Journal of Chromatography, 170 (1979) 498–501

CHROM. 11,544

Note

Visualization of N-protected peptides, amino acids and aminocyclitol antibiotics on a thin-layer chromatogram by ninhydrin

RAMESH C. PANDEY*, RENUKA MISRA and KENNETH L. RINEHART, Jr.
Roger Adams Laboratory, University of Illinois, Urbana, Ill. 61801 (U.S.A.)
(First received May 11th, 1978; revised manuscript received August 21st, 1978)

During the course of our studies with several N-protected peptide antibiotics–antiamoebins I[1] and II[1,2] (1 and 2), emerimicins III and IV (3 and 4)[3], alamethicins I and II (5 and 6)[4], emerimicin II[5] and zervamicins I and II[6] we were faced with the problem of visualizing these compounds. Ninhydrin spray reagent[7–9] did not work due to the absence of any free amino group in these compounds, sulfuric acid spray[10–12] did not give a satisfactory spot and the spots obtained by visualization with iodine were not durable.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1:	Ac	Phe	Aib	Aib	Aib	Iva	Gly	Leu	Aib	Aib	Hyp	Gln	Iva	Hyp	Aib	Pro	Phol
2:	Ac	Phe	Aib	Aib	Aib	Iva	Gly	Leu	Aib	Aib	Hyp	Gln	Iva	Pro	Aib	Pro	Phol
3:	Ac	Phe	Aib	Aib	Aib	Val	Gly	Leu	Aib	Aib	Hyp	Gln	Iva	Hyp	Ala		Phol
4:	Ac	Phe	Aib	Aib	Aib	Val	Gly	Leu	Aib	Aib	Hyp	Gln	Iva	Hyp	Aib		Phol

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
5:	Ac	Aib	Pro	Aib	Ala	Aib	Ala	Gln	Aib	Val	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Glu-α	Gln	Phol
6:	Ac	Aib	Pro	Aib	Ala	Aib	Aib	Gln	Aib	Val	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Glu-α	Gln	Phol

We undertook, therefore, to develop a selective chemical detection method, and report our results here. The new method developed is simple, novel and is not restricted to peptide antibiotics but is also useful, *e.g.*, for N-protected amino acids, amino sugars and aminocyclitol antibiotics[13,14].

EXPERIMENTAL

Precoated silica gel G thin-layer chromatographic (TLC) plates (250 μm thick) were obtained from Analtech (Newark, Del., U.S.A.). The solvents used were of analytical-reagent grade (Mallinckrodt, St. Louis, Mo., U.S.A.). Spray reagent I was

* Present address: NCI Frederick Cancer Research Center, Frederick, Md. 21701, U.S.A.

concentrated hydrochloric acid (≈37%) and spray reagent II was the chromogenic ninhydrin reagent (Ninspray, ninhydrin in an aerosol bomb, an 0.5% solution of ninhydrin in 1-butanol obtained from Nutritional Biochemicals, Cleveland, Ohio, U.S.A.). The test samples were dissolved in methanol or methanol–chloroform (1:1) and *ca.* 10% solutions were applied to the plates with disposable micro-pipettes (3 μl). A sufficient quantity of solution was applied to ensure visualization.

Method

In a typical experiment, after spotting the compounds, the plate is developed in an appropriate solvent and the developed plate is dried at room temperature for 10–15 min. The dried plate is very carefully and thoroughly sprayed with spray reagent I and left at room temperature for 5–10 min. The plate is now covered completely with a clear glass plate and heated at *ca.* 120° for 10–15 min. After cooling to room tempera-

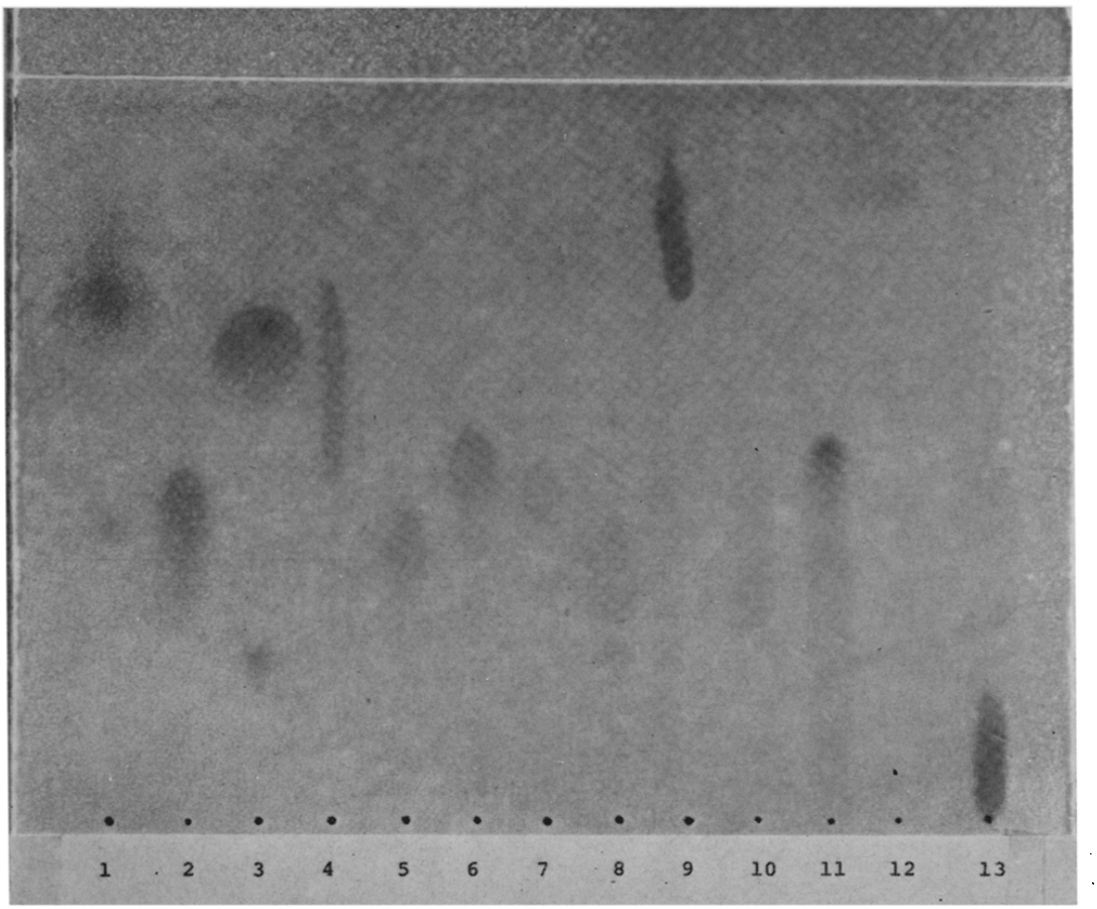

Fig. 1. Thin-layer chromatogram of some selected compounds visualized by the method discussed in the text using a silica gel G plate developed with 1-butanol–acetic acid–water (4:1:5, upper layer). 1 = N-Acetyl-L-leucine; 2 = N-acetyl-L-glutamine; 3 = N-acetyl-L-glutamic acid; 4 = a nonapeptide from antiamoebin I; 5 = antiamoebin I; 6 = antiamoebin I acetate; 7 = emerimicin IV; 8 = alamethicins I and II; 9 = valine- and isoleucine–gramicidins A; 10 = gramicidin J; 11 = nocardamin; 12 = valinomycin; 13 = N-hexaacetylneomycin B.

ture the clear glass plate is removed and the TLC plate is heated again at *ca.* 120° for 10 min. The plate is then sprayed with spray reagent II and kept in an oven at *ca.* 120° for 30–40 min. Sometimes it is necessary to spray more than once with spray reagent II. Spots of different colors develop with different compounds during heating.

RESULTS AND DISCUSSION

This method has been successfully used for N-acetyl-L-leucine, N-acetyl-L-glutamine, N-acetyl-L-glutamic acid, N-acetyl-L-hydroxyprolyl-α-aminoisobutyric acid methyl ester[1], a nonapeptide derived from antiamoebin I[1], antiamoebin I[1,15], antiamoebin I triacetate[1,15], emerimicin IV[3], alamethicins I and II[4], valine- and isoleucine-gramicidins A[15–18], gramicidin J[19], nocardamin[20,21], valinomycin[22–25], N,N'-diacetyldeoxystreptamine[13,14], N,N'-diacetylstreptamine[13,14], N-hexaacetylneomycin B[13,14] and streptidine[13,14], all compounds of known structures.

The principle involved in using two spray reagents to detect the N-protected compounds is that the compound is first hydrolyzed on the TLC plate by spray reagent I and then visualized by spray reagent II. As expected, if the hydrolysis is not complete, spray reagent II does not give good colored spots and it is necessary to give sufficient time and/or heat after spraying with reagent I. Covering the TLC plate with a clear glass plate enhances the hydrolysis by inhibiting loss at 120° of hydrochloric acid. However, before spraying with spray reagent II, it is very important to ensure the complete removal of hydrochloric acid by heating the uncovered plate in an oven at *ca.* 120° for 10 min.

TABLE I

COMPOUNDS TREATED WITH SPRAY REAGENT AND THE COLORS OBSERVED

Solvent systems: A = 1-butanol–acetic acid–water (4:1:5, upper layer); B = pyridine–methyl ethyl ketone (3:7); C = chloroform–acetic acid (1:2); D = chloroform–methanol–water (65:24:4). † = Elongated spot; — = unmoved from origin.

Compound	R_F				*Color of spot*
	A	*B*	*C*	*D*	
N-Acetyl-L-Leu	0.73	0.79	0.72	0.66	Deep orange
N-Acetyl-L-Gln	0.42	0.52	0.48	0.67	Violet
N-Acetyl-L-Glu	0.66	0.63	0.73	0.88	Deep orange
N-Acetyl-L-Hyp–Aib–OMe	0.60	0.77	0.57	0.59	Violet
Nonapeptide from antiamoebin I	0.62	0.91	0.73	0.90	Deep orange
Antiamoebin I	0.40	0.85	0.15	0.83	Violet
Antiamoebin I triacetate	0.50	0.91	0.81	0.85	Violet
Emerimicin IV	0.46	0.86	0.19	0.79	Brown
Alamethicins I and II	0.37	0.57	0.25	0.73	Violet
	0.23			0.46	
Valine- and isoleucine-gramicidins A	0.74†	0.41	Streak	0.82	Violet to dark brown
Gramicidin J	0.48	0.04	—	0.56	Orange to purple
Nocardamin	0.49	0.80	0.21	0.90	Deep yellow
Valinomycin	0.89	0.86	1.0	0.92	Yellow to purple
N,N'-Diacetyldeoxystreptamine	0.14	0.13	†	†	Faint brown
N,N'-Diacetylstreptamine	0.05	0.04	—	—	Brown
N-Hexaacetylneomycin B	0.04†	0.09	0.03	†	Brown
Streptidine	—	—	—	—	Faint brown

The appearance of a spot depends on the nature of the compound. Some compounds show spots within 5 min whereas others may take as long as 45 min of heating. Fig. 1 shows a developed thin-layer chromatogram of some of the compounds selected from Table I. In addition to locating compounds with N-protected groups we feel the method should find wide application in determining if an amide linkage is present in a compound of unknown structure.

ACKNOWLEDGEMENT

This work was supported by grants from the National Institute of Allergy and Infectious Diseases (AI 01278 and AI 04769).

REFERENCES

1 R. C. Pandey, H. Meng, J. C. Cook, Jr. and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 99 (1977) 5203.
2 R. C. Pandey, J. C. Cook, Jr. and K. L. Rinehart, Jr., *J. Antibiot.*, 33 (1978) 241.
3 R. C. Pandey, J. C. Cook, Jr. and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 99 (1977) 5205.
4 R. C. Pandey, J. C. Cook, Jr. and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 99 (1977) 8469.
5 R. C. Pandey and K. L. Rinehart, Jr., unpublished results.
6 S. Tarbox, R. C. Pandey, J. C. Cook, Jr. and K. L. Rinehart, Jr., unpublished results.
7 A. R. Fahmy, A. Niederwieser, G. Pataki and M. Brenner, *Helv. Chim. Acta*, 44 (1961) 2022.
8 A. R. Patton and P. Chism, *Anal. Chem.*, 23 (1951) 1683.
9 E. Kawerau and T. Wieland, *Nature (London)*, 168 (1951) 77.
10 W. L. Anthony and W. T. Beher, *J. Chromatogr.*, 13 (1964) 567.
11 D. F. Jones, J. Macmillan and M. Radley, *Phytochem.*, 2 (1964) 307.
12 H. Metz, *Naturwissenschaften*, 48 (1961) 569.
13 K. L. Rinehart, Jr., and R. M. Stroshane, *J. Antibiot.*, 29 (1976) 319.
14 K. L. Rinehart, Jr., *Pure Appl. Chem.*, 49 (1977) 1361.
15 K. L. Rinehart, Jr., J. C. Cook, Jr., H. Meng, K. L. Olson and R. C. Pandey, *Nature (London)*, 269 (1977) 832.
16 R. Sarges and B. Witkop, *J. Amer. Chem. Soc.*, 86 (1964) 1861.
17 R. Sarges and B. Witkop, *J. Amer. Chem. Soc.*, 86 (1964) 1862.
18 R. Sarges and B. Witkop, *J. Amer. Chem. Soc.*, 87 (1965) 2011.
19 *Brochure*, Nippon Seibutsu Kagaku Kenkyusho Ltd., Tokyo, Japan.
20 W. Keller-Schierlein and V. Prelog, *Helv. Chim. Acta*, 44 (1961) 1981.
21 K. L. Rinehart, Jr., R. C. Pandey, R. J. Hargreaves, R. D. Johnson and D. H. Peterson, *J. Antibiot.*, in preparation.
22 H. Brockmann and H. Geeren, *Justus Liebigs Ann. Chem.*, 603 (1957) 216.
23 M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina and N. A. Aldanova, *Tetrahedron Lett.*, (1963) 351.
24 H. Brockmann, M. Springorum, G. Träxler and I. Höfer, *Naturwissenschaften*, 50 (1963) 689.
25 M. M. Shemyakin, N. A. Aldanova, E. I. Vinogradova and M. Yu. Feigina, *Tetrahedron Lett.*, (1963) 1921.