A NEW UV-SENSITIVE HETEROCYCLIC AMINO ACID FROM PEA SEEDLINGS : 2-ALANYL-3-ISOXAZOLIN-5-ONE.

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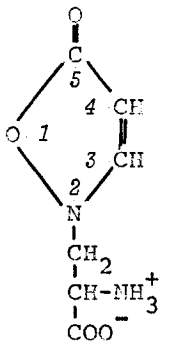
A new α-amino (compound I) has been isolated and purified from pea seedlings. The chemical structure was analyzed by chemical methods and by spectroscopy. The structure was found to be 2-alanyl-3-isoxazolin-5-one. Some properties of this compound were compared to those of synthetic isoxazolin derivatives. The mono-acetyl derivative of I was synthesized. The compound is very sensitive to UV-irradiation.

INTRODUCTION

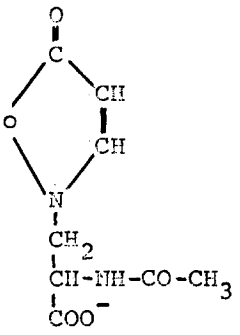
In a previous communication we described the isolation and characterization of 1-alanyl-uracil (willardiine, IV) and 3-alanyl-uracil (*iso*-willardiine) from pea seedlings (1). Meanwhile, the presence and the structure of 3-alanyl-uracil are confirmed by other authors (2).

The quantitatively most important compound (compound I), which appears during germination of *Pisum sativum*, also shows an α-amino-acid character with a specific UV-absorption spectrum. The product was tentatively identified as 3-alanyl-5-ribosyl-6-amino-uracil (3). This preliminary result was based on elementary analysis, colour reactions, UV-spectra and chemical transformation in alkaline and acid media and on UV-sensitivity; the latter properties of compound I were compared with those of a great number of 6-amino-uracil derivatives and of Ψ-uridine.

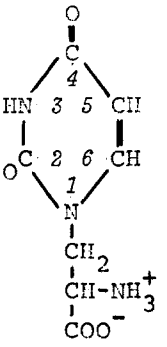
However, further examination by NMR-spectroscopy has demonstrated the absence of a sugar moiety and points to the presence of a heterocyclic ring which is different from a uracil ring. Reexamination of old data, some new experiments, and comparison with recently synthesized products, clearly indicate the following structure : 2-alanyl-3-isoxazolin-5-one (I).



I



N-acetylated I



IV

ISOLATION AND PURIFICATION

The isolation and purification of compound I were described in a previous communication (1). From 565 g 6 days old pea seedlings, we obtained 810 mg product, crystallized from water. Further purification was achieved by recrystallization from a water : ethanol mixture and a water : aceton mixture.

PROPERTIES OF COMPOUND I

Elementary analysis :	found	: C :	41.3 %	N :	16.0 %	H :	4.86 %
	theor.	: C :	41.86 %	N :	16.28 %	H :	4.65 %
Decomposition point :	203-205°C (non corrected)						
UV-spectrum : pH 2 :	λ max :	265 nm	ε max :	12 300			
	λ min :	222 nm	ε min :	1 190			
pH 10 :	λ max :	267 nm	ε max :	13 190			
	λ min :	227 nm	ε min :	1 105			

By spectrophotometric titration, a pK of 7.9 was found. Potentiometric titrations showed pK-values of 7.75 (α-amino-group) and 1.50 (carboxyl-group). In 10 % formaldehyde, the pK-value of the deprotonation of the α-amino-group was lowered to 5.45. Under these conditions, an equivalent weight of 175.6 was determined. Due to the low solubility of compound I in cold water (sat. : 0.3 %, in hot water : 3.7 %) one could only get a rough estimate of theM.W. by cryoscopy, this was found to be 188 (the theoretical value for $C_6H_8N_2O_4$ is 172).

After spraying of paperchromatograms with ninhydrin, spots of I give first a red-orange color, which disappears gradually and become brown-purple. A solution of I gives with ninhydrin reagent (6) a purple color.

PREPARATION AND PROPERTIES OF ACETYLATED COMPOUND I

Compound I, which is not soluble in organic solvents, was acetylated in water. 75 mg crystalline product was dissolved in 5 ml water. Acetic anhydride was added in 100 µl portions under vigorous mixing. The reaction was followed by paperchromatography. After a second addition of acetic anhydride, most of the product was transformed to the monoacetyl derivative. The mixture was dried under vacuum and crystallized twice from absolute ethanol.

14.5 mg pure crystals were obtained.

Elementary analysis :	found	: C :	44.96 %	N :	12.94 %	H : 4.98 %
	theor.	: C :	44.86 %	N :	13.08 %	H : 4.67 %

UV-spectrum : no spectral changes are visible between pH 2 and 12.

λ max : 267 nm	ε max : 12 510
λ min : 232 nm	ε min : 720

The acetylated product has a negative charge at neutral pH and does not react with ninhydrin.

THE α-AMINO ACID MOIETY

The α-amino acid character of compound I was demonstrated by the ninhydrine reaction at room temperature and by paper chromatography in the presence of Cu^{++}-ions. After hydrolysis in 6 N HCl, at 105°C, a diamino acid was found and identified as α,β-diamino-propionic acid by paper chromatography and electrophoresis together with an authentic sample. Reaction with fluoro-2,4-dinitrobenzene, followed by treatment in 6 N HCl at 105°C, resulted in the formation of α-(2,4-dinitrofenyl-amino)-β-amino-propionic acid. The α-aminogroup must therefore be formed as the result of the degradation of the heterocyclic ring.

THE HETEROCYCLIC RING

The heterocyclic ring was studied by spectroscopy, and by comparison of the specific properties with those of synthetic products.

Spectroscopy

Fig. 1 shows the UV-spectra of compound I in water at pH 2 and 10. Spectrophotometric titration indicated only one dissociation (pK = 7.9). The bathochromic shift (2 nm) is due to the deprotonation of the α-aminogroup. A similar shift (3.5 nm) and pK-value (8.0) were obtained for the deprotonation of the α-amino group in 1-alanyl-uracil (compound IV). The UV-spectrum of the N-acetyl derivative of compound I is very similar to the spectrum of the original deprotonated compound and shows no changes between pH 2 and 10. A broad and low absorption mini-

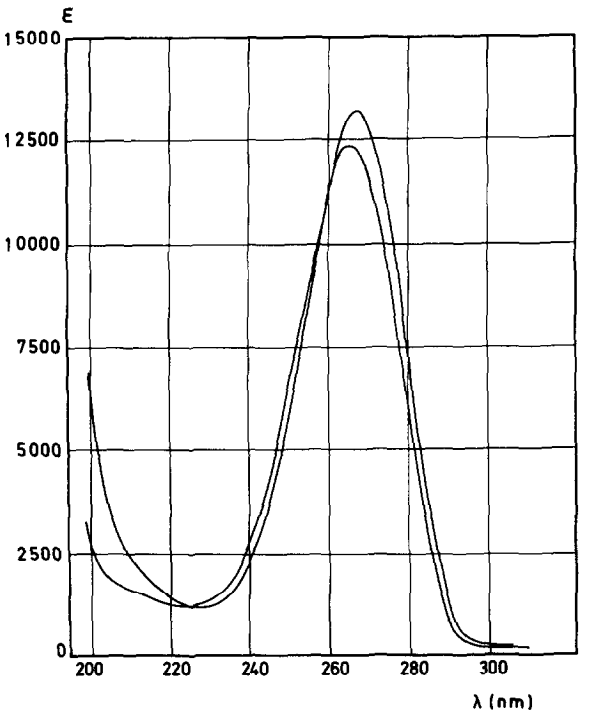


Fig. 1 : UV-spectrum of compound I in water at pH 2 (λmax = 265 nm) and at pH 10 (λmax = 267 nm). In ordinate : the molar extinction ε.

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mum between 210 nm and 240 nm is found in the spectra of all 6-amino-uracil derivatives and, even more characteristically, in the spectra of some 2-substituted isoxazolin-5-one derivatives (*e.g.* 2,4-dimethyl-3-isoxazolin-5-one), which were only recently synthesized (4) (see Fig. 4).

The IR-spectra of I and of its N-acetyl derivative are shown in Fig. 2. The band characteristic for the α-aminogroup at 4.87 μ is not present in the acetyl derivative. The band at 5.84 μ is comparable to the carbonyl-band at 5.80 μ in 2,4-dimethyl-3-isoxazolin-5-one.

The NMR-spectrum of compound I in D_2O (Fig. 3) is comparable with the spectrum of 3-alanyl-uracil (2). The peaks at 3.9 ppm and 4.3 ppm are due to the alanyl moiety. The doublets at 5.23 ppm and 8.13 ppm a due to the protons at the double bound ; their coupling constant of 3.5 cps (7 cps in uracil) indicates that the structure of the ring must be different from a uracil ring.

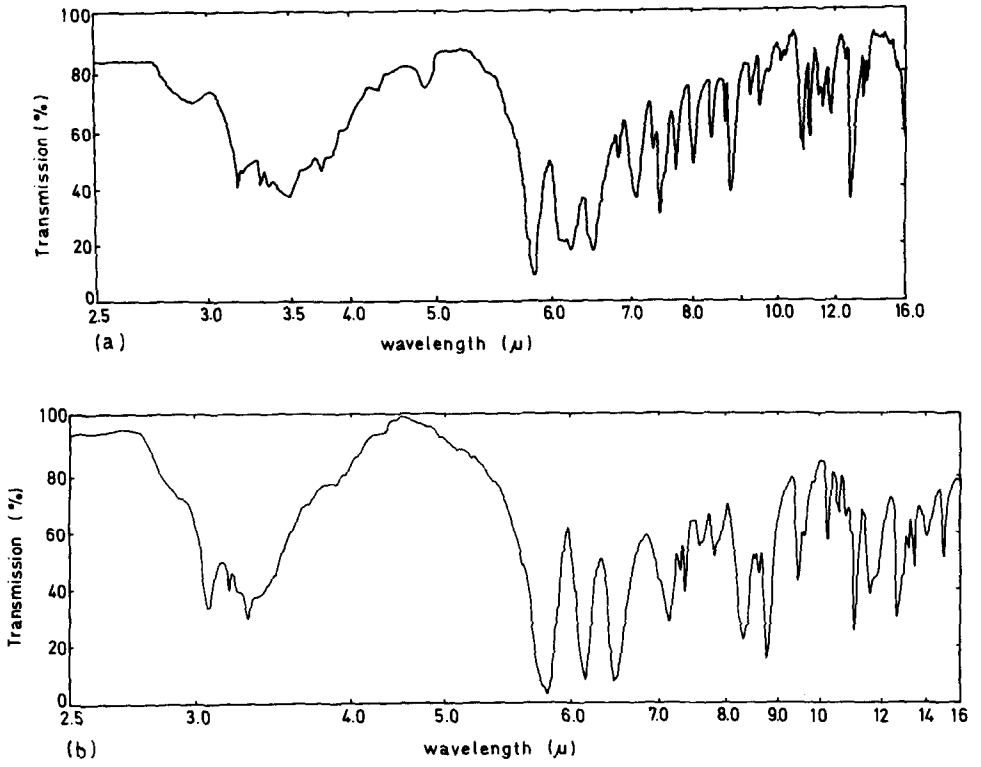


Fig. 2 : IR-spectra of compound I (a) and of the mono-acetyl derivative of compound I (b) in a KBr disc.

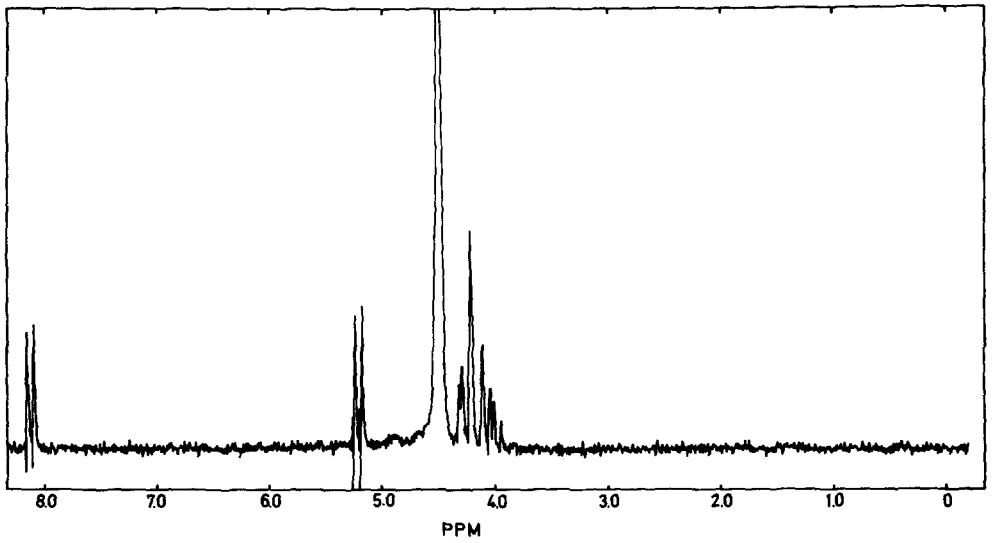
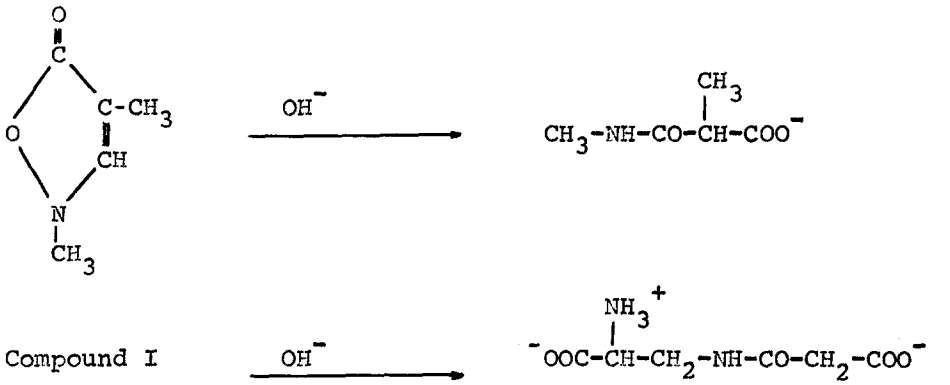


Fig. 3 : NMR-spectrum of compound I at 60 MHz in D_2O, at room temperature. TMS was used as an external standard.

ALKALINE DEGRADATION

Compound I undergoes irreversible transformations at room temperature at pH-values higher than 10. The specific UV-spectrum disappears. One of the products formed is an α-amino acid, with a negative charge at neutral pH. After hydrolysis of this product in 6 N HCl at 105°C, α,β-diamino-propionic acid is found. This behaviour is in agreement with the alkaline ring-opening of N-substituted 3-isoxazolin-5-ones with a proton at position 3. In this case, a malonyl-derivative is formed (5) :



DEGRADATION BY UV-LIGHT

Both compound I and 2,4-dimethyl-3-isoxazolin-5-one, show a very similar sensitivity to UV-irradiation (λ = 253.7 nm)(Fig. 4). The rate of these photochemical transformations is about 20 times higher than the rate of photolysis of uridine, which is commonly used as a standard in photochemical reactions.

DISCUSSION

A new heterocyclic amino acid (compound I) was isolated and purified from young pea seedlings.

The product undergoes photochemical transformation and alkaline degradation, in agreement with the behaviour of isoxazolin-5-one derivatives, recently synthesized (4).

The UV- and NMR-spectra, the elementary analysis, estimation of the molecular weight and the structure of degradation products, very clearly point to the following structure : 2-alanyl-3-isoxazolin-5-one.

The mono-acetyl derivative of compound I was prepared and purified. The elementary analysis of this derivative is also in agreement with the

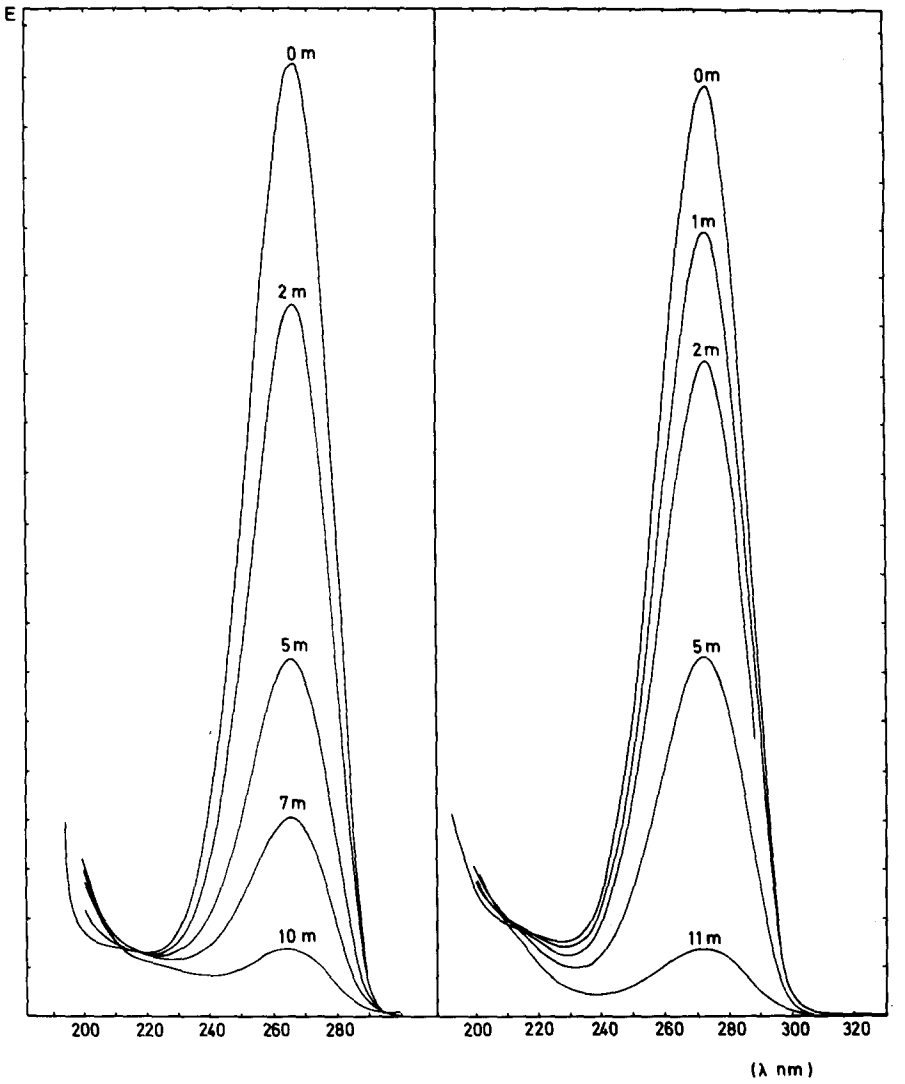


Fig. 4 : Evolution of the UV-spectra of compound I (left) and of 2,4-dimethyl-3-isoxazolin-5-one (right) in distilled water under UV-irradiation (λ = 254 nm). Time of irradiation in minutes (m) is indicated in the figure. The solutions were protected against hihg energy radiation by a CH_3COONa-solution.

proposed structure.

The simultaneous appearance of compound I and of compounds II (3-alanyl-uracil) and IV (1-alanyl-uracil) (1) during the growth of the seedling and the structural relationship between these compounds, suggest a possible biochemical relationship between the uracil and the 3-isoxazolin-5-one rings in the metabolism of the pea seedling.

The uncommon high sensitivity towards UV-light of this natural product may indicate that it plays a role in photobiological mechanisms

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