tration of 20 mg. per 7.5 ml. of medium the growth of one strain of *E. coli* (unidentified strain T) was completely inhibited. The growth of this microörganism was inhibited to 50% of normal by 13 mg. per 7.5 ml. and completely inhibited by 20 mg. The addition of 0.4 microgram of methionine to 20 mg. of this new inhibitor nullified the inhibitory action and permitted growth to 50% of normal; larger amounts of methionine almost completely reversed the toxicity. Typical experimental results are listed in the table.

TABLE I

THE EFFECT OF 2-AMINO-5-HEPTENOIC ACID ON THE GROWTH OF *E. coli*, STRAIN T

Amount of 2-amino-5-heptenoic acid added per 7.5 ml., mg.	Growth of *E. coli* as turbidity, colorimeter units
0	100 (normal growth)
1	100
5	95
10	87
15	25
20	0
25	0
20 plus 0.01γ methionine	0
20 plus 0.1γ methionine	15
20 plus 0.5γ methionine	58
20 plus 1.0γ methionine	82
20 plus 5.0γ methionine	94
20 plus 10.0γ methionine	95

Preliminary results indicate that several other amino acids will also reverse the toxicity of 2-amino-5-heptenoic acid, but methionine seems the most active.

The growth of two other strains of *E. coli* (Unidentified strain N and the strain listed by the American Type Culture Collection as number 9723) and the yeast were either not at all or only slightly affected by 20 mg. per 7.5 ml. of medium.

These results indicate a metabolite antagonistic relationship between methionine and its vinylene analog, but the potency of the antagonist is very low.

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mrs. Virginia Janda, Mrs. Charmion McMillan and Mrs. Hester Purdy McNulty in the microbiological work. We are also indebted to the Shell Development Company for the butenyl chloride sample.

Summary

2-Amino-5-heptenoic acid, the vinylene analog of methionine, has been prepared by the acetamidocyanoacetate method. The structure of the compound has been established.

The new unsaturated amino acid in high concentrations inhibited the growth of one strain of *E. coli* but did not affect the growth of two other strains of *E. coli* nor one strain of yeast. Where the 2-amino-5-heptenoic acid was bacteriostatic, methionine counteracted the toxicity.

BOULDER, COLORADO RECEIVED JUNE 9, 1948

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

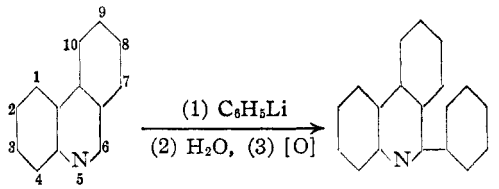
The Reaction of Aryllithium Compounds with 6-Arylphenanthridines

BY HENRY GILMAN AND R. DAVID NELSON

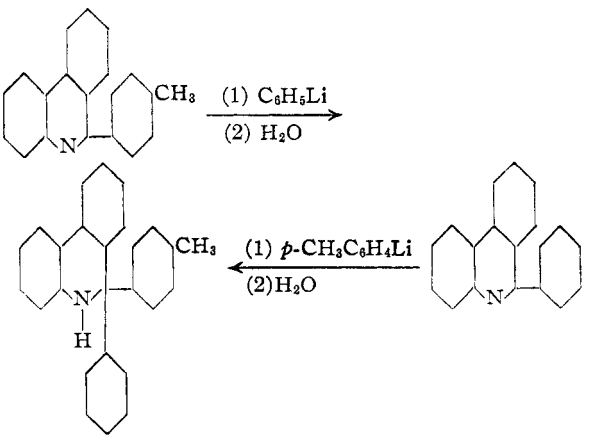
In the study of the addition of organolithium compounds to 2-arylquinolines,[1] 2-aryl-3,4-benzoquinolines (6-arylphenanthridines) were used. These compounds were chosen since 1,4-nuclear addition to the azomethine linkage is hindered because of the blocked 4-position of quinoline. This left only the possibility of 1,2-nuclear or 1,4-binuclear addition to the 6-arylphenanthridines.

Initially, to prove that phenyllithium added 1,2 to phenanthridine, 6-phenylphenanthridine was prepared by the cyclization of *o*-benzamidobiphenyl by means of $POCl_3$[2] in the presence of nitrobenzene, and this authentic specimen was compared with the compound isolated from the reaction of phenyllithium with phenanthridine. The compounds were found to be identical by means of the mixed melting points. Since phenanthridine is obtained in quite poor yields from *o*-formamidobiphenyl,[3] the preferred method for preparing 6-phenylphenanthridine is by the cyclization of *o*-benzamidobiphenyl.

On the basis of work[1] carried out previously in these Laboratories, an aryllithium compound



should add 1,2 to the azomethine linkage of a 6-arylphenanthridine giving, after hydrolysis, a



(1) Gilman and Gainer, THIS JOURNAL, **69**, 877 (1947).
(2) Morgan and Walls, *J. Chem. Soc.*, 294 (1945).
(3) Pictet and Hubert, *Ber.*, **29**, 1182 (1896).

6,6-diaryl-5,6-dihydrophenanthridine. This was proved to be the case by the undepressed mixed melting point of the product from the reaction of 6-phenylphenanthridine with *p*-tolyllithium and of that from the reaction of 6-(*p*-tolyl)-phenanthridine with phenyllithium. In the former reaction there was also formed, in small amounts, a compound melting at 275–277° (dec.) (I).

The reaction was extended to consider the effect of *ortho* substituents in the 6-phenyl group, and the mesityl group was selected for this purpose. In this connection, 6-phenylphenanthridine was treated with mesityllithium. There was unexpectedly formed, in high yield, a light yellow compound melting at 275–277° (dec.), identical, as shown by mixed melting points, with I. Nitrogen analysis and molecular weight determination have shown this compound to be a bis-6-phenylphenanthridine, the complete structure of which has not yet been established.

It may also be mentioned here that treatment of 2-phenylquinoline[4] with phenyllithium, *p*-tolyllithium, *o*-tolyllithium, or mesityllithium gave a bis-2-phenylquinoline; however, the yields of the bis compound were not so great as in the case of 6-phenylphenanthridine. Mesityllithium gave the greatest yield of the bis compound. 2-(*p*-Tolyl)-quinoline also gave a bis compound, in small yield, when treated with an aryllithium compound. It is interesting to note that treatment of 2-(*o*-tolyl)-quinoline, under these conditions, did not give a bis compound.

Experimental

6-Phenylphenanthridine.—To 30 g. (0.168 mole) of phenanthridine[5] in 320 ml. of ether was added (with stirring in a dry nitrogen atmosphere) 0.2 mole of phenyllithium[6] in 500 ml. of ether over a period of one hour. The addition of phenyllithium to the azomethine linkage went very smoothly and rapidly as indicated by color test I.[7] Phenanthridine, being rather insoluble in ether, dissolved as it underwent reaction. Upon addition of excess phenyllithium, the solution became clear, having a green fluorescence. Shortly thereafter a greenish-yellow precipitate was formed. The mixture was hydrolyzed with ice-water after stirring for three hours at room temperature. The ether was removed by distillation, 32 ml. of nitrobenzene added and the solution refluxed for one-half hour (a procedure followed to oxidize any dihydro compound). The nitrobenzene was removed by distillation under reduced pressure (0.5 mm.). There was collected 40 g. of viscous material distilling at 183–184° (0.02–0.03 mm.). The viscous oil was purified by crystallization from a 50–50 benzene–petroleum ether (b. p. 60–68°) solution giving 36 g. (84%) of colorless platelets melting at 104–105°.[8] This compound gave an undepressed mixed melting point with 6-phenylphenanthridine[3] prepared in 85% yield by the cyclization of *o*-benzamidobiphenyl by the method of Morgan and Walls.[2]

6-(*p*-Tolyl)-phenanthridine.—To 30 g. (0.168 mole) of phenanthridine in 300 ml. of ether was added 0.165 mole of *p*-tolyllithium (prepared in 89% yield from *p*-bromotoluene and lithium). The solution became dark green upon adding all the organometallic compound. After stirring for four hours the reaction mixture was hydrolyzed, the ether layer becoming light yellow in color. Subsequent to the removal of the ether by distillation, the residue was refluxed with nitrobenzene for one-half hour. The nitrobenzene was removed by distillation under reduced pressure (0.5 mm.) and the fraction distilling at 205–220° (0.01–0.02 mm.) gave 39 g. (89%) of product. This oil was purified by crystallization from 95% ethanol giving 34 g. (78%) of colorless platelets melting at 107.5–108°.

Anal. Calcd. for $C_{20}H_{15}N$: N, 5.20. Found: N, 5.30.

The picrate crystallized as yellow needles from dioxane and melted at 223–224°.

6,6-Diphenyl-5,6-dihydrophenanthridine.—Two-tenths mole of phenyllithium in 200 ml. of ether was added portion-wise to 20.4 g. (0.08 mole) of 6-phenylphenanthridine in 200 ml. of ether. There was immediately formed a yellow solid which turned white after stirring for a few minutes. After addition of all the phenyllithium, the solution and solid became very dark red, almost black, in color. The mixture was stirred under reflux for twenty-four hours, then hydrolyzed with ice water, the ether layer becoming light red. Attempts to crystallize the residue, after removal of the ether, from benzene were unsuccessful—only an oil resulted. The oil was dissolved in a chloroform–methanol solution from which it, at first, separated as an oil followed by the deposition of a powder. The solid was purified by crystallization from an 80–20 chloroform–methanol solution giving 6 g. (23%) of white powder melting at 147.5–148°.

Anal. Calcd. for $C_{25}H_{19}N$: N, 4.21. Found: N, 4.13.

6-Phenyl-6-(*p*-tolyl)-5,6-dihydrophenanthridine. (A) **Reaction of 6-Phenylphenanthridine with *p*-Tolyllithium.**—Nine-hundredths mole of *p*-tolyllithium in 150 ml. of ether was added to 19 g. (0.075 mole) of 6-phenylphenanthridine in 150 ml. of ether according to the above procedure. The residue, upon treatment with a benzene–petroleum ether (b. p., 60–68°) solution, deposited 8 g. (32%) of brown powder melting at 145–170°. This, upon crystallization from a 50–50 chloroform–methanol solution, gave 4 g. (16%) of light yellow powder melting with decomposition at 275–276° (I). Further removal of the original solvent resulted in the formation of an oil. This was dissolved in ethanol whereupon a solid crystallized, which, after purification by crystallization from ethanol, gave 2 g. (8%) of a white powder melting at 143.5–144°.

(B) **Reaction of 6-(*p*-Tolyl)-phenanthridine with Phenyllithium.**—From 21 g. (0.078 mole) of 6-(*p*-tolyl)-phenanthridine and 0.1 mole of phenyllithium, there was obtained, after purification by recrystallization from ethanol, 14 g. (52%) of a powder melting at 143.5–144°. This was shown to be identical with the low melting product from (A) by mixed melting points.

Anal. Calcd. for $C_{26}H_{21}N$: N, 4.03. Found: N, 4.14.

Reaction of 6-Phenylphenanthridine with Mesityllithium.—Nine-hundredths mole of mesityllithium (prepared in 82% yield from bromomesitylene and lithium) in 190 ml. of ether was immediately added to 20.4 g. (0.08 mole) of 6-phenylphenanthridine in 200 ml. of ether. The solution turned a dark brown, becoming black upon refluxing for forty-eight hours. Following hydrolysis, 17 g. (85%) of yellow material crystallized from the ether solution, m. p. 220–240°. Attempts were made to crystallize this solid from benzene and dioxane, respectively. This gave a material softening at 225–245°. Upon crystallization from a 50–50 chloroform–methanol solution, 13 g. (65%) of a yellow solid melting at 275–277° (dec.) was obtained. A mixed melting point with the high melting compound obtained from the reaction of 6-phenylphenanthridine with *p*-tolyllithium was undepressed.

Anal. Calcd. for $(C_{19}H_{12}N)_2$: N, 5.51. Found: N, 5.48.

The molecular weight was found to be 522 by the freez-

(4) Unpublished work of T. L. Reid and R. D. Nelson.

(5) Morgan and Walls, *J. Chem. Soc.*, 2225 (1932).

(6) Gilman, Zoellner and Selby, THIS JOURNAL, **54**, 1957 (1932).

(7) Gilman and Schultze, *ibid.*, **47**, 2002 (1925).

(8) Crystallization from 95% ethanol gave a melting point of 106.5–107°.

ing-point method using bromoform as the solvent.[9] The molecular weight of a bis-6-phenylphenanthridine is 508.

Summary

6-Phenylphenanthridine adds phenyllithium to the azomethine linkage giving 6,6-diphenyl-5,6-dihydrophenanthridine. The course of the reaction was demonstrated by showing that 6-phenyl-6-(*p*-tolyl)-5,6-dihydrophenanthridine is formed from 6-(*p*-tolyl)-phenanthridine and phenyllithium as well as from 6-phenylphenanthridine and *p*-tolyllithium. In the latter case there is also formed a bis-6-phenylphenanthridine, in particularly good yields when mesityllithium is used.

(9) The authors are indebted to Dr. H. Shine for assistance.

AMES, IOWA RECEIVED MAY 1, 1948

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

X-Ray Molecular Weight of β-Lactoglobulin

BY FREDERIC R. SENTI AND ROBERT C. WARNER[2]

All physical methods of measurement of the molecular weight of proteins in solution are subject to uncertainty because of the tendency of proteins to aggregate or degrade in solution. This uncertainty is minimized or obviated if the protein is examined in the native crystalline state by the X-ray diffraction method. In this method, any denatured protein does not contribute to the sharp interferences of the native protein and therefore may be excluded from consideration.

The X-ray data give directly the volume of an integral number of molecules. This number is restricted to a prescribed set by the space group of the crystal, which is also determined by the X-ray data. Correct choice of this number can be made if an approximate molecular weight is available from other physical measurements, such as osmotic pressure or ultracentrifugal analysis. We may thereby make a precise measurement of the volume of the protein molecule, and this volume, in combination with the density and water content of the protein crystal, yields a value for the anhydrous molecular weight of the protein.

The first X-ray investigation of the orthorhombic modification of β-lactoglobulin was made by Crowfoot and Riley,[3] who reported the unit cell dimensions $a_0 = 63.5$, $b_0 = 63.5$ and $c_0 = 145$ Å. for crystals wet with their mother liquor. Later, Crowfoot[4] revised these values to $a_0 = 67.5$, $b_0 = 67.5$, $c_0 = 154$ Å. Crowfoot and Riley did not calculate the anhydrous molecular weight of β-lactoglobulin from their X-ray data, since they had no direct measure of the hydration of the crystals. McMeekin and Warner[5] have determined the water content of large single crystals of β-lactoglobulin and have also reported a more accurate value for the density of the wet crystals. Using Crowfoot's[4] unit cell dimensions, McMeekin and Warner calculated a value of 33,000 for the anhydrous molecular weight. They computed a value of 35,800 from Crowfoot's unit cell dimensions and their density and water content data for air-dry crystals.

The discrepancy of these molecular weights and the range of values found by other methods (see Table II) made it desirable to obtain X-ray, hydration and density data on crystals from the same preparation.

Experimental

Preparation of Crystalline β-Lactoglobulin.—β-Lactoglobulin was prepared essentially by the method of Palmer as modified by Sørensen and Sørensen.[6] The casein was removed from raw skim milk by isoelectric precipitation. The *p*H of the whey was adjusted to 5.8 to 6.0, and the proteins were fractionated with ammonium sulfate. The protein fraction soluble in 2.2 *M* but insoluble in 3.3 *M* ammonium sulfate was adjusted to *p*H 5.2 and dialyzed. The crystalline lactoglobulin so obtained was recrystallized four times by dissolving in dilute sodium chloride and dialyzing. Two entirely independent preparations (batches A and B) and two preparations of crystals from batch A (A-1 and A-2) were used for the X-ray measurements. Water content and density measurements were made on crystals of batch A only. However, the agreement of the unit cell dimensions and optical properties for batches A and B establishes their identity and justifies the use of the values determined for the crystals of batch A for those of batch B.

Two methods were found for obtaining large crystals. (1) A saturated solution of lactoglobulin in 0.1 *M* sodium chloride was diluted with about two volumes of water and allowed to stand in a closed vessel for several weeks in the refrigerator without being disturbed. Large crystals formed slowly and grew in some cases to be 0.8 to 1 cm. in the greatest dimension. (2) A saturated solution of lactoglobulin in 0.1 *M* sodium chloride was placed in a small beaker containing a Cellophane tube arranged so that distilled water could be slowly run through the tube. The assembly was placed in the cold room, and the rate of flow of water was adjusted so that crystallization began in about eight hours and was complete in forty-eight hours. It is essential that there be no mechanical disturbance until all the protein has crystallized. Crystals produced by this method ranged in size from 0.1 to 2 mm. in their greatest dimension and were well formed and of sufficient strength to be handled easily without breaking. Crystals used for the X-ray measurements were all made by the second method, since those obtained by the first method were too large.

(1) One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

(2) Present address: College of Medicine, New York University, New York, N. Y.

(3) D. Crowfoot and D. Riley, *Nature*, **141**, 522 (1938).

(4) D. Crowfoot, *Chem. Rev.*, **28**, 215 (1941).

(5) T. L. McMeekin and R. C. Warner, THIS JOURNAL, **64**, 2393 (1942).

(6) S. P. L. Sørensen and M. Sørensen, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **23**, No. 7 (1939).