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Received February 14, 1977

The methylation of folic acid (**2**) with excess methyl iodide resulted in the formation of dimethyl 1,3-dimethylfolate (**4**), whereas, methylation of dimethyl folate (**3**) with an equimolar amount of methyl iodide gave mainly dimethyl 3-methylfolate (**6**). Both **4** and **6** underwent the Dimroth rearrangement in base to give the corresponding 2-deamino-2-methylamino folic acids **5** and **7**, respectively. Amination of **2** with hydroxylamine-*O*-sulfonic acid in dilute base gave a low yield of 3-amino folic acid (**8**), which underwent Dimroth rearrangement to give 2-deamino-2-hydrazino folic acid (**9**) in an acidic, but not a basic, medium.

J. Heterocyclic Chem., 14, 885 (1977)

The considerable increase (> 10,000 times) in binding of aminopterin to dihydrofolic reductase relative to folic acid (**2**) has been attributed, at least in part, to the increase in basicity of the pteridine ring of aminopterin (1). Because of this strong binding, aminopterin is a potent inhibitor of dihydrofolic reductase, and it was of interest to prepare 3-aminofolic acid [*N*-[4-[[(2,3-diamino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid] (**8**) to determine if the increased basicity of the latter might also result in the inhibition of this enzyme.

The preparation of **8** by the direct amination of folic acid (**2**) was considered since in the reaction of pyrimidinones and purinones with hydroxylamino-*O*-sulfonic acid in aqueous base, amination occurred at the ring nitrogen adjacent to the oxo function (2). Since only 3-substituted derivatives of pterin can undergo the Dimroth rearrangement (3), amination of folid acid in the 3-position can be confirmed by conversion of the product to the isomeric 2-hydrazino compound. To determine if the side chain of a 3-substituted folic acid was stable to the conditions of the Dimroth rearrangement, the preparation and transformation of 3-methylfolic acid to the corresponding 2-(methylamino) compound was investigated.

Reaction of **2** with an equimolar amount of methyl iodide in dimethylacetamide containing potassium carbonate was unsatisfactory as only a small peak for a *N*-methylated product was observed in the ^{1}H nmr spectrum of the recovered **2**. In contrast, alkylation of **2** with about a 4-molar excess of methyl iodide gave a low yield of a purified sample of a tetramethylated derivative, which was shown by the ^{1}H nmr spectrum to be a dimethyl ester of **2** containing two *N*-methyl groups. Previously, the methylation of 6,7-dimethylpterin (**1**) was reported to give a mixture consisting of the corresponding 3-methyl, 1,3-dimethyl, and 3,8-dimethyl derivatives, suggesting that the methylated product of **2** was either the 1,3-dimethyl- or 3,8-dimethyl- derivative of **3** (4,5). In an acidic medium, the uv spectrum of the cation of the product (255 sh, 297, 334 sh nm) was similar to those of folic acid (247, 297, 334 sh nm) and pterin (243 sh, 312 nm) (6) and dissimilar to those of 8-methylpterin (260, 276, 386 nm) and 3,8-dimethylpterin (262, 278 sh, 393 nm) (7), which provided support for the 1,3-dimethyl derivative **4**. Additional evidence was provided by comparison of the degree of the hypsochromic shift of the high wavelength maxima of the anions of 6,7-dimethylpterin (356 nm), 1,6,7-trimethylpterin (330-340 nm, plateau), and 3,6,7-trimethylpterin (352 nm) (4). These data showed that a greater shift was caused by a 1-substituent, which is similar to that observed for **3** (365 nm) and the alkylated product (342 nm). Because only the 3-methyl group can undergo the Dimroth rearrangement, the presence of this group was shown by rearrangement of the methylated product in base at room temperature, which also resulted in hydrolysis of the ester groups, to give **5** (4). To obtain the desired 3-methyl derivative, preformed dimethyl folate (**3**) was alkylated in dimethylacetamide containing sodium hydride and methyl iodide to give a mono-*N*-methylated product (**6**) contaminated with the di-*N*-methylated product **4** described above. The structure of the 3-methyl com-

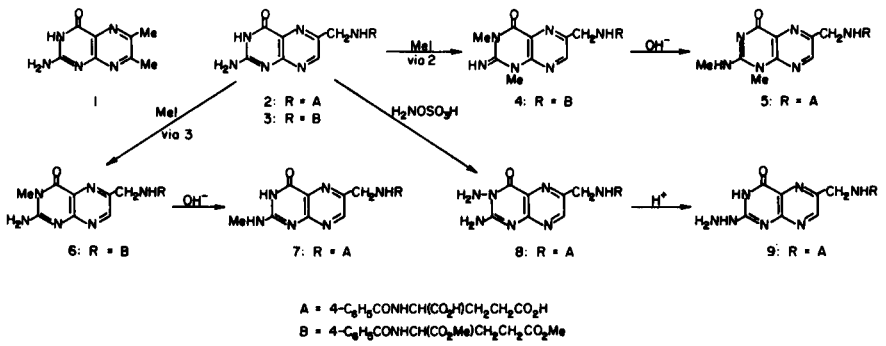

TABLE I

Spectral Properties of Some Folic Acid Compounds

Compound	Uv absorption spectra (a) pH 7	Uv absorption spectra (a) 0.1 *N* NaOH	^{1}H nmr spectral assignments (b), chemical shifts, δ (selected peaks)
2	281 (28.5) 347 (7.45)	256 (26.1) 283 (25.4) 363 (9.20)	3.5 br, 7.1 br, 8.1 d (NH, NH_2), 8.65 (7-CH)
3	282 (27.1) 297 sh (24.5) 348 (7.68)	256 (25.7) 284 (25.3) 365 (9.00)	3.56, 3.60 (CH_3O), 7.7 br, 8.3 d, 8.9 br (NH, NH_2), 8.79 (7-CH)
4	257 (19.8) 292 (22.6) 354 (8.38)	248 (21.3) 286 (23.1) 342 (10.7)	3.3 br, 6.97 t (NH), 3.37 (3-CH_3), 3.52 (1-CH_3), 3.57, 3.62 (CH_3O), 8.58 (7-CH)
5	248 (20.7) 287 (23.2) 341 (11.6)	248 (18.4) 287 (22.8) 342 (10.6)	2.89 br (2-CH_3) (c), 3.59 (1-CH_3), 7.1 br, 7.8 br, 8.1 d (NH) (d), 8.64 (7-CH)
6	244 (17.2) 285 (27.0) 350 (8.21)	247 (17.7) 284 (28.4) 353 (8.17)	3.39 (3-CH_3), 3.57, 3.63 (CH_3O), 6.9 br, 7.5, 8.2 d (NH, NH_2), 8.87 (7-CH) (e)
7	244 (15.3) 283 (29.6) 345 (7.80)	272 sh (27.1) 282 (27.6) 362 (8.12)	2.87 br (2-CH_3), 3.5 br, 6.9 br, 8.1 d (NH) (d), 8.64 (7-CH)
8	250 sh (17.4) 281 (28.9) 354 (8.29)	254 sh (19.8) 282 (28.1) 357 (8.43)	3.7 br, 6.9 br, 8.1 d (NH, NH_2) (d), 5.59 (3-NH_2) (d), 8.67 (7-CH) (f)
9	250 sh (15.7) 281 (27.0) 356 (7.36)	252 sh (16.4) 282 (26.5) 356 (7.36)	5.84, 8.15 d (NH, NH_2) (d), 8.82 (7-CH)

(a) Spectra were determined on a Cary Model 17 spectrophotometer. (b) Spectra were determined in DMSO-d_6 solutions (3-7% w/v) on a Varian XL-100-15 spectrometer with tetramethylsilane as an internal reference. (c) Collapsed to a singlet on addition of deuterium oxide. (d) Exchanged for deuterium on addition of deuterium oxide. (e) Impurity peak(s) indicated that **6** was contaminated with **4** and **7** with **5**. (f) In the hydrochloride, the NH and NH_2 peaks coalesced to give a broad peak centered at 4.7.

pound was confirmed by its rearrangement in base at room temperature to give the 2-methylamino derivative **7**. In the ^{1}H nmr spectrum, **7** showed spin-spin coupling between the Me and NH of the 2-MeNH function. These studies showed that the side-chain moiety was retained under the mild conditions used to effect the Dimroth rearrangement.

The amination of **2** with a large excess of both hydroxylamine-*O*-sulfonic acid and aqueous sodium hydroxide was unsuccessful. Next, the amination of dimethyl folate (**3**) was attempted at a *p*H (9-10) where ester saponification might be minimized, but where the 3-4 ring amide function was ionized (p*K*a 8.2) (8). Although the ^{1}H nmr spectrum of the product of this reaction indicated that amination had occurred, partial ester hydrolysis also occurred and the resulting mixture was not examined further. However, treatment of **2** under these weakly basic conditions gave a pure sample of the amination product **8**. Of interest was the similarity of the chemical shift of the ^{1}H nmr spectrum of the 3-amino group of **8** (δ, 5.59) when compared with that of the 1-amino group of 1-aminoguanosine (δ, 5.47) (2). The unsuccessful amination of **2** in strong base was attributed to the decompostion of hydroxylamine-*O*-sulfonic acid.

In contrast to the 3-methyl derivatives of **4** and **6**, **8** resisted rearrangement to the 2-hydrazino compound **9** in refluxing 0.1 *N* sodium hydroxide. The recovered **8** appeared to contain folic acid, which was probably formed by air oxidation of the hydrazino linkage of the 3-amino compound. The rearrangement of **8** to **9**, however, occurred readily under acidic conditions.

Compound **8** showed no cytotoxicity in the KB cell culture system (9), no activity against leukemia L1210 in mice (9), and no significant activity ($I_{50} > 10^{-6}$ *M*) against dihydrofolic reductase from pigeon liver (10).

EXPERIMENTAL

Melting points were determined on a Mel-Temp or Kofler Heizbank apparatus.

Dimethyl *N*-[4-[[(1,2,3,4-Tetrahydro-2-imino-1,3-dimethyl-4-oxo-6-pteridinyl)methyl]amino]benzyol]-L-glutamate (**4**).

A mixture of **2**·2 H_2O (2.0 g., 4.2 mmoles) and powdered potassium carbonate (2.5 g., 18 mmoles) in dimethylacetamide (40 ml.) containing methyl iodide (1.2 ml.) was stirred under a nitrogen atmosphere at room temperature for 18 hours and evaporated to dryness *in vacuo* at 60°. The resulting residue was suspended in water and adjusted to *p*H 4 (paper) with dilute hydrochloric acid. The insoluble solid was collected by filtration and washed with water to give crude **4**, yield, 0.88 g. This sample was extracted with hot acetonitrile, the extract was evaporated to dryness, and the residue was recrystallized from methanol, yield, 0.15 g., m.p., 125° with presoftening from 105°.

Anal. Calcd. for $C_{23}H_{27}N_7O_6 \cdot H_2O$: C, 53.58; H, 5.67; N, 19.02. Found: C, 53.24; H, 5.38; N, 1901.

N-[4-[[[1,4-Dihydro-1-methyl-2-(methylamino)-4-oxo-6-pteridinyl]-methyl]amino]benzoyl]-L-glutamic Acid (**5**).

A suspension of **4** (100 mg., 0.194 mmole) in oxygen-free 0.1 *N* sodium hydroxide (15 ml.) was stirred at room temperature for 18 hours. The resulting solution was acidified to *p*H 4 (paper) with dilute hydrochloric acid and concentrated *in vacuo* to give a precipitate of **8**·2 HCl, yield, 71 mg. This sample underwent softening and decomposition from 185°.

Anal. Calcd. for $C_{21}H_{23}N_7O_6 \cdot 2$ HCl: C, 46.50; H, 4.65; N, 18.08. Found: C, 46.34; H, 4.74; N, 18.20.

Dimethyl *N*-[4-[[(2-Amino-3,4-dihydro-3-methyl-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-L-glutamate (**6**).

A mixture of 3·2 HCl·0.5 Et_2O (3.0 g., 5.2 mmoles) and 50% sodium hydride (0.72 g., 15 mmoles, dispersed in mineral oil) in dimethylacetamide (150 ml.) containing methyl iodide (0.33 ml.) was stirred at room temperature for 18 hours and evaporated to dryness *in vacuo*. The resulting residue was washed successively with ether and water to give crude **6**, yield, 1.7 g. Extraction of this sample with boiling water (340 ml.) gave an insoluble solid (0.14 g.), which was probably the dimethyl ester of **7** based on its uv spectrum. The aqueous extract was cooled and the precipitate was collected by centrifugation and washed with water, yield, 0.80 g.; this sample underwent presoftening at 95° and partial melting from 150°.

Anal. Calcd. for $C_{22}H_{25}N_7O_6 \cdot H_2O$: C, 52.69; H, 5.43; N, 19.55. Found: C, 52.47; H, 5.23; N, 19.74.

Both the ^1H nmr and mass spectra indicated that this sample was contaminated with **4**.

N-[4-[[[3,4-Dihydro-2-(methylamino)-4-oxo-6-pteridinyl]methyl]-amino]benzoyl]-L-glutamic Acid (**7**).

A suspension of **6**·H_2O (200 mg., 0.415 mmole) in oxygen-free 0.1 *N* sodium hydroxide (30 ml.) was stirred at room temperature for 18 hours. The resulting solution was acidified with dilute hydrochloric acid to *p*H 3 (paper) and evaporated to dryness *in vacuo* at 65°. The residue was recrystallized from water to give **7**, yield, 111 mg. This sample darkened, but melted greater than than 270°.

Anal. Calcd. for $C_{20}H_{21}N_7O_6 \cdot 1.5$ H_2O: C, 49.79; H, 5.01; N,20.32. Found: C, 49.69; H, 5.23; N, 20.25.

The ^1H nmr spectrum indicated that this sample was contaminated with **5**.

N-[4-[[(2,3-Diamino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]-benzoyl]-L-glutamic Acid (**8**).

To a solution of **2**·2 H_2O (4.77 g., 10.0 mmoles) in water (200 ml.) and 1 *N* sodium hydroxide (25 ml.), cooled in an ice bath, was added with stirring hydroxylamine-*O*-sulfonic acid (6.79 g., 60.0 mmoles) followed by the slow addition of 1 *N* sodium hydroxide (~ 70 ml.). The resulting solution was maintained at about *p*H 10 (meter) for 2.5 hours, then adjusted to *p*H 12 and stirred at room temperature for 20 hours. The total amount of 1 *N* sodium hydroxide added was 110 ml. The solution was adjusted to *p*H 5 (meter) with 1 *N* hydrochloric acid, and the colloidal precipitate of **2** containing some **8** was removed by centrifugation. The clear filtrate was adjusted to *p*H 2 with 1 *N* hydrochloric acid, and crude **8** was collected by centrifugation. This sample (1.81 g.) was dissolved in water (75 ml.) by the dropwise addition of 1 *N* sodium hydroxide, and the solution (~*p*H 8) was treated with barium chloride dihydrate (1.75 g.). After the removal of barium sulfate (1.14 g.), the filtrate was adjusted to *p*H 2 with hydrochloric acid. The precipitate was collected by filtration, washed with water, and dried *in vacuo* over phosphorus pentoxide, yield, 0.40 g.; field-desorption mass spectrum: 456 (M^+). This sample contained inorganic salts and was washed with 0.1 *N* hydrochloric acid to give the hydrochloride of **8**: yield, 0.29 g. When the melting point was taken rapidly from 170°, the sample darkened at 175° followed by partial melting and decomposition from 207-215°.

Anal. Calcd. for $C_{19}H_{20}N_8O_6 \cdot$HCl·0.5 H_2O: C, 45.47: N, 4.42; N, 22.33. Found: C, 45.76; H, 4.39; N, 22.27.

N-[4-[[(2-Hydrazino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]-amino]benzoyl]-L-glutamic Acid (**9**).

A solution of **8** (100 mg.) in warm 1 *N* hydrochloric acid (4 ml.) was allowed to stand at room temperature to deposit **9**, yield, 66 mg. When the melting point was taken rapidly from 150°, vapors were evolved, and the sample darkened at 162° and decomposed from about 178°.

Anal. Calcd. for $C_{19}H_{20}N_8O_6 \cdot 1.75$ HCl·2.50 H_2O: C, 40.37; H, 4.77; Cl, 10.98; N, 19.82. Found: C, 40.50; H, 4.51; Cl, 10.97; N, 19.81.

Although no rearrangement of **8** to **9** was observed in refluxing 0.1 *N* sodium hydroxide, the recovered **8** appeared to contain folic acid (tlc).

Acknowledgments.

This investigation was supported by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Contract NO1-CM-43762. The authors are indebted to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute, who performed most of the microanalytical and spectral determinations reported.

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