New Compounds

3′-Fluorofolic Acid[1]

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Halogenated derivatives of 4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid (methotrexate) have been found to be highly effective in prolonging the life span of leukemic (L1210) mice.[2] These results, coupled with our desire to prepare analogs of folic acid which might interfere in the folic acid metabolism but *not* by interfering with folic reductase,[3] led us to prepare 3′-fluoropteroylglutamic acid (3′-fluorofolic acid).

N-(3-Fluoro-4-nitrobenzoyl)glutamic acid, prepared from 3-fluoro-4-nitrobenzoic acid[5] in the usual manner, was reduced catalytically to N-(4-amino-3-fluorobenzoyl)glutamic acid, which was used without purification in the next step, the preparation of 3-fluorofolic acid by the method of Sletzinger, *et al.*[6] Fluoro derivatives of methotrexate have been prepared by a different route.[7]

Experimental

The melting points reported were determined on a Kofler Heizbank and are corrected. The ultraviolet spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer.

3-Fluoro-4-nitrobenzoyl Chloride.—A solution of 3-fluoro-4-nitrobenzoic acid[5] (15.0 g., 81.0 mmoles) in freshly distilled thionyl chloride (30 ml.) was refluxed for 5 hr. Excess $SOCl_2$ was removed by evaporation under reduced pressure with the aid of a warm (50°) water bath, and the residue was freed of volatile substances by the addition and subsequent removal under reduced pressure of several portions of benzene. The residual yellow oil was used as such in the next step. The **amide** was prepared in the usual manner and had m.p. 172°.

Anal. Calcd. for $C_7H_5FN_2O_3$: C, 45.65; H, 2.74. Found: C, 46.06; H, 3.54.

N-(3-Fluoro-4-nitrobenzoyl)glutamic Acid.—3-Fluoro-4-nitrobenzoyl chloride (17.6 g., 86.6 mmoles) and 2 *N* NaOH solution (41.8 ml.) were simultaneously added from separate dropping funnels to a stirred solution of L-glutamic acid (12.3 g., 83.6 mmoles) in 2 *N* NaOH solution (83.6 ml.) during approximately 30 min. The maximum temperature attained was 36°. The mixture was stirred at 50° for 30 min. and was then treated with Norit and filtered through Celite. Concentrated HCl was added to the chilled filtrate until precipitation of yellow oil was complete. Vigorous stirring and cooling soon induced crystallization. The yellow solid was collected, and the filtrate was extracted with ether. Removal of the ether afforded a small additional amount of crude product as a yellow oil that crystallized after being seeded. The two portions of crude product were combined and dissolved in the minimum volume (250–300 ml.) of warm (45–50°) water. The solution was seeded and refrigerated overnight. The yellow crystalline precipitate that separated amounted to 80% yield (21.0 g.) and had m.p. 98–101°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—258 (10.5), pH 7—262 (9.90), pH 13—262 (9.96). Another recrystallization from water afforded 69% yield (18.1 g.) with m.p. 148–149°, and a third recrystallization gave essentially complete recovery (17.8 g.) of pure product with m.p. 152–153°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1—258 (10.6), pH 7—262 (10.2), pH 13—262 (9.86).

Anal. Calcd. for $C_{12}H_{11}FN_2O_7$: C, 45.87; H, 3.53; N, 8.91; neutr. equiv. (dibasic acid), 157.1. Found: C, 45.84; H, 3.74; N, 8.96; neutr. equiv., 156.6.

(4-Amino-3-fluorobenzoyl)glutamic Acid.—(3-Fluoro-4-nitrobenzoyl)gutamic acid (4.00 g., 12.7 mmoles) was catalytically (PtO_2, 0.50 g.) reduced in ethanol (100 ml.) solution at about 2.45 kg./cm.^{2} (35 p.s.i.). The pressure drop corresponding to the theoretical uptake of hydrogen occurred within 5 min. Removal of the solvent from the filtered solution left a colorless viscous syrup that was used as such in the next step.

N^2-Acetyl-3′-fluoropteroylglutamic Acid.—(4-Amino-3-fluorobenzoyl)glutamic acid (640 mg., 2.34 mmoles) was condensed with 2-acetamido-4-hydroxy-6-formylpteridine[6] (400 mg., 1.72 mmoles) in boiling 2-methoxyethanol (15 ml.) containing *p*-thiocresol (1.5 g., 12.1 mmoles) in a nitrogen atmosphere during 3.5 hr. The resultant mixture was diluted with hot (85°) water (210 ml.), and the aqueous mixture was distilled with steam until the distillate became clear. The hot residual solution was treated with Norit and filtered while hot through a Celite mat. The cooled filtrate deposited a yellow-orange crystalline powder which was collected and washed with cold water followed by acetone. The dried (*in vacuo* at 78°) product amounted to 200 mg. The spectral data are found in Table I.

TABLE I
SPECTRAL DATA

pH	λ_{max}, mμ	Abs.	Concn., mg./l.
1	294	0.450	10.016
7	280, 342 (w)	0.553, 0.180	10.016
13	255, 278, 362 (w)	0.552, 0.485, 0.198	10.016

Anal. Calcd. for $C_{21}H_{20}FN_2O_7 \cdot H_2O$: C, 48.55; H, 4.26. Found: C, 48.49; H, 4.56.

A sample of this material was recrystallized from water, and, immediately after being dried *in vacuo* at 110°, gave the following analysis.

Anal. Calcd. for $C_{21}H_{20}FN_7O_7 \cdot 0.5H_2O$: C, 49.41; H, 4.15. Found: C, 49.16; H, 4.24.

The same sample, allowed to equilibrate with atmospheric moisture, was analyzed as follows.

Anal. Calcd. for $C_{21}H_{20}FN_7O_7 \cdot 2H_2O$: C, 46.92; H, 4.50. Found: C, 46.95; H, 4.49.

3′-Fluoropteroylglutamic Acid.—A solution of N^2-acetyl-3′-fluoropteroylglutamic acid monohydrate (68 mg.) in 35 ml. of 0.1 *N* NaOH solution was heated in a N_2 atmosphere at 90° for 30 min. The solution was treated with a little Norit, heated 10 min. longer, and filtered through a Celite mat. The yellow filtrate was reheated to 90° and acidified to pH 3 with HCl. A yellow precipitate separated immediately. The mixture was allowed to cool to room temperature and was then chilled at 5° for 2 hr. The precipitate was collected, washed with water and

TABLE II
SPECTRAL DATA

pH	λ_{max}, mμ	Abs.	Concn., mg./l.
1	296	0.446	10.68
7	278, 346 (w)	0.581, 0.160	10.68
13	256, 283, 365 (w)	0.528, 0.483, 0.194	10.68
	Folic Acid		
1	295	0.419	9.862
7	279, 344 (w)	0.590, 0.155	9.862
13	255, 283, 364 (w)	0.520, 0.514, 0.180	9.862

(1) This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

(2) A. Goldin, S. R. Humphreys, J. M. Venditti, and N. Mantel, *J. Natl. Cancer Inst.*, **22**, 811 (1959).

(3) Many enzymes that use folic acid or derivatives thereof are known. The 4-amino group of methotrexate appears to be essential to its pseudo-irreversible inhibition of folic reductase.[4]

(4) T. H. Jukes and H. P. Broquist in "Metabolic Inhibitors," R. M. Holclester and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963, pp. 481–543.

(5) F. C. Schmelkes and M. Rubin, *J. Am. Chem. Soc.*, **66**, 1631 (1944).

(6) M. Sletzinger, D. Reinhold, J. Grier, M. Beachem, and M. Tishler, *ibid.*, **77**, 6365 (1955).

(7) A. S. Tomcufcik and D. R. Seeger, *J. Org. Chem.*, **26**, 3351 (1961).

acetone, and dried *in vacuo* at 78°. The spectral data are shown in Table II. The yield was 25 mg. of yellow powder that showed a correct analysis for a hemihydrate.

Anal. Calcd. for $C_{19}H_{18}FN_7O_6 \cdot 0.5H_2O$: C, 48.72; H, 4.09; N, 20 93. Found: C, 49.16; H, 4.38; N, 20.71.

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The Synthesis of Some Benzimidazole and Oxygen Analogs of Ethyl Pteroylglutamate

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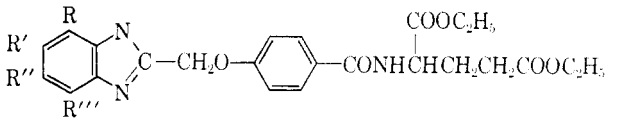
A series of diethyl N-(2-benzimidazolylmethoxy)benzoylglutamates (Table I) was prepared according to Scheme I.

TABLE I

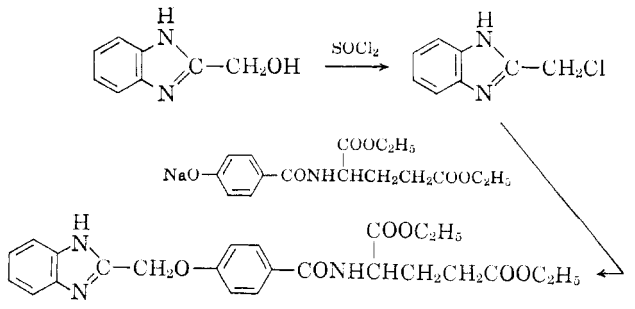
DIETHYL N-(*p*-BENZIMIDAZOLYLMETHOXY)BENZOYLGLUTAMATES



No.	R	R′	R″	R‴	Yield, %	M.p., °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
XXII	H	H	H	H	13	156–157[a]	$C_{24}H_{27}N_3O_6$	63.56	6.00	9.27	63.68	5.89	9.03
XXIII	OCH_3	H	H	OCH_3	11	147.5–148[b]	$C_{26}H_{31}N_3O_8$	60.81	6.09	8.18	60.72	5.97	8.11
XXIV	H	OCH_3	OCH_3	H	11	80–83[c]	$C_{26}H_{31}N_3O_8$	60.81	6.09	8.18	60.52	5.83	8.27
XXV	H	H	OCH_3	H	27	118–120[c]	$C_{25}H_{29}N_3O_7$	62.10	6.04	8.69	61.94	6.27	8.52
XXVI	H	H	CH_3	H	20	126–129[c]	$C_{25}H_{29}N_3O_6$	64.22	6.25	8.99	64.35	6.38	8.76
XXVII	H	CH_3	CH_3	H	11	150.5–151[c]	$C_{26}H_{31}N_3O_6$	64.85	6.49	8.73	64.98	6.69	8.59
XXVIII	H	H	NH_2	H	9	106–115[c]	$C_{24}H_{28}N_4O_6$	61.53	6.02	11.96	61.41	6.13	11.91

[a] Recrystallized from benzene. [b] Recrystallized from ethanol. [c] Recrystallized from ethyl acetate.

SCHEME I



Experimental

All melting points were determined with a Thomas–Hoover melting point apparatus.

2-Hydroxymethylbenzimidazoles were prepared from the corresponding *o*-phenylenediamines and glycolic acid by the procedure described by Phillips.[1] Two of these are new compounds.

2-Hydroxymethyl-4-amino-6-nitrobenzimidazole was isolated in 63% yield, m.p. 256–257° dec.

Anal. Calcd. for $C_8H_8N_4O_3$: C, 46.15; H, 3.87; N, 26.92. Found: C, 46.33; H, 3.95; N, 26.92.

2-Hydroxymethyl-5(6)-aminobenzimidazole Dihydrochloride.—2-Hydroxymethyl-5(6)-nitrobenzimidazole was hydrogenated over platinum in ethanol solution. After removing the catalyst, the dihydrochloride was precipitated by the addition of concentrated HCl and ether. It was recrystallized from methanol–ether; yield 45%, m.p. >350°.

Anal. Calcd. for $C_8H_{11}Cl_2N_3O$: C, 40.69; H, 4.70; N, 17.80. Found: C, 40.70; H, 4.69; N, 17.77.

2-Chlorobenzimidazoles.—The 2-chloromethylbenzimidazoles were prepared from the corresponding 2-hydroxymethylbenzimidazoles by heating with $SOCl_2$ in $CHCl_3$ solution. The addition of ether to the cooled mixture completed the precipitation of the 2-chloromethylbenzimidazole hydrochlorides. In some cases a pure product resulted and recrystallization was not necessary. In a few cases the hydrochlorides were recrystallized from ethyl alcohol.

Condensation of 2-Chloromethylbenzimidazoles with Diethyl *p*-Hydroxybenzoylglutamate. General Method.—Sodium (2 equiv.) was dissolved in dry ethanol. Diethyl *p*-hydroxybenzoylglutamate[2] (1 equiv.) in ethanol was added and then 1 equiv. of solid 2-chloromethylbenzimidazole hydrochloride was slowly added with stirring. The mixture was stirred for 2 hr. at room temperature and then refluxed for 1–4 hr. Quantitative yields of NaCl were obtained by cooling the reaction mixture. In some cases the addition of water to the filtrate gave an oil which solidified on cooling. The products were recrystallized from ethanol or ethyl acetate. A more general procedure was to evaporate the filtrate to an oil. The oil was then treated with ethanol and again evaporated. This was then repeated with dry benzene. Usually this azeotropic removal of volatile impurities caused the oil to solidify. 2-Chloromethyl-5(6)-aminobenzimidazole was used as its dihydrochloride. In this case 3 equiv. of sodium was used. The reflux time was shortened in those cases where the reaction mixture darkened too rapidly. Actually the reactions proceed at room temperature and go to completion if given enough time. The yields of the condensation products were low. It is well known that 2-chloromethylbenzimidazoles undergo self-condensation to form condensation polymers. We believe that this is the cause of the poor yields of desired products.

(1) M. A. Phillips, *J. Chem. Soc.*, 2393 (1928).

(2) E. I. Fairburn, B. J. Magerlein, L. Stubberfield, and D. I. Weishlat, *J. Am. Chem. Soc.*, **76**, 676 (1954).

Myelographic Agents. I. Iodobenzoates

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As part of our search for improved X-ray contrast agents we have synthesized a series of iodinated esters (Table I). These esters are oils or low-melting solids containing aromatic iodine and consequently are suitable for myelography.[1] In liquid form the esters have been injected cisternally into cats and dogs and have been found to permit visualization of details of the spinal

(1) For a review, see J. O. Hoppe in "Medicinal Chemistry," Vol. 6, John Wiley and Sons, Inc., New York, N. Y., 1963, p. 290.