Alkaline Degradation of Lactose in Liquid Ammonia[1]

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Degradation of lactose in a metal amide–liquid ammonia system proceeds similarly to that in aqueous alkali. However, fragmentation occurs to a greater degree in liquid ammonia and D-isosaccharinates are obtained in low yields. At −40 ± 5°, the amides of calcium, potassium, and sodium have nearly equivalent effect on the production of D-isosaccharinates, while at 25° calcium amide is more effective. "β"-D-Isosaccharinic acid is the predominant isomer formed.

Hodge, Karjala, and Hilbert[2] found that the degradations[3] observed in the sodium metal–liquid ammonia methylation procedure of Freudenberg[4] were caused by sodium amide formed in the reaction mixture. Recently Hodge and Nelson[5] reported the degradation of unsubstituted corn starch in a sodium amide–liquid ammonia system.

In this work an examination of the degradation of lactose is made in anhydrous liquid ammonia, using calcium, potassium, and sodium amides as ammono bases. Reactions are conducted at 25° and at −40 ± 5°.

Aqueous alkaline degradation of lactose proceeds by way of fairly well established mechanisms[6] to D-galactose and D-isosaccharinates. The postulated dicarbonyl intermediate has been isolated and characterized.[7] Whereas in calcium hydroxide solution the reaction proceeds comparatively smoothly, in sodium hydroxide solution a competitive reaction becomes dominant which produces extensive amounts of fragmentation products, in addition to isosaccharinates.[8]

In the present work it is observed that lactose degrades in liquid ammonia systems much as it does in aqueous systems. In all cases, however, fragmentation reactions occur to a greater degree in liquid ammonia and isosaccharinates are consequently obtained in low yields. If the normal mechanism for the production of isosaccharinates is extended to the metal amide–liquid ammonia system, D-isosaccharinamide would be the expected product. However, "α"-D-isosaccharinamide is unstable in hot water[9] and as shown here, rapidly converts to the 1,4-lactone under the conditions used for isolation of degradation products. At −40 ± 5°, sodium, potassium, and calcium ions appear to have nearly equivalent effect on the amount of isosaccharinic acid isolated even though a greater percentage of lactose reacts under the influence of calcium amide (Table I). Variation in the amount of potassium amide influences the amount of isosaccharinic acid isolated but does not extend the yield above 1% during an optimum reaction period of seventy-two hours (Table II). Excessive amounts of base decrease the amount of lactose reacting. The low reactivity is mainly a result of the insolubility of the metal lactates and of the heterogeneity of the reaction.

TABLE I

REACTIONS[a] IN LIQUID AMMONIA AT −40 ± 5°

Amide used	Mole amide/ mole lactose	Lactose reacted, %	Yield,[b] % D-gal.[c]	Yield,[b] % "β"-acid[d]
Sodium	12	41	<0.5	1
Potassium	12	34	<0.5	1
Calcium	11	73	<0.5	1

[a] Reaction time was 72 hours, with 10 g. of lactose in 450 ml. of liquid ammonia. [b] Weight percentages based on the amount of lactose added. [c] D-Galactose. [d] "β"-D-Isosaccharinic acid.

TABLE II

EFFECT OF POTASSIUM AMIDE CONCENTRATION ON THE AMOUNT OF DEGRADATION AT −40 ± 5°[a]

Mole amide/ mole lactose	Lactose reacted, %	Yield[b] of "β"-D-isosaccharinic acid, %
1	0	...
4	28	<0.1
9	36	0.5
12	34	1
15	17	Trace

[a] Reaction time was 72 hours, with 10 g. of lactose in 450 ml. of liquid ammonia. [b] Weight percentages based on amount of lactose added.

In liquid ammonia at 25° there is an increase in the amount of lactose reacting and in the percent of isosaccharinic acids isolated (Table III). Furthermore, the unique effect of calcium ion[10] in increasing the amount of isosaccharinic acids is

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(2) J. E. Hodge, S. A. Karjala, and G. E. Hilbert, *J. Am. Chem. Soc.*, **73**, 3312 (1951).

(3) (a) K. H. Meyer, M. Wertheim, and P. Bernfeld, *Helv. Chim. Acta*, **23**, 865 (1940); (b) K. Hess, H. A. Schulze, and B. Krajnc, *Ber.*, **73B**, 1069 (1940).

(4) K. Freudenberg, H. Boppel, and M. Meyer-Delius, *ibid.*, **71B**, 2505 (1938).

(5) J. E. Hodge and E. C. Nelson, Abstracts of Papers, 6-D, Division of Carbohydrate Chemistry, 140th National Meeting, American Chemical Society, Chicago, Illinois, September, 1961.

(6) For a general discussion of the alkaline degradation of carbohydrates see: R. L. Whistler and J. N. BeMiller, *Advan. Carbohydrate Chem.*, **13**, 289 (1958).

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(8) G. Machell and G. N. Richards, *J. Chem. Soc.*, 1924 (1960).

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(10) D. O'Meara and G. N. Richards, *J. Chem. Soc.*, 1944 (1960).

TABLE III

REACTIONS[a] IN LIQUID AMMONIA AT 25°

Amide used	Mole amide/mole lactose	Lactose reacted, %	Yield,[b] %			
			D-glu.[c]	D-gal.[d]	"β"-acid[e]	"α"-lactone[f]
Potassium	6	81	3	5	4	2
Potassium	10	71	4	5	5	1
Calcium	10	63	<0.3	<0.3	9	2

[a] Reaction time was 72 hours, with 2 g. of lactose in 100 ml. of liquid ammonia. [b] Weight percentages based on the amount of lactose added. [c] D-Glucose. [d] D-Galactose. [e] "β"-D-Isosaccharinic acid. [f] "α"-D-Isosaccharino-1,4-lactone.

again evident. Of particular significance in these reactions is the production of "β"-D-isosaccharinic acid which is obtained in much larger ratio to "α"-D-isosaccharino-1,4-lactone than is observed in aqueous degradation of lactose.[11] The mechanism which determines the ratio of isomers is not yet explained.

In all experiments at least a trace of D-glucose is detected which indicates that in addition to the other possible reactions, some form of alkaline hydrolysis also occurs. Other components present in trace amounts and detected by paper chromatography are lactulose, fructose, talose, and an unidentified lactone.

Experimental

Chromatography.—All chromatographic separations were at 25° using the following irrigants: A, ethyl acetate–pyridine–water (10:4:3 v./v.); B, ethyl acetate–acetic acid–formic acid–water (18:3:1:4 v./v.); C, 1-butanol–ethanol–water (40:11:19 v./v.). The components were detected by spraying the paper chromatograms with the following sprays: A, silver nitrate and sodium hydroxide[12]; B, bromothymol blue[13]; C, hydroxamic acid[14]; D, aniline hydrogen phthalate[15]; E, naphthoresorcinol.[16]

Reactions at −40 ± 5°.—Reactions were done in a 1-l., three-necked, round-bottom flask. The center neck contained a ground-glass sealed stirring rod. Reactants were added through the side necks. Gas inlet and outlet was accomplished by means of two further openings controlled by stopcocks. A drying tube containing barium oxide was attached to the gas outlet. The reaction flask was flushed with ammonia gas, placed in a Dry Ice–acetone bath and 450 ml. of liquid ammonia, previously dried over sodium metal, was condensed into the flask. A few milligrams of anhydrous ferric chloride were added to catalyze the conversion of metal to amide. The metal was cut into small pieces under mineral oil, washed with pentane (b.p. 35–37°), the solvent removed with dry nitrogen gas, and then added to the ammonia in several portions. When conversion of metal to amide was complete, as shown by the disappearance of the blue color, anhydrous lactose was added. Stirring was constant during the reaction and the temperature was held at −40 ± 5° by a Dry Ice–acetone bath. At the end of the reaction time, ammonium acetate was added to neutralize the excess amide and the ammonia distilled. Dry nitrogen gas was passed through the flask to remove traces of ammonia. A slurry of 500 ml. of Amberlite IR-120 (H^+) cation exchange resin in 50% ethanol was added to the residue and the mixture stirred for 30 min. The resin was removed by filtration and washed with water until the washings gave a negative orcinol test. Filtrate and washings were combined and concentrated under reduced pressure to a thin sirup. Ethanol was added to a concentration of 80%. The 80% ethanol-insoluble fraction was removed by centrifugation and the D-glucose, D-galactose, and unchanged lactose contained in it determined by the phenol–sulfuric acid method.[17] The 80% ethanol-soluble fraction was further separated on an acid-washed Celite column,[18] using water saturated 1-butanol as developing solvent. D-Glucose, D-galactose, and lactose isolated in this separation were determined as above. Fractions containing D-isosaccharinic acids were further purified by paper chromatography in irrigant A. Fractions containing other substances were combined and kept for identification of trace components.

Reactions were performed using calcium, potassium, and sodium amides, respectively. Reaction times investigated were 36, 72, and 144 hr., but 72 hr. was found optimum. The longer period gave a slight increase in total degradation but no increase in yield of D-isosaccharinates. Effect of varying the base concentration was investigated.

Reactions at 25°.—Reactions were done as described before except that a Parr[19] Series 4000 hydrogention bomb (500 ml.) with glass liner was used as the reaction vessel. After addition of the lactose, the bomb was sealed and the contents agitated by rocking. At the end of the reaction time the pressure was released and the liner removed. Ammonium acetate was added and the ammonia distilled. The residue was treated as above in the low temperature reactions.

Reactions were performed using calcium and potassium amides. Reaction time and amount of base were varied.

"β"-D-Isosaccharinic Acid.—The yield of this acid was determined by concentrating its water solution to a sirup in a tared flask, under reduced pressure. Benzene–ethanol (2:1 w./w.) was repeatedly distilled from the sirup and the flask weighed. It has been shown[20] that a sirup of "β"-D-isosaccharinic acid obtained in this manner is essentially free from water. It had R_g values of 2.0, 2.5, and 2.2 with irrigants A, B, and C, respectively; $[\alpha]^{25}\text{D}$ −1° (*c*, 8.6 in water). An infrared spectra of this material was identical with that of authentic "β"-D-isosaccharinic acid.

The tetrabenzoate was prepared by the method of Whistler and BeMiller[11b]; m.p. 69–70.5, $[\alpha]^{25}\text{D}$ +1° (*c*, 1.0 in chloroform).

"α"-D-Isosaccharino-1,4-lactone.—This substance was present in only small amounts and the yield was determined in the same manner as "β"-D-isosaccharinic acid.

Trace Components.—Fractions containing trace components were combined. Tentative identification was made by paper chromatography in irrigants A and B and with sprays A, B, C, D, and E. Detected were lactulose, fructose, talose, and an unidentified lactone (R_g values of 1.5 and 1.2 in irrigants A and B, respectively).

"α"-D-Isosaccharinamide.—One gram of "α"-D-isosaccharino-1,4-lactone was dissolved in 250 ml. of liquid ammonia, and the ammonia was allowed to evaporate at room temperature. Traces of ammonia were removed by desic-

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(20) R. L. Whistler and J. E. Walsh, unpublished results.

cation over concentrated sulfuric acid. Paper chromatography showed that the resulting sirup contained "α"-D-isosaccharinic acid and its 1,4-lactone as well as a third component with a R_α[21] value in irrigant A of 0.45. This component was separated from the other by paper chromatography. It was obtained as a clear sirup, quite soluble in methanol and water, but insoluble in ethanol. A portion of the sirup was dissolved in 25 ml. of 0.1 *N* hydrochloric acid solution and refluxed for 1 hr. Paper chromatography of this solution indicated only "α"-D-isosaccharino-1,4-lactone.

The remaining sirup was dried for 12 hr. over phosphorus pentoxide in an Abderhalden drier.

Anal. Calcd. for $C_6H_{13}O_5N$: N, 7.8. Found: N, 7.6.

A portion of the "α"-D-isosaccharinamide sirup was dissolved in 500 ml. of water and 250 ml. of Amberlite IR-120 (H^+) cation exchange resin added. This mixture was stirred for 30 min., the resin removed by filtration and washed with 500 ml. of water. Filtrate and washings were combined and concentrated under reduced pressure (45–50°) to a sirup. Paper chromatography of this sirup indicated mainly "α"-D-isosaccharino-1,4-lactone in addition to a small amount of the amide.

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[21] R_α is the rate of movement relative to "α"-D-isosaccharino-1,4-lactone.

Photolysis of Nitrite Esters in Solution. VII. Photochemistry of *dl*-Bornyl and *dl*-Isobornyl Nitrites

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The complete photolysis of either *dl*-bornyl or *dl*-isobornyl nitrite in trichlorotrifluoroethane yielded the following products (determined quantitatively by instrumental methods): *dl*-1,8,8-trimethyl-2-hydroxy-2-azabicyclo[3.2.1]octan-3-one (III) (*ca.* 30%), *dl*-1,6-α-campholenaldehyde (IV) (*ca.* 20%), and *dl*-1,10-α-campholenaldehyde (V) (*ca.* 12%). The photolytically activated *dl*-1-nitroso-α-campholanaldehyde (I) was shown to be the precursor for III.

Robinson, *et al.*,[1] have recently reported the formation of steroidal hydroxamic acids during the photolysis of the following C-17β-nitrite esters: 5α-androstane-3α,17β-diol 3α-acetate 17β-nitrite, testosterone 17β-nitrite, 19-nortestosterone 17β-nitrite, estradiol 3-benzoate 17β-nitrite, and estradiol 3-methyl ether 17β-nitrite. The conditions were not favorable for the Barton reaction. The photolyses of bornyl and isobornyl nitrites were undertaken to determine whether the geometric form of the former would favor hydroxamic acid formation to a greater extent than would the geometric form of the latter compound.

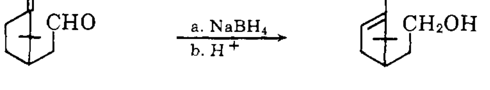
TABLE I
dl-BORNYL AND *dl*-ISOBORNYL NITRITE PHOTOLYSIS[a] PRODUCT COMPOSITION[b]

Products	Yields, % *dl*-Bornyl nitrite	Yields, % *dl*-Isobornyl nitrite
dl-1,8,8-Trimethyl-2-hydroxy-2-azabicyclo[3.2.1]octan-3-one	31	32
dl-1,6-α-Campholenaldehyde	19	20
dl-1,10-α-Campholenaldehyde	12	12
dl-Borneol	1	. . .
dl-Isoborneol	. . .	1
dl-Camphor	<1	<1
dl-1-Nitroso-α-campholanaldehyde	0	0

[a] Photolysis light source λ > 300 mμ. [b] Composition determined by instrumental analysis.

Results

Table I lists the *dl*-bornyl and *dl*-isobornyl nitrite photolysis product composition as determined by quantitative analytical methods when the photolytic radiation consisted of wave lengths greater than 300 mμ from a mercury arc light source. The hydroxamic acid, *dl*-1,8,8-trimethyl-2-hydroxy-2-azabicyclo[3.2.1]octan-3-one, product was characterized by its infrared absorption spectra,[2] color reactions,[3] and comparison with the work of Robinson, *et al.*[1] The *dl*-1,6-α-campholenaldehyde product, containing an *endo* double bond possessing a characteristic infrared absorption band at 12.55 μ[4] was identified by comparison with authentic *dl*-1,6-α-campholenaldehyde prepared by the photolysis of *dl*-camphor.[5] The *dl*-1,10-α-campholenaldehyde product, containing an *exo* double bond possessing a characteristic infrared absorption band at 11.37 μ,[4] was characterized by reaction 1: (a) sodium borohydride reduction to *dl*-1,10-α-campholenyl alcohol; and (b) acid isomerization to the stable *dl*-1,6-α-cam-



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