ACTA CHEMICA SCANDINAVICA 23 (1969) 3393–3397

Biosynthesis of Ergot Alkaloids

Origin of the Side Chain of Ergometrine

U. NELSON and S. AGURELL

Department of Pharmacognosy, Faculty of Pharmacy, Box 6804, 113 86 Stockholm, Sweden

Alanine has been shown to be a precursor of the alaninol side chain of ergometrine, whereas alaninol and α-methylserine are not incorporated. The results suggest that lysergic acid and alanine may form lysergylalanine which is subsequently reduced to ergometrine. The synthesis of specifically labelled alaninol and α-methylserine are described as well as degradation procedures for ergometrine.

Numerous investigations (for reviews *cf.* Refs. 1, 2) during recent years on the biosynthesis of the ergoline skeleton suggest a biosynthetic path from the primary precursors mevalonic acid and tryptophan *via* the lately in nature identified [3,4] 4-dimethylallyltryptophan to chanoclavine-I. Chanoclavine-I is in turn converted to the tetracyclic alkaloid agroclavine which is oxidized, with retention of the hydrogen atom at C-10,[1,5] to elymoclavine and then further to 6-methyl-Δ 8,9-ergolene-8-carboxylic acid (Ref. 1, p. 87) which may isomerize to lysergic acid. The latter compound is incorporated with retention of hydrogen at C-8 into lysergamide and lysergic acid methyl carbinolamide.[6] Less is known about the origin of the side chains of the peptide type ergot alkaloids. However, the biosynthesis of the methyl carbinolamide group of lysergic acid methyl carbinolamide has been investigated and appears to be derived from alanine,[7] not from ethylamine or an acetylated amide.[8] The present report is concerned with the biosynthesis of ergometrine and particularly the origin of the alaninol side chain of ergometrine.

EXPERIMENTAL

L-Alaninol was purchased from Fluka AG, Buchs, ergometrine and lysergic acid from Sandoz AG, Basel, and α-methylserine from Nutrional Biochemical Corp., Cleveland. Radioactive precursors were obtained from the Radiochemical Centre, Amersham, or were prepared earlier.[6,8,9] Experimental techniques are largely described earlier.[9]

Fermentation. A strain (P—72=NRRL 3082) of *C. paspali* obtained from Sandoz AG, Basel,[10] was maintained on a glucose-potato agar medium. Mycelium from a slant was transferred to a flask containing 70 ml of sterile medium C [10] consisting of 45 g/l crude

malt extract (Kebo, Stockholm) in distilled water. This preculture was homogenized and used to inoculate 6—8 alkaloid producing cultures containing 70 ml medium D.[10]

Precursors were added 3—4 days after inoculation and allowed to be metabolized for 3—4 days. Three flasks, usually producing 5—8 mg ergometrine, were used in each precursor experiment.

Isolation and degradation of ergometrine. The extracted alkaloid mixture was chromatographed on formamide-impregnated Whatman 3MM paper developed with benzene-pyridine (6:1) as solvent.[9] Ergometrine was eluted and crystallized from acetone-isopropyl ether with 40—100 mg carrier ergometrine to constant spec. activity.

Ergometrine (25 mg) was hydrolyzed with 1.5 ml conc. HCl at 100° for 24 h in a sealed ampoule. After evaporation, the residue was dissolved in 10 ml water and heated with active charcoal for a few minutes. The filtrate was made slightly alkaline with diethylamine and evaporated to about 0.1 ml to which residue 0.8 ml ethanol was added followed by centrifugation. The amount of l-alaninol present in this solution was determined by GLC (Aerograph 204; 6 ft. × 1/8 in. column, 5 % SE—30 on Gas Chrom P; temp. 80°) and also by TLC on Silica Gel G using isopropanol-conc. NH_3 (9:1) as solvent; spray reagent, ninhydrin. 50 μl (48 mg) of carrier l-alaninol was added to the supernatant and pH adjusted to 9 with diethylamine. Oxalic acid (46 mg) in ethanol was added and alaninol oxalate was allowed to crystallize in the cold overnight (yield about 70 %) and recrystallized from absolute ethanol.[11,12]

Alaninol oxalate (34 mg) was dissolved in 3.0 ml of water to which 3.0 ml 1 N sodium bicarbonate and 3.0 ml of periodic acid reagent according to Reeves [13] were added. The solution was left to react for 1 h. Further reaction and precipitation of the formed acetaldehyde and formaldehyde with dimedone (yield about 90 %) was carried out as described by Reeves.[13] The mixture of formaldehyde and acetaldehyde dimedone derivatives was separated according to Vorländer's method No. 4.[14] Alkaline hydrolysis of ergometrine to yield lysergic acid was carried out with 5 % KOH in 5 % aqueous methanol for 2 h at 100°. Lysergic acid was crystallized after evaporation of methanol and acidification to pH 5.5.[6]

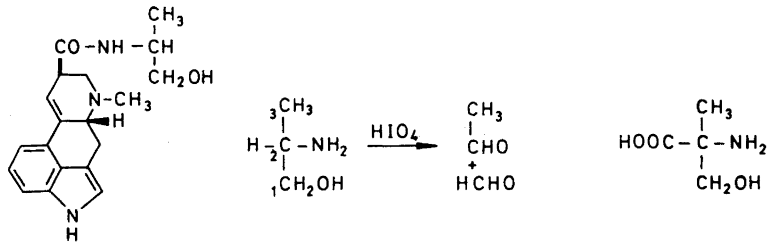
DL-α-Methylserine-^{14}C and -^{3}H.[15] 500 mg L-alanine-U-^{14}C (5.6 mM, 100 μC) was dissolved in 110 ml 0.2 M sodium carbonate. The solution was then treated with 0.56 ml 1 M cupric sulfate and 3.3 ml aqueous formaldehyde (37 %), boiled for 1 h, filtered and acidified with glacial acetic acid. After evaporation to 30 ml it was passed through a Dowex 50 (H^+) column (2 × 35 cm) which was washed with 160 ml water. The column was eluted with 2 N ammonia and the ninhydrin positive fraction was concentrated to 2 ml. After the addition of 10 vol. of ethanol, DL-α-methylserine-^{14}C was allowed to crystallize in the cold and then recrystallized from ethanol-water (3:1). Yield 384 mg (57 %); spec. activity 15.7 μC/mmole. Purity was determined by TLC on cellulose with pyridine-water (4:1) as solvent and by radiochromatogram scanning.

α-Methylserine-methyl-^{3}H was similarly prepared from L-alanine-G-^{3}H. Spec. activity 18.4 μC/mmole.

L-Alaninol-^{3}H.[11,12] L-Alanine ethyl ester (0.25 g) was reduced with 0.10 g lithium aluminium hydride-^{3}H (about 3 mC) in 30 ml dry tetrahydrofurane. The crude l-alaninol was purified by preparative paper chromatography on Whatman 3MM paper using butanol-acetic acid-water (4:1:5) as solvent and further, either on Silica Gel G with isopropanol-conc. NH_3 (9:1) as solvent or by preparative GLC (5 % SE—30 on Chromosorb W—AW—DMCS; temp. 90°). Spec. activity 0.71 mC/mmole.

RESULTS AND DISCUSSION

Earlier we have shown that lysergic acid is the precursor of the lysergic acid part of ergometrine.[16] Several possible routes exist for the biosynthesis of the alaninol (II) side chain of ergometrine (I). We have suggested [1] that a peptide linkage is formed between lysergic acid and alanine and that the carboxyl group of this peptide, lysergylalanine, subsequently is reduced to an alcohol and thus, ergometrine. Another possible route for the biosynthesis of



I II III

ergometrine would also involve alanine, where alanine is reduced to alaninol *before* the formation of the amide linkage.

Interestingly enough, there is also another known biochemical reaction forming alaninol. α-Methylserine (III) is a natural amino acid occurring in the antibiotic amicetin produced by *Streptomyces* fungi [17] and is known to be decarboxylated by some enzyme systems to yield alaninol.[18] α-Methylserine has not been identified so far in ergot. Also the amide formation of α-methylserine and lysergic acid followed by decarboxylation to ergometrine, or still other reactions, although unlikely, could be visualized.

The results of the incorporation experiments with different precursors are given in Table 1.

The distribution of radioactivity in labelled ergometrine obtained from the precursor experiment was determined as follows. Ergometrine was degraded by acid hydrolysis to alaninol (II) which was isolated as the neutral oxalate. Alaninol was further oxidized with periodate to yield formaldehyde (from C-1) and acetaldehyde (from C-2 and C-3). The aldehydes were isolated as dimedone derivatives and separated by conversion of the acetaldehyde derivative to the neutral octahydroxanthon [14] to allow separate radioactivity determinations of C-1 and C-2+C-3 of the side chain. In some experiments also the activity of the lysergic acid moiety was determined after basic hydrolysis to yield lysergic acid.

As Table 1 shows, lysergic acid-^{14}C and known [1,2] precursors of the ergoline skeleton (tryptophan, methionine) are readily incorporated into ergometrine. Lysergic acid-8-^{3}H is also incorporated into ergometrine with retention of the α-hydrogen at C-8 *viz.* without change of configuration of C-8.

It is further evident from Table 1 that alanine, although total incorporation is low, is comparatively well incorporated into the alaninol side chain and, as further degradations show, with little scrambling of label indicating a direct incorporation of alanine or a close derivative there-of. In contrast, acetate is mainly incorporated into the lysergic acid part of ergometrine, presumably *via* mevalonate.[1,2]

The results further show, with reservations for certain factors, such as the varying ability of precursors to penetrate cell membranes and reach the proper enzymatic sites, that α-methylserine is not a precursor or the alaninol side chain of ergometrine and neither is alaninol itself. This was also suggested by experiments carried out by Majer *et al.*[19] Alaninol could possibly, although unlikely,[19] be incorporated after oxidation to alanine but since the label was

Table 1. Incorporation of labelled precursors into ergometrine.

Expt.	Precursor introduced	Incorporation of radioactivity into alkaloid fraction, %[a]	Specific activity				
			Ergometrine[b] dpm/mmole	Degradation products of ergometrine; spec. act. (dpm/mmole) in per cent of spec. act. of ergometrine			
				Lysergic acid	Alaninol[c]	Formaldehyde[d]	Acetaldehyde[e]
221	DL-Tryptophan-3-^{14}C (0.03 mg; 2.5 μC)	4.7	58 800	93	0	—	—
233	D-Lysergic acid-^{14}C (1.1 mg; 0.26 μC)	6.9	50 100	—	—	—	—
234	D-Lysergic acid-8-^{3}H (0.59 mg; 0.44 μC)	2.2	62 000	—	—	—	—
235	D-Lysergic acid amide-^{3}H (2.9 mg; 0.16 μC)	(66.4)	0[g]	—	—	—	—
223	DL-α-Methylserine-^{14}C (9.7 mg; 1.3 μC)	0[h]	0[g]	—	—	—	—
224	DL-α-Methylserine-^{3}H (10.0 mg; 1.5 μC)	0[h]	0[g]	—	—	—	—
255	L-Alaninol-^{3}H (0.41 mg; 4.3 μC)	1.5	0[i]	—	—	—	—
256	L-Alaninol-^{3}H (0.41 mg; 4.3 μC)	1.7	0[i]	—	—	—	—
222	L-Methionine-methyl-^{14}C (0.2 mg; 20 μC)	0.36	33 800	91	4	2	1
245	L-Alanine-G-^{3}H (0.2 mg; 400 μC)	0.03	174 000	—	45	5	42
251	L-Alanine-U-^{14}C (0.05 mg; 50 μC)	0.13	149 000	—	37	11	26
220	Sodium acetate-1-^{14}C (2 mg; 50 μC)	0.28	106 000	92	3	—	—

[a] From crude alkaloid fraction.
[b] Spec. activity after addition of (40—100 mg) carrier ergometrine (m.wt. 325).
[c] Calculated from the spec. activity of the neutral alaninol oxalate (m.wt. 240) and corrected for dilution with carrier alaninol (m.wt. 75).
[d] Determined as the derivate with dimedone (m.wt. 292).
[e] Determined as the anhydride of the dimedone derivative (m.wt. 288).
[f] Recovered unchanged in alkaloid fraction. Negative experiment repeated twice.
[g] Less than 200 dpm/mmole.
[h] In all six negative experiments.
[i] In all eight experiments with no incorporation into ergometrine.

present in hydrogen atoms of the hydroxymethyl group, an oxidation to alanine would eliminate the label.

Thus, the side chain of ergometrine is derived from alanine and further, alanine is not incorporated after reduction to alaninol. This would suggest that our assumption [1] of a peptide formation of lysergic acid and alanine to lysergylalanine followed by a reduction to ergometrine is likely. Recent experiments by Basmadjian *et al.*[20] indeed show that lysergylalanine is a precursor of ergometrine.

Acknowledgements. This investigation was supported by the *Swedish Natural Science Research Council.* The ergot culture was kindly provided by Drs. A. Hofmann and H. Kobel, Sandoz AG, Basel. Suggestions by Dr. E. H. Taylor, Memphis, were greatly appreciated.

REFERENCES

1. Agurell, S. *Acta Pharm. Suecica* **3** (1966) 71.
2. Voigt, R. *Pharm.* **23** (1968) 353, 419.
3. Agurell, S. and Lindgren, J. E. *Tetrahedron Letters* **1968** 5127.
4. Robbers, J. E. and Floss, H. G. *Arch. Biochem. Biophys.* **126** (1968) 967.
5. Floss, H. G., Hornemann, U., Schilling, N., Gröger, D. and Erge, D. *Chem. Commun.* **1968** 105.
6. Agurell, S. *Acta Pharm. Suecica* **3** (1966) 23, 65.
7. Gröger, D., Erge, D. and Floss, H. G. *Z. Naturforsch.* **23b** (1968) 177.
8. Agurell, S. *Acta Pharm. Suecica* **3** (1966) 33.
9. Agurell, S. *Acta Pharm. Suecica* **3** (1966) 11.
10. Kobel, H., Schreier, E. and Rutschmann, J. *Helv. Chim. Acta* **47** (1964) 1052.
11. Karrer, P., Portmann, P. and Suter, M. *Helv. Chim. Acta* **31** (1948) 1617.
12. Stoll, A., Peyer, J. and Hofmann, A. *Helv. Chim. Acta* **26** (1943) 929.
13. Reeves, R. E. *J. Am. Chem. Soc.* **63** (1941) 1476.
14. Vorländer, D. *Z. Anal. Chem.* **77** (1929) 321.
15. Otani, T. T. and Winitz, M. *Arch. Biochem. Biophys.* **90** (1960) 254.
16. Agurell, S. *Abh. Deut. Akad. Wiss. Berlin* **1966** 413.
17. Flynn, E. H., Hinman, J. W., Caron, E. L. and Wolf, Jr., D. O. *J. Am. Chem. Soc.* **75** (1953) 5867.
18. Aaslestad, H. G. and Larson, A. D. *J. Bacteriol.* **88** (1964) 1296.
19. Majer, J., Kybal, J. and Komersova, J. *Folia Microbiol.* **12** (1967) 489.
20. Basmadjian, G., Floss, H. G., Gröger, D. and Erge, D. *Chem. Commun.* **1969** 418.

Received May 14, 1969.