Structures of Sesquiterpenes of *Curcuma aromatica* SALISB. II. 1) Studies on Minor Sesquiterpenes

Masanori Kuroyanagi,*, a Akira Ueno,*, b Kiyotaka Koyama, b and Shinsaku Natort

School of Pharmaceutical Sciences, University of Shizuoka,* 359 Yada, Shizuoka 422, Japan and Meiji College of Pharmacy, 5 Yato-cho, Tanashi, Tokyo 188, Japan. Received June 12, 1989

Further study on the sesquiterpenes from *Curcuma aromatica* (Zingiberaceae) has resulted in the isolation of eleven minor sesquiterpenes, 1-11 having guaiane, secoguaiane and germacrane skeletons, and their structures were elucidated by proton nuclear magnetic resonance (1H-NMR) and 13C-nuclear magnetic resonance (13C-NMR) spectroscopy, as well as chemical investigation. The stereochemistry of these sesquiterpenes was elucidated by 2D NMR techniques such as 1H-1H correlation (COSY) and nuclear Overhauser effect correlation (NOESY), and circular dichroism (CD) spectroscopy.

Keywords *Curcuma aromatica*; Zingiberaceae; sesquiterpene; germacrane-type compound; guaiane-type compound; secoguaiane-type compound; 13C-NMR spectrum; circular dichroism

In the course of our studies on the isolation and the structural elucidation of sesquiterpenes from *Curcuma* spp., we previously reported the structures of ten sesquiterpenes1) isolated from *C. aromatica*. A further study on the sesquiterpenes of the rhizomes of the plant by means of repeated precise silica gel (SiO2) column chromatography and high-performance liquid chromatography (HPLC) has resulted in the isolation of eleven minor sesquiterpenes, named epiprocucumenol (1), isoprocucumenol (2), neprocucumenol (3), (4S)-13-acetoxycoumaridone (4), (4S)-13-hydroxyhydrocoumaridone (5), (4S,5S)-13-hydroxygermacrone 4,5-epoxide (6), (4S,5S)-13-acetoxygermacrone 4,5-epoxide (7), (4S,5S)-12-acetoxygermacrone 4,5-epoxide (8), acetoxycoumaridone (9), curcumadione (10) and isocurcumadione (11) along with the previously known 13-hydroxygermacrone (12),2) isolated from *C. zedoaria*. Their structures were elucidated by means of spectroscopical methods, especially carbon thirteen nuclear magnetic resonance (13C-NMR) and 2D NMR spectroscopy, and chemical investigations.

Epiprocucumenol (1) gave the molecular formula, C15H22O2, from the mass spectrum (MS). The proton nuclear magnetic resonance (1H-NMR) and 13C-NMR spectra of I showed the presence of four methyl groups, one of which was considered to be a tertiary methyl group (δ 1.37 (s)), an olefinic proton (δ 5.91 (br s)) having a long-range coupling with a vinyl methyl group, three vinyl methyl groups (δ 1.84, 1.94 × 2) and the cross-conjugated diene group (δ 196.1) like that found in procucumenol (13),3) whose stereostructure was determined by Kitagawa et al.4) These data indicated that 1 should be a stereoisomer of 13. From the transannular cyclization5) mechanism of transformation of the key intermediate, (4S,5S)-germacrone 4,5-epoxide6) (14), 1 should be the epimer of 13 at C-1 (shown in Chart 2). In the 13C-NMR spectrum of 1, the signals of C-1, C-7 and C-8 were shifted to upper field by a few ppm.

![Chart 1](image)

![Chart 2](image)

© 1990 Pharmaceutical Society of Japan
(4.1, 2.1, 3.8), and that of C-15 was shifted downfield by a few ppm (2.0), compared with those of 13. The other carbon spectral data for 1 appeared at similar chemical shifts (shown in Table 1). From these data, the structure of epiprocurcumenol was supposed to be 1, including the absolute configuration.

Isoprocurcumenol (2), mp 99.5—100.5°C, and neoprocurcumenol (3), mp 77—79°C, showed the same molecular formula, C_{19}H_{22}O_{3}, in the MS. From the 1H- and 13C-NMR spectra, the structures of isoprocurcumenol and neoprocucumenol were supposed to be 2 and 3. The absolute configuration of 3 was confirmed by transformation from 14. The absolute configuration of isoprocurcumenol was also considered to be 2 or its C-1 epimer from the transannular cyclization mechanism as shown in Chart 2. Kitagawa et al. reported the transformation of 14 into procucumenol, GU-2, and GU-3 by treatment with acid, and their structural elucidation included the stereochemistry. They found GU-2 in C. zedoaria. The spectral data of 2 and 3 were identical with the reported data of GU-2 and GU-3. We also obtained 2 and 3 along with the other several products from 14 by treatment with acid and these products (2 and 3) were identical with the natural 2 and 3 (including optical rotations).

(4S)-13-Acetoxydehydrocorticurone (4), mp 77—78°C, gave the molecular formula C_{17}H_{20}O_{4}, which was supported by the 13C-NMR spectrum. The infrared (IR) spectrum of 4 showed the presence of an ester group (1740 cm\(^{-1}\)) and an ordinary carbonyl group (1715 cm\(^{-1}\)). The 13C-NMR spectrum of 4 showed the presence of two carbonyl groups (\(\delta\) 210.3, 206.0), an acetyl group (\(\delta\) 20.8, 170.6) and an acyloxymethyl group (\(\delta\) 64.5). The presence of the acyloxymethyl group was also suggested by the 1H-NMR spectrum (\(\delta\) 2.05 (3H, s), 4.47 (1H, d, J = 12.9 Hz), 4.76 (1H, d, J = 12.9 Hz)). The 13C-NMR spectrum of 4 showed almost the same chemical shifts as those of dehydrocorticurone (17) (Table 1). These data suggested that 4 is an acetoxy derivative of 17 at C-12 or C-13. The 1H-1H correlation (COSY) spectrum of 4 confirmed the assignments of proton signals. The position of the acetoxy group of 4 was determined from the nuclear Overhauser effect correlation (NOESY) spectrum. One of the methylene protons of the acyloxymethyl group had cross peaks with the proton of methylene group at C-6. These data indicated that the acetoxy group is located at C-13. The circular dichroism (CD) spectrum of 4 showed a positive Cotton effect at 317 nm based on the n→π* transition of a \(\beta\),\(\gamma\)-unsaturated ketone, as in the case of 17 (Fig. 1), so the absolute configuration of C-4 of 4 was concluded to be S, like that of 17.

(4S)-13-Hydroxydehydrocorticurone (5), colorless oil, gave the molecular formula C_{19}H_{22}O_{4}, from the MS. The 1H-NMR spectrum of 5 showed the presence of a secondary methyl group (\(\delta\) 1.04 (d, J = 6.6 Hz)), two vinyl methyl groups (\(\delta\) 1.69, 1.84) and an olefin proton (\(\delta\) 5.12 (t, J = 6.5 Hz)). The 13C-NMR spectrum of 5 showed the characteristic signals for dehydrocorticurone (17) except for the presence of a hydroxymethyl group (\(\delta\) 63.2) instead of a methyl group. These results indicated that 5 is a hydroxy derivative of 17. Acetylation of 5 gave an acetate which was identical with 4.

The CD spectrum of 5 showed a similar positive Cotton effect to that of 4 (Fig. 1), and thus 5 was concluded to be (4S)-13-hydroxydehydrocorticurone.

(4S,5S)-13-Hydroxygermacrone 4,5-epoxide (6), an oil, gave the molecular formula C_{19}H_{22}O_{3}, from the MS. The 13C-NMR spectrum of 6 showed the presence of a hydroxymethyl group (\(\delta\) 62.2), an epoxide group (\(\delta\) 61.0, 64.5) and four olefinic carbons (\(\delta\) 129.9, 135.1, 126.2, 136.7). From these data 6 was considered to be the 12- or 13-hydroxy derivative of 14. The 1H-NMR spectrum of 6 also supported this structure. The comparison of the 13C-NMR spectrum of 6 with that of 14 indicated that all carbon chemical shifts of 6 were identical with those of 14 except for those of C-11, C-12 and C-13 (Table 1). 13-Hydroxygermacrone (12) was epoxidized by m-chloroperbenzoic acid to give racemic 13-hydroxygermacrone 4,5-epoxide, which was identical with the natural 6 in terms of thin-layer chromatographic (TLC) and HPLC behavior and 1H-NMR spectrum. These results indicated that 6 is the 13-hydroxy derivative of germacrone 4,5-epoxide (14).

(4S,5S)-13-Acetoxygermacrone 4,5-epoxide (7), an oil, gave the molecular formula C_{19}H_{22}O_{3}, from the MS. The 13C- and 1H-NMR spectra of 7 showed the presence of an acetyl group (\(\delta\) 2.10 (s) and 170.6, 20.6), a tertiary methyl group (\(\delta\) 1.05 (s) and 15.8) on an epoxide carbon, an epoxide group (\(\delta\) 60.6, 64.1), a trisubstituted olefin group (\(\delta\)
5.23 (t, J = 7.5 Hz) and 130.3, 131.9) and a tetrasubstituted olefin group (δ 126.3, 138.3) conjugated with a ketone (δ 203.3). The presence of a conjugated ketone was also supported by the IR spectrum (1680 cm⁻¹). These facts suggested that 7 is the acetoxyl derivative of 14. 13-Hydroxygermacrone 4,5-epoxide (6) was acetylated to give 7, so the structure of 7 was determined to be 13-acetoxygermacrone 4,5-epoxide.

(4S,5S)-12-Acetoxygermacrone 4,5-epoxide (8), an oil, gave the molecular formula C₁₇H₂₃O₄. The 1H- and 13C-NMR spectra of 8 showed the presence of an acetoxymethyl group (δ 2.10 (s) and 63.9, 171.0, 20.7). The 1H- and 13C-NMR spectra of 8 showed similar signal patterns to those of 7. But compound 8 gave a different peak from 7 on HPLC. This indicated that 8 is a geometrical isomer of 7 at the C-7,11 double bond. In a comparison of the 13C-NMR spectra of 4, 5, 13, 6, 7, and 15 (shown in Table I), the vinyl methyl groups of 6 and 7, assignable to 12-CH₃, appeared at 18.0 and 18.4 ppm, respectively. The other hand, a vinyl methyl of 8, assignable to 13-Me, showed a different chemical shift (δ 16.3). It was considered that 6 and 7 were 13-hydroxy- and 13-acetoxygermacrone 4,5-epoxide, respectively, and 8 was 12-acetoxygermacrone 4,5-epoxide. The CD spectra of 6, 7, and 8 gave a similar spectral pattern to that of 14 (shown in Fig. 2). This indicated that the absolute configurations and conformation of the ten-membered ring in 6, 7, and 8 were the same as those of 14.

Acetoxyneocurudine (9) gave the molecular formula C₁₇H₂₃O₄ from the MS. The IR spectrum of 9 showed the presence of an ester group (1740 cm⁻¹) and a ketone (1704 cm⁻¹). The 1H-NMR spectrum of 9 showed the presence of two secondary methyl groups (δ 0.99 (3H, d, J = 6.8 Hz), 1.06 (3H, d, J = 7.0 Hz)), a vinyl methyl group (δ 1.67 (3H, s)), an olefinic proton (δ 5.12 (1H, t, J = 7.0 Hz)) and an acetyl group (δ 2.06 (3H, s)). The 13C-NMR spectrum of 9 showed the presence of an acetoxymethyl group (δ 170.6, 20.7, 67.4), and the carbon chemical shifts except for those of C-7, C-11, C-12 and C-13 were almost the same as those of neocurudine (15) rather than those of curudine (16) (Table I). The CD spectrum of 9 showed a negative Cotton effect due to the β,γ-un saturated ketone, which was superimposable on that of 15, but was of opposite sign to that of 16 (Fig. 3). These facts indicated that 9 is the 12- or 13-acetoxy derivative of neocurudine and the configurations at C-4 and C-7 are 4S and 7R, respectively, the configuration at C-11 remains ambiguous.

Curcumadione (10) and isocurcumadione (11) gave the same molecular formula, C₁₇H₂₃O₂, from the MS (m/z 234 (M⁺)). The 13C-NMR spectrum of 10 and 11 showed the presence of two carbonyl groups (δ 208.1, 205.1 in 10 and 208.1, 191.0 in 11), four olefinic carbons (δ 121.1, 134.7, 140.0, 143.7 in 10 and 126.7, 128.8, 144.8, 162.4 in 11). The 1H-NMR spectrum of 10 showed the presence of two olefinic methyl groups (δ 1.80 (s), 1.99 (s)), an acetyl group (δ 2.14 (s)), a secondary methyl group (δ 1.07, J = 6.8 Hz) and an olefinic proton (δ 5.52, t, J = 6.6 Hz). These facts indicated that curcumadione is a seco-guaianone-type derivative (10) analogous to curcumene. The 1H-NMR spectrum of 11 showed the presence of an acetyl group (δ 2.15 (s)), three vinyl methyl groups (δ 1.88, 1.92, 1.93) and one olefinic proton (δ 5.83 (q, J = 1.3 Hz)) coupled with a vinyl methyl group. These data suggested that the structure of isocurcumadione is 11. The absolute configurations of 10 and 11 have not been determined because the amounts of the samples were insufficient.
Experimental
All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-202 grating infrared spectrometer. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. CD spectra were recorded on a JASCO J-20A spectropolarimeter. \( ^{1}H \), \( ^{13}C \), \( ^{19}F \), and \( ^{1}H \) NMR spectra, as well as \( ^{1}H \) and NOESY spectra were recorded on a JEOL JNM-GX 400 NMR spectrometer with tetramethylsilane as an internal standard (\( \delta \) value, ppm). \( ^{1}H \)- and \( ^{13}C \) NMR spectra were performed on Silicon gel type 60 (Merck). HPLC was performed on a reversed-phase column (YMC D-ODS-7) using various acetonitrile- \( H_{2}O \) solvent systems.

Isolation of the Sesquiterpenes
As described in the previous paper, ten sesquiterpenes were isolated from the chloroform-soluble fraction of the fresh rhizomes of \( C. \) aromatica (2 kg). The residual fraction after the previous isolation was subjected to repeated silica gel column chromatography using hexane-\( \text{AcOEt} \) gradient solvent systems, HPLC (YMC D-ODS-7) using an acetonitrile-\( H_{2}O \) system and PLC using hexane-\( \text{AcOEt} \) solvent systems to give epipicromulen (1) (30 mg), isotricocurmenol (2) (1 mg), neopicrocumenol (3) (60 mg), (45S,13S)-13-hydroxydehydrocurdione (4) (40 mg), (45S,13S)-13-hydroxydehydrocurdione (5) (15 mg), (45S,5S)-13-hydroxygermacrene 4,5-epoxide (6) (30 mg), (45S,5S)-13-acetoxygermacrone 4,5-epoxide (7) (120 mg), (45S,5S)-12-acetoxygermacrene 4,5-epoxide (8) (25 mg), acetoxycornerifuran (9) (45 mg), curcumidione (10) (20 mg), isocurcumidione (11) (15 mg) and 13-hydroxygermacrene (12) (28 mg).

Epipicromulen (1) Viscous oil. Ms \( m/z \): 234.163 (M\(^{+}\)) (Calcd for \( C_{12}H_{22}O_{2} \): 234.162). \( \delta_{\text{IR}} \): 205.6 (c 0.3, MeOH). CD (c 0.009, MeOH): \( \theta \) = +1340, \( \theta \) = -1397.0, \( \theta \) = +4880.1. \( ^{1}H \)-NMR (CDCl\(_{3}\)): 1.37 (3H, s, 14-CH\(_{3}\)), 1.84 (3H, s, 13-CH\(_{3}\)), 1.94 (6H, s, 12-CH\(_{2}\) and 15-CH\(_{2}\)), 5.91 (1H, brs, 9-H). The \( ^{13}C \)-NMR data are given in Table 1.

Neopicrocumenol (2) Colorless needles, mp 77–79°C (hexane).

13-Hydroxygermacrene (12) Colorless needles, mp 99–100°C (hexane).

References and Notes
7) Gu-2 and Gu-3 were named isocurcuminal and neocurcuminal, respectively, with the agreement of Prof. I. Kitagawa, Faculty of Pharmaceutical Sciences, Osaka University.