4 H, J = 7 Hz), 0.91 (dd, 1 H, J = 4, 6 Hz), 1.1 (br s, 2 H), 2.41 (dd, 1 H, J = 4, 6 Hz).

Deamination of 2-Deuteriospiropentylamine Hydrochloride. 2-Deuteriospiropentylamine hydrochloride (0.3 g, 5.2 mmol) was dissolved in 10 mL of glacial acetic acid and solid sodium nitrite (0.36 g, 5.2 mmol) was added in small portions over a 3-h period. After standing overnight, an additional 0.36 g of sodium nitrite was added over a 6-h period. The reaction mixture was allowed to stand 2 h after addition of sodium nitrite was complete; then 45 mL of a 10% sodium hydroxide solution was added cautiously. The still acidic reaction mixture was extracted four times with a total of 75 mL of pentane. The combined organic extracts were washed with a 10% sodium bicarbonate solution until the washings were basic. The solution was then washed once with saturated brine and dried over magnesium sulfate. Pentane was removed through a Vigreux column till approximately 1 mL of solution was left. The residue was analyzed by an SE-30 column, revealing a single peak which was collected: ^{2}H NMR (in $CDCl_3$) δ (relative to Me_4Si) 1.25 (two peaks of equal intensity, 5 D), 3.05 (two peaks of equal intensity, 5 D), 5.10 (two peaks of equal intensity, 1 D). In a separate run, spiropentylamine hydrochloride was deaminated in glacial acetic acid. The ^{1}H NMR spectrum [δ (CCl_4) 0.75 (s), 1.0 (m), 1.13 (t), 1.95 (3 s), 2.2~2.5 (br m), 2.75 (br m), 2.95 (br m), 4.1 (dd), 4.82 (p), 4.90 (m), 5.35 (m)] of the single peak on the SE-30 column indicated three acetates. On a 200 °C DBTCP capillary column a 5:5:1 mixture of three compounds was observed. On a UCON preparative column two peaks in a ~1:1 ratio were observed and collected. One peak was spiropentyl acetate; δ 0.79 (m, 3 H), 1.0 (m, 2 H), 1.14 (t, 1 H, J = 6 Hz), 1.96 (s, 3 H), 4.1 (d of d, 1 H, J = 6, 2 Hz).

The second peak was a mixture of 2- and 3-methylenecyclobutyl acetate in a 1:5 ratio: ^{1}H NMR of mixture, δ 1.97 (s, 3 H), 2.0 (s, 0.6 H), 2.40 (v br m, 0.8 H), 2.75 (br m, 2 H), 2.95 (br m, 2H), 4.80 (p, 2 H, J = Hz), 4.9 (m, 1.4 H), 5.35 (m, 0.2 H).

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Registry No.—2-Deuteriospiropentanecarboxylic acid, 64345-60-4; 2-deuteriospiropentanecarboxylic acid azide, 64345-61-5; 2-deuteriospiropentane isocyanate, 64345-62-6; 2-deuteriospiropentylamine hydrochloride, 64345-63-7; 2-deuteriospiropentylamine, 64345-64-8; 2-deuteriospiropentyl acetate, 64345-65-9; 2-deuteriomethylenecyclobutyl acetate, 64345-66-0; 2-deuterio-3-methylenecyclobutyl acetate, 64345-67-1.

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Mass Spectrometry of Pyrimidine Anhydronucleosides

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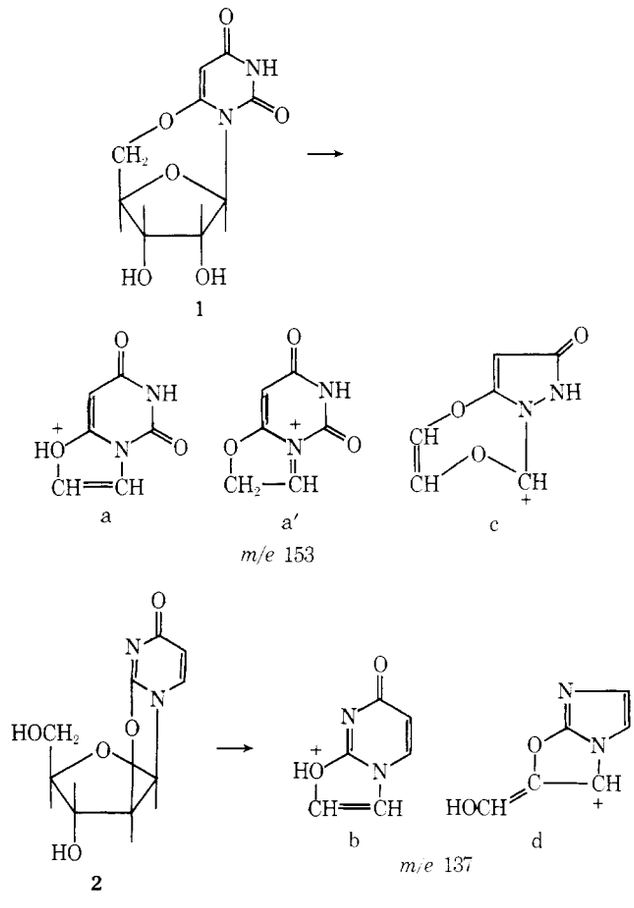
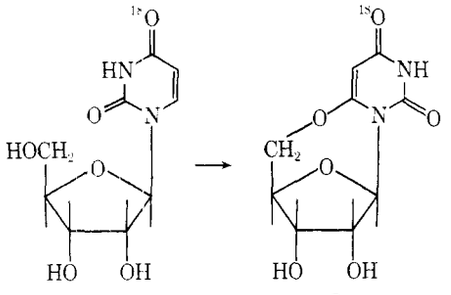
Anhydronucleosides often play an important role in the synthesis of nucleosides,[2,3] and their mass spectra have been shown to be useful for structural characterization (e.g., ref 4–10). The fragmentation reactions of anhydronucleosides are somewhat different from those of conventional nucleosides[11,12] in that conformational rigidity prevents base–sugar hydroxyl interactions that usually generate the primary reaction paths[13] and because of increased complexity of the system due to the anhydro linkage. In the mass spectra of O^6,5′-anhydropyrimidine nucleosides, a decomposition sequence has been proposed in which CO is first eliminated from the molecular ion and in which additional fragmentation of the base proceeds during subsequent reaction steps.[5] The latter rationale contrasts with the general behavior of normal nucleosides in which the heterocyclic base remains intact in initial reaction steps and decomposes only at the stage at which the free base has been generated. However, because initial reaction steps involve loss of neutral species which contain C, H and O but not N, the interpretation of both low- and high-resolution mass spectra is ambiguous, and fragmentation could proceed from either the base or sugar moieties.

The leading examples which clearly demonstrate the principle involved are the intermediate fragment ions m/e 153 ($C_6H_5N_2O_3$) from O^6,5′-anhydrouridine (**1**) and m/e 137 ($C_6H_5N_2O_2$) from O^2,2′-anhydrouridine (**2**). Originally de-



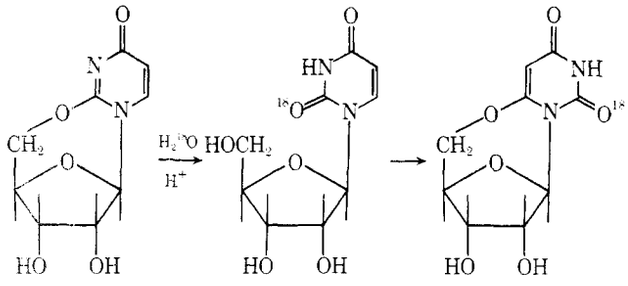
picted as structures a and b,[6] a subsequent and detailed interpretation of the mass spectra led to assignments c and d in which the elements of CO were expelled from the pyrimidine ring.[8] The ion of *m/e* 153 was represented as one step of the sequence *m/e* 242 (M) → 196 → 154 → 153 → 110, in which contraction of the pyrimidine ring was postulated as the first step.

To settle this issue using 1 as a model the ^{18}O-labeled analogue 3 was prepared by cyclization[5,6] of uridine-O^{4}-^{18}O (4).[14]



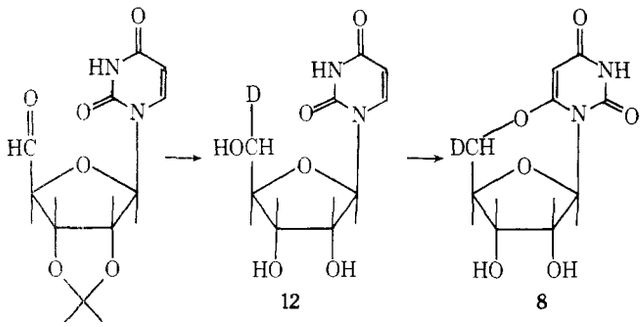
4 3

In order to exclude the additional possibility that *m/e* 153 had lost CO from C-2 rather than from C-4, 5 was obtained by opening of 6[15] in the presence of $H_2{}^{18}O$ and then cyclized to give the O^2-labeled compound 7. Precursors of ion *m/e* 153 in

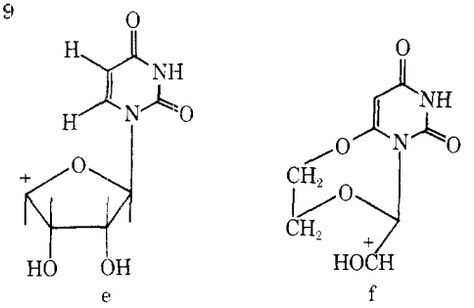


6 5 7

the mass spectrum of 1 were found to be *m/e* 213 (M − CHO), 195, and 154 through detection of metastable ion species by scanning the accelerating voltage with fixed electric sector voltage and fixed magnetic field. Therefore, the monodeuterio model 8 was prepared[16-18] from 9 to distinguish between the assignments previously made as e[6] and f.[8]



9 12 8



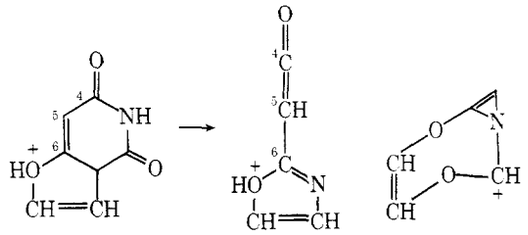
e f

m/e 213

Results and Discussion

Mass spectra of the ^{18}O-labeled compounds 3 and 7 showed essentially complete (~95%) retention of the base labels in all major ions in the upper mass region of the mass spectrum,[8] including *m/e* 153, 154, 195, 196, and 213. Structure a (or its tautomer a') is thus supported and c is excluded. Analogous results were obtained for the *m/e* 137 fragment ion derived from 2-O^{4}-^{18}O.[19] Therefore, structure b is supported and d is excluded, ruling against fragmentation of the pyrimidine ring as a common structural feature. Deuterium labeling at C-5' (8) resulted in a quantitative shift of *m/e* 213, militating against structure e. Ion f or some tautomer is therefore preferred if the original assumption[8] that CHO is expelled from C-3' rather than C-2' is correct. The original preference[8] for f over e was expressed on the basis that e should in principle give rise to abundant ions of the base + H (*m/e* 112) and base + 2H (*m/e* 113) type,[20] which were not observed. Although our isotopic-labeling experiment excludes structure e, the earlier reasoning[8] is not necessarily valid. Ions of the base + H type appear to originate mechanistically from precursors in which the charge resides in the base,[13] which is not the case in structure e. Base + 2H ion species are derived principally from base + CH_2O and base + C_2H_4O precursors,[13] which are not significant in the mass spectrum of 1.

The prominent fragment ion of mass 110 (70% rel int), shown by a metastable transition to be derived from *m/e* 153, was found to contain O^4 and H-5' but lack O^2. Taking into account the structure of ion a, *m/e* 110 is judged to be formed



m/e 153 g h

m/e 110

by retro-Diels–Alder expulsion of HNCO[21] (ion g), excluding structure h[22] proposed earlier.[8] The O^2-linked anhydronucleoside 2 lacks the required cyclohexene-type structure[23] and so *m/e* 110 is absent.

These results further underscore the complexity of electron impact induced reactions of anhydronucleosides, which evidently involve extensive restructuring of the sugar skeleton. However, in spite of a presently incomplete understanding of the reactions involved, mass spectrometry provides a useful means for characterization of anhydronucleoside structure, with the principal exception that O-2'- and O-3'-linked isomers cannot generally be distinguished.[4,6]

Experimental Section

Thin-layer chromatography was performed using Quanta/Gram Q1F plates (Quantum Industries, Fairfield, N.J.) and preparative thin-layer chromatography utilized SIlicAR 7GF plates of 1000- or 2000-μm thickness (Analtech, Inc., Newark, Del.). Plates were developed in ethyl acetate/1-propanol/water (4:1:2; v/v/v; upper phase). Oxygen-18 enriched H_2O (99%) was purchased from Koch Industries (Cambridge, Mass.) and Norsk-Hydro Sales (New York, N.Y.). Sodium borodeuteride (99%) was purchased from Merck Isotopes (St. Louis, Mo.). Mass spectra were acquired using an LKB-9000S mass spectrometer operating at a 70-eV ionizing energy and an ion source temperature of 270 °C. Metastable ion measurements[24] were made using a CEC 21-110B mass spectrometer.

Samples were checked for purity and extent of ^{18}O incorporation by conversion to the trimethylsilyl derivatives and subjecting the samples to gas chromatography–mass spectrometry [3-ft OV-17 (1%) programmed from 180 °C at 4°/min]. Chromatograms from all samples exhibited a single peak. Underivatized samples were examined

for purity by TLC and mass spectrometry (samples introduced by direct probe). Mass spectra of ^{18}O- and deuterium-labeled compounds whose preparations are described below were identical with the exception of isotopic mass shifts to those from the unlabeled materials; peaks due to impurities or starting materials were absent.

Uridine-O^2-^{18}O (5). A solution of O^2,5′-anhydrouridine (**6**)[15] (180 mg) in $H_2{}^{18}O$ (99%) (250 μL) and concentrated HCl (25 μL) was heated at 90 °C for 1.5 h. The reaction mixture was cooled and applied to three 20 × 20 cm preparative TLC plates (2000 μm). The band corresponding to uridine was scraped from the plate and eluted with MeOH until no more UV-absorbing fractions were eluted. The MeOH was evaporated in vacuo. The residue was crystallized from aqueous EtOH (99%) to give 80 mg (40%) of **5**. Mass spectral analysis showed 90% incorporation of ^{18}O (correction for dilution of the $H_2{}^{18}O$ by $H_2{}^{16}O$ of the HCl showed quantitative incorporation of ^{18}O).

5-Iodouridine-O^2-^{18}O (10). A solution of dioxane (9 mL) and 0.5 N HNO_3 (1 mL) containing I_2 (160 mg) and **5** (80 mg) was refluxed for 3 h.[25] The reaction mixture was cooled and applied to a 20 × 20 cm preparative TLC plate. The plate was developed and scraped, and the band corresponding to **10** was eluted with MeOH. After evaporation of the MeOH, the residue was crystallized from EtOH to give 60 mg (50%) of **10**.

O^6,5′-Anhydrouridine-O^2-^{18}O (7). A solution of **10** (60 mg) in dry Me_2SO (10 mL) was added rapidly to a solution of potassium *tert*-butoxide in dry *tert*-butyl alcohol (10 mL) under nitrogen.[5,6] The solution was stirred for 24 h at 70 °C and excess potassium *tert*-butoxide was destroyed with water. The reaction mixture was applied to water-washed Dowex-50 (H^+) (3 mL) and washed with water until no more UV-absorbing fractions were eluted. The eluate was taken to dryness in vacuo and the residue crystallized from EtOH to give 18 mg (46%) of **7**.

5-iodouridine-O^4-^{18}O (11). Prepared like **10** above except that uridine-O^4-^{18}O (90% ^{18}O)[14] (80 mg) was used as starting material, yield 60 mg (50%).

O^6,5′-Anhydrouridine-O^4-^{18}O (3). Prepared like **7** above using **11** (60 mg) as starting material, yield 20 mg (51%).

Uridine-5′-*d* (12). A solution of 2′,3′-*O*-isopropylideneuridine-5′-aldehyde (**9**)[16] (1.6 g) in EtOH (50 mL) and sodium borodeuteride (99%) was stirred at room temperature for 1 h. The solvent was evaporated in vacuo and the residue extracted with hot acetone (3 × 25 mL). The residue, after evaporation of the acetone under reduced pressure, was covered with aqueous trifluoroacetic acid (10 mL) and stirred at room temperature for 10 min.[18] Trifluoroacetic acid was removed under reduced pressure and the oil residue was triturated with ether until an off-white solid was obtained. After decantation of the ether, the solid was crystallized from EtOH to give 750 mg (54%) of **12** from **9**. Mass spectral analysis showed 95% incorporation of deuterium at the 5′ position.

5-Iodouridine-5′-*d* (13). Prepared like **10** above except **12** (100 mg) was used as starting material, yield 70 mg (51%).

O^6-5′-Anhydrouridine-5′-*d* (8). Prepared like **7** above except **13** (78 mg) was the starting material, yield 24 mg (47%).

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Registry No.—**3**, 64235-90-1; **5**, 64235-89-8; **6**, 22329-20-0; **7**, 64252-84-2; **8**, 64235-87-6; **9**, 27999-65-1; **10**, 64235-86-5; **11**, 64235-85-4; **12**, 64235-88-7; **13**, 64235-84-3.

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Enhanced Nucleophilic Reactivity (α Effect) in the Reaction of Peroxobenzoate Anions with *p*-Nitrophenyl Acetate[1]

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Progress in the experimental study of enhanced nucleophilic reactivity (α effect) and the theoretical interpretation of the phenomenon has recently been reviewed by Hudson.[2] Although peroxo anions are recognized as α nucleophiles,[3] few species of this type have been studied,[4,5] probably as a result of the difficulties in preparing the materials, and no systematic investigation of structure–nucleophilic reactivity relationships has been reported. The present work forms part of a study of oxidations by peroxo acids in aqueous solution, relating particularly to hydroperoxidase enzyme systems,[6] in which nucleophilic attack by the peroxo anion may be an important component of the oxidation pathway. We report studies of the nucleophilic reactivity of the peroxobenzoate anion and nine substituted peroxobenzoate anions toward *p*-nitrophenyl acetate, a choice of substrate which permits comparison with a wide range of data in the literature.[4,7]

Experimental Section

Kinetic measurements were made using procedures described in the literature[4] (pseudo-first-order conditions; initial [peroxo acid]/[*p*-nitrophenyl acetate] was >10:1; pH 10 ($NaHCO_3/Na_2CO_3$ buffer); ionic strength 0.1 mol L^{-1} ($NaNO_3$); 25 ± 0.2 °C), recording the release of the *p*-nitrophenolate ion at 402 nm. Peroxobenzoic acid and *m*-chloroperoxobenzoic acid were recrystallized (3:1 v/v, petroleum ether/diethyl ether) to give materials of purity >99%. Other peroxobenzoic acids were ~85% pure, the only significant impurity being the respective parent carboxylic acid. The pK_a values (Table I) were determined by potentiometric titration[8] (25 °C, ionic strength approximately constant at 0.1 mol L^{-1} ($NaNO_3$)). Solutions were checked cerimetrically to ensure that no hydrogen peroxide was present. Calculated second-order rate constants (Table I) were corrected for the "blank rate" in buffer solution alone. EDTA (5×10^{-4} mol L^{-1}) was added in a number of experiments but had no influence on the rate.

Results and Discussion

The nucleophilic reactivity of the meta- and para-substituted peroxobenzoate anions varies systematically with basicity, giving a Brønsted slope of 0.38 (Figure 1, a). The data for *o*-chloroperoxobenzoate falls on the line described by the meta- and para-substituted analogues, whereas *o*-nitro- and

0022-3263/78/1943-0769$01.00/0