[Chem. Pharm. Bull. 18(2) 285—290 (1970)]

UDC 547.831.8.04 : 541.144 : 543.426

Structure of a Product of a Fluorescence Reaction of 3-Hydroxykynurenine[1]

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(Received August 18, 1969)

A product of fluorescence reaction of 3-hydroxykynurenine with *p*-toluenesulfonylchloride was separated and its structure was investigated. A reaction product of 2-amino-3-hydroxyacetophenone, which has the same configuration of carbonyl, amino and hydroxyl groups, with *p*-toluenesulfonylchloride was also studied. It was shown that the product from 3-hydroxykynurenine was *p*-toluenesulfonylester of the 3-hydroxyl group and *p*-toluenesulfonylamide of the aliphatic amino group, and that the product from 2-amino-3-hydroxyacetophenone was *p*-toluenesulfonylester of the 3-hydroxyl group.

3-Hydroxykynurenine (I), a tryptophan metabolite, is known as an endogenous carcinogen,[3,4] and relationship between its urinary excretion and incidence of bladder cancer is discussed.[5,6] Udenfriend[7,8] reported that this compound has strong blue fluorescence in an alkaline aqueous solution. However, this compound has really only very feeble yellow fluorescence and has not been determined by fluorimetry. The authors found that an aqueous solution of 3-hydroxykynurenine exhibited blue fluorescence (extitation: 360 mμ, fluorescence maximum: 460 mμ) by addition of sodium bicarbonate and a solution of *p*-toluenesulfonylchloride in acetone, and applied this reaction to determination of 3-hydroxykynurenine in urine.[9,10] In this paper, structure of the fluorescent product of this reaction is studied.

2-Amino-3-hydroxyacetophenone (II), which has the same configuration of carbonyl, amino and phenolic hydroxyl groups as 3-hydroxykynurenine, also exhibited blue fluorescence by the reaction with *p*-toluenesulfonylchloride in the same condition. This compound was thought to be available as a model compound for investigation of mechanism of this reaction. Therefore, structures of the reaction products of 2-amino-3-hydroxyacetophenone and 3-hydroxykynurenine with *p*-toluenesulfonylchloride were investigated.

Chart 1

1) A part of this work was reported at 87th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 8, 1967.
2) Location: *a*) *Kamiyoga 1–18–1, Setagaya-ku, Tokyo*; *b*) *Hongo 7–3–1, Bunkyo-ku, Tokyo*; *c*) *Takada 3–41–8, Toshima-ku, Tokyo.*
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Experimental

Materials——3-Hydroxy-L-kynurenine was obtained from Senju Seiyaku Co. 2-Amino-3-hydroxyacetophenone was prepared from 2-nitro-3-hydroxyacetophenone.[11] *o*-Aminoacetophenone and *m*-hydroxyacetophenone were obtained from Tokyo Kasei Co.

A Product from 2-Amino-3-hydroxyacetophenone-Compound (A)——A solution of *p*-toluenesulfonylchloride (400 mg) in acetone (40 ml) was added into a solution of 2-amino-3-hydroxyacetophenone (50 mg) and $NaHCO_3$ (500 mg) in H_2O (50 ml). The mixture exhibited blue fluorescence. After 2 hr, a solution of $(NH_4)_2CO_3$ (1 g) in H_2O (10 ml) was added. After 30 min, the fluorescent product was extracted with ether (100 ml). The ether extract was concentrated in vacuum, applied to silicagel H (Merk Co.) plate and developed with benzene. An acetone extract of the fluorescent band (Rf=0.40) was evaporated to dryness. Pale yellow crystals obtained were recrystallized from 95% EtOH. mp 78°. Yield 30 mg. *Anal.* Calcd. for $C_{15}H_{15}O_4NS$ (mole. ratio of the reaction=1:1): C, 59.00; H, 4.95; N, 4.58; S, 10.50. Found: C, 58.87; H, 4.90; N, 4.58; S, 10.09.

A Product from 3-Hydroxykynurenine-Compound (B)——A solution of *p*-toluenesulfonylchloride (500 mg) in acetone (50 ml) was added into a solution of 3-hydroxy-L-kynurenine (120 mg) and $NaHCO_3$ (500 mg) in H_2O (50 ml). The mixture exhibited blue fluorescence. After 2 hr, a solution of $(NH_4)_2CO_3$ (1 g) in H_2O (10 ml) was added. After 30 min, the mixture was acidified with 4N H_2SO_4 and the fluorescent product was extracted with ether (100 ml). Then, the product was transferred to an aqueous phase again by shaking of the extract with a solution of $NaHCO_3$ (1 g) in H_2O (100 ml). This phase was acidified with 4N H_2SO_4 and the product was reextracted with ether (100 ml). The ether extract evaporated to dryness. Pale yellow crystals obtained were recrystallized from EtOH–H_2O (8:1). mp 158°. Yield 25 mg. *Anal.* Calcd. for $C_{24}H_{24}O_8N_2S_2$ (mole. ratio of the reaction=1:2): C, 54.14; H, 4.54; N, 5.26; S, 12.04. Found: C, 53.84; H, 4.43; N, 5.38; S, 11.88.

Fluorescence Emission and Exitation Spectra——Fluorescence emission spectra exited by 366 mμ and exitation spectra of fluorescence at 455 mμ in (A) and in (B) at 465 mμ were measured with Hitachi MPF-2A spectrofluorimeter.

Ultraviolet Absorption Spectra——Hitachi EPS-3T recording photometer was employed.

Fluorescence Quantum Yields——Fluorescence quantum yields were measured by the comparative method.[12,13] Fluorescence emission spectra of 5×10^{-6}M solution of the A or B in various solvents and 10^{-6}M solution of quinine sulfate in 0.1N H_2SO_4 exited by 366 mμ were measured and the quantum yields were calculated from the formula.[14]

$$Q_{(\text{A or B})}=0.55\times\frac{\varepsilon_{366(\text{quinine})}\times C_{(\text{quinine})}\times F_{(\text{A or B})}}{\varepsilon_{366(\text{A or B})}\times C_{(\text{A or B})}\times F_{(\text{quinine})}}$$

Q is the quantum yield, F is the area under the corrected emission spectrum, C is the molecular concentration and ε_{366} is the molar extinction coefficient.

Color Reaction——1) $FeCl_3$ Reaction: Pyridine (0.8 ml) was added to a solution of $FeCl_3$ (100 mg) in $CHCl_3$ (10 ml) and the mixture was filtered. This reagent was added to 1—2% solution of the sample in $CHCl_3$. 3-Hydroxykynurenine, which is insoluble in $CHCl_3$, was dissolved in MeOH and pyridine were added. Violet coloration was regarded as positive result.

2) Ninhydrin Reaction: To a drop of 1—2% solution of the sample, pyridine, 0.5% aqueous solution of ascorbic acid and 1% aqueous solution of ninhydrin were dropped. The mixture was heated at 100° for 20 min. Violet coloration was regarded as positive result.

Infrared Spectra——Infrared spectra were measured in 5% solution in $CHCl_3$. However, those of 2-amino-3-hydroxyacetophenone and 3-hydroxykynurenine, which are not sufficiently soluble in $CHCl_3$, were measured in KBr tablets. Jasco. DS-301 infrared spectrophotometer was employed.

Nuclear Magnetic Resonance Spectra——Nuclear Magnetic Resonance Spectra of A and B were measured in 5% solution in $(CD_3)_2SO$ before and after the addition of D_2O. JEOL C-60HL instrument was employed.

Results

Fluorescence Quantum Yields, Fluorescence Spectra and Absorption Spectra

The fluorescence maxima and quantum yields are shown in Table I. The quantum yields of A and B are largest in the mixture of equal volumes of acetone and water, and relative-

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Table I. Fluorescence Maxima and Flurorescence Quantum Yields

Compounds	Solvents	ε_{366}	Fluorescence max.(corrected) (mμ)(μ^{-1})	Fluorescence quantum yields
A	methanol	4600	455 (2.19)	0.065
	ether	4700	448 (2.23)	0.008
	acetone	4550	448 (2.23)	0.015
	acetone–1% $NaHCO_3$=1:1	4650	455 (2.19)	0.071
	acetone–0.05M Na_2HPO_4-KH_2PO_4 (pH 7.0)=1:1	4650	455 (2.19)	0.072
	acetone–0.25M H_2SO_4=1:1	4650	450 (2.22)	0.047
B	methanol	5900	465 (2.15)	0.040
	ether	6100	440 (2.27)	0.025
	acetone	6100	448 (2.23)	0.037
	acetone–1 %$NaHCO_3$=1:1	5500	465 (2.15)	0.073
	acetone–0.05M Na_2HPO_4-KH_2PO_4 (pH 7.0)=1:1	5500	465 (2.15)	0.075
	acetone–0.25M H_2SO_4=1:1	5500	460 (2.20)	0.058

ly small in the acidic condition. The fluorescence emission and exitation spectra of solutions of A and B in methanol are shown in Fig. 1, and the absorption spectra are shown in Fig. 2. The fluorescence and absorption spectra of A are similar to those of B.

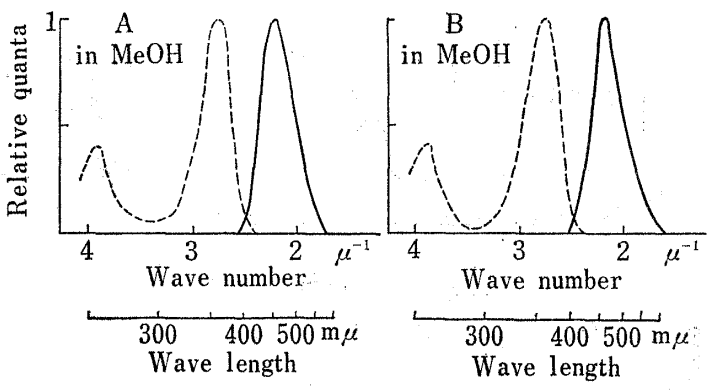

Fig. 1. Fluorescence Spectra

———: emission (exitation at 366 mμ)
- - - - - - - - - - : exitatation (fluorescence at 455 mμ in A, 465 mμ in B)

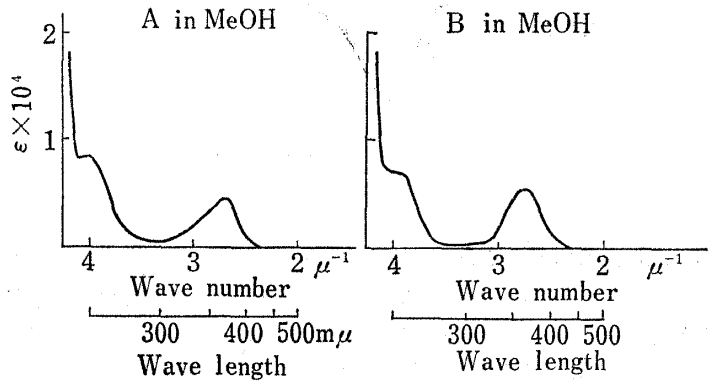

Fig. 2. Absorption Spectra

Color Reaction

The results of the $FeCl_3$ and ninhydrin reactions of A, B and related compounds are shown in Table II. The negative results of the $FeCl_3$ reaction in A and B suggest the absence of a phenolic hydroxyl group in them. The negative result of the ninhydrin reaction in B suggests the absence of an aliphatic amino group in B.

Infrared Spectra

The infrared spectra of A, B and related compounds are shown in Fig. 3. In the spectrum of A, absorption bands at 3350 and 3510 cm^{-1} from NH stretching exist and no broad band in this region from OH stretching exists. From this spectrum, it is shown that A has not a hydroxyl group but an amino group.

In the spectrum of B, absorption bands at 3380 and 3550 cm^{-1} exist. Although this spectrum is not easily interpreted, these bands are presumed to be from NH stretching of an amino or sulfonylamido group.

Table II. Results of the Color Reactions

Compounds	$FeCl_3$ reaction	Ninhydrin reaction
A	−	−
B	−	−
2-Amino-3-hydroxyacetophenone	+	−
3-Hydroxykynurenine	+	+
o-Aminoacetophenone	−	−
m-Hydroxyacetophenone	+	−

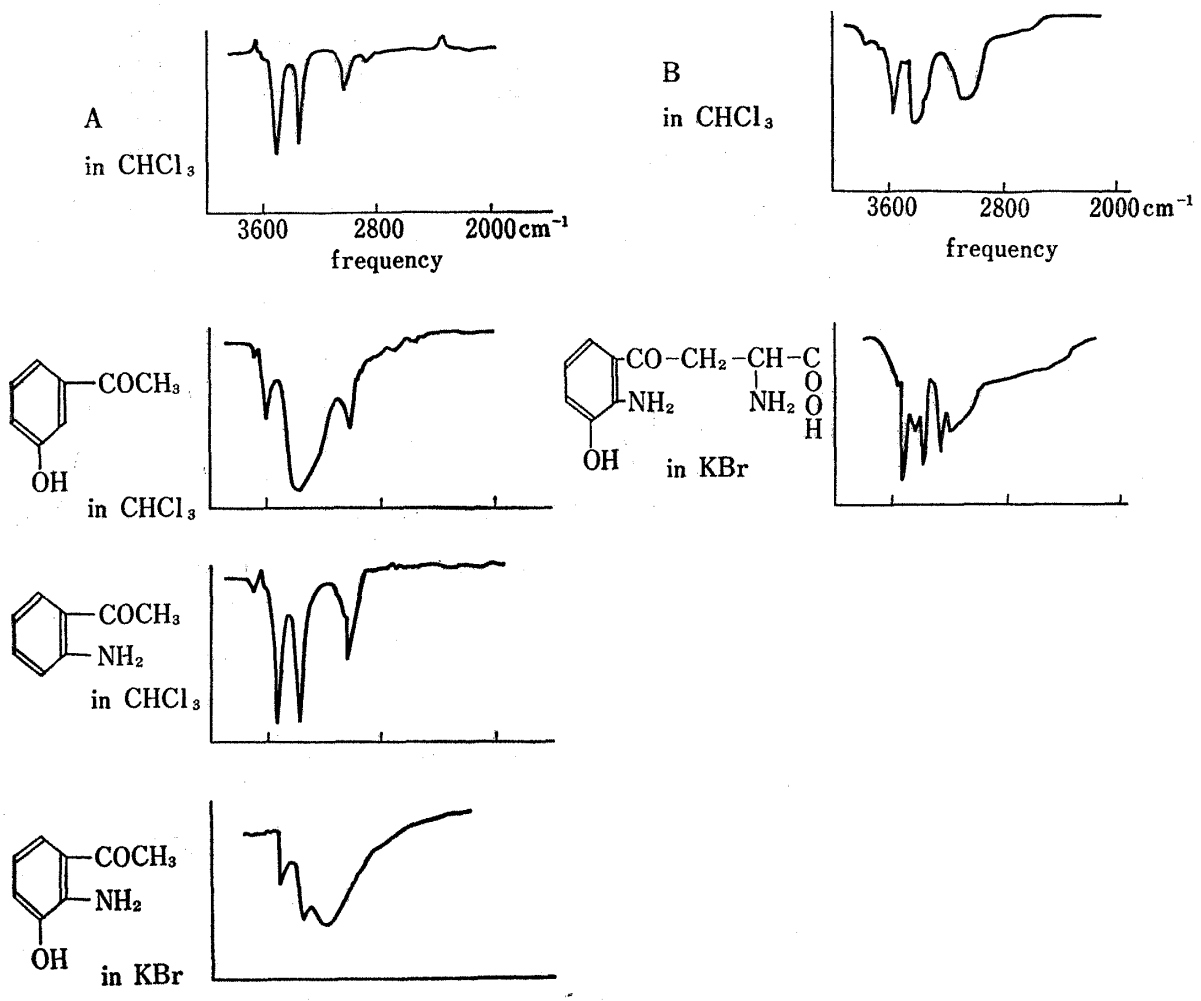

Fig. 3. Infrared Spectra

Nuclear Magnetic Resonance Spectra

The nuclear magnetic resonance spectra of A and B are shown in Fig. 4. In both spectra of A and B, bands at 2.95 τ are disappeared by the addition of deuterium oxide. The band in the spectrum of A is assigned to a signal from the aromatic amino group, because the infrared spectrum has shown that the hydrogen atoms exchangeable for the deuterium atoms in A are those in the aromatic amino group. The existence of the band at the same τ value supports an assumption that B has also an aromatic amino group.

Conclusion

From the elemental analysis, A was shown to be a reaction product from 1 molecule of 2-amino-3-hydroxyacetophenone and 1 molecule of *p*-toluenesulfonylchloride. The results of color reactions suggested lack of a phenolic hydroxyl group and the infrared spectrum showed

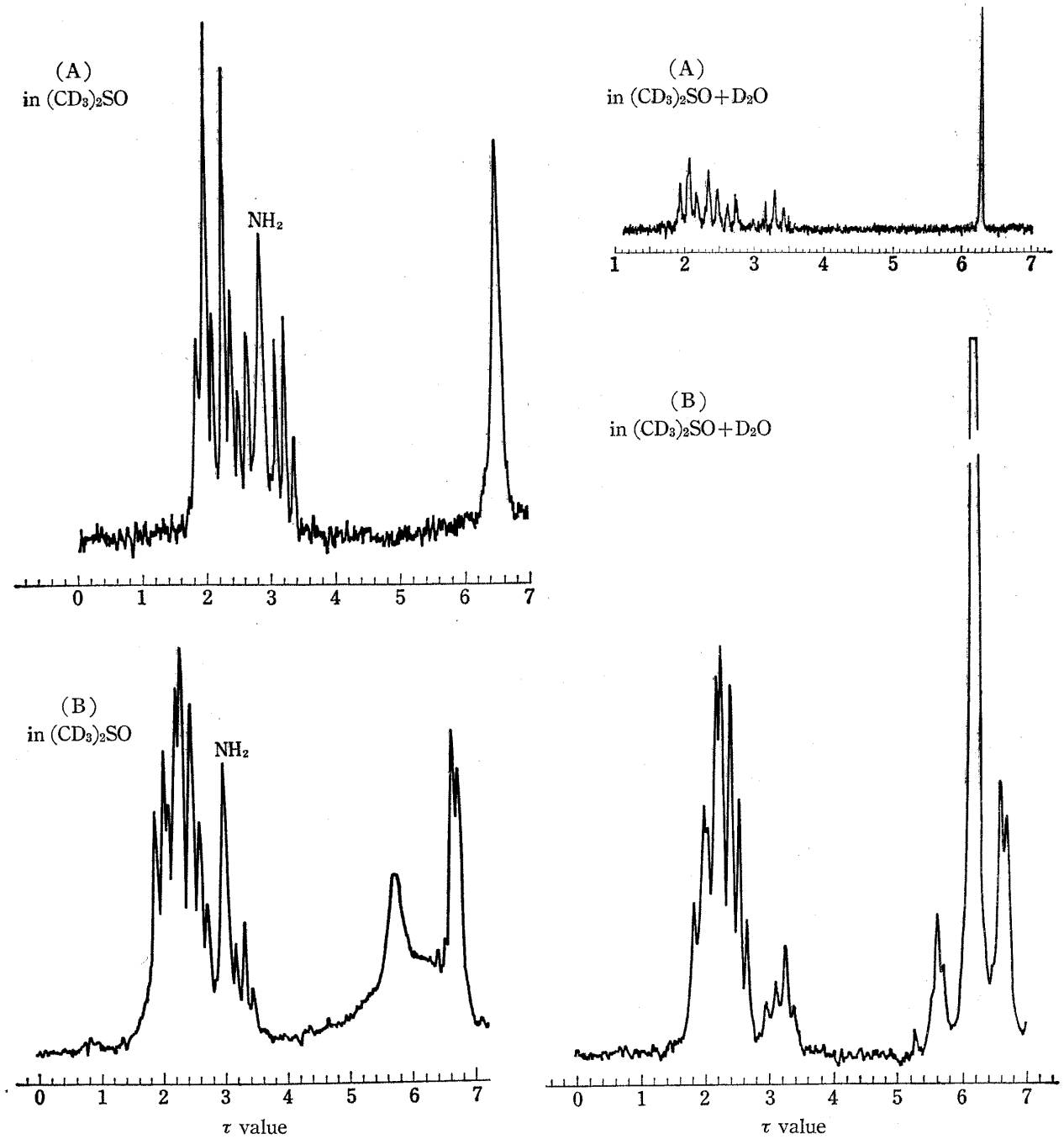

Fig. 4. Nuclear Magnetic Resonance Spectra

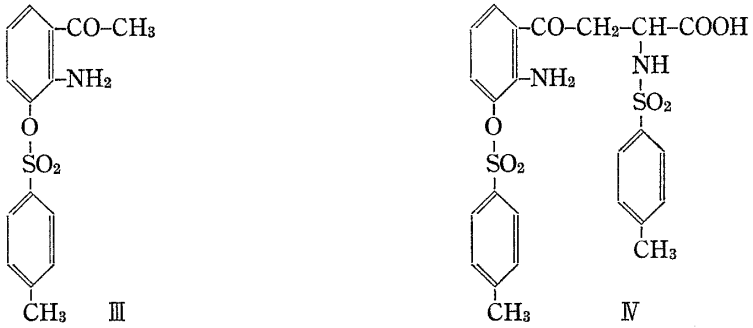

Chart 2

the existence of an amino group and lack of a hydroxyl group. From these facts, the structure of A is concluded to be III.

From the elemental analysis, B was shown to be a reaction product from 1 molecule of 3-hydroxykynurenine and 2 molecules of *p*-toluenesulfonylchloride. The results of color reactions suggested lack of a phenolic hydroxyl group and an aliphatic amino group. From these, it was presumed that these two groups reacted with the reagent and that the aromatic amino group remained. Infrared and nuclear magnetic resonance spectra were not contradictionary to this presumption. Therefore, the structure of B is concluded to be IV.

Discussion

Kynurenine[7,8] and *o*-aminoacetophenone, which have *o*-configuration of carbonyl and amino groups, exhibit blue fluorescence themselves. However, 3-hydroxykynurenine and 2-amino-3-hydroxyacetophenone, which have 3-hydroxyl group, have only very feeble yellow fluorescence in water, and exhibited blue fluorescence by the addition of sodium bicarbonate and a solution of some sulfonylchloride, for instance, methanesulfonylchloride, benzenesulfonylchloride and *p*-toluenesulfonylchloride, in acetone.[10] The strongest fluorescence is obtained by the addition of *p*-toluenesulfonylchloride.

In this paper, it was shown that the fluorescent products of the reaction with *p*-toluenesulfonylchloride were *p*-toluenesulfonylesters of the 3-hydroxyl group. Therefore, it can be presumed that the fluorescence from the *o*-configuration of carbonyl and amino groups is restrained by the existence of 3-hydroxyl group in 3-hydroxykynurenine and 2-amino-3-hydroxyacetophenone, and reappears by the ester formation of the 3-hydroxyl group.

Acknowledgement The authors are grateful to Mr. K. Kanoda and Mr. S. Kojima, National Institute of Hygenic Sciences, for NMR and IR spectra estimation and their kind advice. They are also grateful to Dr. M. Hirobe, the Tokyo University, for his kind advice.