shown in Fig. 1a. When the pH value became about 5.0, the intensity of transmitted light ($\theta=0°$) reached a minimum point and the intensity of light scattered at $\theta=90°$ and 135° reached a maximum point, respectively. These curves show that the precipitation point (which is very probably equal to the iso-electric point) of the protein is pH 5.0. On the other hand, it is found that the curve for $\theta=45°$ is very characteristic and has two maximum points at pH=5.5 and pH=4.5, and the minimum point between them is at pH=5.0.

The protein was, then, fractionated into two fractions, α-crystallin (precipitate) and β-crystallin (supernatant), by adding 0.1*M* hydrochloric acid as much as to reach pH 5.2, according to the method as used by Francois, *et al.*,[11] and the light scattering titration has been carried out for each fraction. Curves for the light intensity at $\theta=0°$, 90°, and 135° showed one of each maximum point at pH=5.0 for both α-crystallin (Fig. 1b) and β-crystallin (Fig. 1c) as it was for the unfractionated protein (Fig. 1a). As for the curve for $\theta=45°$, α-crystallin showed two maxima and one minimum as the unfractionated protein did, but the light intensity at the minimum point was zero on the contrary to the unfractionated one. On the other hand, β-crystallin showed only one maximum at pH=5.0.

The curve of light scattering titration at $\theta=45°$ for unfractionated protein can, therefore, be explained as the superposition of two curves of α- and β-crystallin. Such a characteristic feature of the curve at $\theta=45°$, probably due to the change of shape of the protein molecules, will be useful for further investigation of the protein of this kind.

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Fluorometric Analysis of Pyruvic Acid with 4′-Hydrazino-2-stilbazole

The existing methods for determination of ketones by formation of hydrazones depend on absorption spectroscopy[1~3] and have not sufficient sensitivity for biochemical analysis. In order to establish a more sensitive analytical method, some fluorescent derivatives of hydrazine were investigated.

A reagent specifically selected for determination of α-oxo acids was 4′-hydrazino-2-stilbazole (I) which was synthesized by the route shown in Chart 1. The compound melt at 138° (*Anal.* Calcd. for $C_{15}H_{13}N_3$: C, 73.70; H, 6.16; N, 19.90. Found : C, 73.84; H, 6.11; N, 20.14).

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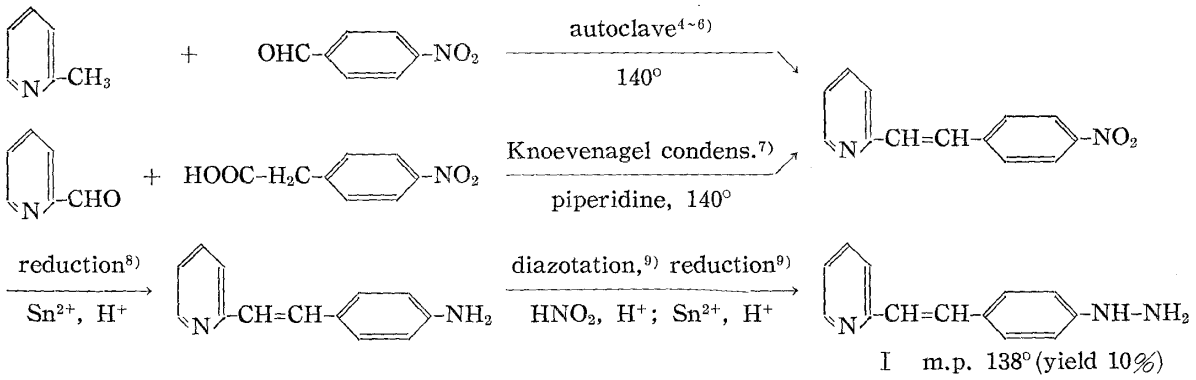
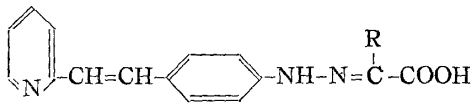


Chart 1. Route of Synthesis of 4′-Hydrazino-2-stilbazole

TABLE I. Hydrazones formed from α-Oxo Acids and 4′-Hydrazino-2-stilbazole



α-Oxo acid	R	m.p.	Analysis (%)					
			Calcd.			Found		
		(°C)	C	H	N	C	H	N
Pyruvic acid	CH_3	203	68.00	5.73	15.04	68.31	5.38	14.94
Oxaloacetic acid	$HOOC-CH_2$	200	62.27	4.80	12.81	62.76	4.65	12.92
2-Oxoglutaric acid	$HOOC-CH_2-CH_2$	195	63.37	5.15	12.10	63.71	5.05	12.38
Phenylpyruvic acid	$C_6H_5-CH_2$	184	73.71	5.41	11.80	73.95	5.36	11.76

I reacted readily with α-oxo acids and exhibited a strong yellow fluorescence in acid solution. Similarly to general hydrazones, the condensates of I with α-oxo acids had 1:1 composition as shwon in Table I.

By the use of pyruvic acid, determination of α-oxo acids with I was examined. The optimal conditions found were as follows:

Reagents

a) Pyruvate solution: Aqueous solution of lithium pyruvate,[10] 2×10^{-7} to $2\times10^{-5}M$ (0.0176~1.76 μg./ml. as pyruvic acid).
b) Acetate buffer solution, pH 4.0 ($\mu=0.1$).
c) 0.01% solution of 4′-hydrazino-2-stilbazole in 0.2% H_2SO_4.
d) $5N$ H_2SO_4.
e) MeOH.

Procedure

To 1 ml. of a) each ml. of b) and c) were added, mixed thoroughly, and allowed to stand for 1 hour at 20~30° in a dark place. To this mixture, each ml. of d) and e) were added, mixed well, and fluorescence intensity was measured at 546 mμ, excited by a light of 460 mμ.

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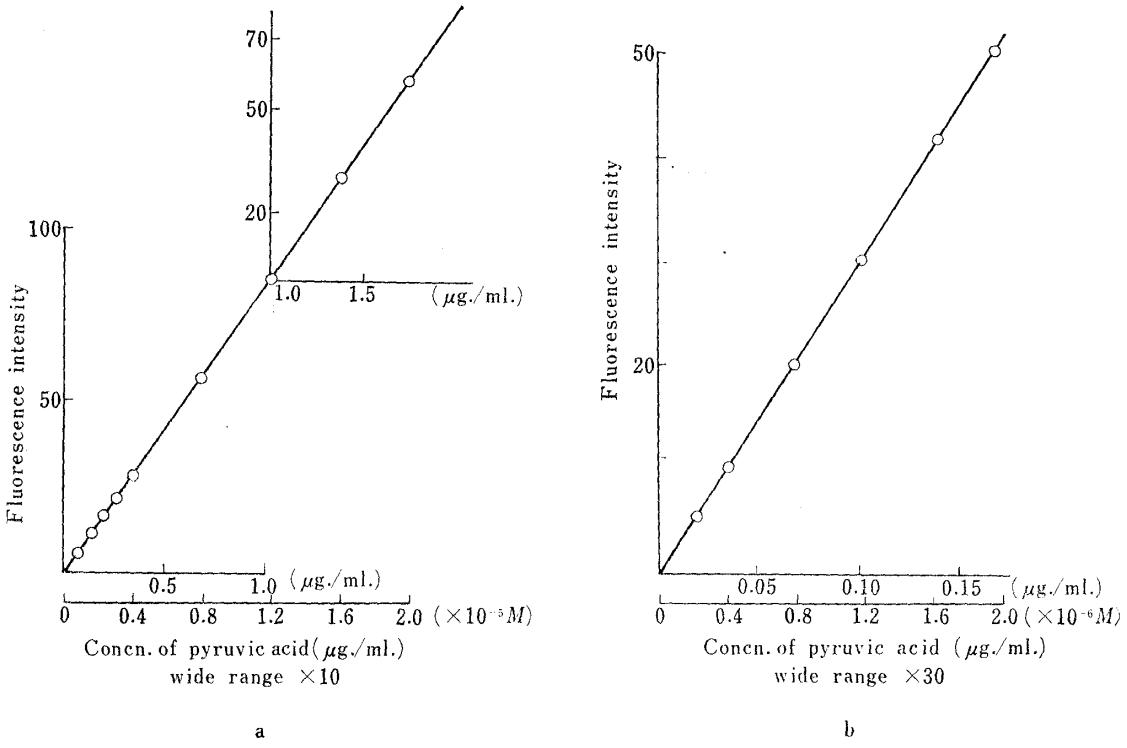

Fig. 1. Calibration Curve of Pyruvic Acid

Apparatus

Turner Fluorometer, Model 110.
Lamp : no. 110—853 (blue lamp) 400～520 mμ.
Filter : Primary no. 110—831, narrow pass, max. 460 mμ.
Secondary no. 110—832, narrow pass, max. 546 mμ.

The calibration curve, as shown in Fig. 1a and b, was linear in the range of 0.0176～1.76 μg./ml. of pyruvic acid.

Other α-oxo acids also showed fluorescence of similar intensity under these conditions, while ketones, aldehydes, and sugars either did not exhibit any fluorescence or showed a very weak fluorescence, as indicated in Table II.

Details of experiment will be reported in the near future.

Table II. Relative Fluorescence Intensity of Other Substances (taken pyruvic acid as 100)

Ketones		Aldehydes		Sugars		Others	
Acetone	0	Formaldehyde	2.5	Glucose	0	Ovalbumin	0
Methyl ethyl ketone	0	Acetaldehyde	0	Xylose	0	Heparin	0
Cyclohexanone	0	Propionaldehyde	0	Sodium 2-oxogluconate	0	Disodium citrate	0
Acetophenone	0	Caprylaldehyde	1.5	Glucuronic acid	0	Lactic acid	0
Diacetyl	0	Capronaldehyde	0	Ascorbic acid	0	Acetoacetic acid	0
Dibenzoyl	0	Benzaldehyde	0.7			Levulinic acid	0
Acetylacetone	0	Salicylaldehyde	4.6				
		p-Anisaldehyde	3.1				
		Glyoxal	27.0				

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Structure and Reactions of Illudin-S (Lampterol)

During our structural studies on lampterol, the antitumor factor isolated[1,2] from the Japanese mushroom, *Lampteromyces Japonicus* (KAWAM.) SING., planer structures (I) and (II) were proposed,[3] respectively, for illudin-S and -M isolated from *Clitocybe illudens.*[4] The same conclusion was reached by our independent investigations, and a subsequent direct comparison[5] showed lampterol and illudin-S to be identical. The structure has received full support from an X-ray analysis[6] on the *p*-iodobenzoate of its acyloin rearrangement product, isoilludin-S, and in conjunction with optical data the relative and absolute configurations have been clarified.[7]

The nature of all twenty protons of illudin-S (III), m.p. 124~126°, $C_{15}H_{20}O_4$ (M^+ peak at 264), UV λ_{max}^{MeOH} mμ (log ε) : 235 (4.10), 320 (3.54); IR ν^{CHCl_3} cm^{-1} : 3629, 3605 (free OH), 3500 (bonded OH of α-ketol), 1698 and 1606 (*cisoid* α,β-unsaturated ketone), and 1651 (C=C); $[\phi]_{589}$ −459°, $[\phi]_{375}$ −4,435° (25°, MeOH, c=0.0069),*1 were easily disclosed by comparing its 100 Mc. nuclear magnetic resonance spectrum*2 with that of the diacetate. Oxidation of illudin-S with aqueous potassium permanganate afforded 1,1-cyclopropanedicarboxylic acid, which together with the high field nuclear magetic resonance peaks established the spirocyclopropane moiety.

When the chloroform solution of illudin-S is passed through a column of alumina it is isomerized to isoilludin-S (IV), m.p. 179~180°, UV λ_{max}^{EtOH} mμ(log ε) : 252 (4.31); IR ν^{KBr} cm^{-1}: 3400 (br), 1697, 1645 (strong *cisoid* $\nu_{C=C}$ band significantly absent); $[\phi]_{589}$ + 499°, $[\phi]_{312}$ +21,380°, $[\phi]_{280}$ −22,440°, $[\phi]_{269}$ 0.00° (25°, MeOH, c=0.0013). This isomer, in contrast to illudin-S, forms a triacetate, m.p. 112~113°, upon conventional treatment with acetic anhydride pyridine. The nuclear magnetic resonance spectra of illudin-S and its isomer (or their acetates) were quite similar excepting that in the case of the isomer the cyclopropyl protons were shifted lower by ca. 0.7 p.p.m. whereas the olefinic proton singlet was shifted higher by 0.5 p.p.m.

Oxidation of isoilludin-S with chromic acid/pyridine at 50° afforded the dihydro-5*H*-indano[5,6-*b*]furan-5,7(6*H*)-dione (VII), m.p. 198~199°, $C_{15}H_{16}O_4$, UV λ_{max}^{EtOH} mμ (log ε) :

*1 ORD curve measured with Japan Spectroscopic Company ORD/UV-5 spectropolarimeter.
*2 NMR spectra measured with Varian A-60 and HR-100 models, $CDCl_3$ solvent, TMS internal reference, chemical shifts in p.p.m. coupling constants in c.p.s.

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