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METABOLITES OF INSECTICIDAL CHROMENES FROM THE MIGRATORY GRASSHOPPER *MELANOPLUS SANGUINIPES*

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Abstract—Three insecticidal acetylchromenes, including encecalin, and precocene II were topically administered to adults of the migratory grasshopper *Melanoplus sanguinipes*. Metabolites formed and excreted via the frass were identified by gas chromatography–mass spectrometry (GC–MS) and by direct comparison with reference compounds obtained by partial synthesis or from plant sources. Of the 12 metabolites found, six compounds were new natural products. Their tentative structure elucidation is described based on MS analysis. The biological importance of the elucidated chromene metabolism in *M. sanguinipes* is discussed for the excretion and detoxification of potentially hazardous compounds.

INTRODUCTION

2,2-Dimethylchromenes (benzopyrans) are characteristic natural products of the Asteraceae [1]. Two chromene derivatives, precocene I and II, that were originally isolated from *Ageratum houstonianum* [2], are known as insect antijuvenile hormones [3]. In addition they are lethal to many insects at doses only slightly higher than those required for antihormonal function [4]. Compounds of the precocene type, however, are rare in the Asteraceae compared to the large group of 2,2-dimethylchromenes exhibiting acetyl substituents in the aromatic ring. Very little in comparison is known on the biological activities of acetyl-chromenes presumably since they lack the antihormonal functions reported for the precocenes [5]. Recently, however, we could show that acetylchromenes exhibit insecticidal activities against a plethora of herbivorous insects including the milkweed bug *Oncopeltus fasciatus* Dallas [5], the migratory grasshopper *Melanoplus sanguinipes* F. [6] and the variegated cutworm *Peridroma saucia* Hübner [7, 8]. The doses required for insecticidal activity of the tested acetylchromenes were usually higher than those needed for the precocenes but still significantly lower than the natural concentrations of these compounds found in many plants.

The metabolic fate of precocene II when incorporated by insects has received considerable attention [9–12]. No information, however, was available to date on the insect metabolism of acetylchromenes. We have now studied the fate of three widespread acetylchromenes and of precocene II in adults of the migratory grasshopper *Melanoplus sanguinipes*, a major pest of North American rangelands and cereal crops, and wish to report on the metabolites identified in the frass of the grasshoppers.

RESULTS AND DISCUSSION

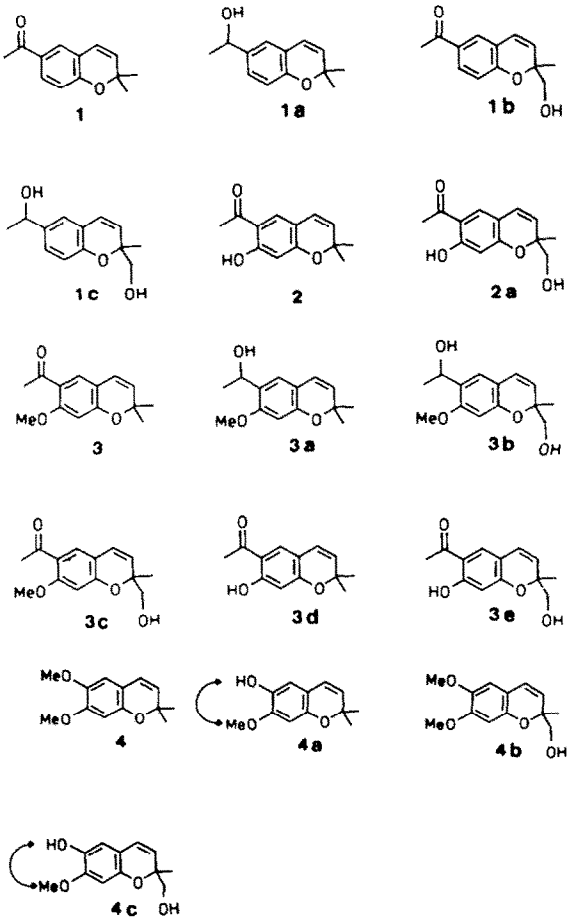
The chromene derivatives demethoxyencecalin (**1**), demethylencecalin (**2**), encecalin (**3**) and precocene II (**4**) were applied to adult grasshoppers topically via the abdomen. Preliminary studies had indicated that the compounds penetrate through the integument within a few hours. Frass produced by the insects within 48 hr after treatment was extracted and analysed by GC-MS. For all applied chromenes the parent compound as well as metabolites produced by the insect could be recovered and tentatively identified based on their mass spectra (Table 1) and on retention indices compared with known chromene derivatives originating from plant sources or obtained by synthetic modification of naturally occurring plant products.

Grasshoppers treated with demethoxyencecalin (**1**) yielded three metabolites along with the applied chromene. Metabolite **1a** was the corresponding 6(1-hydroxyethyl)-derivative of **1**. Its molecular ion was two mass units higher than that of **1** and the mass spectrum was characterized by a strong $[M-15]^+$ fragment (base peak). Additionally no fragment at m/z 43 corresponding to the CH_3CO group of acetylchromenes could be observed. Mass spectrum and retention index were identical to a reference compound obtained by reduction ($LiAlH_4$) of the carbonyl group of demethoxyencecalin (**1**). Metabolite **1b** was the 2-methyl-2-hydroxymethyl-derivative of **1**. Its mass spectrum was characterized by a dominating $[M-31]^+$ fragment. The $[M-15]^+$ fragment, usually the most intensive ion of 2,2-dimethylchromenes, was less than 1% in this case. The identity of this major metabolite of demethoxyencecalin as well as of the one (**2a**) originating from demethylencecalin (**2**) was

Table 1. MS data* and retention indices (RI) of parent chromenes and metabolites

m/z	rel. int.	m/z	rel. int.	m/z	rel. int.	m/z	rel. int.
1 (RI 1605)		**2** (RI 1718)		**3b** (RI 2015)		**4a** (RI 1590)	
202 $[M]^+$	12	218 $[M]^+$	28	394 $[M]^+$	3	206 $[M]^+$	28
187	100	203	100	379	2	191	100
144	23	185	13	305	3	176	20
43	19	43	28	291	100	**4b** (RI 1835)	
1a (RI 1560)		**2a** (RI 1955)		201	4	236 $[M]^+$	11
204 $[M]^+$	14	234 $[M]^+$	6	129	4	205	100
189	100	203	100	117	7	191	27
171	7	185	17	73	23	161	21
145	12	43	6	**3c** (RI 2057)		**4c** (RI 1815)	
115	8	**3** (RI 1815)		248 $[M]^+$	7	222 $[M]^+$	10
1b (RI 1840)		232 $[M]^+$	20	217	100	191	100
218 $[M]^+$	2	217	100	144	10	176	23
187	100	43	26	115	8		
144	24	**3a** (RI 1765)		43	31		
115	10	234 $[M]^+$	20	**4** (RI 1615)			
43	23	219	100	220 $[M]^+$	28		
1c (RI 1785)		201	20	205	100		
220 $[M]^+$	4	189	8	189	8		
189	100			162	8		
174	8						
145	11						

*Only characteristic fragments (rel. int. > 2) are given.
†Only measured as TMS-derivative.



further confirmed by direct comparison with identical biotransformation products formed by cell suspension cultures of *Ageratina adenophora* (Asteraceae) upon feeding of **1** and **2**. Of the biotransformation products enough material was available for high resolution ^1H NMR which supported the structures of **1b** and **2b** (Proksch, P., in preparation). The third metabolite found (**1c**) was the 2-methyl-2-hydroxymethyl-6(1-hydroxyethyl) derivative of **1**. The mass spectrum of **1c** was similar to that of **1b** with the difference that the molecular ion and the base peak $[M-31]^+$ were two mass units higher than the corresponding fragments of **1b** and no *m/z* 43 fragment was observed. Mass spectroscopy of the tetramethylsilane-ether derivative revealed the presence of two tetramethylsilane groups in the molecule and agreed only with the assigned structure. Whereas metabolites **1a** and **1b** are known from plant sources [1], compound **1c** is a new natural product.

Besides the applied chromene demethylencecalin (**2**) only one metabolite could be identified in the frass of the grasshoppers that corresponded to the 2-methyl-2-hydroxymethyl-derivative of **2**. The mass spectrum of **2a** was like that of metabolite **1b** characterized by a small molecular ion, the $[M-31]^+$ fragment being the base peak and a $[M-15]^+$ ion of less than 1% intensity. Compound **2a** is also a new product.

Grasshoppers that had been treated with encecalin (**3**) yielded the largest number of metabolites of the four chromenes studied. Metabolite **3a** was the 6(1-hydroxyethyl) derivative of **3**. Its mass spectrum was characterized by the molecular ion at *m/z* 234 and two large fragments at *m/z* 219 ($[M-15]^+$, base peak) and *m/z* 201 ($[M-15-18]^+$). Also a $[M-18]^+$ fragment was observed. Mass spectrum and retention index of **3a** were identical to those of a reference compound derived from encecalin by reduction ($LiAlH_4$) of the acetyl substituent.

Metabolite **3b** could only be observed after treating the crude frass extract with MSTFA and thus producing the tetramethylsilane-ether derivatives of the metabolites. The structure of **3b** was assigned as the 2-methyl-2-hydroxy-methyl-6(1-hydroxyethyl) derivative of **3** based on the mass spectrum that was characterized by the molecular ion at *m/z* 394 and only two intense ions. One of them represented the trimethylsilyl group at *m/z* 73, the base peak at *m/z* 291 was formed by the loss of a trimethylsilylated hydroxymethyl group, a fragmentation likewise favoured as with the derivatives **1b** and **2a**. A further significant ion at *m/z* 117 corresponded to the silylated hydroxymethyl group at C_6. Compound **3c** was the major metabolite of encecalin found in the frass. Its mass spectrum followed that of the corresponding metabolites **1b** and **2a** arising from demethoxyencecalin (**1**) and demethylencecalin (**2**). The molecular ion of **3c** was observed at *m/z* 248, the base peak at m/z 217 which corresponded to $[M-31]^+$. Metabolites **3d** and **3e** were demethylation products of **3** and **3c** and identical in all aspects to **2** and **2a** respectively. Metabolites **3b** and **3c** are new natural products.

Precocene II (**4**) gave rise to three metabolites tentatively identified in the frass of the grasshoppers. Precocene II was recovered only in minute amounts. Metabolite **4a** was a demethylation product of **4**. The molecular ion of **4a** was consecutively observed at *m/z* 206. The fragment $[M-15]^+$ was the base peak in the mass spectrum. The assignment of the hydroxy substituent either at C-6 or C-7, however, was not possible from the mass spectrum and the same holds true for metabolite **4c**. Metabolite **4b** proved to be the 2-methyl-2-hydroxymethyl derivative of precocene II. Its mass spectrum was in agreement with data published previously for this compound obtained synthetically from precocene II [10]. Compound **4c** was the 6- or 7-demethylanalogue of **4b** as could be followed from the mass spectrum. Both the molecular ion at *m/z* 222 and the base peak at *m/z* 191 ($[M-31]^+$) were 14 mass units lower than the coresponding signals of **4b**. Of the metabolites identified, **4b** and **4c** are new natural products, whereas **4a** is known as a metabolite of precocene II formed *in vitro* on incubation with monooxygenases from fat body homogenates of the cabbage looper *Trichoplusia ni* [10]. When frass extracts from grasshoppers treated with **3** or **4** were partitioned between water and CH_2Cl_2 and the water fractions were hydrolysed under acidic conditions no liberated chromenes could be detected by gas chromatography–mass spectrometry, implying that no methanol-soluble or acid hydrolysable chromene conjugates had been formed.

At first it may seem surprising that no 3,4-diols are among the metabolites identified after treating the insects with chromenes, since the diol-derivatives have been reported frequently as metabolites of precocene II in insects [e.g. 9, 11, 12]. A previous study on precocene metabolism in the haemolymph of *Melanoplus sanguinipes*, however, had likewise shown the absence of diols in this insect [9]. Glucosides of 7-demethylprecocene II were reported as major metabolites instead. The absence of hydrolysable chromene conjugates in the excreta as established by us could perhaps be explained by a hydrolysis of haemolymph-born chromene conjugates during excretion by the Malpighian tubules [13]. Further studies are being carried out.

Of the metabolites originating from **1–4**, those possessing additional hydroxy substituents are significantly less toxic to the grasshoppers that their corresponding parent compounds as could be demonstrated previously with larvae of *Peridroma saucia* [8] and *Melanoplus sanguinipes* [6]. Bioassays to assess toxicity of the prominent 2-methyl-2-hydroxy-methyl-derivatives are in progress. In summary it can be said that the metabolites originating from **1–4** are more polar than the administered parent compounds which will facilitate their excretion via the frass and that at least several, if not all, of the metabolites are also significantly less toxic than the chromenes applied to the insects. Since demethylencecalin (**2**) is the least toxic chromene to the grasshoppers, it is of interest to note that this compound is excreted largely unchanged and only one metabolite is formed which is in significant contrast to the data obtained with the other chromenes. It may thus be that different insecticidal activity of the chromenes is directly correlated to the ability of the insects to excrete incorporated chromenes largely unchanged or only after elaborate enzymatic metabolism. Further experiments involving radioactive chromenes will be important to address the question whether the rate of excretion of an unchanged chromene is correlated to its toxicity.

EXPERIMENTAL

Chromenes **1–3** were isolated and purified from *Encelia* spp. as described earlier [1]. Precocene II was purchased from Sigma. Adults of *Melanoplus sanguinipes* were treated with 60 μg of each chromene (dissolved in 1 μl of Me_2CO) topically at their abdomen. The insects were allowed to feed on wheat blades for 48 hr after the treatment. Frass produced within that period of time was collected and extracted with MeOH. One group of grasshoppers treated in one experiment usually consisted of six males and six females. Frass extracts were taken to dryness and partitioned between H_2O and CH_2Cl_2. The water fractions obtained after partitioning were acidified to give 1 N HCl and subsequently hydrolysed for 1 hr at 100°. After cooling off liberated compounds were extracted by partitioning against CH_2Cl_2. The CH_2Cl_2 fractions were subjected to GC analysis performed on 15 m × 0.23 mm i.d. fused silica glass (quartz) capillary columns coated with the methyl silicone phase DB-1 (J & W Scientific) employing a Perkin-Elmer gas chromatograph F 22 with flame ionization detection. Conditions: temp. 100–300, 6°/min; carrier gas helium, 0.7 bar; split ratio 1:30. Retention indices were calculated using co-chromatographed standard hydrocarbons according to ref. [14]. For GC-MS the same chromatographic system was directly coupled with the mass spectrometer AEI MS 30. Spectra were recorded at an electron energy of 24 eV in combination with the data system AEI DS50/55. Silylation was performed in the usual way using MSTFA.

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