OCCURRENCE OF JUVABIONE-TYPE OR EPIJUVABIONE-TYPE INSECT JUVENILE HORMONE ANALOGUES IN THREE "CZECHOSLOVAKIAN FIRS"*

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The three "Czechoslovakian firs" from the Banská Štiavnica Arboretum, *Abies balsamea* grove, have been examined. Each of these trees appears to be physically distinct; volatile leaf oil compositions indicate that only one tree (No 1) is an *A. balsamea*. This tree contains dehydrojvabione (*II*). The tree No 2 contains epijuvabione (*V*), 4'-dehydroepijuvabione (*VI*), and a third insect juvenile hormone analogue epijuvabiol (*VII*); it is not an *A. balsamea*. The third tree contains juvabione (*I*), 4'-dehydrojuvabione (*II*) and juvabiol (*III*); but it does not contain isojuvabiol (*IV*), it too is not a true balsam fir. Unequivocal assignments of stereochemistry for each compound were made following ^{13}C-NMR and, or, optical rotatory dispersion studies. All juvabione-type compounds isolated in this study are insect juvenile hormone analogues with selective action on the family *Pyrrhocoridae*.

The wood of balsam fir (*Abies balsamea* (L). MILL) contains factors exhibiting juvenile hormone activity on the hemipteran insects of the family *Pyrrhocoridae*. This „paper-factor" effect was first noted by Sláma and Williams[1] with Bowers and coworkers[2] isolating juvabione (*I*) as the active component from balsam fir wood. More recent studies[3,4] have shown that the paper factor is not due to *I* alone. Some other compounds, also responsible for this effect, are 4'-dehydrojuvabione (*II*), juvabiol (*III*) and isojuvabiol (*IV*) (ref.[3,4]). Indirect evidence indicates that the 4'-dehydrojuvabiols may also be partly responsible for this "paper-factor" effect[4].

The first indication that juvenile hormone activity of the wood extracts was not only due to juvabione was provided by Černý and coworkers[5] who published a paper entitled "Dehydrojuvabione – a new compound with juvenile hormone activity from balsam fir". Their work, which did not give evidence for the absolute configura-

* Part V in the Juvabione and its Analogues; Part IV: Can. J. Chem., in press; Part VI: Phytochemistry, in press.

tion of the dehydrojuvabione, was based on the branch wood extract from one of three trees growing in the. "*A. balsamea*" grove in the Arboretum at Banská Štiavnica[6]. While their work effectively extended the field of study at that time, the scope of this extension has but recently been fully understood. It should be noted that their work described not one but two new compounds[3,7]. They were (+)-epijuvabione (*V*) and (+)-4′-dehydroepijuvabione (*VI*) and were the C—1′ epimers of *I* and *II* respectively. Based on the knowledge of the chemical constituents of the native *A. balsamea* some doubt as to the species identity of the tree sampled by Černý and coworkers[5] has been raised by our studies[3,4].

The original intention of the present study was to secure a sample of *V* for ^{13}C-NMR studies of a pair of diastereomers (*I* and *V*) that were virtually identical by more conventional techniques, including $[\alpha]_D$ and melting points of the parent acids. However, several problems had to be overcome, these included precise identification of the originally sampled tree, re-isolation of *V* and possible species identification of the tree sampled by Černý and coworkers[5] (unfortunately the technician who brought the sample to Prague could not specify from which of the three trees he had taken the branches). Historically there can be no doubt as to the female lineage of the three trees in the "*A. balsamea*" grove at Banská Štiavnica – the arboretum from which the original samples of *V* and *VI* were obtained by Černý and coworkers[5,6]. However, these specimens were not native to North America but were grown from seed produced at another European arboretum. It is, therefore, quite probable that some of these seeds were cross pollinated, possibly with *A. alba*.

This study examines the juvabione-type compounds, leaf oil monoterpenes and foliage characteristics (morphological and anatomical[8]) of each tree from the "*A. balsamea*" grove at Banská Štiavnica. Moreover, it identifies the tree sampled by Černý and coworkers[5,6] and extends their work by noting the presence of a third active component in the branch wood extract. A comparison of our findings with the data on the natively growing *A. balsamea* in Canada may provide additional information with respect to the influence of such different biotopes on the chemical and morphological patterns in these plants.

RESULTS

As the results from each tree are different, each is discussed in turn. The tree located left from the road is indicated as tree No 1, the middle as tree No 2 and the last as tree No 3. The measures examined include gas liquid chromatography analysis (GLC) of the leaf oils, physical characteristics of the branch foliage (morphological and anatomical) and identification of juvabione-type compounds.

Tree No 1: As shown in Fig. 1, tree No 1, the branch foliage of this tree is typical of this species, with stomata on lower surfaces, median canals and separate bundles.

PERCENT COMPOSITION
0 5 10 15 20 25 30 35 40
PEAK NUMBER
0 10 20 30 40
TREE 1
PERCENT COMPOSITION
0 5 10 15 20 25 30 35 40
PEAK NUMBER
0 10 20 30 40
TREE 2
PERCENT COMPOSITION
0 5 10 15 20 25 30 35 40
PEAK NUMBER
0 10 20 30 40
TREE 3

Moreover, the distribution of leaf monoterpenes is indicative of the western variety of *A. balsamea*[9,10], (Table I). Extraction of the branchwood with ethanol and subsequent partitioning into light petroleum resulted in small amount of extract for study (Table I). Part of this extract was separated by silica gel column chromatography and 4'-dehydrojuvabione (*II*) was obtained. This compound was initially identified on the basis of its GLC r_t (relative to internal *I*, 1·66), thin layer chromatogram R_F value, and ^{1}H-NMR spectrum. The *R* chirality at C−1' was proven by hydrogenating this sample and noting that the optical rotatory dispersion curve for the generated dihydrojuvabiones was identical with ones from previous studies[3,10]. Although GLC analyses of the light petroleum soluble material indicated the possible presence of juvabione (*I*) and juvabiol (*III* and/or *IV*) there was insufficient quantity of material available to isolate these compounds.

Tree No 2: The results presented for this tree are the averages of three separate analyses of individual branches. The leaves with marginal canals, stomata on lower surface only, bundles separated, and leaf oil analysis (Fig. 1, tree No 2, Table I)

TABLE I

Relative Percentages of Some Diagnostically Important Terpenes of the Leaf Oil of the Three "*Abies*" Trees from the Banská Štiavnica Arboretum, Eastern and Western Canadian *A. balsamea*, and *A. alba*

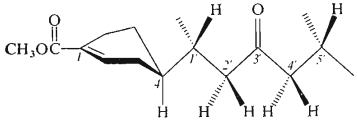
Peak	Terpene	Banská Štiavnica			*A. balsamea*[a]		*A. Alba*	
		1	2	3	eastern	western	A[b]	B[c]
1	santene	2·1	2·6	1·6	1·9	2·8	2·6	2·3
2	tricyclene	0·8	1·5	0·6	1·1	1·1	1·5	1·5
5	α-pinene	9·8	13·2	20·6	9·4	7·5	22·0	26·4
6	camphene	6·1	13·2	4·4	8·5	8·5	12·7	14·8
7	β-pinene	43·6	20·7	11·3	34·6	44·8	10·1	3·6
10	car-3-ene	0·4	tr	tr	11·0[d]	0·1	0·1	6·2
14	limonene	8·8	26·8	1·7	5·3	5·0	28·2	26·3
15	β-phellandrene	7·8	4·5	22·0	2·0	4·7	3·9	1·5
33	bornyl acetate	12·3	7·0	0·3	12·2	12·9	6·5	5·4

[a] Means from several different populations[9]; [b] Means from four trees from Czechoslovakia[11]; [c] Commercial leaf oil[10]; [d] two phenotypes are present in eastern Canadian populations, one with 0—0·5% car-3-ene and other with 7—36% car-3-ene[9].

FIG. 1

Branch Foliage and Leaf Oil Patterns for the Three Trees of the "*A. balsamea*" Grove in the Arboretum at Banská Štiavnica

were not typical of *A. balsamea*[9,10]. Three juvabione-type compounds (Table II), two ketones and one alcohol, were isolated according to established procedures[3,4,11]. On the basis of r_t, R_J, ^{1}H-NMR, $[\alpha]_D$ and mass spectra, the two ketones (r_t 1·00 and 1·66) were found to be identical with those compounds previously reported on by Černý and coworkers[5]. The fact that they both are C–1′ epimers of *I* and *II* respectively was revealed by hydrogenating a small portion of the mixed fractions and recording the optical rotatory dispersion curve. This curve was essentially a mirror image of those obtained previously for hydrogenated samples of *I* and *II* from North American grown *A. balsamea*[3]. These two ketones are epijuvabione (*V*) and 4′--dehydroepijuvabione (*VI*). Their ^{13}C-NMR spectra were in complete accord with *V* and *VI* being C–1′ epimers of *I* and *II* respectively[13].



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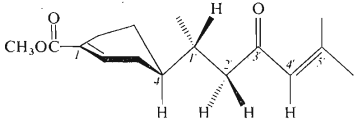

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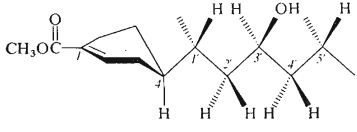

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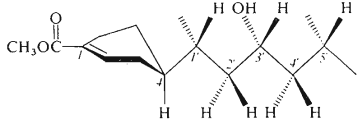

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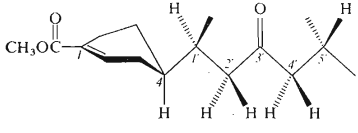

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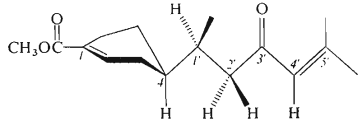

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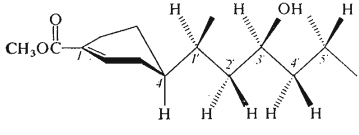
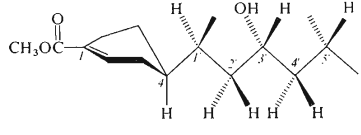


VII



VIII

The third juvabione-type compound was isolated from the polar components by the acetylation technique[4]. A portion of the original alcohol was recovered by deacetylation. On the basis of standard analytical techniques it appeared to be a "juvabiol" (r_t 1·37). High performance liquid chromatography showed only one component. It was identified as a 1′*R*, 3′*R* or 1′*S*, 3′*S* isomer on the basis of its retention volume[4]. ^{13}C-NMR of this alcohol (and its acetate) confirmed the presence of only one isomer and showed it to be epijuvabiol (*VII*) (epijuvabiol *VII* acetate), the major isomer produced by $NaBH_4$ reduction of *V* (ref.[12]). No evidence of isoepijuvabiol (*VIII*) or other related alcohols was noted. The alcohol *VII* was tested for insect juvenile hormone activity. The observed activity, listed in Table III, was lower than noted previously for the epimeric mixture of *III* and *IV* isolated from *A. balsamea*. The ketones *V* and *VI* were re-examined for juvenile hormone activity; the results are also listed in Table III.

Tree No 3: The branch morphology of this tree is unlike that expected for *A. balsamea*. It resembles more closely that of *A. alba*, however, the canals were median, not marginal[8]. In addition, analysis of the leaf oils (Fig. *1c*) clearly indicates that the tree No 3 is unlike either species[9,10]. This tree is markedly different from tree No 2; note that the major leaf oil components are now β-phellandrene (peak 15) and α-pinene (peak 5), whereas tree No 2 had limonene (peak 14) and β-pinene (peak 7) as major components. The branchwood extract of this tree also yielded three juvabione-type compounds (Table II). They were characterised as being *I*, *II* and *III*. Again only one alcohol was found, it was shown to be a 1′*R*, 3′*S* or 1′*S*, 3′*R* isomer by high performance liquid chromatography[4]. ^{13}C-NMR identified it as juvabiol (*III*) (ref.[12]), no evidence of other related alcohols was noted. When the alcohol *III* was tested for juvenile hormone activity, it was found to be slightly more active than the epimeric mixture of *III* and IV obtained from *A. balsamea* (Table III).

TABLE II

Juvabione-type Extractives from the Branchwood of Trees Growing in the "*A. balsamea*" Grove of the Arboretum at Banská Štiavnica

Tree No	Yield (% o.d. wood)		% GLC of light petroleum		
	ethanol	light petroleum	r_t 1·00	1·37	1·66
1	4·56	0·60	5·1	20·9	45·3
2	19·23	1·44	33·1	22·9	55·0
3	35·44	0·94	27·4	8·4	29·8

On the basis of the data presented herein it is apparent that tree No 2 was the tree previously sampled by Černý and coworkers[5]. This tree contains epijuvabione (*V*), 4′-dehydroepijuvabione (*VI*) and a third compound with juvenile hormone activity epijuvabiol (*VII*). This documents that epijuvabione (*V*) had been the compound supplied by Černý to Pawson and coworkers[7], who determined that it had the 4*R*, 1′*S* stereochemistry and believing this compound to be identical with the material commonly found in native *A. balsamea*, inadvertently revised the 4*R*, 1′*R* assignement of stereochemistry[14] for (+)-juvabione to 4*R* 1′*S* (for detailed discussion see[3]). The relatively low juvenile hormone activity of *VII* may account for the fact that Černý and coworkers[5] did not note the occurrence of this compound in their branchwood extract. It now becomes clear that this tree No 2 is not a true *A. balsamea*. On the basis of its leaf oil analysis it could be a hybrid with *A. alba* as the noted concentrations are indicative of contributions from both species. Thus the amount of α-pinene (peak 5) is 13·2% and β-pinene (peak 7) is 20·7%; whereas *A. alba* has 26·4 and 3·6% respectively and *A. balsamea* (western) has 7·5 and 44·8% respectively[9,10]. Also supporting this possible cross are the facts that, whereas *A. balsamea* contains only juvabione-type compounds sith *R* chirality at C−1′[3,4], *A. alba* contains either or both C−1′ epimeric forms[10].

TABLE III

Juvenile Hormone Activity of the Isolated Juvabione-Type Compounds in the Last Instar Larvae of *Pyrrhocoris apterus* L. and *Dysdercus cingulatus* F. (*Pyrrhocoridae*), *Graphosoma italicum* MULL. (*Pentatomidae*), and in Pupae of *Tenebrio molitor* L. (*Tenebrionidae*)

The values indicate amount of the compound in micrograms per specimen which cause in topical application just 50% retention of juvenile morphological characteristics (ID-50 Morph. units)[15]; in.-inactive up to 500 μg per spec.

	Substance	Juvenile hormone activity			
		Pyrrhocoris	*Dysdercus*	*Graphosoma*	*Tenebrio*
I	juvabione	1·7	2·3	in.	in.
V	epijuvabione	4·5	4·3	in.	in.
II	4′-dehydrojuvabione	5·0	2·0	in.	in.
VI	4′-dehydroepijuvabione	7·0	4·0	in.	in.
III	juvabiol	5·0	4·6	in.	in.
VII	epijuvabiol	30·0	12·0	in.	in.
III *IV*	juvabiol and isojuvabiol	5·6	4·7	in.	in.
III *IV*	juvabiol and iso-juvabiol acetates	3·8	4·2	in.	in.
VII *VIII*	epijuvabiol and iso-epijuvabiol acetates	3·2	1·2	in.	in.

It is noteworthy that both trees No 2 and No 3 contain only one juvabione-type alcohol each (*VII* and *III* respectively), whereas *A. alba*[11], *A. balsamea*[4], and *A. lasiocarpa*[12] contain two or more epimeric alcohols. It is also interesting to note that these two alcohols have the same stereochemistry at C−3′(*S*) and that these co-occur in *A. lasiocarpa*[12]. Whereas both trees No 1 and No 3 contain juvabione-type compounds with *R* chirality at C−1′ and therefore conform with being *A. balsamea*[3,4], only tree No 1 appears to be *A. balsamea* on the basis of its foliage characteristics and leaf oil constituents. Tree No 3 differs from typical *A. balsamea* in both its foliage and leaf oil terpenes (Table I). The external appearance of the foliage is characteristic of *A. alba*, but its anatomical features and leaf oil constituents are markedly different. Moreover, the leaf oil pattern is unlike that found for tree No 2, which leaves the species (species hybrid) identification of tree No 3 unresolved. In this respect we have to consider possible influence of the biotic and abiotic factors (different climatic conditions, soil composition, *etc.*) which are associated with the locality of the arboretum trees at Banská Štiavnica.

CONCLUSIONS

The *A. balsamea* grove in the Arboretum at Banská Štiavnica contains three distinctly different trees, only one of which (Tree No 1) is an *A. balsamea*. The remaining two are not true *A. balsamea*, they could be putative crosses. Tree No 1 contains 4′-dehydrojuvabione (*II*) with *R* chirality at C−1′. Tree No 2 contains epijuvabione (*V*), 4′-dehydroepijuvabione (*VI*) and epijuvabiol (*VII*) each has *S* chirality at C−1′ and *VII* has *S* chirality at C−3′. Tree No 3 contains juvabione (*I*), 4′-dehydrojuvabione (*II*) and juvabiol (*III*) each has *R* chirality at C−1′ with *III* having *S* chirality at C−3′. All of the isolated juvabione-type compounds have *R* chirality at C−4 and are active analogues of insect juvenile hormone. Results of this study indicate that arboretum samples may not be good substitutes for the plants growing in their natural biotopes.

EXPERIMENTAL

The general methods and spectral measurements followed the formats outlined previously[3,4,11–13]. Specimen branches were obtained from the Arboretum at Banská Štiavnica. All juvabione-type compounds had physical and spectral measures in accord with the published values. Leaf oil analysis and insect juvenile hormone bioassays were made according to established procedures[9,10,15]. The foliage utilized in the leaf oil terpene analysis was collected during the dormant season (January-March) to ensure strict comparability of the quantitative data.

Tree No 1

4′-Dehydrojuvabione (*II*) (25 mg) isolated by silica gel column chromatography was >90% pure by GLC. Hydrogenation[4,11] resulted in a 26·8% *cis*- and 73·2% *trans*-dihydrojuvabione

mixture. The ORD-molecular amplitude was −21·2 (*c* 0·94, methanol; 23°C) (calculated value −22·8).

Tree No 2

Epijuvabione (*V*) and 4′-dehydroepijuvabione (*VI*) (mixture from silica gel column, <5% impurities) was hydrogenated to give a 29·7% *cis*- and 70·3% *trans*-dihydroepijuvabione mixture. The ORD-molecular amplitude was +22·8 (*c* 0·44, methanol; 23°C) (calculated value for 100% *R* chirality −22·9). Epijuvabiol (*VII*) was an oil and had high performance liquid chromatography r'_t 0·83 (relative to internal *I*) (lit. values[4] for *III* and *IV* are 0·76 and 0·83 respectively). The ^{13}C-NMR (20 MHz, deuteriochloroform) δ 167·8, 139·7, 129·8, 67·3, 51·1, 47·5, 42·0, 38·2, 32·7, 28·1, 25·7, 24·7, 24·3, 22·9, 21·9, and 15·2. Epijuvabiol (*VII*) acetate was an oil and had ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0·909 (6 H, doublet, J = 6·43 Hz), $0{\cdot}900_5$ (3 H, doublet, J = = 6·25); ^{13}C-NMR (20 MHz, $CDCl_3$) δ 170·3, 167·4, 139·1, 129·9, 70·4, 51·0, 44·2, 39·2, 37·8, 32·9, 27·9, 25·7, 24·7, 24·4, 22·7, 22·1, 20·8 and 15·5.

Tree No 3

Juvabione (*I*) and 4′-dehydrojuvabione (*II*) (mixture from silica gel column, <5% impurities) was hydrogenated to give a 28·7% *cis* and 71·3% *trans* dihydrojuvabione mixture. The ORD (*c* 0·38, methanol; 23°C) molecular amplitude was −22·2 (calculated value −22·9). Juvabiol (*III*) was and oil and had high performance liquid chromatography r'_t 0·76. The ^{13}C-NMR (20 MHz, $CDCl_3$) δ 167·7, 139·6, 129·3, 68·0, 51·5, 46·7, 42·8, 37·2, 33·3, 29·5, 24·7, 24·6, 23·8, 23·3, 21·7 and 16·2. Juvabiol (*III*) acetate was an oil and had ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0·909, (3 H, doublet, J = 6·62), $0{\cdot}897_5$ (6 H, doublet, J = 6·25); ^{13}C-NMR (20 MHz, $CDCl_3$) δ 170·5, 167·5, 139·3, 130·1, 71·3, 51·1, 43·1, 38·7, 37·2, 33·2, 29·5, 24·6, 24·4, 23·9, 22·9, 21·8, 20·9, and 15·9.

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