398. *The Isolation of Festuclavine and Two New Clavine Alkaloids from* Aspergillus fumigatus *Fres.*

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Festuclavine, previously isolated from saprophytic cultures of strains of ergot (*Claviceps*) found on certain grasses, has been found in cultures of *Aspergillus fumigatus* Fres. Two new alkaloids, also of the clavine type, have been isolated and characterised, and for them the names fumigaclavine A and B are proposed. Fumigaclavine B, which is deacetylfumigaclavine A, has also been found in *Rhizopus arrhizus* Fischer.

In an investigation on the growth of certain fungi in artificial culture it was decided to examine them for the presence of alkaloids (in the sense of basic materials obtained by solvent-extraction of the basified culture).

As a routine procedure the mycelium was macerated with 2% tartaric acid in 3 : 7 aqueous acetone. After filtration, the acetone was removed under reduced pressure, and the aqueous concentrate was extracted with ether to remove pigments and fats, then made alkaline with aqueous ammonia, and again extracted with ether. Basic material was removed from the ether solution with 0·5N-aqueous hydrochloric acid. These acid extracts were then tested for alkaloids with the usual reagents. Indoles were detected with dimethylaminobenzaldehyde either in a test tube according to the procedure of Allport and Cocking [1] or on filter paper by spraying with Ehrlich's reagent. The results of these tests are summarised in Table 1.

Interest particularly centred around the production of indole alkaloids since these have hitherto only been reported in species of *Claviceps* and in naturally produced *Ustilago zeae*.[2] Whilst the *Phycomyces* gave a barely detectable amount of alkaloid and the *Rhizopus arrhizus* only sufficient subsequently to be identified by paper chromatography as fumigaclavine B, the yield from *Aspergillus fumigatus* (W.R.L. Culture No. CN 1740, originally isolated from a case of mycosis in an osprey) was sufficiently high to suggest that the isolation and characterisation of its alkaloids would be practicable.

[1] Allport and Cocking, *Quart. J. Pharm. Pharmacol.*, 1932, **5**, 341.
[2] Mas, *Bol. Soc. quím. Peru*, 1938, **4**, 3.

TABLE 1. *Alkaloid tests on fungal mycelia.*

Reagents: M = Mayer's; W = Wagner's; D = Dragendorff's; S = Sonnenschein's; STA = silicotungstic acid; E = Ehrlich's.

	Reagents					
	M	W	D	S	STA	E
Phycomycetes						
Absidia corymbifera (Cohn) Sacc.	+	++	++	+	+	—
Absidia ramosa (Lindt) Lendner	+	++	++	+	+	—
Mortierella pusilla Oudem.	+	++	++	+	+	—
Mucor pusillus Lindt	+	+	+	+	+	—
Mucor ramannianus Möller	+	+-+	++	+	—	—
Phycomyces nitens (Agardh) Kunze	+	++	+-+	+	+	*
Rhizopus arrhizus Fischer	+	++	+-	+	+	*
Rhizopus nigricans Ehrenb.	-++	-+++	-+++	-+++	+	*
Syncephelastrum racemosum (Cohn) Schroet.	+	+-+	++	++	+	—
Ascomycetes						
Claviceps paspali Stev. et Hall	+	+	+	+	+	—
Cordyceps ophioglossoides Link	—	—	+	—	—	—
Martinia panamaensis Whetzel	+	+	+	+	+	*
Basidiomycetes						
Flammulina velutipes (Curr. ex Fr.) Karst.	+	+	+	+	+	—
Gymnopilus junonius (Fr.) Orton	+	+	+	+	+	—
Piptoporus betulinus (Bull.) Karst.	+	+	+	+	+	—
Pleurotus cornucopiae (Paulet ex Pers.) Rolland	+	+	+	+	+	—
Pleurotus lignatilis (Pers. ex Fr.) Kummer	+	+	+	+	+	—
Polyporellus squamosus (Huds.) Karst.	+	+	+	+	+	—
Ustilago perennans Rostr.	+	+	+	+	—	—
Ustilago zeae (Beckm.) Unger	+	+	+	+	—	—
Fungi imperfecti						
Acrostalagmus cinnabarinus Cda.	+	++	++	+	+	—
Aleurisma carnis (Brooks et Hansford) Bisby	—	+	+	+	—	—
Aspergillus fischeri Wehmer	++	++	++	++	++	—
Aspergillus flavus Link	++	++	++	++	+	—
Aspergillus fumigatus Fres.	+++	+++	+++	+++	+++	†
Aspergillus niger van Tiegh.	—	+	+	+	—	—
Aspergillus ochraceus Wilhelm	+	++	++	+	+	—
Aspergillus ruber Bremer	+	++	++	++	++	†
Aspergillus sulphureus (Fres.) Thom et Church	—	+	—	—	—	—
Aspergillus ustus (Bain.) Thom et Church	++	++	++	++	+	—
Aspergillus versicolor (Vuill.) Tiraboschi	++	++	++	++	+	—
Calcarisporium arbuscula Preuss	+	++	++	++	—	—
Colletotrichum atramentarium (B. et Br.) Taub.	++	++	+	+	+	—
Colletotrichum biologicum Chaudhuri	++	++	++	++	+	—
Colletotrichum fuscum Laubert	+	++	++	+	+	—
Colletotrichum solanicolum O'Gara	++	++	++	++	+	—
Coniothyrium concentricum (Desm.) Sacc.	—	+	+	+	—	—
Epicoccum nigrum Link	+	++	++	+	+	—
Gliocladium deliquescens Sopp	+	++	++	+	+	—
Phoma herbarum Westend.	+	+	+	+	—	—
Penicillium avellaneum Thom et Church	+	+	+	+	+	—
Penicillium brefeldianum Dodge	++	++	++	++	++	—
Penicillium lilacinum Thom	+	++	++	+	+	—
Penicillium thomii Maire	+	++	++	++	+	—
Scopulariopsis brevicaulis (Sacc.) Bain.	+	+	++	+	+	—
Sepedonium chrysospermum (Bull.) Fr.	+	++	++	+	—	—
Stemphylium asperulum Sacc.	+	++	++	++	+	—
Trichoderma viride Pers. ex Fr.	+	++	++	+	—	—
Trichophyton schoenleini (Leb.) Lang. et Milochev.	+	++	++	+	—	—
Trichothecium roseum Link ex Fr.	+	++	++	+	+	—

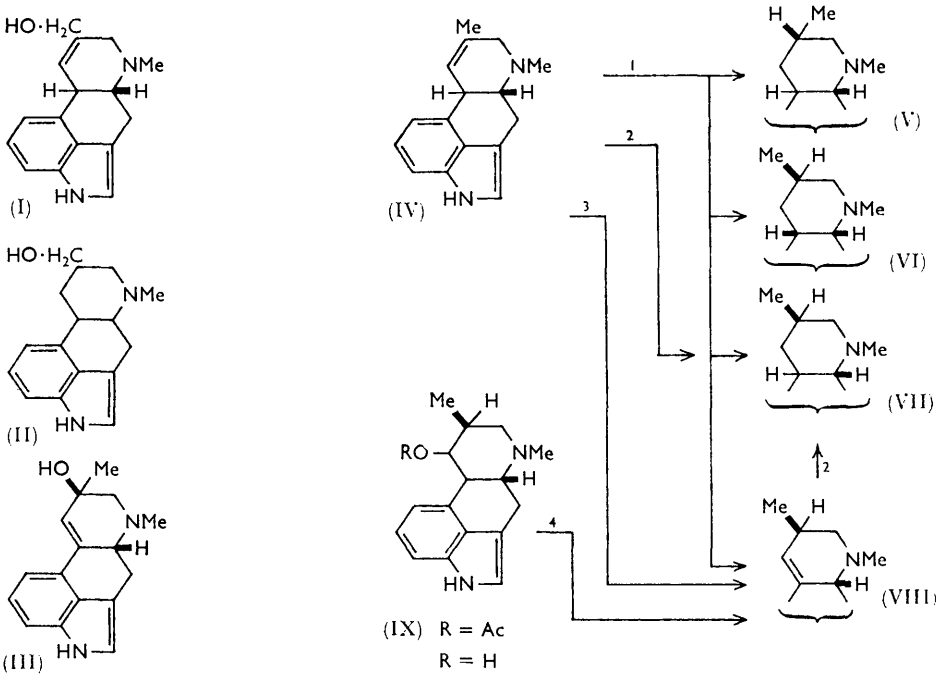
* Pale blue. † Blue.

A malt extract enriched with lactose and asparagine was the medium chosen and the best results were obtained with heavily sporulating cultures as the inoculum; floccose vegetative growth tended to produce lower yields. Preliminary chemical assays of the total alkaloids extracted by chloroform from the cultures, based on the colorimetric estimation of the alkaloids of ergot with dimethylaminobenzaldehyde,[1] showed the alkaloids were present both in the mycelium and in the medium and that maximum production of the order of 150 mg. per l. of total culture was reached after approximately 60 days. The yield was slightly improved by addition of calcium carbonate (0·1%), but addition of tryptophan, apart from stimulating growth in the early stages, had no significant effect. Because of the pathogenicity of the organism, cultures were killed by steaming before extraction, which proved to have an advantage over the use of formaldehyde, as the spores were wetted and their dispersal on harvesting was avoided; also a higher proportion of alkaloid was released into the medium.

It was later found possible to produce the alkaloids in cultures of the fungus grown on sterilised and aerated rye grains. From 1·5 l. of grain 315 mg. of total alkaloids were isolated.

The presence of gliotoxin, a known indole-disulphide metabolite of *A. fumigatus*,[3] could not be demonstrated, but free sulphur was isolated during the extraction.

Paper chromatography was used extensively; the R_F (centre) values quoted, unless



Reagents: 1, Na–BuOH. 2 H_2–PtO_2. 3, Hot NaOBu. 4, Soda-lime.

otherwise stated, refer to descending techniques on Whatman No. 1 paper with the solvent system, sodium chloride (8%) in aqueous acetic acid (2%), and a ten-inch distance of travel of the solvent front. Thus, the crude total alkaloids were found to contain a major component, of R_F 0·62 (fumigaclavine A), and two minor components, of R_F 0·51 (fumigaclavine B) and 0·36 (festuclavine) in the approximate proportions 10 : 0·5 : 1 respectively.

[3] Kidd, *Science*, 1947, **105**, 511; Menzel, Wintersteiner, and Hoogerheide, *J. Biol. Chem.*, 1944, **152**, 419.

A number of yellow, highly fluorescent spots were also found. The three alkaloids were separated by chromatography on alumina washed with acetic acid.

Fumigaclavine A, $C_{18}H_{22}N_2O_2$, gave an intense blue colour with Allport and Cocking's reagent,[1] similar to that given by the alkaloids of ergot, suggesting that the 2-position of the indole nucleus was unsubstituted. An acetyl group was detected and mild alkaline hydrolysis gave deacetylfumigaclavine A, $C_{16}H_{20}N_2O$, identical with fumigaclavine B. Acetylation of fumigaclavine B gave fumigaclavine A, indicating that rearrangement had not occurred during hydrolysis. The easy deacetylation, and strong infrared bands at 1725 and 1241 cm.$^{-1}$ indicated an ester. Fumigaclavine B had one *C*-Me and one *N*-Me group, and the absence of a band at 310 mμ indicated that there was no double bond conjugated with the indole nucleus. Prolonged hydrogenation (Adams catalyst) resulted in the absorption of ~4 mol. of hydrogen.

The sclerotia as well as saprophytic cultures of *Agropyrum*-type ergot fungus parasitic on *Agropyrum semicostatum* Nees, *Trisetum bifidum* Ohwi, *Festuca rubra* L., etc., found growing in Japan, yielded elymoclavine (I), agroclavine (IV), secaclavine [4] (alkaloid X) (II), festuclavine [4] (alkaloid Y) (VII), and, later, pyroclavine (alkaloid Z) (V), costaclavine (alkaloid U) (VI), and isosetoclavine (alkaloid V) (III).[5]

The identity of the festuclavine isolated was proved by analysis and comparison of the physical properties (see Table 2), including ultraviolet and infrared spectra, with those of a sample prepared by hydrogenation of agroclavine.[6,7]

Yamatodani and Abe [8] had shown that reduction of agroclavine (IV) with sodium in butan-1-ol gave, in addition to pyroclavine (V), costaclavine (VI) and festuclavine (VII), an isomer of agroclavine designated lysergine (VIII) which was also obtained, in better yield, by heating agroclavine with sodium butoxide. The change in the ultraviolet spectra (figures not quoted), which now resembled that of lysergic acid, indicated that the double bond had migrated to the position in which it was conjugated with the benzene ring of the indole nucleus (see Table 3).

TABLE 2. *Properties of alkaloids.*

	R_F	M. p.	$[\alpha]_{5461}$ (pyridine)	On paper u.v.	On paper Ehrlich	$\lambda_{max.}$ (mμ)	log ε
Fumigaclavine A	0·62	84—85°	−56·7° *	Blue	Blue		
Fumigaclavine B	0·51	244—245	−113	Blue	Blue	225, 275, 282, 293	4·49, 3·79, 3·82, 3·72
Festuclavine isolated	0·36	238—239	−128	None	Blue	224, 276, 281, —	4·53, 3·81, 3·84, —
,, from agroclavine †	0·36	238—239	−125·3	None	Blue	224, 276, 281, —	4·54, 3·84, 3·86, —
	—	242	−129	None	Blue	225, 275, 282, 292	— — — —
,, from fumigaclavine B	0·36	238—239	−121	None	Blue	224, 276, 281, —	4·54, 3·82, 3·84, —
Pyroclavine	—	204	−105	None	Blue	225, 275, 282, 292	— — — —
Costaclavine [5]	—	182	+59	None	Blue	225, 275, 282, 292	— — — —
Lysergine [5]	—	265—267	+99	Blue	Blue	— — — —	— — — —
Anhydrofumigaclavine B (= lysergine)	0·25	266—267	+98	Blue	Blue	226, 239, 310, —	4·23, 4·20, 3·49, —

* Hydrochloride in MeOH. † Figures in the second row are from refs. 4 and 5.

Heating fumigaclavine B with soda-lime caused dehydration and the ultraviolet spectrum of the product, $C_{16}H_{18}N_2$, showed that a conjugated double bond had been introduced. Further, the physical constants agreed with those recorded for lysergine (see Table 2). Anhydrofumigaclavine B and lysergine are therefore identical. Further proof was obtained, when, as reported by Yamatodani and Abe [8] for lysergine, anhydrofumigaclavine B on catalytic hydrogenation gave festuclavine (VII).

[4] Abe and Yamatodani, *J. Agric. Chem. Soc. Japan*, 1954, **28**, 501.
[5] Abe, Yamatodani, Yamano, and Kusumoto, *Bull. Agric. Chem. Soc. Japan*, 1956, **20**, 59.
[6] Yamatodani and Abe, *Bull. Agric. Chem. Soc. Japan*, 1955, **19**, 94.
[7] Abe, *Ann. Rep. Takeda Res. Lab.*, 1951, **10**, 145.
[8] Yamatodani and Abe, *Bull. Agric. Chem. Soc. Japan*, 1956, **20**, 95.

TABLE 3. *Ultraviolet absorption bands.*

	$\lambda_{max.}$ (mμ)				log ε				Ref.
Agroclavine (IV)	225	—	284	293	3·88	—	3·88	3·81	9
Fumigaclavine B (IX; R = H)	225	275	282	293	4·49	3·79	3·82	3·72	
Anhydrofumigaclavine B (lysergine) (VIII)	226	239	—	310	4·23	4·20	—	3·49	
Setoclavine (isomer of III)	—	243	—	317	—	4·38	—	4·04	*a*
	226	242	—	315	—	—	—	—	5
Lysergic acid (VIII; CO_2H in place of Me)	—	242	—	317	—	—	—	—	*a*

[a] Stoll, Brack, Kobel, Hofmann, and Brunner, *Helv. Chim. Acta*, 1954, **37**, 1815.

The ease of acetylation of fumigaclavine B and the fact that the tertiary hydroxyl group in isosetoclavine (III) is not acetylated,[9] in conjunction with the other evidence described, suggest that the hydroxyl group in fumigaclavine B is secondary and the formulæ for fumigaclavine A and B are therefore (IX; R = Ac and H, respectively).

EXPERIMENTAL

Alumina used for chromatography was supplied by Savory and Moore and deactivated with 10% aqueous acetic acid. Ultraviolet spectra were determined for EtOH solutions with a Hilger Uvispek, and infrared spectra for potassium chloride discs with a Unicam S.P. 100 spectrophotometer.

Growth of the Fungus.—Malt extract was the basic medium used. The most satisfactory one produced was " Kepler " malt extract (10%) enriched with lactose (5%) and asparagine (0·5%). The pH was adjusted to 5·8 and the medium heated in an autoclave for 30 min. at 15 lb./sq. in. Production was carried out in Thomson culture flasks with an average of 250 ml. of medium per flask. Inoculation was carried out with a heavy spore suspension of *A. fumigatus* produced on malt agar and incubated at 23°. Maximum alkaloid concentration was found after 60 days' incubation. The cultures were killed either by the addition of formaldehyde (2%) or by steaming for 1 hr.

Extraction of the Alkaloids.—The mycelium from a total of 2875 flasks of culture was separated in a basket centrifuge, lined with a linen bag, coated with a thin layer of Hyflo supercel to retain the spores. The filtrate was conveniently processed in batches of 75 l. and the mycelium from each such batch extracted separately.

The filtrate (75 l.) was brought to pH 10 with aqueous ammonia (*d* 0·88) and stirred for 6 hr. in a stainless-steel tank with chloroform (75 l.). Some emulsification occurred which slowly dispersed. The aqueous layer was withdrawn and discarded. A second batch of filtrate was introduced, basified, and extracted with the same chloroform solution. After two such extractions the chloroform was replaced with fresh solvent.

The mycelium from 75 l. of culture was macerated with chloroform in a Waring blender and stirred in a stainless-steel tank with chloroform (total, 50 l.) whilst aqueous ammonia (*d* 0·88; 200 ml.) was added. After 6 hr. the mycelium was separated on a centrifuge, returned to the tank, and again stirred with chloroform (50 l.) and aqueous ammonia (200 ml.). This second chloroform extract was used for the next batch of mycelium.

The combined chloroform extracts from the mycelium and filtrate were concentrated under reduced pressure in a circulating-still to a volume of 10 l., then filtered through a thin layer of filter-aid, and the chloroform was removed under reduced pressure. The thick oily residue was dissolved in dry ether (3 l.) and filtered, and a 5% solution of anhydrous oxalic acid in acetone added until precipitation was complete. The oxalate was filtered off, washed with ether, and dried (yield, 55·9 g.). The filtrate slowly deposited sulphur.

A solution of the oxalate (55·9 g.) in water (250 ml.) was filtered, then basified with aqueous ammonia, and the precipitate filtered off, washed with water, and dried (40·2 g.). Extraction of the filtrate with chloroform gave a highly pigmented sticky solid (0·5 g.).

Fractionation of the Alkaloids.—The crude base (20 g.) was passed in chloroform–benzene (1 : 2) down a column of alumina (5 × 25 cm.). The chromatogram was developed with the same solvent mixture and fractions (10 ml.) were collected and examined by paper chromatography, with the following results: 1—10, R_F 0·62 (fumigaclavine A); 11—47 fumigaclavine A + trace R_F 0·51 (fumigaclavine B); 48—76 fumigaclavine B + trace of fumigaclavine A; 80—176 R_F 0·36, festuclavine.

[9] Hofmann, Brunner, Kobel, and Brack, *Helv. Chim. Acta*, 1957, **40**, 1358.

Fractions 11—47 were evaporated and the residue was dissolved in the same solvent mixture and passed down an alumina column (4 × 15 cm.), giving fractions (10 ml.): 1—16 fumigaclavine A; 16—20 fumigaclavine A + trace of fumigaclavine B; 21—50 fumigaclavine B.

Fractions 48—76 from the first and 16—20 from the second column were evaporated and the combined residues were fractionated as before on alumina (2 × 10 cm.), giving fractions (5 ml.): 1—10 fumigaclavine A; 15—20 fumigaclavine B.

Fumigaclavine A (IX; R = Ac) crystallised from aqueous methanol (charcoal) in colourless needles, m. p. 84—85° (12·4 g.) (Found: C, 72·1; H, 7·1; N, 9·2; Ac, 15·0. $C_{18}H_{22}N_2O_2$ requires C, 72·5; H, 7·4; N, 9·4; Ac, 14·8%). Its *hydrochloride* crystallised from ethanol in prisms, m. p. 304—305° (decomp.) (inserted at 300°), $[\alpha]_{5461}^{22}$ −56·7° (*c* 1·5 in MeOH) (Found: C, 64·3; H, 6·8; N, 8·6; Cl, 10·3; Ac, 12·5; NMe, 9·4; OMe, 0. $C_{18}H_{23}ClN_2O_2$ requires C, 64·6; H, 6·9; N, 8·4; Cl, 10·6; Ac, 12·9; NMe, 8·7%).

The hydrochloride, when crystallised from glacial acetic acid, gave *fumigaclavine A monohydrochloride monoacetate*, m. p. *ca.* 306° (decomp.) (Found: N, 7·4; Cl 9·3; Ac, 19·5. $C_{20}H_{27}ClN_2O_4$ requires N, 7·1; Cl, 9·0; Ac, 21·8%).

The *methiodide* formed hair-like crystals (from methanol), m. p. 266° (decomp.), $[\alpha]_{5461}^{22}$ −23·7° (*c* 0·3 in MeOH) (Found: C, 51·5; H, 5·9; N, 6·6. $C_{19}H_{25}IN_2O_2$ requires C, 51·8; H, 5·7; N, 6·4%).

Fumigaclavine B (IX; R = H). Fractions 21—50 from the second and 11—50 from the third column gave a resin (0·65 g.) which crystallised from aqueous ethanol in needles, m. p. 244—245° (at 260° the melt solidified and then remelted at 265—267°), $[\alpha]_{5461}^{22}$ −6·3° (*c* 1·2 in MeOH), −113° (*c* 0·6 in pyridine) (Found: C, 74·9; H, 7·9; N, 11·1. $C_{16}H_{20}N_2O$ requires C, 75·0; H, 7·9; N, 10·9%). The *methiodide* (from ethanol–ether) had m. p. 309—310° (decomp.) (Found: N, 7·1; I, 31·6. $C_{17}H_{23}IN_2O$ requires N, 7·0; I, 31·9%).

Festuclavine. The residue (0·89 g.) from fractions 80—176 of the first column crystallised from light petroleum (b. p. 60—80°)–ether in colourless needles, m. p. 238—239° (decomp.), and then sublimed at 150—160°/0·01 mm. as prisms, m. p. 238—239° (decomp.), $[\alpha]_{5461}^{22}$ −128° (*c* 0·4 in pyridine) (Found: C, 80·2; H, 8·4; N, 11·4. $C_{16}H_{20}N_2$ requires C, 80·0; H, 8·4; N, 11·7%).

Deacetylfumigaclavine A (*Fumigaclavine* B).—(*a*) Fumigaclavine A hydrochloride (0·1157 g.) was dissolved in a 5% solution (10 ml.) of potassium hydroxide in 80% aqueous ethanol. After 12 hr. $[\alpha]_{5461}^{22}$ had fallen from −80·4° to a steady value −6·9°. Re-acetylation of the product (acetic anhydride and pyridine on the steam-bath for 30 min.) and conversion into the hydrochloride gave fumigaclavine A hydrochloride, m. p. 304—305° (decomp.), $[\alpha]_{5461}^{22}$ −56·2° (*c* 0·5 in MeOH) (Found: C, 64·6; H, 6·8%). Racemisation had therefore not occurred during the alkaline hydrolysis.

(*b*) Fumigaclavine A (1·3 g.) was refluxed in methanolic 2N-potassium hydroxide (25 ml.) for 1 hr. Water (25 ml.) was added, the methanol evaporated under reduced pressure, and the crystalline residue filtered off and washed with water. Recrystallisation from aqueous methanol and then ethanol gave colourless needles, m. p. 244—245°, $[\alpha]_{5461}^{22}$ −113·4° (*c* 0·6 in pyridine), −7·0° (*c* 1 in MeOH) (Found: C, 75·3; H, 7·7; N, 10·6; OMe, 4·4%). The mixed m. p. with fumigaclavine B was not depresed and the R_F values (0·51) were identical. The methiodide crystallised from methanol and ether in prisms, m. p. 310—311° (decomp.) (Found: C, 51·3; H, 5·7; N, 6·9; I, 31·6%).

Anhydrodeacetylfumigaclavine A (*Lysergine*) (VIII).—Fumigaclavine B (0·5 g.) was intimately ground with soda-lime (3 g.), covered with soda-lime (1·5 g.), and heated at 260—270° in a slow stream of nitrogen, the gas evolved being passed through dilute hydrochloric acid. After 5 min. the reactants were sublimed at 150—160°/0·01 mm. Paper chromatography of the sublimate revealed a blue-fluorescent spot (R_F 0·25) (giving a blue colour with Ehrlich's reagent), a little unchanged fumigaclavine B (R_F 0·51), and a number of minor pigmented highly fluorescent spots. The solid was triturated with cold chloroform (2·5 ml.), and the residue, recrystallised from ethanol, sublimed at 160—165°/0·01 mm.; this gave prisms, m. p. 266—267°, $[\alpha]_{5461}^{22}$ +98° (*c* 1 in pyridine) (Found: C, 80·5; H, 7·6; N, 11·7. Calc. for $C_{16}H_{18}N_2$: C, 80·7; H, 7·6; N, 11·8%). The methiodide had m. p. 253—254° (from methanol and ether) (Found: C, 53·3; H, 5·9; N, 7·2. Calc. for $C_{17}H_{21}IN_2$: C, 53·7; H, 5·5; N, 7·4%). The contents of the acid trap were evaporated and the small amount of residue identified as methylamine hydrochloride by paper chromatography (Whatman No. 1 paper; butanol–acetic acid–water, 4 : 1 : 5).

Festuclavine (VII).—(a) *From agroclavine.* Agroclavine (0·476 g.) was hydrogenated in

glacial acetic acid (20 ml.) at atmospheric pressure over platinum oxide (0·1 g.) until 1 mol. of hydrogen (44 ml.) had been taken up. On Whatman No. 1 paper, with the usual solvent and a run of 17 cm., the following spots were detected (colours refer to those in ultraviolet light and with Ehrlich's reagent, respectively): R_F 0·40, trace of agroclavine (none, blue); R_F 0·43, major component (none, blue); R_F 0·65, trace (blue, blue). The catalyst was filtered off and the acetic acid evaporated under reduced pressure (nitrogen leak). The residue was dissolved in 1 : 2 chloroform–benzene and fractionated on alumina (2 × 15 cm.). Fractions (5 ml.) 1—10 contained pigment and the material of R_F 0·65 (0·09 g.). Fractions 15—25 gave only festuclavine, R_F 0·43 (0·15 g.), which crystallised from ethanol and then sublimed as prisms, m. p. 238—239°, $[\alpha]_{5461}^{22}$ −125·3° (*c* 0·6 in pyridine).

(b) *From anhydrodeacetylfumigaclavine A* (*lysergine*) (VIII).—Lysergine (0·1 g.) was hydrogenated in glacial acetic acid (10 ml.) over platinum oxide (0·1 g.) at atmospheric pressure until 1 mol. of hydrogen had been taken up. The catalyst was filtered off and the acetic acid evaporated under reduced pressure. The residue was passed in 1 : 2 chloroform–benzene through alumina (1 × 10 cm.). After the initial passage of a narrow blue-fluorescent band pure fractions of a component of R_F 0·43 were obtained. These were combined, evaporated, and sublimed at 150—155°/0·01 mm. to give prisms, m. p. 238—239°, $[\alpha]_{5461}^{22}$ −121° (*c* 0·38 in pyridine). The mixed m. p.s of the three samples of festuclavine were undepressed and the R_F values (0·41) were identical.

The authors thank Miss D. M. Land and Mr. R. Lovegrove for technical assistance, Dr. A. J. Everett for the measurements of the spectra, and Mr. P. R. W. Baker for the microanalyses.

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BECKENHAM, KENT. [*Received, November 11th*, 1960.]