The mechanism of this biotin increase is not yet clear. Wright, *et al.*[4] stated that the biotin antagonists were capable of liberating biotin from the biotin-avidin complex *in vitro*. We believe that the phenomenon observed in the urine of acidomycin-treated rabbits is due to the biotin liberation from the biological protein-biotin complex by the function of acidomycin administered. Wright's observation seems to be the model case of our phenomenon and we intend to study this phenomenon by using avidin preparation.

We are deeply indebted to Mr. A. Miyake of this laboratory for his generous contribution of acidomycin sodium, to Mr. Y. Hamada for acidomycin assay in urine samples, and to Mr. S. Shintani for his statistical treatments. We also express our grateful thanks to Dr. S. Kuwada, Director of this laboratory, for his permission for this investigation and publication.

Summary

In our synthetic medium, we found that neither aspartic acid nor sodium ethyloxalacetate had any influence on biotin assay values, and acidomycin sodium also had no influence in an amount less than 30 γ per cc. concentration. Biotin level in the urine of rabbits increased remarkably after acidomycin treatment. The present observations proved more positively that the anti-acidomycin factor, recently reported, was biotin.

(Received August 5, 1953)

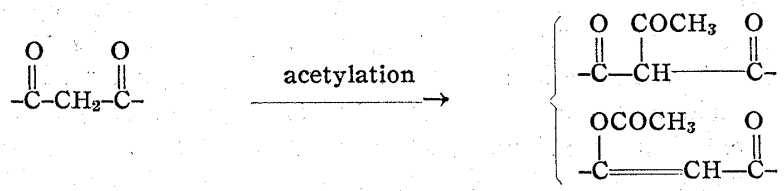
4) L. D. Wright: Arch. Biochem., 12, 27(1947).

---

79. Kiichi Arakawa: Antibacterial Activity of Compounds Possessing a Tricarbonylmethane Group. X[1]. Observations on the Acetylation of 4-Hydroxycoumarin and Synthesis of 3-(α-Aminophenylacetyl)-4-hydroxycoumarins.

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

A series of studies have been carried out by Claisen[2] and Dieckmann[3] on the acetylation of 1,3-dicarbonyl compounds as to the formation of C-acetylated compound by the condensation of the acetyl group with the activated methylene between the two carbonyls or of O-acetylated compounds by the substitution of the acetyl group with one of the enolized carbonyl group.



For example, when ethyl acetoacetate and acetyl chloride are reacted, the use of pyridine or other tertiary amines gives an O-acetylated compound, while the reaction of sodium ethyl acetoacetate and acetyl chloride under the same conditions gives a C-acetyl compound[2]. When the original material is a cyclic diketone, such as hydroresorcinol, acetylation with acetic anhydride alone will give an O-acetyl compound whereas the use of organic bases such as pyridine or tripropylamine will give a C-acetyl compound, but not

* Shirokane-Daimachi, Minato-ku, Tokyo (荒川基一).

1) Part IX: This Bulletin, 1, 255(1953).
2) L. Claisen, H. Haase: Ber., 33, 1242(1900).
3) W. Dieckmann, R. Stein: *Ibid.*, 37, 3370(1904).

with dimethylaniline or quinoline[3]. In the case of cyclic dicarboxylic acids, application of acetic anhydride with pyridine as a catalyst gives a C-acetyl compound[4]. As described above, there is no regularity in the acetylation of these 1,3-carbonyl compounds according to their structures.

Some time ago, Ukita, Nojima, and Matsumoto reported[5] that when pyridine and piperidine are used as solvents in the condensation of 4-hydroxycoumarin and aliphatic acyl chloride, a series of 3-acyl-4-hydroxycoumarins, corresponding to C-acyl compounds, are obtained in good yields. This reaction is assumed to be of the same system as the acetylation of cyclic dicarbonyl compounds described above.

During studies on this series, synthesis of 3-(α-aminophenylacetyl)-4-hydroxycoumarin was attempted as one of the 4-hydroxycoumarins possessing an acyl group with α-amino in the 3-position. Few interesting observations were made during the course of reactions of acid chlorides possessing an aromatic ring with 4-hydroxycoumarins which are described herein.

Benzoyl, cinnamoyl, and phenylacetyl chlorides were respectively reacted with 4-hydroxycoumarin by the method of Ukita and others, using pyridine and piperidine as solvents and boiling in them. However, the objective 3-acyl compounds could not be obtained and the original materials were recovered. In this case, neither 4-acyloxycoumarin, corresponding to O-acyl compounds, could be obtained. The foregoing acid chlorides were then reacted in boiling toluene with metallic sodium but neither the C-acyl nor O-acyl compound was obtained.

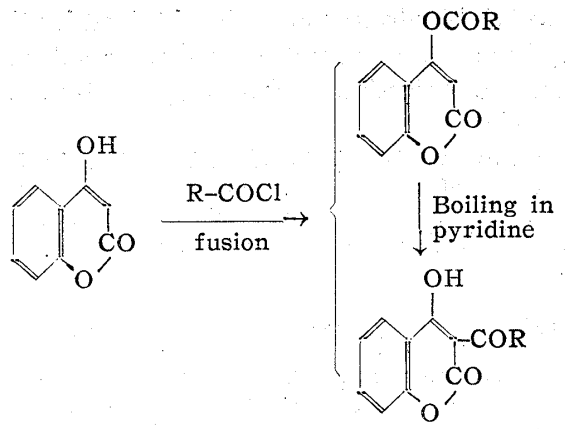
However, when 4-hydroxycoumarin and these acyl chlorides were respectively mixed and directly fused without any solvent, the objective 3-acyl-4-hydroxycoumarins were obtained in a good yield, with 4-acyloxycoumarins as a by-product.

In order to see whether such reactions also occurred with aliphatic acid chlorides, the same reactions were carried out and it was found that 3-acyl-4-hydroxycoumarins were also obtained in a good yield. It was also found that, as shown in the accompanying table, the yield of C-acyl compounds is not so good when the acid chloride molecules are small, irrespective of aliphatic or aromatic group, and a large amount of O-acyl compounds are obtained, but with the increase in the size of acyl molecule, yield of C-acyl compounds increased with attendant decrease in the amount of O-acyl compounds formed.

The O-acetyl compounds obtained in such a manner are known to undergo rearrangement to C-acetyl compounds in the presence of a suitable alkaline catalyst. For example, Claisen and Haase[6] reported that the O-acetyl compound of ethyl acetoacetate easily underwent rearrangement to the C-acetyl compound by alkaline catalyst, such as potassium carbonate or sodium acetate. Such rearrangement is reported to have occurred in the case of hydroresorcinol with sodium acetate. The 4-acyloxycoumarins obtained in the present series of experiments were also found to undergo rearrangement to the C-acyl compounds when heated in pyridine. Such rearrangement does not occur with a good yield when acyl groups are small but with the increase in the size of acyl molecules, the yields were found to become progressively better.

These foregoing experimental results indicated that the acylation of 4-hydroxycoumarin in pyridine-piperidine solvent reported by Ukita, *et al.* (v.s.), resulted in the primary formation of an O-acyl compound which underwent rearrangement as a secondary reaction to produce C-acyl compound[7].

4) W. Dieckmann, F. Breest: *Ibid.*, **37**, 3387(1904).
5) U. Ukita, S. Nojima, M. Matsumoto: J. Am. Chem. Soc., **72**, 5143(1950).
6) L. Claisen, H. Haase: Ber., **33**, 3778(1900).
7) Very recently, K. P. Link and others reported (J. Am. Chem. Soc., **75**, 2044(1953)) the results of their extensive studies on the rearrangement mechanism of the acetyl compounds in the acylation of 3-position of 4-hydroxycoumarin in pyridine-piperidine reported earlier by Ukita, *et al.* (*loc. cit.*)



R=$-C_6H_5$, $-CH_2-C_6H_5$, $-CH_2-CH_2-C_6H_5$, $-CH=CH-C_6H_5$, $-CH_3$, $-(CH_2)_4-CH_3$, $-(CH_2)_6CH_3$, $-(CH_2)_8CH_3$, $-CH_2Cl$.

A special case of this reaction is the condensation of monochloroacetyl chloride with 4-hydroxycoumarin from which only a large amount of O-acyl compound is obtained with a total lack of the C-acyl compound. This O-acyl compound was, moreover, extremely unstable, underwent deacetylation automatically when left in the air to 4-hydroxycoumarin, and failed to undergo rearrangement to the C-acyl compound in pyridine, producing only 4-hydroxycoumarin. Condensation with amino acid chloride was totally unsuccessful under these conditions. The results of these reactions are summarized in the accompanying table.

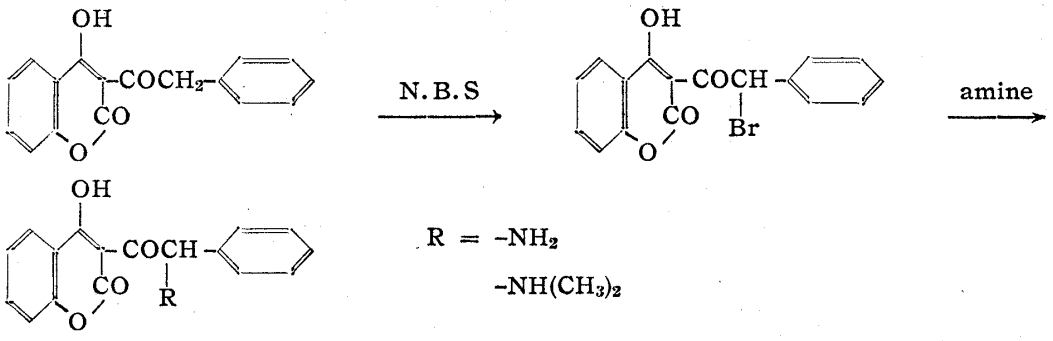
Coumarin	Formula	m.p. (°C)	Yield (%)	C % Calcd.	C % Found	H % Cacld.	H % Found	O→C (%)
3-Benzoyl-4-hydroxy-	$C_{16}H_{10}O_4$	146	10	72.18	(a)	3.76	(a)	1
4-Benzoyloxy-		126	20		72.46		3.92	
3-Phenylacetyl-4-hydroxy-c)	$C_{17}H_{12}O_4$	120	28	72.86	72.84	4.28	4.43	28
4-Phenylacetyloxy-		136	17		73.00		4.31	
3-Phenylpropionyl-4-hydroxy-c)	$C_{18}H_{14}O_4$	117	33	73.40	73.26	4.76	4.58	50
4-Phenylpropionyloxy-		207	7		73.70		4.36	
3-Cinnamoyl-4-hydroxy-	$C_{18}H_{12}O_4$	212	50	—	(a)	—	(a)	—
4-Cinnamoyloxy-		—	—		—		—	
3-Acetyl-4-hydroxy-	$C_{11}H_8O_4$	134	4	64.70	(b)	3.92	(b)	0
4-Acetyloxy-		107	60		64.81		4.19	
3-Caproyl-4-hydroxy-c)	$C_{15}H_{16}O_4$	114	48	69.23	69.41	6.15	6.33	90
4-Caproyloxy-		71	15		69.38		6.26	
3-Octanoyl-4-hydroxy-	$C_{17}H_{20}O_4$	105	72	—	(b)	—	(b)	—
4-Octanoyloxy-		—	—		—		—	
3-Decanoyl-4-hydroxy-	$C_{19}H_{24}O_4$	108	72	—	—	—	—	—
4-Decanoyloxy-		—	—		—		—	
3-Chloroacetyl-4-hydroxy-	$C_{11}H_7O_4Cl$	—	—	55.23	—	2.98	—	0
4-Chloroacetyloxy-d)		136	61		55.36		3.18	

a) No depression of the melting point occurred on admixture with a sample obtained by the cyclization method (Anschutz: Ann., 367, 169(1909)).

b) No depression of the melting point occurred on admixture with the sample obtained by the pyridine-piperidine method of Ukita, *et al.* (cf. Footnote (5)).

c) Coloration with ferric chloride in alcohol:
3-Phenylacetyl-4-hydroxy compound: Yellowish orange
3-Phenylpropionyl-4-hydroxy compound: Yellowish orange
3-Caproyl-4-hydroxy compound: Orange

d) Analytical value of chlorine in 3-chloroacetyloxy compound:
Calcd.: Cl, 15.07. Found: Cl, 15.04.



N-Bromosuccinimide was applied to 3-phenylacetyl-4-hydroxycoumarin obtained by the foregoing experiment and 3-(α-bromophenylacetyl)-4-hydroxycoumarin thereby formed was treated with ammonia or dimethylamine to obtain the objective 3-(α-aminophenylacetyl)- and 3-(α-dimethylaminophenylacetyl)-4-hydroxycoumarin.

The author takes this opportunity to express his gratitude to Prof. Akiya and Prof. Ukita for their unfailing guidance and kind advices during the course of this study, and to Misses Ohta, Kondo, and Ohki, and Mr. Kurihara for carrying out the analyses. A part of the expenses for the present study was defrayed by the Scientific Research Fund granted by the Ministry of Education.

Experimental

General Method of Acylation—One part of 4-hydroxycoumarin and 2.5 parts of acyl chloride are mixed and heated for 2 hours at 140~160°. The cooled and solidified mass is scraped out into ice water and extracted with ether. The ethereal solution is washed with 5% sodium bicarbonate solution, then extracted with 5% sodium carbonate, and the aqueous alkali solution is rendered acid with hydrochloric acid. The recrystallization of the precipitate thereby obtained from alcohol gives the C-acyl compound. The substance which is not extracted with ether is washed with 5% sodium carbonate solution and recrystallized from benzene to the O-acyl compound.

Rearrangement from O-Acyl to C-Acyl compound—The O-acyl compound is dissolved in about 10 volumes of pyridine, refluxed for two hours, and the mixture is poured into cold diluted hydrochloric acid. The precipitate is extracted with ether, the ethereal layer washed with 5% sodium bicarbonate solution, and extracted with 5% sodium carbonate solution from which the C-acyl compound is obtained.

3-(α-Dimethylaminophenylacetyl)-4-hydroxycoumarin—To a solution of 0.56 g. of 3-phenylacetyl-4-hydroxycoumarin dissolved in 20 cc. of dehydrated carbon tetrachloride, 0.36 g. of N-bromosuccinimide was added and the mixture was refluxed for 1.5 hours. Upon cooling, the separated succinimide was removed by filtration, the filtrate concentrated under a reduced pressure to about 5 cc., and cooled by which white brei separated out. This was collected by filtration, pressed and dried on a porcelain plate, and dissolved in 20 cc. of dehydrated benzene. While cooling this solution in water, dry dimethylamine gas was saturated, and the yellowish white brei that separated out was collected by filtration. Recrystallization from alcohol-benzene mixture yielded white sandy crystals of m.p. 192~195°(decomp.). The alcoholic solution of this substance colored reddish brown with ferric chloride. *Anal.* Calcd. for $C_{19}H_{17}O_4N \cdot H_2O$: C, 66.89; H, 5.57; N, 4.10. Found: C, 66.81; H, 5.40; N, 4.22.

3-(α-Aminophenylacetyl)-4-hydroxycoumarin—The same procedures as above carried out with ammonia gas in place of dimethylamine yielded 3-(α-aminophenylacetyl)-4-hydroxycoumarin as white sandy crystals of m.p. 190~192°(decomp.). The alcoholic solution of this substance colored yellowish red with ferric chloride. *Anal.* Calcd. for $C_{17}H_{13}O_4N$: C, 69.15; H, 4.40; N, 4.74. Found: C, 68.84; H, 4.45; N, 4.30.

Summary

By fusing 4-hydroxycoumarin with various acyl chlorides, 3-acyl-4-hydroxycoumarins (C-acyl compounds) were obtained together with 4-acyloxycoumarins (O-acyl compounds). There was found some regularity in the formation of C-acyl and O-acyl compounds by the size of acyl molecules under the same reaction conditions. O-Acyl compounds were found to undergo rearrangement easily when boiled in pyridine to C-acyl compounds and the yield of rearranged products was found to be regulated by the size of the acyl molecule. By the use of 3-phenylacetyl-4-hydroxycoumarin obtained by such a method, 3-(α-aminophenylacetyl)-4-hydroxycoumarins were prepared.

(Received August 15, 1953)