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.

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

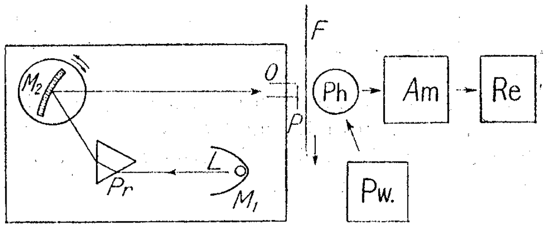
*(Laboratory of Phytochemistry, Kobe Women's College of Pharmacy**)*

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 reaction[1].

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

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* 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.

diaphragm (P). By a microscrew device at M_2, various wave lengths ultraviolet and visible ranges above 2500 Å can be obtained. Movement of F results in absorption of light by the substances present on the filter paper and changes occur in the transmission of light. This change is automatically recorded by the recorder (Re) after amplification by the amplifier (Am). Pw is the constant voltage source supplying current to the photomultiplier (Ph).

Figs. 2 to 5 show the records obtained with chromatograms of flavonoids, potassium salt of alcohol xanthate, hydrolyzate of nucleic acid, and methanolic extracts of the rhizome of *Macleya cordata* or *Polygala Reinii*. It can be seen from these curves that the flavonoids, luteolin, acacetin, and rutin, show distinct absorption in the ultraviolet region.

Fig. 3 is the detection by ultraviolet light at around 3000 Å of papergram obtained from ethanol containing a minute amount of methanol (0.05%) isolated by means of potassium xanthate method previously described by the author[2]. It shows clearly that such a minute amount of methanol could be detected by this method as a distinct peak in the curve.

The hydrolyzate of desoxyribonucleic acid (DNA), obtained from thymus gland, was chromatographed, and the papergram was recorded by various wave lengths from 4000 Å to a shorter wave length region. The results show that the most distinct records are obtained when ultraviolet light of the same wave length as that of absorption maximum is used.

From the results of present series of experiments, it was found that the improvement of the apparatus, the use of precise automatic recorder and perfect monochrometer, with optically uniform filter paper, will allow quantitative results to be obtained.

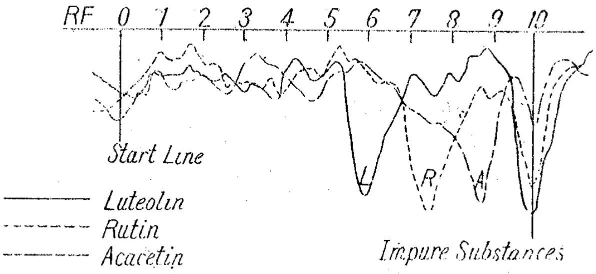

Fig. 2 Ultraviolet papyrograph of luteolin, acacetin, and rutin (ca. 2600Å).

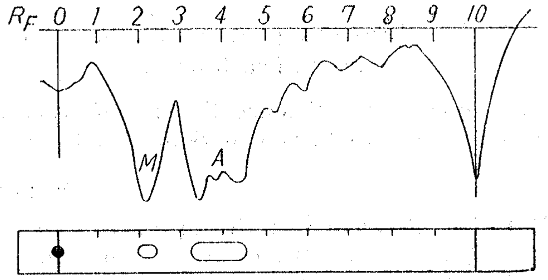

Fig. 3 Ultraviolet papyrograph of methyl and ethyl xanthate (ca. 3000Å).
M: Methyl xanthate
A: Ethyl xanthate

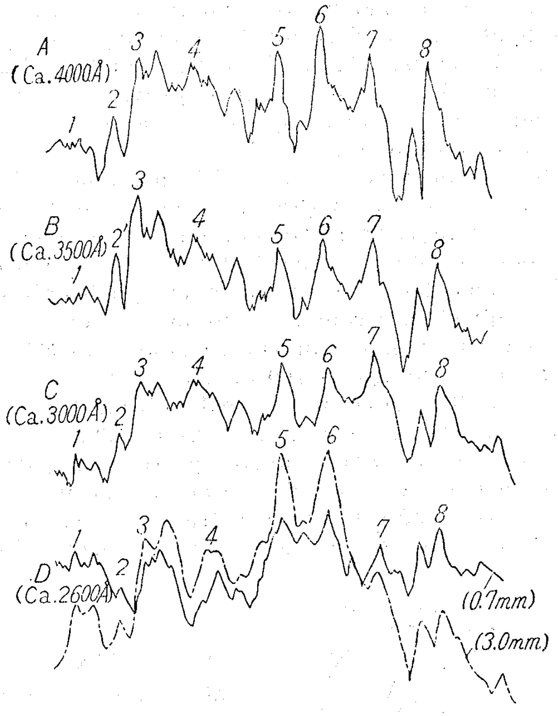

Fig. 4 Variations in papyrograph by changes in wave length (Hydrolyzate of nucleic acid).

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[2] K. Kariyone, Y. Hashimoto, M. Kimura: Nature, 168, 513 (1951).

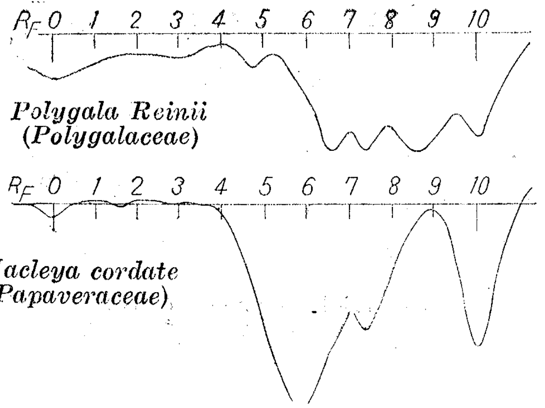

Fig. 5 Ultraviolet papyrograph of plant extracts (ca. 3000Å).

Such detection of papergrams with ultraviolet light makes it possible to detect substances that could not be obtained by the existing method of color reactions or fluorescence, and also gives the correct Rf of substances.

The author expresses his sincere gratitude to Prof. Kariyone of the University of Kyoto for his unfailing guidance throughout the course of this study. The author is also grateful to Mr. Itsuhiko Mori of this Laboratory for making most of the parts used in this apparatus, Mr. Takao Inoue of Pharmacognosy Laboratory, University of Kyoto, Messrs. Mutsuo Kimura and Tamotsu Maeda of this Laboratory, and Mr. Kazuo Amaya of the Science Faculty of Kobe University, for their assistances in carrying out the experiments, and to President Seto of this College for kind encouragements.

Experimental

Detailed structure of the apparatus—The diagram of the apparatus used is shown in Fig. 1. L is the light source, an 80-watt high pressure mercury lamp, the bulb of which had been removed to obtain ultraviolet light. M_1 is the parabolic mirror on which aluminum had been spattered to obtain a parallel light. Dispersion is made by the prism (Pr) made of two quartz plates placed at 60° angle and filled with distilled water. The dispersed light is then reflected by a concave mirror (M_2), an ordinary telescopic mirror on which aluminum has been spattered. The focal distance is made as long as possible, and the diameter of the mirror made as large as possible so as to obtain only the axial rays, and the light that has been dispersed as a parallel light beam by the parabolic mirror (M_1) alone can be collected as the real image. The face of this concave mirror can be moved by a micrometer with 0.01 mm. scale so as to obtain various wave lengths. The light generated from the mercury lamp is selected into various wave lengths such as the ultraviolet (2537, 2987, 3125, 3650 Å), violet (4046 Å) and blue violet (4358 Å), and passed through the orifice O. The orifice O is provided with iris diaphragm P having various holes of from 0.3 to 5 mm. in diameter. By the revolving of this diaphragm, light beam of definite wave length can be taken out (cf. Fig. 6).

The filter paper F is fixed in a frame and the light coming through the slit is projected on the paper. The frame A (Fig. 7) is moved to left or right, and the variation in the transmitted light is changed to variation of current by the photomultiplier for ultraviolet light at Ph, and recorded. In order to obtain a record of the distribution of substances on the papergram in suitable size, the movement of this frame A is synchronized with the revolution of the recorder. Mazda MS-6SY, a six-step, secondary photomultiplier tube, was used for the photoelectric tube. The photomultiplier tube shows anodic sensibility of 275 μA mL m^{-1}, and amplifying rate of 13.1×10^3 when the supplied voltage is 700 V, and about twice the sensibility when the current supplied is raised to 875 V. The current supplied to it must be of constant voltage and, therefore, a constant voltage generator, shown in Fig. 8, is used. This is a modified apparatus using photomultiplier tube of RCA IP21 used in the Raman spectrum apparatus by Heigl3). This gave better results than the use of a battery. In order to obtain the necessary constant voltage, the light source, alternate current B, is adjusted with a transformer. The photomultiplier tube for ultraviolet light is encased in a brass tube, shown in Fig. 9, and placed directly behind the filter paper. The brass tube, R,

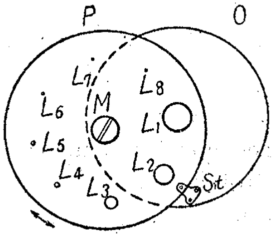

Fig. 6 Pin-hole slit (cf. Fig. 1, P)

O: Orifice P: Pin-hole slit
M: Center screw St: Stopper
L: Light output holes; L_1, dia. 5 mm., L_2, 3 mm., L_3, 1.5 mm., L_4, 1.2 mm., L_5, 1.0 mm., L_6, 0.7 mm., L_7, 0.5 mm., L_8, 0.3 mm.

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2) Heigl: Anal. Chem., 22, 154 (1950).

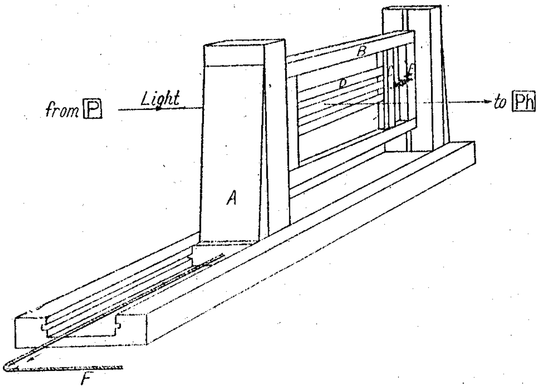

Fig. 7 Filter paper holder (Cf. Fig. 1, F)

A: Holder moving horizontally
B: Holder moving vertically
C: Paper holder
D: Developed papergram
E: Spring
F: String that pulls the holder synchronously with the drum of the recorder.

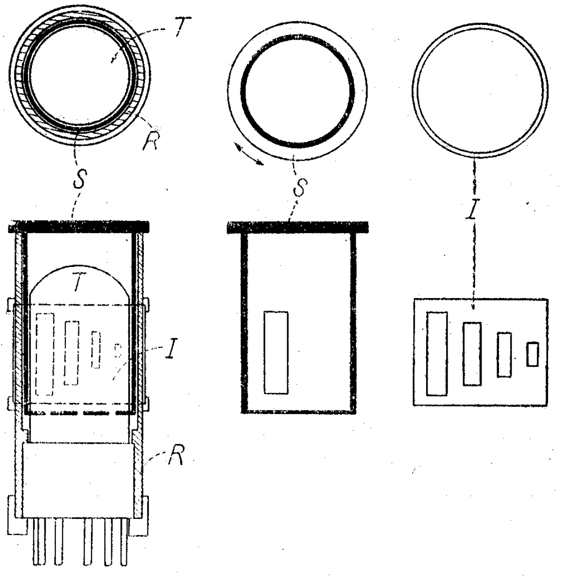

Fig. 9 Case for ultraviolet photomultiplier tube.

R: Outer tube
T: Mazda MS-6SY photoelectric tube
S: Shutter I: Adjustable slit

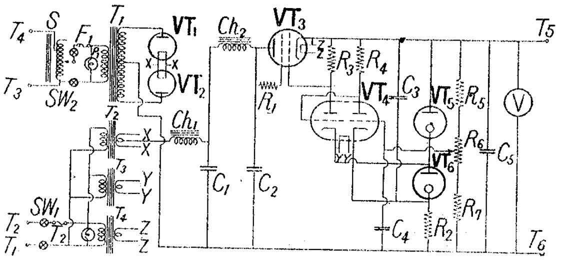

Fig. 8 Regulated power supply source for photomultiplier

Components

T_1: 100 v-1300 v-0-1300 v. 200 mA transformer
T_2: 100 v a.c. -2.5 v. 10 A transformer
T_3: 100 v a.c. -6.3 v. 3 A transformer
T_4: 100 v a.c. -6.3 v. 3 A transformer
R_1: 100 ohm 10 watt resistor
R_2: 40,000 ohm 10 watt resistor
R_3: 500,000 ohm 1 watt resistor
R_4: 500,000 ohm 1 watt resistor
R_5: 25,000 ohm 10 watt resistor
R_6: 30,000 ohm 10 watt resister (variable)
R_7: 50,000 ohm 10 watt resister
C_1: 4 mfd. 7000 volt oil condenser
C_2: 4 mfd. 7000 volt oil condenser
C_3: 0.25 mfd. 600 volt paper condenser
C_4: 2 mfd. 2000 volt oil condenser
C_5: 2 mfd. 3000 volt oil condenser
Ch_1, Ch_2: 10 henry, 150 mA choke
P_1: 100 volt red pilot lamp
P_2: 100 volt green pilot lamp
F_1: 2 amp. fuse
F_2: 3 amp. fuse
VT_1, VT_2: HX 968 Mazda
VT_3: 807 RCA tube
VT_4: 6SL7 RCA tube
VT_5, VT_6: VR 150 RCA tube
Sw_1, Sw_2: toggle switch
S: 100 v-0-130 volt transformer
V: 1000 volt meter

Terminal connection

T_1, T_2, T_3, T_4: 100 volt ac 60 cp. power
T_5: Positive output high voltage connector
T_6: Negative output high voltage connector

is provided with a rectangular window on the side where the light is projected, the size of the window being the exact size of lighted area. This window is covered with a tubular pipe, I, on which windows of various sizes are punched, which act as stops for light adjustment. The shutter, S, is placed on the inside of the tube, R, and the revolution of S to right or left opens or shuts the light orifice.

The divided circuit of current to this multiplier is shown in Fig. 10, and the amplifier through which this weak current generated by the photomultiplier is amplified is shown in Fig. 11. The current therefrom is recorded via the galvanometer. The sensibility of the grid bias, R_9, is adjusted by the use of 2–10 meg. ohms. The galvanometer used for the recorder is a 10^{-9} A one and the variation is recorded on an oscillographic sensitized paper (Fuji Photo). The galvanometer sensibility is adjusted by 2 k. ohm adjustable resistance circuit.

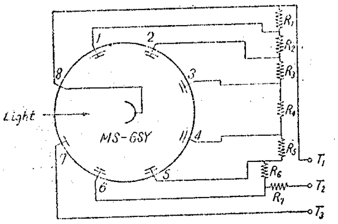

Fig. 10 Photomultiplier Circuit

Components
- R_1 to R_7: 100,000 ohm precision resistors
- Mazda photomultiplier for ultraviolet lights MS-6SY

Terminal Connections
- T_2: Positive connection of 700-875 volt regulated dc
- T_1: Negative connection of 700-875 volt regulated dc
- T_3: to 6SJ7 dc amplifier grid input

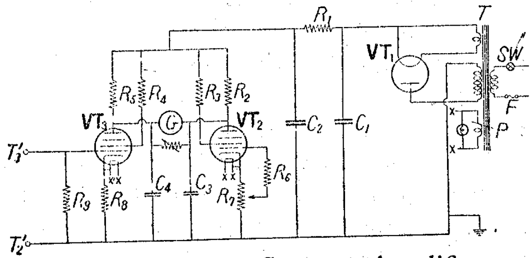

Fig. 11 Direct Current Amplifier (See Fig. 1, Am)

Components
- R_1: 5,000 ohm 3 watt resistor
- R_2,R_5: 250,000 ohm 1 watt resistor
- R_3, R_4: 1 megohms
- R_9: 1 megohms
- R_7: 10,000 ohm variable resistor
- R_8: 10,000 ohm resistor
- R_9: 2-10 megohms
- C_1, C_2: 10 mfd. chemical condenser
- C_3, C_4: 0.1 mfd. paper condenser
- VT_1: Mazda 12 F tube
- VT_2, VT_3: 6SJ7 RCA tube
- T: 100 volt—250 volt 40 mA transformer

Terminal connections
- T_1· to T_2 terminal of photomultiplier
- T_3· to T_3 terminal of photomultiplier
- G, galvanometer attached to recorder

Method of recording—The light beam is projected slightly on the off-side of the start line where the substance is present on the paper chromatogram, and the recording is started from this point. The adjustable resistance, R_7 (Fig. 11), is so adjusted that no current flows through the galvanometer at this position. The filter paper is then moved by the device shown in Fig. 7 by which the transmitted light varies with the presence of a substance developed on the paper chromatogram. This weak variation in the current is amplified, received by the galvanometer, and recorded on a sensitized paper.

The curves in Fig. 2 are the result of detection of the chromatogram of luteolin, acacetin, and rutin, each developed with a mixture of butanol-acetic acid-water (5:1:4), with a light beam of approximately 2600 Å. Luteolin gave a value corresponding to Rf 0.59, acacetin Rf 0.87, and rutin Rf 0.75. These values are the quotient obtained by dividing the distance to the peak of the curve formed by the substances with the distance from the starting line to the peak of a curve formed by the collection of impure substances at the solvent front. In other words

$$\mathrm{Rf} = \frac{\text{Distance from starting line to the peak of substance}}{\text{Distance from starting line to the peak of impurities at the solvent front}}$$

Fig. 3 is the record of the chromatogram of potassium methyl xanthate and potassium ethylxanthate developed with butanol saturated with potassium hydroxide, and a light beam of approximately 3000 Å. Fig. 4 shows the record of a chromatogram of the hydrolyzate of desoxyribonucleic acid and developed with butanol saturated with water. The iris of 0.7 mm. diameter was used (Fig. 6), and the curves A, B, C, and D were obtained with the light beam of 4000, 3500, 3000, and 2600 Å, respectively. The broken line shown in D, is the one obtained with a 3 mm. iris and approximately 2600 Å light beam.

Use of the iris smaller than 0.7 mm. diameter results in the appearance of optical diversity of the filter paper on the record and the complicated unevenness of the paper shows up distinctly. When the iris is opened, the fine dentations due to the unevenness of the paper disappear. Shorter the wave length, the better becomes the recording of substances. When a light beam of approximately 2600 Å was used through a 3-mm. slit, a specific graph of the substance was obtained, as shown by a broken line. By the use of the present apparatus, a mixed light beam containing various spectra ranging from ±50 Å with 0.7 mm. iris, to ±200 Å with 3.0 mm. iris is obtained. It follows, therefore, that the curve shown by broken line in D was recorded by a light beam ranging from 2400 Å to 2900 Å. In this Fig. 4, 1 indicates the substance that remains on the starting line, and the peaks 3, 4, 5, and 6 respectively indicate the presence of guanine, cytosine, adenine, and thymine. In the recorded graph of nucleic acid, it is interesting to see that light absorption does not occur at the spot where the substance is present, and the transmitted light becomes stronger in that region.

Fig. 5 shows the results obtained with methanolic extracts of the rhizomes of *Polygala Reinii* (*Polygalaceae*) and *Macleya cordata* (*Papaveraceae*).

It was assumed that the measurement of area surrounded by the curves or the height of the peak in a curve in these recordings would show a quantitative relationship but for this purpose, there would have to be made an increase in the precision of this apparatus.

Summary

An apparatus was devised whereby paper chromatograms were irradiated with ultraviolet light of a definite wave length and variations in the intensities of transmitted light recorded automatically. This apparatus made it possible to detect unknown substances whose presence cannot be detected by the existing method of chromatography using color reactions or fluorescence.

(Received April 20, 1953)

Errata for Pharm. Bull. Vol. 1, No. 1.

Page	Line	Error	Correction
1	Footnote ↓ 2	Suppl. II: Ibid., 27,	Iibd., 72,
5	„ ↑ 8	m.p. 325°	m.p. 253°
36	„ ↓ 8	possesstion	possession
56	„ ↑ 5	96∼98	96∼98°
56	„ ↑ 6	139∼140	139∼140°
80	„ ↓ 4	J. Chem. Soc. Japan, 71, 67 (1950).	J. Chem. Soc. Japan, 71, 303 (1950).
82	„ ↑ 20	Found: C, 4.8	Found: C, 49.8