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CHEMISTRY OF ANTITUMOR TRIAZINE NUCLEOSIDES. AN IMPROVED SYNTHESIS OF DIHYDRO-5-AZACYTIDINE.

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5-Azacytidine[3] (7, aza-C) is a nitrogen bioisostere of cytidine which has been used effectively for the clinical treatment of leukemia[4]. The severe gastrointestinal toxicity of 7 is best minimized by prolonged continuous infusion,[5,6] but working in opposition to this mode of administration is the instability of aza-C in aqueous formulations[4] which results in a continuously decreasing aza-C concentration and the production of increasing concentrations of hydrolysis products of unknown biological effects. A reduced analog, 5,6-dihydro-5-azacytidine hydrochloride (6, DHaza-C), was

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recently synthesized by reduction of aza-C with sodium borohydride with the goal of eliminating the solution instability while retaining the antitumor activity of the parent drug[7]. Relative to the parent drug, DHaza-C proved to have a greatly enhanced aqueous stability over a broad pH range. DHaza-C also possessed substantial antitumor activity in mouse leukemia test systems.[7,8,9] In order to ensure sufficient quantities of DHaza-C to initiate preclinical pharmacology studies, it was necessary to develop a more direct, economical synthesis. Accordingly, an improved synthesis of DHaza-C is described in this report, and in addition, the results of chemical investigations directed toward reactions at the critical 6-position of the aza-C triazine ring, the vulnerable locus for hydrolytic attack, are presented.

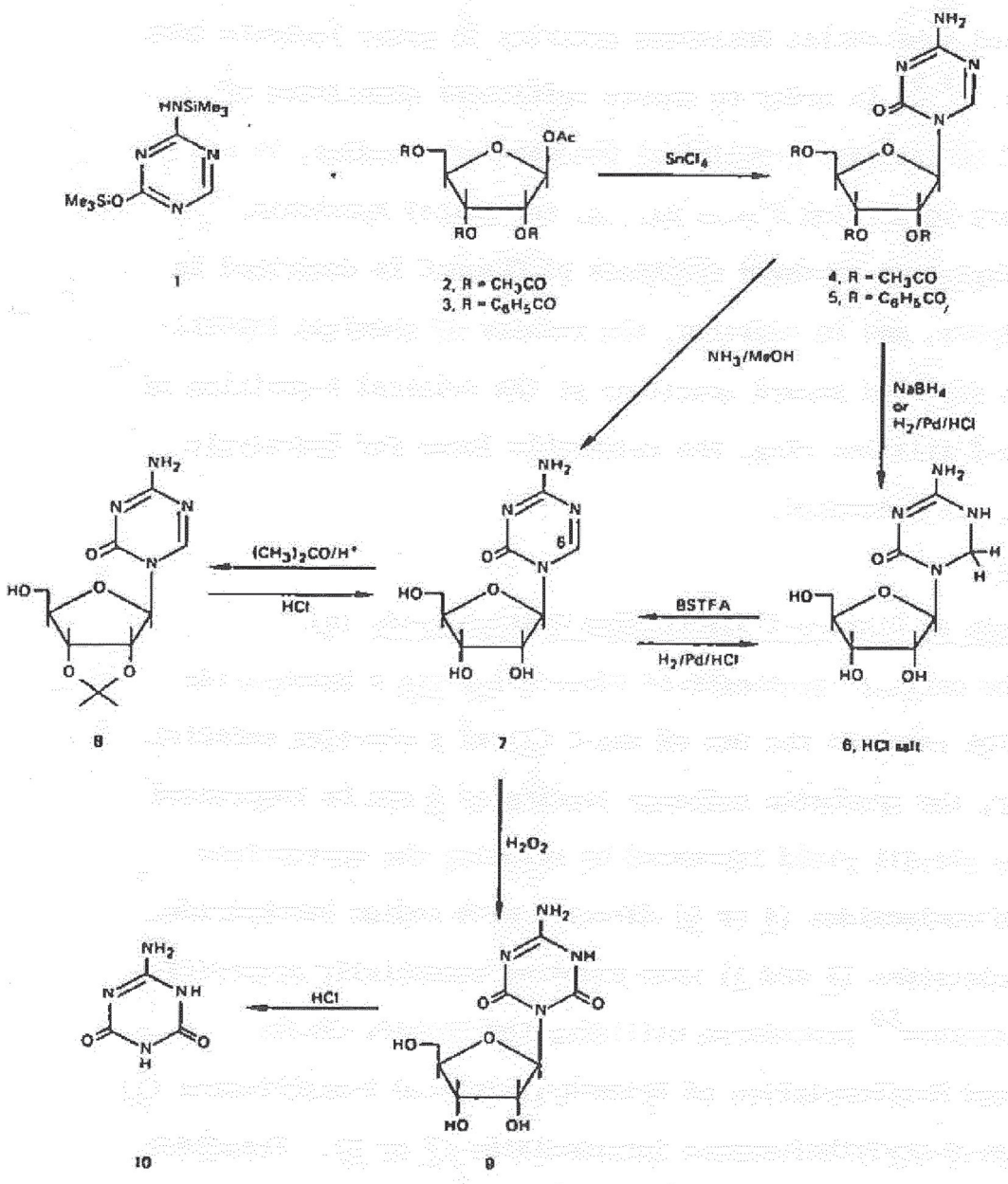
RESULTS

Synthesis of Dihydro-2-azacytidine Hydrochloride (6).

The earlier[9] synthesis of DHaza-C (6) *via* a borohydride reduction required the use of aza-C (7) as a starting material. However, the synthetic sequence leading to 6 can be compressed and the overall yield increased by reducing the appropriate blocked nucleosides (4 or 5) directly with sodium borohydride. The nucleosides (4 and 5) were prepared essentially according to literature[10] procedures utilizing the Friedel-Crafts catalyzed N-ribosylation of trimethylsilylated 5-azacytosine (1) with per-O-acylribofuranose intermediates (2 or 3). Treatment

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of the tri-O-benzoyl nucleoside (5) with sodium borohydride in hexamethylphosphoramide (HMPA) solution led to the reduction of the imine linkage accompanied by the removal of the benzoyl



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protecting groups to provide the reduced and deblocked nucleoside in the form of a boron complex. Without further purification the boron-containing product was hydrolyzed with aqueous acid[9] to give 6 in 72% yield (58% overall yield based on 3). Similarly, the tri-O-acetyl nucleoside (4) was reduced with borohydride to afford an almost identical (73%) yield of 6 (50% overall yield based on 2). Therefore, of the borohydride reductions the former sequence is the method of choice.

It is also possible to reduce and deblock the tri-O-acetyl derivative (4) by catalytic hydrogenation in ethanol under acidic conditions to give 6 in 92% yield (63% overall yield based on 2). However, similar hydrogenation of the tri-O-benzoyl derivative (5) resulted in reduction of the imino group, but the reduction unfortunately was not also accompanied by ethanolysis of the blocking groups.

We have found that the hydrolysis of 5 with methanolic ammonia[11] to give 7 proceeds in 62% yield. Since we demonstrated earlier[9] that 7 can be converted to 6 in 72% yield using a borohydride procedure, a 36% overall yield of 6 based on 3 can be realized with our original synthesis requiring three synthetic steps (3 → 5 → 7 → 6). Alternatively, the reduction of 7 by hydrogenation in ethanol-hydrochloric acid gave 6 in a similar (39%) overall yield based on 3. The present synthesis of DHaza-C requires two steps using the borohydride method (3 → 5 → 6) or the hydrogenation method (2 → 4 → 6) with similar overall yields of 58% and 63%, respectively.

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The Oxidation of 5-Azacytidine (7) to the Oxo-analog (9).

As part of our effort to synthesize hydrolytically stable analogs of 7, we also investigated the conversion of 7 to a derivative having a higher oxidation state at the 6-position than aza-C. It was found that 7 was susceptible to selective oxidation when treated with 30% hydrogen peroxide in acetic acid solution to give the high-melting oxo-5-azacytidine (9). The NMR spectrum of 9 lacked the singlet due to the C-6 aromatic proton characteristic of 7 and the uv spectrum differed considerably from that of 7. The mass spectra of both the trimethylsilylated (TMS) and trifluoroacetylated (TFA) derivatives of 9 were consistent with the addition of one oxygen atom to the triazine ring of 7. Acid hydrolysis of the glycosidic bond of 9 gave ammelide (10) which was identified by elemental analysis and the mass spectrum of its TMS derivative. Although 9, which has the novelty of a higher degree of symmetry in the triazine moiety than 7, is stable in aqueous solution in accord with our expectations, it is devoid of antitumor activity in the L1210 leukemia assay[12] wherein aza-C exhibits high activity.

Since it has been postulated[8,9] that DHaza-C is a pro-drug form[14] of aza-C, it was of interest to investigate the chemical conversion of DHaza-C (6) to aza-C (7) in order to probe the likelihood of this conversion occurring as a result of in vivo metabolism. An acetic acid solution of 6 when treated with hydrogen peroxide at room temperature contained 7 after three days as shown by TLC. At the end of twelve days, the oxidized

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nucleoside (9) was also detectable in the reaction solution suggesting a sequential oxidation process: 6 → 7 → 9. After fifteen days, the reaction was worked-up and the products were derivatized with trifluoroacetic anhydride for MS analysis. Probe introduction of the TFA mixture into the spectrometer gave a composite spectrum of 6 · TFA_4, 7 · TFA_4, 9 · TFA_4 and 10 · TFA. When a few crystals of ferrous sulphate were added as a catalyst to the oxidation system, the reaction rate increased considerably but only 9 and 10 were found (TLC, MS) as products, with 10 as the major product. As well as lending support to the pro-drug hypothesis of 6, these experiments suggest the possibility that the oxidation of 7 to 9 (or 6 to 9) could serve as a detoxification mechanism *in vivo* although 9 has not been identified and reported as a metabolite of 7.

The Dehydrogenation of 6 to Give 5-Azacytidine (7).

Treatment of 6 with bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) in acetonitrile solution at room temperature led smoothly to the introduction of five TMS groups into the nucleoside. The TMS_5 derivative gave a single peak in the GC, which produced a mass spectrum consistent with the proposed structure upon subsequent GC-MS analysis[9]. However, if the silylation solution was heated, a second peak began to appear in the chromatogram due to tetrakis - (trimethylsilyl)-5-azacytidine (7 · TMS_4). This material was identified by MS and was identical to the product of 7 with this silylation reagent. Continued heating ultimately resulted in a complete conversion of 6 · TMS_5 into

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7 · TMS_4 with the formal loss of the elements of $(CH_3)_3SiH$. This unexpected event seemed sufficiently unusual to warrant further investigation to determine its synthetic potential for the preparation of triazine nucleosides which might otherwise be inaccessible and thereby further extend the use of the trialkylsilyl function in synthetic organic chemistry. Therefore, the reaction of 6 with the silylation mixture was repeated on a preparative scale and its progress monitored with GC. When the reaction was complete, the product, 7 · TMS_4, was de-trimethylsilylated by methanolysis to give a 76% yield of 7. The aza-C synthesized in this way was identical to an authentic sample[15]. Applying the silylation reaction to 6 containing a deuterium atom at the 6-position[9] of the triazine resulted in a 2.9:1 mixture (GC-MS) of 6-deutero-5-azacytidine-TMS_4 and 7 · TMS_4 apparently due to a primary deuterium isotope effect. Our earlier[9] positional assignment for the deuterium atom based on NMR spectral characteristics is, therefore, confirmed. Additional synthetic work making use of the silylation-dehydrogenation reaction to prepare nucleosides of biological interest will be reported at a later date.

Preparation of the 2',3'-O-isopropylidene derivative of 7 obtained from the silylation-dehydrogenation procedure was accomplished in acetone solution containing 2,2-dimethyoxypropane and catalyzed by perchloric acid[16]. A good yield of 8 was obtained which was identical in melting point and spectral properties with the isopropylidene derivative prepared in the

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same way from authentic[15] 7. The difference in chemical shift ($\Delta\delta$) of the pair of singlets due to the isopropylidene methyl groups of 8 was observed to be 20 Hz. Since it has been demonstrated that a $\Delta\delta$ > 15 Hz is proof of the β-configuration of the aglycone at C_1, carbon of ribofuranosyl nucleosides[17], spectral confirmation is now at hand for the β-configuration originally assigned[18] to aza-C as well as all of the nucleoside derivatives synthesized during the course of the present investigation.

DISCUSSION

With the synthesis of 9 we have added a third member in a series of triazine nucleosides differing only in the oxidation state of a carbon atom in the heterocyclic moiety. Thus, DHaza-C (6) represents the lowest oxidation state, aza-C (7) is intermediate, and 9 is at the highest oxidation state in the series. In a structure-activity relationship context it is interesting to note that the intermediate oxidation state, 7, has the greatest antitumor potency against murine L1210 leukemia, the lowest oxidation state, 6, has good activity but at a lesser potency, and 9, the highest oxidation state, is completely inactive in the L1210 leukemia test system.

EXPERIMENTAL

Electron impact mass spectra were obtained on a GC-MS system consisting of a Varian Aerograph 2740 gas chromatograph coupled to a DuPont 21-492 mass spectrometer by a glass transfer line and single-stage jet separator; operating conditions were as

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previously described[9]. Gas chromatographic separation of mixtures and determination of isothermal retention indices (IRI)[19] were accomplished on a 1.83m x 2mm i.d. glass column packed with 3% SE-30 on 100/120 mesh Gas-Chrom Q and operated at 220°. Nucleosides and their aglycones were derivatized for GC or MS analysis by either per-trifluoroacetylation[9,20] (direct probe MS) or by per-trimethylsilylation[9] (GC-MS) by using previously reported procedures unless otherwise indicated. Proton NMR spectra were recorded with a Varian T-60 or a Varian HA-100D spectrometer using tetramethylsilane as an internal standard. A Cary Model 15 spectrophotometer was used to obtain UV spectra and a Perkin-Elmer Model 621 was used to record infrared spectra. Optical rotations were measured in a 1-dm cell with a Perkin-Elmer Model 141 polarimeter. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation, NIAMDD, NIH and by Galbraith Laboratories, Inc., Knoxville, Tenn. Compound purity was routinely checked by TLC using 5 x 20 cm plates coated with Baker 1B2-F silica gel. Four solvent systems were employed: butanol-ethanol-water (40:11:19), butanol-acetic acid-water (5:2:3), isopropanol-ammonia-water (7:1:2), isobutyric acid-ammonia-water (66:33:1.5). Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. 5-Azacytosine, 2 and 3 were purchased from the Aldrich Chemical Co., Milwaukee, Wisc. and were used without further purification.

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4-Amino-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)1,3,5-triazin-2(1H)-one (4). With some modifications, which led to an improved yield, the procedure followed was that described by Niedballa and Vorbruggen[10]. To a solution of 1[21] (21.4 mmol) and 2 (5.0 g, 15.7 mmol) in dry acetonitrile (150 ml), cooled to 0°, was added a solution of stannic chloride (3.2 ml, 27.8 mmol) in acetonitrile (80 ml) under anhydrous conditions. The solution temperature was slowly allowed to increase from 0° to 22° over 2 h, then maintained at 22° for 30 min. After work-up[10], concluded by treatment with charcoal and crystallization from ethyl acetate (75 ml), 3.92 g (68%) of 4 was obtained mp 160-161° (lit.[10] mp 160-161°). Using 1,2-dichloroethane as the reaction solvent gave identical results.

4-Amino-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,3,5-triazin-2(1H)-one (5). The condensation of 1 and 3 according to the procedure of Niedballa and Vorbruggen[10] gave the O-benzoylated 5-azacytidine (5) in 81% yield, mp 184-186° (lit.[10] mp 186-187°).

4-Amino-5,6-dihydro-1-β-D-ribofuranosyl-1,3,5-triazin-2(1H)-one Hydrochloride (6). Method A. Borohydride Reduction of Compound 4. A solution of 4 (1.11 g, 3.0 mmol) in 7 ml HMPA was stirred while 300 mg (7.8 mmol) of sodium borohydride was added. The reaction mixture was heated at 50° for 1 h then left at room temperature for 3 h. Excess borohydride was decomposed with water (10 ml) and methanol (10 ml) and the solvent was removed under reduced pressure (bath 30°) after standing

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for 1 h at room temperature. The resulting syrup was triturated successively with ether (50 ml) and acetone (50 ml) to give a white solid which was washed thoroughly with ether and dried. After stirring the solid for 4 h at room temperature with 10 ml 6N hydrochloric acid, the solution was concentrated under vacuum to one third its initial volume and diluted with 50 ml of absolute ethanol. The precipitated inorganic materials were removed by filtration and the filtrate stored overnight at 0° from which 0.48 g of 6 was obtained as white crystals, mp 180-181° dec. The mother liquor, when evaporated and the residue crystallized from methanol-ethanol (1:1), provided an additional 0.14 g of 6 (total yield, 73%), mp 180-181° dec; $[\alpha]^{26}_{D}$ -29° (c 1.0, H_2O); lit.[9] mp 180-181° dec, $[\alpha]^{29}_{D}$ -29° (c 1.0, H_2O).

Method B. Borohydride Reduction of Compound 5. A solution of 5 (557 mg, 1.0 mmol) in 3 ml HMPA was stirred and treated with sodium borohydride (120 mg, 3.1 mmol). The reaction mixture was heated at 50° for 6 h then cooled to room temperature and combined with water (10 ml) and methanol (10 ml). The reaction was worked-up as described in Method A to give 202 mg (72%) of 6, mp 180-181° dec; $[\alpha]^{26}_{D}$ -29.0° (c 1.0, H_2O). The NMR spectrum was identical to that of an authentic[9] sample and the mmp was undepressed.

Method C. Hydrogenation of Compound 4. A solution of 4 (1.0 g, 2.7 mmol) in 160 ml absolute ethanol containing 10 ml of ethanolic hydrogen chloride (saturated at 0°) was slurried with 1.0 g of 10% palladium on charcoal catalyst and hydrogenated

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at 50 psi for 16 h. After removing the catalyst the reaction solution was evaporated under vacuum. The resulting syrup, when triturated with ether, gave a solid which was recrystallized from ethanol to give 0.7 g (92%) of 6, mp 180-181° dec; $[\alpha]^{26}_{D}$ -29° (c 1.0, H_2O). The product was identical with an authentic sample[9] in mp, mmp, NMR and UV.

Method D. Hydrogenation of Compound 7 (aza-C). A solution of 7 (5.0 g, 20.4 mmol) in a mixture of 6N hydrochloric acid (50 ml) and ethanol (50 ml) was combined with 5.0 g of 10% palladium on charcoal and hydrogenated at an initial pressure of 50 psi for 8 h. The catalyst was removed by filtration through a Celite pad which was subsequently washed with ethanol. The filtrate and washings were evaporated under vacuum (30°) and the residue was crystallized from methanol-ethanol (1:1) to give 4.43 g (77%) of 6, mp and mmp 180-181° dec; $[\alpha]^{26}_{D}$ -28° (c 1.0, H_2O). NMR and UV spectra were identical to an authentic sample[9] of 6.

Hydrolysis of 5. 4-Amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1H)-one (7). At room temperature 3 ml of methanolic ammonia (saturated at 0°) was added to 557 mg (1.0 mmol) of 5 giving a complete solution from which crystals began to deposit after a few minutes. After maintaining the temperature at 21° for 90 min in a stoppered flask the solution was stored at -16° overnight. The crystals of 7 (132 mg, mp 230-232° dec) were collected by filtration and washed with methanol. Concentration

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of the mother liquor gave a further 18 mg bringing the total yield to 62%. The mixture melting point of 7 with an authentic[15] sample of 5-azacytidine showed no depression and the NMR spectra were superimposable.

6-Amino-3-β-D-ribofuranosyl-1,3,5-triazine-2,4(1H,3H)-dione (9). A mixture of 30% hydrogen peroxide (80 ml) and glacial acetic acid (80 ml) was stirred with 7 (4.88 g, 20 mmol) for 8 days at 25°. The reaction mixture was concentrated to about one third under vacuum (bath 30-35°), diluted with water (50 ml), and the resulting precipitate removed by filtration. The precipitate was washed with water and methanol and dried to give 2.60 g (50%) of 9 which was recrystallized from water, mp > 360°; UV λ_{max} (H_2O) 221 nm (ε 16300); NMR (D_2O exchanged, Me_2SO-d_6) δ 5.94 (d, J = 4Hz, 1, C_1'H), 4.57-3.30 (m, 5, ribosyl protons); MS (tetrakis - trifluoroacetyl derivative) m/e (rel intensity) 625 (M-F, 0.3), 575 (M-CF_3, 1.1), 530 (M-CF_3CO_2H, 2.7), 461 (M-TFAOH-CF_3,43), 208 (8.9), 155 (8.9), 112 (8.7), 69 (100); MS (pentakis - trimethylsilyl derivative) m/e (rel intensity) 605 (M-CH_3, 1.2), 403 (4.1), 348 (10), 345 (4.5), 344 (4.4), 273 (5.4), 272 (3.1), 245 (29), 217 (21), 73 (100).

Anal. Calcd for $C_8H_{12}N_4O_6$ (260.2): C, 36.92; H, 4.64; N, 21.53. Found: C, 36.66; H, 4.38; N, 21.73.

6-Amino-1,3,5-triazine-2,4(1H,3H)-dione (10). A solution of 9 (600 mg, 2.3 mmol) in 6N hydrochloric acid (15 ml) was heated on a steam bath for 3 h, cooled, and the dark precipitate removed by filtration and washed thoroughly with hot water. The

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filtrate and washings were combined, decolorized with charcoal, and concentrated under vacuum (bath 35-40°) to yield 60 mg (20%) of ammelide (10) as a white powder. The product in 1N ammonium hydroxide (10 ml) solution was treated with 1N hydrochloric acid to pH 7 which gave analytically pure 10, mp > 360°; GC-MS (tris - trimethylsilyl derivative, IRI 1730) m/e (rel intensity) 344 (M^+, 53), 329 ($M-CH_3$, 23), 171 (38), 100 (17), 73 (100).

Anal. Calcd for $C_3H_4N_4O_2$ (128.1): C, 28.13; H, 3.15; N, 43.72. Found: C, 28.44; H, 3.18; N, 43.63.

The Peroxide Oxidation of 6. Method A. Without Catalyst. A solution of 283 mg (1.0 mmol) of 6 in a mixture of 30% hydrogen peroxide (4 ml) and glacial acetic acid (4 ml) was stirred at room temperature. At daily intervals samples were removed, spotted without work-up on TLC plates, and analyzed using four different solvent systems. After 3 days the reaction solution contained 7 as well as starting material, and after 12 days the 2-oxo nucleoside (9) was evident as a third spot. Spot identifications were made by comparison with authentic samples run on the same plate. After a total of 15 days the white precipitate (10 mg) which had accumulated was removed by filtration, washed with water, and dried, mp > 300°. Mass spectroscopic analysis (direct probe) of the product following trifluoroacetyl derivatization showed the product to be a mixture of 6 · TFA_4 (M^+, m/e 630), 7 · TFA_4 (trace, M-19, m/e 609), 9 · TFA_4 (M-19, m/e 625) and 10 · TFA (M^+, m/e 224).

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Method B. Ferrous Catalyst. To a stirred solution of 400 mg (1.4 mmol) of 6 in 2 ml water containing a few crystals of ferrous sulphate was added 2 ml of 30% hydrogen peroxide dropwise at 10°. After the addition the reaction solution was maintained at 10° for 10 min then allowed to come to room temperature for 2 h. The white precipitate (81 mg, mp > 300°) which had separated was removed by filtration and washed with water. MS analysis (direct probe) of the precipitate following treatment with trifluoroacetic anhydride indicated 10 · TFA contaminated with some 9 · TFA_4. TLC analysis of the filtrate from the reaction solution showed the absence of 6 and 7.

4-Amino-1-[2,3-O-(1-methylethylidine)-β-D-ribofuranosyl]-1,3,5-triazin-2(1H)-one (8). The procedure of Zderic et al.[16] was used to convert 244 mg (1.0 mmol) of 7 (obtained via the dehydrogenation reaction described below) to 240 mg (84%) of the isopropylidene derivative (8), mp 279-280°. Recrystallization from dimethylformamide gave 180 mg, mp 279-280°; IR (Nujol) 3410, 3285, 3090, 1688, 1609, 1110, 854, 804 cm^{-1}; NMR (Me_2SO-d_6) δ 8.39 (s, 1, C_6H), 7.56 (broad s, 2, NH_2), 5.72 (d, J=2Hz, 1, C_1,H), 1.37 (d, J=20Hz, 6, Me_2C); GC-MS (bis-trimethylsilyl derivative, IRI 2580) m/e (rel intensity) 428 (M^+, 1.4), 413 (M-CH_3, 6.5), 370 (M-Me_2CO), 1.5), 256 (3.6), 185 (22), 103 (24), 100 (91), 73 (100).

Anal. Calcd for $C_{11}H_{16}N_4O_5$ (284.3): C, 46.47; H, 5.67; N, 19.71. Found: C, 46.66; H, 5.68; N, 19.76.

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An authentic sample[15] of 7 treated similarly gave an isopropylidene derivative with identical spectral properties, mp and mmp as the compound described above.

5-Azacytidine hydrochloride (7 · HCl) was obtained from 8 (100 mg) on treatment with 6N hydrochloric acid (2 ml) at 25° for 4 h. Addition of absolute ethanol (5 ml) to the reaction solution caused the crystallization of 7 · HCl (95 mg, mp 179-181° dec) which was recrystallized from ethanol-benzene, mp 180-181°dec.

Anal. Calcd for $C_8H_{12}N_4O_5 \cdot HCl$ (280.7): C, 34.23; H, 4.67; N, 19.96; Cl, 12.63. Found: C, 33.88; H, 4.89; N, 19.58; Cl, 12.39.

A sample of 7 · HCl prepared from authentic 7 gave the same UV, NMR, mp and mmp as the material described above.

The Dehydrogenation of 6: 4-Amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1H)-one (7). A mixture of BSTFA (17 ml) and dry acetonitrile (30 ml) was refluxed gently with 566 mg (2 mmol) of 6 under anhydrous conditions. As the reaction proceeded the single peak in the gas chromatogram due to 6 · TMS_5 (IRI 2465) was accompanied by increasing concentrations of a second compound with a longer retention time. By GC-MS analysis it was shown to be 7 · TMS_4 (IRI 2620). After refluxing 18 h, the latter compound was the only peak in the chromatogram indicating a complete conversion had occurred. The solvent was removed under vacuum and the syrupy residue was taken up in 100 ml of absolute

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methanol. Removal of the trimethylsilyl groups from the desired product was effected by slow co-distillation of volatile silicon-containing materials with methanol. The methanol volume was renewed twice over the course of the distillation (~3 h) during which time the boiling point increased from 51° to 65° and crystals began to separate from the boiling solution. Crystallization was allowed to continue at room temperature overnight to give 370 mg (76%) of 7, mp 232-234° dec (lit.[18] mp 228-230° dec); $[\alpha]^{26}D$ + 24.4° (c 1.0, H_2O) (lit.[21] $[\alpha]^{26}D$ + 26.6° (c 1.0, H_2O). Mixture melting point with an authentic[15] sample of 7 showed no depression and the NMR and UV spectra were superimposable.

The free base of 6 could also be dehydrogenated to give 7 using the identical reaction conditions described above for the salt.

SUMMARY

Dihydro-5-azacytidine (6) is a hydrolysis-resistant, biologically-active analog of the clinical antitumor agent, 5-azacytidine (7). In order to facilitate the acquisition of sufficient quantities of 6 for preclinical pharmacology studies, a shorter synthesis of 6 was devised giving a substantial improvement in overall yield. As part of a chemical investigation of the reactive 6-position of 7 (*i*) an analog of 7 bearing an oxygen atom at C-6 (9) was synthesized; (*ii*) the peroxide oxidation of 6 was studied and the results related to possible metabolic transformations of 6 and 7; (*iii*) via a per-

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trimethylsilylated derivative, 6 was converted to 7 utilizing a novel thermal elimination reaction. From an NMR analysis of an isopropylidene derivative (8) the β-configuration for 7, and all the nucleoside derivatives described in this report, was confirmed.

REFERENCES

1. Address correspondence to this author at the National Institutes of Health, Building 37, Room 6D19, Bethesda, Maryland 20014.

2. NIH Visiting Postdoctoral Fellow from the National Research Center, Dokki, Cairo, Egypt.

3. J. Skoda in "Antineoplastic and Immunosuppressive Agents", Part II, A. C. Sartorelli and D. G. Johns, Ed., Springer-Verlag, Berlin, 1975, p. 348.

4. D. D. VonHoff, M. Slavik and F. M. Muggia, Ann. Intern. Med., 85, 237 (1976).

5. P. L. Lomen, L. H. Baker, G. L. Neil and M. K. Samson, Cancer Chemother. Rep., 59, 1123 (1975).

6. A. H. Israili, W. R. Vogler, E. S. Mingioli, J. L. Pirkle, R. W. Smithwick and J. H. Goldstein, Cancer Res., 36, 1453 (1976).

7. J. A. Beisler, M. M. Abbasi, J. S. Driscoll and J. A. Kelley, Abstracts (MEDI 72), The 172nd National Meeting of the American Chemical Society, San Francisco, Calif., August 1976.

8. J. A. Beisler, M. M. Abbasi and J. S. Driscoll, Cancer Treat. Rep., 60, 1671 (1976).

9. J. A. Beisler, M. M. Abbasi, J. A. Kelley and J. S. Driscoll, J. Med. Chem., 20, 806 (1977).

10. U. Niedballa and H. Vorbruggen, J. Org. Chem., 39, 3672 (1974).

11. This reaction was discussed by Niedballa and Vorbruggen (ref. 10) but experimental details and yield were not given.

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12. Protocols[13] established by the Division of Cancer Treatment, National Cancer Institute were followed. The test compound (9) was administered by intraperitoneal (i.p.) injection to mice on days 1, 5 and 9 following i.p. implantation of 10^5 L1210 leukemia cells. At dose levels of 200, 100, 50, 25, 12.5, 6.25 and 3.12 mg/kg, no significant increase in survival time over untreated control animals was observed.

13. R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher and B. J. Abbott, Cancer Chemother. Rep., Part 3, 3, 1 (1972).

14. A. J. Repta in "Pro-drugs as Novel Drug Delivery Systems", T. Higuchi and V. Stella, Ed., American Chemical Society, Washington, D. C., 1975, pp. 196-223.

15. 5-Azacytidine was made available by Dr. H. B. Wood, National Cancer Institute, Bethesda, Md. whom we gratefully acknowledge.

16. J. A. Zderic, J. G. Moffatt, D. Kan, K. Gerzon and W. E. Fitzgibbon, J. Med. Chem., 8, 275 (1965).

17. J.-L. Imbach, Ann. N. Y. Acad. Sci., 255, 177 (1975).

18. A. Piskala and F. Sorm, Coll. Czech. Chem. Commun., 29, 2060 (1964).

19. E. Kovats, Helv. Chim. Acta, 41, 1915 (1958).

20. W. A. Koenig, L. C. Smith, P. F. Crain and J. A. McCloskey, Biochemistry, 10, 3968 (1971).

21. M. W. Winkley and R. K. Robins, J. Org. Chem., 35, 491 (1970).

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