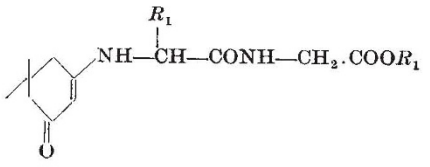
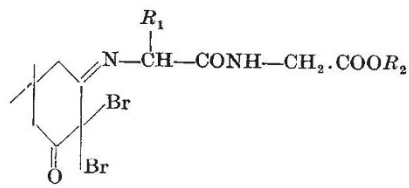
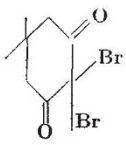


(II)

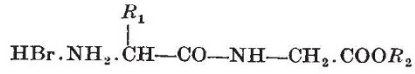


(III)



(IV)

+



(V)

In this way several of the dimedone-peptide esters in Table 1 were hydrolysed. In all cases the isolated peptide esters were found to be chromatographically and analytically pure.

We thank Prof. L. Jackman for taking and interpreting the nuclear magnetic resonance spectra.

B. Halpern*
L. B. James

John Curtin School of Medical Research,
Australian National University,
Canberra.

* Present address: Syntex S.A., Apartado 2679, Mexico D.F., Mexico.

[1] Schwarzenbach, G., and Felder, E., *Helv. Chim. Acta*, **27**, 1701 (1944).
[2] Wieland, T., and Schäfer, W., *Ann.*, **576**, 104 (1952).

Identification of Hexosamines by Two-dimensional Paper Electrophoresis and Chromatography

Glucosamine and galactosamine are usually identified by the ninhydrin degradation method devised by Gardell, Heijkenskjöld and Roch-Norlund[1], and Stoffyn and Jeanloz[2]. The method suffers, however, from the disadvantage that glucosamine and mannosamine are not distinguishable under these conditions, since the two hexosamines are converted into the same pentose, that is, arabinose. This communication describes a new method which permits the identification of the three isomers of the hexosamines.

The proposed method is carried out in two steps, the first being the selective *N*-acetylation[3] of the hexosamines with acetic anhydride on paper impregnated with tetraborate; the second, the two-dimensional electrophoresis and chromatography of the resultant *N*-acetylhexosamines on the same paper. Whatman No. 3 *MM* paper strip with dimensions as shown in Fig. 1 was moistened with 0·025 M potassium or sodium tetraborate solution and then pressed between two sheets of clean filter paper to remove the excess of the tetraborate solution. The strip was then placed in an electrophoretic apparatus and the parts *A* and (another) *A* were dipped in 0·025 M potassium or sodium tetraborate, and the part *C* was hung in air. Sample solution was applied at the origin. The sample spot applied at the origin was spotted with 1 drop of 2 per cent solution of acetic anhydride in acetone. The voltage was connected 10 min later, to complete the *N*-acetylation of the hexosamines. Electrophoresis was carried out by the horizontal open strip method at 200 V for 6 h. Platinum electrodes were used. After electrophoretic run, the strip was dried at room temperature in a current of air for not less than 2 h. The two tags (*A* + *B* and another *A* + *B*) were cut off from the strip. The dry paper strip, now 18 cm × 24 cm, was irrigated in an *n*-butanol–pyridine–water (solvent *A*, 2 : 2 : 1, or solvent *B*, 6 : 4 : 3) at 7° ± 1° C for 16 h by the ascending technique. After development, the strip was dried at room temperature in a current of air. The dry strip was first sprayed with the water-saturated *n*-butanol spray and heated at 95° C for 5 min and then sprayed with the *p*-dimethylaminobenzaldehyde spray[4] at room temperature. The *N*-acetylated hexosamines, that is, resultant *N*-acetylhexosamines, gave violet spots on a white background.

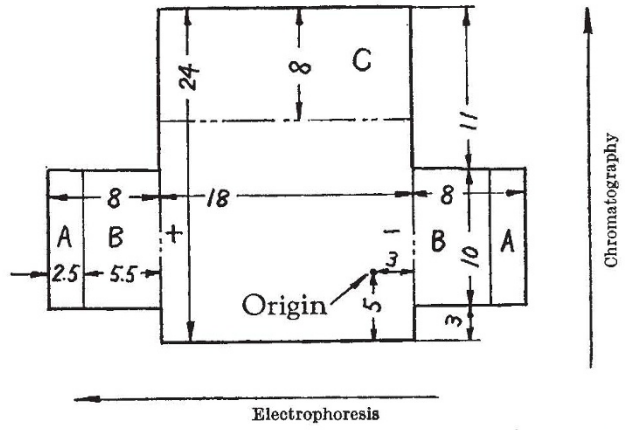


Fig. 1. Dimensions of paper strip used for two-dimensional paper electrophoresis chromatography

Table 1. Two-Dimensional Paper Electrophoresis Chromatography of (*N*-Acetylated) Hexosamines

0·025 M tetraborate	(*N*-acetylated) hexosamine	First dimension electrophoretic mobility. Distance travelled (cm)*	Second dimension chromatographic mobility. Distance travelled (cm)	
			Solvent *A*	Solvent *B*
$K_2B_4O_7$	GlcN	+ 2·7	7·9	6·7
	GalN	+ 4·5	3·0	2·9
	ManN	+ 8·4	3·8	4·1
$Na_2B_4O_7$	GlcN	+ 1·6	8·0	6·5
	GalN	+ 3·7	3·6	3·2
	ManN	+ 7·6	4·9	4·3

The hexosamine hydrochloride solution in concentration of 5 mg/ml. was used in this work. GlcN, glucosamine; GalN, galactosamine; ManN, mannosamine.

* The positive sign denotes movement towards the anode.

The hexosamines were subjected to acetylation treatment and two-dimensional technique already described and the results obtained are summarized in Table 1. The electrophoretic and chromatographic mobilities of *N*-acetylated glucosamine, galactosamine and mannosamine were identical with those of authentic *N*-acetylglucosamine, *N*-acetylgalactosamine and *N*-acetylmannosamine run under the same conditions.

We thank Prof. T. Furuhata for his advice and Drs. M. and S. Watanabe for their support.

Seiichi Ohkuma
Toshiaki Shinohara

Biochemical Laboratory,
Scientific Police Research Institute,
Sanban-cho, Chiyoda-ku, Tokyo.

[1] Gardell, S., Heijkenskjöld, F., and Roch-Norlund, A., *Acta Chem. Scand.*, **4**, 970 (1950).
[2] Stoffyn, P. J., and Jeanloz, R. W., *Arch. Biochem. Biophys.*, **52**, 373 (1954).
[3] Ohkuma, S., and Shinohara, T., *Proc. Japan Acad.*, **39**, 686 (1963).
[4] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).