characteristic infrared diagrams. The structures previously assigned to these compounds are confirmed by this work.

Experimental

Hydrogenolysis of Epimeric Benzyl Ethers. A. *trans*-5-Hydroxy-2-ketocyclohexaneacetic Acid (Ia).—Three and five-tenths grams of *trans*-5-benzyloxy-2-ketocyclohexaneacetic acid, m.p. 109–111°, in solution in 35 ml. of 95% ethanol with 1.8 g. of 5% palladium–charcoal catalyst absorbed the theoretical amount of hydrogen on shaking for an hour at room temperature. The catalyst was filtered and the alcohol removed under reduced pressure at room temperature. A total of 2.0 g. (87%) of product, m.p. 112–115°, was recovered from a cold suspension in ethyl acetate. It was purified by dissolving in a little warm alcohol and adding ethyl acetate. The analytical sample melted at 119–121°.

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.80; H, 7.03. Found: C, 55.82; H, 7.26.

In another similar run in 95% ethanol the absorption of hydrogen was 150% and the yield only 62%. In acetone there was no tendency to over reduce, but the product was usually impure and difficult to separate into its components.

The semicarbazone was purified from water, m.p. 188–189° (gas).

Anal. Calcd. for $C_9H_{15}N_3O_4$: N, 18.33. Found: N, 18.42.

An alkaline solution of the semicarbazone was made by adding 0.2 ml. of 2.16 *N* sodium hydroxide solution to a suspension of 0.050 g. in 0.2 ml. of water. After 30 minutes 0.14 ml. of 3.02 *N* hydrochloric acid precipitated 0.027 g. of the semicarbazone and an additional 0.010 g. was obtained from the mother liquor. Infrared diagrams established the fact that this treatment did not cause isomerization to the epimeric semicarbazone.

B. *cis*-5-Hydroxy-2-ketocyclohexaneacetic Acid (IIa).—Five grams of *cis*-5-benzyloxy-2-ketocyclohexaneacetic acid (liquid isomer of *ca.* 70% purity[2]) in solution in 50 ml. of acetone with 3 g. of 5% palladium–charcoal catalyst absorbed 470 ml. of hydrogen on shaking for 90 minutes at room temperature. The catalyst was filtered and washed with acetone. The solvent was removed at room temperature under reduced pressure, and the crude product washed with cold ethyl acetate. It weighed 1.88 g. (57%) and melted at 140–143°. After further purification by adding ethyl acetate to a hot alcohol solution it melted at 150–152°.

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.80; H, 7.03. Found: C, 55.54; H, 7.22.

The semicarbazone was prepared in and purified from water. After sintering at *ca.* 135°, it resolidified and melted with decomposition at 185°. A mixture with the semicarbazone of *trans*-5-hydroxy-2-ketocyclohexaneacetic acid showed no melting point depression.

Anal. Calcd. for $C_9H_{15}N_3O_4 \cdot H_2O$: C, 43.72; H, 6.93; N, 17.00. Found: C, 43.41; H, 6.68; N, 16.65.

5-Hydroxy-2-ketocyclohexaneacetic Acid Lactone (III).—Five-tenths gram of 5-hydroxy-2-ketocyclohexaneacetic acid, m.p. 149–152°, was melted and the temperature lowered to 130° where partial solidification occurred. The compound was maintained at this temperature for 3 hours. At the end of the first hour the magma had completely liquefied. The oil was then extracted with a benzene–chloroform mixture and the crude lactone–acid mixture dissolved in water and excess calcium carbonate added to react with the acid. Water was removed under reduced pressure with warming and the residue extracted with ethyl acetate. After removal of ethyl acetate the crude product was recovered from a suspension in ether, wt. 0.058 g. (13%), m.p. 95–116°. After purification by adding ether to a benzene solution it melted at 115–118°. The same compound, identified by a mixture melting point determination, was isolated in 21% yield from a similar treatment of the epimeric acid.

Anal. Calcd. for $C_8H_{10}O_3$ (154.2): C, 62.32; H, 6.54. Found: C, 62.56; H, 6.72.

Rast molecular weight determinations on the lactone and its precursor acid were high but the gentler Signer method gave a value of 152.5 for the lactone. The lactone semicarbazone was purified from water and melted at 205–206° (gas).

Anal. Calcd. for $C_9H_{13}N_3O_3$: N, 19.90. Found: N, 19.66.

Lactone Hydrolysis.—To a suspension of 0.052 g. of the lactone semicarbazone in 0.2 ml. of water was added 0.15 ml. of 2.16 *N* sodium hydroxide solution. The solid dissolved in 5 minutes. Acidification with 0.12 ml. of 3.02 *N* hydrochloric acid gave 0.050 g. of *cis*-5-hydroxy-2-ketocyclohexaneacetic acid semicarbazone. This material gave an infrared diagram identical with that from the semicarbazone of the keto acid melting at 150–152°.

Epimerization Experiments. A.—One gram of 5-benzyloxy-2-ketocyclohexaneacetic acid (Ib), m.p. 109–111°, was suspended in 120 ml. of 9.3 *N* hydrochloric acid and the mixture shaken intermittently. After 2 hours, 50 mg. of undissolved solid was removed by filtration. The solution was extracted several times with chloroform and the chloroform removed from the extract at room temperature under reduced pressure. The crude crystalline residue weighed 0.82 g. It was purified by means of an ether suspension chilled by Dry Ice–acetone. The recovered material melted at 104–108° and weighed 0.374 g. The oil, 0.424 g., recovered from the ether could not be induced to crystallize further. Rapid crystallization occurred on triturating with 0.9 ml. of 9.3 *N* hydrochloric acid. The crystals melted at 103–109° and weighed 0.36 g.

B.—A solution of 0.20 g. of *trans*-5-hydroxy-2-ketocyclohexaneacetic acid (Ia), m.p. 118–119°, in 0.4 ml. of 6 *N* hydrochloric stood for one hour at room temperature. The hydrochloric acid was removed with an oil pump at room temperature. Ethyl acetate was added to the residue and crystallized material obtained, wt. 0.11 g., m.p. 139–145°. After further purification it melted at 150–153° and the melting point was not depressed on admixture with a sample of the *cis* isomer.

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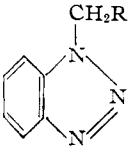
Displacement Reactions in the Benzotriazole Series

BY NORMAN G. GAYLORD

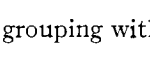
RECEIVED AUGUST 26, 1953

It has been shown that the treatment of benzotriazole with formaldehyde yields 1-hydroxymethylbenzotriazole (I).[1] Catalytic hydrogenation in the presence of Raney nickel or 5% palladium-on-charcoal failed to reduce I to 1-methylbenzotriazole (II). However, conversion of I by means of thionyl chloride to the corresponding halide, followed by reduction with lithium aluminum hydride in refluxing tetrahydrofuran gave II.[1]



I, R = OH
II, R = H

The reaction of a compound containing an



grouping with lithium aluminum hydride

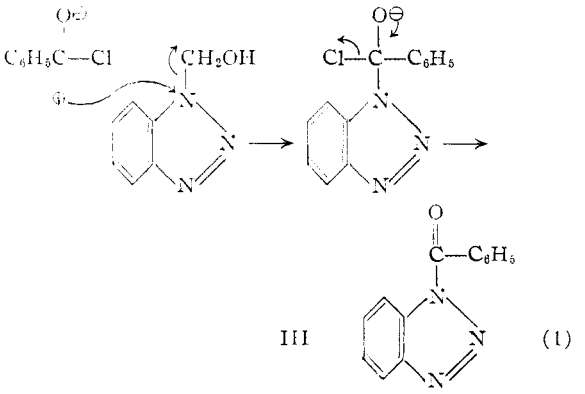
(1) J. H. Burckhalter, V. C. Stephens and L. A. R. Hall, THIS JOURNAL, **74**, 3868 (1952).

has been shown to result in a cleavage of the carbon–oxygen linkage.[2] It was therefore expected that *II* could be obtained directly from I by means of lithium aluminum hydride, without recourse to the halide. Attempts to carry out this reduction in a dioxane–ether–benzene mixture resulted in the immediate precipitation of the complex resulting from the reaction of the hydride and the active hydrogen, and I was recovered from the reaction mixture. The preparation of II *via* the halide was carried out without recourse to the Soxhlet extraction technique[1] with a decreased reaction time and increased yield.

Attempts to prepare the picrate of I by means of a saturated solution of picric acid in alcohol gave a picrate, m.p. 173–174°, identified as the picrate of benzotriazole. On the other hand, the picrate of II had m.p. 150–152°, while the chloromethyl compound could not be induced to form a picrate.

In an effort to eliminate the active hydrogen, I was treated with benzoyl chloride and pyridine in dioxane solution. The resultant product was identified as the benzamide of benzotriazole (III) on the basis of its analysis and identity with the product obtained by the reaction of benzotriazole and benzoyl chloride.

The formation of III can be explained on the basis of three different mechanisms: (a) the elimination of formaldehyde to yield benzotriazole, as indicated in the reaction of I with picric acid, followed by the expected amide formation, (b) the direct displacement of the hydroxymethyl group as shown in equation 1, and (c) the initial formation of the expected benzoate followed by a rearrangement involving a transitory six-membered ring containing the nitrogens in both 1- and 3-positions of the triazole ring.



III (1)

In order to distinguish among these possibilities it will be necessary to carry out the appropriate transformations with suitably substituted benzotriazoles.

Treatment of III with picric acid gave the picrate of benzotriazole, analogous to the reaction of I. Refluxing III with 30% sulfuric acid in 95% ethanol for 9 hours is reported to yield benzotriazole.[3] Similarly, 1-acetyl-5-bromobenzotriazole in concentrated hydrochloric acid,[4] 1-benzenesulfonyl-5-bromonaphtho[1.2]triazole in alcohol[5] and 1-carbamylbenzotriazole-5(or 6)-carboxylic acid in water, alcohol or acetic acid[6] are hydrolyzed to the corresponding benzotriazoles. On the other hand, treatment of 5-hydroxy-1-hydroxymethylbenzotriazole with aqueous sodium hydroxide followed by precipitation with hydrochloric acid results in the transfer of the hydroxymethyl group from the 1-position on the triazole ring to the 4-position on the benzene ring.[7]

The reduction of III with lithium aluminum hydride in ether gave benzyl alcohol and benzotriazole instead of the expected amine. The hydrogenolysis of the acyl group attached to a heterocyclic nitrogen compound under the influence of the hydride, has been reported in the reduction of N-acetylcarbazole,[8] N-acetylskatole,[8] 1,N-diformyl-7-methyltryptamine,[9] dibenzoyl-L-histidine ester[10] and acylated 2-benzylaminothiazole.[11]

Experimental

1-Hydroxymethylbenzotriazole (I).—The procedure of Burckhalter, *et al.*,[1] using benzotriazole, formalin and acetic acid gave 63 g. (85% yield) of recrystallized I, m.p. 147–148°.[12]

The reaction of I with a saturated solution of picric acid in 95% ethanol gave the **picrate**, m.p. 173–174°, which showed no depression on a mixed melting point with the picrate of benzotriazole.

Anal. Calcd. for $C_{12}H_8N_6O_7$: C, 41.39; H, 2.32. Found: C, 41.55; H, 2.73.

Attempted Reduction of 1-Hydroxymethylbenzotriazole (I).—A solution of 14.9 g. (0.1 mole) of I in 180 ml. of dioxane was added over 20 minutes to a solution of 4 g. (0.1 mole) of lithium aluminum hydride in 300 ml. of ether. A white precipitate appeared as soon as I was added to the ethereal hydride solution. Stirring was continued for an additional one-half hour while the refluxing mixture was held at 40–45°. The reaction mixture was refluxed for one-half hour at 45–50° after the addition of 50 ml. of benzene, and decomposed, after cooling with 4 ml. of water, 3 ml. of 20% sodium hydroxide and 14 ml. of water, in that order. The mixture was filtered and the filter cake was washed with benzene and ether. The combined filtrates were dried over magnesium sulfate and concentrated to yield 7.1 g. of I. Extraction of the filter cake in a Soxhlet extractor with dioxane gave an additional 6.9 g. of I, picrate, m.p. 173–174°.

1-Chloromethylbenzotriazole.—The procedure of Burckhalter, *et al.*,[1] using 14.9 g. (0.1 mole) of 1-hydroxymethylbenzotriazole and 45 ml. of thionyl chloride gave 9.8 g. (59%) of product, m.p. 135–137°, reported[1] 136–138°.

1-Methylbenzotriazole (II).—A solution of 6.3 g. (0.038 mole) of 1-chloromethylbenzotriazole in 100 ml. of tetrahydrofuran was added dropwise to a solution of 2 g. of LAH in 80 ml. of tetrahydrofuran. After refluxing for 5 hours, the solution was cooled and decomposed with 2 ml. of water, 3 ml. of 20% sodium hydroxide and 7 ml. of water. After filtration, the filtrate was dried over calcium chloride and concentrated to yield 4.6 g. (86%) of II which solidified readily. Recrystallization from a mixture of benzene and petroleum ether gave plates, m.p. 62–64°. Its picrate melted at 150–152°. These values agree with reported values.[1]

1-Benzoylbenzotriazole (III). A. From I.—A solution of 42.2 g. (0.3 mole) of benzoyl chloride in 25 ml. of dioxane

(2) N. G. Gaylord, *Experientia*, in press.

(3) G. Charrier and A. Beretta, *Gazz. chim. ital.*, **51**, II, 267 (1921).

(4) T. Zincke and C. Campbell, *Ann.*, **255**, 343 (1889).

(5) G. T. Morgan and W. Godden, *J. Chem. Soc.*, **97**, 1702 (1910).

(6) P. Griess, *Ber.*, **15**, 1878 (1882); T. Zincke, *Ann.*, **291**, 313 (1896).

(7) K. Fries, H. Güterbock and H. Kühn, *Ann.*, **511**, 213 (1934).

(8) K. Banholzer, T. W. Campbell and H. Schmid, *Helv. Chim. Acta*, **35**, 1577 (1952).

(9) K. Eiter and O. Svierak, *Monatsh.*, **82**, 186 (1951); **83**, 1453 (1952).

(10) P. Karrer, M. Suter and P. Waser, *Helv. Chim. Acta*, **32**, 1936 (1949).

(11) I. A. Kaye and C. L. Parris, *J. Org. Chem.*, **17**, 737 (1952).

(12) Reference 1 gives m.p. 148–151°; reference 7 gives m.p. 148°.

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[13] 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.

(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

RECEIVED JUNE 25, 1953

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).

(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

RECEIVED SEPTEMBER 8, 1953

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).