THE ISOLATION AND STRUCTURE ANALYSIS OF 2-(2-CYANOETHYL)-3-ISOXAZOLIN-5-ONE, 2-(β,D-GLUCOPYRANOSYL)-3-ISOXAZOLIN-5-ONE AND 2-CARBOXYMETHYL-3-ISOXAZOLIN-5-ONE FROM LATHYRUS ODORATUS.

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Three 2-substituted isoxazolin-5-ones have been isolated from Lathyrus odoratus seedlings. Structural analysis by spectrometric and chemical methods determined their formulas as 2-(2-cyanoethyl)-3-isoxazolin-5-one, 2-(β,D-glucopyranosyl)-3-isoxazolin-5-one and 2-carboxymethyl-3-isoxazolin-5-one. The structures of the first two compounds were confirmed by their chemical synthesis.

INTRODUCTION

The germination of Pisum sativum and of Lathyrus odoratus seedlings is accompanied by the production of a series of strongly UV-absorbing compounds. Their structures were shown to be derivatives of the heterocyclic uracil and isoxazolin-5-one rings (Lambein and Van Parijs, 1968 ; Lambein et al., 1969 ; Lambein, 1973). By comparing the properties of synthetic and of natural isoxazolin-5-ones, the structure of the heterocyclic ring in the natural products was previously confirmed (Van Rompuy et al., 1973). Amongst leguminosae, screened so far, Lathyrus odoratus is the richest source of isoxazolin-5-ones. One of the derivatives reaches a top concentration of 3 % of the dry matter. The purification and structure analysis of three isoxazolin-5-ones from Lathyrus odoratus is described below.

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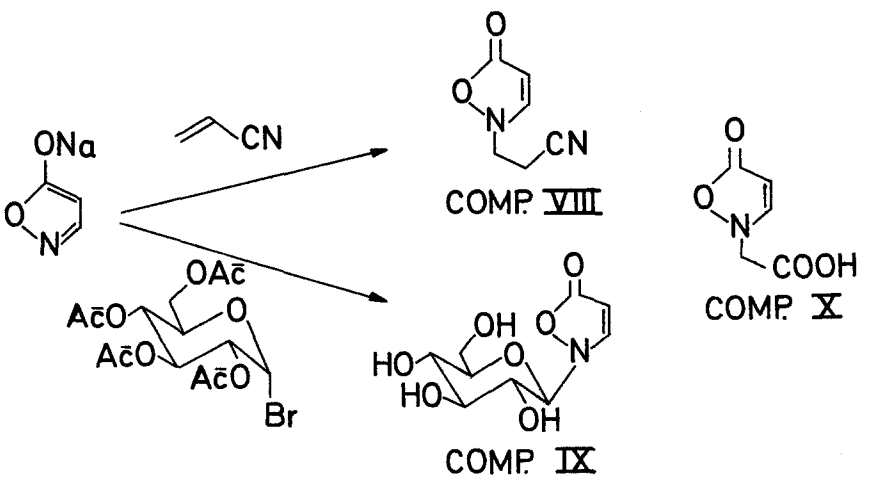


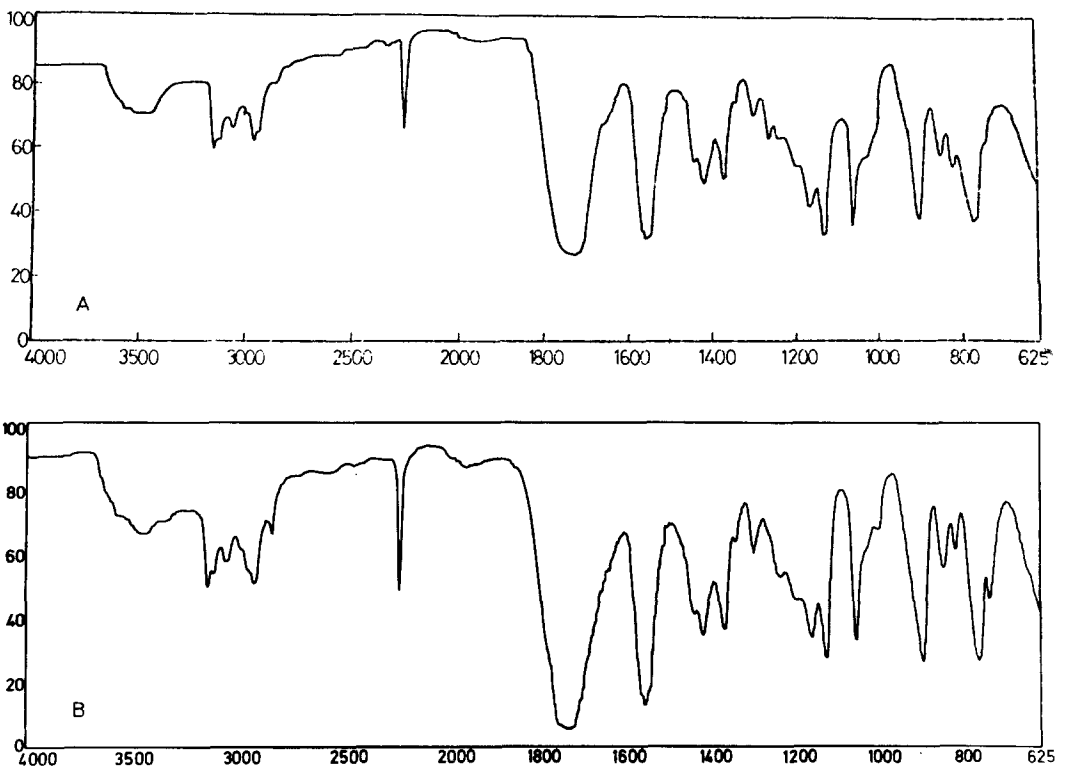
ISOLATION AND PURIFICATION

Six day old seedlings of Lathyrus odoratus (100 g) were homogenized in 70 % ethanol. The mixture was centrifuged at 5000 r.p.m. and the supernatant was brought on a Dowex 50 W (H^+) column (ϕ 5 cm, h 1 m), after removal of the ethanol. The column was eluted with a linear gradient of 10 l (0-2N HCl). The elution pattern was recorded by a UV-detector. The peak emerging at a concentration of 0.5 N HCl, contained component VIII or 2-(2-cyanoethyl)-3-isoxazolin-5-one. Component IX or 2-(β,D-glucopyranosyl)-3-isoxazolin-5-one is not retained on the resin, while component X or 2-carboxymethyl-3-isoxazolin-5-one emerges at a concentration of 0.25 N HCl. The three components were subsequently purified by passing them separately through a Dowex 1 ($HCOO^-$) column, eluted with CO_2-free water. Component VIII was slightly retarded, component IX was not retained, and component X was eluted with 4 N HCOOH after washing the column with water. The aqueous solution of component VIII was repeatedly extracted with chloroform. After drying the chloroform with $MgSO_4$, the chloroform was evaporated to yield component VIII as a pure liquor. Component IX was purified by preparative paper chromatography in butanol : acetic acid : water 60 : 15 : 25 . The product thus obtained was finally passed through Dowex 50 and Dowex 1 columns again. Component X could be crystallized after removal of the formic acid.

STRUCTURAL ANALYSIS

Component VIII : 2-(2-cyanoethyl)-3-isoxazolin-5-one.

This structure was suggested by NMR, UV, IR and mass spectra. Confirmation of the structure was obtained by chemical synthesis. The identity of the natural component VIII and the synthetic 2-(2-cyanoethyl)-3-isoxazolin-5-one is illustrated by comparing their IR spectra (fig. 2). The other spectral data and the photo-



IR. SPECTRUM OF THE NATURAL(A) AND OF THE SYNTHETIC(B) 2-(CYANOETHYL)-3-ISOXAZOLIN-5-ONE

chemical breakdown are published elsewhere (Van Rompuy, 1973).

Component IX : 2-(β,D-glucopyranosyl)-3-isoxazolin-5-one.

The UV spectrum (λ_{max} 260 nm) suggested the presence of a N-substituted isoxazolin-5-one. The positive anthrone reaction pointed to a hexose. By means of the enzymatic reaction with

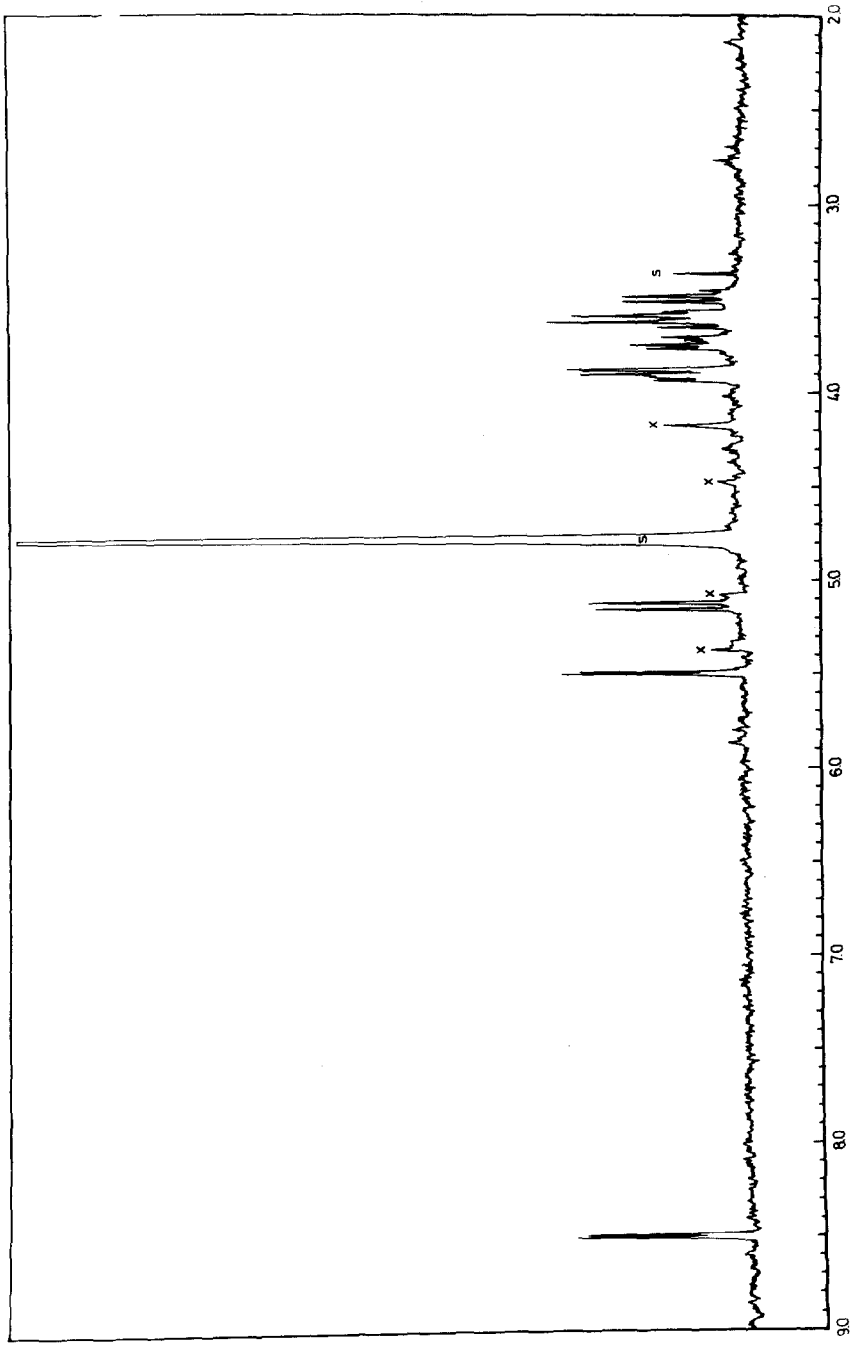


Fig. 3.

300 MHz NMR spectrum of 2-(β,D-glucopyranosyl)-3-isoxazolin-5-one in D_2O.

X = spinning side band, S = solvent peak.

glucose oxidase, the presence of bound D-glucose was established. The 300 MHz NMR spectrum confirmed the structure of the heterocyclic ring, characterized by two doublets at 5.5 and 8.5 p.p.m. (J value 3.5 Hz). From the NMR signals of the glucose moiety it appeared that the glucopyranose ring was attached at C-1'. The β-configuration resulted from the J value (8.5 Hz) of the C-1'-H doublet (5.1 p.p.m.). The structure was confirmed by chemical synthesis. The sodium salt of isoxazolin-5-one reacted with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosylbromide to yield 2-(β,D-glucopyranosyl)-3-isoxazolin-5-one. Component IX and the synthetic compound showed identical R_f values after t.l.c. in three solvents, and p.c. in four solvents. They also showed the same UV spectrum and the same sensitivity towards UV-irradiation. The anthrone reaction, the glucose oxidase reaction and the NMR spectrum of the synthetic glucoside were in agreement with component IX.

Component X : 2-carboxymethyl-3-isoxazolin-5-one.

The UV spectrum (λ_{max} 265 nm) pointed to a N-substituted isoxazolin-5-one. After alkaline degradation of photochemical breakdown, the amino acid glycine was produced. This suggested the carboxymethyl group as a possible substituent. The NMR spectrum showed two doublets at 5.3 and 8.3 p.p.m. (J value 3.5 Hz) from the isoxazolin-5-one ring, and a singlet at 4.8 p.p.m. (2 protons) from the carboxymethyl group.

EXPERIMENTAL

UV-spectra were recorded on a Gilford 2400 or a Cary 14 spectrophotometer. IR-spectra were recorded on a Perkin-Elmer 257 spectrophotometer. NMR spectra were recorded on a Varian T-60 or a Varian HR-300 spectrometer. The anthrone reaction was carried out, according to the procedure, described by S. Colowick and N. Kaplan in Methods in Enzymology III pg 4. The extinction at 260 nm (ε = 10.400) of the untreated component IX was compared with the extinction at 620 nm after the anthrone reaction. The molar ratio UV-absorbing ring : glucose was 1 : 1. The glucose oxidase reaction was carried out after hydrolyzing the glucoside for 2 h in 0.2 N HCl at 100° C. The procedure described by K. Kusai et al. (1960) was followed, using a Boehringer blood sugar TC-M-1 test set. The extinction at 260 nm of untreated component IX was compared

with the extinction at 430 nm after the glucose oxidase reaction. The molar ratio UV-absorbing ring : glucose was 1 ± 1.

Synthesis of 2-(β,D-glucopyranosyl)-3-isoxazolin-5-one :

36 g 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosylbromide was added to 9.2 g of the sodium salt of isoxazolin-5-one (Van Rompuy, 1973), dissolved in 200 ml dry methanol. After stirring overnight, the reaction was stopped by adding a trace amount of water. The solution was deionized with Dowex 50 (H^+) and Dowex 1 (OH^-) resin, avoiding pH values above 8. Water was removed by repeated evaporation from an absolute ethanol/benzene mixture. The acetyl protective groups were then removed in dry methanolic solution by a trace amount of NaOMe. After removal of the methanol, the glucoside was purified by preparative p.c. in butanol : acetic acid : water 60 : 15 : 25 (R_f 0.31). Further treatment is described under isolation and purification of the natural component IX.

R_f values of component IX :

Silica gel t.l.c. :	$CHCl_3$:MeOH 60:40	0.49
	BuOH:$HA\bar{c}$:H_2O 60:15:25	0.43
	IsopropOH:H_2O 90:10	0.65
Whatmann nr.1 p.c.:	BuOH:$HA\bar{c}$:H_2O 60:15:25	0.31
	IsopropOH:H_2O 80:20	0.80
	$Et\bar{A}c$:$HA\bar{c}$:H_2O 70:15:15	0.60
	IsopropOH:BuOH:H_2O 70:10:20	0.54

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