Isoprenoid biosynthesis by *Saccharomyces cerevisiae*[2]

Substrate added	Spec. act. of amino acid, (dpm/mM: I_s)	Spec. act. of metabolite, (dpm/mM: I_m)	Incorporation of ^{14}C (%)	Overall isotope dilution[8] I_s/I_m
[2-^{14}C] glycine (30 μC, 10 mg)	499×10^6	ergosterol (as digitonide): 260×10^3	0.05	2000
		squalene (as hexahydrochloride): 200×10^3	0.04	2500
		saponifiable fraction: 955 cpm/mg		
DL-[3-^{14}C] serine (40 μC) + L-serine (10 mg)	936×10^6	ergosterol (as digitonide): 260×10^3	0.02	4500
		squalene (as hexahydrochloride): 250×10^3	0.02	3700
[1-^{14}C] glycine (40 μC, 10 mg)	667×10^6	no appreciable counts in ergosterol or squalene; saponifiable fraction: 590 cpm/mg		

benzene- was obtained which is known[3] to be derived from the carbons of the A, B, and C rings of ergosterol. In one biosynthetic experiment[4] using [2-^{14}C] glycine (spec. act., 166×10^6 dpm/mM), ergosterol (spec. act., 129×10^4 dpm/mM, sp. incorporation 3.5%) was degraded to this acid (spec. act. 268×10^2 dpm/mM). In view of the known efficiency of the methylene carbon of glycine for methyl transfer in biosynthetic reactions, it is not surprising that most of the radioactivity of ergosterol was accounted for by the 'extra methyl group'. What is significant is that the acid possessed appreciable radioactivity demonstrating thereby that C-2 of glycine was being used efficiently enough in isoprenoid synthesis in spite of competition with side-chain methylation. [1-^{14}C] glycine conferred little reactivity on ergosterol although the fatty acid fraction was labeled nearly as efficiently by this substrate as by [2-^{14}C] glycine.

In subsequent experiments with yeast an even more direct link between glycine and steroid biosynthesis was found. Squalene, the established precursor of many steroids, could be isolated from the metabolites produced by *S. cerevisiae*. Purified through the crystalline hexahydrochloride, this metabolite showed substantial radioactivity (spec. act., 460×10^3 dpm/mM) in an experiment with [2-^{14}C] glycine. In contrast, when [1-^{14}C] glycine was used very little activity was present in the ergosterol or the squalene produced; the saponifiable fraction, however, was appreciably radioactive (see Table).

In another series of experiments rat liver homogenate preparations[5] were incubated with various ^{14}C-labeled substrates; cholesterol from these experiments was found to be radioactive. In case of glycine, the methylene carbon was incorporated nearly 10 times more efficiently than the carboxy carbon; [3-^{14}C] serine also was found to label cholesterol efficiently[6].

In view of our observations reported here on fungi, yeast and rat liver preparations, it is evident that biosynthesis of steroids and terpenoids from amino acids that can produce 'one carbon units' is a general phenomenon. It is therefore important to study the role of amino acids from the protein part of diet in the formation of cholesterol independent of the contribution from fats and carbohydrates. Currently cholesterol is considered to be strongly implicated in atherosclerosis and heart diseases. The recent report by CASPI et al.[7] that in vivo incorporation of the S-methyl carbon of methionine into cholesterol takes place in normal and tumorous rats is further evidence for the hitherto unrecognized pathways from amino acids to isoprenoids.

Zusammenfassung. Während der Biosynthese von Cholesterol mit homogenisierter Rattenleber wird [2-^{14}C] des Glycins viel besser eingebaut als [1-^{14}C]. *Saccharomyces cerevisiae* produziert radioaktives Squalen (ausser Ergosterol mit Radioaktivität des Ringsystems) mit [2-^{14}C] Glycin und mit [3-^{14}C] Serin, aber nicht mit [1-^{14}C] Glycin.

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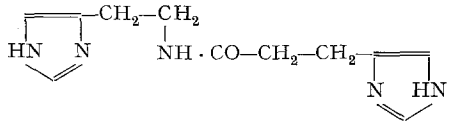
Occurrence of N-Imidazolepropionylhistamine in the Soft Tissues of the Philippine Gastropod *Drupa concatenata* Lam.

The only N-acylated histamine derivative so far found in the living organism is N-acetylhistamine which has been traced in the urine of several mammals, and in some tissues, including nervous tissue[1-5].

This communication describes the occurrence of large amounts of N-imidazolepropionylhistamine in methanol extracts of the total soft tissues of *Drupa concatenata* Lam., a gastropod of the Philippines.

Materials and methods. 1500 specimens of *Drupa concatenata* were collected near Dumaguete City (Negros Oriental). The whole soft tissues (305 g) were removed from the living animals after cautious rupture of the shell and immediately extracted with 5 parts (w/v) of pure methanol. After 10 days the supernatant liquid was decanted and the tissue re-extracted for another 2 days



with 5 parts of 80% methanol. The extracts were mixed and filtered. Part of them was studied as such, but the greatest part, corresponding to 240 g tissue, was submitted to chromatography on alkaline alumina columns which were eluted with descending concentrations of ethanol. Both the crude extracts and the eluates from the alumina columns were submitted to paper chromatography, thin-layer chromatography, high-voltage electrophoresis and bioassay, using the isolated guinea-pig ileum.

Synthetic choline chloride, murexine chloride hydrochloride, senecioylcholine iodide, dihydromurexine dipicrate, urocanic acid, imidazolepropionic acid, the methyl and ethyl esters of these acids, histamine dihydrochloride, N-acetylhistamine and N-imidazolepropionylhistamine were available for comparison. The last histamine derivative was synthetized by one of us (V.).

Results and discussion. Following chromatography on alumina column, the histamine derivative supposed to be N-imidazolepropionylhistamine emerged in the 95–90% ethanol eluates. Its identification as N-imidazolepropionylhistamine was based on the following criteria:

a) both the unknown histamine derivative and synthetic N-imidazolepropionylhistamine showed the same colour shades with the Pauly reagent (pink red) and the Dragendorff reagent (lilac).

b) On high-voltage paper electrophoresis the unknown substance showed the same mobility towards the cathode as synthetic N-imidazolepropionylhistamine: $E_{1.2}$ = 1.1–1.2 histidine; $E_{5.8}$ = 1.7–2 histidine.

c) On paper chromatography the Rf values of the unknown derivative and those of synthetic N-imidazolepropionylhistamine were exactly the same in 5 solvent systems: Rf 0.26–0.28 in *n*·butanol:acetic acid:water (4:1:5), Rf 0.60–0.64 in *n*·butanol:methylamine 35% (8:3), Rf 0.51–0.54 in 1-pentanol:pyridine:water (40:40:10), Rf 0.43–0.46 in methylethylketone:pyridine:water:methylamine 35% (65:15:10:0.5) and finally Rf 0.65–0.72 in 20% KCl. Similar results were obtained in thin-layer chromatography: Rf 0.07–0.1 in *n*·butanol:acetic acid:water (4:1:5), Rf 0.64–0.70 in *n*·butanol:methylamine 35% (8:3), and Rf 0.28–0.32 in *n*·buthanol:ethanol:35% methylamine (22:7:1).

d) Upon hydrolysis with hydrochloric acid (6*N* HCl, 3–6 h at 100 °C) amounts of the unknown derivative and of synthetic N-imidazolepropionylhistamine showing, on paper chromatograms, a Pauly reaction and a Dragendorff reaction of the same intensity, yielded equal amounts of imidazolepropionic acid, as estimated by the Pauly reaction, and of histamine, as estimated by bioassay. For each mole of histamine, one mole of imidazolepropionic acid was liberated. The spasmogenic effect of the hydrolysate on the guinea-pig ileum was completely blocked by mepyramine (0.1–0.2 µg/ml).

The content in N-imidazolepropionylhistamine of the crude extract of total soft tissues was approximately 300 µg per g fresh tissue. In addition to N-imidazolepropionylhistamine, the extracts of *Drupa concatenata* contained more or less conspicuous amounts of choline, imidazolepropionic acid, methyl imidazolepropionate, free histamine and another histamine derivative, the isolation of which is in progress.

Research has been commenced to investigate whether imidazolepropionylhistamine is used by *Drupa concatenata*, a carnivorous gastropod, in the capture of the prey[6].

Riassunto. Gli estratti metanolici dei tessuti molli del mollusco gasteropode delle Fillppine *Drupa cancatenata* Lam. contengono cospicui quantitetivi (circa 300 µg per g di tessuto fresco) di un nuovo derivato dell'istamina, la N-imidazolpropionilistamina.

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Isolation of Different Thermophilic Enzymes from *Bacillus stearothermophilus*

Several reports appeared recently from this laboratory about thermophilic proteolytic enzymes from *Bacillus stearothermophilus* [1–4]. We have now extended our studies and purified some additional enzymes from the same bacillus to a homogenious or nearly homogenious state, to get further information about these thermostable proteins by comparative studies. In this report we describe the method that allows us to purify 7 enzymes out of the same bacillus extract. These enzymes enclose the 3 aminopeptidases I, II and III that occur in *B. stearothermophilus*[5], two glucose-6-phosphatases, a *p*-nitrophenylphosphatase and a glucokinase.

Cells. B. stearothermophilus cells (strain NCIB 8924), grown as described[2], were a generous gift of the Ciba-Geigy AG, Basle.

Enzyme assays. p-Nitrophenylphosphatase: The hydrolyses of 0.01 *M p*-nitrophenylphosphate in 0.05 *M tris*-HCl, pH 9.0 at room temperature, containing 10^{-3} *M* Mg Cl_2 was followed at 405 nm.

Glucose-6-phosphatases: The assay mixture contained 2.5×10^{-2} *M* glucose-6-phosphate and 10^{-2} *M* Mg Cl_2 in 0.05 *M* sodium-N-morpholino-3-propansulfonate, pH 6.9. The released inorganic phosphate was determined by the method of Delsal and Manhouri[6].