Transformation of plant pyrrolizidine alkaloids into novel insect alkaloids by arctiid moths (Lepidoptera)

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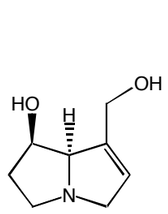
Abstract

Two new pyrrolizidine alkaloids (PAs) were isolated from adults of *Creatonotos transiens*, the larvae of which had ingested retronecine (**1**) or ester alkaloids. The structures were elucidated by GC-MS and ^{1}H and ^{13}C NMR spectroscopy as O^{9}-(2-hydroxy-3-methylpentanoyl)-retronecine (**6**, creatonotine) and its isomer O^{7}-(2-hydroxy-3-methylpentanoyl)-retronecine (**4**, isocreatonotine). The 2-hydroxy-3-methylpentanoic acid obtained by hydrolysis of the creatonotines is the 2S,3S-stereoisomer. The creatonotines as weil as accompanying trace amounts of callimorphine (**5**) were shown to be synthesized by both sexes from dietary retronecine by esterification. If *Creatonotos* larvae had been fed with *Gynura scandens* as PA-source, the insect PAs accounted for about 75% of total PAs isolated from adults, indicating that *Creatonotos* degraded plant PAs and subsequently reesterified the resulting retronecine. This provides definitive proof that an arctiid moth is abie to hydrolyze plant ester alkaloids and reesterify the resulting necine base with acids which are intermediates of insect metabolism. PAs isolated from *Creatonotos* were exclusively present in the form of their *N*-oxides.



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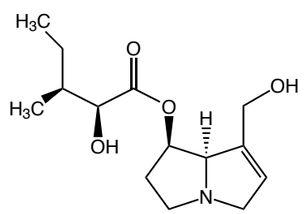

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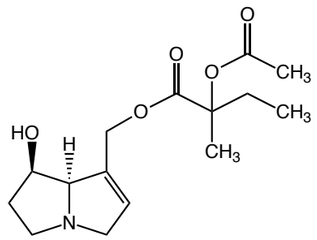

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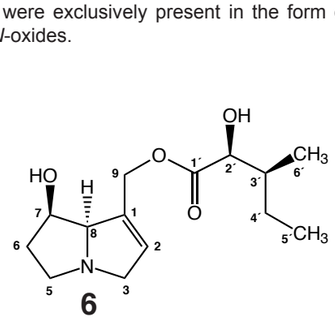

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Introduction

Pyrrolizidine alkaloids (PAs) represent a class of typical plant secondary compounds [11] and have received much attention in the study of insect-plant relationships [2]. Although PA-producing plants are usually avoided by herbivores, a number of insect species have evolved adaptations not only to cope with these compounds but also to utilize them for their own benefit: the insects store PAs obtained from plants and gain protection from predators, and in addition to the use of PAs for defense, males of various Lepidoptera synthesize sex pheromones from PAs of plant origin [see 2-5 for refs.].

„Callimorphine“ (**5**), a PA not known from plant sources, has been isolated from several arctiid moths: *Tyria jacobaeae* [7-9], *Callimorpha dominula* [7], *Arctia caja* [8], *Creatonotos transiens* [10], and *Gnophaela latipennis* [11]. Here we demonstrate the ability of *Creatonotos* to hydrolyze ingested plant PAs and to synthesize novel structures by esterification of the resulting necine with necic acids of insect origin.

Results

GC-Analysis of PAs extracted from adult *Creatonotos transiens*, the larvae of which had ingested retronecine (**1**) as the only source of alkaloids, revealed retronecine plus several retronecine derivatives, all as *N*-oxides (cf. Fig. 1). Two major compounds (**6**, **4**), which by GC- MS-analysis had been identified as isomeric O^{9}- and O^{7}-esters of retronecine, turned out to be novel natural products.

In addition to the creatonotines, callimorphine (**5**) has been found. It was identified by GC-MS by comparison with an authentic sample. Furthermore, among the hydrolysis products of PA extracts 2-hydroxy-2-methylbutanoic acid was detected as the TMS-derivative. This acid is formed during hydrolysis from 2-acetoxy-2-methylbutanoic acid, the acid moiety of 5. 5 accounts for less than 5% of total insect PAs.

Origin of the necic acids

Analyses of *Creatonotos* without access to PAs did not even show traces of retronecine derivatives. Analyses of the organic acid fraction, however, revealed the presence of a number of acids, particularly 2-hydroxy acids. Among those, small quantities of 2-hydroxy-3-methylpentanoic acid were detected and identified by GC-MS comparison with authentic material. Thus, the necic acid of 4 and 6 most likely is a product of insect metabolism.

Transformation of plant PAs into creatonotines

About 14 to 17% of dietary **1** was transformed into **4** and **6** by both sexes of *Creatonotos* (Table 1). In order to see whether the insects are able to produce the creatonotines if they had taken up ester alkaloids instead of the pure necine base, larvae were fed with *Gynura scandens* which contains gynuramine and O-acetylgynuramine as major PAs. GC-analyses of extracts of adult moths revealed **4**, **6** and **9** (gynuramine) as the major PAs, the creatonotines accounting for 60-75% of the total PAs. In addition, components of plant origin, i.e. senecionine, integerrimine and an unknown alkaloid „M^{+} 351“ and insect PAs such as callimorphine and the two isomeric 2-hydroxy-3-methylbutanoyl-retronecines, have been found in minor amounts. O-acetylgynuramine could not be detected in the PA extracts from insects.

If a mixture of five different intact plant PAs were fed more than 45% of total PAs recovered in males were creatonotine and isocreatonotine, but in females only trace amounts of these insect PAs were found (Table 2). Also, there are great differences in the capability to utilize plant PAs as precursors for creatonotines. Monocrotaline, for instance, is an excellent precursor whereas heliotrine is a poor one.

Discussion

Creatonotos moths are able to esterify retronecine (**1**) resulting in creatonotine (**6**), isocreatonotine (**4**) and trace amounts of callimorphine (**5**). **4** and **6** represent new pyrrolizidine alkaloids unknown from plant sources. For the first time it is thus demonstrated that insects are capable of synthesizing their own PAs by "partial biosynthesis", i.e. retronecine, which definitely is of plant origin, is esterified with necic acids which the insects apparently synthesize de novo.

Previously, **5** has been found in arctiid moths which as larvae had ingested PAs of quite different structures from various host plants [7-11]. Recently, labelled **5** could be isolated from pupae of *Tyria jacobaeae* which as larvae received [^{14}C]retronecine [12], indicating the ability of *Tyria* to esterify the necine base. This observation and the results presented above prove that **5** is formed by the insects through reesterification of **1** as suggested by L'Empereur et al. [11], however, the necic acid moiety is of insect and not of plant origin.

Both male and female *Creatonotos transiens* esterify dietary retronecine and there is no significant quantitative difference between the sexes. If *Creatonotos* larvae had ingested complex ester alkaloids, again a large proportion of total PAs found in the adult insects occur as creatonotines. Thus, *Creatonotos* is not only abie to reesterify **1** but also to produce **1** by hydrolyzing plant ester alkaloids. Experiments on the great quantitative differences in the capability of the insects to utilize different plant PAs as precursors for creatonotines are in progress. Although both sexes esterify **1** with the same efficiency, the ability to transform PAs into the creatonotines is expressed much more in males [13], i.e. males seem to be adapted to degrade ester alkaloids into **1**. This might be related to the biosynthesis of the male pheromone (hydroxydanaidal) which also proceeds *via* degradation of ester alkaloids [14].

In any case, two parallels between PA metabolism in plants and insects should be emphasized.
(1) Plants synthesize PAs as *N*-oxides and these are the specific forms of alkaloid translocation and accumulation [15]. The same seems to be true for *Creatonotos*: both the sequestered and the synthesized PAs are present as *N*-oxides. *Creatonotos* possesses the ability to *N*-oxidize PAs. Specific carrier mediated uptake systems for PA-*N*-oxides exist in the plant cell [16] as well as in the midgut of *Creatonotos* [17].
(2) In plants necic acids are preferably derived from the amino acid isoleucine, which is likely to be true also for insect-derived necic acids found in the creatonotines as well as in callimorphine.

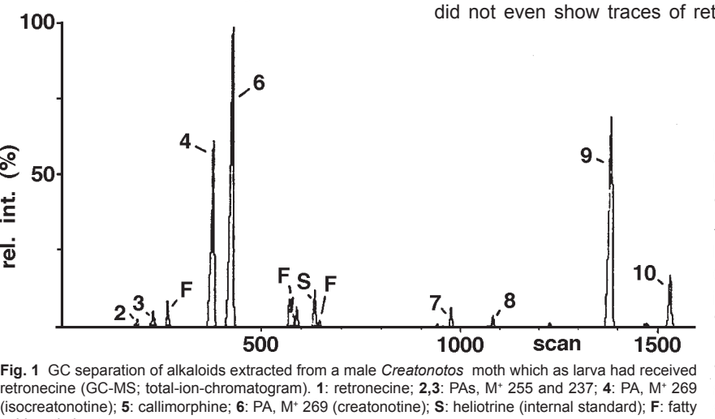

Fig. 1 GC separation of alkaloids extracted from a male *Creatonotos* moth which as larva had received retronecine (GC-MS; total-ion-chromatogram). **1**: retronecine; **2,3**: PAs, M^{+} 255 and 237; **4**: PA, M^{+} 269 (isocreatonotine); **5**: callimorphine; **6**: PA, M^{+} 269 (creatonotine); **S**: heliotrine (internal standard); **F**: fatty acid methyl esters

	Amount µmol/insect	% of fed	Concentration µmol/g fr. wt.
Males (n = 6)			
Creatonotine **6**	0.75 ± 0.24	11.5	3.59 ± 1.14
Isocreatonotine **4**	0.35 ± 0.09	5.3	1.68 ± 0.41
Total	1.10 ± 0.33	16.8	5.27 ± 1.55
Females (n = 6)			
Creatonotine **6**	0.68 ± 0.30	10.4	2.46 ± 1.10
isocreatonotine **4**	0.24 ± 0.13	3.7	0.86 ± 0.45
Total	0.92 ± 0.43	14.1	3.32 ± 1.55

Table 1 Transformation of retronecine (**1**) into creatonotines (**6**, **4**) by *Creatonotos transiens*. Retronecine (6.5 µmol/larva) was fed to last instar larvae; moths were extracted 24 h after emerging from pupae

	fed	Amount nmol/insect recovered	relative abundance	concentration nmol/g fr.wt.
Males (n = 5)				
Creatonotine **6**	—	232 ± 85	35 %	1027 ± 367
Isocreatonotine **4**	—	76 ± 46	11 %	336 ± 204
Heliotrine	640	64 ± 30	10 %	283 ± 133
Monocrotaline	610	96 ± 45	15 %	424 ± 199
Senecionine	536	163 ± 48	24 %	721 ± 212
Seneciophylline	61	27 ± 8	4 %	119 ± 3
Crotalaria PAs	1080	9 ± 3	1 %	41 ± 12
Total	2920	667 ± 265	100 %	2949 ± 1169
Females (n = 4)				
Creatonotine **6**	—	< 5		
Isocreatonotine **4**	—	< 5		
Heliotrine	640	62 ± 18	16 %	187 ± 54
Monocrotaline	610	92 ± 21	24 %	283 ± 63
Senecionine	536	193 ± 62	49 %	581 ± 187
Seneciophylline	61	25 ± 5	6 %	75 ± 6
Crotalaria PAs	1080	17 ± 1	5 %	51 ± 35
Total	2920	391 ± 107	100 %	1171 ± 355

Table 2 Sequestration of different plant PAs by *Creatonotos trensiens* (raised on artificial diet plus a mixture of pure PAs) and transformation of ingested PAs into creatonotine and isocreatonotine

Acknowiedgements

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Selected References

1. Mattocks AR (1986) Chemistry and Toxicology of Pyrrolizidine Alkaloids. Academic Press, London
2. Boppré M (1990) J Chem Ecol 16: 165
3. Boppré M (1986) Naturwissenschaften 73: 17
4. Schneider D (1986) in Perspectives in Chemoreception and Behavior (Chapman RF, Bernays EA, Stoffalano JG, eds) p. 123. Springer Verlag, Berlin & New York
5. Eisner T, Meinwald J (1987) in Pheromone Biochemistry (Prestwich GD & Blomquist GJ, eds) p. 251. Academic Press, Orlando/Fl
7. Edgar JA, Culvonor CCJ, Cockrum PA, Smith LW. Rothschild M. (1980) Tetrahedron Lett 21: 1383
8. Aplin RT, Benn MH, Rothschild M (1968) Nature 219: 747
9. Aplin RT, Rothschild M (1972) in Toxins of Animal and Plant Origin (de Vries A. & Kochva E, eds) p. 579. Gordon & Breach, London
10. Wink M, Schneider D, Witte L (1988) Z Naturforsch 43c: 737
11. L'Empereur KM, Li Y, Stermitz FR (1989) J Nat Prod 52: 360
12. Ehmke A, Witte L, Biller A, Hartmann T (1990) Z Naturforsch (sumitted)
13. Boppré M, Biller A, Witte L, Hartmann T (1990) (in prep.)
14. refs. in Boppré M, Schneider D (1989) Zool J Linn Soc 96: 339; see S. Schulz et al. (1990)
15. Hartmann T, Ehmke A, Eilort U, v. Borstel K, Theuring C (1989) Planta 177: 98.
16. Ehmke A, v. Borstel K, Hartmann T (1988) Planta 176: 83
17. Wink M, Schneider D (1988) Naturwissenschaften 75: 524