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THE BIOSYNTHESIS OF ANGELIC ACID IN *CYNOGLOSSUM OFFICINALE*

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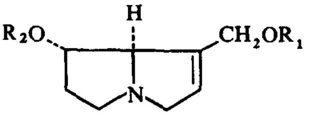
Key Word Index—*Cynoglossum officinale*; Boraginaceae; biosynthesis; heliosupine; angelic acid; tiglic acid; *cis–trans* isomerase system.

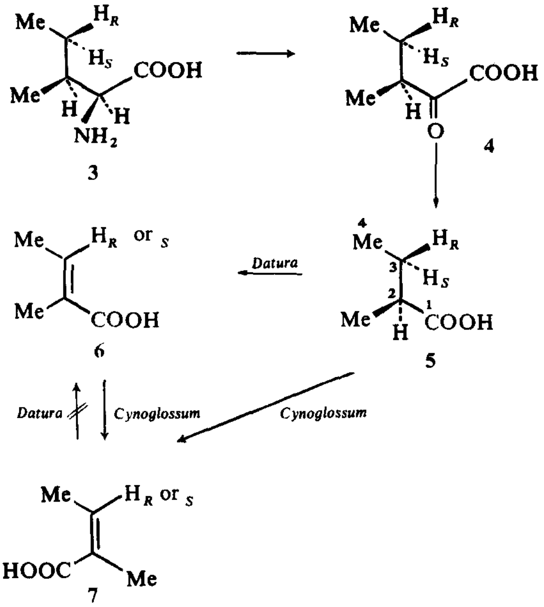
Abstract—Six-month-old *Cynoglossum officinale* plants were fed via the roots with tiglic acid-[1-^{14}C]. After a week the plants were harvested, the alkaloid heliosupine was isolated and hydrolysed to heliotridine and angelic acid. The latter contained all the radioactivity of the original heliosupine showing that angelic acid may be formed from tiglic acid by a *cis–trans* isomerization.

INTRODUCTION

The pyrrolizidine alkaloids are widely distributed being particularly characteristic of the genera *Cynoglossum* and *Heliotropium* (family Boraginaceae), *Senecio* (family Compositae) and *Crotalaria* (family Leguminoase) [1]. Like the tropane and *N*-methylpyrrolidine alkaloids the alkamine moiety is derived from ornithine [1]. The pyrrolizidine bases rarely occur in the free form and are more usually found in ester combination [2]. *Cynoglossum officinale* (the hounds tongue) produces a mixture of pyrrolizidine alkaloids all of which are esters of the necine base heliotridine (**1**) [3], but the major alkaloid is heliosupine (**2**) [3] in which heliotridine (**1**) is esterified with echimidinic and angelic (**7**) acids.



1 $R_1 = R_2 = H$
2 R_1 = echimidinic acid;
R_2 = angelic acid (**7**)



Scheme 1. The formation of tiglic and angelic acids from L(+)-isoleucine in *Cynoglossum* and *Datura*.

Tiglic acid (**6**), the isomer of angelic acid (**7**), has also been found in the Boraginaceae where it occurs as one of the esterifying acids in symphytine (isolated from *Symphytum officinale*) [4]. Tiglic acid (**6**) is derived, in plants, from L(+)-isoleucine (**3**) [5, 6] via 2-methylbutyric acid (**5**) (Scheme 1) [7, 8]. Since L(+)-isoleucine (**3**) has the 2*S*,3*S*-configuration it is thought that (+)-2-methylbutyric acid (**5**), which has the 2*S*-configuration, is the most likely intermediate. However, angelic acid (**7**) has also been shown to be derived from L(+)-isoleucine (**3**) in *Cynoglossum officinale* [9]. This discovery led to the suggestion [10] that angelic acid (**7**) could be the first formed acid in *Datura* and that conversion to tiglic acid (**6**) was effected by a *cis–trans* isomerase system [11]. This was rendered unlikely by Basey and Woolley [10] who found that angelic acid (**7**) was not incorporated into the tigloyl esters of *Datura*. It seemed desirable therefore to explore also the possible role of tiglic acid (**6**) in the biosynthesis of angelic acid (**7**) in *Cynoglossum*.

RESULTS AND DISCUSSION

There are several ways in which angelic acid (**7**) may be formed from (+)-2*S*-methylbutyric acid (**5**) in *Cynoglossum* (Scheme 2). In the simplest form it may involve the loss of a different C-3 proton (*pro-S* or *pro-R*) from that lost in the formation of tiglic acid (**6**) in which case the same dehydrogenation mode is adopted. Alternatively, the same proton could be lost and this would

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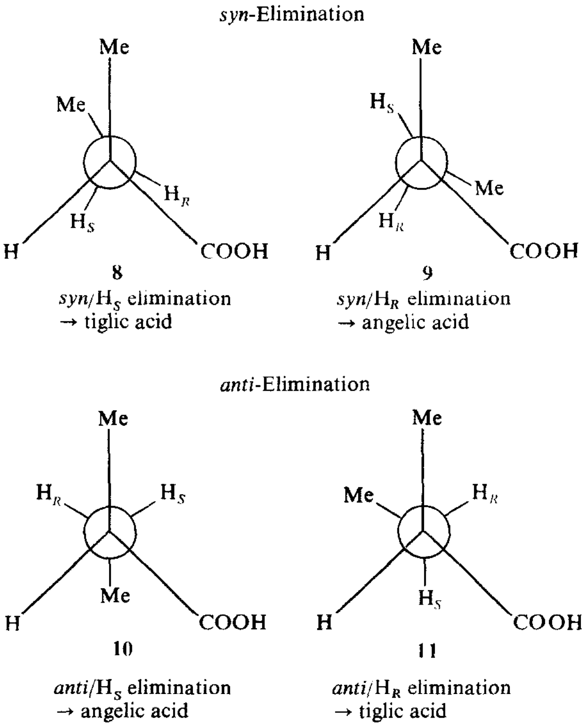

syn/H_S elimination → tiglic acid

syn/H_R elimination → angelic acid

anti/H_S elimination → angelic acid

anti/H_R elimination → tiglic acid

Scheme 2. Newman projections of (+)-2*S*-2-methylbutyric acid showing four possible ways in which it can be dehydrogenated.

involve a different elimination mode with a 180° rotational difference along the C-2, C-3 bond. Lastly, it is possible that in both plants tiglic acid is formed initially and this is later converted to angelic acid by *Cynoglossum*.

To investigate this problem tiglic acid-[1-^{14}C] was prepared [12, 13] and fed to *Cynoglossum officinale* plants. After seven days the plants were harvested and the heliosupine (**2**) was isolated [14]. The heliosupine (**2**) was hydrolysed in alkaline conditions [9] to give angelic acid (**7**).

From Table 1 it appears that *Cynoglossum* can utilize tiglic acid (**6**) in the synthesis of angelic acid (**7**), a result which can only be explained by the presence of a *cis–trans* isomerase system. This does not necessarily mean that the pathway to tiglic acid (**6**) is identical to that of *Datura*. Most enzymes abstract *cis*-protons [15–17], but the free rotation of the C-2, C-3 bond in 2-methylbutyric acid (**5**) allows for two modes this, the eclipsed (*syn*-periplanar) (**8**) and the staggered (*anti*-periplanar) (**11**).

Table 1. Specific activities of heliosupine and its degradation products from *Cynoglossum* plants fed with tiglic acid-[1-^{14}C]

Sp. act. heliosupine picrate dpm/mmol × 10^{-5}	Sp. incorp. (%)*	Sp. act. diluted picrate dpm/mmol × 10^{-5}	Sp. act. angelic acid dpm/mol × 10^{-5} (%recovery in parentheses)
9.32	0.86	1.55	1.47(94.5)

* Calculated as [Sp. act. undiluted picrate (dpm/mmol)]/[Sp. act. precursor (dpm/mmol)] × 100.

EXPERIMENTAL

Cynoglossum officinale plants were grown on open land in Leicester from seed obtained from Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, D.D.R.

Counting procedures. Duplicate samples were counted in commercially available toluene or dioxane based POP/POPOP scintillators in a liquid scintillation spectrometer.

Tracer material. Na-[^{14}CN] for the synthesis of tiglic acid was purchased from the Radiochemical Centre, Amersham.

Tiglic acid-[1-^{14}C], sp. act. 1.08 × 10^8 dpm/mmol, mp and mmp 63°, was synthesized in 29% yield by the previously reported method [12, 13].

Feeding experiments. Tiglic acid-[1-^{14}C] (200 mg), sp. act. 1.08 × 10^8 dpm/mmol was neutralized (dil NaOH), made up to 10 ml with H_2O and distributed to 3 × 6-month-old *C. officinale* plants which had been carefully uprooted and suspended in blackened beakers containing phostrogen soln. After 7 days the plants were harvested and the roots and aerial parts separately dried at 60° for 20 hr.

Isolation of heliosupine [14]. The finely powdered roots (37 g) were extracted by maceration with MeOH. The extract was concd to a small vol. (*ca* 10 ml) at 30° and treated with 2 N H_2SO_4 (10 ml). The acid soln was filtered through a thin layer of kieselguhr and the filtrate was stirred with Zn dust for 10 hr. The mixture was filtered and then extracted with $CHCl_3$ (5 × 10 ml). The $CHCl_3$ soln was dried over $MgSO_4$, filtered and then evapd at 30° onto glass powder (1 g) which was packed onto the top of a column (30 × 1 cm) containing powdered glass (24 g) intimately mixed with pH 8.0 M Pi buffer (6 ml). The column was eluted with petrol–$CHCl_3$ (80:20) that had been equilibrated with the Pi buffer. Fractions (5 ml) were collected and the heliosupine was shown by PC [14] to be in fractions 78–86. The heliosupine was converted to its picrate (5.5 mg), mp and mmp 96°, IR (KBr disc) identical to authentic heliosupine picrate, sp. act. 9.32 × 10^5 dpm/mmol.

Hydrolysis of heliosupine [9]. The diluted heliosupine picrate (30 mg), sp. act. 1.55 × 10^5 dpm/mmol, was converted to its base, a pale yellow gum, and refluxed with 5% $Ba(OH)_2$ soln (6 ml) for 40 min. The soln was made acid with conc HCl and steam distilled. 10 ml of distillate were collected; H_2O being added to replenish the soln as it distilled off. The distillate was titrated against 0.01 N NaOH (2.05 ml) using phenolphthalein as indiator. The resulting soln was concd to 2 ml (at 40°), made acid with dil HCl and extracted with Et_2O (5 × 5 ml) to give angelic acid (1.87 mg) mp 43°, sp. act. 1.47 × 10^5 dpm/mmol (94.5% recovery of label).

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