X-RAY DIFFRACTION PATTERNS OF DINITROPHENYL DERIVATIVES OF AMINO COMPOUNDS[1]

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ABSTRACT

The 2,4-dinitrophenyl derivatives of a number of amino compounds have been prepared and their X-ray powder diffraction measurements made. This appears to be the first time this information has been obtained and it is submitted as a method of identification.

INTRODUCTION

In recent years the reaction of 1-fluoro-2,4-dinitrobenzene with proteins and protein derivatives has been used a great deal in work on the structure of proteins and in similar problems (2, 6, 7, 8, 9, 10, 11). The free amino groups react with the reagent to form dinitrophenyl derivatives and identification of the derivatives formed shows which amino groups were free in the intact protein. X-ray powder diffraction patterns are being used to an increasing degree in the identification of organic compounds (3, 5) and are very useful since a high degree of purity is not required. The 2,4-dinitrophenyl derivatives of a number of amino acids were prepared by the methods described by Abderhalden and Blumberg (1), Sanger (8), and Porter and Sanger (7) as standards of reference for work on the free amino groups of soil organic matter and their X-ray powder diffraction patterns determined.

X-RAY EXAMINATION

Diffraction patterns of the 2,4-dinitrophenyl derivatives of the amino acids were obtained with a Philips Norelco X-ray spectrometer (1950 model). The samples were ground to pass a 350-mesh sieve and placed in spectrometer frames. Considerable difficulty was encountered in sieving the samples and in preparing nonoriented material for the spectrometer examination.

Preliminary investigation showed that Cu $K\alpha$ radiation was too strong for satisfactory spectrometer patterns. Consequently Fe $K\alpha$ radiation ($\lambda = 1.93597$ Å) with manganese filter at 45 kv., 20 ma., and a rate meter setting of 2-06-8 was used. While this was satisfactory for the most prominent lines, it was felt that powder photographs would be better for the weaker lines. Powder samples therefore were made with the material that had been prepared for the spectrometer, using fine glass tubes of 0.2 mm. bore. Philips 114.5 mm. diameter cameras were used with an exposure time of 18 hr.

RESULTS AND DISCUSSION

The analyses of the dinitrophenyl derivatives are reported in Table I. The determinations for carbon, hydrogen, and nitrogen were made in the microanalytical laboratory of Dr. R. Dietrich, Zurich, Switzerland. The nitrogen content was also measured in this laboratory by the Friedrich-Kjeldahl method.

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TABLE I

COMPOSITION AND MELTING POINTS OF DINITROPHENYL DERIVATIVES

Compound	Composition (%)						Melting point (°C.)	
	Found			Calculated			Found	Reported
	C	H	N	C	H	N		
2,4-dinitroaniline	39.5	2.68	22.8	39.3	2.70	22.9	179	176 (188) (4)[2]
dnp[1]-glycine	39.7	2.82	17.4	39.8	2.90	17.4	206	205 (1)
dnp-*dl*-α-alanine	41.2	3.61	16.2	42.3	3.53	16.5	178	178 (1)
dnp-β-alanine	42.5	3.52	16.6	42.3	3.53	16.5	121–5	—
dnp-*dl*-valine	46.5	4.56	14.8	46.6	4.60	14.8	185	185 (1)
dnp-*dl*-serine	40.1	3.40	15.6	39.9	3.30	15.5	200	199 (7)
dnp-*dl*-leucine	48.6	5.19	14.1	48.5	5.10	14.1	126	203 (1)
dnp-*dl*-aspartic acid	40.3	3.16	14.1	40.2	3.00	14.0	190	196 (7)
ε-dnp-*l*-lysine.HCl.H_2O	38.9	5.37	15.4	39.5	5.20	15.4	189	186 (8)
bis-dnp-lysine	45.1	3.68	17.4	45.1	3.77	17.6	174	146 (7)
dnp-*l*-histidine	44.5	2.85	20.0	44.4	2.67	20.1	228	250 (1)
dnp-*l*-asparagine	39.9	3.35	18.7	40.2	3.40	18.8	185	191 (1)

[1]*dnp—dinitrophenyl.*
[2]*Small numbers in brackets indicate literature references.*

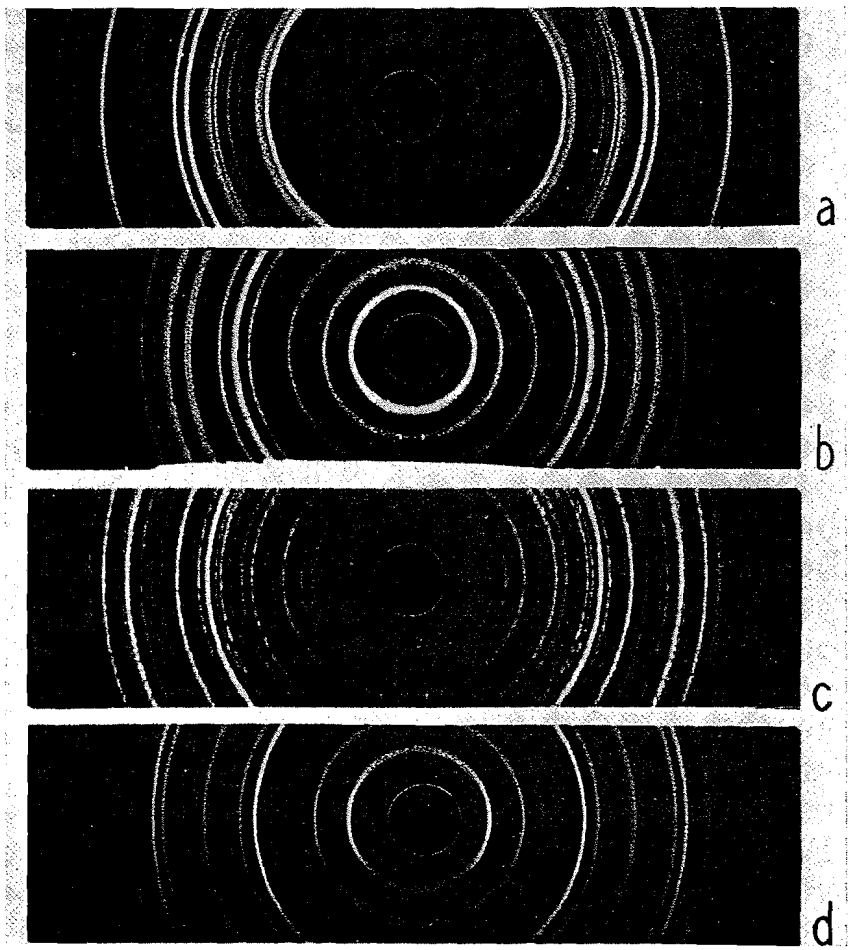

FIG. 1. Typical powder diagrams of dinitrophenyl derivatives with Fe $K\alpha$ radiation.
a. dnp-*dl*-aspartic acid.
b. dnp-*dl*-leucine.
c. dnp-*dl*-serine.
d. dnp-*dl*-valine.

TABLE II

DIFFRACTION DATA*

1. 2,4-dinitro-aniline		2. dnp-glycine		3. dnp-*dl*-α-alanine		4. dnp-β-alanine	
d(Å)	I/I_0	*d*(Å)	I/I_0	*d*(Å)	I/I_0	*d*(Å)	I/I_0
7.78	1	9.68	3	10.4	7	15.1	3
7.00	1	7.39	3	9.83	10(1)	9.79	2
6.40	7	6.36	1	7.99	2	7.60	4
6.26	3	5.91	4	6.63	1	7.24	2
5.72	1	5.11	1	5.99	2	6.37	2
5.24	1	4.73	1	5.39	2	5.85	3
4.79	8(3)	4.57	9	5.14	4	5.41	1
4.55	8(2)	4.36	3	4.98	1	5.05	3
4.32	6	4.21	1	4.66	9(2)	4.92	4
3.83	6	4.10	2	4.46	1	4.70	2
3.75	1	3.98	5	4.22	3	4.54	8(2)
3.58	2	3.75	10(1)	4.10	5	4.29	4
3.40	2	3.66	10(2)	3.92	1	4.21	3
3.32	1	3.52	3	3.75	1	4.12	6
3.20	10(1)	3.26	4	3.67	8(3)	3.87	10(1)
3.10	7	3.18	4	3.46	1	3.77	4
2.98	1	3.12	2	3.33	1	3.59	3
2.94	2	3.08	10(3)	3.29	7	3.51	5
2.86	4	2.93	1	3.20	1	3.39	7(3)
2.74	2	2.84	1	3.12	7	3.27	5
2.68	2	2.70	3	3.06	1	3.23	7(3)
2.63	1	2.62	1	2.97	1	3.16	1
2.56	1	2.55	1	2.90	6	3.01	7(3)
2.51	4	2.51	1	2.75	1	2.93	1
2.46	1	2.44	1	2.64	2	2.76	4
2.44	2	2.40	2	2.58	1	2.64	1
2.39	1	2.28	4*d*	2.50	1	2.54	1
2.33	1	2.18	1	2.41	1	2.44	1*d*
2.28	2	2.15	1	2.32	3	2.34	2
2.24	1	2.13	2	2.05	1	2.27	1
2.20	2	2.09	1	1.97	1	2.24	1
2.15	1	2.06	2	1.72	1	2.20	1
2.08	1*d*	2.03	1			2.15	1*d*
2.06	1	2.00	2			2.03	2
1.94	4	1.94	3			1.99	1
1.91	1	1.92	1			1.94	1
1.88	2	1.87	1			1.92	1
1.83	1	1.83	1			1.85	1
1.79	2	1.81	1			1.80	1
1.75	1	1.79	1			1.63	1
1.72	1	1.75	2				
1.69	2	1.60	1				
1.58	2	1.37	1				
1.54	1						
1.50	1						

**d*(Å) = *interplanar spacings in Ångstroms*; I/I_0 = *estimated relative intensity*; (1) = *strongest line*; (2) = *second strongest line*; (3) = *third strongest line*; *d* = *diffuse*; *dnp* = *dinitrophenyl*.

TABLE II (*Continued*)

5. dnp-*dl*-valine		6. dnp-*dl*-serine		7. dnp-*dl*-leucine		8. dnp-*dl*-aspartic acid	
d(Å)	I/I_0	*d*(Å)	I/I_0	*d*(Å)	I/I_0	*d*(Å)	I/I_0
12.0	9(2)	9.42	3	13.9	10(1)	8.34	2
8.09	5	8.38	4	10.0	2	6.59	1
6.85	3	7.10	5	9.58	6	5.75	10(1)
6.19	1	6.17	2	7.18	1	5.55	2
5.96	1	5.61	5	6.91	7(2)	5.38	5
5.48	1	5.28	4	5.23	6	5.34	1
5.17	10(1)	4.92	3	5.03	1	5.05	2
4.97	1	4.68	5	4.87	7(3)	4.83	3
4.75	7	4.56	5	4.75	5	4.57	4
4.47	2	4.36	10(1)	4.43	6	4.44	3
4.34	1	4.18	5	4.32	1	4.27	5
4.18	1	3.87	1	4.18	1	4.16	5
4.06	2	3.78	6(3)	4.04	2	4.08	3
3.96	5	3.55	3	3.95	2	3.93	1
3.87	2	3.41	3	3.85	3	3.75	9½(2)
3.80	1	3.34	3	3.77	3	3.66	1
3.65	1	3.27	4	3.67	1	3.57	9(3)
3.54	1	3.11	8(2)	3.52	5	3.47	1
3.44	1	3.07	2	3.46	2	3.38	2
3.35	6	3.00	2	3.34	2	3.30	1*d*
3.21	9(3)	2.94	2	3.23	1	3.15	1
3.15	2	2.88	5	3.18	3	3.09	1
2.99	4*d*	2.83	1	3.10	2	3.04	2*d*
2.84	1	2.76	4	3.04	2	2.93	1
2.76	1	2.73	3	2.91	1	2.87	2
2.71	1	2.64	1	2.88	1	2.76	7
2.66	2	2.62	1	2.77	1	2.68	2
2.51	1	2.52	3	2.68	2	2.60	2
2.47	2	2.45	3	2.62	1	2.56	2
2.33	1	2.39	2	2.53	1	2.54	1
2.23	1	2.34	2	2.47	2	2.44	2
2.19	1	2.31	2	2.41	1	2.28	2
2.10	2	2.27	1	2.33	1	2.15	1
1.92	1	2.23	1	2.27	1	1.99	2
1.86	1	2.17	2	2.15	1	1.93	1
		2.13	2	2.11	1	1.90	1
		2.10	3	2.00	1	1.87	1
		2.07	2	1.96	1	1.84	1
		2.04	1	1.91	1	1.79	1
		2.02	1	1.87	1	1.74	1
		1.99	2	1.84	1	1.62	1
		1.94	4	1.39	1	1.60	1
		1.87	2*d*	1.15	1*d*		
		1.80	1	1.09	1*d*		
		1.75	1				
		1.64	1				
		1.57	1				
		1.56	1				
		1.53	1				
		1.50	1				
		1.45	1				
		1.18	1				
		1.17	1				

TABLE II (*Concluded*)

9. ϵ-dnp-*l*-lysine.HCl.H_2O		10. *bis*-dnp-lysine		11. dnp-*l*-histidine		12. dnp-*l*-asparagine	
d(Å)	I/I_0	*d*(Å)	I/I_0	*d*(Å)	I/I_0	*d*(Å)	I/I_0
17.5	5	13.5	2	16.6	1	10.9	2
9.50	10(1)	11.1	5	15.5	4	7.70	7
8.75	1	8.55	3	7.72	1	5.49	5
7.76	1	7.72	1	7.55	3	5.06	4
6.65	3	6.32	4	7.09	7	4.85	7
6.28	5	6.05	2	6.24	8	4.62	8(3)
5.01	1	5.55	10(1)	5.49 } Band	3	4.20	3
4.87	2	5.20	1	5.36 }		3.91	10(1)
4.72	3	4.90	2	5.19	1	3.85	1
4.39	4	4.66	8	5.01	1	3.72	1
4.25	1	4.38	5	4.83	6	3.46	2
4.13	10(2)	4.29	1	4.62	3	3.41	1
3.98	1	4.06	5	4.37	7	3.33	10(2)
3.85	10(3)	3.72	2	4.24	1	3.16	8(3)
3.68	2	3.61	9(2)	4.08	1	3.00	1
3.53	3	3.47	8(3)	4.00	1	2.97	2
3.41	2	3.29	2	3.88	9(2)	2.84	1
3.28	1	3.13	2	3.75	4	2.75	4
3.17	5	3.01	1	3.61	1	2.65	1
3.12	8	2.91	1	3.50	10(1)	2.58	1
2.99	3	2.83	1	3.32	4	2.54	1
2.92	1	2.60	2	3.22	1	2.46	1
2.86	1			3.15	1	2.41	2
2.81	1			3.05	1	2.35	1
2.74	1			2.95	8(3)	2.30	2
2.61	3			2.74	2	2.24	1
2.53 } Band	1			2.37	1	2.18	3
2.47 }				2.23	1	2.14	1
2.35	2			2.17	1	2.12	1
2.30	1			2.10	2	2.08	1
2.25	1			2.00	1	2.00	1
2.21	1			1.39	2*d*	1.94	1
2.14	1			1.15	4*d*	1.84	1
2.10	1			1.09	2*d*		
2.06	3						
2.00	1						
1.93	1						
1.89	1						
1.88	1						
1.83	1						
1.74	1						
1.69	1						
1.57	1						

Most of the compounds melted with some decomposition and, in a few instances, the melting points differed considerably from those recorded in the literature. However, the compounds seemed to be pure when tested chromatographically and a second preparation and purification gave compounds melting at the same temperature.

The results of X-ray analysis are given in Table II and four typical powder diagrams are shown in Fig. 1. The I/I_0 values were estimated visually from 1 to 10 and the three strongest lines are indicated as 1, 2, and 3.

It can be seen from Table II and Fig. 1 that all the compounds examined gave distinct powder patterns; even with such similar compounds as α- and β-alanine, the patterns were quite distinct. In work on protein structure, frequently only small samples of somewhat impure material are available. In such cases, X-ray powder patterns are very useful for purposes of identification.

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