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Note

Separation of amino acids as mansyl derivatives on polyamide layers

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Cory *et al.*[1] introduced the sulphonamide derivative N-methyl-2-aniline-6-naphthalenesulphonyl chloride (mansyl chloride) into the chemistry of N-terminal amino acid labelling of proteins. From the structure of mansyl chloride (see Fig. 1), it would be expected to have the same properties as the commonly used dansyl chloride (see Fig. 1) from the point of view of its sensitivity and reaction with amino acids, and thus would seem to offer no obvious advantage over dansyl chloride in its application. However, a knowledge of the chromatographic behaviour of mansyl derivatives is important in that it would allow the comparison of data on the use of dansyl chloride to suggest the presence of a substance in a sample where only small amounts of material are available. As far as we are aware, no chromatographic data on the fractionation of mansyl derivatives exist[2].

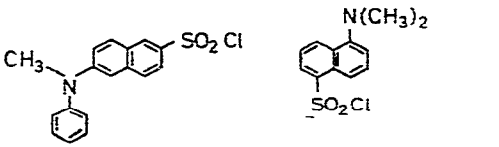

Fig. 1. Structures of dansyl chloride and mansyl chloride[1].

MATERIAL AND METHODS

L-Amino acids and indoles were purchased from Serva (Heidelberg, G.F.R.). Common laboratory chemicals were obtained from E. Merck (Darmstadt, G.F.R.). Mansyl chloride was a gift from Professor Becker, Oregon State University, U.S.A. [1-^{14}C]Leucine was purchased from Amersham Buchler, Braunschweig, G.F.R. (specific activity 50 mCi/nmole).

The method used for the formation and chromatographic separation of mansyl derivatives was similar to that employed for dansyl derivatives[3,4]. Standard amino acids or indoles were each dissolved in 0.05 *M* $NaHCO_3$, pH 10 (adjusted with NaOH), and 10-μl samples (containing approximately 0.06 μmole substance) were mixed

thoroughly with 10 μl of mansyl chloride in acetone (1 mg/ml) in small glass tubes. Mixtures of substances were mansylated in a similar way. Thereafter, the samples were incubated in the dark at 37° for 30 min and were then dried *in vacuo*. Each sample was re-dissolved in 10 μl of acetone–acetic acid (3:2) and an aliquot was applied carefully to the corner of a 3 × 3 cm polyamide layer (Mikropolyamid F 1700, Schleicher & Schüll, Dassel, G.F.R.) with a glass capillary.

Micro-chromatograms were developed by the ascending technique in covered 50-ml beakers. Chromatography in the first dimension was carried out with benzene–acetic acid (9:1) and in the second dimension with ethanol–water–acetic acid (5:5:1). The solvent mixtures were renewed for each run and prepared fresh daily. [^{14}C]Leucine was dansylated and mansylated as described above. These derivatives were subsequently separated from dansyl-OH, dansyl-NH_2 or mansyl-OH and mansyl-NH_2 on 5 × 5 cm polyamide layers using the solvent system described previously for the dansyl derivatives[3,4], and the solvent above for the mansyl derivatives. The derivatives of dansyl-[^{14}C]leucine and mansyl-[^{14}C]leucine were each suspended in a number of different solvents (cyclohexane, chloroform, 2-propanol, acetone, methanol) and the radioactivity in a defined amount was determined by scintillation spectrometry (the efficiency was monitored by the external standard method). As the specificity of the original [^{14}C]leucine was known, the absolute amounts of mansyl-[^{14}C]leucine and dansyl-[^{14}C]leucine in each of the solvents could be established. Thereafter, the fluorescence of each derivative in the different solvents was measured in a Turner Model 210 spectrofluorimeter and related to content of the derivative present (see Table I).

RESULTS AND DISCUSSION

Fig. 2 is a 3 × 3 cm micro-chromatogram showing the separation of a number of mansyl derivatives. Most of the amino acids essential for the chromatographic identification of end-groups of proteins and peptides, or those thought to have transmitter roles[5], are separated by this chromatographic system. Moreover, the neurotransmitter 5-hydroxytryptamine and its metabolites 5-hydroxytryptophan and 5-

TABLE I

RELATIVE FLUORESCENCE OF DANSYL-[1-^{14}C]LEUCINE AND MANSYL-[1-^{14}C]LEUCINE

With dansyl-[1-^{14}C]leucine, excitation was at 350 nm, while mansyl-[1-^{14}C]leucine was excited at 330 nm. All fluorescence measurements were corrected for solvent fluorescence and mean values from 2–3 spectra are reported.

Solvent	*Dansyl-[1-^{14}C]leucine*		*Mansyl-[1-^{14}C]leucine*		*b/a*
	Fluorescence maximum (nm)	*Relative fluorescence (a)*	*Fluorescence maximum (nm)*	*Relative fluorescence (b)*	
Cyclohexane	480–490	314	440	458	1.5
Chloroform	500–510	246	455–460	615	2.5
2-Propanol	510–515	153	455–465	489	3.2
Acetone	510–520	128	475–480	89	0.7
Methanol	513	105	480	119	1.1

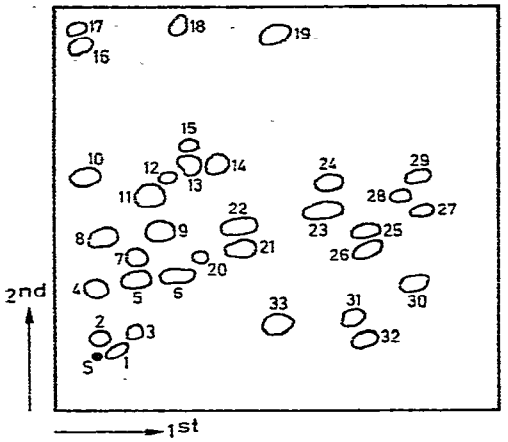

Fig. 2. Micro-chromatogram showing positions of mansyl derivatives. The direction of chromatographic development using benzene–acetic acid (1st dimension) and ethanol–water–acetic acid (2nd dimension) are indicated by the arrows. S = starting point; 1 = mansyl-OH; 2 = mansyltaurine; 3 = mansylhistidine; 4 = mansyl-5-hydroxytryptophan; 5 = mansylserotonin; 6 = mansyltryptophan; 7 = mansylaspartic acid; 8 = mansyltyrosine; 9 = mansylglutamic acid; 10 = mansylcystine; 11 = mansylserine; 12 = mansylthreonine; 13 = mansylglutamine; 14 = mansylcystine; 15 = mansyl-γ-aminobutyric acid; 16 = mansylornithine; 17 = mansylarginine; 18 = mansyllysine; 19 = mansylhistidine; 20 = mansylornithine; 21 = mansyl-5-hydroxyindoleacetic acid; 22 = mansylglycine; 23 = mansylalanine; 24 = mansyl-NH_2; 25 = mansylmethionine; 26 = mansylphenylalanine; 27 = mansylleucine and mansylisoleucine; 28 = mansylvaline; 29 = mansylproline; 30 = mansylputrescine; 31 = mansylhistidine; 32 = mansyltyrosine; 33 = mansyllysine. The individual mansyl derivatives of serotonin, histidine, tyrosine and ornithine were not identified.

hydroxyindoleacetic acid are also easily resolved. All of the mansyl derivatives fluoresce blue under UV light, and the reaction by-products mansyl-OH and mansyl-NH_2 are clearly separated.

Although the optimal reaction conditions between amino acids and mansyl chloride were not examined in detail, they appear to be similar to the reaction between dansyl chloride and amino acids[3,4,6] using identical reaction conditions (reaction time, concentration of reaction products, incubation temperature and pH). Thus, as in the reaction between dansyl chloride and tyrosine[3,4,6], two reaction products of mansyltyrosine were formed (probably N- and bis-compounds). However, the chromatographic mobilities of dansyl and mansyl derivatives of the same compounds were very different. For example, water–formic acid (100:3) is a common chromatographic solvent system for the fractionation of dansyl amino acids on polyamide layers[3,4,7], yet mansyl amino acids did not migrate in this solvent system. It is this difference in the chromatographic properties of mansyl and dansyl substances of the same compounds that seems to be important, for it can be exploited in order to characterize substances in samples available in only limited amounts.

To assess the relative sensitivity of their detection the mansyl and dansyl derivatives dansyl-[^{14}C]leucine and mansyl-[^{14}C]leucine were dissolved in various solvents and the maximum fluorescence was determined with a spectrofluorimeter (Table I). Under the conditions used, the mansyl derivative exhibited a slightly greater fluorescence than dansylleucine in cyclohexane, chloroform and 2-propanol, although this difference would appear to be an insignificant factor. No difference was found

in acetone and methanol, where significant reduction would be expected owing to the greater polarity of the solvents.

Although these results are preliminary, it is clear that mansyl derivatives, like dansyl derivatives, can be fractionated from one another on 3 × 3 cm polyamide layers. Moreover, the sensitivities of the mansyl and dansyl derivatives are of the same order. It is therefore suggested that the value of mansyl chloride lies essentially in its use as an independent derivative for the comparison of data where dansyl chloride has been used, especially when only limited amounts of material are available. Otherwise, mansyl chloride offers no advantage over the commonly used dansyl chloride[3,4,6,7], although it may be possible to produce it commercially with a higher specific activity.

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