

DIRECT SPECTROPHOTOMETRIC EXAMINATION OF 2,4-DINITRO-PHENYLHYDRAZONES ON PAPER CHROMATOGRAMS

D. A. FORSS, P. R. EDWARDS, B. J. SUTHERLAND AND R. BIRTWISTLE

*Division of Dairy Research and
Division of Mathematical Statistics, C.S.I.R.O.,
Melbourne (Australia)*

(Received April 27th, 1964)

INTRODUCTION

Carbonyl compounds, including the recently detected vinyl ketones^{1,2}, are important in the flavours of foods and have been widely studied through their 2,4-dinitrophenylhydrazones. A common first step in the characterization of the 2,4-dinitrophenylhydrazones following separation by paper chromatography is their broad classification according to light absorption maxima (λ_{\max}). This has usually been determined in solution after elution from the paper. However, the direct measurement of λ_{\max} on paper chromatographic spots is considerably more rapid and sensitive. The λ_{\max} of paper chromatographic spots is often reported³ but this is of little value unless the λ_{\max} of several reference compounds for the particular paper chromatographic system is known. The λ_{\max} of the 2,4-dinitrophenylhydrazones of *n*-alkanals, *n*-alk-2-enals, *n*-alka-2,4-dienals and *n*-alkan-2-ones on phenoxyethanol paper chromatograms⁴ and of *n*-nonanal, *n*-non-2-enal and *n*-undecan-2-one on paraffin oil paper chromatograms⁵ have been reported, but for these and other classes of carbonyls, and for other chromatographic systems, additional data is needed.

The spectra of 2,4-dinitrophenylhydrazones of homologous series of *n*-alkanals, *n*-alk-2-enals, *n*-alka-2,4-dienals, *n*-alka-2,6-dienals, *n*-alkan-2-ones, *n*-alk-1-en-3-ones (vinyl ketones) and *n*-alk-3-en-2-ones were measured by transmission through paper and are reported here.

EXPERIMENTAL

Four paper chromatographic systems were studied. In three of the systems the papers were impregnated: with vaseline (GADDIS AND ELLIS⁶), paraffin oil (KLEIN AND DE JONG⁵) and phenoxyethanol (LYNN, STEELE AND STAPLE⁷). Acetylated paper was used in a fourth system described by FORSS AND RAMSHAW⁸. The first two systems are "reversed phase" and the last two systems are "normal phase". The R_F data of the 2,4-dinitrophenylhydrazones are reported separately⁹.

Rectangular strips of paper containing single spots of the 2,4-dinitrophenylhydrazones were held in a special carriage by a spring steel clip and their light absorption measured in a Beckman DK2 spectrophotometer after 1 day and 7 days from completion of the chromatogram.

The spectral data for the C_{2-14} *n*-alkanal, $C_{4-11,16}$ *n*-alk-2-enal, $C_{6-12,14,16,18}$ *n*-alka-2,4-dienal, C_{3-13} *n*-alkan-2-one and C_{4-10} *n*-alk-1-en-3-one 2,4-dinitrophenylhydrazones were examined*. The following assessments were made and are recorded in Tables I-IV.

(a) Whether there were any significant differences between the carbon numbers and if so whether any trend existed. (Where this trend was found to be significant it was always negative, *i.e.* λ_{\max} decreased as the carbon number increased.)

(b) Where trends were found their magnitudes at 1 and 7 days were compared.

(c) The mean values of λ_{\max} for all carbon numbers at 1 and 7 days were compared to see if there was any change between times of measurement. The significance level of this comparison is shown after the bracket linking the two means where:

n.s. denotes "not significant";

s. denotes "significant at the 5 % probability level";

h.s. denotes "significant at the 1 % probability level".

In addition to the above the standard error of the individual means and the coefficients of variation (C.V.) are tabled.

The data for the 2,4-dinitrophenylhydrazones of methanal, propenal and penta-2,4-dienal which are anomalous, and that for the $C_{9,10}$ *n*-alka-2,6-dienals and $C_{6,7,10}$ *n*-alk-3-en-2-ones are found in Table V.

COMPARISON OF THE FOUR SYSTEMS

The value of the procedures depends on the consistency of the λ_{\max} values obtained within each class of 2,4-dinitrophenylhydrazones, on the extent of differentiation between the classes, and on reasonable stability with time.

TABLE I
LIGHT ABSORPTION DATA - VASELINE SYSTEM OF GADDIS AND ELLIS

Compound as 2,4-dinitrophenylhydrazone	Time (days)	Trend with carbon No.	Mean	S.D.	S.E. of mean	C.V. (%)
Alkan-2-ones	1	Negative	362.2	1.86 2.15	0.51 0.66	0.5 0.6
	7	Negative	362.0			
Alka-2,4-dienals	1	Nil	397.4	2.81 2.22	0.60 0.51	0.7 0.6
	7	Nil	394.0			
Alkanals	1	Negative	361.1	2.39 1.88	0.71 0.55	0.7 0.5
	7	Negative	361.3			
Alk-2-enals	1	Negative	380.8	2.21 2.20	0.58 0.73	0.6 0.6
	7	Negative	379.0			
Alk-1-en-3-ones	1	Negative	373.6	3.08 2.90	1.18 1.17	0.8 0.8
	7	Negative	372.8			

Vaseline system of GADDIS AND ELLIS (Table I)

The data for this system give consistent results with a C.V. of the order of 0.6%. Apart from the 2,4-dienals, there is a significant trend with carbon number which is

* The lower carbon number alkanal, alk-2-enal, alka-2,4-dienal and alkan-2-one hydrazones could not be studied by the methods of KLEIN AND DE JONG, and GADDIS AND ELLIS (see ref. 9).

of the same order at both times of measurement. In addition, there is no appreciable change in the means from 1 to 7 days.

The 2,4-dienals depart from the above pattern in all but C.V.; there is little variability between carbon numbers and relatively high variability between replications. GADDIS AND ELLIS⁶ suggested that such variability might be due to the greater instability of the 2,4-dienal 2,4-dinitrophenylhydrazones.

With this system it would be difficult to distinguish the *n*-alkan-2-one from the *n*-alkanal 2,4-dinitrophenylhydrazones but the other three classes could be distinguished from them and from one another.

Paraffin oil system of KLEIN AND DE JONG (Table II)

The data are less consistent than with the vaseline system. The variability is acceptably uniform and corresponds to a C.V. of the order of 0.5%. However, only 3 of the 10 series of observations show a significant trend with carbon number. (This trend also occurred with the *n*-alk-3-en-2-ones). This trend occurred only on measurements per-

TABLE II
LIGHT ABSORPTION DATA - PARAFFIN OIL SYSTEM OF KLEIN AND DE JONG

Compound as 2,4-dinitrophenylhydrazone	Time (days)	Trend with carbon No.	Mean	S.D.	S.E. of mean	C.V. (%)
Alkan-2-ones	1	Negative	356.7	2.59 1.20	0.79 0.40	0.7 0.3
	7	Nil	361.8			
Alka-2,4-dienals	1	Nil	388.8	3.20 1.40	0.73 0.53	0.8 0.4
	7	Nil	385.6			
Alkanals	1	Negative	355.5	1.81 0.83	0.59 0.28	0.5 0.2
	7	Nil	360.2			
Alk-2-enals	1	Nil	374.1	1.87 0.82	0.52 0.33	0.5 0.2
	7	Nil	377.3			
Alk-1-en-3-ones	1	Negative	367.6	3.34 2.59	1.40 1.16	0.9 0.7
	7	Nil	371.8			

formed at one day and could relate to differential effects along the paper arising from the slower drying of the chromatogram developed with dioxane-water (4:1). However, it was not observed in the vaseline system which also uses an aqueous developing solvent, methanol-water (9:1).

In three of the classes there was a highly significant increase in λ_{\max} from the first to the seventh day, but the dienals showed a decrease and the *n*-alk-1-en-3-ones did not change.

It would appear that this method is likely to be less reliable than the vaseline system.

Our results at 7 days for *n*-alkanals (360 $m\mu$), *n*-alkan-2-ones (362 $m\mu$) and *n*-alk-2-enals (377 $m\mu$) agree with the figures of KLEIN AND DE JONG for *n*-nonanal (358 $m\mu$), *n*-undecan-2-one (362 $m\mu$) and *n*-non-2-enal (376 $m\mu$).

The same differentiation between classes observed with the vaseline system is also obtained here; this is slightly affected by the greater change of λ_{\max} with time.

Phenoxyethanol system of LYNN et al. (Table III)

This method gives reasonably consistent results. The variability is fairly uniform and corresponds to a C.V. of the order of 0.4%. In 2 of the 10 sets of data there is a significant trend with carbon number. In all cases there is a significant trend with time, the values of λ_{\max} being lower at 7 days than at 1 day. The data agree well with that of NONAKA *et al.*⁴.

This was the only system which permitted differentiation of the 5 classes.

TABLE III
LIGHT ABSORPTION DATA - PHENOXYETHANOL SYSTEM OF LYNN *et al.*

Compound as 2,4-dinitrophenylhydrazone	Time (days)	Trend with carbon No.	Mean	S.D.	S.E. of mean	C.V. (%)
Alkan-2-ones	1	Nil	374.6	1.09	0.21	0.3
	7	Negative	373.1			
Alka-2,4-dienals	1	Nil	405.0	2.49	0.48	0.6
	7	Nil	402.2			
Alkanals	1	Nil	370.5	1.34	0.29	0.4
	7	Negative	368.2			
Alk-2-enals	1	Nil	388.9	0.79	0.19	0.2
	7	Nil	387.3			
Alk-1-en-3-ones	1	Nil	383.8	1.61	0.45	0.4
	7	Nil	382.4			

Acetylated paper system of FORSS AND RAMSHAW (Table IV)

This method gave the most consistent results. The variability is quite consistent with a C.V. of the order of 0.4%. In no case is there any trend with carbon number and in no case is there any significant difference between the readings at 1 day and 7 days.

TABLE IV
LIGHT ABSORPTION DATA - ACETYLATED PAPER SYSTEM OF FORSS AND RAMSHAW

Compound as 2,4-dinitrophenylhydrazone	Time (days)	Trend with carbon No.	Mean	S.D.	S.E. of mean	C.V. (%)
Alkan-2-ones	1	Nil	367.1	1.25	0.29	0.3
	7	Nil	366.9			
Alka-2,4-dienals	1	Nil	393.0	1.47	0.38	0.4
	7	Nil	392.8			
Alkanals	1	Nil	362.2	1.30	0.28	0.4
	7	Nil	362.8			
Alk-2-enals	1	Nil	377.7	1.35	0.32	0.4
	7	Nil	377.9			
Alk-1-en-3-ones	1	Nil	375.4	1.95	0.47	0.5
	7	Nil	375.8			

TABLE V
 LIGHT ABSORPTION DATA (MEAN AND STANDARD DEVIATION IN $m\mu$) OF MISCELLANEOUS COMPOUNDS

Compound as 2,4-dinitrophenylhydrazone	Vaseline system of Gaddis and Ellis		Paraffin oil system of Klein and De Jong		Phenoxyethanol system of Lynn et al.		Acetylated paper system of Forss and Ramshaw	
	1 day	7 days	1 day	7 days	1 day	7 days	1 day	7 days
Methanal					361.1, 0.64	362.6, 1.06	358.0, 1.73	357.8, 1.92
Propenal					379.2, 1.17	381.1, —*	370.8, 2.05	372.1, 1.27
Penta-2,4-dienal					397.2, 1.09	396.3, 0.75	386.7, 1.94	387.9, 1.45
Nona-2,6-dienal	381.2, 3.19	379.7, 2.81	376.5, 0.84	376.7, 1.21	387.0, —*	385.0, —*	376.8, 0.71	376.8, 1.17
Deca-2,6-dienal	380.5, —*	379.7, 0.58	376.2, 0.45	376.6, 0.89	387.0, —*	385.7, —*	377.4, 0.55	377.6, 0.55
Hex-3-en-2-one	386.3, 3.06	385.0, 1.00	378.2, 2.05	378.7, 1.03	390.2, 0.96	389.7, 1.15	379.0, 0.71	379.5, 1.00
Hept-3-en-2-one	383.7, 3.79	381.0, 1.00	375.8, 2.49	377.0, —*	390.5, 0.57	390.0, 1.73	379.8, 0.84	380.5, 0.57
Dec-3-en-2-one	377.0, 2.10	376.5, 1.38	372.2, 1.86	375.6, 1.14	390.7, 1.03	389.5, 1.29	379.4, 0.97	379.4, 0.55

* With the available data no adequate estimate of standard deviation could be made.

The *n*-alkanals, *n*-alkan-2-ones and *n*-alka-2,4-dienals could be readily distinguished from each other and from the *n*-alk-2-enals and *n*-alk-1-en-3-ones which themselves could not be distinguished.

Miscellaneous compounds on the four systems (Table V)

The propenal and penta-2,4-dienal derivatives could only be studied in the two "normal systems" using phenoxyethanol and acetylated paper. With both systems propenal would be difficult to distinguish from the *n*-alk-1-en-3-ones (vinyl ketones) but penta-2,4-dienal would not be confused with any of the other hydrazones. However, in all four systems, it would not be possible to distinguish by light absorption the *n*-alk-2-enals, *n*-alka-2,6-dienals and *n*-alk-3-en-2-ones.

The λ_{\max} of compounds listed in Table V were unaffected by storage for 7 days except that the *n*-alk-3-en-2-ones on the paraffin oil system showed a decrease of λ_{\max} with increase in carbon number at one day, but this difference became less on storage.

GENERAL DISCUSSION

It is difficult to explain the decrease in λ_{\max} with increase in carbon number which occurred in the three systems using paper impregnated with an involatile organic material. With the "normal phase" phenoxyethanol system it occurred only with the *n*-alkanals and *n*-alkanones (at 7 days) which may be related to the relative instability on some chromatographic systems of the saturated 2,4-dinitrophenylhydrazones¹⁰. With the two "reversed phase" systems using vaseline and paraffin oil the movement of compounds is inversely related to the carbon number and the phenomenon might be due to the developing solvents drying the non-polar stationary phase near the solvent front.

The speed and sensitivity of the direct spectrophotometric examination of 2,4-dinitrophenylhydrazones make this method very attractive. The variability is probably greater than that encountered when 2,4-dinitrophenylhydrazones are dissolved in solvents such as ethanol or chloroform but would be of the same order as when 2,4-dinitrophenylhydrazones are extracted from paper chromatograms. The peaks obtained with 2,4-dinitrophenylhydrazones are flattish and the measurement of λ_{\max} depends more than usual on the subjective judgement of the person making the measurements.

ACKNOWLEDGEMENTS

We wish to thank Dr. ADAM GADDIS, U.S. Department of Agriculture, Beltsville, Md. and Mr. ELTON PIPPEN, U.S. Department of Agriculture, Albany, Calif. for their critical comments on this paper.

SUMMARY

The light absorption maxima of the 2,4-dinitrophenylhydrazones of seven classes of aliphatic carbonyl compounds were measured by transmission on spots obtained from four paper chromatographic systems. Differentiation between classes by these measurements is discussed.

REFERENCES

- ¹ W. STARK AND D. A. FORSS, *J. Dairy Res.*, 29 (1962) 173.
- ² D. A. FORSS, E. H. RAMSHAW AND W. STARK, *J. Am. Oil Chemists' Soc.*, 39 (1962) 308.
- ³ A. CROSSLEY, T. D. HEYES AND B. J. F. HUDSON, *J. Am. Oil Chemists' Soc.*, 39 (1962) 9.
- ⁴ M. NONAKA, E. L. PIPPEN AND G. F. BAILEY, *Anal. Chem.*, 31 (1959) 875.
- ⁵ F. KLEIN AND K. DE JONG, *Rec. Trav. Chim.*, 75 (1956) 1285.
- ⁶ A. M. GADDIS AND R. ELLIS, *Anal. Chem.*, 31 (1959) 870.
- ⁷ W. S. LYNN, Jr., L. A. STEELE AND E. STAPLE, *Anal. Chem.*, 28 (1956) 132.
- ⁸ D. A. FORSS AND E. H. RAMSHAW, *J. Chromatog.*, 10 (1963) 268.
- ⁹ D. A. FORSS AND W. STARK, *Anal. Chem.*, 36 (1964) 941.
- ¹⁰ D. A. FORSS AND E. A. DUNSTONE, *Australian J. Chem.*, 10 (1957) 506.

J. Chromatog., 16 (1964) 460-466