β-hydroxylamine and nitroso compound in the presence of alkali.

5. 2-Azoxy-2,5-dimethylhexane was shown to be non-reactive with methylmagnesium iodide.

6. The hydrolysis of 2-azoxy-2,5-dimethylhexane with dilute hydrochloric acid catalyzed by stannous ion produced nitrogen, octenes and dimethylisoamylcarbinol.

STATE COLLEGE, PENNA. RECEIVED JUNE 9, 1938

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Aliphatic Azoxy Compounds. IV. The Preparation of Certain α-Azoxy Ketones. The Molecular Refractions and Parachors of Aliphatic Azoxy Compounds[1]

BY DAVID E. AILMAN

In order to study the molecular refractions and parachors of aliphatic azoxy compounds, additional liquid azoxy compounds are desirable. Ethyl 2-methyl-2-azoxy-propionate[2] and 2-azoxy-2,5-dimethylhexane[3] have been described elsewhere.

This paper describes the preparation and properties of ethyl α-azoxyisopropyl ketone and α-azoxyisopropyl isobutyl ketone of which only the former is a liquid whose molecular refraction and parachor have been compared with those of the compounds mentioned above.

Experimental

Ethyl α-Azoxyisopropyl Ketone.—Ten grams (0.039 mole, bimolecular basis) of bimolecular ethyl α-nitrosoisopropyl ketone[4] was reduced with 10.8 g. (0.048 mole) of crystalline stannous chloride $SnCl_2 \cdot 2H_2O$, in 15.8 cc. of concentrated hydrochloric acid,[5] keeping the temperature 30–36° for twenty minutes. A titration with permanganate showed that 0.040 mole of stannous ion had been oxidized. After nearly neutralizing the acid solution with sodium carbonate the liquid azoxy compound was removed by solution in ether, dried with potassium carbonate, and distilled, collecting 5.18 g. (0.021 mole; 55% yield), b. p. 126–126.5° (6 mm.), n^{20}_D 1.4587, d^{20}_4 1.0151.

Anal. Calcd. for $C_{12}H_{22}O_3N_2$: C, 59.45; H, 9.16; N, 11.57; mol. wt., 242.3. Found: C, 58.85; H, 9.09; N (Dumas), 11.72; mol. wt. (cryoscopic in benzene), 240, 241.

Reduction of Ethyl α-Azoxyisopropyl Ketone.—Eight grams (0.033 mole) of the azoxy compound on stirring with a solution of 31 g. (0.14 mole) of stannous chloride in 45 cc. of concentrated hydrochloric acid for seven hours at a maximum temperature of 65° (maintained for one hour), oxidized only 46% of the stannous ion (calculated for reduction to ketone and hydrazine[5]). (The excess stannous ion was determined by permanganate titration, which does not distinguish between stannous ion and hydrazine. This has been corrected for.) The isolation of 1.5 g. of a semicarbazone which melted a few degrees below the m. p. of ethyl isopropyl ketone semicarbazone (each recrystallization raised the m. p., but the amounts became too small to handle), together with the isolation of 0.5 g. of dibenzalazine (sharp m. p. and mixed m. p. with an authentic sample) showed that a reduction of the type observed with methyl α-azoxyisopropyl ketone[5] had occurred.

α-Azoxyisopropyl Isobutyl Ketone.—Eight grams (0.025 mole) of the *bis*-nitroso ketone[4] was reduced with 6.9 g. of stannous chloride in 13 cc. of concentrated hydrochloric acid. On chilling, after the reduction was complete, the oil crystallized. The product was recrystallized from lukewarm alcohol–water and then melted at 30–31°. The yield of purified product was 4.0 g. (0.013 mole, 52% yield).

Anal. Calcd. for $C_{16}H_{30}O_3N_2$: C, 64.39; H, 10.14; mol. wt., 298.2. Found: C, 63.97; H, 10.65; mol. wt. (cryoscopic in benzene), 310, 297, 300.

Molecular Refraction and Parachor

In Table I are given the properties of the liquid aliphatic azoxy compounds prepared to date. Densities were determined with a 2-cc. pycnometer. Surface tensions were measured with a DuNouy tensimeter which had been checked against water and alcohol.

TABLE I

No.	Compound	Mol. wt.	n^{20}_D	d^{20}_4	γ_{20}
1	2-Azoxy-2,5-dimethylhexane[3]	270.4	1.4434	0.8564	28.27
2	Ethyl α-azoxyisopropyl ketone	242.3	1.4587	1.0151	35.88
3	Ethyl 2-methyl-2-azoxypropionate[2]	274.3	1.4412	1.0562	33.56

In Table II are given in column 2 the observed molecular refractions; in column 3 the summation of Eisenlohr atomic refractivities,[6] exclusive of the azoxy grouping; and column 4 the refractivity

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[1] Presented in part before the Organic Division of the American Chemical Society at Rochester, N. Y., September, 1937. *Cf.* preceding paper.

[2] Aston and Parker, THIS JOURNAL, **56**, 1387 (1934).

[3] Aston and Ailman, *ibid.*, **60**, 1930 (1938).

[4] Aston and Mayberry, *ibid.*, **57**, 1888 (1935).

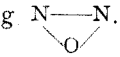
[5] *Cf.* Aston, Menard and Mayberry, *ibid.*, **54**, 1530 (1932).

[6] Eisenlohr, *Z. physik. Chem.*, **75**, 585 (1910); **79**, 129 (1912).

deduced for the atoms of the N_2O group. The index numbers (Col. 1) refer to the compounds identically numbered in Table I.

TABLE II

No.	M. R. exptl. (a)	Σ atomic ref. exclusive of N_2O group (b)	Ref. due to the atoms of the N_2O group (a) − (b)
1	83.772	76.088	7.684
2	65.217	57.638	7.579
3	68.602	60.924	7.678
		Mean	7.647 ± 0.045

We may take the value of N_2 in aliphatic azo compounds as 6.32.[7] From other evidence it is known that the azoxy group in aromatic compounds has the open chain structure. The group refractivity should be obtained by adding the appropriate value for semipolar oxygen to the above value for N_2. Since the existing data do not yield a definite value, as a first try one adds the value for carbonyl oxygen, 2.211. The result is 8.531. A negative exaltation of about 0.9 unit is required to produce agreement with the measured value. Using the value for ether oxygen, 1.643, instead of that for carbonyl oxygen, gives a total of 7.96 units. This value, for no good reason, agrees well with our measured value of 7.65 when we consider that we have not taken into account the loss of the double bond of N=N in forming . It is interesting to note, however, that the molecular refraction of nitrous oxide is 7.58[8] and it has the open chain structure.

In order to calculate the value for aromatic compounds it seems reasonable to add the exaltations observed in aniline and nitrobenzene, 0.85 and 0.78 respectively, to the above value of 8.53, making a total for the azoxy group in aromatic compounds of 10.16. The observed value is 13.25 based on azoxybenzene.[7b] In other words a further positive exaltation of 3.1 units is necessary to produce agreement. Thus consideration of the results on a comparable basis uncovers a discrepancy of the order of 4 units between the experimental group refractivity in aliphatic compounds and in the unsubstituted aromatic compound if both had the same structure. Another method of comparison is to compare the difference of 1.33 between the above values for aliphatic azoxy and azo compounds with the difference of 0.61 between azoxy- and azobenzene.[9] The results obtained with meta substituted aromatic azo and azoxy compounds give a similar result but with ortho and para substituted compounds the difference is as much as two units lower (−1.4).[9] In the face of this big variation among aromatic compounds themselves, it might be said that the difference between azoxy and azo compounds in the aliphatic series is in accord with the difference in the aromatic series. The agreement however may be fortuitous. In view of this the value obtained for the molecular refraction of the azoxy group in aliphatic compounds is difficult to interpret.

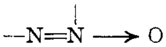
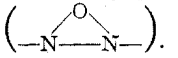
In Table III (Col. 2) are given the parachors (P) deduced from the data of Table I. In column 3 of this table are given the parachors (P_1) calculated using Sugden constants[10] on the basis that the azoxy group has the structure  $-\overset{|}{N}{=}\overset{|}{N}\longrightarrow O$ (*i. e.*, that the oxygen is semi-polar). The parachor value used for semi-polar oxygen was −1.6.

TABLE III

	P	P_1	P_2
2-Azoxy-2,5-dimethylhexane	729.2	724.8	719.9
Ethyl α-azoxy-isopropyl ketone	584.8	586.8	581.9
Ethyl 2-methyl-2-azoxy propionate	625.8	620.4	615.5

In column 4 are given the parachors (P_2) calculated on the basis that the azoxy group has its atoms arranged in a ring .

The agreement is close between the experimental values and those calculated on the basis of the open chain structure.

Similar results have been obtained with aromatic azoxy compounds[10] (p. 119).

The difference between the measured values for aliphatic compounds and those calculated on the basis of a ring might be due to a different parachor value for the heterocyclic ring. This alternative is not particularly appealing but cannot be rejected.

One significant fact bearing on their constitution stands out from the chemical behavior of aliphatic azoxy compounds. It is that the azoxy group does not behave like that in aromatic compounds in which it has unquestionably the unsymmetrical open chain structure. The absence of color in all aliphatic azoxy compounds thus

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[7] (a) Barrick, Drake and Lochte, THIS JOURNAL, **58**, 160 (1936), have measured the molecular refractions of 2,2′-azo-bispropane, 2,2′-azobisbutane, and isopropyl-azo-*s*-butane; (b) Auwers and Heimke, *Ber.*, **61**, 1030 (1938), have studied the methyl and ethyl esters of azoisobutyric acid. From these five compounds we have calculated the mean value of N_2 in azo compounds to be 6.32 ± 0.05.

[8] Brühl, *Z. physik. Chem.*, **25**, 647 (1898).

[9] Auwers, *Ber.*, **61**, 1042 (1928).

[10] Sugden, "The Parachor and Valency," Alfred A. Knopf, New York, 1930, pp. 38 and 116.

far prepared differentiates them from the aromatic azoxy compounds. However, this may be due to absence of conjugation. As a ring structure for the azoxy group in aliphatic compounds is not inconsistent with the data on molecular refraction, one must admit of this possibility, although it requires the assumption of a special parachor value for this ring.

The author wishes to thank Professor J. G. Aston for suggesting this problem.

Summary

1. Ethyl α-azoxyisopropyl ketone and α-azoxyisopropyl isobutyl ketone were prepared by reduction of the corresponding *bis*-nitroso compounds with stannous chloride in concentrated hydrochloric acid.

2. The molecular refractions and parachors of three liquid aliphatic azoxy compounds have been determined and yield a constant value for the azoxy groups.

3. There is no evidence that the azoxy group in aliphatic azoxy compounds has the unsymmetrical open chain structure rather than that of a three-membered ring.

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[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY, UNIVERSITY OF NORTH CAROLINA, AND THE CHICAGO MEDICAL SCHOOL]

Studies on Proteins in Liquid Ammonia. IV. On the Enzymatic Hydrolysis of Proteins Reduced by Metallic Sodium in Liquid Ammonia[1]

BY EVAN W. MCCHESNEY[2] AND RICHARD G. ROBERTS

Previous papers of this series[3] have shown that, of the reactions of proteins in liquid ammonia, perhaps the most interesting is that with metallic sodium. The proteins behave as acids, hydrogen gas is liberated, and sodium salts are formed. From these salts the sodium can be displaced by the addition of ammonium salts such as the chloride or sulfate. During the reaction with sodium reducible groups in the proteins, such as the —S—S— linkages in cystine, undergo reduction. This reaction has been demonstrated by du Vigneaud, Audrieth and Loring.[4]

Goddard and Michaelis[5] have shown that if keratins, such as wool, are treated with sodium thioglycolate in aqueous solution, they undergo reduction of the —S—S— linkages of the cystine residues, forming "kerateines" which are digestible by pepsin and trypsin, even if the resultant —SH groups are reoxidized during the course of the isolation of the kerateine. They postulate that the —S—S— groups act as "very firmly established cross links uniting the elementary fibers of polypeptide chains," and that reduction of the —S—S— groups destroys the fibrous pattern of the protein. Routh[6] has confirmed these observations and has extended them to show that powdered wool, produced by prolonged grinding of wool in a ball mill, is digested about half as fast as casein under comparable conditions.

It occurred to us that it would be of interest to determine whether proteins subjected to reduction by metallic sodium in liquid ammonia are altered with respect to their digestibility by enzymes, and to study proteins of several types, including silk fibroin. This protein is an albuminoid and has been shown[3a] to react rapidly with sodium in liquid ammonia to give a product soluble (as the free acid) in both ammonia and water. Its behavior should be of interest by comparison to wool, since the presence of cystine in the molecule has not been demonstrated.

In this work five substances have been studied: peptone, a substance previously digested by pepsin; egg albumin, a protein not readily digested by trypsin; casein, a protein readily digested by trypsin; wool, an albuminoid containing cystine; and silk fibroin, an albuminoid not containing cystine.[7]

Materials.—Commercial Witte's peptone, egg albumin (Merck impalpable powder), casein (Hammarsten), washed sheep's wool, and silk fibroin (prepared from silk noils supplied by the Cheney Bros. Silk Mills, South Man-

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(1) Presented at the Dallas meeting of the American Chemical Society, April 19, 1938.

(2) Present address: Winthrop Chemical Co., Rensselaer, N. Y.

(3) (a) McChesney and Miller, THIS JOURNAL, **53**, 3888 (1931); (b) Miller and Roberts, *ibid.*, **56**, 935 (1934); (c) Roberts and Miller, *ibid.*, **58**, 309 (1936).

(4) Du Vigneaud, Audrieth and Loring, *ibid.*, **52**, 4500 (1930).

(5) Goddard and Michaelis, *J. Biol. Chem.*, **106**, 605 (1934); **112**, 361 (1935).

(6) Routh, *J. Biol. Chem.*, **123**, Proc., civ (1938).

(7) Analysis by the Sullivan method showed that cystine is entirely lacking in silk fibroin.