hydrobromic acid. The tetrahydrobromide of 1,5,8,12-tetraazacyclohexadecane **7** (80%) was obtained as needles, mp 256.5—257° (dec.). *Anal.* Calcd for $C_{12}H_{32}Br_4N_4$: C, 26.11; H, 5.84; N, 10.15. Found: C, 25.94; H,5.74; N, 10.18. The tetrahydrobromide of 1,5,9,13-tetraazacyxloheptadecane **9** (66%) was obtained as pillars, mp 278—279° (dec.). *Anal.* Calcd for $C_{13}H_{34}Br_4N_4$: C, 27.58; H, 6.05; N, 9.90. Found; C, 27.61; H, 5.75; N, 9.76. The trihydrobromide of 1,5,9-triazacyclotridecane **11** (88%) was obtained as prisms, mp 269.5—272° (dec.). *Anal.* Calcd for $C_{10}H_{26}Br_3N_3$: C, 28.06; H, 6.12; N, 9.82. Found; C, 27.90; H, 5.95; N, 9.79.

[Chem. Pharm. Bull.
28(3) 997—999 (1980)]

Co-occurrence of (—) and (+)-Germacrene-D in *Solidago altissima* L.: Determination of the Optical Rotation of optically Pure Germacrene-D[1]

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(Received October 2, 1979)

Germacrene-D, which has sexual attractant properties similar to those of periplanone-B, has been isolated in an optically impure state from many plants, *i.e.*, (—) and (+)-germacrene-D co-occur in different ratios in various plants. (—)-Germacrene-D isolated from *Solidago altissima* L. in this work was determined to have an optical purity of 18.0%. In addition, the optical rotation of optically pure germacrene-D was estimated to be 305°.

Keywords——sesquiterpene; germacrene; optical rotation; chemical transformation; transannular reaction; biogenesis

Introduction

Germacrene-D, a typical ten-membered ring sesquiterpene, has been found in many plants. (—)-Germacrene-D (**1**) was first isolated by Yoshihara *et al.* from *Pseudotsuga japonica* S. and its structure, including the absolute configuration ($[\alpha]^{23}_{D}$ —240°), was also elucidated.[3] The same authors later obtained (+)-germacrene-D (**2**) ($[\alpha]^{23}_{D}$ +305°) from *Dendropanax trifidus* M.[4] Recently, (—)-germacrene-D ($[\alpha]^{20}_{D}$ —145° in EtOH) was found in the steam distillates of the plant *Solidago altissima* L. collected in Osaka early in September.[5]

In view of the finding that germacrene-D has a sexual stimulant activity similar to that of periplanone-B,[6] which is a sexual stimulant for the American cockroach,[7] we were interested in attempting to isolate germacrene-D from *Solidago altissima* L. on a preparative scale.

Results and Discussion

The fresh leaves of the plant were collected in Nagoya early in August and extracted with benzene at room temperature. The benzene extract was roughly separated by column chro-

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[1] This work was presented at the 99 th Annual Meeting of the Pharmaceutical Society of Japan (Sapporo, August 1979).

[2] Location: *Yagoto-urayama, Tempaku-cho, Tempaku-ku, Nagoya 468, Japan.*

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matography (alumina, benzene), and then rechromatographed on alumina using hexane to provide fairly pure germacrene-D, which was further chromatographed on $AgNO_3$–Al_2O_3 (1 : 9) eluting with hexane–Et_2O (1 : 1), to afford pure germacrene-D as a colorless liquid. Its spectral data were identical with those of an authentic sample. However, its optical rotation ($[\alpha]_D^{23}$ −54.5° in MeOH) was quite small compared with those given in references 3, 4, and 5. Accordingly, it was necessary to determine the optical purity of our sample in addition to the optical rotation of germacrene-D in an optically pure state.

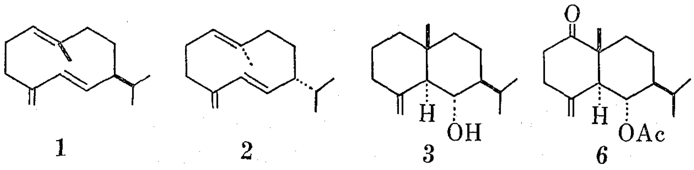

Chart 1

In connection with biomimetic reactions of germacrene-D,[8] we carried out acid-catalyzed reactions of epoxygermacrene-D (**4**) leading to the formation of a selinane-type compound with an acetoxyl group, which was readily converted into (+)-junenol (**3**) having an optical purity of 18.0%, as follows.

The epoxide (**4**),[7,8] readily obtainable from germacrene-D, was treated with 80% aq.AcOH (0°, 1 hr) to give several cyclization products, from which the acetate (**5**, mp 106—108°) was isolated in *ca.* 12% yield (see Chart 2).[8] Further treatment of **5** with pyridinium chlorochromate in CH_2Cl_2 (room temp., 2 hr) gave the corresponding ketone (**6**, mp 132—133°) in 70% yield, which was then subjected to Wolff-Kishner reduction using 80% $NH_2NH_2 \cdot H_2O$–KOH in triethylene glycol to afford the corresponding deoxy compound in 84% yield. The spectral data for this compound, mp 78—79.5° (lit., 77.5—79°)[9] were identical with those of (+)-junenol (**3**, mp 61—61.5°)[10] except for the optical rotation ($[\alpha]_D^{23}$ +9.56° in MeOH), which was quite small as compared with that of **3** ($[\alpha]_D^{23}$ +53.1° in MeOH).[10] Thus, the optical purity of the sample obtained herein was 18.0%. Accordingly, optically pure germacrene-D should have an optical rotation of $[\alpha]_D^{23}=305°$ in MeOH.

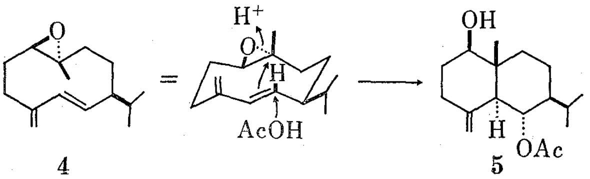

Chart 2. Acid-catalyzed Reaction of Epoxygermacrene-D with 80% aq.AcOH

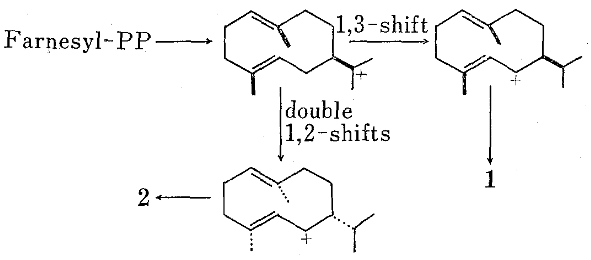

Chart 3. Biogenesis of (−) and (+)-Germacrene-D

Thus, the plant *Solidago altissima* L. collected by us clearly contained a mixture of (−) and (+)-germacrene-D (**1** and **2**) in a ratio of 59 : 41.[11] Many plants appear to have two different enzymatic pathways leading to the formation of (−) and (+)-germacrene-D (**1** and **2**) in different ratios, as shown in Chart 3.

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11) The ratio of (−) and (+)-germacrene-D may vary with season, location or part of the plant.

Experimental[12]

Isolation of Germacrene-D (1 and 2)——The fresh leaves of the plant (20 kg) were collected in Nagoya early in August and immersed in benzene (100 l) at room temperature for 3 weeks, then filtered. The filtrate was concentrated under reduced pressure to leave a greenish oil (*ca.* 450 g). This oil (25 g) was directly chromatographed on alumina (Nakarai Chemicals, 300 mesh, 300 g) with benzene to afford a viscous liquid (7.0 g), which was rechromatographed on alumina (Nakarai Chemicals, 300 mesh, 150 g) using hexane to afford fairly pure germacrene-D (6.0 g). This liquid was further chromatographed on $AgNO_3{-}Al_2O_3$ (1: 9) (200 g), eluting with hexane–Et_2O (1: 1), to afford pure germacrene-D (3.7 g) as a colorless liquid. Its spectral data were identical with those of an authentic sample (IR, NMR, and mass spectra), though its optical rotation ($[\alpha]_D^{23}$ $-54.5°$ ($c=0.66$ in MeOH)) was different.

Acid-catalyzed Reaction of Epoxygermacrene-D (4) with 80% aq. AcOH——A solution of epoxygermacrene-D (1.5 g) in 80% aq. AcOH (20 ml) was stirred vigorously at 0° for 1 hr, and then neutralized with aq. $NaHCO_3$ solution and extracted with large amounts of ether. The ethereal extract was washed with water, and then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave a colorless liquid (1.61 g), which was chromatographed on silica gel (Mallinckrodt, 100 mesh, 200 g) with $CHCl_3$ to give a viscous liquid which was further separated by preparative TLC (Kieselgel PF_{254}) using $CHCl_3$ to afford white crystals of **5** (222 mg), mp 106—108° (from hexane). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3350 br. (OH), 1730 (C=O), 1655 (C=C), and 1255 (Ac–O–C). NMR δ: 0.74 (3H, s, $C{-}CH_3$), 0.87 (3H, d, $J=7$ Hz, $CH{-}C\underline{H}_3$), 0.92 (3H, d, $J=7$ Hz, $CH{-}C\underline{H}_3$), 1.99 (3H, s, $COCH_3$), 3.44 (1H, dd, $J=11$, 5 Hz, $HO{-}C\underline{H}$), 4.55 (1H, br. s, $C{-}C\underline{H}_2$), 4.79 (1H, br. s, $C{-}C\underline{H}_2$), and 5.07 (1H, t, $J=10$ Hz, $AcO{-}C\underline{H}$). MS *m/e*: 280 (M^+) and 220 (Calcd for $C_{17}H_{28}O_3$: *m/e* 280.2038. Found: *m/e* 280.2037).

Oxidation of 5 with Pyridinium Chlorochromate (PCC)——A solution of **5** (85 mg) in CH_2Cl_2 (3 ml) was added to a suspension of PCC (100 mg) in CH_2Cl_2 (2 ml) at room temperature, with stirring. The reaction mixture was stirred for a further 2 hr, and then diluted with anhydrous ether and filtered. The filtrate was concentrated under reduced pressure to leave a white solid (69 mg), which was recrystallized from hexane to give colorless columns of **6**. mp 132—133° (in a sealed tube). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1735 (CH_3COO), 1710 (C=O), 1660 (C=C), and 1250 (Ac–O–C). NMR δ: 0.89 (3H, d, $J=7$ Hz, $CH{-}C\underline{H}_3$), 0.94 (3H, d, $J=7$ Hz, $CH{-}C\underline{H}_3$), 1.04 (3H, s, $C{-}CH_3$), 2.01 (3H, s, $COCH_3$), 4.87 (1H, br. s, $C{=}CH_2$), 5.06 (1H, br. s, $C{=}CH_2$), and 5.21 (1H, t, $J=10$ Hz, $AcO{-}C\underline{H}$). MS *m/e* 278 (M^+) and 218 (Calcd for $C_{17}H_{26}O_3$: *m/e* 278.1882. Found: *m/e* 278.1875).

Wolff-Kishner Reduction of 6——A mixture of **6** (40 mg), 80% $NH_2NH_2\cdot H_2O$ (0.3 ml) and KOH (110 mg) in triethylene glycol (6 ml) was heated at 120° for 1.5 hr with stirring under N_2, and then at 180° for a further 2 hr. As the reaction proceeded, the reduction product gradually sublimed and adhered to the upper glass surface of a condenser. The product was dissolved in ether and washed with water. The ethereal solution was dried over anhydrous Na_2SO_4. Removal of the solvent gave a crystalline solid which was purified by sublimation to afford 26 mg of colorless needles, mp 78—79.5°. The spectral data were identical with those of (+)-junenol (3)[10] except for the $[\alpha]_D$ value: synthetic junenol from germacrene-D, $[\alpha]_D^{23}$ $+9.56°$ ($c=1.88$ in MeOH); (+)-junenol from *l*-santonin, $[\alpha]_D^{23}$ $+53.1°$ ($c=1.07$ in MeOH).

Acknowledgement The authors wish to thank Dr. Y. Hirose (The Institute of Food Chemistry) for providing the IR spectrum of germacrene-D. They are also indebted to Drs M. Toda and H. Niwa (Ono Central Research Institute) for measurements of high resolution mass spectra.

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[12] All mps are uncorrected. IR spectra were taken on a Hitachi 215 spectrophotometer. NMR spectra were obtained on a JEOL JNM-PS 100 NMR spectrometer, using $CDCl_3$ unless otherwise stated. The chemical shifts are given in ppm relative to internal TMS; only prominent signals are cited (d, doublet; m, multiplet; q, quartet; s, singlet; t, triplet). Mass spectra were recorded on a Hitachi RMU-6D mass spectrometer, operating with an ionization energy of 70 eV. The optical rotations were measured on a JASCO DIP-SL automatic polarimeter.