Carbocyclic Analogs of Cytosine Nucleosides

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The carbocyclic analogs of cytidine, 2'-deoxycytidine, and 3'-deoxycytidine were synthesized from the analogous uracil derivatives. The route consists of complete benzoylation of the uracil derivative, selective removal of a benzoyl group attached to the pyrimidine ring, conversion of the 4-oxo to a 4-chloro group with the dimethylformamide-thionyl chloride reagent, and replacement of the chloro group with an amino group in methanolic ammonia. When the total products of the deoxychlorination reaction were employed, the desired cytosine derivatives were frequently accompanied by small amounts of the corresponding *N,N*-dimethylcytosine derivatives, which could be removed by ion-exchange chromatography. Carbodine (VIa), the carbocyclic analog of cytidine, was obtained in 84% yield from the pure 4-chloropyrimidinone intermediate, after the latter was prepared by deoxychlorination in carbon tetrachloride. Carbodine has antileukemic, antiviral, and antibacterial activity.

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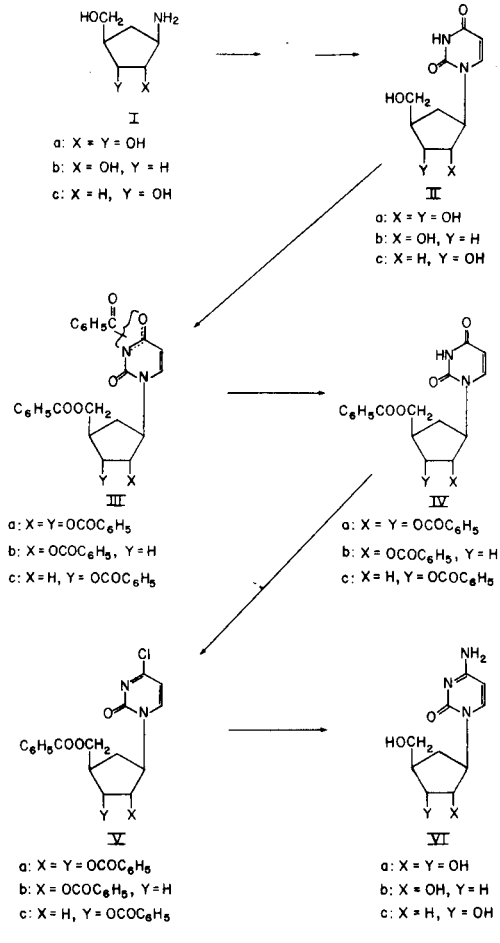
Because of the essential role of cytosine nucleotides in the biosynthesis of RNA and DNA and in other biochemical conversions, structural analogs of cytosine nucleosides are potential chemotherapeutic agents. Currently, the best known example of a therapeutically useful cytosine nucleoside is 1-β-D-arabinofuranosylcytosine (Ara-C, cytarabine); Ara-C has useful anticancer and antiviral activity (reviews 1-3). In addition, a substantial number and variety of other analogs of pyrimidine nucleosides are possessed of antineoplastic or antiviral activity (*e.g.*, reviews 3-6). The prominence of pyrimidine nucleosides in anticancer and antiviral chemotherapy, together with our earlier syntheses and demonstrations of biological activity by carbocyclic analogs of purine nucleosides (*e.g.*, 7-10 and publications cited therein), constituted the general rationale for the synthesis of carbocyclic analogs of cytosine nucleosides. A preliminary account (11) has been given of the synthesis of the carbocyclic analog (VIa, carbodine) of cytidine, together with the antileukemic activity of this analog. In addition, the carbocyclic analog (IXa) of Ara-C has been synthesized and shown to be active against leukemia L1210 (12). We present herein a detailed account of the synthesis and antileukemia evaluation of carbodine and the carbocyclic analogs of 2'- and 3'-deoxycytidine.

The carbocyclic analogs (II) of uracil nucleosides served as intermediates for syntheses of the carbocyclic analogs (VI) of cytosine nucleosides. The uracil derivatives (II) were prepared, as described previously (13), from the prerequisite cyclopentylamines (I) (7,8). The synthesis route is represented by structures II-VI in Chart I. The hydroxyl groups of the uracil derivatives were protected by benzoylation prior to the conversion of the 4-oxo group to a 4-chloro group. The initial benzoylation of the carbocyclic analog (IIc) of 2'-deoxyuridine with 2.2 equivalents of benzoyl chloride in pyridine gave a mixture of the desired dibenzoyl derivative (IVc) and a tribenzoyl derivative (IIIc). Consequently, benzoylation of IIa-c was subsequently carried out with an excess of benzoyl chloride in order to effect complete benzoylation to IIIa-c, and the surplus

CHART I



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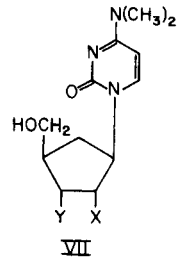
benzoyl group on the pyrimidine ring was then selectively removed in a weakly acidic medium to give IVa-c.

The benzoyl derivatives (IVa-c) were converted to the 4-chloropyrimidinone intermediates (Va-c) by the method of Žemlička and Šorm (14). This procedure included the use of less than one equivalent of DMF plus excess thionyl chloride in chloroform. When the total crude product was employed in the subsequent step, consisting of replacement of the 4-chloro group by an amino group and debenzoylation in methanolic ammonia, the desired cytosine derivatives (VIa-c) were usually accompanied by small amounts of the analogous *N,N*-dimethylcytosine derivatives (VIIa-c). Earlier, Žemlička and Šorm (14) observed the formation of *N,N*-dimethyl derivatives of cytosine and 6-azacytosine nucleosides under certain conditions of formation or use of the chloro derivatives. Typically, the carbocyclic analogs (VIa-c) were isolated by chromatography on a sulfonic acid ion-exchange resin (15). Elution with dilute aqueous ammonia, after neutral impurities had been eluted with water, gave the desired cytosine derivatives. When an *N,N*-dimethylcytosine derivative (VII) was detected, by thin-layer chromatography or by mass spectral analysis, as a contaminant in a cytosine derivative (VI) isolated in this way, pure VI was obtained by chromatographing the mixture on a sulfonic acid ion-exchange resin (16) in the pyridinium salt form and eluting with 0.1*M* pyridinium formate (17). The *N,N*-dimethylcytosine (VII) was eluted first followed by the pure cytosine derivative (VI).

Subsequently, the deoxychlorination of IVa was performed in carbon tetrachloride, and pure 4-chloropyrimidinone Va was isolated in a yield of about 60%. From Va prepared in this way, carbodine (VIa), free of VIIa, was obtained in 84% yield without employing chromatography in pyridinium formate.

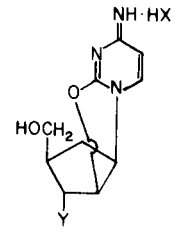
The deoxychlorination procedure previously described (14) is not without difficulties. The reaction of a benzoylated uracil derivative (IV) with 0.6 equivalent of DMF and an excess of thionyl chloride in refluxing chloroform was sometimes incomplete after the usual reaction time (14) of 6 hours, and the mixture of IV and V had to be recycled. As outlined above, the use of the crude 4-chloropyrimidinones (V) for the preparation of the cytosine derivatives (VI), as reported for 6-azacytidine (14), usually also produced small amounts of the *N,N*-dimethylcytosine derivatives (VII) (18). An *N,N*-dimethylcytosine derivative could be formed from dimethylamine, derived from DMF, either during the deoxychlorination reaction or during the subsequent amination process. The tribenzoyl derivative of VIIa was not observed by mass spectral examination of specimens of crude Va. During the amination step, dimethylamine might be formed from residual DMF or from chloromethylenedimethylammonium chloride by transamination with ammonia. Reaction of the generated

CHART 2



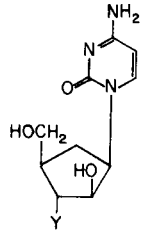
VII

a: X = Y = OH
b: X = OH, Y = H
c: X = H, Y = OH



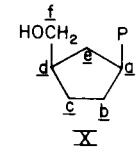
VIII

a: Y = OH
b: Y = H



IX

a: Y = OH
b: Y = H



X

Letters a–f identify protons or carbon atoms for NMR assignments (Experimental)

dimethylamine with the 4-chloropyrimidinone (V) or an amine-exchange reaction of VI with dimethylamine (catalyzed by trace amounts of hydrogen chloride) would produce VII. In order to remove the sources of dimethylamine, the procedure was modified by washing a chloroform solution of the total product of deoxychlorination of IVa with water. Mass spectral analysis of the crude product isolated after the aqueous washing showed the presence of a considerable amount of the tribenzoyl derivative of the 4-ethoxypyrimidinone analog, which must have been formed from Va and ethanol present as a stabilizer in the chloroform. Since 4-alkoxypyrimidinones also undergo amination to 4-aminopyrimidines, the resulting mixture was converted without further purification to VIa. Specimens of VIa obtained by this modification of the usual procedure either contained less, or were free of, detectable VIIa.

These difficulties were avoided by first preparing pure Va by carrying out the deoxychlorination reaction in refluxing carbon tetrachloride.

One method of preparing Ara-C consists of first converting cytidine to 2,2′-anhydrocytidine with the DMF-thionyl chloride reagent (17). The carbocyclic analog (VIIIa) of anhydrocytidine has been prepared by this method and hydrolyzed to the carbocyclic analog (IXa) of Ara-C (12). Application of this two-step sequence to the analog (VIb) of 3′-deoxycytidine gave the corresponding cyclic (VIIIb) and 3′-deoxyarabinosyl (IXb) analogs.

The activity of carbodine (VIa) against leukemia L1210

in vivo and against KB cells *in vitro* was reported earlier (11). The Ara-C analog (IXa) is also active against leukemia L1210 in mice (12). However, at a dose of 200 mg./kg./day, neither the 2'-deoxycytidine analog (VIc) nor the 3'-deoxycytidine analog (VIb) gave evidence of antileukemic activity or of toxicity in the standard test against L1210 leukemia (19), and neither was active against KB cells in culture (ED_{50} > 100 mcg./ml.). Brockman and coworkers (20) have shown that carbodine (VIa) inhibits both DNA and RNA synthesis in L1210 cells and that it is phosphorylated to the mono-, di-, and triphosphate stages. Carbodine has excellent activity against RNA viruses and has also demonstrated activity against DNA viruses and certain bacteria (21).

EXPERIMENTAL

General.

Decomposition and melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (uv) were recorded with a Cary Model 17 spectrophotometer, and maxima are reported in nanometers. Solutions for ultraviolet spectral determinations were prepared by diluting a 5-ml. aliquot of an ethanol or water solution to 50 ml. with 0.1*N* hydrochloric acid, phosphate buffer (*p*H 7), or 0.1*N* sodium hydroxide; absorption maxima of these solutions are reported as being determined in 0.1*N* HCl, at *p*H 7, or in 0.1*N* sodium hydroxide, respectively. Infrared spectra (ir) were recorded with a Perkin-Elmer Model 621 spectrometer from samples in potassium bromide disks; s = strong, sh. = shoulder. Mass spectral data (ms) were taken from low resolution spectra determined at 70 eV with a Varian MAT Model 311A spectrometer equipped with a combination electron-impact, field-ionization, and field-desorption ion source. The peaks listed are those due to the molecular ion (M^+), those attributable to the loss of certain fragments from the molecular ion (M − a fragment), and some other pro minent peaks. Thin-layer chromatography (tlc) was performed on plates of fluorescing silica gel (22), and developed plates were examined with UV light (254 nm) unless indicated otherwise in parentheses. Other pertinent information (amount applied, developing solvent, other methods of detection) is given parenthetically at the appropriate places in the experimental procedures. Proton magnetic resonance spectra (^{1}H-nmr) and carbon-13 nuclear magnetic resonance spectra (^{13}C-nmr) were determined with a Varian Model XL-100-15 spectrometer. Chemical shifts (δ) are in parts per million downfield from internal tetramethylsilane in DMSO-d_6 solution; m = multiplet, d = doublet, s = singlet. Positions for chemical-shift assignments are identified in structure X.

(±)-1-[(1α,2β,3β,4α)-2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]-2,4-(1*H*,3*H*)pyrimidinedione Tribenzoate (IVa).

A solution of benzoyl chloride (3.07 g., 24.6 mmoles) in anhydrous pyridine (12.5 ml.) was added dropwise to a stirred solution of 1.203 g. (4.97 mmoles) of (±)-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)-cyclopentyl]-2,4-(1*H*,3*H*)pyrimidinedione (IIa) in anhydrous pyridine (25 ml.). The solutions of the reactants and the reaction mixture were protected from atmospheric moisture. The reaction was mildly exothermic. After the addition of the benzoyl chloride had been completed, the reaction solution was heated at 57-58° for 48 hours, cooled to room temperature, and poured slowly into an water-ice mixture (500 ml.). The reaction vessel was rinsed twice with 3-ml. portions of pyridine, and these rinsings were added to the cold mixture. After the ice had melted, the mixture was filtered to separate a white solid that was washed thoroughly with water and dried under reduced pressure at room temperature: weight, 3.18 g. (97% yield, calculated as the tetrabenzoyl derivative (IIIa) of compound IIa). A field-desorption mass spectrum (molecular ion at m/e 658; solvent, DMSO; emitter current, 17 mA) and thin-layer chromatography of the white solid confirmed that it was predominantly the tetrabenzoyl derivative (IIIa); a trace amount of the tribenzoyl derivative (IVa) was detected by tlc (40 or 80 mcg., 99:1 chloroform-methanol).

To a solution of 3.165 g. of the tetrabenzoyl derivative (IIIa) in hot ethanol (328 ml.) was added water (164 ml.) and 1*N* hydrochloric acid (18 ml.). This solution was boiled under reflux for 20 hours, cooled to room temperature, and concentrated under reduced pressure to remove ethanol. The resulting mixture was filtered to separate a crystalline solid that was washed thoroughly with water and dried under reduced pressure at 56°: weight, 2.64 g. (96% yield from compound IIa); m.p. 188-191°. Thin-layer chromatography showed that the solid was the desired tribenzoate (IVa) containing a trace quantity of the tetrabenzoyl derivative. Recrystallization of this material from ethanol furnished the tribenzoate (IVa): weight, 2.04 g.; m.p. 191-194°; tlc, 1 spot (80 mcg., 99:1 chloroform-methanol); ir (1800-1300 cm^{-1} region): 1725, 1705, 1685, 1625, 1600, 1582, 1490, 1460 (shoulder), 1450, 1422, 1380, 1350, 1318; field-desorption ms (solvent, DMSO; emitter current, 20 mA): 555 (M + 1), 554 (M), 432 (M − C_6H_5COOH); uv max: 285 (shoulder), 268 (ε 21400), and 235 (ε 31300) at *p*H 7; 267 (ε 9600) and 225 (ε 33800) at *p*H 13.

Anal. Calcd. for $C_{31}H_{26}N_2O_8$: C, 67.14; H, 4.73; N, 5.05. Found: C, 67.15; H, 5.10; N, 4.84.

(±)-4-Chloro-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-2-(1*H*)pyrimidinone Tribenzoate (Va).

To a stirred suspension (protected from atmospheric moisture) of 1.00 g. of the tribenzoate (IVa) in 20 ml. of dry carbon tetrachloride was added 1.42 ml. of re-distilled thionyl chloride and 170 mg. of freshly distilled dimethylformamide. The mixture was boiled under reflux for 4 hours, cooled to room temperature, and filtered to remove the suspended solid. The solid was washed with carbon tetrachloride, recrystallized from acetonitrile, and dried *in vacuo* at room temperature: yield, 610 mg. (59%); m.p., 229-232°; tlc, 1 spot (40 or 80 mcg., 99:1 chloroform-methanol); ir (1800-1300 cm^{-1} region): 1720s, 1710s, 1670s, 1610, 1585, 1515s, 1490, 1460, 1450, 1445, 1400, 1385, 1345 (doublet), 1315, 1305; ms peaks (direct-probe temperature, 270°): m/e 573 (M + 1), 572 (M), 537 (M − Cl), 467 (M − C_6H_5CO), 450 (M − C_6H_5COOH), 345 (M − C_6H_5COOH − C_6H_5CO).

Anal. Calcd. for $C_{31}H_{25}ClN_2O_7$: C, 64.98; H, 4.40; N, 4.89. Found: C, 64.68; H, 4.43; N, 4.80.

(±)-4-Amino-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-2-(1*H*)pyrimidinone (Carbodine, VIa).

A mixture of 3.98 g. of Va and 50 ml. of a 50% solution of anhydrous ammonia in methanol was heated in a stainless steel bomb at 100° for 20 hours. The bomb was cooled and opened, the volatile components of the reaction mixture were evaporated with a current of nitrogen and then *in vacuo*, the residue was dissolved in water (100 ml.), the water solution was extracted with three 50-ml. portions of ethyl acetate to remove benzamide, and the aqueous solution was concentrated to dryness *in vacuo*. A solution of the residual solid in 100 ml. of water was poured onto a column of a sulfonic acid exchange resin (75 ml. of Amberlite CG-120 (15), H^+ form). The column was washed thoroughly with water and then eluted with 2*N* aqueous ammonia. Concentration of the basic eluate to dryness *in vacuo* and trituration of the residual solid with ethanol (20 ml.) afforded 1.40 g. (84% yield) of VIa that was homogeneous according to tlc; uv max, 214 (ε 9940) and 283 (ε 12,900) at *p*H 1. Carbodine (VIa) can be recrystallized, if necessary, from a small amount of water (5-6 ml./g.); m.p. 253-255° dec. (inserted at 225°, 3°/min., progressive darkening); tlc, 1 spot (40 or 80 mcg., 7:3 2-propanol-1*M* ammonium acetate); ms (direct-probe temperature, 230°): m/e 242 (M + 1), 241 (M), 224 (M - OH), 223 (M - H_2O), 213 (M - CO), 210 (M - CH_2OH), 194, 192, 182, 166, 138 (cytosinyl group + C_2H_4), 112 (cytosinyl + 2H), 111 (cytosinyl + H); uv max: 214 (ε 10,000) and 285 (ε 13,600) at *p*H 1, 225 (sh.) and 275 (ε 9300)

at *p*H 7 and *p*H 13; ir (1800-1300 cm^{-1} region): 1660s, 1620 (s, broad), 1570 sh., 1520, 1490, 1455, 1400, 1370, 1340, 1330 sh., 1305; ^{1}H-nmr: δ 1.0-1.5 (m, 1 proton, CH_2 at *e*), 1.7-2.2 (m, 2 protons, CH_2 at *e* + CH at *d*), 3.24-3.56 (m, CH_2 at *f*), 3.62-3.82 (m, CH at *c*), 3.84-4.16 (m, CH at *b*), 4.4-4.76 (m, CH at *a* + OH at *b,c,f*), 5.69 (center of d, position 5 of pyrimidine), 6.94 (NH_2), 7.58 (center of d, position 6 of pyrimidine); ^{13}C-nmr: 28.43 (*e*), 44.95 (*d*), 61.54 (*a*), 62.90 (*f*), 71.73 and 73.50 (*b* and *c*), 93.52 (C5), 143.32 (C6), 156.28 (C2), 165.25 (C4).

Anal. Calcd. for $C_{10}H_{15}N_3O_4$: C, 49.78; H, 6.27; N, 17.42. Found: C, 49.91; H, 6.57; N, 17.60.

Prior to the preparation of pure Va by the procedure described above, the procedure of Žemličkа and Šorm (14) was used to prepare crude Va, and the total product of the latter procedure was used without purification for the preparation of VIa (*cf.* the preparation of VIb). Specimens of VIa obtained in this way usually contained small amounts of the *N,N*-dimethylcytosine derivative (VIIa). The total crude product obtained from the reaction of such crude specimens of Va with methanolic ammonia was chromatographed on a column of a sulfonic acid ion-exchange resin (15), H^+ form. Elution of the column with water to remove neutral contaminants and then with 0.5*N* aqueous ammonia and lyophilization of the ammonia solution gave VIa. When tlc or the mass spectrum showed that VIIa was present in VIa obtained in this manner, the former could be removed by recrystallization or by further chromatography on a sulfonic acid ion-exchange resin (16) in the pyridine salt form. Washing the column with 0.1*M* pyridinium formate first eluted the *N,N*-dimethylcytidine analog (VIIa) and, then, compound VIa. The course of the elution could be followed by an ultraviolet monitor or by tlc. (Compound VIIa moves slightly ahead of VIa during tlc in 2-propanol-water-15*N* ammonia (8:1:1).) Concentration of the appropriate eluate fractions to dryness furnished the pure carbocyclic analogs (VIa and VIIa). The use of pure Va, prepared as described above, obviated the need for chromatographic purification of VIa.

(±)-1-[(1α,2β,3β,4α)-2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]-4-(dimethylamino)-2-(1*H*)pyrimidinone (VIIa).

Chloropyrimidinone Va (150 mg.) was treated with a 50% solution (25 ml.) of anhydrous dimethylamine in methanol by the procedure described for the preparation of VIa. The aqueous layer (15 ml.) resulting from the extraction with ethyl acetate was chromatographed on a column of 15 ml. of a sulfonic acid ion-exchange resin (15), H^+ form, by the procedure described for the isolation of VIa. Trituration of the colorless glass, obtained from the basic eluate, with ethanol-ether (1:1) afforded 69 mg. of crude VIIa; m.p. 198-200° dec. Recrystallization from water gave 40 mg. (57% yield) of pure VIIa: m.p. 198-200° dec. (inserted at 100°, 3°/min.); tlc, 1 spot (40-160 mcg., 8:1:1 2-propanol-water-15*N* ammonia); uv: max, 292 (ε 16,600) and 222 (ε 9100) at *p*H 1, 283 (ε 13,700) and shoulders near 240 and 220 at *p*H 13; ir (1800-1300 cm^{-1} region): 1635s, 1535, 1500, 1465, 1425, 1400, 1395, 1325sh., 1315; ms peaks (direct-probe temperature, 20°): m/e 269 (M), 252 (M − OH), 251 (M − H_2O), 241 (M − CO), 238 (M − CH_2OH), 210, 194, 166 (dimethylaminocytosinyl group + C_2H_4), 140 (dimethylaminocytosinyl group + 2H); ^{1}H-nmr: δ 1.1-1.5 (m, 1 proton, CH_2 at *e*), 1.7-2.2 (m, 2 protons, CH_2 at *e* + CH at *d*), 3.03 (s, 6 protons, CH_3), 3.3-3.5 (m, CH_2 at *f*), 3.66-3.82 (m, CH at *c*), 3.86-4.18 (m, CH at *b*), 4.34-4.86 (m, CH at *a* + OH at *b,c,f*), 6.0 (center of d, position 5 of pyrimidine), 7.68 (center of d, position 6 of pyrimidine).

Anal. Calcd. for $C_{12}H_{19}N_3O_4 \cdot 0.25H_2O$: C, 52.63; H, 7.18; N, 15.35. Found: C, 52.37; H, 7.17; N, 15.34.

Aqueous solutions containing VIIa were obtained, as outlined under VIa, either by ion-exchange chromatography (15) of specimens of VIa (prepared from crude Va) containing small amounts of VIIa or from filtrates resulting from the recrystallization of such specimens of VIa. Lyophilization of an aqueous solution obtained by further ion-exchange chromatography in 0.1*M* pyridinium formate on a sulfonic acid resin (16) in the pyridine salt form, dissolution of the residue in methanol, subsequent evaporation of the methanol, and trituration of the residue with ethyl acetate afforded white crystalline VIIa, identical by m.p. (198-200° dec.), tlc, high-pressure liquid chromatography, and mass spectral analysis with the specimen described above.

(±)-1-[(1α,2β,4α)-2-Hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4-(1*H*,3*H*)-pyrimidinedione Dibenzoate (IVb).

(±)-1-[(1α,2β,4α)-2-Hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4-(1*H*,3*H*)-pyrimidinedione (IIb, 2.826 g., 12.5 mmoles) in anhydrous pyridine (63 ml.) was treated with a solution of benzoyl chloride (5.80 g., 41.3 mmoles) in anhydrous pyridine (31.5 ml.) by the procedure described for the benzoylation of IIa. After 48 hours at 58-60°, the reaction solution was treated with activated charcoal and the tribenzoyl derivative (IIIb) was isolated by the procedure described for the isolation of IIIa: weight, 6.62 g. (98% yield, calculated as tribenzoyl derivative IIIb). This material was shown to be predominantly the tribenzoyl derivative (IIIb) of compound IIb by thin-layer chromatography and by ms analysis (direct-probe temperature, 140°): m/e 539 (M + 1), 433 (M − C_6H_5CO), 416 (M − C_6H_5COOH), 388 (M − C_6H_5COOH − HCN), 311 (M − C_6H_5COOH − C_6H_5CO), 294 (M − $2C_6H_5COOH$).

A solution of 6.6 g. of the tribenzoyl derivative (IIIb), 225 ml. of ethanol, 185 ml. of water, and 21.5 ml. of 1*N* hydrochloric acid was boiled under reflux for 32 hours, cooled to room temperature, and concentrated under reduced pressure to remove ethanol. The resulting mixture was chilled and filtered to separate the precipitate, which was washed thoroughly with water and dried under reduced pressure: weight, 4.91 g. (90% yield from compound IIb). A hot solution of this material in ethanol (250 ml.) was treated with activated carbon, filtered, and cooled. The white crystalline dibenzoate (IVb) was separated by filtration, washed with ethanol, and dried under reduced pressure at 56°, yield, 2.78 g. (51% from IIb); m.p. 180-183°; ir (1800-1300 cm^{-1} region): 1715s, 1690s, 1670s, 1630, 1600, 1580, 1490, 1465, 1450, 1435, 1385, 1355, 1315, 1305; ms peaks (direct-probe temperature, 160°): m/e 435 (M + 1), 312 (M − C_6H_5COOH), 207 (M − C_6H_5COOH − C_6H_5CO), 190 (M − $2C_6H_5COOH$).

Anal. Calcd. for $C_{24}H_{22}N_2O_6$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.09; H, 4.96; N, 6.41.

(±)-4-Amino-1-[(1α,2β,4α)-2-hydroxy-4-(hydroxymethyl)cyclopentyl]-2-(1*H*)pyrimidinone (VIb).

A stirred solution (protected from atmospheric moisture) of 526 mg. of compound IVb, 0.97 ml. of thionyl chloride, 57 mg. of dimethylformamide, and 6 ml. of chloroform was boiled under reflux for 6 hours. The reaction solution was concentrated under reduced pressure, and the solid residue was stored in a high vacuum overnight to complete the removal of volatile materials. The mass spectrum (direct-probe temperature, 20°) of the solid included the following peaks and showed that it was predominantly the desired 4-chloro-2-(1*H*)pyrimidinone derivative (Vb), contaminated with some IVb: m/e 453 (M + 1), 347 (M − C_6H_5CO), 330 (M − C_6H_5COOH). Tlc confirmed the ms results and also revealed the presence of trace amounts of other impurities.

A mixture of the crude solid (Vb) and a solution of anhydrous ammonia in methanol (30 ml. of 50% ammonia-methanol) was heated in a stainless steel bomb at 100° for 18 hours. The bomb was cooled and opened, the volatile components of the reaction mixture were evaporated with a current of nitrogen, and the residue was dissolved in water (20 ml.). The water solution was extracted with three 25-ml. portions of ethyl acetate to remove benzamide. The aqueous layer was concentrated to remove a small amount of ethyl acetate and then poured onto a column of 50 ml. of a sulfonic acid ion-exchange resin (15), H^+ form. Water was passed through the column until the neutral, ultraviolet-absorbing components were eluted. The column was then washed with 0.5*N* aqueous ammonia, and fractions containing the desired product (located with an ultraviolet monitor) were concentrated under reduced pressure to a colorless syrup (weight, 213 mg.). Trituration of the syrup with ether-methanol (9:1) produced a white crystalline solid (VIb), yield, 187 mg. (68%). Both tlc (40 mcg., 8:1:1 2-propanol-water-15*N* ammonia) and the mass spectrum showed that this material contained a small amount of the *N,N*-dimethyl derivative (VIIb).

This product was combined with a similar specimen obtained from

another run, and a solution of the combined specimens (487 mg.) in water (25 ml.) was poured onto a column (50 ml.) of a sulfonic acid ion-exchange resin (16) in the pyridine salt form. Washing the column with 0.1*M* pyridinium formate first eluted the *N,N*-dimethylcytosine derivative (VIIb) and, then, compound VIb. The course of the elution may be followed with an ultraviolet monitor or by thin-layer chromatography. Lyophilization of the eluate solution containing VIb, trituration of the residue with acetonitrile, and recrystallization of the resulting solid (407 mg.) from methanol gave pure VIb: weight, 254 mg. (52% recovery); m.p. 211-214° dec. (inserted at 100°, 3°/min.); tlc, 1 spot (40 or 80 mcg., 7:3 2-propanol-1*M* ammonium acetate); uv max: 285 (ε 13,200) and 214 (ε 10,000) at *p*H 1, 275 (ε 9100) and 225 (sh) at *p*H 7, 275 (ε 9000) and 225-230 (shoulder) at *p*H 13; ms (direct-probe temperature, 20°): m/e 226 (M + 1), 225 (M), 208 (M − OH), 207 (M − H_2O), 197 (M − CO), 196, 194 (M − CH_2OH), 176, 166, 138 (cytosinyl group + C_2H_4), 112 (cytosinyl + 2H); ^{1}H-nmr: 0.92-2.4 (m, 5 protons, CH_2 at *e* and *c*, CH at *d*), 3.2-3.5 (m, CH_2 at *f*), 3.9-4.3 (m, CH at *b*), 4.3-4.68 (m, CH at *a* and OH at *f*), 4.8-5.02 (m, OH at *c*), 5.7 (center of d, position 5 of pyrimidine ring), 6.96 (NH_2), 7.58 (center of d, position 6 of pyrimidine ring).

Anal. Calcd. for $C_{10}H_{15}N_3O_3$: C, 53.32; H, 6.71; N, 18.65. Found: C, 52.92; H, 6.79; N, 18.89.

Isolation of (±)-4-(Dimethylamino)-1-[(1α,2β,4α)-2-hydroxy-4-(hydroxymethyl)cyclopentyl]-2-(1*H*)pyrimidinone (VIIb).

Dibenzoyl derivative IVb (2.05 g.) was treated with thionyl chloride and DMF in chloroform according to the procedure (14) described above for the preparation of crude Vb. Because tlc showed the presence of much unreacted IVb in the isolated material (2.1 g.), the latter was subjected again to the chlorination procedure except that one-half of the usual quantities of thionyl chloride, DMF, and chloroform were used. The product of treatment of the resulting crude 4-chloropyrimidinone derivative (Vb) with methanolic ammonia was chromatographed, as described above, on a sulfonic acid ion-exchange resin (15), H^+ form. The mixture (744 mg.) of compounds VIb and VIIb obtained from the 0.5*N* aqueous ammonia eluate was dissolved in ethanol. The precipitate (366 mg.), collected in two crops, was shown by tlc and ms to be VIb containing a trace amount of VIIb. Pure VIb was obtained from this material by chromatography, as outlined under VIb, in 0.1*M* pyridinium formate on a sulfonic acid resin (16) in the pyridine salt form.

Concentration of the ethanol filtrate afforded a residue (305 mg.) that was chromatographed on a column of the pyridinium salt of a sulfonic acid ion-exchange resin (16), as outlined under VIb. Fractions of the 0.1*M* pyridinium formate eluate containing VIIb were combined and lyophilized to a gummy residue. Pure VIIb was obtained as a white crystalline solid (70 mg.) after methanol (several portions) and acetonitrile had been added to and evaporated *in vacuo* from the gummy residue. Later eluate fractions containing VIb were likewise combined and lyophilized, and the glassy residue was crystallized by trituration with acetonitrile, weight, 150 mg.; m.p. 211-214° dec. Tlc and the ms confirmed that this material was VIb.

Compound VIIb may be recrystallized from ethanol, m.p. 189-194°; mass spectrum (direct probe temperature, 20°): 253 (M), 236 (M − OH), 225 (M − CO), 224, 204, 194, 166 (*N,N*-dimethylcytosinyl group + C_2H_4), 140 (*N,N*-dimethylcytosinyl group + 2H); uv max: 292 (ε 15,800) and 220 (ε 9200) at *p*H 1, 283 (ε 13,200) and shoulders near 240 and 220 at *p*H 7 and 13. Compound VIIb moves slightly ahead of VIb during tlc in 8:1:1 2-propanol-water-15*N* aqueous ammonia.

Anal. Calcd. for $C_{12}H_{19}N_3O_3 \cdot H_2O$: C, 53.12; H, 7.80; N, 15.49. Found: C, 53.54; H, 7.25; N, 15.33.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4-(1*H*,3*H*)-pyrimidinedione Dibenzoate (IVc).

The carbocyclic analog (IIc) of 2′-deoxyuridine was treated with benzoyl chloride in pyridine by the procedure described for the preparation of IVb. A gummy precipitate separated when the reaction solution was poured into a water-ice mixture. The aqueous phase was decanted and the precipitate was dissolved in chloroform. The chloroform solution was washed with 0.5*N* hydrochloric acid and then with aqueous sodium bicarbonate solution, dried with magnesium sulfate, treated with activated charcoal, and concentrated to dryness under reduced pressure. The white residue was shown by tlc and by ms analysis (direct-probe temperature, 160°) to be the tribenzoyl derivative (IIIc) of compound IIc; m/e 538 (M). The material (3.73 g., 95% yield) obtained in this way from 1.653 g. of IIc was dissolved in a solution of 120 ml. of ethanol, 120 ml. of water, and 12.5 ml. of 1*N* hydrochloric acid. This solution was boiled under reflux for 15 hours and concentrated under reduced pressure to remove some of the ethanol, and the resulting mixture was diluted with water (60 ml.) and chilled. A white precipitate was separated by filtration, washed well with water, and dried under reduced pressure at 56°: weight, 2.687 g. (85% yield from IIc); m.p. 189-193° (inserted at 100°, 3°/min.). Recrystallization of this material from ethanol furnished IVc as white platelets: weight, 2.26 g.; m.p. 193-196°; tlc, 1 spot (80 mcg., 99:1 chloroform-methanol); uv max: 267 (ε 13,500) and 232 (ε 24,900) at *p*H 1 or *p*H 7, 266 (ε 9600) and 224 (ε 26,500) at *p*H 13; ir (1800-1300 cm^{-1} region): 1730s, 1695s, 1675s, 1630, 1605, 1585, 1495, 1470, 1455, 1395, 1380, 1360, 1325, 1320, 1315; ms (direct-probe temperature, 110°): 434 (M), 312 (M − C_6H_5COOH), 207 (M − C_6H_5COOH − C_6H_5CO), 190 (M − $2C_6H_5COOH$), 134 (uracilyl + C_2H_4), 113 (uracilyl + 2H), 112 (uracilyl + H).

Anal. Calcd. for $C_{24}H_{22}N_2O_6$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.55; H, 5.32; N, 6.37.

(±)-4-Amino-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2-(1*H*)pyrimidinone (VIc).

Dibenzoate IVc (2.7 g.) was converted to the crude 4-chloro-2-(1*H*)-pyrimidinone derivative (Vc) by the procedure described for the preparation of crude Vb. The mass spectrum of the crude product showed that it contained some unreacted IVc and included the following peaks derived from Vc (direct-probe temperature, 150°): 452 (M), 347 (M − C_6H_5CO), 330 (M − C_6H_5COOH), 225 (M − C_6H_5COOH − C_6H_5CO), 208 (M − $2C_6H_5COOH$), 131 (chloropyrimidinone moiety + 2*H*). Crude Vc was converted to the carbocyclic analog (VIc) of 2′-deoxycytidine by the procedure described for the preparation of VIb. The aqueous layer obtained after extraction of benzamide with ethyl acetate was concentrated to dryness under reduced pressure. The residual solid was chromatographed on a column of a sulfonic acid ion-exchange resin (16) in the pyridine salt form to separate the carbocyclic analog (VIIc) of *N,N*-dimethyl-2′-deoxycytidine. The column was eluted with 0.1*M* pyridinium formate solution, and the elution was followed with an ultraviolet monitor during the collection of 130 fractions of eluate of approximately 25 ml. Fractions 75-124 (selected by ultraviolet monitoring and tlc of the effluent) were combined and concentrated to a residual oil, which was placed in a high vacuum at 50° to remove additional volatile material. Methanol (three 10-ml. portions) was added to and evaporated from the solid product, and the resulting solid was then triturated with acetonitrile, collected by filtration, and dried; weight, 998 mg. Since the ^{1}H-nmr and ms of this material indicated that it contained a formyl derivative (23), it was boiled under reflux in 15*N* ammonia (50 ml.) for 1.5 hours, and the solution was then concentrated under reduced pressure to a white solid (859 mg.). A water solution of 850 mg. of the residual solid was poured onto a column of a quaternary ammonium ion-exchange resin, hydroxide form (24), to remove formate, and the column was eluted further with water. Concentration of the eluate to dryness under reduced pressure furnished a white solid residue (VIc) that was dried further under reduced pressure at 56° for 2 hours and at 110° overnight, weight, 725 mg. (52% yield), m.p. 215-217°; tlc, 1 spot (60 mcg., 8:1:1 ethanol-water-15*N* ammonia); uv max: 284 (ε 13,800) and 214 (ε 10,400) at *p*H 1, 275 (ε 9500) and 225 (sh.) at *p*H 7 and at *p*H 13; ir (1800-1300 cm^{-1} region): 1645s, 1630sh., 1585, 1570, 1520, 1480sh., 1470s, 1395, 1360, 1300; ms (direct-probe temperature, 100°): m/e 225 (M), 208 (M − OH), 194 (M − CH_2OH), 178, 176, 151, 138 (cytosinyl group + C_2H_4), 112 (cytosinyl group + 2H); ^{1}H-nmr: δ 1.1-2.3 (m, 5 protons, CH_2 at *e* and *b*, CH at *d*), 3.2-3.6 (m, CH_2 at *f*), 3.85-4.1 (m, CH at *c*), 4.44-4.74 (m, OH at *f* and *c*), 4.78-5.2 (m, CH at *a*), 5.7 (center of d, position 5 of pyrimidine ring), 6.94 (NH_2), 7.6 (center of d, position 6 of the pyrimidine ring).

Anal. Calcd. for $C_{10}H_{15}N_3O_3$: C, 53.33; H, 6.71; N, 18.66. Found: C, 53.24; H, 6.40; N, 18.87.

(±)-4-(Dimethylamino)-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2(1*H*)pyrimidinone (VIIc).

Fractions 50-59 obtained from the column of sulfonic acid ion-exchange resin (16) described under VIc were combined and concentrated under reduced pressure to a white solid consisting of pyridinium formate and VIIc; ms (direct-probe temperature, 20°): m/e 253 (M of VIIc), 236 (M − OH), 222 (M − CH_2OH), 166 (dimethylcytosinyl + C_2H_4), 140 (dimethylcytosinyl + 2H). Compound VIIc moved slightly ahead of VIc during tlc in 8:1:1 ethanol-water-15*N* ammonia.

Fractions 25-46 obtained from the same column were also combined and concentrated to dryness. The residual solid was shown by tlc and ms to be predominantly, but not entirely, VIIc.

Carbocyclic Analog (VIIIb) of 2,2'-Anhydro-3'-deoxycytidine.

Thionyl chloride (0.1 ml.) was added to redistilled dimethylformamide (1.0 ml.), and the solution (protected from atmospheric moisture) was stirred at room temperature for 0.5 hour after it cooled to room temperature. Compound VIb (100 mg.) was then added to this solution; and, after the exothermic reaction had subsided, the reaction solution was stirred at room temperature for 3 hours. Water (2.5 ml.) was added, the resulting solution was sparged with nitrogen to remove dissolved sulfur dioxide, and the solution was concentrated under reduced pressure to a gummy residue. After several portions of water had been added to and evaporated from the residue, it was chromatographed in 0.1*M* pyridinium formate on a column of a sulfonic acid ion-exchange resin (16) in the pyridine salt form. Elution of the column with 0.1*M* pyridinium formate furnished eluate portions (which were located by ultraviolet monitoring of the effluent) containing VIIIb. These fractions were combined and lyophilized. The residue was azeotroped with methanol, slurried with acetonitrile, concentrated, and dried *in vacuo* at 56°; yield, 58 mg. (63%). Part of this material was hydrolyzed to IXb. The remainder was redissolved in water, the solution was filtered and concentrated to dryness *in vacuo*, and the residue was dried further by evaporating acetonitrile from it and by storing it *in vacuo* at 56° for 3 hours, m.p. 151-154° dec.; ms (direct-probe temperature, 20°): m/e 207 (M), 176 (M - CH_2OH), 156, 139, 112, 111; ir (1800-1300 cm^{-1} region): 1655s, 1590s, 1495s, 1440, 1415, 1370, 1350, 1340, 1310sh.; ^{1}H-nmr: δ 1.6-2.5 (m, 5 protons, CH_2 at *e* and *c*, CH at *d*), 3.1-3.4 (m, CH_2 at *f*), 4.9-5.2 and 5.4-5.7 (m, CH at *a* and *b*), 6.68 (center of doublet, H at position 5 of the pyrimidine ring), 8.21 (center of doublet, H at position 6 of the pyrimidine ring), 8.55 (NH). This material (the formate salt) moved much more slowly (R_f *ca.* 0.5) than VIb and IXb during tlc in 2-propanol-1*M* ammonium acetate (7:3).

Anal. Calcd. for $C_{10}H_{13}N_3O_3 \cdot 1.25$ HCOOH: C, 51.03; H, 5.90; N, 15.87. Found: C, 51.18; H, 5.86; N, 16.52.

(±)-4-Amino-1-[(1α,2α,4α)-2-hydroxy-4-(hydroxymethyl)cyclopentyl]-2-(1*H*)pyrimidinone (IXb).

Compound IXb was obtained by heating VIIIb with 1*N* aqueous ammonia at 80° for 5 minutes and evaporating the volatile material *in vacuo*; ms (direct-probe temperature, 20°): m/e 225 (M), 208 (M − OH), 207 (M − H_2O), 196, 176, 166, 138 (cytosinyl group + C_2H_4), 112 (cytosinyl group + 2H). Compound IXb moved slightly ahead of VIb during tlc in 2-propanol-1*M* ammonium acetate (7:3). Retention times of IXb and VIb during reverse-phase high-pressure liquid chromatography (25) in 98:2 acetonitrile-water (flow rate 1 ml./min.) were 10.9 min. and 8.8 min., respectively.

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REFERENCES AND NOTES

(1) S. S. Cohen in "Progress in Nucleic Acid Research and Molecular Biology", Vol. 5, J. N. Davidson and W. E. Cohn, Eds., Academic Press, New York, N.Y., 1966.

(2) W. A. Creasey in "Antineoplastic and Immunosuppressive Agents", Part II, A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, New York, N.Y., 1975, Chapter 42.

(3) F. Maley in "Cancer", Vol. 5, F. F. Becker, Editor, Plenum Press, New York, N.Y., 1977, Chapter 12.

(4) C. Heidelberger in "Cancer Medicine", J. F. Holland and E. Frei, III, Eds., Lea and Febiger, Philadelphia, Pa., 1973, Chapter XIII-3.

(5) F. M. Schabel, Jr., and J. A. Montgomery in "Chemotherapy of Virus Diseases. The International Encyclopedia of Pharmacology and Therapeutics", Vol. 1, D. J. Bauer, Ed., Pergamon Press, New York, N.Y., 1972, Chapter 4.

(6) W. H. Prusoff and D. C. Ward, *Biochem. Pharmacol.*, **25**, 1233 (1976).

(7) Y. F. Shealy and J. D. Clayton, *J. Am. Chem. Soc.*, **88**, 3885 (1966); *ibid.*, **91**, 3075 (1969).

(8) Y. F. Shealy and C. A. O'Dell, *Tetrahedron Letters*, 2231 (1969).

(9) Y. F. Shealy and J. D. Clayton, *J. Pharm. Sci.*, **62**, 1432 (1973).

(10) Y. F. Shealy, J. D. Clayton, and C. A. O'Dell, *J. Heterocyclic Chem.*, **10**, 601 (1973).

(11) Y. F. Shealy and C. A. O'Dell, *ibid.*, **13**, 1353 (1976).

(12) Y. F. Shealy and C. A. O'Dell, *J. Pharm. Sci.*, **68**, 668 (1979).

(13) Y. F. Shealy and C. A. O'Dell, *J. Heterocyclic Chem.*, **13**, 1015 (1976).

(14) J. Žemlička and F. Šorm, *Collect, Czech, Chem. Commun.*, **30**, 2052 (1965).

(15) Amberlite CG-120, polystrene-sulfonic acid resin, chromatographic grade (Mallinckrodt, Inc., St. Louis, Missouri).

(16) AG-50W-X4, polystyrene-sulfonic acid resin, analytical grade (Bio-Rad Laboratories, Richmond, Calif.).

(17) K. Kikugawa and M. Ichino, *Tetrahedron Letters*, 867 (1970).

(18) There was evidence of the formation of a formyl derivative of VIc during the removal of the by-product VIIc by chromatography in pyridinium formate. Treatment of VIc containing some of the formyl derivative with refluxing aqueous ammonia afforded pure VIc.

(19) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemotherapy Rept., Part 3*, **3**, 1 (1972).

(20) R. W. Brockman, J. W. Carpenter, C. A. O'Dell, Y. F. Shealy, and L. L. Bennett, Jr., *Fed Proc.*, **37**, 234 (1978).

(21) W. M. Shannon and co-workers, unpublished results.

(22) Silica Gel GF precoated thin-layer chromatography plates (fluorescent), 250 microns in thickness, were purchased from Analtech Inc., Blue Hen Industrial Park, Newark, Delaware 19711.

(23) Both VIIc and a formyl derivative of VIc have a molecular ion at m/e 253; however, the ms of VIIc also includes strong peaks at m/e 166 (dimethylcytosinyl + C_2H_4) and 140 (dimethylcytosinyl + 2H) which distinguish VIIc from VIc.

(24) Rexyn 201 (OH), Research Grade (Fisher Scientific Co., Fair Lawn, N.J.).

(25) High pressure liquid chromatography on μBondapak C_{18} (octadecylsilane chemically bonded to porous silica), 10 micron particle size, column 3.9 mm × 30 cm (Waters Associates, Maple Street, Milford, Mass.).