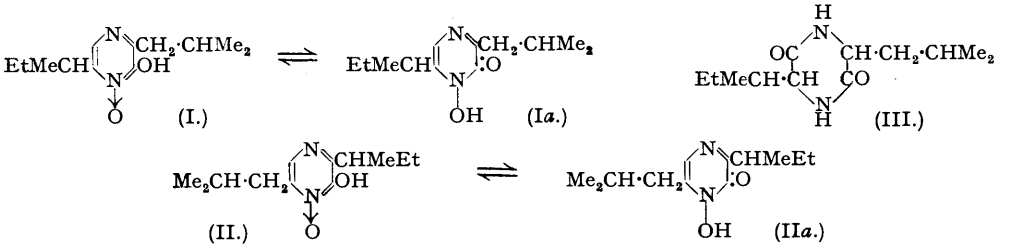
595. *Aspergillic Acid. Part III.* The Synthesis of Racemic Deoxyaspergillic Acid.*

By G. T. Newbold, William Sharp, and F. S. Spring.

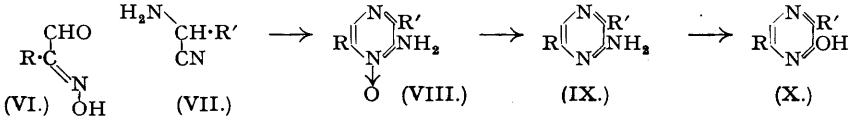
The synthesis of 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine (IV) is described; it is identical with racemic deoxyaspergillic acid. This synthesis establishes the structure of aspergillic acid as 3-*iso*butyl-6-*sec.*-butyl-2-hydroxypyrazine 1-oxide (I).

Degradative experiments have established that the antibiotic aspergillic acid is either 3-*iso*butyl-6-*sec.*-butyl-2-hydroxypyrazine 1-oxide (I or I*a*) or 6-*iso*butyl-3-*sec.*-butyl-2-hydroxypyrazine 1-oxide (II or II*a*). The nature of the alkyl side chains was established by conversion of the antibiotic into a diketopiperazine (III), hydrolysis of which gave a mixture of leucine and *iso*leucine (Dunn, Gallagher, Newbold, and Spring, *J.*, 1949, S 126; Dunn, Newbold, and Spring, *J.*, 1949, S 131).



Reduction of aspergillic acid with hydrazine gives deoxyaspergillic acid (Dutcher, *J. Biol. Chem.*, 1947, **171**, 321) which according to Dunn, Newbold, and Spring (*loc. cit.*) is either 2-*iso*butyl-5-*sec.*-butyl- (IV) or 5-*iso*butyl-2-*sec.*-butyl-3-hydroxypyrazine (V). Deoxyaspergillic acid is readily racemised when heated with alkali (Dunn, Gallagher, Newbold, and Spring, *loc. cit.*). This paper describes a synthesis of racemic deoxyaspergillic acid and its identification as 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine (IV). It follows that aspergillic acid is the corresponding oxide (I).

Condensation of an oximinomethyl ketone with an α-amino-nitrile yields a 3 : 5-disubstituted 2-aminopyrazine 1-oxide, reduction of which with sodium dithionite (hydrosulphite) gives a 3 : 5-disubstituted 2-aminopyrazine (Sharp and Spring, *J.*, 1951, 932). The synthesis of a 2 : 5-disubstituted 3-aminopyrazine by a similar method would require the condensation of an α-oximino-aldehyde (VI) with an α-amino-nitrile (VII). A method has been developed for the synthesis of α-oximino-aldehydes, which so far as we are aware have not been previously



described. Treatment of methylglyoxal diethyl acetal (XI; R = Me) (Dakin and Dudley, *J.*, 1914, **105**, 2453) with hydroxylamine gives α-oximinopropaldehyde diethyl acetal (XII; R = Me) from which α-oximinopropaldehyde (VI; R = Me) was obtained in good yield by hydrolysis with acetic acid–sodium acetate solution. α-Oximinopropaldehyde is very similar in solubility and odour to oximinoacetone but it is less stable, extensive decomposition occurring during a few months' storage under normal conditions. In a similar manner *sec.*-butylglyoxal diethyl acetal (XI; R = Bu^s) (Dakin and Dudley, *loc. cit.*) was converted into the oximino-acetal (XII; R = Bu^s) and thence into β-methyl-α-oximino-*n*-valeraldehyde (VI; R = Bu^s).

Reaction of α-oximinopropaldehyde (VI; R = Me) in chloroform solution with α-amino-

* Part II, *J.*, 1949, S 131.

propionitrile (VII; R′ = Me), liberated from its hydrochloride with 4-methylmorpholine, gives 2-amino-3 : 6-dimethylpyrazine 1-oxide (VIII; R = R′ = Me) which was characterised as its picrate. Like the 3 : 5-disubstituted 2-aminopyrazine 1-oxides described by Sharp and Spring (*loc. cit.*) 2-amino-3 : 6-dimethylpyrazine 1-oxide gives an intense blue colour with aqueous ferric chloride which is discharged on the addition of acid. Reduction of 2-amino-3 : 6-dimethylpyrazine 1-oxide with sodium dithionite gives 3-amino-2 : 5-dimethylpyrazine (IX; R = R′ = Me) characterised as its picrate.

$$\underset{\text{(XI.)}}{(EtO)_2CH\cdot COR} \longrightarrow \underset{\text{(XII.)}}{(EtO)_2CH\cdot \overset{\overset{N\cdot OH}{\|}}{C}R} \longrightarrow \underset{\text{(VI.)}}{OHC\cdot \overset{\overset{N\cdot OH}{\|}}{C}R}$$

Condensation of α-amino-γ-methylvaleronitrile (DL-leucine nitrile) (VII; R′ = Bu^i) with β-methyl-α-oximino-*n*-valeraldehyde (VI; R = Bu^s) gives 2-amino-3-*iso*butyl-6-*sec.*-butylpyrazine 1-oxide (VIII; R = Bu^s, R′ = Bu^i) characterised as its flavianate. Reduction of this amino-oxide with sodium dithionite gives 3-amino-2-*iso*butyl-5-*sec.*-butylpyrazine (IX; R = Bu^s, R′ = Bu^i) characterised as its picrate. Treatment of the amino-pyrazine with nitrous acid gave 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine (X; R = Bu^s, R′ = Bu^i) which gives a 6-phenylazo-derivative. 2-*iso*Butyl-5-*sec.*-butyl-3-hydroxypyrazine is undepressed in melting point when mixed with racemic deoxyaspergillic acid and similarly no depression is observed when its 6-phenylazo-derivative is mixed with that of racemic deoxyaspergillic acid. The identity was confirmed by comparison of the ultra-violet absorption spectra of the two specimens.

Experimental.

The method of Johnson and Cretcher (*J. Amer. Chem. Soc.*, 1915, **37**, 2144) for the preparation of ethyl diethoxyacetate proved unsatisfactory. The following method gave reproducible yields: Ethyl dichloroacetate (162 g.) was added dropwise during 30 minutes to a boiling solution of sodium ethoxide from sodium (46 g.) and ethanol (1 l.). The reaction mixture was heated under reflux for 1 hour and set aside overnight. After removal of salt, the solution was evaporated under reduced pressure. The residue was treated with water (250 c.c.) and extracted with ether (4 × 100 c.c.), and the combined extracts were dried (Na_2SO_4). Removal of the ether followed by distillation gave ethyl diethoxyacetate (40—44 g., 22—25%), b. p. 85—95°/13 mm. Ethyl γ-diethoxyacetoacetate, methylglyoxal diethyl acetal, and *sec.*-butylglyoxal diethyl acetal were prepared as described by Dakin and Dudley (*loc. cit.*).

*α-Oximinopropaldehyde Diethyl Acetal.**—(*a*) Methylglyoxal diethyl acetal (2·4 g.) was heated under reflux in ethanol (100 c.c.) containing hydroxylamine [from the hydrochloride (1·16 g.) and sodium ethoxide from sodium (0·38 g.)] for 2 hours. Removal of the solvent and distillation of the residue gave *α-oximinopropaldehyde diethyl acetal* (1·85 g.) as a colourless oil, b. p. 105°/8 mm., n_D^{14} 1·4388 (Found: C, 52·4; H, 9·3; N, 8·7. $C_7H_{15}O_3N$ requires C, 52·2; H, 9·4; N, 8·7%).

(*b*) A solution of hydroxylamine hydrochloride (7·4 g.) in water (30 c.c.) was added during 15 minutes to a mixture of methylglyoxal diethyl acetal (14·0 g.), methanol (20 c.c.), water (90 c.c.), and anhydrous sodium carbonate (7·7 g.), with stirring at 15°. Stirring was continued for 3 hours and the reaction mixture kept overnight. The mixture was extracted with ether (4 × 50 c.c.), and the extract dried (Na_2SO_4) and evaporated. Distillation of the residue gave the oxime acetal (15·5 g.), b. p. 105°/8 mm.

*α-Oximinopropaldehyde.**—α-Oximinopropaldehyde diethyl acetal (1·8 g.) was warmed on the steam-bath for 10 minutes with a buffer solution (10 c.c.; 1·0M-acetic acid and 0·1M-sodium acetate; pH 3·5), a positive test being then given with Fehling's solution. The cooled solution was saturated with salt and extracted with ether (4 × 10 c.c.). The dried (Na_2SO_4) extract was evaporated and the residue distilled, to give a colourless viscous oil (0·8 g.), b. p. 70—75°/20 mm., which rapidly solidified. Crystallisation from light petroleum (b. p. 60—80°) gave *α-oximinopropaldehyde* as prisms, m. p. 67° (Found: C, 41·1; H, 5·8. $C_3H_5O_2N$ requires C, 41·4; H, 5·7%). α-Oximinopropaldehyde sublimes very readily and decomposes on long storage.

*β-Methyl-α-oximino-*n*-valeraldehyde Diethyl Acetal.*—A well-stirred mixture of *sec.*-butylglyoxal diethyl acetal (30·0 g.; Dakin and Dudley, *loc. cit.*), methanol (250 c.c.), water (120 c.c.), and anhydrous sodium carbonate (12·75 g.) was treated with a solution of hydroxylamine hydrochloride (12·3 g.) in water (60 c.c.). The mixture (pH ~8) was stirred at room temperature for 26 hours and evaporated under reduced pressure at room temperature. The residue was extracted with ether (4 × 50 c.c.), and the extract dried (Na_2SO_4). Removal of the ether followed by distillation of the residue gave *β-methyl-α-oximino-*n*-valeraldehyde diethyl acetal* (29·5 g.) as a colourless oil, b. p. 103°/2 mm., n_D^{12} 1·4459 (Found: C, 59·2; H, 10·0. $C_{10}H_{21}O_3N$ requires C, 59·1; H, 10·4%).

*β-Methyl-α-oximino-*n*-valeraldehyde.*—The diethyl acetal (15·0 g.) was heated in methanol (100 c.c.) and buffer solution (100 c.c.; 1·0M-acetic acid and 0·1M-sodium acetate; pH 3·5) under reflux for 4 hours. Methanol was removed under reduced pressure, water (100 c.c.) added, and the mixture extracted with ether (4 × 50 c.c.). The dried ($MgSO_4$) extract was evaporated and the residue distilled to give *β-methyl-α-oximino-*n*-valeraldehyde* (6·1 g., 64%) as a pale yellow viscous oil, b. p. 70—74°/1·5 mm., n_D^{15} 1·4737 (Found: C, 55·25; H, 8·2. $C_6H_{11}O_2N$ requires C, 55·8; H, 8·6%).

* Preparations marked * were first made in 1945 in Manchester University by Dr. R. A. Baxter.

2-*Amino*-3 : 6-*dimethylpyrazine* 1-*Oxide*.—A suspension of α-aminopropionitrile hydrochloride (2·55 g., 1 mol.) in dry chloroform (18 c.c.) was shaken with 4-methylmorpholine (2·64 c.c., 1 mol.) and α-oximinopropaldehyde (2·10 g., 1 mol.) rapidly added. The mixture was heated under reflux for 4 hours and the chloroform evaporated. The residue was dissolved in water (25 c.c.), hydrochloric acid (3N.; 25 c.c.) added, and the solution extracted with ether (4 × 20 c.c.), the ethereal extract being discarded. The aqueous solution was basified (litmus) with sodium carbonate and extracted with ether (4 × 20 c.c.); the ethereal extract, of which the residue on evaporation did not give a blue colour in ethanol with aqueous ferric chloride, was rejected. The aqueous phase was evaporated and the dried, powdered residue extracted with hot chloroform (5 × 50 c.c.). The combined chloroform extracts were evaporated, the gum (450 mg.) was dissolved in ethanol (5 c.c.) and treated with saturated ethanolic picric acid (10 c.c.), and the precipitate (290 mg.) collected. Three crystallisations from ethanol gave 2-*amino*-3 : 6-*dimethylpyrazine* 1-*oxide picrate* as yellow felted needles, m. p. 220° (decomp.) (Found : C, 39·4; H, 3·3; N, 22·5. $C_6H_9ON_3,C_6H_3O_7N_3$ requires C, 39·1; H, 3·3; N, 22·8%). An ethanolic solution of the picrate gave a greenish-blue colour with aqueous ferric chloride discharged by dilute hydrochloric acid. The picrate (290 mg.) was dissolved in warm hydrochloric acid (3N.; 20 c.c.), and the solution extracted with ethyl acetate (4 × 10 c.c.) to remove picric acid. The aqueous solution was evaporated to dryness, the residue (151 mg.) dissolved in water (1 c.c.), and the solution basified (litmus) with sodium carbonate solution and evaporated to dryness. Sublimation of the residue at 110—120°/10^{-2} mm. gave a crystalline sublimate (85 mg.), m. p. 160—162°. Two further sublimations gave 2-*amino*-3 : 6-*dimethylpyrazine* 1-*oxide* as needles, m. p. 165° (Found : C, 51·7; H, 6·2; N, 29·5. $C_6H_9ON_3$ requires C, 51·8; H, 6·5; N, 30·2%). Light absorption in ethanol : Maxima at 2320 (ε = 23,600) and 3290 A. (ε = 7800). The oxide is readily soluble in water and ethanol and sparingly soluble in ether, light petroleum (b. p. 60—80°), and benzene; its aqueous or ethanolic solution gives an intense blue colour with aqueous ferric chloride, the colour being discharged by addition of dilute hydrochloric acid.

3-*Amino*-2 : 5-*dimethylpyrazine*.—A solution of 2-amino-3 : 6-dimethylpyrazine 1-oxide (35 mg.) in water (2 c.c.) was heated under reflux with sodium dithionite (0·32 g.). After 1 hour the cooled solution was again treated with dithionite (0·32 g.) and the refluxing continued. Six similar additions were made and water was added (2 × 0·5 c.c.) at intervals to maintain complete solution. The reaction mixture was diluted with water to a bulk of 10 c.c., made alkaline (litmus) with 2N-sodium hydroxide, and extracted with ether (6 × 10 c.c.). The dried (Na_2SO_4) ethereal solution was evaporated, the residue dissolved in ethanol (1 c.c.), and the filtered solution treated with saturated ethanolic picric acid (0·5 c.c.) whereupon 3-amino-2 : 5-dimethylpyrazine picrate (10 mg.) separated as yellow prismatic needles, m. p. 205—206° alone or mixed with a specimen prepared as described by Newbold, Spring, and Sweeny (*J.*, 1949, 302).

DL-*Leucine Nitrile Hydrochloride*.—A solution of *iso*valeraldehyde (25 g.) in ether (145 c.c.) was stirred with ammonium chloride (17·4 g.) at 0° and a solution of potassium cyanide (19·4 g.) in water (50 c.c.) was added during 15 minutes. After 4 hours' stirring at 0° the ether was separated and the aqueous phase extracted with ether (3 × 50 c.c.). The combined ethereal solution was dried (Na_2SO_4) at 0° and saturated with dry hydrogen chloride at 0°. The precipitate (11·4 g.) was collected, dried *in vacuo* over sodium hydroxide, and used for the next stage without further purification. A specimen was thrice crystallised from acetone from which DL-*leucine nitrile hydrochloride* separated as plates, m. p. 175—180° with darkening (Found : C 48·9; H, 8·7. $C_6H_{13}N_2Cl$ requires C, 48·5; H, 8·8%).

2-*Amino*-3-iso*butyl*-6-sec.-*butylpyrazine* 1-*Oxide*.—DL-Leucine nitrile hydrochloride (6·65 g.), suspended in dry chloroform (25 c.c.), was shaken with 4-methylmorpholine (4·95 c.c.), and the mixture treated with a solution of β-methyl-α-oximino-*n*-valeraldehyde (5·8 g.) in dry chloroform (7·5 c.c.). The mixture was heated under reflux for 4 hours, cooled, and filtered and the filtrate evaporated. The residue was dissolved in ether (75 c.c.) and extracted with hydrochloric acid (4 × 25 c.c.; 3N.). The hydrochloric acid solution was extracted with ether (2 × 30 c.c.) (the ethereal extract being rejected), basified (litmus) with sodium hydroxide solution, and extracted with ether (4 × 50 c.c.). The ethereal solution was washed with water (3 × 15 c.c.), dried (Na_2SO_4), and evaporated to give a viscous oil (3·12 g.), distillation of which gave 2-*amino*-3-iso*butyl*-6-sec.-*butylpyrazine* 1-*oxide* as a light yellow oil, b. p. 150—155°/0·5 mm., n_D^{15} 1·5410 (Found : C, 64·8; H, 9·9. $C_{12}H_{21}ON_3$ requires C, 64·6; H, 9·4%). It is very soluble in the common organic solvents, insoluble in water, and soluble in 3N-hydrochloric acid; its ethanolic solution gives a deep blue colour with aqueous ferric chloride, discharged by dilute hydrochloric acid. The *flavianate* crystallises from 50% aqueous ethanol as light yellow prisms, m. p. 235° (decomp.) (Found : C, 49·5; H, 5·2; N, 12·4. $C_{12}H_{21}ON_3,C_{10}H_6O_8N_2S$ requires C, 49·2; H, 5·1; N, 13·0%).

3-*Amino*-2-iso*butyl*-5-sec.-*butylpyrazine*.—A solution of 2-amino-3-*iso*butyl-6-*sec*.-butylpyrazine 1-oxide (1·4 g.) in ethanol (15 c.c.) and water (15 c.c.) was treated with sodium dithionite (2 g.) and heated under reflux for 30 hours during which time 20 additions of sodium dithionite (1 g.), ethanol (5 c.c.), and water (5 c.c.) were made at approximately equal intervals. Ethanol was removed under reduced pressure, and the residue made alkaline with 3N-sodium hydroxide and extracted with ether (4 × 30 c.c.). The combined extracts were washed with water (25 c.c.) and dried ($MgSO_4$). Removal of the ether gave an amber-coloured oil, which gave a faint blue colour with aqueous-alcoholic ferric chloride. A solution of the oil in ethanol (5 c.c.) was treated with saturated ethanolic picric acid, and the precipitate (1·15 g.) collected. Crystallisation from ethanol gave 3-*amino*-2-iso*butyl*-5-sec.-*butylpyrazine picrate* as yellow felted needles, m. p. 120—121° (Found : C, 49·9; H, 5·2; N, 19·6. $C_{12}H_{21}N_3,C_6H_3O_7N_3$ requires C, 49·5; H, 5·5; N, 19·3%).

2-iso*Butyl*-5-sec.-*butyl*-3-*hydroxypyrazine*.—A solution of 3-amino-2-*iso*butyl-5-*sec*.-butylpyrazine picrate (400 mg.) in hot ethanol (5 c.c.) was treated with hydrochloric acid (10 c.c.; 3N.), and the ethanol removed by evaporation. The mixture was diluted with water (10 c.c.) and extracted with ethyl acetate

(2 × 10 c.c.). The aqueous phase was evaporated to dryness under reduced pressure, the solid residue dissolved in hydrochloric acid (5 c.c.; 3N.), and the solution cooled to 0° and treated with sodium nitrite (0·25 g.) added in portions over 5 minutes. The reaction mixture was kept at 0° for 30 minutes, heated to 50—60° for 5 minutes, neutralised (litmus) with solid sodium carbonate, and extracted with ether (2 × 10 c.c.). The ethereal extract was washed with potassium hydroxide solution (2 × 5 c.c.; 2N.), and the combined aqueous layers were neutralised (litmus) with hydrochloric acid (3N.). The precipitated solid (65 mg.; m. p. 96—98°) was crystallised from aqueous ethanol, to give 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine as prismatic needles, m. p. 100·5—101·5°. A mixture with racemic deoxyaspergillic acid, m. p. 103—104° (Dunn *et al.*, *loc. cit.*) had m. p. 101·5—102° (Found: C, 69·5; H, 9·6; N, 13·4. Calc. for $C_{12}H_{20}ON_2$: C, 69·2; H, 9·6; N, 13·5%). Light absorption in ethanol: Maxima at 2270 ($\varepsilon = 8000$) and 3240 A ($\varepsilon = 8000$). The 6-phenylazo-derivative separated from aqueous ethanol as red needles, m. p. 188—189° alone or mixed with the 6-phenylazo-derivative of racemic deoxyaspergillic acid, m. p. 188—190° (Found: C, 69·65; H, 7·5. Calc. for $C_{18}H_{24}ON_4$: C, 69·3; H, 7·7%).

THE ROYAL TECHNICAL COLLEGE, GLASGOW.

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