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Color Reaction Product of Urea with Diacetyl Monoxime and Glucuronolactone. II.[1] On the Reaction of Butylurea with Diacetyl, 1-Phenyl-1,2-propanedione, 1-Phenyl-1-hydroxyimino-2-propanone, and 1-Phenyl-2-hydroxyimino-1-propanone

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The reaction products of the color reactions of butylurea and glucuronolactone with 1-phenyl-1,2-propanedione, two positional isomers of its monoxime and diacetyl in aqueous phosphoric acid were investigated. The results showed that all reactions, regardless of the difference in the color developing reagent employed, gave 2,2′-dioxo-4,5′-diimidazolylmethane (I) derivatives as main products, and that the reactions of diacetyl and its monoxime gave tetrahydroimidazo[4,5-*d*]imidazole-2,5-dione (II) derivatives as common products. All of these compounds developed visible colors in acidic media, and the colors received strong hyperchromic shifts by the duration of the keeping time at room temperature. Because every color had close relations with the colors of the respective reaction mixtures, I and II derivatives were indicated to have significant responsibilities for the colorations. In spite of the poor reproducibility of isolation, an isolation of 1,4-dimethyl-4-acetyl-3(or 6)-butylcarbamoylimino-1,2-cyclohexen-6 (or 3)-one (III) from the reaction mixture of butylurea, diacetyl and glucuronolactone was also mentioned, because its responsibility for the color reaction was not excluded.

The influence of glucuronolactone on the formation of the reaction products was shown to be negligible and glucuronolactone was assumed to play its role in the process of the stabilization of the color developed.

Diacetyl,[3] diacetyl monoxime,[4] 1-phenyl-1,2-propanedione,[3] and 1-phenyl-2-hydroxyimino-1-propanone[5] have been reported to be the color developing reagents for the photometric determination of urea derivatives.

In the preceding paper of this series the chemical structures of crystalline reaction products (Ib and Ic) obtained from butylurea and *p*-tolylurea with diacetyl monoxime and glucuronolactone in aqueous phosphoric acid were elucidated. However, important but difficult problems were left for further consideration. These problems were (i) why and how Ib and Ic developed visible colors in acidic media, (ii) whether Ib and Ic were the very products responsible for the color reactions or the transition intermediates to afford the true coloring matters, (iii) the hydroxyimino group does or does not influence the formation of reaction products, and others. In order to obtain reasonable answers, the color reactions of butylurea with 1-phenyl-1,2-dioxo-propane, two positional isomers of its hydroxyimino derivatives and diacetyl in aqueous phosphoric acid were investigated. This paper deals with the results of these color reactions and gives the conclusive answer to the problem (iii) and some suggestions to the problem (ii). The influence of the presence of glucuronolactone on the formation of reaction products was also referred.

1) Part I: Y. Ueda, J. Uchida, J. Kuroki, K. Toyonaga, Y. Watanabe, and T. Momose, *Chem. Pharm. Bull.* (Tokyo), **16**, 2442 (1968).
2) Location: *Katakasu, Fukuoka.*
3) R.C. Dickenman, B. Crafts, and B. Zak, *Am. J. Clin. Path.*, **24**, 981 (1954).
4) A.A. Ormsby, *J. Biol. Chem.*, **146**, 595 (1942); H.S. Friedman, *Anal. Chem.*, **25**, 662 (1953).
5) R.M. Archibald, *J. Biol. Chem.*, **156**, 121 (1944); *idem*, *ibid.*, **157**, 507 (1945).

Result and Discussion

Isolation of Crystalline Reaction Products

A solution of each equimolar amounts of butylurea, 1-phenyl-1-hydroxyimino-2-propanone and glucuronolactone in aqueous phosphoric acid containing small amount of acetic acid — whose concentration and composition were adjusted to be almost identical with those of the practical method of urea determination[6] — was heated in boiling water bath with continuous stirring for 50 minutes. This reaction mixture had purple color (Fig. 1) and its absorption maximum was shown at 524 mμ. The butyl alcohol extract of the reaction mixture showed several spots including three major ones on a thin-layer chromatogram (TLC, Fig. 2). These three spots were considered to correspond to the compounds responsible for the coloration, because they developed visible colors — blue, purple, and pink — by the exposure to the vapor of hydrochloric acid.[7] Therefore, the butyl alcohol extract was subjected to the chromatographic separation on alumina to give faint yellow prisms (Id or Ie) and faint yellow needles (If), both in rather good yield. Id (or Ie) showed the same *Rf* value and color on TLC as those of the blue spot of the butyl alcohol extract, and If had the similar relation to the purple spot. Then,

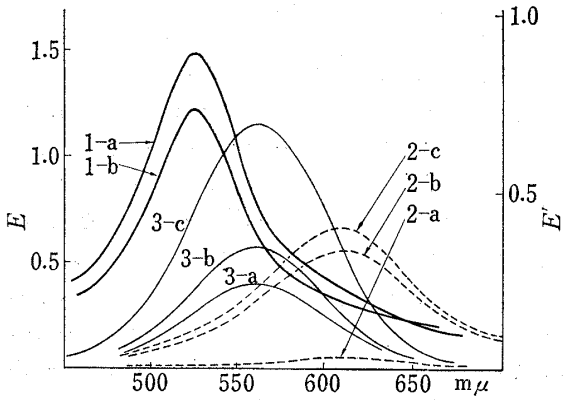


Fig. 1. Absorption Curves of Reaction Mixtures, Id (or Ie) and If

1-a: reaction mixture of 1-phenyl-1-hydroxyimino-2-propanone; λ_{max} 524 mμ
1-b: reaction mixture of 1-phenyl-1,2-propanedione; λ_{max} 524 mμ
2-a—c: Id (or Ie); after 1.5(a), 18.25(b) and 32 hr(c) of dissolution; λ_{max} 607 mμ
3-a—c: If; after 1.5(a), 18.25(b) and 32 hr(c) of dissolution; λ_{max} 560 mμ
The curves should be read against the absorbancy E (1-a and 1-b) and E' (2 and 3).

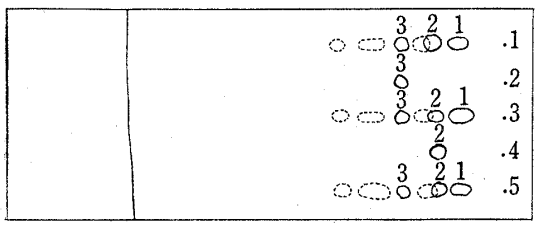


Fig. 2. TLC of Reaction Mixtures

solvent: AcOEt–acetone (1:1)
All spots were visualized with HCl vapor (spot 1: pink, spot 2: purple, spot 3: blue).
1: BuOH extract of the reaction mixture of 1-phenyl-1,2-propanedione
2: reference compound Id (or Ie)
3: BuOH extract of the reaction mixture of 1-phenyl-1-hydroxyimino-2-propanone
4: reference compound If
5: BuOH extract of the reaction mixture of 1-phenyl-2-hydroxyimino-1-propanone

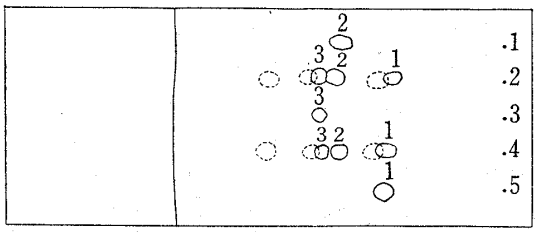


Fig. 3. TLC of Reaction Mixtures

solvent: AcOEt-acetone (3:1)
All spots except 2 and 3 were visualized with HCl vapor (spot 1: pink). Spot 2 was visualized with I_2 vapor (brown) and spot 3 was detected by scorching with H_2SO_4 (brown).
1: reference compound IIIa (or IIIb)
2: BuOH extract of the reaction mixture of diacetyl
3: reference compound IId (or IIe)
4: BuOH extract of the reaction mixture of diacetyl monoxime
5: reference compound Ib

both compounds proved to be the compounds which showed these two spots on TLC. However, one product which was detected as a pink spot could not yet be isolated as crystals.

6) T. Momose, Y. Ohkura, and J. Tomita, *Clin. Chem.*, **11**, 113 (1965).
7) The coloration of this kind of color reaction may be explained by the interaction of proton with coloring matters produced.

Because both Id (or Ie) and If are hardly soluble in water, each of them was dissolved in appropriate amount of ethyl alcohol and 5% aqueous acetic acid and 85% phosphoric acid were successively added in the ratio of 3:1:3 under cooling. The solution of If developed purple color immediately after the addition of phosphoric acid, and the color was intensified gradually by the duration of the time of keeping at room temperature. Though almost equal amount of Id (or Ie) was used, its solution seemed colorless for a little while after the addition of phosphoric acid, but afterward it developed faint blue color, and the color was intensified as similarly as above. A warming of these solutions in water bath accelerated the color developments very much. Fig. 1 shows that the absorption maxima of both solutions did not shift at all during the period of the intensification of colors, and also suggests strongly that both Id (or Ie) and If should be the important constituents of the characteristic reaction products responsible for the coloration, even though they may not be the most important one. But their coloration phenomena mentioned above may stand by the assumption that they must be the transition intermediates to afford the true coloring matters, whose solutions will develop intense colors immediately after the addition of phosphoric acid.

The substitution of 1-phenyl-2-hydroxyimino-1-propanone for 1-phenyl-1-hydroxyimino-2-propanone in this color reaction did not give any alteration to the result. Namely, the reaction mixture of butylurea with 1-phenyl-2-hydroxyimino-1-propanone and glucuronolactone in the same medium had the same color, showed the same absorption curve, showed the same spots on TLC and gave the same crystals as the reaction mixture of 1-phenyl-1-hydroxyimino-2-propanone did. This fact means that the position of hydroxyimino group of the reagent does not affect the condensation reactions of urea derivatives with α-dioxomonoximes, and suggests strongly that the reaction may be preceded by the hydrolysis of hydroxyimino group of the reagent. This consideration was supported strongly by the fact that the color reaction mixture of butylurea with 1-phenyl-1,2-propanedione and glucuronolactone in the same medium had the same color, showed an almost identical absorption curve (Fig. 1), showed the identical spots on TLC and gave Id (or Ie) and If as crystals as the reaction mixture of 1-phenyl-1-hydroxyimino-2-propanone did.

This findings made us to expect the isolation of Ib from the reaction mixture of butylurea, diacetyl and glucuronolactone. The color reaction proceeded almost similarly as that of diacetyl monoxime, and the butyl alcohol extract of the reaction mixture showed several spots including three distinct ones on TLC (Fig. 3). These three spots had the comparative *Rf* values and colors with those of the butyl alcohol extract of the respective reaction mixture of diacetyl monoxime. One of them, spot 1, had the same *Rf* value and color as those of the spot of Ib employed as the reference compound. Therefore, the existence of Ib in the reaction mixture is highly probable, but trials to isolate it from the butyl alcohol extract were without any fruitful result. The reason for this failure may be attributed to the instability[8] of this compound, especially on alumina. The chromatographic separation of this butyl alcohol extract afforded a product (IId or IIe) as colorless needles, which corresponded to the spot 3 on TLC (Fig. 3). The isolation of another compound (IIIa or IIIb) corresponded to the spot 2 was unsuccessful except one time, in spite of the repeated trials. It is difficult to find a suitable reason for the poor reproducibility of isolation, but we would like to describe its isolation and its chemical structure in this paper, because its existence in the reaction mixture was always suggested by the corresponding spot on TLC and it was actually isolated even in only once.

IId (or IIe) was dissolved in ethyl alcohol and 5% aqueous acetic acid and 85% phosphoric acid were successively added in the ratio of 3:1:3 under cooling, because this compound

8) Ib is really not stable enough to store in the atmosphere for a long time, to keep its solution for a few days, and to repeat the chromatographic separation. Its isolation needs some cares even in the case of the reaction of diacetyl monoxime.

p-$CH_3C_6H_4NHCONH_2$+$CH_3COC(=NOH)CH_3$ ⟶ **Ic**+unknown compounds (not investigated)

$C_4H_9NHCONH_2$+$CH_3COC(=NOH)CH_3$ ⟶ **Ib**+*IId* (or *IIe*)+*IIIa* (or *IIIb*)+unknown compounds

$C_4H_9NHCONH_2$+$CH_3COCOCH_3$ ⟶ **IId** (or **IIe**)+*Ib*+IIIa (or IIIb)[b]+unknown compounds

$C_4H_9NHCONH_2$+$CH_3C(=NOH)COC_6H_5$ ⟶ **Id** (or **Ie**)+**If**+unknown compounds

$C_4H_9NHCONH_2$+$CH_3COC(=NOH)C_6H_5$ ⟶ **Id** (or **Ie**)+**If**+unknown compounds

$C_4H_9NHCONH_2$+$CH_3COCOC_6H_5$ ⟶ **Id** (or **Ie**)+**If**+unknown compounds

Chart 1[a]

a) The compound isolated was shown by boldface letters, and the product not isolated but detected on TLC was shown by italic letters.
b) This compound could be isolated only once, but it is always detectable on TLC plates.

is also hardly soluble in water. The solution developed faint orange color immediately after the addition of phosphoric acid, and the color was intensified by the duration of the time of keeping at room temperature. This coloration was also accelerated very much by warming in water bath. Fig. 4 suggests that IId (or IIe) should be one of the important reaction products responsible for the coloration, but the coloration phenomenon in acidic medium mentioned above suggests strongly that it may be the transition intermediate to afford the true coloring matter.

IIIa (or IIIb) was obtained as yellow needles. Its solution in the reaction medium seemed to keep its characteristic yellow color for a rather long time at room temperature, but its absorption curve was found to change very slowly. As an example a solution kept at room temperature for 8 days showed a rather weak absorption peak at 487 mμ. This peak could be superimposed on that of the reaction mixture (Fig. 4). Therefore, IIIa (or IIIb) may also be one of the characteristic transition intermediate. However, it is not clear whether this compound occupies a significant situation in the color reaction or not, because of its poor reproducibility of isolation and of its considerable stability in acidic medium.

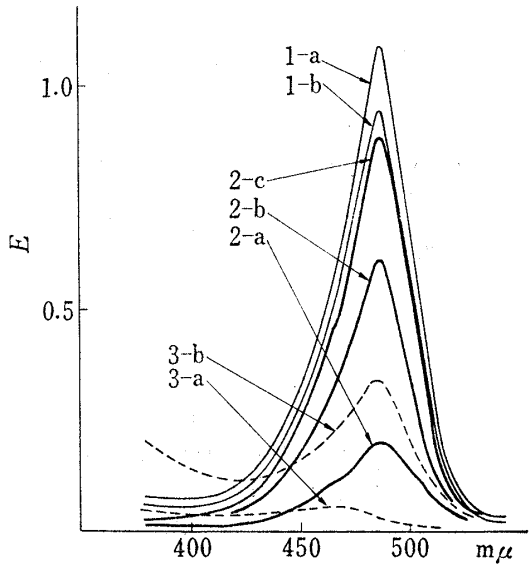


Fig. 4. Absorption Curves of Reaction Mixtures, IId (or IIe) and IIIa (or IIIb)

1-a: reaction mixture of diacetyl; λ_{max} 489 mμ
1-b: reaction mixture of diacetyl monoxime; λ_{max} 489 mμ
2-a—c: IId (or IIe); after 5.5(a), 18.75 (b) and 25 hr(c) of dissolution; λ_{max} 489 mμ
3-a,b: IIIa (or IIIb); after 10 min(a) and 8 days(b) of dissolution; λ_{max} 487 mμ

The results so far mentioned in this and the preceding paper are summarized in Chart 1. This chart shows that I derivatives were always produced in all cases, and that IId (or IIe) and IIIa (or IIIb) were produced commonly in the reactions of both diacetyl and diacetyl monoxime. If we set aside IIIa (or IIIb) from the present consideration, all compounds should be very characteristic reaction products and they may be very important reaction intermediates for the coloration.

The Chemical Structures of the Reaction Products

The micro analysis and the mass spectral data confirmed that both Id (or Ie) and If had the same molecular formula of $C_{27}H_{32}O_2N_4$, and suggested that both compounds may have

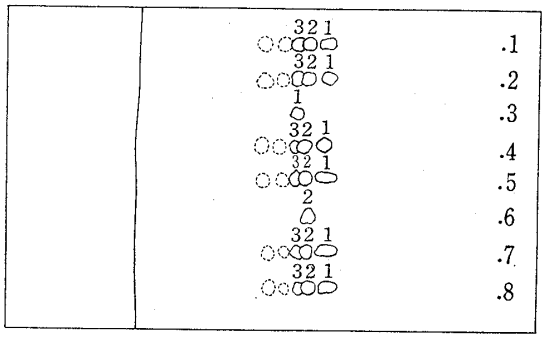


Fig. 5. TLC of BuOH Extracts of Reaction Mixtures (1)

solvent: MeOH–AcOEt–acetone (1:99:100)
All spots were visualized with HCl vapor (spot 1; pink, spot 2; purple, spot 3; blue).
1: butylurea+1-phenyl-2-hydroxyimino-1-propanone
2: 1+glucuronolactone
3: reference compound Id (or Ie)
4: butylurea+1-phenyl-1,2-propanedione
5: 4+glucuronolactone
6: reference compound If
7: butylurea+1-phenyl-1-hydroxyimino-2-propanone
8: 7+glucuronolactone

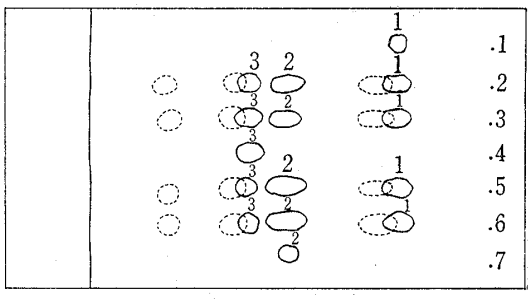
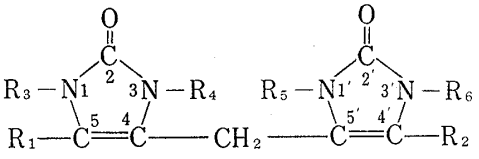


Fig. 6. TLC of BuOH Extracts of Reaction Mixtures (2)

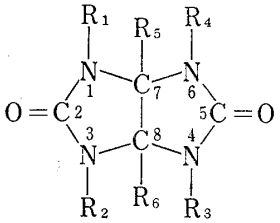
solvent:MeOH–AcOEt–acetone (1:99:100)
All spots except 2 and 3 were visualized with HCl vapor (spot 1; pink). Spot 2 was visualized with I_2 vapor (brown) and spot 3 was detected by scorching with H_2SO_4 (brown).

1: reference compound Ib
2: butylurea+diacetyl monoxime+glucuronolactone
3: butylurea+diacetyl monoxime
4: reference compound IId (or IIe)
5: butylurea+diacetyl+glucuronolactone
6: butylurea+diacetyl
7: reference compound IIIa (or IIIb)



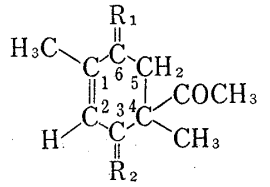
2,2′-dioxo-4,5′-diimidazolylmethane

Ia: $R_1=R_2=CH_3$, $R_3-R_6=H$
Ib: $R_1=R_2=CH_3$, $R_3=R_5=C_4H_9$, $R_4=R_6=H$
Ic: $R_1=R_2=CH_3$, $R_3=R_5=p\text{-}CH_3C_6H_4$, $R_4=R_6=H$
Id: $R_1=R_2=C_6H_5$, $R_3=R_6=C_4H_9$, $R_4=R_5=H$
Ie: $R_1=R_2=C_6H_5$, $R_4=R_5=C_4H_9$, $R_3=R_6=H$
If: $R_1=R_2=C_6H_5$, $R_3=R_5=C_4H_9$, $R_4=R_6=H$



tetrahydroimidazo[4,5-*d*]imidazole-2,5-dione

IIa: $R_1-R_6=H$
IIb: $R_1-R_4=H$, $R_5=R_6=CH_3$
IIc: $R_2=R_3=H$, $R_1=R_4=R_5=R_6=CH_3$ (or $R_2=R_4=H$, $R_1=R_3=R_5=R_6=CH_3$)
IId: $R_2=R_4=H$, $R_1=R_3=C_4H_9$, $R_5=R_6=CH_3$
IIe: $R_2=R_3=H$, $R_1=R_4=C_4H_9$, $R_5=R_6=CH_3$



1,4-dimethyl-4-acetyl-1,2-cyclohexene-3,6-dione

IIIa: $R_1=O$, $R_2=NCONHC_4H_9$
IIIb: $R_1=NCONHC_4H_9$, $R_2=O$

similar chemical structures to Ib, because the difference ($C_{10}H_4$) between this formula and $C_{17}H_{28}O_2N_4$ of Ib could be attributed to the double difference (C_6H_5 and CH_3) between the substituents of the reagents employed. The existence of imidazolone moieties was suggested by their characteristic infrared (IR) absorption bands at 3150, 1680, 1670 cm^{-1} of Id (or Ie) and 3150, 1680, 1670 cm^{-1} of If.[9] The nuclear magnetic resonance (NMR) spectral data tabu-

9) *cf.*, R. Gompper and H. Herlinger, *Chem. Ber.*, **89**, 2825 (1956).

lated in Table I showed no discrepancy for the consideration that the chemical structures of both compounds are nothing else than I derivatives.
Their ultraviolet (UV) absorption spectra were in strong resemblance each other and showed that both compounds are in close relationship in chemical respect. However, Table I showed that in the case of Id (or Ie) either the protons of two terminal methyl groups of two butyl side chains or the protons of two NH groups are in magnetically equivalent conditions, but in the case of If both of them are not in equivalent circumstances. For instance, the NMR spectrum of Id (or Ie) in pyridine showed two methyl signals of the same chemical shift (δ 0.75) and two NH signals of the same chemical shift (δ 12.08), on the contrary the spectrum of If in pyridine showed two methyl signals at δ 0.70 and 0.79 and two NH signals at δ 11.83 and 11.93. From the evidences so far obtained the chemical structure of 1,1′-dibutyl-5,4′-diphenyl-2,2′-dioxo-4,5′-diimidazolylmethane and the alternative structure of 1,3′(or 3,1′)-dibutyl-5,4′-diphenyl-2,2′-dioxo-4,5′-diimidazolylmethane were deduced to If and Id (or Ie), respectively. The mass spectra of both compounds showed almost identical fragmentation patterns each other, and they supported these structures (*cf.* Experimental section).

The elemental composition of IId (or IIe) was established by the micro analysis and the mass spectrometry as $C_{14}H_{26}O_2N_4$. The IR spectrum showed the existence of NH and CO

Table I. Spectral Data of Id (or Ie) and If

	Id (or Ie)		If	
UV λ_{max}^{EtOH} mμ		291		291
IR ν_{max}^{KBr} cm^{-1}		1670, 1680 (C=O) 3150 (NH)		1670, 1680 (C=O) 3150 (NH)
NMR (δ ppm)[a]	in CF_3COOH	in C_5H_5N	in CF_3COOH	in C_5H_5N
C–C–CH_3	0.92(6H, m)	0.75(6H, m)	0.80, 1.08 (6H, m)	0.70, 0.79 (6H, m)
C–$(CH_2)_2$–C	*ca.* 1.0—1.7 (8H,m)	*ca.* 0.8—1.7 (8H,m)	*ca.* 1.0—1.9 (8H,m)	*ca.* 0.9—1.8 (8H,m)
N–CH_2–C	3.73(4H, m)	3.64(4H, m)	3.78(4H, m)	3.72(4H, m)
=C–CH_2–C=	4.41(2H, s)[b]	4.43(2H, s)[b]	4.20(2H, s)[b]	4.02(2H, s)[b]
Arom. H	7.51(10H, m)		7.46(10H, m)	
NH	11.05(2H, b)	12.08(2H, b)	10.95(2H, b)	11.83(1H, b) 11.93(1H, b)

a) Following abbreviations were used: s, singlet; m, multiplet; b, broad. The NMR data of If determined in $CDCl_3$ were given in the experimental section.
b) This signal was somewhat broad.

Table II. Spectral Data of IId (or IIe)

IR ν_{max}^{KBr} cm^{-1}	3200, 3150, 3080 (NH)	1713, 1686 (C=O)
NMR (δ ppm)[a]	in CF_3COOH	in $CDCl_3$[b]
C–C–CH_3	1.00(6H, m)	*ca.* 0.8—1.7(14H, m)
C–$(CH_2)_2$–C	*ca.* 1.6(8H, m)	
N–CH_2–C	3.45(4H, m)	3.14(4H, m)
C–CH_3	1.80(6H, s)	1.49(6H, s)
NH		5.84(2H, b)

a) The same abbreviations were used as those of Table I.
b) Due to the low solubility in $CDCl_3$ good spectrum was not obtained.

Table III. Spectral Data of IIIa (or IIIb)

UV λ_{max}^{EtOH} mμ(ε)	259(97,200)	417(110)
IR ν_{max}^{KBr} cm^{-1}	3180 (NH)	1675 (C=O)
NMR (δ ppm)[a] (in $CDCl_3$)		
	C–C–CH_3	0.95(3H, m)
	C–$(CH_2)_2$–C	*ca.* 1.1—1.8(4H, m)
	N–CH_2–C	3.58(2H, m)
	C–CH_3	1.34(3H, s)
	O=C–CH_3	1.97(3H, s)
	HC=C–CH_3	2.25(3H, d, J=0.8 cps)
	=C–CH_2–C	2.72(2H, s)
	HC=C	5.35(1H, b, J=0.8 cps)
	NH	9.65(1H, b)

a) Following abbreviations were used: s, singlet; d, doublet; m, multiplet; b, broad

group by the absorption bands at 3200, 3150, 3080 cm^{-1} and 1713, 1686 cm^{-1}, respectively. The NMR spectrum determined in trifluoroacetic acid indicated that the molecule has two equivalent butyl groups and two equivalent methyl groups having no proton on the adjacent atoms. The spectrum determined in deuterochloroform showed the existence of two NH groups in the molecule. From these informations and from the fact that a similar reaction of methylurea with diacetyl in hydrochloric acid was reported to produce IIc,[10] the alternative chemical structure of 7,8-dimethyl-1,4 (or 1,6)-dibutyl-tetrahydroimidazo[4,5-*d*]-imidazole-2,5-dione was given to this compound. But the evidences were not enough to select the right one from the two possible structures IId or IIe. Veniamin, *et al.*[11] considered that II derivatives are the chromogens of the carbamidodiacetyl colorimetric assay, but they did not isolate any compound. We consider that II derivatives may not be the sole intermediate responsible for the coloration.

The molecular formula of IIIa (or IIIb) was established by the micro analysis and the mass spectrometry as $C_{15}H_{22}O_3N_2$. This formula shows that this compound should not be the derivative of I or II, and that it may contain only one butylurea residue, $C_4H_9N(H)CON(H)$. Therefore, it remains $C_{10}H_{11-13}O_2$ as the main framework of the molecule which should be formed from diacetyl molecules. The most conclusive evidence of the structure was obtained from its NMR spectrum (Table III). A multiplet (3H) at δ 0.95, a multiplet (4H) at δ 1.1—1.8, and a multiplet (2H) at δ 3.58 could be assigned to the protons of butylurea residue. Two singlets (each 3H) at δ 1.34 and 1.97 may be reasonably assigned to the protons of C–CH_3 and O=C–CH_3 groups, respectively. A doublet (3H, J=0.8 cps) at δ 2.25 may originate from the methyl proton of H_3C–C=CH– group splitted by one methine proton of its allylic position, which showed a somewhat broad peak (1H, multiplet) at δ 5.35, because the irradiation at δ 5.35 decoupled the doublet to a singlet. A singlet (2H) at δ 2.72 and a broad peak (1H) at δ 9.65 may be assigned to the protons of –C–CH_2–C= group and of NH group, respectively. Now it remains C_1O_1 as an unknown part of the molecule. The IR absorption bands at 3180 and 1675 cm^{-1} indicated the existence of NH and CO groups in the molecule. But the band at 1675 cm^{-1} was rather broad and had a few shoulders which could not be dissolved, therefore, a precise interpretation was given up. We had no further information about the molecule, especially on the structure of its unknown part. All of the results mentioned above may well be explained by the alternative structure of 1,4-dimethyl-4- acetyl -3 (or 6)-butylcarbamoylimino-1,2-cyclohexen-6 (or 3)-one, but there is no distinct proof to select the right one from the structures of IIIa and IIIb. The UV spectrum, which indicated the existence of a prolonged conjugation in the molecule, and the mass spectrum gave strong

10) H. Biltz, *Ber.*, **40**, 4811 (1907).
11) M.P. Veniamin and C. Vakirtzi-Lemonias, *Clin. Chem.*, **16**, 3 (1970).

bases to this consideration (*cf.* Experimental section). The isolation of this compound may present an interesting example to the chemical reaction of urea derivatives and α-dioxo-compounds in acidic media, because its structure shows that the condensation reaction of three moles of diacetyl was accompanied with a simultaneous loss of two carbon atoms.

The Influence of Glucuronolactone on the Reaction Products

In order to investigate the influence of glucuronolactone on the color reaction products the reaction of butylurea with diacetyl monoxime was proceeded with or without the presence of glucuronolactone, and the reaction products were examined by TLC. The result (Fig. 5,6) indicated that same products were produced regardless of the presence of glucuronolactone in the reaction medium. Therefore, glucuronolactone may not give any remarkable influence to the formation of color reaction products, though Momose, *et al.*[6] assumed that glucuronolactone may play an important role in the color formation. We assume that glucuronolactone may play its role in the stabilization process of the color formed.

Experimental[12]

Isolation of Id (or Ie) and If——To a mixture of 200 ml of 5% aq. AcOH, 600 ml of *ca.* 85% H_3PO_4 and 600 ml of H_2O were added 5.8 g (0.05 mole) of butylurea, 8.9 g (0.05 mole) of 1-phenyl-1-hydroxyimino-2-propanone and 8.8 g (0.05 mole) of glucuronolactone. The mixture was heated in 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 successively with H_2O, sat. aq. $NaHCO_3$ solution and H_2O, dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The residue (*ca.* 10 g) was dissolved in MeOH–benzene (1:5) and was subjected to the separation on alumina. The column was eluted with the same solvent mixture. The brownish remainings on evaporation of a few early fractions was washed with AcOEt to leave crystals. Recrystallization from MeOH gave *ca.* 2.1 g of faint yellow prisms. Repeated recrystallization from the same solvent afforded pure Id (or Ie) of mp 279° (decomp.). *Anal.* Calcd. for $C_{27}H_{32}O_2N_4$: C, 72.95; H, 7.26; N, 12.60; mol. wt., 444.253. Found: C, 72.83; H, 7.19; N, 12.50; mol. wt. (mass spectrum), 444.251. Mass Spectrum *m/e*: 444.251 (M^+), 427.248 (M^+–OH; calcd. for $C_{27}H_{31}ON_4$: 427.250), 388.189 (M^+–C_4H_8; calcd. for $C_{23}H_{24}O_2N_4$: 388.190), 344.123 (M^+–C_7H_{16}; calcd. for $C_{20}H_{16}O_2N_4$: 344.127), 229.134 (Fragment A[13]; calcd. for $C_{14}H_{17}ON_2$: 229.133), 216.126 (fragment B[13] +1; calcd. for $C_{13}H_{16}ON_2$: 216.126), and 173.065 (fragment A–C_4H_8; calcd. for $C_{10}H_9ON_2$: 173.071). This crystal is slightly soluble in MeOH, EtOH, $CHCl_3$, and sparingly soluble in H_2O, AcOEt, benzene.

The following several fractions gave *ca.* 1.1 g of faint yellow needles. Repeated recrystallization from MeOH afforded pure If of mp 239—240° (decomp.). *Anal.* Calcd. for $C_{27}H_{32}O_2N_4$: C, 72.95; H, 7.26; N, 12.60; mol.wt., 444.253. Found: C, 72.97; H, 7.31; N, 12.60; mol.wt. (mass spectrum), 444.251. NMR (in $CDCl_3$, δ ppm): 0.73, 0.77 (6H, multiplet, C–C–CH_3); *ca.* 0.9—1.6 (8H, multiplet, C–$(CH_2)_2$–C); 3.43 (4H, multiplet, N–CH_2–C); 3.76 (2H, singlet, =C–CH_2–C=), 7.32 (10H, multiplet, arom. H); 9.92 (1H, broad, NH) and 10.43 (1H, broad, NH). Mass spectrum of If had essentially the same fragmentation pattern as that of Id (or Ie). This compound is soluble in MeOH, EtOH, $CHCl_3$ and sparingly soluble in H_2O, AcOEt. Successive fraction gave no crystalline compound.

To a mixture of 150 ml of 5% aqueous AcOH, 450 ml of *ca.* 85% H_3PO_4 and 450 ml of H_2O were added each 0.0025 mole of butylurea (0.29 g), 1-phenyl-2-hydroxyimino-1-propanone (0.40 g) and glucuronolactone (0.44 g). The mixture was treated as above, and the BuOH extract (*ca.* 0.9 g) was dissolved in a small amount of MeOH–benzene (3:1) and subjected to the chromatographic separation on alumina. Recrystallization of the crystals obtained from the MeOH–benzene (1:99) eluates from MeOH gave *ca.* 20 mg of rather pure compound. This compound showed an identical IR spectrum with that of Id (or Ie). The following fractions eluted with MeOH gave *ca.* 10 mg of another compound. Its IR spectrum was identical with that of If.

To the reaction medium (1,260 ml) of the same composition as that of the former case were added each 0.028 mole of butylurea (3.24 g), 1-phenyl-1,2-propanedione (4.13 g) and glucuronolactone (4.91 g). The mixture was treated as above and the reaction products were extracted with BuOH. An addition of a small

12) All melting points were not corrected. UV spectra were taken on Shimadzu MPS-50L Spectrophotometer, IR spectra on Nihon-Bunko DS-301 Spectrophotometer, NMR spectra on JNM C-60-H Spectrometer at 60 Mc with tetramethylsilane as the internal standard, and mass spectra on JMS-01SG Spectrometer.

13) A fission of the bond between C-5 and the adjacent CH_2 group of Id (or Ie) was assumed to give fragment A and B.

amount of MeOH–benzene (1:99) to the extract left crystalline compounds as insoluble substances. Recrystallization of them from MeOH gave *ca.* 150 mg of rather pure compound. This compound showed identical mp and IR spectrum with those of Id (or Ie). The MeOH–benzene (1:99) solution was separated on alumina. A few early fractions eluted with the same solvent mixture gave an additional amount (*ca.* 40 mg) of Id (or Ie). The following fractions eluted with MeOH gave another crystals. Recrystallization of it from MeOH afforded rather pure compound. This compound showed identical mp and IR spectrum with those of If.

Isolation of IId (or IIe)——To a mixture of 200 ml of 5% aq. AcOH, 600 ml of *ca.* 85% H_3PO_4 and 600 ml of H_2O were added 11.6 g (0.1 mole) of butylurea, 8.6 g (0.1 mole) of diacetyl and 17.6 g (0.1 mole) of glucuronolactone. The mixture was treated as similarly as other cases mentioned before. The BuOH extract was dissolved in MeOH–AcOEt (1:99) and was separated on alumina. The column was eluted with the same solvent mixture to give *ca.* 20 mg of colorless needles. Repeated recrystallization from MeOH–AcOEt (1:99) afforded pure IId (or IIe) of mp 256—257°. *Anal.* Calcd. for $C_{14}H_{26}O_2N_4$: C, 59.54; H, 9.28; N, 19.84; mol.wt., 282. Found: C, 59.29; H, 9.07; N, 20.25; mol.wt. (mass spectrum), 282. Mass spectrum *m/e*: 282 (M^+), 267 (M^+-CH_3), 252 ($M^+-2\times CH_3$), 225 ($M^+-C_4H_9$), and 210 ($M^+-C_4H_9-CH_3$).

Isolation of IIIa (or IIIb)——To a mixture of 150 ml of 5% aq. AcOH, 450 ml of *ca.* 85% H_3PO_4 and 450 ml of H_2O were added 7.6 g(0.066 mole) of butylurea, 8.4 g (0.098 mole) of diacetyl and 11.4 g (0.065 mole) of glucuronolactone. The mixture was treated as similarly as other cases mentioned before. The BuOH extract was dissolved in MeOH–AcOEt (1:3) and separated on alumina. Brownish remainings left on evaporation of a few early fractions eluted with the same solvent mixture were washed with AcOEt to give crystalline compounds. Recrystallization of it from AcOEt gave *ca.* 650 mg of yellow needles. Repeated recrystallization from the same solvent afforded pure IIIa (or IIIb) of mp 134°. *Anal.* Calcd. for $C_{15}H_{22}O_3N_2$: C, 64.72; H, 7.97; N, 10.07; mol.wt., 278.163. Found: C, 64.70; H, 7.93; N, 10.07; mol.wt. (mass spectrum), 278.162. Mass Spectrum *m/e*: 278.162 (M^+), 263.137 (M^+-CH_3; Calcd. for $C_{14}H_{19}O_3N_2$: 263.140), 235.146 (M^+-COCH_3; calcd. for $C_{13}H_{19}O_2N_2$: 235.145), 235.111 ($M^+-C_3H_7$; calcd. for $C_{12}H_{15}O_3N_2$: 235.108), 233.132 ($M^+-COCH_3-H_2$; calcd. for $C_{13}H_{17}O_2N_2$: 233.129), 167.117 ($H_3C-C=CH-C=NCONHC_4H_9+H$ or $HC=C(CH_3)-C=NCONH-C_4H_9+H$; calcd. for $C_9H_{15}ON_2$: 167.118), and 111.057 ($H_3C-C=CH-C=NCONH_2+H$ or $HC=C(CH_3)-C=NCONH_2+H$; calcd. for $C_5H_7ON_2$: 111.056). Metastable ions at *m/e* 100.3 and 73.8 supported the fragmentation pathways $278\xrightarrow{100.3}167\xrightarrow{73.8}111$. This compound is soluble in MeOH, EtOH, acetone, benzene and slightly soluble in AcOEt, $CHCl_3$. Successive fraction gave no crystalline compound.

Thin-Layer Chromatography (TLC)——The reaction mixtures were separated on plates (20×20 cm^2). The plates were prepared with 250 μ layers of silica gel (WAKOGEL B-5) and were activated at 115° for 1 hr.

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