

This work was supported by *Statens Medicinska Forskningsråd*.

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Received November 18, 1967.

γ -Glutamyl-phenylalanine and γ -L-Glutamyl-L-tyrosine from Seeds of *Aubrietia deltoidea* DC.

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Previous communications from this laboratory reported results obtained during a systematic investigation of the free amino acids in species of Cruciferae.¹ In the course of these investigations the amino acid content in seeds of *Aubrietia deltoidea* DC. was determined by two-dimensional paper chromatography, and a spot was observed which could not be assigned to any amino acid previously identified in species of Cruciferae. The amino acid in question has now been isolated and identified as γ -L-glutamyl-L-tyrosine. In addition γ -glutamyl-phenylalanine has been isolated from the seeds. The latter compound was present in a concentration so small that it was not observed on the original paper chromatogram.

The fraction of acid amino acids from seeds of *A. deltoidea* DC. (1 kg, purchased from I. E. Ohlsen's Enke, Copenhagen) was

obtained by traditional methods including defatting with carbon tetrachloride, extraction with methanol:water, isolation of the total amino acid fraction on a strongly acid ion-exchange resin in the acid form with subsequent elution of the amino acids with ammonia, and isolation of the acid amino acids on a strongly basic ion-exchange resin in the acetate form with subsequent elution with acetic acid. Final purification was accomplished by ion-exchange chromatography on a strongly basic ion-exchange resin in the acetate form and by use of small ion-exchange columns and preparative paper chromatography as previously described.² Recrystallization from ethanol:water yielded γ -glutamyl-phenylalanine (4 mg, insufficient for the determination of optical rotation) and γ -L-glutamyl-L-tyrosine (101 mg, $[\alpha]_D^{25} + 26.8^\circ$ (c 1.1, H₂O). Lit. value $[\alpha]_D^{25} + 25.5^\circ$ (c 4, H₂O).³ The compounds were identified by comparison with authentic samples³ by use of infra-red absorption spectra and co-chromatography on paper. Furthermore, acid hydrolysis produced glutamic acid and phenylalanine, respectively tyrosine, as determined by co-chromatography on paper. The complete identity of the infra-red absorption spectrum of the isolated phenylalanine derivative with that of authentic γ -L-glutamyl-L-phenylalanine suggests L- (or more unlikely D-) configuration at both centers.

The presence of γ -glutamyl-tyrosine in seeds of *A. erubescens* Griseb. was established by paper chromatography. Traces of this compound may be present also in seeds of *Berteroa incana* (L.) DC. whereas the two γ -glutamyl derivatives have not been identified in any other species of Cruciferae investigated. A number of other γ -glutamyl derivatives are present in the crucifer *Lunaria annua* L.^{1,2}

γ -L-Glutamyl-L-phenylalanine and γ -L-glutamyl-L-tyrosine have been isolated previously from *Glycine max* (soybeans),³ *Lupinus angustifolius*, and *L. albus*.⁴ γ -L-Glutamyl-L-phenylalanine has been isolated also from *Allium cepa* where it occurs together with a number of other γ -glutamyl derivatives.⁵

The authors are indebted to Drs. C. J. Morris and J. F. Thompson for samples of γ -L-glutamyl-L-phenylalanine and γ -L-glutamyl-L-tyrosine.

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Received November 28, 1967.

Alkylation of Maleic Acid and Related Compounds with Alkyldiimine and Azoalkanes

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During the early attempts to synthesize roccellic acid, (+)-2*S*:3*R*)-2-methyl-3-dodecylsuccinic acid,¹ various methods for the stereoselective synthesis of alkylsuccinic acids were investigated. Since diimine reduction is stereospecific² it seemed possible that alkyldiimines and azoalkanes would react stereoselectively with maleic acid and related compounds to give alkyl and dialkylsuccinic acids. Some preliminary experiments along these lines are reported in this communication.

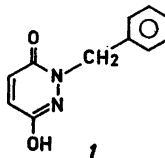
Methyldiimine and phenyldiimine, generated by oxidation of the hydrazines, were found to add to maleic acid to give low yields of methyl- and phenylsuccinic acid, respectively. Similar experiments have recently been carried out by Cohen and Nicholson who also describe an efficient method for generating phenyldiimine.³

To investigate the stereospecificity of the addition, methyldiimine was generated in the presence of acetylenedicarboxylic acid by oxidation of methylhydrazine. A very low yield of methylmaleic and methylfumaric acid was obtained. The high ratio of maleic to fumaric acid (4:1) indicates that the reaction is relatively stereospecific. The low yield makes the reaction unattractive for preparative work. It is possible that the yield is improved if other methods

are used for the generation of methyldiimine (*cf.* Ref. 3). In the early experiments to dialkylate maleic acid derivatives, azo(2-propane) was photolysed in the presence of dimethylmaleate or dimethylfumarate. Under the most favorable conditions a trace of dimethyl diisopropylsuccinate was obtained. Approximately the same ratio of *erythro* and *threo* esters (3:1) was obtained from maleate and fumarate, presumably due to photo isomerisation of fumarate to maleate. When maleic anhydride or maleimide derivatives were used, no dialkylsuccinic acid derivatives could be isolated.

The crude product from the reaction of dimethylmaleate and azo(2-propane) contained nitrogen and rapidly turned brown on contact with air. It therefore seemed possible that some diazetidine was formed, but the attempts to isolate a defined nitrogen containing product were unsuccessful.

Diimine reduction is highly sensitive to steric hindrance.⁴ The failure of azo(2-propane) to give appreciable amounts of dialkylation products in the reaction with dimethyl maleate might be due to steric hindrance. The reaction between azophenylmethane and dimethyl maleate was therefore investigated. Azophenylmethane is an attractive model compound since it may be thermally decomposed at moderate temperature.⁵ In the photochemical reaction of azophenylmethane and dimethylmaleate no dimethyl dibenzylsuccinate was formed (GLC). Dibenzyl, stilbene, and polymers were the main products.



When azophenylmethane was decomposed in the presence of dimethyl maleate, a trace of dimethyl dibenzylsuccinate was formed and also some dibenzyl and stilbene. In addition, 2-benzyl-6-hydroxy-3(2*H*)-pyridazinone (**1**) was formed in about 40 % yield. A similar product is also formed from azo(1-phenylethane) and dimethylmaleate. The mechanism of this reaction is obscure and will be investigated further.