The Synthesis of Deoxyaspergillic Acid.

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Thesis presented to

The University of Glasgow

in fulfilment of the requirements for

the degree of

Doctor of Philosophy

by

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January, 1950.

The author wishes to express his sincere gratitude to Professor F.S. Spring for his supervision and constant interest in the progress of the work, and to Dr. G.T. Newbold for his advice and interpretation of spectroscopic data. Thanks are also due to the Department of Scientific and Industrial Research for financial provision and to The Royal Technical College where the research programme was carried out.

CONTENTS.

HISTORICAL.

INTRODUCTION.

The investigation of penicillin, the antibacterial agent discovered by Fleming (1) in culture filtrates of Penicillium notatum, by Florey, Chain et alia (2), resulting in its isolation, the realisation of its remarkable antibacterial properties and subsequent application to clinical medicine (3) aroused widespread scientific interest in the study of other moulds and bacterial products in general. A considerable number of antibiotics have now been isolated and their structural investigation is providing a growing field of activity for the organic chemist. Various penicillins have been isolated, identified, and a great deal of effort expended in attempts to synthesise not only penicillins but also penicillin analogues (4). Of the other antibiotics isolated and identified, the most notable is streptomycin (5) which is now being widely used, particularly in the chemotherapeutic treatment of tuberculosis (6). The majority of natural antibiotics, so far isolated, do not possess properties to the degree that has established penicillin as an outstanding chemotherapeutic agent. The present work concerns one of these compounds, known as Aspergillic acid.

ASPERGILLIC ACID.

Isolation.

It was shown by White (7) that a mould tentatively identified as Aspergillus flavus, when grown in surface culture on a tryptone-salt medium, yielded filtrates which are bactericidal to some Gram-negative as well as Gram-positive bacteria. White and Hill (8) reported the isolation of a crystalline antibacterial substance from the filtrates. It was named aspergillic acid because the mode of isolation indicated that it was acidic. Tested against Group A β-haemolytic streptococci, aspergillic acid was active in a dilution of 1:40,000. The same authors subsequently gave details of their isolation procedure (9). The mycelium was removed from the culture, the filtrate treated with activated charcoal at pH 4, the charcoal air dried and then exhaustively extracted with ether. Removal of the ether left a gum which was extracted with sodium bicarbonate solution and the resultant solution carefully acidified to give aspergillic acid, as light cream crystals, several samples melting within the range 84-96°, soluble in most organic solvents except petroleum and insoluble in water. The material, which was soluble in both acid and alkali, contained nitrogen and had a molecular formula, $C_{12}H_{20}O_2N_2$.

The culture filtrates from an unidentified mould,

but probably belonging to the Aspergillus genus, were shown by Glister (10) to contain a powerful antibacterial agent with a range considerably greater than penicillin. In addition to the Gram-positive organisms known to be inhibited by penicillin the growth of a number of Gram-megative organisms such as Bact. coli, B. dysenteriae, the typhoid and paratyphoid bacilli and the Vibrio cholerae, was prevented by the new antibiotic, a concentrate of which was claimed to be active in a dilution of 1:200,000. Menzel, Wintersteiner and Rake (11), from the culture filtrates of an Aspergillus flavus strain, isolated a pure aspergillic acid, m.p. 93°, $[\alpha]_D$ + 14° which could be distilled in steam or in vacuo without loss of activity and had a molecular formula, $C_{12}H_{20}O_2N_2$. After a comparison of the physical properties and biological activity of Glister's product (10) with aspergillic acid, Menzel, Wintersteiner and Rake (11) concluded, "the active substance elaborated by Glister's unclassified mould is unquestionably identical with aspergillic acid, m.p. 93°, although the cultural characteristics of this mould are different from those of Aspergillus flavus." In common with White and Hill (8,9), they noted a possible polymorphism in aspergillic acid since sometimes a variety, m.p. 116°, was obtained.

The yields of aspergillic acid obtained from culture filtrates of the mould differ widely. White and Hill (8,9)

reported a yield of only 5 mg./litre of culture filtrate harvested after maximum activity was shown, i.e., after six days growth, though occasional yields of 60 mg./litre were recorded. Assay of culture filtrate activity was rapidly carried out by observation of the quenching of the bioluminescence of Photobacterium fisheri using a serial dilution technique (12). Using the medium which had been adopted by White and Hill (8,9) as their standard and a variant of White's original strain of Aspergillus flavus, Jones Rake and Hamre (12) were able to increase production of aspergillic acid by the mould to 120-250 mg./litre of culture filtrate. The same workers found that higher titres could be obtained by the addition of 2 to 4 per cent of brown sugar to the medium when as much as 400 mg. of crystalline material per litre was obtained. It was subsequently reported by Menzel, Wintersteiner and Rake (11) that such an addition results in the elaboration of another product, hydroxyaspergillic acid, $C_{12}H_{20}O_5N_2$, m.p. 145-146°, $[\alpha]_D$ + 45°, having one tenth of the antibiotic activity of aspergillic acid. Bush, Dickison, Ward and Avery (13) using a strain of Aspergillus flavus Link, and a 2% Difco peptone - 2% lactose medium obtained a titre of approximately 500 mg. of crystalline material per litre of culture filtrate. This was also a mixture of aspergillic acid and hydroxyaspergillic acid. More recently Woodward (14), using a yeast extract-glycerol medium was able to produce

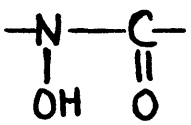
aspergillic acid, m.p. 93°, in consistently good yields of from 800-1000 mg./litre.

A certain amount of confusion exists in the literature concerning the name of this substance. The preempting of the name aspergillin by the pigment of Aspergillus niger (15) decided White, the discoverer, against using this name; hence the name aspergillic acid. Later workers have been less discriminate and have applied the term aspergillin to active materials from Aspergillus flavus and other Aspergillii; Bush and Goth (16) later identified their aspergillin as aspergillic acid (13); Stanley (17), from an unidentified Aspergillus strain isolated an active substance which he designated aspergillin but has subsequently identified it as gliotoxin (18); still another crude product, obtained from Aspergillus fumigatus by Soltys (19) has been called aspergillin but is probably helvolic acid (20). Other antibiotics have been isolated from Aspergillii cultures; amongst them are patulin (21) from Aspergillus clavatus (22) and Aspergillus giganteus (23) and kojic acid from Aspergillus parasiticus (24). An antibiotic flavicin, identical in many respects with penicillin, has been obtained from strains of Aspergillus flavus (25).

Structure of Aspergillic Acid.

A preliminary report summarising the results of their investigation into the structure of aspergillic acid was made by Dutcher and Wintersteiner (26), followed by a more detailed statement (27). Aspergillic acid, $C_{12}H_{20}O_2N_2$ from analysis and molecular weight determinations, crystallises in clusters of pale yellow elongated rods, m.p. 93°, $[\alpha]_D^{24°}$ + 13.4° in ethanol, C = 0.85. It is soluble in most organic solvents, almost insoluble in water, soluble in dilute alkali and can be titrated in alcohol solution with phenolphthalein as indicator. Electrometric titration showed the $p.k'_a$ to be 5.3. It forms a silver and copper salt. It has weakly basic properties giving rise in anhydrous medium to a crystalline hydrochloride, m.p. 182° and with 3:5 - dinitrobenzoic acid forms a salt, m.p. 123°. It contained no methoxyl or methyl imide groups but yielded 2.1 moles of acetic acid in the Kuhn - Roth determination. By the same method DL - isoleucine yielded 1.0 mole of acetic acid. It was indifferent to carbonyl reagents except that in alcoholic solution it formed a crystalline salt with phenylhydrazone. It reacted with an ethereal solution of diazo-methane to give a neutral non-crystalline product which analysed for the methyl ether. It did not reduce Fehling's solution and was recovered unchanged after prolonged refluxing with acid or alkali.

Aspergillic acid is reduced to deoxyaspergillic acid, $C_{12}H_{20}ON_2$, m.p. 102°, $[\alpha]_D^{24°}$ + 15.3° in ethanol, C = 0.5, by dry distillation in the presence of copper chromite catalyst or by the action of hydrazine or hydriodic acid and red phosphorus. Deoxyaspergillic acid is weakly acidic; it dissolves in 10% sodium hydroxide solution but cannot be titrated. It is more basic than aspergillic acid and is readily soluble in normal hydrochloric acid, the hydrochloride has m.p. 207°. It is readily soluble in organic solvents, insoluble in cold water but slightly soluble in hot water. It does not give a copper salt or any trace of colouration with ferric chloride solution. Deoxyaspergillic acid has no antibiotic properties. Since aspergillic acid was not only easily reduced but also formed a green cupric salt, m.p. 198°, and gave a deep-red colouration with ferric chloride, Dutcher (27) suggested that it contained a hydroxamic acid grouping (1). Since aliphatic hydroxamic acids are rather easily hydrolysed or undergo

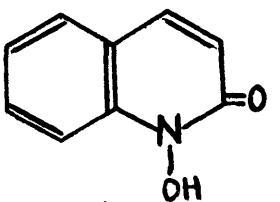


(1)

rearrangement, and aspergillic acid was stable to both acid and alkali, it seemed likely that this hydroxamic acid grouping was present in a heterocyclic system.

At that time very few cyclic hydroxamic acids had

been described in the literature but those that were recorded had properties remarkably similar to aspergillic acid. Oxycarbostyril (11) was obtained by Friedländer and Ostermaier (28) as a minor product of the reduction by ammonium sulphide of ethyl o-nitrocinnamate. Heller and Wunderlich (29) obtained the corresponding oxycarbostyril-3-carboxylic acid (111) by the reduction of o-nitrobenzylidene-malonic acid with zinc and acetic acid whilst Reissert (30) obtained 1:2-dioxyindole (IV) by the reduction of o-nitro-phenylacetic acid with zinc and sulphuric acid. 2-methyl-3-hydroxy-4-keto-3:4-dihydroquinazoline (V) has been obtained by the treatment of acetylanthranil with hydroxylamine (31).



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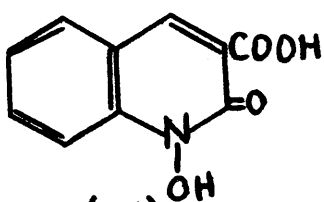

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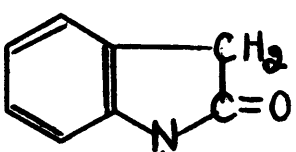

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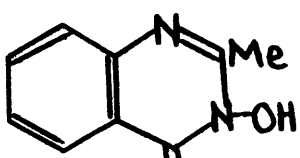


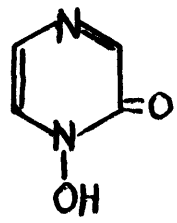
(V)

The identification of aspergillic acid as a cyclic hydroxamic acid (26,27) is, however, the first occasion in which the occurrence of a hydroxamic acid grouping in a natural product has been reported.

The hypothesis has been advanced (32) that formylhydroxamic

acid, the product of activated formaldehyde and inorganic nitrite, is an intermediate in the phytosynthesis of 2-amino-acids and alkaloids but no experimental evidence supporting this view has been forthcoming. A hydroxylamino derivative, canavine, has been isolated from the jack bean (33) and amine oxides are known to occur in fish muscle extracts.

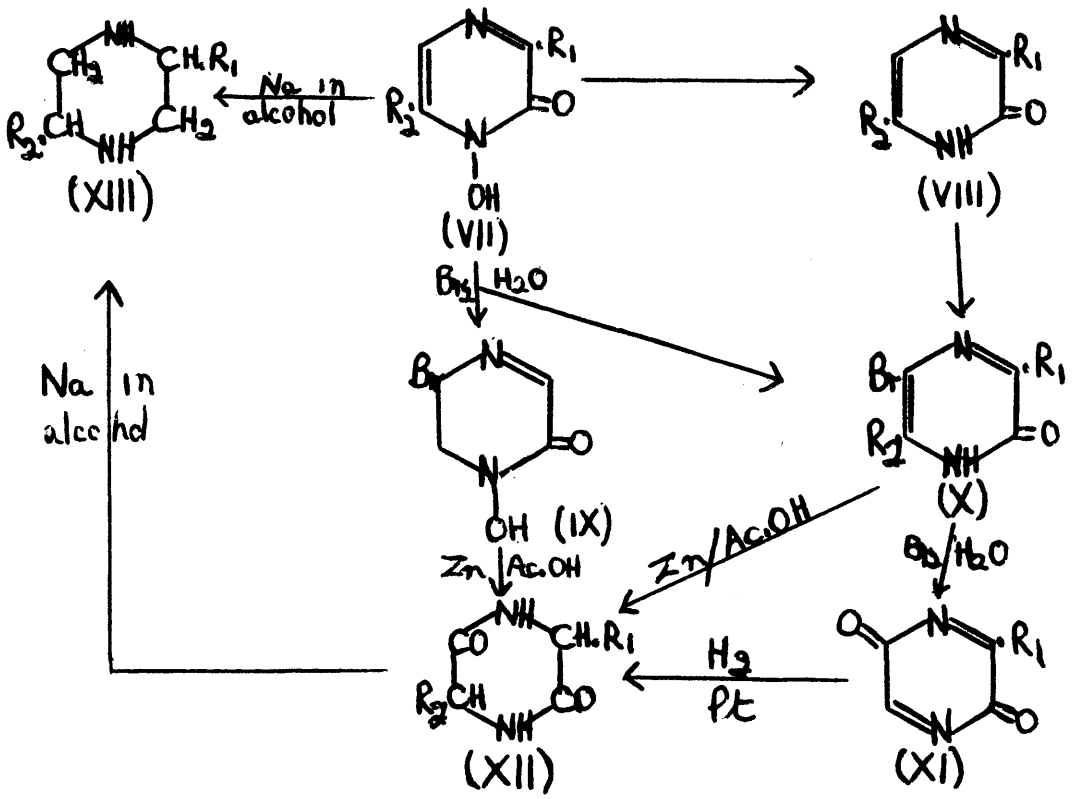
Since aspergillic acid was only weakly basic, did not react with nitrous acid and could not be acylated, it followed that the second nitrogen atom must also be in the heterocyclic system. The formation of a tetrahydro derivative, the indifference to potassium permanganate solution and bromine in carbon tetrachloride or glacial acetic acid solution, and the resistance to hydrolysis indicated that the two double bonds demonstrable by reduction must be presence in a six membered aromatic system, i.e., a diazine nucleus. Ultra-violet absorption spectrum at 3250 A., was not characteristic of any of the monoxypyrimidines none of which had maxima beyond 2900 A.. No ultra-violet absorption spectra for either hydroxypyridazines or hydroxypyrazines had at that time been reported but Dutcher and Wintersteiner (26) considered a pyrazine structure (VI) more likely for a naturally occurring substance than a pyridazine. Absorption spectrum for 2-hydroxypyrazine showed it to have a maximum at 3150 A. ($\mathcal{E}$=4,500). These findings have been amply confirmed by Newbold and Spring (34) who compared the ultra-violet absorption spectra of a



(VI)

number of hydroxypyrazines, including 3-hydroxy-2:5-di-sec.-butylpyrazine with that of deoxyaspergillic acid and concluded that the latter was probably a hydroxypyrazine.

This accounts for $C_4H_4O_2N_2$, which on subtraction from $C_{12}H_{20}O_2N_2$ leaves an aliphatic residue, C_8H_{16}. Since only one position in the nucleus was free for substitution it followed that there were two alkyl groups attached to the nucleus. At least one of these groups must possess an asymmetric carbon atom since aspergillic acid is optically active. The simplest optically active grouping is the sec.-butyl. From a consideration of biogenetic possibilities, Dutcher (26,27) adopted the working hypothesis that the side chains were both sec.- butyl groups and were symmetrically placed at positions 2- and 5- as shown in formula (VII, $R_1 = R_2$ = sec.-butyl). Dutcher (26,27) claims to have verified this structure by converting aspergillic acid (VII) to a 2:5-diketo-3:6-di-sec.-butylpyrazine (isoleucine anhydride) (XII), m.p. 249-250°, via bromo-aspergillic acid (IX) as shown in the diagram.

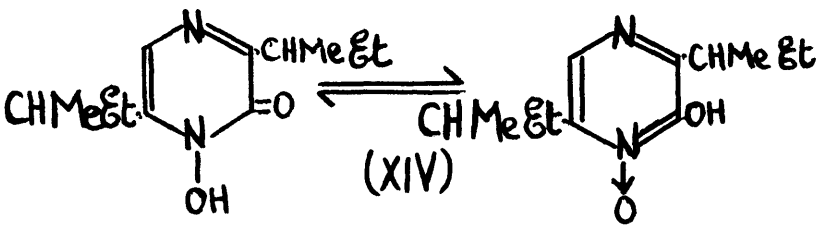


A solution of aspergillic acid (Vll) in 5N hydrochloric acid, on treatment with saturated bromine water, gave rise to a monobromo compound (lX), $C_{12}H_{19}O_2N_2Br$, m.p. 129-130°, which had the properties of a hydroxamic acid yielding a deep red colour with ferric chloride and a crystalline green copper salt. The mother liquor on further examination revealed the presence of another bromo derivative (X), $C_{12}H_{19}ON_2Br$, m.p. 129-130° giving a 35° depression on admixture with bromo-aspergillic acid (lX). Deoxyaspergillic acid (Vlll) in aqueous acetic acid solution was treated with an acetic acid solution of bromine when bromo-deoxyaspergillic acid (X) was isolated, m.p. 129-130° undepressed on admixture with the product from

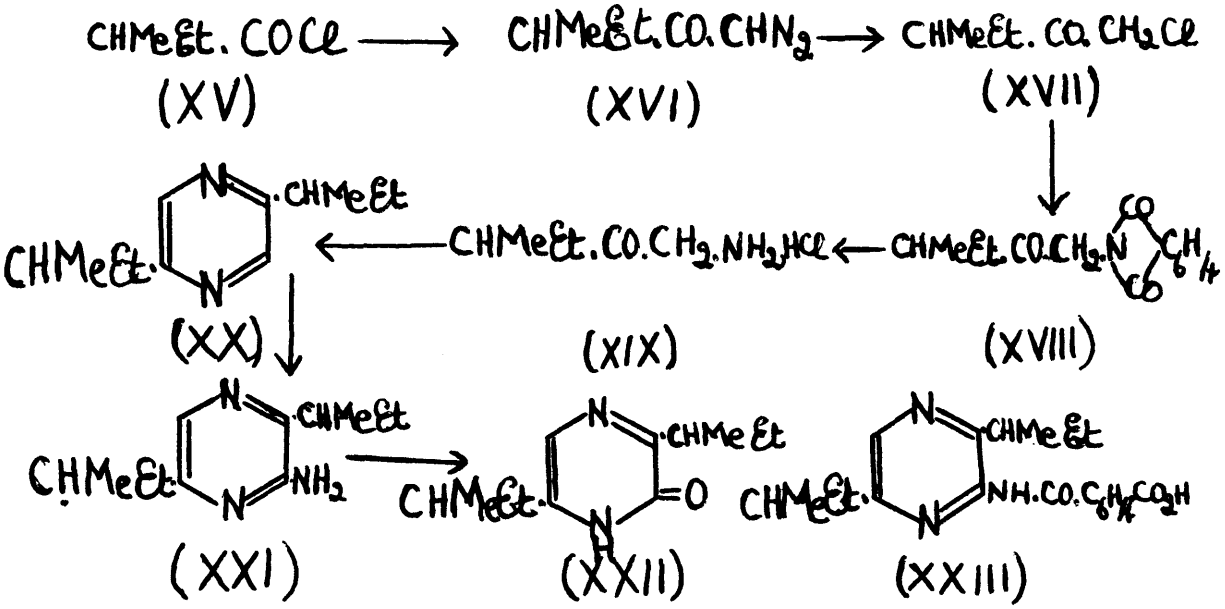
the mother liquor of bromo-aspergillic acid (1X). Further examination of the mother liquor of bromo-deoxyaspergillic acid (X) revealed a quinone-like compound (X1), $C_{12}H_{18}O_2N_2$, m.p. 268-270°. This substitution of a hydrogen atom by bromine in both aspergillic acid (V11) and deoxyaspergillic acid (V111) and the stability of the resulting derivatives (1X and X) to both silver nitrate and sodium hydroxide solution lent force to Dutcher's view that the 6- position was not blocked. Zinc and acetic acid reduction of bromo-aspergillic acid (1X) and bromo-deoxyaspergillic acid (X), in both cases gave a neutral compound (X11), $C_{12}H_{22}O_2N_2$, m.p. 249-250°, which was optically active and showed only end absorption in the ultra-violet region, properties which suggested it might be a diketo-piperazine. This could not be distinguished in appearance and solubility properties from DL-isoleucine anhydride (X11, R_1=R_2= sec.-butyl), m.p. 257°, prepared by a similar method to that adopted by Fischer (35) for DL-leucine anhydride. In a mixed m.p. determination with the natural product no lowering of the m.p. below 249-250° was observed. Dutcher (26,27) also reduced aspergillic acid, the 2:5-diketopiperazine derived from aspergillic acid and the synthetic DL-isoleucine anhydride to piperazine bases (X111) by heating them under reflux with sodium and isoamyl alcohol. From a comparison of simple derivatives of these he concluded that all three piperazine bases were identical and consequently the formula (V11, R_1=R_2= sec.-butyl) put forward by him for aspergillic acid was

correct.

Newbold and Spring (34) postulated a keto-enol tautomeric structure (XlV) for aspergillic acid.



The same authors have described the synthesis of a racemic 3-hydroxy-2:5-di-sec.-butylpyrazine (XXll), m.p. 122-124°, but due to their inability to resolve the latter they were unable to effect any direct comparison with deoxyaspergillic acid (Vlll).

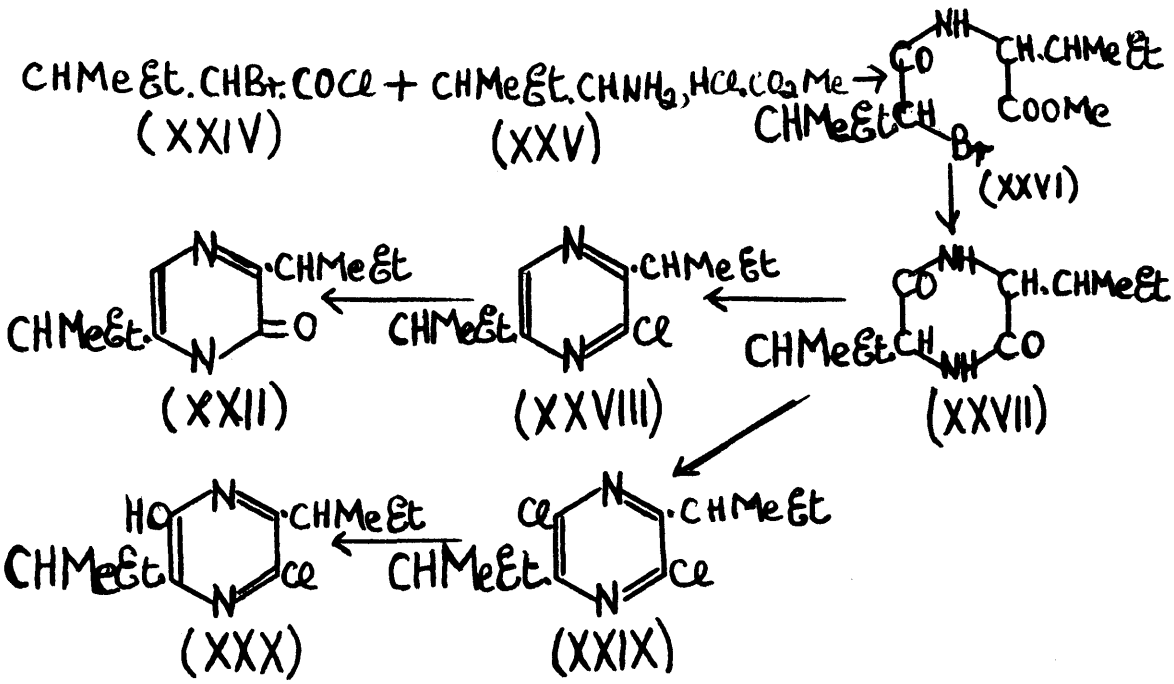


Methylethylacetyl chloride (XV) was converted into 1-chloro-3-methylpentan-2-one (XVll) via the diazo-ketone (XVl). The chloro-ketone was treated in xylene solution with potassium phthalimide to yield 1-phthalimido-3-methyl-pentan-2-one (XVlll)

which was hydrolysed by means of hydrochloric acid to yield 1-amino-3-methylpentan-2-one hydrochloride (XlX). Treatment of the hydrochloride with sodium hydroxide solution followed by oxidation of the reaction product with hydrogen peroxide gave 2:5-di-sec.-butylpyrazine (XX), characterised by the formation of a chloroplatinate. Treatment of 2:5-di-sec.-butylpyrazine in dimethylaniline solution with sodamide gave 3-amino-2:5-di-sec.-butylpyrazine (XXl), characterised by the formation of a picrate. The aminopyrazine, on treatment with nitrous acid gave a racemic 3-hydroxy-2:5-di-sec.-butylpyrazine (XXll), m.p. 122-124°, absorption in ethanol:maxima at 2285 A., ε=9,600 and maxima at 3220 A., ε= 10,000. The absorption spectra for deoxyaspergillic acid were given by Newbold and Spring (34) as maxima at 2295 A., ε=6,700 and 3250 A., ε= 8,000. A mixture of the racemic 3-hydroxy-2:5-di-sec.-butylpyrazine and deoxyaspergillic acid (m.p. 99-100°) melted at 75-85°. In view of the stereochemical complications, this marked depression was not regarded by Newbold and Spring (34) as an indication that deoxyaspergillic acid did not have the structure (Vlll) attributed to it by Dutcher and Wintersteiner (26) and Dutcher (27). Newbold and Spring (34) considered that their 3-hydroxy-2:5-di-sec.-butylpyrazine was a single racemate since fractional crystallisation revealed no signs of heterogeneity. Although it was possible that the racemate did not belong to the same optical series as deoxyaspergillic

acid, $[\alpha]_D^{24°}$ + 15.3°, they attempted to resolve the parent 3-amino-2:5-di-sec.-butylpyrazine by fractional crystallisation of its d-camphorsulphonate and its d-bromocamphor-π-sulphonate but were unsuccessful. A similar lack of success met attempts to resolve 3-phthalamido-2:5-di-sec.-butylpyrazine (XXIII).

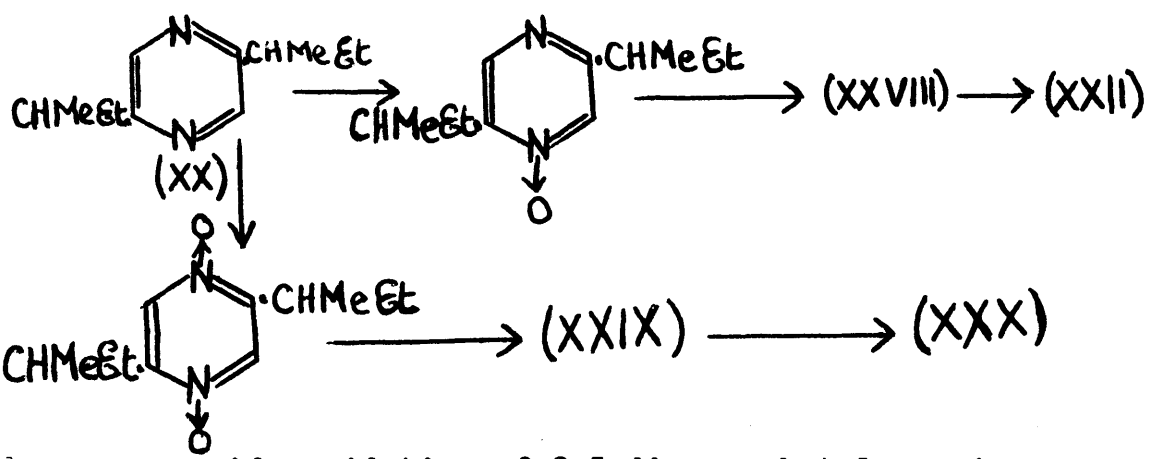
Baxter and Spring (36) have also described the synthesis of racemic 3-hydroxy-2:5-di-sec.butylpyrazine, m.p. 122-124°, undepressed when mixed with a specimen prepared according to Newbold and Spring (34).



2-Bromo-3-methyl-n.-valeryl chloride (XXIV) was condensed with DL-isoleucine methyl ester hydrochloride (XXV) and the resultant product (XXVI) heated with alcoholic ammonia to give a DL-isoleucine anhydride (XXVII) apparently identical with the product obtained when DL-isoleucine was heated under reflux

with ethylene glycol. Treatment of this anhydride with phosphoryl chloride gave a mixture of 3-chloro-2:5-di-sec.-butylpyrazine (XXVlll) and 3:6-dichloro-2:5-di-sec.-butylpyrazine (XXlX). The former was converted into 3-hydroxy-2:5-di-sec.-butylpyrazine (XXll) by heating with powdered potassium hydroxide at 180°.

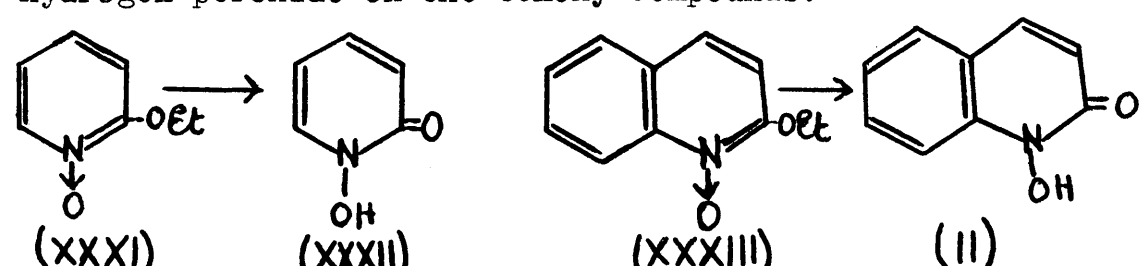
Newbold and Spring (37) obtained 3-hydroxy-2:5-di-sec.-butylpyrazine, identical in all respects with that previously described (34,36).



Hydrogen peroxide oxidation of 2:5-di-sec.-butylpyrazine, dissolved in acetic acid, gave rise to a mixture of the corresponding mono-and di-N-oxides. The mono-N-oxide, on treatment with phosphoryl chloride, gave 3-chloro-2:5-di-sec.-butylpyrazine (XXVlll) identical with that obtained by Baxter and Spring (36) from DL-isoleucine anhydride. The chloropyrazine was characterised by its hydrolysis to 3-hydroxy-2:5-di-sec.-butylpyrazine (XXll) according to the

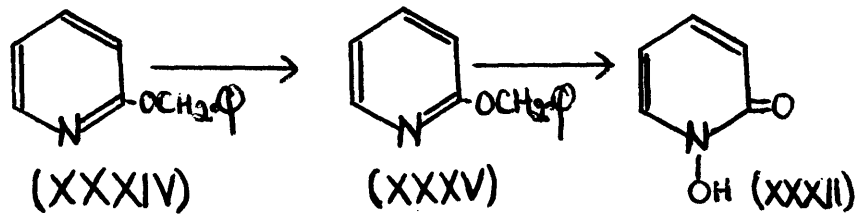
manner of Baxter and Spring (36).

The announcement of the structure of aspergillic acid (26,27) stimulated interest in cyclic hydroxamic acids and several new syntheses have been reported. In attempts to prepare a cyclic hydroxamic acid related to quinoxaline, it was found that 2-hydroxyquinoxaline is smoothly oxidised to 2:3-dihydroxyquinoxaline and, furthermore, that oxidation of 2-ethoxyquinoxaline gave 3-ethoxyquinoxaline 1-oxide and not 2-ethoxyquinoxaline 1-oxide (38). Mild oxidation of 2-chloro and 2-ethoxypyrazine derivatives invariably gave rise to the corresponding 4- oxides and not the required 1- oxides (39). Newbold and Spring (40) have described the syntheses of 1-hydroxy-2-keto-1:2-dihydropyridine (XXXll) and 1-hydroxy-2-keto-1:2-dihydroquinoline ('oxycarbostyril') (11), identical with the product described by Friedländer and Ostermaier (28), by acid hydrolysis of the corresponding ethoxy 1-oxides (XXXl) and (XXXlll), prepared by the action of hydrogen peroxide on the ethoxy compounds.



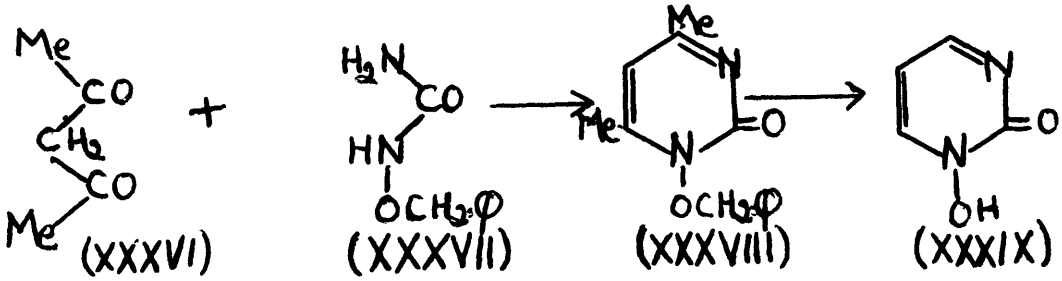
Independent of Newbold and Spring (40), Shaw (41) described the synthesis of 1-hydroxy-2-keto-1:2-dihydropyridine (XXXll)

and the isomeric 3- and 4-hydroxypyridine 1- oxides. 2-Benzyloxypyridine (XXXIV) was oxidised with perbenzoic acid to the corresponding 1- oxide (XXXV) which on catalytic reduction gave the cyclic hydroxamic acid (XXXII).



(XXXIV) (XXXV) (XXXVII)

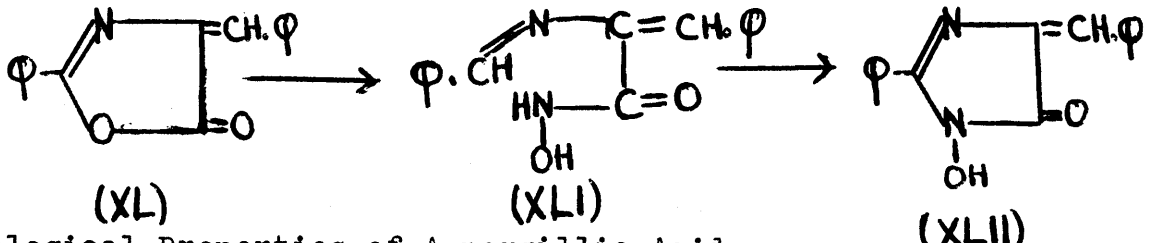
Lott and Shaw (42) have synthesised 1-hydroxy-2-keto-1:2-dihydropyridine, and a number of other cyclic hydroxamic acids in the pyridine series, from 2-hydroxypyridine and related compounds by direct oxidation with perbenzoic acid. The same authors gave details of the synthesis of 1-hydroxy-2-keto-4:6-dimethyl-1:2-dihydropyrimidine (XXXIX). Acetylacetone (XXXVI) was condensed with benzyloxyurea and the resultant product (XXXVIII) cleaved by catalytic hydrogenation to give the cyclic hydroxamic acid (XXXIX).



(XXXVI) (XXXVII) (XXXVIII) (XXXIX)

Shaw and McDowell (43) treated 2-phenyl-4-benzylidene-5-oxazolone (XL) with hydroxylamine obtaining the hydroxamic acid (XLI) which undergoes ring closure, on treatment with dilute mineral acid, to give 1-hydroxy-2-phenyl-4-benzylidene-

-5-imidazolone (XLll).



(XL) (XLI) (XLII)

Biological Properties of Aspergillic Acid.

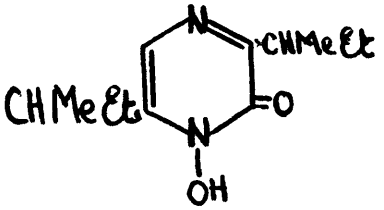
Although aspergillic acid has been shown to have a high inhibitory in vitro activity against some Gram-negative and Gram-positive organisms (7,8,11,12,44) and against M.tuberculosis (45), it possesses such a high toxicity (9) that it is unlikely to find useful application in the treatment of systemic infections. This high toxicity of aspergillic acid for micro-organisms and higher animals is not surprising, in view of its identification as a cyclic hydroxamic acid (26,27), since it has long been known that hydroxlamine and its derivatives are highly potent cell poisons. The lack of antibiotic activity of deoxyaspergillic acid (27) is clear proof that the antibacterial activity of aspergillic acid is due to the hydroxylated nitrogen atom.

THEORETICAL.

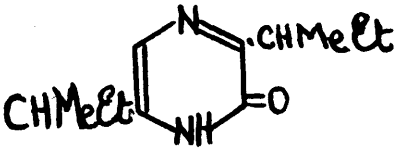
The Theoretical part of this thesis has been divided into two sections. Section I deals with the synthesis of certain degradation products of aspergillic acid, whilst Section II is devoted to model experiments having a bearing upon the synthesis of deoxyaspergillic acid and the preparation of several related compounds

Section I.

Despite the confidence with which Dutcher and Wintersteiner (26,27) ascribed the formula (VII) to aspergillic acid, and formula (VIII) to its primary degradation product, deoxyaspergillic acid, subsequent workers (34,36,37) were unable to obtain synthetic proof of the latter structure (VIII).



(VII)



(VIII)

Comparison of the ultra-violet absorption spectra of deoxyaspergillic acid with those of various hydroxypyrazines, together with the general similarity in the properties, had left little doubt as to the former being a hydroxypyrazine. The differences in the intensities of absorption in the ultra-violet region did not, however, allow of the decision that deoxyaspergillic acid and 3-hydroxy-2:5-di-sec.-butyl-pyrazine are structurally identical.

Absorption Spectra in Ethanol.

	Maxima A.	$\varepsilon_{max.}$	
Deoxyaspergillic acid (27)	2300 , 3200	7,800	7,800
Deoxyaspergillic acid (34)	2295 , 3250	6,700	8,000
3-Hydroxy-2:5-di-sec.-butyl pyrazine	2285 , 3250	9,600	10,000

Dutcher (26,27) had suggested that the 2:5-substituents were both sec.-butyl groups mainly for biogenetic reasons. White and Hill (9) had reported that the production of aspergillic acid by Aspergillus flavus requires the presence of an amino-acid source in the culture medium. Speculation as to a possible precursor of aspergillic acid readily centred around isoleucine since two such amino-acid residues condensing together would give the necessary carbon and nitrogen skeleton. Dutcher (26,27) was further influenced by the fact that the presence of free isoleucine in an Aspergillus had been reported by Woolley and Petersen (46). To support his reasoning as to the nature of the side chains, Dutcher (26,27) produced the following chemical evidence; 1) Kuhn-Roth determinations revealed 2.1 moles. of acetic acid per mole. of aspergillic acid and 1 mole. of acetic acid per mole. of DL-isoleucine; 2) he converted aspergillic acid to a 2:5-di-ketopiperazine, m.p. 249-250°, claimed to be identical with DL-isoleucine anhydride, m.p. 257°, a mixture showing no

depression in melting point; 3) he reduced aspergillic acid, the 2:5-diketopiperazine derived from the former, and DL-isoleucine anhydride to the corresponding piperazine bases and prepared simple derivatives with similar or identical properties.

This, on the surface, is quite convincing evidence but, unfortunately, on closer examination much of it can be discounted. Experiment has failed to disclose the direct utilisation of isoleucine for the formation of aspergillic acid (27). The above Kuhn-Roth determinations can scarcely be regarded as conclusive since the structures of aspergillic acid and DL-isoleucine differ greatly. Dutcher (26,27), although he reduced aspergillic acid, the 2:5-diketopiperazine derived from the former, and DL-isoleucine anhydride to the corresponding piperazine bases and prepared simple derivatives of these, made no direct comparison of either the piperazine bases or the derivatives because of the possible stereochemical complications. The most important evidence adduced in favour of the specific structure (VII) for aspergillic acid is unquestionably the absence of any depression in the melting point of the 2:5-diketopiperazine, derived from aspergillic acid, when mixed with DL-isoleucine anhydride. This tends to lose some of its significance when the work of Abderhalden and Rossner (47) is considered. These authors prepared 2:5-diketo-3-n.-butylpiperazine (A), 2:5-diketo-3-n.-amylpiperazine (B), and 2:5-diketo-3-n.-heptylpiperazine

(C), from the corresponding dipeptides, and carried out a series of mixed melting point observations which are outlined in the table below.

Compound	A	B	C	A + B	B + C	A + C	A+B+C
Melting point	219-220°	221-222°	222°	219°	220°	216-217°	218°

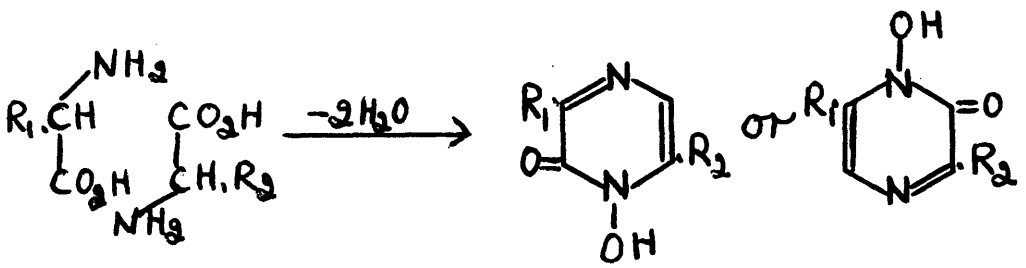
The observed depressions in mixed melting point determinations were slight and in one case no depression was observed. The possibility, therefore, arises that the lack of any depression in the melting point observed when the diketopiperazine derived from aspergillic acid was mixed with DL-isoleucine anhydride is not significant.

At this point it seems advisable to refer to the work of a colleague, Mr. G. Dunn (48). Using a strain of Aspergillus flavus, which was a variant of White's original strain, described by Jones, Rake and Hamre (12), and an aqueous solution of the casein hydrolysate "Pronutrin" and sodium chloride as the culture medium, Mr. G. Dunn (48) isolated aspergillic acid, m.p. 97-98°, $[\alpha]_D^{18°}$ + 13.3° (250 mg. per litre of culture filtrate). He also isolated, from the culture filtrate, in low yield a compound $C_{12}H_{20}ON_2$, m.p. 143-145°, which had all the properties of a hydroxypyrazine and which he subsequently identified as 3-hydroxy-2:5-di-isobutyl-

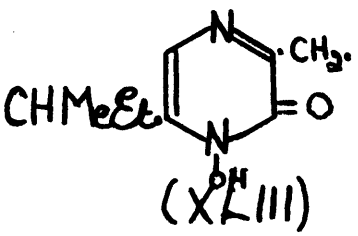
-pyrazine (49). Reduction of aspergillic acid with hydrazine gave deoxyaspergillic acid, characterised by the formation of a phenylazo derivative. This reaction established the presence of an unsubstituted nuclear position in the pyrazine ring and agreed with the formulation of deoxyaspergillic acid as a 3-hydroxy-2:5-dialkyl substituted pyrazine. 3-Hydroxy-2:5-di-sec.-butylpyrazine also gave a 6-phenylazo derivative. When heated with normal alkali at 170° for 24 hours, deoxyaspergillic acid gave a racemate, m.p. 103-104°, which was undepressed in melting point when mixed with dextro-rotary deoxyaspergillic acid but was depressed in melting point when mixed with racemic 3-hydroxy-2:5-di-sec.-butylpyrazine, m.p. 122-124°. The latter compound was unaffected when treated with alkali using the same conditions; if deoxyaspergillic acid and the synthetic isomer differed only in stereochemical orientation, this treatment would have been expected to produce the same racemic mixture. The conclusion must therefore be drawn that the alkyl side chains in deoxyaspergillic acid are not both sec.-butyl groups.

Summarising the evidence before the commencement of this work it is established that deoxyaspergillic acid contains a pyrazine ring, a nuclear hydroxyl group in the 3- position, and at least one unsubstituted nuclear position, most probably the 6- position. The remaining C_8H_{18} fragment is to be distributed either as a single alkyl side chain, which must

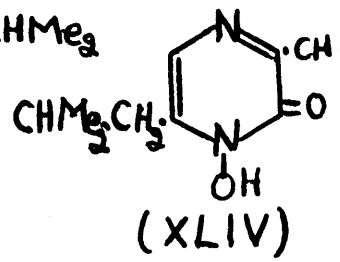
contain at least one asymmetric centre, located at the 2- or the 5- position, or as two alkyl groups, at least one of which must contain an asymmetric centre, located at the 2- and 5- positions. Bearing in mind the facts that the production of aspergillic acid by Aspergillus flavus requires the presence of an amino-acid source in the culture medium (9) and that the side chains are not both sec.-butyl groups (48) it seems likely that aspergillic acid is derived from two different amino-acids thus:



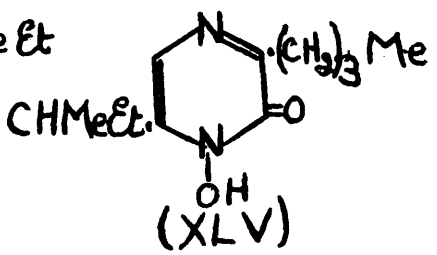
If leucine and isoleucine are assumed to be the parent amino-acids the derived cyclic hydroxamic acid would be (XLIII) or (XLIV); similarly, norleucine with isoleucine would give rise to the cyclic hydroxamic acid (XLV) or (XLVI). If aspergillic acid is (XLIII) or (XLIV) the derived diketopiperazine would be leucylisoleucine anhydride (XLVII)and if the acid is (XLV) or (XLVII) the diketopiperazine would be norleucylisoleucine anhydride (XLVIII).



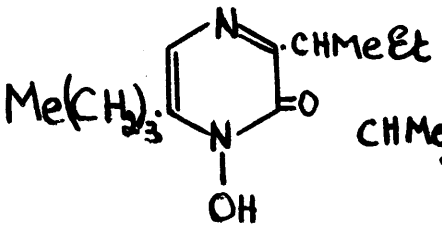
(XLIII)



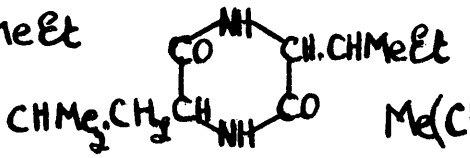
(XLIV)



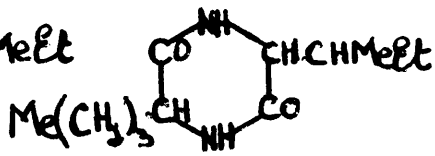
(XLV)



(XLVI)



(XLVII)

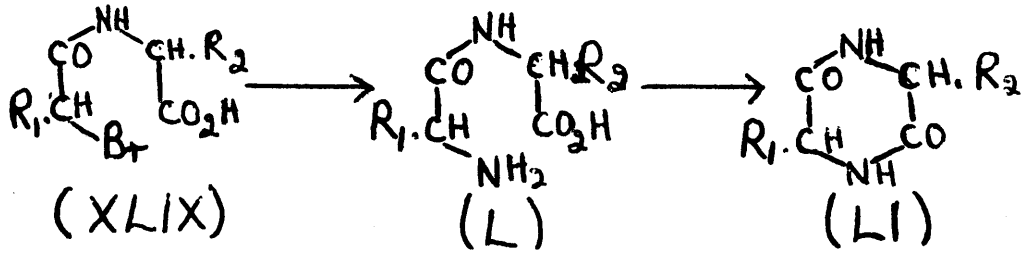


(XLVIII)

In the absence of any real evidence as to the nature of the side chains it was thought advisable to prepare DL-leucyl-DL-isoleucine anhydride (XLVII), DL-norleucyl-DL-isoleucine anhydride (XLVIII) and DL-isoleucine anhydride (XXVII) in order to compare them with the diketopiperazine derived from aspergillic acid (26,27) and as possible intermediates in the synthesis of deoxyaspergillic acid.

A leucylisoleucine anhydride, m.p. 273-277°, $[\alpha]_D$ -15.9°, has been isolated from hog bristles (5) whilst Abderhalden and Hirsch (51) have prepared an active leucylisoleucine anhydride, m.p. 291°, $[\alpha]_D$-35.8°, by heating L-leucyl-D-isoleucine methyl ester hydrochloride with alcoholic ammonia. For the above comparison leucylisoleucine anhydride was prepared by two methods. Condensation of 2-bromo-isohexoyl chloride with DL-isoleucine, according to

Abderhalden Hirsch and Schuler (52), gave 2-bromo-isohexoyl-DL-isoleucine (XLIX, $R_1 = CH_2.CHMe_2$, $R_2 = CHMeEt$.) which was esterified and the ester heated with alcohol and ammonia to give DL-leucyl-DL-isoleucine anhydride (LI, $R_1 = CH_2.CHMe_2$, $R_2 = CHMeEt$) as colourless needles, m.p. 265-267°. 2-Bromo-iso-hexoyl-DL-isoleucine, on treatment with aqueous ammonia gave a racemic dipeptide (L, $R_1 = CH_2.CHMe_2$, $R_2 = CHMeEt$), m.p. 272° (m.p. 266-267° in a sealed tube), together with a gum which could not be crystallised. The yield (33%) of the dipeptide compared very unfavourably with that of Abderhalden et alia (52) (70%) who gave the m.p. as 262-263° (corrected) and 255-256° (uncorrected). Using the method developed by Lichtenstein (53) for the preparation of 2:5-diketopiperazines in which the dipeptide is dehydrated by heating with 2-naphthol, the racemic leucylisoleucine gave in high yield (84%) an anhydride (LI, $R_1 = CH_2.CHMe_2$, $R_2 = CHMeEt$), m.p. 275-277°. A mixture of the latter with DL-leucyl-DL-isoleucine anhydride, m.p. 265-267°, melted at 269-271°. The gum on similar treatment to that of the dipeptide gave a diketopiperazine, m.p. 268-270°, undepressed on admixture with the anhydride obtained via the dipeptide.



DL-Norleucyl-DL-isoleucine anhydride (L1, R_1 = $(CH_2)_3$ Me, R_2 = CHMeEt) was also prepared by two methods. 2-Bromo-n.-hexoyl bromide was condensed with DL-isoleucine in sodium hydroxide solution to give 2-bromo-n.hexoyl-DL-isoleucine (XL1X, R_1 = $(CH_2)_3$Me, R_2 = CHMeEt) as colourless plates, m.p. 168-170°. The bromacylamino acid was esterified and the ester heated with alcohol and ammonia to give the diketopiperazine (L1, R_1 = $(CH_2)_3$Me, R_2 = CHMeEt) as colourless needles, m.p. 258-260°. 2-Bromo-3-methyl-n.-valeryl chloride was condensed with DL-norleucine using the same conditions to give 2-bromo-3-methyl-n.-valeryl-DL-norleucine (XL1X, R_1 = CHMeEt, R_2 = $(CH_2)_3$Me) as colourless needles, m.p. 115-116°. The bromacylamino-acid was esterified and the ester heated with alcohol and ammonia to give the diketopiperazine (L1, R_1 = $(CH_2)_3$ Me, R_2 = CHMeEt), m.p. 259-260°, undepressed on admixture with the specimen described above. From the alcoholic mother liquor, there was isolated another product, which after extensive purification, separated from benzene-light petroleum (b.p. 40-60°) as colourless needles, m.p. 140-170°. The product was insoluble in water and readily soluble in most organic solvents. The analyses and molecular weight could not be interpreted but seemed to indicate some structure closely related to the diketopiperazine (XLV111, R_1 = $(CH_2)_3$Me, R_2 = CHMeEt).

DL-isoLeucine anhydride (L1, $R_1 = R_2 = CHMeEt$) was prepared according to Baxter and Spring (36) by heating DL-isoleucine with ethylene glycol for four hours when the diketopiperazine was obtained as colourless needles, m.p. 280-281°. The solubility properties of DL-isoleucine anhydride, DL-leucyl-DL-isoleucine anhydride, DL-norleucyl-DL-isoleucine anhydride and the compound $C_{12}H_{22}O_2N_2$ from aspergillic acid (26,27) are similar, and they each melt within the range 255-282°. The melting point of these compounds is not a satisfactory criterion; it is complicated by a tendency to sublimation, and varies according to the rate of heating and initial temperature of the bath.

A series of mixed melting point determinations was carried out; the results are tabulated below. These results, whilst confirming Dutcher's observation (26,27)

Name of Compound mixed with $C_{12}H_{20}O_2N_2$	m.p.	Mixed m.p.	m.p. of $C_{12}H_{20}O_2N_2$
DL-isoLeucine anhydride (a)	278°	262-265°	259-261°
DL-isoLeucine anhydride (b)	284°	268-271°	258-260°
Leucylisoleucine anhydride	273-275°	267-270°	260-262°
DL-Leucyl-DL-isoleucine anhydride	266-268°	263-265°	260-262°
DL-Norleucyl-DL-isoleucine anhydride	258-260°	257-259°	259-260°

(a) this thesis.

(b) prepared according to Dutcher (27).

that the melting point of the diketopiperazine derived from aspergillic acid is not depressed when mixed with DL-isoleucine anhydride, when prepared according to either Baxter and Spring (36) or Dutcher (26,27), reveal also that the melting point is not lowered when mixed with leucylisoleucine anhydride. A mixture of the diketopiperazine from aspergillic acid with DL-norleucyl-DL-isoleucine anhydride shows a slight but consistent depression.

In order to clear up the anomaly arising from the above observations, the melting points of a series of mixtures of the synthetic 2:5-diketopiperazines were noted; the results are tabulated below.

Name and m.p. of anhydride.A.	Name and m.p. of anhydride.B.	m.p. of A + B.
Leucylisoleucine, 271-273°	DL-isoLeucine, 280-281°	272-274°
DL-Leucyl-DL-isoleucine, 263.5-265.5°	DL-isoLeucine, 280-282°	266-269°
Leucylisoleucine, 273-275°	DL-Norleucyl-DL-isoleucine, 262-264°	266-268°
DL-Norleucyl-DL-isoleucine, 260-261°	DL-isoLeucine, 280-282°	260-262°

A mixture of DL-isoleucine anhydride, leucylisoleucine anhydride, DL-norleucyl-DL-isoleucine anhydride had m.p. 264-266°. It is apparent from the above results that, where 2:5-diketopiperazines are concerned, a depression in melting

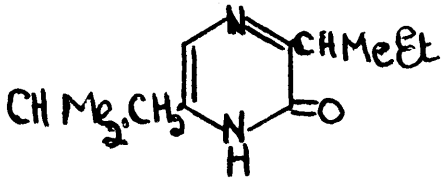
point is observed only when the two components of a mixture have almost identical melting points and that, consequently, the absence of any depression in the melting points of 2:5-diketopiperazines on being mixed is not significant. These observations nullify the most important evidence adduced in favour of the specific structure (Vll) for aspergillic acid.

Kuhn-Roth estimations of the C-Me groups present in deoxyaspergillic acid and a few related compounds were carried out; the results are summarised below. It is evident from

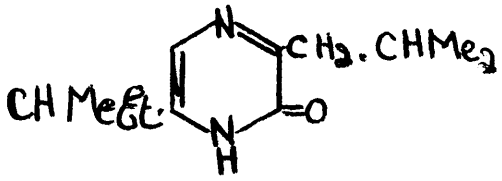
Name of Compound	%age C-Me.	Moles. of acetic acid.
DL-Leucine	9.41	0.82
DL-isoLeucine	16.2	1.43
3-Hydroxy-2:5-di-sec.-butyl-pyrazine	22.4	3.09
3-Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine	17.9	2.46
Deoxyaspergillic acid	17.6	2.42

these results that deoxyaspergillic cannot have the structure (Vlll) attributed to it by Dutcher (26,27) and that the diketopiperazine derived from aspergillic acid is not isoleucine anhydride. The diketopiperazine might be leucylisoleucine anhydride and consequently (XLlll) and XLlV) are possible

structures for aspergillic acid and deoxyaspergillic acid may be either (L11) or (L111).



(L11)



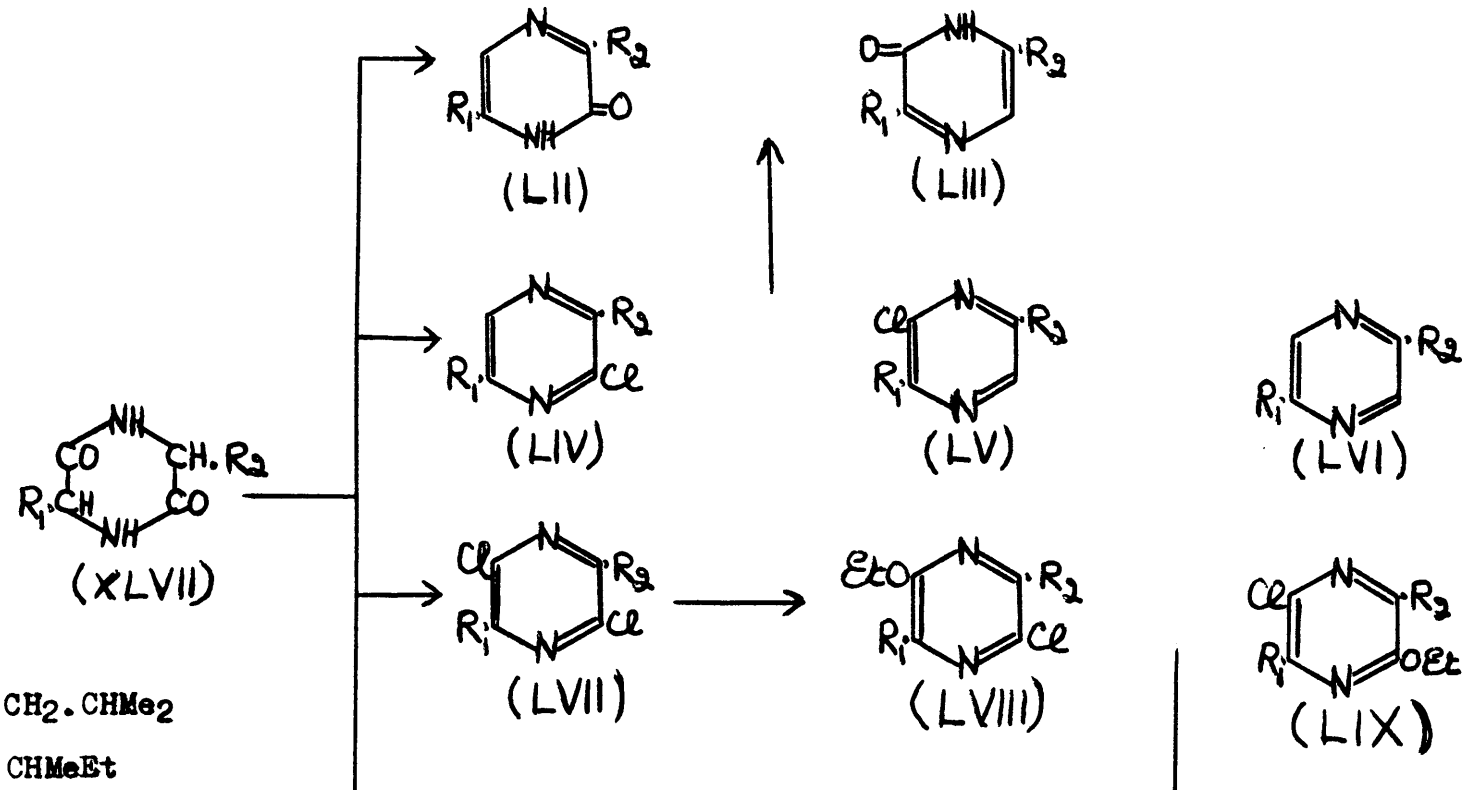
(L111)

Subsequent to the completion of the above work, Dunn, Newbold and Spring (54) identified the side chains of aspergillic acid as a sec.-butyl and an isobutyl group by hydrolysis of the diketopiperazine derived from aspergillic acid. Prolonged refluxing of the diketopiperazine with 48% hydrobromic acid gave a mixture of leucine and isoleucine (or alloisoleucine). The amino-acids were separated by virtue of the difference in solubility of their copper salts in water. Degradation of the amino-acids with ninhydrin gave isovaleraldehyde and methylethylacetaldehyde respectively, identified as their 2:4-dinitrophenylhydrazones. Thus, the structure (XL111) or (XL1V) was established for aspergillic acid.

Following the establishment of the nature of the side chains in aspergillic acid by degradative methods it was desirable to obtain confirmation of the structure of aspergillic acid by the synthesis of either the acid or the primary degradation product, deoxyaspergillic acid. Since, at the time of this work, no method for the synthesis of

pyrazine hydroxamic acids was known it was decided to attempt the conversion of leucylisoleucine anhydride into racemic deoxyaspergillic acid (3-hydroxy-2-isobutyl-5-sec.-butyl-pyrazine or 3-hydroxy-2-sec.-butyl-5-isobutylpyrazine). This method, despite its ambiguity, is the only attractive method of synthesis of 3-hydroxypyrazines containing different alkyl substituents in the 2- and 5- positions. Other possible methods of synthesis of deoxyaspergillic acid are also ambiguous or involve the preparation of hitherto unknown intermediates, the synthesis of which might not be feasible but would certainly be extremely laborious.

Leucylisoleucine anhydride (XLVll), m.p. 275-277°, on treatment with phosphoryl chloride gave a mixture of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (Lll or Llll), a monochloro-2(5)-isobutyl-5(2)-sec.-butylpyrazine (LlV and LV), 2-isobutyl-5-sec.-butylpyrazine (LVl), 3:6-dichloro-2-isobutyl-5-sec.-butylpyrazine (LVll), and 3-chloro-6-hydroxy-2-isobutyl-5-sec.-butylpyrazine (LX) and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-isobutylpyrazine (LXl). The reaction mixture, after removal of the excess phosphoryl chloride, was triturated with ice-water and thoroughly extracted with ether. The ethereal solution (A) was extracted with 3N potassium hydroxide solution and the alkaline phase carefully neutralised with hydrochloric acid when a mixture of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine and 3-chloro-6-hydroxy-2-



R_1 = $CH_2.CHMe_2$

R_2 = CHMeEt

isobutyl-5-sec.-butylpyrazine and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-isobutylpyrazine separated. The former is soluble in 2N hydrochloric acid but the latter is not; hence, the separation of the two products offers no difficulties. 3-Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (L11 or L111) had initially a m.p. 76-80°. Fractional crystallisation from aqueous ethanol gave the hydroxypyrazine as colourless needles, m.p. 97-98°, in admixture with an equal quantity of racemic deoxyaspergillic acid (m.p. 103-104°) it melted at 90-92°. This product was characterised by the formation of a 6-phenylazo derivative which separated from aqueous ethanol as orange needles, m.p. 203-205°. Racemic deoxyaspergillic acid could not be isolated from the mother liquors from the fractional crystallisation but a second product was obtained. This represented approximately half of the original crude material; it crystallised from aqueous ethanol as colourless needles, m.p. 85-87°, in admixture with an equal quantity of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 97-98°) it had m.p. 86-89°. Similarly, in admixture with an equal quantity of racemic deoxyaspergillic acid (m.p. 103-104°) it had m.p. 86-89°. Chromatographic examination of this "mixed" hydroxypyrazine did not result in any further separation of the hydroxypyrazine, m.p. 97-98° or of the racemic deoxyaspergillic acid which appeared to be present. 3-Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine has similar

solubility properties to deoxyaspergillic acid, viz., ready solubility in the common organic solvents, dilute acid and dilute alkali and insolubility in cold water. 3-chloro-6-hydroxy-2-isobutyl-5-sec.-butylpyrazine (LX) and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-isobutylpyrazine (LX1), obtained as a by-product in the above reaction, had initially a m.p. 113-121° but after several crystallisations from aqueous ethanol it separated as colourless needles, m.p. 138-139°. It is soluble in all common organic solvents and in dilute alkali but is insoluble in dilute acid and in water. It is unaffected by alkali under even the most drastic conditions.

The ethereal solution (A) was next extracted with 6N hydrochloric acid, the acid phase made alkaline with 3N potassium hydroxide solution. The mixture was extracted with ether and after drying over sodium sulphate the ether was removed to give 2-isobutyl-5-sec.-butylpyrazine (LV1) which is a colourless oil, b.p. 80°/1mm.. The product, unlike 2:5-di-sec.-butylpyrazine (34), did not form a chloroplatinate.

The ethereal solution (A), after drying over sodium sulphate, was evaporated to give a colourless oil, b.p. 102.5-104°/2mm., which analysed for a mixture of a monochloro-2(5)-isobutyl-5(2)-sec.-butylpyrazine (LIV and LV), and 3:6-dichloro-2-isobutyl-5-sec.-butylpyrazine (LV11). Attempts to fractionally distil the mixture were unsuccessful but showed that the oil was contaminated with the pyrazine base (LV1).

When the base was extracted from the oil with more concentrated hydrochloric acid (d,1.16), it contained a certain amount of halogen containing material, i.e., it gave a positive Beilstein test. It was not, however, possible to fractionate the chlorine containing oil into a monochloro and a dichloropyrazine using concentrated hydrochloric acid. Baxter and Spring (36) were able to separate 3-chloro and 3:6-dichloro-2:5-di-sec.-butylpyrazine, from a mixture of the two, by this method. The oil was heated at 110° for four hours with sodium ethoxide solution and the resultant ethoxy derivatives heated under reflux with 5N hydrochloric acid for 18 hours. The mixture was extracted with ether and the ether removed to give 3-chloro-6-ethoxy-2-isobutyl-5-sec.-butylpyrazine (LVIII) and/or 3-chloro-6-ethoxy-2-sec.-butyl-5-isobutylpyrazine (LIX), as a colourless oil, b.p. 115°/1.5mm.. The oil was stable to 5N hydrochloric acid and 5N sulphuric acid but on heating under reflux with aqueous ethanolic hydrochloric acid it gave 3-chloro-6-hydroxy-2-isobutyl-5-sec.-butylpyrazine and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-isobutylpyrazine, m.p. 116-124°. The product, after several recrystallisations from aqueous ethanol, separated as colourless needles, m.p. 139-140°, undepressed on admixture with the specimen (m.p. 138-139°) obtained directly from leucylisoleucine anhydride by the action of phosphoryl chloride.

The acidic phase was carefully neutralised with

potassium hydroxide solution when a-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine L11 and L111), m.p. 84-86°, separated. After repeated crystallisations from aqueous ethanol, the hydroxypyrazine separated as colourless needles, m.p. 87-88°, in admixture with an equal quantity of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 97-98°) it had m.p. 87-88°, with hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 85-87°) it had m.p. 86-88°, and with an equal quantity of racemic deoxyaspergillic acid it melted at 88-90°. Since this mixture could not be separated by fractional crystallisation, the crude material was adsorbed on a column of alumina and eluted with an ethanol-ether mixture. The first 10% of material collected, on repeated crystallisation from aqueous ethanol, separated as colourless needles, m.p. 97-98°, undepressed on admixture with the specimen (m.p. 97-98°) obtained directly from leucylisoleucine anhydride by the action of phosphoryl chloride. The melting point of the remainder of the material which was eluted could not be raised above 87-88°. This hydroxypyrazine couples smoothly with benzene diazonium chloride to give a 6-phenylazo derivative, m.p. 176-180°, which, after a great many crystallisations from aqueous ethanol, eventually separated as orange needles, m.p. 203-205°, undepressed on admixture with the phenylazo derivative of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 97-98°), m.p. 203-205°. Considerable difficulty was experienced in

raising the melting point above 190°. It may be significant that a mixture of 3-hydroxy-6-phenylazo-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 203-205°, and the phenylazo derivative of racemic deoxyaspergillic acid, m.p. 189-191°, melted at 189-191°. A sample of the phenylazo derivative of racemic deoxyaspergillic acid, m.p. 188-190°, when mixed with 3-hydroxy-6-phenylazo-2:5-di-sec.-butylpyrazine, m.p. 200°, melted at 188-190.5°.

The ultra-violet absorption spectra of the two hydroxypyrazines have been examined in ethanol solution. The positions of the maxima in both the absorption spectrum of each of the synthetic compounds and that of deoxyaspergillic acid are identical. The valuesof the respective molecular extinction coefficients correspond more closely to those of deoxyaspergillic acid than did those of 3-hydroxy-2:5-di-sec.-butylpyrazine.

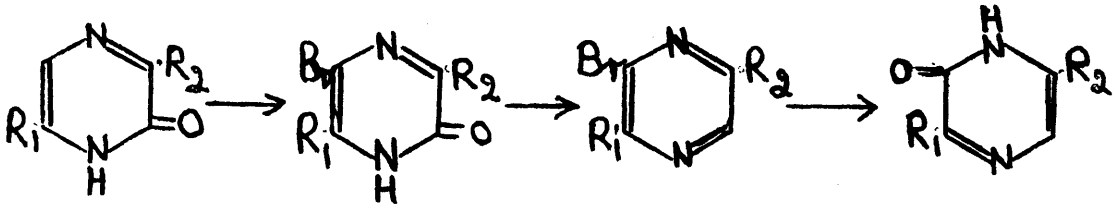
Absorption Spectra in Ethanol.

	Maxima A.		$\varepsilon_{max.}$	
Deoxyaspergillic acid (27)	2300	3200	7,800	7,800
Deoxyaspergillic acid (34)	2295	3250	6,700	8,000
3-Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 97-98°	2290	3250	7,000	7,600
Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 87-88°	--	3250	--	9,500
3-Hydroxy-2:5-di-sec.-butylpyrazine	2285	3250	9,600	10,000

In view of the failure to separate the equilibrium

mixture of the hydroxypyrazines (i.e. product, m.p. 87-88°) it was obviously desirable to prepare a similar mixture by mixing 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 97-98°, and racemic deoxyaspergillic acid. Experiment indicated that such a mixture might occur when the amount of deoxyaspergillic acid present, was between 11% and 21%. Attempts to prepare this mixture, by crystallising mixtures of the two compounds, met with no success, 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 97-98°, being recovered unchanged. Examination of the mother liquors revealed no signs of mixed crystal formation.

After the failure of the foregoing methods to produce racemic deoxyaspergillic acid, an attempt was made to convert 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 97-98°, into the isomeric deoxyaspergillic acid thus:-



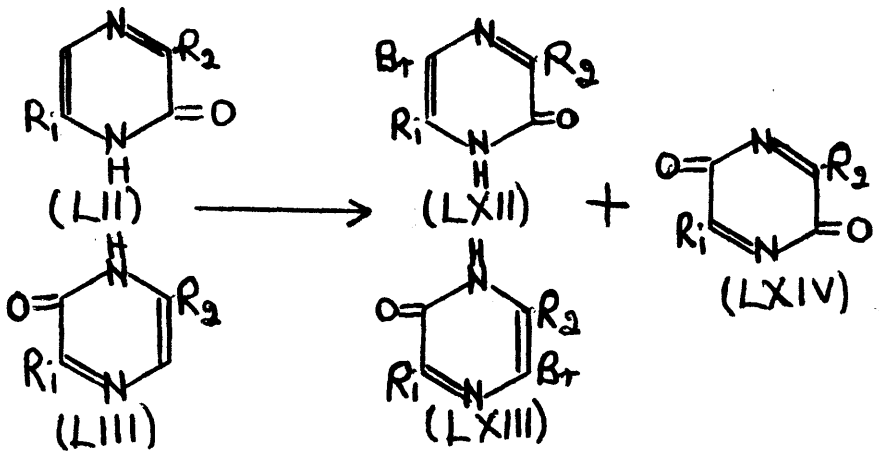
(R_1 = $CH_2.CHMe_2$ or CHMeEt. R_2 = CHMeEt or $CH_2.CHMe_2$. $R_1 \neq R_2$)

It was thought that it might be possible to reduce 3-bromo-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine to 3-bromo-2(5)-isobutyl-5(2)-sec.-butylpyrazine by zinc dust distillation or better the zinc dust fusion method of Clar (55) which was used by him to convert quinones into the corresponding aromatic

hydrocarbons, e.g. anthraquinone on fusion with zinc dust, sodium chloride and zinc chloride gave anthracene. The conversion of the bromopyrazine into the isomeric hydroxypyrazine would have been a simple task, proceeding, either by direct alkaline hydrolysis, or by etherification followed by acid hydrolysis. 3-Bromo-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, on fusion with zinc dust, sodium chloride and zinc chloride, was completely destroyed. No such reduction of a hydroxypyrazine is known, the 3-hydroxy-2:5-diphenylpyrazine which Japp and Knox (56) claim to have reduced to 2:5-diphenylpyrazine was later identified as 2-benzoyl-4(5)-phenylglyoxaline (57). When 3-hydroxy-2:5-dimethylpyrazine was similarly treated the only product identified was ammonia.

An aqueous acetic acid solution, of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (L11 or L111), m.p. 97-98°, was treated with a solution of bromine in acetic acid when 3-bromo-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (LX11 or LX111) separated immediately as colourless needles, m.p. 150-151°. Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 87-88°, when similarly treated gave a bromo derivative which consisted mainly of 3-bromo-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 150-151°, either alone or when mixed with specimen previously described. The aqueous acetic acid mother liquors on evaporation to dryness gave a small quantity of a colourless solid which crystallised from aqueous acetic acid

as colourless needles, m.p. 254-256°, and which analysed for $C_{12}H_{18}O_2N_2$. A mixture with the quinone-like compound (m.p. 268-270°), isolated by Dutcher (26,27) from the mother liquors of bromodeoxyaspergillic acid, had m.p. 256-258°. The ultra-violet absorption spectrum of this compound, 2885 A., closely corresponds to that reported by Dutcher (26,27) for his compound, $C_{12}H_{18}O_2N_2$, 2850 A., whilst the molecular extinction coefficients are almost identical, 22,400 and 22,250 respectively. This completely confirms the identification of the 2- and 5- alkyl substituents of deoxyaspergillic acid as <u>iso</u>butyl and <u>sec</u.-butyl groups but does not indicate which occupies the 2-position.

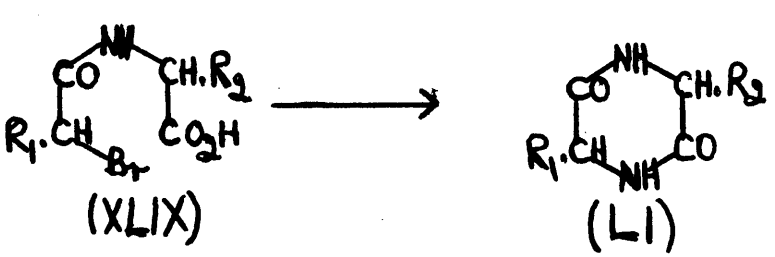


$R_1 = CH_2.CHMe_2$ $R_2 = CHMeEt$

With the examination of the products obtained from the reaction of phosphoryl chloride on leucyl<u>iso</u>leucine anhydride (m.p. 275-277°) and other 2:5-diketopiperazines (see section 2) it became obvious that the products from

such a reaction could not be predicted beforehand. It seemed likely that anyone of the other three racemates of leucyl-isoleucine anhydride might give rise directly to racemic deoxyaspergillic acid. An attempt was therefore made to prepare all four possible racemates. This was not successful, only two racemates being encountered.

Using DL-isoleucine as a starting point, only two racemic anhydrides can be expected since DL-isoleucine is a single racemate although the diastereoisomer DL-alloisoleucine is generally found in small quantities in the mother liquor(58). 2-Bromo-isohexoyl-DL-isoleucine (XLIX, R_1 = $CH_2.CHMe_2$, R_2 = CHMeEt) was prepared according to Abderhalden, Hirsch and Schuler (52) and fractionally crystallised from light petroleum to give two racemates differing only in their m.p.s 178-179° and 131-133°, and their solubility in light petroleum. The individual racemates on esterification and treatment with alcohol and ammonia did not give individual racemic leucyl-isoleucine anhydrides but a mixture of two racemates (LI, R_1 = $CH_2.CHME_2$, R_2 = CHMeEt) differing only in their m.p.s 275-277° and 258-259°, and their solubility in ethanol.



2-Bromo-3-methyl-n.-valerylchloride was condensed with DL-leucine in caustic soda solution according to Abderhalden and Schweitzer (59). The resultant bromacylamino-acid (LlX, R_1 = CHMeEt, R_2 = CH_2.$CHMe_2$) was fractionally crystallised from benzene but only two distinct fractions were obtained, 1) colourless rhombic plates, m.p. 144-145.5°, 2) colourless rods, m.p. 96-98°. In addition, a product with m.p. intermediate between the above two products was obtained but this could not be further fractionated. These individual fractions, on esterification and treatment with alcohol and ammonia gave in low yield mixtures of the two leucyisoleucine anhydrides previously described. From the alcoholic mother liquors of the anhydrides, there was isolated colourless plates, melting points varying from 147-149° to 157-159°. The analyses and molecular weights could not be interpreted but seemed to indicate some structure closely related to the anhydride.

2-Bromo-3-methyl-n.-valeryl-isoleucine, m.p. 143-144.5°, was recovered unchanged after standing in aqueous ammonia for 6 days but when heated with aqueous ammonia at 140° for one hour, the dipeptide isoleucylleucine (or alloisoleucylleucine) was obtained in poor yield as colourless hygroscopic monoclinic prisms, m.p. 272-274°. The bromacylamino-acid, m.p. 96-98°, on similar treatment did not give the dipeptide as expected but gave a mixture of two

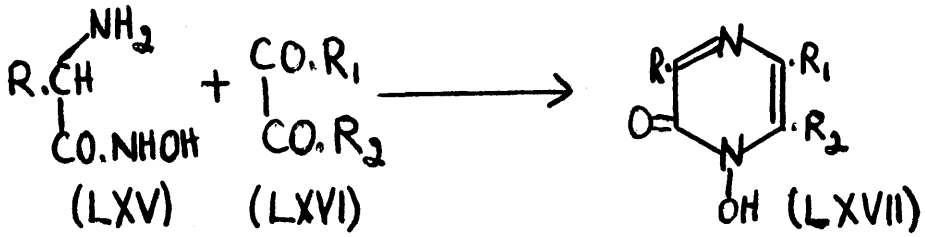
products, one of which was soluble in water. The water insoluble product separated from aqueous ethanol as colourless needles, m.p. 272-273°. The analysis corresponded to that required for leucyl*iso*leucine anhydride but a mixture with the anhydride (m.p. 275-277°) melted at 260-262°. The water soluble product crystallised from benzene as colourless needles, m.p. 146-147°. The analysis could not be interpreted.

The low yields of the diketopiperazine and the dipeptide are due to the difficulty in replacing the bromine atom by an amino-group. A similar case of steric hindrance has been reported by Abderhalden and Zeisset (60) who found that, although the *L*- and *D*-*iso*, *L*- and *D*-*alloiso*-2-bromo-3-methyl-*n*.-valeric acids could be aminated in four to five days by treatment with aqueous ammonia at room temperature, the corresponding bromacylglycines required from thirty to fifty days.

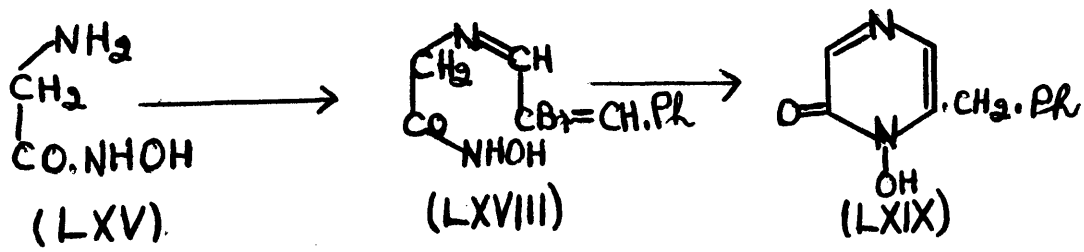
Leucyl*iso*leucine anhydride, irrespective of melting point or method of synthesis, on treatment with phosphoryl chloride behaves in identical manner to that already described.

Although it has not proved possible to achieve a satisfactory synthesis of deoxyaspergillic acid the identities of the alkyl side chains present in the 2- and 5- positions have been rigidly established as *iso*butyl and *sec*.-butyl groups or vice versa.

Methods have been developed by other workers (61) for the syntheses of cyclic hydroxamic acids similar to aspergillic acid and since no satisfactory method exists at present for the preparation of 3-hydroxypyrazines which have different alkyl side chains in the 2- and 5- positions, it seems likely that a synthesis of aspergillic acid will precede that of its primary degradation product, deoxyaspergillic acid.



Condensation of 2-aminohydroxamic acids (LXV) with 1:2-dicarbonyl compounds (LXVl) gives rise to pyrazine hydroxamic acids (LXVll). Reduction with hydrazine gives a 3-5-disubstituted hydroxypyrazine identical with that prepared according to Jones (62) by condensing a 2-amino-acid amide with a 1:2-dicarbonyl compound. Thus, glycine hydroxamic acid (XLV, R = H) condenses with phenylglyoxal (LXVl, R_1 = Ph,R_2 = H) to give 1-hydroxy-2-keto-5-phenyl-1:2-dihydropyrazine (LXVll, R = H, R_1 = Ph, R_2 = H).



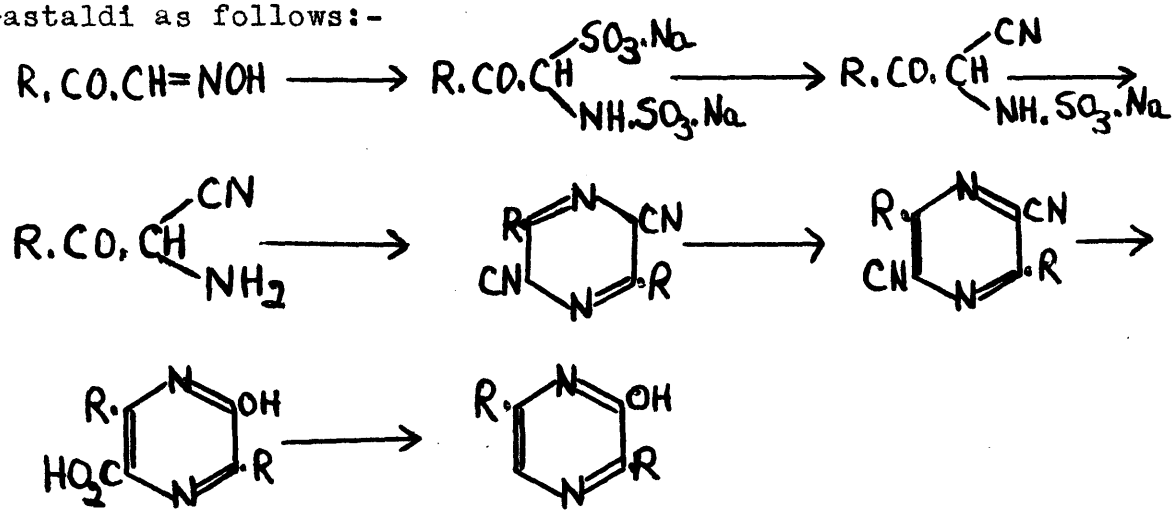
Glycine hydroxamic acid (LXV) reacts with 2-bromocinnamaldehyde to give the Schiff base (LXVlll) which,

on treatment with sodium ethoxide, gives 1-hydroxy-2-keto-6-benzyl-1:2-dihydropyrazine (LX1X). This method, or a simple variant, offers a route to the synthesis of aspergillic acid. The method is attractive in that it allows the introduction of different substituents at the 3- and 6- positions, a feature which will be of value in deciding between the alternative formulae (XL111) and (XL1V) for aspergillic acid.

Section II.

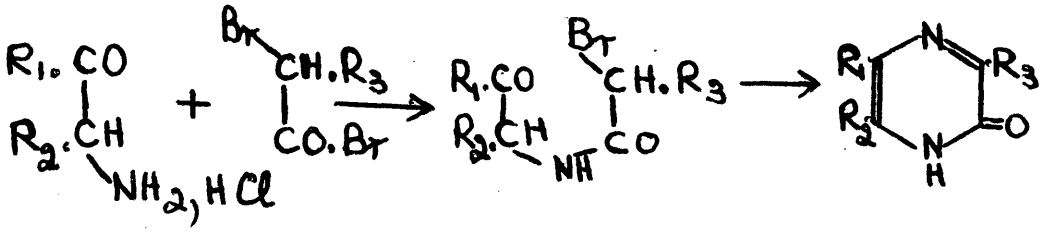
Until the advent of aspergillic acid, its subsequent identification as a cyclic hydroxamic acid and the consequent identification of its primary degradation product as a hydroxpyrazine, little systematic knowledge of methods of synthesis of hydroxypyrazines existed. The methods then existing and subsequently developed will be reviewed briefly.

Gastaldi's method (57) consists in the treatment of the bisulphite compound of an oximinoketone with potassium cyanide, followed by heating the reaction product with hydrochloric acid, whereby a 3:6-dicyano-2:5-disubstituted pyrazine is obtained. When heated with alkali, the dicyano-compound undergoes a remarkable hydrolytic reaction to yield a 2:5-disubstituted-3-hydroxypyrazine-6-carboxylic acid, decarboxylation of which gives the required 2:5-disubstituted-3-hydroxypyrazine. The reaction sequence is formulated by Gastaldi as follows:-



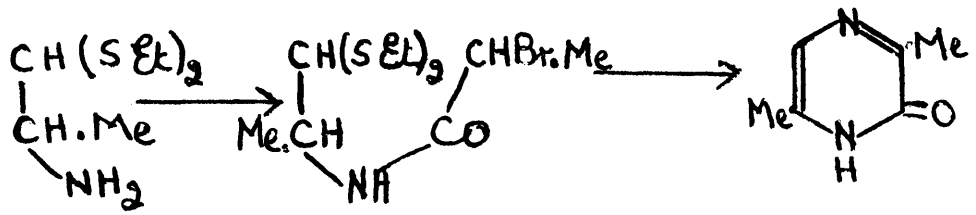
Gastaldi applied the method to oximinoacetone and oximinoacetophenone to obtain 3-hydroxy-2:5-dimethylpyrazine (R = Me) and 3-hydroxy-2:5-diphenylpyrazine (R = Ph) respectively. Sharp and Spring (63) extended this method to oximinomethyl ethyl ketone and obtained 3-hydroxy-2:5-diethylpyrazine (R = Et).

Total and Elderfield (64), by condensing a 2-aminoketone with a 2-halo-acyl halide, followed by treatment of the product with ammonia, were able to prepare 2-hydroxy-5:6-di- and 3:5:6- trisubstituted pyrazines, thus:-



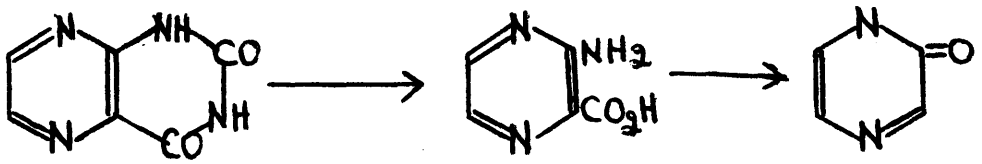
Newbold and Spring (34) have applied this method to the synthesis of 2-hydroxy-3:5:6- trimethylpyrazine ($R_1 = R_2 = R_3$ = Me). Baxter, Newbold and Spring (65) modified the above method and were able to synthesise 3-hydroxy-2:5-dimethylpyrazine. Treatment of 2-aminopropaldehyde diethylacetal with hydrochloric acid and ethyl thiol gave 2-aminopropaldehyde diethylmercaptal which reacted smoothly with 2-bromo-propionyl bromide to give 2-(2-bromo-propionamido) propaldehyde diethylmercaptal. Treatment of the latter with mercuric chloride in the presence of cadmium carbonate, followed by treatment of the product with ammonia gave 3-

hydroxy-2:5-dimethylpyrazine.



Use of aminoacetone in the above reaction instead of the mercaptal gives rise to 3-propionamido-2:5-dimethylpyrazine (66).

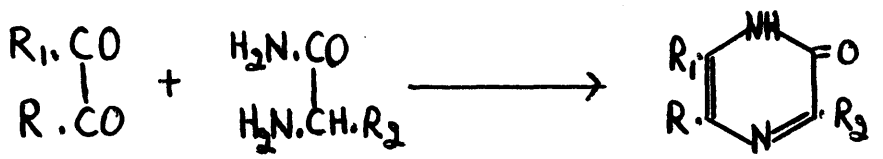
Weijlard, Tishler and Erickson (67) have shown that lumazine can be hydrolysed under acid or alkaline conditions to give aminopyrazine and 2-aminopyrazine-3-carboxylic acid. Drastic alkaline hydrolysis of lumazine or 2-aminopyrazine-3-carboxylic acid gives 2-hydroxypyrazine-3-carboxylic acid, decarboxylation of which yields hydroxypyrazine.



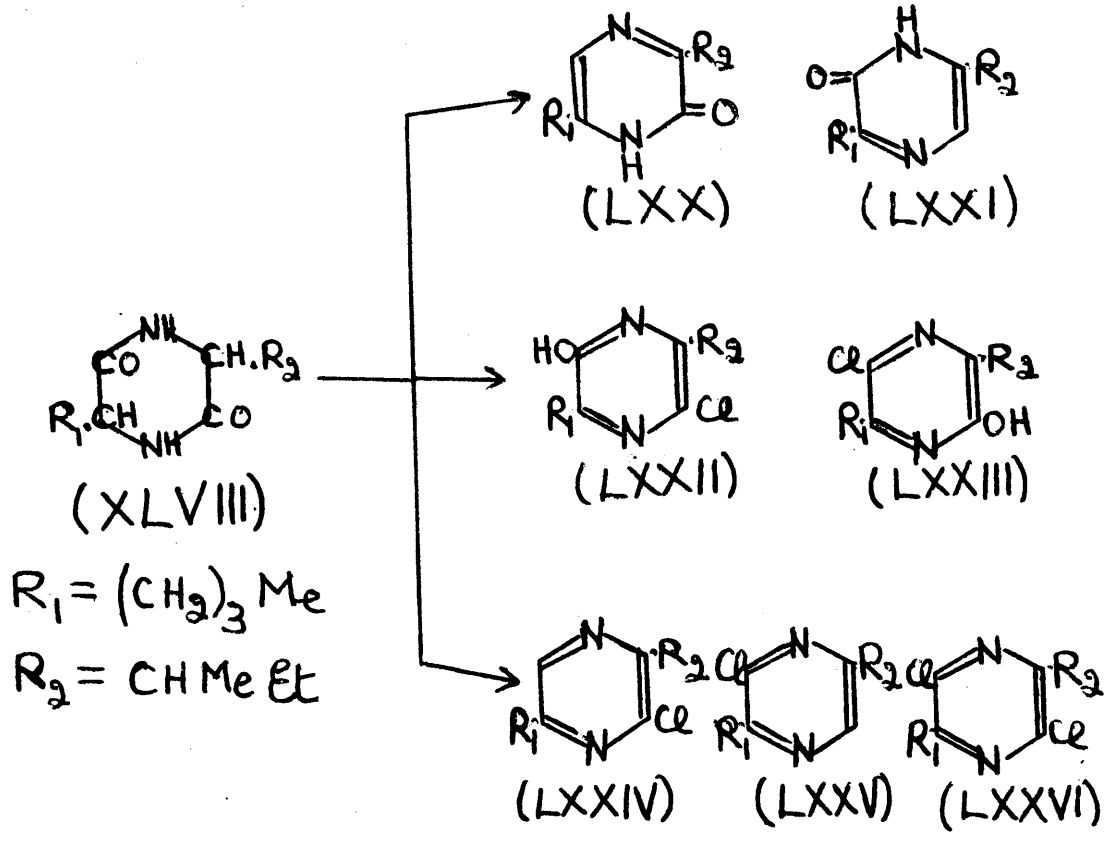
Aminopyrazines, on treatment with nitrosyl sulphuric acid (68) or nitrous acid (27,34,65,66) give the corresponding hydroxypyrazines. 3-chloro-2:5-dimethylpyrazine and 3-chloro-2:5-di-sec.-butylpyrazine have been converted into the corresponding hydroxypyrazines by alkaline hydrolysis (36,37). The most convenient method of synthesis of chloropyrazines is that of Baxter and Spring (36) who obtained mixtures of mono-chloro and dichloropyrazines on treating 2:5-diketopiperazines

with phosphoryl chloride. This section of the thesis is largely concerned with a more complete investigation of this reaction.

The only other noteworthy method of synthesis of hydroxypyrazines is that of Jones (62) who condensed a number of 2-amino-acid amides with 1:2-dicarbonyl compounds and obtained a series of 2-hydroxypyrazines.



DL-Norleucyl-DL-isoleucine (XLVlll), on treatment with phosphoryl chloride gave a mixture of 3-hydroxy-2(5)-n.-butyl-5(2)-sec.-butylpyrazine (LXX or LXXl), 3-chloro-6-hydroxy-2-n.-butyl-5-sec.-butylpyrazine (LXXll) and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-n.-butyl-pyrazine (LXXlll), a monochloro-2(5)-n.-butyl-5(2)-sec.-butyl-pyrazine (LXXlV and/or LXXV), 3:6-dichloro-2-n.-butyl-5-sec.-butylpyrazine (LXXVl). The method of isolation of the various product was exactly the same as that described for the products of the action of phosphoryl chloride on leucylisoleucine anhydride. The presence of 2-n.-butyl-5-sec.-butylpyrazine could not be established but it may have been present in the reaction mixture which contained a considerable amount of resinous material.

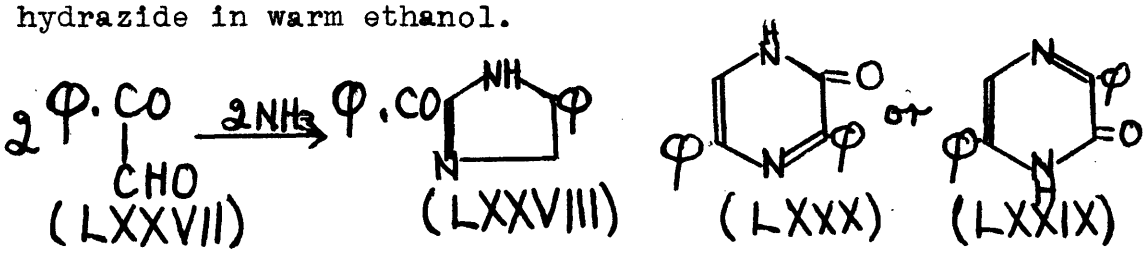


3-Hydroxy-2(5)n.-butyl-5(2)sec.-butylpyrazine (LXX or LXXI), separates from aqueous ethanol as colourless needles, m.p. 78-79°. In the ultra-violet region it exhibits the absorption spectra characteristic of 3-hydroxypyrazines (maxima at 2285 A., ε = 8,500 and 3220 A., ε = 8,500). It is readily soluble in the common organic solvents, dilute acid and dilute alkali but it is insoluble in cold water. 3-chloro-6-hydroxy-2-n.-butyl-5-sec.-butylpyrazine (LXXII) and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-n.-butylpyrazine (LXXIII) separates from aqueous ethanol as colourless needles, m.p. 113-120°. It is readily soluble in the common organic solvents, soluble in dilute alkali but it is insoluble in

2N hydrochloric acid and in water.

In view of the fact that the monochloro-2(5)-isobutyl-5(2)-sec.-butylpyrazine and the 3:6-dichloro-2-isobutyl-5-sec.-butylpyrazine had proved inseparable, no attempt was made to separate the mixture of monochloro-2(5)-n.-butyl-5(2)sec.-butylpyrazine, (LXXIV and/or LXXV), and 3:6-dichloro-2-n.-butyl-5-sec.-butylpyrazine(LXXVI), which was obtained as a colourless oil b.p. 98-102°/0.6mm.. The oil was treated with sodium ethoxide solution and the resultant ethoxy derivatives heated under reflux with 5N hydrochloric acid for 18 hours. The mixture was extracted with ether and the ethereal solution dried over sodium sulphate. Removal of the ether gave 3-chloro-6-ethoxy-2-n.-butyl-5-sec.-butyl-pyrazine and/or 3-chloro-6-ethoxy-2-sec.-butyl-5-n.-butyl-pyrazine as a colourless oil, b.p. 114-118°/1.5mm., which could not be hydrolysed. It was stable to alcoholic hydrochloric acid but was completely destroyed when treated with 10N sulphuric acid. The hydrochloric acid solution was carefully neutralised with 3N alkali when a hydroxy-2(5)n.-butyl-5(2)-sec.-butylpyrazine separated. The hydroxypyrazine separated from aqueous ethanol, even after repeated crystallisations, as colourless needles, m.p. 59-63°. A mixture with 3-hydroxy-2(5)-n.-butyl-5(2)-sec.-butylpyrazine, m.p. 78-79°, melted at 58-62°.

Considerable confusion exists in the literature concerning 3-hydroxy-2:5-diphenylpyrazine (LXXlX). By the action of ammonia on phenylglyoxal (LXXVll), Pinner (69) obtained two isomeric products, $C_{16}H_{12}ON_2$, m.p.s. 202° and 280°. The latter was considered to be 2-benzoyl-4(5)-phenylglyoxaline (LXXVlll) and the former 3-hydroxy-2:5-diphenylpyrazine (LXXlX). The lower melting isomer (*cf* 70) was shown to be identical with a compound obtained by Engler and Hassenkamp (71), by the action of ammonia upon ω-dibromo-acetophenone. It had also been obtained previously by Japp and Miller (72) by treatment of benzil with hydrogen cyanide, and by Minovici (73) by the action of hydrogen chloride on mandelonitrile and was likewise formulated by Japp and Knox (56) as 2-keto-3:6-diphenyl-1:2-dihydropyrazine (equivalent to 3-hydroxy-2:5-diphenylpyrazine). Buch and Foerst (74) also obtained a product identical with that of Engler and Hassenkamp (71) by the self condensation of phenylpyruvyl hydrazide in warm ethanol.

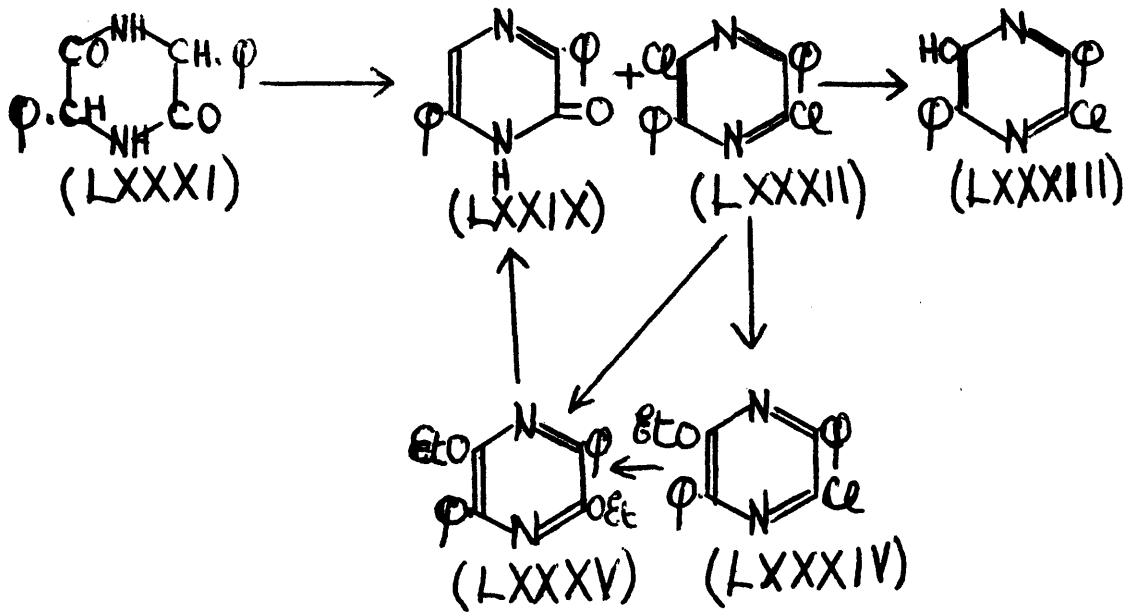


Gastaldi (57) obtained 3-hydroxy-2:5-diphenylpyrazine, m.p. 284°, by a route involving reaction of the hydrogen sulphite derivative of oximinoacetophenone with potassium cyanide followed by treatment of the mixture with hydrochloric acid, whereupon a product, formulated as 3:6-dicyano-2:5-

diphenylpyrazine, was obtained. Alkaline hydrolysis of this gave 3-hydroxy-2:5-diphenylpyrazine-6-carboxylic acid, decarboxylation of which yielded 3-hydroxy-2:5-diphenylpyrazine, m.p. 284°, identical with the compound described by Pinner (69) as 2-benzoyl-4(5)-phenylglyoxaline. Gastaldi's argument is dependent upon the intermediate dicyanide formulated by him as 3:6-dicyano-2:5-diphenylpyrazine; this structure has been supported by Sharp and Spring (63) by the synthesis of 3:6-dicyano-2:5-dimethylpyrazine by an alternative method starting from ethyl 2:5-dimethylpyrazine-3:6-dicarboxylate, the product proving to be identical with the dicyanide obtained from oximinoacetone by Gastaldi's method (57).

An unambiguous synthesis of 3-hydroxy-2:5-diphenylpyrazine from DL-phenylglycine anhydride (LXXXl) has now been effected. The method of preparation of the anhydride was essentially that of Kossel (75). DL-Phenylglycine methyl ester was heated at 140° for 16 hours to give the anhydride which separated from ethylene glycol monoethyl ether or glacial acetic acid as colourless needles, m.p. 292-293° (sintérs 287°). Treatment of the anhydride with phosphoryl chloride gave a mixture of 3-hydroxy-2:5-diphenylpyrazine and 3:6-dichloro-2:5-diphenylpyrazine. The reaction mixture was digested with boiling ethanol and the residue crystallised from dioxam to give 3-hydroxy-2:5-diphenylpyrazine (LXXlX) as yellow prisms, m.p. 285°, undepressed on admixture with a specimen (m.p. 286°) prepared by Dr. J.C. Woods (76)

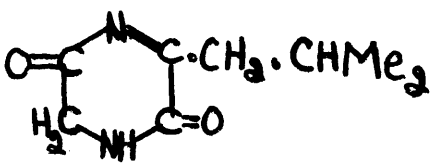
according to Pinner (69). The hydroxypyrazine on warming with glacial acetic acid and bromine gave 3-bromo-6-hydroxy-2:5-diphenylpyrazine which separated from ethanol as yellow felted needles, m.p. 245° undepressed on admixture with a specimen similarly prepared by Dr. J.C. Woods (76). The ethanolic mother liquor on concentration yielded 3:6-dichloro-2:5-diphenylpyrazine (LXXXII) as colourless needles, m.p. 159-160°. The filtrate was evaporated to dryness and the residue digested in the cold with methanol. The solid remaining was recrystallised from glacial acetic acid to give a further quantity of the dichloro compound. The methanolic mother liquor probably contained a certain amount of 3-chloro-2:5-diphenylpyrazine since, on evaporation to dryness, the resultant resin was heated with powdered potassium hydroxide to give a further quantity of 3-hydroxy-2:5-diphenylpyrazine, m.p. and mixed m.p. 284°.



3:6-Dichloro-2:5-diphenylpyrazine, when heated under reflux with sodium ethoxide solution, gave 3-chloro-6-ethoxy-2:5-diphenylpyrazine (LXXXIV) as colourless needles, m.p., 101-102°. Attempted hydrolysis of this compound failed, the ethoxypyrazine being recovered unchanged after prolonged refluxing with alcoholic hydrochloric acid. 3-Chloro-6-hydroxy-2:5-diphenylpyrazine was prepared, however, by heating the dichloro compound with powdered potassium hydroxide; the product separated from aqueous acetic acid as yellow needles, m.p. 253-254°. 3-chloro-ethoxy-2:5-diphenylpyrazine on heating at 150° with a large excess of sodium ethoxide solution gave 3:6-diethoxy-2:5-diphenylpyrazine (LXXXV) and 3-hydroxy-2:5-diphenylpyrazine, m.p. and mixed m.p. 285°. The diethoxy compound separates from ethanol as colourless needles, m.p. 147-149°; the ethanolic solution possesses a strong blue fluorescence. 3:6-Dichloro-2:5-diphenylpyrazine on heating at 150° with a large excess of sodium ethoxide solution gave a similar mixture. 3-chloro-6-ethoxy-2:5-diphenylpyrazine on heating at 150° with the theoretical quantity of sodium ethoxide solution gave in good yield 3:6-diethoxy-2:5-diphenylpyrazine, m.p. and mixed m.p. 142-144°, as the sole product. The diethoxypyrazine was recovered quantitatively after prolonged refluxing with alcoholic hydrochloric acid but on heating under reflux with 55% hydriodic acid for six hours it was converted in low yield into 3-hydroxy-2:5-

diphenylpyrazine, m.p. and mixed m.p. 282-283° and not into the required 3:6-dihydroxy-2:5-diphenylpyrazine.

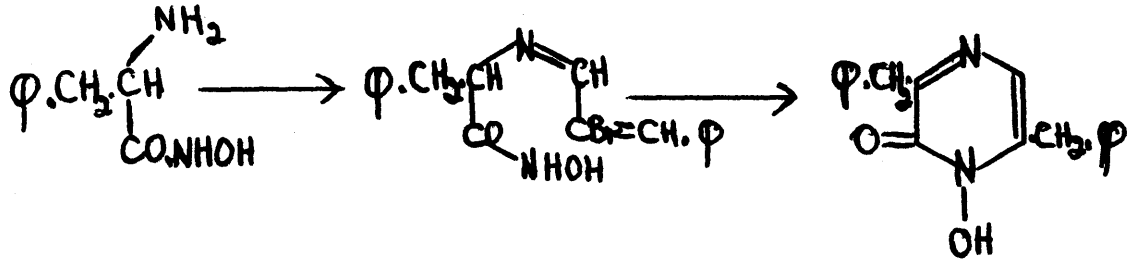
The experiments described on previous page were undertaken with the object of preparing a 3:6-dihydroxypyrazine derivative which was required in connection with an examination of a substance, $C_{12}H_{20}O_3N_2$, obtained together with aspergillic acid from culture filtrates of Aspergillus flavus (11). Such a 3:6-dihydroxypyrazine derivative would be of interest in that it is the simplest oxidation product of a 2:5-diketopiperazine. Dihydroxypyrazines as a class are unknown, although Abderhalden and Rossner (47) have described a dehydro-leucylglycine anhydride, $C_8H_{12}O_2N_2$ (equivalent to 3:6-dihydroxy-2-isobutylpyrazine). Leucylglycine anhydride



was heated under reflux for five hours with synthetic quinoline and charcoal. The resultant product was shaken with normal sodium hydroxide solution for several hours at room temperature in order to hydrolyse the unchanged starting material which formed the greater part of the product. The residue was recrystallised from methanol to give small shiny

needles, m.p. 290°. It analysed for $C_8H_{12}O_2N_2$, showed intensive absorption in the ultra-violet region (maximum at 4200 A., ε = 25,000) and formed a phenylisocyanate. On treatment with bromine and acetic acid it added one molecule of hydrobromic acid which was eliminated on treatment with phenylisocyanate with the formation of a phenylisocyanate derivative identical with that obtained previously.

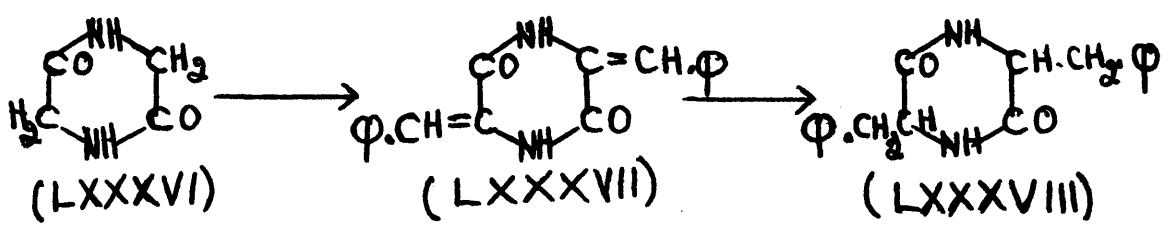
During the course of a general study of the conversion of 2:5-diketopiperazines into 3-hydroxypyrazines, attention was directed to an unambiguous synthesis of 3-hydroxy-2:5-dibenzylpyrazine. A colleague, Mr. D. Ramsey, is at present engaged on the synthesis of 1-hydroxy-2-keto-3:6-dibenzyl-1:2-dihydropyrazine, by the method already described for the preparation of pyrazine hydroxamic acids (61). Mild reduction of the hydroxamic acid with hydrazine would be expected to give 3-hydroxy-2:5-dibenzylpyrazine.



The independent synthesis of this hydroxypyrazine would be of obvious advantage in confirming the location of the benzyl substituents at the 2- and 5- positions. Attempts to convert DL-phenylalanine anhydride into 3-hydroxy-2:5-dibenzylpyrazine

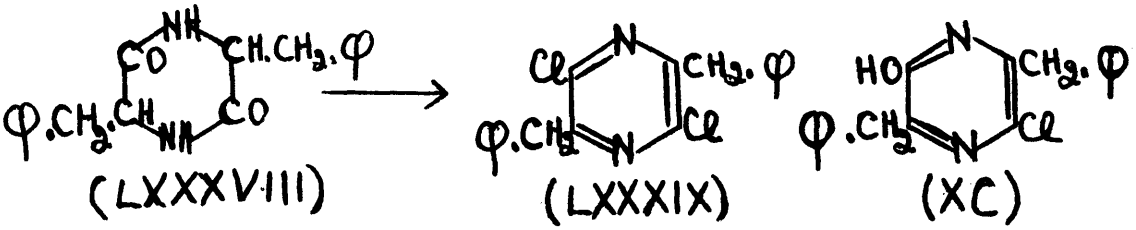
were unsuccessful.

Phenylalanine anhydride was first mentioned in the literature by Schulze and Barbieri (77) who obtained an optically active form by dry distillation of L-phenylalanine. The inactive anhydride has been prepared by the dry distillation of DL-phenylalanine (78), prolonged heating of the methyl (79) or ethyl ester (80) whilst Sasaki (81) has synthesised the anhydride from glycine anhydride (LXXXVl). The latter compound (1 mol.) was condensed with benzaldehyde (2 mols.) in the presence of acetic anhydride and sodium acetate to give 2:5-diketo-3:6-dibenzalpiperazine (LXXXVll) which was then reduced with zinc dust and glacial acetic acid to 2:5-diketo-3:6-dibenzylpiperazine (LXXXVlll).



DL-Phenylalanine anhydride was first prepared by heating DL-phenylalanine under reflux with ethylene glycol, a method developed by Sannié (82) for the preparation of 2:5-diketopiperazines. The anhydride separates from glacial acetic acid as colourless needles, m.p. 296-298°. On treatment with phosphoryl chloride, the anhydride is converted in low yield into 3:6-dichloro-2:5-dibenzylpyrazine (LXXXlX) which crystallises from ethanol or aqueous ethanol as

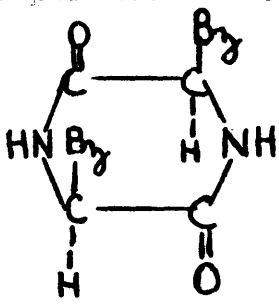
colourless leaflets, m.p. 114-115°. No other product was isolated, the reaction mixture consisted mainly of resinous material which appeared impervious to both alkali and sodium ethoxide solution. When the anhydride was prepared according to **Sasaki** (81) treatment with phosphoryl chloride gave in excellent yield 3-chloro-6-hydroxy-2:5-dibenzylpyrazine (XC), and 3:6-dichloro-2:5-dibenzylpyrazine, m.p. and mixed m.p. 114-115°. The two products were easily separable since 3-chloro-6-hydroxy-2:5-dibenzylpyrazine is almost insoluble in boiling light petroleum (b.p. 40-60°) and the dichloro pyrazine is soluble. The former product separates from ethanol or benzene as colourless needles, m.p. 209°.



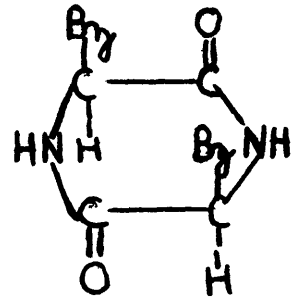
3-Chloro-6-hydroxy-2:5-dibenzylpyrazine was recovered unchanged after fusion with powdered potassium hydroxide, a method used by Baxter and Spring (36) to convert 3-chloro-6-hydroxy-2:5-dimethylpyrazine into 3-hydroxy-2:5-dimethylpyrazine. It seems likely that this reaction is peculiar to the dimethylpyrazine series. 3-chloro-6-hydroxy-2:5-dibenzylpyrazine was unaffected by either vigorous treatment with sodium ethoxide solution or by heating under reflux with sodium hydride in anhydrous medium. The compound was slowly reduced by hydrogen

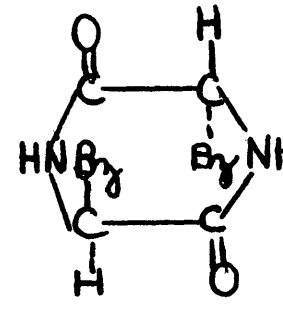
and palladium catalyst but the resultant products were not identified. They could not be isolated pure but it is likely that they were dihydropyrazines since they were slowly oxidised on exposure to the air.

There was a marked difference in the melting points of the 2:5-diketo-3:6-dibenzylpiperazine prepared by the two methods. When prepared according to Sasaki (81) the anhydride melted at 279-281°, a melting point 10° lower than that recorded by Sasaki. A mixture with the anhydride (m.p. 296-298°), prepared by the ethylene glycol method, had a m.p. 285-287°. Other melting points quoted for DL-phenylalanine anhydride are 290-291° (78), 295-296° (79) and 300° (80). The anhydride, which contains two asymmetric centres, is capable of exhibiting geometrical and optical isomerism. Therefore cis and trans forms may result, of which the former would constitute a racemic pair, and the latter an internally compensated meso form.



CIS





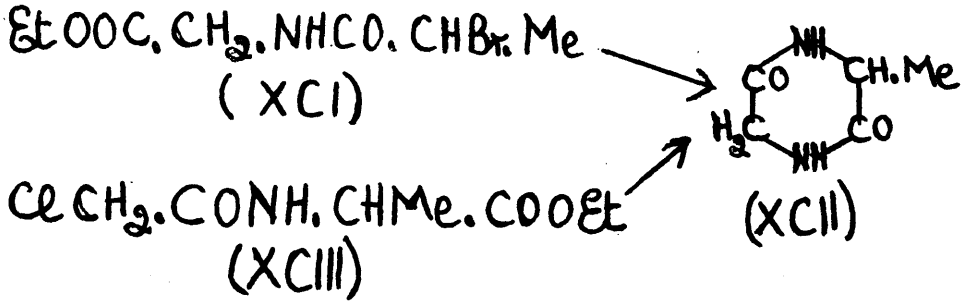
TRANS

It is unlikely that the ethylene glycol method would give an individual cis or trans form but it is conceivable that the method of Sasaki (81) would. If this is the case, it affords

a ready explanation of the poor yield of pyrazine derivatives obtained when the anhydride (m.p. 296-298°) was treated with phosphoryl chloride, viz., either the cis or the trans form is not amenable to such treatment. This method of conversion of 2:5-diketopiperazines into pyrazine derivatives had been applied previously to 3:6-disubstituted compounds only (36,49), i.e. anhydrides capable of exhibiting cis - trans isomerism. The author has attempted the conversion of glycine anhydride and glycyl-DL-alanine anhydride into pyrazine derivatives but was unsuccessful. These 2:5-diketopiperazines exist in only one form (equivalent to cis). This would suggest that 2:5-diketopiperazines undergo such a conversion only when they exist in the trans form. There is, however, little systematic knowledge available concerning 2:5-diketopiperazines and it is possible that the explanation for the lack of success in this reaction lies elsewhere, possibly in the presence of active hydrogens in the ring system.

A number of methods have been described in the literature for the synthesis of 2:5-diketo-3-methylpiperazine. The anhydride has been synthesised from glycyl-D-alanine ethyl ester or its hydrochloride by treatment with alcoholic ammonia (83,84). The inactive anhydride had previously been reported by E. and Otto Fischer (85) who heated chloro-acetyl-DL-alanine ethyl ester with alcoholic ammonia. Other methods of synthesis

are briefly: 1) heating equimolecular quantities of glycine and D-alanine with glycerine (86), 2) the treatment of glycyl-DL-alanine ethyl ester with alcoholic ammonia (87), 3) heating 2-bromo-propionyl-glycinamide with aqueous ammonia (88), 4) heating equimolecular quantities of glycine and DL-alanine with ethylene glycol (82), 5) treating glycyl-DL-alanine ethyl ester with water (89). The isolation of the anhydride from the hydrolysis of Canton silk, New-Chwang silk, Tussah silk (90) and from dog hair (91) has also been reported.

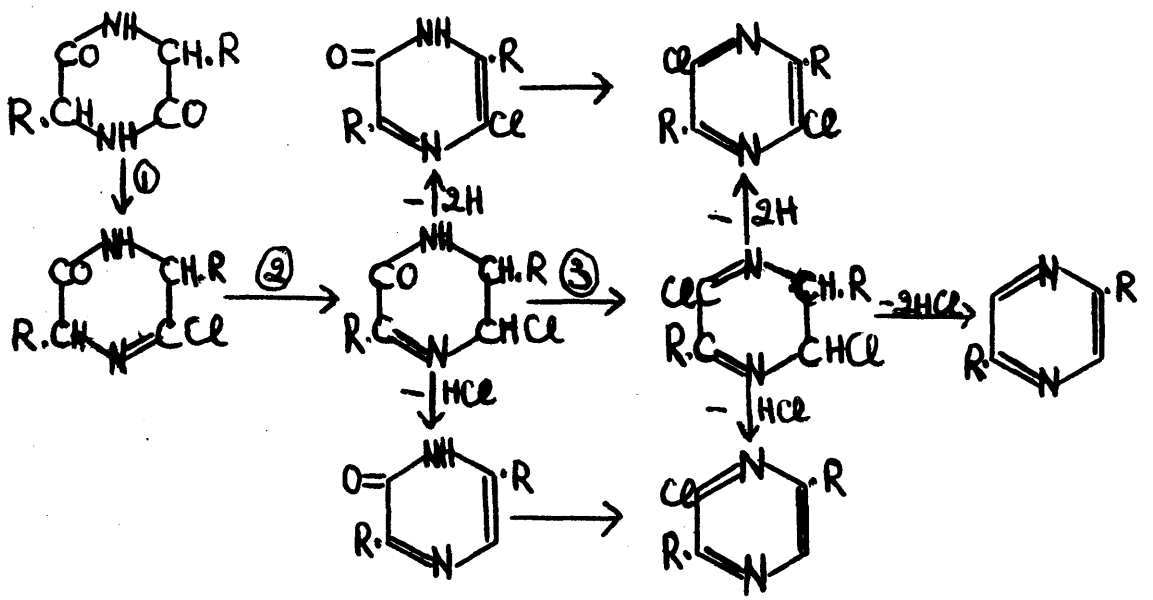


2:5-Diketo-3-methylpiperazine(XCII) was prepared by several methods. 2-Bromopropionyl bromide was condensed with glycine ethyl ester hydrochloride in the presence of a tertiary base to give ethyl 2-bromopropionamido-acetate (XCI)which was heated with alcoholic ammonia to give DL-alanylglycine anhydride (XCII), m.p. 244-245°. Similarly, chloro-acetyl chloride was reacted with DL-alanine ethyl ester hydrochloride to give chloro-acetyl-DL-alanine ethyl ester(XCIII) and the latter heated with alcoholic ammonia to give the diketopiperazine (XCII). Much improved yields were obtained when the chloro-acetyl-DL-alanine ethyl ester was dissolved in alcoholic ammonia and the solution

maintained at room temperature until the anhydride (XCll), m.p. 244-245°, had fully precipitated.

Mechanism for the conversion of 2:5-diketopiperazines into pyrazine derivatives.

Baxter and Spring (36), in their original work on the conversion of 2:5-diketopiperazines into aromatic pyrazine derivatives, attributed the formation of monochloro and dichloropyrazines to the formation of an intermediate dichlorodihydropyrazine which then lost hydrogen chloride to give a monochloropyrazine or was spontaneously oxidised to the dichloropyrazine, a common step in many pyrazine syntheses. Present work has shown that the reaction mechanism is not so simple as that advanced by Baxter and Spring (36) and that products other than monochloro and dichloropyrazine result from the reaction. Accepting the formation of an intermediate dihydropyrazine as a basis the following explanation of the reaction is offered.



Stage (1) involves the usual conversion of a ketone into a dichloride, which in this case loses hydrogen chloride to give the intermediate monochloro-dihydropyrazine. This undergoes a rearrangement of the nitrogen electrons causing the migration of a hydrogen atom and movement of the double bond. Spontaneous oxidation occurs at stage (3) together with the elimination of hydrogen chloride resulting in the formation of a chloro-hydroxypyrazine and a hydroxypyrazine. A repetition of stage (1) also takes place giving an intermediate dichloro-dihydropyrazine. The latter loses hydrogen chloride forming a monochloropyrazine or is spontaneously oxidised to a dichloropyrazine. The hydrogen atoms made available by this oxidation cause the intermediate dichloro-dihydropyrazine to lose two molecules of hydrogen chloride, accept the two hydrogen atoms required for stability and so form a pyrazine base. The latter phase is probably the most striking feature of the reaction and it is not surprising that experiment has only once indicated the existence of this stage.

A dichloropyrazine derivative has been isolated in every conversion examined by the author or by other workers (36,49). A monochloropyrazine has been absent from the products of reaction in only one case (2:5-diketo-3:6-dibenzyl-piperazine) but in view of the high yield obtained (90%) it is unlikely that any product, other than those isolated, was produced. Yields generally are of a much lower order (40-70%).

Since some of the products isolated by the author were present in the reaction mixture in extremely small quantities (e.g. 3-chloro-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine) it is possible that the failure of other workers (36,49) to isolate similar products is due to the small quantities of diketopiperazine used. A colleague, Mr. W. Sweeny, repeated the work of Baxter and Spring (36) and from the action of phosphoryl chloride on DL-alanine anhydride isolated not only the 3-chloro- and 3:6-dichloro-2:5-dimethylpyrazines but also a small quantity of 3-chloro-6-hydroxy-2:5-dimethylpyrazine (92).

SUMMARY.

The statement of Dutcher and Wintersteiner (26, .27). that the alkyl side chains located at the 2- and 5- positions in deoxyaspergillic acid are both sec.-butyl groups has been proved erroneous. Confirmation of the observation of Dutcher that the melting point of the compound $C_{12}H_{22}O_2N_2$ from aspergillic acid is not depressed when mixed with DL-isoleucine anhydride was obtained but it was also found that the former is not depressed in melting point when mixed with DL-leucyl-DL-isoleucine anhydride (2:5-diketo-3-isobutyl-6-sec.-butyl-piperazine). A mixture of the compound $C_{12}H_{22}O_2N_2$ from aspergillic acid with DL-norleucyl-DL-isoleucine anhydride (2:5-diketo-3-n.-butyl-6-sec.-butylpyrazine) on the other hand showed a slight but consistent depression. Kuhn-Roth estimations of the C-Me content of deoxyaspergillic acid and a number of related compound showed that the side chains could not both be sec.-butyl groups but that one might be an isobutyl group and the other a sec.-butyl group. In an attempt to synthesise racemic deoxyaspergillic acid leucyl-isoleucine anhydride has been converted, by treatment with phosphorylchloride, directly into a 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 97-98°) isomeric with deoxyaspergillic acid. A mixture of a monochloro-2(5)-isobutyl-5(2)-sec.-butylpyrazine and 3:6-dichloro-2-isobutyl-5-sec.-butylpyrazine was isolated from the reaction mixture,

together with 3-chloro-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine and 2-isobutyl-5-sec.-butylpyrazine. The monochloropyrazine, which could not be separated from the dichloropyrazine, is probably a mixture of 3-chloro-2-isobutyl-5-sec.-butylpyrazine and 3-chloro-2-sec.-butyl-5-isobutylpyrazine since the mixture on etherification and subsequent treatment with 5N hydrochloric acid gave a chloro-ethoxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine and a hydroxypyrazine (m.p. 87-88°). Experiment revealed the latter to be a mixture of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 97-98°) and racemic deoxyaspergillic acid (m.p. 103-104°), containing 11-21% of the latter, although attempts to prepare a similar mixed crystal, by crystallising mixtures of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 97-98°) and racemic deoxyaspergillic acid from aqueous ethanol were not successful. 3-Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine may be brominated in aqueous acetic acid when it forms 3-bromo-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine and 3:6-diketo-2-isobutyl-5-sec.-butyl-3:6-dihydropyrazine indistinguishable from the diketo-dihydropyrazine isolated by Dutcher (26,27) from the mother liquors of bromo-deoxyaspergillic acid. Deoxyaspergillic acid has thus been identified as either 3-hydroxy-2-isobutyl-5-sec.-butylpyrazine or 3-hydroxy-2-sec.-butyl-5-isobutylpyrazine.

In a series of model experiments having a bearing upon the structure of aspergillic acid and its related compounds a number of pyrazine derivatives were prepared. DL-Norleucyl-DL-isoleucine anhydride was converted into 3-hydroxy-2(5)-n.-butyl-5(2)-sec.-butylpyrazine, a chloro-hydroxy-2(5)-n.-butyl-5(2)-sec.-butylpyrazine, a monochloro-2(5)-n.-butyl-5(2)-sec.-butylpyrazine and 3:6-dichloro-2-n.-butyl-5-sec.-butylpyrazine. An unambiguous synthesis of 3-hydroxy-2:5-diphenylpyrazine from DL-phenylglycine anhydride has been effected. Treatment of the anhydride with phosphoryl chloride gave a mixture of 3-hydroxy-2:5-diphenylpyrazine and 3:6-dichloro-2:5-diphenylpyrazine. Attempts to convert the latter into 3:6-dihydroxy-2:5-diphenylpyrazine were unsuccessful the diethoxy derivative on treatment with 55% hydriodic acid gave 3-hydroxy-2:5-diphenylpyrazine. DL-Phenylalanine anhydride, on treatment with phosphoryl chloride, gave 3:6-dichloro-2:5-dibenzylpyrazine and 3-chloro-6-hydroxy-2:5-dibenzylpyrazine and not the required 3-hydroxy-2:5-dibenzylpyrazine. When glycine anhydride and glycyl-DL-alanine anhydride were similarly treated no identifiable products were isolated.

EXPERIMENTAL.

EXPERIMENTAL.

All m.p.s are uncorrected. Micro-analyses are by Drs. Weiler and Strauss, Oxford, Miss N. Henderson and Mr. Wm. McCorkindale, The Royal Technical College.

SECTION ONE.

sec.-Butyl bromide.

The bromide was prepared by the hydrobromic-sulphuric acid method as described for n.-butyl bromide (Org.Syn., Coll. Vol. 1, p. 28). sec.-Butyl alcohol (880g.) gave sec.-butyl bromide (1120g. ; 69% of theory) as a colourless liquid, b.p. 90-92°.

Ethyl sec.-butylmalonate. (Org.Syn., 21, p. 60).

Ethyl malonate (500g.) was added in a steady stream and with stirring to a sodium ethoxide solution, from sodium (70g.) and dry ethanol (1400c.c.). sec.-Butyl bromide (420g.) was then added at such a rate that the heat of reaction caused refluxing, and the mixture stirred and heated under reflux for 48 hours. The ethanol was removed by distillation and the residue shaken with water (400c.c.). The ester layer, which separated, was distilled to give a colourless oil, b.p. 85-95°/1mm. (527g. or 80% of theory).

2-Bromo-3-methyl-n.-valeric acid (Ibid, p. 61).

The ester (527g.) was added in a steady stream and with vigorous stirring to a hot solution of potassium hydroxide (236g.) in water (500c.c.). The mixture was stirred and heated for five hours and then cooled to 15°. Hydrochloric acid (d., 1.16) was added at such a rate that the temperature did not rise above 20°. After the addition of about 500c.c. of acid, the monopotassium salt separated necessitating stirring by hand until solution again occurred. When the solution was acid to Congo Red, the sec.-butylmalonic acid was extracted with three portion (600c.c.) of ether and the combined extracts dried over calcium chloride. Bromine (16.5c.c.) was added to the clear ethereal solution and the solution stirred until it was decolourised. The remainder of the bromine (100c.c.) was then added dropwise at such a rate that the ether refluxed gently. Water (500c.c.) was added at such a rate that the ether refluxed without frothing. The ether was removed by distillation from the ethereal phase and the residual liquid decarboxylated by heating at 130° for five hours. The bromo-acid was separated from the small quantity of water formed and distilled to give a colourless oil (338g. or 71% of theory), b.p. 95-105°/3mm.

DL-isoLeucine (Ibid, p. 62).

The bromo-acid (338g.) was added to ammonia (1600c.c.

of d., 0.88) and the mixture set aside for one week in a tightly stoppered bottle. The ammonia was removed under reduced pressure and the aqueous solution concentrated to a bulk of 400c.c.. The mixture was cooled to room temperature and the crystals, which had separated, collected and washed with a little ethanol. The filtrate, on further concentration, yielded a second crop of crude material. The combined crude material (101g. or 45% of theory) was recrystallised from water (1300c.c.) and ethanol (660c.c.) when the amino-acid separated as colourless plates, m.p. 266-267° in a sealed tube.

2:5-Diketo-3:6-di-sec.-butylpiperazine (DL-isoleucine anhydride.).

(Baxter and Spring, J., 1947, 1179)

DL-isoLeucine (9g.) was heated under reflux for four hours with anhydrous ethylene glycol (54g.). The mixture was evaporated to dryness under reduced pressure and the residue extracted with boiling chloroform (300c.c.). The residue (1.5g.) was mainly unchanged amino-acid. Removal of the chloroform gave the anhydride as a colourless solid (3g. or 49% of theory). After several crystallisations from ethanol the anhydride separated as colourless felted needles, m.p. 280-281°.

isoButyl bromide.

The bromide was prepared by the hydrobromic-sulphuric acid method as described for n.-butyl bromide (Org.Syn., Coll. Vol. 1, p. 28). isoButyl alcohol (880g.) gave isobutyl bromide (905g. or 56% of theory) as a colourless liquid, b.p. 88-92°.

Ethyl isobutylmalonate.

(Fischer and Schmitz, Ber., 1906, 39, 351).

The method of preparation was essentially that outlined for ethyl sec.-butylmalonate (p. 71). isoButyl bromide (610g.) was condensed with sodio-malonic ester to give ethyl isobutyl-malonate (777g. or 70% of theory) as a colourless liquid, b.p. 105-115°/6mm.

2-Bromo-isohexoic acid.

a) (Fischer and Schmitz, loc.cit.)

The method of preparation was essentially that outlined for 2-bromo-3-methyl-n.-valeric acid (p. 72). Ethyl isobutylmalonate (777g.) was converted to 2-bromo-isohexoic acid (300g. or 40% of theory) which was obtained as a colourless oil, b.p. 107-117°/3mm.

b) (Org.Syn., 21, p. 74).

isoHexoic acid (250g. dried by azeotropic distillation with benzene), dry bromine (116c.c.) and phosphorus trichloride (5c.c.) were heated at 80-85° for twelve hours when the red

colour of the bromine had disappeared from the sides of the condenser. The temperature was then raised to 100-105° and the mixture maintained at this temperature for two hours. 2-Bromo-isohexoic acid distils as a colourless oil, b.p. 104-114°/2mm. (270g. or 64% of theory).

DL-Leucine.

(Ibid, p. 75)

The bromo-acid (120g.) was added to ammonia (600c.c. of d, 0.88) and the mixture set aside for one week in a tightly stoppered flask. The crystals (59g.), which had separated, were collected and washed with ethanol (50c.c.). The filtrate, on concentration to 250c.c. (circa), yielded a second crop of crude material (8g.). The combined crude material (83% of theory) was recrystallised from water (2000c.c.) and ethanol (1500c.c.) when the amino-acid separated as colourless plates, m.p. 282-283° in a sealed tube.

2-Bromo-isohexoyl chloride.

2-Bromo-isohexoic acid (300g.) was heated under reflux for two hours with excess thionyl chloride (300g.). The excess thionyl chloride was removed under reduced pressure and the residue distilled. The fraction distilling at 58-62°/6mm. was 2-bromo-isohexoyl chloride (240g. or 74% of theory) which was obtained as a colourless liquid. (Fischer, Ber.,

1904, 37, 2492, prepared the chloride, b.p. 68-71°/11-12mm. by the action of phosphorus pentachloride on the acid. Fischer and Schmitz, ibid, 1906, 39, 353, gave the b.p. 74-76°/16mm.).

2-Bromo-isohexoyl-isoleucine.

(cf. Abderhalden, Hirsch and Schuler, Ber., 1909, 42, 3398).

2-Bromo-isohexoyl chloride (60g.; 1.2 mol.) and normal sodium hydroxide solution (408c.c.; 1.75 mol.), were added simultaneously over 40 minutes and with vigorous stirring to an ice cold solution of DL-isoleucine (30g.; 1 mol.) in normal sodium hydroxide solution (228c.c.; 1 mol.). The solution was filtered through silaceous earth and the filtrate acidified with 5N hydrochloric acid (81c.c.) when 2-bromo-isohexoyl-DL-isoleucine separated as an oil which quickly solidified and then had m.p. 134-144°. The yield was 67.5g. or 96% of theory. After six recrystallisations from aqueous ethanol the product separated as colourless plates, m.p. 178-179°.

Found: C, 46.8; H, 7.1; N, 4.4%.

$C_{12}H_{22}O_3NBr$ requires C, 46.75; H, 7.1; N, 4.5%.

The crude material (36g.) was digested with boiling petroleum (200c.c.; b.p. 100-120°) and the mixture filtered. The residue, after three such treatments, had m.p. 174-177°. The solid, crystallising from the filtrates, on similar treatment yielded a further crop of material, m.p. 174-177°. Total

yield was 13.6g. or 38% of starting material. After three recrystallisations from aqueous ethanol the product separated as colourless rhombic plates, m.p. 178-179° either alone or when mixed with the specimen described above.

The filtrates, on further examination, yielded a lower melting material, m.p. 128-130° (18.1g. or 50% of starting material). After three recrystallisations from aqueous ethanol the product separated as colourless rhombic plates, m.p. 131-133°.

Found: C, 46.8 ; H, 7.3 ; N, 4.5%.

$C_{12}H_{22}O_3NBr$ requires C, 46.75; H, 7.1 ; N, 4.5%.

Leucylisoleucine.

(Abderhalden, Hirsch and Schuler, loc.cit.).

2-Bromo-isohexoyl-DL-isoleucine, m.p. 134-144° (65g.), was dissolved in ammonia (370c.c.; d, 0.88) and the mixture set aside for one week in a tightly stoppered flask. The ammonia was removed under reduced pressure and the solution concentrated to 100c.c. (circa) and the crystals, which separated, collected. The dipeptide, at this stage, had m.p. 261-262° and amounted to 16g. or 33% of theory. After four recrystallisations from water containing a little ethanol the dipeptide separated as rhombic prisms, m.p. 272° [266-267° in a sealed tube. Abderhalden, Hirsch and Schuler, loc.cit., gave the m.p. as

262-263°(corrected)]. The dipeptide gives a positive ninhydrin reaction which is slow in developing. The colour (grey-blue) is weak compared with the colours produced using either DL-leucine or DL-isoleucine. The dipeptide is hygroscopic and was dried in the usual manner immediately before analysis.

Found: C, 59.1 ; H, 10.25; N, 11.5%.

Calc. for $C_{12}H_{24}O_3N_2$: C, 59.0 ; H, 9.8 ; N, 11.5%.

The mother liquor, on evaporation to dryness, yielded a gum, which, even after further treatment with ammonia (d, 0.88), could not be induced to crystallise.

2:5-Diketo-3-isobutyl-6-sec.-butylpiperazine (leucylisoleucine anhydride.).

a) Leucylisoleucine (15g.) and 2-naphthol (75g.) were heated at 135-150°. The cooled mixture was ground to a fine powder and stirred continuously with ether until all the 2-naphthol dissolved. The insoluble solid, m.p. 272-274° (11.4g. or 84% of theory) was crystallised from ethanol, the anhydride separating as colourless felted needles, m.p. 273-275°. After three recrystallisations from ethyl acetate the anhydride separated as colourless needles, m.p. 275-277° (sealed tube) with some sublimation. A mixture of this diketopiperazine with DL-isoleucine anhydride, m.p. 280-281°(sealed tube), had m.p. 270-272°. A mixture of leucylisoleucine anhydride with the compound $C_{12}H_{22}O_2N_2$, m.p. 260-262°, prepared from

aspergillic acid according to Dutcher (J.Biol.Chem., 1947, 171, 341) had m.p. 263-265°.

Found: C, 63.8 ; H, 9.8 ; N, 12.7%.

$C_{12}H_{22}O_2N_2$ requires C, 63.7 ; H, 9.7 ; N, 12.4%.

The gum, isolated in the previous stage of the synthesis was heated with 2-naphthol in the manner described above when a further batch of leucylisoleucine anhydride was obtained as colourless needles, m.p. 268-270° undepressed on admixture with the specimen described above.

b) A solution of 2-bromo-isohexoyl-DL-isoleucine, m.p. 134-144° (40g.) in dry ethanol (200c.c.) was saturated with hydrogen chloride and heated under reflux for two hours. The alcohol and hydrogen chloride were removed under reduced pressure and the residual gum, ethanol (200c.c.) and liquid ammonia (75c.c.) were heated at 140° for four hours. The alcohol and the ammonia were evaporated under reduced pressure, the residue washed with water (150c.c.) and then crystallised from ethanol when DL-leucyl-DL-isoleucine anhydride separated as long needles m.p. 260-262° (12g. or 41% of theory). The anhydride was recrystallised four times from ethyl acetate from which it separates as colourless needles, m.p. 265-267° undepressed on admixture with the specimen described in (a). A mixture with DL-isoleucine anhydride (m.p. 280-282°, sealed tube) had m.p. 266-269°. A mixture with the compound $C_{12}H_{22}O_2N_2$, m.p. 260-262°, prepared from aspergillic acid according to

Dutcher (loc.cit.) had m.p. 263-265°.

Found: C, 63.4 ; H, 9.7 ; N, 12.4%.

$C_{12}H_{22}O_2N_2$ requires C, 63.7 ; H, 9.7 ; N, 12.4%.

The crude anhydride, on fractional crystallisation from ethanol, yielded two leucylisoleucine anhydrides differing only in melting point and solubility in ethanol; 1) colourless needles, m.p. 275-277° (6.5g.) undepressed on admixture with the specimen described in (a), 2) the more soluble component (5.2g.) which after repeated recrystallisation from ethyl acetate was obtained as colourless needles, m.p. 258-259° undepressed on admixture with the specimen m.p. 258-259° described in (c).

c) 2-Bromo-isohexoyl-isoleucine, m.p. 174-177°(12g.), was treated in identical manner to that described in (b) when two leucylisoleucine anhydrides differing only in melting point and solubility in ethanol were obtained; 1) colourless needles (2.1g.), m.p. 275-277° undepressed on admixture with the specimen described in (a),

Found: C, 63.9 ; H, 10.0 ; N, 12.2%.

$C_{12}H_{22}O_2N_2$ requires C, 63.7 ; H, 9.7 ; N, 12.4%.

2) colourless needles (2.1g.), m.p. 258-259°.

Found: C, 63.4 ; H, 9.9 ; N, 12.6%.

d) 2-Bromo-isohexoyl-isoleucine, m.p. 128-130°(12g.) was treated in identical manner to that described in (b) when two

leucylisoleucine anhydrides differing only in melting point and solubility in ethanol were obtained; 1) colourless needles, m.p. 275-277° (0.75g.) undepressed on admixture with the specimen described in (a),

Found: N, 12.3%.

$C_{12}H_{22}O_2N_2$ requires N, 12.4%.

2) colourless needles, m.p. 258-259° (3.4g.) undepressed on admixture with the specimen, m.p. 258-259°, described in (c).

Found: N, 12.5%.

2-Bromo-3-methyl-n-valeryl chloride.

(Abderhalden, Hirsch and Schuler, loc.cit.)

The chloride was obtained in excellent yield (87% of theory) by the action of excess thionyl chloride on 2-bromo-3-methyl-n.-valeric acid at 60°. The chloride is a colourless liquid, b.p. 71-73°/12mm..

2-Bromo-3-methyl-n.-valerylleucine.

(cf. Abderhalden and Schweitzer, Z. physiol.Chem., 1932, 206, 116).

2-Bromo-3-methyl-n.-valeryl chloride (40g.; 1.2 mol.) and normal sodium hydroxide solution (267c.c.; 1.75 mol.) were added simultaneously over 45 minutes and with vigorous stirring to an ice cold solution of DL-leucine (20g.; 1 mol.) in normal sodium hydroxide solution (153c.c.; 1 mol.). The solution was acidified with 5N hydrochloric acid (54c.c.) when 2-bromo-3-methyl-n.-valeryl-DL-leucine separated as an oil which

quickly solidified and then had m.p. 115-125°. The yield was 42.7g. or 91% of theory.

The crude material (26g.) was fractionally crystallised from benzene when two distinct fractions were obtained; 1) colourless rhombic plates, m.p. 140-142°(6.2g.), which after repeated crystallisations from benzene or aqueous ethanol had m.p. 144-145.5°,

Found: C, 46.7 ; H, 7.0 ; N, 4.5%.

$C_{12}H_{22}O_3NBr$ requires C,46.75;H,7.1; N,4.5%.

2) colourless rods, m.p. 91-99°(10.5g.) which after eight crystallisations from light petroleum (b.p. 60-80°) had m.p. 96-98° (flows to form pool at 111°).

Found: C, 46.9 ; H, 7.2 ; N, 4.5%.

In addition, a fraction with a melting point intermediate between the above materials was obtained. This was not further purified.

isoLeucylleucine.

a) 2-Bromo-3-methyl-n.-valerylleucine (5g.), m.p. 142-4°, was dissolved in ammonia (30c.c.; d, 0.88) and the solution set aside in a tightly stoppered flask for six days. The ammoniacal solution was evaporated to dryness and the residue crystallised from aqueous ethanol (80c.c.) when colourless rhombic plates (3.2g.) separated, m.p. 143-144.5° either alone or when mixed with a pure specimen of starting material.

Examination of the mother liquor gave a colourless gummy solid which could not be purified.

b) 2-Bromo-3-methyl-n-valerylleucine (5g.), m.p. 91-99°, treated as above gave a colourless gum which could not be induced to crystallise.

c) 2-Bromo-3-methyl-n-valerylleucine (5g.), m.p. 142-144°, was heated at 140° for one hour with ammonia (30c.c.; d. 0.88). The ammoniacal solution was evaporated to dryness and the residue digested with boiling acetone (80c.c.). The filtrate was concentrated to 40c.c. (circa) and chilled at 0° for 16 hours. The mixture was filtered and the residue washed with water (5c.c.) and ethylacetate (10c.c.) to give colourless needles, m.p. 266-270° (0.9g. or 23% of theory). After three recrystallisations from water the dipeptide separated as colourless monoclinic prisms, m.p. 272-274°. The product is insoluble in water, ether and benzene, and slightly soluble in acetone and alcohol. The dipeptide is hygroscopic and was dried in the usual manner immediately before analysis.

Found: C, 58.6 ; H, 9.9 ; N, 11.7%.

$C_{12}H_{24}O_5N_2$ requires C, 59.0 ; H, 9.8 ; N, 11.5%.

d) 2-Bromo-3-methyl-n.-valerylleucine (5g.), m.p. 91-99°, was heated at 140° for one hour with ammonia (30c.c.; d, 0.88). The solution was evaporated to dryness and the residue digested with boiling acetone (80c.c.). The filtrate was concentrated to a bulk of 40c.c. and chilled at 0° for one hour when colourless needles (0.23g.), m.p. 248-252°, were deposited. After

repeated crystallisations from aqueous ethanol the product separated as colourless needles, m.p. 272-273°. A mixture with leucylisoleucine anhydride, m.p. 275-277°, melted at 260-262°.

Found: C, 63.25 ; H, 10.0 ; N, 12.5%.

$(C_6H_{11}ON)_n$ requires C, 63.7 ; H, 9.7 ; N, 12.4%.

The acetone mother liquor was concentrated to 25c.c. (circa), a few drops of water added and the solution chilled at 0° for seven days when a mixture of a colourless product and a tar separated. This was dissolved in ethyl acetate (10c.c.) and the solution chilled at 0° for 48 hours to give a colourless product (0.15g.), m.p. 138-192°. After several crystallisations from water, there separated a small quantity of colourless needles (5mg.), m.p. 270-271° undepressed on admixture with the specimen described above. Colourless crystals (20mg.), m.p. 138-141°, were isolated from the aqueous mother liquor. After two recrystallisations from benzene, the product was obtained as colourless needles, m.p. 146-147°.

Found: C, 61.6 ; H, 10.6 ; N, 11.7%.

The analyses of these two products could not be interpreted. Although the analysis of the first corresponded with that of leucylisoleucine anhydride the depression observed in the melting point of a mixture of the two was not only substantial but consistent.

2:5-Diketo-3-isobutyl-6-sec.-butylpiperazine.

a) A solution of 2-bromo-3-methyl-n.-valeryl-leucine (13.5g.), m.p. 142-144°, in dry ethanol (150c.c.) was saturated with

hydrogen chloride and heated under reflux for three hours. The alcohol and hydrogen chloride were removed under reduced pressure to give a pale yellow gum. A sample was crystallised four times from light petroleum (b.p. 60-80°) when the ester separated as colourless needles, m.p. 85.5-86.5°.

Found: C, 49.9 ; H, 8.0 ; N, 4.5%.

$C_{14}H_{26}O_3NBr$ requires C, 50.0 ; H, 8.0 ; N, 4.2%.

The crude ester, ethanol (150c.c.) and liquid ammonia (30c.c.) were heated at 140° for four hours. The alcohol and ammonia were evaporated under reduced pressure, the residue washed with water (80c.c.) and then fractionally crystallised from ethanol. Two leucyl<u>iso</u>leucine anhydrides, differing only in melting point and solubility in ethanol, were isolated; 1) colourless needles (0.7g.), m.p. 275-277° undepressed on admixture with leucyl<u>iso</u>leucine anhydride, m.p. 275-277° described on p.78. 2) colourless needles (0.9g.), m.p. 259-260° either alone or when mixed with the specimen of leucyl<u>iso</u>leucine anhydride, m.p. 258-259°, described on p. 80.

The alcoholic mother liquor was diluted with water (200c.c.), thoroughly extracted with chloroform (200c.c.) and the extract dried over sodium sulphate. The chloroform was removed and the residual brown solid crystallised from ether-light petroleum (b.p. 60-80°) to give grey plates (2.43g.), m.p. 140-142°. Repeated crystallisations from benzene gave colourless plates, m.p. 147-149°. The product is insoluble in

3N potassium hydroxide solution, soluble in 3N hydrochloric acid from which it may be recovered unchanged on neutralisation, soluble in ethanol, ether and chloroform, sparingly soluble in benzene and water and insoluble in light petroleum.

Found: C, 63.1 ; H, 10.35 ; N, 12.8%. Mol.Wt.,226.

b) 2-Bromo-3-methyl-n.-valeryleucine (7.1g.), m.p. 91-99°, was treated in identical manner to that described in (a) to give 1) colourless needles (0.1g.), m.p. 275-277° either alone or when mixed with the specimen described in (a),

Found: N, 12.3%.

$C_{12}H_{22}O_2N_2$ requires N, 12.4%.

2) colourless needles (0.6g.), m.p. 259-260° either alone or on admixture with the specimen, m.p. 259-260° described in (a),

Found: N, 12.1%.

3) colourless plates (0.9g.), m.p. 147-149° undepressed on admixture with the specimen, m.p. 147-149°, described in (a),

Found: C, 63.1 ; H, 10.6%. Mol.Wt.,301.

c) 2-Bromo-3-methyl-n.-valerylleucine (13g. of intermediate fraction from fractional crystallisation) was treated in identical manner to that described in (a) to give 1) colourless needles (0.5g.), m.p. 275-277° either alone or when mixed with the specimen, m.p. 275-277° described in (a), 2) colourless needles (0.25g.), m.p. 259-260° undepressed on admixture with the specimen, m.p. 259-260° described in (a), 3) colourless plates (2.57g.), m.p. 157-159°. A mixture with the product,

m.p. 147-149° described in (a) melted at 147-150°.

Found: C, 62.8 ; H, 10.15 ; N, 12.9%. Mol.Wt., 268.

2-Bromo-n.-hexoyl bromide.

(Abderhalden, Froehlich and Fuchs, Z.physiol.Chem., 1913, 86, 454; Auwers and Wegener, J.prakt.Chem., 1923, 106, 226).

Dry bromine (100c.c.; 1.9 mol.) was added dropwise over three hours to freshly distilled n.-hexoic acid (120g.; 1 mol.) at 75°,the temperature raised to 100° and the mixture maintained at this temperature for two hours. The bromide distils as a colourless liquid (247g. or 96% of theory), b.p. 90-95°/12-13mm..

2-Bromo-n.-hexoic acid.

(Abderhalden, Froehlich and Fuchs, loc.cit.).

The bromide (150g.) was added dropwise to boiling water (100c.c.). The bromo-acid separated quantitatively as a colourless oil, b.p. 128-131°/10mm.

DL-Norleucine.

(Org.Syn., Coll.Vol.1, p.48).

2-Bromo-n.-hexoic acid (110g.) was added to ammonia (600c.c.; d, 0.88) and the mixture set aside for four days in a tightly sealed flask. The amino-acid separated as colourless plates (36.3g.), m.p. 294-296°. The filtrate, on concentration to 150c.c.(circa), yielded a second crop of crystals (9.4g.). Total yield was 45.7g. or 62% of theory.

2-Bromo-n.-hexoyl-DL-isoleucine.

2-Bromo-n.-hexoyl bromide (70g.; 1.2 mol.) and normal sodium hydroxide solution (408c.c.; 1.75 mol.) were added intermittently over one hour and with vigorous shaking to an ice-cold solution of DL-isoleucine (30g.; 1 mol.) in normal sodium hydroxide solution (228c.c.; 1 mol.). The solution was filtered through silaceous earth and the filtrate acidified with 5N hydrochloric acid when 2-bromo-n.-hexoyl-DL-isoleucine separated as an oil which quickly solidified and then had m.p. 120-130°. Yield was 62g. or 88% of theory. After repeated crystallisations from aqueous ethanol the bromacyl-amino-acid separated as colourless plates, m.p. 168-170°. The product is soluble in ethanol and ether but insoluble in light petroleum and water.

Found: C, 47.1 ; H, 6.9 ; N, 4.5%.

$C_{12}H_{22}O_3NBr$ requires C, 46.75; H, 7.1 ; N, 4.5%.

2-Bromo-3-methyl-n.-valeryl-DL-norleucine.

2-Bromo-3-methyl.n.-valeryl chloride (40g.; 1.2 mol.) and normal sodium hydroxide solution (332c.c.; 1.75 mol.) were added intermittently over a period of 40 minutes and with vigorous shaking to an ice cold solution of DL-norleucine (20g.; 1 mol.) in normal sodium hydroxide solution (152c.c.; 1 mol.). The solution was washed with a little ether (50c.c.), acidified with 5N hydrochloric acid (54c.c.) and the mixture thoroughly extracted with chloroform. The dried (sodium sulphate) extract was evaporated to give a clear yellow gum

(45g.) which solidified on chilling at 0° for 24 hours. The product was crystallised three times from ether-light petroleum (b.p. 60-80°) when the bromacyl-amino-acid separated as colourless needles, m.p. 115-116°. The product is readily soluble in all the common organic solvents except light petroleum and is also insoluble in water.

Found: C, 47.0 ; H, 7.3 ; N, 4.4%.

$C_{12}H_{22}O_3NBr$ requires C, 46.75; H, 7.1 ; N, 4.5%.

2:5-Diketo-3-n.-butyl-6-sec.-butylpiperazine.

a) A solution of 2-bromo-n.-hexoyl-DL-isoleucine (1g.) in dry ethanol (20c.c.) was saturated with hydrogen chloride and heated under reflux for two hours. The alcohol and hydrogen chloride were removed under reduced pressure and the residue heated at 140° for four hours with ethanol (20c.c.) and liquid ammonia (5c.c.). The mixture was concentrated to 10c.c. (circa), cooled and the crystalline solid (240mg.), m.p. 253-255°, which separated, collected. Repeated crystallisations from ethyl acetate gave the anhydride as felted needles, m.p. 258-260°. The anhydride is moderately soluble in alcohol and acetone, slightly soluble in ether and ethyl acetate and insoluble in water. This diketopiperazine is undepressed in melting point when mixed with DL-leucyl-DL-isoleucine anhydride, m.p. 265-267°. A mixture of DL-norleucyl-DL-isoleucine anhydride and the compound $C_{12}H_{22}O_2N_2$ from aspergillic acid (m.p. 259-260°) had m.p. 257-259°.

Found: C, 64.0 ; H, 9.7 ; N, 12.2%.

$C_{12}H_{22}O_2N_2$ requires C, 63.7 ; H, 9.7 ; N, 12.4%.

b) A solution of 2-bromo-3-methyl.n.-valeryl-DL-norleucine (40g.) in dry ethanol (250c.c.) was saturated with dry hydrogen chloride and heated under reflux for four hours. The ethanol and hydrogen chloride were removed under reduced pressure and the residue heated at 140° for four hours with ethanol (250c.c.) and liquid ammonia (70c.c.). The mixture was evaporated under reduced pressure, the residue washed with water (100c.c.) and crystallised from ethanol when the diketopiperazine separated as colourless felted needles, m.p. 252-254° (6.2g.). After four recrystallisations from aqueous ethanol the diketopiperazine was obtained as colourless needles, m.p. 259-260° undepressed when mixed with the specimen described under (a). A mixture with the compound $C_{12}H_{22}O_2N_2$ (from aspergillic acid), m.p. 260-262°, had m.p. 256-258°.

Found: C, 63.6 ; H, 9.6 ; N, 12.2%.

$C_{12}H_{22}O_2N_2$ requires C, 63.7 ; H, 9.7 ; N, 12.4%.

The alcoholic mother liquor was diluted with water (200c.c.) and thoroughly extracted with ether (4 x 50c.c.) and the ether dried over sodium sulphate. Removal of the ether left a reddish-brown gum (7g.) which after six crystallisations from benzene-light petroleum (b.p. 40-60°) separated as colourless needles, m.p. 140-170°. The product was insoluble in water and

readily soluble in most organic solvents with the exception of light petroleum.

Found: C, 62.9 ; H, 10.0 ; N, 11.4%. Mol.Wt. 260.

Action of phosphoryl chloride on 2:5-diketo-3-isobutyl-6-sec.-butylpiperazine.

Leucylisoleucine anhydride, irrespective of the melting point or method of synthesis, behaves in the manner described below, on heating with excess of phosphoryl chloride.

2-isoButyl-5-sec.-butylpyrazine.

Leucylisoleucine anhydride (8.9g.) was heated under reflux at 120° for two hours with phosphoryl chloride (90c.c.). The excess phosphoryl chloride was removed under reduced pressure and the residue triturated with ice-water (100c.c.). The mixture was just neutralised with 3N potassium hydroxide solution and thoroughly extracted with ether (200c.c.). The ethereal solution (A) was concentrated to 70c.c. (circa) and thoroughly extracted with 3N potassium hydroxide solution (2 x 25c.c.; 2 x 10c.c.) and then thoroughly extracted with 6N hydrochloric acid (4 x 25c.c.). The acidic phase was made just alkaline with 3N potassium hydroxide solution, the mixture extracted with ether (3 x 25c.c.) and the ethereal solution dried over sodium sulphate. Removal of the ether gave a red oil which distilled to give a colourless oil (0.21g.)

b.p. 80°/1mm; index of refraction at 16°, 1.4845. The product gave no simple derivatives.

Found: C, 74.2;74.7; H,10.3,10.2; N,14.6,14.7%.

$C_{12}H_{20}N_2$ requires C, 75.0 ; H,10.4 ; N,14.6 %.

Monochloro-and 3:6-dichloro-2(5)-isobutyl-5(2)-sec.-butylpyrazine.

The ethereal solution (A) was dried over sodium sulphate and the ether removed leaving a reddish-brown oil which distilled to give a colourless oil (5.0g.), b.p. 102.5-104°/2mm.; index of refraction at 15°, 1.5120.

Found C,58.8; H,7.5; N,12.6; Cl,25.9%.

The oil was subjected to several fractional distillations (arbitrary cuts) and two fractions analysed a) the highest boiling fraction, b.p. 105°/1mm.; index of refraction at 16°, 1.5155.

Found: C, 57.4; H, 7.5; N, 12.8%.

$C_{12}H_{18}N_2Cl_2$ requires C, 55.2; H, 6.9; N, 10.7%.

$C_{12}H_{19}N_2Cl$ requires C, 63.6; H, 8.4; N, 12.4%.

b) the lowest boiling fraction, b.p. 85°/0.5mm.; index of refraction at 16°, 1.4995.

Found: C, 65.9; H, 8.8; N, 12.6%.

$C_{12}H_{19}N_2Cl$ requires C, 63.6; H, 8.4; N, 12.4%.

$C_{12}H_{20}N_2$ requires C, 75.0; H,10.4; N, 14.6%.

Analyses indicated that the oil consisted mainly

of 3:6-dichloro-2-isobutyl-5-sec.-butylpyrazine and a monochloro-2(5)-isobutyl-5(2)-sec.-butylpyrazine contaminated with 2-isobutyl-5-sec.-butylpyrazine.

3-Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine.

The alkali extract was now just neutralised with hydrochloric acid (d, 1.16) and the mixture thoroughly extracted with ether (3 x 50c.c.). The ethereal solution (B) was now extracted with 2N hydrochloric acid (3 x 50c.c.) and the acidic phase just neutralised with 3N potassium hydroxide solution when the hydroxypyrazine separated as a colourless amorphous material (1.04g.), m.p. 76-80°. It sublimed rapidly at 95°/0.002mm. to give a colourless sublimate (0.9g.), m.p. unchanged. Repeated crystallisations from aqueous ethanol gave colourless needles, m.p. 97-98°. A mixture with racemic deoxyaspergillic acid, m.p. 103-104° (48) had m.p. 90-92°. The product is readily soluble in all organic solvents, insoluble in cold water but very slightly soluble in hot water. It is soluble in 3N potassium hydroxide solution and 2N hydrochloric acid. Light absorption in ethanol: maxima at 2290 A., $\mathcal{E}$= 7,000 and 3250 A., $\mathcal{E}$= 7,600

Found: C, 69.4; H, 9.3; N, 13.3%.

$C_{12}H_{20}ON_2$ requires C, 69.2; H, 9.6; N, 13.5%.

Examination of the mother liquors revealed a further quantity of material which crystallised from aqueous ethanol as colourless needles, m.p. 85-87°. Repeated crystallisations

from aqueous ethanol and filtration through a column of alumina (15 x 0.5" dia.) failed to raise the above melting point. A mixture with the specimen, m.p. 97-98° described above had m.p. 86-89°. A mixture with racemic deoxyaspergillic, m.p. 103-104° melted at 86-89°.

Found: N, 13.7%.

$C_{12}H_{20}ON_2$ requires N, 13.5%.

3-Chloro-6-hydroxy-2-isobutyl-5-sec.-butylpyrazine and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-isobutylpyrazine.

The ethereal solution (B) was now extracted with 3N potassium hydroxide solution (3 x 10c.c.). The alkaline extract was just neutralised with hydrochloric acid (d, 1.16) and the mixture chilled at 0° for one hour. Filtration gave a colourless product (50g.) which sublimed rapidly at 95°/0.002mm. to give a colourless sublimate (25mg.), m.p. 113-121°. Repeated crystallisations from aqueous ethanol yielded colourless needles, m.p. 138-139° either alone or when mixed with a specimen of chloro-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 139-140° described on p. 97.

Found: N, 11.9%.

$C_{12}H_{19}ON_2Cl$ requires N, 11.5%.

3-Chloro-6-ethoxy-2-isobutyl-5-sec.-butylpyrazine and/or 3-chloro-6-ethoxy-2-sec.-butyl-5-isobutylpyrazine.

The chlorine containing oil (3.95g.) was heated at 110° for four hours with sodium ethoxide solution (0.4g. sodium in 25c.c. of ethanol). The ethanol was removed by

distillation and the residue treated with water (100c.c.), the mixture just neutralised with hydrochloric acid (d, 1.16) and thoroughly extracted with ether (2 x 50c.c.). The ether was removed and the colourless residual oil was heated under reflux for 18 hours with 5N hydrochloric acid (100c.c.). The acid was extracted with ether (2 x 25c.c.) and the ethereal solution dried over sodium sulphate. The ether was removed and the residue distilled to give a colourless oil (2.15g.) b.p. 115°/1mm.; index of refraction at 13°, 1.4995.

Found: C, 62.1; H, 8.6; N, 10.8%.

$C_{14}H_{23}ON_2Cl$ requires C, 62.1; H, 8.5; N, 10.4%.

Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine.

The acidic phase was just neutralised with 3N potassium hydroxide solution and the resulting mixture chilled at 0° for one hour when a grey solid (1g.) m.p. 76-80°, separated. The product sublimed rapidly at 95°/0.001mm. to give a colourless sublimate, m.p. 85-86°. After repeated crystallisations from aqueous ethanol the product separated as colourless needles, m.p. 87-88° undepressed on admixture with the specimen, m.p. 85-87° described on p.93. A mixture of the above product with 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. 97-98° melted at 87-89°. A mixture with racemic deoxyaspergillic acid, m.p. 101-102° had m.p. 88-90°. Light absorption in

ethanol: maxima at 2280 A., ε= 9,000 and 3240 A., ε = 10,500.

Found, C, 69.3; H, 9.5; N, 13.1%.

$C_{12}H_{20}ON_2$ requires C, 69.2; H, 9.6; N, 13.5%.

A solution of the above crude product (0.5g.) in dry benzene (50c.c.) was added to a column of alumina (grade II; 15 x 0.5" dia.) and the column eluted with a further quantity of benzene (250c.c.). The column was then eluted with dry ether (500c.c.) but no product was isolated from the elutriate. The ether was replaced by an ethanol-ether mixture (5 pts.: 95 pts.) and the elutriate collected in 200c.c. portions. Evaporation of the first portion and sublimation of the residue at 95°/0.001mm. gave a colourless sublimate (45mg.), m.p. 73-78°, which after five crystallisations from aqueous ethanol separated as colourless needles, m.p. 97-98° undepressed on admixture with 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butyl-pyrazine, m.p. 97-98° (p.93.). All subsequent fractions when similarly purified gave colourless needles m.p. 87-88° either alone or when mixed with a pure specimen of starting material.

3-Chloro-6-hydroxy-2-isobutyl-5-sec.-butylpyrazine and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-isobutylpyrazine.

a) 3-Chloro-6-ethoxy-2-isobutyl-5-sec.-butylpyrazine and/or 3-chloro-6-ethoxy-2-sec.-butyl-5-isobutylpyrazine (1g.) was heated under reflux for 16 hours with 6N sulphuric acid (30c.c.) when the oil was recovered unchanged.

b) The chloro-ethoxypyrazine (1g.) was heated under reflux for 16 hours with 10N sulphuric acid but no identifiable product was isolated.

c) The Chloro-ethoxypyrazine (1g.) was dissolved in 65% ethanol (70c.c.), saturated with hydrogen chloride and heated under reflux for five hours. The mixture was evaporated under reduced pressure, the residue treated with water (20c.c.), the resultant mixture extracted with ether (3 x 10c.c.) and the ethereal solution dried over sodium sulphate. Removal of the ether left a pale yellow oil which crystallised on standing to give colourless needles. These were freed from contaminating oil by pressing them on porous tile. The product (0.23g.), m.p. 116-124°, was crystallised repeatedly from aqueous ethanol from which it separated as colourless needles, m.p. 139-140°. The product is readily soluble in all organic solvents, soluble in 3N potassium hydroxide solution, insoluble in water and 2N hydrochloric acid. Light absorption in ethanol: maxima at 2320 A., $\mathcal{E}$= 8,000 and 3240 A., $\mathcal{E}$= 6,000.

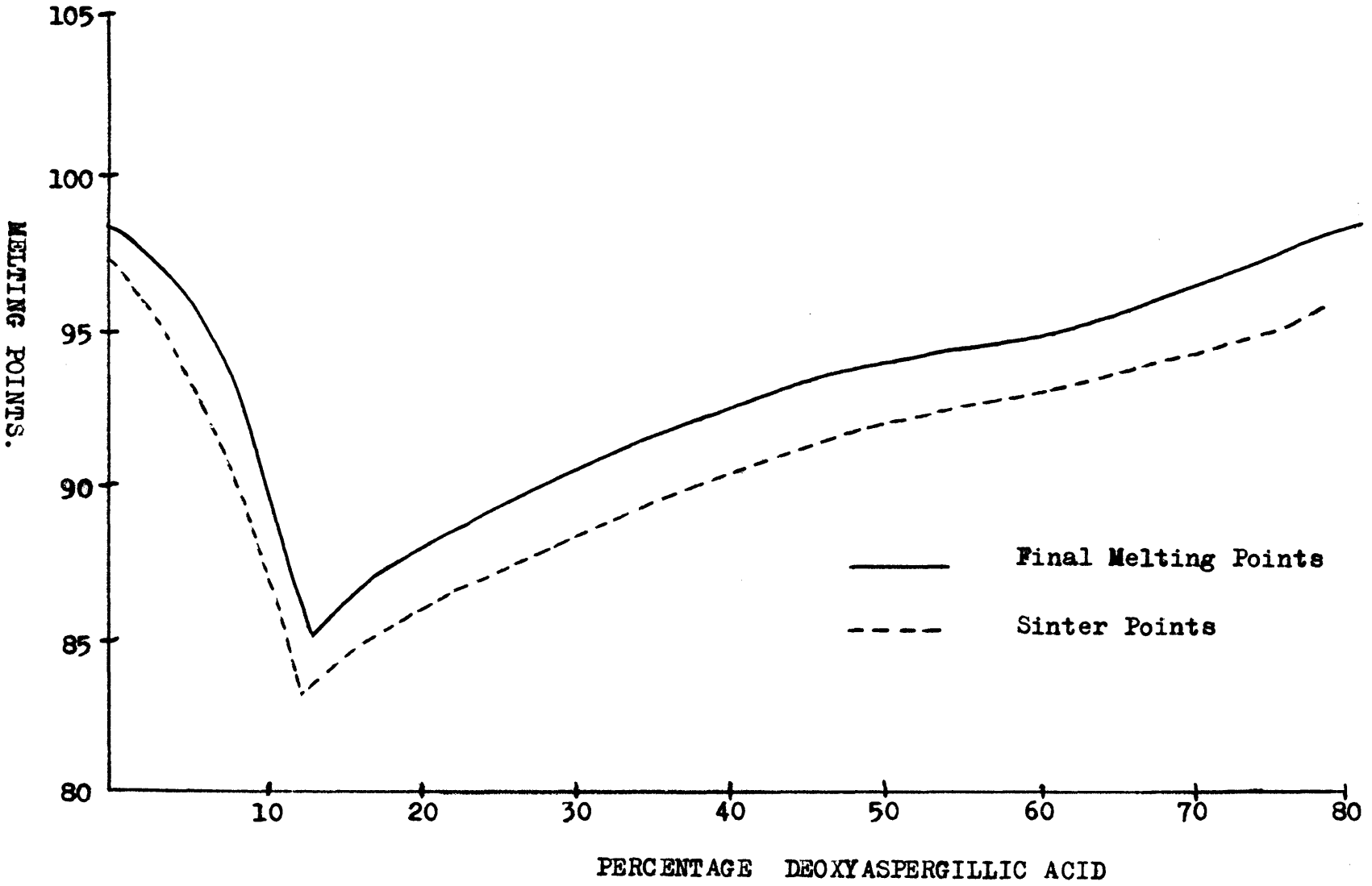
Found: C, 60.0,58.8; H, 7.8,8.0; N, 11.3,11.3%.

$C_{12}H_{19}ON_2Cl$ requires C, 59.3 ; H, 7.8 ; N, 11.5%.

Attempted synthesis of a mixed crystal of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine and racemic deoxyaspergillic acid.

The melting points of various mixtures of 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (m.p. 97-98°) and racemic deoxyaspergillic acid were plotted against the percentage of racemic deoxyaspergillic acid present in the

mixture. The resulting graph indicates that m.p.s below 88° occur when the amount of racemic deoxyaspergillic acid present is between 11% and 21%. A eutectic at or about 13% is indicated. Attempts to prepare a mixed crystal (m.p. 87-88°) by crystallising mixtures of the two components (12%, 18%, 22% and 25%) from aqueous ethanol met with no success, 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine, m.p. and mixed m.p. 97-98° being recovered after several crystallisations. Examination of the mother liquors revealed no evidence of mixed crystal formation nor could the deoxyaspergillic acid be recovered.


105
100
95
90
85
80
MELTING POINTS.
10
20
30
40
50
60
70
80
PERCENTAGE DEOXYASPERGILLIC ACID
Final Melting Points
Sinter Points

3-Hydroxy-6-phenylazo-2(5)-isobutyl-5(2)-sec.-butylpyrazine

a) A solution of aniline (0.4c.c.) in hydrochloric acid (3c.c. of d, 1.16) and water (3c.c.) was treated with a solution of sodium nitrite (0.45g.) in water (8c.c.) and the resulting diazonium solution added to 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (0.2g.), m.p. 97-98°, dissolved in ice-cold sodium hydroxide solution (6c.c. of 3N). The orange precipitate, which formed almost immediately was collected after five minutes, washed with ice-water and suspended in hot water (20c.c.). The mixture was made acid to Congo Red with dilute hydrochloric acid and vigorously shaken for five minutes. The free hydroxy-azo derivative (0.31g. or 92% of theory) was collected and crystallised from aqueous ethanol when it separated as orange-red needles, m.p. 198-200° with decomposition. After three recrystallisations from aqueous ethanol the product separated as orange needles, m.p. 203-205°(decomp.). A mixture with the phenylazo derivative of racemic deoxyaspergillic acid (m.p. 189-191°) had m.p. 189-191°.

Found: C, 69.2; H, 7.7; N, 18.3%.

$C_{18}H_{24}ON_4$ requires C, 69.2; H, 7.7; N, 17.9%.

b) Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (0.5g.) m.p. 87-88°, was coupled with benzene diazonium chloride as described above to give a crude phenylazo derivative (0.6g. or 80% of theory), m.p. 176-180° with decomposition. Chromatographic examination of the crude material revealed no signs of heterogeneity. The melting point was raised above 190° only with the greatest difficulty since the melting point tended

to remain at 188-190° (the melting point of the phenylazo derivative of deoxyaspergillic acid). After a great many crystallisations from aqueous ethanol with heavy loss of material, the product separated as orange needles, m.p. 203-205° (decomp.) undepressed on admixture with the specimen described in (a).

Found: N, 17.7, 17.8%.

$C_{18}H_{24}ON_4$ requires N, 17.9%.

3-Bromo-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine.

a) A solution of bromine (0.4g.) in glacial acetic acid (1c.c.) was added to 3-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (0.5g.), m.p. 97-98°, dissolved in glacial acetic acid (10c.c.) and water (6c.c.). The bromo derivative separated immediately as colourless needles (0.4g.), m.p. 147-148°. After several crystallisations from aqueous ethanol the product separated as colourless needles, m.p. 150-151°. The product is soluble in all organic solvents but insoluble in water. Light absorption in ethanol: maxima at 2325 A., ε= 9,700 and 3315 A., ε= 7,100.

Found: C, 50.1; H, 6.9 ; N, 9.3%.

$C_{12}H_{19}ON_2Br$ requires C, 50.2; H, 6.6 ; N, 9.75%.

b) -Hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine (0.45g.), m.p. 87-88°, when treated as above gave the bromo derivative as colourless needles (0.28g.), m.p. 126-135°. After one recrystallisation from aqueous ethanol the product separated

as colourless needles (0.2g.), m.p. 147-148°. Further crystallisations from the same solvent raised the melting point to 150-151° alone or when mixed with the specimen described in (a).

3:6-Diketo-2-isobutyl-5-sec.-butyl-3:6-dihydropyrazine.

The combined aqueous acetic acid mother liquors from the previous experiments were evaporated to dryness under reduced pressure and the residue crystallised from aqueous acetic acid to give a colourless microcrystalline product m.p. 245-249°. After four recrystallisations from aqueous acetic acid the product separated as minute needles, m.p. 254-256°. A mixture with the diketo-dihydropyrazine (m.p. 268-270°) prepared from deoxyaspergillic acid according to Dutcher (27) had m.p. 256-258°. Light absorption in ethanol: maximum at 2885 A., ε = 22,400.

Found: C, 64.5 ; H, 8.3; N, 12.5%.

$C_{12}H_{18}O_2N_2$ requires C, 64.85; H, 8.1; N, 12.6%.

Attempted reduction of 3-bromo-6-hydroxy-2(5)-isobutyl-5(2)-sec.-butylpyrazine.

(cf Clar, Ber., 1939, 72B, 1645.)

The bromo compound (0.7g.), zinc dust (1g.), sodium chloride (1g.) and zinc chloride (4g.) were ground together in an intimate mixture and heated to 350° in 20 minutes. The mixture fused at 230-240°. No volatile product was isolated

nor was any identifiable product recovered from the fused mass.

Section II.

3-Hydroxy-2(5)-n.-butyl-5(2)-sec.-butylpyrazine.

DL-Norleucyl-DL-isoleucine anhydride (8.4g.) was heated under reflux at 120° for two hours with phosphoryl chloride (85c.c.). The excess phosphoryl chloride was removed under reduced pressure, the residue triturated with ice-water, the mixture carefully neutralised with 3N potassium hydroxide solution and thoroughly extracted with ether (250c.c.). The ethereal solution (A) was concentrated to a smaller bulk (70c.c.) and extracted with 3N potassium hydroxide solution (4 x 25c.c.). The alkali was just neutralised with hydrochloric acid (d, 1.16) and the mixture thoroughly extracted with ether (150c.c.). The ethereal solution (B) was extracted with 2N hydrochloric acid (5 x 25c.c.), the acid phase just neutralised with 3N potassium hydroxide solutionand the resultant mixture thoroughly extracted with ether (100c.c.). The ether, after drying over sodium sulphate, was removed by distillation and the residue sublimed at 95°/0.003mm. to give a slightly yellow sublimate (0.27g.), m.p. 48-53°. After five crystallisations from aqueous ethanol the hydroxypyrazine separated as colourless needles, m.p. 78-79°. Light absorption in ethanol: maxima at 2285 A., ε= 8,500 and 3220 A., ε= 8,500

Found: C, 68.9 ; H, 9.7 ; N, 13.3%.

$C_{12}H_{20}ON_2$ requires C, 69.2 ; H, 9.6 ; N, 13.5%.

3-Chloro-6-hydroxy-2-n.-butyl-5-sec.-butylpyrazine and/or 3-chloro-6-hydroxy-2-sec.-butyl-5-n.butylpyrazine.

The ethereal solution (B) was dried over sodium sulphate and the ether removed by distillation. The residue was sublimed at 150°/0.1mm. to give an almost colourless slightly oily sublimate (130mg.) which after two crystallisations from aqueous ethanol separated as colourless needles, m.p. 113-120°.

Found: C, 60.1 ; H, 7.7 ; N, 11.0%.

$C_{12}H_{19}ON_2Cl$ requires C, 59.3 ; H, 7.8 ; N, 11.5%.

Monochloro-2(5)-n.-butyl-5(2)-sec.-butylpyrazine and 3:6-dichloro-2-n.-butyl-5-sec.-butylpyrazine.

The ethereal solution (A) was extracted with 6N hydrochloric acid (2 x 10c.c.) and the ethereal solution dried over sodium sulphate. Removal of the ether left a reddish-brown oil which distilled to give a colourless oil (4.65g.), b.p. 98-102°/0.6mm.. Index of refraction at 20°, 1.5100.

Found: C, 59.7 ; H, 8.4 ; N, 11.1%.

$C_{12}H_{18}N_2Cl_2$ requires C, 55.2 ; H, 6.9 ; N, 10.7%.

$C_{12}H_{19}N_2Cl$ requires C, 63.6 ; H, 8.4 ; N, 12.4%.

The acid extract was neutralised with 3N potassium hydroxide solution, the mixture extracted with ether (40c.c.) and the ethereal solution dried over sodium sulphate. Removal

of the ether left a reddish-brown residue which, when heated to 150°/0.1mm., yielded one drop of clear distillate which did not form a chloroplatinate.

3-Chloro-6-ethoxy-2-n.-butyl-5-sec.-butylpyrazine and/or 3-chloro-6-ethoxy-2-sec.-butyl-5-n.-butylpyrazine.

The oil (4.2g.) was heated under reflux for four hours with sodium ethoxide solution (0.5g. of sodium in 30c.c. of ethanol). The solution was evaporated to dryness, the residue treated with water (100c.c.) and the mixture extracted with ether (100c.c.). The ether was removed by distillation and the residue heated under reflux with 5N hydrochloric acid for 18 hours. The mixture was extracted with ether (2 x 25c.c.) and the ethereal solution dried over sodium sulphate. The ether was removed and the residue distilled to give a colourless oil (2.6g.), b.p. 114-118°/1.5mm.. Index of refraction at 20°, 1.5005.

Found: C, 62.2 ; H, 8.7 ; N, 10.7%.

$C_{14}H_{23}ON_2Cl$ requires C, 62.1 ; H, 8.5 ; N, 10.4%.

Hydroxy-2(5)-n.-butyl-5(2)-sec.-butylpyrazine.

The hydrochloric acid solution was carefully neutralised with 3N potassium hydroxide solution, the mixture chilled at 0° for one hour and the grey solid (0.5g.), m.p. 54-60°, collected. Repeated crystallisations from aqueous ethanol,

from which the product separated as colourless needles, failed to raise the melting point above 59-63°. A mixture with 3-hydroxy-2(5)-n.- butyl-5(2)-sec.-butylpyrazine (m.p. 78-79°) melted at 58-62°.

Found: C, 69.8 ; H, 9.5 ; N, 13.1%.

$C_{12}H_{20}ON_2$ requires C, 69.2 ; H, 9.6 ; N, 13.5%.

Attempted hydrolysis of 3-chloro-6-ethoxy-2-n.-butyl-5-sec.-butylpyrazine and/or 3-chloro-6-ethoxy-2-sec.-butyl-5-n.-butylpyrazine.

a) The oil (2.5g.) was dissolved in ethanol (20c.c.), the solution saturated with hydrogen chloride and heated under reflux for 12 hours when the starting material was recovered unchanged.

b) When the oil was heated under reflux for 16 hours with 10N sulphuric acid, no identifiable product was isolated.

DL-Phenylglycine.

(Org.Syn., 22, p. 23.)

A solution of benzaldehyde (212g.) in methanol (400c.c.) was added to a solution of ammonium chloride (118g.) and potassium cyanide (132g.) in water (400c.c.) and the mixture stirred for two hours. The mixture, after dilution with water (1000c.c.), was extracted with benzene (1000c.c.) and the benzene layer washed with water (3 x 50c.c.). The

amino-nitrile was extracted from the benzene by shaking with hydrochloric acid (d, 1.16; 600c.c., 2x300c.c.). The combined acid extracts were heated under reflux for two hours, diluted to two litres and subjected to vacuum distillation (20-30mm.) to remove any volatile substances (volume maintained at 2 litres throughout). The mixture was boiled with activated charcoal and filtered. Ammonia (d, 0.88) was added dropwise to the filtrate and when the solution was faintly alkaline the amino-acid separated as yellow crystals. The crystals were washed with water (1000c.c.), ether (150c.c.), hot ethanol (3 x 50c.c.) and finally with water (500c.c.). The product was dissolved in normal sodium hydroxide solution (800c.c.), ethanol (500c.c.) added and the solution filtered. The filtrate was heated to the boil and 5N hydrochloric acid (160c.c.) added slowly and with stirring. The crystals (100g. or 33% of theory), which separated on cooling, were washed with ethanol (100c.c.) and water (200c.c.). The product sublimes at 256° without melting.

DL-Phenylglycine methyl ester hydrochloride.

(Kossel, Ber., 1891, 24, 4146).

DL-Phenylglycine (45g.) and dry methanol (225c.c.) were saturated with hydrogen chloride. The acid dissolved with the evolution of heat as the esterification proceeded.

On concentration and cooling, colourless needles separated. The product was heated in an evaporating dish on a water-bath to remove free hydrogen chloride. The material (45g. or 75% of theory) had m.p. 216-217°.

DL-Phenylglycine anhydride.

(Kossel, loc.cit.)

The hydrochloride (45g.) was dissolved in the minimum amount of water and covered with a layer of ether. The theoretical amount of 5N potassium hydroxide solution (11.9g. of potassium hydroxide) was added, the solution extracted with ether (3 x 50c.c.) and the ethereal solution dried over sodium sulphate. The ether was removed and the residue heated at 140° for 16 hours when the anhydride was obtained as a red solid. The crude product was triturated with ether and crystallised from ethylene glycol monoethyl ether when the diketopiperazine (10.8g. or 39% of theory) separated as colourless needles, m.p. 292-293° (sinters 287°).

Found: C, 72.5; H, 5.7%.

Calc. for $C_{16}H_{14}O_2N_2$: C, 72.2; H, 5.3%.

3-Hydroxy-2:5-diphenylpyrazine.

DL-Phenylglycine anhydride (10g.) was heated under reflux at 120° for two hours with phosphoryl chloride (50c.c.). The excess phosphoryl chloride was removed in vacuo and the

residue triturated with ice-water. The solid was collected and digested with boiling ethanol (500c.c.) to give a green solid, m.p. 275-280° (Filtrate A). This was recrystallised from glacial acetic acid to give yellow prisms (0.53g.), m.p. 283°. After four recrystallisations from dioxan and water the compound was obtained as yellow prisms, m.p. 285° undepressed on admixture with a specimen, m.p. 286°, prepared by Dr. J.C. Woods (76) according to Pinner (69).

Found: C, 77.0 ; H, 4.9 ; N, 11.7%.

Calc. for $C_{16}H_{12}ON_2$: C, 77.4 ; H, 4.8 ; N, 11.3%.

3:6-Dichloro-2:5-diphenylpyrazine.

The alcoholic filtrate A was concentrated to 200c.c. and on cooling yielded pale yellow crystals (2.96g.), m.p. 156-157° (Filtrate B). On repeated crystallisations from ethanol the dichloro compound separated as colourless needles, m.p. 159-160°. The compound is insoluble in dilute acid, dilute alkali and in water, sparingly soluble in ethanol and glacial acetic acid and readily soluble in acetone.

Found: C, 63.8 ; H, 3.5 ; N, 8.9%.

$C_{16}H_{10}N_2Cl_2$ requires C, 63.8 ; H, 3.3 ; N, 9.3%.

The filtrate B was evaporated to dryness and the amorphous solid residue digested with methanol (50c.c.) and the solid collected. This was recrystallised from glacial acetic acid when 3:6-dichloro-2:5-diphenylpyrazine separated

as colourless needles (0.6g.), m.p. 157-158° undepressed on admixture with the specimen described above.

The methanolic mother liquor was evaporated to dryness and the resultant resin heated at 200° for two hours with powdered potassium hydroxide (10g.). The mixture was diluted with water (100c.c.) and acidified with hydrochloric acid (d, 1.16) when a yellow solid separated. The solid was collected, boiled with glacial acetic acid and filtered. The filtrate deposited 3-hydroxy-2:5-diphenylpyrazine as yellow prisms (0.41g.), m.p. 284° undepressed on admixture with the specimen previously described.

3-Bromo-6-hydroxy-2:5-diphenylpyrazine.

3-Hydroxy-2:5-diphenylpyrazine (0.6g.), suspended in glacial acetic acid (4c.c.), was warmed with an equal weight of bromine in glacial acetic acid (7c.c.). After solution occurred, the solution was allowed to cool when pale yellow felted needles (0.8g.), m.p. 243-244°, separated. After five recrystallisations from ethanol the product had m.p. 245°, undepressed on admixture with a specimen prepared by Dr. J.C. Woods (76).

Found: C, 58.9 ; H, 3.5%.

Calc. for $C_{16}H_{11}ON_2Br$: C, 58.7 ; H, 3.4%.

3-Chloro-6-Ethoxy-2:5-diphenylpyrazine.

3:6-Dichloro-2:5-diphenylpyrazine (2g.) was heated

under reflux for 9 hours with sodium ethoxide solution (0.5g. of sodium in 20c.c. of ethanol). On cooling colourless rectangular prisms (1.95g.), m.p. 101-102°, separated. The melting point remained constant on a subsequent crystallisation. The product is sparingly soluble in ethanol and in ether but insoluble in water.

Found: C, 69.8 ; H, 5.0 ; N, 9.0%.

$C_{18}H_{15}ON_2Cl$ requires C, 69.6 ; H, 4.8 ; N, 9.0%.

3-Chloro-6-hydroxy-2:5-diphenylpyrazine.

a) 3-Chloro-6-ethoxy-2:5-diphenylpyrazine (0.25g.) was heated under reflux for 12 hours with ethanol (20c.c.) saturated with hydrogen chloride when colourless needles were obtained, m.p. 101-102° undepressed on admixture with a pure sample of starting material.

b) 3:6-Dichloro-2:5-diphenylpyrazine (0.5g.) and powdered potassium hydroxide (5g.) were heated at 180° for 6 hours. The mixture was diluted with water (20c.c.) and the resultant solution acidified with hydrochloric acid (d, 1.16) when yellow prisms (0.2g.), m.p. 248-249°, separated. Four crystallisations from aqueous acetic acid yielded yellow needles, m.p. 253-254°. Light absorption in ethanol: maxima at 2640 A., ε= 12,500 and 3500 A., ε= 15,000.

Found: C, 67.51 ; H, 4.0 ; N, 9.6%.

$C_{16}H_{11}ON_2Cl$ requires C, 67.96 ; H, 3.9 ; N, 9.9%.

3:6-Diethoxy-2:5-diphenylpyrazine.

a) 3-Chloro-6-ethoxy-2:5-diphenylpyrazine (1g.) was heated at 140° for 15 hours with a large excess of sodium ethoxide solution (0.6g. of sodium in 25c.c. of ethanol). The ethanol was removed under reduced pressure, the residue treated with water (30c.c.), the solid collected (Filtrate A) and crystallised from ethanol to give colourless needles (0.3g.), m.p. 140-143°. After four recrystallisations from ethanol colourless needles, m.p. 147-149°, were obtained. An ethanolic solution of the diethoxy compound displays a strong blue fluorescence. Light absorption in ethanol : maxima at 2660.A., ε= 8,500 and 3700 A., ε= 11,000.

Found: C, 75.3 ; H, 6.7 ; N, 8.70%.

$C_{20}H_{20}O_2N_2$ requires C, 75.0 ; H, 6.3 ; N, 8.75%.

Filtrate A was neutralised with hydrochloric acid (d, 1.16) when a yellow product separated, crystallising from glacial acetic acid as yellow prisms (0.2g.), m.p. 285° undepressed on admixture with a specimen of 3-hydroxy-2:5-diphenylpyrazine, m.p. 285°.

b) 3:6-Dichloro-2:5-diphenylpyrazine (0.5g.) was heated at 150° for 7 hours with a large excess of sodium ethoxide solution (0.6g. of sodium in 25c.c. of ethanol) and the reaction mixture worked up as described in (a). 3:6-Diethoxy-2:5-diphenylpyrazine (0.36g.) was isolated as colourless needles, m.p. 147-149°, undepressed on admixture with the

specimen described in (a).

Found: C, 75.4 ; H, 6.4 ; N, 8.33%.

$C_{20}H_{20}O_2N_2$ requires C, 75.0 ; H, 6.3 ; N, 8.75%.

3-Hydroxy-2:5-diphenylpyrazine (0.12g.) was isolated from the filtrate as yellow prisms, m.p. 284-285° undepressed on admixture with an authentic specimen.

c) 3-Chloro-6-ethoxy-2:5-diphenylpyrazine (0.5g.) was heated at 150° for 24 hours with a very slight excess of sodium ethoxide solution (50mg.of sodium in 25c.c.of ethanol). The mixture was filtered and the solid washed with water to give the diethoxy compound (0.45g.) as colourless needles, m.p. 140-143°. After one crystallisation from ethanol, the compound had m.p. 142-144°, undepressed on admixture with the specimen described in (a).

Hydrolysis of 3:6-diethoxy-2:5-diphenylpyrazine.

a) 3:6-Diethoxy-2:5-diphenylpyrazine (0.25g.) was heated under reflux for 12 hours with ethanol (20c.c.) saturated with hydrogen chloride, when colourless needles were obtained, m.p. 143-145°, undepressed on admixture with a pure sample of starting material.

b) 3:6-diethoxy-2:5-diphenylpyrazine (0.5g.) was heated under reflux for 6 hours with 55% hydriodic acid (15c,c,). The insoluble material was collected and washed with dilute alkali (5c.c.) to remove traces of free iodine to give a yellow amorphous solid (110mg.), m.p. 264-270°. The product

crystallised from glacial acetic acid as yellow prisms, m.p. 282-283°, undepressed on admixture with a specimen of 3-hydroxy-2:5-diphenylpyrazine, m.p. 285°.

Glycine anhydride.

(Sannié, Bull.Soc.chim., 1942, 9, 487).

Glycine (150g.) was heated under reflux with ethylene glycol (900 g.) for 45 minutes and the resultant mixture chilled at 0° for 16 hours. The anhydride separated as reddish-brown crystals (54g.) which were recrystallised from water to give colourless needles, m.p. 310° (decomp.).

2:5-Diketo-3:6-dibenzalpiperazine.

(Sasaki, Ber., 1921, 53B., 164).

Glycine anhydride (44g.), benzaldehyde (103g.), anhydrous sodium acetate (128g.) and acetic anhydride (198g.) were heated at 120-130° for 8 hours. The solid material was collected, digested with hot water (400c.c.) and washed with methanol (500c.c.), water (500c.c.) and finally with methanol (200c.c.) to give yellow-brown plates (82g. or 73% of theory), m.p. 292-294°. After three recrystallisations from glacial acetic acid the diketopiperazine separated as yellow rhombic plates, m.p. 294-296°.

Found: C, 74.3 ; H, 4.9 ; N, 9.50%.

Calc. for $C_{18}H_{14}O_2N_2$: C, 74.5 ; H, 4.8 ; N, 9.65%.

DL-Phenylalanine.

a) (Org.Syn., 21, p. 99-102)

Benzyl chloride (158g.) was condensed with sodiomalonic ester to give ethyl benzylmalonate (cf. ethyl sec.-butylmalonate p.71) as a colourless liquid (161g. or 52% of theory), b.p. 144-146°/4mm.. The ester was hydrolysed with 55% potassium hydroxide solution, the acid brominated in ethereal solution before being decarboxylated in usual manner to give 2-bromo-3-phenylpropionic acid (cf. 2-bromo-3-methyl-n.- valeric acid p.72). The crude bromo-acid was added to ammonia (2250c.c.; d, 0.88) and the mixture set aside for one week in a tightly stoppered flask. The ammonia was removed under reduced pressure and the aqueous solution concentrated to a bulk of 1200c.c., boiled with activated charcoal, filtered, methanol (500c.c.) added and the solution chilled at 0° for 16 hours when the amino-acid separated as colourless plates, m.p. 262-263° in a sealed tube. A further crop was obtained on concentrating the mother liquor. Yield was 48.2g. or 47% of theory.

b) (Sasaki, loc.cit.)

2:5-Diketo-3:6-dibenzalpiperazine (20g.) red phosphorus (14g.) and hydriodic acid (140c.c.; d, 1.7) were heated under reflux for six hours. The mixture was diluted with water (250c.c.) and filtered. The filtrate was

evaporated to dryness under reduced pressure and the residue dissolved in water (150c.c.). The solution was just neutralised with 3N sodium hydroxide solution, chilled at 0° for one hour, the solid material collected and crystallised from water to give colourless plates (18.8g. or 83% of theory), m.p.263-264° undepressed on admixture with the specimen described in (a).

2:5-Diketo-3:6-dibenzylpiperazine.

a) DL-Phenylalanine (45g.) was heated under reflux for 4 hours with anhydrous ethylene glycol (270g.). The anhydride separated as colourless needles (23.8g. or 59% of theory), m.p. 292-294°. The diketopiperazine was recrystallised three times from dioxan when it separated as colourless needles, m.p. 296-298° with decomposition.

Found: C, 72.7 ; H, 6.2 ; N, 9.6%.

Calc. for $C_{18}H_{18}O_2N_2$: C, 73.5 ; H, 6.1 ; N, 9.5%.

b) (Sasaki, loc.cit.)

2:5-Diketo-3:6-dibenzalpiperazine (40g.) was dissolved in boiling glacial acetic acid (1800c.c.) and zinc dust (100g.) added portion-wise over 30 minutes. The mixture was heated under reflux for 11 hours and then filtered. On cooling, the filtrate deposited colourless needles (37.5g. or 92% of theory), m.p. 273-277° with decomposition. After three recrystallisations from glacial acetic acid the diketopiperazine

separated as colourless needles, m.p. 279-281° with decomposition. A mixture with the specimen described in (a) melted at 285-287° with decomposition.

Found: C, 73.0 ; H, 6.4 ; N, 9.0%.

Calc. for $C_{18}H_{18}O_2N_2$: C, 73.5 ; H, 6.1 ; N, 9.5%.

3-Chloro-6-hydroxy-2:5-dibenzylpyrazine.

DL-Phenylalanine anhydride (5g; according to Sasaki, loc.cit.) and phosphoryl chloride (25c.c.) were heated at 120° for 2 hours. The excess phosphoryl chloride was removed under pressure and the residue triturated with ice-water. The resulting solid (5.5g.) was collected and dried over concentrated sulphuric acid in a vacuum desiccator. The product was twice digested with boiling light petroleum (100c.c.; b.p. 40-60°) to give a colourless amorphous product, m.p. 192-197°. This was crystallised from ethanol when colourless needles, (3·7g.) m.p. 207-208°, separated. After three recrystallisations from ethanol the product was obtained as colourless needles, m.p. 209°. It is slightly soluble in ethanol and in benzene, almost insoluble in light petroleum and insoluble in water.

Found: C, 69.8 ; H, 5.0 ; N, 9.0%.

$C_{18}H_{15}ON_2Cl$ requires C, 69.6 ; H, 4.8 ; N, 9.0%.

3:6-Dichloro-2:5-dibenzylpyrazine.

a) The light petroleum mother liquors were concentrated

to small bulk (circa 10c.c.) when the dichloropyrazine separated as yellow prisms (1.3g.), m.p. 112-114°. The product was recrystallised four times from ethanol when it separated as colourless leaflets, m.p. 114-115°, undepressed on admixture with the specimen described in (b). It is insoluble in acid and alkali, soluble in ether, acetone, chloroform and glacial acetic acid, almost insoluble in ethanol, methanol and light petroleum and insoluble in water.

Found: N, 8.4%.

$C_{18}H_{14}N_2Cl_2$ requires N, 8.5%.

b) DL-Phenylalanine anhydride (10g.; ethylene glycol method), phosphoryl chloride (50c.c.) were heated at 120° for two hours. The excess phosphoryl chloride was removed under reduced pressure and the residue triturated with ice-water. The resulting solid was collected by filtration and dried over concentrated sulphuric acid in a vacuum desiccator to give a sticky brown solid (12.8g.). This was extracted with ether (500c.c.) and the ethereal solution evaporated. The residue was washed with methanol (50c.c.) and crystallised from ethanol to give pale yellow prisms (3.05g.) m.p. 106-108°. After five such crystallisations the dichloro compound separated as colourless leaflets, m.p. 114-115°.

Found: C, 65.4; H, 4.3; N, 8.4%.

$C_{18}H_{14}N_2Cl_2$ requires C, 65.7; H, 4.3; N, 8.5%.

Attempted dehalogenation of 3-chloro-6-hydroxy-2:5-dibenzylpyrazine.

a) 3-Chloro-6-hydroxy-2:5-dibenzylpyrazine (0.5g.) was heated at 180-200° with powdered potassium hydroxide (4g.). The mixture was treated with water (50c.c.) and filtered. The filtrate was neutralised with hydrochloric acid (d, 1.16), the precipitated solid collected and crystallised from ethanol to give colourless needles (0.4g.), m.p. 207-208° either alone or when mixed with starting material.

b) The compound (0.5g.) was heated at 150° for 16 hours with sodium ethoxide solution (0.5g. of sodium in 40c.c. of ethanol). The solution was evaporated to dryness, treated with water (50c.c.) and neutralised when a colourless product was obtained, m.p. 206-207° undepressed on admixture with a pure sample of starting material.

c) The compound (0.5g.) and sodium hydride (0.1g.) were heated under reflux for four hours with anhydrous benzene (50c.c.). The mixture was filtered when the filtrate deposited colourless needles, m.p. 206-207° either alone or when mixed with starting material.

d) 3-Chloro-6-hydroxy-2:5-dibenzylpyrazine (1g.) was shaken with ethanol (100c.c.), palladium/barium carbonate catalyst (3g.) in an atmosphere of hydrogen. After five minutes, 60c.c. of hydrogen had been adsorbed. At the end of three hours a

total of 145c.c. of hydrogen had been adsorbed. The mixture was heated to the boil and filtered. The filtrate deposited colourless needles (0.34g.), m.p. 198-200°, raised to 200-202° on one crystallisation from ethanol. The melting point was not depressed on admixture with a pure specimen of starting material. The mother liquor seemed to contain a mixture of several other compounds, none of which could be isolated pure. Their separation was complicated by the fact that they were undergoing a slow but continual air oxidation.

N-Methyl morpholine.

(Atherton, Orpenshaw and Todd, J., 1945, 660).

Paraform (105g.; 3.5 mols.) was added to morpholine (261g.) and the mixture well shaken. It was kept under a reflux condenser for a short time (approx. 10 minutes) when a vigorous reaction set in and the paraform dissolved. Formic acid (170g.; 98%; 3.5 mols.) was then added dropwise over two hours, carbon dioxide being steadily evolved after which the mixture was heated on a water-bath for three hours, gas evolution then being slight. The reaction mixture formed two layers. Sodium hydroxide (200g.) was added with cooling, the upper layer separated and further treated with sodium hydroxide until no more separation of the aqueous phase took place. The base was then diluted with benzene (200c.c.) and kept overnight over potassium hydroxide pellets. It was then

distilled (Fenske) giving N-methyl morpholine, b.p. 114-115° (225g. or 74% of theory) which was stored over sodium wire.

2-Bromopropionic acid.

(Bischoff, Annalen, 1882, 214, 55; Zelinsky, Ber., 1887, 20, 2026).

Bromine (182c.c.) was added dropwise over six hours to propionic acid (200g.) at 95° in the presence of red phosphorus (10g.). The bromo-acid boiled at 113°/25mm.. The yield was 232g. or 56% of theory.

2-Bromopropionyl bromide.

(cf Weinig, Annalen, 1894, 280, 247; Anwers and Bernhardi, Ber., 1891, 24, 2219).

The more volatile fraction and the residue from the previous reaction were treated with bromine (25c.c.) in the presence of red phosphorus as before. This yielded 2-bromopropionyl bromide (150g.), b.p. 48-52°/5mm..

DL-Alanine.

(Org.Syn., Coll. Vol. 1., p. 24).

2-Bromopropionic acid (232g. at 1-4°) was added dropwise and with constant shaking to ammonia (7000c.c. at 1-4°; d, 0.88) and the solution set aside for 6 days in a tightly sealed flask. The ammonia was removed under reduced

pressure and the aqueous solution concentrated to 500c.c.. The amino-acid separated as colourless plates (104g. or 78% of theory), m.p. 266-267°.

DL-Alanine ethylester hydrochloride.

(Curtius and Goebel, J. prakt. Chem., 1888, 37, 159; Curtius and Koch, ibid., 1888, 38, 487.)

DL-Alanine (104g.) was suspended in dry ethanol (500c.c.), the mixture saturated with hydrogen chloride and heated under reflux for two hours. The hydrochloride separated quantitatively as colourless needles, m.p. 64-68°.

Glycine ethyl ester hydrochloride.

(Curtius and Goebel, ibid., 37, 433.)

Glycine (50g.) was suspended in dry ethanol (200c.c.), the mixture saturated with hydrogen chloride and heated under reflux for two hours. The hydrochloride separated quantitatively as colourless needles m.p. 144°.

Ethyl 2-bromopropionamido-acetate.

2-Bromopropionyl bromide (80g.; 1 mol.) was added dropwise and with stirring to glycine ethyl ester hydrochloride (58g.; 1.14 mols.) suspended in dry chloroform (600c.c.) and surrounded by an ice-bath. N-Methyl morpholine (61.5g.; 2 mols.) diluted with dry chloroform (40c.c.) was added dropwise over one hour. The ice-bath was removed and stirring

continued for one hour. The chloroform solution was washed with water, normal hydrochloric acid, twice normal sodium carbonate solutionand water (100c.c. each) and the solution dried over calcium chloride. The chloroform was removed under reduced pressure to give a light-brown oil which on chilling at 0° for 16 hours gave long colourless needles (35.8g. or 37.4% of theory), m.p. 40-43°. After four recrystallisations from light petroleum (b.p. 40-60°) the ester separated as long colourless silky needles, m.p. 49-50°.

Found: C, 35.5 ; H, 5.1 ; N, 6.1%.

$C_7H_{12}O_3NBr$ requires C, 35.3 ; H, 5.0 ; N, 6.2%.

Chloro-acetyl-DL-alanine ethyl ester.

(cf. Fischer, E. and Otto, Ber., 1903, 36, 2113.)

Freshly distilled chloro-acetyl chloride (98g.) was condensed with DL-alanine ethyl ester hydrochloride (152g.) to give chloro-acetyl-DL-alanine ethyl ester (93.6g.or 56% of theory) in the manner described above for ethyl-2-bromo-propionamido-acetate. The ester separated from ether-light petroleum (b.p. 40-60°) as colourless needles, m.p. 40-41°.

2:5-Diketo-3-methylpiperazine.

a) (Fischer, E and Otto, loc.cit.)

Chloro-acetyl-DL-alanine ethyl ester (20g.) was heated with alcoholic ammonia (200c.c.) at 100° for four

hours. The mixture was filtered and the residue extracted with acetone (250c.c.), the acetone removed by distillation and the residue crystallised fromalcohol to give colourless needles (3.5g. or 26% of theory). After four such recrystallisations the diketopiperazine had m.p. 244-245°. It was not possible to work up the mother liquor due to the amount of charring that had occurred.

Found: C, 47.0 ; H, 6.0%.

Calc. for $C_5H_8O_2N_2$: C, 46.9 ; H, 6.3%.

b) Chloro-acetyl-DL-alanine ethyl ester (20g.) was dissolved in dry ethanol (200c.c.) and saturated with ammonia at 0°. The solution was maintained at room temperature for three days, by which time the diketopiperazine had precipitated quantitatively as colourless needles, m.p. 233-234°. After four crystallisations from ethanol the anhydride separated as colourless needles, m.p. 244-245° undepressed on admixture with specimen described in (a).

Found: C, 47.1 ; H, 6.3%.

Calc. for $C_5H_8O_2N_2$: C, 46.9 ; H, 6.3%.

c) Ethyl-2-bromopropionamido-acetate (10g.) was heated with alcoholic ammonia (150c.c.) at 130° for four hours. The ammonia and alcohol were removed under reduced pressure and the residue extracted with acetone (300c.c.). After removal of the acetone, the product was crystallised from ethanol to give small colourless needles (2.5g. or 45% of theory), m.p.

233-234°. After four such recrystallisations the product had m.p. 244-245° undepressed on admixture with the specimen described in (a).

Found: C, 46.9 ; H, 6.2 ; N, 22.2%.

Calc. for $C_5H_8O_2N_2$: C, 46.9 ; H, 6.3 ; N, 21.9%.

Action of phosphoryl chloride on 2:5-diketo-3-methylpiperazine.

2:5-Diketo-3-methylpiperazine (5g.) was heated at 75° for 10 minutes with phosphoryl chloride (50c.c.). The excess phosphoryl chloride was removed under reduced pressure at as low a temperature as possible and the residue triturated with ice-water. Extraction of the mixture with ether, before and after neutralisation with sodium carbonate, yielded only small quantities of black intractable material. This was the case, irrespective of the method of preparation of the diketopiperazine. Similar results were obtained when glycine anhydride was treated with phosphoryl chloride under these and other conditions.

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