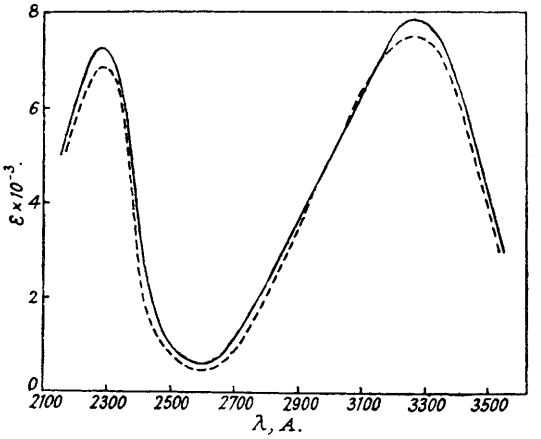
545. *Synthesis of Flavacol, a Metabolic Product of* Aspergillus flavus.

By George Dunn, G. T. Newbold, and F. S. Spring.

Flavacol, obtained together with aspergillic acid from culture filtrates of *Aspergillus flavus*, is shown by synthesis to be 3-*hydroxy*-2 : 5-*di*iso*butylpyrazine* (I).

Culture filtrates of the mould *Aspergillus flavus* grown on a medium containing a casein hydrolysate and sodium chloride give a crude acidic product separable into two components, one of which, aspergillic acid, is soluble in sodium hydrogen carbonate solution; the other, which we now name *flavacol*, $C_{12}H_{20}ON_2$, is soluble in sodium hydroxide solution but insoluble in sodium hydrogen carbonate solution. Flavacol does not give a coloration with ferric chloride and in its general properties, particularly its ultra-violet absorption spectrum, it resembles the isomeric

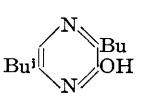


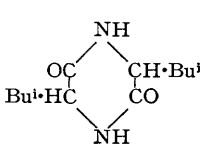
——— *Flavacol.* – – – – 3-*Hydroxy*-2 : 5-*di*iso*butylpyrazine.*

deoxyaspergillic acid and 3-hydroxy-2 : 5-di-*sec.*-butylpyrazine. It is, however, not identical with either of these two compounds nor is it identical with racemic deoxyaspergillic acid (Dunn, Gallagher, Newbold, and Spring, this vol., p. S 126).

Flavacol has now been identified by synthesis as 3-*hydroxy*-2 : 5-*di*iso*butylpyrazine* (I). The method selected for this synthesis was that developed by Baxter and Spring (*J.*, 1947, 1179) in which a diketopiperazine is treated with phosphoryl chloride to yield a 2-chloropyrazine (together with a 2 : 5-dichloropyrazine), treatment of which with alkali, or better with sodium ethoxide followed by reaction of the 2-ethoxypyrazine with mineral acid, yields the required 2-hydroxypyrazine.

Treatment of dl-leucine anhydride (II) with phosphoryl chloride gives a mixture of products from which 3-hydroxy-2 : 5-di*iso*butylpyrazine (I) was directly isolated. It is identical with flavacol. The direct dehydration of a diketo-piperazine to a hydroxy-pyrazine has now been observed in three different cases: reaction of dl-phenylglycine anhydride with phosphoryl chloride gives 3-hydroxy-2 : 5-diphenylpyrazine (Gallagher, Newbold, Spring, and Woods, this vol., p. 910), and similar treatment of dl-leucyl-dl-*iso*leucine anhydride yields an hydroxy-2-*iso*butyl-5-*sec.*-butylpyrazine (Gallagher, Newbold, and Spring, unpublished observation).

(I.) 



(II.)

Treatment of dl-leucine anhydride with phosphoryl chloride gives, in addition to 3-hydroxy-2 : 5-di*iso*butylpyrazine, a mixture of chloropyrazine derivatives, probably 3-chloro-2 : 5-di*iso*butylpyrazine and 3 : 6-dichloro-2 : 5-di*iso*butylpyrazine, which could not be separated directly. The presence of the monochloro-pyrazine was shown by reaction of the mixture with

sodium ethoxide followed by hydrolysis of the product with mineral acid, 3-hydroxy-2 : 5-di*iso*butylpyrazine being obtained.

Experimental.

DL-*Leucine Anhydride.*—DL-Leucine (30 g.) was heated under reflux with ethylene glycol (180 c.c.), and after 20 minutes solution was complete. Heating was continued for 70 minutes and the mixture then kept at 0° for 24 hours. The solid was separated off, washed with cold water (2 × 150 c.c.) and ethanol (2 × 150 c.c.) and dried (P_2O_5) (yield, 14·6 g.). Crystallisation from ethanol gave DL-leucine anhydride as needles, m. p. 267—270° (sealed tube) (Found: C, 63·5; H, 9·7; N, 12·7. Calc. for $C_{12}H_{22}O_2N_2$: C, 63·7; H, 9·7; N, 12·4%). Fischer (*Ber.*, 1901, **34**, 433) gave m. p. 271° (corr.) for DL-leucine anhydride prepared from the ethyl ester of DL-leucine.

3-*Hydroxy*-2 : 5-*di*i*so*butylpyrazine.—DL-Leucine anhydride (12 g.) was heated under reflux for one hour with phosphoryl chloride (120 c.c.), the mixture was evaporated, the residue treated with water (100 c.c.), and the solution neutralised (litmus) by the addition of 2N-sodium hydroxide. The solution was extracted with ether (6 × 150 c.c.), the extract dried, and the ether evaporated. The residue partly crystallised; the solid was separated (filtrate A) and washed with small portions of cold ether (yield, 2·15 g.). Recrystallisation from ethanol yielded 3-*hydroxy*-2 : 5-*di*i*so*butylpyrazine as large needles, m. p. 144·5—147° (Found: C, 69·4; H, 9·7; N, 13·7. $C_{12}H_{20}ON_2$ requires C, 69·2; H, 9·6; N, 13·5%). A mixture with flavacol (m. p. 144—146·5°) had m. p. 144—146·5°. 3-Hydroxy-2 : 5-di*iso*butylpyrazine is insoluble in water and in sodium hydrogen carbonate solution but is soluble in 3N-hydrochloric acid and in 3N-sodium hydroxide. It does not give a coloration with aqueous ferric chloride and it sublimes readily at 120°/10^{-2} mm. Its ultra-violet absorption spectrum in ethanol together with that of flavacol is shown in the figure.

Distillation of filtrate A at 8 mm. gave a pale yellow oil (5·3 g.), redistillation of which gave a colourless oil, b. p. 115—119°/3—4 mm. (Found: C, 59·7; H, 7·6; N, 11·0; Cl, 21·15. $C_{12}H_{19}N_2Cl$ requires C, 63·6; H, 8·4; N, 12·4; Cl, 15·7. $C_{12}H_{18}N_2Cl_2$ requires C, 55·2; H, 6·9; N, 10·7; Cl, 27·2%). Attempts to separate the mixture by means of concentrated hydrochloric acid, a method which leads to the separation of 3-chloro-2 : 5-di-*sec.*-butylpyrazine from 3 : 6-dichloro-2 : 5-di-*sec.*-butylpyrazine (Baxter and Spring, *loc. cit.*), were unsuccessful. The oil, b. p. 115—119°/3—4 mm. (1·5 g.), was heated in an autoclave at 180° for 4 hours with ethanolic sodium ethoxide (from 0·6 g. of sodium and 25 c.c. of ethanol). The mixture was diluted with water and extracted with ether, the extract was evaporated, and the residue heated under reflux with 6N-hydrochloric acid (15 c.c.) for 24 hours. The solution was diluted with water (15 c.c.) and neutralised (litmus) with 3N-sodium hydroxide. The solid which separated (250 mg.) was sublimed in a high vacuum and the sublimate crystallised from aqueous ethanol; 3-hydroxy-2 : 5-di*iso*butylpyrazine separated as needles, m. p. 147—149° undepressed when mixed with the specimen described above.

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