a colorless oil, which was almost saturated to $C(NO_2)_4$ and $Br_2$.

**Chromatography of Atractylol; Separation of Atractylol into Two Components**—Atractylol, m.p. 61~63° (5.0 g.), was dissolved in light petroleum (b.p. 40~70°) and adsorbed on a column of active alumina (120 g.). Elution of the column with light petroleum liberated no material. Light petroleum-benzene yielded (a) stout needle crystals (1.1 g.), which, after repeated crystallisation from hydr. MeOH, formed white needles, m.p. 59~60°, $[\alpha]_D$ −40.2(c=10.0). *Anal.* Calcd. for $C_{15}H_{16}O$ : C, 81.02; H, 11.79. Found : C, 80.84, 80.77; H, 11.53, 11.65.

Elution with benzene gave (b) white mass of crystals (2.3 g.), which was a mixture of (a) and (c). Elution with EtOH gave (c) as colorless vitreous substance (1.6 g), which slowly crystallised into a solid mass, m.p. 60~72°. Several crystallisations of (c) from hydr. MeOH gave white needles, m.p. 79~80°, unchanged by further crystallisation, $[\alpha]_D$ +58.5°(c=10.0). *Anal.* Calcd. for $C_{15}H_{26}O$ : C, 81.02; H, 11.79. Found : C, 80.80; H, 11.83.

This was identified with eudesmol by m.p., mixed m.p., specific rotation, and infrared absorption.

**Hinesol-Eudesmol Mixture; Regeneration of Atractylol**—A mixture of hinesol (104 mg., 0.38 mole) and eudesmol (186 mg., 0.62 mole), melted together, showed $[\alpha]_D$ +20.2°(c=12.9), and recrystallised once from hydr. MeOH to white needles, m.p. 60~62°, $[\alpha]_D$ +22.0°(c=10.0). The mixed crystal obtained by this method was identical in mixed m.p. test and comparison of infrared spectra with atractylol derived from the original oil.


## Summary

The sesquiterpenoid alcohol, the so-called atractylol, a major constituent of the essential oil from the rhizomes of *Atractylodes lancea* De Candolle (Compositae), was separated into the known eudesmol and a new sesquiterpenoid alcohol, hinesol, which were characterised. Association of the two alcohols was established unambiguously by the regeneration of the so-called atractylol from them.


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## 62. Sadao Iguchi, Kazuhito Hisatsune, Michiko Himeno, and Shigeteru Muraoka : Studies on Pyrone Derivatives. III.*1 On the Reaction of Dehydroacetic Acid to Amino Acids. (1).

(*Pharmaceutical Institute, Medical Faculty, University of Kyushu**2)

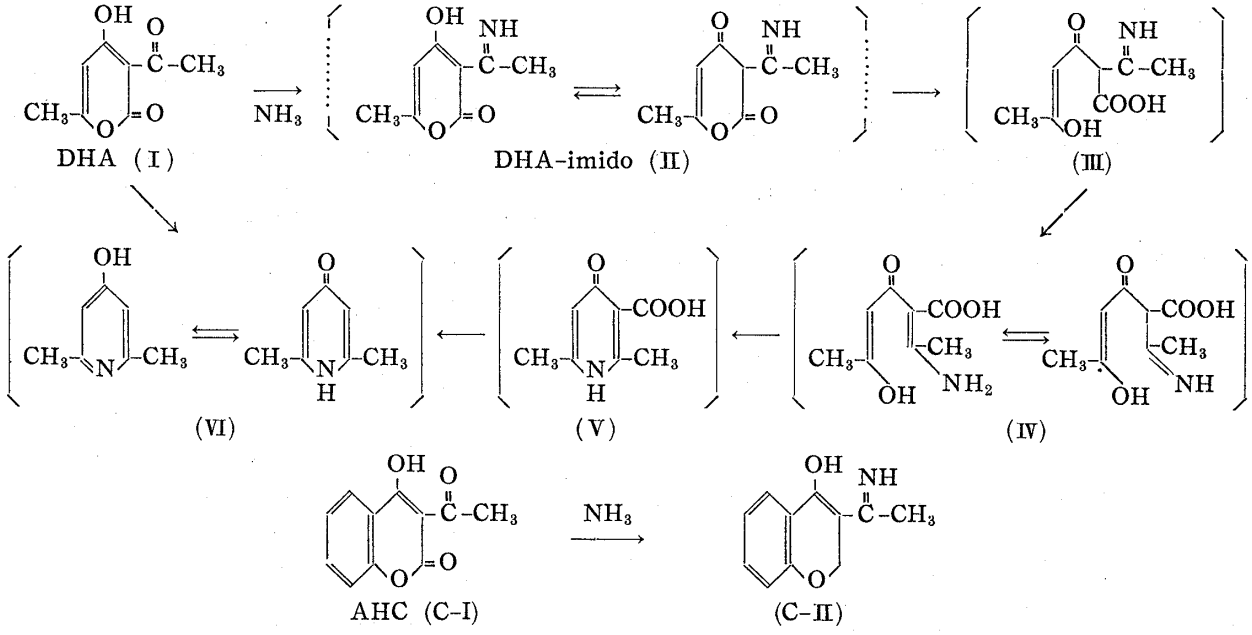
As was briefly reported in a previous communication,[1] it is thought interesting, in considering the powerful antibacterial and antifungal properties, that both dehydroacetic acid (DHA) and 3-acetyl-4-hydroxycoumarin (AHC), analogical in their structure, react with ammonia, amines, various amino acids, and with some aromatic amino compounds forming reaction products under a mild condition. In this paper, details of such a reaction between DHA or AHC and amino compound are described and the structure of reaction products is also discussed.

It was already reported by Oppenheim, *et al.*[2~5] that DHA reacted with conc. ammonia, producing 3-(1-iminoethyl)-4-hydroxy-6-methyl-2-pyrone(DHA-imide)(II), but not with dil. ammonia. However, it was found that this reaction also proceeded under a milder condition, i.e. in dil. aqueous ammonia (below 5%) at room temperature and the

*1 Part II : Yakugaku Zasshi, **77**, 98(1957).
*2 Katakasu, Fukuoka (井口定男, 久恒和仁, 姫野道子, 村岡重輝).
1) S. Iguchi, K. Hisatsune : Yakugaku Zasshi, **77**, 1258(1957).
2) Oppenheim, Precht : Ber., **9**, 1100(1876).
3) Collie, Myers : J. Chem. Soc., **63**, 128(1893).
4) Feist : Ann., **257**, 253~297(1890).
5) Petrenko-Kritschenko, Schöttle : Ber., **44**, 2830(1911); *ibid.*, **45**, 3231(1912).

reactive carbonyl group in $\alpha$-position of 3-acyl side chain of DHA readily reacted with ammonia to yield the 3-(1-iminoalkyl) compound (II).[6] The same was true with AHC and 3-(1-iminoethyl)-4-hydroxycoumarin (C-II) was obtained easily.[6]

The structure of DHA was verified by Feist,[4] Oppenheim,[2] and others, after many discussions and experiments, utilizing its transformation[2,4,7~9] to 2,6-dimethyl-4-hydroxypyridine (VI), and the validity of this structure was also recognized later by Rassweiler and Adams.[10] In such cases, (VI) was obtained from DHA after a long heating (100°) or treatment in a bomb (130°, under pressure) with conc. ammonia, and the reaction process was later explained as passing through (II), (III), (IV), and (V) as intermediates.[4] From these facts, the reaction of DHA with other substances,[11] e.g. aniline, toluidine, phenylhydrazine, etc., was thought to be similar to that with ammonia. Then the transformation reaction was examined using DHA-imide (II) as a starting material, and 2,6-dimethyl-4-hydroxypyridine(VI) was also obtained in a good yield. This supports the validity of the structure of (II), especially the position of nitrogen in DHA-imide molecule.




As a result of present experiments with various other amino compounds, it was also observed that both DHA and AHC had remarkable affinity to amino compounds in general. For example, DHA reacted readily with methylamine, arginine, or glycine ethyl ester, even when kept in aqueous alkaline solution at room temperature, reaction products began to precipitate immediately after mixing, and were obtained in a good yield after 24 hours. In the case of glycine, DHA gave similar reaction product after addition of one equivalent of NaOH or KOH into the test solution and keeping in incubator (37°) for 24 hours. It was proved later, however, that sodium salt of DHA (DHA-Na) always reacted similarly as DHA itself and therefore DHA-Na was chiefly used thereafter.

These reaction products were also identified by paper chromatography using the Dragendorff and ninhydrin as spraying reagents. The former was for the detection of tertiary amino group present in the substances and the latter for amino acids recovered

6) S. Iguchi, *et al.* : Yakugaku Zasshi, **77**, 98(1957).
7) Haitinger : Ber., **18**, 452(1885); Monatsh., **6**, 103(1885).
8) Conrad, Guthzeit : Ber., **20**, 159(1887).
9) Collie : J. Chem. Soc., **77**, 973(1900).
10) Rassweiler, Adams : J. Am. Chem. Soc., **46**, 2758(1924).
11) Beilstein's, 4te Aufl., **17**, 564.

by hydrolysis of condensation products. As a developing solvent, butanol-acetic acid-water (4:1:5) system was chiefly used and ascending method was adopted. Application of paper chromatography made it easy to investigate the effect of pH variation, and also the possibility of a reaction between DHA and many other amino acids. By this method it was clearly observed that glycine, the most frequently used representative amino acid, reacted with DHA-Na already at pH 5.3 (buffer solution) and more markedly with increasing pH after setting the solution in incubator for 24 hours. It was found from this experiment that following amino acids reacted with DHA-Na in a buffer solution (pH 6.8~8.2): Arginine, lysine, glycine, glutamic acid, valine, leucine, methionine, histidine, asparagine, and aspartic acid. With cysteine, tryptophan, and γ-aminobutyric acid no reaction could be observed. Besides these amino acids, some aromatic amino compounds and sulfanilamide derivatives, i.e. *p*-aminobenzoic acid (PABA) and its ester (PABA-Et), *p*-aminobenzenesulfonamide (sulfanilamide), N-acetyl-*p*-aminobenzenesulfonamide (sulfacetamide), and *p*-aminomethylbenzenesulfonamide (homosulfanilamide) also reacted with DHA and yielded condensation products. The Rf values of these reaction products on paper chromatogram are listed in Table I.

In the range of the present investigation, similar phenomenon was also observed with AHC.

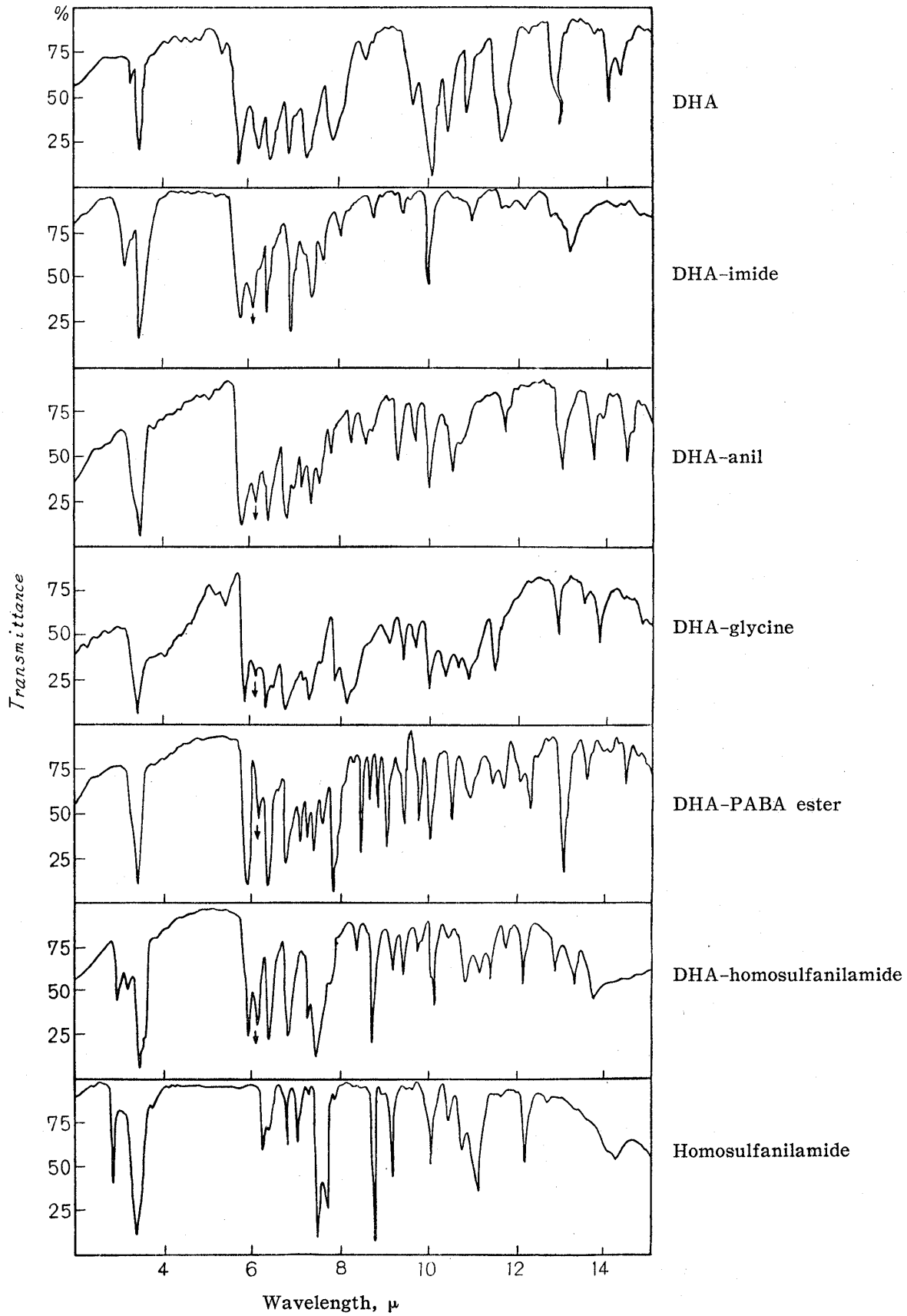
Table I. Rf Values of Reaction Products from DHA-Na and Amino Compounds

| Amino compd. | Rf[a] | Spray. reagent[b] | Develop. solvent[c] | pH of test solution[d] |
|---|---|---|---|---|
| (DHA-Na) | (0.95) | ($FeCl_3$) | (B) | (7.2) (K) |
| *dl*-Alanine | 0.82 (0.22) | N. D | B | 8.2 (K) |
| Arginine (monohydrochloride) | 0.44 (0.08) | N. D. S | B | 6.8 (K) |
| Asparagine | 0.52 (0.13) | N. D | B | 8.2 (K) |
| Aspartic acid | 0.57 (0.14) | N. D | B | 8.2 (K) |
| Glutamic acid | 0.58 (0.19) | N. D | B | 8.2 (K) |
| Glycine | 0.68 (0.14) | N. D | B | 7.2 (K) |
| Glycine ethyl ester (hydrochloride) | 0.66 | N. D | B | 6.8 (K) |
| Histidine (hydrochloride) | 0.42 (0.10) | N. D | B | 7.2 (K) |
| Leucine | 0.61 (0.43) | N. D | A | 7.2 (K) |
| Lysine (monohydrochloride) | 0.56 (0.09) | N. D | B | 7.2 (K) |
| *dl*-Methionine | 0.85 (0.46) | N. D | B | 7.2 (K) |
| *dl*-Valine | 0.51 (0.31) | N. D | A | 7.2 (K) |
| Methylamine hydrochloride | 0.66 | N. D | B | 7.2 (K) |
| Ethylamine hydrochloride | 0.66 (0.35) | N. D | B | 7.2 (K) |
| PABA | 0.87 | D. | B | (EtOH) |
| PABA-Et | 0.91 | D. | B | (EtOH) |
| Homosulfanilamide | 0.76 (0.55) | N. E | A | 7.4 (G) |

*a*) Rf values of original amino compds. themselves are given in parentheses.
*b*) N: Ninhydrin D: Dragendorff E: Ehrlich S: Sakaguchi
*c*) A: *iso*-AmOH-pyridine-$H_2O$ (1:1:1) B: BuOH-AcOH-$H_2O$ (4:1:5)
*d*) K: Kolthoff's buffer soln. ($KH_2PO_4$-borax).
G: Gifford's buffer soln. ($Na_2CO_3$-borax-KCl).

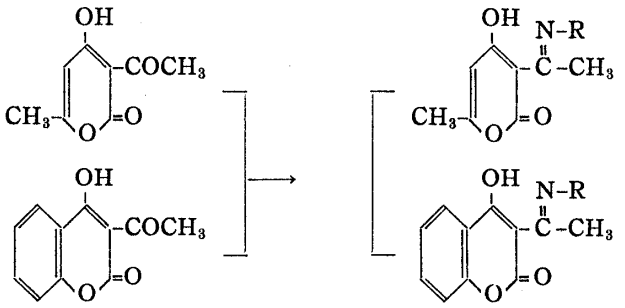
The structure of these substances was assumed to be a type of compounds of Schiff base derived from a combination between reactive >C=O group of 3-acyl side chain in DHA or AHC and $NH_2$ group in amino compounds. The results of elementary analyses of the isolated substances agreed well with this assumption and it seemed to be further supported by the following experimental results: (1) Infrared spectral analysis showed that there existed the specific absorption band of C=N bond in the region of 6.00~6.10 μ, commonly in these pyrone derivatives. Some typical examples are shown in Fig. 1. (2) Not only from free type of glycine or *p*-aminobenzoic acid but also from their ester types, condensation products of similar type were obtained by reaction with DHA or AHC. Therefore, there seems to be little doubt that $NH_2$-group of these compounds take part in this reaction. (3) The condensation product of DHA and glycine ethyl

Fig. 1. Infrared Spectra of DHA and its Derivatives (Nujol mull)
(Koken DF-201 infrared spectrophotometer, NaCl prism)



ester could be saponified to DHA-glycine condensate, which dissolved itself in aqueous $NaHCO_3$ solution with foaming and was hydrolyzed to original DHA by HCl solution. (4) No products could be obtained from reaction between these amino compounds and 4-hydroxycoumarin or 3-alkyl-4-hydroxycoumarin having no acyl in their 3-position.

From these findings, it was concluded that these reaction products are not salts but compounds of Schiff base-type, structure of which was assumed to be quite analogous to the afore-mentioned case of ammonia.



| R | $-CH_3$ | $-C_2H_5$ | $-CH_2COOH$ | $-CH_2COOC_2H_5$ | $-C_6H_4COOH$ |
|---|---|---|---|---|---|
| DHA | (X) | (XI) | (XII) | (XIII) | (XIV) |
| AHC | (C-X) | (C-XI) | (C-XII) | (C-XIII) | (C-XIV) |

| R | $-C_6H_4COOC_2H_5$ | $-C_6H_4SO_2NH_2$ | $-C_6H_4SO_2NHAc$ | $-CH_2C_6H_4SO_2NH_2$ |
|---|---|---|---|---|
| DHA | (XV) | (XVI) | (XVII) | (XVIII) |

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## Experimental

**Conversion of 3-(1-Iminoethyl)-4-hydroxy-6-methyl-2-pyrone (II) into 2,6-Dimethyl-4-hydroxypyridine (VI)**—A mixture of 1.0 g. of (II) and 40 cc. of conc. $NH_4OH$ was heated at 130~140° in a sealed tube for 10 hr. The reaction mixture was evaporated to leave a yellowish residue which was recrystallized from water to white needles, m.p. 229~230°. This substance was identical with 2,6-dimethyl-4-hydroxypyridine synthesized directly from DHA and $NH_4OH$ after the method of Oppenheim, *et al.*[2]

**3-(1-Methyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (X)**—0.3 g. of DHA was added to a mixed soln. of $CH_3NH_2 \cdot HCl$ (2 g.) and NaOH soln. (1 g. in 10 cc. of $H_2O$) and clear soln. obtained was kept in incubator (37°). With passage of time crystals gradually began to precipitate. After 48 hrs., precipitated crude crystals were collected and recrystallized from AcOEt to colorless prisms. Yield, 0.25 g. Hydrate., m.p. 108~110°. *Anal.* Calcd. for $C_9H_{11}O_3N \cdot 2H_2O$: C, 49.8; H, 6.9; N, 6.4. Found: C, 50.33; H, 7.05; N, 7.04.

Anhydrate (obtained by drying in a vacuum desiccator), m.p. 242~244°.

**3-(1-Methyliminoethyl)-4-hydroxycoumarin (C-X)**—To a mixture of $CH_3NH_2 \cdot HCl$ (6 g.) and NaOH soln. (3 g. in 20 cc. of $H_2O$), EtOH soln. of AHC (0.5 g. in 15 cc.) was added and the mixture was kept in an incubator for 10 days. On cooling the soln. in ice water, crystals separated out and were recrystallized from AcOEt to colorless prisms, m.p. 181~183°; yield, 0.42 g. *Anal.* Calcd. for $C_{12}H_{11}O_3N$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.25; H, 5.21; N, 6.59.

**3-(1-Ethyliminoethyl)-4-hydroxycoumarin (C-XI)**—Obtained by the same procedure as (C-X). Colorless prisms, m.p. 149~150°. *Anal.* Calcd. for $C_{13}H_{13}O_3N$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.78; H, 5.92; N, 6.06.

**3-(1-Carboxymethyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (XII)**—i) To the soln. of glycine (1 g. in 10 cc. of $H_2O$), 1.0 g. of DHA-Na was added and the mixture was kept in an incubator for 48 hrs. After unchanged DHA was extracted with $Et_2O$, the soln. was acidified with 5% HCl, the deposited crude product was washed with $Et_2O$, and recrystallized from dioxane, m.p. 247~248° (decomp.). Yield, 0.95 g. *Anal.* Calcd. for $C_{10}H_{11}O_5N$: C, 53.33; H, 4.92; N, 6.22. Found: C, 52.91; H, 5.06; N, 6.51.

ii) DHA (1.0 g.) was added to the soln. of glycine (1.0 g.) and KOH (0.8 g. in 10 cc. of $H_2O$), and the reaction mixture was worked up as in (i). Yield, 0.68 g.

**3-(1-Carboxymethyliminoethyl)-4-hydroxycoumarin (C-XII)**—To a mixture of 1.0 g. of glycine and KOH soln. (0.8 g. in 10 cc. of $H_2O$), 0.8 g. of AHC was added and treated similarly as for (XII). m.p. 233~234°(decomp.). Yield, 0.99 g.

**3-(1-Ethoxycarbonylmethyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (XIII)**—To the soln. of $NH_2CH_2COOC_2H_5 \cdot HCl$ (1.0 g.) and KOH (0.4 g. in 20 cc. of $H_2O$), 1.0 g. of DHA was added and the reaction mixture was allowed to stand at room temperature for 24 hr. With passage of time crystals gradually deposited. The crystals were collected and recrystallized from dehyd. EtOH to colorless prisms, m.p. 150~152°. Yield, 1.17 g. *Anal.* Calcd. for $C_{12}H_{15}O_5N$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.80; H, 5.93; N, 5.69.

**3-(1-Ethoxycarbonylmethyliminoethyl)-4-hydroxycoumarin (C-XIII)**—0.5 g. of AHC was added to a solution of $NH_2CH_2COOC_2H_5 \cdot HCl$ (1.0 g.) and KOH (0.4 g. in 10 cc. of $H_2O$), and the reaction mixture was treated similarly as for (XIII). m.p. 157~158°. Yield, 0.68 g. *Anal.* Calcd. for $C_{15}H_{15}O_5N$: C, 62.28; H, 5.23; N, 4.82. Found: C, 62.63; H, 5.72; N, 5.18.

**3-(1-*p*-Carboxyphenyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (XIV)**—A solution of 1.0 g. of DHA and 0.8 g. of *p*-aminobenzoic acid dissolved in 10 cc. of dehyd. EtOH was refluxed on a steam bath for 8 hr. Crystals gradually deposited as the solution cooled. The crude crystals obtained were recrystallized from dehyd. EtOH to colorless needles, m.p. 256~258°(decomp.). Yield, 0.56 g. *Anal.* Calcd. for $C_{15}H_{13}O_5N$: C, 62.71; H, 4.56; N, 4.88. Found: C, 63.21; H, 4.81; N, 4.90.

**3-(1-*p*-Ethoxycarbonylphenyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (XV)**—Analogous to (XIV), EtOH soln. of DHA (1.0 g.) and ethyl *p*-aminobenzoate (1.0 g.) was refluxed for 8 hr. Recrystallization from dehyd. EtOH gave colorless needles, m.p. 149.5~151.5. Yield, 0.61 g. *Anal.* Calcd. for $C_{17}H_{17}O_5N$: C, 64.75; H, 5.43; N, 4.43. Found: C, 64.67; H, 5.75; N, 4.61.

**3-(1-*p*-Carboxyphenyliminoethyl)-4-hydroxycoumarin (C-XIV)**—EtOH soln. (10 cc.) of AHC (0.8 g.) and *p*-aminobenzoic acid (0.53 g.) was heated for 8 hr. as for (XIV). Recrystallized from dioxane to colorless prisms, m.p. 255.5~257.5°(decomp.). Yield, 1.0 g. *Anal.* Calcd. for $C_{18}H_{13}O_5N$: C, 66.85; H, 4.05; N, 4.33. Found: C, 67.31; H, 4.43; N, 4.66.

**3-(1-*p*-Sulfamylphenyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (XVI)**—Sulfanilamide (1 g.) and DHA (1 g.) were dissolved in 5 cc. of warm dehyd. EtOH and refluxed on a steam bath. Reaction product began to precipitate gradually after about 2 hr. After 6 hr.' heating, the crude product obtained was filtered while warm and recrystallized from EtOH to white leaflets, m.p. 253~255° (decomp.). Yield, 1.3 g. *Anal.* Calcd. for $C_{14}H_{14}O_5N_2S$: C, 52.16; H, 4.38; N, 8.69. Found: C, 52.21; H, 5.00; N, 8.66.

**3-(1-*p*-Acetylsulfamylphenyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (XVII)**—A solution of sulfacetamide (3.3 g.) and DHA (2.4 g.) dissolved in hot dehyd. EtOH (10 cc.) was refluxed for 9 hr. Raction product was obtained as for (XVI). Recrystallization from EtOH-pyridine (10:1) gave white cubic crystals, m.p. 241~243°(decomp.). Yield, 2 g. *Anal.* Calcd. for $C_{16}H_{16}O_6N_2S$: C, 52.60; H, 4.42; N, 7.69. Found: C, 52.55; H, 4.58; N, 8.11.

**3-(1-*p*-Sulfamylbenzyliminoethyl)-4-hydroxy-6-methyl-2-pyrone (XVIII)**—Homosulfanilamide-HCl and DHA-Na were dissolved in distilled water or buffer solution (pH 5~8) in equimolar concentrations. After keeping the solutions for several hours at room temperature or in incubator (37°), needle-like crystals began to precipitate and were obtained almost quantitatively after longer standing. Heating accelerated this reaction. When relatively high concentrations of solutes were used, reaction product was obtained immediately. White needles (from EtOH), m.p. 229~230°(decomp.). *Anal.* Calcd. for $C_{15}H_{16}O_5N_2S$: C, 53.50; H, 4.78; N, 8.33. Found: C, 53.52; H, 4.96; N, 8.00.

**Hydrolysis of (XIII) to (XII)**—0.1 g of (XIII) was added to 10 cc. of 1% ethanolic KOH soln. and white precipitate formed after about 5 min. This crude product was dissolved in 10 cc. of $H_2O$, the solution was acidified with dil. HCl, and the white crystals so formed were recrystallized from dioxane to colorless prisms, m.p. 247~248°(decomp.), identical with (XII).

**Paper Chromatographic Procedure**—Test solution: 0.1~1% Solution of DHA-Na and amino compound was kept in incubator (37°) for suitable hours (generally for 24 hr.). Distilled water or Kolthoff's buffer solution was chiefly used as a solvent, but in the case of PABA and its ester, EtOH solution was prepared.

Method of developing: Toyo Roshi No. 50 was used and developed for 16 hr. at 18~20° by the ascending method. BuOH-AcOH-$H_2O$ (4:1:5) system was chiefly used as developing solvent and *iso*-AmOH-pyridine-$H_2O$ (1:1:1) system was used in some cases. For spraying, ninhydrin and Dragendorff reagents were generally used and for special cases, Ehrlich and Sakaguchi reactions were also used.

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