

Ozonolysis of 9-Octadecylenedioic Acid (V).—Ozonized oxygen in excess was passed through an acetic acid solution (60 ml.) of V (75 mg.), m.p. 99–100°, at room temperature. After evaporation of approximately 50 ml. of the solvent in a current of dry nitrogen at temperatures no higher than 45°, 10 ml. of 30% hydrogen peroxide (Merck Superoxol) and 5 ml. of water were added, and the mixture was allowed to stand overnight at room temperature. The azelaic acid cleavage product, after isolation and crystallization from water and from ethyl acetate, melted alone or admixed with authentic azelaic acid at 105–106°. The yield was 74 mg. or 81%.

cis-9-Octadecenedioic Acid (VI) from 9-Octadecylenedioic Acid (V).—A solution of 6.2 g. (0.020 mole) of 9-octadecylenedioic acid (melting a few degrees lower than the analytical sample) in 170 ml. of absolute methanol containing 150 mg. of 10% palladium-on-carbon that had been saturated previously with hydrogen was stirred at room temperature under one atmosphere of hydrogen. Hydrogen corresponding to 98% of the theoretical for one mole was absorbed after one-half hour. No further absorption was evident after another 15 minutes of stirring. The catalyst was removed by filtration through a sintered glass funnel and was washed with two small portions of absolute methanol. Removal of all solvent from the combined filtrates by evaporation in a stream of dry nitrogen at room temperature left 6.18 g. of a white solid, m.p. 67–68°. Two crystallizations of this material from ethyl acetate gave 4.3 g. of product, m.p. 69–70°. Reworking the mother liquors gave an additional 1.6 g. with the same melting point. The total yield of *cis*-acid VI was 94%.

Anal. Calcd. for C₁₈H₃₂O₄: C, 69.2; H, 10.3; neut. equiv., 156. Found: C, 69.3; H, 10.3; neut. equiv., 156.

Phloionic Acid (VII) from cis-9-Octadecenedioic Acid.¹⁵—To a solution of 6.2 g. (0.020 mole) of 9-octadecenedioic acid in 40 ml. of 98–100% formic acid was added 3 ml. of aqueous hydrogen peroxide containing 0.023 mole of peroxide by iodimetric titration. An exothermic reaction was noted. The flask was kept for one hour in a 40° water-bath.

The reaction mixture was poured into 150 ml. of 4 *N* sodium hydroxide solution, and the solution was boiled for one-half hour. Concentrated hydrochloric acid was added to bring the pH to 2, and the acid solution was allowed to stand at 0° for 18 hours. The solids were collected by filtrations, washed with cold water and air-dried. Two crystallizations from ethyl acetate raised the melting points from 114–117° to 121–122°, and afforded 6.4 g. (93%) of phloionic acid. A third crystallization did not change the melting point.

Anal. Calcd. for C₁₈H₃₄O₆: C, 62.4; H, 9.9; neut. equiv., 173. Found: C, 62.2; H, 10.0; neut. equiv., 173, 175.

The melting point of a mixture of this synthetic phloionic acid with phloionic acid isolated from cork (*vide infra*) was 120.5–122°.

meso-9,10-Dihydroxyoctadecanedioic Acid (VII) from cis-9-Octadecenedioic Acid (VI).¹⁸—A solution of 3.1 g. (0.010 mole) of acid VI in 20 ml. of water containing 1 g. of potassium hydroxide was cooled to 5°, finely powdered potassium permanganate (1 g. or 0.0063 mole) was added, and the mixture was allowed to stand without cooling for ten minutes. Sulfur dioxide was bubbled into the mixture until all the precipitated manganese dioxide dissolved. The resulting mixture (pH 4) was cooled, and the white precipitate was collected on the funnel, washed with 4 ml. of cold water, and dried by drawing air through the funnel. The crude product was boiled with 40 ml. of ethyl acetate, and the hot solution gravity filtered to remove some insoluble material. The filtrate, kept at 0°, deposited 1.9 g. of white crystals, m.p. 152–155°. Two additional crystallizations from ethyl acetate (25 and 20 ml.) gave 1.54 g. of material melting at 156–157°. Another crystallization did not change the melting point.

Anal. Calcd. for C₁₈H₃₄O₆: C, 62.4; H, 9.9; neut. equiv., 173. Found: C, 62.5; H, 10.1; neut. equiv., 173, 177.

The total yield of *erythro*-9,10-dihydroxyoctadecanedioic acid, m.p. 156–157°, including material obtained on reworking mother liquors, was 1.91 g. (55%).

Phloionic Acid (VII) from Cork.—Isolation of phloionic acid from cork was carried out as described by Guillemonat and Cesaire¹⁷ except that the dry mixture of phloionic acid and calcium sulfate (from 500 g. of powdered cork) was continuously extracted in a Soxhlet apparatus with 350 ml. of ethyl acetate for two days. The extract was concentrated to 200 ml. by distillation, and was cooled first to room temperature and then to 0° where it was held for two days. The precipitate (8.6 g., m.p. 115–119°) was collected and was recrystallized twice from ethyl acetate. Decolorizing carbon (Darco G-60) was used in one of the crystallizations. Phloionic acid (5.43 g.) was obtained in this way as white platelets, m.p. 121–122°. Reworking mother liquors gave an additional 0.48 g. with the same melting point; neut. equiv., calcd., 173; found, 172, 177.

Other isolation experiments starting with 500-g. batches of cork dust afforded 6.8, 7.0 and 7.2 g. of phloionic acid, m.p. 121–122°.

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[CONTRIBUTION FROM THE COLLEGE OF PHARMACY, UNIVERSITY OF MICHIGAN]

The Reaction of the Chloromagnesium Derivative of Chloromagnesium Phenylacetate with Isocyanates, Carbamyl Chlorides and Isothiocyanates

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The Ivanov reagent, obtained from phenylacetic acid and isopropylmagnesium chloride, reacted with isocyanates and carbamyl chlorides to yield *N*-substituted α -phenyl- α -carbamylacetic acids. The products isolated when the Ivanov reagent reacted with isothiocyanates were *N*-substituted thioamides of phenylacetic acid.

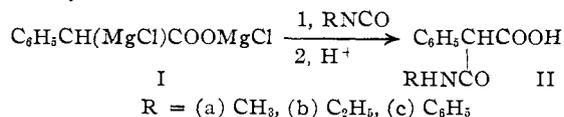
Since it is known that the common type Grignard reagents react with isocyanates,³ it was to be expected that the Ivanov reagent, C₆H₅CH(MgCl)-COOMgCl (I), prepared from phenylacetic acid and isopropylmagnesium chloride, would react with isocyanates in the manner indicated below. By

(1) This paper represents part of a dissertation submitted by Harold Zinnes in partial fulfillment of the requirements for the Ph.D. degree in the University of Michigan, 1955.

(2) American Foundation for Pharmaceutical Education Fellow.

(3) See M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Nonmetallic Substances," Prentice-Hall, Inc., New York, N. Y., 1954, p. 1199.

interaction of compound I with isocyanates, three *N*-substituted α -phenyl- α -carbamylacetic acids (II) were obtained; they were converted into their methyl esters.



The Ivanov reagent I reacted with diethylcarbamyl chloride and with diphenylcarbamyl chloride to form α -phenyl- α -(diethylcarbamyl)-acetic (III)

and α -phenyl- α -(diphenylcarbonyl)-acetic (IV) acids, respectively; methyl esters also were prepared from these acids.

The five acids (Table I) mentioned above decompose slowly at room temperature, but rapidly when heated, into acid amides of phenylacetic acid.

When methyl α -phenyl- α -(diethylcarbonyl)-acetate was treated with lithium aluminum hydride, β -phenyl- γ -diethylaminopropanol was obtained.

The hydrochloride of the β -diethylaminoethyl ester of IIb was prepared by ester exchange and of IIc by use of the Horenstein-Pählicke⁴ procedure.

When compound I was allowed to react with ethyl isothiocyanate the reaction product, α -phenyl- α -(ethylthiocarbonyl)-acetic acid, underwent decarboxylation during the isolation procedure and α -phenyl-N-ethylthioacetamide was obtained. Similarly, after reaction of I with phenyl isothiocyanate, α -phenylthioacetanilide was isolated as the reaction product.

Experimental

N-Substituted α -Phenyl- α -carbonylacetic Acids (Table I).—Isopropylmagnesium chloride was prepared from 10.7 g. of magnesium, 50 cc. of isopropyl chloride and 200 cc. of ether. After the addition of 400 cc. of benzene, the mixture was stirred and 27.2 g. of phenylacetic acid dissolved in 100 cc. of benzene was added, dropwise. The mixture was refluxed for 18 hours, 0.24 mole of the required isocyanate or substituted carbonyl chloride, dissolved in 125 cc. of benzene, was added and the material was refluxed for 4 hours.

The following procedure was used for the isolation of IIb and III:

(A) The mixture was poured into 400 cc. of ice-cold, 10% ammonium chloride solution. After separation and extraction of the organic layer with water, the combined aqueous layers were washed with ether. The aqueous solution was cooled in an ice-bath, stirred and an ice-cold mixture of 20 cc. of concentrated sulfuric acid and 150 cc. of water was added slowly. The precipitated oil was extracted with ethyl acetate. The extract was washed with water, dried over magnesium sulfate and the solvent was removed under an air jet. The residue was stirred with 300 cc. of diisopropyl ether, and the mixture was refrigerated for 24 hours. The products, after they had been washed several times with ice-cold diisopropyl ether, were practically pure. They were obtained only in poor yield after recrystallization.

Compounds IIc and IV were isolated by the following process: (B) The reaction mixture was poured into a stirred, ice-cold mixture of 300 cc. of concentrated sulfuric acid and 500 cc. of water and then shaken with 300 cc. of ethyl acetate. After separation of the layers, the aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with water. The organic solution was extracted with a solution of 40 g. of potassium carbonate in 500 cc. of water and the alkaline extract was washed with ether. Upon the addition of an ice-cold mixture of 30 cc. of concentrated sulfuric acid and 200 cc. of water, the gummy product precipitated. It was treated as described above (A).

For the preparation of IIa, all of the benzene, except that required to dissolve the phenylacetic acid, was replaced by ether. After the addition of the methyl isocyanate, the mixture was refluxed for 20 hours. The product was isolated according to procedure A.

Methyl Esters (Table I).—The acid (0.15 mole) dissolved or suspended in 50 cc. of ethyl acetate, was treated with an excess of ethereal diazomethane. After 30 minutes, the excess diazomethane was destroyed with a few drops of acetic acid, the solvent was removed under reduced pressure, diisopropyl ether was added to the residue and the mixture was refrigerated for 12 hours.

Decarboxylation of Acids.— α -Phenyl- α -(methylcarbonyl)-acetic acid was heated in an oil-bath at 150–160° for 2

hours and the α -phenyl-N-methylacetamide produced was recrystallized from petroleum ether (40–60°); m.p. 56–57°.⁵
 α -Phenyl- α -(phenylcarbonyl)-acetic acid yielded α -phenylacetanilide, m.p. 118–119°⁶ after recrystallization from ethanol.

α -Phenyl- α -(diphenylcarbonyl)-acetic acid was converted into α -phenyl-N,N-diphenylacetamide, m.p. 75–76° after recrystallization from petroleum ether (30–40°).

α -Phenyl- γ -diethylaminopropanol.—Methyl α -phenyl- α -(diethylcarbonyl)-acetate (37.4 g.), dissolved in 400 cc. of hot ether, was treated with 6.8 g. of lithium aluminum hydride suspended in 150 cc. of ether. The mixture was refluxed for 40 hours, 7 cc. of water was added and the mixture was filtered through a sintered glass funnel. The product (22.5 g., 72%) boiled at 159–161° (17 mm.).

Anal. Calcd. for C₁₃H₂₁ON: N, 6.76. Found: N, 6.59.

The hydrochloride was recrystallized from methyl ethyl ketone; m.p. 93–94°.

Anal. Calcd. for C₁₃H₂₀ONCl: N, 5.75; Cl, 14.55. Found: N, 5.52; Cl, 14.33.

The *p*-nitrobenzoate hydrochloride melted at 178–180° dec. after recrystallization from methyl ethyl ketone.

Anal. Calcd. for C₂₀H₂₅O₄N₂Cl: N, 7.13; Cl, 9.02. Found: N, 6.98; Cl, 8.96.

β -Diethylaminoethyl α -Phenyl- α -(phenylcarbonyl)-acetate Hydrochloride.— β -Diethylaminoethyl chloride (5.4 g.), dissolved in 5 cc. of benzene, was added dropwise during a period of an hour to a stirred, refluxing solution of 11.2 g. of α -phenyl- α -(phenylcarbonyl)-acetic acid in 60 cc. of isopropyl alcohol. The mixture was refluxed for 6 hours, the hot mixture was filtered and ether was added to the filtrate. After 12 hours in a refrigerator, the precipitate was recrystallized from isopropyl alcohol; yield 9.0 g. (58%), m.p. 153–155°.

Anal. Calcd. for C₂₁H₂₇O₃N₂Cl: N, 7.17; Cl, 9.07. Found: N, 7.05; Cl, 8.92.

β -Diethylaminoethyl α -Phenyl- α -(ethylcarbonyl)-acetate Hydrochloride.—Methyl α -phenyl- α -(ethylcarbonyl)-acetate (11.0 g.) was refluxed with 60 cc. of β -diethylaminoethanol and 0.25 g. of sodium for 36 hours. After distillation of the mixture, the fraction which boiled at 90–125° (0.5 mm.) solidified. After one recrystallization from diisopropyl ether–petroleum ether (40–60°), the ester base (m.p. 66–69°) was converted into the hydrochloride, m.p. 108–110° after recrystallization from methyl ethyl ketone diisopropyl ether, yield 6.0 g.

Anal. Calcd. for C₁₇H₂₇O₃N₂Cl: N, 8.17; Cl, 10.34. Found: N, 7.90; Cl, 10.30.

Reaction of Compound I with Ethyl and Phenyl Isothiocyanate.—When compound I, prepared from 0.10 mole of phenylacetic acid, was allowed to react with 0.12 mole of ethyl isothiocyanate using the same reaction conditions and isolation procedure A employed in the analogous reaction with ethyl isocyanate,⁸ there was obtained 8.6 g. of product which was recrystallized from diisopropyl ether; the colorless needles, α -phenyl-N-ethylthioacetamide, melted at 82–84°.⁹

Anal. Calcd. for C₁₀H₁₃NS: C, 66.98; H, 7.31; S, 17.89. Found: C, 67.08; H, 7.22; S, 17.87.

α -Phenyl-N-ethylthioacetamide was synthesized also in the following manner. To benzylmagnesium bromide,¹⁰ prepared from 17.1 g. of benzyl bromide, there was added 8.7 g. of ethyl isothiocyanate. The mixture was refluxed for 4 hours, decomposed with ammonium chloride solution, the layers were separated and the aqueous layer was extracted with ether. The product obtained by evaporation of the combined ether solution was washed with petroleum ether (30–40°) and then crystallized from 25 cc. of diisopro-

(5) H. J. Taverne, *Rec. trav. chim.*, **16**, 34 (1897), report m.p. 58°.

(6) A. W. Hofmann, *Ber.*, **13**, 1225 (1880), give m.p. 117°.

(7) W. Wislicenus and R. Erbe, *Ann.*, **421**, 157 (1920), report m.p. 73–74°.

(8) However, in this instance, the residue was stirred with 50 cc. of diisopropyl ether instead of 300 cc.

(9) Y. Sakurada, *Bull. Chem. Soc. Japan*, **2**, 307 (1927); *Chem. Zentr.*, **98**, 1, 683 (1927), stated that he obtained this compound as a yellow, viscous oil.

(10) The method used was analogous to that employed by H. R. Henze, B. B. Allen and W. B. Leslie, *J. Org. Chem.*, **7**, 326 (1942), for the preparation of allylmagnesium bromide.

(4) H. Horenstein and H. Pählicke, *Ber.*, **71**, 1644 (1938).

TABLE I

		N-SUBSTITUTED α -PHENYL- α -CARBAMYLACETIC ACIDS AND THEIR METHYL ESTERS $C_6H_5\overset{CONRR'}{C}HCOOH$											
R	R'	Reagent used	M.p., °C. ^b	Yield, ^c %	Formula	Carbon, %		Hydrogen, %		Neut. equiv.			
						Calcd.	Found	Calcd.	Found	Calcd.	Found		
1	CH ₃	H	CH ₃ NCO ^a	112-114	29	C ₁₀ H ₁₁ O ₃ N	62.16	62.16 ^d	5.74	5.83	193.2	193.6	
2	Methyl ester of 1			121-123		C ₁₁ H ₁₃ O ₃ N	63.75	63.84	6.32	6.32			
3	C ₂ H ₅	H	C ₂ H ₅ NCO	110-112	57	C ₁₁ H ₁₃ O ₃ N	63.75	63.52	6.32	6.11	207.2	208.0	
4	Methyl ester of 3 ^e			81-83		C ₁₂ H ₁₅ O ₃ N	65.13	65.43	6.85	7.08			
5	C ₆ H ₅	H	C ₆ H ₅ NCO	132-133	67	C ₁₅ H ₁₃ O ₃ N	70.58	70.83	5.13	5.10	255.3	256.0	
6	Methyl ester of 5 ^f			111-113 ^g									
7	C ₂ H ₅	C ₂ H ₅	(C ₂ H ₅) ₂ NCOCI	91-92	71	C ₁₃ H ₁₇ O ₃ N	66.36	66.49	7.28	7.29	235.3	236.1	
8	Methyl ester of 7			64-66		C ₁₄ H ₁₉ O ₃ N	67.44	67.48	7.68	7.66			
9	C ₆ H ₅	C ₆ H ₅	(C ₆ H ₅) ₂ NCOCI	121-122	47	C ₂₁ H ₁₇ O ₃ N	76.12	76.19	5.17	5.26	331.4	331.9	
10	Methyl ester of 9			113-115		C ₂₂ H ₁₉ O ₃ N	76.50	76.75	5.55	5.68			

^a Prepared by the method of J. Colucci, *J. Can. Research*, **23B**, 111 (1945). ^b All of the acids melted with the evolution of a gas. ^c Yields based on phenylacetic acid. ^d Because of the instability of this compound, it was necessary to analyze it immediately after isolation. ^e Calcd.: N, 6.33. Found: N, 6.27. ^f Calcd.: N, 5.20. Found: N, 5.16. ^g H. Staudinger and H. Hurzel, *Ber.*, **50**, 1031 (1917), report m.p. 109°.

pyl ether; yield 9.5 g. After further recrystallization from diisopropyl ether, the compound melted at 82-84°, mixed m.p. 82-84°.

Compound I was treated with 0.12 mole of phenyl isothiocyanate in the manner described above; the product was isolated by procedure B. The crude material was triturated with 50 cc. of diisopropyl ether and then refrigerated for 12 hours; yield 13.5 g. The yellow α -phenylthioacetan-

ilide melted at 86.5-87.5°¹¹; after recrystallization from diisopropyl ether, mixed m.p. with an authentic sample^{11,12} 86.5-87.5°.

(11) F. Sachs and H. Loewy, *Ber.*, **37**, 875 (1904), m. p. 87°.

(12) We obtained the product in 80% yield by the method of Sachs and Loewy.¹¹

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Physical Chemical Studies of Soluble Antigen-Antibody Complexes. V. Thermodynamics of the Reaction between Ovalbumin and its Rabbit Antibodies¹

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Solutions of soluble complexes formed between ovalbumin (as antigen) and its rabbit antibodies have been subjected to electrophoresis and ultracentrifugation. It has thus been possible to determine the equilibrium concentration of uncombined antigen (Ag) in a given solution of known total antigen and total antibody (Ab) content. With the aid of the Gold-berg theory, the data have been interpreted to give the following information for the reaction $Ag + AgAb \rightleftharpoons (Ag)_2Ab$ in veronal-NaCl buffer, pH 8.5, $\Gamma/2$ 0.3, at 0°: $K = (3.1 \pm 0.5) \times 10^4$, $\Delta F^\circ = -5.6 \pm 0.2$ kcal./mole, $\Delta H^\circ = 0 \pm 2$ kcal./mole, and $\Delta S^\circ = +20 \pm 8$ e.u. These data are remarkably similar to those previously found for the rabbit antibody serum albumin system, and their significance is discussed.

In previous papers of this series,² ultracentrifugal and electrophoretic studies were described with the soluble complexes formed between bovine serum albumin (BSA) and its rabbit antibodies. A general method was introduced^{2b,c} for the evaluation of thermodynamic data for antigen (Ag)-antibody (Ab) reactions, which involves essentially the electrophoretic determination of the amounts of both the total Ag and the free Ag in equilibrium in a solution. The method was applied to the rabbit anti-BSA system,^{2c} and independent tests confirmed its validity.^{2d} We have now investigated the system containing ovalbumin (OA) and its precipitating rabbit antibodies. The thermodynamic data for these two systems are remarkably similar, and suggest that a basic mechanism, more detailed than previously recognized, may be common to all anti-

gen-antibody reactions. This suggestion is further implemented in the following paper.³

Materials and Methods

Ovalbumin.—The protein was a four-times recrystallized preparation⁴ which was lyophilized for storage. About 95% of the protein redissolved in neutral buffers to give an ultracentrifugally homogeneous material. Upon centrifuging off the insoluble residue, the solution remained clear for the time necessary to perform the following experiments.

Solutions of Soluble Complexes.—Two preparations, OA-I and OA-II, were made as previously described for solution VI in the rabbit anti-BSA system,^{2c} except that the pooled hyperimmune antisera were never frozen. In dissolving the specific Ag-Ab precipitate in excess OA, it was found necessary to use no more than gentle stirring in order to avoid denaturation of OA.

Solutions OA-I-1 through OA-I-6, in increasing OA excess, were prepared by weight by the addition of a standard OA solution to OA-I.^{2c} Solution OA-I-4a, used in the re-

(1) This work was supported in part by grants from the Rockefeller Foundation and the United States Public Health Service.

(2) (a) S. J. Singer and D. H. Campbell, *THIS JOURNAL*, **74**, 1794 (1952); (b) **75**, 5577 (1953); (c) **77**, 3499 (1955); (d) **77**, 3504 (1955).

(3) S. J. Singer, L. Eggman and D. H. Campbell, *ibid.*, **77**, 4855 (1955).

(4) S. P. L. Sørensen and M. Høyrup, *Compt. rend. trav. lab. Carlsberg*, **12**, 12 (1915/1917).