TABLE IV. Physical and Analytical Data of 2-Benzyl-1,3-indandiones

X	Appearance (recryst. solv.)	mp (°C) (lit. mp (°C))	Analysis (%) Calcd. (Found) C	H	N or Cl	IR ν_{max}^{KBr} cm^{-1}	UV λ_{max}^{MeOH} nm (log ε)
H	pale yellow prisms (EtOH)	95—96 (96—97[a])	81.34 (81.46)	5.12 (4.92)		1713 (CO)	
p-NO_2	pale yellow needles (EtOH)	139—141 (142[a])	68.32 (68.29)	3.94 (3.97)	4.88 (4.76)	1703 (CO) 1505 1348 (NO_2)	252(3.46)
m-NO_2	pale yellow needles (EtOH)	121—122	68.32 (68.33)	3.94 (4.00)	4.88 (4.77)	1700 (CO) 1532 1348 (NO_2)	278(3.36)
p-Cl	pale yellow needles (MeOH)	116—118 (119—120[a])	71.01 (70.74)	4.10 (4.18)	13.10 (13.30)	1702 (CO)	
m-Cl	pale yellow needles (MeOH)	138—139	71.01 (70.78)	4.10 (3.98)	13.10 (12.98)	1705 (CO)	
p-CH_3O	pale yellow neeldes (MeOH)	101—102 (102—104[a,b])	76.67 (76.71)	5.30 (5.44)		1704 (CO)	
m-CH_3O	pale yellow needles (MeOH)	58—59	76.67 (76.54)	5.30 (5.28)		1706 (CO)	
p-OH	pale yellow needles (MeOH)	181—183	76.18 (76.35)	4.80 (4.86)		1698 (CO) 3230 (OH)	
m-OH	prisms (MeOH)	114—116	76.18 (76.07)	4.80 (4.92)		1701 (CO) 3348 (OH)	

a) J. Strandings, E. Ermanc, T. Dumpis, J. Linabergs, and G. Vanags, *Zh. Organ. Khim.*, **1** (2), 388 (1965)
b) G. Vanags and T. Dumpis, *Doklady. Akad. Nauk.*, **135**, 549 (1959)

stirring. A stream of dry air free from CO_2 was passed through the reaction mixture so as to check emission of CO_2 by $Ba(OH)_2$ solution. Heating was continued until emission of CO_2 was almost ceased. The reaction solution was concentrated under reduced pressure to remove triethylamine and excess of TEAF. Trituration of the resulting residue with water gave fine powder which was recrystallized from appropriate solvent to give the corresponding 2-benzyl-1,3-indandione.

Reaction time and yield of the products are shown in Table II. Analytical and physical data of the products are summarized in Table IV.

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An Improved Method for the Analysis of Dansyl Polyamines

KAZUEI IGARASHI, IKUE IZUMI, KATSUKO HARA, and SEIYU HIROSE

Faculty of Pharmaceutical Sciences, Chiba University[1]

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Recently, increasing attentions have been directed to the significance of polyamines in various biological processes including DNA replication, RNA synthesis, and protein synthesis.[2]

1) Location: *Yayoi-cho, Chiba.*
2) H. Tabor and C.W. Tabor, *Ann. Rev. Pharmacol.*, **16**, 245 (1964); U. Bachrach, *Ann. Rev. Microbiol.*, **24**, 109 (1970); S.S. Cohen, "Introduction to the Polyamines," Prentice-Hell, Englewood Cliffs, N. J., 1971; T.A. Smith, *Endeavour*, **31**, 22 (1972).

Among the methods for polyamine analysis, the dansylation method developed by Seiler and Weichmann[3] and modified by Dion and Herbst[4] is the most sensitive one. However, the separation of the dansyl polyamines by Silica gel G thin-layer chromatography (TLC) is not satisfactory. Accordingly, Gibson and Roizman[5] developed the Silica gel G TLC plates three times to attain better separation of dansyl polyamines.

This paper describes an improved method for the analysis of dansyl polyamines. The procedure utilizes only a single development of Kieselguhr G TLC plates yet gives better separation of dansyl polyamines.

Experimental

Materials——The sources of materials used were as follows: Silica gel G (Lot No. 70168911) and Kieselguhr G (Lot No. 70121418), E. Merck; dansyl chloride, Seikagaku Fine Biochemicals; spermine-4HCl, spermidine-3HCl, and putrescine-2HCl, Sigma Chemical Co.; cadaverine-2HCl and 1,3-propanediamine-2HCl, Nakarai Chemicals, Ltd. Other chemicals used were of reagent grade from commercial sources.

Dansylation of Polyamines——Dansylation of polyamines was performed by the modified method of Dion and Herbst.[4] Polyamine solution (0.2 ml of 0.2 N perchloric acid solution containing 5 to 40 nmoles of polyamines) was dansylated by the addition of 0.4 ml of dansyl chloride (10 mg/ml acetone) and 50 mg of $Na_2CO_3 \cdot 10H_2O$. Dansylation was allowed to proceed for 16 hr in the dark at room temperature. Excess dansyl chloride was then converted to dansyl proline by reaction of the preparation with 0.1 ml of proline (50 mg/ml H_2O) for 30 min in the dark. Dansyl polyamines were then extracted by adding 0.5 ml of benzene to the preparation and shaking this mixture for 5 min.

TLC Separation of Dansyl Polyamines——After an aliquot (20 μl) of benzene extract was applied to an activated (1 hr at 110°) Kieselguhr G TLC plate (250 μ in thick), the plate was chromatographed with ethyl acetate–cyclohexane (4: 13). When the solvent front was 10—12 cm from the starting line (usually 40 min), the plate was taken out of the jar, and sprayed with triethanolamine–isopropanol (1: 4). The plate was dried over butanol and P_2O_5 for about 30 min in the dark.

Extraction and Estimation of Dansyl Polyamines——The position of the polyamine derivatives, detected by fluorescence under ultraviolet light were marked. These marked patches of adsorbent were scraped off the plate and the polyamine derivatives were extracted with 5 ml of methanol–28% NH_4OH (95: 5) by shaking the mixture for 10 min. After the Kieselguhr G was removed by centrifugation at $3000 \times g$ for 10 min, the relative intensity of fluorescence of the polyamine derivatives at 522 nm was measured by activation at 342 nm in a Hitachi MPF-2A fluorescence spectrophotometer. Quinine sulfate (0.15 μg/ml 0.01 N H_2SO_4) was used as a reference.

Measurement of Polyamine Concentration in Rat Liver——For the measurement of polyamine concentration in rat liver, the method of standard additions was used. Three 50 mg (wet weight) of rat liver were weighed exactly. Different additions of polyamines (20 and 60 nmoles) were made to two 50 mg samples. To the third sample no polyamine was added. Each sample was then homogenized in 0.4 ml of 0.2 N perchloric acid, centrifuged at $3000 \times g$ for 10 min at room temperature, and the supernatant fluid was collected. An aliquot (0.2 ml) of this extract was then subjected to the polyamine analysis as described above. Curves were drawn of the relative intensity versus the amount of polyamines added. The intercepts of the curves on the polyamine axis give the polyamine contents in 50 mg of the liver.

Result and Discussion

The Quantitation of Dansyl Polyamines

A comparison of *Rf* values obtained by the method of Dion and Herbst[4] (A) and the present method (C) is shown in Table I. The results obtained by the modified method of Dion and Herbst are also shown (B). The modification was done by changing the ethyl acetate to cyclohexane ratio from 2: 3 to 8: 9 to attain better separation of dansyl polyamines. As shown in the table, dansyl putrescine expressed the smallest *Rf* value among the dansyl derivatives of putrescine, cadaverine and 1,3-propanediamine, and was clearly sepa-

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4) A.S. Dion and E.J. Herbst, *Ann. N. Y. Acad. Sci.*, 171, 723 (1970).
5) W. Gibson and B. Roizman, *Proc. Natl. Acad. Sci. U.S.* 68, 2818 (1971).

TABLE I. Comparison of *Rf* Values of Various Dansyl Derivatives Obtained by Three TLC Methods

Amines	*Rf* values		
	(A)	(B)	(C)
Ammonia	0.45	0.57	0.93
1,3-Propanediamine	0.39	0.53	0.83
Cadaverine	0.35	0.48	0.81
Putrescine	0.36	0.50	0.76
Spermidine	0.27	0.41	0.56
Spermine	0.16	0.33	0.39

(A): Silica gel G TLC plate was developed with ethyl acetate-cyclohexane (2:3) (the method of Dion and Herbst[4]).
(B): Silica gel G TLC plate was developed with ethyl acetate-cyclohexane (8:9) (the modified method of Dion and Herbst).
(C): Kieselguhr G TLC plate was developed with ethyl acetate-cyclohexane (4:13) (the present method).

TABLE II. The Concentration of Spermine, Spermidine, and Putrescine in Rat Liver

Rat	Experiment		Concentration of polyamines (μmoles/g wet liver)		
			Spermine	Spermidine	Putrescine
1	1		0.72	0.50	0.06
	2		0.66	0.52	0.05
	3		0.69	0.53	0.06
		mean	0.69	0.52	0.06
2	4		0.65	0.54	0.04
	5		0.68	0.56	0.03
	6		0.71	0.59	0.04
		mean	0.68	0.56	0.04

Male Wistar rats weighing 150—200 g were used. The liver was perfused as described previously.[a] For other details of the procedure, see the text.
a) K. Igarashi, K. Hikami, K. Sugawara and S. Hirose, *Biochim. Biophys. Acta*, 299, 325 (1973)

rated by our method from the latter two compounds, while the resolution of these three compounds was hardly attained by the method of Dion and Herbst with or without the modification of the solvent system. The dansyl derivative of ammonia was clearly separated by our method from the other dansyl derivatives also. In addition, the resolution of the dansyl derivatives of spermine, spermidine, and putrescine by our method was better than that by the former method. Therefore, our method is superior to the method of Dion and Herbst for the TLC isolation of dansyl polyamines.

The linear relationship between the relative intensity of fluorescence and the concentration of dansyl polyamines over the range of 0.2—1.6 nmoles is shown in Fig. 1.

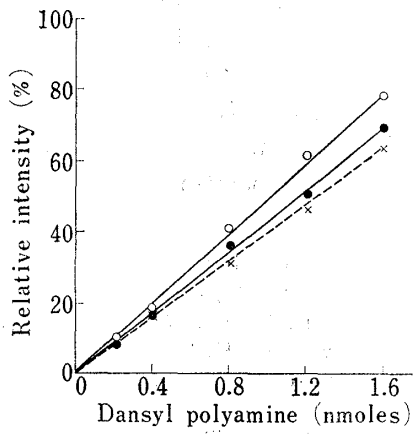

Fig. 1. Standard Curve of Dansyl Polyamines
●—●: spermine; ○—○: spermidine; ×----×: putrescine
For details of the procedure, see the text.

The Concentration of Spermine, Spermidine, and Putrescine in Rat Liver

The concentration of polyamines in the liver of two rats was determined by our method. As shown in Table II, the duplicability of the method was quite good. The mean values of spermine, spermidine, and putrescine per gram of liver were 0.69, 0.52, and 0.06 in the first rat, and 0.68, 0.56, and 0.04 in the second rat, respectively. These data are similar to those reported by other workers[6] using electrophoretic analysis. The present method has proved to be rapid and reliable for the estimation of polyamine content in *E. coli* also.

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Solvent Effects on Comparative Dissolution of Pharmaceutical Solvates[1]

Ho-Leung Fung and Thomas Nealon

Pharmaceutics Department, School of Pharmacy, State University of New York at Buffalo[2]

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Two potential applications of organic solvates in pharmaceutical formulation have been suggested, *viz.*: (i) as intermediates to achieve particle size reduction and (ii) as pharmaceutical derivatives to promote dissolution of poorly soluble drugs. While there are evidences which testify to the success of the first application to drugs such as griseofulvin[3] and chloramphenicol,[4] conflicting data exist in the literature about the general ability of solvates to show enhanced dissolution rates over their non-solvated compounds. Thus, while some solvates[5,6] showed higher *in vitro* dissolution rates when compared to their non-solvated forms, others[7,8] demonstrated the reverse behavior. This confusing picture is further complicated by the lack of standardization of dissolution techniques used to study solvate dissolution. In particular, in the dissolution studies of water insoluble drugs, it is often expedient to employ mixed aqueous organic solvent systems to facilitate data collection.[5] This practice does not necessarily lead to ready extrapolation of comparative solvate dissolution data in these media to those in aqueous solutions. This is primarily due to the probable difference in the rate or extent of dissociation of the solvate in these solvent systems, which in turn provides a dif-

1) Partly presented at the 119th Annual Meeting of the American Pharmaceutical Association, Houston, Texas, April 24, 1972.
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