Biosynthesis of tetraponerine-6. Evidence that two different pathways are operating in the biosynthesis of the two tetraponerine skeletons

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Evidence is presented showing that tetraponerine-6 2a from *Tetraponera* sp. ants is biosynthesized from two molecules of putrescine and a seven-carbon moiety coming from an eight-carbon polyacetate chain through decarboxylation, in contrast with tetraponerine-8 1a, which derives from one putrescine unit and a twelve-carbon polyacetate precursor.

The tetraponerines are a group of eight tricyclic alkaloids isolated from the poison gland of the Neo Guinean ant *Tetraponera* sp.[1] Structural and synthetic work from our laboratories[2–4] showed that these compounds are distributed into two families, which are based either on the 5-alkyldecahydropyrido[1,2-*c*]pyrrolo[1′,2′-*a*]pyrimidine† skeleton **1** (tetraponerines-3, -4, -7 and -8) or on the 5-alkyldecahydrodipyrrolo[1,2-*a*:1′,2′-*c*]pyrimidine† skeleton **2** (tetraponerines-1, -2, -5 and -6) (Fig. 1). In each family, the compounds differ from each other by the length of the alkyl chain (propyl or pentyl) and (or) by the configuration of the carbon atom to which it is attached.

Incorporation experiments with *Tetraponera* sp. ants using sodium [1-^{14}C]- and [2-^{14}C]-acetate, l-[U-^{14}C]glutamic acid, l-[U-^{14}C]ornithine hydrochloride, and [1,4-^{14}C]putrescine dihydrochloride, followed by chemical degradation of radioactive tetraponerine-8 (T-8) **1a**, the major alkaloid of the venom, allowed us to propose a biosynthetic pathway for this alkaloid.[5] It was found that acetate is a precursor of the entire carbon skeleton of T-8, but it is incorporated through two different pathways: the pyrrolidine ring of T-8 comes from acetate by its conversion, *via* the tricarboxylic acid cycle, into l-glutamic acid **3**, l-ornithine **4** and putrescine **5**, whereas the twelve remaining carbon atoms appear to be derived from a polyacetate precursor, for which a compound such as **6** is a likely candidate (Scheme 1).

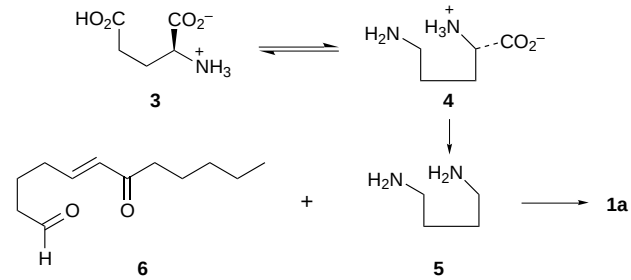
The presence of alkaloids based on two different, albeit closely related, skeletons in one and the same organism is intriguing, particularly with respect to their biosynthesis. We now report our results from biosynthetic studies conducted on tetraponerine-6 (T-6) **2a**, which show that the two tetraponerine skeletons **1** and **2** are assembled *via* two different pathways.

We had to repeat the incorporation experiments with [1,4-^{14}C]putrescine dihydrochloride, since the sample of T-6 **2a** isolated from the first experiment[5] decomposed before it could be submitted to chemical degradation. Thus, 1000 *Tetraponera* sp. ants were starved for three days, then fed with a 1 mol dm^{-3} sucrose solution containing [1,4-^{14}C]putrescine dihydrochloride (specific activity: 114 Ci mol^{-1}; total activity: 50 μCi). They were killed ten days later by dropping them into methanol. Using the already described[2] extraction and purification scheme, four of the tetraponerines (T-8 **1a**, T-4 **1b**, T-6 **2a** and T-5 **2c**) could be isolated in pure state. All were radioactive. Their specific activity and specific incorporation rate (SIR) are reported in Table 1 (experiment 2), together with the values measured from the first incorporation experiment[5] for T-8 (experiment 1). Table 1 shows that the specific incorporation rates of the four alkaloids isolated from experiment 2 are of the same order of magnitude as that of T-8 **1a** from the first experiment.[5] A closer inspection, however, shows that, in experiment 2, the specific incorporation rates of T-6 **2a** and T-5 **2c** are nearly twice that of T-8. Compound T-6 **2a** (6.50 mg), which in this experiment was found to be the major alkaloid of the venom, was then diluted with racemic synthetic[4] material (23.91 mg) as carrier, and divided into two parts, each of which was separately submitted to a degradation scheme analogous to that devised for T-8[5] (Scheme 2). This procedure allowed us to split the molecule into three moieties:‡ *N*-methylpyrrolidine **9**, comprising C-4 to C-7, *N*-tosyl-*p*-bromophenacylprolinate **10**, comprising C-1 to C-3, C-9 and C-10, and *p*-bromophenacylhexanoate **11**, comprising C-8 and C-11 to C-15 of T-6 (Scheme 2). The specific activities and relative specific activities (RSA) of compounds **9** to **11**, and of the two

1a 9β-H; R = C_5H_{11} (T-8)
b 9β-H; R = C_3H_7 (T-4)
c 9α-H; R = C_5H_{11} (T-7)
d 9α-H; R = C_3H_7 (T-3)

2a 9β-H; R = C_5H_{11} (T-6)
b 9β-H; R = C_3H_7 (T-2)
c 9α-H; R = C_5H_{11} (T-5)
d 9α-H; R = C_3H_7 (T-1)

Fig. 1 Structures of the eight tetraponerines

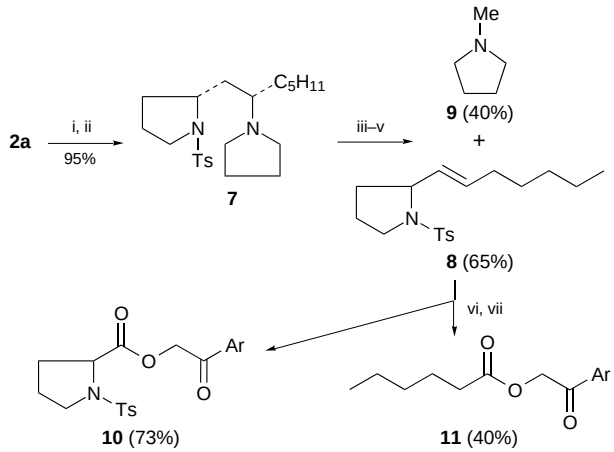


Scheme 1 Biogenetic scheme proposed for tetraponerine-8 **1a**

Table 1 Incorporation of [1,4-^{14}C] putrescine (50 μCi, 114 Ci mol^{-1}) into the tetraponerines

Experiment	Compound	Amount/mg	TA[a]/μCi	10^2 SA[b]/Ci mol^{-1}	10^2 SIR[c] (%)
1	**1a** (T-8)	6.4	1.01	4.0	3.7
2	**1a** (T-8)	5.70	0.68	3.0	2.6
2	**2a** (T-6)	6.70	1.44	5.1	4.5
2	**1b** (T-4)	0.81	0.12	3.3	2.9
2	**2c** (T-5)	0.22	0.05	4.9	4.3

[a] Total activity. [b] Specific activity. [c] Specific incorporation rate.



Scheme 2 Degradation scheme of tetraponerine-6 **2a**. *Reagents and conditions*: i, H_2, PtO_2, EtOH, HCl; ii, TsCl, pyridine; iii, MeI, MeCN; iv, OH^-; v, 160 °C, 3 h; vi, $KMnO_4$, $NaIO_4$, 24 h; vii, *p*-$BrC_6H_4COCH_2Br$, MeCN, reflux, 1 h (Ts = *p*-$MeC_6H_4SO_2$, Ar = 4-BrC_6H_4).

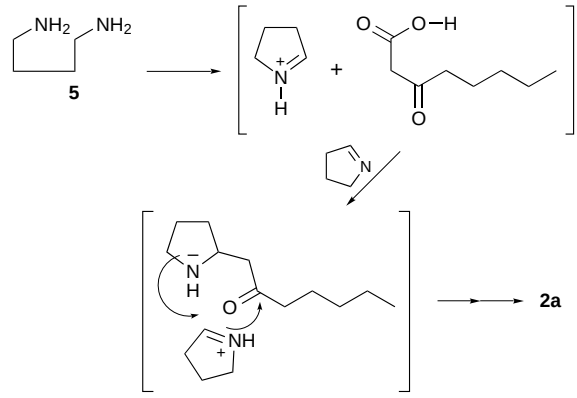
Table 2 Observed distribution of label within the degradation products of T-6 **2a**[a]

Compound	10^3 SA/ Ci mol^{-1}	RSA[b] (%)
2a[c]	10.9	–
7	10.7	100.0
8	5.0	47.5
9	5.0	46.5
10	4.7	44.5
11	0	0

[a] Mean of two separate experiments. [b] Relative specific activity, that of **7** being taken as 100%. [c] 6.50 mg diluted with 23.91 mg of cold synthetic (±)-**2a**.

degradation intermediates **7** and **8** (Scheme 2), calculated by using a 100% value for the RSA of **7**, are reported in Table 2.

Surprisingly, the radioactivity of T-6 **2a** was equally divided between *N*-methylpyrrolidine **9** and the olefin **8**. This was in complete contrast with our previous results on putrescine incorporation in T-8 **1a** (experiment 1),[5] where the olefin corresponding to **8** was not labelled at all. Moreover, after oxidative cleavage of the double bond of **8**, only the *N*-tosyl-*p*-bromophenacylprolinate **10** was found to be radioactive, whereas no activity was found in **11**. It turns out that, during the biosynthesis of T-6 **2a**, putrescine serves as precursor to both pyrrolidine rings. To confirm that different pathways are indeed operating in the biosynthesis of T-6 and T-8, the sample of T-8 **1a** (5.70 mg) isolated from the second incorporation experiment was also diluted with cold synthetic material,[6] and submitted to the usual degradation scheme. The results obtained were in complete agreement with those already reported for this alkaloid,[5] confirming that all the label from putrescine was located in the pyrrolidine ring of T-8, with no radioactivity detected at any other position of the molecule.



Scheme 3 Biogenetic scheme proposed for tetraponerine-6 **2a**

These results allow us to propose a biogenetic hypothesis for the formation of tetraponerine skeleton **2** in *Tetraponera* sp. ants. Thus, whereas skeleton **1** presumably originates from the condensation of a twelve-carbon polyacetate precursor with one putrescine unit (Scheme 1), skeleton **2** is formed by reaction of two putrescine units with an eight-carbon polyacetate chain, with concomitant decarboxylation (possibly under enzyme control, Scheme 3).

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Footnotes

† IUPAC numbering. For convenience, we will use the numbering of earlier publications.

‡ All compounds isolated from the degradation scheme were characterized by EIMS, ^1H NMR, ^{13}C NMR and IR spectroscopy, and, in some cases, by HREIMS.

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