nitude of the absorption suggests, on first examination, that these changes constitute only a very small part of the total reaction. However, the addition of alkali immediately increases the absorption by approximately 6 to 8 times, as is shown by a comparison of the ratios of absorptions to peroxide values in columns 2 and 3 of Table I.

TABLE I

RATIOS OF $E^{1\%}_{1\ \mathrm{cm.}}$ TO MEASURED PEROXIDE VALUES AT 2775 Å.

Temp., °C.	Alcohol	KOH 15 min.	KOH 48 hr.
40	0.00067	0.0056	0.0212
60	.00079	.0065	.0177
80	.00089	.0069	.0163
100	.00131	.0077	.0166

It is evident therefore that secondary products, apparently consisting of diene ketones that possess either linear conjugation or cross conjugation, are formed in appreciable amounts. These are presumably enolized by alkali into conjugated triene structures.

The magnitude of the absorption at 2775 Å. suggests, though the evidence is not conclusive, that these secondary products may be in a large measure formed by action of the oxygen that is represented in the differences between the experimental and theoretical peroxide values in Fig. 3. There does not appear to be sufficient evidence available to define the reaction or reactions by which these chromophores are formed, although several possibilities are evident. The constancy of the proportion of conjugated diene peroxides irrespective of temperature, the straight line relationships between spectral absorption at 2775 Å. and peroxide values, and the dependence of the latter relationships on temperature all taken together suggest that the chromophores absorbing at 2775 Å. are not formed from the stable peroxides either by decomposition or by subsequent oxidation, but that they are formed in reactions that are concurrent with the formation of the stable peroxides.

A comparison of the figures in columns 2 and 3 also suggests that not only the amount but the chemical nature of these lesser products is to some extent dependent on the temperature of the autoxidation. Thus, even in its earliest stages, the autoxidation of methyl linoleate appears to be a complicated mixture of reactions, among which the predominant reaction is the formation of stable peroxides by attack on the methylene carbon between the double bonds.

Summary

A study of the early stages of the autoxidation of pure methyl linoleate at 40, 60, 80 and 100° has been made.

Most but not all of the absorbed oxygen may be found in the form of relatively stable linoleate peroxides. A small fraction of the absorbed oxygen at any given temperature is not found in the peroxides, but this fraction increases with increasing temperature.

At all levels of oxidation up to 300 m. e./kg. of peroxide and at all temperatures between 40 and 100°, a constant fraction of the total peroxides is present as conjugated dienes; all of the conjugated dienes are present as peroxides.

In addition, secondary products showing absorption at 2775 Å. are formed in proportion to the oxygen uptake. The proportion of these chromophores increases with increasing temperature. Changes in absorption upon the addition of alkali suggests that they are largely ketonic in character, and that their character is to some extent dependent on the temperature of autoxidation. They appear not to be formed from the stable peroxides but by reactions concurrent with the formation of stable peroxides.

AUSTIN, MINNESOTA RECEIVED NOVEMBER 18, 1946

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF TEXAS]

The Ammonolysis of Ethyl Iodide by Liquid Ammonia[1, 1a]

BY GEORGE W. WATT AND JOHN B. OTTO, JR.

The rate of reaction between ethyl iodide and liquid ammonia and the nature of the reaction products have been the subject of conflicting reports in the chemical literature. White, Morrison, and Anderson[2] reported that the reaction is very slow at −33°, but they did not isolate or identify any of the reaction products. Picon[3] stated that the reaction was complete in twenty-four hours at "ordinary temperatures," and after four hours yielded 28% $C_2H_5NH_2$, nearly an equal quantity of $(C_2H_5)_2NH$ and a very small quantity of $(C_2H_5)_3N$. Markova and Shatenshtein[4] considered the reaction at 25° to be sufficiently slow to permit rate measurements and reported primary amine as the only reaction product. None of these studies has accounted for more than 50% of the ethyl iodide employed in the reactions and all leave the impression that the reaction is slow.

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[1] This work was supported, in part, by grants from the University Research Institute, Project No. 25.

[1a] Presented at the Southwestern Regional Meeting of the American Chemical Society, Dallas, Texas, December 13, 1946.

[2] White, Morrison and Anderson, THIS JOURNAL, **46**, 963 (1924).

[3] Picon, *Bull. soc. chim.*, [4] **35**, 979 (1924).

[4] Markova and Shatenshtein, *Compt. rend. acad. sci. U. R. S.S.*, **35**, 68–70 (1942).

Experimental

Materials.—Ethyl iodide was prepared as described by Hunt[5] and purified by fractional distillation, b. p. 72.0° (71.9–72.0[6]), d^{20}_4 1.9367 (1.9371[7]), n^{21}_D 1.5121 ($n^{18.5}_D$ 1.5133[6]). Ammonia was purified and subjected to prolonged drying over excess sodium amide in the manner recommended by Johnson and Fernelius.[8] Ethylamine hydrochloride was purified by recrystallization from ethanol–water mixtures, m. p. 110° (109°[9]). Diethylamine was purified by fractional distillation, b. p. 55.2° (55.5–56.0°[10]); d^{20}_4 0.7056 (d^{15}_4 0.7094[11]), $n^{20.5}_D$ 1.3850 (n^{19}_D 1.3871[12]), as was triethylamine, b. p., 89.0° (89–90°[10]), d^{20}_4 0.7293 (d^{15}_4, 0.7320[11]), $n^{20.8}_D$ 1.4000 (n^{19}_D 1.4005[12]).

The Ammonolytic Reaction.—All ammonolyses were carried out in sealed *Pyrex* tubes calibrated to contain a total liquid volume of 20.0 ml. and thoroughly dried in an oven at 110° and cooled and maintained in an atmosphere of dry ammonia until sealed. Into each tube, 7.51 g. of ethyl iodide was introduced and liquid ammonia was thereafter condensed up to the calibration mark in a Dry Ice–acetone-bath. There was no appreciable evidence of mixing of the two liquid phases during these operations. The sealed tube was transferred to a thermostat maintained at 0.0 ± 0.5° and allowed to warm to the bath temperature before the reactants were mixed. About two minutes after mixing, a vigorously exothermal reaction was evidenced by boiling of the reaction mixture, refluxing of the ammonia, and a pronounced turbidity. The reaction subsided after about fifteen minutes and the only evidence of further change over an additional period of fifteen minutes was the separation of two liquid phases. The tube was returned to the Dry Ice–acetone-bath and opened in a manner dependent upon the procedure employed in the treatment of the reaction products.

Identification of Products.—In preliminary experiments designed to establish the extent of ammonolysis, tubes were cooled, drawn out to a fine capillary, and the solvent ammonia allowed to escape. The residual material was analyzed gravimetrically for iodide ion as silver iodide. Iodide ion equivalent to 7.51 g. of ethyl iodide amounts to 0.0482 gram-atom and that found by analysis of a typical sample was 0.0479 gram-atom. In separate blank runs it was established that the iodide ion found did not result from hydrolysis.

The absence of tetraethylammonium iodide was demonstrated on the basis of the complete solubility of the ammolysis product in concentrated sodium hydroxide solution.[13] Mono, di and triethylamines were identified in terms of the derivatives listed in Table I and the identity of each derivative was established by determination of the melting temperatures of mixtures with authentic samples.

Separation and Estimation of Ethylamines.—Several methods for the separation and estimation of ethylamines were investigated in considerable detail and each was evaluated by the use of known mixtures of ethylamine hydrochlorides (alone as well as in the presence of a large excess of ammonium chloride) compounded from carefully purified materials.[16] The method described below is that found to be the most reliable and the least time-consuming. It consists of a combination and extension of the familiar Hinsberg procedure[17] and the method of Bertheaume.[18]

TABLE I

DERIVATIVES OF ETHYLAMINES

Ethyl-amine	Deriv.	M. p., °C. (cor.) From ammonolysis	Authentic sample	Mixt.	Lit.
Mono	Benzenesulfonyl	56.7	56.7	56.7	57–58[14]
Di	Benzenesulfonyl	41.5	41.3	41.5	42–43[14]
Tri	Picrate	174.9	174.9	174.9	173[15]

After completion of the ammonolytic reaction as described above, the entire reaction mixture was collected in an excess of dilute hydrochloric acid solution. A substantially quantitative separation of amine hydrochlorides from ammonium chloride was accomplished by evaporation of the acidic solution to dryness on the steam-bath, desiccation of the residue, and extraction (Soxhlet) with dry chloroform over a period of twelve hours under strictly anhydrous conditions. The chloroform was distilled from the extract and the residual amine hydrochlorides were dissolved in water.

The aqueous solution was agitated vigorously in a separatory funnel with 35 ml. of 12 *M* sodium hydroxide solution and 6 ml. of benzenesulfonyl chloride in a total solution volume of 100 ml. over a period of ten minutes at 0–5°. This reaction mixture was then extracted three times with 50-ml. portions of cold ethyl ether and the combined ether extracts were in turn extracted three times with 25-ml. portions of cold 1 *M* hydrochloric acid solution. The sodium hydroxide, ether, and hydrochloric acid solutions were treated separately as follows.

The ether extract was found to contain the benzenesulfonyl diethylamide, some dibenzenesulfonyl ethylamide and the excess benzenesulfonyl chloride. After evaporation of the ether, the excess of benzenesulfonyl chloride was hydrolyzed to sodium benzene sulfonate and the disubstituted primary amine was hydrolyzed to the monosubstituted derivative by warming the residual oil for half an hour with 10 ml. of 6 *M* sodium hydroxide together with enough ethanol to keep the oil in solution. Thereafter, the solution was cooled to the temperature provided by a mixture of ice and calcium chloride, seeded, diluted slowly and with stirring with about 100 ml. of sodium chloride solution (30 g. sodium chloride/liter) and allowed to stand overnight. The benzenesulfonyl diethylamide was removed by filtration, washed with cold saturated sodium chloride solution, and transferred quantitatively to a Kjeldahl digestion flask. The nitrogen content as determined by the usual Kjeldahl procedure was interpreted as equivalent to the secondary amine content of the original reaction mixture.

The filtrate from the separation of benzenesulfonyl diethylamide was found to contain a small fraction of the benzenesulfonyl ethylamide. Hence this filtrate was combined with the sodium hydroxide solution that contained the bulk of the primary amine as the monobenzenesulfonyl derivative. The resulting solution was diluted to 500 ml. and a 50-ml. aliquot was transferred to a Kjeldahl flask and evaporated to dryness by heating the flask with steam and passing a slow stream of air over the sample.[19] Thereafter, primary amine content of the original reaction mixture was calculated on the basis of the nitrogen content (by Kjeldahl) of the aliquot.

The 1 *M* hydrochloric acid solution contained the tertiary amine fraction and the absence of other than traces of ammonium ion was established by the method of Budai.[20] Accordingly, nitrogen in the acid extract was determined (Kjeldahl) and calculated as triethylamine.

Typical results obtained in the application of the fore-

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[5] Hunt, *J. Chem. Soc.*, **117**, 1592 (1920).

[6] Cottrell and Rogers, *Am. Chem. J.*, **21**, 64 (1899).

[7] Patterson and Thompson, *J. Chem. Soc.*, **93**, 371 (1908).

[8] Johnson and Fernelius, *J. Chem. Ed.*, **6**, 443 (1929).

[9] Curtius and Hille, *J. prakt. Chem.*, [2] **64**, 410 (1901).

[10] Hofmann, *Ber.*, **22** 705 (1889).

[11] Swift, THIS JOURNAL, **64**, 115 (1942).

[12] Gladstone, *J. Chem. Soc.*, **45**, 246 (1884).

[13] "Allen's Commercial Organic Analysis," 5th edition, Vol. V, P. Blakiston's Son and Co., Philadelphia, Pa., 1927, p. 293.

[14] Ginzberg, *Ber.*, **36**, 2706 (1903).

[15] Jerusalem, *J. Chem. Soc.*, **95**, 1281 (1909).

[16] The details of these studies are too extensive for inclusion here; see J. B. Otto, Jr., M. A. Thesis, The University of Texas, June, 1943.

[17] Shriner and Fuson, "Identification of Organic Compounds," 2nd ed., John Wiley and Sons, Inc., New York, 1940, p. 48.

[18] Bertheaume, *Compt. rend.*, **150**, 1251 (1910).

[19] In experiments involving samples of known composition, this step was found necessary in order to avoid loss of primary amine by steam distillation following addition of concentrated sulfuric acid.

[20] Budai, *Z. physiol. Chem.*, **86**, 107 (1913).

going procedure to the estimation of ethylamines in mixtures of known composition are given in Table II.

TABLE II

ESTIMATION OF ETHYLAMINES IN MIXTURES OF KNOWN COMPOSITION

Ethylamine	5.0 g. NH_4Cl present Present, g.	 Found, g.	NH_4Cl absent[a] Present, g.	 Found, g.
Mono	1.03	0.95	1.03	0.99
Di	0.49	0.48	0.49	0.49
Tri	0.30	0.27	0.30	0.28

[a] Chloroform extraction step omitted.

Table III includes representative data from studies on the ammonolysis of ethyl iodide. Yields are expressed on the basis of 7.51 g. of ethyl iodide employed in each ammonolytic reaction and the total for each run represents the percentage of the starting material accounted for by analysis.

TABLE III

ESTIMATION OF ETHYLAMINES FROM THE AMMONOLYSIS OF ETHYL IODIDE

Ethylamine	Yield, % 1	 2	 3	 Average
Mono	46	45	46	46
Di	31	31	32	31
Tri	17	16	17	17
Total	94	92	95	94

Discussion

In contrast to the somewhat fragmentary reports from earlier investigations, the present work shows conclusively that the ammonolysis of ethyl iodide by excess liquid ammonia at 0° is a rapid and vigorously exothermal reaction that is complete in about fifteen minutes and leads to the formation of mono, di, and tri-ethylamines. The absence of tetraethylammonium iodide is not surprising since this product would be expected only if the ethyl iodide were present in excess or if the reaction were slow and tetraethylammonium iodide were of low solubility in liquid ammonia.

With regard to the methods employed in the separation and estimation of ethylamines, carefully conducted experiments involving samples of known composition showed that the procedure gives surprisingly accurate measurement of the secondary amine content of the mixtures. These studies showed also that the results for primary and tertiary amines are always low but too variable from one experiment to another to warrant application of a correction factor without sufficient data to establish a reliable average deviation. A comparison of the two sets of data in Table II shows that the chloroform extraction step (separation of amine hydrochlorides from ammonium chloride) entails a small but significant loss of primary amine. Finally, it should be emphasized that the known mixtures used to evaluate the different procedures were ones which simulated approximately the composition of the reaction mixture. Accordingly, it is not claimed that the procedures described above would lead to equally satisfactory results when applied to mixtures containing widely different mole ratios of mono, di and triethylamine.

Summary

1. The ammonolysis of ethyl iodide by an excess of liquid ammonia under strictly anhydrous conditions at 0° has been shown to be a rapid exothermal reaction that is complete in less than fifteen minutes and yields 46% ethylamine, 31% diethylamine and 17% triethylamine.

2. Methods have been devised for the separation and estimation of ethylamines from mixtures containing a large excess of ammonium salts. The reliability of these methods has been established in terms of samples of composition known to approximate that of the gross product of the ammonolytic reaction.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Activity of the Red Pigment from Leguminous Root Nodules[1]

BY HENRY N. LITTLE AND R. H. BURRIS

The root nodules of leguminous plants that are actively fixing nitrogen contain a red pigment. Without the evidence of isolation and characterization, Pietz[2] erroneously suggested that the compound was an oxidation product of 3,4-dihydroxyphenylalanine. Kubo[3] reported that observation of suspensions of crushed nodules showed upon shaking an absorption band at 555 and 575 mμ. He concentrated the pigment by ammonium sulfate fractionation and demonstrated that it was a hemoprotein. He observed that the absorption maxima of a number of derivatives of the nodule pigment closely resembled corresponding derivatives from hemoglobin, and that the pigment yielded hemin crystals apparently identical with those obtained from horse hemoglobin. Addition of the pigment stimulated oxygen uptake by suspensions of *Rhizobium japonicum* oxidizing sodium succinate. Burris and Haas[4] found an absorption maximum for the reduced pigment at 555 mμ. and in addition strong

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[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the Wisconsin Alumni Research Foundation.

[2] J. Pietz, *Zentr. Bakt. Parasitenk.*, II, **99**, 1 (1938).

[3] H. Kubo, *Acta phytochim., Japan*, **11**, 195 (1939).

[4] R. H. Burris and E. Haas, *J. Biol. Chem.*, **155**, 227 (1944).