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CHEMICAL ALTERATION OF NUCLEIC ACIDS AND THEIR COMPONENTS—XIII[1]

REACTION OF NUCLEOSIDES WITH DIACYL PEROXIDES

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Abstract—Diacyl peroxides reacted at room temp with cytidine and adenosine. The former gave 4-acyl and 3-oxido derivatives and the latter gave 6-acyl and 1-oxido derivatives. At 90°, diacetyl peroxide reacted with guanosine, adenosine, cytidine, and uridine by a homolytic process to give their C-methylated derivatives. The latter reaction was accelerated by the presence of a ferrous ion.

It is known that diacyl peroxides undergo homolytic cleavage of the –O–O– bond to form acyloxy free radicals.[2,3] These undergo ready conversion to carbon dioxide and alkyl radicals, which in turn may abstract an atomic hydrogen or add to an electron-deficient sp^2 carbon.[4] In addition, by heterolytic cleavage diacyl peroxide can be expected to react with nucleophiles in two ways, resulting in acylation and acyloxylation of the nucleophilic centers of the nucleophiles. Thus, diacyl peroxide can transfer its acyl group to an amino or hydroxyl group, and in some cases it reacts with nucleophiles to give acyloxylated derivatives.[3,5,6]

This paper describes the reaction of nucleosides with dibenzoyl peroxide (**1**) and diacetyl peroxide (**2**). This study was undertaken in order to obtain basic knowledge on the chemical modification of the gene material of nucleic acids by these peroxides, since the toxic effect of these peroxides on organisms is possibly related to their giving rise to genetic disturbance such as mutation and cancerization.[7]

RESULTS

Experiments were carried out under two different sets of reaction conditions, one for the heterolytic (ionic) reaction and the other for the homolytic reaction.

Under heterolytic reaction conditions

Peroxide **1** or **2** was added to a dimethyl formamide (DMF) solution of a nucleoside at room temperature, and the mixture was kept overnight at room temperature. The resulting mixture was then separated into its components by thin-layer or paper chromatography, and the products were identified by co-chromatography with authentic specimens.

Equimolar amounts of cytidine and **1** gave 4-benzoylcytidine (**3**), cytidine 3-oxide (**4**) and 3-benzoyloxycytidine (**5**) in yields of 46, 5 and 33%, respectively. The recovery of cytidine was 16%. **5** was readily hydrolyzed to **4** in quantitative yield. The structure of **5** was confirmed by co-chromatography with a sample prepared by treatment of **4** with benzoic anhydride in pyridine. The treatment of cytidine with **2** produced N^4-acetylcytidine (**6**), **4**, and the starting material in yields of 56, 10 and 30%, respectively. 3-Acetoxy derivative was not detected in this case. 3-Acetoxycytidine, which was prepared by the treatment of 3-oxide (**4**) with acetic anhydride in pyridine, was too labile to be isolated from protic solvents. It is worth noting that the 4-acyloxyamino or 4-hydroxyamino derivative was not detected in these cases and that the 3-acyl derivative was not obtained either.

Adenosine appeared to be less reactive than cytidine toward diacyl peroxides. Equimolar amounts of adenosine and **1** gave adenosine 1-oxide (**7**) in 15% yield with 85% recovery of the starting material under the same reaction conditions chosen for cytidine. The reaction with **2** afforded **7** and N^6-acetyladenosine (**8**) in yields of 10 and 15%, respectively. Neither the hydroxyamino nor the 1-acyl derivative was found, just as in the reaction of cytidine with these peroxides.

Guanosine, thymidine, and uridine were completely inert under the conditions chosen in the present study.

The results thus obtained (summarized in Chart 1) indicate that under the heterolytic conditions, diacyl peroxides react as typical electrophiles with the nucleophilic centers of basic nucleosides such as cytidine and adenosine, and that the two electrophilic centers in the reagents, the acyl-carbonyl carbon and the peroxide oxygen, tend to specifically attack the substituent *exo*-NH_2 and the ring-N (3-N of cytidine and 1-N of adenosine), respectively. This specificity may be related to the reaction mechanisms for the specific attack of acylating agents at the *exo*-NH_2[8] and the predominant attack of alkylating agents at the *endo*-N of cytidine (3-N) and adenosine (1-N).[9]

Under homolytic reaction conditions

The reaction was carried out by treatment of an aqueous solution of the nucleoside with an equimolar amount of diacyl peroxide in the presence of 0·1–0·2 M equivalents of $FeSO_4$ at 90° for 30 min. The pH of the mixture was around 6. At the end of the reaction, the pH has dropped to about 4·5. The products were separated by thin-layer or paper chromatography, and were identified by co-chromatography with authentic specimens.

All the nucleosides examined were recovered after treatment with **1**, whereas they reacted with **2** to give various types of products. All the reactions with **2** proceeded, both in the presence and absence of $FeSO_4$, but the yields of products were in all cases lower in the absence of ferrous ion.

Reaction of purine nucleosides with **2** gave the corresponding C-Me derivatives without production of appreciable amounts of by-products; 8-methylguanosine (**9**) from guanosine (39% yield), and 2-methyladenosine (**10**) and 8-methyladenosine (**11**) from adenosine (6 and

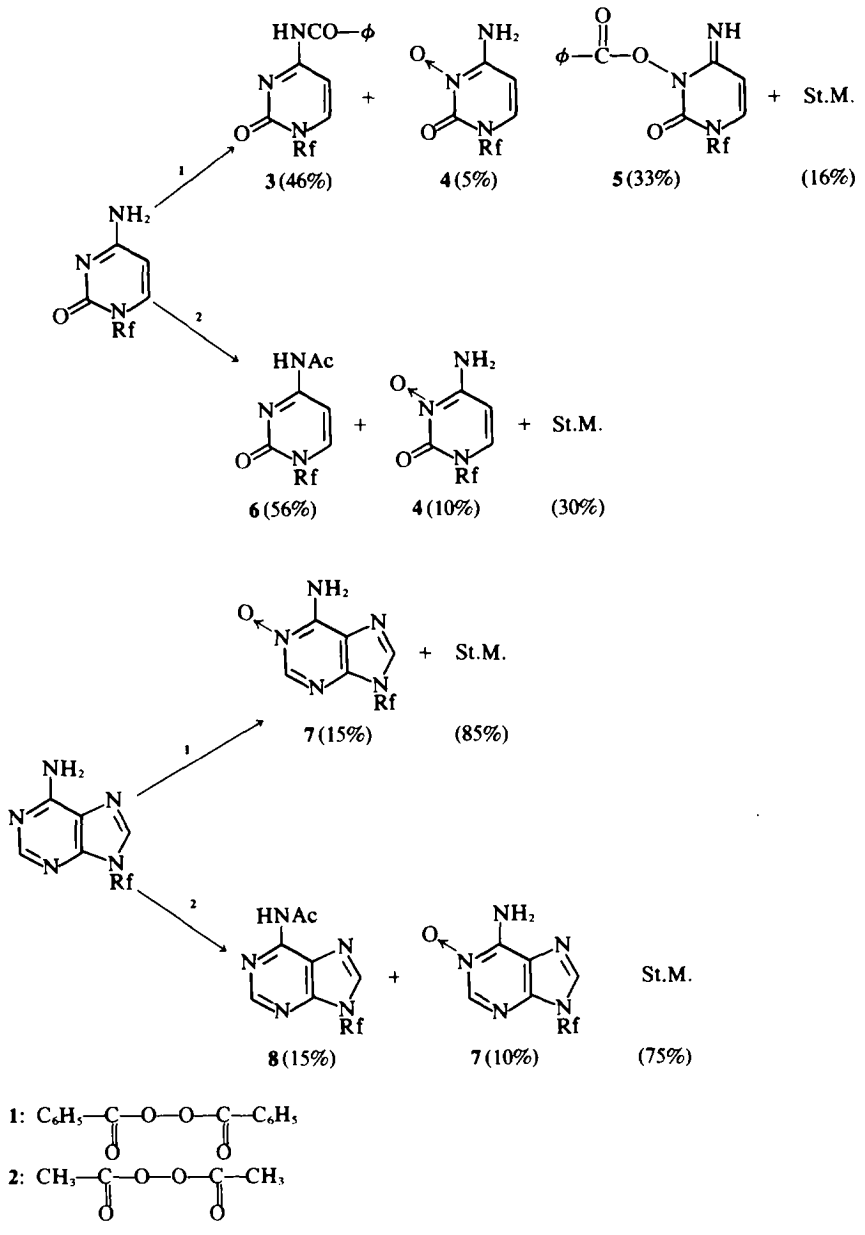

Chart 1. Reactions of nucleosides with an equimolar amount of dibenzoyl peroxide (1) or diacetyl peroxide (2) in dimethylformamide at room temperature (Rf: robofuranosyl; St. M.: the starting material).

11% yield, respectively). Guanosine and adenosine were recovered in 54 and 83% yield, respectively. The mechanism is considered to involve a nucleophilic attack by the Me free radical produced from acetoxy radical.[4] As we previously reported, the same methylated derivatives are also obtained on treatment of purine nucleosides with t-butyl hydroperoxide in the presence of ferrous ion through a similar mechanism.[10,11]

The treatment of cytidine with **2** afforded N^4-acetylcytidine (**6**) (11% yield), 6-methylcytidine (**12**) (8% yield), and deglycosylated cytosine (25% yield), besides recovered starting material (53%). Methylation at position-6 may have proceeded through nucleophilic attack by the Me radical produced from the acetoxy radical.[4] Chromatographic analysis of the mixture revealed the presence of an unidentified product in less than 5% yield. The structure of this was assumed from NMR and UV data to be 5- or 6-methyl-5,6-dihydropyrimidine riboside derivative.

The reaction of uridine with **2** gave 5-methyluridine (**13**) in 10% yield, besides deglycosylated uracil (10% yield) and recovered starting material (56% yield). As in the reaction with cytidine, an unidentified product was isolated in about 10% yield by preparative paper chromatography, the structure of which was assumed to involve a 5- or 6-methyl-5,6-dihydropyrimidine moiety from NMR and UV spectroscopy. Thymidine reacted with **2**, but apart from recovered starting material (60%) the only product identified was thymine (12% yield). It is assumed that a considerable part of the thymidine consumed was degraded via a 5,6-dihydropyrimidine derivative because the only spots detected on the

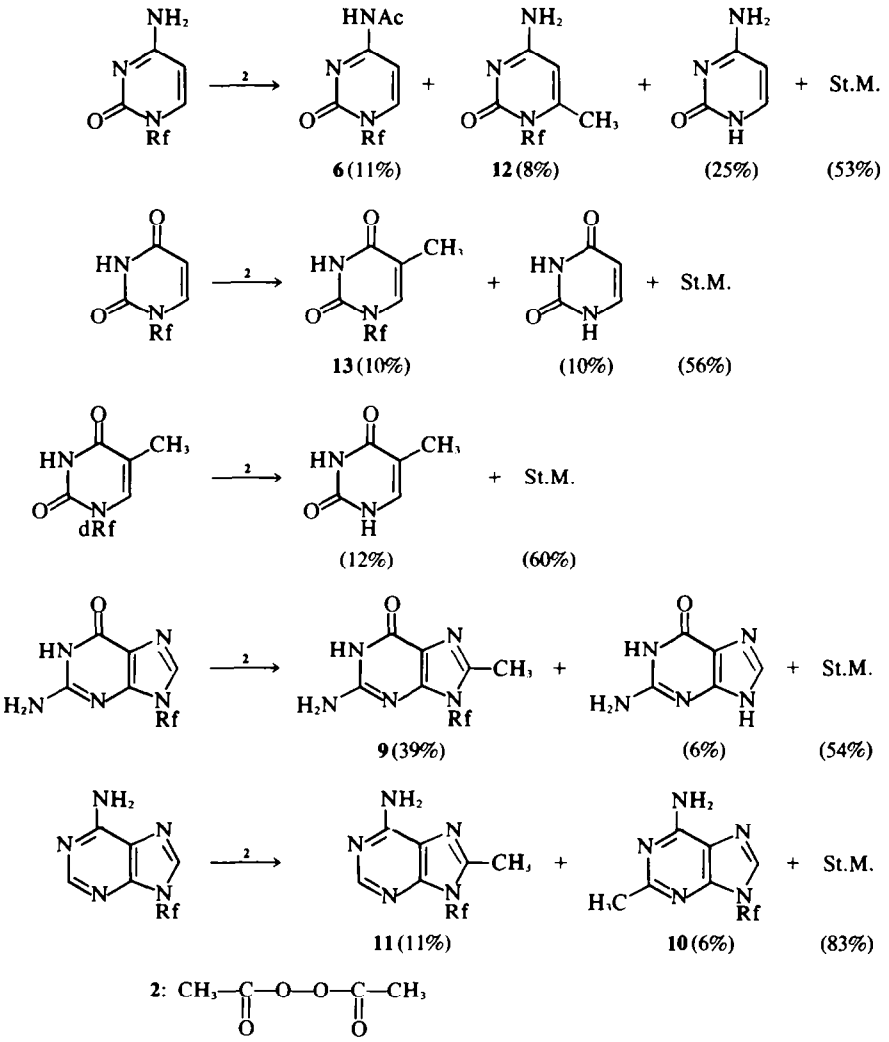

Chart 2. Reactions of nucleosides with an equimolar amount of diacetyl peroxide (2) in the presence of ferrous sulfate at 90°.

thin-layer plate by UV irradiation were those of thymine and thymidine.

DISCUSSION

As expected, diacyl peroxides reacted with nucleosides in both heterolytic and homolytic ways leading to chemical modification of the base moiety of the nucleosides. These reactions of diacyl peroxides do not seem to be peculiar to nucleosides. In fact, quinoline N-oxide was produced by the treatment of quinoline with **2** in benzene at room temperature, whereas 4-methylquinoline was produced when refluxed in the same solvent for 1 hr. These types of modifications should be taken into account when considering the mechanism for the DNA lesion induced by toxic peroxides, although there may be no direct correlation between this and the data presently obtained.

EXPERIMENTAL

Materials. Nucleosides were purchased from Kojin Co., Tokyo, and Sigma Chemical Co., St. Louis, U.S.A. **2** was freshly prepared by the method reported.[12]

Chromatography. TLC was carried out using Avicel-SF cellulose plates (Funakoshi Co., Tokyo) or silica gel-60-F 254 plates (E. Merck, Darmstadt). Paper chromatography for preparative separation was made using Whatman-3MM papers. The solvent systems for elution were (A) i-PrOH–conc. NH_4OH–H_2O (7:1:2) and (B) MeOH–conc. HCl–H_2O (7:2:1).

Reaction under heterolytic conditions

Cytidine with **1**. A soln of cytidine (121 mg; 0·5 mmol) and **1** (242 mg; 1 mmol) in 2 ml DMF was stirred at room temp. for 3 days. The mixture was evaporated to dryness under reduced pressure at 45° and washed with Et_2O. The residue was separated by paper chromatography into **3**, **4**, and **5** in yields of 46, 45 and 33%, respectively. **3** and **4** were identified with authentic samples[8,13] by TLC and NMR spectroscopy. R_f values of **4**, cytidine, **5**, and **3** on Avicel plate were (solvent system A): 0·28, 0·48, 0·63, and 0·88, respectively. **5** was identical with a sample prepared by treating 24 mg of **4** with 23 mg of benzoic anhydride in 0·3 ml of pyridine at room temp. for several hr, followed by evaporation of the solvent and washing of the residue with Et_2O.

Cytidine with **2**. The reaction was carried out with 121 mg (0·5 mmol) of cytidine and 118 mg (1 mmol) of **1** as described above. Chromatographic separation gave **4**, **6**[8] and cytidine in yields of 10, 56 and 30%, respectively. R_f values of **4**, cytidine, and **6** on Avicel plate (solvent system A): 0·3, 0·5 and 0·7, respectively.

Adenosine with **1**. The reaction was carried out with 54 mg (0·2 mmol) of adenosine and 121 mg (0·5 mmol) of **1** as described above. **7**[14] was isolated in 15% yield with 85% recovery of the starting material.

Adenosine with **2**. The reaction was carried out with 54 mg

(0·2 mmol) of adenosine and 59 mg (0·5 mmol) of **2** as described above. TLC separation indicated that the reaction mixture consisted of **7**, **8**, and adenosine in a relative ratio of 10:15:75. Preparative paper chromatography showed that the total yield of the three materials identified on TLC was almost quantitative.

Reaction under homolytic conditions

Guanosine with **2** *at* 90°. A soln of 283 mg of guanosine (1 mmol) and 40 mg of $FeSO_4 \cdot 7H_2O$ in 15 ml of H_2O was warmed at 90°. To this soln 118 mg (1 mmol) of **2** dissolved in 1 ml of DMF was added dropwise with stirring. The mixture was kept at 90° for 30 min with stirring. TLC separation (solvent system B) indicated that the mixture consisted of **9**,[10,11] guanine, and guanosine in a relative ratio of 39:6:54. Preparative paper chromatography showed that the total yield of the three materials identified on TLC was almost quantitative.

Adenosine with **2** *at* 90°. The reaction was carried out with 276 mg (1 mmol) of adenosine, 40 mg of $FeSO_4 \cdot 7H_2O$, and 118 mg (1 mmol) of **2** in as described above. TLC separation (solvent system B) indicated that the mixture consisted of **10**, **11**,[11] and adenosine in a relative ratio of 6:11:83. Preparative paper chromatography showed that the total yield of the three materials thus identified was almost quantitative.

Cytidine with **2** at 90°. To a soln of 972 mg (4 mmol) of cytidine and 130 mg of $FeSO_4 \cdot 7H_2O$, 470 mg (4 mmol) of **2** dissolved in 1 ml of DMF was added dropwise at 90° with stirring, and the mixture was kept at this temp. for 30 min with stirring. TLC and paper chromatography (solvent system A) showed that the products consisted of **6**, **12**, cytosine, cytidine, and an unidentified product (R_f 0·54), in a relative ratio of 11:8:25:53:3, respectively. The structure of the unidentified product was suggested by NMR to be a 5- or 6-methyldihydropyrimidine ribose derivative, but this was not definitely confirmed. In addition, the recovery of the materials thus identified on TLC indicated that some other products might have been produced in less than 5% yield.

Uridine with **2** *at* 90°. The reaction was carried out with 600 mg (2·5 mmol) of uridine and 100 mg of $FeSO_4 \cdot 7H_2O$ in 6 ml of H_2O, and 295 mg (2·3 mmol) of **2** in 1 ml of DMF in the same way as described above. The mixture was separated by paper chromatography (solvent system A) into **13** (10% yield), uracil (10% yield), uridine (56% yield), and two unidentified products, **14** and **15** (50 mg and 44 mg, respectively). **13** was identical with an authentic sample[15] which was kindly supplied by Drs. S. Nishimura and Z. Ohashi of this Institute. The unidentified product **14** (R_f 0·60) was assumed to be a 5- or 6-methyldihydropyrimidine riboside derivative, but this was not definitely confirmed. The other product, **15** (R_f 0·13), was similar to 5'-carboxyuridine in nature (UV, NMR and electrophoresis) but was shown not to be identical with it by co-chromatography.

Thymidine with **2** at 90°. The reaction was carried out with 50 mg of thymidine and 50 mg of $FeSO_4 \cdot 7H_2O$ in 3 ml of H_2O and 240 mg of **2** in 1 ml of DMF as described above. UV absorption at 260 nm decreased to 75% of optical density of the starting material used. Paper chromatography (solvent system A) gave only thymine and thymidine in yields of 12 and 60%, respectively.

Reaction of quinoline with **2**. (i) A soln of 129 mg of quinoline and 118 mg of **2** in 2 ml of benzene was kept overnight at room temp. After the mixture was washed with water, it was put on an Al_2O_3-column and eluted with benzene and then with $CHCl_3$ to give quinoline (89% yield) and quinoline 1-oxide (11% yield), respectively. (ii) A benzene solution (1 ml) containing 100 mg of quinoline and 200 mg of **2** was refluxed for 1 hr. The main product was isolated by Al_2O_3-chromatography and identified to be 4-methylquinoline (10% yield).

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