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1-Deoxy-1-(3,4-dihydro-8-hydroxy-2,4-dioxopyrimido[4,5-*b*]quinolin-10-(2*H*)-yl)-D-ribitol (7,8-didemethyl-8-hydroxy-5-deazariboflavin), the flavin moiety of *Methanobacterium* coenzyme F_{420}, and its 7-methyl analog were prepared by acid-catalyzed reaction of appropriately substituted 6-(*N*-D-ribitylanilino)uracils with trimethyl or triethyl orthoformate followed by deprotection.

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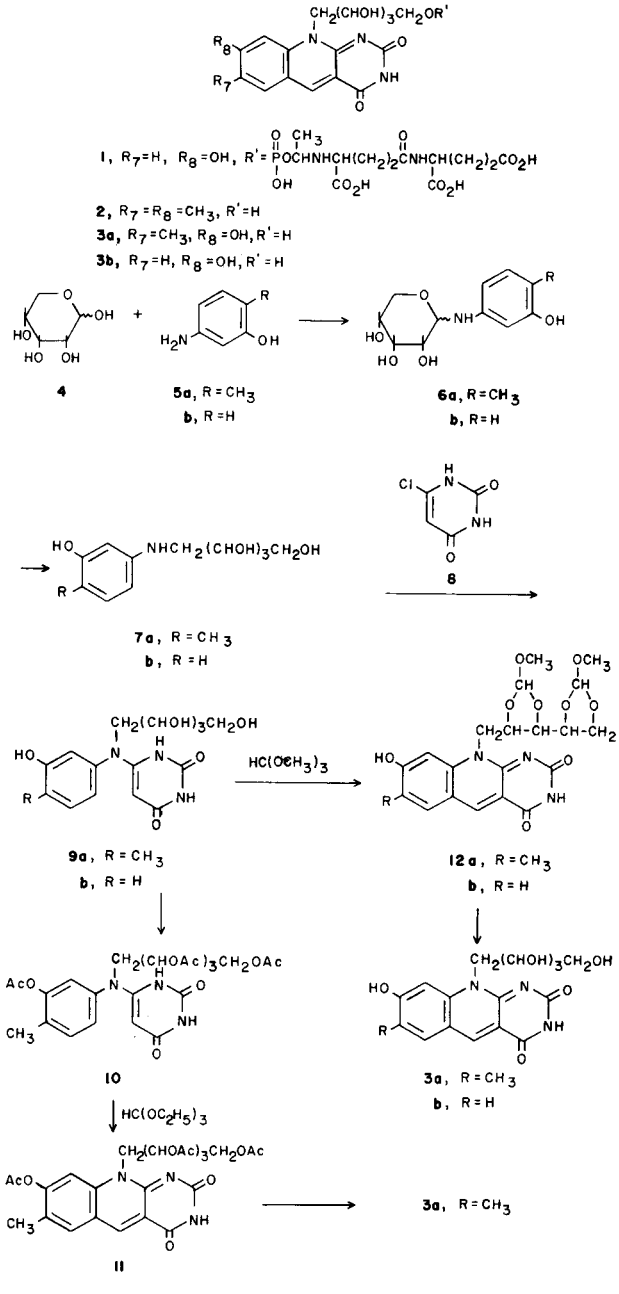
Methanogenic bacteria possess an apparently unique coenzyme known as F_{420}, which serves as an electron carrier in the NADP-linked hydrogenase system of these organisms (1-3). Coenzyme F_{420} was proposed to have the structure **1** containing an 8-hydroxy-5-deazaisoalloxazine moiety (4). Since we had already developed a convenient synthesis of 5-deazariboflavin (**2**) (5), we undertook the preparation of 8-demethyl-8-hydroxy-5-deazariboflavin (**3a**) and 7,8-didemethyl-8-hydroxy-5-deazariboflavin (**3b**). A preliminary account of these synthetic studies included spectral, chemical, and biochemical studies confirming that **3b** is identical to the deazaflavin moiety of coenzyme F_{420} (6). We now report full details of the synthesis of **3**.

Condensation of D-ribose (**4**) with the appropriate aminophenol **5** yielded *N*-ribosyl derivatives **6** (isolated only in the case of **6b**). Whereas **6a** was successfully hydrogenated to the *N*-ribityl compound **7a** (7) in the presence of Raney nickel, attempted hydrogenation of **6b** using either Raney nickel or palladium catalyst resulted in extensive reduction of the aromatic ring. However, reduction of **6b** with sodium cyanoborohydride afforded **7b** in good yield. Reaction of **7** (3 equivalents) with 6-chlorouracil (**8**) (8) in water gave the 6-(*N*-ribitylanilino)-uracil derivative **9**.

When initially prepared, **9a** was not isolated. Instead, the reaction residue containing **9a** and unreacted **7a** was acetylated, and the resulting product **10** was isolated by chromatography. Acid-catalyzed ring closure with triethyl orthoformate yielded **11**. Upon deprotection with concentrated hydrochloric acid, **3a** was obtained. Alternatively, crude **9a** was purified by cation-exchange chromatography and reacted directly with trimethyl orthoformate to give **12a**, which was converted to **3a** with dilute hydrochloric acid.

Similarly, **9b**, isolated by cation-exchange chromatography, was cyclized to **12b** with trimethyl orthoformate. Mild acid hydrolysis of **12b** afforded **3b**. The synthesis of **3b** proceeded more smoothly and in better yield than that of **3a** at virtually every step.



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EXPERIMENTAL

^{1}H Nmr spectra were obtained with a Varian T-60 or T-60A spectrometer, using tetramethylsilane as internal standard. Signals due to the ribityl moiety are omitted here. UV-visible spectra were recorded on a Carey 118C spectrophotometer. Mass spectra were obtained on a Varian MAT 731 instrument or an LKB 9000 gc-mass spectrometer. Melting points (uncorrected) were determined in open capillary tubes with a Thomas Hoover apparatus. High pressure liquid chromatography runs were carried out on a Waters Prep 500 instrument. Silica gel GF plates were used for preparative and analytical thin layer chromatography. Compounds showed satisfactory purity by tlc in the indicated solvent systems.

N-(3-Hydroxyphenyl)-D-ribosylamine (**6b**).

A mixture of 37.5 g. (0.25 mole) of D-ribose (**4**), 27.25 g. (0.25 mole) of *m*-aminophenol (**5b**), and 150 ml. of methanol was stirred at reflux under nitrogen for 4 hours. The resulting solution was concentrated to small volume and diluted with 20 ml. of 2-propanol. The solid which crystallized was collected on a filter and washed with small quantities of 2-propanol and then with ether to give 48 g. (79%) of off-white crystals, m.p. 144° dec. (from cold methanol); tlc in 80:20:2 chloroform-methanol-water. This material was unstable and was used directly in the next reaction.

1-Deoxy-1-[(3-hydroxy-4-methylphenyl)amino]-D-ribitol (**7a**) (7).

A solution of 21.0 g. (0.14 mole) of D-ribose (**4**) and 17.2 g. (0.14 mole) of **5a** (9) in 180 ml. of methanol was stirred at reflux under a drying tube. After 2 hours, tlc in 80:20:2 chloroform-methanol-water indicated nearly complete conversion to **6a**. The cooled solution was treated with 14 g. of Raney nickel and shaken with hydrogen (600 psi) at 70° for 7 hours. The catalyst was removed by filtration, and the filtrate was concentrated to give 39.0 g. (108%) of viscous, yellow-brown oil. After standing for several days, this solidified to a light brown, amorphous material, m.p. 107-115° (preliminary softening). A sample purified by hplc (80:20:2 chloroform-methanol-water) had m.p. 119-122°; tlc in 80:20:2 chloroform-methanol-water; nmr (DMSO-d_6): δ 1.90 (s, 3H, CH_3), 5.9-6.1 (m, 2H, ArH), 6.70 (d, J = 8 Hz, 1H, ArH), 8.60 (s, 1H, NH).

Anal. Calcd. for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44. Found: C, 56.02; H, 7.63; N, 5.27.

1-Deoxy-1-[(3-hydroxyphenyl)amino]-D-ribitol (**7b**).

A mixture of 96 g. (0.4 mole) of **6b**, 5 ml. of acetic acid, and 1500 ml. of dry methanol was stirred under nitrogen in an ice bath at 10° as 40 g. (0.64 mole) of sodium cyanoborohydride was added in small portions. The mixture was then stirred at room temperature for 16 hours. Next the *p*H was adjusted to 2 with concentrated hydrochloric acid. (CAUTION: evolution of hydrogen cyanide and hydrogen.) After gas evolution had subsided, the *p*H was readjusted to 4 by addition of solid lithium hydroxide in small portions. This solution was stirred with 300 ml. of AG 50W-X8 cation-exchange resin (100-200 mesh, H^+ form) until renewed effervescence had subsided. This entire mixture was layered on top of an additional 300 ml. of pre-washed resin in a column (8 cm. diameter). After washing the column with 4 l. of water, the product was eluted with 1% ammonium hydroxide solution. Fractions containing the desired material were combined and concentrated *in vacuo* at 40°. The residue was redissolved in methanol, treated with charcoal, and filtered. The residue obtained on evaporation of the filtrate was washed with acetone to give 78 g. (80%) of light gray solid, m.p. 131-134°, suitable for use in the next step. The analytical sample purified by hplc (80:20:2 chloroform-methanol-water) had m.p. 133-135° dec.; tlc in 80:20:2 chloroform-methanol-water; nmr (DMSO-d_6): δ 5.9-6.2 (m, 3H, ArH), 6.7-7.1 (m, 1H, ArH), 8.87 (s, 1H, NH).

Anal. Calcd. for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.05; N, 5.76. Found: C, 54.46; H, 6.90; N, 5.84.

1-Deoxy-1-[(3-hydroxy-4-methylphenyl)(1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinyl)amino]-D-ribitol (**9a**).

A mixture of 3.15 g. (12 mmoles) of **7a** (purified by cation-exchange chromatography as in procedure for **7b**), 0.58 g. (4 mmoles) of 6-chlorouracil (**8**), and 15 ml. of water was stirred at reflux under nitrogen for 24 hours. The cooled solution was then applied to a column containing 40 ml. of AG 50W-X8 cation-exchange resin (100-200 mesh, H^+ form) pre-washed with water. After elution with water, fractions containing the product were combined and concentrated to give 0.28 g. of amorphous solid (19% based on **8**; 17% based on recovered **7a**, which finally was eluted from the column with 1% ammonium hydroxide). The analytical sample obtained by preparative tlc (70:30:3 chloroform-methanol-water) had m.p. 112° dec.; nmr (DMSO-d_6): δ 2.14 (s, 3H, CH_3), 4.11 (s, 1H, uracil C(5)-H), 6.71 (d, J = 8 Hz, 1H, ArH), 6.75 (s, 1H, ArH), 7.18 (d, J = 8 Hz, 1H, ArH).

Anal. Calcd. for $C_{16}H_{21}N_3O_7 \cdot 0.5H_2O$: C, 51.06; H, 5.89; N, 11.16. Found: C, 50.95; H, 5.82; N, 10.91.

1-Deoxy-1-[(3-hydroxyphenyl)(1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinyl)amino]-D-ribitol (**9b**).

A solution of 33 g. (0.136 mole) of **7b** and 6.4 g. (0.044 mole) of 6-chlorouracil (**8**) (8) in 50 ml. of water was stirred at reflux under nitrogen for 14 hours. After cooling, the solution was added to a column containing 300 ml. of pre-washed AG 50W-X8 cation-exchange resin (100-200 mesh, H^+ form). Upon elution with 3 l. of water, fractions containing the product were combined and concentrated under nitrogen to yield 7.1 g. of glassy residue (46% based on **8**; 70% based on recovered **7b**, which subsequently was eluted from the column with 1% ammonium hydroxide). This material was suitable for use in the next step. The analytical sample purified by preparative tlc (70:30:3 chloroform-methanol-water) had m.p. 131° dec.; tlc in 80:20:2 chloroform-methanol-water; nmr (DMSO-d_6): δ 4.08 (s, 1H, uracil C(5)-H), 6.6-6.9 (m, 3H, ArH), 7.1-7.4 (m, 1H, ArH, partly superimposed on broad NH hump).

Anal. Calcd. for $C_{15}H_{19}N_3O_7 \cdot 0.25H_2O$: C, 50.35; H, 5.49; N, 11.74. Found: C, 50.55; H, 5.58; N, 11.48.

1-Deoxy-1-[(3-acetoxy-4-methylphenyl)(1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinyl)amino]-D-ribitol 2,3,4,5-Tetraacetate (**10**).

A solution of 16.8 g. (0.065 mole) of unpurified **7a** and 3.0 g. (0.0204 mole) of 6-chlorouracil (**8**) (8) in 80 ml. of water was stirred under reflux for 7 hours. After cooling, the water was removed *in vacuo*. The residue was taken up in ethanol and again concentrated to a viscous syrup. This material was dissolved in 100 ml. of pyridine, cooled in an ice bath, and treated with 40 ml. of acetic anhydride. The mixture was stirred under nitrogen in the ice bath for 16 hours and then concentrated *in vacuo*. The residue was dissolved in chloroform and washed with water and with cold dilute hydrochloric acid. The chloroform solution was dried with anhydrous sodium sulfate, filtered, and concentrated to give 23 g. of viscous oil. This material was applied to a column of silica gel (60 x 8 cm.) packed in methylene chloride. Successive elutions were carried out with methylene

chloride (3 l.), methylene chloride-methanol 200:1 (3 l.), 100:1 (3 l.), and 50:1 (3 l.). Fractions of 25 ml. were collected. Fractions 230-350, which contained the product, were combined and evaporated *in vacuo* to give 6.1 g. (52% based on **8**) of residual oil; tlc in 9:1 chloroform-methanol; ms: m/e 578 ($M^+ + 1$); nmr (deuteriochloroform): 2.00, 2.03, 2.11 (overlapping s, one s buried, total 12H, CH_3), 2.20, 2.33 (s, each 3H, CH_3), 4.89 (s, 1H, uracil C(5)-H), 6.97-7.10 (m, 2H, ArH), 7.35 (d, J = 8 Hz, 1H, ArH). The analytical sample purified by preparative tlc (19:1 chloroform-methanol) was obtained after vacuum drying as a glassy solid with no distinct m.p.

Anal. Calcd. for $C_{26}H_{31}N_3O_{12}\cdot H_2O$: C, 52.43; H, 5.58; N, 7.06. Found: C, 52.24; H, 5.33; N, 7.00.

1-Deoxy-1-(3,4-dihydro-8-acetoxy-7-methyl-2,4-dioxopyrimido-[4,5-*b*]quinolin-10-(2*H*)-yl)-D-ribitol 2,3,4,5-Tetraacetate (**11**).

A mixture of 5.6 g. (0.0097 mole) of **10**, 0.22 g. of *p*-toluenesulfonic acid monohydrate, 50 ml. of triethyl orthoformate, and 12 ml. of dimethyl sulfoxide was heated at 95° for 16 hours and finally at 115° for 8 hours. Then the solution was cooled and concentrated *in vacuo* to small volume. The residue was taken up in chloroform and washed twice with water. After drying over magnesium sulfate, the chloroform solution was filtered and evaporated *in vacuo*. The residual oil (4.7 g.) was applied to a column containing 500 g. of silica gel packed in methylene chloride. Successive elutions were carried out with methylene chloride (3 l.), methylene chloride-methanol 200:1 (3 l.), 100:1 (3 l.), 200:3 (3 l.), and 50:1 (3 l.). Fractions of 25 ml. were collected. Fractions 90-160, which contained the product, were combined and evaporated to give 1.4 g. of viscous, amber residual oil. This material was crystallized from ethanol to give 0.52 g. (9.1%) of yellow solid, m.p. 180-184° (preliminary softening). The analytical sample recrystallized from methanol had m.p. 187-189° (preliminary softening); tlc in 9:1 chloroform-methanol; uv (methanol): λ max (ε) 262 (29,800), 323 (8,300), 397 (13,900); ms: m/e 587 (M^+), 588 ($M^+ + 1$); nmr (deuteriochloroform): δ 1.74, 2.07, 2.23, 2.29, 2.36, 2.45 (s, each 3H, CH_3), 7.60, 7.76 (s, each 1H, C(6), C(9)-H), 8.79 (s, 1H, C(5)-H), 9.06 (br s, 1H, exchangeable, NH).

Anal. Calcd. for $C_{27}H_{29}N_3O_{12}\cdot 0.75H_2O$: C, 53.95; H, 5.11; N, 6.99. Found: C, 53.89; H, 5.15; N, 7.02.

2,3:4,5-Bis-*O*-methoxymethylene-1-deoxy-1-(3,4-dihydro-8-hydroxy-7-methyl-2,4-dioxopyrimido[4,5-*b*]quinolin-10-(2*H*)-yl)-D-ribitol (**12a**).

A mixture of 280 mg. (0.76 mmole) of **9a**, 20 mg. of *p*-toluenesulfonic acid monohydrate and 8.5 ml. of trimethyl orthoformate was stirred at reflux under nitrogen. Nearly all of the material dissolved on heating, and this was followed shortly by precipitation. After 15.5 hours, the mixture was cooled and decanted away from some dense, dark pellets, which were discarded. The product was collected on a filter and washed with methanol until the washings were only light yellow, giving 110 mg. (31%) of golden, fluorescent solid, m.p. 253-255° dec. (preliminary softening); nmr (DMSO-d_6): δ 2.30 (s, 3H, C(7)-CH_3), 3.13, 3.28 (s, each 3H, OCH_3), 5.97, 5.99 (overlapping s, total 2H, orthoformyl CH), 7.24 (s, 1H, C(9)-H), 7.88 (s, 1H, C(6)-H), 8.78 (s, 1H, C(5)-H), 10.91, 11.39 (br s, each 1H, NH, OH).

Anal. Calcd. for $C_{21}H_{23}N_3O_9$: C, 54.66; H, 5.02; N, 9.11. Found: C, 54.55; H, 5.05; N, 9.06.

2,3:4,5-Bis-*O*-methoxymethylene-1-deoxy-1-(3,4-dihydro-8-hydroxy-2,4-dioxopyrimido[4,5-*b*]quinolin-10-(2*H*)-yl)-D-ribitol (**12b**).

A mixture of 2.00 g. (5.67 mmoles) of **9b**, 0.14 g. of *p*-toluenesulfonic acid monohydrate, and 60 ml. of trimethyl orthoformate was stirred at reflux under nitrogen. Precipitation began within a few minutes. After 18 hours, the mixture was cooled. The solid was collected on a filter and washed with small volumes of methanol until the washings were only light yellow, giving 1.05 g. (41%) of golden-yellow, fluorescent solid, m.p. >251° dec.; nmr (DMSO-d_6): δ 3.11, 3.17, 3.27, 3.34 (s, total 6H, OCH_3), 5.91 (br s, 2H, orthoformyl CH), 7.0-7.5 (m, 2H, C(7), C(9)-H), 7.99 (d, J = 8 Hz, 1H, C(6)-H), 8.88 (s, 1H, C^5-H), 10.93, 11.27 (br s, each 1H, NH, OH) (10).

Anal. Calcd. for $C_{20}H_{21}N_3O_9$: C, 53.69; H, 4.73; N, 9.39. Found: C, 53.29; H, 4.68; N, 9.17.

8-Demethyl-8-hydroxy-5-deazariboflavin or 1-Deoxy-1-(3,4-dihydro-8-hydroxy-7-methyl-2,4-dioxopyrimido[4,5-*b*]quinolin-10-(2*H*)-yl)-D-ribitol (**3a**).

Method A.

A solution of 235 mg. (0.4 mmole) of **11** in 4 ml. of concentrated hydrochloric acid was allowed to stand at room temperature for 23 hours. Then the filtered solution was diluted gradually with water until crystallization began. After standing, the solid was collected on a filter and washed thoroughly with water. Additional washings with methanol and acetone were collected separately. The first crop of yellow, fluorescent solid thus obtained amounted to 50 mg., m.p. 311-313° dec. (preliminary softening). From partial evaporation of the combined methanol-acetone washings were obtained a second crop of 30 mg., m.p. 313-314° (preliminary softening), and a third crop of 20 mg., m.p. 307-308° (preliminary softening), giving a total yield of 100 mg. (66%); tlc in 80:20:2 chloroform-methanol-water; uv (0.1*N* sodium hydroxide): λ max (ε) 244 (51,000), 288 (shoulder, 8,800), 425 (52,900); field desorption ms: m/e 378 ($M^+ + 1$); nmr (DMSO-d_6): δ 2.30 (s, 3H, CH_3), 7.57 (s, 1H, C(9)-H), 8.01 (s, 1H, C(6)-H), 8.95 (s, 1H, C(5)-H), 11.11 (s, 1H, exchangeable, NH).

Anal. Calcd. for $C_{17}H_{19}N_3O_7\cdot 0.5H_2O$: C, 52.85; H, 5.22; N, 10.88. Found: C, 53.05; H, 5.36; N, 10.75.

Method B.

A suspension of 78 mg. (0.17 mmole) of **12a** in 4 ml. of 1*N* hydrochloric acid was heated on a steam bath, resulting in nearly complete dissolution followed shortly by crystallization of product. After 30 minutes, the mixture was diluted to 12 ml. with water, reheated on the steam bath for 5 minutes, and then cooled. The solid was isolated by filtration and washed with water, then with acetone, yielding 49 mg. (72%) of golden-yellow, fluorescent crystals, m.p. 312-314° (slight preliminary softening and darkening). By tlc and nmr, this material matched that prepared by Method A.

7,8-Didemethyl-8-hydroxy-5-deazariboflavin or 1-Deoxy-1-(3,4-dihydro-8-hydroxy-2,4-dioxopyrimido[4,5-*b*]quinolin-10-(2*H*)-yl)-D-ribitol (**3b**).

A suspension of 894 mg. (2 mmoles) of **12b** in 40 ml. of 1*N* hydrochloric acid was heated on a steam bath, resulting in fairly rapid dissolution. After an additional 30 minutes on the steam bath, the hot solution was filtered. The filtrate was diluted to 100 ml. with water, reheated to dissolve the precipitated solid, and then allowed to cool slowly. After standing, the crystallized product was collected on a filter, washed with water, then with acetone, and finally vacuum-dried at 100° to give 611 mg. (83%) of golden-yellow, fluorescent crystals, m.p. 284-286° dec.; tlc in

70:30:3 chloroform-methanol-water; uv (0.1*N* sodium hydroxide): λ max (ε) 232 (51,200), 287 (10,200), 419 (51,100); field desorption ms: m/e 364 ($M^+ + 1$); nmr (DMSO-d_6): δ 7.04 (d, J = 9 Hz, 1H, C(7)-H), 7.40 (br s, 1H, C(9)-H), 8.01 (d, J = 9 Hz, 1H, C(6)-H), 8.89 (s, 1H, C(5)-H), 11.01 (s, 1H, exchangeable, NH), 11.2 (v br hump, 1H, exchangeable, OH); $[\alpha]_D^{25}$ + 38° (c, 0.5, 0.1*N* sodium hydroxide).

Anal. Calcd. for $C_{16}H_{17}N_3O_7 \cdot 0.25H_2O$: C, 52.24; H, 4.80; N, 11.42. Found: C, 52.35; H, 4.81; N, 11.35.

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(10) The complexity of the methoxymethylene signals in the nmr spectrum may be explained at least in part by the presence of a pair of diastereomers, but the possibility of other positional isomers such as 2,4:3,5-bis-*O*-methoxymethylene cannot be excluded.