Table 1. Mortality rate of *Goniobasis livescens* to heat exposure per 10 individuals.

Temp (°C)	1 min		5 min		10 min		20 min		30 min		60 min		120 min		180 min		240 min	
	S*	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
41	10																	
40		0		3		5												
39	8	0	9	3	10	4	10		10									
38	8	1	10	1	9	2	10	9	9	9		9						
37	3		5		5	2	6	2	8	7								
36	1	1	2		2	0	1	0	4	4	8	5						
35	1		0		1		0	0	0	1	1	2	2	0				
34	1		1		1		1	0	1	0	1	0	1	1	1	2	0	2
33	1		1				0	0	0	0								
32	1		1		0		1		0	0	0	0						
31	0				0													
30	0				0													
29	0																	

* S represents the slowly heated snails; R represents rapidly heated snails.

raised by increasing the temperature of the water 1°C every 5 min. Both groups were exposed to the ultimate temperature for periods ranging from 1 min to 4 hr. At the end of the exposure period, they were immediately transferred to lake water at room temperature, and individuals that were alive after 3 days were considered to have withstood heat death. Ten snails were tested under each set of conditions, and the numbers that died are shown in Table 1.

The minimum heat death temperature for the majority of individuals is indicated to be approximately 36°C. since most of the snails were killed by 1-hr exposure at this temperature, whereas only a minority were killed by 1-hr exposure at 35°C. The mortality increased with longer exposure at 36°C but not with longer exposure up to 2 hr at 35°C (Table 1). While there was great individual variation, the heat death temperatures for 50 percent of the rapidly heated snails exceeded 40° at 5 min, 40° at 10 min, between 37° and 38° at 20 min, between 36° and 37° at 30 min, and 36° at 60 min. The heat death temperatures for 50 percent of the slowly heated snails were between 37° and 38° at 1 min, 37° at 5 and 10 min, between 36° and 37° at 20 and 30 min, and between 35° and 36° at 60 and 120 min.

The heat death exposure times for 50 percent of the rapidly heated snails were 10 min at 40°, between 10 and 20 min at 39° and 38°, between 20 and 30 min at 37°, and 60 min at 36°. The heat death exposure times for 50 percent of the slowly heated snails were less than 1 min at 38°, 5 to 10 min at 37°, and between 30 and 60 min at 36°. These results indicate that the heat death exposure time decreased with increase of temperature above 36°C and that the heat death temperature decreased with increase of exposure time up to 60 min.

The results indicate that there is a differential in the effects of slow and rapid heating on the heat death temperatures and exposure times, although the differential was the reverse of that expected. Instead of the heat death temperatures and exposure times being greater for the gradually heated snails than for the rapidly heated snails, they were less. We believe that this results from the longer total exposure time to high temperatures of the slowly heated snails. It is evident that a temperature increase of 1°C every 5 min is not sufficiently slow to permit heat acclimatization of *Goniobasis livescens*. At this rate of heating, the temperature is raised before acclimatization to the preceding temperature increment is achieved. The fact that the heat death temperatures and exposure times are effected by the rate of heating indicates that, in a comparison of these characteristics of different organisms, the rates of heating should be given.

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Aromatic Biosynthesis. XI. The Aromatization Step in the Synthesis of Phenylalanine*

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A wide variety of benzenoid compounds are produced from nonaromatic materials in the plant and microbial kingdoms. In none of these biosyntheses,

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however, has the actual aromatization reaction been accessible to experimental study; for though hydroaromatic precursors of certain aromatic metabolites have been recognized [shikimic acid and related compounds (*1*); *meso*-inositol (*2*)], these precursors are clearly several steps removed from aromaticity.

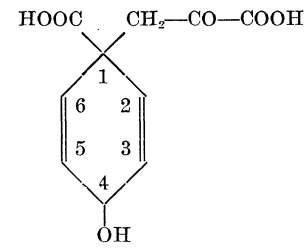
We now wish to report the isolation of a non-aromatic biosynthetic intermediate, prephenic acid (PPA) (*3, 4*), that appears to be the immediate precursor of an aromatic one. PPA is excreted by phenylalanine-requiring mutants of *Escherichia coli* (for example, strain 83–5) (*4, 5*). Exposure to even very mild acidity (for example, *p*H 6) converts it into phenylpyruvic acid (*4*); and we have now observed that this conversion can also be effected enzymatically by extracts of wild-type *E. coli* but not by comparable extracts of strain 83–5, which is blocked between PPA and phenylpyruvic acid. PPA is nutritionally inactive, but the foregoing enzymatic observations imply that this inactivity is due to an accessibility barrier and, hence, offers no obstacle to considering this compound a true intermediate.

PPA was isolated in crystalline form from culture filtrates of mutant 83–5 by charcoal chromatography, precipitation as the barium salt from aqueous solution with methanol, and repeated precipitation from water with methanol or pyridine. The progress of purification was followed by acid-catalyzed conversion to phenylpyruvic acid, which was assayed spectrophotometrically in alkaline medium at 320 mμ. The apparent molecular weights, based on the assays, dropped on recrystallization from about 750 to constant values of 396–410. Elementary analysis is compatible with the formula $C_{10}H_{10}O_7Ba$ [found (*6*): C 31.66, H 2.82, Ba 36.02 percent; calculated: C 31.64, H 2.66, Ba 36.19 percent; mol. wt. 379.5]. The acid-catalyzed conversion of PPA to phenylpyruvic acid ($C_9H_8O_3$), which takes place in the absence of any ready electron acceptor (including oxygen), is accompanied by release of one equivalent of CO_2. It would follow that PPA is a C-10 dicarboxylic acid.

An aromatic structure is excluded for PPA on several grounds. On catalytic hydrogenation over platinum, the compound took up between three and four molar equivalents of hydrogen, whereas phenylpyruvic acid took up the expected one molar equivalent. The infra-red spectrum shows no trace of the band at wavelength 6.74 μ (wave number, 1485 cm^{-1}) that is present, and with identical shapes (*7*) in the spectra of cinnamic, β-phenylpropionic, and phenylpyruvic acids. Finally, although PPA shows strong end-absorption in the ultraviolet, reduction of its carbonyl group with $NaBH_4$ decreased this absorption to a level ($\varepsilon_{260} = 20$) incompatible with aromaticity or the presence of a conjugated double-bond system in the ring; and the reduced compound takes up two molar equivalents of Br_2.

The product of $NaBH_4$ treatment was also decarboxylated and aromatized by acid, yielding a substance provisionally identified as phenyllactic acid. This observation excludes the possibility that the decarboxylation depends on the presence of a β-keto acid grouping.

The following structure is proposed for PPA:



This structure is compatible with the aforementioned facts and is supported by the following further observations. (i) No optical activity could be detected. (ii) The product of catalytic hydrogenation lactonizes in acid solution, as is shown by treatment with hydroxylamine followed by $FeCl_3$. (iii) The presence of a hydroxyl group, plus a molecule of water of crystallization in the Ba^{++} salt (as required by its empirical formula), is supported by the demonstration that 3 hydrogen atoms exchange rapidly with D_2O (*8*). (iv) Degradation of shikimic acid and tyrosine, obtained from organisms grown on C^{14}-labeled glucose, has shown that the carboxyl of the former compound and the side-chain of the latter occupy the same site on the ring (*9, 10*).

The structure proposed can account for the ease of aromatization and decarboxylation under the influence of acid. This reaction is presumably initiated by proton attack on the ring hydroxyl group. PPA has a half-life, in aqueous solution at room temperature, of 130 hr at *p*H 7.0, 13 hr at 6.0, and 1.0 min in 1*N* HCl.

Further details of this work are to be published.

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