tion of ethanol occurred over 10 minutes. The reaction was maintained at 190–200° for an additional 15 minutes. When cool, the reaction product was dissolved in 50 ml. of ether and after removal of the residue was distilled to give 2.3 g. (77%) of product, b.p. 104–110° (13 mm.), which partially crystallized. The crystals were separated by decantation and on recrystallization (hexane) melted at 67–85°. Analysis indicated that the thioamide (compound 8, Table II) had been formed.

N-(d-α-Methylphenethyl)-p-chlorophenylmercaptoacetamide (Compound 5, Table II).—A mixture of 11.5 g. (0.050 mole) of ethyl p-chlorophenylmercaptoacetate and 8.0 g. (excess) of d-α-methylphenethylamine was heated under reflux. Over 5 hours the internal reaction temperature fell from 182 to 133°. When cool the product crystallized. It was separated, washed with hexane, then water, and yielded 7.35 g., m.p. 126–130°.

The N-(d-α-Methylphenethyl)-benzylmercaptoacetamide (Compounds 6 and 6a, Table II).—The mixture of isomers obtained by reaction of 0.105 mole of d-α-methylphenethylamine with 0.05 mole of benzylmercaptoacetyl chloride weighed 13.8 g. (93%). It was dissolved in 1.1 l. of boiling hexane and allowed to cool. On standing, small hard crystals deposited. The solution decanted from these crystals

and seeded afforded an additional crop which was collected. This step was repeated. The three portions of hard crystals were combined (2.0 g.), m.p. 80° after softening at 65°. Recrystallization from 100 ml. of boiling hexane gave 1.4 g. (10%), m.p. 88–90° (compound 6, Table II), αD(CHCl₃) −5.0°.

On prolonged standing, the fluffy needles deposited from the hexane filtrate were separated (6.65 g.), m.p. 63–67°. This was dissolved in 1.1 l. of hexane at 45°, and on standing gave 2.7 g., m.p. 66–67°, αD(CHCl₃) −40.0°.

The mother liquor, stored at 10°, gave an additional crop of 2.6 g. and 0.85 g., m.p. 66–67°; total 6.15 g. (41%) of compound 6a. The ultraviolet absorption spectra of compounds 6 and 6a were identical; λmax (shoulder) 255–258, (ε540) (methanol). Since it is surprising that racemization could have occurred, the isolation of two forms of this compound requires additional clarification.

Pteridine Chemistry. IV. Structure of the Reaction Products Obtained from Acrylonitrile and 2-Amino-4-hydroxypteridines
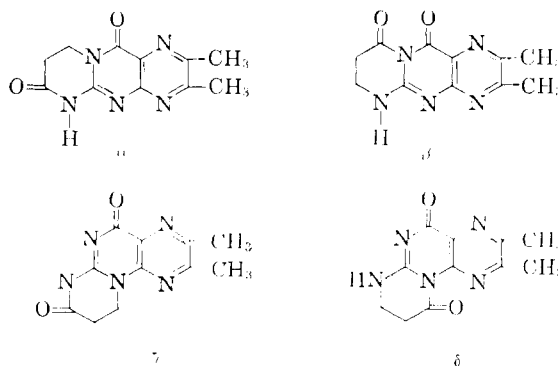
By Robert B. Angier and William V. Curran

The structure of the product obtained by the reaction of 2-amino-4-hydroxy-6,7-dimethylpteridine (Ia) with excess acrylonitrile in a pyridine–water solution has been proved to be 2,3-dimethyl-8,9-dihydro-11H-pyrimido[2,1-b]pteridine-7(6H),11-dione (IIa), a representative of a previously unknown ring system. At pH 9.2 hydrolysis of the lactam linkage of IIa gave 2-amino-3-(2-carboxyethyl)-6,7-dimethyl-4-pteridone (IIIa) while in a sodium hydroxide solution a rearrangement occurred to produce 2-(2-carboxyethylamino)-4-hydroxy-6,7-dimethylpteridine (IVa). The application of this reaction to several other 2-amino-4-hydroxypteridines also was studied. The reaction of 2-methylmercapto-4-hydroxy-6,7-dimethylpteridine (VII) with acrylamide also gave IIa. This constituted the displacement of a methylmercapto group from the pteridine nucleus under unusually mild conditions.

While attempting to synthesize N¹⁰-cyanoethylpteroylglutamic acid by direct cyanoethylation,[1] it was observed that under basic conditions the acrylonitrile attacked the pteridine portion of the molecule. Since the p-aminobenzoylglutamic acid moiety of pteroylglutamic acid was not involved, we resorted to the use of some simple 2-amino-4-hydroxypteridines in order to study the reaction and determine the structure of the products.

The 2-amino-4-hydroxy-6,7-dimethylpteridine (Ia) having been suspended in a water–pyridine solution,[2] was treated with a large excess of acrylonitrile and heated on a steam-bath until the reaction was complete as shown by paper chromatography. When the isolation procedure involved the use of slightly acidic conditions, the product had elemental analyses which indicated the addition of a C₃H₂O residue to Ia while the infrared absorption spectra exhibited no nitrile band. This could be explained by assuming a normal addition reaction between Ia and acrylonitrile involving any one of the three nitrogen atoms of the pyrimidine portion of the pteridine (Ia). A ring closure followed by hydrolysis of the resulting imino compound would then produce one of the four isomeric pyrimidopteridines (Chart I).

(1) A number of attempts to cyanoethylate pteroylglutamic acid under acidic conditions were entirely unsuccessful.

(2) Sodium hydroxide or Triton B also could be used as catalysts if the pH was maintained between 7 and 10.5. However, a pyridine-water solution was more satisfactory.

CHART I

Mild alkaline treatment of this pyrimidopteridine in 0.5 N sodium hydroxide solution at 90° for 30 minutes gave a new product (IVa) which was shown to be 2-carboxyethylamino-4-hydroxy-6,7-dimethylpteridine by elemental analysis and comparison of its ultraviolet absorption spectra with the spectra of a sample of 2-methylamino-4-hydroxy-

6,7-dimethylpteridine.[3,4] This originally suggested that the precursor pyrimidopteridine had structure β or δ since the conversion of either of these compounds to IVa would require only the simple hydrolysis of a lactam. However, it was discovered subsequently, as we have recently described,[4] that 2-amino-3,6,7-trimethyl-4-pteridone in dilute sodium hydroxide rearranges to 2-methylamino-4-hydroxy-6,7-dimethylpteridine. Therefore, α also had to be considered as a possible structure for the pyrimidopteridine since hydrolysis of the lactam linkage of α followed by a rearrangement would produce IVa. In contrast to this rearrangement it was known[4] that 2-amino-1,6,7-trimethyl-4-pteridone was hydrolyzed by dilute alkali to 1,6,7-trimethyl-2,4-pteridinedione. This eliminated γ as a possible structure since it would not be converted to IVa in an alkaline solution.

A choice among structures α, β and δ was made possible when it was found that the pyrimidopteridine when dissolved in 0.1 M sodium borate (pH 9.2) for several days and then carefully acidified at 5° gave a new crystalline product (IIIa) which had ultraviolet absorption spectra identical to the spectra of 2 - amino - 3,6,7 - trimethyl - 4 - pteridone.[4] The only one of the four pyrimidopteridines (Chart I) which could give such spectra after a mild hydrolysis at pH 9.2 would be α. The pyrimidopteridine was thus shown to be 2,3-dimethyl-8,9-dihydro-11H-pyrimido(2,1-b)pteridine-7(6H),11-dione (α) (IIa), a representative of a previously unknown ring system. Confirmation for the latter structure was obtained when it was found that treatment of 2-methylmercapto-4-hydroxy-6,7-dimethylpteridine (VII) with acrylamide in the usual manner gave, somewhat unexpectedly,[5] the same product (IIa) previously obtained from Ia (all numbers now refer to Chart II). This reaction presumably involved ring closure of the intermediate, 3-(2-carboxamidoethyl)-2-methylmercapto-6,7-dimethyl-4-pteridone, with the elimination of methylmercaptan to give IIa. This unique reaction is being investigated in more detail.

Compound IIIa, although chromatographically pure, was not obtained analytically pure. However, when 2-amino-4-hydroxypteridine-6-carboxylic acid (Ib) was treated with acrylonitrile or acrylamide[6] the pyrimido(2,1-b)pteridine (IIb) was obtained in 80–90% yield; IIb treated with 0.1 M sodium borate (pH 9.2) gave a crystalline product which analyzed as a hemihydrate of 2-amino-3-(2-carboxyethyl)-4-oxo-3,4-dihydropteridine-6-carboxylic acid (IIIb); IIIb was readily cyclized again to the pyrimido(2,1-b)pteridine (IIb) by brief warming in a dilute acid solution. On the other hand, the pyrimido(2,1-b)pteridine (IIb) with dilute sodium hydroxide was rearranged to 2-(2-carboxyethylamino)-4-hydroxypteridine-6-carboxylic acid (IVb). Pteroylglutamic acid was

also converted to a pyrimido(2,1-b)pteridine and subsequently to its 2-(2-carboxyethylamino) analog by these same procedures.

When this acrylonitrile reaction was applied to the 2-alkylamino-4-hydroxypteridines (IVb and IX) they were converted to the corresponding 6-alkylpyrimido(2,1-b)pteridines (V and X). However, it was noted that the rate of the reaction of acrylonitrile with the 2-alkylamino-4-hydroxypteridines was significantly slower than with the 2-amino-4-hydroxypteridines (I).

Compounds V and X underwent hydrolysis of the lactam linkage in dilute alkali more readily than IIa and IIb. In fact, in order to obtain ultraviolet absorption spectra of V or X in a basic solution it was necessary to dissolve the compounds in 0.1 M sodium borate solution. In 0.1 N sodium hydroxide V and X underwent immediate conversion to the open chain compounds VI and XI, whereas with II this ring-opening required several hours. Compound XI was not isolated, but its ultraviolet absorption spectra in acid and alkali were identical to those of 2-propylamino-3,6,7-trimethyl-4-pteridone.[4] This verified the structure of XI as 3-(2-carboxyethyl)-6,7-dimethyl-2-methylamino-4-pteridone.

As previously suggested, the conversion of 2-amino-4-hydroxypteridines (I) to pyrimido(2,1-b)-pteridine-7,11-diones (II) by the use of acrylonitrile must proceed through 7-imino or 7-amino analogs of II. The usual work-up of the reaction mixture involved an acidification which might be expected to hydrolyze any such intermediate. However, in one case the physical properties of the 7-amino or imino derivative facilitated its isolation. When the 2-amino-4-hydroxy-6-methylpteridine (Ic) was heated with excess acrylonitrile in a pyridine–water (1:5) solution for two hours after Ic had dissolved, cooling of the solution gave a nicely crystalline product which analyzed for 7-amino-2-methyl-8,9-dihydro-11H-pyrimido(2,1-b)-pteridine-11-one (XII) or its imino tautomer. As will be discussed later, its ultraviolet absorption spectra indicated that it was a 7-amino derivative. Compound XII was hydrolyzed readily by dilute acetic acid to its 7-oxo analog IIc while hot 1 N sodium hydroxide gave the expected 2-(2-carboxyethylamino)-4-hydroxy-6-methylpteridine (IVc).

Although the isomeric pyrimidopteridine (γ) resulting from the attack of acrylonitrile on the 1-nitrogen of the pteridine ring was considered a likely product in these reactions, no such compound was ever isolated.[7]

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectra of the pyrimido(2,1-b)pteridines

(3) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **73**, 2864 (1951).

(4) W. V. Curran and R. B. Angier, *ibid.*, **80**, 6095 (1958).

(5) It was originally intended that VII should react with acrylamide to produce 3-(2-carboxamidoethyl)-2-methylmercapto-6,7-dimethyl-4-pteridone. Treatment of the latter compound with ammonia would then presumably have given the pyrimido(2,1-b)pteridine (IIa).

(6) Acrylamide or ethyl acrylate can be substituted for acrylonitrile in this reaction to give the same product.
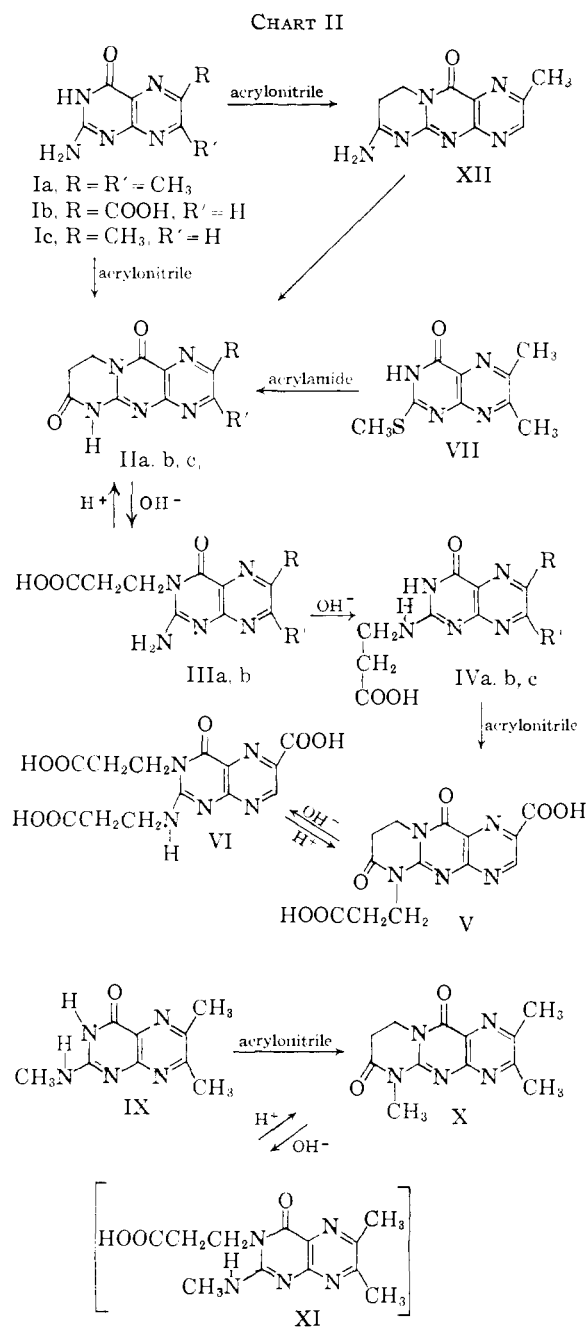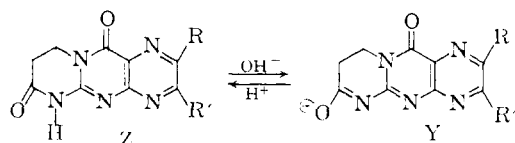
(7) The yields of pyrimido(2,1-b)pteridines in most of the reactions described were not very high. Thus, it must be considered possible that some of the isomeric pyrimidopteridines may have been formed. However, the solubilities of these isomers should not differ greatly from the isolated materials and therefore one would have expected to have obtained mixtures during the isolation processes. Furthermore, the conversion of 2-amino-4-hydroxypteridine-6-carboxylic acid (Ib) to the corresponding pyrimido(2,1-b)pteridine (IIb) gave a 90% yield of product which was chromatographically pure. In addition, further reactions carried out on IIb gave good yields of products in which no impurities could be detected either by chromatography or by ultraviolet absorption spectra. Therefore, it would appear likely that alkylation of 2-amino-4-hydroxypteridines with acrylonitrile or acrylamide occurs almost entirely on the 3-nitrogen.

CHART II

Ia, R = R' = CH₃
Ib, R = COOH, R' = H
Ic, R = CH₃, R' = H

(II) unsubstituted in the 6-position exhibit an unusually strong absorption band in basic solutions at 300 mμ (ϵ 27,000 in 0.1 N sodium hydroxide and ϵ 21,000 in 0.1 M sodium borate for IIa) which is missing in neutral or acidic solutions and which is not found in 2-acetamido-3,6,7-trimethyl-4-pteridone even in basic solutions. This strong band probably is due to the fact that II exhibits the type of enol–ketol tautomerism shown and that the longer chromphore in Y causes the in-

creased absorption in a basic solution. This is verified by the fact that 2,3,6-trimethyl-8,9-di-hydro - 11H - pyrimido(2,1-b)pteridine - 7,11-dione (X) which cannot undergo this type of tau-tomerism does not have this intense absorption at 300 mμ in 0.1 M sodium borate solution. The spectra of X in acidic, neutral and weakly basic solutions are essentially identical to one another and to the spectra of IIa in methanol indicating that in methanol IIa exists in the keto form (Z). This ability of pyrimido(2,1-b)pteridines of type II to form enol anions undoubtedly explains the fact that they are more stable in alkali than the pyrimido(2,1-b)pteridines V and X which cannot form such anions.

The 7-amino derivative XII might also exist as an imino tautomer. However, XII in both 0.1 M sodium borate and methanol exhibits the same in-tense absorption at 300 mμ that is shown by the anion of IIa. In acid this absorption remains strong (ϵ 24,000) although shifted to 280 mμ. This indicates the structure to be as shown (XII) with the double bond in conjugation with the pteridine ring system.

Acknowledgment.—The authors are indebted to Mr. Louis Brancone and staff for the microanalyti-cal data and to Mr. William Fulmor and Mr. George Morton for the spectral data.

Experimental

Paper Chromatography.—Paper chromatography was used routinely to follow reactions and to identify products. Chromatograms were viewed under ultraviolet absorption light of λ 254 mμ. All of the pyrimido(2,1-b)pteridines of type II gave greenish-blue fluorescent spots when run in 0.5% Na₂CO₃. Running ahead of the greenish-blue spot was a small deep blue spot due to ring opening of the pyri-midopteridine in the Na₂CO₃ solution. When these same compounds were run in 3% NH₄Cl the spots were difficult to see until they were exposed to ammonia vapors when a green-ish-blue fluorescent spot appeared. Only one spot was present in NH₄Cl.

All of the 3-substituted-2-amino-4-pteridones gave deep blue spots while most of the parent 2-amino-4-hydroxypteri-dines gave bright blue fluorescent spots. These various colors were helpful in identifying the type of compound in any reaction mixture.

2-Methylmercapto-4-hydroxy-6,7-dimethylpteridine (VII). —2-Methylmercapto-4-hydroxy-5,6-diaminopyrimidine[8] (1.0 g., 58 mmoles) was dissolved in 100 ml. of hot absolute eth-anol. One ml. of biacetyl was added and the solution re-fluxed for three hours. The solution then was concentrated *in vacuo* to approximately one-half of the original volume. After chilling, the product was collected by filtration and washed with cold ethanol and ether; yield 0.9 g. (70%), m.p. 283° dec. For analysis a portion of this product was recrys-tallized twice from 1-propanol; R_f 0.56 in 0.5% Na₂CO₃; ultraviolet absorption spectra in 0.1 N NaOH, λmax 263 mμ (ϵ 20,400), 347 mμ (ϵ 8,730); 0.1 N HCl, λmax 228 mμ (ϵ 11,000), 282 mμ (ϵ 15,300), 334 mμ (ϵ 8,330).

Anal. Calcd. for C₉H₁₀N₄OS (222): C, 48.7; H, 4.54; N, 25.2. Found: C, 48.8; H, 4.58; N, 25.3.

7(6H),11-Dioxo-6,7,8,9-tetrahydro-11H-pyrimido(2,1-b) pteridine-2-carboxylic Acid (IIb). Method A.—2-Amino-4-hydroxypteridine-6-carboxylic acid (20.7 g., 0.1 mole), 40 g. of acrylamide, 500 ml. of water and 500 ml. of pyridine were added to a 3-l. flask and heated to reflux using a Glas-col heater. After 4 hours and again after 8 hours of heating 40-g. portions of acrylamide were added. At 12 hours 20 g. of acrylamide was added. After a total of 20 hours refluxing the solution was evaporated *in vacuo* to a sirup, water (300 ml.) was added and the evaporation was repeated. The

(8) T. B. Johnson, C. O. Johns and F. W. Heyl, *Am. Chem. J.*, **36**, 173 (1906).

sirup then was dissolved in one liter of water, treated with Norit and filtered. The filtrate was heated to 85°, acidified with 100 ml. of acetic acid to pH 4.0–4.5, then, while still hot, 24 ml. of concentrated hydrochloric acid was added to give a solution with a pH of approximately 2. This was cooled overnight and the crystalline product then was collected, washed with water, acetone and ether and dried; yield 23.4 g. (90%). This product was dissolved in 1500 ml. of hot water by adding 11 ml. of pyridine. The solution was treated with Norit and filtered. The warm filtrate was acidified with 14 ml. of concentrated hydrochloric acid to give a crystalline product; yield 21.2 g. (81%).

Anal. Calcd. for $C_{10}H_7N_5O_4$ (261): C, 46.0; H, 2.7; N, 26.8. Found: C, 45.8; H, 3.4; N, 26.7.

The same product was obtained when acrylonitrile or ethyl acrylate was used in place of the acrylamide.

Method B.—2-Amino-3-(2-carboxyethyl)-4-oxo-3,4-dihydropteridine-6-carboxylic acid (100 mg., 0.36 mmole, IIIb) was dissolved in 40 ml. of boiling water. This solution was acidified with 3 ml. of concentrated hydrochloric acid and cooled to give 75 mg. of crystalline material which was identical in all respects to the product obtained by method A; R_f 0.73 (0.5% Na_2CO_3), 0.56 (3% NH_4Cl) (not fluorescent in acid, fuming with NH_3 brings out greenish-blue fluorescence); ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 272 mμ (ϵ 12,800), 313 mμ (ϵ 25,300), 358 mμ (ϵ 11,200); 0.1 M $Na_2B_4O_7$, λ_{max} 248 mμ (ϵ 10,400), 266 mμ (ϵ 10,800), 313 mμ (ϵ 21,100), 355 mμ (ϵ 9,900); 0.1 N HCl, λ_{max} 248 mμ (ϵ 16,700), 300 mμ (ϵ 13,000), 333 mμ (ϵ 10,700).

2,3-Dimethyl-8,9-dihydro-11H-pyrimido(2,1-b)pteridine-7(6H), 11-dione (IIa). Method A.—2-Amino-4-hydroxy-6,7-dimethylpteridine (5.0 g., 26.2 mmoles) was added to 450 ml. of water containing 50 ml. of pyridine and 10 ml. of acrylonitrile. The mixture was refluxed on a steam-bath for 32 hours and 50 ml. of acrylonitrile was added in five portions during this period. Removal of the solvents *in vacuo* gave an oil which was dissolved in water and again evaporated *in vacuo*. The resulting oil was dissolved in 30 ml. of hot water, treated with Norit, and filtered. The filtrate was acidified while hot to pH 1.5–2 with 5 ml. of glacial acetic acid and 3 ml. of concentrated hydrochloric acid. Cooling the solution gave a crystalline product which was collected and dried; yield 2.1 g. (32.8%). For analysis a portion of this product was recrystallized from water and absolute alcohol; R_f 0.62 (0.5% Na_2CO_3); ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 250 mμ (ϵ 15,700), 300 mμ (ϵ 27,000), 352 mμ (ϵ 8,700); 0.1 N HCl, λ_{max} 240 mμ (ϵ 14,900), 283 mμ (ϵ 14,300), 332 mμ (ϵ 8,250); methanol, λ_{max} 240 mμ (ϵ 15,200), 283 mμ (ϵ 16,100), 332 mμ (ϵ 7,800); 0.1 M $Na_2B_4O_7$, λ_{max} 246 mμ (ϵ 14,700), 299 mμ (ϵ 20,800), 345 mμ (ϵ 7,600).

Anal. Calcd. for $C_{11}H_{11}N_5O_2$ (245): C, 53.8; H, 4.52; N, 28.6. Found: C, 53.9; H, 4.72; N, 28.6.

Method B.—2-Methylmercapto-4-hydroxy-6,7-dimethylpteridine (2.2 g., 10 mmoles) was added to 40 ml. of 50% aqueous pyridine containing 1.4 g. of acrylamide. The solution was refluxed for 101 hours during which time 7 g. of acrylamide was added in several portions. The solution was then taken down to an oil *in vacuo*, dissolved in water and again evaporated *in vacuo*. This procedure was repeated twice using absolute alcohol and the resulting oil was extracted with absolute alcohol and filtered from some insoluble material. The filtrate was treated with Norit and evaporated to about 20 ml. to give crystals. After chilling, the crystals were filtered and dried; yield 0.65 g. Evaporation of the mother liquor gave crops of 0.30 and 0.25 g. (49% total). Combining the three crops and recrystallizing from water gave 0.90 g. (41%). Comparison of the ultraviolet and infrared spectrum of this product with that obtained by method A showed them to be the same.

7-Amino-2-methyl-8,9-dihydro-11H-pyrimido(2,1-b)pteridine-11-one (XII).—A mixture of 5.0 g. (23.2 mmoles) of 2-amino-4-hydroxy-6-methylpteridine, 200 ml. of water, 40 ml. of pyridine and 10 ml. of acrylonitrile was heated on the steam-bath. Ten-ml. portions of acrylonitrile were added after 2.5 and 5 hours heating. After 5.5 hours everything had dissolved and after a total of 7.5 hours of heating the solution was treated with Norit, filtered and cooled overnight to give a white crystalline product; yield 2.3 g. (43%); ultraviolet absorption spectra in 0.1 M $Na_2B_4O_7$ (pH 9.2), λ_{max} 252 mμ (ϵ 13,100), 300 mμ (ϵ 25,800), 345 mμ (ϵ 7,900); methanol, λ_{max} 253 mμ (ϵ 12,200), 304 mμ (ϵ 25,800), 345

mμ (shoulder) (ϵ 7,400); 0.1 N HCl, λ_{max} 225 mμ (ϵ 9,900), 281 mμ (ϵ 24,000), 328 mμ (ϵ 11,500).

Anal. Calcd. for $C_{10}H_{10}N_6O$ (230): C, 52.2; H, 4.4; N, 36.5. Found: C, 52.0; H, 4.6; N, 36.8.

2-Methyl-8,9-dihydro-11H-pyrimido(2,1-b)pteridine-7(6H) 11-dione (IIc).—A solution of 600 mg. (2.6 mmoles) of XII in 12 ml. of 10% acetic acid was heated to boiling for 4 minutes. This was treated with Norit, filtered, and reheated to boiling at which time a solid began to appear. The mixture was cooled overnight and the crystalline product was collected; yield 370 mg. (61%); R_f 0.64 (0.5% Na_2CO_3), 0.57 (3% NH_4Cl); ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 248 mμ (ϵ 13,400), 300 mμ (ϵ 31,300), 359 mμ (ϵ 7,400); 0.1 M $Na_2B_4O_7$, λ_{max} 245 mμ (ϵ 12,900), 298 mμ (ϵ 22,400), 351 mμ (ϵ 6,200); methanol, λ_{max} 241 mμ (ϵ 14,500), 280 mμ (ϵ 16,900), 335 mμ (ϵ 6,300); 0.1 N HCl, λ_{max} 240 mμ (ϵ 16,100), 280 mμ (ϵ 16,500), 335 mμ (ϵ 6,700).

Anal. Calcd. for $C_{10}H_9N_5O_2$ (231): C, 52.0; H, 3.9; N, 30.3. Found: C, 51.7; H, 4.2; N, 30.1.

N-[4-(N-[(7,11-Dioxo-6,7,8,9-tetrahydro-11H-pyrimido [2,1-b]pterid-2-yl)-methyl]-amino)-benzoyl]-glutamic Acid (XIII).—Pteroylglutamic acid (6.0 g., 13.6 mmoles) and 6.0 g. of acrylamide were dissolved in a solution of 120 ml. of water and 20 ml. of pyridine and heated on the steam-bath for 12 hours (3-g. portions of acrylamide were added after 4 and 8 hours heating). The solution then was treated with Norit and filtered. The filtrate was warmed to 70° and acidified with 30 ml. of concentrated hydrochloric acid. After cooling overnight the product was collected, washed with water, acetone and ether and dried; yield 3.8 g. (54%).

This material was dissolved in 500 ml. of boiling water, treated with Norit, filtered, reheated to boiling and acidified with 0.5 ml. of concentrated hydrochloric acid. This was cooled overnight; yield 2.7 g. (38%) of yellow, needle-like crystals; ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 305 mμ (ϵ 41.500), 360 mμ (ϵ 8,900); 0.1 M $Na_2B_4O_7$, λ_{max} 302 mμ (ϵ 37,400), 355 mμ (ϵ 8,200); 0.1 N HCl, λ_{max} 242 mμ (ϵ 18,300), 288 mμ (ϵ 28,200).

Anal. Calcd. for $C_{22}H_{21}N_7O_7$ (495): C, 53.3; H, 4.3; N, 19.8. Found: C, 52.9; H, 4.6; N, 19.6.

2-Amino-3-(2-carboxyethyl)-4-oxo-3,4-dihydropteridine-6-carboxylic Acid (IIIb).—A solution of 4.0 g. (15.3 mmoles) of 7,11-dioxo-6,7,8,9-tetrahydro-11H-pyrimido(2,1-b)pteridine-2-carboxylic acid (IIb) in 4 liters of 0.1 M sodium borate was allowed to stand at room temperature for 3 days. It then was cooled to 0° and acidified with 80 ml. of concentrated hydrochloric acid. A solid appeared rather quickly. The mixture was cooled for 30 minutes and the crystalline product was collected; yield 3.1 g. (72%), m.p. 274–276° dec.

If this material was dried at elevated temperatures it was shown by chromatography that some ring closure had occurred to give IIb. Therefore, an analytical sample was prepared by drying three hours at room temperature; R_f 0.82 (0.5% Na_2CO_3); ultraviolet absorption spectra in 0.1 m $Na_2B_4O_7$, λ_{max} 247 mμ (ϵ 13,500), 291 mμ (ϵ 15,800), 358 mμ (ϵ 8,100); 0.1 N HCl, λ_{max} 245 mμ (ϵ 15,100), 300 mμ (ϵ 12,900), 330 mμ (ϵ 9,500).

Anal. Calcd. for $C_{10}H_9N_5O_5 \cdot 1/2 H_2O$ (288): C, 41.7; H, 3.5; N, 24.3; H_2O, 3.1. Found: C, 41.9; H, 3.8; N, 24.6; H_2O, 4.2.

2-Amino-3-(2-carboxyethyl)-5,6-dimethyl-4(3H)-pteridone (IIIa).—Five hundred mg. of IIa was added to 50 ml. of 0.1 m sodium borate solution with stirring. Approximately 10 minutes after adding IIa, and before it had all dissolved, a precipitate of white needles came out which re-dissolved on stirring. After standing 50 hours, paper chromatography in 0.5% sodium carbonate showed only one spot R_f 0.77 (blue fluorescence). A 10-ml. aliquot of the solution was withdrawn and chilled to 0–2°, then acidified to pH 5 with glacial acetic acid and further to pH 2–2.5 with 6N hydrochloric acid. Crystals appeared immediately. They were collected and dried after chilling the solution; yield 80 mg. This product gave one spot, R_f 0.77 (blue fluorescence), when chromatographed in 0.5% sodium carbonate. The ultraviolet spectra in 0.1 N sodium hydroxide and in 0.1 N hydrochloric acid were practically identical to spectra of 2-amino-3,6,7-trimethyl-4(3H)-pteridone[4]; ultraviolet spectra in 0.1 N NaOH, λ_{max} 243 mμ (ϵ 15,800), 278 mμ (ϵ 13,500), 352 mμ (ϵ 7,240); 0.1 M $Na_2B_4O_7$, λ_{max} 243 mμ (ϵ 15,300), 278 mμ (ϵ 13,300), 352 mμ (ϵ 7,240);

0.1 N HCl, λ_{max} 220 mμ (ϵ 18,050), 244–252 mμ (ϵ 8,600), 323 mμ (ϵ 9,800).

2-(2-Carboxyethylamino)-4-hydroxypteridine-6-carboxylic Acid (IVb).—A solution of 3.0 g. (11.5 mmoles) of 7,11-dioxo-6,7,8,9-tetrahydro-11H-pyrimido(2,1-b)pteridine-2-carboxylic acid (IIb) in 120 ml. of 0.5 N sodium hydroxide was warmed to 50–60° for one hour, treated with Norit and filtered. The filtrate was heated to 75° and acidified with 5.5 ml. of concentrated hydrochloric acid. The product crystallized while still warm. The product was collected after cooling; yield 2.6 g. (81%); R_f 0.86 (0.5% Na$_2$CO$_3$), 0.79 (3% NH$_4$Cl); ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 273 mμ (ϵ 24,000), 370 mμ (ϵ 10,600); 0.1 N HCl, λ_{max} 241 mμ (ϵ 10,300), 257 mμ (ϵ 9,800), 301 mμ (ϵ 17,000), 337 mμ (ϵ 7,700).

Anal. Calcd. for C$_{10}$H$_9$N$_5$O$_5$ (279): C, 43.0; H, 3.2; N, 25.1. Found: C, 42.9; H, 3.7; N, 25.2.

2-(2-Carboxyethylamino)-4-hydroxy-6,7-dimethylpteridine (IVa).—One hundred mg. (0.41 mmole) of 2,3-dimethyl-8,9-dihydro-11H-pyrimido(2,1-b)pteridine-7(6H),11-dione (IIa) was added to 4 ml. of 0.5 N sodium hydroxide and heated at 60–70° for one hour. The solution was acidified at 85° with 0.2 ml. of concentrated hydrochloric acid, chilled overnight and filtered; yield 100 mg. (92.5%). Recrystallization of this material from 10 ml. of water (Norit) gave 80 mg. (75%) of product, R_f 0.83 (0.5% Na$_2$CO$_3$); ultraviolet spectra in 0.1 N NaOH, λ_{max} 260 mμ (ϵ 17,650), 365 mμ (ϵ 6,780); 0.1 N HCl, λ_{max} 218 mμ (ϵ 15,100), 250 mμ (ϵ 8,900), 283 mμ (ϵ 5,450), 322 mμ (ϵ 6,120).

Anal. Calcd for C$_{11}$H$_{13}$N$_5$O$_3$ (263): C, 50.2; H, 4.98; N, 26.6. Found: C, 50.5; H, 5.20; N, 26.8.

2-(2-Carboxyethylamino)-4-hydroxy-6-methylpteridine (IVc).—A solution of 600 mg. (2.6 mmoles) of 7-amino-2-methyl-8,9-dihydro-11H-pyrimido(2,1-b)pteridine-11-one (XII) in 10 ml. of 0.5 N sodium hydroxide was heated on the steam-bath for 50 minutes. This was treated with Norit, filtered and acidified with 0.7 ml. of acetic acid and 0.5 ml. of concentrated hydrochloric acid. Cooling overnight gave 320 mg. of crystalline product which was recrystallized from 6 ml. of water; yield 250 mg. (38%); R_f 0.82 (0.5% Na$_2$CO$_3$), 0.75 (3% NH$_4$Cl); ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 252 mμ (ϵ 24,000), 362 mμ (ϵ 7,000); 0.1 N HCl, λ_{max} 237 mμ (ϵ 14,200), 326 mμ (ϵ 6,800).

Anal. Calcd. for C$_{10}$H$_{11}$N$_5$O$_3$ (249): C, 48.2; H, 4.4; N, 28.1. Found: C, 47.9; H, 4.9; N, 27.9.

N-[4-(N-[(2-[2-Carboxyethylamino]-4-hydroxy-6-pteridyl)-methyl]-amino)-benzoyl]-glutamic Acid (N^2-Carboxyethyl-pteroylglutamic Acid).—A solution of 20 g. (40 mmoles) of the pteroylglutamic acid–acrylamide product (XIII) in 800 ml. of 1.0 N sodium hydroxide was heated to 50° for 30 minutes. This was treated with Norit and filtered. Concentrated hydrochloric acid (40 ml.) was added, the solution was heated to 70° and then acidified with another 40 ml. of hydrochloric acid. The solution was cooled overnight, the product collected, washed with water, acetone and ether and dried; yield 11.7 g. (56%), chemical assay 78%.[9]

A portion of this material (9.7 g.) was dissolved in 45 ml. of concentrated hydrochloric acid, treated with Norit and filtered. The filtrate was poured into 550 ml. of warm water and cooled; yield 4.5 g.

A sample (1.5 g.) of this product was dissolved in 400 ml. of water by adding 10 N sodium hydroxide. This was reacidified with hydrochloric acid, warmed to 70° and magnesium oxide added to pH 8-9. Norit (0.5 g.) was added and after several minutes the mixture was filtered at 65°. The filtrate was acidified until a solid appeared. The temperature was then raised to 90° and hydrochloric acid added until complete solution was obtained. Cooling gave 1.0 g. of crystalline product. Chemical assay on dried material was 96.5% as its hemihydrate; ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 268 mμ (ϵ 32,400), 281 mμ (shoulder) (ϵ 29,800, 373 mμ (ϵ 9,500); 0.1 N HCl, λ_{max} 288 mμ (ϵ 22,300); pH 7.0, λ_{max} 285 mμ (ϵ 27,700), 360 mμ (ϵ 6,400).

Anal. Calcd. for C$_{22}$H$_{23}$N$_7$O$_8$·^{1}/$_2$H$_2$O (522): C, 50.5; H, 4.6; N, 18.8. Found: C, 50.2; H, 4.8; N, 18.7.

2,3,6-Trimethyl-8,9-dihydro-11H-pyrimido(2,1-b)pteridine-7(6H),11-dione (X).—A solution of 4.7 g. (23 mmoles) of 2-methylamino-4-hydroxy-6,7-dimethylpteridine,[3,4] 10 ml. of acrylonitrile and 110 ml. of pyridine in 110 ml. of

water was heated on a steam-bath for 48 hours. An additional 35 ml. of acrylonitrile was added in 5-ml. portions at intervals during the heating. The solution then was evaporated to an oil *in vacuo*, water added and the solution evaporated again. The oil was dissolved in 90 ml. of water, heated to boiling, treated with Norit and filtered. The filtrate was acidified with 2 ml. of acetic acid and then concentrated hydrochloric acid was added to pH 2. The solution was heated to boiling and then cooled overnight; yield of crystalline product (needles) 2.4 g. (40%).

A portion of this material (200 mg.) was recrystallized from 5 ml. of water; yield 150 mg. This material was a hydrate. After drying at 130° in a pistol it had a m.p. of 249–251° (cor.). The material before drying and after drying had the same chromatography and absorption spectra; R_f 0.69 (3% NH$_4$Cl); ultraviolet absorption spectra in 0.1 M Na$_2$B$_4$O$_7$, λ_{max} 240 mμ (ϵ 17,100), 282 mμ (ϵ 16,000), 328 mμ (ϵ 8,300); methanol, λ_{max} 240 mμ (ϵ 15,800), 282 mμ (ϵ 16,000), 330 mμ (ϵ 7,900); 0.1 N HCl, λ_{max} 242 mμ (ϵ 16,000) 284 mμ (ϵ 15,300), 330 mμ (ϵ 8,000).

Anal. Calcd. for C$_{12}$H$_{13}$N$_5$O$_2$ (259): C, 55.6; H, 5.1; N, 27.0. Found: C, 55.4; H, 5.3; N, 27.1.

When X was dissolved in 0.1 N NaOH the ultraviolet absorption spectra in alkali and acid became essentially the same as for 2-propylamino-3,6,7-trimethyl-4-pteridone,[4] 0.1 N NaOH, λ_{max} 245 mμ (ϵ 14,700), 280 mμ (ϵ 15,800), 357 mμ (ϵ 7,200); 0.1 N HCl, λ_{max} 221 mμ (ϵ 16,000), 253 mμ (shoulder) (ϵ 9,600), 280–285 (plateau) (ϵ 5,800), 320 mμ (ϵ 7,800), 330 mμ (shoulder) (ϵ 7,200). Thus alkali converted X into 3-(2-carboxyethyl)-2-methylamino-6,7-dimethyl-4-pteridone (XI).

6-(2-Carboxyethyl)-7,11-dioxo-6,7,8,9-tetrahydro-11H-pyrimido(2,1-b)pteridine-2-carboxylic Acid (V).—2-(2-Carboxyethylamino)-4-hydroxypteridine-6-carboxylic acid (8.4 g., 30 mmoles) was dissolved in a solution of 200 ml. of water, 200 ml. of pyridine and 10 ml. of acrylonitrile and heated to reflux for 52 hours. During this period of refluxing, a 10-ml. portion of acrylonitrile was added every 8 hours. Samples were removed at intervals and chromatograms were run in order to determine when the reaction was complete. After 52 hours the solution was evaporated to a sirup, water added and the solution evaporated a second time. The resulting sirup was dissolved in 100 ml. of warm water, treated with Norit and filtered. The filtrate was acidified with 10 ml. of acetic acid and 6.5 ml. of concentrated hydrochloric acid to pH 1.5. After cooling overnight the crystalline product was collected, washed with water, acetone and ether and dried; yield 6.2 g. (62%). This was redissolved in 180 ml. of hot water, treated with Norit and filtered. The filtrate was cooled a short time until it became cloudy. It was decolorized again with Norit and filtered. The filtrate at 40° was acidified with 1 ml. of concentrated hydrochloric acid and cooled overnight; yield of yellow crystalline product 4.7 g., m.p. 267–269° dec.; melts and resolidifies when placed in bath at 200°. Chromatography showed that this was a mixture of the cyclic compound V and the related open-chain compound VI.

This mixture was suspended in 115 ml. of 1 N hydrochloric acid and heated to boiling for 5 minutes. The solid dissolved and a new crystalline product appeared. The mixture was cooled and the product collected; yield 3.8 g. (38%) of a cream-colored solid, m.p. 276–277° dec.; does not melt when placed in bath at 200°. Chromatography indicated that this was the pure cyclic compound V.

A portion (0.4 g.) of this material was recrystallized from 35 ml. of water; yield 0.3 g., m.p. 277–278° dec. (cor.), R_f 0.86 (3% NH$_4$Cl); ultraviolet absorption spectra in 0.1 M Na$_2$B$_4$O$_7$, λ_{max} 249 mμ (ϵ 15,650), 292 mμ (ϵ 15,650), 330 mμ (ϵ 9,800); 0.1 N HCl, λ_{max} 253 mμ (ϵ 16,100), 300 mμ (ϵ 14,300), 330 mμ (ϵ 11,200). The spectra could not be determined in 0.1 N NaOH due to the very facile ring-opening to VI.

Anal. Calcd. for C$_{13}$H$_{11}$N$_5$O$_6$ (333): C, 46.8; H, 3.3; N, 21.0. Found: C, 46.5; H, 3.3; N, 20.7.

2-(2-Carboxyethylamino)-3-(2-carboxyethyl)-4-oxo-3,4-dihydropteridine-6-carboxylic Acid (VI).—A solution of 100 mg. (0.3 mmole) of V and 300 mg. of Na$_2$B$_4$O$_7$ in 2 ml. of water (pH 8.4) was allowed to stand at room temperature for three weeks. The solution then was cooled in an ice-bath and acidified with 1.5 ml. of 1.0 N hydrochloric acid. The product crystallized quickly; yield 80 mg.; ultraviolet absorption spectra in 0.1 N NaOH, λ_{max} 246 mμ (ϵ 11,000),

(9) B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705 (1947).

297 mμ (ε 18,300), 362 mμ (ε 8,300); 0.1 N HCl, λ_{max} 244 mμ (ε 9,800), 303 mμ (ε 17,300), 361 mμ (ε 7,700).

Attempts to dry this compound for analysis caused some ring closure to V. When a sample was recrystallized from a dilute acid solution it also was converted again to the pyrimidopteridine (V), as shown by ultraviolet absorption spectra.

2-Acetamido-3,6,7-trimethyl-4(3H)-pteridone.—One gram (4.9 mmoles) of 2-amino-3,6,7-trimethyl-4(3H)-pteridone[4] was refluxed for 4 hours in 20 ml. of acetic anhydride protected with a tube of Drierite. The hot solution was filtered from a small amount of insoluble material. On cooling, the filtrate deposited crystals; yield 0.25 g. (20.5%), m.p. 196.5–199°. An additional 0.30 g. (45% total), m.p. 195–

198°, was obtained by concentrating the mother liquor. For analytical purposes a portion of the product was recrystallized from 95% ethanol: R_f 0.87 (yellow-green) in 0.5% Na_2CO_3; ultraviolet spectra in 0.1 N NaOH, λ_{max} 246 mμ (ε 15,920), 287 mμ (ε 12,450), 340 mμ (ε 7,160); 0.1 N HCl, λ_{max} 239 mμ (ε 15,680), 281 mμ (ε 9,130), 316 mμ (ε 7,750); methanol, λ_{max} 242 mμ (ε 12,600), 275 mμ (ε 10,600), 323 mμ (ε 7,300).

Anal. Calcd. for $C_{11}H_{13}N_5O_2$ (247): C, 53.4; H, 5.3; N, 28.3. Found: C, 53.8; H, 5.6; N, 28.7.

Pearl River, N. Y.

The 1,2,4-Thiadiazine Ring System. I. The Synthesis of 1,2,4,2H-Thiadiazine-3,5(4H,6H)-dione-1,1-dioxide[1]

By R. L. Hinman[2] and Louis Locatell, Jr.[3]

1,2,4,2H-Thiadiazine-3,5(4H,6H)-dione-1,1-dioxide (I) has been synthesized *via* the sequence: sulfoacetic acid (III), chlorosulfonylacetic acid (V), ethyl sulfamylacetate (VII), carbethoxymethanesulfonylurea (VIb) and base-catalyzed ring closure of the last compound to I. The product I, which was characterized by analysis, neutralization equivalent and infrared spectrum, is a stable, crystalline solid, resembling barbituric acid (II) in its properties, particularly its marked acidity (pK_a' 2.7). Other attempted syntheses of I are discussed.

Analogs of naturally-occurring pyrimidines and purines have provoked considerable interest because of their potential activity as antimetabolites of the naturally-occurring heterocycles. We have been interested in preparing pyrimidine and purine analogs in which one or more ring carbons have been replaced by sulfur atoms. As our first approach, we chose the synthesis of 1,2,4,2H-thiadiazine-3,5(4H,6H)-dione-1,1-dioxide (I), which is formally related to barbituric acid (II).

Although little has been reported on the 1,2,4-thiadiazine ring system, the synthesis of I has been the subject of three previous investigations.[4–6] Since these reports conflict on several points, they will be summarized briefly before the present work is discussed. Bodendorf and Senger[4] investigated several approaches to the synthesis of I, modeled after the classical synthesis of barbituric acid. They found that in the condensation of urea with either the diethyl ester or diacid chloride of sulfoacetic acid (III), the urea was acylated only by the carboxyl end of the sulfoacetic acid derivative.[7]

(1) Taken from the Ph.D. thesis of Louis Locatell, Jr., State University of Iowa, June, 1957. Presented before the Organic Division of the American Chemical Society at the Chicago Meeting, September, 1958.

(2) Union Carbide Research Institute, 32 Depot Plaza, White Plains, N. Y.

(3) Bakelite Co. Fellow, 1956–1957.

(4) K. Bodendorf and N. Senger. *Ber.*, **72B**, 571 (1939).

(5) J. B. Dickey, U. S. Patent 2,466,396, April 5, 1949 (to Eastman Kodak Co.); *C. A.*, **43**, 4868d (1949).

(6) P. N. Rylander and E. Campaigne, *J. Org. Chem.*, **15**, 249 (1950).

(7) Since alkyl esters of sulfonic acids are generally alkylating rather than acylating agents, acylation by the sulfonic end would not be expected in any case.
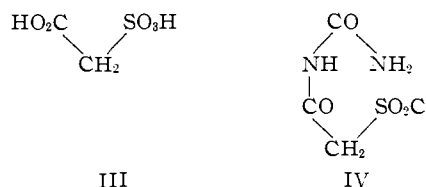
Thus, the reaction of urea with sulfoacetic diacid chloride yielded IV. Attempts to effect thermally the cyclization of IV to I resulted only in decomposition of IV. Seeking to take advantage of the greater reactivity of the carboxylic function, the authors assumed that partial hydrolysis of the diacid chloride of sulfoacetic acid would yield chlorosulfonylacetic acid (V), which might react with urea to give VIa, the isomer of IV. Ring closure to I
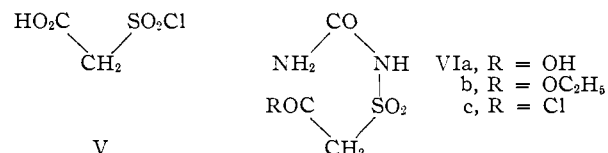
might then be effected through the more reactive carboxylic acid ester VIb or acid chloride VIc. Partial hydrolysis of the diacid chloride of sulfoacetic acid yielded a compound which analyzed for V. However, treatment of this product with urea or aniline yielded products in which the nitrogen had been acylated by the carboxylic acid end. Bodendorf and Senger concluded that the sulfonyl chloride group of the diacid chloride of sulfoacetic acid is hydrolyzed more easily than the carboxylic acid chloride end.[8] They abandoned the project at this point.

(8) Subsequent work has shown that this conclusion is erroneous, and that the sulfonyl chloride end is, as would be expected, the more stable under hydrolytic conditions. See R. Vieillefosse. *Compt. rend.*, **208**, 1406 (1939), and the remainder of the present paper.