Tetrahedron Letters No.46, pp. 3437-3444, 1964. Pergamon Press Ltd.
Printed in Great Britain.

# α-PYRONE-6-CARBOXYLIC ACID DERIVATIVES. II.

# SYNTHESES OF DL-STIZOLOBINIC ACID, DL-STIZOLOBIC ACID AND DL-β-(6-CARBOXY-α'-PYRON-5-YL)ALANINE

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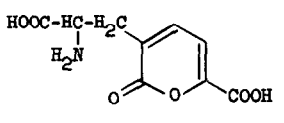
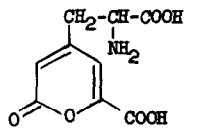
(Received 23 September 1964)

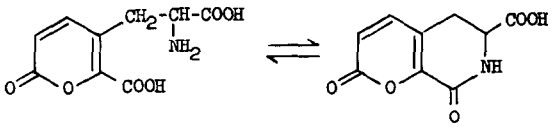
The preceding paper (1) has shown that the amino acids (2) isolated from Stizolobium hassjoo, stizolobinic acid (I) and stizolobic acid (II),



Stizolobinic Acid (I)



Stizolobic Acid (II)

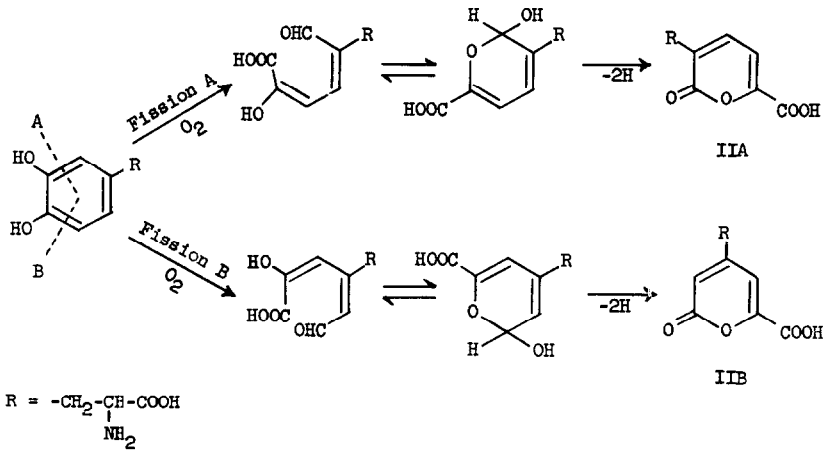


IIIA IIIB

β-(6-Carboxy-α'-pyron-5-yl)alanine (III)

on paper electrophoresis; $\lambda_{max}^{pH\ 5.0}$ 233, 306 mμ (log ε 3.41, 4.00). Ozone oxidation of stizolobinic acid in aqueous solution produces aspartic acid. Therefore, stizolobinic acid is considered to have a structure closely related to that of stizolobic acid.

Since all the reported properties of this acid can be explained as readily by an α-pyrone structure (II) and since α-pyrone-6-carboxylic acid derivatives may be formed from catechol compounds by the oxidative cleavage reaction of the "metapyrocatechase" type (2), as indicated by the hypothetical biogenesis shown below, the structure of stizolobic and stizolobinic acid has been reexamined. Because the amino acids were present in very small amounts, sufficient quantities were not available for the usual structural determinations.

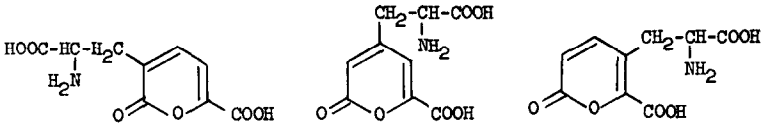



R = $-CH_2-\underset{\underset{NH_2}{|}}{CH}-COOH$

In order to determine whether the two natural amino acids had a γ- or α-pyrone structure, several γ- (3,4) and α-pyronecarboxylic acid derivatives (5,6,7) were synthesized as model substances and their acid-base dissociation constants, pKa', and ultraviolet absorption spectra at each

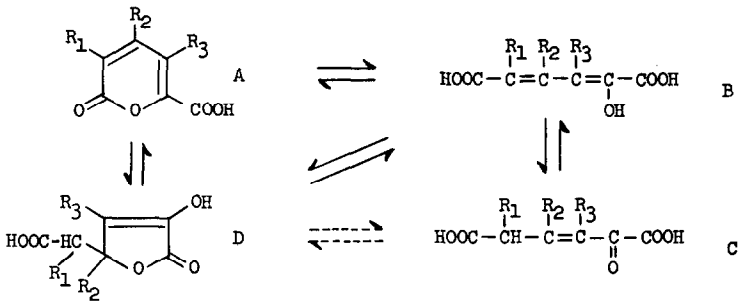
dissociation step were compared. Stizolobic and stizolobinic acids resembled α-pyrone-6-carboxylic acid derivatives (Table 1).

In general, both α-pyrones and γ-pyrones undergo facile ring opening with strong base at room temperature. In γ-pyrones ring opening is reversible under acidic conditions, whereas, in α-pyrones, it is irreversible and other products are formed. The two natural amino acids behaved like α-pyrones under these conditions. Thus, stizolobic acid and stizolobinic acid represent two of the three α-pyrone-6-carboxylic acids shown below.



IIA IIB IIC

The behavior of α-pyrone-6-carboxylic acid and its three methyl homologs was examined under various conditions, and it was found that they underwent the following four isomerization reactions (8).



These reactions were considerably affected by the position of the side-chain substituent groups $R_1$, $R_2$, and $R_3$ and by temperature, time and pH. Thus it was possible to deduce the position of the side-chain substituent by an examination of these isomerization reactions (8).

Table 1 pKa' and Ultraviolet Spectra of α- and γ-Pyrone Derivatives

| Compounds | pKa' [a] | $\lambda_{max}$ in mμ (log ε) pH 1.4[b] | pH 5.0[c] | pH 12.4[d] |
|---|---|---|---|---|
| 2-Carboxy-γ-pyrone (3) | 1.6 | ——— | 260 (4.0 )[e] | ——— |
| 2,6-Dicarboxy-γ-pyrone (4) | <2<br>2.2 | 223 (4.06)<br>273 (3.94) | 223 (4.10)<br>274 (3.99) | 385 (4.38) |
| 5-Carboxy-α-pyrone | 3.3 | 245 (3.90)<br>288 (3.61) | 239 (3.81)<br>291 (3.68) | 323 (3.96) |
| 6-Carboxy-α-pyrone (5) | 1.9 | 229 (3.34)<br>301 (3.89) | 227 (3.51)<br>303 (3.88) | 350 (>4.37) |
| 3-Methyl-6-carboxy-α-pyrone (6) | 2.2 | 239 (3.48)<br>301 (4.06) | 234 (3.53)<br>302 (4.01) | ca.<br>330 (>4.13) |
| 4-Methyl-6-carboxy-α-pyrone (6) | 2.0 | 230 (3.36)<br>300 (3.90) | 228 (3.34)<br>300 (3.89) | 258 (3.92) |
| 5-Methyl-6-carboxy-α-pyrone (7) | 2.4 | 232 (3.43)<br>305 (3.87) | 228 (3.57)<br>308 (3.87) | 345 (>4.17) |
| Stizolobic acid | <2<br>2.2<br>8.3 | 301 (3.85) | 303 (3.86) | 348 (4.07) |
| Stizolobinic acid | <2<br><3<br>8.9 | 234 (3.38)<br>304 (4.00) | 233 (3.41)<br>306 (4.00) | ca.<br>330 (>4.11) |

a Measured with Titrigraph Type SBR 2 / SBU 1 (Radiometer).
b 0.1 N aqueous hydrochloric acid solution.
c 0.1 M phosphate buffer solution.
d 0.1 N aqueous sodium hydroxide solution. Maximum absorption of the cleaved product that appears during gradual ring-cleavage reaction of the pyrone ring at room temperature under these conditions.
e Aqueous solution.

The opening of the α-pyrone ring in the three methyl α-pyrone-6-carboxylic acids was followed by ultraviolet spectroscopy in 0.1 N alkali at room temperature. The resulting spectral changes (Fig. 1) are characteristic and different for each of the three methyl homologs. The analogous data for stizolobic and stizolobinic acids are shown in Fig. 2.

Comparison of Figs. 1 and 2 demonstrates the similarity between stizolobinic acid and 3-methyl-6-carboxy-α-pyrone. Together with the ultraviolet data shown in Table 1, examination of isomerization under different conditions indicates that stizolobinic acid has a side-chain in the 3-position. Stizolobinic acid would then be β-(6-carboxy-α'-pyron-3-yl)alanine (IIA).

The behavior of stizolobic acid under base conditions did not, however, correspond with any of the side-chain substituted α-pyrone-6-carboxylic acids. Also the ultraviolet spectrum did allow a definite differentiation between the 4- or 5-substitution. The failure of stizolobic acid to form a lactam is, however, evidence against formula IIC since a compound of this structure should easily form a lactam.

The reason for the anomalous behavior of stizolobic acid was found when the two amino acids were boiled in concentrated hydrochloric acid. Stizolobinic acid (IIA) is quite stable, but stizolobic acid is abnormally labile and easily undergoes decarboxylation in the presence of oxygen to form 4-methylpicolinic acid. Investigation of this reaction suggested the isomerization scheme depicted below. If this reaction is carried out under nitrogen, intermediate B is formed via intermediate A by decarboxylation. In air, further decarboxylation occurs with the formation of 4-methylpicolinic acid. Intermediate A resembles the alkaline cleavage product of stizolobic acid. Intermediate B has the spectral properties of a dihydropyridine derivative. 4-Methylpicolinic

Fig. 1

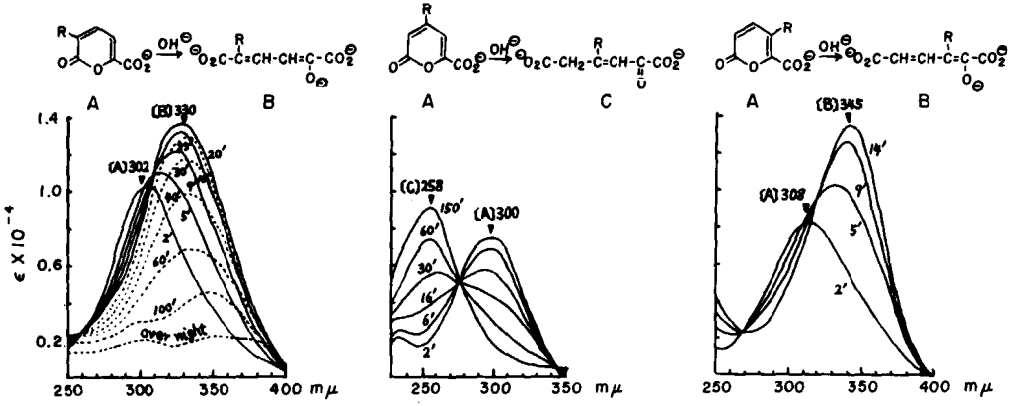
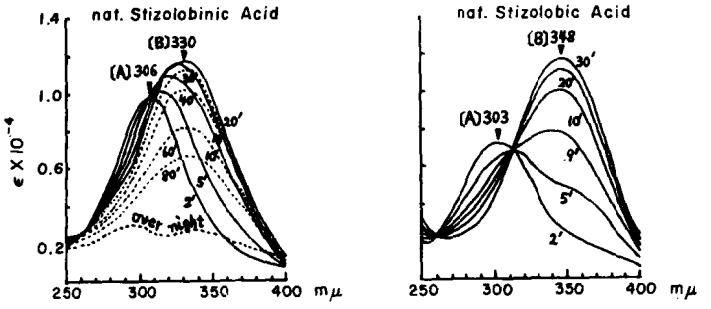
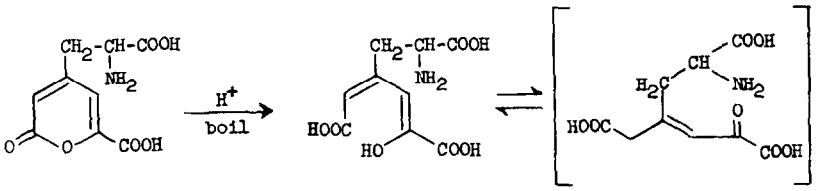



Fig. 2

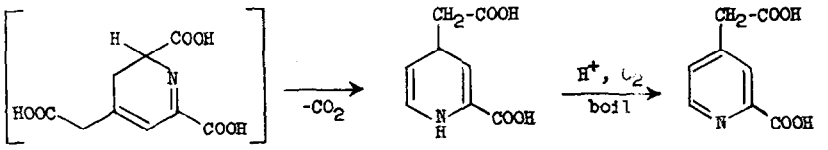



Spectral changes of monosubstituted α-pyrone-6-carboxylic acid derivatives in $^{N}/_{10}$ NaOH at r.t.

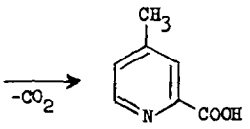
acid was identified by comparison with an authentic sample.



Intermediate A
$\lambda_{max}^{pH\ 1.4}$ 302 mμ
$\lambda_{max}^{pH\ 12.5}$ 348 mμ



Intermediate B
$\lambda_{max}^{pH\ 1.4}$ 288 mμ
$\lambda_{max}^{pH\ 12.5}$ 258 mμ



$\lambda_{max}^{pH\ 1.4}$ mμ (log ε): 221 (3.63), 257 infl. (3.17), 263 (3.76), 269 infl. (3.71).

$\lambda_{max}^{pH\ 12.5}$ mμ (log ε): 257 infl. (3.38), 264 (3.46), 270 infl. (3.42).

pKa' 2.1, 6.3

The alanyl side-chain in stizolobic acid must be in the 4-position of α-pyrone-6-carboxylic acid and the structure of stizolobic acid is best expressed by formula IIB, β-(6-carboxy-α'-pyron-4-yl)alanine. Accordingly, both stizolobic and stizolobinic acid have structures in agreement with the above proposed biogenetic pathway.

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