A Simple Oxidation of Formycin to Oxoformycin and Oxoformycin B. Synthesis of 6-Methyloxoformycin, a *C*-Nucleoside Analog of Doridosine

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A simple, high-yield procedure has now been developed for the direct oxidation of formycin to oxoformycin and oxoformycin B. Treatment of formycin (**1**) with bromine/water provided oxoformycin (**8**). A similar treatment of formycin B (**4**) gave oxoformycin B (**6**). Upon prolonged exposure of either **1** or **8** to bromine/water at reflux temperature, conversion to **6** occurred in good yield. Application of this procedure to 1-methylformycin (**2**), 1-methylformycin B (**5**) and 2-methylformycin (**17**) gave 1-methyloxoformycin (**9**), 1-methyloxoformycin B (**7**) and 2-methyloxoformycin (**18**), respectively. Deamination of **8** and **9** with nitrosyl chloride also gave **6** and **7**, respectively. This selective oxidation of 6-methylformycin gave 7-amino-6-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (**10**), a *C*-nucleoside analog of doridosine. A similar oxidation of 1,6-dimethylformycin B (**11**) gave 1,6-dimethyloxoformycin B (**12**). This direct introduction of the 5-oxo function into the pyrazolo[4,3-*d*]pyrimidine ring appears to be due to the attack of Br^+ at N(4), followed by the addition of water to C(5) and subsequent elimination of hydrogen bromide from the transient intermediate **3**.

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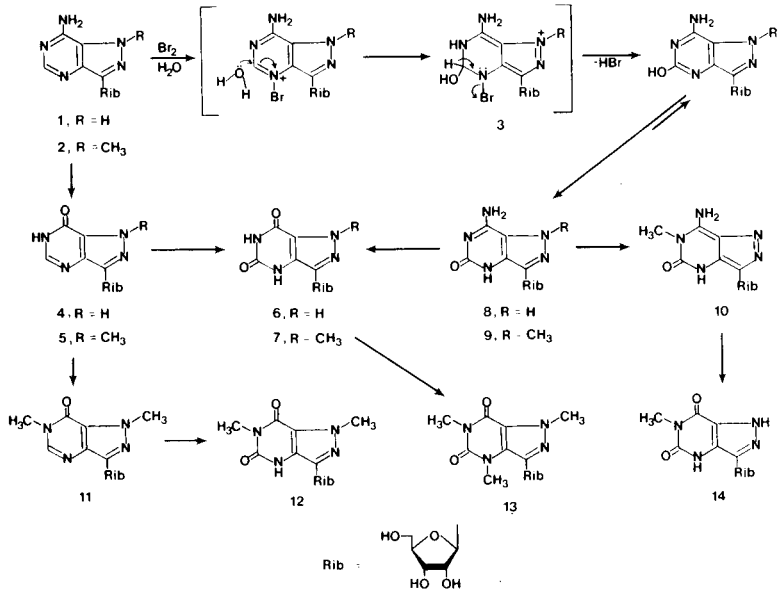
The pyrazolo[4,3-*d*]pyrimidine nucleosides, formycin (**1**) [1], formycin B [2] (laurusin [3] or oyamycin [4], **4**) and oxoformycin B (**6**) [5] are a group of naturally occurring *C*-nucleoside antibiotics which are stable to purine nucleoside phosphorylase [6-9]. This resistance to glycosidic cleavage provides a distinct advantage of these nucleosides in clinical trials. The structural elucidation of formycin and formycin B was first reported from our laboratory [10]. Formycin, a cytotoxic analog of adenosine, exhibits diverse biological activities [1,8], and is readily deaminated to formycin B, an analog of inosine, by adenosine deaminase [2,11,12]. Formycin B has recently generated renewed interest due to its potent antiparasitic activity against *Leishmania donovani* [13-16], *Leishmania tropica* [17] and *Trypanosoma cruzi* [18] *in vitro*. Mouse and rabbit liver aldehyde oxidase oxidizes formycin B to the xanthosine analog oxoformycin B (**6**) [19,20]. Originally oxoformycin B was prepared chemically [21,22] by a multistep synthesis involving 1,3-dipolar addition of 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyldiazomethane with dimethyl acetylenedicarboxylate, albeit in low yield.

A search of the literature revealed that in addition to oxoformycin B, only a handful of 5-substituted derivatives of formycin and formycin B have been reported [23-25]. We have been interested in developing a method for the direct introduction of the 5-oxo function chemically into the pyrazolo[4,3-*d*]pyrimidine ring, which would also be applicable to the synthesis of the C-nucleoside analog of doridosine (1-methylisoguanosine). The present work describes a unique, high-yield, one-flask procedure for the preparation of oxoformycin B (**6**) from formycin by treatment with bromine-water. The previously unknown intermediate oxoformycin (**8**) was also isolated in good yield under milder conditions. This unusual oxidation is similar to the biochemical conversion of IMP to XMP by IMP dehydrogenase [26].

Treatment of formycin monohydrate (**1**) with bromine-water at room temperature for 4 days gave a 65% yield of 7-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(1*H*,4*H*)-one (oxoformycin, **8**) (Scheme I). The structure of **8** was established from the ^1H nmr spectrum by observing the disappearance of C(5) aromatic proton at δ 8.17 ppm and appearance of an additional proton resonance at δ 13.76 ppm due to ring N(4)*H*. The mass spectral pattern of **8** was also consistent with that reported for the carbon-linked nucleosides [27] where, in addition to the molecular ion peak (m/e 283), a major base peak at m/e 180 (B + 30) was found. Deamination of **8** with liquid nitrosyl chloride in DMF gave 3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7(1*H*,4*H*,6*H*)-dione (oxoformycin B, **6**) in good yield. On prolonged exposure (> 24 hours) of either **1** or **8** to bromine-water at reflux temperature, conversion to **6** occurred in good yield. When formycin B (**4**) was treated with bromine-water at room temperature for 4 days, oxoformycin B (**6**) was formed in more than 90% yield. This selective oxidation of formycin B with bromine-water is found to give a better yield of **6** than that with formycin. Compound **6**, prepared by these methods, was found to be identical with oxoformycin B previously reported [5,21,22]. This direct synthesis of oxoformycin B from either formycin or formycin B is very unique and could be prepared in laboratory scale with yields comparing very favorably with those of the reported multistep procedures [21,22]. In view of the current interest in 5,7-disubstituted pyrazolo-[4,3-*d*]pyrimidine *C*-nucleosides [24,25], the presently described procedure should provide a direct route to the synthesis of hitherto inaccessible 5-substituted formycin or formycin B.

SCHEME I



A plausible mechanism of this simple oxidation may be visualized simply as occurring by the direct attack of Br^+ at N(4) followed by the addition of water to C(5). It has been shown by X-ray crystallography that the N(4), C(5) double bond in formycin hydrobromide (bond length 1.284 Å) [28,29] is shorter than that in formycin (1.313 Å) [30] or formycin B (1.310 Å), and is > 0.03 Å shorter than in the normal purine nucleoside [corresponding N(3), C(2) double bond]. It is presumed that this increased double bond character leads to the formation of **3**, followed by spontaneous elimination of hydrogen bromide to give **8**.

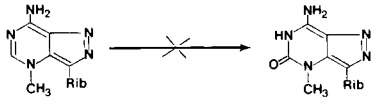
This facile reaction was also found to be applicable to methyl derivatives of formycin and formycin B. As mentioned above, formycin is a substrate for adenosine deaminase [31]. Consequently the biological activity of formycin is reduced by its ready conversion to relatively inactive formycin B. Recently it has been observed that 1-methylformycin is quite resistant to deamination by calf intestinal and human erythrocytic adenosine deaminase [32,33]. In view of this observation, we also undertook the synthesis of various methyl derivatives of oxoformycin and oxoformycin B. Treatment of 1-methylformycin (**2**) [34] and 1-methylformycin B (**5**) [35,36] with bromine-water under identical conditions gave 7-amino-1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (1-methyloxoformycin, **9**) and 1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7(4*H*,6*H*)-dione (1-methyloxoformycin B, **7**) in 81% yield, respectively. Deamination of **9** with nitrosyl chloride in DMF at 0-5° also gave **7** in 90% yield. The absence of a C(5) aromatic proton resonance around δ 8.20 ppm in the ^1H nmr, mass spectrum and elemental analysis confirmed the structure of **7** and **9**.

Methylation of formycin B or 1-methylformycin B [35] with excess of *N,N*-dimethylformamide dimethyl acetal in DMF at 80-95° for 2 days, followed by the treatment of the reaction product with ammonium hydroxide, gave a dimethylated compound identified as 1,6-dimethylformycin B (**11**). The methylated compound **11** obtained from formycin B (Method A) and 1-methylformycin B (Method B) was found to be identical in all respects. The fact that compound **11** is indeed 1,6-dimethylformycin B is confirmed by further methylation of 6-methylformycin B [36] with *N,N*-dimethylformamide dimethyl acetal in DMF (Method C), followed by deacylation of the intermediate product. The isolated yield of **11** from all three methods is over 85%. Treatment of **11** with bromine-water, as in the case of **8**, gave 1,6-dimethyloxoformycin B (**12**) in excellent yield. The disappearance of C(5) aromatic proton at δ 8.19 ppm in the ^1H nmr spectrum of **12** indicated that the oxidation had indeed taken place at position 5.

1,4,6-Trimethyloxoformycin B (**13**) is of particular interest since it may be viewed as a C-nucleoside analog of caffeine. Methylation of either oxoformycin B (**6**) or 1-methyloxoformycin B (**7**) with large excess of *N,N*-dimethylformamide dimethyl acetal in DMF, under the conditions described by Zemlicka [34] gave a 90% yield of 1,4,6-trimethyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7-dione (**13**). Although similar methylation of xanthosine at 100° is reported to give both intra- and intermolecular alkylation products [37], our data [in ^1H nmr spectrum three sharp singlets for the three N-CH_3 groups between δ 3.25-4.10, strong carbonyl absorption at 1700 cm^{-1} in the ir spectrum and elemental analysis] suggests that the product is a tri-N-methylated derivative of oxoformycin B. Moreover, the

mass spectrum of **13** also revealed a molecular ion peak at m/e 325 and the characteristic B + 30 peak at m/e 223.

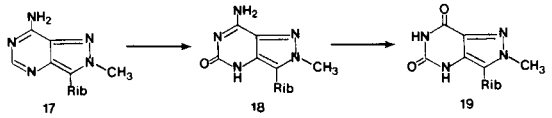
This unique simple oxidation procedure was also found to be suitable for the synthesis of 7-amino-6-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (**10**), which is a *C*-nucleoside analog of doridosine (1-methylisoguanosine) [38]. 1-Methylisoguanosine has recently been isolated from the marine sponge *Tedania digitata* [39], from the digestive glands of a Nudibranch *Anisodoris nobilis* [38], and from the coral tissue of *Madracis mirabilis* [40]. The total chemical synthesis of doridosine has recently been reported [41,42]. Doridosine has shown significant muscle relaxant, anti-inflammatory and other pharmacological activities [43,44]. Direct methylation of oxoformycin (**8**) with excess methyl iodide in DMF, under the conditions used to methylate formycin [36], gave a complex reaction mixture from which **10** was isolated in 22% yield after silica gel column chromatography. However, oxidation of 6-methylformycin [36] using bromine-water under our reaction conditions, raised the yield of **10** to more than 90%. The products **10** obtained from both the methods are virtually identical. The fact that the starting material 6-methylformycin has the established structure [36], and only position 5 is available for oxidation, proved the structural assignment of **10**. This structural assignment was further corroborated by spectroscopic and elemental analysis (see experimental). Deamination of **10** with liquid nitrosyl chloride in DMF gave 6-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7-(1*H*,4*H*)-dione (6-methyloxoformycin B, **14**). Compound **14** was also prepared by the selective oxidation of 6-methylformycin B [36] with bromine-water.



15 16

Attempts to oxidize 4-methylformycin (**15**) [36] with bromine-water under these reaction conditions to obtain 7-amino-4-methyl-1-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(6*H*)-one (**16**), resulted in the isolation of the unchanged starting material. This failure of selective oxidation at position 5 of **15** lends additional support to the proposed mechanism that the increased double bond character of N(4), C(5) linkage is probably necessary for this kind of oxidation. However, this selective oxidation is found to proceed with equal ease in the presence of a 2-methyl group (Scheme II). Thus, treatment of 2-methylformycin (**17**) [45] with bromine-water gave a 78% yield of 7-amino-2-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (2-methyloxoformycin, **18**). A similar treatment of 2-methylformycin B [35] afforded 2-methyloxoformycin B (**19**) in 80% yield. Alternatively, deamination of

Scheme II



17 18 19

18 with nitrosyl chloride in DMF readily gave **19** in rather low yield.

The site of glycosylation and anomeric configuration of all the nucleosides synthesized in this study were confirmed, since the structural assignment of the starting nucleosides were already established. This newly observed apparent oxidation by addition and elimination sequence with formycin, formycin B and certain of their *N*-methyl derivatives provides a straightforward procedure for the preparation of 5,7-disubstituted pyrazolo[4,3-*d*]pyrimidine *C*-nucleosides.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H nmr) spectra were determined at 89.6 MHz with a JEOL FX 90Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of water as indicated by elemental analysis was verified by ^{1}H nmr. Infrared spectra (ir) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (uv, sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee, and Robertson Labs, Florham Park, New Jersey. Thin-layer chromatography (tlc) was run on silica gel 60 F-254 plates (EM Reagents). J. T. Baker silica gel (70-230 mesh) was used for column chromatography. All solvents used were reagent grade. Detection of components on tlc was by uv light and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°.

7-Amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(1*H*,4*H*)-one (Oxoformycin) (**8**).

To a suspension of formycin monohydrate [46] (**1**, 2.85 g, 10 mmoles) in water (50 ml) was added an aqueous solution of bromine (3 ml of bromine in 300 ml of water) over a period of 45 minutes with stirring at room temperature. After stirring for 4 days at ~22°, the reaction mixture was purged with nitrogen and concentrated to ~50 ml. The solid that separated on cooling (ice-bath) was collected by filtration, washed with methanol (2 × 50 ml) and air-dried. An aqueous solution of the solid was neutralized with Dowex 1-X8 OH^- resin. The resin was filtered and washed repeatedly with distilled water. Evaporation of the combined aqueous solutions gave a solid, which was crystallized from water/methanol (1:1, v/v) to yield oxoformycin, 1.84 g (65%); mp 175-176° dec; ir (potassium bromide): ν 1620 (C=O), 3170 and 3340 (OH, NH_2) cm^{-1}; uv: λ max (nm) (ε × 10^{-3}) *p*H 1, 305 (5.0), 246 sh (5.1); *p*H 7, 294 (5.8), 244 (8.8); *p*H 11, 293 sh (4.7), 264 (9.0); ^{1}H nmr (DMSO-d_6): δ 4.85 (d, 1, $J_{1',2'}$ = 5.5 Hz, $C_{1'}H$), 5.85 (br s, 2, NH_2), 11.01 (br s, 1, ring N*H*), 13.76 (br s, 1, ring N*H*); ms: m/e 283 (M^+), 180 (B + 30).

Anal. Calcd. for $C_{10}H_{13}N_5O_5$ (283.24): C, 42.41; H, 4.63; N, 24.73. Found: C, 42.35; H, 4.78; N, 24.80.

7-Amino-1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (1-Methyloxoformycin) (**9**).

In the same manner as for **8**, 1-methyloxoformycin was prepared using 1-methylformycin [34] (**2**, 1.0 g, 3.5 mmoles) and bromine/water (2 ml of

bromine in 200 ml of water). The product was crystallized from water/-methanol (1:1, v/v) to give 0.85 g (81%), mp 175-176°; ir (potassium bromide): ν 1620 (C=O), 3200 and 3340 (OH, NH_2) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1, 315 (7.9), 258 (10.2); *p*H 7, 301 (8.9), 253 (12.9); *p*H 11, 305 (8.0), 250 sh (11.4); ^1H nmr (DMSO-d_6): δ 4.05 (s, 3, C*H*$_3$), 4.74 (d, 1, $J_{1',2'}$ = 5.5 Hz, $C_{1'}$*H*); ms: m/e 297 (M^+), 194 (B + 30).

Anal. Calcd. for $C_{11}H_{15}N_5O_5 \cdot H_2O$ (315.28): C, 41.90; H, 5.43; N, 22.21. Found: C, 42.17; H, 5.10; N, 22.05.

3-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidin-5,7(1*H*,4*H*,6*H*)-dione (Oxoformycin B) (**6**). Method A.

In the same manner as for **8**, treatment of a suspension of formycin B (**4**, 2.68 g, 10 mmoles) in water (50 ml) with bromine/water (3 ml of bromine in 250 ml of water) at room temperature for 4 days gave, after crystallization from water, 2.50 g (90%) of oxoformycin B, mp 284° (lit [22] mp 284-286°); ir (potassium bromide): ν 1680, 1700 (C=O), 3080-3400 (OH) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1, 286 (7.0); *p*H 7, 287 (7.1); *p*H 11, 297 (6.1), 245 sh (7.2); ^1H nmr (DMSO-d_6): δ 4.83 (d, 1, $J_{1',2'}$ = 5.0 Hz, $C_{1'}$*H*); ms: m/e 284 (M^+), 181 (B + 30).

Anal. Calcd. for $C_{10}H_{12}N_4O_6$ (284.23): C, 42.26; H, 4.26; N, 19.71. Found: C, 41.99; H, 4.46; N, 19.60.

This compound was identical in all respects with oxoformycin B reported in the literature [22].

Method B.

Liquid nitrosyl chloride (5 drops) was added to a cooled (ice-salt bath) and stirred suspension of **8** (0.10 g, 0.35 mmoles) in dry DMF (5 ml). After 15 minutes, additional nitrosyl chloride (5 drops) was added and the stirring was continued for an additional 30 minutes. The reaction mixture was allowed to warm to room temperature before it was purged with nitrogen. The contents of the flask were poured over ice water (25 ml) and evaporated to dryness. The residual solid was crystallized from water to yield 60 mg (60%) of the title compound, which was found to be identical to that obtained by Method A.

1-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7(4*H*,6*H*)-dione (1-Methyloxoformycin B) (**7**) Method A.

In the same manner as for **8**, 1-methyloxoformycin B was prepared using 1-methylformycin B [35] (**5**, 0.50 g, 1.77 mmoles) and bromine/water (0.6 ml of bromine in 50 ml of water). The product was crystallized from water/methanol (1:1, v/v) to yield 0.42 g (81%), mp 244°; ir (potassium bromide): ν 1705 (C=O), 2850-3400 (OH, NH) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1 and 7, 293 (2.4), 240 sh (1.8), 218 (4.8); *p*H 11, 312 (2.5), 240 sh (2.7), 212 (9.8); ^1H nmr (DMSO-d_6): δ 4.10 (s, 3, C*H*$_3$), 4.78 (d, 1, $J_{1',2'}$ = 5.5 Hz, $C_{1'}$*H*), 10.65 (br s, 1, ring N*H*); ms: m/e 297 (M-1)$^+$, 195 (B + 30).

Anal. Calcd. for $C_{11}H_{14}N_4O_6 \cdot \frac{1}{2}H_2O$ (307.25): C, 43.00; H, 4.92; N, 18.23. Found: C, 43.29; H, 4.88; N, 18.09.

Method B.

Deamination of **9** (0.15 g, 0.5 mmole) with liquid nitrosyl chloride (5 drops), in the same manner as for **6** (Method B) gave the title compound, 0.13 (90%), which was found to be identical to that prepared by Method A.

7-Amino-6-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (**10**). Method A.

To a solution of 6-methylformycin [36] (0.50 g, 1.8 mmoles) in water (10 ml) was added bromine/water (1 ml of bromine in 100 ml of water) over a period of 30 minutes, with stirring at room temperature. After stirring for 30 hours at ~22°, the reaction mixture was purged with nitrogen and evaporated to dryness. The residue was triturated with ether (2 × 25 ml), the ether insoluble solid was dissolved in water (20 ml) and neutralized with Dowex 1-X8 OH^- resin. The resin was removed by filtration and washed repeatedly with water. Evaporation of the solvent gave a solid which was crystallized from aqueous ethanol to yield 0.48 g (90%), mp 217°; ir (potassium bromide): ν 1635, 1675 (C=O), 3200-3400 (OH, NH_2) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1, 308 (5.2), 250 (5.8); *p*H 7, 278 (5.9); *p*H 11, 327 sh (1.8), 265 (6.3), 228 (16.8); ^1H nmr (DMSO-d_6): δ 3.36 (s, 3, C*H*$_3$), 4.86 (d, 1, $J_{1',2'}$ = 5.5 Hz, $C_{1'}$*H*); ms: m/e 297 (M^+), 194 (B + 30).

Anal. Calcd. for $C_{11}H_{15}N_5O_5 \cdot \frac{1}{2}H_2O$ (306.28): C, 43.13; H, 5.26; N, 22.86. Found: C, 43.43; H, 5.08; N, 22.57.

Method B.

To a solution of **8** (0.20 g, 0.7 mmole) in dry DMF (5 ml) was added methyl iodide (0.5 ml), and the mixture was stirred at room temperature for 48 hours. The reaction mixture was evaporated to dryness on a steam bath and the residue was co-evaporated several times with ethanol (4 × 20 ml). The resulting solid was purified on a silica gel column (1.5 × 25 cm) using methanol/chloroform (4:6, v/v) as the solvent, to yield 45 mg (22%) of the title compound, which was found to be identical to **10** prepared by Method A in all respects.

6-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7(1*H*,4*H*)-dione (6-Methyloxoformycin B) (**14**). Method A.

To a cold (ice-salt bath) solution of **10** (0.10 g, 0.3 mmole) in dry DMF (20 ml) was added liquid nitrosyl chloride (5 drops) and stirred for 10 minutes. Additional (5 drops) was added and stirring was continued for another 30 minutes before the temperature was raised to 60°. After 3 hours at 60° the solvent was evaporated to drynes. The residue was triturated with water and again evaporated to dryness. An aqueous solution of the residue was neutralized with Dowex 1-X8 OH^- resin. The resin was removed by filtration and the filtrate was evaporated to dryness. The residue was crystallized from water to yield 30 mg (30%) of the title compound, mp 284-285°; ir (potassium bromide): ν 1640, 1700 (C=O), 3020-3480 (OH, NH) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1, 284 (6.7); *p*H 7, 284 (6.4); *p*H 11, 295 (4.6); ^1H nmr (DMSO-d_6): δ 3.20 (s, 3, C*H*$_3$), 4.86 (d, 1, $J_{1',2'}$ = 5.5 Hz, $C_{1'}$*H*), 11.01 (br s, 1, ring N*H*), 13.80 (br s, 1, ring N*H*); ms: m/e 298 (M^+), 195 (B + 30).

Anal. Calcd. for $C_{11}H_{14}N_4O_6$ (298.25): C, 44.30; H, 4.73; N, 18.78. Found: C, 44.17; H, 4.73; N, 18.71.

Method B.

In the same manner as for **8**, 6-methyloxoformycin B was prepared using 6-methylformycin B [36] (0.10 g, 0.3 mmole) and bromine/water (0.2 ml of bromine in 20 ml of water). The crude product was purified on a silica gel column (1.5 × 25 cm) using chloroform/methanol (6:2) as the solvent, to yield 75 mg (75%), after crystallization from water. This compound was found to be identical to **14** prepared by Method A.

1,6-Dimethyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7-one (1,6-Dimethylformycin B) (**11**). Method A.

A mixture of formycin B (**4**, 1.0 g, 3.7 mmoles), DMF (10 ml) and *N,N*-dimethylformamide dimethyl acetal (10 ml) was heated at 80-95° for 48 hours. The reaction mixture was evaporated to dryness and the residue was co-evaporated with water (2 × 25 ml). The residual semi-solid was dissolved in concentrated ammonium hydroxide (25 ml) and stirred at room temperature for 48 hours. Evaporation of the solution gave an oil which, on trituration with absolute ethanol, gave a solid. Crystallization of the solid from aqueous ethanol gave the title compound, 0.95 g (90%), mp 165-166°; ir (potassium bromide): ν 1690 (C=O), 3400 (OH, NH) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1 and 7, 273 (7.4); *p*H 11, 273 (7.7); ^1H nmr (DMSO-d_6): δ 3.50 (s, 3, N_6-C*H*$_3$), 419 (s, 3, N_1-C*H*$_3$), 4.92 (d, 1, $J_{1',2'}$ = 6.0 Hz, $C_{1'}$*H*), 8.19 (s, 1, C_8-*H*); ms: m/e 296 (M^+), 193 (B + 30).

Anal. Calcd. for $C_{12}H_{16}N_4O_5$ (296.28): C, 48.65; H, 5.44; N, 18.91. Found: C, 48.58; H, 5.62; N, 18.67.

Method B.

In the same manner as for **11** (Method A), methylation of 1-methylformycin B [35] (0.50 g, 1.8 mmoles) with *N,N*-dimethylformamide dimethyl acetal (10 ml) in DMF (10 ml) gave the title compound, yield 0.45 g (85%), which was found to be identical to **11** prepared by Method A.

Method C.

In the same manner as for **11** (Method A), methylation of 6-methylformycin B [36] (0.50 g, 1.8 mmoles) with *N,N*-dimethylformamide dimethyl

acetal (10 ml) in DMF (10 ml) gave the title compound, yield 0.45 g (85%), which was found to be identical to **11** prepared by Methods A and B.

1,6-Dimethyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7(4*H*)-dione (1,6-Dimethyloxoformycin B) (**12**).

In the same manner as for **8**, 1,6-dimethyloxoformycin B was prepared using **11** (0.50 g, 1.7 mmoles) and bromine/water (1 ml of bromine in 100 ml of water). The product was crystallized from aqueous ethanol to yield 0.47 g (88%) of the title compound, mp 206-207°; ir (potassium bromide): ν 1660, 1700 (C=O), 3340 (OH, NH) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1 and 7, 287 (6.1), 240 sh (5.9); *p*H 11, 315 (6.9); ^1H nmr (DMSO-d_6): δ 3.21 (s, 3, N_6-CH_3), 4.08 (s, 3, N_1-CH_3), 4.82 (d, 1, $J_{1',2'}$ = 5.0 Hz, $C_1'H$), 11.10 (s, 1, ring N*H*); ms: m/e 312 (M^+), 209 (B + 30).

Anal. Cacld. for $C_{12}H_{16}N_4O_6$ (312.28): C, 46.15; H, 5.16; N, 17.94. Found: C, 45.95; H, 5.25; N, 17.65.

1,4,6-Trimethyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7-dione (1,4,6-Trimethyloxoformycin B) (**13**).

A mixture of oxoformycin B (**6**, 1.0 g, 3.5 mmoles) or 1-methyloxoformycin B (**7**, 1.0 g, 3.35 mmoles), DMF (25 ml) and *N,N*-dimethylformamide dimethyl acetal (15 ml) was heated at 80-90° for 50 hours. The reaction mixture was evaporated to dryness and the residue was co-evaporated with water (2 × 50 ml). The residual solid was dissolved in concentrated ammonium hydroxide (50 ml) and stirred at room temperature for 48 hours. Evaporation of the reaction mixture gave a solid, which was crystallized from ethyl acetate (charcoal) to yield 1.0 g (90%) of the title compound; mp 135° (softens), 175-176° dec: ir (potassium bromide): ν 1660, 1700 (C=O), 3400 (OH) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1, 288 (5.4), 241 sh (4.4); *p*H 7, 288 (5.9), 240 sh (5.1); *p*H 11, 288 (6.5), 241 sh (6.0); ^1H nmr (DMSO-d_6): δ 3.25 (s, 3, N_6-CH_3), 3.58 (s, 3, N_4-CH_3), 4.10 (s, 3, N_1-CH_3), 4.92 (d, 1, $J_{1',2'}$ = 6.0 Hz, $C_1'H$); ms: 325 $(M-1)^+$, 223 (B + 30).

Anal. Calcd. for $C_{13}H_{18}N_4O_6$ (326.31): C, 47.85; H, 5.56; N, 17.17. Found: C, 47.56; H, 5.82; N, 16.90.

7-Amino-2-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (2-Methyloxoformycin) (**18**).

In the same manner as for **8**, 2-methyloxoformycin was prepared using 2-methylformycin [45] (**17**, 1.0 g, 3.5 mmoles) and bromine/water (2 ml of bromine in 200 ml of water). The product was crystallized from aqueous methanol to give 0.81 g (78%) of the title compound, mp 200-202° dec; ir (potassium bromide): ν 1630 (C=O), 3180 and 3330 (OH, NH_2) cm^{-1}; uv: λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1, 310 (5.2), 257 (6.5); *p*H 7, 300 (6.8), 254 (10.2); *p*H 11, 300 (6.5), 254 (9.8); ^1H nmr (DMSO-d_6): δ 3.90 (s, 3, CH_3), 4.98 (d, 1, $J_{1',2'}$ = 5.0 Hz, $C_1'H$), 7.62 (br s, 2, NH_2), 9.81 (br s, 1, ring N*H*); ms: m/e 297 (M^+), 194 (B + 30).

Anal. Calcd. for $C_{11}H_{15}N_5O_5 \cdot 2H_2O$ (333.30): C, 39.64; H, 5.74; N, 21.01. Found: C, 39.49; H, 5.48; N, 21.15.

2-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-5,7(4*H*,6*H*)-dione (2-Methyloxoformycin B) (**19**). Method A.

In the same manner as for **8**, 2-methyloxoformycin B was prepared using 2-methylformycin B [35] (0.50 g, 1.77 mmoles) and bromine/water (0.7 ml of bromine in 75 ml of water). The product was crystallized from aqueous methanol to yield 0.41 g (80%) of the title compound, mp 280-281° dec; ir (potassium bromide): ν 1700 (C=O), 2850-3400 (OH, NH) cm^{-1}; uv λ max (nm) ($\epsilon \times 10^{-3}$) *p*H 1, 290 (8.4), 239 (2.1); *p*H 7, 291 (5.6), 243 (1.1); *p*H 11, 307 sh (5.1), 244 (3.2); ^1H nmr (DMSO-d_6): ν 3.92 (s, 3, CH_3), 4.99 (d, 1, $J_{1',2'}$ = 5.0 Hz, $C_1'H$), 10.41 (br s, 1, ring N*H*), 10.86 (br s, 1, ring N*H*); ms: m/e 298 (M^+), 195 (B + 30).

Anal. Calcd. for $C_{11}H_{14}N_4O_6 \cdot \frac{3}{4}H_2O$ (311.75): C, 42.30; H, 5.01; N, 18.15. Found: C, 42.19; H, 4.82; N, 18.43.

Method B.

Deamination of **18** (0.20 g, 0.68 mmole) with liquid nitrosyl chloride (10 drops) in DMF (20 ml), in the same manner as for **6** (Method B) gave the title compound, 0.12 g (60%), which was found to be identical to that prepared by Method A.

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