79. *Studies in Relation to Biosynthesis. Part XXVII.* The Biosynthesis of Ergot Alkaloids.*

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New degradations of agroclavine (I; R = H) and elymoclavine (I; R = OH) have been developed which confirm the presence of a biogenetic isoprene unit in each alkaloid.

Radioactivity from [2-^{14}C]mevalonic lactone, [1-^{14}C]acetic acid, and [2-^{14}C]acetic acid is incorporated by a *Claviceps* species found on *Pennisetum typhoideum* into the ergot alkaloids agroclavine (I; R = H) and elymoclavine (I; R = OH). We have shown[1] that the labelling at $C_{(8)}$ and $C_{(17)}$ in the various radioactive samples is consistent with the

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* Part XXVI, *J.*, 1962, 370.

[1] Birch, McLoughlin, and Smith, *Tetrahedron Letters*, 1960, No. 1, 1.

presence of an isoprene unit located as shown in the dissection of structure (I). Similar incorporations had been recorded by Weygand and his colleagues,[2,3] but were not supported by degradative evidence. Since tryptophan has also been implicated[4] as a biosynthetic precursor of various ergot alkaloids, including the above, all of the skeletal atoms of the ergoline nucleus can be accounted for. We considered that our evidence for the part-terpenoid nature of the nucleus would be made virtually conclusive if we could isolate all of the radioactivity in agroclavine and elymoclavine derived from [2-^{14}C]-mevalonic lactone and show that it is located only at $C_{(7)}$ and $C_{(17)}$. Additionally, we wished to investigate the extent of randomisation of label between the 7- and the 17-position from this precursor. Theoretical considerations (*e.g.* ref. 5) indicate that only the 17-position [derived from the methyl group of isopentenyl pyrophosphate (III; $* = {}^{14}C$)] should carry a label unless labelling has been randomised during biological oxidation. This non-randomisation has been proved for the *gem*-dimethyl group of methylgeraniolene.[6] Our preliminary results[1] appeared to indicate a surprising degree of randomisation, and we felt that the conclusion should be checked by more reliable experimental procedures.

In order to isolate $C_{(7)}$, $C_{(8)}$, and $C_{(17)}$ as a unit we first split the 6,7-bond in each alkaloid by Emde-type fission of the methiodides by using sodium in liquid ammonia. The alkali metal–liquid ammonia fission of alkyl groups from nitrogen in quaternary ammonium salts has long been known,[7] and evidence has recently been obtained[8] that the alkyl group, depending on its structure, is cleaved as a radical or an anion through the addition of one or two electrons, respectively, from the metal. In either event we expected the 6,7- rather than the 5,6-linkage to be preferentially split by this reduction of agroclavine and elymoclavine methiodides because of the stabilisation of the intermediate radical or anion by allylic resonance. The fission product from agroclavine methiodide was characterised as a hydrochloride, $C_{17}H_{22}N_2,HCl$, identical in ultraviolet absorption spectrum with agroclavine and therefore containing an "isolated" indole chromophore. On ozonolysis in chloroform the free base gave acetone and must presumably have the expected structure (II; R = H). The ozonolysis product contained also some formaldehyde and acetaldehyde. The mixture of derivatives was separated by chromatography.[9]

The reductive fission of the methiodide of elymoclavine (I; R = OH) followed a similar course giving what is presumably the indole (II; R = OH) characterised as the hydrochloride which contained, as required, one new *C*-methyl group. It is notable that experimental completion of the bond fission requires the addition only of just over two atoms of sodium to the methiodide in liquid ammonia. Apparently, therefore, under these conditions elymoclavine methiodide preferentially undergoes carbon–nitrogen bond fission rather than salt formation on the indole nitrogen or alkoxide formation by, or allylic fission of, the $C_{(17)}$-hydroxyl group. The indole (II; R = OH) upon ozonolysis in chloroform gave an intractable product possibly because of the basic nitrogen present. Accordingly, the indole (II; R = OH) was ozonolysed as the methochloride. The product, after sodium borohydride reduction of the ozonide,[10] was apparently the expected propane-1,2-diol since oxidation with sodium metaperiodate[11] gave a mixture of formaldehyde and acetaldehyde isolated, respectively, as the dimedone derivative and the octahydroxanthone (IV)[12] (from the cyclodehydration of the acetaldehyde dimedone derivative).

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[2] Weygand, quoted in a discussion, *Angew. Chem.*, 1959, **71**, 1383.

[3] Gröger, Mothes, Siman, Floss, and Weygand, *Z. Naturforsch.*, 1959, **156**, 141; see also Taylor and Ramstad, *Nature*, 1960, **188**, 494.

[4] Taber and Vining, *Chem. and Ind.*, 1959, 1218; ref. 3 and references cited therein.

[5] Birch, Rickards, Smith, Harris, and Whalley, *Tetrahedron*, 1959, **7**, 241.

[6] Birch, Kocor, Sheppard, and Winter, unpublished work.

[7] Thompson and Cundall, *J.*, 1888, **53**, 761; Schlubach and Ballauf, *Ber.*, 1921, **54**, 2811; Clayson, *J.*, 1949, 2016.

[8] Grovenstein and Stevenson, *J. Amer. Chem. Soc.*, 1959, **81**, 4850; cf. Birch, *Quart. Rev.*, 1950, **4**, 69.

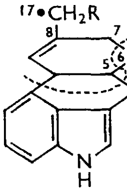
[9] Carson, *J. Amer. Chem. Soc.*, 1951, **73**, 4652.

[10] Sonsa and Bluhn, *J. Org. Chem.*, 1960, **25**, 108.

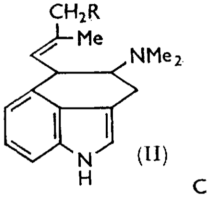
[11] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.

[12] Vorlander, *Z. analyt. Chem.*, 1929, **77**, 241, 321.

Degradation by the procedure above of [2-^{14}C]mevalonate-derived agroclavine (I; R = H) gave acetone (from $C_{(7)}$, $C_{(8)}$, and $C_{(17)}$) containing all of the radioactivity of the parent alkaloid. Kuhn–Roth oxidation of the alkaloid and degradation [13] of the resulting acetic acid through the Schmidt reaction gave carbon dioxide (from $C_{(8)}$) containing no radioactivity and methylamine (from $C_{(17)}$) containing 78% of the total activity. From our earlier degradations of the same sample, we had concluded that $C_{(17)}$ possessed only 31% of the total activity. In this case the Kuhn–Roth-derived acetic acid had been degraded, through pyrolysis of the lithium salt, to lithium carbonate and acetone.[14] Recent work in these laboratories has shown that this procedure may sometimes give anomalous results (cf. ref. 15) and we have abandoned its use. We therefore consider that these results should be disregarded, the present data being much more reliable.



(I)



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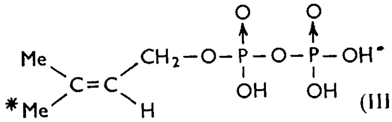

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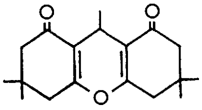

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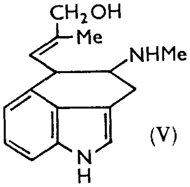
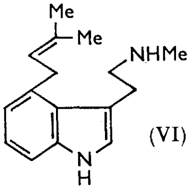


(V)



(VI)

Degradation of [2-^{14}C]mevalonate-derived elymoclavine (I; R = OH) as above gave formaldehyde (from $C_{(17)}$) and the octahydroxanthone (IV) which contained, respectively, 86% (from $C_{(17)}$) and 8·6% (from $C_{(8)}$) of the total activity, thus accounting for 94·6% of the total activity. Schmidt degradation of the acetic acid obtained by Kuhn–Roth degradation of the radioactive indole (II; R = OH) gave carbon dioxide (from $C_{(8)}$) and methylamine (from $C_{(7)}$) which contained, respectively, 0·3 and 4·2% of the total activity.

The results of the two degradations do not agree as quantitatively as might have been expected; it is probable that the dimedone reactions do not completely separate the formaldehyde and acetaldehyde derivatives. The general picture with both alkaloids is, however, quite clear. Essentially all of the radioactivity is distributed between the 8- and the 17-position, with the latter activity greatly preponderant as expected. The slight randomisation observed could have taken place during the biological oxidation stages necessary for the cyclisations.

The minor alkaloids [16,17] can be accounted for by slight modifications of the system (bond migration or reduction) except chanoclavine (secaclavine) (V) which may represent a biosynthetic precursor. It is a reasonable speculation that the most primitive precursor containing the complete skeleton is (VI) and the cyclisations may be brought about by allylic oxidation processes. The first of these is presumably that leading to the skeleton of (V) and must be stereospecific in view of lack of any marked randomisation of $C_{(7)}$ and $C_{(17)}$. Another fungal product, echinulin, is known which has been proved to contain two

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[13] Anker, " Methods in Enzymology," ed. Colowick and Kaplan, Academic Press, New York, Vol. IV, 1957, p. 805.

[14] Cornforth, Hunter, and Popjak, *Biochem. J.*, 1953, **54**, 597.

[15] Popjak, Hunter, and French, *Biochem. J.*, 1953, **54**, 238.

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

[17] Abe, Yamatodani, Yamano, and Kusumato, *Bull. Agric. Chem. Soc. Japan*, 1955, **19**, 92; Hofmann and Brunner, *Helv. Chim. Acta*, 1954, **37**, 1815; Semonsky, Beran, and Macek, *Coll. Czech. Chem. Comm.*, 1958, **23**, 1364.

"introduced" isoprene units on an indole ring,[18] although these are in the 5- and the 7-position.

EXPERIMENTAL

The general methods and equipment are the same as those used in previous parts of this series.

[^{14}C]Agroclavine and [^{14}C]Elymoclavine.—A *Claviceps* species was grown for 30 days as previously described [16] except that after 16 days [2-^{14}C]mevalonic lactone (150 μc to 2 l. of culture medium) was added. Working up in the known manner gave [^{14}C]agroclavine (200 mg.; 1·5 μc; 1%), m. p. 208—210°, and [^{14}C]elymoclavine (473 mg.; 2·1 μc; 1·4%), m. p. 228—230°.

Degradation of [^{14}C]Agroclavine.—(*i*) Kuhn–Roth oxidation of [^{14}C]agroclavine (r.m.a. $\times 10^{-3}$, 50·9) gave acetic acid (75% for 1 *C*-Me) which was converted [13] through the Schmidt reaction into barium carbonate (Me) (r.m.a. $\times 10^{-3}$, 39·6) and barium carbonate (CO_2H) (r.m.a., 0).

(*ii*) [^{14}C]Agroclavine (0·63 g.; r.m.a. $\times 10^{-3}$, 46·4) was added to methyl iodide (12 c.c.), the mixture was kept at room temperature with occasional swirling for 30 min., and the excess of methyl iodide was removed under reduced pressure. The residue, in anhydrous liquid ammonia, was treated with sodium (0·18 g.), which was added in small pieces with stirring during 10 min., the blue colour being allowed to discharge between each addition. On completion of the reaction, water (300 c.c.) was added and the mixture was extracted with ether (3 × 200 c.c.). The combined extracts were washed with water, dried, and the solvent was evaporated under reduced pressure in nitrogen. The amorphous residue (0·66 g.) had light absorption maxima at 224, 283·5, and 293·5 mμ. An aliquot part (0·1 g.) with 0·5N-hydrochloric acid (2 c.c.) gave a precipitate (0·101 g.), which was recrystallised from acetone to give N-*methyl*-6,7-seco*agroclavine hydrochloride*, m. p. 213—215° (decomp.) (Found: C, 70·15; H, 7·7. $C_{17}H_{23}ClN_2$ requires C, 70·2; H, 7·9%), identical in ultraviolet absorption spectrum with agroclavine. Ozonised oxygen was passed into a further portion (0·31 g.) in ethanol-free chloroform (15 c.c.) for 15 min. The solvent was evaporated under reduced pressure, water (100 c.c.) was added, and the mixture was steam-distilled. The distillate (500 c.c.) was collected in Brady's reagent. The dinitrophenylhydrazones were filtered off and the filtrate was extracted with chloroform. The chloroform extract after evaporation and trituration with chloroform (5 c.c.) gave 2,4-dinitrophenylhydrazine, the mother liquors from which contained a mixture of 2,4-dinitrophenylhydrazones which was combined with the precipitate obtained directly from the steam-distillate. The total mixture of 2,4-dinitrophenylhydrazones in benzene (10 c.c.) was adsorbed on silica gel (200 g.) (200—300 mesh) pretreated as described by Carson [9] except that ether was substituted for acetone when washing the adsorbent. Elution with hexane–ether (6 : 1) gave, successively, an unidentified oil, acetone 2,4-dinitrophenylhydrazone, a mixture shown by paper chromatography (stationary phase dimethylformamide; mobile phase decalin saturated with dimethylformamide) to contain acetaldehyde and formaldehyde 2,4-dinitrophenylhydrazones, and formaldehyde 2,4-dinitrophenylhydrazone. After recrystallisation from a small volume of methanol the acetone 2,4-dinitrophenylhydrazone (22 mg.) (r.m.a. $\times 10^{-3}$, 46·8) had m. p. 125—126°.

Degradation of [^{14}C]Elymoclavine.—[^{14}C]Elymoclavine (0·5 g.; r.m.a. $\times 10^{-3}$, 74·8) was refluxed for 1 hr. in methyl iodide (12·5 c.c.). The excess of methyl iodide was removed under reduced pressure and the residue in anhydrous liquid ammonia (500 c.c.) was treated with sodium (92 mg.), added in small pieces during 10 min. with stirring. Ammonium chloride and water (300 c.c.) were added and the mixture was worked up as before to give a residue (0·395 g.) which after recrystallisation from ether had m. p. 149—150°. A portion (0·1 g.) with 0·5N-hydrochloric acid (2 c.c.) gave a precipitate (15 mg.) which was recrystallised from acetone to give N-*methyl*-6,7-seco*elymoclavine hydrochloride*, m. p. 175—177° (Found: Cl, 11·7. $C_{17}H_{23}ClN_2O$ requires Cl, 11·6%). Kuhn–Roth oxidation of the fission product gave acetic acid (100% for 1 *C*-Me) which was degraded [13] to barium carbonate (Me) (r.m.a. $\times 10^{-3}$, 0·2) and barium carbonate (CO_2H) (r.m.a. $\times 10^{-3}$, 3·13). A further portion (0·133 g.) of the fission product was refluxed with methyl iodide for 3 hr. The residue from removal of the solvent was taken up in water (20 c.c.) and triturated with freshly prepared silver chloride (400 mg.) at 60° for 10 min. The solids were removed by filtration through "Norit," and the filtrate was evaporated under reduced pressure to a glass which was taken up in dry methanol (5 c.c.).

[18] Part XXIV, Birch, Blance, David, and Smith, *J.*, 1961, 3128; Casnati, Piozzi, Quilico, and Ricca, *Chimica e Industria*, 1961, 412.

A stream of ozonised oxygen was passed through this solution at 0° for 4 min., and sodium borohydride (190 mg.) in methanol was added. After 5 min. water was added and the solution freed from cations and borohydride by passage through sulphonated polystyrene (Amberlite IR 100, acid form). It was evaporated under reduced pressure to 5 c.c. and 3·6% aqueous sodium metaperiodate was added. After 5 min. the solution was treated successively with 2N-hydrochloric acid (3 c.c.), 1·2N-disodium hydrogen arsenite (2 c.c.), M-sodium acetate (2 c.c.), and 8% ethanolic dimedone (1 c.c.), and was heated on the steam-bath for 10 min. and set aside overnight. The precipitate was filtered off, washed with water, and refluxed for 6 hr. in glacial acetic acid.[12] Removal of the solvent under reduced pressure gave a residue which was shaken with ether and 5% aqueous sodium carbonate. Removal of the solvent from the ether layer gave a residue which was recrystallised from aqueous ethanol to give the octahydroxanthone (IV),[12] m. p. 175—178° (11 mg.; r.m.a. × 10^{-3}, 64·3). Acidification of the sodium carbonate solution gave a precipitate which was recrystallised from methanol to give formaldehyde dimedone derivative, m. p. 193—194° (18 mg.; r.m.a. × 10^{-3}, 6·4).

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