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Color Reaction Product of Urea with Diacetyl Monoxime and Glucuronolactone. I. On the Reaction of Urea Derivatives with Diacetyl Monoxime and Glucuronolactone[1]

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The chemical structures of the color reaction products (Ib and Ic) obtained from butyl- and *p*-tolyl-urea with diacetyl monoxime and glucuronolactone in phosphoric acid were elucidated by their physical properties. Ib develops immediately a red color in phosphoric acid and is thought to be one of the main reaction product or one of the main reaction intermediate responsible for the color reaction.

Ib gave two kinds of diacetate, and their structures were also established.

Diacetyl monoxime gives an intense orange color with urea when heated in the presence of glucuronolactone in phosphoric acid, and this coloration has been proved to be very suitable for the determination of urea in biological fluids.[3] This paper deals with the chemical structures of crystalline reaction products obtained from urea derivatives, diacetyl monoxime, and glucuronolactone.

Isolation of Crystalline Reaction Products

In this study butyl- and *p*-tolylurea were used, because the isolation of crystalline reaction product of urea was found to be considerably difficult.

Butylurea was heated with each equimolar amount of diacetyl monoxime and glucuronolactone in a moderately concentrated aqueous phosphoric acid for 50 minutes. This reaction condition was almost identical with that of the proposed practical method of urea determination. The reaction mixture was extracted with butyl alcohol, and the extract was separated chromatographically on alumina to give faint yellow needles (Ib) in rather good yield.

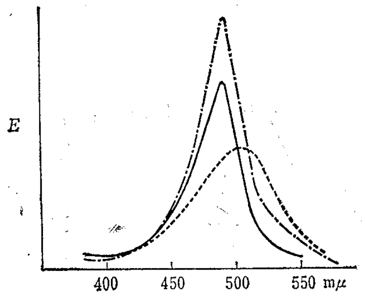

Fig. 1. Absorption Curves in Reaction Medium

——— reaction mixture, λ_{max} 489 mμ

··········· Ib afrer 2 days of dissolution, λ_{max} 507 mμ

– – – – Ib after 2 days of dissolution, λ_{max} 492 mμ

This crystal develops a red color immediately when dissolved in an acidic medium whose composition is as same as that of the reaction medium. The shape and absorption maximum of the absorption curve becomes almost identical with those of the reaction mixture when standing for a rather long period at room temperature (Fig.1). Therefore, it may be one of the main reaction product or one of the main reaction intermediate responsible for the color reaction.

p-Tolylurea gave faint yellow needles (Ic) by the similar treatments. Ic also develops a red color in the same medium.

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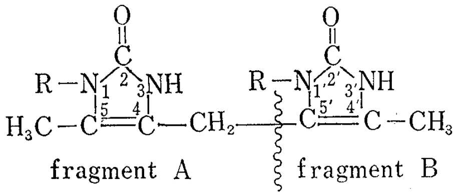
1) This paper forms Part LXIX of "Organic Analysis," by T. Momose. Preceding paper, Part LXVIII: T. Momose, Y. Ohkura, and J. Shiota, *Chem. Pharm. Bull.* (Tokyo), **16**, 2370 (1968).

2) Location: *Katakasu, Fukuoka.*

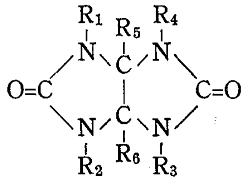
3) T. Momose, Y. Ohkura, and J. Tomita, *Clin. Chem.*, **11**, 113 (1965).

Structure of Ib and Ic

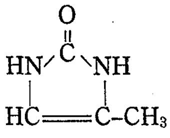
The molecular formulae of Ib and Ic were established from micro analyses and mass spectral data as $C_{17}H_{28}O_2N_4$ and $C_{23}H_{24}O_2N_4$, respectively. These results indicated that the basic skeleton of the molecules Ib and Ic should have a molecular formula of $C_9H_{12}O_2N_4$ (Ia).



Ia : R=H
Ib : R=C_4H_9
Ic : R=p-$CH_3C_6H_4$



IIa : R_1—R_6=H
IIb : R_1—R_4=H, R_5=R_6=CH_3
IIc : R_2=R_3=H or R_2=R_4=H,
R_1=R_4=R_5=R_6=CH_3 or
R_1=R_3=R_5=R_6=CH_3



III

In the literatures, tetrahydroimidazo [4,5-*d*] imidazole-2,5-dione (IIa) and its methyl derivatives (IIb and c) were reported to be obtained in acidic media from glyoxal and urea,[4] from diacetyl monoxime and urea,[5] and from diacetyl and methyl urea,[6] respectively. However, these structures could not be applied to the products obtained here. Our results clearly showed that Ib should be formed from each two moles of diacetyl monoxime and butylurea, and moreover, one carbon atom should be removed at the time of condensation. In this respect, the elucidation of the structures of Ib and Ic was thought to be significant not only in analytical aspect, but also in chemical reaction.

The infrared (IR) spectrum of Ib showed the presence of NH and CO groups by absorption bands at 3160, and 1695, 1675, 1665 cm^{-1}, respectively. Ic also showed similar IR spectral characteristics at 3150, 1688, and 1677 cm^{-1}. These absorption bands were assumed to be associated with the imidazolone moiety of the molecule, because some imidazolone derivatives have been reported to show NH and CO absorption bands at 3135—3165 and 1684—1677 cm^{-1} [7] and, in fact, 4-methyl-2-imidazolone (III) showed these bands at 3180, 1689 and 1678 cm^{-1}.

Table I.[a] NMR Data of Ib, Ic, and III

	Ib		Ic	III
	in $CDCl_3$	in CF_3COOH	in CF_3COOH	in CF_3COOH
C–CH_3	0.89 (*m*) 0.95 (*m*) } 6H	1.03 (*m*) 1.12 (*m*) } 6H		
C–CH_2	1.40 (8H, *m*)	1.58 (8H, *m*)		
=C–CH_3	1.98 (*s*[b]) 2.04 (*s*[b]) } 6H	2.35 (6H, *d*)	1.63 (6H, *s*)	2.23 (3H, *d*, $J=1.7$ cps)
Arom. CH_3			2.48 (6H, *s*)	
=C–CH_2–C= N–CH_2	3.42 (*m*) 3.50 (*m*) } 6H	3.93 (*m*) 3.80 (*m*) } 6H	3.60 (2H, *m*)	
=C–H				6.45 (1H, *m*)
Arom. H			7.40 (8H, *m*)	
NH	9.79 (1H, *m*) 10.03 (1H, *m*)	10.5 (2H, *m*)	9.77 (2H, *m*)	10.02 (2H, *m*)

a) Following abbreviations are used; *s*: singlet, *d*: doublet, *m*: multiplet. Chemical shifts are given in δ (ppm).
b) These signals were practical singlet.

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4) J. Nematollahi and R. Ketcham, *J. Org. Chem.*, **28**, 2378 (1963).
5) H. Biltz, *Ber.*, **41**, 1882 (1908).
6) H. Biltz, *Ber.*, **40**, 4811 (1907).

The presence of double bonds in Ib could be detected by a tetranitromethane test, but no conjugated unsaturation was suggested by the ultraviolet (UV) spectrum of Ib, which was transparent in longer wavelength region than 220 mμ. III also has no absorption band in this region.

The nuclear magnetic resonance (NMR) spectral data of Ib, Ic and III were tabulated in Table I. The results indicated that Ib had two butyl side chains, two vinylic methyl groups, one deshielded methylene group, which is probably attributable to $=C-CH_2-C=$, and two NH groups. If two butyl side chains were located at 1 and 3′ or 3 and 1′ of I, chemical shifts of two NH protons would be same, and so would be those of methyl protons in two butyl side chains and those of methyl protons in two vinylic methyl groups.

Now, the evidences so far obtained together with the similarities of spectral characteristics and coloration[8] between Ib and III suggested that the chemical structure of Ib might be 1,1′-dibutyl-5,4′-dimethyl-2,2′-dioxo-4,5′-diimidazolylmethane. The similar consideration led the chemical structure of Ic as shown before.

The following mass spectral data of Ib and Ic strongly supported these structures.

Ib: *m/e*; 321 (M^+ +1), 320(M^+), 305 ($M^+ - CH_3$), 263 ($M^+ - C_4H_9$), 206 ($M^+ - C_4H_9NCONH$ or $M^+ - 2 \times C_4H_9$), 168 (fragment A+1), 167 (fragment A), 154 (fragment B+1), 153 (fragment B).

c: *m/e*; 389 (M^+ +1), 388 (M^+), 373 ($M^+ - CH_3$), 296 ($M^+ - C_7H_7$), 240 ($M^+ - C_7H_7NCONH$), 201 (fragment A+1), 200 (fragment A), 189 (fragment B+1), 188 (fragment B).

Ib gave two diacetates (IV and V). The spectral characteristics of them were tabulated in Table II. The UV spectra of both acetates showed that the conjugated unsaturation were formed by the acetylation. The IR spectum of IV showed only one absorption maximum associated with carbonyl group, therefore, together with the result of UV spectrum the ex-

Table II.[a] Spectral Characteristics of Ⅳ and V

		Ⅳ	V
UV (λ_{max}^{EtOH} mμ)		272	234, 268
IR (ν_{max}^{KBr} cm^{-1})		1665 (C=C)	1652 (C=C)
		1715 (C=O)	1680 (C=O) 1720 (C=O) 1754 (C=O)
NMR (δ ppm)		(in CCl_4)	(in $CDCl_3$)
	$C-CH_3$	0.98 (3H, *m*) 1.06 (3H, *m*)	0.98 (3H, *m*) 1.08 (3H, *m*) 1.19 (3H, *d*, *J*=6.4 cps)
	$C-CH_2$	1.45 (8H, *m*)	1.53 (8H, *m*)
	$=C-CH_3$	1.93 (3H, *s*[b]) 2.15 (3H, *s*[b])	1.97 (3H, *d*, *J*=1.2 cps)
	$CO-CH_3$	2.60 (6H, *s*)	2.50 (3H, *s*) 2.60 (3H, *s*)
	$N-CH_2$	3.55 (4H, *m*)	3.60 (4H, *m*)
	$=C-CH_2-C=$	3.96 (2H, *m*)	
	$C-CH$		4.68 (1H, *q-d*, *J*=6.4 and 2.1 cps)
	$=CH-$		5.45 (1H, *m*)

a) Following abbreviations are used; *s*: singlet, *d*: doublet, *q*: quartet, *m*: multiplet.
b) These signals were practical singlet.

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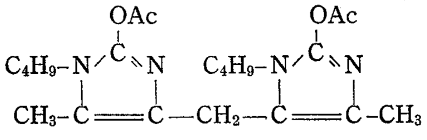
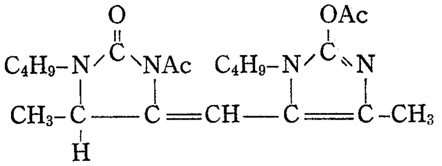
7) R. Gompper and H. Herlinger, *Chem. Ber.*, **89**, 2825 (1956).
8) III develops a pink color in acidic medium.



IV



the one of the four possible structures of V

istence of two equivalent -N=C-OAc groups might be most probable. The NMR spectrum of IV also indicated that two methyl protons of $COCH_3$ groups were magnetically equivalent, and that no big modification of chemical structure had occurred by the acetylation. From the above interpretations chemical structure of IV was assumed as shown below.

In contrast with IV the IR spectrum of V showed two additional absorption maxima associated with carbonyl groups. The band at 1680 cm^{-1} and the other at 1754 cm^{-1} might probably be assigned to carbonyl groups of $NCOCH_3$ group and of N-acetyl-N′-substituted imidazolidone or imidazolone moiety of the molecule, respectively.[7,9] The NMR spectrum of V clarified the following points: one of the two vinylic methyl proton received a large diamagnetic shift and was adjacent to one proton; the molecule had two different $COCH_3$ groups, one of which corresponded to that of IV; a methine proton neighbouring the methyl group mentioned above received an allylic coupling and gave a quartet-doublet signal; and the molecule had one vinylic proton. There are four possible structrutures which satisfy the above spectral characteristics, because both imidazolone rings have similar probabilities to give either N-acetate or O-acetate and to give either $=C-CH_3$ group or $-CH-CH_3$ group. The choice of the right structure from them is not an easy problem from evidences so far available. Therefore, only one of those structures is given here.

Experimental[10]

Isolation of Ib——To a mixture of 50 ml of 5% aqueous AcOH, 150 ml of *ca.* 85% H_3PO_4 and 150 ml of H_2O were added 10.0 g (0.087 mole) of butylurea, 8.8 g (0.087 mole) of diacetyl monoxime and 15.2 g (0.087 mole) of glucuronolactone. The solution was heated on a boiling water bath with continuous stirring for 50 min. After cooling in running water, reaction products were extracted with BuOH, BuOH layer was washed with H_2O, dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved in MeOH–AcOEt (1:3) and was separated chromatographically on alumina. Brownish crystals left on evaporation of a few early fractions were washed with acetone and recrystallized from MeOH to afford about 800 mg of faint yellow needles. No successive fraction gave crystalline compound. Repeated recrystallization from the same solvent gave pure Ib of mp 230—231° (decomp.). *Anal.* Calcd. for $C_{17}H_{28}O_2N_4$: C, 63.72; H, 8.81; N, 17.49; mol.wt., 320. Found: C, 63.65; H, 8.91; N, 17.60; mol.wt. (mass spectrum), 320. Ib is soluble in MeOH, EtOH, BuOH, and AcOH, less soluble in $CHCl_3$ and THF, and sparingly soluble in H_2O, AcOEt, acetone, ether, benzene, and CCl_4.

Isolation of Ic——To the same mixture employed in the isolation of Ib were added 4.5 g (0.03 mole) of *p*-tolylurea, 3.0 g (0.03 mole) of diacetyl monoxime and 3.4 g (0.02 mole) of glucuronolactone. The suspension became a clear solution after heating on a boiling water bath, the heating was continued with stirring for 50 min. The reaction mixture was treated similarly as above. Repeated chromatographic separation of the BuOH extract on alumina with MeOH–AcOEt (1:4) as eluate gave about 300 mg of faint yellow needles. Repeated recrystallization from MeOH–AcOEt afforded pure Ic of mp 314—315° (decomp.). *Anal.* Calcd. for $C_{23}H_{24}O_2N_4$: C, 71.11; H, 6.23; N, 14.42; mol.wt., 388. Found: C, 70.73; H, 6.37; N, 14.30; mol. wt. (mass spectrum), 388.

Acetylation of Ib——A solution of 400 mg of Ib in 5 ml of Ac_2O was heated in an oil bath under refluxing for 1 hr. The reaction mixture was evaporated *in vacuo*, H_2O was added, neutralized with aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhyd. Na_2SO_4, and the solvent was removed. The residue was extracted with petro. ether. On condensation of the solvent 130 mg of V were given as colorless prisms. Further condensation of the solvent afforded 100 mg of IV as

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[9] H.K. Hall Jr. and R. Zbinden, *J. Am. Chem. Soc.*, **80**, 6430 (1958).

[10] All melting points were not corrected. UV spectra were taken on Shimadzu MPS-50 Spectrophotometer, IR spectra on Nihon-Bunko DS-301 Spectrophotometer, NMR spectra on Hitachi H-20, JNM 3H-60, and JNM C-60-H Spectrometer at 60 Mc with tetramethylsilane as internal standard. Mass spectra were kindly taken on RMU-6E Mass Spectrometer in Naka Factory, Hitachi, Ltd.

colorless prisms. Repeated recrystallization of V from petro. ether gave pure crystals of mp 109—110°. *Anal.* Calcd. for $C_{21}H_{32}O_4N_4$: C, 62.35; H, 7.97; N, 13.85. Found: C, 62.29; H, 8.00; N, 13.87. Repeated recrystallization of IV from petro. ether gave pure crystals of mp 77—78°. *Anal.* Calcd. for $C_{21}H_{32}O_4N_4$: C, 62.35; H, 7.97; N, 13.85. Found: C, 62.50; H, 7.98; N, 13.81.

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