising trichloroacetic acid in benzene, in which fat is freely soluble. They also differentiated between saturated and unsaturated carbonyl compounds by measuring the absorbance at two wavelengths. LOHMAN[12] separated the DNPs from the excess of dinitrophenylhydrazine by extraction into petroleum ether, in which solvent the

reagent is insoluble. He noted that the DNP is more stable in neutral than in alkaline solution.

A reaction column technique was first employed by POOL AND KLOSE[14] for the estimation of monocarbonyl compounds in rancid foods. They deposited dinitrophenylhydrazine on the top section of an alumina column, using benzene to elute the DNPs and the lower part of the alumina column to retain unchanged reagent.

The column technique was further developed by HAVERKAMP BEGEMANN AND DE JONG[15], who compared a number of methods for preparing DNPs on a micro scale. Combining the best features of each, they advocated percolation of a petroleum solution of the carbonyl compound through a column composed of 2,4-dinitrophenylhydrazine hydrochloride supported on Celite. It was later shown by HORIKX[18] that this column causes quantitative decomposition of oleate hydroperoxides into monocarbonyl compounds and therefore is not applicable to carbonyl determination in oxidising lipids.

SCHWARTZ *et al.*[19] proposed a column of dinitrophenylhydrazine–phosphoric acid–Celite (SCHWARTZ column) for the quantitative conversion of carbonyls to DNPs, claiming that it does not convert hydroperoxides to carbonyl compounds. They also outlined a sequence of column chromatographic techniques whereby monocarbonyl compounds were isolated from fat, unchanged reagent and other classes of carbonyl compounds and then sequentially separated according to class and chainlength into individual components.

Of all the methods for carbonyl determination published to date, that of SCHWARTZ *et al.*[19] appears to be the most comprehensive and logical. However, when used in this laboratory for the examination of oxidising animal fats, a number of problems arose which could not be explained readily on the basis of published information. In the present paper, a critical evaluation is presented of a number of factors that can influence the results obtained when using the SCHWARTZ method.

EXPERIMENTAL

DNP–phosphoric acid–Celite columns were prepared as described by SCHWARTZ AND PARKS[20]. Dinitrophenylhydrazine used for the preparation of SCHWARTZ columns was purified by recrystallisation from benzene. The concentration of the phosphoric acid was determined by titration and the amount used for the preparation of a SCHWARTZ column adjusted to be equal to that recommended by SCHWARTZ AND PARKS[20]. Two-dimensional thin-layer chromatography (TLC) of DNPs was effected by the method of CRASKE AND EDWARDS[21]. The ion-exchange technique for isolation of DNPs was that of SZONYI[22]. Merck neutral alumina was employed for the isolation of DNPs and was prepared by drying overnight at 130°, deactivating by the addition of 20% (w/w) water and equilibrating overnight before use. Carbonyl-free solvents were prepared by the methods of SCHWARTZ AND PARKS[20], HENICK *et al.*[13], and HORNSTEIN AND CROWE[23] and variations of these methods as mentioned in the text. The method adopted for the assessment of SCHWARTZ column stoichiometry was as follows: Immediately prior to use, the columns (one each for sample and blank) were washed with carbonyl-free benzene saturated with DNP (100 ml) to remove therefrom spurious DNPs and other decomposition products. Benzene was displaced from the column with carbonyl-free cyclohexane (50 ml). The sample was applied in 80 ml

of the same batch of cyclohexane and the eluate collected in a 250-ml volumetric flask. Further solvent was applied until a total of 250 ml eluate had been collected. The flow rate was maintained at 1 ml/min. The yield of DNP was assessed on aliquots of the final eluate either by the method of SZONYI or by direct spectrophotometric measurement with an allowance for the blank reading.

RESULTS AND DISCUSSION

Solvent stability

To achieve familiarity with the various steps in the method published by SCHWARTZ *et al.*[19], a preliminary experiment was carried out by processing a sample of highly rancid beef stearin (P.V. *ca.* 200, carbonyl value *ca.* 600 as measured according to LOHMAN[12], strong paint-like rancid smell). The aim was the generation of a large amount of DNPs from a small sample of fat (0.1 g) and hence initially to minimise any problems that might arise in isolating DNPs from fat. Using as solvent petroleum ether rendered carbonyl-free by the method of SCHWARTZ AND PARKS[20], a bright yellow eluate was obtained from the DNP–phosphoric acid column which, however, continued to elute yellow in colour even after extended percolation of solvent. When left overnight and restarted the following morning, the initial eluate was quite strong in colour and, on further washing, decreased to an equilibrium value, but never became colourless. Careful purification of the column dinitrophenylhydrazine by repeated recrystallisation from a number of solvents did nothing to alleviate the problem. After the majority of the yellow band had been eluted, further washing of the column with a small volume of benzene eluted a strong band of yellow colour and thereafter the column could be eluted nearly colourless with petroleum ether. The yellow colour was not due to highly polar dicarbonyl compounds present in the strongly rancid fat and only slightly soluble in the non-polar eluting solvent since a similar phenomenon occurred in a blank column to which no fat had been applied. Using the two-dimensional TLC method of CRASKE AND EDWARDS[21] and by IR spectrophotometric examination it was shown that the offending yellow compound was acetone DNP which had arisen from decomposition of the petroleum ether used as a solvent. It is presumed, although without unequivocal proof, that acetone is generated by decomposition of branched and/or unsaturated hydrocarbons in the petroleum ether. HAVERKAMP BEGEMANN AND DE JONG commented on the presence in petroleum ether of acetone, which could not be removed by processing through one of their columns and distilling. In the light of the evidence presented above, and coupled with a demonstration reported later in this paper that acetone is quantitatively converted to its DNP on a SCHWARTZ column, it seems probable that this problem also arose as a result of decomposition of the petroleum ether used as solvent.

Further evidence of solvent decomposing to carbonyl compounds was obtained when 2,2,4-trimethylpentane (selected for its high degree of branching) was rendered "carbonyl-free" by SCHWARTZ's method, allowed to remain several days in a SCHWARTZ column and then eluted therefrom with further "carbonyl-free" trimethylpentane. Concentration of the yellow-coloured eluate followed by TLC on a Carbowax-impregnated plate yielded predominantly one compound with an R_F value slightly less than that of 2-octanone DNP. It is presumed that this compound was a branched-

chain carbonyl compound produced from the trimethylpentane in much the same way as acetone was produced from the petroleum ether. However, as concurrent work demonstrated that cyclohexane is a satisfactory solvent, it did not seem constructive to elucidate the exact nature of this DNP. When cyclohexane was used as the solvent, only a very small smount of colour was obtained from the column and by two-dimensional TLC this was identified as cyclohexanone DNP.

It is pertinent to pose the question why this problem should have arisen in the present work when the SCHWARTZ method has been used by a number of workers in the United States without apparent trouble. It is suggested that the severity of the problem could well differ from locality to locality dependent upon the source of the petroleum solvent and its method of purification. It seems reasonable to presume that, in the United States, with a very large throughput and using sophisticated stills, the fraction produced for solvent could well consist largely of *n*-hexane and be free of hydrocarbons unstable in the present analysis. In work carried out subsequent to this finding, cyclohexane was used as solvent, being of a polarity similar to that of petroleum ether, readily obtainable in a pure form, easy to render carbonyl-free and stable in the SCHWARTZ column. Furthermore, any oxidation that might take place would generate cyclohexanone only and, if this were found in an actual fat analysis, it could be immediately ignored as a solvent artifact. Acetone generated spuriously cannot be differentiated from acetone that might have been genuinely produced in the oxidising lipid.

Preparation of carbonyl-free solvents

For the reasons detailed above, no method could be found for rendering locally available petroleum ether carbonyl-free. Even repeated treatment through a SCHWARTZ column followed by distillation did not yield a satisfactory product. On the other hand, cyclohexane could be prepared carbonyl-free both by the method of HENICK *et al.*[13] and by that of SCHWARTZ AND PARKS[20]. The most convenient method has been found to be that of HORNSTEIN AND CROWE[23], which involves percolation of the solvent through a column of sulphuric acid supported on Celite. Solvent so prepared is ready to use, requiring no distillation or other treatment prior to use. When analysed by means of a SCHWARTZ column, the DNP colour formed is very low and has invariably been found to consist solely of cyclohexanone DNP. Such a sulphuric acid column has been used in this laboratory for many months for the preparation of carbonyl-free cyclohexane and, whilst it is now almost black in colour, it still delivers excellent solvent.

Attempts to produce carbonyl-free benzene gave rise to many frustrations and the reason again was found to be the presence of unstable impurities present in the commercial grade used as starting material. When use was made of crystallisable benzene (*i.e.* benzene from which unstable impurities had been removed by treatment with strong sulphuric acid) the majority of troubles disappeared. It is now customary to assess the benzene for suitability as a raw material for preparing carbonyl-free solvent by testing for substances darkened by sulphuric acid[24]. However, the method of HORNSTEIN AND CROWE[23] is not applicable to the preparation of carbonyl-free benzene. During percolation through the column, a small amount of benzene is sulphonated and use of this solvent in a SCHWARTZ column would rapidly change its nature with unknown effect on the fate of hydroperoxides and carbonyls during

analysis. The SCHWARTZ column can be used for benzene purification, but is inconvenient as dinitrophenylhydrazine is moderately soluble in benzene and this limits the yield of solvent obtainable after distillation. The method of HENICK *et al.*[13] has been found to be most convenient and the yield of benzene can be markedly increased without any effect on quality by reducing the amount of dinitrophenylhydrazine by a factor of five. It has been further found that the yield and quality of the solvent can be improved if dissolved water and water of reaction is removed during the initial refluxing period by adding a Dean and Stark water trap to the stillhead.

Although cyclohexane and benzene can be readily made carbonyl-free and are comparatively stable, they will decompose to some extent when allowed to stand in a SCHWARTZ column for any lengths of time between analyses. In order to remove spurious DNPs, it is therefore essential to condition the column immediately prior to every analysis by percolating through it 100 ml of carbonyl-free benzene followed by 50 ml of the solvent in which the analysis is to be carried out.

Isolation of DNPs

The eluate from the SCHWARTZ column contains, in addition to the required monocarbonyl DNPs, the DNPs of glyceridocarbonyls and those of more polar carbonyl compounds, some unchanged reagent and fat. SCHWARTZ *et al.*[19] isolated the DNPs from the fat and unchanged reagent by chromatography on magnesium oxide and then separated monocarbonyl DNPs from those of glyceridocarbonyls using a column of alumina.

Whilst magnesium oxide has been found to be a satisfactory adsorbent for working up fat samples that are only slightly oxidised, a problem arises when the fat is extensively degraded. In this case it is difficult to separate the polar oxidised lipids from the required DNPs. Two difficulties were encountered when attempts were made to recover model compounds by chromatography of their DNPs on alumina. In the first place, any unchanged reagent was held strongly by the adsorbent and formed a reaction column with alumina. (Dinitrophenylhydrazine was used as a reaction column by POOL AND KLOSE[14].) It was thus necessary to ensure that all solvents used were carbonyl-free, failing which spurious DNPs were formed and yields higher than theoretical obtained. On the other hand, recoveries considerably less than theoretical were often obtained and, whilst the precise reason for the low yields was not ascertained, the problem was shown to be less acute when the adsorbent was greatly deactivated with water (20% addition) and when long-chain, low-polarity DNPs, which elute quickly, were processed.

The ion-exchange method of SZONYI[22] was found to give better quantitative recoveries and offered the further advantages that isolation required one column less and that more sophisticated separation of classes of carbonyl compounds could be achieved.

The Schwartz column as an equilibration column

When investigating the stoichiometry of the DNP–phosphoric acid column, SCHWARTZ *et al.*[19] used oximes and semicarbazones of aldehydes and ketones, which can be readily purified and are stable to oxidation, thereby circumventing the problem of applying to the column micro-molar quantities of labile aldehydes in accurately known amounts. Using derivatives of a wide range of carbonyl compounds and

alumina chromatography to isolate the DNPs for estimation, they obtained yields ranging from 93 to 106%. Whilst the approach is ingenious, the scatter in the results is too great to allow the claim made that the column is quantitative and, from the evidence presented, the reason for the deviation from 100% conversion cannot be determined with certainty.

One probable reason for the variability of the results is that alumina chromatography was used for the isolation of the DNPs formed. Experience in this laboratory has shown that, using alumina as an adsorbent, scatter of the order published by SCHWARTZ can be expected, high results being due to contamination with spurious carbonyls and low results to destruction of the DNP on the column. More reliable results can be obtained either by using the ion-exchange technique of SZONYI[22], which has been shown to effect 98–100% recovery of a wide variety of monocarbonyl compounds. In the case of model experiments, where there is no fat to be separated and where a non-polar solvent such as cyclohexane is used for elution, it is possible to take a direct reading of the column eluate, making allowance for a blank reading.

Although this could explain the whole of the variation published, it is pertinent also to question whether it is permissible to assume that a derivative applied to a column will behave in exactly the same fashion as would the parent carbonyl compound under the same experimental conditions. This problem can be avoided if it is assumed that the column is an equilibration column as well as a reaction column, *i.e.* it is proposed that the column serves two major functions, *viz.* to supply dinitrophenylhydrazine to allow derivative formation and to act as a catalyst to ensure that equilibrium is achieved in the reversible reaction:

$$\text{Dinitrophenylhydrazine} + \text{carbonyl} \rightleftarrows \text{DNP} + \text{water}$$

If the theory is valid that the column acts as an equilibration column, it should be possible to achieve the same eluate composition either by adding a carbonyl compound or by adding its DNP, thereby approaching the equilibrium in the reverse direction. PARSONS[25] suggested that the reaction is reversible and demonstrated that when a cyclohexane solution of butanone DNP was applied to a column containing phosphoric acid, but no dinitrophenylhydrazine, the solution was decolorised. However, his proof is not conclusive because he did not identify butanone in the eluate, nor did he determine that the yellow material left behind on the column was dinitrophenylhydrazine and not butanone DNP.

That the column is indeed reversible was shown in the following way. Acetone DNP in cyclohexane was applied to a phosphoric acid column, whereby a yellow band remained near the top of the column and a colourless eluate was obtained. This eluate was shown to contain acetone by passing it through a normal column to produce a yellow-coloured compound, identified by TLC as acetone DNP. The yellow colour left in the phosphoric acid column was shown to be dinitrophenylhydrazine as it was readily eluted with cyclohexane containing 1% of pentanone and this eluate was shown by TLC to contain pentanone DNP.

The generality of this phenomenon was demonstrated in a series of similar experiments, using a variety of DNPs, the only difference being that in the case of all DNPs except that of acetone, 1% acetone in cyclohexane was used to elute the dinitrophenylhydrazine from the phosphoric acid column. In all cases the expected

TABLE I

HYDROLYSIS OF DNPs ON A PHOSPHORIC ACID COLUMN

DNP	*Hydrolysis (%)*
Acetone	96.4
Butanone	98.2
Pentanone	82.3
Heptanone	47.8
Decanone	6.2
Acetaldehyde	18.4
Propionaldehyde	14.4
Octadienal	5.5

DNPs were identified by TLC. The results obtained for degree of hydrolysis by the phosphoric acid column are shown in Table I.

The varying degree of hydrolysis reflects the differing partition coefficient of the DNPs between cyclohexane and 66% phosphoric acid. The shorter-chain DNPs are of more limited solubility in cyclohexane and much more soluble in the phosphoric acid. They are thus more subject to hydrolysis during the time taken to traverse the column. By contrast, it is evident that the longer-chainlength carbonyl compounds will tend to form DNPs more rapidly and completely when processed in the normal manner through a column containing DNP reagent. Thus if it be shown that a short-chain carbonyl can be quantitatively converted to its DNP, it can be assumed that longer-chain homologues will also be quantitatively converted.

The hypothesis was further evaluated by processing butanone, butanone DNP and (to check the validity of SCHWARTZ's assumption) butanone semicarbazone. In each case approximately 16 μmoles were processed through the SCHWARTZ column and one fifth of the eluate was purified by ion exchange. Butanone yielded 96.5% DNP, and the DNP and semicarbazone 100.4% and 101.8%, respectively. When handling crystalline derivatives, it is felt that the experimental technique is accurate to approximately $\pm$ 2%. The somewhat low yield of DNP from butanone can be explained by the difficulty of avoiding losses of highly volatile liquid while making up the ketone solution for application to the column.

The technique of using the DNP as a means of evaluating the stoichiometry of a SCHWARTZ column offers the following advantages:

(1) It is applicable to all carbonyl compounds that form a DNP (providing it has been demonstrated that the carbonyl class reacts reversibly on the SCHWARTZ column).

(2) The experimental work is extremely simple and it is not necessary to know the extinction coefficient of the DNP to calculate the column conversion. Indeed it is not even necessary to know the identity of the compound under investigation as it is quite feasible to evaluate a solution of an unknown isolate for both stoichiometry and reversibility without the need to purify it to a crystalline form.

Using this method, the conversions of the carbonyl compounds listed in Table II were determined using a column prepared according to SCHWARTZ *et al.*[19] (cyclohexane solvent, DNPs not isolated from unchanged reagent, corrected for blank reading).

It is evident that in most cases the conversion is very close to quantitative.

TABLE II

CONVERSION OF CARBONYL COMPOUNDS BY A SCHWARTZ COLUMN

Chainlength	*Conversion (%)*			
	Anal	*2-Enal*	*2,4-Dienal*	*2-One*
1	98.1			
2	99.3			
3	98.0	5.5–14.2		102.9
4		98.7		100.4
5		97.8	60.6	
6	97.2	99.2	96.1	
7			98.6	
11			99.5	

Two notable exceptions are propenal and pentadienal. In a number of experiments carried out with propenal DNP, the yield varied considerably and on examination of the eluate by TLC, a number of coloured spots other than propenal DNP were evident. It is suggested that carbonyl compounds with vinyl groups conjugated to the carbonyl group are polymerised by the SCHWARTZ column and are therefore not amenable to analysis by this method. In confirmation, methyl vinyl ketone DNP was recovered only to the extent of 1.8%. Hex-5-en-2-one DNP, which has a vinyl group not conjugated to the carbonyl group gave a 95.0% yield—low by comparison to the majority of the monocarbonyl DNPs examined, but not as low as those of the conjugated vinyl carbonyl DNPs.

ACKNOWLEDGEMENTS

We are grateful for discussions with Mrs. C. SZONYI; technical assistance by Mrs. J. VAN WEIJE and Miss K. FRANCIS is gratefully acknowledged.

REFERENCES

1 W. O. LUNDBERG (Editor), *Autoxidation and Antioxidants*, Interscience, New York, 1961.
2 H. T. BADINGS, *Neth. Milk Dairy J.*, 14 (1960) 215.
3 D. SWERN, in K. S. MARKLEY (Editor), *Fatty Acids*, Vol. 2, Interscience, 1961, p. 1387.
4 A. M. GADDIS, R. ELLIS AND G. T. CURRIE, *Food Res.*, 24 (1959) 283.
5 N. P. WONG, S. PATTON AND D. FORSS, *J. Dairy Sci.*, 41 (1958) 1699.
6 F. D. HILL AND E. G. HAMMOND, *J. Amer. Oil Chem. Soc.*, 42 (1965) 1148.
7 S. S. CHANG, *J. Amer. Oil Chem. Soc.*, 38 (1961) 669.
8 J. DE BRUYN AND J. C. M. SCHOGT, *J. Amer. Oil Chem. Soc.*, 38 (1961) 40.
9 C. H. LEA AND P. A. T. SWOBODA, *Chem. Ind. (London)*, (1958) 974.
10 O. W. PARKS, M. KEENEY AND D. P. SCHWARTZ, *J. Dairy Sci.*, 44 (1961) 1940.
11 A. M. GADDIS, R. ELLIS AND G. T. CURRIE, *J. Food Sci.*, 29 (1964) 6.
12 F. H. LOHMAN, *Anal. Chem.*, 30 (1958) 972.
13 A. S. HENICK, M. F. BENCA AND J. H. MITCHELL, *J. Amer. Oil Chem. Soc.*, 31 (1954) 88.
14 M. F. POOL AND A. A. KLOSE, *J. Amer. Oil Chem. Soc.*, 28 (1951) 215.
15 P. HAVERKAMP BEGEMANN AND K. DE JONG, *Rec. Trav. Chim.*, 78 (1959) 275.
16 R. L. SHRINER AND R. C. FUSON, *The Systematic Identification of Organic Compounds*, John Wiley, New York, 1948, p. 171.
17 G. R. LAPPIN AND L. C. CLARK, *Anal. Chem.*, 23 (1951) 541.
18 M. M. HORIKX, *J. Appl. Chem.*, 14 (1964) 50.
19 D. P. SCHWARTZ, H. S. HALLER AND M. KEENEY, *Anal. Chem.*, 35 (1963) 2191.
20 D. P. SCHWARTZ AND O. W. PARKS, *Anal. Chem.*, 33 (1961) 1396.

21 J. D. CRASKE AND R. A. EDWARDS, *J. Chromatogr.*, 51 (1970) 237.
22 C. SZONYI, in preparation.
23 I. HORNSTEIN AND P. F. CROWE, *Anal. Chem.*, 34 (1962) 1037.
24 J. A. RIDDICK AND E. E. TOOPS, JR., in A. WEISSBERGER (Editor), *Technique of Organic Chemistry*. Vol. VII. *Organic Solvents*, 2nd ed., Interscience, New York, 1955, p. 318.
25 A. M. PARSONS, *Analyst*, 91 (1966) 297.