constant (368 cc.), the catalyst was filtered, washed with acetic acid and the filtrate evaporated under reduced pressure. The oily residue was heated with acetic anhydride (40 cc.) and pyridine (20 cc.) for 5 hr. on the steam-bath. Evaporation *in vacuo* with addition of toluene (repeated 5 times) yielded the diacetate **XL**, which was partially saponified with 0.324 g. of sodium hydroxide in methanol (60 cc.) by heating under reflux for 4 hr. and keeping for 10 hr. at room temperature. The methanol was removed, the residue was partitioned between ether and water and the yellowish aqueous phase extracted with ether. Evaporation of the washed and dried ether extract yielded a colorless gum (2.06 g.), the infrared spectrum of which exhibited a strong amide band at 6.15 μ and only a very small acetate band. A portion (1.9 g.) of the hydroxy amide **XLI** was left at room temperature for 4 days with 7.58 g. of *p*-toluenesulfonyl chloride and 125 cc. of dry pyridine and after heating for 5 hr. on the steam-bath, water was added cautiously and the product extracted with ether. Evaporation of the washed and dried ether solution yielded the tosylate XLII as a gum ($\lambda_{max}^{CHCl_3}$ 7.35 and 8.5 μ), which was dissolved in 200 cc. of dimethyl sulfoxide,[42] 20 cc. of pyridine and 100 cc. of benzene and distilled very slowly over a period of 48 hr. Dilution with water, ether extraction and chromatography on 175 g. of neutral alumina gave in the benzene–ether (3:1 and 1:1) fractions 0.96 g. of crystals (m.p. 136–140°) of the olefin **XXXIX**. Recrystallization from hexane or ethyl acetate furnished the analytical specimen, m.p. 144–145°, $[\alpha]_D$ −45° (*c* 1.10), $\lambda_{max}^{CHCl_3}$ 6.1 and 12.2 μ (trisubstituted double bond), n.m.r. signal at 306 c.p.s. (deuteriochloroform with added tetramethylsilane as internal standard) due to the single olefinic proton.

Anal. Calcd. for $C_{22}H_{35}NO$: C, 80.19; H, 10.71. Found: C, 80.37; H, 10.43.

Catalytic hydrogenation of 47 mg. of the olefin XXXIX in 10 cc. of acetic acid and 70 mg. of 10% palladized charcoal catalyst gave, after recrystallization from hexane, the saturated amide **XXXVIII**, m.p. 141–142°, $[\alpha]_D$ −55° (*c* 0.63). The melting point was not depressed upon admixture with its precursor XXXIX, but the infrared spectrum showed the complete disappearance of the 12.2 μ band; furthermore, gas phase chromatography (SE-30 column, 268°) readily separated a mixture of XXXIX, XXXVIII and XXXVII.

Anal. Calcd. for $C_{22}H_{35}NO$: C, 80.19; H, 10.71. Found: C, 80.37; H, 10.54.

[CONTRIBUTION FROM THE FACULTY OF PHARMACY, UNIVERSITY OF TORONTO, TORONTO, CAN.]

Biosynthesis of Ergot Alkaloids: Incorporation of Mevalonic Acid into Ergosine[1]

BY ROSS M. BAXTER, S. I. KANDEL AND A. OKANY

RECEIVED MARCH 16, 1962

The degradation of radioactive ergosine, isolated from a culture of *Claviceps purpurea*, PRL 1578, fed DL-mevalonic acid-2-C^{14}, revealed that almost all the activity was localized in the carboxyl carbon (C-17) of ergosine. This constitutes strong evidence that the C-2 of mevalonic acid becomes the C-17 of lysergic acid. The small percentage of radioactivity recovered from the C-7 was considered to indicate a possible randomization of the C-2 of mevalonate. The manner in which this may take place has been discussed.

Studies on the biosynthesis of ergot alkaloids in the saprophytic culture of *Claviceps purpurea* have revealed that the indole nucleus and carbons 4, 5, nitrogen 6 of ergoline originate from tryptophan[2] without prior conversion to the general metabolites, 5-hydroxytryptophan,[2d,e] tryptamine[3] or kynurenine[3] and that the carboxyl group is lost during the incorporation.[2a] Preliminary experiments[3] have shown that both formate and the S-methyl group of methionine are incorporated into clavine alkaloids. The larger percentage incorporation of the latter suggests that the N-methyl group of the clavine alkaloids arises from methionine *via* transmethylation. Evidence has been presented that carbons 7, 8, 9, 10[4] and the substituent at the carbon 8 of ergoline arise from mevalonic acid (3,5-dihydroxy-3-methylpentanoic acid) as was suggested earlier by Birch[5a] and Mothes.[5b]

If it is assumed that prior or subsequent to decarboxylation, mevalonic acid is incorporated as a unit,[4a,b] the C^{14} from DL-mevalonic acid-2-C^{14} should appear[6] at C-17 ($I \xrightarrow{a} II$) or at C-7 ($I \xrightarrow{b} II$) of the ergot alkaloids. The distribution of the 2-C^{14} of mevalonate between C-7 and C-17 might also occur to some degree. Degradation[4a] of agroclavine and elymoclavine isolated from mevalonic acid lactone-2-C^{14} fed cultures has indicated that about 30% of the radioactivity was present at the C-17 in agroclavine and about 90% in the case of elymoclavine. This would appear to suggest that different biosynthetic pathways exist for the two alkaloids which is unlikely in light of their closely related structures.[4d]

In the present work a partial degradation was devised whereby the C-7 and the C-17 of lysergic acid derived from ergosine were isolated individually as carbon dioxide. The radioactive ergosine (II) obtained from *Claviceps purpurea* PRL 1578 fed with DL-mevalonic acid-2-C^{14} was degraded by the sequence shown in Fig. 1. The first step was the conversion of ergosine (II) into D-lysergic acid

(1) A preliminary report of part of this work has appeared in *Tetrahedron Letters*, **No. 17**, 595 (1961). This investigation was supported in part by a grant from the National Research Council of Canada.

(2) (a) D. Groger, H. J. Wendt, K. Mothes and F. Weygand, *Z. Naturforsch.*, **14b**, 335 (1959); (b) W. A. Taber and L. G. Vining, *Chemistry & Industry*, 1218 (1959); (c) H. Plieninger, R. Fischer, W. Lwowski, A. Brack, H. Kobel and A. Hofmann, *Angew. Chem.*, **71**, 383 (1959); (d) R. M. Baxter, S. I. Kandel and A. Okany, *Chemistry & Industry*, 266 (1960); (e) H. Plieninger, R. Fischer, G. Keilich and H. D. Orth, *Liebigs Ann.*, **642**, 214 (1961).

(3) R. M. Baxter, S. I. Kandel and A. Okany, *Chemistry & Industry*, 1453 (1961).

(4) (a) A. J. Birch, D. J. McLoughlin and H. Smith, *Tetrahedron Letters*, **No. 7**, 1 (1960); (b) D. Groger, K. Mothes, H. Simon, H. G. Floss and F. Weygand, *Z. Naturforsch.*, **15b**, 141 (1960); (c) E. H. Taylor and E. Ramstad, *Nature*, **188**, 494 (1960); (d) subsequent to the preparation of our manuscript, a paper has been published reporting the finding of 78% of the incorporated radioactivity from mevalonic lactone-2-C^{14} at C-17 in agroclavine (S. Bhattacharji, *et al.*, *J. Chem. Soc.*, 421 (1962).

(5) (a) A. J. Birch in Ciba Foundation Symposium on "Amino Acids and Peptides with Antimetabolic Activity," G. E. W. Westenholme and C. M. O'Connor, Editors, J. and A. Churchill Ltd., London, 1958, p. 247; (b) K. Mothes, F. Weygand, D. Groger and H. Grisebach, *Z. Naturforsch.*, **13b**, 41 (1958).

(6) The carbon atom linked to C-8 of ergoline is denoted as C-18 according to Birch, *et al.*[4a]

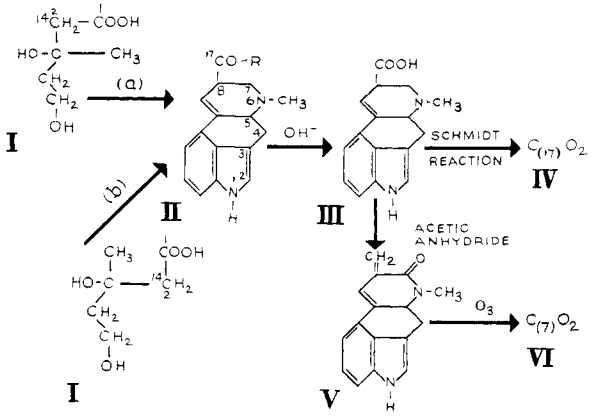

Fig. 1.—Degradation of the radioactive lysergic acid: R = peptide side chain of ergosine.

(III) by alkaline hydrolysis. The Schmidt reaction of lysergic acid (III) afforded the C-17 as carbon dioxide (IV). Another part of the lysergic acid (III) was converted into the lactam V by treatment with acetic anhydride. The lactam so obtained was subjected to ozonolysis and the formaldehyde so produced was oxidized yielding the C-7 as carbon dioxide (VI).

Experimental

Growth of *C. purpurea*, PRL 1578, and the Administration of the Tracer.—The preparation of the subcultures, inocula and the growth of *C. purpurea* were carried out in a manner similar to that described by Taber and Vining[7a,b] with the exception that the inoculum was not washed with saline. Mevalonic acid-2-C^{14}-N,N'-dibenzylethylene diamine salt[8] (200 μc.) was dissolved in water (20 ml.) and shaken with 10 ml. of Dowex 50-H ion exchange resin to liberate the free acid from its salt. A quantity of 1 ml. of the mevalonic acid solution (sterilized by filtration and containing approximately 10 μc.) was fed to 20-day old cultures (50 ml. of nutrient medium per culture, total 20 cultures). The cultures were stored in an incubator (25–27°) under stationary conditions.

Isolation of Ergosine.—The 60-day old cultures were homogenized in a Waring Blendor and lyophilized. The dry residue was treated with concentrated aqueous ammonia (1 ml. per flask) and was extracted with peroxide-free ether (30 ml. per flask). The ether extracts were combined and evaporated to 200–220 ml. in a nitrogen stream and shaken with 2% aqueous tartaric acid solution (7 × 20 ml.). The aqueous solution, made alkaline with concentrated aqueous ammonia, was shaken vigorously with peroxide-free ether (5 × 40 ml.). The ethereal solution was dried over anhydrous sodium sulfate and gradually evaporated to dryness by a nitrogen stream in a 15-ml. centrifuge tube. The residue was dissolved in a minimum of a 3:1 mixture of chloroform and isopropyl alcohol and chromatographed (at room temperature) on formamide impregnated Whatman No. I papers after a prior equilibration period of 6–8 hours with a solvent system of benzene–petroleum ether, b.p. 100–120°, 4:1.[9a,b] The alkaloids were detected by ultraviolet light and the spots corresponding to ergosine[10] were cut out of each sheet and eluted with ammoniacal peroxide-free ether (2 × 20 ml. each spot). The combined eluate was evaporated to dryness, 1.6 g. of unlabeled ergosine was added and the mixture was dissolved in acetone, filtered and reduced to dryness *in vacuo*.

Degradation of Ergosine. (a) **Hydrolysis.**—The crude diluted ergosine was hydrolyzed according to the procedure of Jacobs and Craig[11] and the lysergic acid (360 mg.) so obtained was recrystallized from water to constant specific activity (m.p. 231–234°, undepressed by admixture with authentic lysergic acid).

(b) **Formation of Lysergic Acid Lactam (V).**—The lactam was prepared from the lysergic acid by a method similar to that of Stoll, Hofmann, *et al.*[12] Lysergic acid (143 mg.) was added to 6 ml. of boiling, freshly distilled acetic anhydride. The reaction mixture was refluxed for 2 minutes whereupon the lysergic acid dissolved. The brownish solution was evaporated to dryness *in vacuo* and the traces of acetic anhydride were removed by storage overnight *in vacuo* over potassium hydroxide. The dry residue was taken up in 4 ml. of chloroform containing 0.5% ethanol. The filtered solution was chromatographed on a column of 10 g. of aluminum oxide (Brockmann). The blue fluorescent band observed with ultraviolet light was eluted with chloroform containing 0.5% absolute ethanol. The solution was reduced to a small volume and the yellow crystalline precipitate which formed was collected and recrystallized from methanol to constant specific activity (yield 30 mg.). The pure compound was identified by its ultraviolet spectrum.[12]

(c) **Ozonolysis (V → VI).**—Ozonized oxygen was passed through an ice-cooled solution of lactam (30 mg.) in aldehyde-free chloroform (20 ml.). After 30 minutes the dark brown reaction mixture was evaporated *in vacuo* and water (50 ml.) was added to the dry residue. The water was distilled into ice-cold sodium hypoiodite solution (5 ml. of 1 *N* sodium hydroxide and 6 ml. of 0.05 *N* iodine) resulting in the conversion of the formaldehyde into formic acid. The formic acid so obtained was oxidized to carbon dioxide according to the procedure of Wood, *et al.*[13] After 1 hour at room temperature the hypoiodite–formic acid solution was acidified with a minimum excess of 2 *N* sulfuric acid and the excess iodine was reduced by titrating the solution with 0.1 *N* sodium thiosulfate; then 1 *N* aqueous acetic acid (1 ml.) was added. The carbon dioxide was precipitated as barium carbonate following the addition of 0.3 *N* aqueous mercuric acetate solution (4 ml.) and after refluxing in a stream of nitrogen for 2 hours.

Counting Procedures. (a) **Organic-C^{14} samples** were combusted by the method of Van Slyke, *et al.*[13] The carbon dioxide was collected in an ionization chamber and counted.[15]

(b) **Barium Carbonate Samples.**—The radioactive barium carbonate samples were suspended in 1 ml. of water and treated with concentrated sulfuric acid and the carbon dioxide collected and counted as described above.

Results and Discussion

In Table I are shown the specific activities of lysergic acid (III), the lactam V and its degradation products [$C_{(17)}O_2$(IV) and $C_{(7)}O_2$(VI)]. These data indicate that almost all the activity is localized in the carbonyl carbon, C-17, of ergosine which constitutes strong evidence that the C-2 of mevalonic acid (I) becomes the C-17 of lysergic acid (I $\xrightarrow{a}$ II). The percentage of radioactivity recoverable from the C-7, although small, is not negligible and may indicate randomization of the C-2 of mevalonate to a small extent. One of the hypotheses which assumes this randomization prior to the incorporation is depicted in Fig. 2.

Reported data indicate that one of the two precursors (isopentenyl pyrophosphate (VII), dimethylallyl pyrophosphate (IX)) of squalene and cholesterol is also involved in the biosynthesis of the

(7) (a) W. A. Taber and L. C. Vining, *Can. J. Microbiol.*, **3**, 1 (1957); (b) W. A. Taber and L. C. Vining, *ibid.*, **4**, 611 (1958).

(8) Purchased from New England Nuclear Corp., Boston, Mass.

(9) (a) K. Macek, A. Cerny and M. Semonsky, *Pharmazie*, **9**, 388 (1959); (b) W. A. Taber and L. C. Vining, *Can. J. Microbiol.*, **3**, 55 (1957).

(10) Ergosine was identified by the comparison of the R_f value with that of an authentic sample of ergosine and the paper chromatographic identification of the amino acids obtained from its acidic hydrolysate; see ref. 2d.

(11) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **104**, 547 (1934).

(12) A. Stoll, A. Hofmann and F. Troxler, *Helv. Chim. Acta*, **32**, 5061 (1948).

(13) H. G. Wood, N. Lifson and V. Lorbes, *J. Biol. Chem.*, **159**, 475 (1945).

(14) D. D. Van Slyke, J. Plazin and J. R. Weisiger, *ibid.*, **191**, 249 (1957).

(15) Dynacon electrometer, Nuclear Chicago Corp., Boston, Mass.

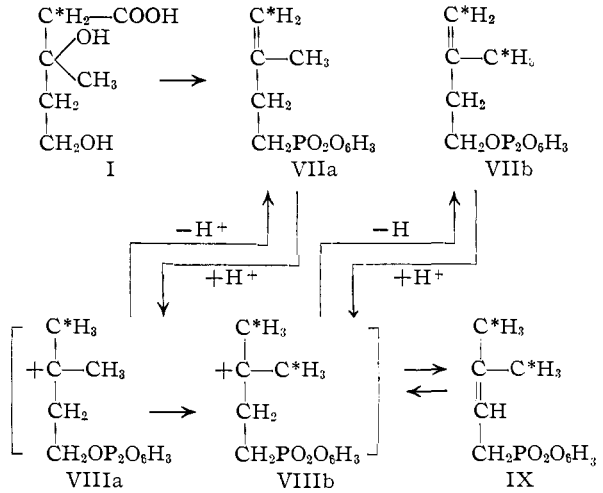

Fig. 2.—The possible mechanism of the randomization of mevalonic acid-2-C^{14} before its incorporation into ergot alkaloids. * The position of the C^{14} label.

ergot alkaloids[2e,16] and that the two pyrophosphate metabolites are in an equilibrium in the yeast cells.[17] If it is assumed that a similar equilibrium exists in the ergot alkaloids producing organism, the randomization of the 2-C^{14} of mevalonic acid-2-C^{14} might be formulated chemically through the following

TABLE I

	Lysergic acid	Lactam	$C_{(17)}O_2$	$C_{(7)}O_2$
D.p.m./mM.	2.38×10^4	2.38×10^4	2.39×10^4	1.51×10^3
Recovery, %			100.4	6.3
Incorporation, %	0.016			

subsequent steps. Mevalonic acid (I) is converted into isopentenyl pyrophosphate (VII) which in turn yields its isomer dimethylallyl pyrophosphate (IX) through an intermediate cation VIIIa, as was suggested by Cornforth and Popjak.[18] The radiocarbon originally present in the second carbon of mevalonate (I) might randomize by the free ro-

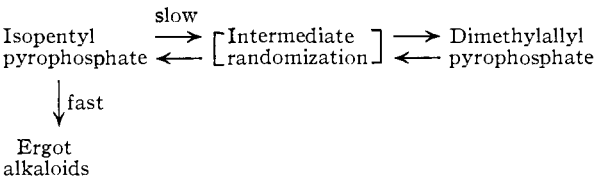

Fig. 3.

tation of the supposed intermediate cation (VIIIa → VIIIb). The radioactivity in the dimethylallyl pyrophosphate obtained by this mechanism will be equally distributed between its two geminal carbons. Similarly, equal distribution of the radiocarbon will appear in the isopentenyl pyrophosphate (VIIb) through the same cation (VIIIb) by the reversal of the latter reaction sequence (IX → VIIIb → VIIb). Under these conditions equal radioactivity would be expected to appear in the C-17 and C-7 of ergosine from both mevalonate metabolites. If, however, the rate of formation of isopentenyl pyrophosphate and/or ergosine proceeds at a greater rate than isomerization (Fig. 3), a disproportionation of the radioactivity will take place resulting in the appearance of a smaller proportion of the radioactivity from 2-C^{14}-mevalonic acid at the C-7 of ergosine. The labeling pattern reported from squalene[19b,c] and cholesterol[19a] neither supports nor negates such a possibility. The possibility also exists that randomization may occur after the incorporation of the mevalonic acid metabolite.[20] Further data are required to elucidate more clearly the manner in which the distribution of radioactivity takes place into the C-17 and C-7 ergosine from 2-C^{14}-mevalonic acid.

(16) R. M. Baxter, S. I. Kandel and A. Okany, *Tetrahedron Letters*, **No. 17**, 595 (1961).

(17) W. Agranoff, H. Eggerer, U. Henning and F. Lynen, *J. Biol. Chem.*, **235**, 326 (1960).

(18) J. W. Cornforth and G. Popjak, *Tetrahedron Letters*, **No. 19**, 29 (1959).

(19) (a) O. Isler, R. Ruigg, L. Chopard-Dit-Jean, L. Wagner and K. Bernhard, *Helv. Chim. Acta*, **39**, 897 (1956); (b) J. W. Cornforth, R. H. Cornforth, G. Popjak and I. Y. Gore, *Biochem. J.*, **69**, 146 (1958); (c) F. Dituri and S. Gurin, *J. Am. Chem. Soc.*, **79**, 2650 (1957).

(20) Evidence has been presented lending support to this assumption that there is no randomization of the label of 2-C^{14}-mevalolactone in some triterpenes assumed to be derived from squalene (D. Arigoni in Ciba Foundation Symposium on "Biosynthesis of Terpenes and Sterols," G. E. W. Westenholme and C. M. O'Connor, Editors, J. and A. Churchill Ltd., London, 1959, p. 231). A similar conclusion was reached by Birch and Smith in the case of certain triterpenoid compounds (A. J. Birch and Henchel Smith, *ibid.*, p. 245).