Synthesis of an Isomer of Pteroic Acid, 4-[(2-Amino-4-hydroxypyrimido[5,4-*d*]pyrimidin-6-ylmethyl)amino]benzoic Acid[1]

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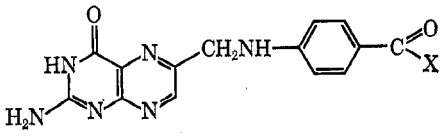
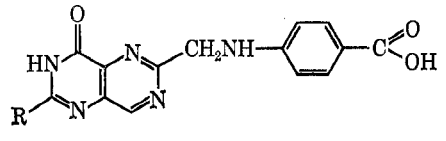
4-[(2-Amino-4-hydroxypyrimido[5,4-*d*]pyrimidin-6-ylmethyl)amino]benzoic acid (**3**) and its 2-hydroxy analog (**4**), in which C_7 and N_8 of the pteridine nucleus are interchanged, were synthesized in seven steps from 5-bromo-2-hydroxymethyl-4-pyrimidinecarboxylic acid. Efforts to condense *p*-carbethoxyanilinoacetamidine hydrochloride (**6**) with mucobromic acid did not succeed.

Many chemically modified analogs of pteroylglutamic acid (**2**) and pteroic acid (**1**) are known to antagonize the growth-promoting activity of the vitamin **2** which is known to function in its tetrahydro form.[3]



1, X = OH
2, X = HNCHCO$_2$H
CH$_2$CH$_2$CO$_2$H

Recent interest in this field has been centered on the preparation and biochemical study of deazapteridine analogs in efforts to determine the structural requirements for the biological activity.[4] No attempt has heretofore been made, however, to investigate the effect on the activity of changing the relative positions of the nitrogen atoms in the pteridine nucleus.

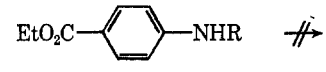


3, R = NH$_2$
4, R = OH

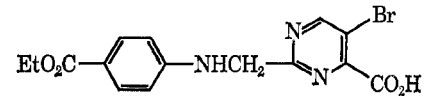
We have been particularly interested in an isomer in which C_7 and N_8 of the pteridine ring of pteroic acid are interchanged. This paper describes the synthesis of **3**, in which this interchange has been accomplished.

An initial approach to this synthesis involved the preparation of 2-amino-6-methylpyrimido[5,4-*d*]pyrimidin-4-ol and unsuccessful efforts to attach *p*-aminobenzoic acid at the 6-methyl site.[5] It seemed more promising, therefore, to prepare first a pyrimidine ring with the desired side chain followed by construction of the second pyrimidine ring. Accordingly, condensation of *p*-carbethoxyanilinoacetamidine hydrochloride (**6**) with mucobromic acid was attempted. Then the expected bromopyrimidinecarboxylic acid **8** might lead to the desired **3** by an approach previously developed in this laboratory.[5] Preparation of the required intermediate is depicted in Scheme I. *p*-Carbethoxyanilino-

SCHEME I



5, R = CH$_2$CN
6, R = CH$_2$C(NH$_2$)=NH·HCl
7, R = CH$_2$CONH$_2$



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acetonitrile (**5**) was obtained in excellent yield from ethyl *p*-aminobenzoate by the method of Takeda.[6] An attempt to displace the chlorine of chloroacetonitrile with ethyl *p*-aminobenzoate was not successful. The conversion of **5** into **6** was carried out according to the method of Schafer and Peters,[7] which consists of treatment of the nitrile with dry methanol in the presence of sodium methoxide followed by addition of an equivalent amount of ammonium chloride. The structure of the resultant amidine was supported by elemental analysis, infrared spectrum, and its conversion into *p*-carbethoxyanilinoacetamide (**7**) by alkaline hydrolysis. Our efforts to condense **6** with mucobromic acid, however, did not succeed under the various conditions applied. This lack of success might be attributable, at least in part, to the nucleophilic character of the anilino nitrogen, which interferes in the reaction of the amidine with mucobromic acid.

At this stage, our attention turned to a preparation of a 5-bromo-4-pyrimidinecarboxylic acid whose 2 substituent could be converted into a *p*-carboxyanilinomethyl group. After an unsuccessful effort to obtain 5-bromo-2-chloromethyl-4-pyrimidinecarboxylic acid from chloroacetamidine and mucobromic acid, 2-(*p*-tolylsulfonyloxymethyl)-5-bromo-4-pyrimidinecarboxylic acid (**11**) was prepared as shown in Scheme II from the corresponding 2-hydroxymethylpyrimidine **10a**, itself obtained from hydroxyacetamidine (**9**)[8] and mucobromic acid by a modified Budesinsky procedure.[9] An incorporation of the *p*-carboxyanilinomethyl group into the pyrimidine was thereupon achieved by allowing

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(3) (a) L. Delmonte and T. H. Jukes, *Pharm. Rev.*, **14**, 91 (1962); (b) R. P. Rao, *J. Sci. Ind. Res.*, **26**, 333 (1967).

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(5) C. E. Cook, Ph.D. Thesis, University of North Carolina, 1960.

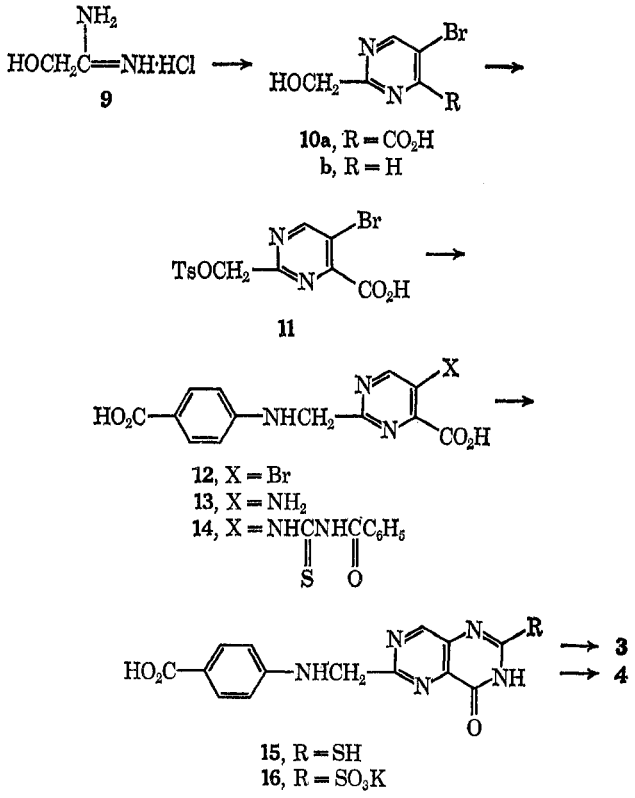
(6) A. Takeda, *J. Org. Chem.*, **22**, 1096 (1957).

(7) F. E. Schaefer and G. A. Peters, *ibid.*, **26**, 412 (1961).

(8) G. E. McCasland and D. S. Tarbell, *J. Amer. Chem. Soc.*, **68**, 2393 (1946).

(9) Z. Budesinksy, *Collect. Czech. Chem. Commun.*, **14**, 223 (1949).

SCHEME II



11 to react with *p*-aminobenzoic acid in dimethylformamide to give 5-bromo-2-(*p*-carboxyanilino)methyl-4-pyrimidinecarboxylic acid (**12**). Treatment of **12** with aqueous ammonia in a sealed tube afforded the corresponding 5-aminopyrimidinecarboxylic acid **13**.

Construction of the second pyrimidine ring was achieved in excellent yield by refluxing a basic solution of the reactive intermediate **14**,[10] which was obtained by treatment of **13** with 1 molar equiv of benzoyl isothiocyanate.

Displacement of the 2-mercapto group of **15** by ammonia had to be accomplished indirectly,[5,11] first converting the mercapto group into the labile sulfonate. Thus, oxidation of **15** with the stoichiometric amount of potassium permanganate[13] afforded the reactive sulfonate **16**. The infrared spectrum of **16** exhibited bands at 1049 and 1238 cm^{-1} which are ascribed to the sulfonate group. The introduction of the amino group was brought about by heating a solution of **16** in aqueous ammonia at 100° in a closed vessel. Since no suitable recrystallization solvent could be found for the product, purification was achieved by dissolution in dilute base followed by precipitation with an acid, giving a pale yellow powder whose analysis[14] indicated it to be the hemihydrate of **3**. The uv spectrum of **3** was similar to that of pteroic acid,[17] exhibiting its absorption maxima at 258 mμ (ε 16,900), 280 (24,400), and 349 (4000) in 0.1% aqueous sodium hydroxide solution, and the nmr spectrum run in basic deuterium oxide solution exhibited its C_8 proton signal at τ 1.88 (s), its *para*-disubstituted phenyl ring protons at τ 2.58 and 3.73 as an AA′XX′ pattern, and its C_6 methylene protons at τ 6.03 (s).

Warming a slightly acidic aqueous solution of the sulfonate **16** on a steam bath caused the loss of sulfur dioxide and afforded N-[(2,4-dihydroxypyrimido[5,4-*d*]-6-pyrimidyl)methyl]-4-aminobenzoic acid (**4**).[18]

Experimental Section

Elemental analyses were performed by Triangle Chemical Laboratories, Inc., Chapel Hill, N. C., and Micro-Tech Laboratories, Skokie, Ill. Melting points were uncorrected. Ir spectra were obtained in KBr pellets using a Perkin-Elmer Infracord Model 137 spectrophotometer, uv spectra were recorded on a Perkin-Elmer Model 202 spectrophotometer, and nmr spectra were obtained on a Varian Model A-60 spectrometer using Me_4Si as internal standard.

***p*-Carbethoxyanilinoacetonitrile** (**5**).—An aqueous solution of KCN (21.5 g in 60 ml) was added to a hot solution obtained by dissolving 84.4 g of sodium *p*-carbethoxyanilinomethanesulfonate in 150 ml of water, and the resulting mixture was refluxed for 80 min. **Sodium *p*-carbethoxyanilinomethanesulfonate** was prepared from ethyl *p*-aminobenzoate and sodium hydroxymethanesulfonate according to a literature method.[6] Chilling of the reaction mixture caused separation of a precipitate, which was collected on a filter and washed with water. The crude product was recrystallization from 50% EtOH with charcoal treatment to give 61.2 g of needlelike crystals: mp 92–93.5°; nmr ($CDCl_3$) δ 1.33 (t, 3, CH_3CH_2), 4.28 (m, 4, CH_3CH_2, CH_2), and 4.70 ppm (s, 1, NH).

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.49; H, 5.92; N, 13.72. Found: C, 64.70; H, 5.89; N, 13.95.

Hydrolysis of **5** with aqueous NaOH solution afforded *p*-carbethoxyanilinoacetamide (**7**): mp 145–146°; ir 3455 (NH), 1686 (ester C=O), and 1662 cm^{-1} (amide C=O).

Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.61. Found: C, 59.87; H, 6.43; N, 12.60.

***p*-Carbethoxyanilinoacetamidine Hydrochloride** (**6**).—The procedure of Schaefer and Peters[7] was employed with the following modifications. The reaction period of the base-catalyzed imino ether formation from **5** was extended to 7 hr, and subsequent conversion of the imino ether into the amidine hydrochloride by NH_4Cl was carried out at 57 ± 1° for 48 hr. The crude product was recrystallized from absolute EtOH and 2-butanone to give a crystalline product, yield 71%, mp 189–190°.

Anal. Calcd for $C_{11}H_{16}ClN_3O_2$: C, 51.27; H, 6.26; N, 16.31. Found: C, 51.82; H, 6.49; N, 16.17.

When **6** was hydrolyzed in aqueous KOH solution, the amide **7** was obtained in 60% yield, mp 143–145°. A mixture melting point with the authentic sample obtained from **5** was not depressed.

Hydroxyacetonitrile was obtained according to the procedure of McCasland and Tarbell.[8] A continuous ether extraction of the product from the reaction mixture increased the reported yield more than twofold, bp 79–81° (7.5 mm) [lit.[8] bp 102° (14 mm)].

5-Bromo-2-hydroxymethyl-4-pyrimidinecarboxylic Acid (**10a**).—Mucobromic acid (25.8 g, 0.1 mol) was dissolved in dry MeOH and diluted with MeOH to 40 ml. Compound **9**[8] (19 g, 0.172 mol) was dissolved in 140 ml of dry MeOH in a 500-ml three-neck flask equipped with a mechanical stirrer and a thermometer. The reaction flask was immersed in an oil bath at 50–55°. Under

(10) No attempt was made to fully characterize this seemingly unstable intermediate, although it was isolated from the reaction mixture.

(11) Although it has been amply demonstrated by Tayler and Cain[12] that the 2-mercapto group of pteridine derivatives can be replaced by amines, rather strenuous reaction conditions coupled with low yields induced us to search for an alternative route which circumvents the limitations of the direct replacement method.

(12) E. C. Taylor and C. K. Cain, *J. Amer. Chem. Soc.*, **73**, 4384 (1951); **74**, 1644, 1788 (1952).

(13) The presence of excess oxidizing agent appears to yield an undesirable, further oxidized compound, whose identification was not attempted.

(14) As was the case in analyses of pteridines,[15] **4** showed a lower value than the calculated value for nitrogen by as much as 1.6% by a convention micro Dumas method. Satisfactory results were obtained by adding a small amount of silver oxide to the combustion tube when a sample was charged.[16]

(15) A. Albert, *Quart. Rev.* (London), **6**, 197 (1952).

(16) L. M. Brancone and W. Fulmor, *Anal. Chem.*, **21**, 1147 (1949).

(17) C. W. Waller, B. L. Hutchings, J. H. Mowat, E. L. R. Stokstad, J. H. Boothe, R. B. Angier, J. Semb, Y. Suffarow, D. B. Cosulich, M. J. Fahrenback, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, and J. P. Sickels, *J. Amer. Chem. Soc.*, **70**, 19 (1948).

(18) Pyrimidinesulfonic acids are known to transform into hydroxy derivatives in an acidic medium; see D. J. Brown, "The Pyrimidines," Interscience Publishers, Inc., New York, N. Y., 1962, pp 17, 297.

vigorous stirring, 72 ml of methanolic NaOMe solution which was obtained by dissolving 6.9 g of Na in absolute MeOH to give a total volume of 120 ml was added rapidly to the amidine solution followed by an immediate addition of 24 ml of the mucobromic acid solution over a period of 5 min. A mild exothermic reaction took place. When the temperature of the reaction mixture decreased to the original state, the rest of the NaOMe solution was added followed by an addition of the remaining mucobromic acid solution. The stirring was continued for 2 hr. The inorganic salts were removed by filtration and washed with absolute EtOH. The filtrate and washings were combined and evaporated under reduced pressure to dryness. The black residue was dissolved in 50 ml of 2 *N* HCl and extracted continuously with Et_2O for 24 hr. Evaporation of the Et_2O extract afforded crystalline product, which was collected on a filter and washed with Me_2CO–Et_2O (2:1) and then with dry Et_2O to give 8.23 g (35%) of **10a**: mp 152–153.5° dec; ir 3600 (OH) and 1720 cm^{-1} (C=O).

Anal. Calcd for $C_6H_5BrN_2O_3$: C, 30.93; H, 2.17; N, 12.02. Found: C, 31.28; H, 2.73; N, 12.41.

Addition of a large excess of Me_2CO to a solution obtained by dissolving **10a** in an equimolar aqueous KOH solution caused precipitation of the K salt of **10a**. Recrystallization from Me_2CO–H_2O (4:1) afforded needlelike crystals: mp 251–252° dec; ir 1612 and 1390 cm^{-1} (CO_2^-).

Anal. Calcd for $C_6H_4BrN_2O_3K$: C, 26.58; H, 1.49; N, 10.33. Found: C, 26.53; H, 1.45; N, 10.41.

Heating of **10a** in boiling Me_2CO for 0.5 hr resulted in decarboxylation to give 5-bromo-2-hydroxymethylpyrimidine (**10b**). Purification by sublimation *in vacuo* at 66 ± 0.5° (oil-bath temperature) afforded white crystals, mp 93–94°, no carbonyl absorption band in the ir.

Anal. Calcd for $C_5H_5BrN_2O$: C, 31.77; H, 2.67; N, 14.82. Found: C, 31.78; H, 2.89; N, 14.73.

2-(*p*-Toluenesulfonyloxymethyl)-5-bromo-4-pyrimidinecarboxylic Acid (11).—*p*-Toluenesulfonyl chloride (8.5 g) dissolved in 30 ml of Et_2O was added slowly to a solution obtained by dissolving **10a** in 70 ml of 1 *N* aqueous NaOH solution under vigorous stirring. The stirring was continued for 3 hr, during which 15 ml of additional Et_2O was added. The Et_2O layer was removed, and the aqueous layer was washed with Et_2O. Careful acidification of the aqueous solution with 2 *N* HCl under cooling caused separation of the product, which was collected on a filter and washed with water several times: yield 2.28 g (30%); mp 150 151°; ir 1723 (C=O), 1357, and 1166 cm^{-1} (SO_2).

Anal. Calcd for $C_{13}H_{11}BrN_2O_5S$: C, 40.32; H, 2.86; N, 7.24. Found: C, 40.19; H, 2.84; N, 7.30.

5-Bromo-2-(*p*-carboxyanilino)-methyl-4-pyrimidinecarboxylic Acid (12).—A solution obtained by dissolving 9.33 g of **11** and 17 g of *p*-aminobenzoic acid in 47 ml of dry DMF was stirred at 50–60° (oil-bath temperature) for 48 hr. After most of the DMF was removed *in vacuo*, the residual oil was added dropwise to 140 ml of cold water, whereby precipitation occurred. The precipitate was collected on a filter and washed with water and then with Et_2O repeatedly to give 13.4 g of crude product, mp 240–250°. This was used directly in the following debromoamination reaction.

5-Amino-2-(*p*-carboxyanilino)methyl-4-pyrimidinecarboxylic Acid (13).—A mixture of 13.4 g of crude **12**, 0.5 g of $CuSO_4 \cdot 5H_2O$, and 100 ml of NH_4OH was charged in a steel bomb, and the latter was kept in boiling water for 2.5 hr. After the mixture had cooled to room temperature, the excess ammonia was evaporated under a mild stream of air. Insoluble material was removed by filtration and the filtrate was acidified with 2 *N* HCl to pH *ca.* 1 under cooling. The crystals so obtained were collected on a filter and washed with water. Recrystallization of the crude product from a large excess of water with treatment of charcoal afforded 1.6 g of needlelike crystals, mp 235–236° dec.

Anal. Calcd for $C_{13}H_{12}N_4O_4$: C, 54.18; H, 4.20; N, 19.44. Found: C, 53.89; H, 4.33; N, 19.63.

5-(3-Benzoyl-2-thioureido)-2-(4-carboxyanilinomethyl)-4-pyrimidinecarboxylic Acid (14).[10]—To a solution obtained by dissolving 1.4 g of **13** in 9 ml of dry DMF at 50–60° (oil-bath temperature) was added freshly distilled benzoyl isothiocyanate[19] over a period of 5 min under vigorous stirring. The stirring and warming was continued for 3.5 hr. The reaction mixture was added dropwise to 200 ml of cold water, and the resulting mixture was stirred at 0° for 0.5 hr. The precipitate was collected on a filter and washed with water to give 2.1 g of product, mp 215–218° dec. This was used directly in the following ring-closure reaction.

4-[(4-Hydroxy-2-mercaptopyrimido[5,4-*d*]pyrimidin-6-ylmethyl-amino]benzoic Acid (15).—A solution obtained by dissolving 2.1 g of **14** in 6.8 ml of hot aqueous 3 *N* KOH solution was refluxed for 7 min. Acidification of the reaction mixture with 2 *N* HCl to pH *ca.* 2 under vigorous stirring caused separation of a fine powder, which was collected on a filter and washed with 0.1% aqueous NaCl, 0.05% aqueous NaCl, and water, successively. The filter residue was triturated with Et_2O repeatedly after being dried *in vacuo*, giving 1.6 g (98%) of product which did not melt below 360° but charred at *ca.* 300°. For purification the crude product was dissolved in warm aqueous $NaHCO_3$ solution, treated with charcoal, and filtered. Acidification of the filtrate with HOAc caused separation of hardly filterable fine particles. The resulting mixture was digested for 20 min on a steam bath and cooled slowly to room temperature. After the supernatant liquid was removed by careful decantation, the product was collected on a filter and washed with water: ir 3508, 3225, 2942, 1720, and 1700 cm^{-1}.

Anal. Calcd for $C_{14}H_{11}N_5O_3S \cdot H_2O$: C, 48.42; H, 3.77; N, 20.17. Found: C, 49.04; H, 3.40; N, 19.71.

4-[(2-Amino-4-hydroxypyrimido[5,4-*d*]pyrimidin-6-ylmethyl)-amino]benzoic Acid (3).—To a solution obtained by dissolving 0.5 g of **15** in 5 ml of 1 *N* aqueous KOH was added dropwise 43 ml of aqueous $KMnO_4$ solution (10 mg/ml) with vigorous stirring and chilling (1 ± 1°) over a period of 1 hr. The MnO_2 was removed by filtration and washed with water. A small amount of $Na_2S_2O_5$ was added to the filtrate. The filtrate was then heated to boiling, treated with charcoal, and filtered. Neutralization and subsequent slow cooling of the filtrate caused separation of precipitate, which was collected on a filter and washed with water, giving 0.63 g (80%) of **potassium 6-(4-carboxyanilinomethyl)-4-hydroxypyrimido[5,4-*d*]pyrimidin-2-sulfonate (16)**: mp >360°; ir 3400, 3182, 1708, 1604, 1238, 1170, and 1049 cm^{-1}.

A mixture of 34 ml of concentrated NH_4OH and 0.43 g of **16** was charged in a steel bomb and heated at 100° for 2.5 hr. After being cooled to room temperature, the bomb was opened and the excess NH_3 was evaporated. Neutralization of the remaining solution with 2 *N* HCl caused separation of fine particles, which were collected on a filter to give 0.22 g (68%) of product, mp >360°. For purification, the product was dissolved in 40 ml of hot 0.03 *N* aqueous NaOH solution, treated with charcoal, and filtered. Careful acidification of the hot filtrate with 1 *N* HCl to pH *ca.* 3 caused separation of the product in a colloidal state. After digestion for 30 min on a steam bath, the product was filtered and washed with 0.1 *N* aqueous NaCl solution and then with water twice. Repeated purification by the above manner afforded an analytical sample, which did not melt below 360° but turned to brown from *ca.* 300°: ir 3400, 3310, 3100 (br), 1670 (br), and 1600 cm^{-1}.

Anal. Calcd for $C_{14}H_{12}N_6O_3 \cdot {}^1/_2H_2O$: C, 52.33; H, 4.09; N, 26.22. Found: C, 52.62; H, 4.22; N, 26.05.

4-[(2,4-Dihydroxypyrimido[5,4-*d*]pyrimidin-6-ylmethyl)amino]-benzoic Acid (4).—Digestion of **16** in slightly acidic medium at *ca.* 95° for 20 min (an odor of SO_2 was noticed during the digestion) resulted in formation of **4**. Repeated purification of the product by a similar manner used for **3** afforded a pale yellow powder which did not melt below 360°: ir 3400, 2100 (br,) 1700 (br), and 1600 cm^{-1}; uv max (0.1 *N* NaOH) 282 mμ (ε 32,400) and 344 (4500).

Anal. Calcd for $C_{14}H_{11}N_5O_4 \cdot {}^1/_2H_2O$: C, 52.17; H, 3.77; N, 21.78. Found: C, 51.85; H, 3.93; N, 21.49.

Registry No.—**3**, 22433-07-4; **4**, 22487-49-6; **5**, 22433-08-5; **6**, 22433-09-6; **7**, 22487-50-9; **10a**, 22433-10-9; **10a** (potassium salt), 22433-11-0; **10b**, 22433-12-1; **11**, 22433-13-2; **12**, 22433-14-3; **13**, 22433-15-4; **14**, 22433-16-5; **15**, 22433-17-6; **16**, 22433-18-7.

(19) J. C. Ambelang and T. B. Johnson, *J. Amer. Chem. Soc.*, **61**, 6321 (1939).