

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°, reported³ 112°.

Anal. Calcd. for C₁₃H₈N₂O: 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 C₁₂H₈N₆O₇: 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*_D²⁰ 1.5410. The α -naphthylurethan had m.p. 134° reported¹⁸ 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 C₈H₆N₂: C, 60.49; H, 4.23; N, 35.28. Found: C, 60.75; H, 4.21; N, 35.36.

(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

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The method recently reported¹ 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 described¹ is used to remove casein and crude whey globulin, and to crystallize β -lactoglobulin. The clear, yellow supernatant liquid (9 l.) at pH 5.2, from which the β -lactoglobulin crystals were centrifuged, is adjusted to pH 4.0 by the drop-

(1) W. G. Gordon and W. F. Semmett, *THIS JOURNAL*, **76**, 328 (1953).

wise 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 H₂O, and *N* NH₄OH is added dropwise to pH 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 pH 4.0 by the dropwise addition of *N* H₂SO₄, 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 pH 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 pH 8.5; its mobility at this pH, 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-cystine content of α -lactalbumin was determined in both 6 *N* HCl and HCl-urea hydrolyzates³ by means of the phosphotungstic acid reaction as used by Kassell and Brand.⁴ Slightly higher results (6.4 \pm 0.1%) were obtained with the HCl-urea hydrolyzates than with HCl alone (6.3 \pm 0.1%). Although about 1.5% cystine was found in the HCl-urea hydrolyzates, it is probable that this was formed as a result of interaction of cystine and tryptophan⁵ (α -lactalbumin contains about 7% tryptophan¹). 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.⁶

The methionine content of α -lactalbumin was found to be 0.95 \pm 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).

(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

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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}

(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).

TABLE I
 2-HYDROXY-3-ALKYLQUINOXALINES

R	M.p., °C. uncor.	Yield, %	Nitrogen, %		Absorption maxima and mol. extinction coefficients		
			Calcd.	Found	0.1 N NaOH	λ , $m\mu$ ($\epsilon \times 10^{-3}$) 0.1 N HCl	95% Ethanol
-CH ₂ CH ₂ CH ₃	184-185	15	14.88	14.94 14.85	238(26.32)	229(19.69) 254(7.28) 290(5.98)	230(20.86) 280(6.01)
-CH ₂ (CH ₃) ₂	233-234	14	14.88	15.06 14.75	238(24.67)	228(18.51) 252(6.42) 288(5.46)	229(21.75) 279(6.18)
-(CH ₂) ₃ CH ₃	154-155	34	13.85	13.78 13.72	238(24.69)	228(18.40) 252(6.88)	229(21.73) 280(6.28)
-(CH ₂) ₄ CH ₃	151-152	8	12.95	12.70 13.06	238(26.39)	228(19.15) 252(6.54)	229(22.39) 280(6.65)
-(CH ₂) ₅ CH ₃	140-141	31	12.16	12.11 12.25	238(27.51)	Very insoluble	230(21.40) 280(6.31)

Both Motylewski³ and Hinsberg⁶ demonstrated that *o*-phenylenediamines and α -haloacids could be condensed to give mixtures of various products, including 2-hydroxy-3-alkyl-3,4-dihydroquinoxaline and its oxidation product, 2-hydroxy-3-alkylquinoxaline. More recently, Wear and Hamilton⁷ showed that a basic solution of hydrogen peroxide was an excellent reagent for the oxidation of 2-hydroxy-3-alkyl-3,4-dihydroquinoxalines to 2-hydroxy-3-alkylquinoxalines. The 2-hydroxy-3-alkylquinoxalines reported in this paper were prepared by combining the above methods into one general reaction, giving colorless crystalline products which were soluble in basic solutions, moderately soluble in organic solvents, and relatively insoluble in water and acid solutions.

Table I lists the physical properties and ultraviolet absorption characteristics of the new compounds prepared.

Experimental Procedures

2-Hydroxy-3-alkylquinoxalines. General Procedure.—Into a 1-l., three-necked flask equipped with a reflux condenser and paddle stirrer were placed 10.8 g. (0.1 mole) of *o*-phenylenediamine and 0.066 mole of α -haloacid. The contents of the flask was stirred and heated on a steam-bath for 8 hours, then to this solution was added 8 g. of sodium hydroxide, 100 ml. of water and 10 ml. of 30% hydrogen peroxide solution. Heating and stirring were continued an additional 4 hours. The solution was treated with 1 g. of charcoal and filtered hot.

The cooled filtrate was extracted with three 100-ml. portions of ether to remove undesired tars; all ether extractions were discarded. The filtrate was then heated on a steam-bath to drive off any ether which still remained in solution, and the cooled filtrate was brought to pH 4 with acetic acid, whereupon the 2-hydroxy-3-alkylquinoxaline precipitated as a tan powder in 50 to 80% yield, melting approximately 25–30° below the melting point of the pure material.

The crude material was purified for analysis by sublimation at 1 mm. and at a temperature about 10° below the crude melting point, recrystallized 4 times from hot 95% ethanol, and again sublimed to give white crystals (except 2-hydroxy-3-*n*-butylquinoxaline which remained a light yellow in color despite repeated recrystallizations from dioxane, acetone and benzene, in addition to the alcohol recrystallizations) in 8 to 34% yield.

Absorption Spectra.—The ultraviolet absorption spectra, condensed in Table I, were obtained on a Beckman Model DU quartz spectrophotometer.

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(6) O. Hinsberg, *Ann.*, **248**, 77 (1888).

(7) R. L. Wear and C. S. Hamilton, *THIS JOURNAL*, **72**, 2893 (1950).

Addition Compounds of Silicon Tetrachloride with Amides

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Silicon tetrachloride is unusual in the paucity of addition compounds it forms. Ammonia appears to ammonolyse it, and only amines form compounds with integral molar ratios. Three such amine adducts have been known for over fifty years,^{2,3} and many more have recently been described in a thorough series of investigations by Trost⁴⁻⁶: all contain one, two or four moles of amine per mole of silicon tetrachloride. Trost also reports that silicon tetrachloride forms addition compounds with ketones, acids, esters and alcohols,⁸ but gives no information about the composition or structure of such substances. An unsuccessful attempt to prepare addition compounds of silicon tetrachloride with amides was reported by Harold,⁹ who passed the vapor of the tetrachloride into some fused amides and observed no combination. Despite the affinity of silicon for oxygen, compounds of silicon tetrachloride with oxygen-containing substances are still more rare: the compounds with ether, dioxane, tetrahydrofuran and tetrahydropyran that might have been anticipated have not been obtained.¹⁰⁻¹²

We now find that compounds of the formulas $\text{SiCl}_4 \cdot 10\text{HCONH}_2$ and $\text{SiCl}_4 \cdot 5\text{HCON}(\text{CH}_3)_2$ may be prepared by mixing solutions of the components in anhydrous organic solvents. These compounds are white solids and are quite stable in the absence of moisture. They are somewhat soluble in excess of the amide but are insoluble in common organic solvents. The bonding between the central silicon

(1) Procter and Gamble Predoctoral Fellow at Harvard University, 1952-1953.

(2) A. Hardin, *J. Chem. Soc.*, **61**, 40 (1887).

(3) F. Lengfeld, *Am. Chem. J.*, **21**, 531 (1899).

(4) W. R. Trost, *Can. J. Chem.*, **29**, 877 (1951).

(5) W. R. Trost, *ibid.*, **29**, 1075 (1951).

(6) W. R. Trost, *ibid.*, **30**, 835 (1952).

(7) W. R. Trost, *ibid.*, **30**, 842 (1952).

(8) W. R. Trost, *Nature*, **169**, 289 (1952).

(9) J. F. X. Harold, *THIS JOURNAL*, **20**, 13 (1898).

(10) H. H. Sisler, H. H. Batey, B. Pfabler and R. Mattair, *ibid.*, **70**, 3824 (1948).

(11) S. Kennard and P. A. McCusker, *ibid.*, **70**, 1039 (1948).

(12) T. J. Laue, P. A. McCusker and B. C. Curran, *ibid.*, **64**, 2076 (1942).