# The Absolute Configuration of Juvenile Hormone III Bisepoxide

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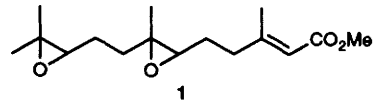
The absolute configuration of juvenile hormone III bisepoxide, the putative characteristic juvenile hormone of higher dipteran insects, has been established as 2*E*,6*S*,7*S*,10*R* by HPLC and cyclodextrin-modified CE comparison of synthetic stereoisomers with biosynthetically $^3$H-labelled hormone from the Australian sheep blowfly *Lucilia cuprina*.

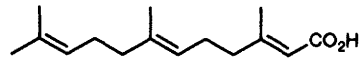
The neurohormonal control of development and reproduction of many insect species is mediated by juvenile hormones of the farnesate group.[1] In 1989 a new member of this group, juvenile hormone III bisepoxide ($JHB_3$, **1**), was identified as the principal metabolite secreted during *in vitro* culture of ring glands dissected from *Drosophila melanogaster* ('fruit' fly) larvae.[2] The same metabolite is released from corpora allata separated from adult *D. melanogaster* ring glands,[3] and from corpora allata or ring glands from larval and adult stages of related insects including *Musca domestica* (house fly),[2] *Sarcophaga bullata* (flesh fly),[2] *Calliphora vicina*[2] and *C. vomitoria*[4] (blowflies) and *Lucilia cuprina* (Australian sheep blowfly).[5,6] In conjunction with biochemical and physiological studies,[2,3,4,7,8] these observations support the view that $JHB_3$ **1** is the predominant and characteristic juvenile hormone of higher dipteran insects.[2] Despite its significance, however, neither the absolute nor the relative configuration of $JHB_3$ has been established, reflecting *inter alia* the difficulties imposed by the minute (pmol) levels of unstable natural hormone available.

The hormone is probably derived biosynthetically by enzymic methylation and epoxidation of (2*E*,6*E*)-farnesic acid **2**, since addition of this acid to cultured ring glands of *D. melanogaster*[2,8] and *L. cuprina*[5] dramatically promotes its formation. Accordingly we expected the olefinic configurations of this farnesic acid to be conserved in $JHB_3$. In particular the 6,7-epoxide should carry *trans* carbon chains, whilst the hormone is already known to retain unchanged 2*E* geometry.[2] These configurational restrictions reduce the number of stereoisomers to be considered for $JHB_3$ to four, namely the (6*S*,7*S*,10*R*)-**3** and (6*R*,7*R*,10*R*)-**4** diastereoisomers and their (6*R*,7*R*,10*S*)- and (6*S*,7*S*,10*S*)-enantiomers. We have synthesised recently these four reference compounds in high stereoisomeric purity,[9] and now describe their use to define the absolute configuration of $JHB_3$ produced by ring glands of *L. cuprina*.

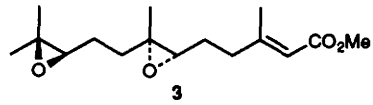
The mixture from non-stereospecific epoxidation of methyl (2*E*,6*E*)-farnesate contained these four stereoisomers of $JHB_3$ **1** in approximately equal amounts, and was separable by HPLC into two racemates.[5] In the presence of this mixture as a carrier and reference for UV detection, radioactivity from biosynthetically labelled [*methyl*-$^3$H]$JHB_3$, obtained from excised *L. cuprina* ring glands incubated in a medium containing L-[*methyl*-$^3$H]methionine, co-eluted with the slower eluting racemate.[5] Comparison with the synthetic stereoisomers identified this racemate as the (6*RS*,7*RS*,10*SR*) pair (**3** and *ent*-**3**), the faster eluting racemate being the (6*RS*,7*RS*,10*RS*) pair (**4** and *ent*-**4**). This defines the relative configuration of $JHB_3$ as (6*RS*,7*RS*,10*SR*). Of these two enantiomers, it was likely that $JHB_3$ has the (6*S*,7*S*,10*R*) absolute configuration **3** corresponding at C-10 to that of juvenile hormone III (JH III, **5**),[10] the analogous 10,11-



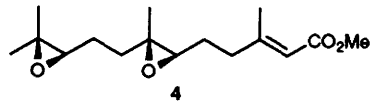

**1**



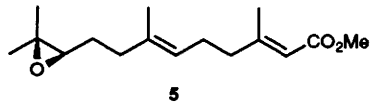

**2**



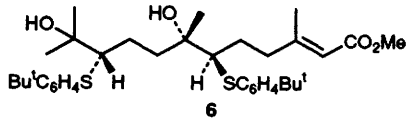

**3**




**4**




**5**




**6**

monoepoxide of methyl (2*E*,6*E*)-farnesate. JH III occurs with $JHB_3$ in *L. cuprina*[5] and probably also in other higher diptera,[2,3,4],† and there is evidence that it may be a precursor of $JHB_3$.[2,5]

In order to prove the absolute configuration of $JHB_3$, however, it was necessary to be able to separate the enantiomers of the (6*RS*,7*RS*,10*SR*)-racemate. This was achieved without the need for conversion into diastereoisomeric derivatives by micellar electrokinetic capillary chromatography (MECC), using sodium borate as the running buffer, sodium dodecyl sulfate as the micellar phase, and β-cyclodextrin as the chiral host solute.[11] Under these conditions the (6*S*,7*S*,10*R*)-enantiomer **3** eluted faster than the (6*R*,7*R*,10*S*)-enantiomer (*ent*-**3**) (Fig. 1).‡ Biosynthetically labelled [*methyl*-$^3$H]$JHB_3$ was then mixed with the carrier racemate and subjected to MECC under conditions in which the migration times of the two enantiomers were extended to *ca.* 79 and 85 min, respectively, in order to maximise the separation. Fractions defined by UV absorption of the carriers were collected by a manual stopped-flow technique. Since the pressure injection process[11] introduces only a small amount of the sample solution onto the column in any run, the respective fractions from 14 such chromatograms were combined. Radioassay of half of each combined fraction showed that 93% of the total radioactivity on the column had migrated with the faster eluting (6*S*,7*S*,10*R*)-enantiomer **3**.§ The remaining half of each fraction was used to validate the collection procedure, since it had been necessary to make allowance for the time taken for solute to travel from the MECC UV detector to the capillary outlet. In order to compensate for the lower sensitivity of the HPLC analysis required for this validation, each MECC fraction was treated with sodium *p-tert*-butylthiophenoxide (MeOH–$H_2O$, room temp., 8 days). The resulting strongly chromophoric bissulfides (**6** and *ent*-**6**) formed by $S_N2$ ring opening of the carrier diepoxides (**3** and *ent*-**3**) were detected readily by HPLC, and only in the two expected MECC fractions.

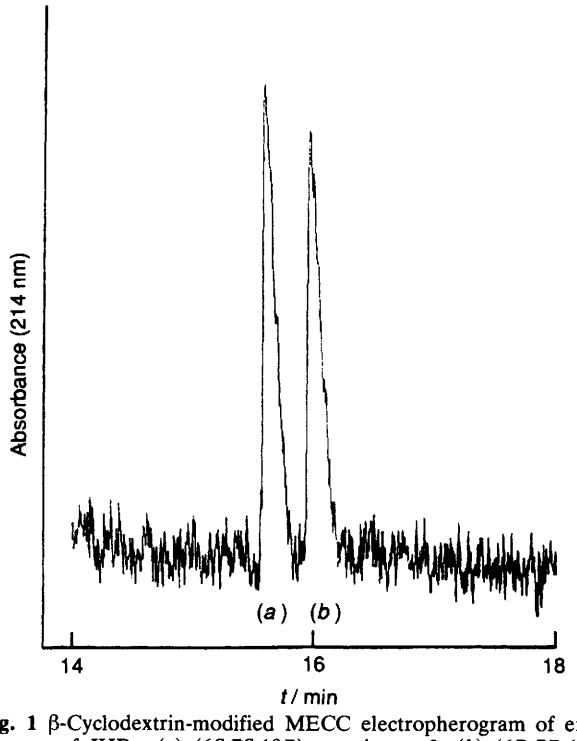



**Fig. 1** β-Cyclodextrin-modified MECC electropherogram of enantiomers of $JHB_3$: (*a*) (6*S*,7*S*,10*R*)-enantiomer **3**, (*b*) (6*R*,7*R*,10*S*)-enantiomer (*ent*-**3**)

† The identification of JH III in these cases, however, is less secure, being based only on chromatographic behaviour.

‡ All four stereoisomers from non-stereospecific epoxidation of methyl (2*E*,6*E*)-farnesate were separable by β-cyclodextrin-modified MECC.

§ Of the remaining radioactivity, some 2% eluted prior to the faster enantiomer, 2% in final washings of the capillary, and 3% coeluted with the slower (6*R*,7*R*,10*S*)-enantiomer (*ent*-**3**). The radioactivity carried with this (6*R*,7*R*,10*S*)-enantiomer did not represent contamination by the faster enantiomer, since two intervening fractions (and the succeeding fraction) were inactive. Although this coelution may be fortuitous, it may also reflect imperfect enantiospecificity of the enzymes responsible for $JHB_3$ synthesis, particularly in view of the extreme resolving power of MECC.[11]

These results establish that $JHB_3$ produced by *L. cuprina* has the absolute configuration (2*E*,6*S*,7*S*,10*R*)-**3**. This configuration corresponds at C-2 and C-10 to that of JH III **5**,[10] its probable biosynthetic precursor,[2,5] which in a wide variety of insects has constant (2*E*,6*E*,10*R*)-stereochemistry.[10,12] It is likely that $JHB_3$ from other higher dipteran insects where it is the characteristic hormone also has the same absolute configuration as that defined here.

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