324. Kenkichi Tomita: The Antibacterial Properties of Compounds Containing the Tricarbonyl-methane Group. V.* The Syntheses of 3-Acyl-2,4-dihydroxyquinolines and of the Related Compounds, with respect to their Antibacterial Properties.

(Institute for Infectious Diseases, University of Tokyo**)

Ukita and his co-workers synthesized a series of 3-acyl-4-hydroxycoumarins (I)¹⁾, tested their antibacterial properties against various bacteria *in vitro* and found that 3-decanoyl derivative (I, R=C₉H₁₉) was highly active against *Staph. aureus*.²⁾ From their studies, it seemed likely that the following

(I)

three factors played an important role in the antibacterial activities of these compounds: (1) The number of carbon atoms in the R of RCO-groups in the 3-position; (2) the arrangement of three carbonyl groups around the carbon atom number 3 as the centre (tricarbonyl methane group); and (3) the existence of a benzene

ring.

The present investigation was undertaken to study further the relationship between the chemical structures and antibacterial properties of the compounds of this type in more detail with respect to the significance of pyrone ring. Some quinoline compounds (II) and (III), possessing the above three important factors assumed to be responsible for the antibacterial activities, and containing a nitrogen atom instead of an oxygen atom of coumarin ring of (I), were synthesized to compare the activities against bacteria with those of I-series.

(II)

(III)

nor-Dictamnol (II, R=H), the most simplest member of II-series, had been derived from 2,4-dihydroxyquinoline (IV),³⁾ the nucleus of II-series, by the Reimer-Tiemann reaction,⁴⁾ but the introduction of any acyl group into the 3-position of (IV) had never been reported. The general acylation procedure of 4-hydroxycoumarin¹⁾ was not available, because the treatment of (IV) with benzoyl chloride at 37° overnight only led to the formation of the known monobenzoate (V, R=C₆H₅), m.p., 228-229°⁵⁾. Under similar conditions, however, acetyl chloride was hardly reactive and the reaction was not effected even by heating for 3 hrs. on a water bath. The monoacetate (V, R=CH₃), m.p., 214-217°, was prepared with acetic anhydride containing a few drops of pyridine⁶⁾.

* See Footnote 1), 2) for previous papers. ** Shirokane-Dai-machi, Minato-ku, Tokyo. (富田謙吉)

1) T. Ukita, S. Nojima and M. Matsumoto: J. Am. Chem. Soc., **72**, 5143 (1950).

2) T. Ukita, D. Mizuno, T. Tamura, T. Yamakawa and S. Nojima: This Journal, **71**, 234 (1951).

3) R. Camps: Arch. Pharm., **239**, 690 (1901); cf. E. H. Hurtress and J. Bornstein: J. Am. Chem. Soc., **71**, 745 (1949).

4) Y. Asahina and M. Inubuse: This Journal, **52**, 120 (1932).

5) G. Heller and W. Tischner: Ber., **42**, 4564 (1909).

6) J. N. Achley, W. H. Perkin, Jr. and R. Robinson: J. Chem. Soc., 388 (1930).

These compounds were easily hydrolyzable by dil. caustic alkali solution, but the chemical structure, particularly as to which of two hydroxyl groups had participated in the esterification, was not thoroughly investigated.

On the other hand, the Friedel-Crafts reaction of 2,4-dihydroxyquinoline (IV) with benzoyl or acetyl chloride furnished a benzoyl, m.p., 258°, or an acetyl derivative m.p., 255°. Each of them was the functional group isomer of the above monobenzoate or monoacetate, differing only in the position of the acyl group.

For the identification of these isomers, the ultraviolet absorption spectra* of the related compounds were studied. The results were as follows: (1) The curve of the monoacetate (V, $R=CH_3$) resembled that of 2,4-dihydroxyquinoline (IV), almost identical with that of 2-hydroxyquinoline (IX), but quite different from that of 4-hydroxyquinoline (X); (2) the bathochromic shifts of absorption maxima were observed when 2,4-dihydroxyquinoline was acylated by the Friedel-Crafts reaction and the curves bore no resemblance to any of the above-mentioned compounds; (3) similar bathochromic shifts were observed when one aldehyde group was introduced into (IV) (*nor*-dictamnol); and (4) 4-hydroxycoumarin derivatives also showed similar shifts when certain acyl groups were introduced into the 3-position. (Figs. 1, 2 and 3).

From these facts, it was presumed that the compounds acylated by the Friedel-Crafts reaction were the desired 3-substituted derivatives and that the above monoacylated compounds would be the ester of the 4-hydroxyl group.

Since 2,4-dihydroxyquinoline was thus acylated with acetyl or benzoyl chloride by the Friedel-Crafts reaction, similar acylated compounds were expected to be derived also from the monoacetate or monobenzoate (V), probably an ester of a kind of phenol, by the Fries rearrangement, and this came up to the expectation.

Fig. 1.

- a: 3-Butyryl-2,4-dihydroxyquinoline
- b: 4-Hydroxyquinoline
- c: 2-Hydroxyquinoline
- d: 2,4-Dihydroxyquinoline monoacetate

* All spectroscopic measurements were made with a Beckman model DU quartz spectrophotometer using 1-cm. cells. Solvent: alcohol. Concentration: 1×10^{-4} M.

Fig. 2.

- a: 2,4-Dihydroxyquinoline
 b: 3-Acetyl-2,4-dihydroxyquinoline
 c: *nor*-Dictamnol (3-Formyl-2,4-dihydroxyquinoline)
 d: 3-Benzoyl-2,4-dihydroxyquinoline

Fig. 3.

- a: 4-Hydroxycoumarin
 b: 3-Acetyl-4-hydroxycoumarin
 c: 3-Propionyl-4-hydroxycoumarin
 d: 3-Decanoyl-4-hydroxycoumarin

It was by the cyclization method that these acylated compounds obtained by the above two reactions were confirmed to be 3-acyl-2,4-dihydroxyquinolines (II). The thermal condensation of ethyl anthranilate (VI) with ethyl acetoacetate (VII, $R=CH_3$) or benzoylacetate (VII, $R=C_6H_5$) furnished ethyl *O*-(acetoacetyl)-aminobenzoate (VIII, $R=CH_3$) or ethyl *O*-(benzoylacet)-aminobenzoate (VIII, $R=C_6H_5$), respectively, the cyclization

of which was effected with sodium in xylene to yield 3-acetyl- (II, $R=CH_3$), m.p., 255° , or 3-benzoyl-2,4-dihydroxyquinoline (II, $R=C_6H_5$), m.p. 258° . The m.p., mixed m.p. and elementary analyses showed that they were quite identical with the corresponding ones obtained by the foregoing reactions.

In a similar way, the propionyl, butyryl, isovaleryl, caproyl, octanoyl, and decanoyl derivatives prepared by the Friedel-Crafts reaction were proved to be 3-acyl-2,4-dihydroxyquinolines on the ground of the identities with the corresponding ones synthesized by the ring closure method. Of these compounds, 3-propionyl derivative, however, was not attempted by this method because of the reported difficulty of obtaining pure ethyl propionylacetate.⁷⁾ Moreover, both the thermal condensation of ethyl anthranilate (VI) with β -ketoic esters (VII) into (VIII), and the subsequent cyclization, gradually grew harder with the increase in the number of carbon atoms contained in those esters, so that 3-octanoyl derivative was obtained only in a small amount and 3-decanoyl compound not obtained at all.

In addition, some *N*-methyl- and *N*-ethyl-3-acyl-4-hydroxyquinolones- (2) were synthe-

7) Bouveault and Bongert: Bull. soc. chim., (3) 27, 1089 (1902).

sized, because these compounds derived from *N*-alkyl-4-hydroxyquinolone-(2), which seemed structurally somewhat more similar to 4-hydroxycoumarin than 2,4-dihydroxyquinoline, were anticipated to possess interesting antibacterial actions.

N-Methyl-4-hydroxyquinolone-(2) (XIII, $R' = \text{CH}_3$), as the material for some kind of dyes, had been synthesized by the condensation of monomethylaniline with ethyl malonate at high temperatures,⁸⁾ but the cyclization of ethyl *N*-acetyl-*N*-methylantranilate (XII, $R' = \text{CH}_3$) was much more convenient and, similarly, *N*-ethyl-4-hydroxyquinolone-(2) (XIII, $R' = \text{C}_2\text{H}_5$), was derived from ethyl *N*-acetyl-*N*-ethylantranilate (XII, $R' = \text{C}_2\text{H}_5$).

The chemical behaviors of these quinolones were about the same as those of 2,4-dihydroxyquinoline. The acylation of *N*-methylquinolone-(2) with acetic anhydride or benzoyl chloride in pyridine yielded a monoacetate (XIV, $R = R' = \text{CH}_3$), m.p., 142–144°, or monobenzoate (XIV, $R = \text{C}_6\text{H}_5$, $R' = \text{CH}_3$), m.p., 170–171°, respectively. Both of them were readily hydrolyzable with dil. caustic alkali solution and are presumably the 4-OH-acylated compounds.*

The Friedel-Crafts reaction of this quinolone (XIII, $R' = \text{CH}_3$) with acetyl or benzoyl chloride produced an acetyl, (III, $R = R' = \text{CH}_3$), m.p., 143–145°, or benzoyl compound (III, $R = \text{C}_6\text{H}_5$, $R' = \text{CH}_3$), m.p., 182–184°, both of which were readily soluble in dil. caustic alkali solution and quite different from the above monoacetate or monobenzoate because of the depression of mixed m.p., though the melting points of the two acetyl isomers were almost the same.

Likewise, propionyl, butyryl, and isovaleryl derivatives were prepared by the Friedel-Crafts reaction. They were all readily soluble in dil. caustic alkali solution. Their melting points were much lower than those of the corresponding ones in II-series, and, therefore, the compounds with the longer carbonyl side-chains than isovaleryl could not be obtained because of the difficulty to recrystallize and also by their poor yield.

The direct thermal condensation of ethyl *N*-methylantranilate (XI, $R' = \text{CH}_3$) with ethyl acetoacetate to ethyl *N*-acetoacetyl-*N*-methylantranilate (XV, $R' = \text{CH}_3$), which would be cyclized by sodium to *N*-methyl-3-acetyl-4-hydroxyquinolone-(2) (III, $R = R' = \text{CH}_3$), did not proceed as in the case of ethyl anthranilate. In boiling xylene, however, both the condensation of the two esters and cyclization were carried out simultaneously to produce the desired 3-acetyl compound, which was proved by the mixed fusion to be quite identical with the acetyl derivative prepared by the Friedel-Crafts reaction, confirming that any acyl group could be introduced into the 3-position of this kind of quinolones by the Friedel-Crafts reaction.

3-Acetyl, and 3-benzoyl compounds were also derived from the acetate (XIV, $R = R' = \text{CH}_3$) and benzoate (XIV, $R = \text{C}_6\text{H}_5$, $R' = \text{CH}_3$) by the Fries rearrangement as in the case of II-series.

8) H. Waldmann: J. prakt. Chem., (2) **147**, 322 (1937).

* It was assumed that there would be little chance for the type (XIII') to contribute to the resonance system of (XIII) during the reactions.

Similarly, with *N*-ethyl-4-hydroxyquinolone-(2) (XIII, $R' = C_2H_5$), the Friedel-Crafts reaction yielded 3-acetyl (III, $R = CH_3$, $R' = C_2H_5$), and 3-benzoyl compounds (III, $R = C_6H_5$, $R' = C_2H_5$). The latter was also derived from the monobenzoate (XIV, $R = C_6H_5$, $R' = C_2H_5$) by the Fries reaction and the former was identical with that prepared by the treatment of ethyl *N*-ethylanthranilate (XI, $R' = C_2H_5$) with ethyl acetoacetate in boiling xylene.

Finally, the Reimer-Tiemann reaction with these quinolones (XIII) also caused the introduction of one aldehyde group, the position of which was presumed to be 3 on the analogy of *nor*-dictamnol.

Table I
Antibacterial Properties of 3-Acyl-2,4-dihydroxyquinolines and Related Compounds

Bac- teria	No.	CH ₃	C ₂ H ₅	C ₃ H ₇	<i>i</i> C ₄ H ₉	C ₅ H ₁₁	<i>i</i> C ₅ H ₁₁	C ₇ H ₁₅	C ₉ H ₁₉	C ₆ H ₅	H
<i>Staph. aureus</i>	I	B.S. 3,500	B.S. 4,000	B.S. 4,000	B.S. 16,000		K. 256,000	K. 2,560,000	K. 64,000,000		
	II	K. <800*	K. <800*	K. <800*	K. <800	K. <800*		K. <10,000	K. <10,000	K. <800*	K. 6,400
	IIIa	K. 6,400	K. <800*	K. <800*	K. 12,800					K. <800	K. 1,600
	IIIb	K. <800*								K. <800*	K. 3,200
<i>Esch. coli</i>	I	B.S. 1,600	B.S. 2,000	B.S. <1,000	B.S. <1,000		B.S. <1,000	B. <10,000	B. <10,000		
	II	K. <800	K. <800	K. <800	K. <800	K. <800		K. <10,000	K. <10,000	K. <800	K. 1,600
	IIIa	K. <800	K. <800	K. <800	K. <800					K. <800	K. 1,600
	IIIb	K. <10,000								K. <10,000	K. <10,000

The values are expressed as the maximum dilution at which the test organisms failed to grow.

B.: Broth. K.: Knight's synthetic medium. B.S.: Broth streak method.

*: On standing after dilution, the compound tested slightly crystallized out, but the multiplication of organisms was confirmed by the subsequent subcultivation on agar plate.

The antibacterial activities of these synthesized compounds against *Staph. aureus* and *Esch. coli* *in vitro* were compared with those of I-series (Table I).^{*} All the compounds belonging to II-series were almost inactive against the two bacteria and no effect of the elongation of carbonyl side-chain was observed, but III-series were thought to have a tendency to gradually become more active against *Staph. aureus*. To the author's

* Cf. Footnote 2), as to the bacteriological procedure.

regret, however, the difficulty of obtaining derivatives having a larger acyl group than isovaleryl prevented him from confirming this tendency.

From these facts, it was shown that the coumarin ring was an essential factor for the antimicrobial activities of these compounds, and that the replacement of oxygen atom by nitrogen was of little importance for the increase of activities. If the activities of III-series, unlike II-series, should increase, as imagined, with the elongation of carbonyl side-chain, it was supposed that the presumable difficulty of 2-carbonyl group of III-series to take an enol form, as in I-series, had a significance for this difference.

The author wishes to express his deep appreciations to Prof. Dr. S. Akiya and Assist. Prof. Dr. T. Ukita for much helpful advices and encouragement, and to Miss R. Matsuda for her assistances throughout this investigation. The author also wishes to thank Miss T. Tamura for carrying out antibacterial tests, and to Miss R. Ota, Miss E. Kondo and Mr. B. Kurihara for carrying out microanalysis.

Experimental

Monoacetate (V, $R=CH_3$) and monobenzoate (V, $R=C_6H_5$) of 2,4-dihydroxyquinoline: The monoacetate⁶⁾, colorless fine needles (from alcohol), m.p., 214–217°. *Anal.* Calcd. for $C_{11}H_9O_3N$: C, 65.02; H, 4.46; N, 6.89. Found: C, 65.41; H, 4.37; N, 6.79.

The benzylation of 2,4-dihydroxyquinoline (IV) with benzoyl chloride in pyridine solution for 24 hrs. furnished the monobenzoate⁵⁾, as colorless needles (from alcohol), m.p., 228–229°. Yield, 57% of the theory. *Anal.* Calcd. for $C_{16}H_{11}O_3N$: C, 72.44; H, 4.18; N, 5.28. Found: C, 72.28; H, 4.25; N, 5.50.

Under similar conditions, however, acetyl chloride was hardly reactive, and the quinoline (IV) was recovered from the reaction mixture (m.p., 340°, from a large amount of alcohol).

These compounds were easily hydrolyzed by 10% caustic soda solution at room temperature in about 0.5 hr. and only gave a light yellow coloration with ferric chloride in alcoholic solution, in contrast to the orange coloration of 2,4-dihydroxyquinoline.

Acylation of 2,4-dihydroxyquinoline by the Friedel-Crafts reaction: 3-Decanoyl-2,4-dihydroxyquinoline, for example, was prepared as follows: To a solution of 0.5 g. (1.2 mol. equiv.) of aluminum chloride in 5 cc. of nitrobenzene were added 0.7 g. (1.2 mol. equiv.) of capryl chloride and 0.5 g. (1 mol. equiv.) of 2,4-dihydroxyquinoline, and the mixture was warmed on a steam bath for 3 hrs. The order of addition of the reagents was not closely examined, because the reaction was slow. After decomposing with hydrochloric acid, the solvent was removed with steam, and the resulting dark brown oily substance was separated from the warm colorless aqueous solution, from which the starting material (IV) crystallized out in fine needles after cooling. The oily substance was recrystallized from alcohol with charcoal.

This procedure was available also for the syntheses of other members of this series. As to the quantities of catalyst, some variations might be necessary, because, with 2 mol. of aluminum chloride, the yield of 3-acetyl derivative was higher than with 1 mol. of the catalyst (65% as compared with 39%).

Most of them were colorless fine needles recrystallizable from alcohol, with the exception of 3-benzoyl derivative, which formed glazing yellow needles from alcohol or acetic acid. They were all soluble in 5% caustic soda solution and gave orange-yellow to orange-red coloration with ferric chloride in alcohol, like 2,4-dihydroxyquinoline. Their melting points were much lower than that of (IV), and gradually grew lower with the increase in the number of carbon atoms contained in their side-chains (Table II).

These compounds hardly produced any derivative, in spite of the presence of many active atom groups. The 3-acetyl compound, for example, resisted benzylation, and most of them gave no authentic phenylhydrazones, the only exception being the phenylhydrazone of 3-butyryl compound obtained as yellow needles (from alcohol), m.p., 235.5°. *Anal.* Calcd. for $C_{19}H_{19}O_2N_3$: C, 71.01; H, 5.91; N, 13.08. Found: C, 70.90; H, 6.06; N, 13.47.

3-Formyl-2,4-dihydroxyquinoline (nor-dictamnol) (II, $R=H$): This aldehydequinoline was synthesized according to Asahina and Inubuse's report⁴⁾, obtained as colorless fine needles, m.p., over 360°, from acetic acid with charcoal and then from a large amount of alcohol (Table II). This also gave orange-yellow coloration with ferric chloride in alcohol, but, unlike the other members of II-series, this was insoluble in 5% caustic soda solution. Moreover, an analytically pure phenylhydrazone could not be obtained and, besides, it melted about 20° lower than Asahina's (235°) notwithstanding repeated recrystallizations.

Table II
3-Acyl-2,4-dihydroxyquinolines

3-Acyl-group	Formula	m.p., °C	Yield, %	Analyses, C, H, N, %	
				Calcd.	Found
Formyl-	C ₁₀ H ₇ O ₃ N	>360	40	C 63.48, H 3.73, N 7.41	C 63.50, H 3.76, N 7.71
Benzoyl-	C ₁₆ H ₁₁ O ₃ N	258	12	C 72.44, H 4.18, N 5.28	C 72.22, H 4.13, N 5.28
Acetyl-	C ₁₁ H ₉ O ₃ N	255-256	39	C 65.02, H 4.43, N 6.90	C 64.76, H 4.60, N 6.78
Propionyl-	C ₁₂ H ₁₁ O ₃ N	225-226	38	C 66.35, H 5.10, N 6.45	C 66.37, H 5.10, N 6.47
Butyryl-	C ₁₃ H ₁₃ O ₃ N	218-219	25	C 67.52, H 5.67, N 6.06	C 67.71, H 5.91, N 5.90
Isovaleryl-	C ₁₄ H ₁₅ O ₃ N	195-198	26	C 68.55, H 6.16, N 5.71	C 68.75, H 6.46, N 5.87
Caproyl-	C ₁₅ H ₁₇ O ₃ N	183-184	12	C 69.48, H 6.61, N 5.40	C 69.25, H 6.65, N 5.37
Octanoyl-	C ₁₇ H ₂₁ O ₃ N	168-169	17	C 71.05, H 7.37, N 4.84	C 70.83, H 7.33, N 5.01
Decanoyl-	C ₁₉ H ₂₅ O ₃ N	127-130	10	C 72.35, H 7.99, N 4.44	C 72.38, H 7.84, N 4.09

The Fries rearrangement of the monoacetate or monobenzoate of 2,4-dihydroxyquinoline : Using nitrobenzene as a solvent, the monoacetate (V, R=CH₃) and aluminum chloride in equimolar quantities were warmed on a water bath for 4 hrs., then the reaction mixture was treated in the same manner as in the above Friedel-Crafts reaction, and the crude product was recrystallized from alcohol to colorless fine needles, m.p., 252-255°. In admixture with an authentic sample no depression of m.p. was observed. *Anal.* Calcd. for C₁₁H₉O₃N: C, 65.02; H, 4.43; N, 6.90. Found: C, 64.84; H, 4.75; N, 7.35.

Similarly, from the monobenzoate (V, R=C₆H₅), yellow fine needles, m.p., 255-260° (alcohol), were obtained and m.p. was not depressed in admixture with an authentic sample by the Friedel-Crafts reaction. *Anal.* Calcd. for C₁₆H₁₁O₃N: C, 72.44; H, 4.18; N, 5.28. Found: C, 72.41; H, 4.55; N, 5.61.

Ethyl acylacetates (VII) : The aliphatic β -ketonic esters were prepared according to the method of synthesizing ethyl benzoylacetate⁹⁾, with reference to the reports of Asano¹⁰⁾ and of Asahina and Nakayama¹¹⁾.

To 3.45 g. of powdered sodium in 340 cc. of dry benzene was added 19.5 g. of ethyl acetoacetate and the mixture was boiled for 12 hrs. under stirring. To the resulting ethyl sodioacetoacetate, after cooling, was added 0.01 mol. equiv. of fatty acid chloride and the mixture was refluxed further for 8 hrs. under stirring. After adding 3.5 g. of ice to the cooled reaction mixture and stirring, the benzene layer was separated from the aqueous layer and washed with a small amount of 5% sodium bicarbonate solution. After drying over sodium sulfate, the solvent was removed and the residual ester distilled under diminished pressure (Table III). The hydrolyses of ethyl acylacetoacetates thus obtained to β -ketonic esters were always effected by dry ammonia, because the ammonium chloride-ammonia method was unsatisfactory. For example, ethyl isovalerylacetoacetate was recovered unchanged even after this treatment.

Into an ice-cooled solution of ethyl acylacetoacetate in equal volume of dry ether, dry ammonia gas was introduced until the absorption subsided, and then the reaction mixture was allowed to stand overnight. After removing the solvent and ammonia, the residue was washed with 10% hydrochloric acid solution, taken up in ether, dried, and then distilled under diminished pressure. The β -ketonic esters thus obtained were used for the next reaction without further purification. The boiling points and yields are summarized in Table III.

Ethyl O-(acylaceto)-aminobenzoates (VIII) : The general procedure for the condensation of the above β -ketonic esters (VII) with ethyl anthranilate (VI) into (VIII) was as follows: To 4 mol. of β -ketonic ester previously heated up to 160-180°, was added dropwise 1 mol. of ethyl anthranilate under stirring. After the foaming subsided, the mixture was kept at the same temperature for 30 min. and then, under reduced pressure, were recovered the unreacted ethyl anthranilate and the excess of β -ketonic ester, with which the same reaction was repeated several times. The combined residues were purified either by recrystallization, in the case of R=CH₃ or C₆H₅, or by repeated distillation under diminished pressure, when R was higher than

9) "Org. Synth." Col. Vol. II, 760. cf. *ibid.*, 23, 35.

10) M. Asano: This Journal, 44, 79 (1924).

11) Y. Asahina and H. Nakayama: *Ibid.*, 45, 1059 (1925).

Table III.

Acyl group	Ethyl acylacetoacetates			Ethyl acylacetates		
	b.p., °C/mm.	Yield, %	Coloration by FeCl ₃ in alcohol	b.p., °C/mm.	Yield, %	Coloration by FeCl ₃ in alcohol
Benzoyl	142-150/6	61.2	red	132-140/4	81.6	red
Butyryl	108-112/16	42.3	red	89-95/15	70.2	reddish-violet
Isovaleryl	100-110/8	48.4	red	90-103/20	60.0	red
Caproyl	128-131/8	51.0	red	128-130/20	54.0	red
Octanoyl	120-123/2	48.9	red	118-122/5	46.8	red
Decanoyl	132-139/1	58.6	red	147-155/10	39.9	red

C₃H₇ and the condensed product was a viscous oily liquid.

As β -ketonic ester grew higher, the condensation gradually became more difficult to obtain an analytically pure product, especially on a smaller scale, but after being purified as much as possible by distillation, the product was successfully used for the next reaction.

The crystalline acetyl or benzoyl derivative gave purplish coloration with ferric chloride in alcoholic solution. The other compounds with higher alkyl groups than butyryl also gave similar colorations, which, however, might be contaminated, more or less, by the coloration of β -ketonic ester which could not be removed only by distillation (Table IV).

Table IV

Ethyl O-(acylaceto)-aminobenzoates

Acyl group	m.p., °C (solvent) or b.p., °C/mm.	Appearance	Coloration by FeCl ₃ in alcohol	Yield, %	Formula	Analyses, C, H, N, %	
						Calcd.	Found
Benzoyl	126 (alcohol)	colorless fine needles	violet	20.2	C ₁₅ H ₁₇ O ₄ N	C 69.43, H 5.50	C 69.71, H 5.76
Acetyl	65-66 (ligroin)	colorless fine cubes	reddish violet	38.6	C ₁₃ H ₁₅ O ₄ N	N 5.62	N 5.95
Butyryl	162-163/0.5	viscous yellow liquid	violet	86.5	C ₁₅ H ₁₉ O ₄ N	C 64.96, H 6.91 N 5.05	C 65.29, H 6.76 N 5.37
Isovaleryl*	156-161/0.3	" "	reddish violet	41.5	C ₁₅ H ₁₉ O ₄ N	C 64.96, H 6.91	C 63.39, H 6.80
Caproyl	147-150/0.1	" "	brownish violet	35.4	C ₁₇ H ₂₃ O ₄ N		
Octanoyl	168-171/0.25	" "	violet	23.3	C ₁₉ H ₂₇ O ₄ N		
Decanoyl	160-180/0.15	" "	" "	31.7	C ₂₁ H ₃₁ O ₄ N		

*Only this one is the methyl ester.

In the above reactions, the excess of β -ketonic ester was used as a solvent for higher yield. For the purpose of simplifying the procedure, ethyl acetoacetate and anthranilate in equimolar quantities were treated in boiling xylene, but the result was unsatisfactory. To 7.2 g. of ethyl acetoacetate in 25 cc. of boiling xylene was added gradually 8.3 g. of ethyl anthranilate under stirring. Taking advantage of the difference of boiling points, the resultant alcohol was removed from the system as it was produced during the reaction. Only 1.7 cc. of alcohol distilled out as compared with the theoretical amount of 3 cc. After removing the solvent and the unreacted ester, the residue was recrystallized from petroleum ether as colorless platelets, m.p., 64-66°. No depression was observed in admixture with the authentic sample, but the yield was only 1.7 g. (14% of the theory).

The cyclization of ethyl O-(acylaceto)-aminobenzoate: To 2 mol. of powdered sodium in dry xylene was added 1 mol. of the above ester (VIII) and the mixture refluxed for 1.5 hrs. The resultant sodium salt was separated and dissolved in hot water, which was acidified with acetic acid. The resulting precipitate was collected and recrystallized from alcohol. Generally speaking,

3-acyl-2, 4-dihydroxyquinolines obtained by this cyclization method were rather hard to purify, and their melting points were a little lower than those of the corresponding ones obtained by the Friedel-Crafts reaction. Because of the poor yield, only the mixed m.p. test was carried out in the case of 3-octanoyl derivative, and no decanoyl compound was obtained at all (Table V).

Table V
3-Acyl-2,4-dihydroxyquinolines by the Cyclization Method

Acyl group	m.p., °C	Mix. m.p., °C	Coloration by FeCl ₃ in alcohol	Yield, %	Formula	Analyses, C, H, N, %	
						Calcd.	Found
Benzoyl	258	258	orange-red	26	C ₁₆ H ₁₁ O ₃ N	C 72.44, H 4.18, N 5.28	C 72.02, H 4.44 N 5.57
Acetyl	253	254	„ „	30	C ₁₁ H ₉ O ₃ N	C 65.02, H 4.43, N 6.90	C 65.06, H 4.62 N 6.95
Butyryl	216-218	216-218	orange	59	C ₁₃ H ₁₃ O ₃ N	C 67.52, H 5.67	C 67.55, H 5.78
Isovaleryl	193-194	195-196	„	14	C ₁₄ H ₁₅ O ₃ N	C 68.55, H 6.16	C 69.04, H 6.22
Caproyl	181-182	182-184	orange-red	14	C ₁₅ H ₁₇ O ₃ N	C 69.48, H 6.61	C 69.31, H 6.72
Octanoyl	169	168-169	orange		C ₁₇ H ₂₁ O ₃ N	N 4.84	N 5.25

Ethyl *N*-alkyl-*N*-acetyl-anthranilates (XII)—Dimethyl or diethyl sulfate with sodium carbonate was used for the alkylation of anthranilic acid¹²). As the reaction with diethyl sulfate was rather slow at lower temperature, refluxing was necessary from the beginning. One part of the alkylated acid thus obtained was esterified by refluxing with 2 parts of conc. sulfuric acid and absolute alcohol on a steam bath for 4 hrs. (Table VI).

Table VI
N-Alkyl Anthranilic Acids and their Ethyl Esters

Alkyl	Acids			Esters	
	Crystals (solvent)	m.p., °C	Yield, %	b.p., °C/mm.	Yield, %
Methyl	colorless needles (alcohol)	178-179	54.5	153/32	63
Ethyl	colorless needles (dil. alcohol)	150-152	36.4	149-153/17	51.5

The esters (XI) thus obtained were acetylated by the agency of acetic anhydride (Table VII).

Table VII
Ethyl *N*-Alkyl-*N*-acetyl-anthranilates

Alkyl	b.p., °C/mm.	Yield, %	Analyses, C, H, %	
			Calcd.	Found
Methyl	165-167/10	89	C 65.14 H 6.83	C 64.97 H 7.00
Ethyl	170-173/13	98	C 66.36 H 7.28	C 66.07 H 7.39

***N*-Alkyl-4-hydroxyquinolones-(2) (XIII)**—As in the case of 2,4-dihydroxyquinoline, the cyclization of 1 mol. of the above ester was effected by refluxing with 2 mol. of powdered sodium in xylene for 4-5 hrs. The sodium salt was separated after cooling, taken up in hot water, which was acidified with hydrochloric acid after filtering. The resultant precipitate was collected and recrystallized from alcohol. These quinolones were more easily soluble in alcohol and recrystallizable than 2,4-dihydroxyquinoline and, besides, the yields were also better (Table VIII).

12) J. Houben and W. Brassert: Ber., **39**, 3233 (1906).

Table VIII
N-Alkyl-4-hydroxyquinolones-(2)

Alkyl	Formula	m.p., °C	Crystals	Yield, %	Analyses, C, H, N, %	
					Calcd.	Found
Methyl	C ₁₀ H ₉ O ₂ N	255-268	colorless cubes	76	C 68.56 H 5.18 N 8.00	C 68.87 H 5.09 N 7.64
Ethyl	C ₁₁ H ₁₁ O ₂ N	255-264	colorless platelets or cubes	58	C 69.82 H 5.86 N 7.40	C 69.42 H 6.11 N 7.51

Acetate and benzoate of *N*-alkyl-4-hydroxyquinolone-(2) (XIV)—According to the usual method, the acetate was prepared by warming (XIII) with 3-5 parts of acetic anhydride containing a few drops of pyridine, and the benzoate by the treatment with 3 parts of benzoyl chloride in pyridine at 37° overnight, or at 100° for several hrs. (Table IX). The acetate of *N*-methyl-4-hydroxyquinolone-(2) was recrystallized from water to colorless fine needles, m.p., 142-144°. Yield, 40% of the theory. *Anal.* Calcd. for C₁₂H₁₁O₃N: C, 66.35; H, 5.12; N, 6.45. Found: C, 66.40; H, 4.98; N, 6.08. The acetate of *N*-ethyl-4-hydroxyquinolone-(2) had a lower m.p. and was hardly purifiable in a small amount. They were all readily hydrolyzed by dil. caustic alkali solution.

Table IX
 Benzoates of *N*-Alkyl-4-hydroxyquinolones-(2)

Alkyl	Formula	m.p., °C	Crystals (solvent)	Yield, %	Analyses, C, H, N, %	
					Calcd.	Found
Methyl	C ₁₇ H ₁₃ O ₃ N	170-171	colorless prisms (alcohol)	44	C 73.11 H 4.69 N 5.02	C 73.09 H 4.56 N 5.06
Ethyl	C ₁₈ H ₁₅ O ₃ N	149-150	colorless needles or cubes (ether or alcohol)	52	C 73.70 H 5.15 N 4.78	C 73.57 H 5.08

The Friedel-Crafts reaction of *N*-alkyl-4-hydroxyquinolone-(2)—This reaction proceeded exactly as in the case of 2,4-dihydroxyquinoline. *N*-Methyl-3-acetyl-4-hydroxyquinolone-(2), for example, was prepared as follows: To a solution of 2.5 g. (1.5 mol. of equiv.) of aluminum chloride in 15 cc. of nitrobenzene were added 1.4 g. (1 mol. equiv.) of acetyl chloride and 2.0 g. (1 mol. equiv.) of *N*-methyl-4-hydroxyquinolone-(2) and the mixture was warmed on a steam bath for 4 hrs. After decomposing with hydrochloric acid, the solvent was removed with steam, the resulting dark brown oily substance was separated from the warm colorless aqueous solution, and, recrystallized from 50% alcohol with charcoal to colorless fine needles, m.p., 143-145° (mixed m.p. with the monoacetate, m.p., 142-144°, of this quinolone was 10-20° lower). Yield, 1.4 g. (56.5% of the theory, as compared with 40% yield in the same reaction with 1 mol. equiv. of the catalyst).

The melting points of the acyl derivatives grew lower with the elongation of the carbonyl side-chain. Moreover, the isovaleryl derivative of *N*-methyl-4-hydroxyquinolone-(2) was already hardly recrystallizable because of its low m.p., and, therefore, it was purified by distillation under diminished pressure.

Though little difference of m.p. was observed between *N*-methyl-, and *N*-ethyl-4-hydroxyquinolone-(2), the acetyl derivative of the latter melted much lower than that of the former, and, therefore, only the acetyl and benzoyl compounds were synthesized by this reaction.

Both benzoyl derivatives formed light yellow fine needles from alcohol in contrast to the other members (Table X).

Table X
N-Alkyl-3-acyl-4-hydroxyquinolones-(2)

Alkyl group	3-Acyl-group	Formula	m.p., °C or b.p., °C/mm.	Coloration by FeCl ₃ in alcohol	Yield, %	Analyses, C, H, N, %			
						Calcd.		Found	
Methyl	Formyl	C ₁₁ H ₉ O ₃ N	175-177	orange-red		C 65.02	H 4.46 N 6.89	C 64.77	H 4.29 N 7.15
"	Benzoyl	C ₁₇ H ₁₃ O ₃ N	182-184	red	36.5	C 73.11	H 4.69 N 5.02	C 73.38	H 4.83 N 5.01
"	Acetyl	C ₁₂ H ₁₁ O ₃ N	143-145	"	56.5	C 66.35	H 5.10 N 6.45	C 66.25	H 5.28 N 6.33
"	Propionyl	C ₁₃ H ₁₃ O ₃ N	150-151	orange	45.4	C 67.52	H 5.67 N 6.06	C 67.54	H 5.70 N 6.37
"	Butyryl	C ₁₄ H ₁₅ O ₃ N	97-98	orange-red	21.0	C 68.55	H 6.16 N 5.71	C 68.35	H 6.28 N 5.55
"	Isovaleryl	C ₁₅ H ₁₇ O ₃ N	150-158/ 0.035	red	16.0	C 69.48	H 6.61 N 5.40	C 69.28	H 6.74 N 5.40
Ethyl	Formyl	C ₁₂ H ₁₁ O ₃ N	130-132	red		C 66.35	H 5.10 N 6.45	C 66.37	H 5.41 N 6.36
"	Benzoyl	C ₁₈ H ₁₅ O ₃ N	135-136	"		C 73.70	H 5.15 N 4.78	C 73.37	H 5.47 N 4.89
"	Acetyl	C ₁₃ H ₁₃ O ₃ N	115-117	"		C 67.52	H 5.67 N 6.06	C 67.77	H 6.06 N 6.32

The Reimer-Tiemann reaction of N-alkyl-4-hydroxyquinolone-(2)—All the procedure was quite similar to the synthesis of *nor*-dictamnol. To 1 g. of N-methyl-4-hydroxyquinolone-(2) in 85 cc. of 15% sodium hydroxide solution was added gradually 14 g. of chloroform under stirring and refluxing. During the initial violent reaction, there appeared a pink gummy substance, which was separated after refluxing for further 1.5 hrs. and recrystallized from alcohol to colorless fine needles, m.p., 175-177°.

The introduction of an aldehyde group into N-ethyl-4-hydroxyquinolone-(2) by the similar procedure furnished colorless fine needles, m.p., 130-132° (from dil. alcohol) (Table X).

The Fries reaction of monoacetate and monobenzoate of N-alkyl-4-hydroxyquinolone-(2)—The monoacetate of N-methyl-4-hydroxyquinolone-(2) also underwent the Fries rearrangement in the presence of aluminum chloride as catalyst. The resulting acetyl compound was colorless fine needles, m.p., 142-144° (from alcohol), which was proved, by mix. fusion, to be quite identical with that obtained by the Friedel-Crafts reaction. *Anal.* Calcd. for C₁₂H₁₁O₃N: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.12; H, 5.22; N, 6.56.

Similarly, from the benzoates of N-methyl- and N-ethyl-4-hydroxyquinolone-(2) were derived the benzoyl derivatives quite identical with those prepared by the Friedel-Crafts reaction (Table XI).

Table XI
The Benzoyl Derivatives by the Fries Reaction from the Monobenzoates
of N-alkyl-4-hydroxyquinolones-(2)

Alkyl group	m.p., °C	Mix. * m.p., °C	Formula	Analyses, C, H, N, %					
				Calcd.			Found		
Methyl	182-184	182-184	C ₁₇ H ₁₃ O ₃ N	C 73.11	H 4.69	N 5.02	C 73.53	H 4.67	N 5.21
Ethyl	135	134-135	C ₁₈ H ₁₅ O ₃ N	C 73.70	H 5.15	N 4.78	C 73.60	H 5.24	N 4.72

* m.p. in admixture with those obtained by the Friedel-Crafts reaction.

N-Alkyl-3-acetyl-4-hydroxyquinolone-(2) by the cyclization method—The thermal condensation of ethyl N-methylantranilate with ethyl acetoacetate, using the excess of the latter as a

solvent, was unsuccessful, because no foaming was observed during the heating and no product was obtained after removing the unreacted reagents.

In contrast, by the treatment of the two esters in equimolar quantities in boiling xylene there remained a small amount of substance after recovering the unreacted reagents. This residue boiled at 167–172° (4–5 mm.), readily solidified and recrystallized from alcohol to colorless fine needles, m.p., 143–145°. The m.p., mix. m.p., and elementary analyses showed that it was quite identical with the acetyl derivative obtained by the previous two reactions and, in return, these facts established that they were all *N*-methyl-3-acetyl-4-hydroxyquinolones-(2). *Anal.* Calcd. for $C_{12}H_{11}O_3N$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.07; H, 5.13; N, 6.23.

Similarly, from ethyl *N*-ethylanthranilate and ethyl acetoacetate was derived *N*-ethyl-3-acetyl-4-hydroxyquinolone-(2), b.p., 144–154° (3 mm.) and m.p., 116–117° (from alcohol). *Anal.* Calcd. for $C_{13}H_{13}O_3N$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.49; H, 5.85; N, 6.63.

Summary

1) 3-Acyl-2,4-dihydroxyquinolines were synthesized by the Friedel-Crafts reaction of 2,4-dihydroxyquinoline, and similarly, *N*-alkyl-3-acyl-4-hydroxyquinolones-(2) by the same procedure of *N*-alkyl-4-hydroxyquinolone-(2). Some of them were also prepared by the cyclization method to confirm the chemical structures of the products obtained by the Friedel-Crafts reaction.

2) 3-Acetyl and 3-benzoyl derivatives of both 2,4-dihydroxyquinoline and *N*-alkyl-4-hydroxyquinolone-(2) were also derived from their acetates and benzoates by the Fries rearrangement.

3) 3-Formyl derivatives were prepared by the Reimer-Tiemann reaction.

4) The ultraviolet absorption spectra of these synthetic compounds and of the related substances were examined for the identification of their chemical structures.

5) Most of these acylated compounds were found to be less active against *Staph. aureus in vitro* than 3-acyl-4-hydroxycoumarins.

6) *N*-Methyl-3-acyl-4-hydroxyquinolones-(2) were found to have a slight tendency to increase their antimicrobial activities with the elongation of their carbonyl side-chains.

(Received June 14, 1951)

325. Tutou Aihara: On the Principles of *Xanthoxylum piperitum* DC. IV. The Structure of Sanshoamide.

(Pharmaceutical Institute, Medical Faculty, University of Tokyo*)

In the first paper¹⁾ of this study, the late Prof. M. Asano and the author reported a new crystalline substance melting at 110–111° occurring in the fresh unripe fruits of *Xanthoxylum piperitum* DC., and named it sanshoamide. In connection with studies on sanshoöls^{2,3)} some efforts were made thereafter to determine the chemical structure of sanshoamide.

This substance was obtained from the non-volatile fraction of the ethereal extract of the fresh fruits collected in the early summer.

* Bunkyo-ku, Tokyo. (相原 傳)

1) M. Asano and T. Aihara: This Journal, **69**, 79 (1949).

2) T. Aihara: Ibid., **70**, 43 (1950).

3) T. Aihara: Ibid., **70**, 47 (1950).