mit diesem Hydrierprodukt an. Das Gaschromatogramm zeigte neun Banden und war deckungsgleich mit dem Gaschromatogramm in Fig. 2.

Tabelle I enthält die Retentionsindices der neun isomeren Cyclohexantricarbonsäuren, die nach dem Verfahren von KOVATS[3,4] unter Zuhilfenahme der Retentionszeiten des *n*-Octadecans und des *n*-Eikosans berechnet wurden.

Institut und Lehrstuhl E. BENDEL
für Technische Chemie und Petrolchemie, W. MELTZOW
Technische Hochschule Aachen (Deutschland) V. VOGT

1 G. E. MCCASLAND, M. O. NAUMANN UND L. J. DURHAM, *J. Org. Chem.*, 31 (1966) 3079.
2 R. WILLSTÄTTER, D. HATT AND J. JAQUET, *Ber. Deut. Chem. Ges.*, 45 (1912) 1476; *ibid.*, 51 (1918) 767.
3 A. WEHRLI UND E. KOVATS, *Helv. Chim. Acta*, 42 (1959) 2709.
4 R. KAISER, *Chromatographie in der Gasphase*, 3. Teil, Hochschultaschenbücher-Verlag Bd. 24/24a.

Eingegangen am 22. August 1968

J. Chromatog., 38 (1968) 133–136

CHROM. 3739

Gas chromatography of cytokinins

We have sought a rapid sensitive means of identifying cytokinins in plant extracts as an adjunct to bioassay methods. The trimethylsilyl (TMS) derivatives of bases and nucleosides have been successfully separated by gas chromatography[1–3]. We therefore examined the possibility of separating the TMS derivatives of isopentenyladenine (2iP), dihydrozeatin, zeatin, isopentenyladenosine (2iPA) and zeatin riboside by this method. Pyrene, kinetin and kinetin riboside were included as standards.

Retention times were determined with a F and M 5750 dual column gas chromatograph fitted with flame ionization detectors. Silanized glass columns (2 m × 6 mm O.D.) were packed with 3% SE 52 (phenyl methyl silicone gum rubber) on 80–100 mesh Diataport S (a silanized diatomaceous earth). The flow-rate of helium was 60 ml/min. The injection port was maintained at 230° and the flame detector at 310°. Retention times were determined both isothermally and by temperature programming at 10°/min from 150° to 300° and then holding at 300° for 5 min.

The free bases and ribosides were dried over P_2O_5 to avoid the formation of small side peaks due to hydrolysis of the TMS derivatives[3]. A mixture of bis(trimethylsilyl) acetamide and methyl cyanide in the ratio 1:2 was added at the rate of 1 μl reagent to 1 μg of sample. The mixture was heated at 60° for 5 min, then centrifuged before sampling for injection into the gas chromatograph. Results are expressed as (a) the absolute retention time in min, (b) the retention time relative to pyrene (R_e Pyrene), and (c) the retention time relative to kinetin (R_e Kinetin). The separation achieved with temperature programming is shown in Table I and isothermally in Table II.

TABLE I

GLC RETENTION TIMES AND RELATIVE RETENTION TIMES OF CYTOKININ BASES AND RIBOSIDES ON 3% SE 52

Compound	*Temperature programmed*			*Retention temperature (°C)*
	Time (min)	R_e *Pyrene*	R_e *Kinetin*	
Pyrene	6.3	1.00	—	213
2iP	6.9	1.09	0.90	219
Kinetin	7.7	1.21	1.00	227
Dihydrozeatin	8.9	1.42	1.16	239
Zeatin	9.7	1.54	1.27	247
2iPA	13.3	2.11	1.74	283
Kinetin riboside	14.0	2.20	1.82	290
Zeatin riboside	15.3	2.42	2.00	300*

* There were no signs of decomposition at 300°.

Using pure compounds, and on the basis of a 1% full scale deflection, the limit of detection was approximately 0.005 μg. Detection of smaller quantities was limited by bleeding from the septa and O rings.

These results show that the three cytokinin bases and the two ribosides can be separated by gas chromatography as the TMS derivative. HASHIZUME AND SASAKI[4] have separated ribonucleotides by gas chromatography. It may also be possible for cytokinin ribonucleotides to be detected by the technique described here.

TABLE II

GLC RETENTION TIMES OF CYTOKININ BASES AND RIBOSIDES ON 3% SE52

Compound	*Isothermal at 210°*			*Isothermal at 250°*		
	Time (min)	R_e *Pyrene*	R_e *Kinetin*	*Time (min)*	R_e *Pyrene*	R_e *Kinetin*
Pyrene	2.5	1.00	—	1.11		
2iP	2.8	1.14	0.77			
Kinetin	3.7	1.47	1.00	1.3	1.18	1.00
Dihydrozeatin	5.7	2.29	1.55			
Zeatin	7.8	3.11	2.11			
2iPA	—			7.1	6.56	5.52
Kinetin riboside	51.9	20.8	14.1	8.8	8.16	6.86
Zeatin riboside	—			15.3	14.1	11.9

The use of combined gas chromatography–mass spectrometry for the identification of gibberellins in plants has been described by MACMILLAN *et al.*[5]. We suggest that it may be possible to couple a mass spectrometer to the gas chromatograph to facilitate the identification of microgram quantities of cytokinins in plant extracts.

Acknowledgements

The cytokinins were given as gifts to Dr. NITSCH from Dr. X. G. MONSEUR (isopentenyladenine), Dr. LETHAM and Dr. SHAW (zeatin), Dr. KOSHIMIZU (dihydro-

zeatin), Dr. HALL (isopentenyladenosine) and Dr. SHAW (zeatin riboside). We wish to thank the donors of the cytokinins and Dr. NITSCH for generously providing samples for our use. One of us (B.H.M.) wishes to acknowledge his appreciation to Dr. NITSCH for facilities and hospitality at Laboratoires de Physiologie Pluricellulaire.

Tate and Lyle Research Centre,
Keston, Kent (Great Britain)

B. H. MOST*
J. C. WILLIAMS
K. J. PARKER

1 R. L. HANCOCK, *J. Gas Chromatog.*, 4 (1966) 363.
2 J. MACGEE, *Anal. Biochem.*, 14 (1966) 305.
3 Y. SASAKI AND T. HASHIZUME, *Anal. Biochem.*, 16 (1966) 1.
4 T. HASHIZUME AND Y. SASAKI, *Anal. Biochem.*, 15 (1966) 199.
5 J. MACMILLAN, R. J. PRYCE, G. EGLINTON AND A. MCCORMICK, *Tetrahedron Letters*, (1967) 2241.

Received August 12th, 1968

* Present address: Laboratoire de Physiologie Pluricellulaire, Phytotron, 91 Gif-sur-Yvette, Essonne, France.