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2-(2-CARBOXYETHYL)-3-ISOXAZOLIN-5-ONE, A NEW ISOXAZOLINONE
DERIVATIVE FROM *LATHYRUS ODORATUS*¹⁾

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A new 3-isoxazolin-5-one derivative, 2-(2-carboxyethyl)-3-isoxazolin-5-one (1), was isolated from the immature seeds and pods of *Lathyrus odoratus*. The structure was determined by spectroscopic methods and by chemical degradation.

KEYWORDS — *Lathyrus odoratus* ; Leguminosae ; sweet pea ;
2-(2-carboxyethyl)-3-isoxazolin-5-one ; 3-isoxazolin-5-one derivative

A number of 3-isoxazolin-5-one derivatives have been isolated from legume seedlings belonging to the genera *Pisum*, *Lathyrus* and *Lens*.^{2,3)} Two of these compounds, 2-(3-amino-3-carboxypropyl)-3-isoxazolin-5-one (2) and 2-(2-cyanoethyl)-3-isoxazolin-5-one (3), are major components in sweet pea (*Lathyrus odoratus*) during development and growth⁴⁾ and have been shown to cause different forms of lathyrism.^{5,6)}

Our current interest in the distribution and the biosynthesis of these lathyrogenic compounds 2 and 3 in seeds and seedlings of *Lathyrus* species led to the isolation of a new 3-isoxazolin-5-one derivative from *L. odoratus*. We wish to report the isolation and the structure of this natural product.

A 75% aqueous ethanol extract of 160 g of fresh immature seeds and pods was concentrated to dryness and the residue dissolved in dist. water. The resulting solution was then applied on a column of Dowex 50W-X4 ([H⁺], 100-200 mesh, 4.8 x 85 cm). The column was washed with dist. water and then eluted with a linear HCl-gradient (0 - 2N HCl) monitoring by UV-detection at 265 nm. The unknown acidic compound 1 was eluted at about 0.2N HCl. The fraction containing 1 was concentrated, applied on a Biogel P-2 column (1.2 x 130 cm)

eluting with 0.3 M AcOH, and then chromatographed over Dowex 1-X4 ($[\text{HCOO}^-]$, 100 - 200 mesh, 4.8 x 30 cm) eluting with a linear HCOOH-gradient (0 - 2 N HCOOH). 1 was eluted at about 1.8 N HCOOH. Further purification was performed by preparative TLC on Si-gel 60GF₂₅₄ (0.5 mm thick, Merck) developed with BuOH — AcOH — H₂O (12: 3: 5, v/v) and then by a second Biogel P-2 column chromatography.

The final purification of 1 was performed by crystallization from water — acetone yielding colorless needles (3.0 mg). The purified 1 did not react with ninhydrin, but formed a yellow color with Bromocresol green. It behaved as an acidic compound on ion exchange resin and on paper electrophoresis at pH 3.5 using the following buffer system: AcOH — pyridine — H₂O (5: 0.5: 95, v/v).

The structure was analyzed by IR spectrum (KBr) showing bands at 2700-3400 cm^{-1} (COOH), 1720 cm^{-1} (C=O), 1580 cm^{-1} (C=C) and at 1420 cm^{-1} (CH₂), by UV spectrum ($\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 265 nm) and by MS(EI) with M^+ at m/e (rel. int.) 157(48) and peaks at 98(100), 71(36), 45(48) and 43(53) which are characteristic of the 2-substituted, 3,4-unsubstituted 3-isoxazolin-5-one ring.^{2,7)} High resolution EI-MS measurement gave the molecular formula of $\text{C}_6\text{H}_7\text{NO}_4$, M^+ 157.0366 (calcd. 157.0375). The ¹H-NMR spectrum (100 MHz, D₂O, TMS) of 1 showed two olefinic signals at δ 8.25 ppm (1H, d, J =3.5 Hz, 3-H) and δ 5.17 ppm (1H, d, J =3.5 Hz, 4-H), which are characteristic of the 3-isoxazolin-5-one ring.^{2,7)} The high field signals in the ¹H-NMR spectrum of 1 were comparable to those of 3.⁷⁾ Thus, the signals at δ 2.76 ppm (2H, t, J =6.4 Hz, CH₂-COO⁻) and δ 4.17 (2H, t, J =6.4 Hz, CH₂-N) of 1 were assigned to a carboxyethyl group at the 2-position of the ring.

The structure of 1 was further confirmed by chemical degradation. Treatment of 1 with 1 N HCl at 105-110°C for 60 min yielded one ninhydrin-positive substance. It was identified as β -alanine by comparison with authentic material on TLC and on an amino acid analyzer. Photolysis of 1 by UV-irradiation in aqueous solution also yielded β -alanine. This behaviour is similar to that of other natural 3-isoxazolin-5-ones described before.²⁾

From these results we propose the structure of 1 as 2-(2-carboxyethyl)-3-isoxazolin-5-one. The lower homologue of 1, 2-carboxymethyl-3-isoxazolin-5-one, had been isolated before from sweet pea seedlings.²⁾ It seems likely that 1 is a metabolite of 3 in sweet pea plants.

The chemical synthesis and the biosynthesis of this compound are in progress.

REFERENCES AND NOTES

- 1) This is the twelfth natural heterocyclic compound to be found in the ongoing study of *Lathyrus* and *Pisum* species; besides isowillardine and willardiine all compounds contain the 3-isoxazolin-5-one ring.
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