Anal. Calcd. for $C_{27}H_{36}O_4N_2 \cdot C_{20}H_{16}O_{10}N_8$ (980.97): C, 57.54; H, 5.34; N, 14.28. Found: C, 57.45; H, 5.59; N, 14.25.

In a similar manner the reduction of 0.50 g. of the dehydro base lactam XXI was also performed. The product (410 mg.) appeared to be a mixture of *dl*-emetine and *dl*-isoemetine. It gave 230 mg. of a hydrochloride which melted over the range 225–238° dec. after several crystallizations from ethanol–ethyl acetate. By treatment with ammonia this derivative yielded 120 mg. of an amorphous base, m.p. 60–68°, in which the presence of *dl*-emetine was readily demonstrated by the infrared spectrum and also by the formation of its oxalate, m.p. 159–161°, after recrystallization from methanol–ether.[3f]

From the reduction of 0.65 g. of the dehydro base lactam XXIb was obtained 0.35 g. of a hydrochloride, m.p. 236–240° dec. after recrystallization from ethanol–ethyl acetate, as reported.[3f] The infrared spectrum of the regenerated free base was very similar to that of (−)-emetine; however, it also contained a number of small but distinct differences.

Formation of the Tricyclic Ester Base XIX from the 191° Lactam Acid XVI. A.—To 35 ml. of refluxing absolute ethanol containing 200 mg. of the lactam acid XVI, 3 g. of sodium was added, in small pieces. After the reaction was complete the mixture was concentrated *in vacuo*, acidified with dilute hydrochloric acid, and extracted with three 25-ml. portions of benzene–ether (1:1). The aqueous phase was evaporated to dryness under reduced pressure, and 50 ml. of absolute ethanol was added. The solution was filtered, saturated with hydrogen chloride at 20°, and allowed to stand overnight at this temperature. After evaporation of the solvent under reduced pressure the residue was treated with 20 ml. of 5% aqueous ammonia and re-extracted with chloroform. Evaporation of the chloroform furnished 45 mg. of an ester (λ_{max} 5.77 μ; no lactam absorption) which was purified by chromatography on neutral alumina (Woelm, activity grade 1) and then treated with anhydrous hydrogen chloride in ethanol–ether. The resulting hydrochloride (12 mg.), m.p. 192–194°[3d] after recrystallization from ethanol–ethyl acetate, crystallized with some difficulty. The regenerated free ester (XIX, R = Et) was evaporatively distilled, b.p. (bath temp.) 145–150° (0.0004 mm.), and then crystallized from petroleum ether (b.p. 40–60°) as fine needles, m.p. 65–66° (hot-stage) (lit.[3c,h] 66–66.5°). The perchlorate crystallized from ethanol–ether in stout needles, m.p. 145–146°[3h] (raised to 167–169° after being dried *in vacuo* at 80°). A mixed m.p. of this derivative with an authentic specimen graciously provided by Dr. A. R. Battersby[3h,20] was undepressed.

The corresponding methyl ester (XIX, R = Me), m.p. 78–97°[5a] (hydrochloride m.p. 204–206°[5a]), could also be obtained from the reduction product in similar fashion. The infrared spectrum of this ester and that of an authentic sample kindly supplied by Professor E. E. van Tamelen[5a,20] were identical.

B.—Alternatively, and somewhat more satisfactorily, the lactam function in XVI could be reduced at high pressure (4500 p.s.i.) and elevated temperature (230°) with Adkins copper chromium oxide catalyst[23] in freshly purified dioxane. By this means, the hydrogenation of 100 mg. of XVI in a 20-ml. micro glass liner with 80 mg. of high-activity catalyst[23] in 5 ml. of dioxane afforded, after esterification of the basic product with ethanol and chromatography on alumina, 21 mg. of the hydrochloride of XIX (R = Et), m.p. 192–194°. The regenerated ester had m.p. and mixed m.p. 65–66° with the preceding preparation, and the infrared spectra were also identical.

CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY, UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL, N. C.]

The Pyrolysis of 2-(N-β-Acyloxyethylanilino)-4,6-dialkoxy-*s*-triazines[1]

BY RICHARD G. HISKEY,[2] JEROME HOLLANDER[3] AND J. F. BUNNETT

RECEIVED APRIL 1, 1960

The pyrolysis of 2-(N-β-acyloxyethylanilino)-4,6-dialkoxy-*s*-triazines proceeds smoothly, in the absence of solvent, giving high yields of 2-N-vinylanilino-4-hydroxy-6-alkoxy-*s*-triazines and the corresponding alkyl ester. Pyrolysis of the triazines in the presence of added anions and decomposition of carbonyl oxygen-18 labeled 2-(N-β-acetoxyethylanilino)-4,6-dimethoxy-*s*-triazine indicates the reaction involves the formation of a free acid anion. The unusual ease of pyrolysis, 250°, suggests the formation of the acid anion may proceed by an intramolecular process involving a nitrogen atom of the triazine ring. A mechanism compatible with the observed experimental results is proposed.

Numerous examples in the literature describe the pyrolytic decomposition of esters containing a β-hydrogen in the alkyl portion of the molecule. The accepted interpretation[4] of the process depicts the formation of the observed reaction products, an acid and an olefin, by a cyclic transition state. Schaefer, *et al.*,[5] however, have described the pyrolysis of several esters which did not yield the expected products. For example, pyrolysis of 2-(N-β-acetoxyethylanilino)-4,6-diethoxy-*s*-triazine (Ib) at 250°, resulted in the formation of ethyl acetate (IIIb) and a product assigned as 2-N-vinylanilino-4-hydroxy-6-ethoxy-*s*-triazine (IIb). Likewise, pyrolytic decomposition of the dimethoxy-*s*-triazine acetate (Ia) was reported to yield IIa and presumably IIIa, although the latter ester was not characterized. In contrast 2,4,6-tris-(N-β-acetoxyethylanilino)-*s*-triazine was stable when heated to 350° alone or in the presence of 2,4,6-trimethoxy-*s*-triazine. These observations led to the suggestion[5] that the products were produced by an unknown type of intramolecular decomposition. The present report concerns a more detailed study of these interesting results in an effort to clarify the course of the pyrolysis reaction.

The dialkoxy-*s*-triazine esters (Ia–d) were prepared in good yield using cyanuric chloride and N-phenylethanolamine[6] as starting materials. Etherification of the resulting 2-(N-β-hydroxyethylanilino)-4,6-dichloro-*s*-triazine with the appropriate alcohol in base afforded the 2-(N-β-hydroxyethylanilino)-4,6-dialkoxy-*s*-triazines which were esterified with the desired acid chloride in pyridine solution.

(1) Presented in part before the Division of Organic Chemistry. Abs. of Papers, 136th Meeting Amer. Chem. Soc., Atlantic City, N. J., September, 1959, p. 11-P. Supported in part by the National Science Foundation (Grant No. NSF-G2359).

(2) To whom inquiries should be addressed.

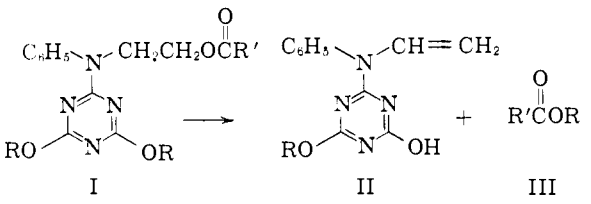
(3) Abstracted from the Ph.D. Dissertation of Mr. Jerome Hollander, January, 1960.

(4) (a) C. D. Hurd and F. H. Blunck, THIS JOURNAL, **60**, 2419 (1938); (b) D. H. R. Barton, *J. Chem. Soc.*, 2174 (1949); (c) C. H. DePuy, R. W. King and D. H. Froemsdorf, *Tetrahedron*, **7**, 123 (1959).

(5) F. C. Schaefer, J. R. Dudley and J. T. Thurston, THIS JOURNAL, **73**, 3004 (1951).

(6) J. R. Dudley, J. T. Thurston, F. C. Schaefer, D. Holm-Hansen, C. J. Hull and P. Adams, *ibid.*, **73**, 2986 (1951).

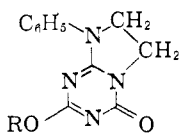
Pyrolysis of the dialkoxy-*s*-triazine esters was accomplished by heating the esters, in the absence of solvent, to 200–250° for 1–3 hours. In this manner pyrolysis of Ia afforded a 75% yield of IIa and a 62.3% yield of IIIa. Pyrolysis of Id yielded 71% of IIId.



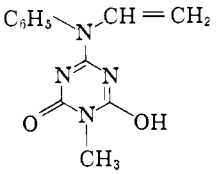
I II III

a, R = R′ = CH_3
b, R = $-C_2H_5$, R′ = CH_3
c, R = CH_3, R′ = C_3H_7
d, R = C_2H_5, R′ = C_3H_7

The structure of the non-volatile pyrolysis product of Ia was previously[5] assigned as IIa, primarily on the basis of the elemental analysis and spectral data. However, the substance was reported to be insoluble in dilute alkali and inert to the action of bromine in carbon tetrachloride. In view of these apparent discrepancies more conclusive evidence regarding the structure of the pyrolysis product was desirable. At least two alternative formulations of IIa were possible from the available evidence. The substituted imidazolidine IV would not be expected to add bromine and should be insoluble in dilute alkali. Also the triazine V must be considered in view of the O to N alkyl migrations observed in other alkoxy-*s*-triazines[7] and aryl-N-phenyliminobenzoates.[8]



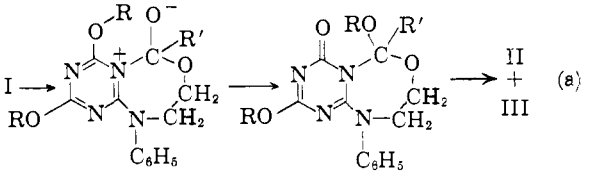
IV



V

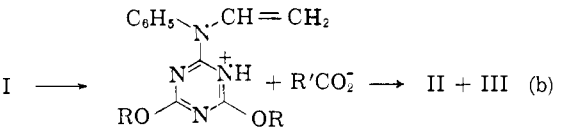
The non-volatile pyrolysis product failed to decolorize solutions of potassium permanganate in acid and neutral solvents and yielded a perbromide salt when treated with bromine in acetic acid. Attempted oxidation of the substance with chromium trioxide afforded no tractable products and catalytic hydrogenation, using Adams catalyst, furnished only starting material. Acid hydrolysis of the substance, however, provided evidence for the presence of the N-vinylanilino grouping. When the pyrolysis product was refluxed in 50% sulfuric acid for 11 hours, acetaldehyde could be readily identified. Further evidence in favor of either IIa or V was obtained from an active hydrogen determination which indicated the presence of a single active hydrogen. The present data do not allow a decision between structures IIa and V although a closely related compound, 2-N-methylanilino-4,6-dimethoxy-*s*-triazine, was recovered unchanged after heating at 185–205°.[7] 2-Alkoxy-6-hydroxypyrimidines are reported to exist as the alkoxypyrimidone tautomer in aqueous solution[9] and thus in addition to V, 2-N-vinylanilino-6-methoxy-4-*s*-triazone is a possible representation. However, since this assignment has no bearing on the ensuing discussion, structure IIa has been employed.

A priori, the mechanism of pyrolysis of I might involve (a) an intramolecular process wherein the alkyl and acyloxy (or alkoxy and acyl) groups *from the same molecule* combined to form a simple ester (III); or (b) an intermolecular process in which an alkyl, alkoxy, acyl or acyloxy group was released, later to combine with the necessary moiety *from the same or another molecule* to form a simple ester III. A plausible intramolecular mechanism may be represented as



(a)

An attractive intermolecular process involves intramolecular E2 elimination of $R'CO_2^-$, followed by alkylation of this carboxylate ion by the ether function of the original or another *s*-triazine molecule



(b)

VI

In order to distinguish between the two possible reaction paths, a mixture of Ia and Id was pyrolyzed. The volatile mixture of esters, obtained from the pyrolysis, was analyzed by means of a vapor fractometer and was found to consist of approximately equal parts of methyl acetate, ethyl acetate, methyl *n*-butyrate and ethyl *n*-butyrate. Control experiments indicated that only negligible amounts of ethyl acetate and methyl *n*-butyrate were formed by ester interchange under the conditions employed for the pyrolysis. The formation of four esters during pyrolysis is consistent with an intermolecular process (path b) involving, at some stage in the reaction, a free carboxylate anion. Additional evidence for path b was obtained by the pyrolysis of Ic in the presence of added sodium acetate. The vapor fractogram of the volatile pyrolysis products indicated the presence of 26.6% methyl acetate and 73.4% methyl *n*-butyrate. Likewise when Ia was pyrolyzed in the presence of sodium phenoxide the distillate consisted of 34.4% methyl acetate and 56.5% anisole.

Further evidence against the intramolecular mechanism was provided by study of the pyrolysis of carbonyl oxygen-18 labeled Ia. The labeled ester was prepared by acetylation of VII with oxygen-18 labeled acetyl chloride, a portion of which was separately ethanolized to ethyl acetate. Pyrolysis of the labeled Ia produced, in 96% yield, methyl acetate of oxygen-18 analysis approximately

(7) F. C. Schaefer, J. R. Dudley and J. T. Thurston, THIS JOURNAL., **73**, 2996 (1951).

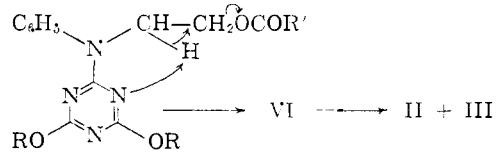
(8) A. W. Chapman, *J. Chem. Soc.*, 1743 (1927).

(9) J. R. Marshall and J. Walker, *ibid.*, 1004 (1951).

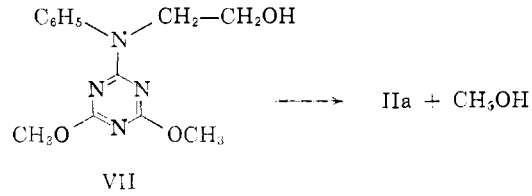
equal to this ethyl acetate. This shows that all the carbonyl oxygen of Ia appears in IIIa, as expected from either mechanism. However, hydrolysis of the methyl acetate from pyrolysis gave methanol of unchanged oxygen isotope ratio. This means that the two oxygen atoms of the volatile pyrolysis product have equal isotopic composition, and suggests that the two oxygens were chemically equivalent at some stage in pyrolysis. This result is compatible with intermolecular path b but not with the intramolecular mechanism.

These two lines of evidence strongly suggest that the volatile ester produced in this reaction arises by attack of the carboxylate anion on the alkoxy carbon atom of the intermediate triazinium salt VI. Biomolecular decompositions of this type are analogous to the thermal transformation of iminoester hydrohalides to amides and alkyl halides.[10,11] Also a number of similar ether cleavages have been reported in various heterocyclic systems.[12]

The observation[5] that acylation of 2,4-bis-(N-β-hydroxyethylanilino)-6-methoxy-*s*-triazine with refluxing acetic anhydride yielded only 2-N-vinylanilino-4-(N-β-hydroxyethylanilino)-6-methoxy-*s*-triazine rather than the anticipated diacetate prompted a study of the pyrolysis of Ia at lower temperatures. When Ia was pyrolyzed at 100° for 2 hours a 7.4% yield of ester IIIa was obtained; heating at 100° for 5 hours afforded 11.1% of IIIa. Since the normal pyrolysis of primary acetate esters does not usually occur below 400–450° another factor, responsible for the unusual ease of pyrolysis, was sought. It appeared likely that the nitrogen atoms of the triazine ring were assisting in the elimination of the carboxylate ion. To test this possibility the carbon analog of Ia, N,N-diphenylaminoethyl acetate, was pyrolyzed at 250 and 350°. No acetic acid was observed at either temperature and, in fact, only when the pyrolysis was conducted at 500° was acetic acid (6.1%) detected. A mechanism consistent with these results would involve intramolecular β-elimination initiated by attack of a nuclear nitrogen atom on a hydrogen atom beta to the acyloxy group, forming the triazinium salt VI and the carboxylate ion. Decomposition of VI, in the manner previously discussed, would afford the observed products.



The results obtained from the pyrolysis of 2-(N-β-hydroxyethylanilino)-4,6-dimethoxy-*s*-triazine (VII) are interesting in this regard. When VII was heated at 200–260° for 1.5 hours, methanol (43.8% yield) and IIa were the observed products. Thus the hydroxyl group may also function as a leaving group in the elimination process. Further studies



VII

on this rather unique pyrolytic elimination reaction are in progress.

Acknowledgment.—The authors are grateful to Dr. C. A. Bunton, University College, London, who kindly performed the oxygen-18 analyses and to Professor R. L. McKee for his valuable suggestions.

Experimental[13]

2-(N-β-Hydroxyethylanilino)-4,6-dimethoxy-*s*-triazine (VII).—Following the procedure of Dudley, *et al.*,[5] 2-(N-β-hydroxyethylanilino)-4,6-dimethoxy-*s*-triazine was obtained by the reaction of 40.0 g. (0.14 mole) of 2-(N-β-hydroxyethylanilino)-4,6-dichloro-*s*-triazine with 140 ml. of absolute methanol and 11.2 g. (0.28 mole) of sodium hyroxide. The product, 21.0 g. (54.3%), melted at 144–145.5°, reported[6] m.p. 143–145°. The ultraviolet spectrum, in ethanol, exhibited an absorption maximum at 243 mμ, ε 19,500.

2-(N-β-Acetoxyethylanilino)-4,6-dimethoxy-*s*-triazine (Ia).—To a cold solution of 5.52 g. (0.02 mole) of the dimethoxy-*s*-triazine alcohol (VII) in 31 ml. of pyridine was added 1.74 g. (0.022 mole) of acetyl chloride, dropwise with mechanical stirring. The reaction mixture was stirred at room temperature for 3 hours and then poured into 1 *N* hydrochloric acid solution. The white solid which formed was dissolved in ether, washed in 1 *N* hydrochloric acid and water and dried over sodium sulfate. Removal of the ether, *in vacuo*, yielded 5.87 g. (92.2%) of the dimethoxy-*s*-triazine acetate as a colorless oil.

The dialkoxy-*s*-triazine esters (Ia, c, d) resisted numerous attempts at crystallation. The analytical results were obtained on liquid samples of the esters prepared in the following manner: The ester was dissolved in ether, washed well with water and dried. Removal of the ether *in vacuo* yielded an oil which was redried *in vacuo* and submitted for analysis. The infrared spectrum, determined in Nujol, showed absorptions at 1740 and 1230 cm.$^{-1}$. The ultraviolet spectrum, in ethanol, exhibited an absorption maximum at 242 mμ, ε 13,100.

Anal. Calcd. for $C_{15}H_{18}N_4O_4$: C, 56.61; H, 5.69; N, 17.59. Found: C, 56.11; H, 5.73; N, 17.29.

2-(N-β-*n*-Butyroxyethylanilino)-4,6-dimethoxy-*s*-triazine (Ic).—Using a procedure similar to that employed for the preparation of Ia, 3.0 g. (0.01 mole) of the dimethoxy-*s*-triazine alcohol afforded 1.95 g. (51.4%) of 2-(N-β-*n*-butyroxyethylanilino)-4,6-dimethoxy-*s*-triazine as a colorless oil.

Anal. Calcd. for $C_{17}H_{22}N_4O_4$: C, 58.95; H, 6.41; N, 16.17. Found: C, 58.98; H, 6.67; N, 16.27.

2-(N-β-*n*-Butyroxyethylanilino)-4,6-diethoxy-*s*-triazine (Id).—By the procedure described for the preparation of VII, 33.5 g. (64%) of 2-(N-β-hydroxyethylanilino)-4,6-diethoxy-*s*-triazine was obtained from 50.0 g. (0.175 mole) of 2-(N-β-hydroxyethylanilino)-4,6-dichloro-*s*-triazine, 200 ml. of absolute ethanol and 14.0 g. (0.35 mole) of sodium hydroxide. The crude product melted at 84–88° and was used directly in the preparation of 2-(N-β-*n*-butyroxyethylanilino)-4,6-diethoxy-*s*-triazine. The butyrate ester was obtained using the procedure described for the preparation of Ia; 3.0 g. (0.01 mole) of the crude diethoxy-*s*-triazine alcohol afforded 2.80 g. (74.6%) of ester when treated with

(10) S. M. McElvain and B. E. Tate, THIS JOURNAL, **73**, 2233 (1951).

(11) C. L. Stevens, D. Morrow and J. Lawson, *ibid.*, **77**, 2341 (1955).

(12) E. Wasser and K. Sander, *Helv. Chim. Acta*, **8**, 106 (1925); G. E. Hilbert and T. B. Johnson, THIS JOURNAL, **52**, 1152 (1930); G. Illuminati and H. Gilman, *ibid.*, **71**, 3349 (1949); H. Gilman, K. E. Lentz and J. A. Beel, *ibid.*, **74**, 1081 (1952).

(13) The melting and boiling points are uncorrected unless otherwise noted. Elemental analysis by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and Micro-Tech Laboratories, Skokie, Ill. Active hydrogen determinations by Clark Microanalytical Laboratory, Urbana, Ill.

1.17 g. (0.011 mole) of *n*-butyryl chloride. The product was obtained as a colorless oil.

Anal. Calcd. for $C_{19}H_{26}N_4O_4$: C, 60.95; H, 7.00; N, 14.96. Found: C, 60.90; H, 7.05; N, 15.27.

Pyrolysis of 2-(N-β-Acetoxyethylanilino)-4,6-dimethoxy-s-triazine (Ia).—Following the procedure of Schaefer, *et al.*,[5] a 5.87-g. (0.018 mole) sample of 2-(N-β-acetoxyethylanilino)-4,6-dimethoxy-*s*-triazine was heated in a 25-ml. distilling flask, under a nitrogen atmosphere, for 1.25 hours at 200–240°. A Wood metal-bath was used to maintain the desired temperature range. The distillate, 0.81 g. (62.3%), was collected in a Dry Ice trap and identified as methyl acetate, b.p.(cor.) 54.8°, $n^{28}D$ 1.3572; reported b.p. 57.5°,[13a] $n^{20}D$ 1.3594.[13b]

The residue in the distilling flask was recrystallized from dioxane to yield 3.3 g. (75%) of 2-N-vinylanilino-4-hydroxy-6-methoxy-*s*-triazine as white needles, m.p. 255–255.5° reported[5] 252–255°. The ultraviolet absorption spectrum of the non-volatile pyrolysis product determined in ethanol exhibited an absorption maximum at 262 mμ, ε 23,500. The spectrum in 1 *N* sodium hydroxide showed a maximum at 252 mμ, ε 3,100.

Anal. Calcd. for $C_{12}H_{12}N_4O_2$: C, 59.01; H, 4.95. Found: C, 59.23; H, 4.88.

A sample of purified Ia from another pyrolysis experiment was analyzed for the number of active hydrogens per molecule; calcd. for one active hydrogen 0.21, found 0.28.

Pyrolysis of 2-(N-β-*n*-Butyroxyethylanilino)-4,6-diethoxy-s-triazine (Id).—A 1.0 g. (0.0027 mole) sample of 2-(N-β-*n*-butyroxyethylanilino) - 4,6 - diethoxy - *s* - triazine, when heated to 250–260° for 1.5 hours under a nitrogen atmosphere, furnished 0.22 g. (71%) of ethyl *n*-butyrate, b.p. (cor.) 122.4°, $n^{26}D$ 1.3900; reported b.p. 119.9–120.1°,[13a] $n^{18}D$ 1.3931.[14]

Hydrolysis of 2-N-Vinylanilino-4-hydroxy-6-methoxy-s-triazine (IIa).—A 0.5 g. (0.002 mole) sample of 2-N-vinylanilino-4-hydroxy-6-methoxy-*s*-triazine was dissolved in 50 ml. of 50% sulfuric acid and heated at reflux for 11 hours. The volatile hydrolysis product was collected by distillation and identified as acetaldehyde by the following derivatives: acetaldehyde-2,4-dinitrophenylhydrazone, m.p. 146–148°, reported[15a] m.p. 147°; acetaldehyde bis-menthone, m.p. 140–141.5°; reported[15b] 141–142°, octahydroxanthene derivative, m.p. 175–176°, reported[15b] 176–177°. Mixture melting points of the derivatives obtained from the distillate and authentic samples of each derivative, prepared from acetaldehyde, were not depressed.

Pyrolysis of a Mixture of 2-(N-β-Acetoxyethylanilino)-4,6-dimethoxy-s-triazine (Ia) **and 2-(N-β-*n*-Butyroxyethylanilino)-4,6-diethoxy-s-triazine** (Id).—A mixture of 1.72 g. (0.0054 mole) of Ia and 2.80 g. (0.0075 mole) of Id was heated under a nitrogen atmosphere at 200–250° for 1.5 hours. The distillate, collected in a Dry Ice trap, weighed 0.9 g. A sample of the distillate was analyzed in a vapor fractometer. The results indicated the presence of 17.7% IIIa, 22.1% of IIIb, 33.4% IIIc and 26.8% of IIId (Perkin–Elmer column A, 130°, helium flow 75 cc./min.; elution times, 2.7, 4.1, 7.0 and 10.2 min.).

Pyrolysis of 2-(N-β-*n*-Butyroxyethylanilino)-4,6-dimethoxy-s-triazine (Ic) **with Sodium Acetate.**—A mixture of 1.95 g. (0.006 mole) of Ic and 0.5 g. (0.006 mole) of anhydrous sodium acetate was heated in a distillation flask, under a nitrogen atmosphere, at 200–250° for 1.5 hours. The distillate, 0.4 g., was analyzed by means of a vapor fractometer. The results indicated the presence of 26.6% IIIa and 73.4% IIIc. The residue from the pyrolysis, after recrystallization from dioxane, yielded 1.40 g. (96%) of 2-N-vinylanilino-4-hydroxy-6-methoxy-*s*-triazine (IIa), m.p. 254–255°.

Pyrolysis of 2-(N-β-Acetoxyethylanilino)-4,6-dimethoxy-s-triazine (Ia) **with Sodium Phenoxide.**—A mixture prepared from 1.27 g. (0.004 mole) of 2-(N-β-acetoxyethylanilino)-4,6-dimethoxy-*s*-triazine and 0.46 g. (0.004 mole) of sodium phenoxide was pyrolyzed at 200–260° for 3 hours. The distillate, 0.3 g., was analyzed by means of a vapor fractometer and consisted of 34.4% IIIa and 56.5% anisole (Perkin–Elmer column A, 150°, helium flow 140 cc./min., elution times 1.2 and 8.6 min.).

Exchange Reactions of Methyl Acetate and Ethyl *n*-Butyrate. A. Methyl Acetate and Ethyl-*n*-Butyrate.—A mixture of 0.5 ml. each of methyl acetate and ethyl *n*-butyrate was heated at 200–260° for 1 hour in a sealed tube. Analysis of the mixture using the vapor fractometer indicated only IIIa and IIId were present.

B. Methyl Acetate and Ethyl-*n*-Butyrate with Quinoline and 2-Hydroxy-4-methylquinoline.—A mixture of 0.5 ml. of methyl acetate and 0.8 ml. of ethyl *n*-butyrate was heated at 250° for 1.5 hours in the presence of 0.31 ml. of quinoline and 0.24 g. of 2-hydroxy-4-methylquinoline. The reaction was carried out in a sealed tube. Analysis of the mixture in the vapor fractometer indicated the presence of 40.27% IIIa, 56.1% IIId, 2.14% IIIc and 1.54% IIIb.

C. Methyl Acetate and Ethyl *n*-Butyrate with 2-N-Vinylanilino-4-hydroxy-6-methoxy-s-triazine.—A mixture of 0.125 ml. of methyl acetate and 0.2 ml. of ethyl *n*-butyrate was heated at 250° for 1.5 hours, in a sealed tube, with 0.356 g. of 2-N-vinylanilino-4-hydroxy-6-methoxy-*s*-triazine. Analysis of the mixture in a vapor fractometer indicated the presence of 42.17% IIIa, 54.9% IIId, 1.80% IIIc and 1.21% IIIb.

Pyrolysis of Carbonyl Oxygen-18 Labeled 2-(N-β-Acetoxyethylanilino)-4,6-dimethoxy-s-triazine.—A 16.0 g. (0.05 mole) sample of carbonyl oxygen-18 labeled dimethoxy-*s*-triazine acetate, prepared in 77.3% yield from oxygen-18 labeled acetyl chloride and 2-(N-β-hydroxyethylanilino)-4,6-dimethoxy-*s*-triazine, was pyrolyzed under the conditions previously described. The methyl acetate collected weighed 3.57 g. (96.2%).

Anal. Found: atom per cent. excess O^{18}, 0.294.

The residue from the pyrolysis afforded 11.3 g. (92.6%) of 2-N-vinylanilino-4-hydroxy-6-methoxy-*s*-triazine, m.p. 258–264°. Recrystallization from dioxane lowered the melting point to 256–259°.

Carbonyl Oxygen-18 Labeled Ethyl Acetate.—Carbonyl oxygen-18 labeled ethyl acetate was prepared for a portion of the oxygen-18 labeled acetyl chloride used to prepare the labeled dimethoxy-*s*-triazine acetate, and absolute ethanol.

Anal. Found: atom per cent. excess of O^{18}, 0.24 (this analysis was disturbed by a minor mishap which caused the result to be somewhat low).

Hydrolysis of Methyl Acetate Obtained from Pyrolysis of Labeled 2-(N-β-Acetoxyethylanilino)-4,6-dimethoxy-s-triazine.—A 2.97 g. (0.04 mole) sample of oxygen-18 labeled methyl acetate, obtained from pyrolysis of the labeled dimethoxy-*s*-triazine acetate, was heated in 12 ml. of 20% sodium hydroxide solution for 12 hours. The methanol, collected by distillation, weighed 0.48 g. (37.5%), b.p. 64–64.6°.

Anal. Found: atom per cent. excess O^{18}, 0.300.

Carbonyl Oxygen-18 Labeled Methyl Acetate.—Another portion of the labeled acetyl chloride was treated with absolute methanol. The resulting methyl acetate boiled at 57–58°.

Anal. Found: atom per cent. excess O^{18}, 0.261 (this analysis was performed with a different type of amplifier on the mass spectrometer, and therefore is not strictly comparable with those above).

A 3.5 g. (0.047 mole) sample of labeled methyl acetate was heated at reflux with 16 ml. of 20% sodium hydroxide for 10.75 hours. The methanol, removed by distillation, weighed 0.63 g. (49.3%), b.p. 65–70°.

Anal. Found: atom per cent. excess O^{18}, 0.003.

Equilibration of Carbonyl Oxygen-18 Labeled Methyl Acetate.—A 4.14-g. (0.056 mole) sample of the labeled methyl acetate prepared above was heated in a sealed tube at 250° for 1.5 hours and hydrolyzed as previously described. Distillation yielded 0.63 g. (35%) of methanol, 64–70°.

Anal. Found: atom per cent. excess O^{18}, 0.011.

A 4.14 g. (0.056 mole) sample of the labeled ester was heated as described above with 3 ml. of quinoline and 1.0 g. of 2-hydroxy-4-methylquinoline. The methanol obtained by hydrolysis weighed 0.40 g. (22%), b.p. 64–65°.

Anal. Found: atom per cent. excess O^{18}, 0.070.

Pyrolysis of 2-(N-β-Acetoxyethylanilino)-4,6-dimethoxy-s-triazine (Ia) **at 100°.**—A 1.15-g. (0.0036 mole) sample of

(13) (a) T. W. Richards, W. N. Stull, J. H. Mathews and C. L. Speyers, THIS JOURNAL, **34**, 987 (1912); (b) F. Eisenlohr, *Z. physik. Chem.*, **75**, 592 (1911).

(14) K. G. Falk, THIS JOURNAL, **31**, 808 (1909).

(15) (a) C. F. H. Allen, *ibid.*, **52**, 2955 (1930); (b) E. C. Horning and M. G. Horning, *J. Org. Chem.*, **11**, 95 (1946).

Ia was pyrolyzed at 100°. After 2 hours of heating, 0.02 g. (7.41%) of methyl acetate was collected. After 5 hours of heating a total of 0.03 g. (11.1%) of ester was obtained.

Preparation of N,N-Diphenylaminoethyl Acetate.—Following the procedure of Paul,[16] 18.8 g. (0.088 mole) of N,N-diphenylaminoethanol[17] and 10.2 g. (0.1 mole) of acetic anhydride were refluxed for 2 hours. The reaction mixture was then diluted with water and the *p*H of the solution adjusted to 9 with sodium hydroxide solution. The alkaline solution was extracted with ether, the combined extracts washed with water and dried over sodium sulfate. Removal of the ether followed by distillation afforded 15.1 g. (67.4%) of N,N-diphenylaminoethyl acetate, b.p. 174.5–175° at 3 mm., sp. gr. 20/20 1.1120, $n^{22.5}$D 1.5821; *MR* calcd. 74.60, found 74.31; reported[16] b.p. 140–145° at 1–2 mm.

Anal. Calcd. for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.40; H, 6.34; N, 5.51.

Pyrolysis of N,N-Diphenylaminoethyl Acetate. A. At 250°.—A 5.1-g. sample of N,N-diphenylaminoethyl acetate was heated, under a nitrogen atmosphere, at 250° for 2 hours. No volatile products were collected.

B. At 500°.—A 8.9-g. (0.036 mole) sample of the ester was passed through a Vycor tube packed with 1/8 inch helices and heated to 500° by a furnace. The ester was passed through the tube at a rate of 1.5 g. per minute. The pyrolysate, collected in a Dry Ice trap, was extracted with water and the aqueous solution diluted to 100 ml. A 10-ml. aliquot, titrated with 0.0111 *N* sodium hydroxide solution using a Beckman model G *p*H meter, consumed 19.8 ml. of base and indicated that 0.132 g. (6.1%) of acetic acid was produced. The water-insoluble, brown oil from the pyrolysate was chromatographed on an alumina column but no N,N-diphenylvinylamine could be isolated.

Pyrolysis of 2-(N-β-Hydroxyethylanilino)-4,6-dimethoxy-*s*-triazine (VII). Run No. 1.—A 3.0-g. (0.01 mole) sample of the dimethoxy-*s*-triazine alcohol was pyrolyzed at 200–260° for 1.5 hours. No volatile products were observed. The residue in the distillation flask, after recrystallization from dioxane, afforded 1.56 g. (65%) of 2-N-vinylanilino-4-hydroxy-6-methoxy-*s*-triazine (IIa), m.p. 254–256°. A mixture melting point with a sample of IIa from pyrolysis of Ia was not depressed.

Run No. 2.—A 20.0-g. (0.07 mole) sample of VII was pyrolyzed under the same conditions. The distillate, 1.0 g. (43.8%), was identified as methanol, b.p. (cor.) 63.3°, n^{20}D 1.3296; reported b.p. 64.6°,[18a] $n^{20.5}$D 1.3288.[18b]

(16) P. T. Paul, U. S. Patent 2,401,658, June 4, 1946.

(17) Generously supplied by the Naugatuck Chemical Co.

(18) (a) W. R. G. Atkins and T. A. Wallace, *J. Chem. Soc.*, **103**, 1469 (1913); (b) F. H. Getman and V. L. Gibbons, THIS JOURNAL, **37**, 1995 (1915).

[CONTRIBUTION FROM DEPT. OF BIOCHEMISTRY RESEARCH, ROSWELL PARK MEMORIAL INSTITUTE, BUFFALO, NEW YORK]

Nature of the Combining Site of Antibody against a Hapten Bearing a Positive Charge[1]

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Previous work indicated that the specific site of antibody against the positively charged phenyltrimethylammonium ion contains a negative charge. The present report emphasizes this and shows that the negative charge is apparently a carboxylate group since esterification ofthe antibody carboxyl groups by diazoacetamide destroys binding activity. This destruction is not due to the esterification which occurs elsewhere than in the specific site. Moroever esterification of antibody to a negative hapten does not affect its ability to bind hapten.

One approach to the exact composition of the antibody site, *e.g.*, which chemical groups contribute to the intimate structure of the site, is to modify the site chemically by use of reagents for reactive groups in protein. This approach has been used with varying success.[2–8] Reagents which react with only one type of residue are few[9,10] and reaction conditions must be mild enough to permit retention of biological activity. Care must be taken to assure that reaction in other parts of the large antibody molecule does not obscure effects on the site or confuse the interpretation of data. Thus acetylation of antihapten[5–7] or antiprotein[3,4,7] antibodies has been shown to reduce or prevent precipitation by antigen but this is due mainly to reaction with amino groups in regions elsewhere than in the combining site.[6] Specific protection of the site by hapten during treatment with reagent provides a means of proving that the destructive reaction is in the site.[2] It was by such methods that acetylation, when extensive, was shown to affect the combining site.[6]

The use of antihapten sera has a distinct advantage over the use of antiprotein sera in such studies in that information concerning the combining site of antibodies is more easily interpreted in the former case. This is because, unlike antibodies against complex antigens such as proteins, the antihapten antibodies are directed against a known chemical configuration. Such systems have also been of value in indicating the presence of a charged group in the antibody site; *i.e.*, a positively charged group is in the site homologous for the negatively charged *p*-(*p*-azobenzene)-azobenzoate (X_p)[11] and 4-azophthalate[12]; also a negative charge is in the site homologous for the positively charged *p*-azophenyltrimethylammonium ion (A_p).[13] Measurement of hapten binding by dialysis equilibrium allows detection of interaction at the site without the com-

(1) Presented at the 136th National Meeting of the American Chemical Society, Atlantic City, Sept. 1959. Supported in part by a grant from the National Institute of Allergy and Infectious Diseases, USPHS.

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