Tetrahedron Letters No.46, pp. 3431-3436, 1964. Pergamon Press Ltd.
Printed in Great Britain.

STRUCTURE OF STIZOLOBIC ACID AND STIZOLOBINIC ACID*; TWO NOVEL AMINO ACIDS IN STIZOLOBIUM HASSJOO

Siro Senoh, Shoji Imamoto and Yoshiko Maeno

The Institute of Food Chemistry, Osaka, Japan

Takashi Tokuyama and Takeo Sakan

Faculty of Science, Osaka City University, Osaka, Japan

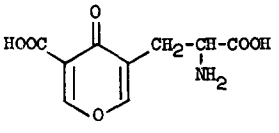
Atsushi Komamine and Shizuo Hattori

Department of Botany, University of Tokyo, Tokyo, Japan

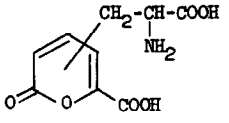
(Received 23 September 1964)

In 1959, Hattori and Komamine isolated two new amino acids from the sap of etiolated epicotyl tips of Stizolobium hassjoo and named them stizolobic acid and stizolobinic acid (1), respectively.

Stizolobic acid is a dibasic α-amino acid of m.p. 231-233°, $\lambda_{max}^{pH\ 5.0}$ 303 mμ (log ε 3.89), with a molecular formula of $C_9H_9NO_6$, and its structure was presumed to be β-(3-carboxy-γ'-pyron-5-yl)alanine (I) (1).



I



II

Stizolobinic acid does not decompose under 300°, gives positive ninhydrin reaction, forms a Cu complex, and behaves like a dibasic amino acid

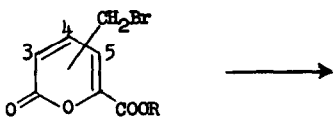
*α-PYRONE-6-CARBOXYLIC ACID DERIVATIVES. I.

are α-pyrone-6-carboxylic acid derivatives with the alanyl side-chain in the 3- and 4-positions, respectively.

The structures of stizolobinic acid (I) and of stizolobic acid (II) have now been conclusively established by the syntheses of the three possible isomers (I, II, and III) of β-(6-carboxy-α'-pyronyl)alanine.

For the synthesis of DL-stizolobinic acid (I), ethyl 3-methyl-α-pyrone-6-carboxylate (3), m.p. 111.5-112.5°, was refluxed with N-bromosuccinimide in carbon tetrachloride to afford ethyl 3-bromomethyl-α-pyrone-6-carboxylate (IV), m.p. 135.5-136.5° (Found: C, 41.80; H, 3.67; Br, 30.38. $C_9H_9O_4Br$ requires C, 41.40; H, 3.48; Br, 30.66%. $\lambda_{max}^{50\%\ EtOH}$ 303 mμ (log ε 4.03)). Condensation of IV with diethylacetamido-sodiomalonate in benzene afforded V, m.p. 153.5-155° (Found: C, 54.61; H, 6.07; N, 3.46. $C_{18}H_{23}NO_9$ requires C, 54.40; H, 5.83; N, 3.53%. λ_{max}^{EtOH} 237, 308 mμ (log ε 3.36, 4.01)). When V was refluxed in a mixture of acetic and hydrochloric acid DL-β-(6-carboxy-α'-pyron-3-yl)-alanine (VI) was obtained in good yield. The free acid did not melt below 300° (Found: C, 47.70; H, 3.97; N, 6.20. $C_9H_9NO_6$ requires C, 47.58; H, 3.83; N, 6.16%. $\lambda_{max}^{pH\ 1.4}$ 234, 304 mμ (log ε 3.38, 4.02); $\lambda_{max}^{pH\ 5.0}$ 234, 306 mμ (log ε 3.42, 4.01)).

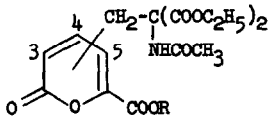
The analogous synthesis of stizolobic acid (II) started with ethyl 4-methyl-α-pyrone-6-carboxylate (3), m.p. 71-72°, which was refluxed with N-bromosuccinimide in carbon tetrachloride under irradiation or in the presence of benzoyl peroxide as a catalyst. The reaction product was chromatographed over silica gel and eluted with chloroform. A dibromide (21% yield) and a monobromide (42% yield) were obtained, in addition to unreacted ester. The dibromide is probably ethyl 4,4-dibromomethyl-α-pyrone-6-carboxylate, m.p. 72-73° (Found: C, 32.02; H, 2.78; Br, 47.02.



IV: Side-chain in 3-position, R = C_2H_5

VII: Side-chain in 4-position, R = C_2H_5

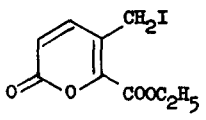
X: Side-chain in 5-position, R = CH_3 or C_2H_5



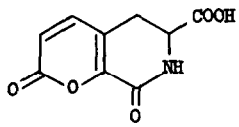
V: Side-chain in 3-position, R = C_2H_5

VIII: Side-chain in 4-position, R = C_2H_5

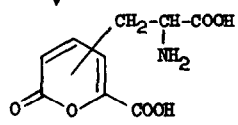
XI: Side chain in 5-position, R = CH_3



XII



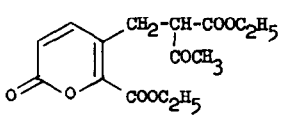
IIIB



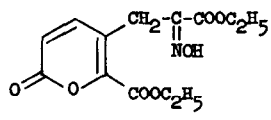
VI: Side-chain in 3-position

IX: Side-chain in 4-position

IIIA: Side-chain in 5-position



XIII



XIV

$C_9H_8O_4Br_2$ requires C, 31.79; H, 2.37; Br, 47.01%. $\lambda_{max}^{50\% \text{ EtOH}}$ 306 mμ (log ε 3.86)). The dibromide is also obtained on further bromination of the monobromide. The monobromide, ethyl 4-bromomethyl-α-pyrone-6-carboxylate (VII), m.p. 68-69.5° (Found: C, 41.89; H, 3.80; Br, 30.66. $C_9H_9O_4Br$ requires C, 41.38; H, 3.48; Br, 30.66%. $\lambda_{max}^{50\% \text{ EtOH}}$ 305 mμ (log ε 3.86)) was refluxed with diethylacetamido-sodiomalonate in ether and afforded the condensation product (VIII), m.p. 111-113° (Found: C, 54.51; H, 6.04; N, 3.51. $C_{18}H_{23}NO_9$ requires C, 54.40; H, 5.83; N, 3.53%. $\lambda_{max}^{50\% \text{ EtOH}}$ 306 mμ (log ε 3.87)). When VIII was heated in a sealed tube with glacial acetic acid, saturated with hydrogen chloride gas, for several days at 100° there was obtained in good yield, DL-β-(6-carboxy-α'-pyron-4-yl)alanine (IX), free acid, decomposition above 270° (Found: C, 47.44; H, 3.92; N, 6.01. $C_9H_9NO_6$ requires C, 47.58; H, 3.83; N, 6.16%. $\lambda_{max}^{pH\ 1.4}$ 301 mμ (log ε 3.85); $\lambda_{max}^{pH\ 5.0}$ 303 mμ (log ε 3.86)). Hydrolysis under ordinary conditions failed to produce IX due to the characteristic isomerization reactions reported in the preceding paper (1).

The two amino acids, VI and IX, obtained by the foregoing methods corresponded, respectively, with the natural stizolobinic and stizolobic acids, I and II, with respect to R_f values (paper) in five different solvent systems, behaviour on paper electrophoresis, ultraviolet spectra at various pH values and in chemical properties.

When the synthesis of the remaining isomer, DL-β-(6-carboxy-α'-pyron-5-yl)alanine (III) was carried out in an analogous fashion, the product was the cyclic lactam (IIIB). The free amino acid was obtained by a different route described below.

For the synthesis of the cyclic lactam (IIIB), methyl 5-methyl-α-pyrone-6-carboxylate (4), m.p. 131-133°, was refluxed with N-bromosuccinimide in carbon tetrachloride, with irradiation or with benzoyl peroxide

as a catalyst, to form the monobromide (X), m.p. 40-50° (Found: C, 38.66; H, 3.11. $C_8H_7O_4Br$ requires C, 38.99; H, 2.86%. $\lambda_{max}^{50\% \text{ EtOH}}$ 305 mμ (log ε 3.82)). This monobromide was refluxed with diethylacetamido-sodiomalonate in benzene to afford XI, m.p. 118-119° (Found: C, 53.64; H, 5.66; N, 3.78. $C_{17}H_{21}NO_9$ requires C, 53.26; H, 5.52; N, 3.65%. $\lambda_{max}^{50\% \text{ EtOH}}$ 304 mμ (log ε 3.87)). When XI was heated with glacial acetic acid in a sealed tube saturated with hydrogen chloride for several days at 100° the lactam (IIIB) was obtained in a quantitative yield, m.p. 249-251° (decomp.) (Found: C, 51.70; H, 3.54; N, 6.84. $C_9H_7NO_5$ requires C, 51.68; H, 3.37; N, 6.70%. $\lambda_{max}^{pH\ 1.4}$ 317 mμ (log ε 3.93); $\lambda_{max}^{pH\ 5.0}$ 319 mμ (log ε 3.91)).

Since opening of this lactam (IIIB) to the free amino acid (IIIA) has not been successful, the synthesis of the amino acid (IIIA) was achieved by another route.

Ethyl 5-bromomethyl-α-pyrone-6-carboxylate (X) was converted to the corresponding iodide (XII) with sodium iodide in acetone, m.p. 77.5-78.5° (Found: C, 35.00; H, 3.03. $C_9H_9IO_4$ requires C, 35.09; H, 2.94%). The iodide was condensed with ethyl sodio-acetoacetate in ether and the product (XIII) chromatographed on silica gel. Elution with chloroform containing 3% dioxane afforded XIII as a viscous oil. Nitrosation of XIII with butyl nitrite in 85% sulphuric acid in the cold gave ethyl α-hydroxyimino-β-(6-ethoxy-carbonyl-α'-pyron-5-yl)propionate (XIV), m.p. 120-122° (Found: C, 52.77; H, 5.34; N, 4.65. $C_{13}H_{15}NO_7$ requires C, 52.52; H, 5.09; N, 4.71%. λ_{max}^{EtOH} 309 mμ (log ε 3.84)). The α-hydroxyimino ester (XIV) was reduced with stannous chloride and hydrochloric acid in acetic acid. The reaction mixture was concentrated in vacuo, hydrochloric acid added, and the mixture warmed on a water bath to effect hydrolysis. The reaction mixture was treated with hydrogen sulfide. The

free amino acid, DL-β-(6-carboxy-α'-pyron-5-yl)alanine (IIIA), crystallized on concentration in vacuo. The free amino acid dehydrated at 178-180°, and melted with effervescence at 251-252°. The ninhydrin reaction was bluish purple. (Found: C, 44.39; H, 4.53; N, 5.71. $C_9H_9NO_6 \cdot H_2O$ requires C, 44.08; H, 4.53; N, 5.92%. $\lambda_{max}^{acid\ aq.\ soln.}$ 303 mμ (log ε 3.82)). This amino acid is easily converted to the cyclic lactam (IIIB) by fusion above 250°.

The optical resolution of the three isomeric racemic amino acids and the determination of their absolute configurations will be reported in subsequent papers.

REFERENCES

1. S. Senoh, S. Imamoto, Y. Maeno, T. Tokuyama, T. Sakan, A. Komamine and S. Hattori, This Journal (1964).

2. S. Hattori and A. Komamine, Nature 183, 1116 (1959).

3. L. Higginbothum and A. Lapworth, J. Chem. Soc. 123, 1325 (1923).

4. J. Fried and R. C. Elderfield, J. Org. Chem. 6, 566 (1941).