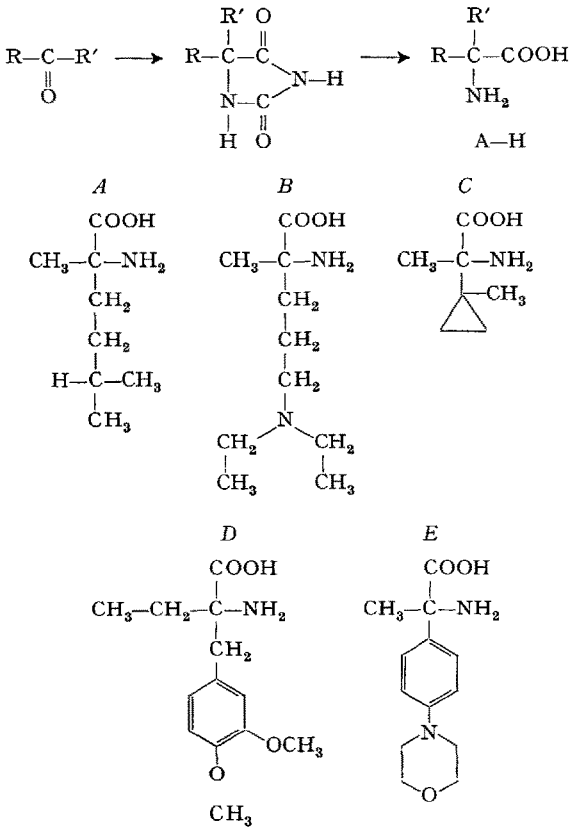
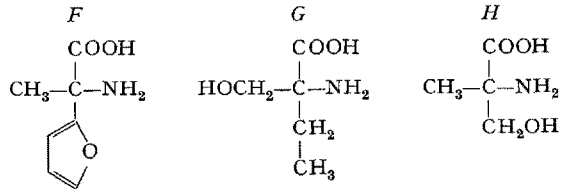
Several New α-Alkyl Amino Acid Analogues

For biological investigation, 8 amino acids (of which 5 are new compounds) were synthesized from the corresponding ketones via the hydantoins.

A preliminary examination of these compounds was made in a bacterial system and also in a mammalian system. It was hoped that some of these compounds would inhibit microbial growth and would have no toxic effect in vivo in a mammal, thus initiating a study of metabolism difference between species.





The synthesis of the hydantoins and amino acids (form DL) was carried out as previously described for α-(2-furyl)alanine and its hydantoin[1]. Table I contains the data.

A stock culture of *Escherichia coli* 9723 was employed. The salts-glucose medium described by ANDERSON[4] was modified as described previously[5] before use. The inoculation and incubation methods employed were as described previously[5]. The compounds to be tested were weighed into sterile test-tubes and dissolved in sterile water. The solutions were adjusted to pH 7 and added aseptically to the assay tubes. A dilute microbial cell suspension with log-phase cells was utilized for inoculation of the assay; 1 drop was added to each tube. The incubation was at 37 °C for 18 h. The amount of growth was determined turbidimetrically by means of a nephelometer.

Toxicity effects could not be demonstrated with compounds A, C, D, E, F and G up to the maximum concentrations that solubility would allow in this technique. Compounds B and H showed definite toxicity at 50 and 20 mg/5 ml assay respectively. Attempts were made to reverse the toxicity of these 2 compounds with 18 natural amino acids: L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophane, L-tyrosine and

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[4] E. H. ANDERSON, Proc. natn. Acad. Sci. *32*, 120 (1946).
[5] C. J. ABSHIRE, J. LAROUQUERE and L. BERLINGUET, Can. J. Biochem. *45*, 557 (1967).

Table I. α-Alkyl amino acids and corresponding hydantoins

Compound	Yield (%)	Nitrogen (%) Calculated	Found	m.p. (°C)	Rf (pyridine-H_2O, 80:20)
A	54	8.80	8.68	300	0.79
Hydantoin of A	98	15.21	14.98	150–151	
B	55	12.23	12.32	200–210	0.80
Hydantoin of B	99	18.50	18.24	134–135	
C	57	9.78	10.00	285–286	0.76
Hydantoin of C	59	16.66	16.60	163–164	
D	77	5.81	5.74	243–244	0.89
Hydantoin of D	65	10.07	10.17	233–234	
E	74	11.06	11.20	230–231	0.74
Hydantoin of E	63	15.27	15.32	212–213	
F	82	9.14	9.15	195–199[a]	0.71
Hydantoin of F	76	15.55	15.63	179–180[b]	
G	36	10.53	10.50	258–260[c]	0.67
Hydantoin of G	84	17.72	17.88	166–167[d]	
H	34	11.76	11.94	240–241[e]	0.54
Hydantoin of H	99	19.44	19.38	198–199	

[a] Reported 194–200°[1], [b] reported 176–178°[2], [c] reported 257–259°[1], [d] reported 165–168°[1], [e] reported 243°[3].

L-valine. At low concentrations of the natural amino acids, reversal of toxicity of B was obtained only with proline. Table II shows the results of varying quantities of proline. B appears to be a competitive inhibitory analogue of proline, at least within a certain concentration range. The inhibitory ratio (B/L-proline) is approximately 20:1.

The toxicity of compound H on the other hand was reversed by serine and methionine. Table III shows the data. Increasing concentrations of the natural amino acids cause greater toxicity reversal, but the inhibitory ratio is not stable as in the previous case.

The comparative toxicity of the substances towards mice was examined in the following manner. Albino male mice of 20 g were employed. Each of the compounds was injected into the mice at a dose of 500 mg/kg; 0.2 ml of a saline solution of the compound at pH 7 was injected i.p.

Table II. Reversal of toxicity of B by proline

B, mg/5 ml	Nephelometer readings L-proline (γ/5 ml) 0	2	5	10	20	50	100
0	30	28	28	29	30	30	29
10	32						
20	24	27	27				
50	[0]	[7]	18	21	23	25	26
100	0	0	[0]	12	17	21	22
200			0	[0]	[0]	[0]	[0]

Table III. Reversal of toxicity of H by serine and methionine

H, mg/5 ml	Nephelometer readings L-serine (γ/5 ml) 0	0.5	1	10	20	100	L-methionine (γ/5 ml) 0.001	0.01	0.05	0.1	2	5	500
0	64	67	62	61	58	57	60	61	61	59	59	58	54
5	73	72					70						
10	40	59	65	71			52	68	71				
20	[0]	[0]	12	41	70		[7]	14	32				
50	0	0	[0]	[0]	24	61	0	[0]	[0]	50	70	61	51
100			0	0	[0]	[0]	0	0	0	12	24	36	48
200					0	0				[0]	[0]	12	27

in an aseptic manner once a day for 6 consecutive days; a minimum of 6 mice were employed for each compound. The animals were then allowed to rest for 8 days. There was no difference in weight change of the animals compared to controls.

Résumé. Synthèse a été faite de 8 acides aminés non naturels et des 8 hydantoïnes respectives. Cinq de ces acides aminés et les hydantoïnes correspondantes sont des composés nouveaux. Dans toute la série étudiée, B et H sont toxiques chez *Escherichia coli* et non chez les souris.

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Effect of Hypophysectomy on the Incorporation of Proline in the Collagen Fractions[1]

Although hypophysectomy is known to influence the content[2] and the biosynthesis[3-5] of collagen in experimental animals, the mechanism has not been investigated in more detail.

The rats were hypophysectomized by the method of Sato and Yoneda[6] at the age of 1 month (weight 48–53 g), 2–3 weeks before the experiments. The control animals included normal rats of the same weight and of the same age. All the data are averages of 2 rats, which differed by less than 5%. Porcine somatotrophin (STH) or L-thyroxine were injected to some of the hypophysectomized animals for 4 days. The control animals received the same volume of solvent (0.15 *M* NaCl). The single dose of [^{3}H]proline-G (0.5 μCi/g, The Radiochemical Centre, Amersham, England) was injected i.p., simultaneously with the first injection of the hormones.

During the 84-h experimental period, the average weight gains were in the hypophysectomized rats 0.5 g (initial weight 56 g), in the normal rats of the same weight 12.5 g and in the normal rats of the same age 16.5 g (initial

[1] Supported by institutional grants from the Sigrid Jusélius Foundation and U.S. Department of Agriculture, Foreign Research and Technical Programs Division.

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