Nachweis mit Quecksilber–Fluorescein-Reagens[5]

Äquimolare Mengen von Quecksilber(II)-acetat und Fluorescein werden getrennt in Eisessig unter Erwärmen gelöst. Noch warm wird die Fluoresceinlösung in die Quecksilberacetatlösung filtriert. Dabei fällt in der Regel das Präparat bereits aus. Zur vollständigen Auskristallisation des Additionsproduktes wird der Ansatz längere Zeit im Eisschrank belassen. Man saugt ab, wäscht mit Eiswasser und trocknet unter Vakuum über Blaugel. 20 mg von dieser Substanz werden in 10 ml 1 *N* Kalilauge in der Kälte gelöst. Das Sprühreagens ist etwa eine Woche haltbar.

Mit dieser Lösung wird das Chromatogramm besprüht und nach 5 min ausgewertet. Es zeigen sich rote Flecke auf hellrotem Grund. Empfindlicher gestaltet sich der Nachweis im U.V.-Licht.

Nachweis mit Natriumsulfidlösung (Lösung I) und Eisen(III)-chloridlösung (Lösung II)

Lösung I. 5.0 g Natriumsulfid (9 H_2O) werden unter leichtem Erwärmen in 20 ml Wasser gelöst und nach dem Abkühlen mit Äthanol (96 %) zu 100 ml aufgefüllt.

Lösung II. 40 ml 5 %-ige wässrige Eisen(III)-chloridlösung werden zu 100 ml mit 20 %-iger abgekochter Salpetersäure aufgefüllt.

Das Chromatogramm wird mit Lösung I besprüht, über einer heissen Kochplatte sorgfältig getrocknet (beschleunigte Bildung von Alkalirhodanid) und anschliessend mit Lösung II besprüht. Es erscheinen rote Flecke auf weissem Grund.

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Paper chromatography of 5-substituted tryptophans*

Serotonin, one of the brain neurohumeral mediators, is formed biosynthetically from tryptophan *via* 5-hydroxytryptophan (5-HTP). On administration of either of these three compounds, only 5-HTP crosses the blood-brain barrier readily. A study was inaugurated to see if some isosteres of 5-HTP would interfere with the transport of this amino acid into the brain. If any of these 5-substituted tryptophans compete

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with the transport of 5-HTP across the blood-brain barrier, alterations of brain serotonin levels would be noted.

Accordingly, a series of tryptophans substituted in the 5-position were synthesized and purity was determined by paper chromatography. Two independent solvent systems, butanol–acetic acid–water (B–A–W) and phenol–water (P–W) and two color developing reagents (ninhydrin and Ehrlich's reagent) were used. Surprisingly, some of the compounds with different polarities had identical R_F values. Using P–W, it was not possible to separate and hence to characterize some of the 5-substituted tryptophans by R_F values from paper chromatography alone.

Experimental

(a) *Synthesis of amino acids.* The DL-amino acids were synthesized by methods which appear in the literature. References to these procedures are given in Table I.

(b) *Paper chromatography.* Classical procedures, as described by SMITH[1], were used. The amino acids were dissolved in a solvent, spotted, dried, chromatographed in one dimension on 1.5 × 11 in. Whatman No. 1 paper strips. Experiments were repeated a minimum of five times. The apparatus used was the Gordon-Misco chromatographic tubes, as recommended by GORDON[2].

The tubes were equilibrated for a minimum of 1 h and thereafter were kept saturated. Paper strips were saturated for 20 min prior to use in all cases. The chamber temperature was constant during a run. At the end of the experiment time, usually 3 or 4 h, the papers were removed and dried overnight in a slow current of air. To develop color, one strip was sprayed with ninhydrin (0.2% w/v acetone); the other with Ehrlich reagent (1 vol. of *p*-dimethylaminobenzaldehyde 10% w/v in conc. HCl +4 vol. acetone).

The solvents were either butanol–acetic acid–water (60:15:25 v/v); or phenol–water (40 g:10 ml). All amino acids were run individually and then in pairs. DL-Tryptophan was added to the mixture of pairs to serve as a reference. For statistical evaluation the runs were repeated from six to thirty-three times. The data in Table I give the mean R_F values of the individually run amino acids and the standard deviation.

Table II gives the mean of the R_F values of compounds which were run in pairs. In cases where no separation was noted, that observation was verified by three consecutive runs.

TABLE I

R_F VALUES FOR DL-5-SUBSTITUTED TRYPTOPHANS

Substituent	*R_F value ± S.D.*				*Lab temp. (°C)*	*Exp. time (h)*	*Ref. to synthesis*
	B–A–W	*± S.D.*	*P–W*	*± S.D.*			
None	0.45	0.018	0.73	0.013	22	3	—
Amino	0.17	0.024	0.21	0.028	24	4	3
Nitro	0.41	0.021	0.66	0.008	24	4	3
Methyl	0.53	0.022	0.77	0.007	23	3	4
Hydroxy	0.58	0.026	0.87	0.019	22	3	Commercial
Fluoro	0.52	0.016	0.73	0.014	23	4	5
Bromo	0.58	0.007	0.73	0.028	23	4	6
Benzyloxy	0.59	0.017	0.90	0.016	24	4	Commercial

TABLE II

R_F VALUES OF MIXTURES OF TRYPTOPHANS

Substituent	*R_F value**				*Lab. temp.* (°C)	*Exp. time* (*h*)
	B–A–W		*P–W*			
	N	*E*	*N*	*E*		
Methyl	0.53	0.56	no separation		22	3
None	0.44	0.45				
Hydroxy	0.54	0.57	0.89	0.90	22	3
None	0.48	0.41	0.74	0.75		
Hydroxy	0.63	0.61	no separation		23	4
Methyl	0.51	0.51				
Nitro	0.39	—	—	—	24	4
None	0.48	0.45	—	0.74		
Amino	0.16	0.17	0.20	0.21	24	4
None	0.45	—	0.73	0.74		
Benzyloxy	0.60	0.58	0.92	0.90	24	4
None	0.44	0.43	0.74	0.73		
Methyl	0.50	0.52	0.78	0.77	23	4
Amino	0.15	0.22	0.15	0.17		
Amino	0.17	0.18	0.23	0.23	23	4
Benzyloxy	0.60	0.61	0.88	0.92		
Nitro	0.44	—	no separation		23	4
Methyl	0.53	—				

* N = ninhydrin; E = Ehrlich's reagent.

Results and conclusions

The R_F values are given in Table I, which were run on an individual basis. Table II gives the data for amino acids run in pairs. In each of these cases, DL-tryptophan was added for a reference. From Table II it can be concluded that it is not possible to separate and, hence, to characterize the following pairs when phenol–water is the solvent: 5-methyl from tryptophan, 5-hydroxy- or 5-nitro. No difficulty was encountered when butanol–acetic acid–water was used.

The pair, 5-bromo and 5-fluoro, had like R_F values in both solvent systems and thus cannot be separated.

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