CHROM. 5039

Separation of dansylated products of L-seryltyrosine and their identification

Dansyl chloride has been increasingly utilized in labeling proteins and peptides for the identification and characterization of N-terminal amino acids of those proteins and peptides[1] as it reacts with amino groups to form highly fluorescent derivatives which can be detected in extremely small amounts (10^{-3}–10^{-4} μmole). Dansyl derivatives of amino acids have been prepared as standards and a mixture of them was separated and identified by thin-layer chromatography (TLC)[2].

Confusing results may be obtained where compounds with two reactive places in the molecule are to be separated. Besides serine, tyrosine, threonine,cysteine, lysine, ornithine, arginine and histidine which present at least two spots upon dansylation, peptides offering the possibility of N- and O-dansyl derivatives are of particular interest.

The scope of the present work was to work out a method of separation and identification of the N-dansyl and N,O-didansyl derivatives of small molecular weight peptides. L-Seryltyrosine, which results from the chymotryptic digest of ACTH is of particular practical interest and was used as the model substance during this study.

Experimental

Dansyl chloride (206 mg) dissolved in acetone (6.5 ml) was added to a solution of seryltyrosine (90.3 mg, Cyclo Chemical Co.) in 5 % sodium bicarbonate solution (6 ml). The mixture was stirred at room temperature in the dark. After 1 h, some precipitate formed which was redissolved by the addition of acetone (5 ml) and water (5 ml). Then the extent of the reaction was checked by taking an aliquot (5 μl) of the mixture and running it on a TLC plate (Silica Gel G) with solvent system I (chloroform–ethyl alcohol–acetic acid (60:40:2.5)). Four fluorescent spots were observed under UV light at R_F values of 0.4 (blue), 0.76 (yellow), 0.88 (yellow) and 1.0 (yellow). When the plate was sprayed with ninhydrin reagent, a purple spot appeared at the origin, indicating that the reaction was not yet complete. After 4 h, the acetone from half of the reaction mixture was evaporated under nitrogen; the aqueous residue was processed as described in the flow-sheet.

Aqueous solution
↓
ether extraction 5 × 7 ml ——→ ether extract (A)
↓
solution
↓
acetic acid (1:1) pH 4.8, centrifuged ——→ solid (B)
↓
aqueous solution (C)
↓
kept cold overnight, centrifuged ——→ solid (D)
↓
aqueous solution
↓
ether extraction 6 × 7 ml ——→ ether extract (E)
↓
aqueous solution
↓
ethyl acetate extraction 6 × 7 ml ——→ ethyl acetate extract (F)
↓
aqueous solution

A small aliquot of each fraction was tested on TLC (Silica Gel G) using solvent system I; the R_F values of the fluorescent spots are given in Table I. Similar results were obtained on the other half of the reaction mixture after 22 h of reaction.

TABLE I

TLC OF THE FRACTIONS FROM THE AQUEOUS SOLUTION AFTER REACTION WITH DANSYL CHLORIDE

Fractions	R_F			
	1.0 (yellow)	*0.43–0.44 (blue)*	*0.77–0.79 (yellow)*	*0.85–0.89 (yellow)*
A	++	—	—	—
B[a]	—	—	—	++
C	—	++	++	+
D	—	++	+	+
E	—	++	++	+
F[b]	—	++	++	—

[a] Several minor spots.
[b] Two faint spots between the two reported.

Fraction A (yellow) and the blue fluorescent spot were identified as dansyl amine and dansyl acid respectively, by comparison with standards. The yellow material of R_F 0.77 and 0.85 in fractions B, C, D, E and F was purified by preparative TLC using a 0.5 mm thick Silica Gel H plate and solvent system II, consisting of chloroform–ethyl alcohol–acetic acid (85:15:1), as well as system I. Spots with an R_F value of 0.21 (designated X) and 0.46 (designated Y) in system II corresponded to R_F of 0.77 (X) and 0.85 (Y) in system I, respectively. The products were eluted with acetone, concentrated under nitrogen and dried *in vacuo* yielding glassy masses. It has been observed that fraction Y when treated with dilute sodium hydroxide solution for 24 h at room temperature gave fraction X and dansyl acid, identified by TLC with solvent system II. The nature of the mobilities and the results of this reaction indicate that the fractions X and Y are N-dansyl-seryltyrosine and N,O-didansyl-seryltyrosine respectively.

Further proof of their identities was obtained in the following manner. Fraction X was hydrolyzed with 6 *N* HCl in an atmosphere of nitrogen for 24 h. The solution was evaporated to dryness and the residual material was dissolved in methanol. TLC using solvent system II yielded two yellow fluorescent spots (R_F 0.23 and 0.79) and a ninhydrin spot (purple, R_F 0.0). The fluorescent spots corresponded exactly with those obtained by hydrolysis of dansyl-L-serine and the ninhydrin spot corresponded to L-tyrosine itself. The fast moving fluorescent material (R_F 0.79) was obtained from dansyl-L-serine during hydrolysis by β-elimination of the hydroxyl group. Fraction Y, in a similar way, was hydrolyzed and chromatographed. In system II, it gave three fluorescent spots of R_F 0.09, 0.23 and 0.79. The spot of R_F 0.09 corresponded to that obtained from hydrolyzed product of N-acetyl-O-dansyl-L-tyrosine. The other two spots of R_F 0.23 and 0.79 corresponded to those obtained from dansyl-L-serine hydrolysis. The material of R_F 0.09 was also ninhydrin positive.

Hence, it has been definitely established that the fractions X and Y are N-dansyl and N,O-didansyl seryltyrosine respectively. The fraction X was utilized as our standard in our ACTH work.

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Medical Foundation of Buffalo,
Buffalo, N.Y. 14203 (U.S.A.)

N. KUNDU
SUJATA ROY

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Chromatography and 77°K luminescence of some hydrocarbons on thin layers of microcrystalline nylon–polytetrafluoroethylene (Aviamide-6–Fluoroglide 200)*

The separation, identification, and quantitative measurement of hydrocarbons is of particular interest in studies of air pollutants, tobacco-smoke components, and chemical carcinogenesis. Low-temperature luminescence has been shown to be a sensitive means to detect organic compounds on thin-layer chromatograms[1]. This technique was used to evaluate new layer materials for thin-layer chromatography (TLC). The evaluation of particulate polytetrafluoroethylene as a layer material for TLC and its usefulness for separating metal ions have been described[2,3]. A mixed layer that consists of the polytetrafluoroethylene Fluoroglide 200 and the new microcrystalline nylon Aviamide-6 has been found suitable for separating hydrocarbons.

Ten hydrocarbons (aromatic and heterocyclic) and a mixture that contained the ten were chromatographed on thin layers of Aviamide-6–Fluoroglide 200 (4:1) developed with *n*-propanol. The resulting chromatograms were observed with 254- and 366-nm UV light under liquid nitrogen (77°K). Eight of the ten hydrocarbons were resolved. Presumably, the separation is based on the differences in the solubilities of the hydrocarbons in *n*-propanol. The hydrocarbons show distinctive luminescent properties by which they can be identified; even the unresolved hydrocarbons were detectable in the presence of each other. The intensity of the phosphorescence of certain of the compounds is particularly striking and possibly may be a means to their sensitive quantitative measurement.

Materials

Fluoroglide 200, chromatography grade TWO218 from Chemplast, Inc., 150 Dey Road, Wayne, N.J. 07470.

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