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A New Fluorometric Analysis of Dulcin using Sodium Nitrite. III.[1,2] Structural Determination of the Fluorescent Compound by the Single Crystal X-Ray Diffraction Method[3]

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The structure of the fluorescent compound (A) obtained from the reaction of dulcin and sodium nitrite was previously presumed to be 1,3-bis(4-ethoxyphenyl)-5-tetrazolone. In this paper, (2-bromoethoxy)phenyl derivative (B) which has the same structural skeleton of compound A was synthesized and analized by the single crystal X-ray diffraction method to determine the structure more conclusively. The structure of compound B was discussed on the basis of the result of the two-dimensional analysis. The structure of the fluorescent compound A was consequently concluded as 1,3-bis(4-ethoxyphenyl)-5-tetrazolone.

In previous papers the authors reported a fluorometric determination of dulcin using sodium nitrite and the isolation of a fluorescent compound from the reaction mixture. The structural formula of this compound A was presumed to be 1,3-bis(4-ethoxyphenyl)-5-tetrazolone (Chart 1).

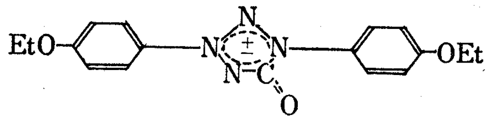

Chart 1

Since compound A was found to be formed by *p*-phenetidine instead of using dulcin, (2-bromoethoxy)phenyl derivative (compound B) which was presumed to have the same structural skeleton as compound A was synthesized from 4-(2-bromoethoxy)aniline in order to apply the heavy atom method in single crystal X-ray diffraction analysis.

4-(2-Bromoethoxy)aniline was prepared by deacetylation of 4-acetaminophenyl 2-bromoethyl ether with hydrobromic acid. This ether was beforehand synthesized by the reaction of 4-N-acetylaminophenol with dibromoethane in the presence of potassium carbonate. Crystals of compound B grown from ethyl acetate solution were pale yellow leaflets, mp 167—168°.

The structural resemblance between compound A and compound B was confirmed as follows. As shown in Fig. 1, the nuclear magnetic resonance (NMR) spectrum of compound B shows two triplets due to the protons of four methylenes of the two bromoethoxyl groups at 3.66 and 4.36 ppm, and two sets of A_2B_2 quartet due to the protons of the two benzene rings at 7.01, 7.03, 8.01 and 8.05 ppm (J obs.=9.15 cps). The elemental analysis indicated a formula $C_{17}H_{16}O_3N_4Br_2$. From these facts, compound B was supposed to have two 4-(2-bromoethoxy)phenyl groups.

The infrared (IR) spectrum of compound B showed that most of the bands are similar to those of compound A, as shown in Fig. 2. The ultraviolet (UV) spectrum of compound B in chloroform, which showed three absorption maxima at 285 mμ (log ε: 4.04), 295 mμ (4.00) and 345 mμ (4.25) was close to that of compound A, in which three absorption maxima

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1) Part I: S. Uchiyama, T. Kondo, and I. Kawashiro, *Yakugaku Zasshi*, **89**, 828 (1969).
2) Part II: S. Uchiyama, H. Tanabe, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **20**, 357 (1972).
3) This work was presented at the symposium of absorption spectrochemical analysis and fluorometric analysis at Gifu, November, 1970.
4) Location: *a) Hongo, Bunkyo-ku, Tokyo; b) 1-18-1, Kamiyoga, Setagaya-ku, Tokyo.*

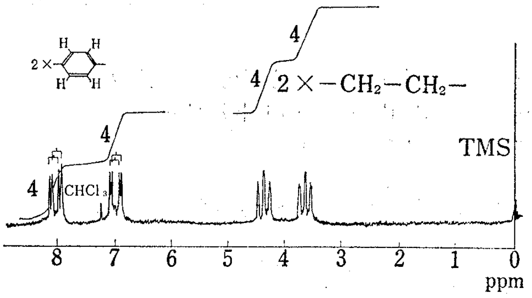

Fig. 1. NMR Spectrum of Compound B in $CDCl_3$

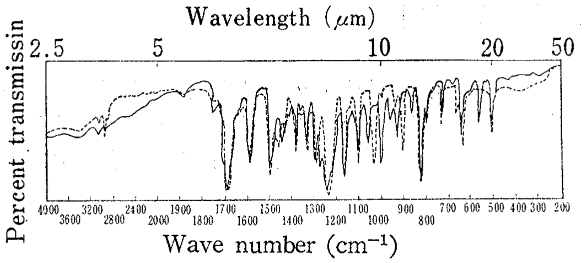

Fig. 2. Infrared Spectra of Compound A and Compound B

------: compound A
——: compound B

at 287 mμ (log ε: 4.01), 296 mμ (3.99) and 348 mμ (4.25) were also observed. The fluorescence spectrum of compound B (excitation maximum at 352 mμ, emission maximum at 440 mμ in chloroform) was similar to that of compound A (excitation maximum at 354 mμ, emission maximum at 446 mμ). These facts indicated that compound B must have the same structural skeleton as compound A. It was therefore concluded that the structural determination of compound B might reasonably substitute for that of compound A.

The structure of the crystal projected on (010) was solved by the heavy atom method. The positions of the two bromine atoms, (0.054, 0, 0.5) and (0.527, 0, 0.168), were at first determined by the two-dimensional Patterson function, $P(u,w)$. The resulting Fourier map, $\rho(x,z)$, phased by these two atoms revealed a gross feature of the molecule. The eight atoms belonging to the ethoxy phenyl groups, C(9), C(10), C(12), O(13), C(20), C(21), O(22), and C(23), were located on the map. At this stage, no atom in the tetrazolone ring was taken as phasing atoms to avoid any prejudice imposed on the structure. Successive Fourier and difference Fourier syntheses revealed the whole structure of the molecule as shown in Fig. 3. Nitrogen atoms were distinguished from carbon by refering the proposed structures (which were presented in chart 1 of part II[2]). Final positional and thermal parameters of the atoms are listed in Table I. Table II shows a comparison of observed and calculated structure factors. Refinement of the parameters was carried out by the difference Fourier method until the R factor reached to 0.16 for 111 reflections. No further refinement was attempted because of the poor quality of the intensity data. A model, which fited most adequately to

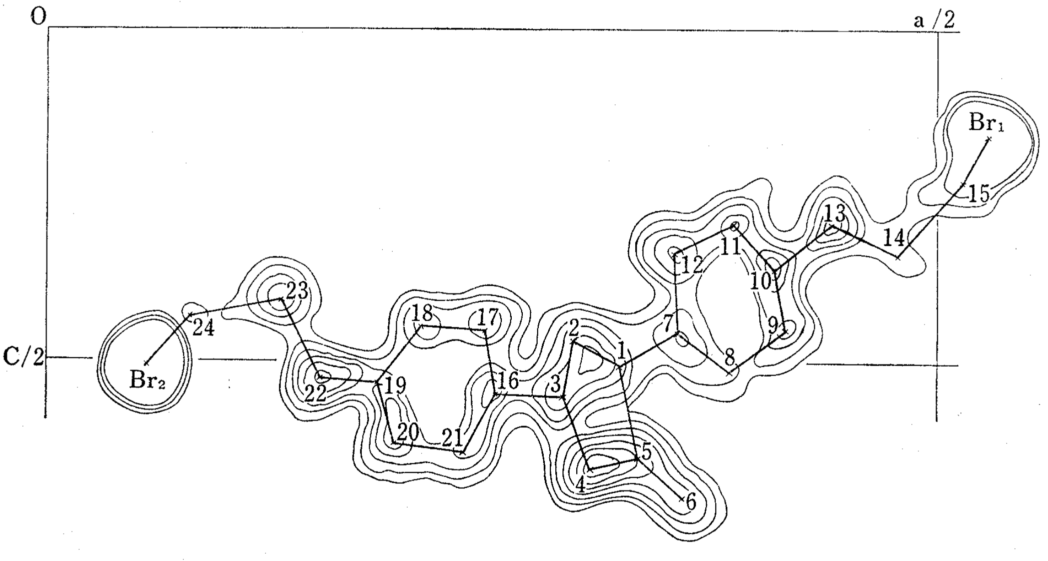

Fig. 3. *b*-Axis Projection of the Electron Density Distribution Function of Compound B

Contours are drawn at intervals of 1e./Å² starting at 2e./Å². Those for bromine are not shown.

Table I. Final Atomic Parameters

	x	z		x	z
Br(1)	0.5265	0.168	C(12)	0.350	0.340
Br(2)	0.0535	0.500	O(13)	0.440	0.290
N(1)	0.321	0.505	C(14)	0.476	0.339
N(2)	0.295	0.470	C(15)	0.515	0.230
N(3)	0.290	0.550	C(16)	0.250	0.547
N(4)	0.308	0.655	C(17)	0.247	0.452
C(5)	0.333	0.645	C(18)	0.210	0.445
O(6)	0.358	0.705	C(19)	0.184	0.530
C(7)	0.349	0.453	C(20)	0.195	0.620
C(8)	0.380	0.525	C(21)	0.232	0.635
C(9)	0.410	0.460	O(22)	0.150	0.525
C(10)	0.408	0.368	C(23)	0.132	0.405
C(11)	0.385	0.290	C(24)	0.080	0.435

An overall temperature factor of 4.5 Å^2 was assigned for all atoms.

the final electron density map, was then constructed. This model shows that the molecule is almost planar except for the terminal bromine atoms and that each bromine atom is situated at the gauche position about the ethoxyl C-C bond with respect to the oxygen atom. The torsion angles about the bonds joining the tetrazolone and benzene rings may not exceed 20°. The planar configuration of the five-membered ring including its exocyclic carbon atoms, C(7) and C(16), bonded to N(1) and N(3) respectively, indicates the resonance structure of tetrazolone.

On the basis of the result of the present two-dimensional X-ray analysis and of the chemical study, the structure of the fluorescent compound A is now concluded to be formulated as 1,3-bis (4-ethoxyphenyl)-5-tetrazolone.

Experimental5)

Fluorescent Compound obtained from *p*-Phenetidine——To a solution of *p*-phenetidine hydrochloride (10 g) in H_2O (2000 ml) was added carefully conc. HCl (200 ml) and 10% $NaNO_2$ (1000 ml), during 30 min at room temperature. The reaction mixture was made alkaline with 20% NaOH (1000 ml), stood for 40 min at room temperature, and extracted twice with $CHCl_3$. The combined extracts were dried over anhyd. Na_2SO_4, concentrated to a few ml and chromatographed through a column of silica gel (Mallinchrodt, 100 mesh, 100 g) by eluting with $CHCl_3$. The fluorescent fraction was concentrated and rechromatographed on a thin layer prepared from silica gel (Kiesel gel G, 500 μ, activated at 100°) using a mixture of $CHCl_3$ and EtOH (=50: 3) as a solvent. From the fluorescent spot, colorless crystals were obtained, mp 183—184°, and were identified with compound A by comparison of thin-layer chromatography (TLC), IR, UV, and fluorescence spectra, and by a mixed fusion.

4-Acetaminophenyl 2-Bromoethyl Ether——This compound was synthesized from 4-acetaminophenol and ethylene bromide in the presence of K_2CO_3 by Weigel's6) method as colorless crystals, mp 130—131°, (Reported, 130°7)). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3300, 1658, 1546 (-CONH), 1240, 1029 (phenylether), 515 (C-Br).

4-(2-Bromoethoxy)aniline Hydrobromide——This compound was synthesized from 4-acetaminophenyl 2-bromoethyl ether and hydrobromic acid by Jacobs's method8) as colorless leaflets, mp 227—228°, (recrystalized from ethanol), (Reported, 227—228°). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 2870, 1510 (-NH_3^+), 1250, 1010 (phenylether), 507 (C-Br).

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5) Melting points were determined in a Yanagimoto melting point apparatus and are not corrected; The UV spectra were taken in $CHCl_3$ solution on a Hitachi EPU-2 Spectrophotometer; The fluorescence spectra were taken in $CHCl_3$ solution on a Hitachi MPF-2A Fluorescence Spectrophotometer; The IR spectra were measured on a Diffraction Grating Infrared Spectrophotometer DS-403 G of Japan Spectroscopic Co, in KBr discs; The NMR spectrum was run on a Japan Electro Optics Lab. JNM-C-60HL High Resolution NMR Instrument (60MC) and recorded in ppm values with TMS as the internal standard.

6) W. Weigel, *J. Prakt. Chem.*, (4) **4**, 79 (1956); *Chem. Abstr.*, **51**, 12023 (1957).

7) O. Hinsberg, *Ann. Chem.*, **305**, 276 (1899).

8) W.A. Jacobs and M. Heidelberger, *J. Am. Chem. Soc.*, **39**, 2418 (1917).

Table II. Observed and Calculated Structure Factors

H	K	L	|F(OBS)|	|F(CAL)|
4	0	0	77.95	94.32
6	0	0	68.47	74.89
8	0	0	98.98	74.77
10	0	0	140.45	91.96
12	0	0	113.11	106.38
14	0	0	18.77	52.88
18	0	0	49.80	48.55
20	0	0	44.98	29.04
22	0	0	47.38	44.77
24	0	0	37.56	49.16
26	0	0	45.62	55.05
4	0	1	93.84	78.54
6	0	1	126.79	112.63
8	0	1	146.21	117.60
10	0	1	125.02	107.18
12	0	1	182.26	175.80
14	0	1	138.82	128.66
16	0	1	102.19	103.43
18	0	1	17.74	14.62
20	0	1	27.05	19.35
22	0	1	85.94	74.91
24	0	1	48.67	63.75
26	0	1	32.35	41.59
0	0	2	192.55	192.84
2	0	2	105.67	92.90
4	0	2	173.54	133.88
6	0	2	179.38	137.89
8	0	2	151.87	120.72
10	0	2	96.31	85.98
12	0	2	33.67	43.26
14	0	2	70.68	68.96
16	0	2	87.31	78.84
18	0	2	85.08	95.67
20	0	2	105.29	107.01
22	0	2	61.01	56.57
24	0	2	27.86	43.24
2	0	3	180.91	166.13
4	0	3	203.06	169.36
6	0	3	199.92	189.45
8	0	3	230.23	200.05
10	0	3	70.46	59.15
14	0	3	20.59	49.90
16	0	3	27.12	22.95
18	0	3	20.57	17.03
20	0	3	64.03	29.29
22	0	3	38.09	48.91
24	0	3	28.37	3.32
0	0	4	137.74	130.81
2	0	4	82.25	65.24
4	0	4	78.94	75.68
6	0	4	72.79	85.87
8	0	4	104.26	75.44
10	0	4	104.50	88.06
12	0	4	57.53	40.93
14	0	4	43.73	33.17
16	0	4	104.23	99.12
18	0	4	104.50	118.75
20	0	4	80.54	79.15
22	0	4	24.02	35.61
2	0	5	47.74	48.18
4	0	5	68.93	70.43
6	0	5	58.78	57.98
8	0	5	95.47	81.70
10	0	5	89.11	82.98
12	0	5	112.85	107.66
14	0	5	57.24	64.02
16	0	5	80.98	70.83
18	0	5	26.06	34.64
20	0	5	33.55	23.74
22	0	5	39.19	43.12
24	0	5	57.36	57.26
0	0	6	167.35	202.84
2	0	6	107.40	114.48
4	0	6	40.30	61.92
6	0	6	52.25	45.97
8	0	6	78.72	78.81
10	0	6	73.39	65.91
12	0	6	53.50	56.75
14	0	6	50.09	51.87
16	0	6	32.11	19.11
2	0	7	22.80	25.32
4	0	7	55.34	70.35
6	0	7	80.69	69.40
8	0	7	27.02	19.21
10	0	7	59.78	57.14
12	0	7	95.71	101.73
14	0	7	76.32	66.22
16	0	7	55.54	45.67
20	0	7	29.54	26.97
0	0	8	54.55	69.45
2	0	8	25.10	30.45
4	0	8	28.34	27.22
6	0	8	28.78	36.30
8	0	8	47.38	62.25
10	0	8	30.10	32.24
12	0	8	19.56	28.35
14	0	8	31.78	35.97
16	0	8	35.71	43.10
18	0	8	42.12	58.37
20	0	8	41.38	46.53
22	0	8	33.94	43.42
2	0	9	44.23	54.88
4	0	9	42.38	55.63
6	0	9	58.99	81.06
8	0	9	41.14	63.76
2	0	10	39.89	52.92
4	0	10	35.86	34.47
6	0	10	20.83	23.78
8	0	10	29.71	34.75
10	0	10	29.98	37.73
12	0	10	30.22	27.41

2-Bromoethoxy Derivative (Compound B)——To a solution of 4-aminophenyl 2-bromoethyl ether hydrobromide (20 g) in H_2O (2000 ml) was added carefully conc. HCl (200 ml) and 10% $NaNO_2$ (1000 ml), during 30 min at room temperature. The reaction mixture was made alkaline with 20% NaOH (1000 ml) at room temperature, and extracted with $CHCl_3$ after 40 min. The $CHCl_3$ extract was chromatographed under the same conditions as fluorescent compound A obtained from *p*-phenetidine. Crude crystals were refined using a column prepared from a mixture of Celite and charcoal (1: 1). The product was recrystallized from ethyl acetate to give pale yellow crystals (41.7 mg), mp 167—168°. *Anal.* Calcd. for $C_{17}H_{16}O_3N_4Br_2$: C, 42.17; H, 3.33; N, 11.57; Br, 33.01. Found: C, 42.66; H, 3.17; N, 11.75; Br, 33.11.

Two-dimensional X-ray Analysis of Fluorescent Compound B——A thin flaky crystal [flattened on (100)] of compound B mounted in (010) was used for X-ray diffraction work. Size of the crystal was about $0.7 \times 0.2 \times 0.01$ mm and the crystal was mounted along the longest edge. Weissenberg photographs of $h0l$ and $h1l$ layers and precession photographs of $hk0$ and $0kl$ were taken with Cu*Ka* radiation. The lattice constants were determined from the two equatorial precession photographs. The space group was determined to be $Pna2_1$ from the systematic absences; $h0l$ when $h \neq 2n$, $0kl$ when $k+l \neq 2n$, $00l$ when $l \neq 2n$. The density measured by the flotation method was in good agreement with the value calculated by assuming four molecules in the unit cell. Since the number of asymmetric units in the unit cell of $Pna2_1$ is four, no symmetry is imposed on the molecule despite the apparent high symmetry of some of the proposed structures. Crystal data: 1,3-bis [4-(2-bromoethoxy)phenyl]-5-tetrazolone: M. W. 484, mp 167—168°.

Orthorhombic, $Pna2_1$: $a=32.8 \pm 0.3$, $b=4.60 \pm 0.03$, $c=12.3 \pm 0.1$ Å; $U=1860$ Å^3; $Dm=1.731$ g·cm^{-3}, $Dx=1.738$ g·cm^{-3}; $Z=4$.

Intensity data were collected from Weissenberg photographs using the multiple film technique. However, it was not possible to measure accurate intensities from the photographs, since all the crystals grown from ethyl acetate solutions were quite thin and their faces were curved so that even on the equatorial layer line some of the diffraction spots were extended as much as about 3 mm in the direction of the film translation. In the 1st layer line, the spots were much more elongated and diffused to form almost continuous lines along the row lines parallel to $\boldsymbol{a}^*$, which makes almost impossible to measure the intensity for each spot. The intensities were therefore measured only for $h0l$ reflections with the aid of microdensitometer.

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