Tetrahedron Letters No.12, pp. 1053-1057, 1967. Pergamon Press Ltd. Printed in Great Britain.

DEHYDROJUVABIONE - A NEW COMPOUND WITH JUVENILE HORMONE ACTIVITY FROM BALSAM FIR

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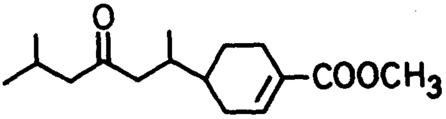
(Received 16 January 1967)

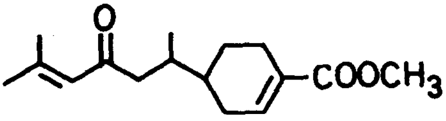
The wood of the balsam fir (Abies balsamea L.) contains factors exhibiting high juvenile hormone activity on the bug Pyrrhocoris apterus L. (1). Recently, Bowers et al. (2) have reported isolation of an active component from the wood of the balsam fir. They have identified it as a methyl ester of the known todomatsuic acid (3,4) and have named it juvabione. The American authors pointed out that juvabione (I) was the only component with significant juvenile hormone activity in the extracts prepared from the balsam fir of American origin.

In the course of simultaneous work on the same problem, we have found in the wood of a balsam fir growing in Czechoslovakia two active components, one of them identical with juvabione. However, the main component of the active fraction is another substance with high juvenile hormone activity, the isolation and structure of which we describe in the present communication.

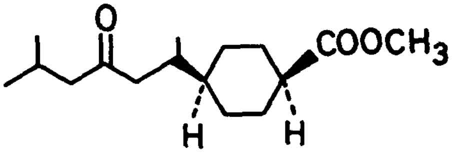
The wood of the balsam fir was pulverized and extracted with acetone at room temperature. The solvent was evaporated under vacuum and the residue chromatographed on silica gel in petroleum ether solution. A subsequent elution with benzene yielded the active fraction, which was



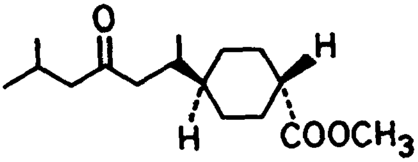
I



II

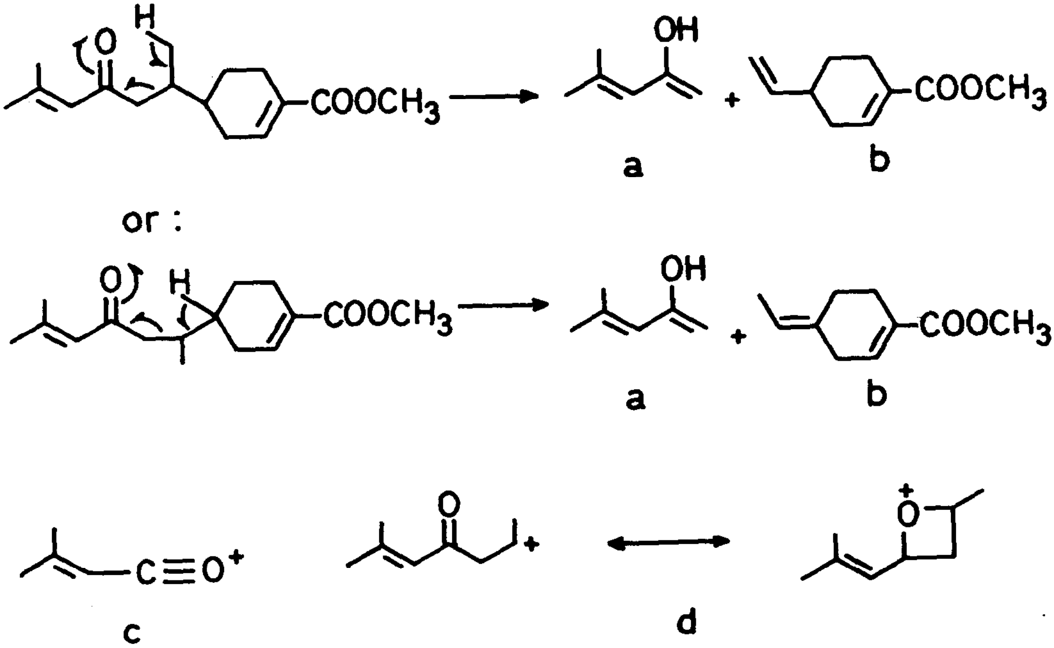


III



IV

SCHEME 1



separated by thin layer chromatography into two components. The less polar minor component proved to be identical with juvabione, according to the physical and physiological properties. The IR spectrum (CCl_4) showed bands characteristic of a $-\overset{|}{C}=\overset{|}{C}-COOCH_3$ grouping: 1439, 1653, 1719 cm^{-1}; the mass spectrum: M=266, $\frac{m}{e}$ = 234, 207, 206, 177, 167, 166, 139, 134, 127, 107, 85, 79, 59, 57, and $[\alpha]_D^{20}$ +79.5° ($CHCl_3$, c 3.5). The juvenile hormone activity was tested on freshly molted last instar larvae of Pyrrhocoris apterus in topical applications. It has been found that a dose of 1 μg per specimen causes adultoids with partly larval, partly adult epidermal structures. A dose of 10 μg causes perfect extra-larvae. This is fully in agreement with the activity of juvabione (2).

The major component, $[\alpha]_D^{20}$ +102.5 ($CHCl_3$, c 3.6), which does not contain any juvabione according to the gas liquid chromatography, is inactive when applied in a dose of 1 μg per specimen, however, the 10 μg dose causes adultoids with at least half of the epidermal structures retaining the larval form.

The elemental analysis was consistent with the composition of dehydrojuvabione (Calc. for $C_{16}H_{24}O_3$, m.w. 264.3, C. 72.69%; H, 9.15. Found: C, 72.41%; H, 9.02%). The IR spectrum (CCl_4) proved the compound to be an α,β-unsaturated carboxylic acid methyl ester (bands at 1437, 1652, 1717 cm^{-1}) and an α,β-unsaturated ketone in s-cis conformation (1621, 169 cm^{-1}) (5). The UV spectrum showed the following characteristics: $\lambda_{max_1}^{EtOH}$ 229 mμ (log ε , 4.31); $\lambda_{max_2}^{EtOH}$ 318 mμ (log ε , 2.08).

100 Mc n.m.r. spectrum ($CDCl_3$) revealed the presence of a secondary methyl group (a doublet, 0.875 δ, J 7 c.p.s.) and two olefinic protons at 6.06 δ and 6.94 δ while frequency swept decoupling experiments showed the absence of mutual long range coupling. The olefinic proton at 6.06 δ is part of a grouping $(CH_3)_2C=CHCO$ characterized further by the presence of two methyl doublets at 2.125 and 1.865 δ (long range coupling of allylic type, J ∼ 1.5 c.p.s.). The olefinic proton at 6.94 δ belongs to

the grouping $CH_3OOC\overset{|}{C}{=}CH$ characterized also by the signal of the carbomethoxy methyl at 3.71 δ .

The above data thus indicated the structure II which could be confirmed by further spectrometric data. In the mass spectrum of this compound the molecular ion was found at $\frac{m}{e}$ 264. The peaks in the higher mass range of the spectrum at $\frac{m}{e}$ 232, 205, and 204 correspond to the elimination of methanol, carbomethoxy radical, and methyl formate, respectively. The intense peaks at $\frac{m}{e}$ 166 and 98 arise by McLafferty mechanism (scheme1). The fragment of $\frac{m}{e}$ 166 eliminates further methanol to afford the ion of $\frac{m}{e}$ 134. The characteristic ion c ($\frac{m}{e}$ 83, base peak) arises, at least to some extent, from ion a by splitting off a methyl radical ($\frac{m^*}{e}$ 70.5). According to a metastable transition ($\frac{m^*}{e}$ 36.4), the ion c eliminates carbon monoxide to give the fragment C_4H_7 ($\frac{m}{e}$ 55). The fragment d ($\frac{m}{e}$ 125) arises by β-cleavage to the carbonyl group and its occurrence supports the position of the secondary methyl group in the side chain.

Eventually, the substance II was correlated with juvabione (I) in the following manner. Catalytic hydrogenation of II on $Pd/CaCO_3$ catalyst in methanol solution yielded a mixture of two tetrahydroderivatives [m.w. 268, mass spectr.; IR-bands(CCl_4): 1739, 1438, 1173 cm^{-1} ($COOCH_3$), 1716 cm^{-1} (CO)] formulated as III and IV. As evidenced by gas chromatographic analysis, both isomers are present in the mixture in about equal quantities. Under the same conditions, hydrogenation of juvabione (I) led to the same mixture as shown by IR spectroscopy and gas liquid chromatography.

Acknowledgement. Our thanks are due to Mr. Z. Samek for n.m.r. measurements and valuable comments and to Mrs. J. Smolíková for IR measurements and useful discussion.

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