$Na_2S_2O_3$ solution. The solid was collected, washed with water and recrystallized from MeOH–pyridine to give pale yellow prisms (54 mg.), m.p. 310°(decomp.). One spot by TLC (Rf : 0.37). *Anal.* Calcd. for $C_{30}H_{17}O_9(OMe)$: C, 67.39; H, 3.65; OMe, 5.62. Found : C, 67.73; H, 3.91; OMe, 7.18.

ii) A mixture of cryptomerin A and B (200 mg.) was treated with PhOH (100 mg.) and HI (12 ml.) as described above. Before recrystallization the yellow solid (200 mg.) gave two spots by TLC (Rf : 0.26, 0.37). Two recrystallizations from a EtOH–pyridine mixture furnished the pure monomethyl ether (isocryptomerin, Rf : 0.37) as pale yellow prisms (100 mg.), m.p. and mixed m.p. 310° (decomp.) giving the same IR spectrum. The solvent was distilled off *in vacuo* from the mother liquor separated from the crystals and the yellow materials obtained were treated with hot EtOH and recrystallized twice from a mixture of MeOH (4 ml.) and pyridine (0.4 ml.) to give yellow crystals (70 mg.), m.p. 336° (decomp.). One spot by TLC (Rf : 0.26) and the same IR spectrum with hinokiflavone.

Isocryptomerin Tetraacetate——Isocryptomerin (100 mg.) was refluxed with Ac_2O (1 ml.) and AcONa (100 mg.) for 1 hr. Two recrystallizations from AcOEt yielded colorless crystals (65 mg.), m.p. 211°(softening at 208°). *Anal.* Calcd. for $C_{30}H_{13}O_5(OMe)(OAc)_4$: C, 65.00; H, 3.91. Found : C, 64.55; H, 3.81. NMR δ p.p.m. : 3.86 (3H, OMe); 2.45 (3H, OAc); 2.41 (3H, OAc); 2.25 (6H, OAc). IR (KBr) cm^{-1} : $\nu_{C=O}$ 1769, ν_{C-O} 1197, 1170, 1136.

Isocryptomerin Tetraethyl Ether——Isocryptomerin (100 mg.) was ethylated with diethyl sulfate (1 ml.) and 30% KOH solution at 50～60°, filtered, washed with water and recrystallized twice from EtOH to yellow crystals (35 mg.), m.p. 260°(softening at 254°). *Anal.* Calcd. for $C_{30}H_{13}O_5(OMe)(OEt)_4 \cdot H_2O$: C, 68.61; H, 5.61. Found : C, 69.06; H, 5.35.

Methylation and Demethylation of Isocryptomerin——i) Isocryptomerin (100 mg.) was methylated with dimethyl sulfate and the crude product was recrystallized from 70% dioxane to pale yellow crystals (20 mg.), m.p. and mixed m.p. with hinokiflavone pentamethyl ether 259～260°.

ii) A mixture of isocryptomerin (15 mg.), PhOH (300 mg.), Ac_2O (1.5 ml.), and HI (15 ml.) was refluxed for 1 hr. A homogeneous compound was obtained and identified with hinokiflavone by TLC.

Partial Demethylation under Controlled Conditions——Compound (100 mg.) was treated as described above in the case of isocryptomerin under the condition shown in Table II. The reaction product washed with water and dried was examined by TLC.

[Chem. Pharm. Bull. 15(2) 235～238 (1967)]

UDC 612.398.1-083 : 598.617 : 591.465

Tatsuzo Fujii,*1 Toshiko Shimano,*1 and Tomio Fujii*2 : Dissociative Behavior of High-Density Lipoprotein of Egg Yolk.

(*Department of Biochemistry, Gifu University School of Medicine**1 *and Department of Physiology, Faculty of Medicine, Kagoshima University**2)

(Received May 27, 1966)

It was first reported by Joubert and Cook,[1] Fujii[2] and Sugano[3] that lipovitellin, a high-density lipoprotein of hen egg yolk which has a sedimentation constant of 11 S, is reversibly dissociated in alkaline medium to form a 7 S subunit with half a molecular weight of the original lipovitellin. It was also revealed that the degree of such a dissociation increases with increasing alkalinity. Later, it was found by Bernardi and Cook[4] that the lipovitellin is, in reality, a mixture of two high-density lipoproteins, α- and β-lipovitellin, which can be distinguished by their protein phosphorus content

*1 Tsukasamachi, Gifu (藤井達三, 嶋野淑子).
*2 Yamashitacho, Kagoshima (藤井富男).

1) F. J. Joubert, W. H. Cook : Can. J. Biochem. Physiol., **36**, 389 (1958).
2) T. Fujii : Acta Embryol. Morphol. Exptl., **3**, 260 (1960).
3) H. Sugano, I. Watanabe : J. Biochem., **50**, 473 (1961).
4) G. Bernardi, W. H. Cook : Biochim. et Biophys. Acta, **44**, 96 (1960).

and the pH value required for equivalent dissociation; α-lipovitellin requires pH 10.5 and β- pH 7.8 to produce 50% dissociation (Burley and Cook[5,6]). Furthermore, Radomski, Wallace and Cook[7] reported a significant difference in the dissociative behaviors between avian and amphibian lipovitellins, showing that, in carbonate buffer at pH 11.0 (I 0.2), α-lipovitellin of hen egg was 72% dissociated, while lipovitellin of frog egg, having a sedimentation constant of 10.7 S, was 40% dissociated into a 6.8 subunit.

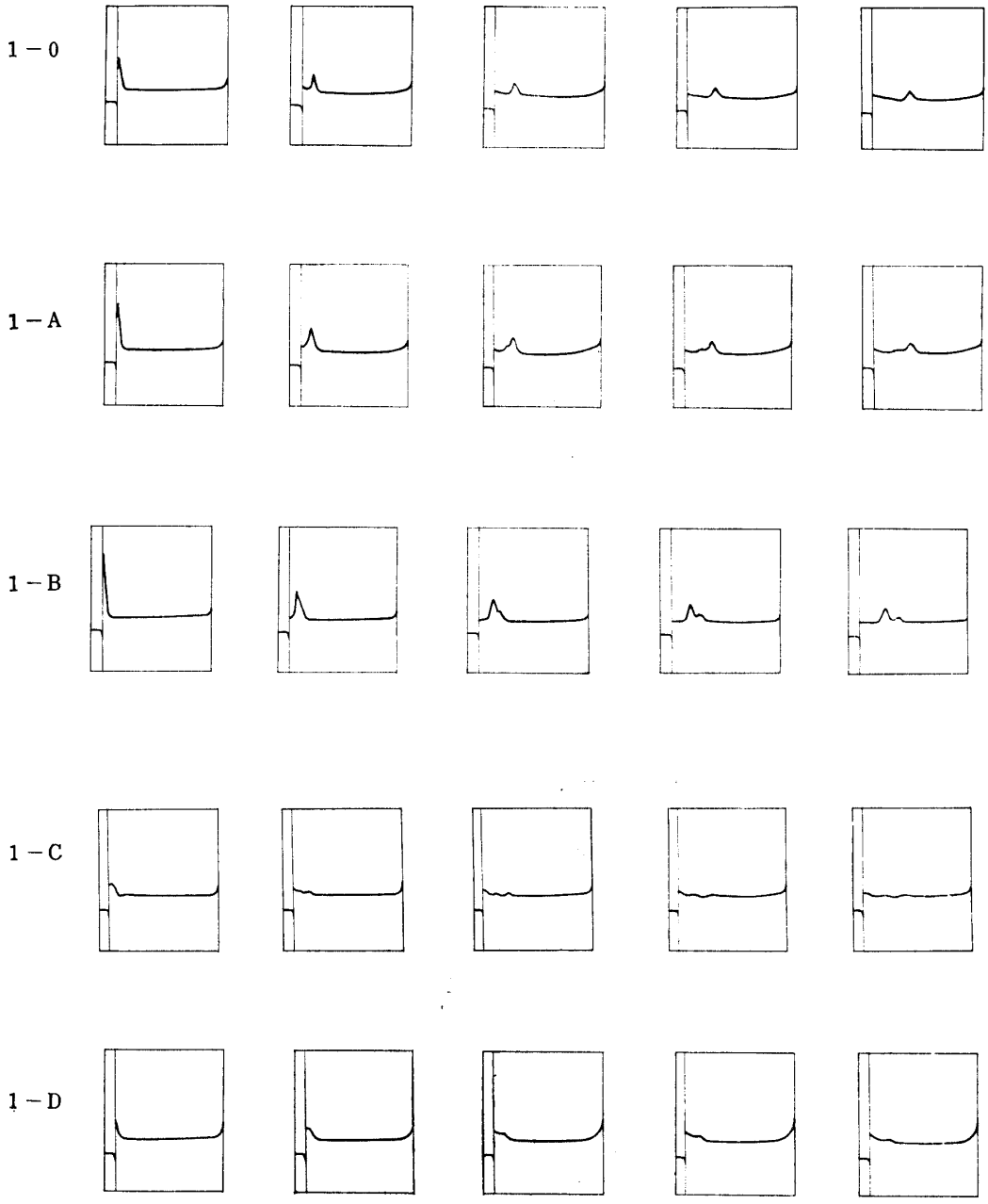


Fig. 1. Sedimentation Patterns of Lipovitellin before and after Certain Treatment

1-0: before treatment (control) 1-A: alkaline medium (pH 11.0)
1-B: incubation (37°, 24 hrs.) 1-C: detergent (0.25% desoxycholate)
1-D: oxidant (1% H_2O_2)
Buffer used: 1-0, 1-B, 1-C and 1-D in 0.8*M* NaCl-0.05*M* phosphate buffer (pH 7.0); 1-A in carbonate-bicarbonate buffer (pH 11.0, I 0.2)
Ultracentrifugation: at 60,000 r.p.m. (227,000 ×g.); photographs taken at 8 minutes intervals.

5) R. W. Burley, W. H. Cook: Can. J. Biochem. Physiol., **40**, 363 (1962).
6) *Idem*: *Ibid.*, **40**, 373 (1962).
7) M. W. Radomski, R. A. Wallace, W. H. Cook: Biochim. et Biophys. Acta, **70**, 600 (1963).

From these facts, it is understood that any avian and amphibian lipovitellin so far studied exists as a dimer form in natural state, while in alkaline medium, a portion of it is dissociated into monomer form, the degree of the dissociation varying considerably on respective lipovitellin.

Investigating on the influence of detergent and oxidant treatments and of incubation on the hen egg lipovitellin molecule, the present authors found that, by means of certain kinds of them, lipovitellin is irreversibly split into a molecular species which almost corresponds to the above-mentioned monomer reversibly formed in alkaline environment, and the result is the subject of the present note.

Hen egg lipovitellin was prepared and purified*3 by fractional precipitation[2] followed by agar-gel filtration.[8] This preparation was subjected to each one of the following treatments and subsequently analyzed by ultracentrifugation.

A) Dissolved in carbonate-bicarbonate buffer (pH 11.0, I 0.2).

B) Incubated at 37° for 24 hours in 0.8*M* NaCl-0.05*M* phosphate buffer (pH 7.0) containing phenylmercuric acetate (10 μg./ml.) to prevent microbial contamination.

C) It was treated with 0.25% sodium desoxycholate solution at room temperature for 10 minutes, and after removing insoluble denatured protein, the supernatant was filtered through 4% granulated agar-gel column immediately to remove the detergent.

D) Treated with 1% hydrogen peroxide solution at room temperature for 10 minutes (in this case, too, significant amount of insoluble product was formed).

E) Irradiated by ultrasonic wave (10 KC, 100 W) in 0.8*M* NaCl-0.05*M* phosphate buffer (pH 7.0).

Fig. 1 shows the sedimentation patterns of the purified lipovitellin before (Fig. 1-0) and after (1-A through 1-D) respective treatment. $S_{20,w}$ values were calculated from these diagrams and indicated on the TABLE I, together with the ratio of S value of the formed component (LV-M) *versus* that of the original lipovitellin (LV-D), and also the ratio of molecular weight*4 of LV-M *versus* LV-D.

TABLE I. The Ratio of Sedimentation Constant and of Molecular Weight of Dissociation Product (LV-M) *versus* the Original Lipovitellin (LV-D)

Treatment	Component	$S_{20,w}$	$S_{20,w}$ ratio	$M^{a)}$ ratio
None (intact LV)	LV-D	11.0		
A) Alkaline medium (pH 11.0)	〃	10.9	1	1
	LV-M	7.1	0.65	0.52
B) Incubation (37°, 24 hr.)	LV-D	10.7	1	1
	LV-M	7.1	0.66	0.54
C) Detergent (0.25% desoxycholate)	LV-D	10.2	1	1
	LV-M	6.5	0.64	0.51
D) Oxidant (1% H_2O_2)	(LV-D$^{b)}$)	(10.1)	1	1
	LV-M	6.1	0.60	0.47
E) Ultrasonic issadiation	LV-D	10.7		

a) M=molecular weight, calculated according to the following equation:

$$M=\frac{4690(S)^{3/2}[\eta]^{1/2}}{(1-\bar{V}\rho)^{3/2}}=\frac{4690(S)^{3/2}(0.070)^{1/2}}{(1-0.744\times0.998)^{3/2}} \qquad \mathrm{Log}\,M=\mathrm{Log}\,S+3.9789$$

were S: sedimentation constant at 20° in water
$[\eta]$: intrinsic viscosity
$\bar{V}$: partial specific volume
ρ: density of water at 20°

b) After this treatment, the original lipovitellin does not exist.

*3 No attempt was made to separate the lipovitellin into α- and β-components.

*4 Calculated by an equation cited under Table I, using the values of necessary physicochemical constants reported previously.[2]

8) T. Fujii, *et al.*: J. Biochem., in press.

From these data, it is disclosed that, except the ultrasonic treatment which brought about no apparent influence on the physical state of the lipovitellin molecule, all the treatments similarly yielded a smaller component of which molecular weight corresponds to about one half (0.47～0.54) of the original lipovitellin. It is worth to note that the treatment B, C and D all irreversibly give rise to such a monomer-like component as reversibly formed in alkaline medium (A). Particularly, an oxidation treatment by means of hydrogen peroxide yielded the 6 S component solely, without leaving the original 10 S component (though a significant amount of insoluble denaturant was yielded at the same time).

Therefore, it is supposed that hen lipovitellin in a dimer form is easily split into its monomer, reversibly or irreversibly, by certain treatments, though the mechanism is not clear yet. In this respect, it is recalled that the frog lipovitellin exists in two molecular forms, 11 S and 6 S components, in the course of its embryonal development (detected at the rotation stage) as reported previously,[2] whereas in unfertilized egg it is present only as a dimer form. Significance of such a dimer-monomer dissociation with reference to the utilization of lipoproteins during the course of development remains to be clarified.

[Chem. Pharm. Bull. 15(2) 238～240 (1967)]

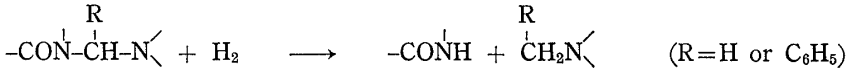
UDC 542.941 : 547.554.04

Minoru Sekiya and Masayasu Tomie : Reaction of Amide Homologs. XVII.*1 Catalytic Hydrogenolysis of N,N′-Benzylidenebisamides.

(*Shizuoka College of Pharmacy**2)

(Received May 28, 1966)

Previously, facile hydrogenolysis of N-amidomethyl[1,2] or N-α-amidobenzyl compound[1] attached to secondary amine has been reported from this laboratory to proceed as in the following, generally by catalytic hydrogenation under high hydrogen



pressure at elevated temperature using Raney nickel catalyst. However, in the study[2] on the scope of the reaction for variety of N-amidomethyl compounds, N,N′-methylenebisamides have been shown to resist to the hydrogenolysis under the above conditions and N,N′-benzylidenebisamides were shown as well. Lately, it was found that the hydrogenolysis of N,N′-benzylidenebisamide was effected in acidic environment chiefly to afford N-benzylamide and amide. The conditions were that the hydrogenation was carried out in acetic acid under high hydrogen pressure at elevated temperature using palladium-on-charcoal catalyst.

A series of N,N′-benzylidenebisamides, *i.e.*, N,N′-benzylidenebisformamide, -bisacetamide, -bispropionamide, and -bisbenzamide were elected. Table I shows the

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*1 Part XVI : M. Sekiya, N. Yanaihara : This Bulletin, **15**, 108 (1967).

*2 Oshika, Shizuoka (関屋 実, 冨江正泰).

1) M. Sekiya, K. Ito : This Bulletin, **11**, 892 (1963).

2) *Idem* : *Ibid.*, **14**, 996 (1966).