NOTES

The *in vivo* Synthesis of Diethylriboflavin Phosphate[1,2]

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Diethylriboflavin has been shown to be a very potent antagonist of riboflavin in the rat.[3] Since part of the biological activity of diethylriboflavin appears to be due to its ability to displace riboflavin from tissues, it was felt that a study of the tissues of animals fed this riboflavin homolog should be undertaken. It was believed that to be an effective displacing agent the diethylriboflavin must be converted to some form resembling those products which are normally derived from riboflavin. For this purpose the livers of rats fed diethylriboflavin were examined for diethylriboflavin and its derivatives. Rats of Group 1 (seven animals) received 2 mg. of diethylriboflavin each day until they died or for a maximum of 36 days, and those of Group II (six animals) received a flavin-free diet for 36 days. The diet and the mode of administration of the diethylriboflavin have been described.[3]

After death or termination of the experiment, the livers were removed, frozen, lyophilized and ground to a powder in a porcelain mortar. The liver powders of animals of Group I were combined, as were those of Group II, and the flavin concentration per gram of each group was determined fluorometrically.[4] Protein-free and salt-free extracts, containing 44.4 μg. of flavin, were prepared by the method of Yagi[5] and aliquots, containing equal amounts of flavin (4.4–8.8 μg.), of each of the extracts were placed on Whatman No. 1 paper and chromatographed using the upper phase of a water–*n*-butanol–acetic acid (5:4:1) solvent system.

Riboflavin, riboflavin phosphate and flavin adenine dinucleotide (R_f 0.30, 0.10 and 0.05, respectively) were detected in the chromatograms of Groups I and II. Two spots of R_f 0.19 and 0.54 were noted in the Group I chromatograms and were not present in those of Group II. The two spots had a yellow–green fluorescence when viewed under ultraviolet light as is characteristic of flavin compounds. The two new spots had R_f values identical with synthetic diethylriboflavin 5′-phosphate (0.19) prepared by the use of the Flexser and Farkas method[6] for the synthesis of riboflavin 5′-phosphate, and synthetic diethylriboflavin (0.54) prepared by the method of Lambooy.[7] Furthermore, when two-dimensional paper chromatography was employed, the two new spots behaved identically with diethylriboflavin 5′-phosphate and diethylriboflavin. Solvent 1 was used for the first dimension and solvents 2, 3 or 4 for the second dimension (Table I).

Table I

R_f Values (Ascending)

Solvent[a]	1	2	3	4
Riboflavin	0.30	0.34	0.77	0.66
Diethylriboflavin	.54	.34	.84	.77
Riboflavin 5′-phosphate	.10	.50	.18	.06
Diethylriboflavin 5′-phosphate	.19	.53	.21	.11
Flavin adenine dinucleotide	.05	.40	.22	.17
FAD-X[8]	.05	.40	.47	.30
Riboflavin phosphate-X[8]	.13	.54	.50	.15
Lumichrome[8]	.68	.07	.88	.72
Lumiflavin[8]	.48	.18	.94	.68
Riboflavinyl glucoside[8]	.22	.40	.60	.50

[a] Solvent (1) water:*n*-butanol:acetic acid 5:4:1 (top phase); (2) 5% Na_2HPO_4 in water and isoamyl alcohol[9]; (3) 160 g. phenol:30 ml. *n*-butanol:100 ml. water (lower phase)[10]; (4) collidine saturated with water.

The failure to detect the diethylriboflavin homolog of flavin adenine dinucleotide in the tissues of animals fed diethylriboflavin may be due to its having an R_f value similar to FAD in the solvents used or because the FAD homolog was not formed.

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(2) Reference was made to this study in a paper presented at The National Vitamin Foundation Symposium on Antimetabolites, New York City, March 1, 1955.

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(8) These R_f values taken from reference 8.

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The Structure of Macrocyclic Glycine Peptides

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The synthesis of two macrocyclic glycine peptides, "cyclo-(triglycyl)"[1] and cyclo-(hexaglycyl)[2] have been reported recently. Comparison of these materials in this laboratory has shown them to be identical and to possess the cyclo-(hexaglycyl) structure.

The assignment of the cyclo-(triglycyl) structure to the compound prepared by the cyclization of diglycylglycine azide[1] appears to have been made on the basis of the method of preparation only, no confirmatory molecular weight evidence being described. The cyclo-(hexaglycyl) structure proposed for our material (prepared from N-carboxyglycine anhydride) was established by many reproducible molecular weight determinations by the vapor pressure technique of Menzies,[3] adapted to

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