Communications to the Editor

[Chem. Pharm. Bull. 32(6)2450—2451(1984)]

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

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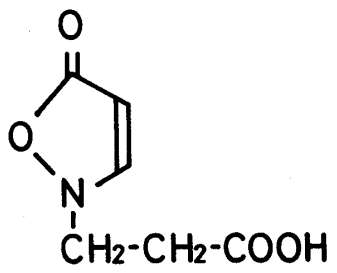
A new 3-isoxazolin-5-one derivative, 2-(2-carboxyethyl)-3-isoxazolin-5-one (1), was isolated from the immature seeds and pods of *Lathyrus odoratus*. The structure was determined by spectroscopic methods and by chemical degradation.

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 growth[4)] 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.



1

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 ([$HCOO^-$], 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 $60GF_{254}$ (0.5 mm thick, Merck) developed with BuOH — AcOH — H_2O (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 Bromcresol 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 — H_2O (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} (CH_2), by UV spectrum ($\lambda_{max}^{H_2O}$ 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 $C_6H_7NO_4$, M^+ 157.0366 (calcd. 157.0375). The ^{1}H-NMR spectrum (100 MHz, D_2O, 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 ^{1}H-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, CH_2-COO^-) and δ 4.17 (2H, t, *J*=6.4 Hz, CH_2-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 isowillardiine and willardiine all compounds contain the 3-isoxazolin-5-one ring.
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(Received April 10, 1984)