SYNTHESIS OF TRITIATED JUVENILE HORMONES

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SUMMARY

General procedures for the tritiation of juvenile hormone analogues are described. Methyl epoxyfarnesoate, ethyl dichlorofarnesoate, epoxygeranyl-p-ethylphenyl ether, and ethyl 3,7,11-trimethyl-2,4-dodecadienoate were prepared with specific activities in the 50 mCi/mmole range by simple reactions that can be carried out directly in the biochemical laboratory without need for special equipment.

Introduction

The mechanisms of insect juvenile hormone (JH) biosynthesis, transport, metabolism, and mode of action have attracted increasing attention in the last year. Implicit in these kinds of studies has been the need for a wide variety of radiolabelled hormones as fundamental research tools, but unfortunately no general procedures still exist for the convenient preparation of such compounds in the biological or the biochemical laboratory.

As an adjunct to ongoing studies on the substrate specificity of the enzymes that metabolize JH and JH mimetics in several insect species,[1] it became of interest to explore radiosynthetic alternatives capitalizing on the existence of separate structural units within the JH molecules, which could be labelled readily with tritium and reassembled without benefit of specialized radiochemical know-how or equipment. For this purpose we adapted procedures for the radiosynthesis of trisubstituted double bonds that had been applied already in the preparation of radiolabelled squalene[2]

and other terpenoidal olefins.[3] This work presents the detailed laboratory protocols for the preparation of four tritiated JH's (Fig. 1).

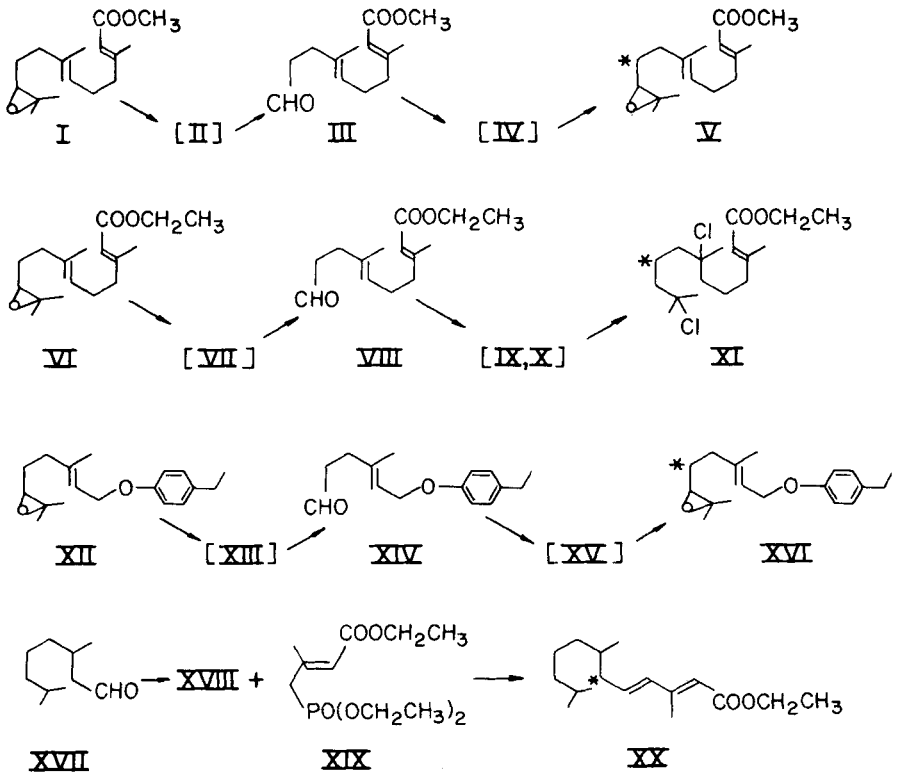

Figure 1. Flow chart of synthetic steps for the preparation of tritiated JH compounds

Experimental

Instrumentation. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian A-60 spectrometer at 60 MHz using $CDCl_3$ solutions with tetramethylsilane as internal standard. Chemical shifts are reported in ppm (δ). Analytical gas-liquid chromatography (GLC) was performed on a flame ionization detector Barber-Coleman model 5,000 apparatus fitted with 6 foot glass columns,

0.125 in diameter, and with N_2 as carrier gas. The carrier stream was split so that 1/20 passed through the detector while the remainder entered a Packard model 852 fraction collector chilled with dry ice-acetone. Radiolabelled fractions from analytical runs were taken up in Aquasol and counted as previously described.[1] We carried out preparative thin layer chromatographic (TLC) separations in a Desaga BN-continuous elution chamber with the solvent systems used before[1] unless otherwise specified.

Methyl 10,11-oxido-3,7,11-trimethyl-2,6-trans,trans-[9-^{3}H]-dodecadienoate (V). Epoxide Hydration: A solution of I (100 mg, 0.376 mmole), prepared according to Anderson et al.[4] in 5 ml freshly distilled tetrahydrofuran (THF), was stirred with an equal volume of 0.1 N aqueous H_2SO_4 at room temperature.[5] After 4 hr TLC showed complete disappearance of starting material and a single spot for the product diol. We transferred the reaction mixture in 25 ml diethyl ether to a separatory funnel containing 25 ml of half-saturated brine and extracted it 3 times with diethyl ether. The combined organic extracts were washed with bicarbonate (10%) and then with water until neutral, dried, and concentrated. The residue was taken up in 10 ml 1:1 acetone water. Then, while the slightly clouded solution was being stirred at room temperature under nitrogen, solid sodium metaperiodate (280 mg, 0.66 mmole) was added in one portion. After 1 hr a heavy white precipitate had formed. We diluted the reaction mixture with 20 ml half-saturated brine and extracted five times with 10 ml ether. The combined organic extracts were washed once with brine, dried, and concentrated to an oil. III (50 mg, 90%), which was homogeneous by TLC.

Aldehyde Tritiation: A solution of III (10 mg, 47 μmole) and tritiated water (20 μl, specific activity 90 mCi/mmole, Amersham-Searle Corporation) was placed in a 2.5 ml capacity glass tube which was sealed and heated at 50° for 14 hr. We cooled the tube to room temperature, broke it open, and carefully diluted the re-

action mixture with 2.5 ml 0.5 M phosphate buffer, pH 7.0. Extraction of the aqueous phase six times with 10 ml of diethyl ether, followed by drying and concentration of the solvent, afforded tritiated starting material (IV) as an oil (7.6 mg). The product obtained in this manner co-chromatographed with III in two TLC systems and was used without further purification.

Epoxide Regeneration: This reaction is optimally carried out in a 5 ml two necked flask while following all the procedural precautions outlined by Nadeau and Hanzlik.[2] We prepared the required sulfonium ylid by addition of phenyl lithium (50 µl, 0.1 mmole) through a syringe to isopropyldiphenylsulfonium fluoroborate (5 mg, 0.102 mmole) suspended in 0.5 ml dry THF at -78°C under nitrogen. After 10 minutes a solution of tritiated aldehyde IV (5 mg, 0.024 mmole) in 0.5 ml THF was transferred carefully again by syringe and added dropwise through a rubber septum to the ylid reagent. The mixture was stirred at -78°C for 5 min and allowed to warm to room temperature with stirring for an additional 20 min at which time we added 1 ml of water followed by 1 ml of saturated brine. Extraction with ether (2 ml portions, 5 times) yielded, after drying and concentration, crude product contaminated with diphenylsulfide. Purification by TLC on a 20 x 5 cm silica gel plate, 250 µ thick, eluted with 1:4 ether-hexane, afforded pure V (3.1 mg, 46%).

Ethyl 7,11-dichloro-3,7,11-trimethyl-2-trans-[9-^{3}H]-dodecenoate (XI). We converted the ethyl epoxyester VI[4] via the 10,11-diol (VII) to the aldehyde (VIII) and then to the tritiated aldehyde IX in the manner just described. IX was converted to ethyl [9-^{3}H]-3,7,11-trimethyl-2,6-trans,trans-10-dodecatrienoate (X) by treatment with an isopropyl triphenylphosphonium ylid.[2] 0.04 ml of 2 M methyl lithium in ether was added to a suspension of isopropyl triphenylphosphonium iodide (35.5 mg, 0.084 mmole) in 0.5 ml THF stirred at 0°C under nitrogen. Next we added tritiated alde-

hyde IX (5 mg, 0.021 mmole) in 0.3 ml THF to the ylid reagent. The mixture was stirred for 2 hr at 0°C, then for 6 hr at room temperature. Filtration of the resulting phosphine oxide through a Celite pad on a Büchner funnel under suction followed by three washes with 10 ml CH_2Cl_2 afforded the tritiated olefin X, after drying and concentration of the organic solvents.

A solution of crude X in 0.5 ml absolute ethanol was chilled to 0°C and treated next with 0.5 ml ice cold ethanol previously saturated with dry gaseous HCl. After 5 min stirring the reaction mixture was concentrated on a rotary evaporator, and the residue percolated through a 2.5 x 0.8 cm column of alumina (Activity IV) in 25 ml hexane. Concentration of the hexane left 3 mg of XI as a yellow oil.

3,7-dimethyl-6,7-oxido-2-trans-[5-^{3}H]-octenyl-4'-ethylphenyl ether (XVI). The epoxide XII was hydrated and cleaved to the corresponding aldehyde XIV, which in turn was tritiated as described (vide supra). Subsequent treatment of XV with the ylid of isopropyldiphenylsulfonium fluoroborate gave XVI in 40% yield after TLC purification.

Ethyl 3,7,11-trimethyl-2,4-[6-^{3}H]-dodecadienoate (XX). We made the starting aldehyde XVII in 85% yield as described for the preparation of n-octanal.[6] It was tritiated in the usual manner.

Allylic bromination of dimethyl acrylic acid methyl ester with N-bromosuccinimide in carbon tetrachloride[7] afforded after fractional distillation through an efficient Vigreux column a 1:9 mixture of cis to trans bromo esters (b.p. 89-91°C/10 mm). Eight grams of this material and 6.4 g of triethylphosphite were combined with stirring at 100-110°C.[8] We then warmed the mixture to 150°C over a 30 min period and maintained it at that temperature for 1 hr. The product (XIX) was obtained in 65% yield by fractional distillation (b.p. 112-114°C/0.01 mm).

A solution of XIX (50 mg, 0.2 mmole) in 0.5 ml THF was added

by syringe to a vigorously stirred suspension of washed NaH (50 mg, 0.21 mmole) in 0.5 ml THF under N_2. After one hour of stirring, a flocculent suspension resulted to which we added the tritiated aldehyde XVIII (5 mg, 32 μmole) in 0.5 ml THF. Six hours later the product, XX, was obtained through a chemical workup identical to the one used in the recovery of XI from its reaction mixture. The yield of purified material after TLC was 60%.

Results and Discussion

Radiosyntheses: Chemical Yield. Figure 1 illustrates the overall approach to the preparation of radiolabelled juvenile hormones. The specific synthetic steps used throughout the preparation of both the intermediates and the final products proved to be simple and reproducible. Yields, as reported in the Experimental Procedure, were consistently high in the case of all epoxide hydrations, diol cleavages, and base catalyzed tritiations. As an average of three trials we obtained compounds II-IV, VII-IX, XIII-XV and XVIII in better than 80% yield each time.

Yields of the Wittig type reactions, however, proved to be lower and more erratic in their reproducibility. Thus, after three attempts, compounds V, X, XVI, and XX could only be obtained in 50% average yield with individual results ranging from 30 to 60%. Such variability is in part accounted for by the general technical difficulty resulting from the need to maintain strictly anhydrous conditions during the generation of the aforementioned ylid reagents on a micro scale. We found also in this connection that attempts by other workers to repeat with respectable yields this particular set of synthetic reactions failed most often due to carelessness in the exclusion of moisture from the experimental system.

Radiochemical yields, listed in Table 1, ranged between 50 and 70% of the theoretical total radioactivity which would have resulted

Table 1.

Hormone	Specific activity mCi/mmole (average of 3 trials)	Biological activity MD_{50} µg/insect	Insect tested and mode of application
V	60±6	4	Tenebrio molitor pupae - topical
XI	58±6	0.005	Pyrrhocoris apterus fifth instar - topical
XVI	68±10	0.004	Tenebrio molitor pupae - topical
XX	44±4	0.002	Manduca sexta larvae - topical

from the complete exchange of the two protons positioned alpha to the carbonyl function in compounds III, VIII, XIV, and XVII.

Purity of Intermediates and Products. We monitored the course of each reaction outlined in Figure 1 as well as the purity of the resulting products. TLC analyses were performed routinely and in most instances GLC checks were also carried out. Products obtained on a 50 to 100 mg scale were also examined by NMR spectroscopy (Table 2). Table 2 illustrates the pertinent chromatographic data. It should be noted in particular that in the case of compounds I, V, XII, and XVI, both unlabelled and labelled materials co-chromatographed by TLC or GLC with identical R_f or R_t values.

We found V to be at least 92% pure trans,trans isomer by GLC. XVI, derived through XII from Palma Rosa geraniol (Givaudan) was at least 97% trans by GLC. Although the dihydrochloro ester XI chromatographed as a single spot on TLC, its GLC behavior indicated extensive sample thermal decomposition. Compound X, the immediate precursor of XI, however, was found to be 90% trans,trans isomer. GLC of XX showed two peaks in a 2:3 ratio representing the 2-trans-4-cis and 2-trans-4-trans isomers, respectively. Both resulted

Table 2. Chromatographic data

Compound	TLC		GLC[a]	
	Solvent[b]	R_f	Column	R_t (min)
I, V	A	0.65	OV 1	24
	B	0.45	-	-
II	A	0.30	OV 1	17
	C	0.65	-	-
III, IV	D	0.55	-	-
VI	B	0.45	-	-
VII	A	0.30	OV 1	20
VIII, IX	D	0.50	-	-
X	E	0.40	OV 17	7
XI	E	0.40	-	-
XII, XVI	B	0.45	OV 1	15
XIII	C	0.60	OV 1	10
XIV, XV	D	0.55	-	-
XVII, XVIII	F	0.65	-	-
XX	E	0.60	OV 1[c]	9

a GLC conditions were as follows: column, 175°, unless otherwise noted; injector, 210°; detector, 250°; flow rate, 40 ml/hr nitrogen.

b The solvent systems used were A, ether; B, 1:2 ether-hexane; C, 1:2 ethyl acetate-ether; D, 1:1 hexane-ether; E, 1:19 ether-hexane; F, 1:9 ethyl acetate-benzene.

c Column temperature was 160°.

from the addition of the predominantly trans phosphonate XIX to the tritiated aldehyde XVIII. The isomer ratio obtained here is in keeping with the findings of Corey et al.[9] on the stereochemical course of a similar phosphonate ylid reaction.

Whenever NMR spectra were obtained, they agreed with postulated structures of the compounds submitted to analysis. Pertinent data for three such spectra are reproduced in Table 3. These further attest to the identity and purity of a characteristic diol intermediate (II) and its periodate cleavage product (III). Of particular interest, also, is the heretofore unpublished spectrum of the trans phosphonate XIX.

Table 3. Selected nuclear magnetic resonance spectral data

Compound	Solvent	Chemical shift (ppm)	Type	No. of protons
II	$CDCl_3$	1.25, 1.30	singlets	2 x 3
		1.62	singlet	3
		2.16	singlet	3
		1.90-2.25	multiplet	8
		3-3.3	broad singlet	2
		3.72	singlet	3
		5.1-5.4	multiplet	2
III	$CDCl_3$	1.60	singlet	3
		2.13	singlet	3
		1.8-2.3	multiplet	8
		3.7	singlet	3
		5.1-5.45	multiplet	2
		9.72	sharp doublet (J = 1 Hz)	1
XIX	CCl_4	1.28-1.31	overlapping triplets (J = 7 Hz)	9
		2.30	sharp doublet (J = 1 Hz)	3
		2.69	doublet	2
		4.1	overlapping quartets (J = 7 Hz)	4
		4.25	quartet (J = 7 Hz)	2
		5.83	broad singlet	1

Finally, as an overall check on the identity and purity of the tritiated hormones generated according to Figure 1, we tested their biological performance in several of the most sensitive biological assays for JH activity. In each case, as seen in Table 1, the labelled hormone showed biological activity comparable to that of unlabelled material, tested concurrently, and in good agreement with activity values reported in the literature.

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