688. *Hydropteridines. Part V.*[1] *7,8-Dihydro-6-hydroxypteridine-4-carboxylic Acids.*

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The preparation of some 2-substituted 7,8-dihydro-6-hydroxypteridine-4-carboxylic acids and their ethyl esters is described.

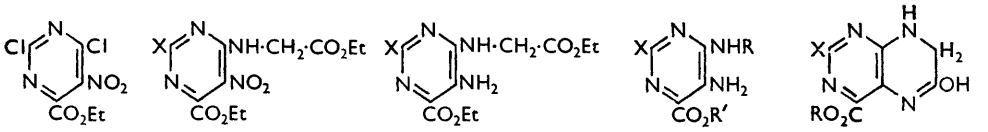
Many pteridine-6- and -7-carboxylic acids have been described. Some were isolated from natural sources [2] while others have been synthesised. Synthetical routes include condensation of 4,5-diaminopyrimidines with dicarbonyl compounds such as ethyl mesoxalate,[3,4] and reaction of 4-amino-5-nitrosopyrimidines with ethyl malonate or cyanoacetate.[5] Pteridine-6- and -7-carboxylic acids have also been obtained by oxidation

[1] Brook and Ramage, Part IV, *J.*, 1957, 1.
[2] Forrest and Mitchell, *J. Amer. Chem. Soc.*, 1955, **77**, 4865. Viscontini, Schoeller, Loeser, Karrer, and Hadorn, *Helv. Chim. Acta*, 1955, **38**, 397.
[3] Purrmann, *Annalen*, 1941, **548**, 284; Elion, Hitchings, and Russell, *J. Amer. Chem. Soc.*, 1950, **72**, 78. Elion and Hitchings, *ibid.*, 1953, **75**, 4311.
[4] Whittle, O'Dell, Vandenbelt, and Pfiffner, *J. Amer. Chem. Soc.*, 1947, **69**, 1786.
[5] Osdene and Timmis, *J.*, 1955, 2036.

of suitable 6- or 7-substituted pteridines.[4,6,7] These acids have been used as reference compounds for the identification of degradation products of pteridine pigments [7,8] and pteroylglutamic acid and related compounds.[4,9]

Although many 6- and 7-carboxylic acids are well known, no pteridine-2- or -4-carboxylic acid has been reported. The lack of suitable pyrimidine intermediates has been partly responsible but 2,6-dichloro-5-nitropyrimidine-4-carboxylic esters [10] now provide starting materials for the synthesis of pteridine-4-carboxylic acids.

Ethyl 2,6-dichloro-5-nitropyrimidine-4-carboxylate (I) has been converted into 7,8-dihydro-6-hydroxypteridine-4-carboxylic acids by a route which is essentially that used by Boon, Jones, and Ramage [11] to prepare 6-hydroxypteridines. The dichloro-compound (I) was condensed with ethyl aminoacetate, and the 2-chlorine atom of the product (II; X = Cl) replaced by a variety of groups. An amino- or dimethylamino-group was introduced by reaction with the appropriate amine, and a hydroxyl group by reaction with boiling acetate buffer, whilst treatment with sodium sulphide and sodium hydrogen carbonate yielded the 2-mercapto-derivative (II; X = SH). The corresponding 2-ethoxy-compound was obtained by boiling the chloro-compound with ethanol and an equivalent of pyridine.



(I) (II) (III) (IV) (V)

Use of Raney nickel and hydrogen to reduce the 5-nitropyrimidines was less satisfactory than for similar pyrimidines with no ester group,[11] and this reflected the experience of Clark and Ramage [10] in reductions of 6-2′-chloroethylamino-5-nitropyrimidine-4-carboxylic esters. Reduction by sodium dithionite in sodium hydrogen carbonate solution gave good results except for the 2-chloro-compound (II; X = Cl) which was better reduced by zinc and a little acetic acid in methanol. Chloroform solutions of some 5-aminopyrimidines (III; X = Cl, OH, or OEt) exhibited a strong blue and others (III; X = NH_2 or NMe_2) a strong green fluorescence in ultraviolet light but the 2-mercapto-compound (III; X = SH) was not fluorescent. Fluorescence appears to be a general property of 5,6-diaminopyrimidine-4-carboxylic esters for it was shown by the above compounds, by 5-amino-6-2′-chloroethylaminopyrimidine-4-carboxylic esters (IV; R = CH_2·CH_2Cl),[10] by 6-acetonylamino-5-aminopyrimidine-4-carboxylic esters (IV; R = CH_2Ac),[12] and by 5,6-diaminopyrimidine-4-carboxylic esters (IV; R = H).[12] The pronounced effect, frequently visible in daylight, appeared immediately reduction of the 5-nitro-compounds was commenced and so was unlikely to be due to oxidative self-condensation reactions which can lead to fluorescent impurities in 5-amino-4-substituted-aminopyrimidines kept in non-reducing conditions.

The ethyl 5-amino-6-ethoxycarbonyl-4-pyrimidylaminoacetates (III) were sufficiently stable to be crystallised without interference by cyclisation and therefore differed from similar compounds with no ester group.[11] The less ready cyclisation of these compounds may have been due to the base-weakening effect of the ester group on the 5-amino-group

[6] Cain, Mallette, and Taylor, *J. Amer. Chem. Soc.*, 1948, **70**, 3026; Forrest and Walker, *J.*, 1949, 79, 2077; Backer and Houtman, *Rec. Trav. chim.*, 1951, **70**, 725.
[7] Tschesche and Korte, *Chem. Ber.*, 1951, **84**, 77.
[8] Hirata, Nawa, Matsuura, and Kakizawa, *Experientia*, 1952, **8**, 339.
[9] Stokstad, Hutchings, Mowat, Boothe, Waller, Angier, and Subba Row, *J. Amer. Chem. Soc.*, 1948, **70**, 5; Mowat, Boothe, Hutchings, Stokstad, Waller, Angier, Semb, Cosulich, and Subba Row, *ibid.*, p. 14.
[10] Clark and Ramage, *J.*, 1958, 2821.
[11] Boon, Jones, and Ramage, *ibid.*, 1951, 96.
[12] Clark, unpublished results.

and to hydrogen bonding. The compound (III; X = Cl), in which the base-weakening effect of the ester group was supplemented by that of a 2-chlorine atom, was the most stable.

With the exception of the last-named compound, which was recovered unchanged from boiling ethanol, water, or 2-ethoxyethanol, the 5-amino-4-pyrimidylaminoacetates were cyclised in water or aqueous ethanol. The chloro-compound (III; X = Cl) required dilute acetic acid to promote the reaction.

The resulting 7,8-dihydro-6-hydroxypteridinecarboxylic esters were readily hydrolysed by alkali to the corresponding acids but it was necessary to use mild conditions to prevent the easy cleavage of the dihydropyrazine rings of some of these compounds.

Experimental

Ethyl 2-Chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (II; X = Cl).—Ethyl aminoacetate hydrochloride (15 g.) was added, with shaking, during 20 min. to a mixture of ethyl 2,6-dichloro-5-nitropyrimidine-4-carboxylate [10] (27 g.) in chloroform (250 c.c.) and sodium hydrogen carbonate (25 g.) in water (100 c.c.). Shaking was continued for a further 15 min. before the chloroform layer was separated, washed with water, dried, and evaporated. *Ethyl 2-chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate* (29 g.) crystallised from ethanol as needles, m. p. 99° (Found: C, 39·6; H, 4·0. $C_{11}H_{13}O_6N_4Cl$ requires C, 39·7; H, 3·9%).

Ethyl 5-Amino-2-chloro-6-ethoxycarbonyl-4-pyrimidylaminoacetate (III; X = Cl).—To a stirred solution of ethyl 2-chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (4·5 g.) in methanol (120 c.c.) was added acetic acid (30 c.c.) immediately followed by powdered zinc (15 g.). The mixture was well stirred for 5 min. and quickly filtered. The residue was washed with warm methanol, and the combined filtrates concentrated under reduced pressure. Water (200 c.c.) was added, and *ethyl 5-amino-2-chloro-6-ethoxycarbonyl-4-pyrimidylaminoacetate* (3 g.) was filtered off and crystallised from ethanol as prisms, m. p. 178—180° (Found: C, 43·5; H, 4·9. $C_{11}H_{15}O_4N_4Cl$ requires C, 43·6; H, 5·0%).

Ethyl 2-Chloro-7,8-dihydro-6-hydroxypteridine-4-carboxylate (V; X = Cl, R = Et).—A solution of ethyl 5-amino-2-chloro-6-ethoxycarbonyl-4-pyrimidylaminoacetate (1 g.) in dilute acetic acid (50 c.c.) was heated under reflux for 1 hr. *Ethyl 2-chloro-7,8-dihydro-6-hydroxypteridine-4-carboxylate* (0·62 g.) was filtered off and crystallised from aqueous ethanol, forming needles which gradually decomposed above 250° (Found: C, 41·5; H, 3·5; Cl, 13·8. $C_9H_9O_3N_4Cl$ requires C, 42·1; H, 3·5; Cl, 13·8%).

An *acetyl* derivative crystallised from aqueous ethanol as blades, m. p. 153—154° (Found: C, 44·0; H, 3·7. $C_{11}H_{11}O_4N_4Cl$ requires C, 44·2; H, 3·7%); the picrate dissociated on recrystallisation.

Ethyl 2-Ethoxy-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (II; X = OEt).—Ethyl 2-chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (5 g.), ethanol (100 c.c.), and pyridine (1·3 c.c.) were heated under reflux for 3 hr. The cooled solution was acidified with N-hydrochloric acid and diluted with water, and *ethyl 2-ethoxy-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate* (4·6 g.) was washed with water and crystallised from ethanol, forming needles, m. p. 93—94° (Found: C, 46·0; H, 5·3. $C_{13}H_{18}O_7N_4$ requires C, 45·6; H, 5·3%).

Ethyl 2-Ethoxy-7,8-dihydro-6-hydroxypteridine-4-carboxylate (V; X = OEt, R = Et).—To a well-stirred solution of ethyl 2-ethoxy-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (4·6 g.) in acetone (100 c.c.) was added sodium hydrogen carbonate (20 g.) in water (100 c.c.), followed by sodium dithionite (20 g.) added during 5 min. After a further 10 min. the mixture was diluted with water (200 c.c.) and extracted with chloroform. The extract was washed with water, dried, and evaporated to yield *ethyl 5-amino-2-ethoxy-6-ethoxycarbonyl-4-pyrimidylaminoacetate* (3·2 g.); a sample of which crystallised from benzene–cyclohexane as pale cream needles, m. p. 155° (with rapid heating) (Found: C, 50·0; H, 6·5. $C_{13}H_{20}O_5N_4$ requires C, 50·0; H, 6·5%).

This amine (3 g.) was heated in refluxing aqueous ethanol during 1 hr.; *ethyl 2-ethoxy-7,8-dihydro-6-hydroxypteridine-4-carboxylate* (2·2 g.) was filtered off and crystallised from aqueous ethanol, forming needles which gradually decomposed above 240° (Found: C, 49·1; H, 5·2. $C_{11}H_{14}O_4N_4$ requires C, 49·6; H, 5·3%).

A *monoacetyl* derivative crystallised from aqueous ethanol as needles, m. p. 150° (Found:

C, 50·4; H, 5·1; N, 18·5. $C_{13}H_{16}O_5N_4$ requires C, 50·6; H, 5·2; N, 18·2%). The *picrate* crystallised from ethanol, containing a little picric acid, as yellow needles, m. p. 182° (decomp.) (Found: C, 40·8; H, 3·5. $C_{11}H_{14}O_4N_4,C_6H_3O_7N_3$ requires C, 41·2; H, 3·5%).

Ethyl 2-Dimethylamino-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (II; X = NMe_2).—A solution of 26% aqueous dimethylamine (2·2 c.c.) in ethanol (5 c.c.) was added dropwise to a stirred solution of ethyl 2-chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (2 g.) in ethanol (30 c.c.), and the mixture stirred for a further 15 min. *Ethyl 2-dimethylamino-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate* was filtered off and crystallised from ethanol, forming needles (1·9 g.), m. p. 135° (Found: C, 46·1; H, 5·4. $C_{13}H_{19}O_6N_5$ requires C, 45·8; H, 5·6%).

Ethyl 2-Dimethylamino-7,8-dihydro-6-hydroxypteridine-4-carboxylate (V; X = NMe_2, R = Et).—Ethyl 2-dimethylamino-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (5 g.) was reduced as described for the corresponding 2-ethoxy-compound, except that the resulting amino-derivative (3·6 g.) was recovered by filtration. *Ethyl 5-amino-2-dimethylamino-6-ethoxycarbonyl-4-pyrimidylaminoacetate* crystallised from benzene as greenish-yellow needles, m. p. 139° (Found: C, 50·3; H, 6·6. $C_{13}H_{21}O_4N_5$ requires C, 50·1; H, 6·8%). The *acetyl* derivative crystallised from water as needles, m. p. 123° (Found: C, 48·5; H, 6·7. $C_{15}H_{23}O_5N_5,H_2O$ requires C, 48·5; H, 6·8%).

The amine (1·0 g.) was cyclised in boiling water during 3 hr. and the resulting *ethyl 2-dimethylamino-7,8-dihydro-6-hydroxypteridine-4-carboxylate* (0·5 g.) crystallised from dioxan, forming greenish-yellow needles which gradually decomposed above 260° (Found: C, 49·6; H, 5·8. $C_{11}H_{15}O_3N_5$ requires C, 49·8; H, 5·7%).

A *monoacetyl* derivative crystallised from water as yellow needles, m. p. 177—178° (Found: C, 51·1; H, 5·8; N, 22·4. $C_{13}H_{17}O_4N_5$ requires C, 50·8; H, 5·6; N, 22·8%). The *picrate* crystallised from ethanol, containing a little picric acid, as yellow needles which gradually decomposed above 170° (Found: C, 41·5; H, 3·6. $C_{11}H_{15}O_3N_5,C_6H_3O_7N_3$ requires C, 41·3; H, 3·7%).

Ethyl 2-Amino-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (II; X = NH_2).—A solution of ethyl 2-chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (5 g.) in ethanol (25 c.c.) was treated with ammonia (1·67 c.c., s.g. 0·88) and the solution heated under reflux for ½ hr. *Ethyl 2-amino-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate* (4 g.) was filtered off and crystallised from ethanol, forming needles, m. p. 161° (Found: C, 41·8; H, 4·8. $C_{11}H_{15}O_6N_5$ requires C, 42·2; H, 4·7%).

Ethyl 2-Amino-7,8-dihydro-6-hydroxypteridine-4-carboxylate (V; X = NH_2, R = Et).—Ethyl 2-amino-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (3 g.) was reduced as described for the corresponding 2-ethoxy-compound and yielded *ethyl 2,5-diamino-6-ethoxycarbonyl-4-pyrimidylaminoacetate* (2·1 g.) which crystallised from benzene as pale yellow needles, m. p. 171—172° (with rapid heating) (Found: C, 46·5; H, 5·8. $C_{11}H_{17}O_4N_5$ requires C, 46·6; H, 6·1%).

The diamine (1·25 g.) was cyclised in boiling water during 2 hr. and yielded ethyl 2-amino-7,8-dihydro-6-hydroxypteridine-4-carboxylate (0·55 g.) as a pale-brown amorphous powder (decomp. >230°) which did not dissolve in the usual solvents. Attempts to purify the material by precipitation from alkali led to hydrolysis of the ester group and so it was characterised as the corresponding carboxylic acid (below).

Ethyl 6-Ethoxycarbonyl-2-hydroxy-5-nitro-4-pyrimidylaminoacetate (II; X = OH).—A mixture of ethyl 2-chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (2·5 g.), sodium acetate trihydrate (4 g.), water (7·5 c.c.), and acetic acid (7·5 c.c.) was heated under reflux during 15 min. *Ethyl 6-ethoxycarbonyl-2-hydroxy-5-nitro-4-pyrimidylaminoacetate* (1·6 g.) was filtered off from the cooled solution; a sample crystallised from water as needles, m. p. 190° (decomp.) (Found: C, 42·2; H, 4·4. $C_{11}H_{14}O_7N_4$ requires C, 42·0; H, 4·5%).

Ethyl 7,8-Dihydro-2,6-dihydroxypteridine-4-carboxylate (V; X = OH, R = Et).—Ethyl 6-ethoxycarbonyl-2-hydroxy-5-nitro-4-pyrimidylaminoacetate (1·6 g.) was reduced as described for the corresponding 2-ethoxy-compound, except that it was unnecessary to use acetone. The resulting *ethyl 5-amino-6-ethoxycarbonyl-2-hydroxy-4-pyrimidylaminoacetate* (0·82 g.) crystallised from tetrahydrofuran as prisms which gradually decomposed above 150° (Found: C, 46·1; H, 5·7. $C_{11}H_{16}O_5N_4$ requires C, 46·5; H, 5·7%).

The amine (1 g.) was cyclised in boiling water during 2 hr. and yielded *ethyl 7,8-dihydro-2,6-dihydroxypteridine-4-carboxylate* (0·5 g.) which crystallised from water as plates which

gradually decomposed above 240° (Found: C, 45·0; H, 4·0. $C_9H_{10}O_4N_4$ requires C, 45·4; H, 4·2%).

The *picrate* crystallised from water as yellow needles which gradually decomposed above 190° (Found: C, 38·6; H, 2·8. $C_9H_{10}O_4N_4,C_6H_3O_7N_3$ requires C, 38·6; H, 2·7%).

Ethyl 7,8-Dihydro-6-hydroxy-2-mercaptopteridine-4-carboxylate (V; X = SH, R = Et).—Ethyl 2-chloro-6-ethoxycarbonyl-5-nitro-4-pyrimidylaminoacetate (5 g.) was stirred with a solution of sodium sulphide nonahydrate (4 g.) and sodium hydrogen carbonate (1·3 g.) in water (200 c.c.) and acetone (5 c.c.) for 3 hr; undissolved material was filtered off (*A*). Sodium hydrogen carbonate (18 g.) and sodium dithionite (20 g.) were added and after 10 min., *ethyl 5-amino-6-ethoxycarbonyl-2-mercapto-4-pyrimidylaminoacetate* (3·4 g.) was filtered off. A sample crystallised from ethanol as yellow prisms, m. p. 161—162° (Found: C, 43·4; H, 5·4. $C_{11}H_{16}O_4N_4S$ requires C, 44·0; H, 5·4%).

This amine (1 g.) was cyclised as described for the corresponding 2-amino-compound and the product, ethyl 7,8-dihydro-6-hydroxy-2-mercaptopteridine-4-carboxylate (0·7 g.), was a similarly intractable solid (yellow) which gradually decomposed above 230°. It was not possible to purify it by crystallisation or reprecipitation and it was characterised by hydrolysis to the 4-carboxylic acid (below).

(*A*) If the solution was acidified (5N-hydrochloric acid) at this point, *ethyl 6-ethoxycarbonyl-2-mercapto-5-nitro-4-pyrimidylaminoacetate* (II; X = SH) (4·5 g.) was precipitated. It crystallised from carbon tetrachloride as yellow prisms, m. p. 127—128° (Found: C, 39·8; H, 4·1. $C_{11}H_{14}O_6N_4S$ requires C, 40·0; H, 4·3%).

2-Substituted 7,8-Dihydro-6-hydroxypteridine-4-carboxylic Acids (V; R = H).—The corresponding ethyl esters (above) were hydrolysed with 2N-sodium hydroxide for 2 min., then cooled as necessary and acidified with dilute hydrochloric acid. The carboxylic acids were purified by repeated precipitation from sodium hydroxide solution or from a solution of a monosodium salt which had been crystallised from water.

Although no undue difficulty had been experienced with the corresponding esters, the carboxylic acids tended to be difficult to burn and held water strongly even when dried over phosphoric oxide *in vacuo* at 150° for 4 hr. The structures of hydrates of 2- and 6-hydroxypteridine and some of their derivatives have been discussed in detail.[13]

2-Substituted 7,8-dihydro-6-hydroxypteridine-4-carboxylic acids (V).

			Found, %			Reqd., %			
X	Decomp.	Formula	C	H	N	C	H	N	Remarks
Cl	>220°	$C_7H_5O_3N_4Cl$	37·1	2·3		36·8	2·2		Dried at 100°
NH_2	>260	$C_7H_7O_3N_5,H_2O$	37·0 36·8	4·1 3·9		37·0	4·0		Monohydrate after being dried at 150°
NMe_2	>240	$C_9H_{11}O_3N_5,H_2O$	42·9	5·0		42·4	5·1		Air dried
	>240	$C_9H_{11}O_3N_5,\frac{1}{2}H_2O$	43·9 44·1	4·8 4·7		43·9	4·9		Dried at 150°
OH	>250	$C_7H_6O_4N_4$	39·4	3·0	26·2	40·0	2·9	26·7	Dried at 150°
OEt	>220	$C_9H_{10}O_4N_4,5H_2O$	32·2	5·9		32·9	6·1		Air dried
	>220	$C_9H_{10}O_4N_4,\frac{1}{2}H_2O$	43·8	4·3		43·7	4·5		Dried at 150°
SH	>275	$C_7H_6O_3N_4S$	36·8	2·8		37·2	2·7		Dried at 100°
	>280	$C_7H_5O_3N_4SNa,H_2O$	31·8	2·8	21·1	31·6	2·7	21·0	Monosodium salt

Aqueous solutions of these acids generally exhibited a blue or purple fluorescence in ultraviolet light. This was less pronounced than that of the uncyclised amines, being imperceptible in daylight. The mercapto- (V; X = SH, R = H) and dimethylamino- (V; X = NMe_2, R = H) derivatives were exceptional; the former was non-fluorescent while the latter was fluorescent only as its mono- and di-sodium salts.

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[13] Brown and Mason, *J.*, 1956, 3443; Albert, Lister, and Pederson, *J.*, 1956, 4621; Fidler and Wood, *J.*, 1957, 3980.