[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Pteridines. XVIII. A Direct Synthesis of 2-Aminopyrazine-3-carboxamides[1,2]

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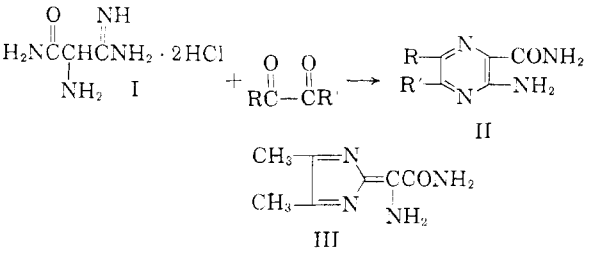
Aminomalonamidamidine dihydrochloride (I) was condensed with several α,β-dicarbonyl compounds in aqueous ammonium hydroxide to give 2-aminopyrazine-3-carboxamides (II) directly and usually in satisfactory yield. Methylglyoxal and phenylglyoxal led exclusively to the corresponding 5-substituted 2-aminopyrazine-3-carboxamides (II, R′ = –H). This orientation is of considerable interest, since subsequent cyclization of these intermediates yields 6-substituted pteridines. Condensation of I with biacetyl yielded a mixture of 2-amino-5,6-dimethylpyrazine-3-carboxamide and a higher melting, extremely insoluble isomeric product to which structure III has been provisionally assigned.

The synthesis of pteridines by ring closure of pyrazines has enjoyed only restricted application because of the paucity of synthetic methods available for the preparation of the requisite intermediates. The first synthesis of pyrazines suitable for further cyclization to pteridines was somewhat circuitous, since it involved the initial formation of a 2,4-dihydroxypteridine (lumazine) by the conventional route *via* pyrimidine intermediates,[4] followed by cleavage under hydrolytic[5,7] or aminolytic[6,7] conditions to give a 2-aminopyrazine-3-carboxylic acid or carboxamide, respectively. A more direct synthesis of pyrazines which could be converted to intermediates suitable for cyclization to pteridines was developed by Jones,[8] who prepared 2-hydroxypyrazine-3-carboxamides by the condensation of α,β-dicarbonyl compounds with aminomalondiamide. Subsequent conversion of these intermediates either to 2-chloro-3-cyanopyrazines[9] or to 2-chloro-3-methoxycarbonylpyrazines[10] followed by fusion with reagents such as guanidine or urea yielded pteridines. Although Jones claimed to have obtained 2-hydroxy-5-methylpyrazine-3-carboxamide from the condensation of methylglyoxal with aminomalondiamide, later work[10,11] has shown that the 6-methyl isomer is formed exclusively in this reaction. Finally, a synthesis of 2-aminopyrazine-3-carboxamides by the reductive ring cleavage of the –N–N– bond of 3-hydroxy-1-pyrazolo[b]pyrazines has recently been reported.[12,13]

We now wish to report a direct synthesis of 2-aminopyrazine-3-carboxamides (II) by the condensation of α,β-dicarbonyl reagents with aminomalonamidamidine dihydrochloride (I),[14–16] which we consider the method of choice for the preparation of these intermediates. The reaction is best carried out in dilute ammonium hydroxide (*p*H 8–9) at 0–20°. Thus, the condensation of aminomalonamidamidine dihydrochloride (I) with glyoxal bisulfite yielded 2-aminopyrazine-3-carboxamide (II, R = R′ = –H) directly in 76% yield. The product was identical with a sample of 2-aminopyrazine-3-carboxamide prepared by the Raney nickel cleavage of 3-hydroxy-1-pyrazolo[b]pyrazine,[13] and dilute alkaline hydrolysis converted it to 2-aminopyrazine-3-carboxylic acid, identical with an authentic sample prepared by the method of Weijlard, Tishler and Erickson.[5]



Condensation of I with methylglyoxal yielded 2-amino-5-methylpyrazine-3-carboxamide (II, R = $-CH_3$, R′ = –H). The position of the methyl substituent was definitely established by hydrolysis of the amide to 2-amino-5-methylpyrazine-3-carboxylic acid. It is of considerable interest that the only isomer formed in this condensation is the 5-methyl derivative, since subsequent cyclization of this intermediate then yields a 6-methylpteridine.

The condensation of I with phenylglyoxal was not as satisfactory from the standpoint of yield, but again the 5-substituted isomer was the only isolable product. The structure of the product was established as 2-amino-5-phenylpyrazine-3-carboxamide (II, R = $-C_6H_5$, R′ = –H) by alkaline hydrolysis to a phenyl-2-aminopyrazine-3-carboxylic acid, m.p. 196° dec., which was not identical with 2-amino-6-phenylpyrazine-3-carboxylic acid, m.p. 225° dec., the only previously reported isomer.[17] Moreover, treatment of our phenyl-2-aminopyrazine-3-carboxylic acid with nitrosylsulfuric acid yielded 2-hydroxy-5-phenylpyrazine-3-carboxylic

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acid, identical with an authentic sample[18] kindly supplied by Dr. H. C. S. Wood. A mixture melting point determination with an authentic sample of 2-hydroxy-6-phenylpyrazine-3-carboxylic acid[18] showed a marked depression; the green fluorescence of our isomer agrees with the reported[17,18] fluorescence of the 5-phenyl isomer. Our hydrolysis product, m.p. 196° dec., is thus firmly established as the hitherto unknown 2-amino-5-phenylpyrazine-3-carboxylic acid, and it follows that the initial condensation product was also the 5-phenyl isomer.

The orientation observed in the above reactions is readily understandable, since condensation at *p*H 8–9 between the more reactive (aldehyde) carbonyl grouping of the substituted glyoxal with the most basic center of aminomalonamidamidine, *e.g.*, the amidine grouping, would lead to a 2-amino-5-substituted pyrazine-3-carboxamide. The opposite orientation observed with aminomalondiamide[10,11] results from the fact that the most basic center of this intermediate is the aliphatic amino group, and preliminary condensation of this center with the more active (aldehyde) carbonyl grouping of the substituted glyoxal would thus lead to a 6- rather than a 5-substituted isomer.

In contrast to the above condensations, which gave only one product, the reaction of aminomalonamidamidine dihydrochloride (I) with biacetyl yielded two isomeric products, A and B, in a ratio of approximately 2:1. The more soluble, lower melting isomer A was shown to be the expected 2-amino-5,6-dimethylpyrazine-3-carboxamide (II, R = R′ = $-CH_3$) by comparison with an authentic sample prepared by the Raney nickel cleavage of 3-hydroxy-5,6-dimethyl-1-pyrazolo(b)pyrazine[13] and by alkaline hydrolysis to the known 2-amino-5,6-dimethylpyrazine-3-carboxylic acid.[5] The second product B was a high melting, extremely insoluble yellow compound which was best purified by long extraction with hot ethanol (to remove A) followed by repeated recrystallization from aqueous dimethylformamide. The compound could be recovered unchanged after treatment for one hour with boiling 6 *N* hydrochloric acid, but it was rapidly destroyed on heating in dilute sodium hydroxide. A molecular weight determination on this material was not carried out because of its insolubility, but microanalysis showed that it was isomeric with 2-amino-5,6-dimethylpyrazine-3-carboxamide. On the basis of the above observations, we therefore suggest III as a tentative structure for this second isomer B, formed by the condensation of biacetyl with the amidine grouping of I.

Since the condensation of α-ketoaldehydes with aminomalonamidamidine dihydrochloride (I) appears to yield 5-substituted 2-aminopyrazine-3-carboxamides exclusively, the possibility that this reaction might be utilized for the preparation of naturally-occurring pteridines, e.g., folic acid derivatives, biopterin, which bear alkyl substituents on position 6 (formed by cyclization of the above 5-substituted pyrazines) is immediately apparent. Experiments directed toward this end are currently in progress.

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Experimental[19]

2-Aminopyrazine-3-carboxamide.—To a solution of 30 g. of aminomalonamidamidine dihydrochloride in 300 ml. of water at 0° was added 45 g. of dry glyoxal bisulfite, and 40 ml. of concentrated ammonium hydroxide added dropwise over a period of 15 minutes. When neutrality was reached, a light cream-colored solid started to separate. The reaction mixture was then stirred overnight at room temperature and the suspended solid collected by filtration, washed with water and dried in a vacuum oven; crude yield, 16.6 g. (76%), m.p. 233–238°. The product may be readily purified by recrystallization from water or by vacuum sublimation at 180° (0.01 mm.), m.p. 241–242°. This compound is reported to melt at 239.3°,[20] and at 238–239°.[21] When commercial glyoxal (30% aqueous solution) rather than glyoxal bisulfite was employed in the above condensation, the yield of 2-aminopyrazine-3-carboxamide was lowered to 32%.

2-Aminopyrazine-3-carboxylic Acid.—A suspension of 3.1 g. of 2-aminopyrazine-3-carboxamide in 20 ml. of 3 *N* sodium hydroxide was heated under reflux for 1.5 hours. Solution was achieved after 10 minutes and ammonia evolution had essentially ceased after one hour. The hot solution was acidified with concentrated hydrochloric acid to *p*H 3, whereupon the free amino acid started to separate in almost colorless flakes. The mixture was chilled and filtered and the collected solid washed with a small amount of ice-cold water and dried to give 2.43 g. (78%), m.p. 196° dec. The melting point was unchanged after recrystallization from water. The reported decomposition point for this material is 201°.[5]

2-Amino-5-methylpyrazine-3-carboxamide.—To a solution of 19 g. (0.1 mole) of aminomalonamidamidine dihydrochloride in 200 ml. of water at 10° was added a solution of 7.2 g. (0.1 mole) of pyruvaldehyde in 60 ml. of water, and the *p*H was adjusted to 8–9 by the addition of 10 ml. of concentrated ammonium hydroxide. The reaction mixture was then stirred overnight, chilled to 0° and filtered to give 8.3 g. (54%) of light cream-colored crystals, m.p. 180–185°. The product was purified for analysis by sublimation at 180° (0.01 mm.) followed by recrystallization from methanol to give pale yellow flakes which exhibited a strong bluish fluorescence even in the solid state; m.p. 203–204°.

Anal. Calcd. for $C_6H_8N_4O$: C, 47.4; H, 5.3; N, 36.8. Found: C, 47.2; H, 5.2; N, 36.8.

2-Amino-5-methylpyrazine-3-carboxylic Acid.—A suspension of 1.52 g. of 2-amino-5-methylpyrazine-3-carboxamide in 10 ml. of 3 *N* sodium hydroxide was heated under reflux for 1.5 hours. The resulting solution was adjusted to *p*H 3 with concentrated hydrochloric acid and chilled overnight at 0°. Filtration yielded 0.92 g. (60%) of yellow crystals, m.p. 166–168° dec. Recrystallization from 20 ml. of water raised the melting point to 171–173° dec. The reported melting point for this isomer is 171.5–172°[22] and 173°.[23] 2-Amino-6-methylpyrazine-3-carboxylic acid is reported to melt at 210° dec.[23] and at 211–212° dec.[5]

2-Amino-5-phenylpyrazine-3-carboxamide.—To a solution of 7.5 g. of aminomalonamidamidine dihydrochloride in 250 ml. of ice-cold water was added a solution of 7.0 g. of phenylglyoxal monohydrate in 150 ml. of ice-cold water. The resulting solution was maintained at 0–5° by means of an ice-bath while ammonium hydroxide was added, with continual stirring, until the *p*H reached 8–9. Additional ammonium hydroxide was added from time to time over the first 30 minutes as required to maintain the *p*H at 8–9. The mixture was then stirred at room temperature overnight and the light yellow solid which separated was collected by filtration and dried. Recrystallization from absolute ethanol yielded 3.1 g. (36.6%) of yellow crystals, m.p. 239–240°.

(19) Microanalyses were performed by Dr. Joseph F. Alicino, Metuchen, N. J., and by Drs. Weiler and Strauss, Oxford, England. All melting points are uncorrected.

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Anal. Calcd. for $C_{11}H_{10}N_4O$: C, 61.7; H, 4.7. *Found*: C, 61.4; H, 4.5.

2-Amino-5-phenylpyrazine-3-carboxylic Acid.—A mixture of 3.1 g. of 2-amino-5-phenylpyrazine-3-carboxamide in 200 ml. of 1 *N* sodium hydroxide was heated under reflux for 8 hours and the resulting clear solution adjusted to *p*H 3 with concentrated hydrochloric acid to yield 2.19 g. (70%) of yellow needles, m.p. 196° dec. Recrystallization from aqueous methanol did not change the melting point. The reported melting point for 2-amino-6-phenylpyrazine-3-carboxylic acid is 225° dec.[17]

Anal. Calcd. for $C_{11}H_9N_3O_2$: C, 61.4; H, 4.2. Found: C, 61.4; H, 4.5.

2-Hydroxy-5-phenylpyrazine-3-carboxylic Acid.—A solution of 0.511 g. of 2-amino-5-phenylpyrazine-3-carboxylic acid in 15 ml. of cold concentrated sulfuric acid was treated with a solution of 0.25 g. of sodium nitrite in 5 ml. of cold concentrated sulfuric acid. The resulting deep red solution was held at 0° for four hours, at room temperature for four hours, and then poured into ice. The frothy suspension of yellow solid was stirred at room temperature overnight and filtered, and the collected solid recrystallized first from water and then from ethanol to give 0.455 g. (88.5%) of long light yellow needles, m.p. 210° dec. The material exhibited a bright green fluorescence in dilute aqueous solution. A mixture melting point with an authentic sample of 2-hydroxy-5-phenylpyrazine-3-carboxylic acid, m.p. 203° dec. (reported[17,18] m.p. 200° dec.) was 208–209° dec.; infrared spectra of the two samples were identical. A mixture melting point with an authentic sample of 2-hydroxy-6-phenylpyrazine-3-carboxylic acid, m.p. 224° dec. (reported[17] m.p. 208–209° dec.; 217° dec.[18]) was 196–199° dec.

2-Amino-5,6-dimethylpyrazine-3-carboxamide (A).—A solution of 28.5 g. of aminomalonamidamidine dihydrochloride in 300 ml. of water at 10° was added slowly, with external cooling, to a solution of 13 g. of biacetyl in 60 ml. of ethanol. When the exothermic reaction had subsided, 30 ml. of concentrated ammonium hydroxide was added slowly, with concomitant separation of a heavy, bright lemon-yellow solid. The reaction mixture was stirred for several hours, cooled to 0° and filtered to yield 23.0 g. (92%) of a mixture of A and B, m.p. 255–260° dec. Ten grams of this material was placed in a soxhlet cup and extracted with absolute ethanol for five days. Evaporation of the ethanol extracts yielded 7.47 g. of a yellow solid which consisted predominately of 2-amino-5,6-dimethylpyrazine-3-carboxamide (A) but which contained a small amount of B. Vacuum sublimation readily separated these compounds and yielded A as a light yellow, crystalline sublimate, m.p. 255°.

Anal. Calcd. for $C_7H_{10}N_4O$: C, 50.6; H, 6.1. Found: C, 50.7; H, 6.3.

Higher Melting Isomer B.—The solid residue remaining in the soxhlet cup above was extracted for 10 minutes with 50 ml. of boiling 50% aqueous dimethylformamide. The cooled extract yielded 1.13 g. of a light yellow solid which proved to be a mixture of A and B by examination of its infrared spectrum. The residue from the above extraction was then recrystallized from 300 ml. of boiling 50% aqueous dimethylformamide to give 1.19 g. of pure B. This compound decomposes slowly above 280°; decomposition is complete between 320–330°. Since it does not have a characteristic melting or decomposition point, its purity was determined by solubility measurements in boiling 50% aqueous dimethylformamide (0.5 g. in 100 ml.) and by examination of its infrared spectrum. The sample was judged pure when it was shown that the above obtained 1.19 g. was completely extracted by eight successive 30-ml. portions of boiling 50% aqueous dimethylformamide, each portion yielding 0.15 g. ±0.02 g. of product with identical infrared absorption spectra; $\lambda_{max}^{0.1N\ HCl}$ 244, 377 mμ; log ε 4.01, 4.06.

Anal. Calcd. for $C_7H_{10}N_4O$: C, 50.6; H, 6.1. Found: C, 50.3; H, 6.0.

2-Amino-5,6-dimethylpyrazine-3-carboxylic Acid.—A mixture of 5.8 g. of 2-amino-5,6-dimethylpyrazine-3-carboxamide (A) (the material obtained directly from the ethanol extraction described above may be used) and 40 ml. of 3 *N* sodium hydroxide was heated under reflux for 1.5 hours, chilled and the clear solution acidified to *p*H 3 with hydrochloric acid to yield 4.7 g. (81%) of cream-colored crystals, m.p. 208° dec. Recrystallization from water did not raise the melting point. This compound is reported[5] to melt with decomposition at 209–210°.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY, AND THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Pteridines. XIX. A Synthesis of 8-Substituted Pteridine-6-carboxylic Acids

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A new route to pteridine-6-carboxylic acids is described in which a 4,5-diaminopyrimidine is treated with alloxan in dilute alkaline solution. The condensation proceeds *via* the intermediate formation of a *spiro* pteridine (XV) which undergoes subsequent cleavage with alkali. Utilization of 2,5-diamino-4-hydroxy-6-substituted aminopyrimidines leads to 2-amino-4-hydroxy-7-keto-8-substituted-7,8-dihydropteridine-6-carboxylic acids, and by this procedure both the 8-(D-1-sorbityl) and 8-(D-1-ribityl) derivatives are prepared. It is shown that initial condensation of the pyrimidine and alloxan in acidic solution leads *via* a deep purple anil (similar to I) to a 9-substituted pyrimido(5,4-g)pteridine (XXX), which cleaves under the reaction conditions to the 7-keto-8-substituted-7,8-dihydropteridine-6-carboxylic acid. Reduction under Clemmensen conditions then yields the corresponding 2-amino-4-hydroxy-8-substituted-7,8-dihydropteridine-6-carboxylic acid.

Discussion

Although the existence of pteridine glycosides in nature has never been demonstrated, the possibility that pteridines might be carried through their metabolic pathways with a sugar attached remains an intriguing possibility. The demonstrated *in vitro*[2–4] and *in vivo*[5] conversion of purines into pteridines implies that appropriate conversions of nucleosides would provide pteridine glycosides, at least as initial products; a mechanism for solubilizing the extremely insoluble pteridines would be found; and the close relationship between purines and pteridines, and between the pteridines and the flavins (such as riboflavin) would be strengthened. Striking support for the latter relationship is found in the recent isolation from *Eremothecium ashbyii* of the 8-ribityl derivative of dimethyllumazine (2,4-dihydroxy-6,7-dimethylpteridine) and its implication as an intermediate in the biosynthesis of riboflavin,[6,7] and in the isolation of the 8-ribityl deriva-

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