Phytochemistry, 1977, Vol. 16, pp. 1967–1971. Pergamon Press. Printed in England.

OCCURRENCE OF JUVABIONE-TYPE AND EPIJUVABIONE-TYPE SESQUITERPENOIDS IN *ABIES ALBA**

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(*Received* 30 *April* 1977)

Key Word Index—*Abies alba*; leaf oil monoterpene; wood extractives; sesquiterpenoids; insect juvenile hormone analogues; biosynthesis; stereoconfigurations; ^{13}C NMR; ORD; juvabione.

Abstract—The petrol-soluble fractions from the branchwood of four *Abies alba* trees were examined. Only two trees contained sufficient amounts of 'juvabione-type' insect juvenile hormone analogues for isolation and characterization. The first contained juvabione (4*R*, 1′*R*), 4′-dehydrojuvabione (4*R*, 1′*R*) and its 4*R*, 1′*S* diastereomer in a ratio of 3 : 1, and juvabiol (4*R*, 1′*R*, 3′*S*), isojuvabiol (4*R*, 1′*R*, 3′*R*) and epijuvabiol (4*R*, 1′*S*, 3′*S*) in an approximate ratio of 7 : 3 : 2. 4′-Dehydroepijuvabione (4*R*, 1′*S*) was the only 'juvabione-type' compound ioslated from the second tree. If it is accepted that juvabione and epijuvabione are enzymatically reduced forms of dehydrojuvabione and dehydroepijuvabione, respectively; then for these two *A. alba* our results indicate that only one enzyme which is specific for *R* chirality at C-1′ is present, since epijuvabione is not observed.

INTRODUCTION

A number of studies on the extractive composition of Canadian coniferous species has shown the occurrence of (+)—juvabione (4*R*, 1′*R*) (**1**) [1] and several other related insect juvenile hormone analogues (IJHAs) [2–5]. With one exception, all 'juvabione-type' sesquiterpenoids isolated from North American trees have *R* chirality at C-1′. That exception, epijuvabiol (4*R*, 1′*S*, 3′*S*) (**2**) with *S* chirality at C-1′, was found in the petrol-soluble fraction of the whole wood of a number of alpine fir (*Abies lasiocarpa*) trees [5].

A recent study [6] of three trees from the '*A. balsamea*' grove in the Banská Štiavnica arboretum showed that one tree contained epijuvabione (4*R*, 1′*S*) (**3**), 4′-dehydroepijuvabione (4*R*, 1′*S*) (**4**) and **2** [3, 7–9]. This result contrasted with previous studies on *A. balsamea* which revealed only 4*R*, 1′*R* diastereomers to be present in this species [3, 4]. In addition, the study of the three Czechoslovakian firs revealed that only the female parent was known; the seed came from another European arboretum. Because *A. alba* is the widespread European fir, it was considered as the likely male parent of these 'Czechoslovakian firs' and thus the genetic source of the 4*R*, 1′*S* forms of juvabione.

The study was undertaken to ascertain if these 'juvabione-type' sesquiterpenoids occurred in *A. alba* trees and if the *S* configuration at C-1′ was general for firs of European origin.

RESULTS

Quantitative analysis of volatile leaf-oil monoterpenes (listed in Table 1) [10] showed a significant tree-to-tree variation, but the characteristic terpenes of *A. alba* were present in the expected relative amounts [11]. The petrol-soluble materials from the branchwood of four *A. alba* trees were examined. Table 2 lists percent yields for each of the four trees examined. The general isolation

Table 1. Percentage compositions of leaf oils isolated from C.S.S.R. *Abies alba*

Monoterpene	Tree 1	2	3	4	Commercial oil
Santene	2.0	2.6	3.5	1.9	2.3
Tricyclene	1.0	1.5	2.4	1.2	1.6
α-Pinene	12.8	8.6	28.6	37.8	26.4
Camphene	9.0	11.8	19.7	10.4	14.8
β-Pinene	15.8	5.1	14.6	4.8	3.6
Myrcene	1.6	1.6	0.9	1.4	1.1
3-Carene	0.05	0.2	0.05	tr	6.2
Limonene	43.5	33.3	5.6	30.3	26.3
β-Phellandrene	(< 1)	12.3	2.4	0.5	1.5
Bornyl acetate	4.4	10.1	9.4	1.9	5.4

Table 2. Yields of some 'juvabione-type' extractives from the branchwood of *Abies alba* trees

Tree	Site*	Yield (% o.d. wood) EtOH	Yield (% o.d. wood) Petrol	%(GLC of petrol-solubles with *RR*, 1.00	1.35	1.66
1	1	15.4	1.6	33	4	63
2	2	9.3	0.6	<0.5	<0.5	83
3	2	1.7	0.3	<1	<1	7
4	2	1.8	0.3	<0.5	<0.5	6

*Site locations: (1) a planted forest near the arboretum at Banská Štiavnica, C.S.S.R., and (2) a natural forest near Hrabičov, C.S.S.R.

*Part 6 in the series 'Juvabione and its analogues', for Part 5, see ref. [6].

and purification procedures have been previously reported [2–5]. The purity (>90%) and tentative identities of each 'juvabione-type' compound were determined by GLC, TLC, IR, MS and PMR. The final identification and assignment of absolute configuration for each 'juvabione-type' compound from each tree was made after measurement of the ORD (of a hydrogenated sample) and/or, the ^{13}C-NMR spectra.

Tree 1

This tree contained juvabione (**1**) (RR_t = 1.00). The ^{13}C-NMR spectrum of this material showed it to be homogeneous and was in complete agreement with published values for **1** [9]. The *R* chirality at C-1′ was proven by comparing the ORD-molecular amplitude (MA) of a hydrogenated portion of this material with that calculated for the same ratio (GLC) of *cis* and *trans* isomers of dihydrojuvabione (**8** and **9**) (1′*R*). Since the agreement was well within experimental error, it confirmed the presence of only the 4*S*, 1′*R* isomer of juvabione. It should be noted that ^{13}C-NMR cannot distinguish between the 4*R*, 1′*R* and 4*S*, 1′*S* enantiomeric forms of juvabione and $[\alpha]_D$ values cannot be used to differentiate between the 4*R*, 1′*R* and 4*R*, 1′*S* forms of (+)—juvabione [3, 8].

What appeared to be 4′-dehydrojuvabione (**5**) (RR_t = 1.66) was also isolated from this tree by Si gel column chromatography. It was chromatographically pure and exhibited physical parameters expected for compound **5** [3]. However, the ^{13}C-NMR spectrum of this syrup revealed the presence of a second, minor component as evidenced by the number (20) of distinct carbon resonances; compound **5** has only 15 [9]. The minor resonances at δ 48.4, 33.0, 28.0, 25.7 and 15.9 were readily assignable to those carbon resonances of **4** which differ in chemical shift from those in **5** by more than 0.1 ppm [9]. In this manner, we were able to fully assign the carbon shifts observed. Thus, this unresolved material was identified as a 3:1 mixture of **5** and **4**. The proportions were determined by ^{13}C-NMR from the intensity values for the C-3 resonances of each isomer. It is possible to extract fairly accurate quantitative (5%) information by measuring intensities of the same carbon resonance of each component in an isomeric mixture. This has been verified by mixing known amounts of two of the pure compounds and determining their percentage composition by ^{13}C-NMR spectroscopy under the same experimental conditions as mentioned below.

The isomeric ratio of 3:1 was confirmed by measuring the ORD–MA of a hydrogenated portion of this syrup. The value was one-half that calculated for 100% *R* chirality at C-1′.

Also obtained from this Si gel column separation was

Table 3. ^{13}C-NMR data for the 'juvabione-type' alcohols and their acetates from *A. alba* (tree No. 1)

Alcohols			Alcohol acetates		
δ	Intensity	Carbon (compound(s))	δ	Intensity	Carbon (compound(s))
167.8	8	7 (**2**, **6**, **7**)	170.5	7	acetate
139.7	60	2 (**2**, **6**, **7**)	167.8	7	7 (**2**, **6**, **7**)
129.9	21	1 (**2**, **6**, **7**)	139.4	69	2 (**2**, **6**, **7**)
102.7	21	—*	139.0	7	—*
68.3	65	3′ (**6**)	130.1	14	1 (**2**, **6**, **7**)
67.4	20	3′ (**2**, **7**)	71.5	60	3′ (**6**)
51.2	81	8 (**2**, **6**, **7**)	70.8	18	3′ (**2**, **7**)
47.6	27	4′ (**2**, **7**)	51.3	69	8 (**2**, **6**, **7**)
46.6	60	4′ (**6**)	44.3	25	4′ (**2**, **7**)
42.2	80	2′ (**2**, **6**, **7**)	43.3	73	4′ (**6**)
38.3	24	4 (**2**, **7**)	39.4	21	2′ (**2**)
37.2	68	4 (**6**)	39.2	14	2′ (**7**)
33.2	69	1′ (**6**)	38.9	81	2′ (**6**)
32.8	20	1′ (**2**, **7**)	38.2	7	4 (**7**)
29.7	72	3 (**6**)	37.9	15	4 (**2**)
29.5	32	3 (**7**)	37.5	67	4′ (**6**)
28.2	20	3 (**2**)	33.4	71	1′ (**6**)
25.8	22	5 (**2**)	33.1	24	1′ (**2**, **7**)
24.7	96	6 (**2**, **6**, **7**)	29.7	78	3 (**6**)
24.6	63	5′ (**7**)	29.5	30	3 (**7**)
24.4	61	5′ (**6**); 5 (**7**)	29.4	11	—*
24.3	73	5′ (**2**)	28.1	20	3 (**2**)
23.8	66	5 (**6**)	25.9	22	5 (**2**)
23.3	65	7′ (**6**)	24.8	104	5 (**7**); 6 (**2**, **7**)
23.0	30	7′ (**2**, **7**)	24.6	84	6 (**6**); 5′ (**2**, **6**, **7**)
22.0	26	6′ (**2**, **7**)	24.1	77	5 (**6**)
21.7	52	6′ (**6**)	23.8	8	—*
18.3	48	—*	23.1	66	7′ (**6**)
17.8	25	—*	22.8	33	7′ (**2**, **7**)
16.2	65	8′ (**6**)	22.3	29	6′ (**2**, **7**)
15.3	17	8′ (**2**, **7**)	22.0	70	6′ (**6**)
			21.1	41	acetate
			16.1	66	8′ (**6**, **7**)
			15.7	18	8′ (**2**)

*Impurity.

an alcohol fraction (RR_t = 1.35). Sitosterol crystallized from this solution leaving a syrup that appeared to be juvabiol (4*R*, 1'*R*, 3'*S*) (**6**) or a mixture of **6** and isojuvabiol (4*R*, 1'*R*, 3'*R*) (**7**) as observed for *A. balsamea* [4]. Comparative analysis of the ^{13}C-NMR chemical shift data (Table 3) of this alcohol fraction, or that of an acetylated portion, with those values published [5] was only consistent with a three-component mixture. This mixture consisted of **6**, **7**, and **2** in an approximate ratio of 7:3:2. No evidence of the fourth isomer (isoepijuvabiol) was detected.

Tree 2

Dehydroepijuvabione (4*R*, 1'*S*) (**4**) (RR_t = 1.66) [3, 6, 7, 9] was the only 'juvabione-type' compound isolated from the branchwood of this tree. It was identified by GLC, TLC, PMR and ^{13}C-NMR. The *S* chirality at C-1' was confirmed by measuring the ORD–MA for a hydrogenated sample. The absolute value was within experimental error, but the sign was opposite, as expected for an enantiomer.

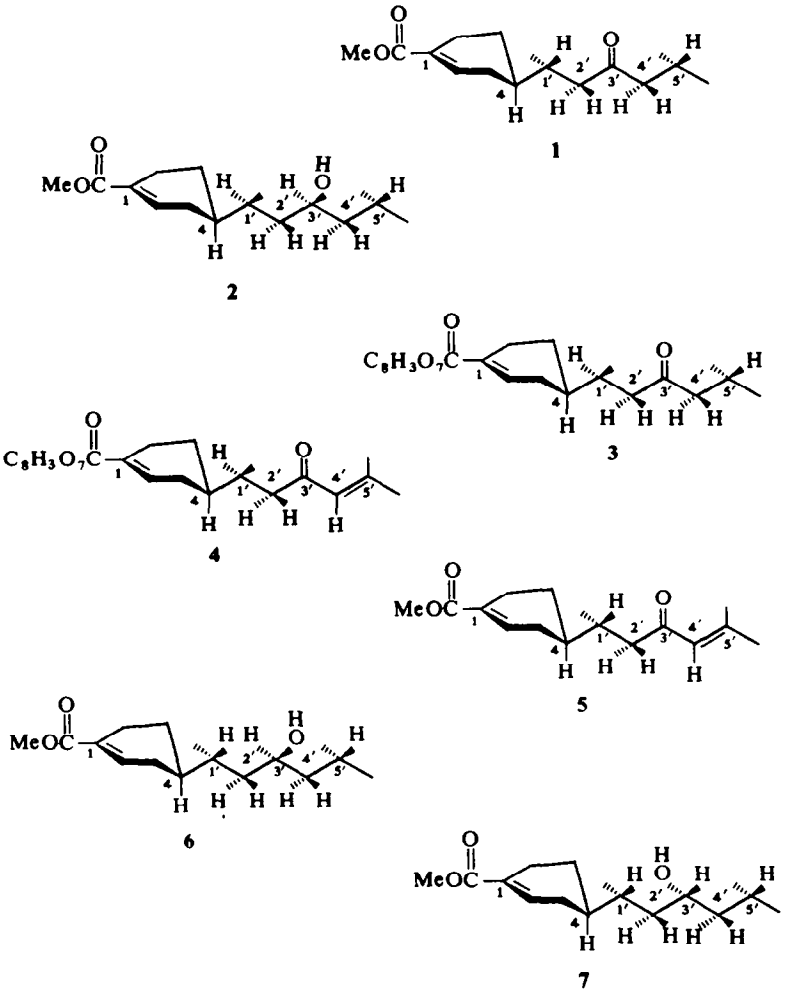
Trees 3 and 4

No 'juvabione-type' compounds were isolated from the branchwood of these two trees. Although traces were possibly present (see Table 2), they were not isolated due to the complexity of the petrol-soluble material.

DISCUSSION

The morphological and physiological characters of *Abies alba* are known to vary greatly from one tree to another [12]. This we find to hold true also for the leaf-oil terpene composition of the four trees analyzed in this study (Table 1). However, an overall pattern of similarity is evident, and the relative percentages of santene (2–3.5%), tricyclene (1–2.5%), camphene (9–20%) and bornyl acetate (2–10%) are useful in characterizing *A. alba* individuals. The relatively high α-pinene and limonene (except tree 3) percentages and correspondingly lower β-pinene and especially β-phellandrene percentages may also have some diagnostic value [11]. Comparison of the very low percentages of 3-carene in all four trees with about 6% in the commercial leaf oil indicates that two phenotypes (one with low, the other with relatively high percentages) may be present, just as was found in *A. balsamea* populations of eastern Canada [10]. Other phenotypic pairs may be present as well, but a large number of trees from natural stands will have to be analyzed to characterize these. Trees 1 and 2, 3 and 4 show such phenotypic relationships with respect to α-pinene. The relationship of the leaf oil terpenes of *A. alba*, *A. balsamea*, *A. sibirica* and *A. lasiocarpa* (santene–tricyclene–camphene–bornyl acetate type) and those of other North American true firs was discussed previously [11].

Table 2 shows a clear distinction between two types of



A. alba trees. Trees 1 and 2 both correspond to the 'juvabione-type' in that the majority of the petrol-soluble material is accounted for by these sesquiterpenoids. In contrast, the nature of the petrol-soluble from trees 3 and 4, which were from the same site as tree 2, were clearly different: less than 10% of the petrol-soluble material could be attributed to juvabione-type compounds. Based on the lipid-extract fraction, trees 3 and 4 would appear to conform with that expected [13] for *A. alba*. However, since Grandos and co-workers did not mention the number of trees examined in their study, our results may be more typical.

Based on this study, it is apparent that *A. alba* trees have a more pronounced quantitative variability of occurrence of 'juvabione-type' IJHAs than was found in either *A. balsamea* [3, 4] or *A. lasiocarpa* trees [5]. Moreover, due to the occurrence of either, or both, diastereomeric forms of these 'juvabione-type' compounds in individual *A. alba* trees, it is neither sufficient nor possible to identify these compounds by chromatographic techniques or by PMR.

The utility of ^{13}C-NMR for identifying diastereomers, but not enantiomers, has been well established and was used extensively in studies on these IJHAs isolated from *Abies* species [5, 9]. For example, it would have been extremely difficult to identify the three-component-alcohol fraction by other means. The quantitative information obtained by ^{13}C-NMR, using the methods already discussed, is fairly accurate and for the ketones was confirmed by ORD–MA measurements (on hydrogenated samples).

The technique of hydrogenating the 'juvabione' and/or 'dehydrojuvabione' samples to remove the chiral centre at C-4 is most useful, as it will not affect the stereoconfiguration at C-1'. The ORD curves of the 1'*R* or 1'*S* enantiomers are easily distinguished, since they are mirror images. Moreover, the ORD–MAs of the enantiomers, or mixtures of enantiomers, can be used for quantitative analysis of the C-1' stereoconfigurational composition. In contrast, the ORD curves of the diastereomers (juvabione and epijuvabione) are very similar [8], as are the $[\alpha]_D$ values [1, 3, 6, 7]; leaving the assignment of C-1' configuration doubtful.

This combination of ^{13}C-NMR and ORD–MA measurements of hydrogenated samples made possible the complete identification of each juvabione-type compound. Without exception, all juvabione-type IJHA compounds isolated from *Abies* species have the *R* chirality at C-4, but can have either *R* or *S* chirality at C-1'.

The observation of both C-1' stereoconfigurations of '4'-dehydrojuvabione' (**5** and **4**), but only the C-1' stereoconfiguration of juvabione (**1**) in the same *A. alba* tree, was not expected. On the basis of previous isolations [3–7] from other *Abies* species, 'juvabione' and 'dehydrojuvabione' were expected to have identical stereoconfigurations at C-1', either both *R* or both *S*. In addition, the observation of only 4'-dehydroepijuvabione (**4**) in the second tree, together with a 1:3 mixture of **4** and **5** in the first tree, could indicate that **4** and **5** are each produced by a specific enzyme. It is unlikely that one enzyme could produce both diastereomers, in different trees of the same species, in just those ratios (1:3 and 1:0) that would be expected on the basis of possible genetic inheritance.

If one accepts the hypothesis that 'juvabione' is the dihydro analogue of 'dehydrojuvabione', then it follows that *A. alba* has only the enzyme system which reduces the C-4' double bond of **5**. In addition, this enzyme appears to be specific for *R* chirality C-1', since epijuvabione (**3**) is not observed, even though two trees contain 4'-dehydroepijuvabione (**4**).

It is concluded that juvabione-type sesquiterpenoid IJHAs appear to be normal constituents of *A. alba* trees; they occur in the 4*R*, 1'*R* and/or the 4*R*, 1'*S* diastereomeric forms in *A. alba*; they appear to be widespread in the genus *Abies*; and their occurrence may be genetically controlled. This class of sesquiterpenoids could prove valuable in possible future chemosystematic studies of the genus *Abies*. *Abies alba* could be the 'source' of the epijuvabione found in the 'Czechoslovakian fir' [6, 7].

EXPERIMENTAL

Materials. Branches, from four *Abies alba* Mill. trees, complete with vigorous foliage were kindly supplied by Dr. L. Greguss, Director of the Arboretum at Banská Stiavnica, C.S.S.R. The samples were from locations noted in Table 2.

Volatile leaf oil analyses. These were carried out according to an established procedure [10]. The foliage samples were collected during the dormant season (January–March) to ensure strict comparability of the quantitative data. Leaf-oil monoterpenes percentage composition is listed in Table 1. The general methods and spectral measurements followed the format outlined previously [2–5]. All compounds isolated in this study had GLC, TLC and PMR values in accord with published values. The ^{13}C-NMR spectra were recorded for samples in *ca* M $CDCl_3$ solns on a Varian CFT-20 spectrometer at 20 MHz. Chemical shift (±0.1 ppm) is given with respect to Me_4Si using $CDCl_3$ (δ = 76.9 ppm) as internal standard. Generally, a pulse width of 10 μsec (α = 45°), 8k data points and sweep width of 4000 Hz (digital resolution ±0.98 Hz = ±0.05 ppm) were used. All spectra were multiplied with an exponential function (sensitivity enhancement = −0.4 sec) prior to Fourier transformation.

Cis *and* trans *dihydrojuvabiones* (**8** *and* **9**). These compounds were obtained previously [3] by hydrogenating an authentic sample of **1**. They were separated by Si gel column chromatography using petrol–Et_2O (3:1) as eluent. They had ORD curves as follows: **8** (*cis*-dihydrojuvabione) with 0.4% **9** (*c* 0.476, MeOH; 23°) $[\Phi]_{450} \simeq 0°$, $[\Phi]_{350}$ − 135°, $[\Phi]_{307}$ − 833°, $[\Phi]_{290}$ 0°, $[\Phi]_{264}$ + 1577°; molecular amplitude after correcting for **9** = −24.1; **9** (*trans*-dihydrojuvabione) with 4.2% **8** (*c*, 0.468, MeOH; 23°) $[\Phi]_{450} \simeq 0°$, $[\Phi]_{350}$ − 149°, $[\Phi]_{307}$ − 801°, $[\Phi]_{291}$ 0°, $[\Phi]_{264}$ + 1443°; molecular amplitude after correcting for **8** = −22.4.

Tree 1, *juvabione* (4R, 1'R) (**1**). Juvabione was isolated by column chromatography and was 95.8% pure (by GLC;—it contained 4.2% 'dehydrojuvabione'—actually 3.1% **5** and 1.1% **4**). ^{13}C-NMR: δ 209.8, 167.3, 138.8, 129.9, 52.1, 51.0, 47.4, 37.4, 32.3, 29.4, 24.5, 24.2, 22.2 and 16.2; identical to that observed previously [8] for North American isolations. Hydrogenation (in MeOH over 5% Pd on charcoal) of a sample gave a mixture of 30.2% *cis*- and 69.8% *trans*-dihydrojuvabiones, as determined by GLC, which had an ORD–MA of −22.2 (*c* 0.536, MeOH; 23°). The expected MA for this *cis*:*trans* ratio was (0.302 × −24.1 + 0.698 × −22.4) = −22.9; but correcting for contribution due to dehydroepijuvabione present in the original sample (i.e. subtracting twice the %*S* form present due to the dehydroepijuvabione), this calculated value becomes 0.978 × −22.9 = −22.4, which is within experimental error of the observed value.

Tree 1,4'-*dehydrojuvabione* (4R, 1'R) (**5**) *and* 4'-*dehydroepijuvabione* (4R, 1'S) (**4**). This mixture was isolated as before [3]. It was 93% pure (GLC) and contained 1.3% juvabione and had $[\alpha]_D^{23}$ +77.7° (lit. value +49.0° for **5** [4] and +102° for (**4**) [7]. ^{13}C-NMR: δ 200.0, 167.1, 154.2, 138.7, 129.7, 123.7, 50.8, 48.4, 48.2, 37.4, 33.0, 32,8, 29.3, 28.0, 27.0, 25.7, 24.4, 20.1, 16.1, and 15.9. The ratio of intensities of the peaks at δ 29.3 and 28.0 (due to C-3 of each isomer [9]) indicated a 3:1 ratio of **5** and **4**. Hydrogenation resulted in 31.1% *cis* and 68.9% *trans* isomers of 'dihydrojuvabione' and a measured ORD–MA of −11.5 (*c* 0.544, MeOH, 23°)

the calculated value, assuming 75% *R* chirality at C-1', is −11.5.

Tree 1, '*Juvabiols*'. A mixture of alcohols was isolated from this column and the sitosterol fraction separated by crystallization. Table 3 lists the ^{13}C-NMR intensities and δ values observed for this mixture of 'juvabiols'. By comparing the δ values with those previously published [5], it is possible to identify three isomers of 'juvabiol'. They are juvabiol (**6**) isojuvabiol (**7**), and epijuvabiol (**2**). No evidence for the occurrence of isoepijuvabiol (4*R*, 1'*S*, 3'*R*) was noted. The relative amounts of each isomer were determined from the observed intensities for the C-3 resonances. A portion of this syrup was acetylated in the usual manner and further purified by Si gel column chromatography. The intensities and δ values for the carbon resonances observed for these 'juvabiol acetates' are also listed in Table 3. They confirm the results obtained from the alcohols.

Tree 2, *Dehydroepijuvabione* (**4**) (4R, 1'S). This isolation was 92.8% pure by GLC. ^{13}C-NMR: δ 200.1, 167.2, 154.4, 138.8, 129.8, 123.7, 50.9, 48.5, 37.3, 33.1, 28.1, 27.1, 25.8, 24.6, 20.2 and 16.0. Hydrogenation of a sample resulted in 30.8% *cis* and 69.2% *trans* isomers of dihydroepijuvabione and a measured ORD-MA of +23.1 (*c* 0.452, MeOH, 23°); the calculated value for 100% *R* chirality is −22.9 and therefore for 100% *S* chirality a value of +22.9 is expected.

Acknowledgements—We are grateful to Mrs. C. D. Kriz for technical assistance and to the Chemistry Department, University of British Columbia, for use of their CFT-20 NMR spectrometer.

REFERENCES

1. Bowers, W. S., Fales, H. M., Thompson, M. J. and Uelbel, E. C. (1966) *Science* **154**, 1020.
2. Rogers, I. H., Manville, J. F. and Sahota, T. (1974) *Can. J. Chem.* **52**, 1192.
3. Manville, J. F. (1975) *Can. J. Chem.* **53**, 1579.
4. Manville, J. F. (1976) *Can J. Chem.* **54**, 2365.
5. Manville, J. F. and Kriz, C. D. (1977) *Can. J. Chem.* **55**, 2547.
6. Manville, J. F., Greguss, L., Sláma, K. and von Rudloff, E. (1977) *Coll. Czech. Chem. Commun.* in press.
7. Černý, V., Dolejš, L., Lábler, L., Šorm, F. and Sláma, K. (1967) *Coll. Czech. Chem. Commun.* **32**, 3926.
8. Pawson, B. A., Cheung, H. C., Gurbaxani, S. and Saucy, G. (1970) *J. Am. Chem. Soc.* **92**, 336.
9. Manville, J. F. and Bock, K. (1977) *Org. Mag. Res.* in press.
10. Hunt, R. S. and von Rudloff, E. (1974) *Can. J. Botany* **52**, 477.
11. von Rudloff, E. (1975) *Biochem. Syst. Ecol.* **2**, 131.
12. Sauer, A., Kleinschmit, J. and Lunderstädt, J. (1973) *Silvae Gen.* **22**, 149.
13. Granados, R., Ribo, J. M. and Torres, E. (1973) *Phytochemistry* **12**, 1496.