Studies Concerning the Biogenesis of Natural Derivatives of Lysergic Acid

Little is known about the biogenesis of the side chains of lysergic acid (the prefix D is omitted) derivatives, and their biogenetic interrelationships. The carbinolamide moiety of lysergic acid α-hydroxyethylamide (I) arises probably from alanine or a closely related nitrogenous compound[1–4]. It has been suggested that ergometrine (II) could be a precursor of ergotamine (III)[5]. We have tested the ability of 4,5-^{3}H-lysergic acid (spec. act. 9.35 μc/mg)[6] and of 4,5-^{3}H-ergometrine (4.22 μc/mg)[6] to act as intermediates in the biogenesis of the alkaloids in *C. paspali* and in *C. purpurea*, as well as the efficiency of L-alanine-U-^{14}C (57.7 μc/mg) and of L-alaninol-U-^{14}C (0.95 μc/mg)[7] as precursors of the side chains of I, II, and of the α-hydroxy-α-aminoacid fragment of III and of ergokryptine (IV).

Strain 22 of *C. paspali* produced from 75 up to 135 μ*M* of lysergic acid derivatives (I and II, the latter being 5–10% of the total alkaloids produced) per flask containing 50 ml of the mannitol-ammonium succinate medium[8]. ^{3}H-Lysergic acid (5 mg per flask) was efficiently incorporated into the alkaloids (9.5% of dose). Upon feeding of ^{3}H-ergometrine (5 mg per flask) the incorporation rate of tritium into lysergic acid amide (obtained by degradation of I, followed by paper and thin layer chromatographic separation from ^{3}H-ergometrine) was, in 3 experiments, in the range 2.0–3.2%. When non-radioactive lysergic acid (5 mg) was fed together with ^{3}H-ergometrine, the incorporation of tritium was 1.1% of the dose, while free lysergic acid, as recovered at the end of the fermentation and purified by chromatography on silicic acid and on paper to constant specific radioactivity, contained 2.8% of added tritium.

When ^{14}C-alanine or ^{14}C-alaninol were added to the cultures on the 3rd day of fermentation, different incorporation data into the side chains of I and II were obtained (Table I). Acetaldehyde was formed by degradation of I and isolated as the 2.4-dinitrophenylhydrazone[8]. L-Alaninol was obtained by hydrolysis of II with 6*N* hydrogen chloride and purified by paper chromatography to constant specific radioactivity. Alaninol was determined colorimetrically by the 1.2-naphthoquinone-4-sulfonate method[9].

Strain 275 of *C. purpurea* produced 60–100 μ*M* of a mixture of the 2 epimeric pairs ergotamine-ergotaminine (75%) and ergokryptine-ergokryptinine (25%), per flask containing 50 ml of the sucrose-ammonium citrate medium[10]. Upon feeding of ^{3}H-lysergic acid (5 mg per flask) a considerable incorporation (15.8% of administered radioactivity) was found into III and IV, both alkaloids showing the same specific activity. However, when ^{3}H-ergometrine (5 mg per flask) was fed to the cultures, no incorporation of the label was found although a measurable amount (3.5%) of ^{3}H-ergometrine appeared to be present in the mycelium at the end of the fermentation.

A good incorporation of the label was found in III and IV when ^{14}C-alanine and ^{14}C-alaninol were fed to cultures of strain 275 on the 3rd day of fermentation. Pyruvic and dimethylpyruvic acid, formed from the α-hydroxy-α-aminoacid fragments of ergotamine and ergokryptine on alkaline treatment[11], were isolated as the 2.4-dinitrophenylhydrazones and purified to constant specific activity by chromatography on silicic acid and on paper[12]. The results are shown in Table II.

It seems unlikely that II be a precursor of I, because of the lower incorporation rate of ^{3}H-ergometrine into I (as compared with that of ^{3}H-lysergic acid), and the formation of ^{3}H-lysergic acid from ^{3}H-ergometrine during the fermentation. This conclusion is supported by the different incorporation data of ^{14}C-alanine and ^{14}C-2-aminopropanol into the side chains of I and II, indicating no direct relationship in the formation of the side chains of the 2 alkaloids in vivo. The equivalence of the 2 labelled compounds as precursors of the side chain of I suggests that the incorporation of ^{14}C-alaninol may be a consequence of its conversion to pyruvic acid or to a related 3 carbon atoms compound. The much greater ability of ^{14}C-alaninol to be a source of the side chain carbon atoms of II, as compared with ^{14}C-alanine, indicates that alaninol is probably the direct precursor of the same residue in II. If this is true, the small value of the incorporation of ^{14}C-alanine into the side chain of II, i.e. into endogenous L-alaninol, suggests that the latter

Table I. Incorporation rate of label from L-alanine-U-^{14}C and from L-alaninol-U-^{14}C into the alkaloids by *C. paspali* strain 22, and specific activity of the degradation products

Precursor	Dose (μc/flask)	Alkaloids μ*M*	i.r. %	Acetaldehyde (μc/m*M*)	Alaninol (μc/m*M*)
^{14}C-alanine	20.00	124	2.13	0.90 (4.5)[a]	0.39 (1.9)
^{14}C-alaninol	0.18	135	2.91	0.012 (5.6)	0.06 (33.9)

[a] Specific activity expressed as percent of dose.

Table II. Incorporation rate of the label from L-alanine-U-^{14}C and from L-alaninol-U-^{14}C into the alkaloids produced by strain 275, and specific activity of the degradation products

Precursor	Dose (μc/flask)	Alkaloids μ*M*	i.r. %	Pyruvic acid (μc/m*M*)	Dimethylpyruvic acid (μc/m*M*)
^{14}C-alanine	20.00	63	1.32	1.34 (6.7)[a]	2.26 (11.3)
^{14}C-alaninol	0.69	93	1.93	0.082 (11.8)	0.078 (11.2)

[a] Specific activity expressed as percent of dose.

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compound does not arise directly from the 3 carbon atoms units such as alanine itself or pyruvic acid. The origin of this molecule is unknown, although it could take place by decarboxylation of an hypothetical α-methyl-L-serine, which has been found in nature as a constituent of the molecule of the antibiotic amicetin[13], and which has been shown to be non enzymically decarboxylated by pyridoxal[14].

II is not an intermediate in the biogenesis of III and IV as can be deduced by the lack of incorporation of ^{3}H-ergometrine, in contrast with the high incorporation rate of ^{3}H-lysergic acid. The relative incorporation rates of ^{14}C-alanine into the α-hydroxy-α-aminoacid fragments of III and IV would be in agreement with the proposed mechanism of the formation in vivo of the corresponding non-hydroxylated aminoacids[15]. However, the efficiency of ^{14}C-alaninol to act as a source of the carbon atoms of these fragments suggests that the formation of the said α-hydroxy-α-aminoacids in vivo may follow a different route.

Riassunto. Dall'esame della incorporazione di possibili precursori negli alcaloidi prodotti da *C. paspali* e *C. purpurea* risulta che non vi è una diretta correlazione fra la biogenesi delle catene laterali della ergometrina e della α-idrossietilammide dell'acido lisergico, e che l'ergometrina non è un precursore dei derivati peptidici dell'acido lisergico.

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Temperature and Acidity for Maximal Fluorescence of Serotonin and Serotonin-O.P.T.

Numerous techniques are available for the determination of serotonin[1]; however, fluorometry has emerged as the technique of choice due to its inherent sensitivity and selectivity[2]. The most commonly employed fluorometric method is that of Bogdanski et al.[3]; however, recently Maickel and Miller[4] have reported increased detection sensitivity after reaction of serotonin standards with *o*-phthaldialdehyde (O.P.T.). The fluorescence of a compound is profoundly influenced by the temperature and pH of the solution read[5]. Control of these 2 parameters will result in greater method sensitivity and precision. Since no detailed data are available in the literature describing pH and temperature effects on the fluorescence of serotonin or serotonin-O.P.T. these were studied.

Instrumentation. Fluorescence was recorded by using a spectrophotofluorometer (S.P.F.)[6]. Activation wavelengths were 295 nm and 360 nm, respectively, for the serotonin-direct and serotonin-O.P.T. methods. Wavelengths versus fluorescence diagrams (spectra) were recorded[7] from 200–800 nm. Serotonin-direct and serotonin-O.P.T. fluorescence were read at 545 nm and 470 nm wavelengths, respectively.

Experimental, serotonin-direct. 13 analytical duplicates of serotonin standards were prepared from a 520 ng/ml standard in 0.003 N HCl. 5 ml aliquots were diluted with 4 g/100 ml (w/v) ascorbic acid, double distilled water (D.D.W.) and conc. HCl. Final concentrations obtained were 200 ng/ml serotonin, $5.6 \times 10^{-3} M$ ascorbic acid and graded normalities from 1.50–6.00 HCl. Individual blanks were also prepared containing $5.6 \times 10^{-3} M$ ascorbic acid for each normality. Each of the 13 standards and blanks were divided into 8 fractions and refrigerated at 4 °C until tested.

Experimental, serotonin-O.P.T. 12 analytical duplicates of serotonin standards were prepared from a 1560 ng/ml standard in 0.008 N HCl. 1.59 ml of this standard were diluted with 0.4 ml of 0.05 g/100 ml (w/v) O.P.T., D.D.W. and 10 N HCl. Final concentrations obtained were 200 ng/ml serotonin and graded normalities of HCl from 4.50–7.50. Individual blanks were prepared from 0.008 N HCl and were treated similarly. Specimens were complexed with O.P.T.[4]. Each of the 12 standards and blanks were then divided into 5 fractions and refrigerated at 4 °C until tested.

Fluorometry. In sequence, a single set of 13 or 12 samples and blanks representing the entire normality ranges of the direct and O.P.T. series were placed in a waterbath to bring the tubes to the desired temperature (5–50 °C range). The waterbath was connected to the flow-through compartment of the S.P.F. cuvette housing to maintain temperature. Dry, filtered nitrogen gas was used to continuously flush the cuvette housing to reduce condensate formed on the cuvettes at low temperatures and to reduce optical scattering. Samples were permitted to equilibrate in the waterbath for 5 min prior to fluorometry.

Results. Serotonin-direct. Results corrected for individual blanks (Figure 1) indicated that (a) maximal serotonin fluorescence was achieved at an acidity of 3.25–3.50 N HCl; this was found to be independent of temperature. (b) There was an inverse relationship between temperature and serotonin fluorescence for all normalities tested. At the normality of maximal fluorecence (3.5 N HCl), this relationship was exponential between 5–20 °C (Figure 3). At temperatures greater than 20 °C, this relationship was no longer linear due to serotonin destruction. In support of this, the curve obtained from samples at 50 °C (Figure 1) was irregular; this irregularity was still present when these samples were cooled and reread at 20 °C.

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