Table 1. *N*-Terminal sequence of winged bean trypsin inhibitor 3 compared with soybean, acacia (*Acacia elata*) and silk tree (*Albizia julibrissin*) trypsin inhibitors

Winged bean	Glu – Pro	Leu – Leu – Asp	Ser	Glu	Gly	Glu – Leu – Val – Arg	Asn – Gly – Gly – Thr – Tyr – Tyr
Soybean	Asp – Phe – Val	Leu – Asp	Asn	Glu	Gly	Asn – Pro – Leu – Glu	Asn – Gly – Gly – Thr – Tyr – Tyr
Acacia	Lys – Glu	Leu – Leu – Asp	Ala	Asp	Gly	Asp – Ile – Leu –	
Silk tree	Lys – Glu	Leu – Leu – Asp	Ala	Asp	Gly	Asp – Ile – Leu – Leu	Asn – Gly – Gly – * – Tyr – Tyr

Identical residues are in the boxes.
*Residue not determined.

and purified as described [1, 2]. The inhibitors were reduced with dithioerythritol (0.1 M) and alkylated with iodoacetic acid [10]. The alkylated inhibitors were desalted on Sephadex G-25 (100 × 1 cm) in 0.1 M ammonium bicarbonate buffer, pH 8, and recovered by freeze-drying.

The alkylated inhibitors (0.1 μmol) were subject to sequence analysis in a protein sequenator as described elsewhere [11]. The thiazolinone derivatives were converted to phenylthiohydantoin derivatives and were identified by TLC on Si gel plates [12] and by HPLC [13].

Acknowledgement—The assistance of John Burns in the purification of the winged bean inhibitors is gratefully acknowledged.

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Phytochemistry, Vol. 22, No. 3, pp. 768–770, 1983.
Printed in Great Britain.

0031–9422/83/030768–03$03.00/0
Pergamon Press Ltd.

GLUCOSIDASE AND TREHALASE INHIBITION BY 1,5-DIDEOXY-1,5-IMINO-D-MANNITOL, A CYCLIC AMINO ALDITOL FROM *LONCHOCARPUS SERICEUS*

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(*Revised received* 19 *August* 1982)

Key Word Index—*Lonchocarpus sericeus*; Leguminosae; glucosidase; trehalase; 1,5-dideoxy-1,5-imino-D-mannitol.

Abstract—1,5-Dideoxy-1,5-imino-D-mannitol, a cyclic amino alditol isolated from *Lonchocarpus sericeus* has been found to be a potent inhibitor of certain α- and β-glucosidases and insect-derived trehalase. In structure and biological activity it resembles nojirimycin (5-amino-5-deoxy-D-glucopyranose) and deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol), two glucosidase inhibitors previously isolated from bacteria.

INTRODUCTION

Nojirimycin, (5-amino-5-deoxy-D-glucopyranose, **1**) an antibiotic isolated from species of *Streptomyces* was the first naturally occurring 5-amino sugar to be discovered. This compound differs structurally from glucose only in the replacement of oxygen in the ring by nitrogen, and is a

Table 1. Concentration of inhibitor required to produce 50% inhibition of enzyme activity under the stated conditions

	Nojirimycin bisulphite	LU1	D-Glucono-1,5-lactone
β-Glucosidase (emulsin)	2.8×10^{-6} M	7.3×10^{-6} M	1.1×10^{-5} M
α-Glucosidase (yeast)	3.3×10^{-4} M	6.5×10^{-6} M	2.1×10^{-3} M
Trehalase (insect)	NI	5.5×10^{-5} M	NI

NI = no inhibition at up to 10^{-2} M.

potent inhibitor of β-glucosidases (ex. *Trichoderma viride* and apricot emulsin) and of α-glucosidases (microbial and mammalian) [1–3]. Subsequently, 1-deoxynojirimycin, (1,5-dideoxy-1,5-imino-D-glucitol, **2**) was isolated from species of *Bacillus* and *Streptomyces* [2, 4]. The deoxy compound inhibits several glucosidase enzymes but differs from nojirimycin in that it is a less effective inhibitor of emulsin and a more effective inhibitor of certain α-glucosidases (such as intestinal maltase and α-glucoamylase) [1, 2]. Deoxynojirimycin also inhibits a microbial trehalase [4]. A comparable molecule recently isolated from higher plants is 1,5-dideoxy-1,5-imino-D-mannitol, (LU1, **3**) present in seed of *Lonchocarpus sericeus* [5]. In the present study, LU1 has been compared with nojirimycin and D-glucono-1,5-lactone (**4**) as an inhibitor of certain glycosidase enzymes. We now report that LU1 is a potent inhibitor of α-glucosidase (yeast), β-glucosidase (emulsin) and insect trehalase.

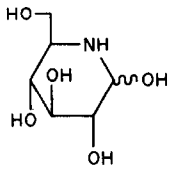
RESULTS

Table 1 shows the concentration of LU1, nojirimycin bisulphite and D-glucono-1,5-lactone required to give 50% inhibition of each enzyme under the assay conditions stated (see Experimental). None of the three compounds tested showed any inhibition (at concentrations up to 10^{-2} M) of α-mannosidase (*Canavalia ensiformis*), α- and β-galactosidase (*Aspergillus niger*) and β-glucuronidase (*Helix pomatia*).

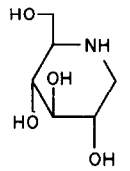
Kinetic studies have revealed that LU1 is a competitive inhibitor of β-glucosidase. A Lineweaver–Burk plot for β-glucosidase at different concentrations of LU1 showed typical competitive inhibitory effects on the enzyme activity.

DISCUSSION

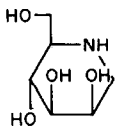
The relationship between the structure and inhibitory actions of nojirimycin and D-glucono-1,5-lactone on various glucosidases has been discussed previously [3]. In the present study it has been shown that LU1 (an amino mannitol) is a more potent inhibitor of α-glucosidase than nojirimycin bisulphite (an amino glucose) and that LU1 does not inhibit α-mannosidase despite the structural analogy of LU1 to mannose. Clearly, further work is required before one can predict with certainty which sugar analogues are likely to inhibit which glycosidases. The finding that LU1 inhibits insect trehalase is of ecological interest. Trehalase occupies a central role in the carbohydrate metabolism of insects [6] and the presence of a trehalase inhibitor may protect a plant against phytophagous insects. LU1 can be considered as a sugar analogue or as an alkaloid and it may be of significance that the poisoning of cattle by swainsonine, the alkaloid



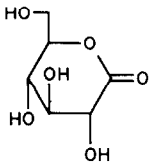
5-Amino-5-deoxy-D-glucopyranose
(Nojirimycin)
1



1,5-Dideoxy-1,5-imino-D-glucitol
(1-Deoxynojirimycin)
2



1,5-Dideoxy-1,5-imino-D-mannitol
(LU 1)
3



D-Glucono-1,5-lactone
4

from *Swainsona canescens*, has been attributed to its inhibition of α-mannosidase [7]. Inhibition of carbohydrate metabolism in potential predators by secondary compounds is possibly a widespread defensive strategy among plants.

EXPERIMENTAL

LU1 was isolated as the hydrochloride as described previously [5]. Nojirimycin bisulphite [1] and trehalase from *Sarcophaga barbata* [8] were donated (see Acknowledgements). All other enzymes and chemicals were purchased from Sigma, Poole, U.K.

Enzyme assays. LU1, nojirimycin bisulphite and D-glucono-1,5-lactone were incorporated into assay buffers where appropriate to give a final concn range 10^{-8}–10^{-2} M.

Trehalase. Assayed as described in ref. [8].

β-Glucosidase (Sigma G-8625; almonds). 200 μl 50 mM trisodium citrate, pH 4.8; 200 μl 2 mM *p*-nitrophenyl-β-D-glucoside; 200 μl enzyme (5 μg/ml). Incubated 15 min, 25°. Added 400 μl 0.1 M NaOH. Read at 400 nm.

α-Glucosidase (Sigma G-5003; yeast). 200 μl 50 mM trisodium citrate, pH 6.8; 200 μl 1 mM *p*-nitrophenyl-α-D-glucoside; 200 μl enzyme (5 μg/ml). Incubated 15 min, 25°. Added 400 μl 0.1 M NaOH. Read at 400 nm.

α-Mannosidase (Sigma M-7257; Jack bean). 200 μl 50 mM trisodium citrate, pH 4.5; 200 μl 2 mM *p*-nitrophenyl-α-D-mannoside; 200 μl enzyme (5.5 μg/ml). Incubated 15 min, 25°. Added 400 μl 0.1 M NaOH. Read at 400 nm.

β-Galactosidase (Sigma G-9007; *Aspergillus niger*). 200 μl 50 mM trisodium citrate, pH 4.0; 200 μl 1 mM *o*-nitrophenyl-β-D-galactoside; 200 μl enzyme (0.85 μg/ml). Incubated 10 min, 25°. Added 400 μl 0.2 M NaOH. Read at 400 nm.

α-Galactosidase (Sigma G-1932; *Aspergillus niger*). 200 μl 50 mM trisodium citrate, pH 4.0; 200 μl 4 mM *o*-nitrophenyl-α-D-galactoside; 200 μl enzyme (1.41 μg/ml). Incubated 10 min, 25°. Added 400 μl 0.2 M NaOH. Read at 400 nm.

β-Glucuronidase (Sigma G-0751; *Helix pomatia*). 700 μl 0.1 M sodium acetate, pH 5.0; 700 μl 1.2 mM phenolphthalein glucuronide; 100 μl enzyme (2 mg/ml). Incubated 30 min, 37°. Added 5 ml 0.2 M glycine buffer, pH 10.4. Read at 540 nm.

Acknowledgements—We are grateful to the following: Dr. K. Clifford (Shell Biosciences Laboratory, Sittingbourne, U.K.) for his trehalase preparation from *Sarcophaga barbata*; to Dr. T. Niwa (Meiji Seika Kaisha Ltd., Yokohoma, Japan), for a sample of nojirimycin bisulphite; and to the S.E.R.C. for financial support (S.V.E.).

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Phytochemistry, Vol. 22, No. 3, pp. 770–772, 1983.
Printed in Great Britain.

0031-9422/83/030770-03$03.00/0

THE INTRAMOLECULAR FORMATION OF EPITHIOALKANENITRILES FROM ALKENYLGLUCOSINOLATES BY *CRAMBE ABYSSINICA* SEED FLOUR

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(*Received* 17 *June* 1982)

Key Word Index—*Crambe abyssinica*; Cruciferae; allylglucosinolate; 3-butenylgluco[1-^{35}S]sinolate; 3,4-epithiobutanenitrile; 4,5-epithiopentanenitrile; sinigrin; episulphide; thiirane; biosynthesis.

Abstract—Enzymatic degradations of mixtures of potassium 3-butenylgluco[1-^{35}S]sinolate and allylglucosinolate by aqueous suspensions of *Crambe abyssinica* seed flour led to the formation of 4,5-epi[^{35}S]thiopentanenitrile and essentially unlabelled 3,4-epithiobutanenitrile. The formation of epithioalkanenitriles from alkenylglucosinolates is, therefore, deduced to be an intramolecular process.

Thiiranes (1,2-episulphides) seem to be of rare natural occurrence. To date, the only compounds of this type of which we are aware are a few epithioalkanenitriles formed as autolysis products of alkenylglucosinolates [1–4], three sesquiterpenes from hops [5, 6] and a marine sponge product [7]. We have interests in the toxicology [8] and