ISOXAZOLIN-5-ONES

CHEMISTRY AND BIOLOGY OF A

NEW CLASS OF PLANT PRODUCTS[†]

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A group of uv-sensitive heterocyclic compounds
characterized as isoxazolin-5-ones were found in some
legume seedlings. Their isolation, purification and
structural identification are explained. With emphasis
on the particular properties of the natural products
- chemical and photochemical degradation - the chemistry
of N-substituted isoxazolin-5-ones is reviewed. The
second part of this paper deals with the distribution,
metabolism and with some biological aspects of the
title compounds. Their possible origin and function
are discussed.

In some legume seedlings isoxazolin-5-one derivatives are pre-
sent in high concentrations. The natural occurrence of the isoxa-
zolin-5-one ring was first recognized in 1969[1]. From a small number
of plant sources, eight derivatives have now been characterized,
four of these are α-amino acids.

[†] Dedicated to Professor Tsunematsu Takemoto on the occasion of his retirement.

Legume seeds form a rich source of non-protein amino acids[2-6], even so that chemotaxonomic studies can be based on their distribution. Seedlings of the garden pea (Pisum sativum) have been frequently used to study nitrogen metabolism, nitrogen transport[6-8] or to study the occurrence and the metabolism of the free amino acids[9-16], particularly homoserine[17-24].

There is little doubt that some unknown compounds mentioned by a number of authors may be identical to the heterocyclic amino acids studied in our laboratory[1,25,26]. Other authors who used dilute alkali in their extraction procedure may have destroyed the isoxazolin-5-one amino acids in the isolation process[10-12], or they may have used older plant material where the isoxazolin-5-one derivatives are virtually absent[17,24].

In the authors' laboratory, two uracil amino acids and eight isoxazolin-5-one derivatives have been isolated and characterized. Besides their chemical name, they are also designated by a Roman numeral (see Fig. 1), avoiding the introduction of new trivial names.

Isolation and identification

Van Parijs and coworkers, while looking for nucleic acid precursors in the elongating axis of pea seedlings, found four products with promising uv spectra and having α-amino acid properties[27]. Two of these products were rather stable, eventually it was concluded that they were the isomeric β-uracil-alanines : willardiine (IV) and isowillardiine (II)[25]. Two other products (I and III) were very sensitive to uv-irradiation and to dilute alkali. The uv-spectra of I and III in water at different pH were very similar to those of 6-amino uracil derivatives and it
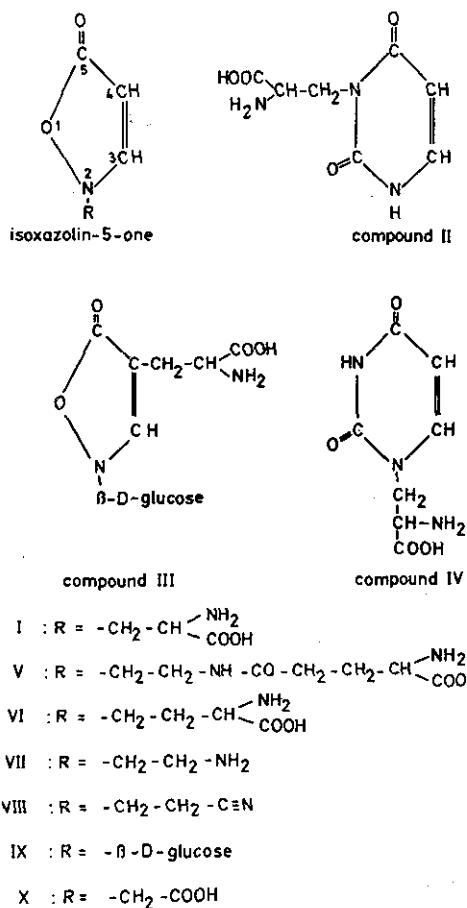
Fig. 1 : Structures of heterocyclic compounds isolated from 6-day-
old seedlings of pea (Pisum sativum) (I-IV) and from 10-day-old
seedlings of sweet pea (Lathyrus odoratus) (I, III, V-X).

I : β-(isoxazolin-5-one-2-yl)-alanine, II : β-(uracil-3-yl)-alanine
or isowillardiine, III : β-(2-β-D-glucopyranosyl-isoxazolin-5-one-
4-yl)-alanine, IV : β-(uracil-1-yl)-alanine or Willardiine,
V : 2-(γ-glutamyl-aminoethyl)-isoxazolin-5-one, VI : α-amino-γ-
(isoxazolin-5-one-2-yl)-butyric acid, VII : 2-aminoethyl-isoxazolin-
5-one, VIII : β-(isoxazolin-5-one-2-yl)-propionitrile, IX : 2-β-
D-glucopyranosyl-isoxazolin-5-one, X : 2-carboxymethyl-isoxazolin-
5-one.

was suggested that these products bear some structural relationship to the plant pyrimidines vicine and convicine[28]. When the nmr spectra became available the presence of a five membered heterocyclic ring became clear. After comparison of the chemical and spectral properties of the natural products I and III with synthetic N-substituted isoxazolin-5-ones obtained as a gift from De Sarlo[29] the pieces of the puzzle fell into place.

For the isolation of the isoxazolin-5-one derivatives, 6-day-old dark grown pea seedlings or 10-day-old dark grown sweet pea (Lathyrus odoratus) seedlings were generally used. For preparative isolation the cotyledons were discarded because of the much lower concentration on a dry weight basis. The tissue was extracted by cold 70 % ethyl alcohol, 1 N perchloric acid or 5 % trichloro acetic acid ; or the pressed out juice was dialyzed against water. The extracts were separated by cation exchange chromatography on Dowex 50 W (H^+), from which IX, X and VIII were eluted out with water in this order. The Dowex column was eluted further with a linear gradient of HCl (0-2 N), total volume 10 l for a bed volume of about 1.25 l. The peak containing III was eluted out after 3.3 l. (0.6 N). I, II and IV were eluted out together at about 1 N HCl. V and VI were eluted out at 1.5 N HCl as a double peak, the front part containing mainly V. VII was eluted out at about 2 N HCl. Further purification was achieved by passing the products, dissolved in H_2O, through a Dowex 1 ($HCOO^-$) column, where only VIII was slightly retained but was eluted out with H_2O and the acidic X was eluted out with 2 N HCOOH. Crystallization as a purification step could be used with I, II, III, V, VI and X. VII and IX were purified by paper chromatography. VIII could be easily purified because of the non-ionic adsorption on both Dowex 1 and Dowex 50 resins and the solubility in chloroform.

The products can be identified by paper chromatography : the Rf values in four solvents were reported[27]. After reacting with a ninhydrin spray, compound I gives an unusual red-orange color which changes gradually to purple after 1 or 2 hours. VII gives a similar but stable color. In the same conditions VIII gives a faint turquois color like β-amino propionitrile. The use of a conventional amino acid analyzer, combining uv-detection (254 or 260 nm) with ninhydrin detection, permits the localization of the compounds I to VIII (see Fig. 2).

The identity of the isoxazolin-5-one derivatives can be confirmed by using their specific properties : a high sensitivity to uv-irradiation and a rapid degradation in dilute alkali. Furthermore, the uv-photolysis products can be easily identified.

CHEMISTRY

Uv spectra The natural occurring 2-substituted-3-isoxazolin-5-ones have only one tautomeric form, which makes the notation 3 superfluous. The chromophore is very similar to the chromophore of some important natural pyrimidines hence the uv-spectra are very similar.(λ_{max} around 265 nm, λ_{max} IX at 260 nm). In alkaline solution a bathochromic shift of 2 nm is observed in the uv spectra of I and III, with a spectrophotometric pK-value of 7.9[1] and about 8[26] respectively. This coincides with the deprotonation of the α-amino group in the side chain. In the uv-spectrum of VI a shift of 1 nm is observed at alkaline pH.

Alkaline degradation In alkaline medium the natural isoxazolin-5-ones are unstable. At room temperature the uv absorption disappears irreversibly with a half life time of about 2 hours at pH
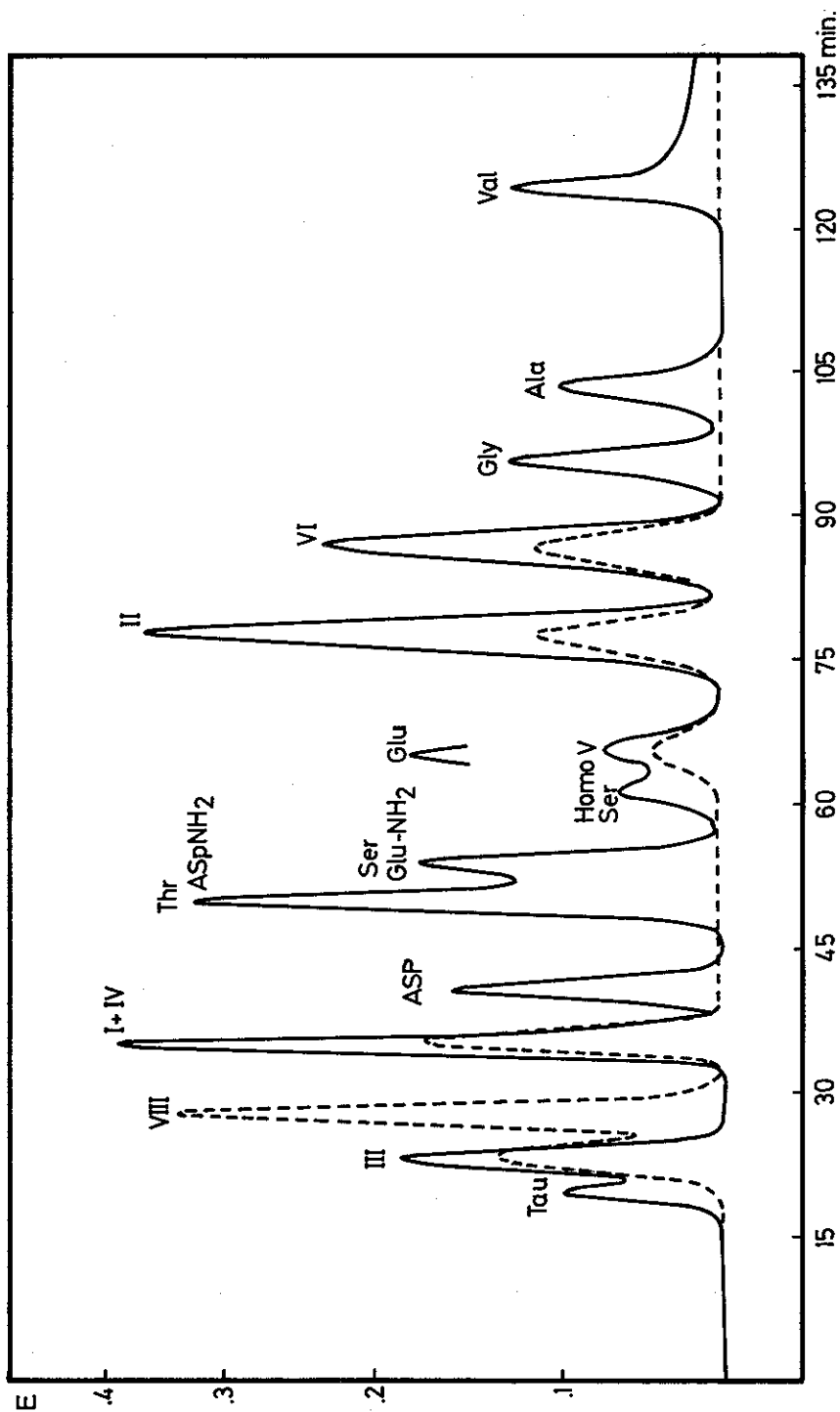
Fig. 2: Localization of the heterocyclic amino acids present in pea or in sweet pea seedlings, relative to some common acidic and neutral ones separated by automated amino acid analysis. Full line indicates absorbance after ninhydrin reaction, the broken line the uv-absorbance.

10 and about 6 min at pH 12. At pH 12 the half life time of I,
III and 2,4-dimethyl isoxazolin-5-one is 6 min, 37 sec and 8 min
respectively. In the presence of 4 % formaldehyde the half life
time is extended three to tenfold[31]. The heterocyclic ring is
hydrolyzed in 6 N HCl at 100°C for 18 hrs. After heating in
1 N HCl or H_2SO_4 for 1 hr, I was almost unaltered but III was
partially degraded[31].

In 0.25 M $NaIO_4$ at room temperature, the side chain of I
and II is slowly oxidized, using about 2 eq. of IO_4^- after
7 days, while the heterocyclic ring is unchanged[31]. On the
other hand catalytic hydrogenation of isoxazoles leads to ring
opening at the N-O bond. [32,54]

While 3,5-disubstituted isoxazoles are very stable to
bases[32], other isoxazoles can undergo different modes of ring
cleavage by alkaline reagents depending upon the nature of the
substituents[33,34]. The alkaline ringopening of 2-methyl-isoxazolin-
5-ones occurs at room temperature only when position 3 is not
substituted, yielding N-methyl monoamides of the corresponding
malonic acid derivatives[35]. After alkaline treatment of I, the
β-malonyl derivative of α,β-diaminopropionic acid was found[1].

Photolysis The photochemical reactions of isoxazoles[36] and
benzisoxazoles[37] with the formation of oxazoles have been repor-
ted. From 3,5-diphenylisoxazole, 2,5-diphenyloxazole is formed
over a three membered intermediate azirine[36], depending on the
wavelength of the irradiation. This reaction seems to be rever-
sible[38]. When the 3-position of the isoxazol ring is not substi-
tuted, the azirine intermediate gives rise to an aliphatic
product instead of an oxazole[39]. In the photochemical conversion
of benzisoxazole to benzoxazole, no azirine intermediate is formed[37].

The natural isoxazol ibotenic acid (α-amino-α-(3-hydroxy-isoxazoyl-5-yl)-acetic acid) was converted by uv light into the natural oxazol muscazone (α-amino-α (2(3H) -oxazolonyl)-acetic acid)[40].

When a 10^{-4}M solution of I or 2,4 dimethylisoxazolin-5-one is irradiated with a 253.7 nm light source, shielded from high energy radiation with a 60 % saturated Na-acetate solution, the uv-spectrum disappears with an isobestic point at around 215 nm[1]. No new unsaturated heterocyclic product seems to be formed.

Since the chromophores of the natural isoxazolin-5-ones are similar to those of the natural pyrimidines uracil and thymine, their photochemical behaviours were compared.

The photohydration of uracil and uridine is a well documented phenomenon[41,42]. A water addition at the 5-6 double bond occurs, yielding 5-hydro-6-hydroxy derivatives with a quantum yield (Φ) of 21.6 x 10^{-3} for uridine. On heating in the absence of uv-light 90 % of the absorbance at 260 nm can be regenerated. The photohydration reaction is much slower in D_2O[43] where 70 % of the absorbance was regenerated[42].

The photolysis of the natural isoxazolin-5-ones and the synthetic 2,4-dimethyl-isoxazolin-5-one show quantum yields about 20 times higher than the quantum yield of uridine (see Table 1).

To our knowledge, the natural isoxazolin-5-ones appear to be the most uv-sensitive natural products. High quantum yields (0.35) have also been reported for the photoconversion of azirines to isoxazoles by irradiation at 334 nm[36]. The assumed photohydrate product of N-substituted isoxazolin-5-ones must be unstable, giving rise to a number of fragments. In analogy with the photohydrate of uridine, a 3-hydroxy-4-dihydro substitution is proposed for the photohydrate of N-substituted isoxazolin-5-ones[44,45].

Table 1. Relative quantum yield of the photolysis of several
natural and one synthetic isoxazolin-5-ones, compared to uridine
as a standard[a][26,31].

Compound	Φ in H_2O	Φ in D_2O
I	.49	.38
III	.34	.28
VI	.62	
VIII	.49	
X	.46	
2,4-dimethylisoxazolin-5-one	.46	.33
uridine	.0216	.010

a.   Mercury light source (Hanovia TUV, 6 W, shielded with 60 %
     sat. NaAc solution).

The identification of the photoproducts of the natural iso-
xazolin-5-ones supports the proposed photoreaction and was a very
helpful tool in the structure analysis. From every product
isolated, at least one photoproduct was identified.

From an irradiated solution of I, three new products were
isolated in small amounts and in low yield (5 %, 8 % and 12 %
as determined by ninhydrin staining). These photoproducts were
identified by paperchromatographic and electrophoretic comparison
with synthetic compounds as α,β-diaminopropionic acid, α-amino-
β-malonylamino propionic acid and α-amino-β-acetylamino propionic
acid respectively[31] (see Fig. 3).
The malonyl derivative was identical with the product of alkaline
ringopening. Diaminopropionic acid was also found after acid
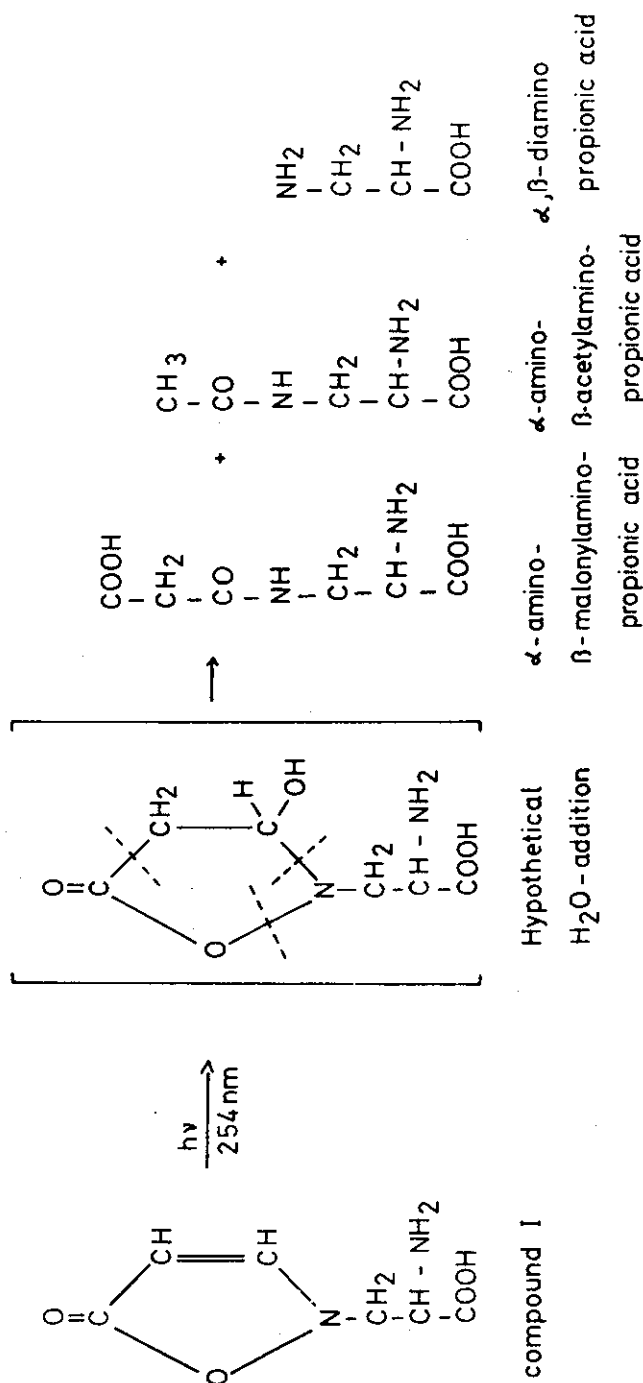hydrolysis of I[1].

Fig. 3 : The photodegradation scheme of compound I by uv-light from a mercury light source. The water addition product was not isolated.

Photolysis of III yielded D-glucose and glutamic acid together with three unidentified ninhydrin reacting products[26]. Glucose and glutamic acid were also formed by acid hydrolysis of III. The formation of glutamic acid as a photoproduct of III can be explained by the same scheme that was valid for I : the 4-substituent alanine forms one fragment with the ring carbons 3 and 4 (or 4 and 5) which became the C-4 and C-5 of the glutamic acid molecule. Glucose accounts for 48 % of the weight of the molecule III[26].

Photolysis of V yielded two unidentified ninhydrin reacting products. On the basis of their electrophoretic mobility, they are supposed to be the γ-glutamyl derivatives of the photoproducts of VII. After mild hydrolysis V yielded VII and glutamic acid[30].

Photolysis of VI yielded α,γ-diaminobutyric acid and α-amino-γ-acetylaminobutyric acid. These are the homologues of photoproducts of I, indicating the homology between I and VI.

Photolysis of VII yielded ethylene diamine and its monoacetyl derivative as indicated by amino acid analysis, where the diamine was eluted with a 1 N citrate buffer at pH 6.18[30]. Both the natural[46] and the synthetic[47] compound VIII show identical sensitivity for uv-irradiation. In the two cases β-aminopropionitrile was the photoproduct.

Similarly the natural and the synthetic compound IX produced D-glucose as photoproduct[48]. Perhaps the unstable 1-aminoglucose was also formed in small amounts as suggested by the automatic amino acid analysis of an irradiated solution of IX or III.

The one acidic compound X yielded glycine as the only ninhydrin reacting photoproduct[48]. Until now no attempt has been made to identify malonic acid or volatile components which eventually might be formed during the photochemical ringopening of the isoxazolin-5-ones.

<u>Nmr-spectra</u>   Spectroscopic examination of the natural isoxazolin-5-ones and of some synthetic 2-substituted ones shows some points of similarity which are summarized in Table 2. The coupling constant (J-value) of the olefinic protons is consistently 3.5 Hz, quite different from the 7 Hz for the olefinic protons in the uracil rings of II and IV. The infrared spectra of the N-substituted-3,4-unsubstituted isoxazolin-5-ones show bands at ca. 1550 (C=C), 1720-1730 (C=O), 3060-3080 (3-H) and 3120-3150 cm^{-1} (4-H)[49]. In the mass spectra major peaks occur with m/e = 99 (ring + 15) or m/e = 98[49,50].

<u>Synthesis</u>   At the time that the natural isoxazolin-5-ones were dis-covered, no description of N-substituted-3,4-unsubstituted isoxazolin-5-ones was found in the literature. While the isoxazol ring was often synthesized[52], we know of only a few accounts on N-substituted isoxazolin-5-ones[29,35,49,53-55]. A number of products were synthe-sized by condensation of N-alkyl hydroxylamines with β-oxo esters[29,53,54] or by alkylation of N-unsubstituted compounds[56]. Synthesis of N-substituted-3,4-unsubstituted derivatives was achieved only by direct substitution of the free ring[49,50,54,57].

The free isoxazolin-5-one ring was first isolated after the reaction of hydroxylamine with ribonucleic acid[58,59]. In the reaction of hydroxylamine with pyrimidines, the N-O of the isoxazole ring originates from the hydroxylamine[60]. Chemical synthesis of the unstable ring was achieved by cyclization of the ethyl ester of 3-oximino-propionate formed by reaction of hydroxylamine with ethylformylacetate[55], or with ethylpropiolate[50]. The Na-salt of the ring is more stable.

Table 2. uv and nmr spectral data of natural and synthetic isoxazo-lin-5-one derivatives and two natural uracil derivatives (II and IV)[a].

Compound	uv λ_{max} in nm	Nmr δ values in ppm				Ref.
		3-H	4-H	J 3-4	R:C1'H	
I	265	8.1	5.2	3.5 Hz	4.3	1
II	260	7.8	6.2	7 Hz	4.7	25
III	265	8.3			5.0	26
IV	263	7.6	5.8	7.5 Hz		51
V	265	8.2	5.2	3.5 Hz	4.0	30
VI	265	8.3	5.3	3.5 Hz	4.0	30
VII	265	8.3	5.3	3.5 Hz	4.2	30
*VIII	265	8.0	5.3	3.5 Hz	4.0	49
IX	260	8.5	5.5	3.5 Hz	5.1	48
X	266	8.7	5.7	3.5 Hz	4.8	48
2-methyl Is	264	7.7	5.1	3.5 Hz	3.4	49
*2-ethyl Is	265	7.8	5.1	3.5 Hz	3.7	49
*2-OH ethyl Is	265	8.0	5.0		3.9	49
*2,4-dimethyl Is	273	8.0			3.6	26

a. Uv-spectra were recorded in water. Nmr-spectra were recorded in D_2O or in $CDCl_3$ for the products marked *.

For the uracil derivatives (II and IV) the signals for the corresponding olefinic protons 6-H, 5-H and J 5-6 are given. Is stands for isoxazolin-5-one.
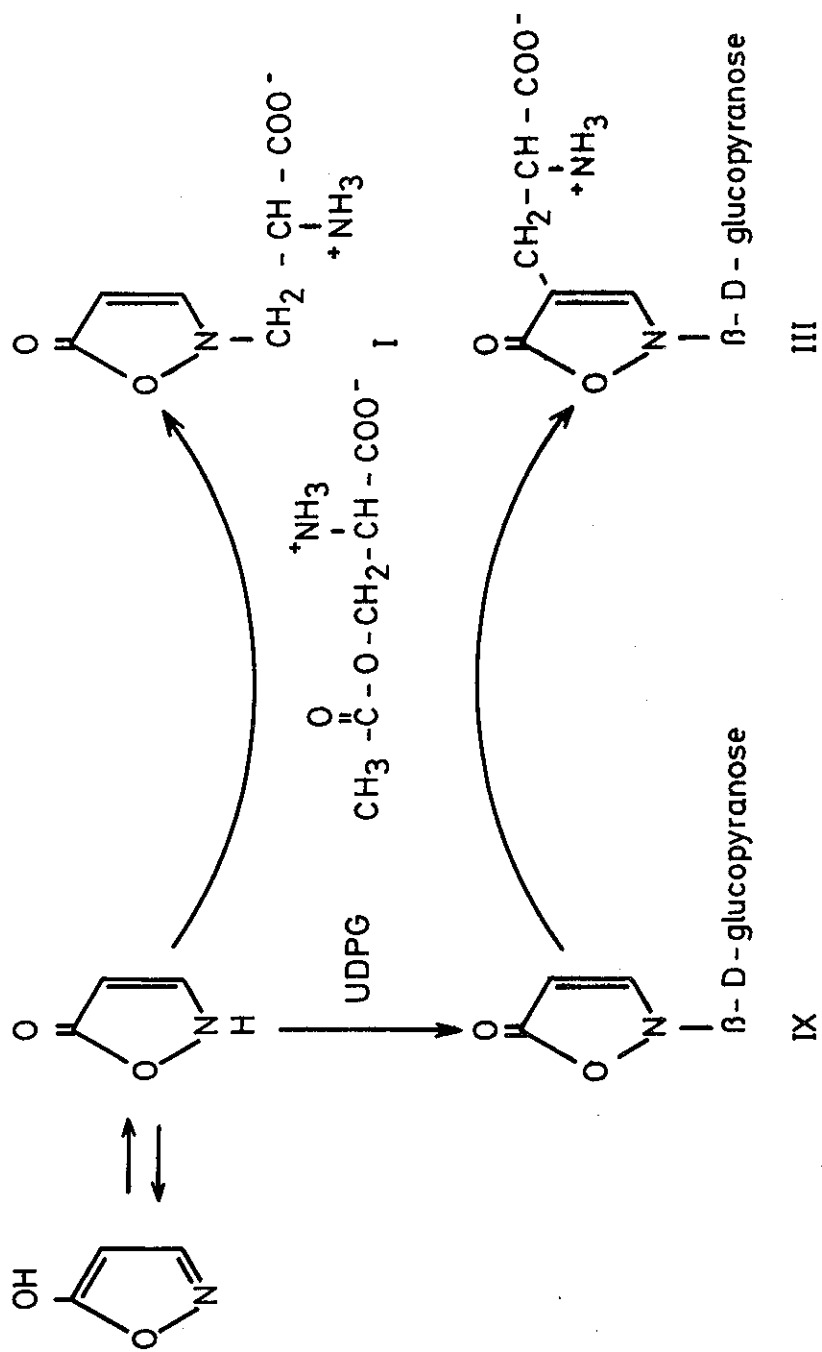
Fig. 4 : Scheme for the enzymatic synthesis of compounds I, III and IX. Crude protein extracts from seedlings were used as enzyme sources.

A number of N-alkyl-isoxazolinones have been synthesized,
including two natural products. Compounds VIII (2-cyanoethyl-
isoxazolin-5-one)was obtained by reaction of acrylonitrile and
the free ring in 0.1 M Na-acetate buffer, pH 4.25 at room tempe-
rature[49]. The glucoside (IX) was prepared by reacting 2,3,4,6-
tetra acetyl-α-glucopyranosylbromide with isoxazolin-5-one Na-
salt in dry methanol[48]. The two natural products VIII and IX
were produced in poor yield ($\pm$ 1 %), while the alkylations of
the ring had much higher yield (20-50 %). The production of
[14]C labelled natural isoxazolin-5-ones by substitution of the
free labelled ring is therefore uneconomical[57].

Attempts to synthesize the other natural isoxazolin-5-ones
have been unsuccessful thus far[50,57].

BIOLOGICAL ASPECTS

The heteroaromatic N-substituted isoxazolin-5-one derivatives
have been found in high concentration in a small number of legume
species,belonging to the Vicieae. No chemotaxonomic screening has
been done. Compound I was identified in Pisum sativum, Pisum arvense,
Lathyrus odoratus, Lathyrus latifolius, Lens culinaris and Vicia
sativa. Compound III was found together with I, except in L. lati-
folius, of which only a small sample of seedlings was used. The
other isoxazolin-5-ones have thus far only been isolated from
L. odoratus, except VIII that was found also in L. latifolius.

The concentration of heterocyclic amino acids and its
evolution during the development of pea seedlings has been studied
by paper chromatography[44] and by amino acid analysis[61]. On a dry
weight basis, maximum concentrations of I and II are reached after
6 to 8 days of germination. In different parts of the seedling
axis, 1 to 2 % of I and about 0.1 % of II is reached. No great

differences are observed whether the seedlings are grown in the dark or under continuous light[44]. A maximum amount per plant or per plant part is reached in a later stage. After two weeks of germination, about 3 μmoles of I is found per seedling (without the cotyledons) and about 2 μmoles in a pair of cotyledons[61].

In 10-day-old seedlings of Lathyrus odoratus (without cotyledons) about 10 % of the dry weight consists of N-substituted isoxazolin-5-one derivatives. The major compound in this material is VI, making up 3.5 % of the dry weight, followed by I (1.9 %), V (1.2 %) and VIII (0.8 %)[30].

Recently, isoxazolin-5-one derivatives have been found in the root exudate of axenic seedlings of peas and sweet peas[61]. The concentration of the products have been determined in the exudates and in the corresponding roots after varying periods of germination under an artificial light cycle. In the exudate from pea seedlings I and II were major constituents ; in the exudate from sweet pea seedlings, VIII was the main component while I, V and VI were present as well. In the sweet pea root exudates, there was a pronounced rise of VIII as compared to the concentration in the root. The exuded common amino acids seemed to be reabsorbed by the root[67] in aseptic conditions, especially homoserine from the pea exudate.

The biosynthesis of the natural isoxazolin-5-one derivatives has been studied in vitro by Murakoshi and coworkers. The chemically unstable free isoxazolin-5-one ring seems to be the starting point for the enzymatic synthesis of the alanine derivative I[63] and the glucoside IX[64]. The donor of the alanine side chain is O-acetylserine while UDP-glucose is the donor of the glucoside substituent. After glucosidation of the ring-N, the C-4 of the ring seems to have the right properties to receive

an alanine substitution, hereby producing III[65] (see Fig. 4).
Perhaps the same enzyme system is involved in the two alanylation
reactions.

Previously it was found that the in vivo synthesis of II also
involves N-substitution of the uracil ring with serine, producing
again the β-substituted heterocyclic alanine derivative[66]. Later
work showed that O-acetylserine was a more effective precursor for
the side chain and that uracil as well as uridine and orotic acid
are precursors for the heterocyclic ring of both the isomeric
compounds II and IV[67].

The lathyrogenic compounds α,γ-diaminobutyric acid and
β-aminopropionitrile are the photoproducts of VI and VIII respecti-
vely. Therefore VIII was tested for its potential toxicity.
When weanling rats received 0.4 % of VIII (half of the concentration
in Lathyrus seedlings) in their diet, similar symptoms developed
as if the osteolathyrogenic β-aminopropionitrile was consumed
in the same concentration[68]. After examining the urine of the
experimental animals it was concluded that VIII was metabolized
in the rat, thereby liberating β-aminopropionitrile as toxic
principle.

When a fungicide containing an isoxazol-5-one ring
(4-(2-chlorophenylhydrazono)-3-methylisoxazol-5-one) is fed to
mammals, metabolic opening of the isoxazol ring also occurred[69].
If this holds true for VI, then this compound may have neurolathy-
rogenic toxicity like its photoproduct α,γ-diaminobutyric acid
(review on lathyrism[70]).

We did not search in the literature for synthetic isoxazol
derivatives and their metabolism. From the information at hand
it seems that in animals the isoxazol ring is metabolized through
ringopening[68,69]. In plants isoxazol derivatives seem to be either

alanylated at the N or the C-4 of the ring[63,65], or glucosylated at the ring-N or at OH substituents on the ring[64,71-73].

Compounds I and II have been tested by Watkins for their activity on neurones of vertebrates. It was found that I was a weak neuronal excitant of the glutamate type, while II had no activity[74]. However, the related plant product quisqualic acid (β-(3,5-dioxo-oxadiazolidine-2-yl)-alanine) discovered and described by Takemoto and coworkers[75,76] had a much higher activity[77].

It should be noted that several natural products with an isoxazol ring and with biological activity have been isolated from other organisms. The antibiotic cycloserine (D-4-amino-3-isoxazolidone) was isolated from the soil organism Streptomyces orchidaceus.[78] From the poisonous mushroom Amanita muscaria the halucinogens muscimol (5-aminoethyl-3-hydroxy-isoxazole) and ibotenic acid were isolated[79]. From the mushroom Tricholoma muscarium the insecticide tricholomic acid (2,3-dihydro derivative of ibotenic acid) was isolated[80].

Recently, two natural isoxazole amino acids, resembling ibotenic acid, have been isolated from Streptomyces sviceus. The products are code numbered U-42126 or NSC 163501 (α-amino-3-chloro-4,5-dihydro-5-isoxazole acetic acid) and U-43795 or NSC 176324 (α-amino-3-chloro-4-hydroxy-4,5-dihydro-5-isoxazole acetic acid). Both heterocyclic amino acids have antibiotic and antitumor activity[81]. They are selective antagonists of L-glutamine[82]. Similar but less specific properties are attributed to ibotenic acid[83]. The structures of natural isoxazoles from bacterial or fungal origin are given in Fig. 5.
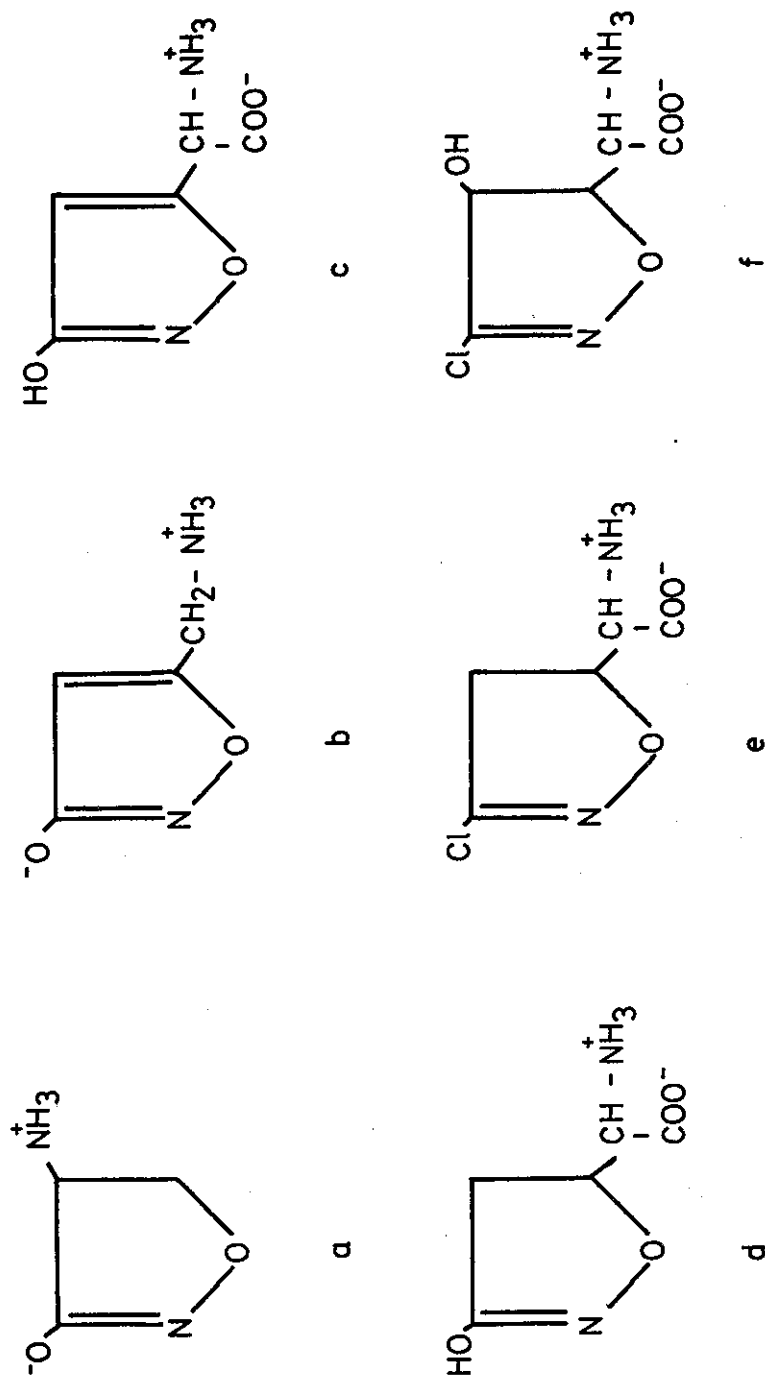
Fig. 5 : Structures of natural occurring isoxazol-derivatives.

a : cycloserine or oxamycine, B : muscimol, c : ibotenic acid,

d : tricholomic acid, e : U-42126, f : U-43795.

DISCUSSION

From the results reviewed in this paper it is clear that the natural isoxazolinone derivatives have some points of interest to the chemist as well as to the biologist.

The instability of the natural isoxazolinones in alkaline solution may be the main reason why these products were not described earlier as constituents of otherwise popular material in botanical research. We have found that if 2 % NH_4OH is included in the extraction procedure, virtually no isoxazolinone derivatives were isolated as compared to neutral or acidic preparations. The efficiency of the photochemical ringopening ($\Phi = 0.5$) and the high UV-absorption ($\varepsilon = 12,400$) of the isoxazolinones make them the natural products with the highest sensitivity to uv light. There is no indication however that these unique properties have any physiological impact.

The high amount of isoxazolinones being found in the seedling tissue raises questions about their origin, function and fate. Because their synthesis in the seedling is little influenced by the light and no effective precursor for the isoxazol ring has yet been found, we assume that the isoxazolinone derivatives originate from the carbon and nitrogen reserves in the cotyledons (seed globulines). The formation of the isoxazolin-5-one ring from the chemical reaction of hydroxylamine with ribonucleic acids might suggest that RNA, which is present in rather large amounts in the cotyledons, should be considered as a possible origin for the isoxazolinone ring. However the amount of RNA per pea seed ($\pm$ 1 mg)[84,85], which is only partially broken down during the germination, can hardly provide all the material needed for the synthesis of the high concentrations of I in the seedling and in the exudate ($\pm$ 0.95 mg).

The compounds may have some function in the transport of carbon and nitrogen from the storage cotyledons to the growing plantlets. Since the growing plant requires a high water influx[86] and thus a high osmotic pressure, a physiological role as osmotic regulator in the expanding shoot and root is also plausible for these small molecules.

The different composition of the root exudates of the closely related pea and sweet pea seedlings may indicate that the influence of the root exudate on the microorganisms in the rhizosphere (discussed by Rovira [87])is rather specific. The specific exudation of the toxic compound VIII from sweet pea roots suggests the possibility that the seedling acquires some protection against root infection by secreting VIII.

From the enzymatic synthesis of I, IX and III, it seems that the plant synthesizes the N-substituted-3,4-unsubstituted isoxazolin-5-ones, in the same way as the chemist, by direct substitution of the free ring. This is also true for a range of heterocyclic β-substituted alanines from plants, where however the free ring was present.[88] This indicates that the free ring may be found in plants where the substituted ring is present. Also, those plant species containing III should contain also its precursor IX albeit in minute quantities.

The assumption that VI and VIII are precursors for the lathyrogens α,γ-diaminobutyric acid[89] and β-aminopropionitrile is not supported by our findings : L. odoratus is not neurolathyrogenic and probably does not contain α,γ-diaminobutyric acid[68]. On the other hand the neurolathyrogenic species L. latifolius contains α,γ-diaminobutyric acid in the seeds[70] but does not synthesize VI in the seedlings. Incorporation studies with ^{14}C-β-aminopropionitrile did not show its metabolic relation with VIII[57].

Some metabolic relationship between isoxazolinones can be readily expected, such as the conversion of I to VII by decarboxylation (the same relation exists between ibotenic acid and muscimol) and the conversion of VII to V by the γ-glutamyl transpeptidase enzyme. In plants metabolic pathways leading from an amino acid over an oxime intermediate to a nitrile with one carbon less have been described[90]. This is a possible metabolic relationship between VI and VIII. Oxidation of the nitrile and loss of HCN may lead to a carboxylic acid[90], this is a possible metabolic relationship between VIII and X.

The α-aminobutyric acid side chain of VI has recently been found in other natural products : an unusual nucleotide in transfer ribonucleic acid 3-(3-amino-3-carboxypropyl)uracil, the higher homologue of II[91]; an antibiotic amiclenomycin α-amino-γ-(4-amino, 2,5-cyclohexadienyl)-butyric acid[92] and two plant products N-(3-amino-3-carboxypropyl)azetidine-2-carboxylic acid and N-(3-amino-3-carboxypropyl) nicotinic acid[93]. Azetidine-2-carboxylic acid has been proposed as the precursor for this side chain[93].

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