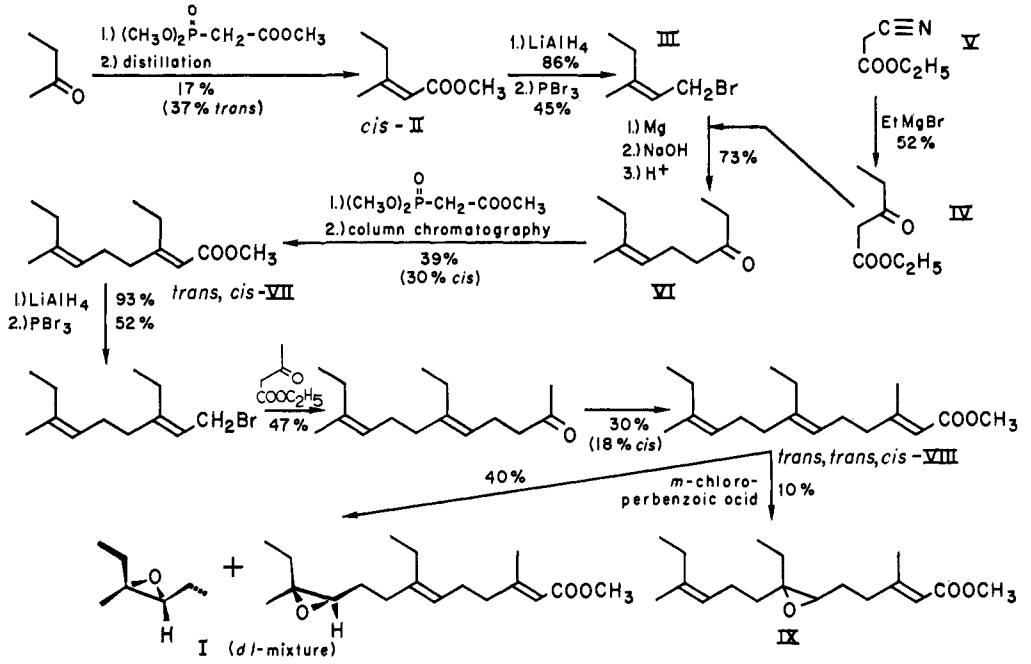
The Juvenile Hormone. V. Synthesis of the Racemic Juvenile Hormone

Sir:

We have previously reported the identification of the juvenile hormone isolated from *Hyalophora cecropia*[1,2] as methyl *trans,trans,cis*-10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (I).[3,4] In this communication we wish to describe the synthesis of I and its comparison with the natural product. The synthesis which we devised allowed separation and stereochemical assignment of *cis–trans* isomers after the introduction of each double bond (Scheme I).

Scheme I

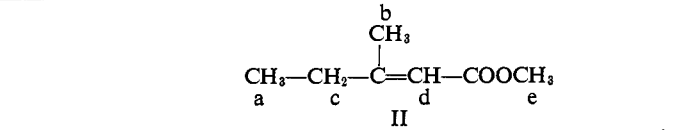


Methyl *cis*-3-methyl-2-pentenoate (*cis*-II) was isolated by distillation on a 60-cm Teflon spinning-band column from the *cis–trans* mixture obtained by treating 2-butanone with the sodium salt of trimethylphosphonoacetate. *cis*-II was converted with lithium aluminum hydride to the alcohol and with phosphorous tribromide in pyridine to *cis*-1-bromo-3-methyl-2-pentene (III). Reaction of III with ethyl 3-oxopentanoate (IV), prepared from ethyl cyanoacetate (V), and subsequent saponification and decarboxylation gave *cis*-7-methyl-6-nonen-3-one (VI). Reaction of VI with the sodium salt of trimethylphosphonoacetate yielded the *cis,cis–trans,cis* mixture of the C_{12}-methyl esters from which the latter (*trans,cis*-VII) was separated by column chromatography on silica gel. Continuing from *trans,cis*-VII, we synthesized the *trans,trans,cis*-C_{17}-methyl ester (*trans,trans,cis*-VIII) by repeating the sequence of steps described, with the exception that ethyl 3-oxobutanoate was substituted for IV (see Scheme I). VIII was allowed to react with *m*-chloroperbenzoic acid in ether, and I (40%), VIII (10%), IX (10%), and the diepoxy compound (10%) were isolated from the reaction mixture by thin layer chromatography.

Assignment of stereochemistry (*cis* and *trans*) to the pairs of 2,3-unsaturated esters II, VII, and VIII is based on nmr data (Table I). In β-substituted α,β-unsaturated esters, the β group *cis* to the carbonyl experiences a larger deshielding effect than the one *trans*.[5] Thus, in *trans*-II and *trans,trans,cis*-VIII the β-methyl group appears 0.27 and 0.28 ppm, respectively, at lower field than in their corresponding 2-*cis* isomers (see Table I), and the methylene quadruplet of the β-ethyl group of *trans,cis*-VII appears approximately 0.5 ppm at lower field than its *cis,cis* isomer. In all of the intermediates, the retention of the *cis* configuration at the terminal double bond was indicated by the lower field position of the methyl group on this double bond compared to the previously synthesized *trans* isomers.[4,6] Furthermore, in this series of compounds, the allylic coupling of the vinyl methyl groups with the olefinic protons follows the general trend of cisoid being larger than transoid.[7,8] This assignment of stereochemistry

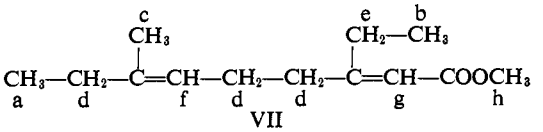
(1) H. Röller and J. S. Bjerke, *Life Sci.*, 4, 1617 (1965).
(2) H. Röller, J. S. Bjerke, D. W. Norgard, and W. H. McShan, Proceedings of the International Symposium on Insect Endocrinology, Brno, Czechoslovakia, 1966, Academic Press Inc., New York, N. Y., in press.
(3) H. Röller, K. H. Dahm, C. C. Sweeley, and B. M. Trost, *Angew. Chem.*, 79, 190 (1967); *Angew. Chem. Intern. Ed. Engl.*, 6, 179 (1967).
(4) K. H. Dahm, H. Röller, and B. M. Trost, submitted for publication.
(5) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Ltd., London, 1959.
(6) J. W. K. Burrell, R. F. Garwood, L. M. Jackman, E. Oskay, and B. C. L. Weedon, *J. Chem. Soc., Sect. C*, 2144 (1966).
(7) S. Sternhell, *Rev. Pure Appl. Chem.*, 14, 15 (1964).
(8) In general, cisoid allylic coupling is larger than transoid; how-

Table I. Nmr Spectral Data[a]



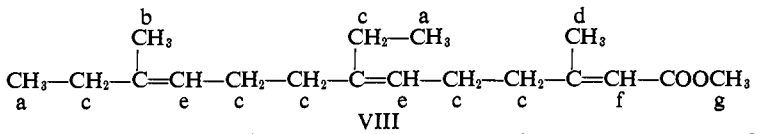
II

	a	b	c	d	e
cis	1.06, t, J = 7.5	1.87, d, J = 1.5	2.63, q, J = 7.5	5.58, bs	3.60, s
trans	1.06, t, J = 7.5	2.14, d, J = 0.8	2.15, q, J = 7.5	5.58, q, J = 0.8	3.60, s



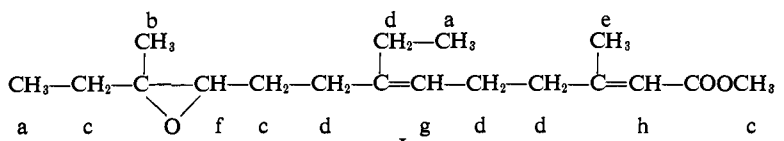
VII

	a	b	c	d	e	f	g	h
cis,cis	0.98, t, J = 7.0	1.08, t, J = 7.0	1.69, d, J = 1.0	1.8-2.8, m		5.14, bt, J = 7.0	5.59, bs	3.64, s
trans,cis	0.97, t, J = 7.0	1.07, t, J = 7.0	1.70, d, J = 1.0	1.8-2.3, m	2.64, q, J = 7.0	5.07, m	5.60, bs	3.64, s

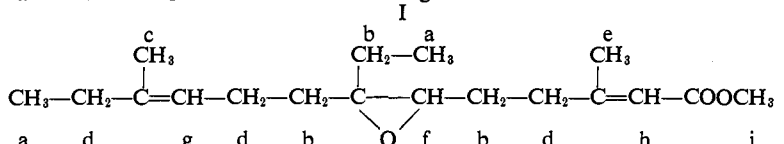


VIII

	a	b	c	d	e	f	g
cis,trans,cis	0.97, t, J = 7.0	1.67, bs	1.8-2.8, m	1.88, d, J = 1.7	5.09, m	5.60, bs	3.62, s
trans,trans,cis	0.97, t, J = 7.0	1.67, bs	1.8-2.3, m	2.16, d, J = 0.8	5.08, m	5.65, bs	3.66, s



I



IX

	a	b	c	d	e	f	g	h	i
trans,trans,cis-10-epoxy	0.97, t, J = 7.0	1.20, s	1.25-1.70, m	1.8-2.3, m	2.16, d, J = 0.8	2.53, t, J = 6.0	5.14, m	5.60, bs	3.66, s
6-epoxy	0.98, t, J = 7.0	1.2-1.8, m	1.68, bs	1.8-2.4, m	2.18, d, J = 0.8	2.60, t, J = 6.0	5.09, m	5.68, bs	3.65, s

[a] In each column, the chemical shift in parts per million relative to TMS as an internal standard is listed first, the multiplicity second, and the coupling constant in cycles per second third. The spectra were recorded at 60 Mc in CCl_4; s = singlet; bs = broad singlet; d = doublet; t = triplet; bt = broad triplet; q = quadruplet; m = multiplet.

is confirmed by chromatographic data. On gas–liquid partition chromatography (XE-60, SE-30, and Carbowax) in every case the *trans* isomer had the higher retention time. On thin layer plates with the system used (Table II) the *trans* isomers have consistently lower R_f values: *cis*-II 0.54, *trans*-II 0.50; *cis,cis*-VII 0.70, *trans,cis*-VII 0.67; *cis,trans,cis*-VIII 0.73, *trans,trans,cis*-VIII 0.71. The position of the epoxy function in I and IX was easily determined by nmr (see Table I) by observing the position of absorption for the C_{11}-methyl group in the products.

I is identical with the natural juvenile hormone in the following respects: mass spectrum, retention time on gas–liquid partition chromatography, R_f value on thin layer chromatography (Table II), and biological activity (Table II). The nmr spectrum is virtually identical with that of the natural material. The data reported here (Table I) were obtained at 60 Mc, whereas those reported for the natural product were obtained at 100 Mc. The differences in chemical shifts observed are those expected from the differences in field strength. The question of the *d–l* isomerism of the natural hormone remains unsolved. The bioassay results indicate that the *d* and the *l* forms have a similar specific activity; the maximum difference between the two could not be greater than twofold. Experiments with the isolated natural juvenile hormone demonstrated that this single molecular species possessed the morphogenetic, prothoracotropic, and gonadotropic activities attributed to the secretion of the corpora allata.[2] In preliminary experiments we have been able to show that all synthesized compounds listed in Table II had a significant morphogenetic effect in lepidopterous larvae.

ever, in some instances the differences are negligible or the magnitudes very slightly reversed. See L. M. Jackman and R. H. Wiley, *J. Chem. Soc.*, 2881, 2886 (1960); R. R. Fraser and D. E. McGreer, *Can. J. Chem.*, **39**, 505 (1961); D. R. Davis and J. D. Roberts, *J. Am. Chem. Soc.*, **84**, 2252 (1962).

Table II. Specific Activity and R_f Values of Natural Juvenile Hormone and Several Synthesized Compounds

Compound	Spec act., TU/μg[a]	R_f[b]
Natural juvenile hormone	5000	0.40
t,t,c-10-epoxy-C_{17}-methyl ester (synthesized *dl* juvenile hormone) (I)	5000	0.40
t,t,t-10-epoxy-C_{17}-methyl ester[4]	2000	0.40
t,t,c-C_{17}-methyl ester (VIII)	500	0.67
t,t,t-C_{17}-methyl ester[4]	200	0.67

[a] TU/μg, *Tenebrio* units per microgram, as previously defined.[1,2] [b] Thin layer chromatography on silica gel G (E. Merck), activated 2 hr at 120°, benzene–ethyl acetate 15:1.

Supernumerary larval molts in *Galleria mellonella* could be produced by injection of 2000–4000 TU of each compound per animal. This dosage level is equivalent to that required to obtain a similar response with the natural juvenile hormone. Further biological experiments with these compounds in insects of different orders are currently in progress.

Acknowledgments. The authors are indebted to Dr. C. C. Sweeley, Department of Biochemistry, University of Pittsburgh, for providing the mass spectra. These studies were aided by grants from the National Science Foundation (GB-5475 and GB-6244) and the National Institutes of Health (GM-13598-02) to the University of Wisconsin.

(9) Department of Zoology.
(10) Alfred P. Sloan Foundation Fellow; Department of Chemistry.

Karl Heinz Dahm,[9] Barry M. Trost,[10] Herbert Röller[9]
Departments of Zoology and Chemistry
University of Wisconsin, Madison, Wisconsin 53706
Received July 28, 1967

Steroids and Steroidases. V.[1] On the Mechanism of Action of the Δ^5-3-Ketoisomerase of *Pseudomonas testosteroni*

Sir:

During our investigations on the Δ^5-3-ketoisomerase of *P. testosteroni*, which effects the isomerization of Δ^5-3-keto steroids to the corresponding Δ^4-3-ketones, it became of interest to compare the acid-, base-, and enzyme-catalyzed reactions.

Previous studies on base-catalyzed isomerizations of Δ^5-3-keto steroids have been hindered by multiple product formation.[2] However, when solutions of buffers, such as Tris–HCl, pH range 8.5–9, or aqueous sodium hydroxide, pH >10, were used, clean isomerizations to Δ^4-3-ketones were observed. Using aqueous sodium hydroxide solutions in the pH range 10.6–11.7, androst-5-ene-3,17-dione isomerized with over-all second-order kinetics, the reaction being first order with respect to both steroid and hydroxide ion concentrations.

Loss of the C_4 hydrogen atom has been shown to be the rate-determining step for both acid- and enzyme-catalyzed isomerizations of androst-5-ene-3,17-dione.[3] A comparison of the pH 10 isomerization of androst-5-ene-3,17-dione with that of its 4,4-dideuterio analog showed a primary kinetic isotope effect of 3.2, thus indicating C_4 proton abstraction to be rate determining for the base-catalyzed reaction also.

The Δ^5-3-ketoisomerase of *P. testosteroni* is the most active enzyme known,[4] and the rates of the acid-, base-, and enzyme-catalyzed reactions of androst-5-ene-3,17-dione are in the ratio $1:700:(17 \times 10^6)$. In an attempt to ascertain where the enzymic isomerization gains its advantage over those catalyzed by acid and base, the activation parameters were determined.[5] As seen from the values recorded in Table I, the facility of the enzymic process is mainly due to an extremely low enthalpy of activation. The differences in the entropies of activation for the three processes are not very great, and the $\Delta S^{\ddagger}$ value of -16.8 cal deg^{-1} mole^{-1} for the enzymic catalysis is consistent with the development of charge separation in the transition state as required by the mechanism proposed by Ringold and Malhotra.[3]

Table I. Activation Parameters for the Isomerization of Androst-5-ene-3,17-dione[a]

	Catalyst		
	HCl, pH 0.88[b]	Tris–HCl, pH 8.82[b]	Enzyme[c]
Enthalpy of activation, $\Delta H^{\ddagger}$, kcal mole^{-1}	14.0 ± 0.1	11.4 ± 0.1	5.0 ± 0.1
Entropy of activation, $\Delta S^{\ddagger}$, cal deg^{-1} mole^{-1}	-19.6 ± 0.4	-15.5 ± 0.4	-16.8 ± 0.4

[a] Data obtained with 1.6% aqueous methanol solutions and a steroid concentration of 0.05 μmole/ml. [b] Temperature range 15–40°. [c] Temperature range 15–30°.

Acknowledgments. We are grateful to the National Research Council of Canada and to the Province of Ontario, Department of University Affairs, for financial support of this work, and to the Canadian Universities Foundation for the award (to D. C. W.) of a Commonwealth Scholarship. We also thank Dr. P. Talalay for providing us with a starter culture of *P. testosteroni* and Mr. D. G. Glass for its large-scale growth.

(5) Direct comparison of the activation parameters for the acid-, base-, and enzyme-catalyzed reactions is considered to be reasonable since the same rate-determining step is involved in each case.

J. Bryan Jones, Donald C. Wigfield
Department of Chemistry, University of Toronto
Toronto 5, Canada
Received July 24, 1967

Correlations between Carbon-13 and Boron-11 Chemical Shifts. I. The Alkanes and Analogous Boron–Nitrogen Compounds[1]

Sir:

Chemical shift data for carbon-13 and boron-11 nuclei are not very extensive because of a number of well-known features which are unfavorable toward magnetic resonance measurements.[2] A clear correlation of chemical shift between the two nuclei in related types of compounds would have considerable value from both a practical as well as a theoretical viewpoint.

The amine boranes and the closely related compounds, the diborazanes and cycloborazanes, may be viewed as the inorganic analogs of the alkanes in which one or more carbon–carbon linkages have been replaced by a corresponding number of isoelectronic boron–

(1) Part IV: J. B. Jones, J. M. Zander, and P. Price, *J. Am. Chem. Soc.*, **89**, 94 (1967).
(2) W. R. Nes, E. Loeser, R. Kirdani, and J. Marsh, *Tetrahedron* **19**, 299 (1963).
(3) H. J. Ringold and S. K. Malhotra, *J. Am. Chem. Soc.*, **87**, 3228 (1965).
(4) F. S. Kawahara, S-F. Wang, and P. Talalay, *J. Biol. Chem.*, **237**, 1500 (1962).

(1) The support of the University of North Carolina Materials Research Center under Contract SD-100 with the Advanced Research Projects Agency and of the donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.
(2) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 2, Pergamon Press, New York, N. Y., 1966, p 969.