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.

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

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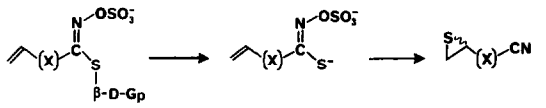
Key Word Index—*Crambe abyssinica*; Cruciferae; allylglucosinolate; 3-butenylgluco[1-^{35}S]sinolate; 3,4-epithiobutanenitrile; 4,5-epithiopentanenitrile; sinigrin; episulphide; thiirane; biosynthesis.

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

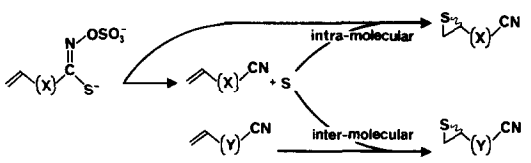
biosynthesis [9] of the thiirane function, and it is to an aspect of the latter that we address ourselves in this report: the biosynthesis of the epithioalkanenitriles.

Several mechanisms have been considered for the biosynthesis of thiiranes [5, 6, 10] and it may very well be that there is more than one way in which they are formed, i.e. that the way in which the function is constructed in the marine natural product is different from that involved in making the hop derivatives, or the epithioalkanenitriles. So far as the latter are concerned, it is known that they are generated from the corresponding a alkenylglucosinolates by the reactions summarized in Scheme 1 [9, 11]. Implicit



Scheme 1. The formulation of epithioalkanenitriles from alkenylglucosinolates.

in this is the idea that the sulphidic sulphur of the aglucone becomes the epithio sulphur via an intra-molecular process. However, there has been no direct evidence to support this idea. In fact, since it is known that glucosinolate aglucones can disproportionate to nitriles and elemental sulphur [10–12], and some evidence has been provided for the formation of the hop thiiranes from the sesquiterpene alkenes and sulphur [5, 6], it could be hypothesized that the epithioalkanenitriles are formed entirely, or in part, by an inter-molecular sulphur-transfer process, i.e. as in Scheme 2.



Scheme 2. Inter- and intra-molecular alternatives for epithioalkanenitrile formation.

We now report the results of an investigation of the formation of 3,4-epithiobutanenitrile (**1a**) and 4,5-epithiopentanenitrile (**1b**) from the corresponding glucosinolates which provided a test of the intra- or inter-molecularity of the process.

Mixtures of potassium allylglucosinolate (**2a**) and 3-butenylgluco[1-^{35}S]sinolate (**2b**) were treated with aqueous suspensions of *Crambe abyssinica* Hochst ex. R. E. Fries var. Prophet seed flour (conditions known to convert these two glucosinolates to the corresponding episulphides [9]). The diethyl ether extractable reaction products were analysed by GC and GC/MS and shown to consist largely of the two episulphides **1a** and **1b**, accompanied by small amounts of (*R*)- and (*S*)-3-hydroxy-4,5-epithiopentanenitrile (**1c**) (formed from endogenous 2-hydroxy-3-butenylglucosinolate present in the seed [3, 4]), allyl isothiocyanate (**3a**) and 3-butenyl isothiocyanate (**3b**). Traces of allyl thiocyanate (**4**) were found, but 3-butenenitrile and 4-pentenenitrile were not detected. The episulphides were separated, and isolated by prep. GC. The amount of ^{35}S-label which appeared in the fraction corresponding to **1a** was at about the level of the background and, at most, *ca* 2% of the activity contained in the **1b** fraction (see Experimental). Epithioalkanenitrile formation from alkenylglucosinolates is thus deduced to be a highly intra-molecular process.

EXPERIMENTAL

General instrumental conditions have been described before [9]. The radioactivity measurements were performed with a LKB Rackbeta LS instrument, the samples being counted in 10 ml toluene cocktail containing PPO (4 g/l.) and POPOP (0.05 g/l.). Prep. GC was performed on a 2 m × 4 mm (i.d.) glass column packed with 20% OV-17 on Chromosorb W (80–100 mesh) using N_2 carrier gas and a flow-rate of *ca* 30 ml/min and temp. programming from 180 to 240°.

Potassium 3-butenylgluco[1-^{35}S]*sinolate* (**2b**). This compound was prepared from [^{35}S]thiourea (1 mCi, 0.5 mg, New England Nuclear) diluted with thiourea (900 mg), via 2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl[^{35}S]mercaptan and 5-nitro-1-pentene according to published procedures [13, 14] for the unlabelled compounds. The ^{1}H NMR, IR, mp and $[\alpha]_D$ data for all the intermediates were in agreement with the published values and consistent with the proposed structures. As obtained from the crystalline tetra-*O*-acetyl precursor, **2b** was a glassy solid with an activity of 1.4×10^6 cpm/mg: PC (*n*-BuOH–HOAc–H_2O, 4:1:4) R_f 0.7 (relative to **2a** $R_f = 1.0$); ^{1}H NMR (200 MHz, D_2O): δ5.85–6.10 (1H, *m*, H-4), 5.0–5.25 (3H, *m*, H-5A, H-5B, H-1'), 3.92 (1H, *dd*, $J_{6'A,5'} = 2$ Hz, $J_{6'A,6'B} = 12$ Hz, H-6'A), 3.70 (1H, *dd*, $J_{5'6'B} = 6$ Hz, $J_{6'A,6'B} = 12$ Hz, H-6'B), 3.4–3.6 (4H, *m*, H-2'–H-5'), 2.84 (2H, *t*, $J_{2,3} = 7$ Hz, H-2), 2.50 (2H, *m*, H-3); and $[\alpha]_D^{28}$ −26° (H_2O; *c* 0.24).

Enzymatic conversion of the glucosinolates to epithioalkanenitriles. Aq. solns of mixtures of **2a** (10, 30 or 150 mg) and [1-^{35}S]**2b** (30 mg) were treated with a *C. abyssinica* var. Prophet seed flour preparation according to a previously published procedure [9]. A single Et_2O extraction recovered *ca* 56% of the radioactivity from the aq. soln and a further four extractions yielded another 21%. After removing the Et_2O from the dried ($MgSO_4$) extracts, under a N_2-jet, the residue was taken up in CH_2Cl_2 (1.5 ml). GC/MS analyses (2 m × 4 mm, 3% OV-17, 100–240° at 16°/min, 70 eV) revealed the presence of **1a**, **1b** and **1c**, as well as trace amounts of **3a**, **3b** and **4**. (The identity of these compounds being confirmed by comparison with the data for reference compounds.) Collecting the effluent from prep. GC separations of the reaction products, so as to trap **1a**, **1b** and **1c** (as well as inter-peak and background collections), and counting these revealed the following relative activities which were, within experimental error, independent of the **2a**:**2b** ratio in the alkenylglucosinolate mixtures: **1a** (2.3 ± 0.3), **1b** (97.2 ± 2.3) and **1c** (0.5 ± 0.2). Part, at least, of the activity seen in the **1a** collection was due to contamination from [^{35}S]**3b**, which eluted shortly before the epithioalkanenitrile; and the activity seen in the **1c** collection is at the level of background counts on either side of this, i.e. **1a** and **1c** are surely even less active than the counts suggest.

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γ-LACTONES FROM *MEZILAURUS SYNANDRA**

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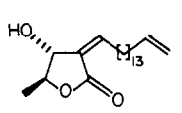
Key Word Index—*Mezilaurus synandra*; Lauraceae; benzylisoquinoline alkaloids; α-alkyl-β-hydroxy-γ-methyl-γ-lactone.

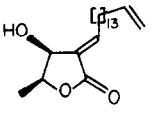
Abstract—The trunk wood of *Mezilaurus synandra* contains the known alkaloids coclaurine, corytuberine and norcinnamolaurine, besides the novel (2*R*,3*R*,4*S*)-2-dodec-ω-enyl-3-hydroxy-4-methylbutanolide.

The trunk wood of *Clinostemon mahuba* (A. Samp.) Kuhlm. et A. Samp. has been shown to contain 22 butanolides, e.g. **1** and **2** [2]. The genus *Clinostemon* belongs to the chemically little known [3] subtribe Beilschmiediineae [4]. An additional genus of this subtribe, *Mezilaurus*, is widespread in Amazonia. One of the species, *M. synandra* (Mez) Kosterm., occurs in the drier *terra firma* forests in the Manaus region [5]. As shown in the present work, its trunk wood contains a butanolide (**3a**). Compound **4** would be expected to be less polar than **3a**. Since **4** was eluted from a Si gel column in succession to **3a** it may well be an artificial dehydration product of this β-hydroxy-γ-lactone.

The mass spectrum of **3a** showed all the characteristic peaks previously assigned [2] to the lactone moieties of the hydrogenation products of **1** (**5a**) and **2** (**6a**) (Table 1). While the constitution of these moieties should thus be identical, the side chains not only differ by the number of methylenes ([M]$^+$ **3a** 282, **5a** and **6a** 340) but also by the presence of a terminal vinyl group (*ca* δ 5.6 and 4.95, respectively, one and two hydrogens) in **3a**. The comparison of ^{1}H NMR data indicate **3a** to be analogous to **5a** and not to **6a** (Table 2) with respect to relative



1



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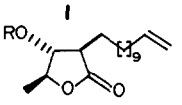
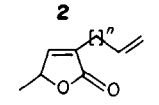
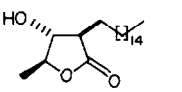


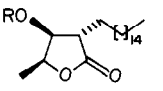
3a R = H
3b R = Ac



4



5a R = H
5b R = Ac



6a R = H
6b R = Ac

*Part 72 in the series "The Chemistry of Brazilian Lauraceae". For Part 71 see ref. [1]. Taken from part of the M.Sc. thesis submitted by R.S. to Universidade Federal de Minas Gerais.