Sincere gratitude is expessed to Prof. Imaizumi of the School of Medicine, and Prof. Hano of the School of Pharmacy, Osaka University for warmful encouragements, and also to Ono Pharmaceutical Co., Ltd; Kyowa Hakko Kogyo Co., Ltd. for syntheses of samples.

Summary

Following Walker's extracting method of kidney transamidinase, brain transamidinase the whole brain of the rabbit.

Then enzymic reaction was carried out, and reaction mixture was investigated with paper chromatography as a sole experimental technique, and it was found out that the following reaction might be possible.

1. 4-guanidinobutyric acid+glycine $\rightleftharpoons$ 4-aminobutyric acid+guanidinoacetate
2. 3-hydroxy-4-guanidinobutyric acid+glycine $\rightleftharpoons$ 3-hydroxy-4-aminobutyric acid +guaninoacetate
3. arginine+glycine $\rightleftharpoons$ ornithine+guanidinoacetate

(Received December 10, 1961)

UDC 547.812.5 : 547.292 : 615.778.4

173. Sadao Iguchi and Atsuko Inoue : Studies on Pyrone Derivatives. VII. On the Syntheses of Dehydroacetic Acid Analogue having Aroyl Group in its Side Chain.

(*Institute of Pharmaceutical Sciences, Faculty of Medicine, Kyushu University*[*1])

Numerous reports were already published as to the relationship between the chemical structures and antibacterial properties[1~3] of the dehydroacetic acid (DHA) type compounds. Since in the previous studies, our main interest was in synthesizing a compound possessing the acyl group in the 3-position of DHA nucleus [triacetic acid lactone (TAL)], the derivatives having the aroyl instead of the acyl group in the 3-position have never been synthesized. In order to investigate the activity which the carbonyl group in their side chain displays for of various primary amines and their antibacterial activity, some new DHA type derivatives possessing the aroyl group were attempted to synthesize, and the details of these synthesis and their reaction processes are described below.

It was already attempted[3] to synthesize 3-benzoyl derivative of DHA by Friedel-Crafts reaction of TAL (I) and benzoyl chloride in the presence of a suitable condensing agent, for example, a few drops of conc. sulfuric acid, pyridine-piperidine or an equivalent mole of aluminum chloride, but without success.

In the cases when pyridine-piperidine or an equivalent mole of aluminum chloride was used as the condensing agent, a monobenzoate (IIIa), m.p. 91°, was obtained, while when conc. sulfuric acid, a dibenzoate, m.p. 242~243°, was formed.

Treatment of (IIIa) with an excess of aluminum chloride led to the productien of a new compound, m.p. 109~111°. This substance was found to be a 3-benzoyl derivative of DHA (IIa) prepared by Fries rearrangement. It was also proved that the 3-benzoyl derivative (IIa) could be directly prepared from (I) using an equivalent mole of benzoyl chloride in the presence of an excess of aluminum chloride in nitrobenzene.

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*1 Katakasu, Fukuoka (井口定男, 井上敦子).

1) M. Namiki, *et al.* : Nippon Nōgei-Kagaku Kaishi, **26**, 178 (1952).
2) K. Tamari, *et al.* : *Ibid.*, **29**, 190 (1955).
3) T. Miyagi, *et al.* : Yakugaku Zasshi, **75**, 43 (1955).

Since when an excess of aluminum chloride was not used, the above reaction did not occur, it seems reasonable to assume that the amount of aluminum chloride used is the most important factor in this reaction.

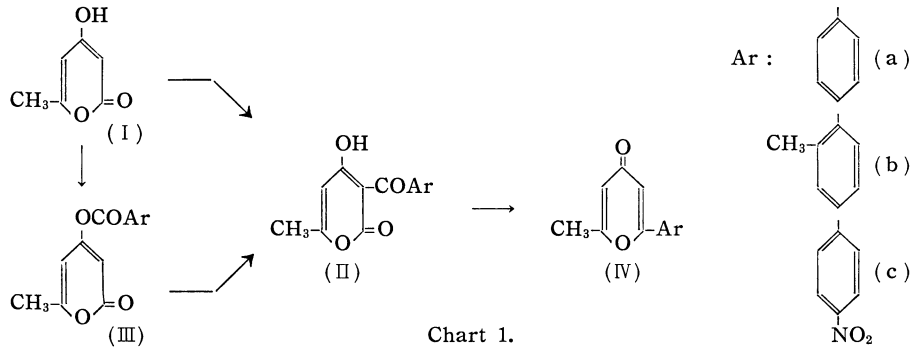


Chart 1.

In a similar manner *o*-methylbenzoyl and *p*-nitrobenzoyl derivatives (IIb and IIc) were prepared not only by Fries rearrangement but also by Friedel-Crafts reaction.

The validity of the structure assignments of all these compounds was proved by their infrared and ultraviolet absorption spectral as well as elemental analytical data (Fig. 1, 2, and 3).

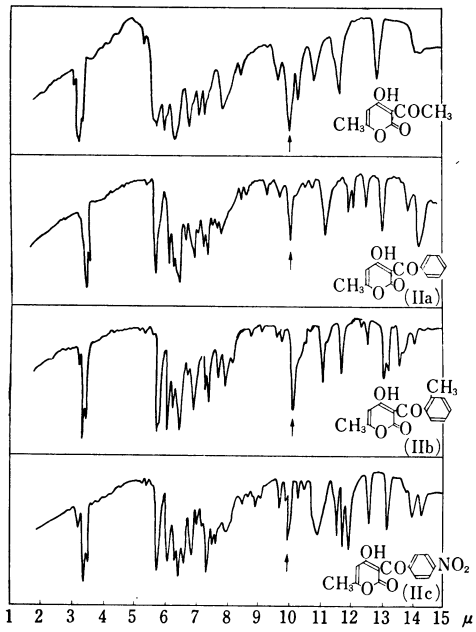


Fig. 1. Infrared Spectra (Nujol)

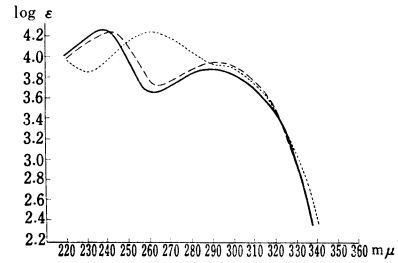
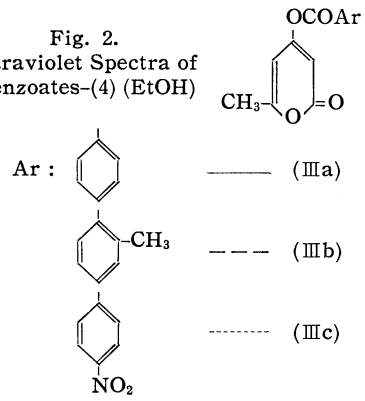


Fig. 2.
Ultraviolet Spectra of
Benzoates-(4) (EtOH)



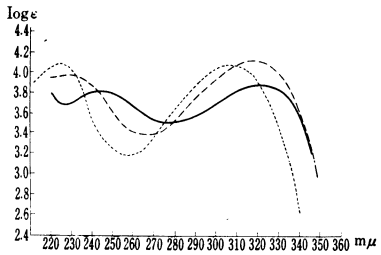
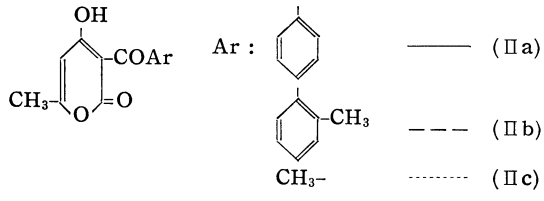


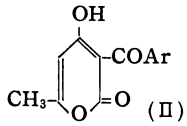
Fig. 3. Ultraviolet Spectra of Dehydroaroylacetic Acid (EtOH)



Furthermore, that these compounds display the following characteristic chemical reactions may serve as an additional evidence for their assigned structures : i) transformation to γ-pyrone type compound (IV), ii) formation of anilide.

The experimental conditions and the analytical data of the compounds are summarized in Tables I and II.

TABLE I.



(II)

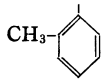
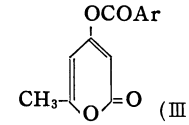
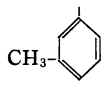
Ar.	Reaction	Amount of acyl chloride (mol.)	Amount of $AlCl_3$ (mol.)	Time (hr.)	Yield (%)	Anilide m.p. (°C)	Color reaction with $FeCl_3$
 (IIa)	Fries	—	2	4	40	177~178	reddish orange
	Friedel-Crafts	1	2	4	43		
 (IIb)	Fries	—	2	5.5	40	97~ 98	reddish orange
	Friedel-Crafts	1	2	5.5	31		
	Fries	—	4	8	38	180~181	orange
	Friedel-Crafts	1	4	8	20		
NO_2 (IIc)		1	2	4	×	/	/

TABLE II.



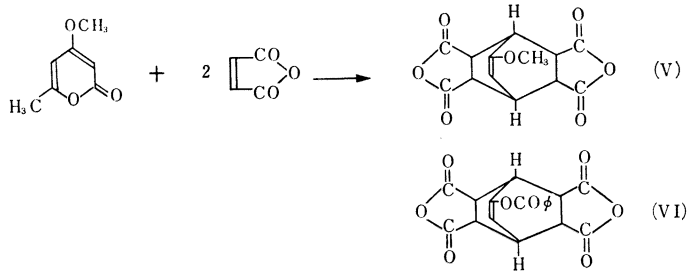
(III)

Ar.	M.p. (°C)	Molecular formula	C (%)		H (%)		N (%)	
			Calcd.	Found	Calcd.	Found	Calcd.	Found
 (IIIc)	90~ 91	$C_{13}H_{10}O_4$	67.82	68.01	4.35	4.48	/	/
 (IIIb)	75~ 77	$C_{14}H_{12}O_4$	68.85	68.74	4.91	4.88	/	/
 NO_2 (IIIc)	175~177	$C_{13}H_9NO_6$	56.72	56.78	3.27	3.35	5.09	4.95

On the other hand, the problem on the structure of (IIa) still remains undetermined, though it was proposed to be the 4- or 2-benzoate in the previous papers.[3] However, from the fact that this compound did not form a picrate nor a hydrochloride in our experiment, it was considered likely to be the 4-benzoate. Moreover, upon treatment of (IIa) with maleic anhydride, the new compound (VI), m.p. 242~243° of the same type as the compound (V) prepared by Bu'Lock *et al.*,[4] was obtained. This showed definitely that the compound (II) was not the 2-benzoate but the 4-benzoate.

The presence of the 3,4-dibenzoate already reported in the previous papers[3] was found to be doubtful. Because the literatures[5~8] pointed out that when acyl group

4) J.D. Bu'Lock, *et al.* : J. Chem. Soc., **1960**, 502.
5) G.G. Badcock, *et al.* : J. Chem. Soc., **1960**, 903.
6) S. Iguchi : Yakugaku Zasshi, **72**, 122 (1952).
7) C.E. Spencer, *et al.* : J. Am. Chem. Soc., **80**, 140 (1958).
8) K. Okumura : Yakugaku Zasshi, **80**, 525 (1960).



was introduced into the 3- or 4-position of the compound of this type, the resulted product could not undergo further acylation at the 4- or 3-position.

In fact, we tried many times to get the "dibenzoate," (m.p. 242~243°), by treating TAL (I) or the monobenzoate (IIIa) with an excess of benzoyl chloride without success.

Finally, the antibacterial activities of these newly synthesized compounds against Bacillus subtilis, Staphylococcus aureus, Escherichia coli B and Aspergillus niger were examined, but none of them was found to be effective as DHA against these bacteria and mold.

Experimental

Synthesis of Dehydroaroylacetic Acids

A. 3-Aroylation of Triacetic Acid Lactone by Friedel-Crafts Reaction : (i) Dehydrobenzoylacetic Acid (IIa)——To a solution of nitrobenzene (75 ml.) containing $AlCl_3$ (6.3 g., 0.047 mol.) were added BzCl (3.5 g., 0.025 mole) and TAL (3.3 g., 0.026 mole), and the mixture was warmed on a steam-bath for 4 hr. After decomposing the excess of $AlCl_3$ with HCl, the solvent was removed by steam distillation. Crude colorless crystals separated out when the remaining aqueous solution was cooled with ice-water. These crystals were dissolved in Et_2O, and extracted with 5% Na_2CO_3 solution. The alkaline solution was acidified with conc. HCl, and extracted with Et_2O. The extract was dried and Et_2O was removed to leave a residue, which was recrystallized from MeOH to afford (IIa) as colorless prisms, m.p. 109~111°. Yield, 2.6 g. (43%). *Anal.* Calcd. for $C_{13}H_{10}O_4$: C, 67.8; H, 4.3. Found : C, 67.81; H, 4.30.

(ii) Dehydro-*o*-methylbenzoylacetic Acid (IIb)——(IIb) was also obtained by a similar procedure. Colorless brilliant prisms, m.p. 97~98°, from MeOH. Yield, 40%. *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.08; H, 4.9. Found : C, 68.74; H, 5.03.

(iii) Dehydro-*p*-nitrobenzoylacetic Acid (IIc)——This compound was analogously obtained by the above described procedure, but in this case it was necessary to use 4 equivalent moles of $AlCl_3$. Lemon yellow needles, m.p. 245~248°, from AcOEt. Yield, 20%. *Anal.* Calcd. for $C_{13}H_9NO_6$: C, 56.7; H, 3.3; N, 5.1. Found : C, 56.72; H, 3.40; N, 5.14.

B. Fries Rearrangement of the Monobenzoates (III) : (i) Synthesis of Monobenzoate-(4) (IIIa)——This compound was already prepared by Miyagi *et al.*,[3] by the use of pyridine-piperidine as a condensing agent, but it was found that the following method afforded better yield. A mixture of sodium metal (1 g., 0.044 mole) in abs. EtOH (10 ml.) and TAL (I) (5.5 g., 0.044 mole) was stirred for 30 min. at room temperature and then BzCl (6.1 g., 0.043 mole) was added gradually under cooling and stirring for 2 hr. After removing the EtOH under reduced pressure, water was added to this mixture to decompose the excess of BzCl. Then Et_2O was added and the acidic substance was extracted by 5% Na_2CO_3 solution. The remaining ethereal solution was dried and then evaporated. The residue was crystallized from EtOH to afford colorless brilliant scaly crystals, m.p. 90~91°. Yield, 7.2 g. (72%). *Anal.* Calcd. for $C_{13}H_{10}O_4$: C, 67.8; H, 4.3. Found : C, 68.01; H, 4.48.

(ii) *o*-Methyl-benzoate-(4) (IIIb)——This compound was obtained by the same procedure as described above. White needles, m.p. 74~75°. Yield, 93.4%. *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.8; H, 4.9. Found : C, 68.74; H, 4.88.

(iii) *p*-Nitro-benzoate-(4) (IIIc)——This compound was obtained by a different method. When pyridine-piperidine was used as the condensing agent, (IIIc) was obtained successfully. Lemon yellow needles, m.p. 177~178°, Yield, 46%. *Anal.* Calcd. for $C_{13}H_9NO_6$: C, 56.7; H, 3.3; N, 5.1. Found : C, 56.78; H, 3.35; N, 4.95.

(iv) Rearrangement of (III) to (II)——The monobenzoate-(4) (III) (1 mole) and 2 equivalent moles of $AlCl_3$ in nitrobenzene were warmed on a steam bath for 4 hr., and then the reaction mixture was

treated exactly in the same way as in the case of Friedel-Crafts reaction. The average yield of (II) from (III) by the rearrangement was ca. 40%.

Diels-Alder Reaction of Monobenzoate-(4) (IIIa) to (VI)——Maleic anhydride (2.0 g., 0.02 mole) and monobenzoate-(4) (2.2 g., 0.01 mole) were heated under reflux in toluene (20 ml.) for 13 hr. The deposited adduct (8-benzoyloxy-1-methyl-1,2-ethenocyclohexane-2,3,5,6-dicarboxylic dianhydride) (VI) was filtered off, and recrystallized from EtOH-AcOEt as white powder, m.p. 256~257°(decomp.). *Anal.* Calcd. for $C_{20}H_{14}O_8$: C, 62.8; H, 3.7. Found : C, 62.69; H, 3.72.

The Rearrangement of Dehydroaroylacetic Acid (II) to γ-Pyrone (IV)——(II) (1 g.) was refluxed with conc. HCl (100 ml.) for 15 hr. and then kept overnight at room temperature. On concentrating the reaction mixture to 10 ml. under reduced pressure, colorless crystals separated out. These crude crystals were washed with ice-water and dissolved in hot benzene. The benzene solution was dried and evaporated. After recrystallization of the residue from hexane, γ-pyrone was obtained. γ-Pyrone (IVa) thus obtained was the same compound as already reported by S. Ruhemann[9] as 2-methyl-6-phenyl-γ-pyrone. The structures of the other γ-pyrones synthesized this time were deduced by their spectral (Fig. 4 and 6) as well as analytical data (Table III).

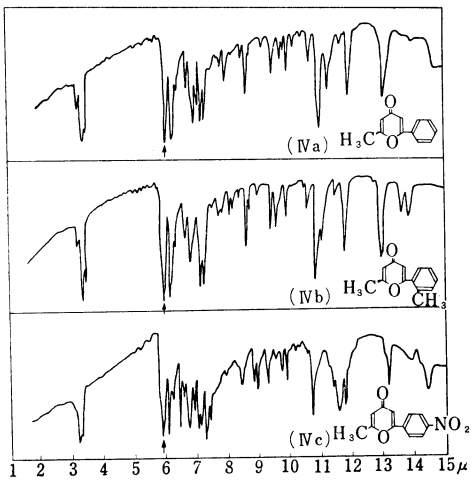


Fig. 4. Infrared Spectra (Nujol)

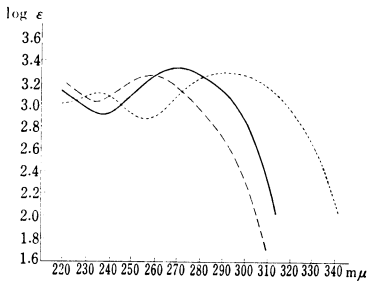
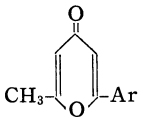


Fig. 5. Ultraviolet Spectra of γ-Pyrones (90% EtOH)



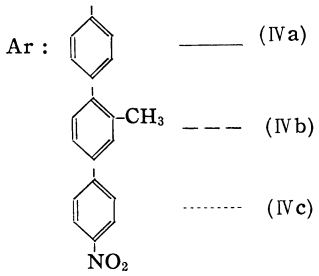
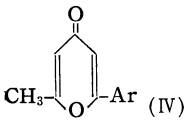
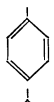
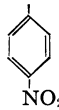


TABLE III.



Ar.	M.p. (°C)	Molecular formula	C (%) Calcd.	C (%) Found	H (%) Calcd.	H (%) Found	N (%) Calcd.	N (%) Found
 (IVa)	85~ 87*	$C_{12}H_{10}O_2$	77.42	77.50	5.38	5.56	—	—
CH_3-  (IVb)	93~ 95	$C_{13}H_{12}O_2$	78.00	77.95	6.00	6.19	—	—
 NO_2 (IVc)	227~228	$C_{12}H_9NO_4$	62.33	62.48	3.90	3.98	6.06	5.89

* monohydrate m.p. 50~53°

9) S. Ruhemann : J. Chem. Soc., **93**, 431 (1908).

Antibacterial and Antifungal Test——The antibacterial test was made by the dilution method after 24 hr. and 48 hr. The bacteria used were Bacillus subtilis, Staphyrococcus aureus and Escherichia coli B. These were incubated at 37° in the Biuillon medium by the usual method.

In the case of Aspergillus niger, the Biuillon medium containing glucose (4%) was used (pH 7.0) and after 72 hr. (at 37°) the results were observed.

The authors are grateful to Prof. H. Matsumura of Kyushu University for his encouragement throughout this work. Thanks are also due to Prof. T. Toda and Dr. K. Hisatsune, Department of Bacteriology of this University, for the antibacterial and antifungal tests. They are also indebted to Mrs. S. Matsuba, Mr. M. Shirōzu and Miss S. Indō for the microanalyses and to Messrs. H. Yano, H. Matsui and K. Hikita for infrared and ultraviolet spectral measurements.

This work was supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education, to which they are also grateful.

Summary

1) Dehydroaroylacetic acids were directly obtained by Friedel-Crafts reaction of triacetic acid lactone (TAL) and aroyl chloride in the presence of an excess of aluminum chloride. They were also derived from the corresponding 4-benzoates of TAL by Fries rearrangement using the excessive aluminum chloride.

2) The monobenzoate of TAL synthesized by the usual method as also reported in the previous reports was definitely shown to be the 4-benzoate rather than the 2-benzoate.

3) None of these new compounds was superior to DHA in the activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli B and Aspergillus niger *in vitro*.

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UDC 612.398.145 : 547.964

174. Morio Ikehara, Akihiro Yamazaki, and Toshiko Fujieda : Studies on Coenzyme Analogs. XIII.[*1] Oxidation of Methylmercaptopurine and its Nucleoside by means of N-Chloro- and N-bromosuccinimide.

(*Faculty of Pharmaceutical Sciences, School of Medicine, Hokkaido University*[*2])

Recently several investigators reported the reaction of nucleophilic reagent with thiolated purine riboside.[1,2] In these instances, methylmercapto group situated on the 6-position of purine nucleus reacted readily with nucleophiles, such as mono-and di-alkylamine or ammonia. However the latter reagent failed to react with 6-methylmercapto group when the same nucleus was substituted with additional electron-releasing group on position 2, even in the drastic conditions.[3]

In efforts to increase the susceptibility of this type of methylmercapto group against

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*1 Part XII. T. Ueda, *et al.* : This Bulletin, **10**, 788 (1962).

*2 Kita 12-jo, Nishi 5-chome, Sapporo (池原森男, 山崎晤弘, 藤枝聰子).

1) J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr : J. Am. Chem. Soc., **80**, 1669 (1958).
2) M. Ikehara, T. Ueda, S. Horikawa, A. Yamazaki : This Bulletin, **10**, 665 (1962).
3) M. Ikehara, A. Yamazaki, T. Fujieda : Unpublished results.