Tetrahedron Vol 39, No 13, pp 2303 to 2306, 1983
Printed in Great Britain

0040-4020/83 $3.00 + .00
© 1983 Pergamon Press Ltd

SYNTHESIS AND BIOLOGICAL ACTIVITY OF OPTICALLY ACTIVE FORMS OF (*E*)-3, 7-DIMETHYL-2-OCTENE-1, 8-DIOIC ACID (CALLOSOBRUCHUSIC ACID)

A COMPONENT OF THE COPULATION RELEASE PHEROMONE (ERECTIN) OF THE AZUKI BEAN WEEVIL†

KENJI MORI* and TERUHITO ITO
Department of Agricultural Chemistry, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

and

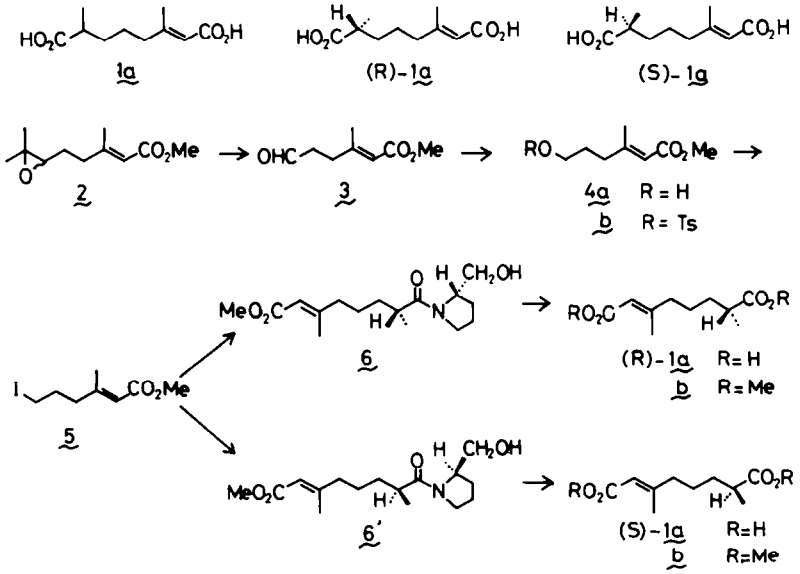
KAORU TANAKA, HIROSHI HONDA and IZURU YAMAMOTO
Department of Agricultural Chemistry, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156, Japan

(*Received in Japan* 11 *June* 1982)

Abstract—Both enantiomers of callosobruchusic acid were synthesized, confirming its proposed plane structure as (*E*)-3, 7-dimethyl-2-octene-1, 8-dioic acid. Both of them were biologically active as the copulation release pheromone of *Callosobruchus chinensis* L.

IN 1981 Yamamoto *et al.* isolated and identified the copulation release pheromone of the azuki bean weevil, *Callosobruchus chinensis* L., which induces the male to extrude his genital organ and to attempt copulation.[1] They named it erectin and found it to consist of two synergistically acting fractions. One was a mixture of methyl-branched hydrocarbons such as 11, 15-dimethyltritriacontane. The other was a new monoterpene dicarboxylic acid, which was named callosobruchusic acid.[1] Since the amount of this acid in the insect was only 15 ng per female, it was impossible to isolate it in quantity sufficient for the measurement of various physical properties. Indeed such data as m.p., $[\alpha]_D$, IR and NMR spectra of the acid were unavailable due to the scarcity of the material. The only available spectral information was the MS of the corresponding methyl ester. Based on this and other MS data, Yamamoto *et al.* proposed the structure of callosobruchusic acid to be (*E*)-3, 7-dimethyl-2-octene-1, 8-dioic acid **1a** without assigning the absolute configuration at C-7.[1] We therefore became interested in synthesizing optically active forms of the acid, (*R*)-**1a** and (*S*)-**1a**, with known absolute configura-

†Pheromone Synthesis-54. Part 53, K. Mori, H. Nomi, T. Chuman, M. Kohno, K. Kato and M. Noguchi, *Tetrahedron* **38**, 331, (1982). This work was presented as a part of K. M.'s lecture at Rattanakosin (Bangkok) Bicentennial Seminar on Chemistry of Natural Products, Bangkok, Thailand (Aug. 1982).



FORMULA 1.

tion so as to confirm the assigned structure as well as to know the relationship between stereochemistry and pheromone activity.

Our synthesis started from methyl (±)-epoxygeranate 2[2] and used Evans' method of enantioselective alkylation as the key-step. Methyl (±)-epoxygeranate 2 was treated with HIO_4 to give an aldehyde 3, which, without purification, was reduced with $NaBH_4$ to an alcohol **4a**. Treatment of the corresponding tosylate **4b** with NaI yielded an iodide 5. This was employed for the alkylation of the chiral amide enolate derived from (*R*)- or (*S*)-prolinol.[3] (*S*)-Prolinol propionamide[3] was converted to its enolate anion by treatment with 3 eq of LDA in THF. Then it was alkylated with iodide 5 at $-100°$ in the presence of HMPA. After 15 hr at $-100 \sim -95°$, the reaction was quenched to give 6 in 46% yield with diastereomeric purity of 96.6% (96.6:3.4) as revealed by glc analysis. The amide 6 was heated with 1N HCl to give crystalline (*R*)-**1a** in 44% yield. The (*R*)-configuration was given to this product in analogy with Evans' result.[3] This was recrystallized from ether-petroleum ether to give pure (*R*)-callosobruchusic acid **1a**, m.p. $91 \sim 92°$, $[\alpha]_D^{23} -11.75°$ ($c = 1.105$, $CHCl_3$). The optical purity of (*R*)-**1a** was thought to be ~93% (96.6–3.4) on the assumption that no significant racemization took place during the hydrolysis of 6 as had been observed in the cases reported by Evans.[3] The MS of the corresponding dimethyl ester (*R*)-**1b** was entirely identical with that of the dimethyl ester derived from the natural product. This firmly established the correctness of the assigned plane structure **1a**. In the same manner, by alkylating (*R*)-prolinol propionamide with 5, 6' was obtained whose diastereomeric purity was also ~96% (~96 : ~4). Acid hydrolysis of 6' gave (*S*)-**1a**, m.p. $90 \sim 91°$, $[\alpha]_D^{21} +10.5°$ ($c = 0.10$, $CHCl_3$), with ~92% (~96 – ~4) optical purity.

It is known that in most cases samples with >85% optical purity are suitable for bioassay to study the enantio-specificity of pheromone perception.[4,5]† Our synthetic enantiomers, (*R*)-and (*S*)-**1a** with $92 \sim 93\%$ optical purity, were thus assayed on male azuki bean weevils to compare their activities with that of the natural **1a**. The result is shown in Table 1. (*S*)-**1a** was as active as the natural pheromone itself at 15 ng dose. However, (*R*)-**1a** was also active, although its potency was slightly lower than that of (*S*)-**1a**. To exclude the possibility that the bioactivity of (*R*)-**1a** was due to the contaminating (*S*)-**1a**, we carried out a further biological test to see the relationship between copulation release activity and the dose of (*R*)- or (*S*)-**1a** given to the insects. The result is shown in Table 2. It enabled us to calculate the dose necessary for 50% copulation release (10 responded males out of 20; ED_{50}). The ED_{50} for (*S*)-**1a** was 6.5 ng, while that for (*R*)-**1a** was 11.4 ng. This means that our (*R*)-**1a** is almost a half as active as (*S*)-**1a**. Since the contaminating amount of the (*S*)-isomer in our sample of (*R*)-**1** is only $3 \sim 4\%$, the bioassay clearly indicates that (*R*)-**1a** is also bioactive.‡ The present case is another example of those pheromones whose both enantiomers are biologically active.[8,9] It is well-established that even the unnatural enantiometer is bioactive in the case of the boll weevil pheromone [grandisol, (1*R*, 2*S*)-(+)-1-methyl-1-(2-hydroxy)ethyl-2-isopropenylcyclobutane][10] or the German cockroach pheromone [(3*S*, 11*S*)-(+)-3, 11-dimethylnonacosan-2-one].[11]

The above bioassay result posed a difficulty in determining the absolute configuration of the natural callosobruchusic acid. In the case of grandisol, its $[\alpha]_D$ value was known, and there was no difficulty in establishing the absolute configuration of the natural product.[12] In the case of the German cockroach pheromone, its m.p. and $[\alpha]_D$ value were available, and the final identification of the natural isomer as (3*S*, 11*S*) was based on the mixture m.p. determination with the synthetic (3*S*, 11*S*)-isomer.[11] In the present case, m.p. and $[\alpha]_D$ value of the natural

†For example, Ohloff's enantiomers of ipsdienol (2-methyl-6-methylene-2, 7-octadien-4-ol) with 80% [(*S*)-isomer] or 91% [(*R*)-isomer] optical purity[6] was sufficiently pure to establish very interesting species-specific enantio-specificity existing among bark beetles: *Ips calligraphus* responded to (*R*)-ipsdienol, while *Ips paraconfusus* was attracted by the (*S*)-enantiomer.[7]

‡It was also found that the synthetic callosobruchusic acid alone could induce the copulation release in the absence of the hydrocarbon mixture obtained from the insect. The required dose ($1.5 \sim 4.7\ \mu g$), however, was $100 \sim 1000$ times larger than that ($4.7 \sim 15$ ng) necessary in the presence of the hydrocarbon mixture. On the other hand, the hydrocarbon mixture alone was biologically inactive even with $>120\ \mu g$ dose. The role of the hydrocarbon mixture is therefore only the synergist.

Table 1. Copulation release activity of natural and synthetic callosobruchusic acid **1a** on *Challosobruchus chinensis* L

Sample	No. of responded males
Natural 1a*	16.3 ± 3.5**
(R)-1a*	14.3 ± 1.5***
(S)-1a*	16.0 ± 1.0***

* 15ng (1 female equivalent) of 1a with 12μg of the hydrocarbon mixture obtained from the female insects.

** Average of four replications using 20 males. This bioassay had been done previously in the course of Yamamoto's structural work.[1]

*** Average of three replications using 20 males.

Table 2. Relationship between the copulation release activity and dose of the synthetic enantiomers of callosobruchusic acid **1a** given to *Callosobruchus chinensis* L

Sample	No. of responded males* dose of 1a (ng) 0.48	1.5	4.8	15	150	1500
(R)-1a**		2.7 ±1.5	5.3 ±3.5	14.3 ±1.5	17.0 ±4.4	17.0
(S)-1a**	2.3 ±2.1	8.0 ±0	6.7 ±1.5	16.0 ±1.0	18.7 ±0.6	19.0

* Out of 20 males.

** Assayed after the addition of 12 μg of the hydrocarbon mixture obtained from the female insects.

pheromone were unavailable.[1] At present we are therefore unable to establish the absolute configuration of callosobruchusic acid. Reisolation of the natural pheromone will be attempted to settle the matter. The problem may be solved by analyzing the methyl ester or some other derivatives of the natural acid by GLC using an efficient chiral stationary phase coupled with co-injection experiments with synthetic samples of known absolute configuration. This technique for determining the absolute configuration of a trace amount of sample has recently been reported by us in the cases of lineatin[13] and phoracantholides I and J.[14]

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra were determined as films for liquids and as Nujol mulls for solids on a Jasco A-102 spectrometer. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer unless otherwise stated. Optical rotations are measured on a Jasco DIP-140 automatic polarimeter.

Methyl (E)-3-*methyl*-6-*oxo*-2-*hexenoate* **3**. A soln of $HIO_4 \cdot 2H_2O$ (17.43g) in THF (20 ml) was added dropwise to a stirred and ice-cooled soln of **2** (12.61 g) in ether (100 ml) at 0 ~ 5°. The mixture was stirred for 30 min at 0 ~ 5°, poured into ice-water, and extracted with ether. The ether soln was washed with water, sat $NaHCO_3$ aq, $Na_2S_2O_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 7.27 g (73.2%) of crude **3**, ν_{max} 2700 (m), 1720 (s), 1650 (s), 1220 (s), 1150 (s) cm^{-1}; δ (CCl_4) 2.14 (3H, s), 2.40 ~ 2.60 (4H, m), 3.60 (3H, s), 5.59 (1H, br. s), 9.75 (1H, br. s). This was employed for the next step without further purification. Attempted distillation caused significant loss of the product in one occasion (b.p. 125°/22mm).

Methyl (E)-6-*hydroxy*-3-*methyl*-2-*hexenoate* **4a**. A soln of $NaBH_4$ (0.499 g) in H_2O (10 ml) was added dropwise to a stirred and ice-cooled soln of **3** (6.00 g) in EtOH (70 ml) at 0 ~ 5°. After stirring for 10 min, the mixture was acidified with 0.5N HCl to pH 5. The mixture was concentrated *in vacuo* to remove EtOH and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 4.43 g (53% from **2**) of **4a**, b.p. 96 ~ 98°/0.2 mm, $n_D^{22.8}$ 1.4726; ν_{max} 3400 (br. s), 1720 (s), 1650 (s), 1230 (s), 1150 (s) cm^{-1}; δ (CCl_4) 1.40 ~ 2.00 (2H, m), 2.14 (3H, d, J = 1Hz), 2.24 (2H, t, J = 7Hz), 3.54 (2H, t, J = 6Hz), 3.62 (3H, s), ~3.2 (1H, -OH), 5.65 (1H, br. s); glc (Column, PEG 20M, 2m × 4 mm at 170°; Carrier gas, N_2 1.2 kg/cm^2): R_t 8.5 min (94.8%). Found: C, 60.29; H, 8.92. Calc. for $C_8H_{14}O_3$: C, 60.74; H, 8.99%.

Methyl (E)-6-*tosyloxy*-3-*methyl*-2-*hexenoate* **4b**. *p*-TsCl (5.79 g) was added portionwise to a stirred and ice-cooled soln of **4a** (4.001 g) in dry C_5H_5N (20 ml). After the addition, the mixture was left to stand at 10° for 12 hr. Then it was poured into ice-dil HCl and extracted with ether. The ether soln was washed with sat $CuSO_4$ aq, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 7.27 g (92.0%) of crude **4b**, ν_{max} 1720 (s), 1650 (m), 1600 (m), 1360 (s), 1230 (s), 1190 (s), 1180 (s), 1150 (s), 1100 (s) cm^{-1}; δ (CCl_4) 1.46 ~ 2.00 (2H, m), 2.02 (3H, br. s), 2.10 (2H, t, J = 7Hz), 2.39 (3H, s), 3.52 (3H, s), 3.90 (2H, t, J = 6Hz), 5.42 (1H, br. s), 7.30 (2H, d, J = 8 Hz), 7.65 (2H, d, J = 8 Hz). This was employed for the next step without further purification.

Methyl (E)-6-*iodo*-3-*methyl*-2-*hexenoate* **5**. NaI (6.72 g) was added to a stirred soln of **4b** (7.00 g) in dry acetone (70 ml) and the mixture was stirred overnight at room temp. Then it was concentrated *in vacuo*. The residue was diluted with water and extracted with ether. The ether soln was washed with $Na_2S_2O_3$ aq, water, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 6.19 g of crude **5**. This was chromatographed over SiO_2 (120 g). Elution with n-hexane–ether (95:5) gave 5.39 g (79% from **4a**) of **5**, ν_{max} 1720 (s), 1650 (s), 1220 (s), 1150 (s) cm^{-1}; δ (CCl_4) 1.70 ~ 2.55 (4H, m), 2.17 (3H, d, J = 1Hz), 3.20 (2H, t, J = 7Hz), 3.16 (3H, s), 5.71 (1H, br. s); MS: *m/z* 268 ($M^+ = C_8H_{13}O_2I$), 237 (M^+-OMe), 155, 141.

(S)-(−)-*Prolinol propionamide*. Propionic anhydride (783 mg) was added to stirred and ice-cooled (*S*)-(−)-prolinol (603 mg). The mixture was stirred at 65° for 2 hr. After cooling, it was chromatographed over SiO_2 (25 g). Elution with CH_2Cl_2 yielded 637 mg (67.8%) of the amide, b.p. 120°/0.16 mm, $[\alpha]_D^{22}$ −69.6° (*c* = 1.18, $CHCl_3$), ν_{max} 3375 (br. s), 1620 (s), 1050 (s), cm^{-1}; δ (CCl_4) 1.04 (3H, t, J = 7Hz), 1.50 ~ 2.20 (4H, m), 2.23 (2H, q, J = 7 Hz), 3.00 ~ 3.72 (4H, m), 3.70 ~ 4.20 (2H, br), 4.20 ~ 4.70 (1 H, br).

(R)-(+)-*Prolinol propionamide*. In the same manner as described above (*R*)-(+)-prolinol (303 mg) yielded 298 mg (63.3%) of this amide, $[\alpha]_D^{22}$ + 71.1° (*c* = 0.813, $CHCl_3$). The IR and NMR spectra of this compound were identical with those of the (*S*)-enantiomer.

1-[(R, E)-2', 6'-*Dimethyl*-7'-*methoxycarbonyl*-6'-*heptenoyl*]-(*S*)-2-*hydroxymethylpyrrolidine* **6**. A soln of LDA was prepared from i-Pr_2NH (3.0 ml) and n-BuLi (1.6 N, 7.6 ml) in dry THF (20 ml) under Ar at 0 ~ 5°. The mixture was stirred for 1 hr at 0 ~ 5°. Then a soln of (*S*)-prolinol propionamide (637 mg) in THF (4 ml) was added dropwise to the mixture. The stirring was continued for 1 hr at room temp. HMPA (2.0 ml) was added to the mixture and it was cooled to −100°. To the stirred mixture was added a soln of **5** (1.631 g) in THF (3 ml) at −100 ~ −95°. The mixture was stirred at this temp for 15 hr. After warming to room temp. It was poured into water and extracted with ether. The ether soln was washed with dil HCl, $Na_2S_2O_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.29 g of crude **6**. This was

chromatographed over SiO_2 (40 g). As earlier eluted fractions 0.45 g of **5** was recovered. Elution with $CHCl_3$ yielded 543 mg (46.0% based on prolinol propionamide) of **6**, $[\alpha]_D^{23}$ −44.2° (*c* = 1.035, $CHCl_3$), ν_{max} 3400 (m), 1720 (s), 1640 (s), 1620 (s), 1225 (s), 1150 (s), 750 (s) cm^{-1}; δ (CCl_4) 1.05 (3H, d, J = 7 Hz), 1.18 ~ 1.65 (~4H, m), 1.65 ~ 2.00 (~4H, m), 2.10 (3H, br. s), 2.10 ~ 2.30 (2H, br), 3.30 ~ 3.60 (3H, m), 3.57 (3H, s), 3.80 ~ 4.20 (1H, m), 4.20 ~ 4.70 (1H, br), 5.52 (1H, br. s); MS: *m/z* 297 (M^+ = $C_{16}H_{27}O_4N$), 279 (M^+-H_2O), 266 (M^+-OMe), 234, 197; GLC (Column, OV-101, 30 m × 0.25 mm at 150–280° (+2°/min); Carrier gas, He): Rt 52.4 min (3.4%), 53.2 min (96.6%). Diastereomeric purity = 96.6%.

1-[(S, E)-2′, 6′-*Dimethyl*-7′-*methoxycarbonyl*-6′-*heptenoyl*]-(R)-2-*hydroxymethylpyrrolidine* **6′**. In the same manner as described above, (*R*)-prolinol propionamide (298 mg) and **5** (1018 mg) yielded 246.4 mg (43.5%) of **6′**, $[\alpha]_D^{22}$ +42.7° (*c* = 0.709, $CHCl_3$). Its spectral data were identical with those of **6**. GLC analysis of **6′** was carried out as described for **6**. The diastereomeric purity was calculated to be 96 ~ 96.5%.

(R, E)-3, 7-*Dimethyl*-2-*octene*-1, 8-*dioic acid* (R)-**1a**. A mixture of **6** (100 mg) and 1N HCl (3 ml) was stirred and heated under reflux for 2 hr. After cooling, the mixture was extracted with ether. The ether soln was extracted with 1.3 N NaOH (20 ml × 3). This basic aq soln was acidified with 6N HCl to pH 4 and extracted with ether. The ether soln was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* to give 49.1 mg of crude (*R*)-**1a**. This was further purified by prep TLC (double development with $CHCl_3$–MeOH (9:1) on Merck silica gel; R_f 0.51) to give 29.5 mg (44.0%) of (*R*)-**1a**. Further recrystallization from ether-petroleum ether yielded 23 mg of pure (*R*)-**1a**, m.p. 91 ~ 92°, $[\alpha]_D^{23}$ −11.75° (*c* = 1.105, $CHCl_3$); ν_{max} (Nujol) ~ 3600 – ~ 2200 (br. m), 1695 (s), 1640 (s), 1420 (m), 1300 (m), 1240 (m), 1180 (w), 1160 (m), 1120 (w), 1080 (w), 1040 (w), 950 (m), 905 (w), 870 (m), 830 (w), 800 (w), 750 (w), 710 (m) cm^{-1}, δ (400 MHz, $CDCl_3$) 1.20 (3H, d, J = 7.0 Hz), 1.41 ~ 1.48 (1 H, m), 1.50 ~ 1.58 (2 H, m), 1.63 ~ 1.73 (1H, m), 2.16 (3H, d, J = 1.1 Hz), 2.19 (2H, t, J = 7.0 Hz), 2.44 ~ 2.57 (1H, m), 5.70 (1H, br. s), 7.5 ~ 10.0 (2H, br). (Found: C, 59.88; H, 8.05. Calc. for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05%).

(S, E)-3, 7-*Dimethyl*-2-*octene*-1, 8-*dioic acid* (S)-**1a**. In the same manner as described above **6′** (100 mg) yielded 28.5 mg (43%) of (*S*)-**1a**, m.p. 90 ~ 91°, $[\alpha]_D^{21}$ +10.5° (*c* = 0.10, $CHCl_3$). The IR and NMR spectra were identical with those of (*R*)-**1a**. (Found: C, 60.22; H, 8.13. Calc. for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05%).

Dimethyl (R, E)-3, 7-*dimethyl*-2-*octene*-1, 8-*dioate* (R)-**1b**. Treatment of (*R*)-**1a** (1 mg) with ethereal CH_2N_2 yielded (R)-**1b**, which was purified by prep TLC (Merck silica gel, ether-n-hexane 1:4, R_f 0.33) to give pure (*R*)-**1b**, MS: *m/z* 197 (M^+-OMe), 196 (M^+-MeOH), 168 (M^+–60), 164(M^+–64), 136(M^+–92), 109(M^+–119, base peak). The mass spectrum was identical with that of the Me ester derived from the natural product.

Dimethyl (S, E)-3, 7-*dimethyl*-2-*octene*-1, 8-*dioate* (S)-**1b**. In the same manner as described above, (*S*)-**1a** yielded (*S*)-**1b**, whose MS was identical with that of (*R*)-**1b**.

Acknowledgements—We thank Mr. M. Ikunaka, University of Tokyo, for his help in the early phase of this work. We are indebted to Kuraray Co. (geraniol) and Ajinomoto Co. (proline) for the gifts of starting materials. Elemental analyses were carried out by Mrs. Y. Naito, University of Tokyo, to whom we express our thanks.

REFERENCES

[1]K. Tanaka, K. Ohsawa, H. Honda and I. Yamamoto, *J. Pesticide Sci.* **6**, 75 (1981).
[2]Y. Ichikawa, M. Komatsu, T. Takigawa, K. Mori and K. J. Kramer, *Agric. Biol. Chem.* **44**, 2709 (1980).
[3]D. A. Evans and J. M. Takacs, *Tetrahedron Letters* **21**, 4233 (1980).
[4]K. Mori, *The Synthesis of Insect Pheromones* in *The Total Synthesis of Natural Products* (Edited by J. ApSimon) Vol. 4, pp 109–168. Wiley, New York (1981).
[5]K. Mori, *The Significance of Chirality: Methods for Determining Absolute Configuration and Optical Purity of Pheromones and Related Compounds* in *Technique of Pheromone Researches* (Edited by H. E. Hummel) Springer Verlag, New York, in press.
[6]G. Ohloff and W. Giersch, *Helv. Chim. Acta* **60**, 1496 (1977).
[7]J. P. Vité, G. Ohloff and R. F. Billings, *Nature* **272**, 817 (1978).
[8]Ref. 4, p. 166.
[9]K. Mori, *Yakagaku Zasshi* **102**, 899 (1982).
[10]K. Mori, S. Tamada and P. A. Hedin, *Naturwissenschaften* **65**, 653 (1978).
[11]K. Mori, S. Masuda and T. Suguro, *Tetrahedron* **37**, 1329 (1981).
[12]P. D. Hobbs and P. D. Magnus, *J. Am. Chem. Soc.* **98**, 4595 (1976).
[13]D. Klimetzek, V. Schurig, R. Weber and K. Mori, *Naturwissenschaften* in press.
[14]T. Kitahara, K. Koseki and K. Mori, *Agric. Biol. Chem* **47**, 389 (1983).