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# Absolute Configuration of Cecropia Juvenile Hormone

**(Horeau's method/glycol/epoxide hydration/PMR/mass spectroscopy)**

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**ABSTRACT** **The absolute configuration of the predominant Cecropia hormone, methyl 12,14-dihomojuvenate, has been determined to be methyl (*E,E*)-(10*R*,11*S*)-(+)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (I). The less abundant hormone, methyl 12-homojuvenate, can be presumed by analogy to have the corresponding 3,7,11-trimethyldienoate structure (II). The assignment has been established with microamounts of substance by applying Horeau's method to the glycol derivative (III) of the hormone.**

**The course of the perchloric acid-catalyzed epoxide ring opening of I was checked by conducting the conversion in $^{18}O$-labeled water. It has been ascertained that the configuration at the secondary hydroxyl group of the resulting III remained unchanged. On the other hand, the hydration proceeded with a surprisingly high rate of *cis* opening.**

The *relative* configurations of the two Cecropia juvenile hormones (I, II) have been known for some time (1, 2). The two double bonds in the molecules have the *trans* or *E* configuration, whereas the principal carbon chain is *cis* oriented at the oxirane. This implies *R* configuration on one of the carbon atoms of the epoxide and *S* configuration on the other. Recently we reported optical rotatory dispersion data for a mixture of the two natural hormones, and thereby demonstrated that they are not racemic (3). Hence, it became of interest to determine the absolute configuration of their vicinal chiral centers. The assignment (10*R*,11*S*) derived from the molecular rotation was considered not entirely reliable (3). Therefore, we sought to establish the configuration more firmly by applying Horeau's method (4) involving kinetic resolution of α-phenylbutyric anhydride by a chiral alcohol.

TABLE 1. *Optical rotatory dispersion measurement of the recovered α-phenylbutyric acid and its optical yield after acylation of 2.5 μmol of glycol III*

| λ (nm) | α* (milli-degree) | $[M_{exp}]$† (degree) | $[M]$‡ (degree) | Observed optical yield§ (%) | Optical yield, actual¶ (%) |
|---|---|---|---|---|---|
| 365 | +14 | +47.0 | +469 | 10.0 | |
| 436 | +7 | +23.5 | +280 | 8.4 | |
| 546 | +4 | +13.4 | +154 | 8.7 | |
| 578 | +3.5 | +11.8 | +135 | 8.7 | |
| Sign: positive | | | Mean: | 9.0 | 11.2 |
| | | | (SE) | ±0.7 | ±1.2 |

* Observed rotation (±3 millidegrees) of the acid fraction of the reaction mixture diluted in methanol to 840 $mm^3$ and measured in a 1.000-dm tube at 29°C.

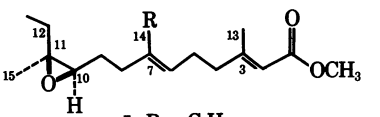
† Observed rotation expressed per 1 mol of reacting glycol and dilution of acid fraction to 100 $cm^3$.

‡ Molecular rotation of pure enantiomeric α-phenylbutyric acid in methanol (*c* 0.58%) at 27°C (see text).

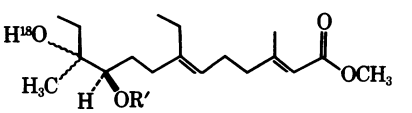
§ Quotient $[M_{exp}]/[M]$.

¶ Corrected for esterification efficiency of 80 ± 2%.

Though the hormones possess no acylable hydroxyl group at the chiral centers, they can be converted to such compounds by hydration of their epoxide function. Long and Pritchard (5) have shown that unsymmetrically alkyl-substituted oxiranes undergo attack in aqueous acid almost exclusively at the more substituted carbon atom. For the interpretation of our experiment, it has been crucial to ascertain that the reaction with juvenile hormone takes the same course. Thus, the acid-catalyzed hydrolysis was conducted in $^{18}O$-labeled water. The procedure was fashioned after a hydrolysis method for methyl juvenate (unpublished results) kindly provided by Prof. E. E. van Tamelen of Stanford University. We are likewise indebted to Dr. J. B. Siddall who communicated his similar, equally efficient procedure to us.



I, R = $C_2H_5$
II, R = $CH_3$



III, R′ = H
IV, R′ = (*R*)-(−)-$C_9H_{11}CO$
V, R′ = $CH_3CO$
*a*, ---$^{18}OH$; *b*, —$^{18}OH$

## RESULTS AND DISCUSSION

In a preliminary experiment, the conversion was done with a sample of synthetic racemic methyl 12,14-dihomojuvenate ((±)-I) (6). The resulting *vic*-diol ((±)-III) was isolated and examined by mass spectrometry; in particular the $^{18}O$-content of both hydroxyl groups was assessed. Abundant ions, arising from the predictable cleavage of the C-10,11 bond, are found at *m/e* 75.0691 ($C_4H_9{}^{18}O^+$, calcd 75.0695) and 239.1647 ($C_{14}H_{23}O_3^+$, calcd 239.1647), and so are the analogous ions in the spectrum of the bistrimethylsilyl ether derivative at *m/e* 147.1086 and 311.2036. Moreover the M −

15 ion, typical of trimethylsilyl derivatives, is exhibited at *m/e* 443.2913 ($C_{23}H_{45}O_3{}^{18}OSi_2{}^+$, calcd 443.2900). These data demonstrate that the glycol and its derivative each contain one atom of $^{18}O$, which is predominantly located at C-11. The distribution of the $^{18}O$ can be evaluated more precisely by measuring the relevant peak heights in low-resolution spectra (7). This was done in the case of the glycol, the fragment ion at *m/e* 239 being compared with the less abundant ion two atom mass units (amu) higher, in which $^{16}O$ at C-10 is replaced by $^{18}O$. Accordingly, at least 94% of the incorporated $^{18}O$ atoms are present in the tertiary hydroxyl group of the glycol. We therefore have concluded that, on hydration of I, the bonding around C-10 remains (in the main) unaltered, and what can be learned about the configuration at this center by an apposite reaction of the glycol is equally valid for the structure of the hormone.

While the configuration on C-10 has been thus fixed, it became evident that the neighboring tertiary carbon atom of the hormone molecule has undergone hydroxylation with less steric selectivity than had been anticipated (compare ref. 8). The proton magnetic resonance (PMR) spectrum of the diol III exhibits two maxima of different intensities in the region of slightly deshielded methyl protons. The peaks at $\delta$ 1.00 and 1.045 ppm are ascribed to the methyl attached to the carbinol group C-11, and their height ratio of 5:2 indicates the approximate proportion of the two epimeric glycols. (A lower homolog of (±)-III with the isopropyl terminal of regular terpenes, obtained from methyl (±)-juvenate, elicits two singlets at $\delta$ 1.06 and 1.10 ppm.) In keeping with Cavill *et al.*'s (9) correlation in the case of 3-methylnonane-3,4,7-triol, we have assigned the intense resonance at higher field to the 10,11-*threo* ((±)-III*a*) and the one at lower field to the 10,11-*erythro* epimer ((±)-III*b*). Hence, *trans* opening was the predominant, but by no means the sole, mode of hydrolysis.

The glycol mixture (III) behaved on silica thin-layer chromatography (TLC) and on gas–liquid chromatography (GLC) like a homogeneous substance, except for a slight impurity ($\leqslant 2\%$) detected by GLC. Separation of the 11-epimeric glycols, however, has not been necessary for the present investigation, as the configuration at C-10 of *both* constituents is identical with that of the hormone molecule from which they have been derived. The formation of the pair of epimeric glycols was somewhat unexpected, because the presently prevailing notion holds that acid-catalyzed hydration of simple aliphatic epoxides proceeds mostly through *trans* opening (*e.g.*, 8). On the other hand, formation of epimeric glycol mixtures has been well-documented in cases of aryl-substituted epoxides (*inter al.* 10).

Because of the scarcity of the natural hormones (11), we had to perform the Horeau reaction with a few micromoles of the glycol. This meant a scaling down of the usual 100-μmol procedure (4) by some 40 times. The conditions were first tested on two 17β-hydroxy steroids, 5α-androstan-17β-ol (VI) and testosterone (VII). As long as an adequate excess of α-phenylbutyric anhydride was present, only minimal quantities of unesterified steroid, as analyzed by GLC, remained in the reaction mixtures. Acylation has thus gone practically to completion ($\geqslant 99.5\%$) in each instance. To arrive at the *optical* yield of the reaction, a standard of reference applicable to the select wavelengths of our polarimeter (see Table 1) was required. In the absence of a sample of resolved α-phenylbutyric acid, the pertinent rotatory dispersion data of Sjöberg (12, p. 293) were interpolated in a Heller plot (13). The optical yields of each individual experiment, as thus determined at the four wavelengths in question, stayed within quite acceptable precision limits. In experiments in which 30 μmol of steroid, or more, were allowed to react with 2 equivalents of anhydride, the mean optical yields amounted to 42 ± 0.3% for VI and 39 ± 0.3% for VII. These values are in substant al agreement with those originally reported (4). When the quantities of steroids were further reduced, a larger excess of anhydride (*e.g.* 4 equivalents) was necessary for complete esterification. Yet, up to 1/3 lower optical yields were obtained in these experiments, presumably as a result of enhanced racemization. Since, however, it is the sign of rotation rather than its magnitude that is of diagnostic significance, optimal conditions for the reaction on a microscale were not elaborated. In any case, prolonging the reaction time beyond 16 hr also led to diminution of the optical yields, even in the larger scale experiments. Horeau showed in 1962 that optically active α-phenylbutyric acid is slowly racemized in the presence of unresolved anhydride.

At last we were ready to conduct the reaction sequence with a sample of natural pure Cecropia hormone. Methyl 12,14-dihomojuvenate (I) was hydrolyzed by 0.07 N $HClO_4$ in tetrahydrofuran–$H_2{}^{18}O$. The isolated glycol (III) was subjected to the Horeau reaction, and the products were isolated by silica gel column chromatography. The phenylbutyrate ester (IV) was obtained in 75% yield. The approximately 7 parts of *threo* (10*R*,11*R*, IV*a*) and 2 parts of *erythro* epimers (10*R*,11*S*, IV*b*) were separated by silica TLC. A small quantity (5%) of a slightly more polar conversion product (V) has been identified as the monoacetates of glycol mixture III. Their mass spectrum comprises an array of ions that appears to be displaced downscale by 104 amu when compared with the spectra of IV*a* and IV*b*. Substantiation for the presence of an acetyl group has been provided by the PMR spectrum ($\delta$ 2.05 ppm), which moreover suggests that the mixture has preponderantly *erythro* configuration (V*b*) (intense resonance at $\delta$ 1.03 ppm). Since acetic anhydride was used in the preparation of the α-phenylbutyric anhydride reagent (see ref. 14), which was used under the present conditions in a large excess, formation of the acetate side product can be readily visualized. Finally, 13% of unchanged glycol (III), with a composition of 4 parts of *threo* and 1 part of *erythro* epimers, was recovered. Its mass spectrum, including the distribution of $^{18}O$, is virtually identical with that of the racemic glycol (see above). Considering the total recovery (93%) of material, we estimate the efficiency of the esterification reaction III → IV as 80%.

From the acid fraction of the reaction mixture, α-phenylbutyric acid was recovered and its optical rotatory dispersion was measured (Table 1). The material was dextrorotatory, implying (see ref. 4) *R* configuration at the chiral center C-10 of III. The optical yield of the 2.5-μmol experiment amounted to 11%. In view of the stereochemical relationship between the hormone and its glycols discussed above, we have concluded that the structure of methyl 12,14-dihomojuvenate is methyl (*E*,*E*)-(10*R*,11*S*)-(+)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (I).

## COMMENTS

It may be of interest to compare Cecropia hormone with some naturally occurring terpene oxides in regard to their configura-

tions. (*E,E,E*)-14,15-epoxygeranylgeraniol, a chemoprophylactic agent against schistosomiasis, has been isolated from the fruits of *Pterodon pubescens* by Mors *et al.* (15). A sample of the nearly pure compound was kindly donated to us by Prof. Walter Mors of the Federal University of Rio de Janeiro. The optical rotatory dispersion curve of the specimen has been determined in chloroform to be plain positive; $[\alpha]_D^{29}$ +4.0°, $[M]_D^{29}$ +12.2° (*c* 3.35%). Since (*R*)-(+)-2,3-epoxy-2,5-dimethylhexane is dextrorotatory ($[M]_D^{16}$ +11.5°, see ref. 3), the diterpene also has *R* configuration.

On the other hand, the important biosynthetic intermediate 2,3-epoxysqualene may be presumed to have *S* configuration, as can be deduced from the configuration of its principal cyclization product, lanosterol. The micromethod described in this report should be of service in experimentally corroborating the assignment. In this context, it seems appropriate to mention the studies with the unnatural stereoisomeric homologs of epoxysqualene in which the terminal carbon atom of the oxirane (C-2) is substituted with a methyl and an ethyl grouping, as is the case with C-11 of the Cecropia juvenile hormones. Only the 1-homoepoxysqualene with presumably the 2*S*,3*S* configuration reacts in presence of the cyclase from mammalian tissues and is nearly completely converted to 4$\alpha$-homolanosterol (16). Moreover, the 1-norepoxysqualene analogs of either configuration at C-2 are converted by microsomal preparations to glycols (16). One may possibly see in these findings a rationale for the particular configuration of the Cecropia hormones and for the unusual homologization in nature of their basic farnesate structure. If analogy with the squalene series holds up, the mentioned structural characteristics of the hormones could provide a measure of protection to the metabolically active oxirane function against indiscriminate hydrolytic breakdown, as well as from proton attack leading to cyclization.

## EXPERIMENTAL SECTION

Liquid–solid chromatography was performed on 4-mm diameter columns of silica gel (Davison, grade 932) that had been exhaustively washed in a Soxhlet extraction apparatus with benzene and then methanol, dried in an oven at 135°C, and deactivated with 8% of its weight of water. "Chromar" (7G) silica TLC plates were developed in benzene–ethyl acetate 7:3. GLC was done on a 1% OV-225 column (175 × 0.3 cm) at 193°C and a carrier flow of 50 ml of argon per min. Solvents were of "Nanograde" quality; glass-distilled water was extracted with ether before use. $\alpha$-Phenylbutyric acid (puriss.) and testosterone were purchased from Fluka, androstanol came from Schwarz-Mann, and the $^{18}O$-enriched normalized water from Miles Laboratories.

Optical rotations were observed by means of a Perkin-Elmer 141 polarimeter with digital readout. Mass spectra were recorded at 70 eV on a Hitachi Perkin-Elmer RMU 6-L and on a CEC 110B instrument (resolving power 20,000); the samples were introduced directly into the ion source. Additional spectral data of the compounds were obtained from their solutions in "Instra-analyzed" carbon tetrachloride: the PMR spectra in a 100-MHz field, generally by Fourier transform, the substances being dissolved in 300 $\mu$l of solvent containing 0.15% of tetramethylsilane; the IR absorptions were determined from 0.10 M solutions (see ref 11). Only the most significant spectral data are noted in this report.

*Glycol III of methyl 12,14-dihomojuvenate.* Methyl 12,14-dihomojuvenate (I) (1.09 mg or 3.71 $\mu$mol containing 2.5% of II, from the fourth batch of silk moth, ref. 11) was dissolved in 20 $\mu$l of tetrahydrofuran and hydrolyzed with 20 $\mu$l of 70% $HClO_4$ that had been diluted with exactly 50 times its weight of $H_2{}^{18}O$ (97.9 atom % $^{18}O$, 1.1 atom % $^{16}O$). The $H_2{}^{18}O$–$H_2{}^{16}O$ ratio of the medium was about 60:1. The reaction mixture was left at ice-water temperature for 22 hr. The residue of 1.17 mg from the processed ether extract of the reaction mixture was chromatographed on 1.2 gm of silica gel, from which the glycol (III) was eluted with 12.0 ml of benzene that contained 9 vol % of ethyl acetate. The fraction weighed 795 $\mu$g net (2.52 $\mu$mol). As judged by GLC, the material was identical with the sample from synthetic (±)-I that had been hydrolyzed in the same way.

The latter glycol ((±)-III) has been characterized as follows. TLC $R_f$ 0.27; GLC $t_R$ 6.55 min, also an impurity ($\leqslant$2%) with $t_R$ 5.1 min; PMR (by computer of average transients technique) $\delta$ 0.90 (t, *J* 7.5 Hz, $C\underline{H}_3CH_2$), 1.00 and 1.045 ppm (s, $C\underline{H}_3COH$), total of 9 H; $\delta$ 2.25 (broad, 0.13 M solution at 5°C, 2 CO$\underline{H}$), 3.23 ppm (qa, 1 C$\underline{H}$OH); IR $\nu$ 3572 (m/st) and 3510 (shoulder, hydrogen-bonded O—H), 1072 (m, C—O), 863 $cm^{-1}$ (w, RC=C$\underline{H}$ *trans*). The mass spectral data (see text) are also consistent with structure III.

*Esterification of III with $\alpha$-phenylbutyric anhydride.* The entire fraction of the natural glycol (III) was transferred into a 3-ml glass-stoppered tube and dried for 5 hr over $P_2O_5$ in a vacuum desiccator. The material was then allowed to react at room temperature for 16 hr with 0.10 ml of an anhydrous pyridine solution containing 14.3 mg (18 equiv) of freshly prepared (±)-$\alpha$-phenylbutyric anhydride (14). Subsequently, 20 $\mu$l of water was added to hydrolyze excess anhydride at 36°C for 2 hr. The reaction mixture was then dissolved in 0.8 ml of benzene, made alkaline to phenolphthalein with 0.45 ml of 0.20 N aqueous NaOH, diluted with an additional 1.2 ml of benzene, and the neutral phase was separated from the alkaline solution, which was twice reextracted with benzene. Upon acidification of the aqueous phase with 0.30 ml of 2.0 N HCl, $\alpha$-phenylbutyric acid was recovered with 2 × 2.0 ml of benzene, and the solution was freed from HCl by washing with water. The benzene solution was evaporated, the remaining *acid fraction* was dissolved in methanol to a volume of 840 $mm^3$, and the rotation was measured without delay (Table 1).

The residue from the washed, neutral phase was fractionated on a silica gel column (1.0 g, 13 cm high). Benzene containing 2 vol % of ethyl acetate eluted 870 $\mu$g (1.89 $\mu$mol) of *phenylbutyrate ester IV*. The material was separated by TLC into two fractions, with $R_f$ of 0.66 and 0.58, from which 481 $\mu$g of *threo* (IV*a*) and 139 $\mu$g of *erythro* ester (IV*b*) were secured. The two epimers have many spectral characteristics in common, e.g. PMR $\delta$ 3.61 (s, 1 $C\underline{H}_3OCO$), 4.7 (mt, 1 C$\underline{H}$OR'), 4.9 (mt, 1 $CH_2C\underline{H}$=C), 5.59 (s, 1 COC$\underline{H}$=C), 7.25 ppm (s, $CHC_6\underline{H}_5$). Their mass spectra are nearly identical, *m/e* 460 ($M^+$), 440 (M − $H_2{}^{18}O^+$), 276 (M − $H_2{}^{18}O$ − $C_9H_{11}CO_2H^+$), 91 ($C_9H_{11}$—$C_2H_4^+$, base peak). The two esters can be distinguished in some regions of their IR spectra; IV*a* $\nu$ 3577 (w, O—H), 1220 (m/st), 1195 (m/st), 1023 $cm^{-1}$ (w); IV*b* $\nu$ 3585 (w, O—H), 1244 (m/st), 1219 (st), 1006 $cm^{-1}$ (m/w). The PMR spectral data that do not coincide in both compounds are difficult to interpret in the absence of decoupling and other analyses [e.g. the methyl region or $\delta$ 3.4 (mt) in IV*a* versus 3.58 ppm (s!) in IV*b*, both these resonances presumably due to the $C\underline{H}C_6H_5$].

When, in the chromatographic development, the ethyl acetate content of the benzene eluent was raised to 6%, 47 μg (0.13 μmol) of an additional product came off the column. The fraction has been characterized as the mixture of epimeric *acetates V*. TLC $R_f$ 0.41; GLC $t_R$ 6.95 min; PMR δ 0.90 (t, *J* 7.5 Hz, $C\underline{H}_3CH_2$), 0.96 and 1.03 (s, $CH_3COH$), 2.05 ppm (s, 1 $C\underline{H}_3COOC$); IR ν 3410 (st and broad, intramolecular hydrogen-bonded O—H), 1720 (st) and 1734 (shoulder, C=O), 1453 (m), 1367 (m), 1223 $cm^{-1}$ (m/st, broad); mass spectrum *m/e* 356 ($M^+$), 336 ($M - H_2{}^{18}O^+$), 276 ($M - H_2{}^{18}O - CH_3CO_2H^+$). Finally, 105 μg of glycol mixture III was recovered in the chromatographic eluent containing 9 vol % of ethyl acetate in benzene. The most important mass spectral and PMR data of the fraction have been mentioned in the text; otherwise the material is identical with (±)-III.

## NOTE ADDED IN PROOF

Prof. William S. Johnson was kind enough to send us a copy of his manuscript in which the synthesis of the optically active form of methyl 12,14-dihomojuvenate is described (Loew, P., and W. S. Johnson, *J. Amer. Chem. Soc.*, in press).

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