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PREPARATION AND ACTIVITY OF OPTICALLY ACTIVE C_{16}-JUVENILE HORMONE

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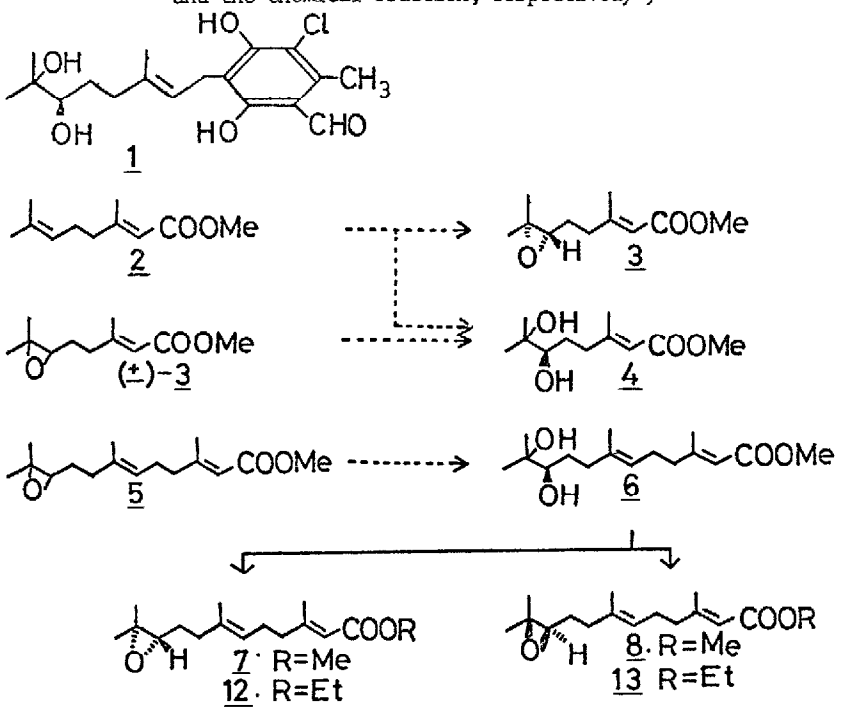
Since then the 10R,11S stereochemistry of natural (+)-C_{18}-juvenile hormone (JH) was established,[1] the question has been raised whether or not the (-) antipode of the hormone is really biologically active This problem is important for clarifying a chiral nature of the hormone receptor of insects, but remains unsolved because of the extreme difficulty in preparing the optically pure (-) enantiomer The synthetic specimen of (-)-C_{18}-JH by Loew and Johnson[1a] was contaminated approximately 8% by its (+) isomer We have prepared both enantiomers of C_{16}-JH by metabolizing stereoselectively the racemic hormone with a fungus, Helminthosporium sativum[2] However, the considerable enantiomeric contamination in the prepared sample made the conclusion ambiguous[3] Optically active C_{17}-JH was also prepared by a similar way,[4] but a lack of determination of the exact enantiomeric purity made the sample unsuitable for the present purpose We wish now to describe our successful preparation of optically active C_{16}-JH employing either a fungal metabolism or a chemical resolution method, and their hormone activity assayed on the silkworm is also reported

We selected for the present study a fungus, Colletotrichum nicotianae, instead of H sativum Contrary to the previously used fungus, the new fungus was expected to produce an optically plus glycol, as suggested by its normal metabolite, colletochlorin (1), which contains (+)-6,7-dihydroxygeranyl moiety, $[\alpha]_D$ +11 6°[5] Actually, when methyl geranate (2) was metabolized with this fungus, that is, the substrate (2) was shaken with the precultured mycelia in the modified Czapek-Dox medium for 9 hr by the same way as in the case of H sativum,[6] two metabolites were obtained as expected, i e , S(-)-methyl 6,7-epoxygeranate (3),[7] $[\alpha]_D$ -12 4° (c 1 7, MeOH), yield 19 6%, and R(+)-methyl 6,7-dihydroxygeranate (4),[7] $[\alpha]_D$ +26 1° (c 1 4, MeOH), yield 15 6% Furthermore, the metabolism of a longer period (24 hr) produced only the glycol (4) with the isolated yield as high as 85%[8] By the similar metabolic experiment upon racemic methyl 6,7-epoxygeranate, (±)-3, for 12 hr, the glycol (4), $[\alpha]_D$ +26 0° (c 1 8, MeOH) was obtained, accompanied by the unchanged but partially optically active substrate, $[\alpha]_D$ +3 8° (c 4 7, MeOH) The enantiomeric purity of 4 obtained from the both substrates was determined by nmr method using a chiral shift reagent (csr), tris[3-(trifluoromethylhydroxymethylene)d-camphorato]europium (III) At first, when racemic 4 was measured in CCl_4 at the reagent/substrate ratio of 0 3 (mole/mole), the C_7-dimethyl signals of its S and R components shifted downfield appearing at δ 3 35 and 4 00, and δ 3 40 and 3 95, respectively Under the same conditions, the isolated 4 did not show the signals corresponding to the S enantiomer, indicating that the glycol (4)

Scheme I (The arrows, ---> and ⟶ , indicate the fungal metabolism and the chemical reaction, respectively)

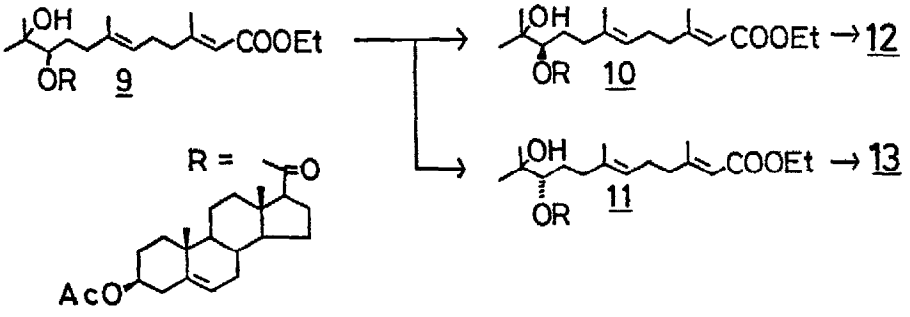


obtained in this way must be optically pure

With the success in the stereoselective conversion of methyl epoxygeranate, (±)-3, we conducted the fungal transformation of racemic methyl 10,11-epoxyfarnesate (5) Although the 24 hr metabolism transformed the substrate (5) into the more modified product,[9] the desired optically active glycol, R(+)-methyl 10,11-dihydroxyfarnesate (6), $[\alpha]_D$ +17 8° (c 1 8, MeOH) was obtained in the 12% isolated yield after 6 hr incubation Its enantiomeric purity was determined by HSLC (Hitachi gel 3040, 15% Et_2O in n-hexane, 2 1 mm × 500 mm, 0 6 ml/min) after conversion into (+)-α -methoxy-α -trifluoromethylphenylacetyl (MTPA) ester (Rt of the R ester 20 min, and of the S ester 23 min) The metabolite (6) was shown to contain 5% of the S isomer Then, optically active C_{16}-JH was chemically prepared starting from this glycol (6) Mesylation (MsCl, pyridine) followed by base treatment (0 5 N methanolic KOH) afforded S(-)-C_{16}-JH (7), $[\alpha]_D$ -5 44° (c 0 7, MeOH), and successive reactions of acetylation (Ac_2O, pyridine), bromination (PBr_3, pyridine) and base treatment (0 5 N methanolic KOH) gave R(+)-C_{16}-JH (8), $[\alpha]_D$ +5 75° (c 0 4, MeOH) As the racemization was assumed not to occur in the conversion of 6 into 7 or 8,[10] the both enantiomers thus chemically derived should have the enantiomeric composition of 95 5

As our goal is to obtain optically pure C_{16}-JH, attempts were then made to prepare via a chemical resolution method 3β-Acetoxyetienyl as well as (+)-MTPA esters of racemic ethyl 10,11-dihydroxyfarnesate were found to be suitable, especially the former to be superior, for

the resolution Thus, the etienyl ester (9) was subjected to repeated preparative TLC (Kiesel gel PF_{254}, 20% ethyl acetate in n-hexane) until each component, 10 and 11, was obtained completely in pure state The purity of the isolated diastereomers was confirmed by HSLC (Hitachi gel 3040, 30% Et_2O in n-hexane, 2 1 mm x 250 mm, 2 0 ml/min, Rt of 10 12 7 min and of 11 8 5 min) The pure specimen of 10 or 11 was stereospecifically converted into S(-)- and R(+)-C_{16}-JH (12 and 13), respectively, upon reductive cleavage of the etienyl ester ($LiAlH_4$ in Et_2O, -72°) followed by mesylation and base treatment by the same way as before The specimen of C_{16}-JH[11] thus prepared in the form of ethyl ester[12] must be optically pure because of the stereospecific reactions carried out on the optically pure glycols, although the enantiomeric purity of the final products has not been determined



JH activity of the chemically prepared both enantiomers was determined on the allatectomized 4th instar bombyx larvae, and their results are shown in Table The natural (+) form[13] of the hormone exhibited the strong activity, whereas its (-) enantiomer in question showed the

Table JH Activity* of Synthetic Enantiomers of C_{16}-JH Ethyl Ester on the Silkworm, Bombyx mori[14]

Dose(μg)	R(+)-C_{16}-JH					S(-)-C_{16}-JH				
	No of animal	++	+	-	activity (%)	No of animal	++	+	-	activity
100	5	5	0	0	100	16	4	5	7	40 7
10	15	7	3	5	56 6	13	2	3	8	26 9
1	14	3	3	8	32 1	14	1	1	12	10 7
0 1	17	0	0	17	0	-	-	-	-	-
control	12	0	0	12	0	12	0	0	12	0

*A test compound dissolved in 0 5 μl of peanut oil was injected to the fourth instar larvae immediately after the corpora allata was extirpated The effects were evaluated after the next moulting, being classified into three grades, ++ (perfect 5th instar larvae), + (larval-pupal intermediate type) and - (precocious pupae) The activity (%) was calculated using scores (1, 0 5 and 0, assigned to the grades, ++, + and -, respectively) as the percent of total sum of the scores to the number of animal tested

weak activity about one fiftieth that of the (+) hormone This activity should originate from the (-) enantiomer itself The remarkable difference in the activity between the both enantiomers clearly indicated that there must be the strict relationship between the stereochemistry of the hormone and the chiral nature of the receptor molecule of insects

We thank Professor S Tamura of the University of Tokyo for his generous gift of a strain of C nicotianae used in this work

References and Notes

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3 Y Suzuki, K Imai, S Marumo and T Mitsui, Agric Biol Chem , 36, 1849 (1972), the biological activity concluded in it should be revised as described in this communication, based on our later findings that the enantiomeric purity of the JH sample previously used was low (ca 65%) from the optical rotation

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5 Y Kosuge, A Suzuki, S Hirata and S Tamura, Agric Biol Chem , 37, 455 (1973)

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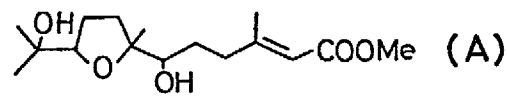
7 The absolute configuration of 3 was determined after conversion by acid catalyzed hydration into (-)-methyl 6,7-dihydroxygeranate, whose absolute stereochemistry was determined by applying the csr-nmr method already established [2]

8 Yield and optical purity of the glycollic metabolites seem to depend on the metabolic condition, e g , when the young mycelia precultured for 2 5 days were employed, the metabolism was fast and after 24 hr an optically pure specimen was obtained in 85% yield, whereas use of the old mycelia (3 5 days) made the metabolism slow and the optical rotation of the obtained 4 decreased as low as 80%

9 The metabolic product was the tetrahydrofurane derivative (A)



(A)

10 There was the possibility of racemization to occur in synthesis of R(+)-JH by acetyl migration from C_{10}-sec to C_{11}-tert-hydroxyl group, however, its optical rotation excluded this possibility, when compared with the optical rotation of S(-)-JH, during whose synthesis no racemization should occur

11 Both enantiomers of C_{16}-JH were completely identical in nmr and mass spectra with the racemic one synthesized from ethyl farnesate upon epoxide formation The S enantiomer had $[\alpha]_D$ -5 2 ± 0 6° (MeOH), which supported a high purity of the prepared enantiomer, although the strict conclusion can not be obtained from the optical rotation

12 Ethyl ester, but not methyl ester of the hormone was preferred for bioassay, because the former exhibits the stronger activity on the silkworm than the latter

13 K. J Judy, D A Schooley, L L Dunham, M S Hall, B J Bergot and J B Siddal, Proc Nat Acad Sci , 70, 1509 (1973)

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