On the structure of lysopine, a new amino acid isolated from crown gall tissue*

One of us (C.L.) has recently reported that crown-gall tissue of salsify (*Scorzonera hispanica*), tobacco (*Nicotiana tabacum*), Virginia creeper (*Parthenocissus tricuspidata*) and Jerusalem artichoke (*Helianthus tuberosus*), cultivated *in vitro* contains large quantities of an "unknown" amino acid, not found in normal tissue and present only in the free state[1]. This substance was obtained in crystalline form [m.p. 157–160°, followed by decomposition at 240°); $[\alpha]_D + 18 \pm 1°$ in water (*c*, 1.40)] after purification on Permutite C 50 and by preparative paper chromatography; with ninhydrin, this compound gives a violet color and has the following R_F values: 0.09 in butanol–acetic acid–water (4:1:5) and 0.75 in phenol/NH_3[1,2].

On a Moore and Stein column of Amberlite CG-120[3], it is eluted like alanine, between glycine and valine.

A preliminary investigation of this new amino acid has shown that it was neutral, that it was not an α-amino acid, and that half of its nitrogen was present as an $-NH_2$ group[2].

A more detailed chemical examination revealed the probable presence of two carboxyl groups, one $-NH_2$ group, and one CH_3–C grouping and absence of OCH_3 and N–CH_3.

The determination of the exact molecular formula was difficult because of the presence of water or alcohol of crystallisation which was very tenaciously retained and because neither on acetylation nor on methylation homogenous crystalline preparations were obtained. Nevertheless, the molecular formula $C_9H_{18}N_2O_4$ was considered the most probable.

An examination of the ethyl ester of the amino acid by mass spectrometry with a CEC 21–103 C mass spectrometer equipped with a heated inlet system operated at 140° gave the following information:

The spectrum of the ester exhibited intense peaks at m/e 84, 156, 184, 201 and a small peak at 274, accompanied by an even smaller one at 275, the intensity of which, relative to the others, showed the changes, on variation of the ionizing conditions, characteristic[4] for $M + 1$ peaks. The molecular weight of the ester is therefore 274. This is confirmed by the strong peak at 201, which corresponds to the loss of the carbethoxy group, one of the most important fragmentation processes for ethyl esters of α-amino acids. This fragment decomposes further to mass 184, confirmed by the presence of a metastable peak[5] at 169 (calcd. 168.4), corresponding to the loss of NH_3 which indicated the presence of a primary amino group. The fragment 184 decomposes further to mass 156 (loss of 28 mass units), suggesting the presence of a second carbethoxy group ($-COOC_2H_5 \rightarrow -COOH + C_2H_4$). The corresponding metastable peak for this process is found at 133 (calcd. 132.3).

The most intense peak of the spectrum is found at mass 84 ($C_5H_{10}N$) indicating that the two nitrogen atoms are originally attached to a common C_5-segment, probably like in lysine, where the strongest peak also is mass 84 (ref. 6). This fragment may arise via cleavage of the α-carbon-carbon bond of the lysine moiety of the diester, followed by the loss of the elements of alanine ethyl ester.

* Third communication on applications of mass spectrometry to structure problems. 2nd communication: K. BIEMANN AND J. SEIBL, *J. Am. Chem. Soc.*, 81 (1959) 3149.

The absence of a peak at 102 ($-CH(NH_2)COOC_2H_5$) excludes a primary α-amino ester[6] whereas the presence of a peak at mass 30 suggests a $-CH_2NH_2$ group. Furthermore, the amino and ester groups can not be in a 1,6 relationship to each other, because it was found that in such a case (ornithine) only the spectrum of the corresponding lactam is obtained[6].

All this evidence is most compatible with the structure (I). The only alternative formula, having the $-NH_2$ group at the γ-carbon is eliminated because of the virtual absence of a peak at mass 58 ($CH_3-CH_2-CH-NH_2$) which would have to be observed instead of mass 30.

Structure (I) has now been confirmed by synthesis. N-ε-carbobenzoxy-L-lysine was condensed with D, L-α-bromopropionic acid in presence of $Ba(OH)_2$[7].

After decarbobenzoxylation of the resulting condensation product and purification on Amberlite IRC-50 a crystalline compound was obtained, needles softening at about 160° with decomposition at 240°, $[\alpha]_D$ + 16 ± 1° in water (c, 1.1). (Found: C, 46.29; H, 8.92; N, 11.57. $C_9H_{18}N_2O_3 \cdot H_2O$, requires C, 45.75; H, 8.53; N, 11.86).

This substance was identical with the natural amino acid by the following criteria: R_F values in 3 solvents; electrophoresis at 900 V at pH 3.6 and pH 6.5; retention volume on a Moore and Stein column; infrared spectrum (in Nujol) and finally the mass spectrum of the diethyl ester.

The synthetic compound is probably still a mixture of diastereo-isomers, having the α-carbon of the alanine moiety respectively in the D and L configuration. We hope to report soon the exact stereochemistry of the natural amino acid.

We propose the name *lysopine* for the new amino acid (I) as it is the lysine analogue of octopine (II), a characteristic component of octopus muscle.

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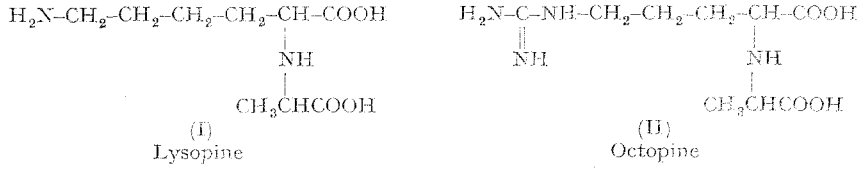

(I) Lysopine (II) Octopine

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass. (U.S.A.) K. BIEMANN

Laboratoire de Photosynthèse, Gif-sur-Yvette (France) C. LIORET

Institut de Biologie Physico-Chimique, Paris (France) J. ASSELINEAU
E. LEDERER
MRS. J. POLONSKY

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