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SYNTHESIS OF BOTH OPTICAL ISOMERS OF INSECT JUVENILE HORMONE III AND THEIR AFFINITY FOR THE JUVENILE HORMONE-SPECIFIC BINDING PROTEIN OF *Manduca sexta*

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Summary: A procedure is described for resolution of synthetic juvenile hormone III [methyl (2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate] into 10*R* and 10*S* enantiomers, using high resolution liquid chromatographic separation of diastereomeric esters formed from (+)-α-methoxy-α-trifluoromethylphenylacetic acid and the 10,11-diol derivative of JH III. Chemical conversion of the diastereomers into chiral epoxides resulted in 8% epimerization at C-10. The binding properties of racemic JH III and the 10*R* and *S* antipodes were studied with the hemolymph juvenile hormone binding protein of *Manduca sexta* (Lepidoptera:Sphingidae). Competition studies against [*methoxy*-^{3}H]-10*R*,*S*-JH III revealed that the order of binding activity is 10*R*>10*R*,*S*>>10*S*. The binding observed for the 10*S* enantiomer (∿1/14 of affinity of 10*R*) may be due largely to its 8% contamination with 10*R*.

Insect juvenile hormones are produced by the corpus allatum and are involved in the control of morphogenesis and vitellogenesis (1). To date, three homologous juvenile hormones have been isolated and identified (2, 3, 4). A specific JH binding protein has been discovered (5, 6) in the hemolymph of *Manduca sexta*. The apparent role of this binding protein is the protection of JH from general esterases, although a JH-specific esterase can hydrolyze JH even when "protected" by the specific binding protein (7).

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Abbreviations: JH, juvenile hormone; JHBP, JH binding protein; TLC, thin-layer chromatography; HRLC, high-resolution liquid chromatography; mesyl, methanesulfonyl; MTP, α-methoxy-α-trifluoromethylphenylacetic (acid or ester).

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Binding studies have shown that the *M. sexta* JHBP has a relatively high affinity for the JH in order of their lipophilicity (JH I > JH II > JH III), and that the ester methyl function, the epoxide ring, and the aliphatic side chains are required for binding (5, 8, 9). Further studies of the binding properties of the four geometrical isomers of JH III (10) and the eight geometrical isomers of JH I (11) have shown that within each group, the natural isomer has highest affinity for the JHBP. We now report the chemical synthesis of both 10*R* and 10*S* antipodes of JH III and the binding properties of these enantiomers with the JHBP of *M. sexta*.

Materials and Methods

Synthesis of JH III Enantiomers: Methyl (2*E*,6*E*)-(10*R*,*S*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate (JH III) was obtained from a batch of material synthesized by Anderson *et al.* (12). Acidic hydration of the epoxide to the (10*R*,*S*)-10,11-diol derivative 2, and subsequent esterification of the diol with the acid chloride derived from (+)-(*R*)-α-methoxy-α-trifluoromethylphenylacetic acid, was performed according to established procedures (4). The MTP acid (Aldrich) was from a single batch which was found to have >99.9% enantiomeric purity (13).

The diastereomeric MTP ester derivatives 3 were separated by preparative HRLC using a liquid chromatograph assembled from components (13, 14). Preparative HRLC columns (2.2 cm i.d. x 50 or 100 cm long), were packed with 20 μm LiChrosorb SI 60 (E. Merck) using a balanced density slurry packing technique (15) with CH_2Br_2-isooctane as solvent. Approximately 100 mg quantities of the mixture of 10*R*,*S* diastereomers were chromatographed on a 100 x 2.2 cm LiChrosorb column eluted with 40% diethyl ether in pentane at 20 ml/min. Fractions were collected and analyzed for enantiomeric composition on the same instrument using a 22 x 0.4 cm Zorbax-SIL column (DuPont, pre-packed) eluted with water-saturated 10% ethyl acetate in pentane at 1.7 ml/min. The faster eluting 10*R* diastereomer 3a had enantiomeric purity of >99.5%, but the slower eluting 10*S* diastereomer 3b was less pure and was rechromatographed preparatively (50 x 2.2 cm LiChrosorb eluted with 8% ethyl acetate in pentane, 50% water-saturated). Suitable fractions were analyzed and found to exceed 99.5% enantiomeric purity.

Both resolved diastereomeric esters were hydrolyzed to the chiral diol using base under phase transfer catalysis conditions: 0.09 *M* MTP ester in dichloromethane was treated with 1/2 volume of a 10% solution of tetrapentylammonium chloride in 50% aqueous KOH for 10 minutes at ambient temperature. The reaction was acidified, extracted, and the residue purified by preparative TLC to afford 11 mg (73%) of the diol, together with 1.9 mg (14%) of epoxide.

Each diol 2a and 2b was converted to the mono-methanesulfonate (mesyl) ester by standard methods (to 0.16 *M* diol in ether was added 3.5% triethylamine and 10% molar excess of mesyl chloride). The mesyl ester was treated with a slight excess of KOH in methanol at 5° for 10 minutes. The solution was neutralized and the epoxide isolated by extraction. The epoxides were then purified by preparative TLC and HRLC (22 x 0.46 cm Zorbax-SIL, 8% ether in pentane, 50% water-saturated).

Analysis of Enantiomeric Purity: Aliquots of the resolved epoxides were converted to the methoxyhydrin derivatives as described previously (14), followed by esterification of the 10-hydroxy group with optically pure

MTPCl. The diastereomeric MTP esters of the methoxyhydrins were analyzed by HRLC under conditions (3 series-connected 22 x 0.46 cm Zorbax-SIL columns eluted with 12% ether in pentane, 50% water saturated) where the closely eluting diastereomers [separation factor α = 1.07 (15)] were totally resolved [resolution R_s = 2.2 (15)]. The chiral diol intermediates were also converted to their MTP monoesters for enantiomeric purity analysis by HRLC (22 x 0.46 cm Zorbax-SIL eluted with 12.5% ethyl acetate in pentane, 100% water-saturated).

Binding Protein Assay Conditions: Juvenile hormone binding protein was obtained from hemolymph of fourth instar *Manduca sexta* larvae and partially purified as described previously (8). A recently developed assay was employed which utilizes hydroxylapatite for separation of bound and unbound JH (11).

Radiolabeled Juvenile Hormone III: A sample of [*methoxy*-^{3}H]JH III (~1 Ci/mmol) was obtained from Dr. B.D. Hammock and was purified by a combination of TLC and HRLC procedures (8).

Results and Discussion

Acidic hydration of synthetic JH III to its 10,11-diol, followed by formation of the monoester of (+)-α-methoxy-α-trifluoromethylphenylacetic acid, affords diastereomeric derivatives which can be readily separated by silica gel HRLC. In earlier studies at Zoecon, we utilized this technique to determine the absolute configuration of carrier-diluted, biosynthetic [*methoxy*-^{14}C]JH III, (4), and noted that the faster eluting diastereomer with (+)MTP acid has the natural 10*R* configuration. We found that on a larger scale (~100 mg) these esters can be separated by preparative HRLC only with some difficulty. For preparative resolution we originally hoped to regenerate the epoxide from the MTP ester directly *via* base catalyzed intramolecular displacement of the MTP anion, with concomitant inversion of configuration at C-10 (analogous to behavior of mesyl esters, Fig. 1). Unfortunately, a number of different base-solvent combinations generated the epoxide only as a minor product giving the 10,11-diol as the major product. In order to accumulate sufficient mass of material for bioassay and binding studies, we chose to hydrolyze the resolved esters to the 10*R*,11 diol and 10*S*,11 diol. Under the phase transfer catalysis conditions employed, a 73% yield of diol was realized with negligible hydrolysis of the carbomethoxy moiety. Each diol next was converted in high yield to the mesyl ester, followed by *in situ* base treatment to give the epoxide of opposite configuration at C-10. Reaction impurities and minor amounts of geometrical isomers formed on base treatment were removed by TLC and HRLC.

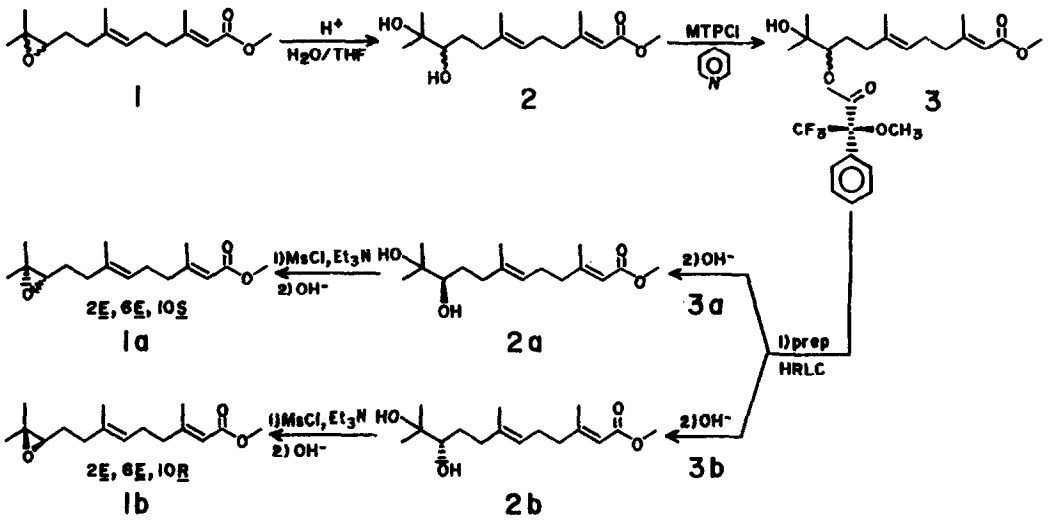

Fig. 1. Scheme showing procedure used for enantiomeric resolution of JH III.

The 10*R* and 10*S* epoxides were subjected to methanolysis (14) and the resultant 11-methoxy-10-hydroxy derivatives were converted to MTP esters for HRLC analysis. [While these diastereomers are much more difficult to separate by HRLC than the 11-hydroxy series, acidic hydrolysis of a pure 10*R* epoxide would generate 96% 10*R*, 4% 10*S* diol (16), rendering this approach unsuitable for enantiomeric purity analysis.] The resultant chromatograms revealed that each diastereomeric derivative contained 92% of the desired C-10 enantiomer contaminated with 8% of the opposite enantiomer. Samples of the diols 2a and 2b from hydrolysis were then reconverted to MTP esters and analyzed by HRLC, again showing a 92:8 mixture of enantiomers. Therefore some racemization occurred in the base hydrolysis of the MTP esters.

A similar procedure was recently reported by Imai *et al.* for synthesis of both antipodes of the ethyl ester of JH III (17), but using acetoxyetienic acid instead of MTP acid. These workers used selective reduction with lithium aluminum hydride to convert the resolved diastereomeric esters to the chiral 10,11-diols, as reduction should leave the C-10 configuration unchanged. In our hands, the procedure resulted in reduction of the methyl ester moiety as

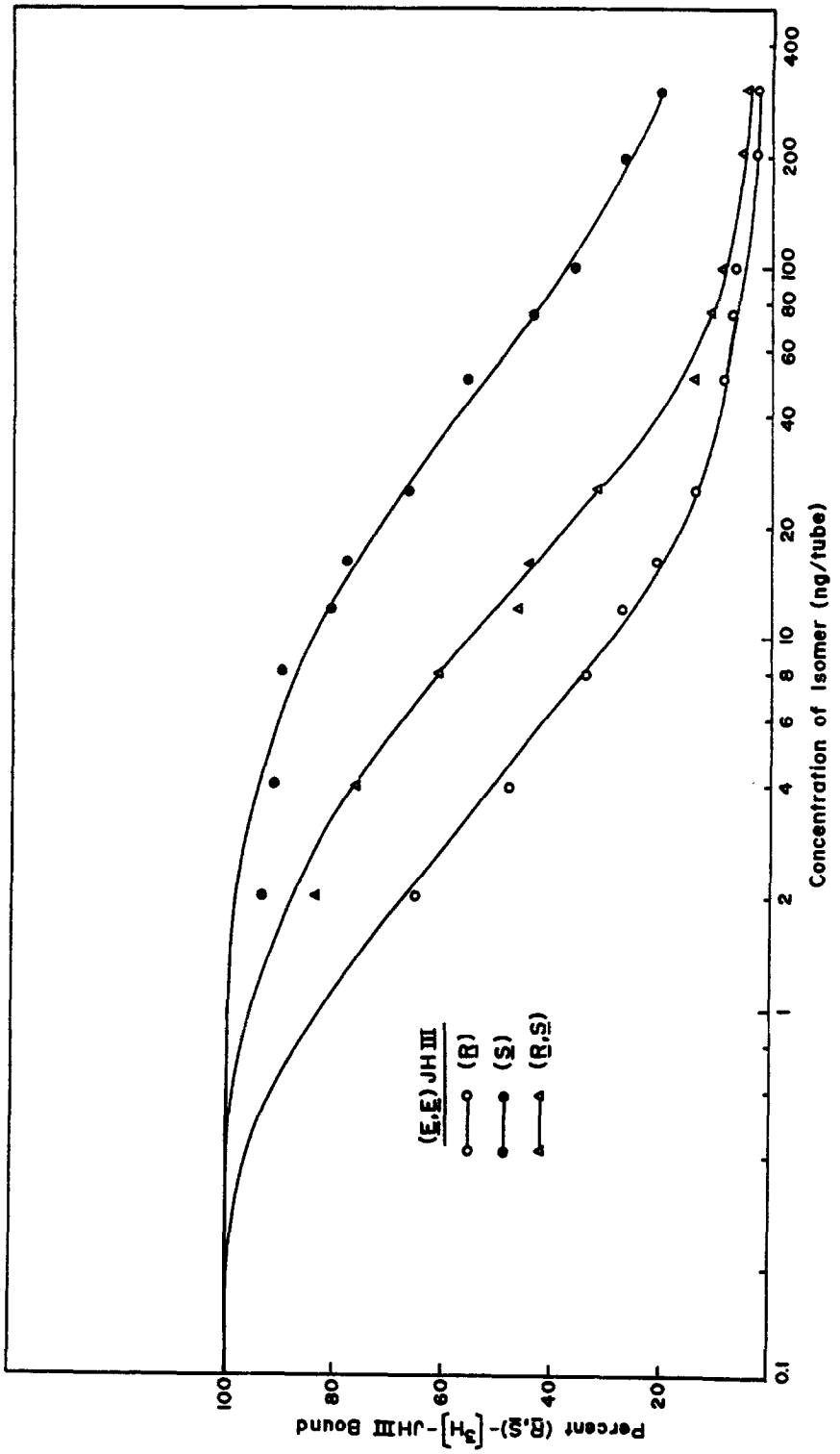

Fig. 2. Competitive displacement of [*methoxy*-^{3}H]JH III (2*E*,6*E*,10*R*,*S*) from the binding protein by optical isomers of JH III.

well, perhaps as a function of small scale.

The JHBP was obtained and partially purified, as previously described (8). A fixed concentration of 10*R*,*S* [*methoxy*-^{3}H]JH III (∿7 ng) and varying amounts (0-300 ng) of 10*R*, 10*R*,*S* and 10*S* JH III were added to assay tubes. JHBP (3 ng of partially purified preparation) was added to the tubes and incubated. Analysis of competition was performed by the recently described hydroxylapatite method (11). Previous evidence has demonstrated that the kinetics of binding for the partially purified protein are the same as for a homogenous preparation of JHBP (11). The data are plotted in Fig. 2. It is apparent that the order of binding is 10*R*>10*R*,*S*>>10*S*, and that the binding of 10*R* at 50% saturation is about 14 times that of 10*S*.

This strongly suggests that most of the binding activity of the 10*S* antipode is due to its 8% contamination with natural 10*R* antipode. Clearly the binding site has a highly stereoselective requirement for the epoxide ring, and in this context it is of interest that the unepoxidized precursor does not bind significantly (9).

The samples of predominantly 10*R* and 10*S* JH III were bioassayed on *Galleria* pupae (18). The specific biological activities were estimated at 57 and 670 pg/*Galleria* unit, respectively, *vs.* values of 144 and 197 pg/*Galleria* unit for two simultaneously assayed 10*R*,*S* samples. The 12-fold difference in biological activity is remarkably (if not fortuitously) close to the ratio of binding constants. We attribute this to the likelihood that the JH receptors also require a chiral epoxide for recognition. Under the conditions of the *Galleria* bioassay, hemolymph transport of exogenous JH is not needed, so JHBP interactions with JH can probably be ruled out in contributing to the bioassay results. It has been reported that the (+) isomer of JH I is about nine times more active on *Galleria* than partially pure (-) isomer [containing 8-10% (+) isomer] (19).

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References

1. Gilbert, L.I. and King, D.S. (1973) in Physiology of Insecta, Vol. 1, ed. Rockstein, M., pp. 249-370, Academic Press, New York.
2. Röller, H., Dahm, K.H., Sweeley, C.C. and Trost, B.M. (1967) Angew. Chem. Int. Ed. Engl. 6, 179-180.
3. Meyer, A.S., Schneiderman, H.A., Hanzmann, E. and Ko, J.H. (1968) Proc. Nat. Acad. Sci. USA 60, 853-860.
4. Judy, K.J., Schooley, D.A., Hall, M.S., Bergot, B.J. and Siddall, J.B. (1973) Proc. Nat. Acad. Sci. USA 70, 1509-1513.
5. Kramer, K.J., Sanburg, L.L., Kezdy, F.J. and Law, J.H. (1974) Proc. Nat. Acad. Sci. USA 71, 493-497.
6. Goodman, W. and Gilbert, L.I. (1974) Amer. Zool. 14, 1289.
7. Sanburg, L.L., Kramer, K.J., Kezdy, F.J. and Law, J.H. (1975) J. Insect Physiol. 21, 873-887.
8. Goodman, W., Bollenbacher, W.E., Zvenko, H.L. and Gilbert, L.I. (1976) in The Juvenile Hormones, ed. Gilbert, L.I., pp. 75-95, Plenum Press, New York.
9. Kramer, K.J., Dunn, P.E., Peterson, R.C. and Law, J.H. (1976) in The Juvenile Hormones, ed. Gilbert, L.I., pp. 327-341 , Plenum Press, New York.
10. Peterson, R.C., Reich, M.F., Dunn, P.E., Law, J.H. and Katzenellenbogen, J.A. (1977) Biochemistry 16, 2305-2311.
11. Goodman, W., Schooley, D.A. and Gilbert, L.I. (1978) Proc. Nat. Acad. Sci. USA, in press.
12. Anderson, R.J., Henrick, C.A., Siddall, J.B. and Zurflüh, R. (1972) J. Amer. Chem. Soc. 94, 5379-5386.
13. Bergot, B.J., Anderson, R.J., Schooley, D.A. and Henrick, C.A. (1978) J. Chromatog., in press.
14. Bergot, B.J., Schooley, D.A., Chippendale, G.M. and Yin, C.-M. (1976) Life Sci. 18, 811-820.
15. Snyder, L.R. and Kirkland, J.J. (1974) Introduction to Modern Liquid Chromatography, pp. 36-37, 190-192, J. Wiley and Sons, New York.
16. Nakanishi, K., Schooley, D.A., Koreeda, M. and Dillon, J. (1971) Chem. Commun. 1971, 1235-1236.
17. Imai, K., Marumo, S. and Ohtaki, T. (1976) Tetrahedron Letters 1976, 1211-1214.
18. de Wilde, J., Staal, G.B., de Kort, C.A.D., de Loof, A. and Baard, G. (1968) Kon. Ned. Akad. Wetensch. Proc. Ser. C 71, 321-326.
19. Loew, P. and Johnson, W.S. (1971) J. Amer. Chem. Soc. 93, 3765-3766.