The Percarbobenzoxylation of L-Arginine

The complex nature of the highly basic arginine has afforded considerable difficulty in the development of synthetic procedures for preparing peptides which incorporate this amino acid. This difficulty has, to some extent, been overcome through the nitration of arginine, to permit coupling with its α-amino group (1), whereas the α-monocarbobenzoxylated derivatives of arginine hydrohalide or nitroarginine have served for condensations involving the carboxyl group (2). It is the purpose of the present communication to report the preparation and utility of the previously undescribed tricarbobenzoxy-L-arginine for coupling reactions of the latter type.

In an attempt to secure the dicarbobenzoxy derivative of L-arginine by treatment of a *strongly alkaline* solution of the amino acid with 2–4 equivalents of carbobenzoxy chloride under Schotten-Baumann conditions, an insoluble material precipitated which was filtered cold, washed with Na_2CO_3 solution, and the wet cake taken up in alcohol-free chloroform, dried, and concentrated *in vacuo*; on treatment with ether, the residue solidified. This material unexpectedly revealed elemental analyses conforming to sodium tricarbobenzoxy-L-argininate (I). The yield, when 4 equivalents of carbobenzoxy chloride was employed, was 70%. (*Analysis*, calculated for $C_{36}H_{31}O_8N_4Na$: N, 9.4; Na, 3.8. Found: N, 9.4; Na, 3.8.)[1] That this sodium salt was, in reality, a mixture of at least two isomeric forms was suggested by the fact that soon after the solution of some 12 g. in 100 ml. ethanol, 5.8 g. precipitated as a crystalline material (II) which, although now only sparingly soluble in alcohol, showed analyses identical with that of the parent mixture, and which, upon neutralization, yielded the corresponding crystalline free acid; $[\alpha]_D^{25} = +15.5°$ (1% in alcohol-free chloroform). (*Analysis*, calculated for $C_{30}H_{32}O_8N_4$: C, 62.5; H, 5.6; N, 9.7. Found: C, 62.2; H, 5.7; N, 9.8.) Upon concentration of the ethanolic mother liquors, an exceedingly alcohol-soluble material was obtained which, after acidification and recrystallization from methanol, analyzed for the dicarbobenzoxylated amino acid (III). Yield, 3.2 g.; m.p., 150°C.; $[\alpha]_D^{25} = -10.0°$ (1% in pyridine). (*Analysis*, calculated for $C_{22}H_{26}O_6N_4$: C, 59.7; H, 5.9; N, 12.6. Found: C, 59.3; H, 6.0; N, 12.5.)

That the precursor of dicarbobenzoxy-L-arginine (III) was, in fact, a highly alkali-susceptible isomer of II, was indicated, aside from elemental analyses, by the following: (a) carbobenzoxylation of III, in alkaline solution, proceeded with remarkable facility to yield the sodium salt of the tricarbobenzoxy derivative which, upon fractionation with alcohol, again yielded II and III; and (b) although prolonged treatment of II with ethanol led to no apparent change in its constitution, its conversion to III could be readily achieved upon reaction with one equivalent of alkali in cold ethanol.[2]

The coupling of tricarbobenzoxy-L-arginine with either glycine benzyl ester or

[1] Although the use of 2 equivalents of carbobenzoxy chloride led to the separation of sodium tricarbobenzoxy-L-argininate, the yields were necessarily lower than when more of the reagent had been employed.

[2] Complete conversion of I (the crude mixture of tricarbobenzoxy-L-arginine) to III (pure dicarbobenzoxy-L-arginine) may be accomplished by reaction of I with 1 equivalent of alkali in methanolic solution at 25°.

L-glutamic acid dibenzyl ester, via the mixed anhydride procedure, gave condensation products whose elemental analyses were consistent with those expected for the corresponding tricarbobenzoxylated dipeptide esters. With the former ester, (*Analysis*, calculated for $C_{39}H_{41}O_9N_5$: C, 64.7; H, 5.7; N, 9.7. Found: C, 64.1; H, 6.0; N, 9.6), whereas that derived from the latter ester, (*Analysis*, calculated for $C_{49}H_{51}O_{11}N_5$: C, 66.4; H, 5.8; N, 7.9. Found: C, 65.9; H, 5.8; N, 7.9).[3]

The present findings indicate that arginine readily reacts with 3 equivalents of an acyl radical, a phenomenon which it may be advisable to consider in the synthesis of peptides containing this amino acid.

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Received September 25, 1956

[3] We have reason to believe that the tricarbobenzoxylated arginines, under certain conditions, are capable of transferring a single carbobenzoxy moiety to another amino acid, comparable to the acyl transfer reactions earlier described for diacylated histidines [Bergmann, M., and Zervas, L., *Z. physiol. Chem.* **175,** 145 (1928)].

S-Methyl-L-Cysteine as a Naturally Occurring Metabolite in *Neurospora crassa*[1]

Evidence that an *S*-methyl derivative of cysteine might be a naturally occurring amino acid has recently been reported by Morris and Thompson (1, 2), who isolated *S*-methyl-L-cysteine sulfoxide from turnip roots. They point out, however, that the sulfoxide probably arises from oxidation of *S*-methyl-L-cysteine (SMC) in the plant. More recently, Zacharius *et al.* (3) have reported the isolation of SMC from the nonprotein nitrogen of the bean. We have found SMC in Neurospora and in addition have shown for the first time that SMC will support growth of strains of this organism.

[1] Report of research conducted under a grant from the Robert A. Welch Foundation.