Notes

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Ryozo Hirata : Experimental Anticancer Studies. I. The Preparation of 6-(2'-Hydroxy-3',5'-dibromophenylazo)-4-hexylresorcinol and Related Compounds, with Brief Reference to their Anticancer Activity.

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Within the space of a few years, many reports have appeared on agents for chemotherapy of malignant tumors.[1)]

On the basis of the observations made by Ito *et al.*[2)] in 1948 that 2,2'-dihydroxyazobenzene** exhibits a very potent inhibiting effect against the production of streptolysin S of *Streptococcus hemolyticus* in the medium containing ribonucleic acid,[3)] experimental studies in chemotherapy of cancer have been carried out for these two years.

2,2'-Dihydroxyazobenzene was obtained by Weselsky and Benedikt,[4)] in 1878, by the alkali fusion experiment of *o*-nitrophenol. They further prepared two halogen derivatives of this azo compound, 3,5,5'-trichloro-2,2'-dihydroxyazobenzene and 3,3',5,5'-tetrabromo-2,2'-dihydroxyazobenzene. Later, Orton[5)] reported the preparation of (2'-hydroxy-3',5'-dihalophenylazo)-2-naphthol by coupling 4,6-dihalo-*o*-diazophenol with *β*-naphthol. Quite recently, Koshimura[6)] of our laboratory prepared bis(2'-hydroxy-3',5'-dibromophenylazo)-N^a-benzoyl-*l*-histidine and bis(2'-hydroxy-3',5'-dibromophenylazo)-*l*-tyrosine by coupling 4,6-dibromo-*o*-diazophenol, suspended in alkaline water, with N^a-benzoyl-*l*-histidine and -*l*-tyrosine respectively.

Extending this study, a number of azo compounds belonging to 2,2'-dihydroxyazobenzene series, a potent chelating agent, were synthesized, and tested for their activity upon Yoshida sarcoma and Ehrlich ascites sarcoma.

The purpose of this report is to describe the preparation procedures and physical and chemical properties of the azo compounds that were used in the biological experiments in this line of work.

According to the procedure of Koshimura, attempt was first made to couple 4,6-dibromo-*o*-diazophenol with either one of the following nine phenols : *p*-Nitrophenol, *p*-acetylaminophenol, *p*-sulfophenol, ethyl *p*-hydroxybenzoate, thymol, 4-chlorothymol, resorcinol, 4-hexylresorcinol, and phloroglucinol. Although the coupling experiments failed with *p*-nitrophenol, *p*-acetylaminophenol, *p*-sulfophenol, and ethyl *p*-hydroxybenzoate, the coupling occurred easily with all other phenols with 4,6-dibromo-*o*-diazophenol, giving rise to a type of azo compounds shown in Table I.

In the biological test on rats inoculated with Yoshida sarcoma, it was observed that of the seven azo compounds, 6-(2'-hydroxy-3',5'-dibromophenylazo)-4-hexyl-

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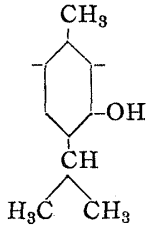
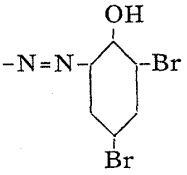
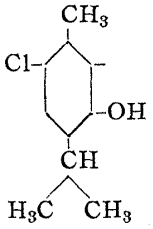
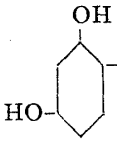
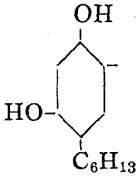
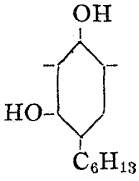
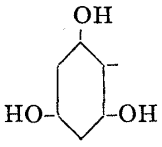
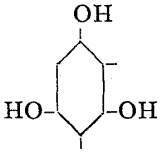
** cf. "Experiments on the Influence of Various Derivatives in the Dihydroxyazobenzene Series upon the Production of Streptolysin S by *Streptococcus hemolyticus* in the Resting Cell System." Part 2. by Himeno (Annual Report of the Research Institute of Tuberculosis, Kanazawa University, Japan, Vol. 12, No. 3, 111 (1954)).

1) J. P. Greenstein : "Biochemistry of Cancer," Academic Press, N. Y., 276(1954); M. Ishidate, T. Yoshida, Y. Sakurai, *et al.* : Proc. Japan Acad., **27**, 493(1951).
2) R. Ito, *et al.* : Japan. Med. J., **1**, 260(1948).
3) H. Okamoto : Japan. J. Med. Sci., **IV**. Pharmacol., **12**, 167(1940).
4) P. Weselsky, R. Benedikt : Ann., **196**, 346(1878).
5) Orton : J. Chem. Soc., **83**, 804(1887); **91**, 1566(1895).
6) S. Koshimnra : Japan. J. Exptl. Med., **21**, 343(1951).

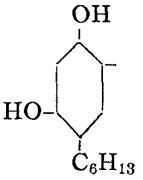
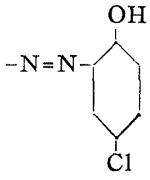
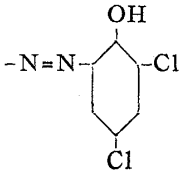
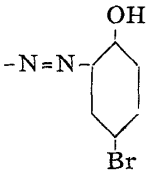
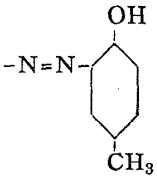
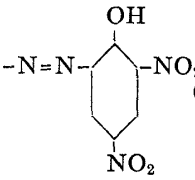
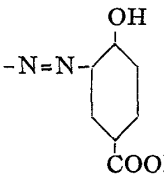
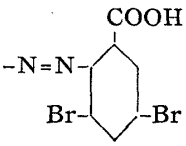
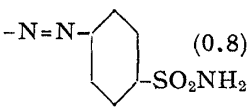
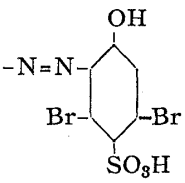
TABLE I.

No. of Azo Compd.	Coupling component (g.)	Diazo component (g. of base)	m.p. (°C)	Appearance (Solvent for recrystn.)	Yield (g.)	Formula	N% Found	N% Calcd.
1	 (0.7)	 (3.3)*	226 ~227	reddish brown needles (EtOH)	2.3	$C_{22}H_{18}O_3N_4Br_4$	7.93	7.90
2	 (1.8)	〃	(〃)*	226 (de-comp.)	reddish brown needles (EtOH)	2.1	$C_{16}H_{15}O_2N_2Br_2Cl$	6.05	6.57
3	 (1.0)	〃	(〃)*	209 ~211	reddish brown needles (EtOH)	2.8	$C_{12}H_8O_3N_2Br_2$	7.22	7.41
4	 (2.0)	〃	(〃)*	174 ~176	orange yellow needles (benzene)	3.3	$C_{18}H_{20}O_3N_2Br_2$	5.93	5.64
5	 (1.0)	〃	(〃)*	211	brown needles (benzene)	0.5	$C_{24}H_8O_4N_4Br_4$	5.66	5.85
6	 (1.3)	〃	(〃)*	>300	red cryst. (EtOH)**	3.5	$C_{12}H_8O_4N_2Br_2$	6.93	7.29
7	 (0.7)	〃	(〃)*	>300	red cryst. (EtOH)**	2.5	$C_{18}H_{10}O_5N_4Br_4$	8.21	7.92

* As a hydrochloride.

** The crude material was dissolved in 5% KOH-EtOH with warming and acidified with 5% HCl-EtOH.

TABLE II.

No. of Azo Compd.	Coupling component (g.)	Diazo component (g. of base)	m.p. (°C)	Appearance (Solvent for recrystn.)	Yield (g.)	Formula	N% Found	N% Calcd.
8	 (1.0)	 (0.9)*	193 ~194	orange needles (benzene)	0.7	$C_{18}H_{21}O_3N_2Cl$	8.03	7.77
9	〃 (2.0)	 (2.1)*	165	orange yellow needles (benzene)	2.7	$C_{18}H_{20}O_3N_2Cl_2$	7.31	7.38
10	〃 (1.0)	 (1.0)*	206 (de-comp.)	brown needles (benzene)	0.8	$C_{18}H_{21}O_3N_2Br$	7.13	7.19
11	〃 (〃)	 (0.8)*	209	orange red needles (benzene)	1.2	$C_{19}H_{24}O_3N_2$	8.53	8.50
12	〃 (〃)	 (2.0)	204	brown needles (EtOH)	1.2	$C_{18}N_{20}O_7N_4$	13.86	13.50
13	〃 (〃)	 (1.0)*	240	orange red (EtOH)	1.4	$C_{18}N_{22}O_5N_2$	8.09	7.61
14	〃 (〃)	 (1.0)	181 ~182	fine red needles (benzene)	0.5	$C_{19}H_{20}O_4N_2Br_2$	5.60	6.15
15	〃 (〃)	 (0.8)	166 ~167	fine red needles	0.8	$C_{18}H_{23}O_4N_3S$	13.42	13.60
16	〃 (〃)	 (1.0)	238 (de-comp.)	orange yellow needles (water)	0.7	$C_{18}H_{20}O_6N_2Br_2S$	5.07 (Br, 28.90;	4.82 29.05%)

* As a hydrochloride.

resorcinol was especially active in causing the destruction of tumor cells when injected intraperitoneally.

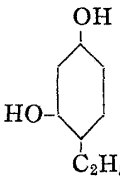
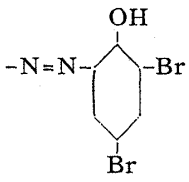
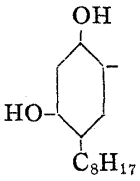
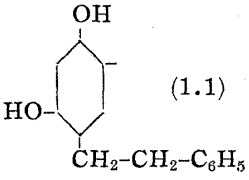
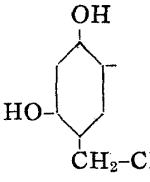
In view of this observation, it seemed worth while to investigate the activity of azo compounds of hexylresorcinol series. Thus, nine azo compounds shown in Table II were synthesized. Here, it is notable that eight azo compounds, No. 8 to No. 15, were found to be preparable in good yield by coupling 4-chloro-, 4,6-dichloro-, 4-bromo-, 4-methyl-, 4,6-dinitro-, and 4-carboxy-2-aminophenol, 3,5-dibromo-2-aminobenzoic acid, and sulfamide with 4-hexylresorcinol in the usual way, and that 6-(2′-hydroxy-4′,6′-dibromo-5′-sulfophenylazo)-4-hexylresorcinol (No. 16) was obtained by coupling the diazotized 1-amino-2,4,6-tribromobenzene-3-sulfonic acid and 4-hexylresorcinol in the presence of sodium carbonate, as in the case of the synthesis of 1-(2′-hydroxy-4′,6′-dibromo-5′-sulfophenylazo)-2-naphthol by Noeliting and Battegy.[7]

The results of biological experiment with these azo hexylresorcinols may be summarized as follows:

a) 6-(2′-Hydroxy-5′-chlorophenylazo)-4-hexylresorcinol (No. 8), 6-(2′-hydroxy-3′,5′-dichlorophenylazo)-4-hexylresorcinol (No. 9), and 6-(2′-hydroxy-5′-bromophenylazo)-4-hexylresorcinol (No. 10) were proved to be somewhat less active in affecting tumor cells than 6-(2′-hydroxy-3′,5′-dibromophenylazo)-4-hexylresorcinol (No. 4).

b) Azohexylresorcinol derivatives having either $-NO_2$, $-CH_3$, $-SO_3H$, or $-COOH$ in the benzene ring of the azo component were all found to be quite innocuous to the tumor cells.

Table III.

No. of Azo Compd.	Coupling component (g.)	Diazo component (g. of base)	m.p. (°C)	Appearance (Solvent for recrystn.)	Yield (g.)	Formula	N% Found	N% Calcd.
17	 (1.5)	 (3.3)*	222 ~223	red needles (EtOH)	2.0	$C_{14}H_{12}O_3N_2Br_2$	6.73	6.64
18	 (0.4)	〃 (0.6)*	168 ~171	orange red needles (benzene)	0.2	$C_{20}H_{24}O_3N_2Br_2$	5.60	5.62
19	 (1.1)	〃 (1.6)*	178 ~179	red needles (EtOH)	2.0	$C_{20}H_{16}O_3N_2Br_2$	5.69	5.66
20	 (1.8)	〃 (3.3)*	258 (de-comp.)	red needles (EtOH)	2.7	$C_{14}H_{12}O_5N_2Br_2$	6.25	6.01

* As a hydrochloride.

7) E. Noeliting, M. Battegy: Ber., **39**, 83(1906).

As may be seen from Table III, four azo compounds, in which the C_6H_{13}- group in 4-position of 6-(2'-hydroxy-3',5'-dibromopheylazo)-4-hexylresorcinol is replaced by either $-C_2H_5$, $-C_8H_{17}$, or $-C_2H_4C_6H_5$, or by $-CH_2COOH$, were also synthesized; none was found to be strongly active.

It should here be added that numerous experiments performed recently by Koshimura *et al.*, have shown that intraperitoneal administration of both 6-(2'-hydroxy-3',5'-dibromophenylazo)-4-hexylresorcinol and 6-(2'-hydroxy-3',5'-dichlorophenylazo)-4-hexylresorcinol, caused a consistent prolongation of the life of mice transplanted with Ehrlich ascites sarcoma.

The details of these biological test experiments will be published in the near future.

The author is greatly indebted to Prof. Dr. H. Okamoto and Dr. S. Koshimura for valuable helps and advices.

Experimental

As there is no essential variation in the experimental procedure employed in the preparation of these azo compounds, only a few examples will be given for the preparation of 6-(2'-hydroxy-3',5'-dibromophenylazo)-4-hexylresorcinol, 6-(2'-hydroxy-3',5'-dichlorophenylazo)-4-hexylresorcinol and 6-(2'-hydroxy-4',6'-dibromo-5'-sulfophenylazo)-4-hexylresorcinol. The quantities of materials used in the synthesis, the physical constants, and analysis are listed in Tables I, II, and III.

6-(2'-Hydroxy-3',5'-dibromophenylazo)-4-hexylresorcinol (No. 4)—a) Diazotization of 4,6-dibromo-2-aminophenol: 3.3 g. of 4,6-dibromo-2-aminophenol hydrochloride was dissolved in a mixture of 150 cc. of water and 25 cc. of *N* HCl, and diazotized with 0.8 g. of $NaNO_2$ dissolved in 10 cc. of water, at 0° to 5°, with starch iodide paper as an indicator. After standing the reaction mixture for 20 mins. in an ice bath, the precipitate produced was collected by filtration, washed thoroughly with chilled water. The precipitate of 4,6-dibromo-*o*-diazophenol thus obtained was suspended in 100 cc. of water and the suspension was directly used for coupling experiment described below.

b) Coupling with 4-hexylresorcinol: 2.0 g. of 4-hexylresorcinol was dissolved in a mixture of 100 cc. of water and 40 cc. of *N* NaOH and cooled well. To the mixture was added, with stirring, the above-mentioned suspension of 4,6-dibromo-*o*-diazophenol, and the reaction mixture, after being kept at a temperature of 0° to 5° for 3 hrs., was allowed to stand in an ice box over night. The deep reddish solution resulted was filtered through a paper to remove a small amount of unchanged 4,6-dibromo-*o*-diazophenol, and the filtrate was acidified with HCl, which caused voluminous, reddish precipitate to form. The precipitate was collected, washed with water, pressed on a clay plate, and the crude product was recrystallized from benzene; yield, 3.3 g., m.p. 174~176°. *Anal.* Calcd. for $C_{18}H_{20}O_3N_2Br_2$: N, 5.93. Found: N, 5.64.

6-(2'-Hydroxy-3',5'-dichlorophenylazo)-4-hexylresorcinol (No. 9)—a) Diazotization of 4,6-dichloro-2-aminophenol: 2.1 g. of 4,6-dichloro-2-aminophenol hydrochloride was dissolved in a mixture of 75 cc. of water and 25 cc. of *N* HCl, and diazotized with 0.8 g. of $NaNO_2$ in the same manner as described above. The crystalline paste, 4,6-dichloro-*o*-diazophenol, obtained was then suspended in 100 cc. of water, and cooled to 0°.

b) Coupling with 4-hexylresorcinol: 2.0 g. of 4-hexylresorcinol was dissolved in a mixture of 40 cc. of *N* NaOH and 10 cc. of water, and to this solution, after cooling to 0°, was added all at once the above suspension of 4,6-dichloro-*o*-diazophenol. The reaction mixture was stirred continuously for 3 hrs. at 0° to effect good mixing, and then placed in an ice box over night. The deep reddish reaction mixture, after passing through a paper to remove a small amount of unchanged diazophenol, was acidified with HCl. The precipitate was collected, washed with water, dried, and recrystallized from benzene; yield 2.7 g., m.p. 165°. *Anal.* Calcd. for $C_{18}H_{20}O_3N_2Cl_2$: N, 7.31. Found: N, 7.38.

6-(2'-Hydroxy-4',6'-dibromo-5'-sulfophenylazo)-4-hexylresorcinol (No. 16)—2.0 g. of 1-amino-2,4,6-tribromobenzene-5-sulfonic acid was dissolved in a mixture of 5 cc. of *N* NaOH and 10 cc. of water, and to this solution was added 20 cc. of *N* HCl, and diazotized with 0.5 g. of $NaNO_2$ dissolved in 5 cc. of water at 0° to 5°. The yellowish fine needles produced was washed thoroughly with chilled water, and dissolved in a mixture of 2.0 g. of Na_2CO_3 and 100 cc. of water. On the other hand, 1.0 g. of 4-hexylresorcinol was dissolved in 20 cc. of *N* NaOH, and to this solution was added the above-mentioned solution of carbonate-treated diazo compound. The reaction mixture was stirred continuously for 3 hrs. at 0°. The reddish reaction mixture resulted was acidi-

fied with HCl, which caused orange precipitate to form. The precipitate was collected and recrystallized from water. Yield 0.7 g., m.p. 238°(decomp.). *Anal.* Calcd. for $C_{18}H_{20}O_6N_2Br_2S$: N, 5.07; Br, 28.90. Found : N, 4.82; Br, 29.05.

Summary

A total of 20 azo compounds related to 6-(2′-hydroxy-3′,5′-dibromophenylazo)-4-hexylresocinol were prepared. The effect of these newly synthesized azo compounds upon Yoshida sarcoma and Ehrlich ascites sarcoma is described briefly.

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Shoji Shibata, Kôtaro Takahashi, and Yôko Tanaka (nèe Hiizumi) : Decomposition of Usnic Acid. V.* Pyrolysis of Dihydrousnic Acid.(2). Some Observations on Dihydrousnic Acid.

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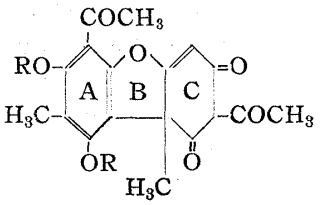
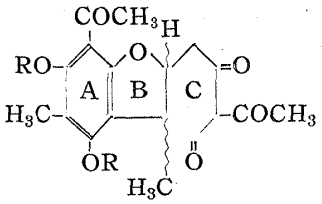
The structural formula of usnic acid, which was previously advanced by Robertson[1] and Schöpf,[2] has recently been established by Barton and his co-workers[3] by their elegant synthesis of (±)usnic acid which was accomplished by the oxidative condensation of methylphloroacetophenone.



(I) R=H Usnic acid
(II) R=$COCH_3$ Diacetylusnic acid



(III) R=H Dihydrousnic acid
(IV) R=$COCH_3$ Diacetyldihydrousnic acid

It should be noted, however, that dihydrousnic acid (III) showed some peculiar behavior on pyrolysis, on which we elucidated previously.[4]

The earlier workers[5] described that usnic acid resists direct catalytic hydrogenation and dihydrousnic acid could only be prepared through diacetylusnic acid which is hydrogenated catalytically to give diacetyldihydrousnic acid.

In this reaction process a conversion of optical rotation occurs in giving (−)dihydrousnic acid from (+)usnic acid, and (+)dihydrousnic acid from (−)usnic acid.

The present communication concerns direct hydrogenation of usnic acid and an isomerization of dihydrousnic acid.

Using tetrahydrofuran as a solvent and palladium-black as a catalyst, dihydrousnic acid was obtained directly from usnic acid in a sufficient yield. The optical conversion was also observed in this case as in the indirect hydrogenation.

On the other hand, during the course of examination on the pyrolysis of dihydro-

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