

BIOSYNTHESIS OF THE TIGLIC ACID MOIETY
OF METELOIDINE IN DATURA METELOIDES

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Tiglic acid is esterified with the amino-alcohol teloidine in the alkaloid meteloidine, which occurs with hyoscyamine and hyoscyne in the plant Datura meteloides. Several other tiglic acid esters have been found in higher plants(1). Robinson *et al.* (2) discovered that tiglic acid was formed from isoleucine in animal tissues, and it was suggested that α -keto- β -methylvaleric acid and

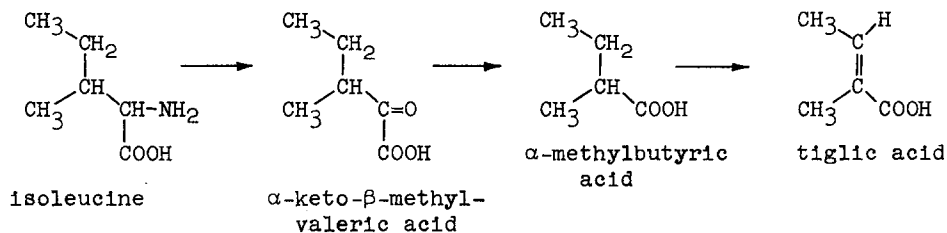


Figure 1. Hypothetical scheme for the biosynthesis of tiglic acid

α -methylbutyric acid were intermediates in this transformation, as illustrated in Fig. 1. Preliminary experiments reported by Evans (3) and Wooley (4) are consistent with a similar metabolic sequence occurring in higher plants. They fed L-isoleucine- $\text{U-}^{14}\text{C}$ to D. meteloides and obtained radioactive meteloidine which was labeled only on its tiglic acid moiety. On the other hand meteloidine

isolated from plants which had been fed L-isoleucine-1- ^{14}C was inactive. We (5) suggested that tiglic acid is formed by the reduction and dehydration of β -keto- α -methylbutyric acid produced by the C-methylation of acetoacetic acid. This hypothesis was rendered improbable by Wooley (4) who isolated inactive meteloidine from plants which had been fed acetate-2- ^{14}C . However too much emphasis must not be placed on this negative result for the following reason. Acetate is incorporated into the tropane ring system of the related alkaloid hyoscyamine (6), and if one assumes that teloidine is produced by a similar metabolic route one would have expected the tropane moiety of meteloidine to be labeled following the administration of acetate-2- ^{14}C .

We have now confirmed and extended the work of Evans and Wooley by feeding DL-isoleucine-2- ^{14}C * (10 mg, 1.6×10^7 d.p.m.) to eight 3-month-old *D. meteloides* plants growing in soil in a greenhouse (August 1966). The tracer, dissolved in

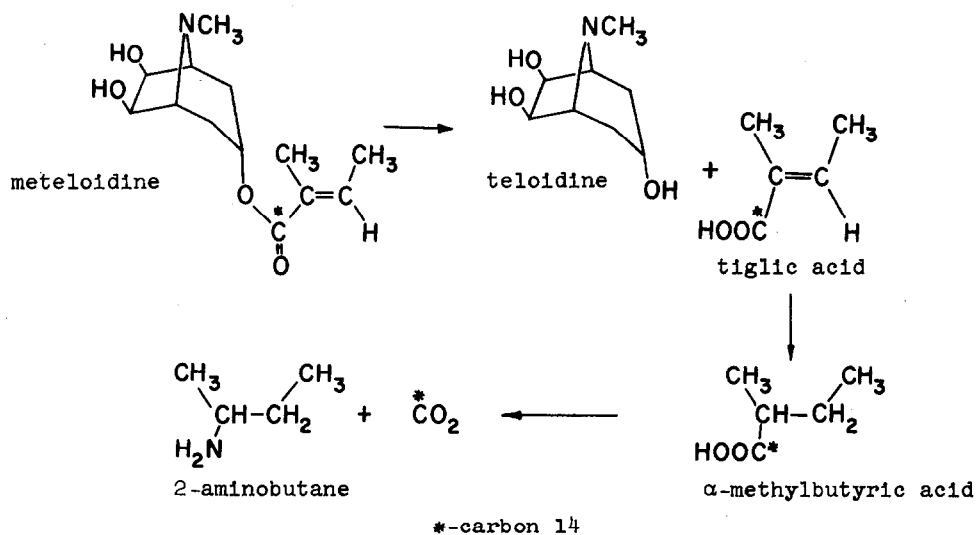


Figure 2. Degradation of the radioactive meteloidine

*The DL-isoleucine-2- ^{14}C was prepared by the method of Greenstein and Winitz (7). The sodium salt of ethyl acetamidocyanoacetate-2- ^{14}C was condensed with 2-bromobutane and the product hydrolysed with hydrochloric acid. The isoleucine was purified by thin layer chromatography on silica gel G.

water, was administered via cotton wicks inserted into the stems of the plants which were about 70 cm tall at the time of feeding. The plants (fresh wt. 2.6 kg) were harvested one week after feeding and the crude alkaloids isolated using a procedure similar to the one used for the extraction of the alkaloids of Datura stramonium (8). Meteloidine (62.5 mg, 2.1×10^4 d.p.m./mM.) was separated from the other alkaloids by thin layer chromatography. On silica gel G, developing with a mixture of 95% ethanol and chloroform (1:1), meteloidine had an R_F of 0.40 (detected by spraying with alkaline permanganate solution). The radioactive meteloidine was degraded by the scheme illustrated in Fig. 2. Hydrolysis with barium hydroxide (9) yielded teloidine, isolated as its hydrobromide salt, and tiglic acid. The tiglic acid was hydrogenated in the presence of platinum yielding α -methylbutyric acid which was subjected to a Schmidt reaction, using sodium azide and concentrated sulfuric acid, affording carbon dioxide collected as barium carbonate and 2-aminobutane collected as its N-benzoyl derivative. The activities of these degradation products are recorded in Table I, and it is apparent that all the activity of the tiglic acid was located on its carboxyl carbon, strongly supporting the biosynthetic scheme illustrated in Fig. 1.

Table I

Activities of Meteloidine and its degradation products

	Activity (d.p.m./mM $\times 10^{-4}$)	Relative Activity
Meteloidine	2.1	100
Teloidine hydrobromide	<0.03	<2
Tiglic acid	1.8	86
Barium carbonate	1.8	86
N-Benzoyl-2-aminobutane	<0.02	<1

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