The Acylation of 3-Methylcytosine.

By G. W. KENNER, C. B. REESE, and SIR ALEXANDER R. TODD.

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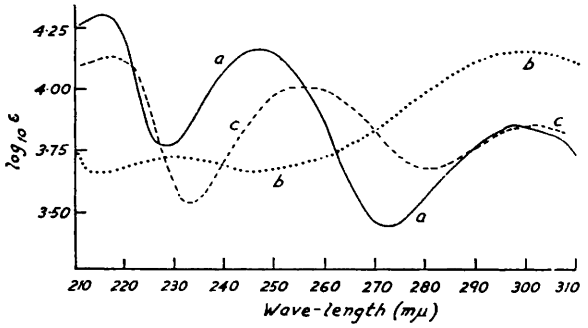
In the monoacetyl and monobenzoyl derivatives of 3-methylcytosine the acyl residue must be attached to the exocyclic nitrogen atom, since methylation by diazomethane yields the acetyl and the benzoyl derivative respectively of 1 : 3-dimethylcytosine (II; R = Me, R′ = Ac or Bz). From comparisons of spectra and dissociation constants it is deduced that 3-methylcytosine and its acyl derivatives all exist predominantly in the tautomeric form corresponding to (I) and not to (II).

MICHELSON and TODD (*J.*, 1954, 34) have remarked that it is uncertain whether $N_{(1)}$ or $N_{(6)}$ bears the acetyl group in *N*-acetyl derivatives of cytidine. The bond between $N_{(3)}$ and the sugar residue is apparently weaker in these *N*-acetyl derivatives than in the parent bases and it therefore seemed worth attempting to resolve the ambiguity by a study of the analogous 3-methylcytosine compounds. Our investigation acquired additional interest when Flynn, Hinman, Caron, and Woolf (*J. Amer. Chem. Soc.*, 1953, **75**, 5867) announced that part of the structure of amicetin, an antibiotic, resembles that of *N*-benzoyl-3-methylcytosine, to which they assigned the structure (I; R = H, R′ = Bz) without comment. Our work now justifies the American authors' assumption and excludes the alternative structure (II; R = Bz, R′ = H).

Acetylation of 3-methylcytosine with hot acetic anhydride and pyridine gave a crystalline monoacetyl derivative, the ultra-violet absorption of which (see Figure, curve *a*) closely resembled that of *NO*$^{3'}$-diacetyldeoxycytidine (Michelson and Todd, *loc. cit.*). The same material, together with 3-methylcytosine, was produced by the reaction between 6-methoxy-3-methyl-2-pyrimidone and sodium acetimide in molten

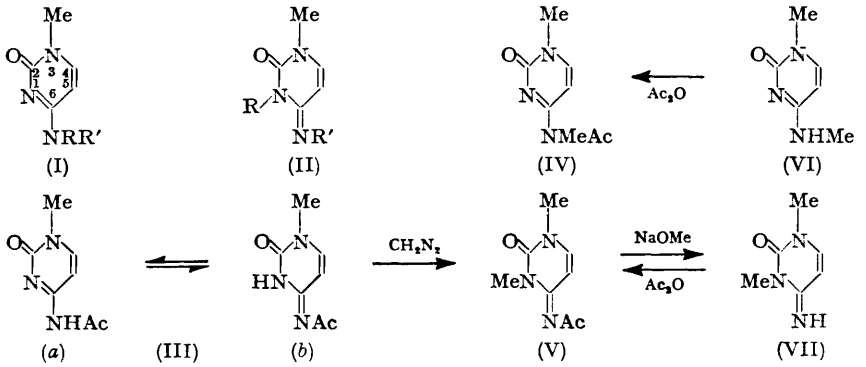
acetamide. This is consistent with its formulation as (III), but can hardly be adduced as proof of the structure since acyl migration might have occurred. An alcoholic solution of the acetyl compound reacted easily with ethereal diazomethane, giving a monomethyl derivative, m. p. 156°, with a very different ultra-violet absorption (curve *b*). This substance was converted by sodium methoxide into 1 : 3-dimethylcytosine (VII), and was shown by direct comparison to be identical with the acetyl derivative of 1 : 3-dimethylcystosine prepared by Dr. S. Varadarajan in these laboratories. The structure of 1 : 3-dimethylcytosine rests securely on the work of Hilbert (*J. Amer. Chem. Soc.*, 1934, **56**, 190), who transformed it into 5-bromo-1 : 3-dimethyluracil, and the compound of m. p. 156° must therefore be (V). Unless acyl migration took place during the very mild diazomethane treatment, the acetyl group must be attached to $N_{(6)}$ in monoacetyl 3-methylcytosine itself which is therefore (III) and not (II; R = Ac, R′ = H). Monobenzoyl-3-methylcytosine was likewise methylated by diazomethane in alcohol and ether;



Ultra-violet spectra in ethanol:
(*a*) N⁶-*acetyl*-3-*methylcytosine* (III*a*).
(*b*) N⁶-*acetyl*-1 : 3-*dimethylcytosine* (V).
(*c*) N⁶-*acetyl*-3 : N⁶-*dimethylcytosine* (IV).

the product was identified by mixed melting point with the benzoyl derivative of 1 : 3-dimethylcytosine, kindly given to us by Dr. Varadarajan.

N^6-Acetyl-3-methylcytosine has two tautomeric forms (III*a* and *b*). As diazomethane substitutes a methyl group directly for an acidic hydrogen atom (Arndt, "Organic Analysis," Vol. I, p. 207, Interscience Publ., 1953), the compound (V) came from (III*b*) and it might be tempting to assume that this is the predominant tautomer. That the reverse is actually the case was shown by acetylation of 3 : N^6-dimethylcytosine (VI) and comparison of the ultra-violet spectrum (curve *c*) of the product, m. p. 196°, with the spectra of (III) and (V). The correspondence between curves *c* and *a* strongly suggests



that the compound of m. p. 196° is (IV) and that (III*a*) is the predominant tautomer. The form (III*b*) is therefore the more strongly acidic and reacts faster with diazomethane. Indeed we have been unable to obtain the compound (IV) from (III*a*); no reaction occurred when a solution of acetyl 3-methylcytosine in tetrahydrofuran was kept for two weeks with ethereal diazomethane in absence of alcohol. The situation is essentially that described as case A by Arndt (*op. cit.*, p. 211). The benzoyl series of compounds showed

the same relations of spectra, structure, and tautomerism; the structure (I; R = H, R′ = Bz) assigned by Flynn *et al.* (*loc. cit.*) to benzoyl-3-methylcytosine is therefore correct, with respect to, not only the location of the benzoyl residue, but also that of the mobile hydrogen atom.

Although our main object was the study of the acyl derivatives, we were also interested in the tautomerism of the parent 3-methylcytosine. It has recently been demonstrated in a number of instances that heterocyclic amines of the amidine type have preferentially an exocyclic amino- rather than an imino-group (*e.g.*, Angyal and Angyal, *J.*, 1952, 1461; Brown and Short, *J.*, 1953, 331). The same conclusion is drawn in the same way from physical data in the present instance. Thus 3 : N^6 : N^6-trimethylcytosine (I; R = R′ = Me) is a fairly weak base (pK_a 4·20), whereas 1 : 3 : N^6-trimethylcytosine (II; R = R′ = Me) and 1 : 3-dimethylcytosine (II; R = Me, R′ = H) (pK_a 9·29) are strong bases. Naturally the compounds capable of tautomerism, 3-methylcytosine and 3 : N^6-dimethylcytosine, adopt the more stable structure (I) and are weak bases; and their ultraviolet absorption in alcoholic solution also closely resembles that of (I; R = R′ = Me), when allowance is made for the bathochromic effect of methyl groups (cf. Brown and Short, *loc. cit.*). In acidic solution all the compounds show generally similar absorption corresponding to the resonant cation. The apparent course of *acylation*, direct substitution of an acyl residue for a hydrogen atom in (I), contrasts with Pyman's rule (*J.*, 1923, 3359) that *alkylation* of an amidine occurs at the "imino-nitrogen." Angyal (*Austral. J. Sci. Res.*, 1952, **5**, *A*, 375) has shown that sulphonyl halides do attack preferentially the ring nitrogen atoms of several heterocyclic amines and that the more stable isomer can be formed from the initial product by acyl migration. On the other hand it may be that acylation takes place *via* the resonant anion or *via* the small proportion of the strongly basic tautomer of type (II). It is noteworthy that methylation of 3 : N^6-dimethylcytosine by methyl iodide gives 1 : 3 : N^6-trimethylcytosine, whereas acetylation gives the 6-acetyl derivative.

Our principal starting material was 6-methoxy-3-methyl-2-pyrimidone, which was converted by ammonia, methylamine, or dimethylamine into the amines of type (I). 1 : 3-Dimethylcytosine (II; R = Me, R′ = H) is most conveniently made by Hilbert's method (*loc. cit.*) but can also be obtained by the action of diazomethane on 3-methylcytosine, which evidently reacts, like its acetyl derivative, as the less stable, more acidic tautomer. The importance of the 2-keto-group in promoting methylation of $N_{(1)}$ is shown by the inertness towards diazomethane of 4-acetamidopyrimidine. This compound was obtained from 2-chloro-4-methoxypyrimidine, but the method was less convenient than that described by Brown (*J. Soc. Chem. Ind.*, 1950, **69**, 353; Brown and Short, *loc. cit.*). 2-Chloro-4-methoxypyrimidine is easily accessible from 2 : 4-dichloropyrimidine owing to the greater reactivity of the chlorine at position 4 (cf. Chapman and Rees, *J.*, 1954, 1190) and its hydrogenolysis to 4-methoxypyrimidine proceeds smoothly.

During some preliminary experiments conditions were established for the conversion of 3-methyluracil into 6-chloro-3-methyl-2-pyrimidone by means of phosphoryl chloride.

Experimental

M. p.s are corrected.

3-Methylcytosine (I; R = R′ = H).—The following conditions give a slightly higher yield than those of Flynn, Hinman, Caron, and Woolf (*J. Amer. Chem. Soc.*, 1953, **75**, 5871). A solution of 6-methoxy-3-methyl-2-pyrimidone (3·5 g.; Hilbert and Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 2005) in methanol (20 c.c.; saturated at 0° with ammonia) was heated for 8 hr. at 150°. On cooling, the product separated in large crystals (3 g.; m. p. 300°), pK_a 4·57. Light absorption in 95% EtOH : $\lambda_{max.}$ 275—276 mμ (log ε 3·85), $\lambda_{min.}$ 252 (log ε 3·64); in 0·1N-HCl : $\lambda_{max.}$ 213, 282 (log ε 4·00, 4·09), $\lambda_{min.}$ 241 (log ε 3·07).

3 : N⁶-Dimethylcytosine (I; R = Me, R′ = H).—A solution of 6-methoxy-3-methyl-2-pyrimidone (2 g.) in 33% aqueous methylamine (14 c.c.) was heated for 8 hr. at 150°. After evaporation to dryness the *3 : N⁶-dimethylcytosine* was separated from 3-methyluracil by crystallisation from ethanol (once) and ethyl acetate (twice) and had m. p. 179°, pK_a 4·47 (Found, in material dried at 100°: C, 51·6; H, 6·5; N, 30·5. $C_6H_9ON_3$ requires C, 51·8; H,

6·5; N, 30·2%). Light absorption in 95% EtOH: $\lambda_{max.}$ 275 mμ (log ε 4·03), $\lambda_{min.}$ 251 (log ε 3·84); in 0·1N-HCl: $\lambda_{max.}$ 218, 285—286 (log ε 3·98, 4·13), $\lambda_{min.}$ 244 (log ε 3·26).

3 : N^6 : N^6-*Trimethylcytosine* (I; R = R′ = Me).—Repetition of the foregoing experiment, using 35% aqueous dimethylamine instead of methylamine, gave 3 : N^6 : N^6-*trimethylcytosine* as needles, m. p. 179°, pK_a 4·20 (Found, in material dried at 80°: C, 55·0; H, 7·2; N, 27·5. $C_7H_{11}ON_3$ requires C, 54·9; H, 7·2; N, 27·4%). Light absorption in 95% EtOH: $\lambda_{max.}$ 282 mμ (log ε 4·04), $\lambda_{min.}$ 240 (log ε 3·73); in 0·1N-HCl: $\lambda_{max.}$ 221, 290 (log ε 3·93, 4·17), $\lambda_{min.}$ 247 (log ε 3·26).

1 : 3-*Dimethylcytosine* (II; R = Me, R′ = H).—A solution of 3-methylcytosine (0·2 g.) in ethanol (25 c.c.) was kept for 3 days with a solution of diazomethane (about 0·7 g.) in ether (25 c.c.). On evaporation of the solvent much unchanged material crystallised but evaporation and sublimation of the liquors at 80°/0·2 mm. gave 1 : 3-dimethylcytosine (0·03 g.; m. p. 145°, undepressed by authentic material), pK_a 9·29. Light absorption in 95% EtOH: $\lambda_{max.}$ 223, 273 mμ (log ε 4·00, 3·93), $\lambda_{min.}$ 244 (log ε 3·40); in 0·1N-HCl: $\lambda_{max.}$ 281 (log ε 4·06), $\lambda_{infl.}$ 212 (log ε 3·99), $\lambda_{min.}$ 243 (log ε 3·23).

1 : 3 : N^6-*Trimethylcytosine* (II; R = R′ = Me).—Methyl iodide (1·5 c.c.) was added to a solution of 3 : N^6-dimethylcytosine (0·36 g.) in methanol (2 c.c.). The mixture was kept in the dark for 7 days and then evaporated to a dark yellow mass, which was dissolved in the minimum of water and made alkaline with concentrated aqueous sodium hydroxide. The oil was extracted with chloroform, from which 1 : 3 : N^6-*trimethylcytosine* was obtained by evaporation and two sublimations at 45°/0·5 mm. as colourless hygroscopic crystals, m. p. 79° (Found: C, 55·3; H, 7·2; N, 27·1. $C_7H_{11}ON_3$ requires C, 54·9; H, 7·2; N, 27·4%). Its aqueous solution was strongly alkaline. Light absorption in 95% EtOH: $\lambda_{max.}$ 222, 285—286 (log ε 4·09, 3·97), $\lambda_{min.}$ 248 (log ε 3·39); in 0·1N-HCl: $\lambda_{max.}$ 212, 287 (log ε 3·81, 3·98), $\lambda_{min.}$ 248 (log ε 3·16).

N^6-*Acetyl*-3-*methylcytosine* (I; R = Ac, R′ = H).—Finely powdered 3-methylcytosine (0·5 g.) was dissolved in a boiling mixture of acetic anhydride (1·2 c.c.) and pyridine (11 c.c.), which was then kept at 100° for 1 hr. The *acetyl derivative* (0·5 g.) was collected after the mixture had been cooled to 0° and recrystallised from ethanol in needles, m. p. 268° (Found, in material dried at 100°: C, 50·5; H, 5·5; N, 25·0. $C_7H_9O_2N_3$ requires C, 50·3; H, 5·4; N, 25·1%). Light absorption in 95% EtOH (curve *a*): $\lambda_{max.}$ 215, 246—247, 299—300 mμ (log ε 4·30, 4·16, 3·83), $\lambda_{min.}$ 228, 272 (log ε 3·76, 3·42); in 0·1N-HCl: $\lambda_{max.}$ 214, 311 mμ (log ε 4·10, 4·19), $\lambda_{infl.}$ 224—228 (log ε 3·92), $\lambda_{min.}$ 262 (log ε 3·00).

Reaction between Sodium Acetimide and 6-*Methoxy*-3-*methyl*-2-*pyrimidone.*—A solution of sodium (0·2 g.) in methanol was evaporated to dryness. Acetamide (4·2 g.) and the pyrimidone (1 g.) were added to the residue and the mixture was kept for 1 hr. at 100°/15 mm. in a stream of nitrogen. Ammonium chloride (0·5 g.) was then added to the solution, which was cooled after 20 minutes' further heating and extracted with hot ethanol (50 c.c.). On concentration to 15 c.c. and cooling, this yielded 3-methylcytosine (0·1 g.), m. p. 294—295°. Paper chromatography of the liquor in *n*-butanol saturated with water showed that it contained approximately equal amounts of 3-methylcytosine (R_F 0·28) and of N^6-acetyl-3-methylcytosine (R_F 0·5), identified by the shape of its ultra-violet absorption curve.

N^6-*Acetyl*-3 : 6-*dimethylcytosine* (I; R = Ac, R′ = Me).—3 : 6-Dimethylcytosine (0·067 g.), acetic anhydride (0·070 g.), and pyridine (1 c.c.) were boiled until solution was complete and then kept at 100° for 1 hr. The *acetyl derivative* (0·030 g.), which separated from the cooled mixture, recrystallised from ethanol in needles, m. p. 196° (Found, in material dried at 80°: C, 53·0; H, 6·4; N, 23·4. $C_8H_{11}O_2N_3$ requires C, 53·0; H, 6·1; N, 23·2%). Light absorption in 95% EtOH (curve *c*): $\lambda_{max.}$ 216, 255, 302 mμ (log ε 4·13, 4·01, 3·83), $\lambda_{min.}$ 232, 280 (log ε 3·52, 3·65).

N^6-*Acetyl*-1 : 3-*dimethylcytosine* (II; R = Me, R′ = Ac).—Diazomethane (about 1 g.) in ether (40 c.c.) was added to a solution of N^6-acetyl-3-methylcytosine (0·25 g.) in ethanol (50 c.c.). The solution was kept at room temperature for 18 hr. and then concentrated. The *product* (0·23 g.) crystallised in needles and, purified by sublimation at 90°/0·2 mm., had m. p. 156° (Found: C, 53·2; H, 6·3; N, 22·9. $C_8H_{11}O_2N_3$ requires C, 53·0; H, 6·1; N, 23·2%). Light absorption in 95% EtOH (curve *b*): $\lambda_{max.}$ 228—229, 299 mμ (log ε 3·72, 4·13), $\lambda_{min.}$ 215, 244 (log ε 3·66, 3·66); in 0·1N-HCl: $\lambda_{max.}$ 214, 242, 315 mμ (log ε 4·03, 3·91, 4·14), $\lambda_{min.}$ 226, 270 (log ε 3·68, 3·21).

Deacetylation of N^6-*Acetyl*-1 : 3-*dimethylcytosine.*—The acetyl compound (0·056 g.) was heated under reflux for 2 hr. with a solution from sodium (0·02 g.) in methanol (10 c.c.). The methanol was then evaporated and the residue extracted with dry benzene, which was concentrated to small bulk and cooled. 1 : 3-Dimethylcytosine crystallised in needles (0·011 g.)

and, purified by sublimation at 80°/0·2 mm., had m. p. and mixed m. p. 147—147·5° (Found: N, 30·0. Calc. for $C_6H_9ON_3$: N, 30·2%).

N^6-*Benzoyl*-3-*methylcytosine* (I; R = Bz, R′ = H).—Finely powdered 3-methylcytosine (0·5 g.) was boiled with benzoyl chloride (0·6 c.c.) and pyridine (8 c.c.) until dissolution was complete and then kept at 100° for 1½ hr. The product (0·6 g.), which separated from the cooled solution, was recrystallised from ethanol, and had m. p. 222° (Flynn, Hinman, Caron, and Woolf, *J. Amer. Chem. Soc.*, 1953, **75**, 5871, give m. p. 221—222°). Light absorption in 95% EtOH: $\lambda_{max.}$ 259, 304—305 mμ (log ε 4·35, 3·98), $\lambda_{min.}$ 232, 283 (log ε 4·00, 3·85).

N^6-*Benzoyl*-3: N^6-*dimethylcytosine* (II; R = Bz, R′ = Me).—3: N^6-Dimethylcytosine (0·14 g.) and benzoyl chloride (0·18 g.) were dissolved in boiling pyridine (1·3 c.c.). The solution was kept at 100° for 1 hr. before being evaporated to dryness. Recrystallisation of the residue from ethanol afforded the *benzoyl derivative* (0·03 g.), m. p. 145° (Found, in material dried at 80°: C, 64·4; H, 5·6; N, 17·6. $C_{13}H_{13}O_2N_3$ requires C, 64·2; H, 5·4; N, 17·3%). Light absorption in 95% EtOH: $\lambda_{max.}$ 265, 305 mμ (log ε 4·09, 3·94), $\lambda_{min.}$ 251, 289 (log ε 4·05, 3·87).

N^6-*Benzoyl*-1: 3-*dimethylcytosine* (II; R = Me, R′ = Bz).—Diazomethane (about 0·7 g.) in ether (25 c.c.) was mixed at room temperature with a solution of N^6-benzoyl-3-methylcytosine (0·25 g.) in ethanol (20 c.c.). After 18 hr. the solution was concentrated to small bulk. The N^6-benzoyl-1: 3-dimethylcytosine (0·2 g.), which separated on cooling, recrystallised from ethanol in needles, m. p. 156° (Found, in material dried at 60°: C, 63·9; H, 5·6; N, 17·0. $C_{13}H_{13}O_2N_3$ requires C, 64·2; H, 5·4; N, 17·3%). Light absorption in 95% EtOH: $\lambda_{max.}$ 245, 318 mμ (log ε 4·07, 4·25), $\lambda_{min.}$ 224, 270 (log ε 3·93, 3·84).

2-*Chloro*-4-*methoxypyrimidine*.—A solution of sodium (0·23 g.) in methanol (7 c.c.) was added slowly to a stirred solution of 2: 4-dichloropyrimidine (1·5 g.) in methanol (13 c.c.). After the mixture had been stirred for 40 min. further, ether (30 c.c.) was added to complete the precipitation of the salt, which was removed by filtration. Evaporation of the filtrate and crystallisation of the residue from light petroleum (b. p. 40—60°) afforded 2-*chloro*-4-*methoxypyrimidine* (0·6 g.), m. p. 55° (Found, in material sublimed at 35°/0·2 mm.: C, 41·7; H, 3·2; N, 19·3. $C_5H_5ON_2Cl$ requires C, 41·5; H, 3·5; N, 19·4%).

4-*Methoxypyrimidine*.—A solution of 2-chloro-4-methoxypyrimidine (3 g.) in methanol (25 c.c.) was shaken with magnesium oxide (1 g., "AnalaR"), 5% palladised barium sulphate (1 g.), and hydrogen (1 atm.). The theoretical quantity of hydrogen (470 c.c. at N.T.P.) was absorbed in 5 hr. The residue, obtained by filtration and evaporation, was dissolved in water (20 c.c.), which was then extracted with ether (200 c.c.). Distillation of the dried (Na_2SO_4) extract yielded 4-methoxypyrimidine (1·6 g.), b. p. 71°/33 mm. (Brown and Short, *J.*, 1953, 336, give b. p. 69—70°/30 mm.). When a small amount was added to alcoholic picric acid, the *picrate* was immediately precipitated and, recrystallised from ethanol, had m. p. 122·5° (Found, in material dried at 60°: C, 39·1; H, 3·0; N, 20·4. $C_5H_6ON_2,C_6H_3O_7N_3$ requires C, 38·9; H, 2·7; N, 20·6%).

4-*Acetamidopyrimidine*.—A solution of 4-methoxypyrimidine (1·07 g.) in methanol (9 c.c.), saturated at 0° with ammonia, was heated at 150—160° for 7½ hr. Evaporation of the solvent gave a yellow solid mass. Acetylation of this impure 4-aminopyrimidine by Brown and Short's technique (*J.*, 1953, 337) afforded 4-acetamidopyrimidine (0·5 g.), which, sublimed at 120°/0·5 mm., had m. p. 203° (Brown and Short record m. p. 198—200°) (Found: C, 52·3; H, 5·3; N, 30·9. Calc. for $C_6H_7ON_3$: C, 52·5; H, 5·2; N, 30·6%).

6-*Chloro*-3-*methyl*-2-*pyrimidone*.—Phosphoryl chloride (8 c.c.) and 3-methyluracil (2 g.) were heated in a bath at 135° under reflux for 4½ hr. The excess of phosphoryl chloride was distilled under reduced pressure and some 2: 4-dichloropyrimidine removed by sublimation. Ice (20 g.) was added to the remaining resin, followed gradually by ammoniacal methanol until the pH rose to 8. The mixture was warmed to 30° and filtered. When kept at 0° it deposited almost pure 6-*chloro*-3-*methyl*-2-*pyrimidone* (0·63 g.), which after sublimation had m. p. 207—208° (Found: C, 41·7; H, 3·3; N, 19·1. $C_5H_5ON_2Cl$ requires C, 41·5; H, 3·5; N, 19·4%). On being heated at 150° for 8 hr. with saturated methanolic ammonia, this substance yielded 3-methylcytosine, m. p. 298° undepressed by an authentic specimen and with the same ultraviolet absorption.

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UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, October 27th*, 1954.]