was added dropwise to a mixture of 29.1 g. (0.195 mole) of I, 31.6 g. (0.4 mole) of pyridine and 100 ml. of dioxane. The reaction temperature was maintained at 15–20° during the course of the addition. The reaction mixture was heated to 50–55° for one hour, cooled and diluted with 750 ml. of ether. The mixture was stirred for one-half hour while the flask was immersed in an ice-bath. The ethereal solution was decanted and washed successively with aqueous solutions of sodium carbonate, hydrochloric acid, sodium bicarbonate and water. The volatile solvents were removed under vacuum and the residual solid recrystallized from ethyl acetate. A total of 28.9 g. (66% yield) of III, m.p. 97–103°, was obtained which on further recrystallization had m.p. 110–113°, reported[3] 112°.

Anal. Calcd. for $C_{13}H_9N_3O$: C, 69.94; H, 4.06. Found: C, 70.22; H, 4.13.

The reaction of III with picric acid gave the picrate, m.p. 171–173°.

Anal. Calcd. for $C_{12}H_8N_6O_7$: C, 41.39; H, 2.32. Found: C, 41.75; H, 2.69.

B. **From Benzotriazole.**—III was prepared from benzotriazole by the above procedure, m.p. 110–112°. No depression was obtained in a mixed melting point with III obtained from I.

Reduction of 1-Benzoylbenzotriazole (III).—A solution of 4 g. (0.1 mole) of LAH in 300 ml. of ether was refluxed beneath a Soxhlet thimble containing 12.65 g. (0.05 mole) of III. All the III was dissolved after 3 hours. Refluxing was continued for an additional hour. The mixture was cooled and decomposed with 4 ml. of water, 3 ml. of 20% sodium hydroxide and 14 ml. of water, with vigorous stirring. The filtrate obtained after suction filtration was dried over magnesium sulfate and concentrated to yield 3.2 g. (59% yield) of benzyl alcohol, n^{25}_D 1.5410. The α-naphthylurethan had m.p. 134° reported[13] 134°, and was not depressed on admixture with the authentic derivative.

The filter cake was dissolved in 160 ml. of 10% hydrochloric acid and the aqueous solution extracted several times with ether and benzene. The combined extracts were evaporated to yield 5.6 g. (84%) of benzotriazole. Several recrystallizations from a mixture of benzene and hexane gave the product, m.p. 97–99°, which was not depressed on admixture with authentic benzotriazole and was converted to III on treatment with benzoyl chloride.

Anal. Calcd. for $C_6H_5N_3$: C, 60.49; H, 4.23; N, 35.28. Found: C, 60.75; H, 4.21; N, 35.36.

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[13] R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 227.

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Crystalline α-Lactalbumin: An Improved Method for Its Isolation. Sulfur Distribution

BY WILLIAM G. GORDON, WILLIAM F. SEMMETT AND JACOB ZIEGLER

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The method recently reported[1] for the isolation of crystalline α-lactalbumin from cows' milk whey has been modified. The present procedure is simpler and gives much better yields.

It has been found that all the sulfur in α-lactalbumin is present as cystine and methionine.

Experimental

Preparation of Crystalline α-Lactalbumin.—Starting with 15 gallons of raw skimmed milk, the procedure previously described[1] is used to remove casein and crude whey globulin, and to crystallize β-lactoglobulin. The clear, yellow supernatant liquid (9 l.) at *p*H 5.2, from which the β-lactoglobulin crystals were centrifuged, is adjusted to *p*H 4.0 by the dropwise addition of *N* HCl. A relatively small precipitate, which contains α-lactalbumin, is formed, but it is not removed at this point. Ammonium sulfate (187 g. per liter) is added until a concentration of 1.3 *M* is reached, whereupon more α-lactalbumin is precipitated. The precipitate is centrifuged off, and the supernatant fluid is discarded.[2] The precipitate is suspended in about 600 ml. of H_2O, and *N* NH_4OH is added dropwise to *p*H 8.0. Practically all the protein dissolves, although the solution may still be turbid. The solution is clarified by filtration through a thin layer of diatomaceous silica, and the clear filtrate is adjusted to *p*H 4.0 by the dropwise addition of *N* H_2SO_4, with efficient stirring. The precipitated α-lactalbumin is centrifuged off, and the supernatant fluid is discarded. Crystallization and further purification are carried out as described before,[1] except that reprecipitations are done at *p*H 4.0 instead of 4.6. The yield of once recrystallized α-lactalbumin is 18.5 g. (anhydrous, salt-free basis). This may be increased by about 20%, as indicated in footnote 2. The yield of 18.5 g. is more than four times that previously reported.[1] Crystalline α-lactalbumin isolated by this procedure is electrophoretically homogeneous at *p*H 8.5; its mobility at this *p*H, under the same conditions used before, is −4.2, a figure identical with that obtained for the original preparation.[1]

Sulfur Distribution.—The total cystine–cysteine content of α-lactalbumin was determined in both 6 *N* HCl and HCl–urea hydrolyzates[3] by means of the phosphotungstic acid reaction as used by Kassell and Brand.[4] Slightly higher results (6.4 ± 0.1%) were obtained with the HCl–urea hydrolyzates than with HCl alone (6.3 ± 0.1%). Although about 1.5% cysteine was found in the HCl–urea hydrolyzates, it is probable that this was formed as a result of interaction of cystine and tryptophan[5] (α-lactalbumin contains about 7% tryptophan[1]). Sulfhydryl groups in unhydrolyzed α-lactalbumin could not be detected with the nitroprusside test even when the protein was dissolved in 8 *M* guanidine hydrochloride solution. In this respect, α-lactalbumin resembles lysozyme and chymotrypsinogen.[5]

The methionine content of α-lactalbumin was found to be 0.95 ± 0.05% by the method of Bakay and Toennies.[6]

Thus, the total sulfur of α-lactalbumin, 1.91%,[1] is satisfactorily accounted for in terms of cystine (6.4% cystine = 1.71% S) and methionine (0.95% methionine = 0.20% S).

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[1] W. G. Gordon and W. F. Semmett, THIS JOURNAL, **75**, 328 (1953).

[2] The supernatant fluid contains additional α-lactalbumin. If maximal yields are sought, the ammonium sulfate concentration is increased to 2.0 *M*, whereupon a second large precipitate separates. This is handled in the same way as the first precipitate at 1.3 *M*. The final yield of α-lactalbumin can be increased by about 20% if this fraction is worked up.

[3] E. Brand and B. Kassell, *J. Gen. Physiol.*, **25**, 167 (1941).

[4] B. Kassell and E. Brand, *J. Biol. Chem.*, **125**, 115 (1938).

[5] H. S. Olcott and H. Fraenkel-Conrat, *ibid.*, **171**, 583 (1947).

[6] B. Bakay and G. Toennies, *ibid.*, **188**, 1 (1951).

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Quinoxaline Studies. VI. The Preparation and Physical Properties of Some 2-Hydroxy-3-alkylquinoxalines

BY MORTON GOLDWEBER[1] AND HARRY P. SCHULTZ

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A series of 2-hydroxy-3-alkylquinoxalines has been prepared, and their physical properties have been determined. Three 2-hydroxy-3-alkylquinoxalines have previously been reported.[2-5]

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[1] Abstracted from a thesis by Morton Goldweber, presented to the graduate faculty of the University of Miami in partial fulfillment of the requirements for the degree of Master of Science in Chemistry, June, 1953.

[2] O. Hinsberg, *Ann.*, **292**, 248 (1896).

[3] S. Motylewski, *Ber.*, **41**, 800 (1908).

[4] A. Gowenlock, G. Newbold and F. Spring, *J. Chem. Soc.*, 622 (1945).

[5] Y. L'Italien and C. Banks, THIS JOURNAL, **73**, 3246 (1951).