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# A New Reductone derived from D-Gluculonolactone by Alkali. II.[1]
## Further Studies on the Properties of the Reductone

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3-Keto-4,5-dideoxy-*trans*-4,5-dehydro-glucuronic acid is to exhibit various tautomeric forms in the solution. The possible tautomerism in acidic and alkaline medium was discussed on the basis of polarographic and absorption spectrographic data. Oxidation of the reductone by oxygen in alkaline solution gave disodium 2,3-diketo-4,5-dehydro-adipate, while oxidation of the reductone with iodine in acidic solution gave 4,5,6-trioxo-2,3-dehydro-caproic acid which was isolated as bisphenylhydrazone.

In the preceding paper[1] the authors described that a reductone isolated from the reaction-product of D-glucuronolactone with potassium hydroxide has the structure of 3-keto-4,5-dideoxy-*trans*-4,5-dehydro-glucuronic acid. It was predicted that the reductone has an ene-diol grouping in the molecule on account of its strong reducing power and the color reaction with ferric chloride in acidic solution. In the present polarographic study carried out excluding air, it was found that the reductone gave a marked anodic wave, thus affording a further evidence on the presence of an ene–diol grouping. The decrease of anodic wave and the increase of reduction wave in acidic solution during the time course suggested gradual transformation of the ene–diol to a α-ketol grouping (Fig. 2). This suggestion was supported by the absorption spectral data. Thus, in acidic solution, the intensity of an absorption band of the reductone at 340 mμ decreased with an increase of that of another band at 230 mμ (Fig. 1-A). Shift of the absorption band at 340 mμ to 390—400 mμ in neutral or alkaline solution as shown in Fig. 1-B, C, and D might be due to the dissociation of one of the two hydroxyl groups in the reductone, and that to 418—420 mμ in the more alkaline solution than 0.1 N sodium hydroxide solution might be due to the dissociation of the two hydroxyl groups. Furthermore, the appearance of an absorption band at 280 mμ (Fig. 1-E) on the oxidation of the reductone by oxygen in alkaline solution would account for the formation of 2,3-diketo-4,5-dehydro-adipic acid (VII).

Oxidation of the reductone by iodine in acidic solution gave 4,5,6-trioxo-2,3-dehydro-caproic acid (VIII) which was isolated as the bisphenylhydrazone (IX).

### Experimental

**Ultraviolet and Visible Spectra of the Reductone**——1) In Nonaqueous Solvent: The reductone exhibited a high-intensity band near 340 mμ and a low-intensity band at 415 mμ as shown in Table I. The intensities of these absorption bands decreased rapidly on standing in all the solvents used in the experiment even under nitrogen.

2) In Aqueous Solution: The following buffer solutions were used; 1/15 mole $KH_2PO_4$–$Na_2HPO_4$ buffer solutions of pH 5.0—9.0, 1/15 mole $Na_2HPO_4$–NaOH buffer solutions of pH 10.0—12.0, 0.1 N HCl and 0.1 N

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1) Part I: M. Kawata, Y. Mizutani, N. Shinriki, M. Kimura and M. Ishidate, *Chem. Pharm. Bull.* (Tokyo), **18**, 50 (1970).

2) Location: *Nishi-6-chome, Kita-12-jo, Sapporo*; *a*) Present address: *Government Industrial Development Laboratory, Higashi-tsukisamu, Sapporo*; *b*) Present address: *National Institute of Hygienic Sciences, Tamagawayoga-machi, Setagaya-ku, Tokyo.*

Table I. Absorption Spectra of the Reductone in Nonaqueous Solvents

| Solvent | $\lambda_{\max}$, mμ (ε) | $\lambda_{\max}$, mμ (ε) |
|---|---|---|
| Acetone | 343 (33200) | 415 (400) |
| Ethylacetate | 345 (41700) | 415 (500) |
| Methanol | 345 * | * |
| Ethanol | 345 (35500) | 415 (600) |
| Dioxane | 338 (25900), 349 (27600) | * |
| Pyridine | 356 (31700) | * |

All absorption spectra were obtained in 45 sec. after dissolving I in solvents.
* Too rapid decrease in absorption band.

NaOH. 1.0 mg of the reductone was dissolved in 1.5 ml of MeOH, diluted to 100 ml with a buffer solution for the measurement of the absorption bands at 230 and 340 mμ (A and B in Table II) in acidic solution, and to 50 ml with a buffer solution for the absorption bands at 220, 280 and 400 mμ (C, D and E in Table II) in alkaline solution. The absorption spectra measurements were carried out on Hitachi Recording Spectrophotometer EPS-3T.

In 0.1 N HCl and buffer solutions of pH 5.0, 6.0 and 7.0, two absorption bands were observed: a high-intensity band at 339—342 mμ decreased rapidly while a low-intensity band at 230 mμ increased ($\varepsilon=5200$ in 0.1 N HCl after standing at room temperature for 24 hr) under both nitrogen and air. The case of 0.1 N HCl is shown in Fig. 1-A. In the more alkaline solution than pH 7.0, another band at 400—420 mμ was observed. This band was more weak than a band at 340 mμ at pH 7.5 (Fig. 1-B), which was observed as a shoulder of the band at 400 mμ at pH 8.0 (Fig. 1-C). In the more alkaline solution than pH 9.0, the reductone produced a band at 280 mμ, which did not appear under nitrogen (Fig. 1-D) together with the decrease of the bands at 400 mμ and 220 mμ under air (Fig. 1-E). The band at 400 mμ in buffer solutions of pH 7.0—12.0 shifted to 418 mμ in 0.1 N NaOH. The effect of pH on the five absorption bands is shown in Table II.

Table II. Effect of pH on Absorption Maxima of the Reductone

| Absorption band | $\lambda_{\max}$, mμ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | | | | | | | | |
| | 0.1 N HCl | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 0.1 N NaOH |
| A | 230 | 230 | 230 | 230 | | | | | | |
| B | 339 | 340 | 340 | 342 | | | | | | |
| C | | | | 220 | 220 | 220 | 220 | 220 | 220 | 220 |
| D | | | | | | 278 | 280 | 280 | 280 | 280 |
| E | | | | 390 | 393 | 395 | 395 | 395 | 400 | 418 |

**Polarography of the Reductone**——5.0 mg of the reductone was dissolved in 25 ml McIlvain buffer of pH 3.0 and 5.0, and nitrogen was bubbled for 12 min at 25±0.2°. The polarograms were obtained on Yanagimoto Pen-recording Polarograph Model P-B4 (at $D=1$, $S=0.080$ μA/mm). The results are shown in Fig. 2 and Table III.

**Preparation of Disodium 2,3-Diketo-4,5-dehydro-adipate (VII)**——After bubbling of oxygen through 4.8 ml of 0.2 N NaOH for 5 min, a solution of the reductone (50 mg) in 5 ml MeOH was added, and the bubbling was further continued for 20 min at 0°. Then, pH of the solution was adjusted to 7.0 by addition of Amberlite IR-120 ($H^+$). After filtration of the ion exchanger, the resulting pale yellow solution was concentrated to 2 ml under reduced pressure at 30°, and 10 ml of EtOH was added, and again concentrated to 3 ml. Yellow precipitates were filtered off after standing for 2 hr at 0°, washed with EtOH, and dried to give VII, decomp. 95—97°. *Anal.* Calcd. for $C_6H_2O_6Na_2$: Na, 21.28. Found: Na, 21.54.

**Reaction of 4,5,6-Trioxo-2,3-dehydro-caproic Acid (VIII) with Phenylhydrazine**——4 ml of 1 N iodine solution was added dropwise to a solution of the reductone (300 mg) and $CH_3COONa$ (600 mg) in 3 ml MeOH at 0°, then 1 ml of phenylhydrazine was added under stirring. The resulting gum was washed with EtOH, and the reaction product hardly soluble in EtOH was obtained as red crystals, decomp. 154—155°. *Anal.* Calcd. for $C_{18}H_{16}O_3N_4$: C, 64.27; H, 4.80; N, 16.66. Found: C, 64.21; H, 5.13; N, 16.49.

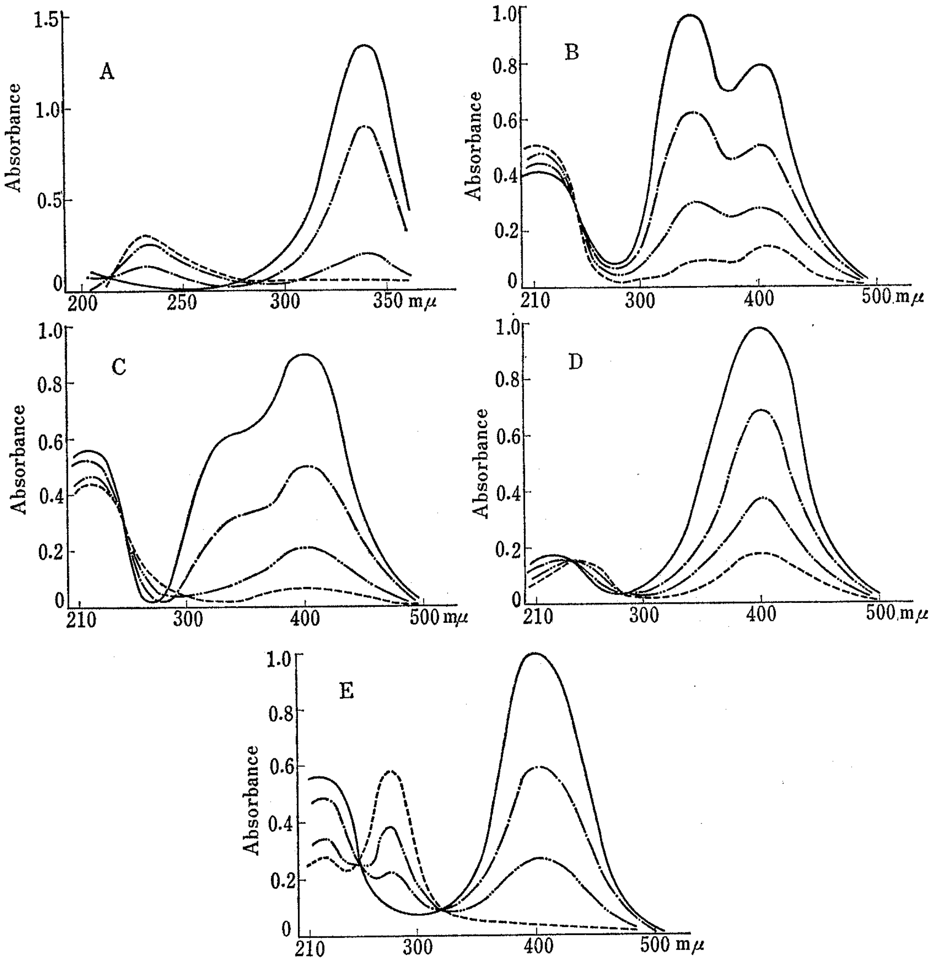

Fig. 1. Absorption Spectra of the Reductone under Nitrogen except E

A: in 0.1N HCl (———: 45 sec, —·—: 30 min, —··—: 210 min, ---------: 23 hr),
B: in solution of pH 7.5 (———: 2 min, —·—: 3 min, —··—: 6 min, ---------: 9 min),
C: in solution of pH8.0 (———: 2 min, —·—: 3 min, —··—: 6 min, ---------: 14 min),
D: in solution of pH 10.0 (———: 45 sec, —·—: 1 min, —··—: 2 min, ---------: 6 min),
E: in solution of pH 10.0 under air (———: 45 sec, —·—: 1.5 min, —··—: 2.5 min, ---------: 30 min)

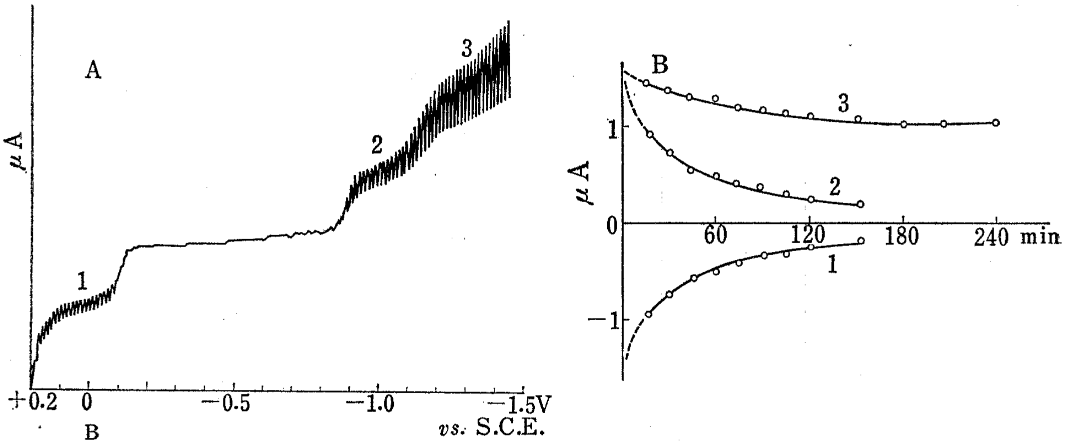

Fig. 2. A: Polarogram of the Reductone under Nitrogen in the Buffer Solution of pH 5.0; B: Time Course of the Polarographic Wave Hights

1: anodic wave, 2: first reduction wave, 3: second reduction wave

Table III. Half-wave Potentials of the Reductone in the Buffer Solutions of pH 3.0 and 5.0

| pH | $E_{1/2}$ (Volts) *vs.* S.C.E. | | |
|---|---|---|---|
| | Oxidation wave | Reduction | |
| | | First wave | Second wave |
| 3.0 | +0.05 | −0.73 | −1.05 |
| 5.0 | −0.10 | −0.89 | −1.16 |

## Results and Discussion

### On the Structure of the Reductone in Acidic Solutions

The reductone has an absorption band at a longer wave length ($\lambda_{\max}$ 340 m$\mu$ in acidic solution and $\lambda_{\max}$ 338—356 m$\mu$ in nonaqueous solvents as shown in Table I and II) than that of L-ascorbic acid ($\lambda_{\max}$ 245 m$\mu$ at pH 2.0 and $\lambda_{\max}$ 265 m$\mu$ at pH 6.4)[3] and triose reductone, HCO–C(OH)=CH(OH) ($\lambda_{\max}$ 266 m$\mu$ in acidic solution and $\lambda_{\max}$ 310 m$\mu$ in alkaline solution).[4] The assignment of the absorption band at 340 m$\mu$ to formula (I-A) or (I-B) is supported by the

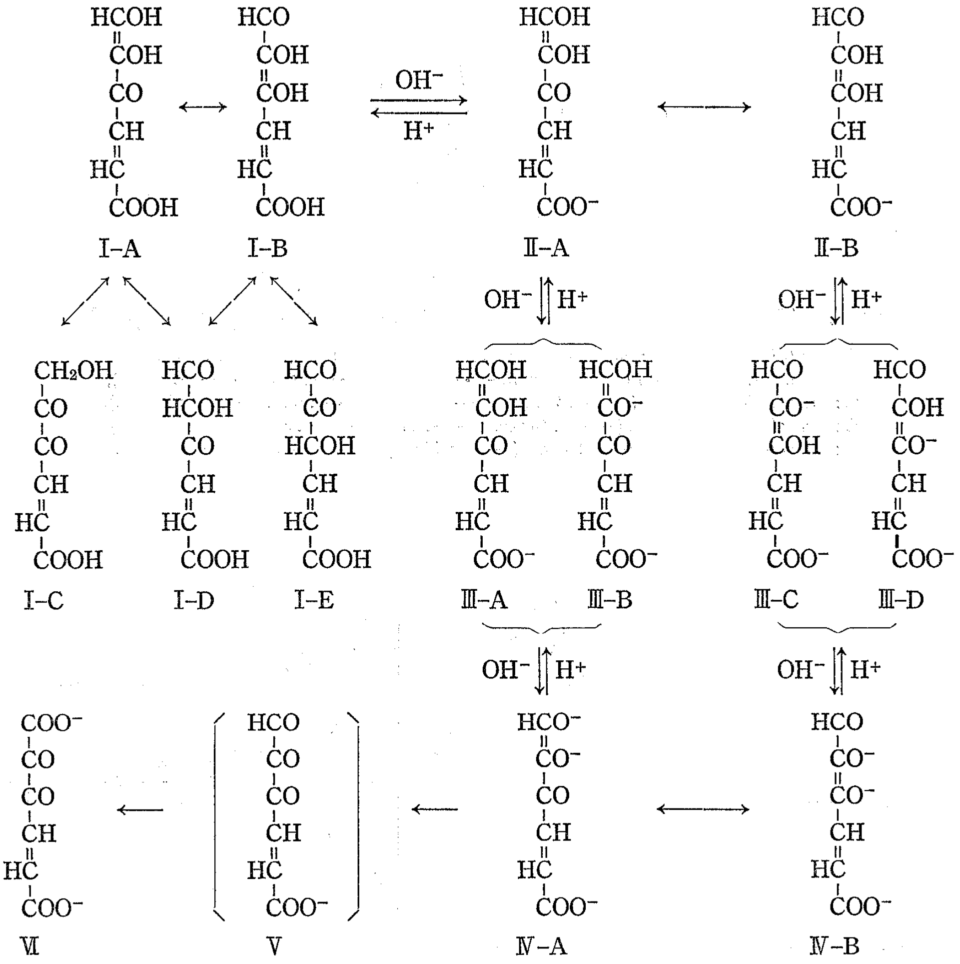

Chart 1

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[3] P. Karrer, H. Salomon, R. Morf and K. Schöpp, *Biochem. Z.*, **258**, 8 (1933); G. Carteni, *Helv. Chim. Acta*, **21**, 1031 (1938).

[4] H. von Euler, H. Hasselquist and G. Hanshoff, *Z., Naturforshg.*, **86**, 636 (1953).

known relation between structure and location of the absorption maximum, that is, a compound containing a conjugated dienone substituted with two hydroxyl groups would have a calculated absorption band at 333 m$\mu$ in ethanol.[5]

On the other hand, the decrease in the polarographic anodic wave of the reductone in a solution of pH 5.0 in Fig. 2 as well as pH 3.0, accounts for the change of $-C(OH)=C(OH)-$ in I-A or I-B to a $-CO-CH(OH)-$ grouping in acidic solutions on standing. This fact is consistent with the result obtained on the titration of the reductone with iodine and 2,6-dichloroindophenol sodium as mentioned in the preceding paper.[1] The decrease in the first reduction wave and the increase in the second reduction wave correspond to the change of a more easily reducible conjugated dienone grouping in I-A or I-B to a less easily reducible carbonyl group in I-C, I-D or I-E as shown in Chart 1. In consideration of tautomerism, formulas (I-C, D and E) are possible in acidic solution as in the case of methylreductone.[6] The presence of I-D is supported by the fact that the reductone reacted with three moles of hydroxylamine in acidic solution as reported in the preceding paper.[1] The increase in the intensity of the absorption band at 230 m$\mu$ (band A in Table II) in acidic solutions might be ascribed to the transformation of I-A or I-B to I-C or I-D, not to I-E, in comparision with ethyl 4-oxo-2-pentenoate ($\lambda_{max}$ 220 m$\mu$, log $\varepsilon=4.1$ in EtOH)[7] and 3-oxo-6,7-secocholest-4-ene-6,7-dienic acid ($\lambda_{max}$ 236 m$\mu$, log $\varepsilon=4.0$ in EtOH),[8] $pKa_1=3.1$ and $pKa_2=7.0$ for the reductone were obtained by the titrimetric method. The former corresponds to the carboxyl group of the reductone in comparision with fumaric acid ($pKa_1=3.0$, $pKa_2=4.1$) and 4-oxo-glutaconic acid ($pKa_1$, $pKa_2=3.2$). On the complete transformation of (I-A or B) to (II-A or B) in a solution of pH 5.0, no shift was observed in absorption maximum as shown on band B in Table II.

**On the Structure of the Reductone in Neutral and Alkaline Solutions**

Bathochromic displacement by 60 m$\mu$ on absorption maximum of the reductone between $\lambda_{max}$ 400 m$\mu$ (band E in Table II) in alkaline solution and $\lambda_{max}$ 340 m$\mu$ (band B in Table II) in acidic solution, is in good agreement with the displacement by 44 m$\mu$ in the case of triose reductone due to the dissociation of its ene–diol grouping in alkaline solution. $pKa_2=7.0$ for the ene–diol grouping of the reductone obtained by the titrimetric method is also compatible with the result from the absorption spectral data that the concentration of the reductone represented in formulas (II-A and B) is nearly identical with that in formulas (III-A to D) in solution of pH 7.0. In 0.1 N NaOH and in the more alkaline solutions, the reductone has an absorption band at $\lambda_{max}$ 418 to 420 m$\mu$ (band E in Table II), and then might exist in formula (IV-A) or (IV-B). In the more alkaline solution than pH 8, the absorption band at 280 m$\mu$ (band D in Table II) increased under air, not nitrogen, as shown in Fig. I-E, on account of the oxidation of (IV-A or B) to VI. Disodium 2,3-diketo-4,5-dehydro-adipate (VII) which has an absorption band at 280 m$\mu$ in alkaline solution, was isolated by the oxidation of the reduc-

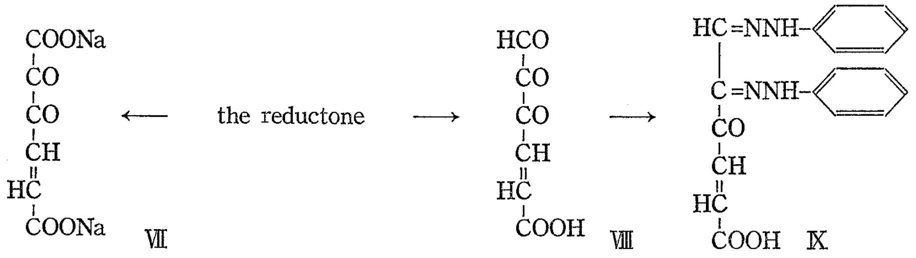

Chart 2

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5) L.F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Corp., New York, 1949.

6) G. Hesse and H. Stahl, *Chem. Ber.*, **89**, 2424 (1965).

7) S. Raymond, *J. Am. Chem. Soc.*, **72**, 4304 (1950).

8) L.F. Fieser, *J. Am. Chem. Soc.*, **75**, 4386 (1951).

tone with oxygen in aqueous methanol solution of NaOH. The reductone was oxidized to VIII by iodine in acidic solution. Treatment of VIII with two moles of phenylhydrazine gave bisphenylhydrazone which was assumed to have formula (IX) as shown in Chart 2.

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