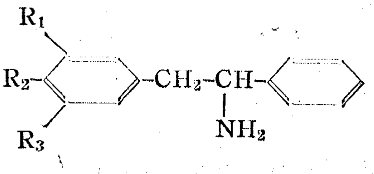
42. **Sakahiko Owari:** Effect of α,β-Diphenylethylamines upon Yoshida Sarcoma.*

(*Iatro-chemical Institute of the Pharmacological Research Foundation***)

The fact that colchicine has a remarkable inhibiting action upon cell mitosis has raised much interest in biochemistry, as it would be interesting to know which part of its molecular constitution plays the chief rôle in the peculiar mitotic effect. In 1943, Lettré[1] showed that some derivatives of α,β-diphenylethylamine (stilbylamine), which were believed at that time to have a partial constitution of Windaus' formula of colchicine, had more or less inhibitory effect on mitosis of tissue-cultured fibroblast cells of chick embryo. However, Rapoport[2] completed the total synthesis of *dl*-N-acetylcolchicinol methyl ether and accordingly, the B-ring of colchicine molecule was decided as seven-membered.

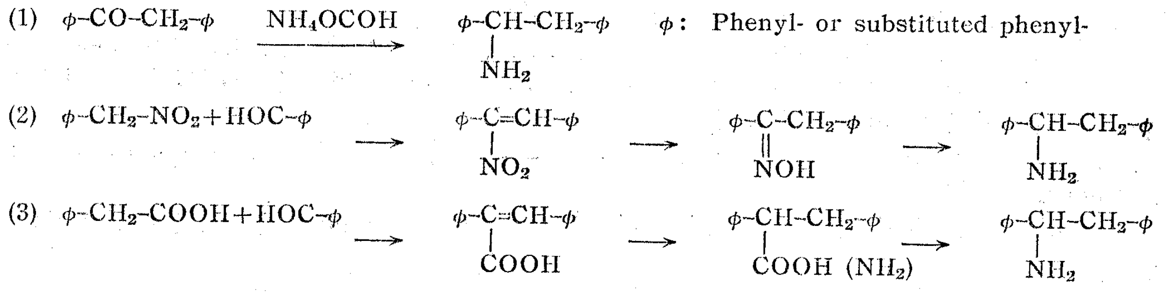
Although, according to this aspect, the derivatives of α,γ-diphenylpropylamine should be the compounds in question instead of α,β-diphenylethylamines, it was also found by Lettré[3] that no derivatives of benzhydrylamine or α,γ-diphenylpropylamine exhibit any effect on mitotic cells.

Five stilbylamines shown in the following were synthesized.



Compd. No.	I	II	III	IV	V
R_1	H	H	CH_3O-	CH_3O-	$CH_2<\begin{smallmatrix}O-\\O-\end{smallmatrix}$
R_2	H	CH_3O-	CH_3O-	CH_3O-	
R_3	H	H	H	CH_3O-	H

In order to prepare these compounds, the following three routes of synthesis were adopted. Out of them, the method (3) is not found so far in literature and the details are given in the Experimental Part.



(1) $\phi\text{-CO-CH}_2\text{-}\phi \xrightarrow{NH_4OCOH} \phi\text{-}\underset{NH_2}{\underset{|}{CH}}\text{-CH}_2\text{-}\phi$ ϕ: Phenyl- or substituted phenyl-

(2) $\phi\text{-CH}_2\text{-NO}_2+\text{HOC-}\phi \longrightarrow \phi\text{-}\underset{NO_2}{\underset{|}{C}}\text{=CH-}\phi \longrightarrow \phi\text{-}\underset{NOH}{\underset{||}{C}}\text{-CH}_2\text{-}\phi \longrightarrow \phi\text{-}\underset{NH_2}{\underset{|}{CH}}\text{-CH}_2\text{-}\phi$

(3) $\phi\text{-CH}_2\text{-COOH}+\text{HOC-}\phi \longrightarrow \phi\text{-}\underset{COOH}{\underset{|}{C}}\text{=CH-}\phi \longrightarrow \phi\text{-}\underset{COOH\ (NH_2)}{\underset{|}{CH}}\text{-CH}_2\text{-}\phi \longrightarrow \phi\text{-}\underset{NH_2}{\underset{|}{CH}}\text{-CH}_2\text{-}\phi$

These compounds had already been investigated by Lettré and the compounds (II) and (V) were reported to be active against mitosis of fibroblast cells at the concentration of $4\sim6\,\gamma$/cc., but (III) and (IV) were inactive. The aim of the present experiments was to study their effect on the mitosis of Yoshida sarcoma cells, especially in relation to the studies on chemotherapy of cancer.

The cytological experiments were carried out by Prof. Tomizo Yoshida (Tohoku University) and his collaborators, and the details will be published elsewhere. In short, the results obtained with Yoshida sarcoma were somewhat different from that of Lettré's. These five compounds were toxic in similar degrees against rats by intraperitoneal administration (M.L.D. 100 mg./kg.) and no remarkable difference was observed between each compound in their cytological effect.

---

* M. Ishidate, Y. Sakurai: Studies on Cancerocidal Substances. III.

** 33 Nishigahara, Kita-ku, Tokyo (尾張栄彦).

1) H. Lettré: Z. physiol. Chem. (Hoppe-Seyler's), 278, 175 (1943).

2) H. Rapoport: J. Am. Chem. Soc., 72, 3324 (1950); 73, 1896 (1951).

3) H. Lettré: Z. physiol. Chem. (Hoppe-Seyler's), 281, 139 (1944); Angew. Chem., 63, 421 (1951).

The tumor cells remained intact when 20 mg./kg. of the compound was injected into peritoneal cavity of animals with Yoshida sarcoma. A slight pycnotic degeneration of nuclei and spontaneous appearance of leucocytes in ascites were observed within 6 hours after injection of the increased dose (50∼70 mg./kg.), which however, did not last so long***. Neither colchicine-like inhibition of mitosis nor the prolongation of life-length of tumor animals occured. A wide-spread infiltration of tumor around mesenterium and omentum were observed on autopsy, i.e. all animals died of typical tumor death.

On the other hand, Prof. Bungo Wada (Tokyo University) has also examined the effects of these compounds aginst mitotic cells of young staminal hair of *Tradescantia reflexa*. The results of his experiment were the same as in the case of Yoshida sarcoma cells, that is, the compounds exhibited some necrobiotic change in nuclei but no colchicine-like inhibition. It was found, however, that the toxicity of the compounds against these plant mitotic cells decreased with the substitution of the benzene ring in the compounds with methoxyl groups.

The author expresses his deep gratitude to Prof. Tomizo Yoshida and to Prof. Bungo Wada for their kind advices during the course of this experiment.

Experimental

1) **α,β-Diphenylethylamine**—A mixture of 5 g. of desoxybenzoin and 10 g. of ammonium formate is heated in a sealed tube for 4 hours at 220∼230°[4]. Hydrochloride of α,β-diphenylethylamine melts at 253°. Yield, 83%[5].

2) **α-Phenyl-β-(4-methoxyphenyl)-ethylamine**—ω-Nitrotoluene is prepared in a good yield by the method of Wiselicenus and Endres. A mixture of 2 g. each of ω-nitrotoluene and anisaldehyde is added with a small quantity of alcohol and methylamine, and kept at room temperature for a week[6]. α-Nitro-α-phenyl-β-(4-methoxyphenyl)-ethylene crystallizes from the mixture as yellow needles, m.p. 151°. 1.5 g. of the latter is dissolved in 10 cc. of pyridine and shaken with Pd-carbon in hydrogen stream without heating[7]. Two molar equivalents of hydrogen is quantitatively absorbed and 4-methoxydesoxybenzoin oxime is obtained which melts at 132°. This is again dissolved in alcohol and shaken with Pd-carbon catalyst in hydrogen at 60°. α-Phenyl-β-(4-methoxyphenyl)-ethylamine is obtained as the hydrochloride, m.p. 208∼209°[7]. Yield, 0.6 g.

3) **α-Phenyl-β-(3,4-dimethoxyphenyl)-ethylamine**—This is obtained by the same process as described in (2), using veratrum aldehyde instead of anisaldehyde. However, being the condensation of the aldehyde with ω-nitrotoluene the former method is rather drastic in this case and it is necessary to keep the reaction mixture in ice-box for 24 hours which is sufficient for completion of the reaction in this case. The free base of α-phenyl-β-(3,4-dimethoxyphenyl)-ethylamine and its bioxalate melt respectively at 66∼67° and 176°[7].

4) **α-Phenyl-β-(3,4,5-trimethoxyphenyl)-ethylamine**—3,4,5-Trimethoxybenzaldehyde is prepared by the Rosenmund reduction of 3,4,5-trimethylgallic acid chloride. The condensation and reduction are carried out as for compounds (2) and (3). Hydrochloride of this amine comes as colorless fine needles, m.p. 224°[7].

5) **α-Phenyl-β-(3,4-methylenedioxyphenyl)-ethylamine**— a) α-Phenyl-β-(3,4-methylenedioxyphenyl)-acrylic acid: A mixture of piperonylaldehyde (7.0 g.), sodium phenylacetate (9.0 g.), and acetic anhydride (35.0 g.) is heated at 150∼160° for 10 hours. The reaction mixture is allowed to stand in an ice-box after decomposing the remaining acetic anhydride by warming with water. The sodium salt of α-phenyl-β-(3,4-methylenedioxyphenyl)-acrylic acid separated thereby is collected and dissolved in hot water, from which the free acid gradually crystallizes by acidification with dil. hydrochloric acid. Light yellow needles, m.p. 233°. Yield, 8.5 g. *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.51; H, 4.48.

b) α-Phenyl-β-(3,4-methylenedioxyphenyl)-propionic acid: It is obtained quantitatively by the catalytic reduction of acrylic acid, as colorless prisms, m.p. 126°, and its acid amide, m.p. 145°, which is easily prepared in the usual manner. *Anal.* Calcd. (for the acid) $C_{16}H_{14}O_4$: C, 71.11; H, 5.15. Found: C, 70.81; H, 5.15. Calcd. (for the amide) $C_{16}H_{15}O_3N$: C, 71.37; H, 5.58; N, 5.21. Found: C, 71.53; H, 5.61; N, 5.49.

*** For the method of animal experiment, cf. T. Yoshida, M. Ishidate, Y. Sakurai, H. Sato: Gann, **41**, 93 (1951).

4) R. Leuchart, H. Jansen: Ber., **22**, 1409 (1889).

5) W. Wislicenus, A. Endres: Ber., **35**, 1755 (1902).

6) E. Knoevennagel: Ber., **37**, 4509 (1904).

7) B. Reicht, W. Hoffmann: Chem. Zentr., **1936**, (4909).

c) Hofmann's reaction of propionic acid amide: 5.6 g. of the amide is added to the solution of 3.5 g. of bromine, 6.5 g. of KOH, and 120 cc. of water. The mixture is warmed on a boiling water bath for 5 minutes and after cooled, is made strongly alkaline with solid KOH. The solution is extracted with ether and the ether extract is evaporated to dryness. The residue becomes a solid when added with a small quantity of hydrochloric acid. α-Phenyl-β-(3,4-methylenedioxyphenyl)-ethylamine hydrochloride crystallizes as colorless prisms, m.p. 258~259°7). Yield, 4 g. *Anal.* Calcd. for $C_{15}H_{16}O_2NCl$: C, 64.87; H, 5.77; N, 5.05. Found: C, 64.69; H, 5,72; N, 4.80.

It was also confirmed that the compounds (II) and (III) can be obtained by Hofmann's reaction with the corresponding acid amides, but the yields were not always appreciable in these cases.

Summary

Several derivatives of α,β-diphenylethylamine were synthesized and their cytological effect on Yoshida sarcoma cells was examined. These compounds were able to induce karyolysis and pycnosis of cell nuclei in a comparatively large dose but no colchicine-like inhibition of mitosis was observed.

(Received April 21, 1953)

---

43. **Yohei Hashimoto:** Ultraviolet Papyrography*. An Apparatus for detecting Substances on Papergrams by Ultraviolet Light.

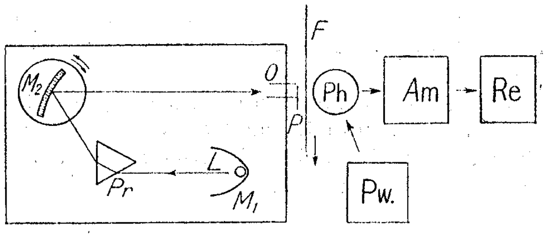
(*Laboratory of Phytochemistry, Kobe Women's College of Pharmacy***)

The detection of substances on papergram, in paper chromatography or paper electrophoresis, is made by color reaction or by fluorescence with ultraviolet light (3650 Å), but there are still many substances which cannot be detected by these means.

Many organic compounds possess absorption maxima in the ultraviolet region and the author's attention was drawn to the fact that the irradiation of such maximal wave length to the compounds produced a remarkable variation in the intensities of transmitted or reflected light. Therefore, photoelectric apparatus to record such variation in intensity was devised. By this means, it became possible to detect various substances without the use of complicated color reaction1).

General construction of the apparatus is shown in Fig. 1. The light rich in ultraviolet rays from high pressure mercury lamp (L) is reflected by the parabolic mirror (M_1) for ultraviolet light and the parallel light thereby obtained is dispersed by the quartz prism (Pr). The dispersed light is reflected by the aluminum-spattered concave mirror (M_2) and is projected on the filter paper (F) after passing through the orifice (O) and iris

Fig. 1 Diagram of Apparatus



L: High pressure mercury lamp (Glider SHL-80)
M_1: Parabolic mirror (Aluminum-spattered)
Pr: Quartz prism
M_2: Concave mirror (Aluminum-spattered) with microscrew. f=142 cm.)
O: Orifice
P: Iris diaphragm
F: Filter paper (finished chromatogram)
Ph: Photomultiplier Mazda MS-6SY
Am: Amplifier
Re: Recorder
Pw: Regulated power supply source for photomultiplier

---

* Tatsuo Kariyone: Microchemical Studies on Plant Components. XIV.

** Motoyama, Higashinada-ku, Kobe (橋本庸平).

1) Nature, 170, 1024 (1952); Paper read before the Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1952.