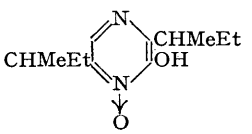
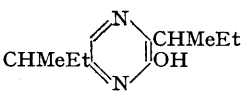
221. *Pyrazine Derivatives. Part III. Conversion of Diketopiperazines into Pyrazine Derivatives. Synthesis of* 2-*Hydroxy*-3 : 6-*di*-sec.-*butylpyrazine from* iso*Leucine.*

By R. A. Baxter and F. S. Spring.

A method for the conversion of diketopiperazines (α-amino-acid anhydrides) into pyrazine derivatives is described. *iso*Leucine anhydride (IX) has been converted into 2-chloro-3 : 6-di-*sec.*-butylpyrazine (XI) and thence into 2-hydroxy-3 : 6-di-*sec.*-butylpyrazine (II), and similarly alanine anhydride (III) has been converted into 2-chloro- (IV) and 2-hydroxy-3 : 6-dimethylpyrazine (VII).

The antibacterial substance aspergillic acid, for which the structure (I) has been suggested, is obtained from culture filtrates of the mould *Aspergillus flavus* using a medium containing an

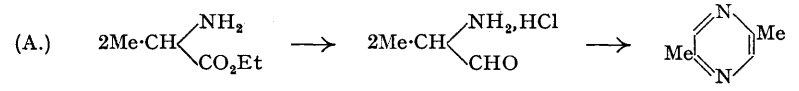
(I.) 

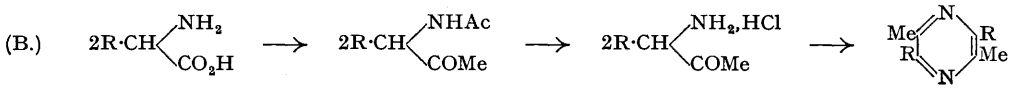
 (II.)

amino-acid source such as tryptone. The present paper describes some preliminary experiments undertaken with the object of developing a synthetic route, starting from *iso*leucine, to

aspergillic acid or to the related deoxyaspergillic acid for which structure (II) has been suggested (for literature see Newbold and Spring, this vol., p. 373).

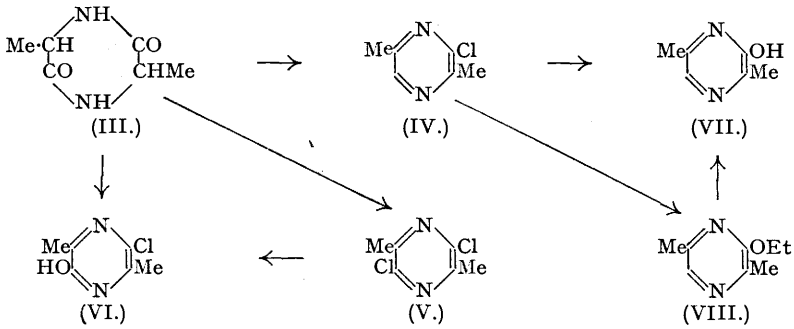
Two syntheses of pyrazine bases from α-amino-acids have been described. Reduction of the ethyl ester of alanine with sodium amalgam in the presence of hydrochloric acid gave the hydrochloride of α-aminopropaldehyde which when treated with alkali and mercuric chloride yielded 2 : 5-dimethylpyrazine in poor yield (A) (Neuberg, *Ber.*, 1908, **41**, 956; Fischer, *ibid.*, p.





1019). The second synthesis (B) consists in treatment of an α-amino-acid with acetic anhydride and pyridine to yield an α-acetamido-ketone which, when hydrolysed with hydrochloric acid, gave the corresponding α-amino-ketone hydrochloride. The latter when condensed with alkali in the presence of an oxidising agent gave a fully substituted pyrazine base (Dakin and West, *J. Biol. Chem.*, 1928, **78**, 745, 757).

Our method of approach to the problem consisted in a study of methods for converting a diketopiperazine (α-amino-acid anhydride) into an aromatic pyrazine derivative. The simplest reaction of this type would involve oxidation of a diketopiperazine to a dihydroxypyrazine, but so far as we are aware no transformation of this type has been reported. Abderhalden (*Z. physiol. Chem.*, 1924, **140**, 52; 1925, **143**, 128; 1925, **144**, 234) has shown that oxidation of glycine anhydride with potassium permanganate or with hydrogen peroxide and ferrous sulphate yields oxamide (cf. Ludtke, *Biochem. Z.*, 1925, **143**, 158), and according to Arai (*ibid.*, 1930, **226**, 233) glycine anhydride and phenylalanine anhydride on prolonged shaking with oxygen both yield urea. Goldschmidt and Steigenwald (*Ber.*, 1925, **58**, 1346) have shown that treatment of alanine anhydride with hypobromite yields an imidazolone derivative (cf. Goldschmidt, Wiberg, Nagel, and Martin, *Annalen*, 1927, **456**, 1). The present paper describes a method for the conversion of α-amino-acid anhydrides into halogen substituted pyrazine derivatives and the characterisation and conversion of these into hydroxypyrazine derivatives.



Treatment of *dl*-alanine anhydride (III) with phosphoryl chloride gives a mixture of 2-*chloro*-3 : 6-*dimethylpyrazine* (IV) and 2 : 5-*dichloro*-3 : 6-*dimethylpyrazine* (V). Separation of the two reaction products is relatively simple since the dichloro-derivative is not basic, whereas 2-chloro-3 : 6-dimethylpyrazine is soluble in 3N-hydrochloric acid. The formation of the monochloro-derivative (IV) from alanine anhydride does not involve an oxidation step, whereas the formation of 2 : 5-dichloro-3 : 6-dimethylpyrazine involves the oxidation of an intermediate dihydropyrazine derivative, a common step in many pyrazine syntheses. Treatment of *dl*-alanine anhydride with phosphoryl chloride in the presence of a tertiary base (dimethylaniline) gives only the monochloro-derivative, the intermediate dichlorodihydropyrazine presumably losing hydrogen chloride and thereby yielding the stable aromatic 2-chloro-3 : 6-dimethylpyrazine. 2-Chloro-3 : 6-dimethylpyrazine was not isolated from the reaction product obtained by treatment of *dl*-alanine anhydride with a mixture of phosphoryl chloride and phosphorus pentachloride (as oxidising agent), the reaction giving 2 : 5-dichloro-3 : 6-dimethylpyrazine in poor yield, together with a small quantity of 2-*chloro*-5-*hydroxy*-3 : 6-*dimethylpyrazine*

(VI); the latter was the only isolated product of the reaction between *dl*-alanine anhydride and phosphorus pentachloride.

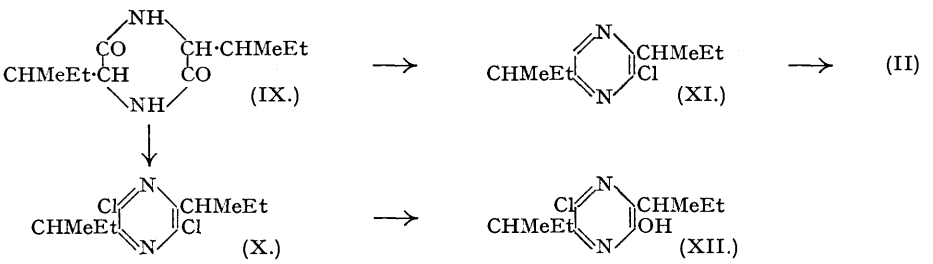
Prolonged treatment of 2-chloro-3 : 6-dimethylpyrazine with concentrated potassium hydroxide solution gives 2-hydroxy-3 : 6-dimethylpyrazine (VII) identical with that previously obtained by different synthetic methods (Baxter, Newbold, and Spring, this vol., p. 370). Less drastic methods for the replacement of the halogen atom by hydroxyl were sought, but so far we have not succeeded in replacing the halogen by an amino-group. Treatment of 2-chloro-3 : 6-dimethylpyrazine with alcoholic sodium ethoxide yields *2-ethoxy-3 : 6-dimethylpyrazine* (VIII), hydrolysis of which with hydrochloric acid gives 2-hydroxy-3 : 6-dimethylpyrazine (VII).

Treatment of 2 : 5-dichloro-3 : 6-dimethylpyrazine with concentrated aqueous alkali yields 2-chloro-5-hydroxy-3 : 6-dimethylpyrazine (VI). This compound is soluble in alkali and reprecipitated from alkaline solution on acidification: its ultra-violet absorption spectrum is very similar to those of the simple hydroxypyrazine derivatives examined by Newbold and Spring (this vol., p. 373). 2-Chloro-5-hydroxy-3 : 6-dimethylpyrazine was recovered unchanged after treatment under drastic conditions with alcoholic ammonia. Although attempts to convert it into 2-hydroxy-3 : 6-dimethylpyrazine by reduction in alkaline solution with Raney alloy [Schwenk and Papa, *J. Org. Chem.*, 1944, **9**, 1; *Ind. Eng. Chem.* (*Anal.*), 1943, **15**, 576] were unsuccessful, this transformation was effected by heating 2-chloro-5-hydroxy-3 : 6-dimethylpyrazine with solid potassium hydroxide. Treatment of 2-chloro-5-hydroxy-3 : 6-dimethylpyrazine with phosphoryl chloride using conditions which successfully convert *dl*-alanine anhydride into a mixture of 2-chloro- and 2 : 5-dichloro-3 : 6-dimethylpyrazine gives a very small yield of 2 : 5-dichloro-3 : 6-dimethylpyrazine, the starting material being largely recovered unchanged.

The reaction stages described above constitute a conversion of *dl*-alanine into 2-hydroxy-3 : 6-dimethylpyrazine. It was of great interest, therefore, to attempt to convert *dl-iso*leucine into 2-hydroxy-3 : 6-di-*sec.*-butylpyrazine in order to compare the product with the racemate previously obtained by Newbold and Spring (*loc. cit.*) by a different synthetic route.

Ehrlich (*Ber.*, 1907, **40**, 2550) obtained an *iso*leucine anhydride by heating *d-iso*leucine and commented that the compound was probably a mixture of isomers. Dutcher and Wintersteiner (*J. Biol. Chem.*, 1944, **155**, 359) describe a racemic *iso*leucine anhydride prepared from *dl-iso*leucine. Using a modification of the method developed by Sannié (*Bull. Soc. chim.*, 1942, **9**, 487) for the preparation of amino-acid anhydrides in which the α-amino-acid is heated with ethylene glycol, *dl-iso*leucine gave a *dl-iso*leucine anhydride in 49% yield. In a second method, α-bromo-β-methylvaleryl chloride was reacted with *dl-iso*leucine methyl ester hydrochloride and the product heated with alcoholic ammonia to yield a *dl-iso*leucine anhydride. Although the specimens of anhydride obtained by the two methods appear to be identical, it is clear that they may comprise different mixtures of isomers.

Treatment of *dl-iso*leucine anhydride (IX) with phosphoryl chloride gives a mixture of 2 : 5-*dichloro*-3 : 6-*di*-sec.-*butylpyrazine* (X) and *2-chloro*-3 : 6-*di*-sec.-*butylpyrazine* (XI). The halogen substituents in these pyrazine derivatives are even less reactive than those in the corresponding dimethyl homologues. Thus 2-chloro-3 : 6-di-*sec.*-butylpyrazine was recovered unchanged after prolonged refluxing with 20% potassium hydroxide solution, conditions which



converted 2-chloro-3 : 6-dimethylpyrazine into 2-hydroxy-3 : 6-dimethylpyrazine. When heated to 180° with powdered potassium hydroxide, however, 2-chloro-3 : 6-di-*sec.*-butylpyrazine yielded 2-hydroxy-3 : 6-di-*sec.*-butylpyrazine (II) identical with the racemate obtained previously from di-*sec.*-butylpyrazine. 2 : 5-Dichloro-3 : 6-di-*sec.*-butylpyrazine was also unchanged after treatment with concentrated aqueous alkali, but when heated with powdered potassium hydroxide it gave *2-chloro-5-hydroxy*-3 : 6-*di*-sec.-*butylpyrazine* (XII).

Experimental.

2 : 5-*Dichloro*-3 : 6-*dimethylpyrazine*.—A mixture of 2 : 5-diketo-3 : 6-dimethylpiperazine prepared from *dl*-alanine by the method described by Sannié (*loc. cit.*) (5 g.) and phosphoryl chloride (50 c.c.) was heated at 120° (bath temp.) for 20 minutes. Excess of phosphoryl chloride was removed under reduced pressure and the residue triturated with ice–water. The solid was collected (filtrate *A*), washed with water, and dried over phosphoric oxide (yield, 1·3 g.). Sublimation of this material at 60—80°/2 mm. gave 2 : 5-*dichloro*-3 : 6-*dimethylpyrazine* as prisms, m. p. 73° (Found: C, 41·1; H, 3·7; N, 15·4. $C_6H_6N_2Cl_2$ requires C, 40·7; H, 3·4; N, 15·8%). Light absorption in alcohol: Maximum at 2975 A., $\epsilon = 810$.

2-*Chloro*-3 : 6-*dimethylpyrazine*.—The filtrate *A* was treated with sodium carbonate solution until just alkaline and the mixture extracted with ether. The extract was dried, the ether removed, and the residue distilled to yield 2-*chloro*-3 : 6-*dimethylpyrazine* as an oil, b. p. 78°/15 mm. (1·4 g.), $n_D^{19°}$ 1·5284 (Found: C, 50·2; H, 4·8. $C_6H_7N_2Cl$ requires C, 50·5; H, 4·9%). 2-Chloro-3 : 6-dimethylpyrazine is soluble in 3N-hydrochloric acid. It forms a picrate, m. p. approx. 100°, and a chloroplatinate, m. p. > 360°, which are extremely soluble in the common solvents; this extreme solubility precluded their satisfactory purification.

Treatment of alanine anhydride (5 g.) with phosphoryl chloride (30 c.c.) and dimethylaniline (10 c.c.) for 20 minutes at 120° gave 2-chloro-3 : 6-dimethylpyrazine (1 g.). Difficulty was experienced in separating the product from the dimethylaniline; this may account for the low yield.

2-*Chloro*-5-*hydroxy*-3 : 6-*dimethylpyrazine*.—(*a*) A mixture of *dl*-alanine anhydride (5 g.), phosphorus pentachloride (5 g.), and phosphoryl chloride (15 c.c.) was refluxed for 30 minutes. Excess of phosphoryl chloride was removed by distillation and the residue triturated with water to give 2 : 5-dichloro-3 : 6-dimethylpyrazine (0·5 g.), m. p. and mixed m. p. 72—73°. The mother liquor was neutralised with sodium carbonate and extracted with chloroform. Evaporation of the dried (Na_2SO_4) extract gave a solid (0·8 g.) which, after crystallisation from benzene (needles) followed by sublimation, gave 2-*chloro*-5-*hydroxy*-3 : 6-*dimethylpyrazine* as needles, m. p. 224°. Although sparingly soluble in water, it is soluble in dilute sodium hydroxide and precipitated from this solution on acidification (Found: C, 45·6; H, 4·7. $C_6H_7ON_2Cl$ requires C, 45·4; H, 4·4%).

(*b*) A solution of 2 : 5-dichloro-3 : 6-dimethylpyrazine (0·5 g.) in dioxan (4 c.c.) and aqueous potassium hydroxide (10 c.c.; 20%) was refluxed for 20 hours. The solution was acidified with hydrochloric acid, the crystalline solid collected and purified by sublimation at 140°/1 mm. to give 2-chloro-5-hydroxy-3 : 6-dimethylpyrazine (0·35 g.) as needles, m. p. 224°, either alone or when mixed with the specimen described above (Found: N, 17·5. $C_6H_7ON_2Cl$ requires N, 17·7%). Light absorption in alcohol: Maxima at 2285 A., $\epsilon = 10{,}000$ and 3330 A., $\epsilon = 6{,}800$.

dl-iso*Leucine Anhydride*.—(*a*) *dl-iso*Leucine (9 g.) was refluxed with ethylene glycol (54 c.c.) for 4 hours. The crude anhydride (2 g.) which separated on standing was purified by extraction with chloroform and evaporation of the chloroform filtrate. On crystallisation of the residue from alcohol, *dl-iso*leucine anhydride formed felted needles, m. p. 270° (sintering at 259°) (yield 49%) (Found: C, 63·5; H, 9·6. Calc. for $C_{12}H_{22}O_2N_2$: C, 63·7; H, 9·7%).

(*b*) An ice-cooled suspension of *dl-iso*leucine methyl ester hydrochloride (6·2 g.) in chloroform (80 c.c.) and α-bromo-β-methylvaleryl chloride (8 g.) was stirred and treated during 1 hour with a solution of *n*-butylpiperidine (22 g.) in chloroform (20 c.c.). The ice-bath was removed and the mixture stirred for a further hour. The chloroform solution was washed successively with water, dilute hydrochloric acid, sodium carbonate solution, and water. Evaporation of the dried chloroform solution under reduced pressure gave a semi-solid mass which did not crystallise completely. It was dissolved in ethanol (150 c.c.), the solution saturated with ammonia at 0° and heated at 180° for 8 hours. The reaction mixture was evaporated to dryness and the residue extracted with chloroform. The chloroform extract was evaporated and the product (1·4 g.) crystallised from alcohol to yield *dl-iso*leucine anhydride as needles, m. p. 270°, sintering at 260°. A mixture of this anhydride with a specimen prepared by method (*a*) softened at 260° and melted at 270° (Found: C, 64·2; H, 9·6; N, 12·6. Calc. for $C_{12}H_{22}O_2N_2$: C, 63·7; H, 9·7; N, 12·4%).

2 : 5-*Dichloro*-3 : 6-*di*-sec.-*butylpyrazine*.—*dl-iso*Leucine anhydride (2·0 g.) was refluxed with phosphoryl chloride (20 c.c.) for one hour. The phosphoryl chloride was removed under reduced pressure and the product triturated with ice–water. The mixture was extracted with ether, the extract dried (Na_2SO_4), and the solvent removed. Distillation of the residue gave an oil (1·2 g.), b. p. 118°/12 mm. The oil was shaken with concentrated hydrochloric acid (15 c.c.) and the crystalline mass collected (0·15 g.) (filtrate B) and sublimed at 50°/1 mm. to yield 2 : 5-*dichloro*-3 : 6-*di*-sec.-*butylpyrazine* as plates, m. p. 58—60° (Found: C, 55·3; H, 6·9; N, 10·9. $C_{12}H_{18}N_2Cl_2$ requires C, 55·2; H, 6·9; N, 10·7%).

2-*Chloro*-3 : 6-*di*-sec.-*butylpyrazine*.—The filtrate B was diluted with water and neutralised with sodium carbonate solution. The mixture was extracted with ether and the extract dried and distilled; 2-*chloro*-3 : 6-*di*-sec.-*butylpyrazine* (0·8 g.) was thus obtained as a colourless oil; b. p. 116°/12 mm., $n_D^{15°}$ 1·5030 (Found: C, 64·2; H, 8·5; N, 12·0. $C_{12}H_{19}N_2Cl$ requires C, 63·6; H, 8·4; N, 12·4%).

2-*Ethoxy*-3 : 6-*dimethylpyrazine*.—2-Chloro-3 : 6-dimethylpyrazine (1 g.) was treated with an alcoholic solution of sodium ethoxide (from 0·4 g. of sodium and 10 c.c. of ethanol) and the mixture heated under reflux for 4 hours. The solution was concentrated, diluted with water, and extracted with ether. The dried extract was distilled to give 2-*ethoxy*-3 : 6-*dimethylpyrazine* (0·7 g.) as a colourless oil, b. p. 81°/15 mm., $n_D^{15°}$ 1·4993 (Found: C, 62·8; H, 7·7; N, 18·5. $C_8H_{12}ON_2$ requires C, 63·2; H, 7·9; N, 18·4%). The *picrate* separated as prismatic needles from methanol, m. p. 108—109° (Found: C, 44·4; H, 4·2; N, 18·8. $C_{14}H_{15}O_8N_5$ requires C, 44·1; H, 3·9; N, 18·4%).

2-*Hydroxy*-3 : 6-*dimethylpyrazine*.—(*a*) 2-Chloro-3 : 6-dimethylpyrazine (1 g.) was heated under reflux with aqueous potassium hydroxide (20%; 10 c.c.) for 18 hours. The solution was neutralised (litmus) with hydrochloric acid and evaporated to dryness under reduced pressure. The residue was extracted with benzene and the extract concentrated; 2-hydroxy-3 : 6-dimethylpyrazine (0·6 g.) then

separated as needles. After recrystallisation from the same solvent it had m. p. 208—210° either alone or when mixed with an authentic specimen.

(*b*) A solution of 2-ethoxy-3 : 6-dimethylpyrazine (0·6 g.) in hydrochloric acid (5N, 20 c.c.) was heated under reflux for 18 hours. The solution was evaporated to dryness, the residue dissolved in water, and the solution neutralised by the addition of sodium carbonate solution and evaporated to dryness. The residue was extracted with benzene and the extract concentrated; 2-hydroxy-3 : 6-dimethylpyrazine (0·4 g.), m. p. and mixed m. p. 208—210°, then separated as needles.

(*c*) 2-Chloro-5-hydroxy-3 : 6-dimethylpyrazine (0·5 g.) was heated at 180—200° for 5 hours with powdered potassium hydroxide (2 g.). The cold reaction product was dissolved in water, and the solution neutralised with dilute hydrochloric acid and then evaporated to dryness (reduced pressure). Extraction with benzene in the usual manner gave 2-hydroxy-3 : 6-dimethylpyrazine (0·2 g.) as needles, m. p. and mixed m. p. 208—210°. The picrate separated as blades from methanol, m. p. 180—182°, undepressed when mixed with the specimen described by Baxter, Newbold, and Spring (*loc. cit.*).

2-*Hydroxy*-3 : 6-*di*-sec.-*butylpyrazine*.—2-Chloro-3 : 6-di-*sec*.-butylpyrazine (0·5 g.) was heated with powdered potassium hydroxide (2 g.) for 4 hours at 180°. The reaction mass was dissolved in water and the solution extracted with ether. The alkaline solution was acidified; 2-hydroxy-3 : 6-di-*sec*.-butylpyrazine (0·3 g.) then separated. It was collected and purified by sublimation at 110°/1 mm., and so obtained as small needles, m. p. 122—124°, not depressed when mixed with a specimen, m. p. 122—124°, prepared as described by Newbold and Spring (*loc. cit.*). Light absorption in alcohol: Maxima at 3250 A., $\epsilon = 10{,}000$ and 2290 A., $\epsilon = 9{,}100$.

2-*Chloro*-5-*hydroxy*-3 : 6-*di*-sec.-*butylpyrazine*.—2 : 5-Dichloro-3 : 6-di-*sec*.-butylpyrazine (0·3 g.) was heated with powdered potassium hydroxide (2 g.) for 4 hours at 160—180°. The mass was dissolved in water and unchanged dichloro-compound (0·2 g.) isolated by extraction with ether. Acidification of the solution with hydrochloric acid gave a solid which after sublimation at 90°/1 mm. gave 2-*chloro*-5-*hydroxy*-3 : 6-*di*-sec.-*butylpyrazine*, m. p. 105—106°. It is freely soluble in the common organic solvents, but insoluble in water (Found: C, 59·4; H, 8·0. $C_{13}H_{19}ON_2Cl$ requires C, 59·4; H, 7·8%).

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