Pteridine Derivatives. Part I. A New Synthesis of 2-Amino-4-hydroxypteridines.

By G. P. G. Dick and H. C. S. Wood.

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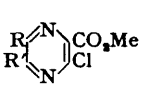
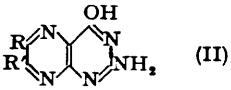
A new method is reported for the synthesis of 2-amino-4-hydroxypteridines, namely, condensation of 2-chloro-3-methoxycarbonylpyrazines with guanidine salts.

Only two methods have been reported in the literature for the synthesis of pteridine derivatives from pyrazine intermediates. The first, due to Gabriel and Sohn (*Ber.*, 1907, **40**, 4857), consisted in the treatment of pyrazine-2 : 3-dicarboxyamide with potassium hypobromite to give 2 : 4-dihydroxypteridine in poor yield. In the second, Albert, Brown, and Cheeseman (*J.*, 1951, 474) synthesised 4-hydroxy- and 4-mercapto-pteridine by heating 2-aminopyrazine-3-carboxyamide and the 3-thiocarboxyamide with ethyl orthoformate and acetic anhydride, a reaction since extended to the synthesis of *N*-substituted pteridines (*idem*, *J.*, 1952, 4219; Taylor, *J. Amer. Chem. Soc.*, 1952, **74**, 1651; Taylor, Carbon, and Hoff, *ibid.*, 1953, **75**, 1904).

The pyrazinecarboxyamides required for these syntheses were obtained by degradation of more complex molecules. We now report a pteridine synthesis using pyrazine intermediates which are readily available from aliphatic compounds. In addition, this method will enable the 2-position in a 2-amino-4-hydroxypteridine molecule to be labelled isotopically at the final stage in the synthesis.

Condensation of 2-chloro-3-methoxycarbonylpyrazines (I) with guanidine salts provides a convenient synthesis of 2-amino-4-hydroxypteridine derivatives (II). The chloro-esters are prepared from the 2-hydroxypyrazine-3-carboxyamides (Jones, *ibid.*, 1949, **71**, 80), which are readily available by condensation of aminomalonamide with 1 : 2-dicarbonyl compounds.

(I) 

 (II)

2-Hydroxypyrazine-3-carboxyamide was hydrolysed to the acid, the methyl ester of which with phosphorus oxychloride gave 2-chloro-3-methoxycarbonylpyrazine (I; R = R′ = H) in good yield. Condensation of the chloro-ester with guanidine was catalysed by sodium methoxide in boiling methanol or by potassium *tert.*-butoxide in boiling *tert.*-butyl alcohol, to give 2-amino-4-hydroxypteridine (II; R = R′ = H) in 20% yield. However, heating the chloro-ester and guanidine carbonate at 170° for 30 minutes gave an 89% yield. A similar fusion with urea did not give any 2 : 4-dihydroxypteridine.

The synthesis of 2-amino-4-hydroxy-6 : 7-diphenylpteridine (II; R = R′ = Ph) from the pyrazine (I; R = R′ = Ph) was carried out by fusion with guanidine; use of sodium methoxide, under the conditions used for the parent compound, gave none of the required pteridine, but good yields of 2-methoxy-5 : 6-diphenylpyrazine-3-carboxylic acid.

This new method could be applied to the synthesis of 2-amino-4-hydroxy-6-methylpteridine (II; R = Me, R′ = H), which is an intermediate in one of the most flexible methods for the synthesis of pteroylglutamic acid and its analogues (Boothe *et al.*, *ibid.*, 1948, **70**, 27. Roche Products Ltd., B.P. 624,394/1949). The starting material, 2-hydroxy-5-methylpyrazine-3-carboxyamide, has been obtained by the condensation of methyl glyoxal with aminomalonamide (Jones, *loc. cit.*). That this type of reaction gives only 3 : 5-disubstituted 2-hydroxypyrazines, and none of the isomeric 3 : 6-disubstituted compounds was confirmed by Spring and his co-workers (*J.*, 1949, 2707) who investigated the condensation of methylglyoxal and α-amino-hydroxamic acids.

In our hands, the condensation of methylglyoxal with aminomalonamide gave exclusively 2-hydroxy-6-methylpyrazine-3-carboxyamide. Aldehyde- and ketone-binding reagents (such as sodium hydrogen sulphite and hydrazine) which influence the orientation

when unsymmetrical dicarbonyl compounds are used in the synthesis of pteridines (Seeger, Cosulich, Smith, and Hultquist, *J. Amer. Chem. Soc.*, 1949, **71**, 1753; Forrest and Walker, *J.*, 1949, 2077) did not affect the orientation of our reaction. The use of sodium hydrogen sulphite facilitated the condensation however, and 2-hydroxy-6-methylpyrazine-3-carboxyamide was obtained in the absence of the usual basic catalyst.

Hydrolysis of the 6-methyl-amide gave 2-hydroxy-6-methylpyrazine-3-carboxylic acid which was converted into 2-chloro-3-methoxycarbonyl-6-methylpyrazine (I; R = H, R′ = Me) *via* the hydroxy-ester in the usual way. Fusion of the chloro-ester with guanidine carbonate gave 2-amino-4-hydroxy-7-methylpteridine (II; R = H, R′ = Me) in 80% yield.

The identity of the 2-amino-4-hydroxypteridines was confirmed in each instance by comparison of the infra-red spectrum with that of a specimen prepared by methods in the literature (cf. Mowat *et al.*, *ibid.*, 1948, **70**, 14; Cain, Mallette, and Taylor, *ibid.*, 1946, **68**, 1996; Forrest and Walker, *J.*, 1949, 83). The infra-red spectrum of 2-amino-4-hydroxy-7-methylpteridine (II; R = H, R′ = Me) was different from that of the 6-methyl isomer (II; R = Me, R′ = H) (*idem*, *ibid.*, p. 2080).

Experimental

Yields of substances that have no definite m. p. refer to the stage when they appeared homogeneous in paper chromatography in butanol–5N-acetic acid (7 : 3) on being viewed in ultra-violet light of wave-lengths 254 and 365 mμ.

Aminomalonamide.—Ethyl aminomalonate, the precursor of aminomalonamide, is best prepared on a large scale by the catalytic reduction of ethyl (hydroxyimino)malonate (Schipper and Day, *J. Amer. Chem. Soc.*, 1952, **74**, 350). It is readily converted into aminomalonamide by treatment with alcoholic ammonia (Jones, *J. Amer. Chem. Soc.*, 1949, **71**, 80).

2-Hydroxy-6-methylpyrazine-3-carboxyamide.—(*a*) Freshly prepared methylglyoxal (Riley, Morley, and Friend, *J.*, 1932, 1875) was treated with aminomalonamide as described by Jones (*loc. cit.*). The yellow sodium salt which separated was collected after 2 days at 0°, washed with a small volume of water, and suspended in moist acetone (250 c.c.). The mixture was adjusted to pH 4 with concentrated hydrochloric acid, and the buff solid (13·0 g., 70%) which separated was collected, washed with ice-cold water and acetone, and dried. Rigorous chromatographic examination of the product, and the mother-liquors, did not reveal any fluorescent material other than the solid isolated. Recrystallization from methanol (charcoal) gave *2-hydroxy-6-methylpyrazine-3-carboxyamide* as pale yellow needles, m. p. 219—220° (decomp.) (Found: C, 46·9; H, 4·7; N, 28·0. $C_6H_7O_2N_3$ requires C, 47·1; H, 4·6; N, 27·5%).

(*b*) Freshly prepared methylglyoxal (12 g.) in water (30 c.c.) was treated with sodium hydrogen sulphite (10 g.), and the mixture set aside at room temperature for 30 min. Finely powdered aminomalonamide (20 g.) was added, and the mixture was heated on a water-bath until the solid had dissolved. Refrigeration gave a semi-solid buff mass which was purified *via* the sodium salt as before [yield 70%; m. p. 219—220° (decomp.)].

2-Hydroxypyrazine-3-carboxylic Acids.—(*a*) 2-Hydroxypyrazine-3-carboxyamide (1·0 g.) (Jones, *loc. cit.*) and sodium hydroxide (1·0 g.) in ethanol (30 c.c.) were heated in a steel bomb at 170° for 6 hr. After cooling, the ethanol was evaporated, the residue dissolved in aqueous sodium hydrogen carbonate, and the solution treated with charcoal and filtered. The filtrate was acidified with concentrated hydrochloric acid to pH 4 and allowed to cool. The solid was collected, washed with ice-cold water, and dried at 100° (0·61 g., 60%). The m. p. was 218—219° (decomp.), undepressed when mixed with a specimen prepared from 2-aminopyrazine-3-carboxylic acid (Spoerri and Fibel, *ibid.*, 1948, **70**, 3908).

(*b*) Similar hydrolysis of the diphenyl-amide gave *2-hydroxy-5 : 6-diphenylpyrazine-3-carboxylic acid* (91%) as golden needles (from aqueous acetone), m. p. 216—217° (decomp.) (Found: C, 69·7; H, 3·9; N, 9·4. $C_{17}H_{12}O_3N_2$ requires C, 69·8; H, 4·1; N, 9·6%)

(*c*) The sodium salt of 2-hydroxy-6-methylpyrazine-3-carboxyamide (3·0 g.) was dissolved in 5N-sodium hydroxide (20 c.c.), and the solution was refluxed until evolution of ammonia had ceased (30 hr.). The solution was stirred and treated with concentrated hydrochloric acid until the pH was 7—8, then some silica was filtered off. The filtrate was taken to pH 4—5, treated with charcoal, and concentrated *in vacuo* to 10 c.c. in an atmosphere of nitrogen. The dark violet solution deposited needles on cooling (1·3 g., 50%). Crystallization from methanol containing a few drops of water gave 2-hydroxy-6-methylpyrazine-3-carboxylic acid as thick clear needles which became opaque on drying, m. p. 188—189° (decomp.) (Found: C, 47·0;

H, 4·0; N, 18·3. Calc. for $C_6H_6O_3N_2$: C, 46·8; H, 3·9; N, 18·2%), recorded by Jones (*loc. cit.*) as a tan powder, m. p. 183—184°.

2-Hydroxy-3-methoxycarbonylpyrazines.—(*a*) 2-Hydroxy-5 : 6-diphenylpyrazine-3-carboxylic acid (7·5 g.) in dry boiling methanol (300 c.c.) was treated with dry hydrogen chloride until it had completely dissolved (about 20 min.), and the solution was refluxed for a further 2 hr. When the reaction mixture was cooled, *2-hydroxy-3-methoxycarbonyl-5 : 6-diphenylpyrazine* was deposited as yellow needles. These were collected, and the mother-liquors concentrated to *ca.* 50 c.c. *in vacuo*, a second crop being obtained. Recrystallization from methanol gave the hydroxy-ester as fine yellow needles (6·65 g., 89%), m. p. 204—205° (Found : C, 70·7; H, 4·7; N, 8·9. $C_{18}H_{14}O_3N_2$ requires C, 70·6; H, 4·6; N, 9·1%).

(*b*) 2-Hydroxy-6-methylpyrazine-3-carboxylic acid was esterified as above, to give the *methyl ester* (100%) as clear needles (from methanol); m. p. 174—175° (decomp.) (Found : C, 49·8; H, 4·7; N, 16·6. $C_7H_8O_3N_2$ requires C, 50·0; H, 4·8; N, 16·7%).

2-Chloro-3-methoxycarbonylpyrazines.—(*a*) 2-Hydroxy-3-methoxycarbonylpyrazine was converted into the chloro-ester by treatment with phosphorus oxychloride. [This preparation was first carried out in the Department of Medical Chemistry, the Australian National University; Albert, Brown, and Wood, unpublished work.]

(*b*) 2-Hydroxy-3-methoxycarbonyl-5 : 6-diphenylpyrazine (3·5 g.) and redistilled phosphorus oxychloride (23 c.c.) containing one drop of concentrated sulphuric acid were mixed in a Carius tube. The mixture was heated at 110° (bath) for 10 min.; evolution of hydrogen chloride had then ceased. The tube was then sealed and heated at 160° for 5·5 hr. The dark yellow solution was poured on cracked ice (200 g.), and stirred for 30 min., during which a buff solid separated. This was collected, washed with water, and recrystallised from methanol, to give small plates of *2-chloro-3-methoxycarbonyl-5 : 6-diphenylpyrazine* (3·0 g., 81%), m. p. 113—114°. Recrystallization from methanol–light petroleum (b. p. 60—80°) followed by sublimation gave a sample of m. p. 116—116·5° (Found : C, 66·6; H, 4·0; N, 8·9; Cl, 11·5. $C_{18}H_{13}O_2N_2Cl$ requires C, 66·6; H, 4·0; N, 8·6; Cl, 10·9%).

The yield at 150° was only 50%, and at 190° 14%. The use of a mixture of phosphorus oxychloride and diethylaniline (cf. Kenner, Lythgoe, Todd, and Topham, *J.*, 1943, 574), or of phosphorus oxychloride and phosphorus pentachloride (Karmas and Spoerri, *J. Amer. Chem. Soc.*, 1952, **74**, 1580), was unsuccessful.

(*c*) 2-Hydroxy-3-methoxycarbonyl-6-methylpyrazine (0·3 g.) was refluxed with redistilled phosphorus oxychloride (6 c.c.) containing one drop of concentrated sulphuric acid for 5 hr. The mixture was poured on cracked ice (100 g.) and stirred for 30 min. Ethyl acetate (30 c.c.) was added to the ice-cold solution, and the pH was adjusted to 8 with concentrated aqueous ammonia. The aqueous layer was saturated with sodium chloride and was extracted with ethyl acetate for 24 hr. The extract was dried and evaporated to dryness *in vacuo* to give a brown gum. Crystallisation from methanol (charcoal) gave *2-chloro-3-methoxycarbonyl-6-methylpyrazine* (0·2 g., 61%) as small yellow needles, m. p. 82—83°. Recrystallization from light petroleum (b. p. 60—80°) gave plates, m. p. 84—85° (Found : C, 45·7; H, 3·4. $C_7H_7O_2N_2Cl$ requires C, 45·1; H, 3·8%).

2-Amino-4-hydroxypteridine.—(*a*) 2-Chloro-3-methoxycarbonylpyrazine (1·0 g.), finely powdered and mixed with guanidine carbonate (2·0 g.), was heated at 170° (bath) for 30 min., with occasional stirring; effervescence took place, and the mixture became brown. On cooling, the residual solid was dissolved in boiling water (50 c.c.), and the solution was filtered. After treatment with charcoal, and filtration, the boiling solution was brought to pH 5 with 3N-hydrochloric acid. The pale yellow solid which separated, was collected at 90—100°, washed with boiling water (25 c.c.) and ethanol (15 c.c.), and dried at 100° to give 2-amino-4-hydroxypteridine (0·84 g., 89%), m. p. > 360°.

A sample (0·37 g.) was dissolved in hot 2N-sodium hydroxide (1·8 c.c.), the solution filtered hot, and 10N-sodium hydroxide (1·8 c.c.) added to the filtrate. The yellow sodium salt, which separated on cooling, was collected, washed with 2·5N-sodium hydroxide, and air-dried to give fine yellow needles. These were dissolved in boiling water (9 c.c.), and the hot solution was poured slowly into boiling 3N-acetic acid (30 c.c.). The pale yellow solid which separated was collected, washed as before, and dried at 135° (Found : C, 43·9; H, 3·0; N, 42·6. Calc. for $C_6H_5ON_5$: C, 44·2; H, 3·1; N, 42·9%).

(*b*) Guanidine hydrochloride (1·11 g.) was added to a solution of sodium (1·06 g.) in dry methanol (40 c.c.). The solution was filtered from sodium chloride, and 2-chloro-3-methoxycarbonylpyrazine (2·0 g.) was added. A clear, bright scarlet solution resulted on shaking, and this was refluxed for 30 hr., during which further precipitation of sodium chloride took place.

The filtered solution was diluted with water (60 c.c.), and the pH of the boiling solution was adjusted to 4·5 with warm glacial acetic acid. The yellow bulky precipitate (0·375 g., 20%) was collected and purified as above. The yield fell when the reaction was carried out at higher temperatures in a sealed tube, or when the period of refluxing was reduced.

2-Amino-4-hydroxy-6 : 7-diphenylpteridine.—2-Chloro-3-methoxycarbonyl-5 : 6-diphenylpyrazine (0·2 g.) was fused with guanidine carbonate (0·4 g.) as before. The mixture was extracted with boiling water to leave an orange solid (0·230 g.). This was purified by dissolution in hot 2N-sodium hydroxide (20 c.c.), filtration, and pouring of the warm filtrate into boiling glacial acetic acid (10 c.c.). The yellow solid which separated was collected at 90—100°, washed with warm water, and ethanol, and dried to give 2-amino-4-hydroxy-6 : 7-diphenylpteridine (0·13 g., 70%), m. p. > 360°. Recrystallization from dimethylformamide gave a yellow microcrystalline solid which was dried at 135° (Found : C, 68·3; H, 4·0; N, 22·4. Calc. for $C_{18}H_{12}ON_5$: C, 68·6; H, 4·2; N, 22·2%).

2-Amino-4-hydroxy-7-methylpteridine.—2-Chloro-3-methoxycarbonyl-6-methylpyrazine (0·135 g.) was fused with guanidine carbonate (0·4 g.) as before. The product was isolated as in the case of the unsubstituted compound to give 2-amino-4-hydroxy-7-methylpteridine (0·105 g., 80%), m. p. > 360°. This was purified *via* the sodium salt and dried at 135° (Found : C, 44·9; H, 4·2. Calc. for $C_7H_7ON_5,0{\cdot}5H_2O$: C, 45·2; H, 4·3%).

Authentic material was prepared from 2 : 4 : 5-triamino-6-hydroxypyrimidine (Forrest and Walker, *J.*, 1949, 83), and dried at 135° (Found : C, 45·0; H, 4·3%).

2-Methoxy-5 : 6-diphenylpyrazine-3-carboxylic Acid.—To a solution of sodium (0·06 g.) in dry methanol (7 c.c.) were added guanidine hydrochloride (0·06 g.) and 2-chloro-3-methoxycarbonyl-5 : 6-diphenylpyrazine (0·2 g.). The mixture was refluxed for 12 hr., during which a white bulky precipitate separated. This was collected and dissolved in boiling water, and the filtrate was brought to pH 4 with glacial acetic acid. Crystallization occurred on cooling, to give fluffy white needles (0·096 g.), m. p. 179° (decomp.). The filtrate from the reaction mixture was concentrated *in vacuo*, warm water was added to dissolve any solid material, and the solution was filtered from traces of slime. The clear filtrate was adjusted to pH 4 with glacial acetic acid, and, on cooling, a further deposit of crystals was formed (0·04 g.), m. p. 175° (decomp.) (total yield 73%). Recrystallization from aqueous methanol gave *2-methoxy-5 : 6-diphenylpyrazine-3-carboxylic acid* as small white needles, m. p. 180—181° (decomp.) (Found : C, 70·7; H, 4·3; N, 8·9. $C_{18}H_{14}O_3N_2$ requires C, 70·6; H, 4·6; N, 9·2%). Care must be taken during the preparation of this material that it does not become mixed with its sodium salt which crystallizes readily from water as white plates, m. p. 254—256° (decomp.). The same methoxy-acid, m. p. and mixed m. p. 178—180° (decomp.), is obtained from 2-chloro-3-methoxycarbonyl-5 : 6-diphenylpyrazine and sodium methoxide in the absence of guanidine.

The methoxy-acid (0·2 g.) was esterified with methanol and dry hydrogen chloride, by the method employed for the esterification of the corresponding hydroxy-acid. *2-Methoxy-3-methoxycarbonyl-5 : 6-diphenylpyrazine* (0·2 g., 95%) recrystallized from methanol as fluffy white needles, m. p. 118·5—119° (Found : C, 71·2; H, 5·0; N, 8·6. $C_{19}H_{16}O_3N_2$ requires C, 71·2; H, 5·0; N, 8·8%).

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