Studies on the Constituents of *Tussilago farfara* L. II.[1] Structures of New Sesquiterpenoids Isolated from the Flower Buds

Masao KIKUCHI* and Noriko SUZUKI

Tohoku College of Pharmacy, 4–4–1 Komatsushima, Aoba-ku, Sendai-shi 981, Japan. *Received April 17, 1992*

Four new notonipetranone-type sesquiterpenoids, 7β-(3-ethyl *cis*-crotonoyloxy)-14-hydroxy-notonipetranone, 14-acetoxy-7β-angeloyloxy-notonipetranone, 14-acetoxy-7β-senecioyloxy-notonipetranone and tussilagolactone, were isolated together with 7β-(3-ethyl *cis*-crotonoyloxy)-14-hydroxy-1α-(2-methylbutyryloxy)-notonipetranone and 7β-(3-ethyl *cis*-crotonoyloxy)-1α-(2-methylbutyryloxy)-3,14-dehydro-*Z*-notonipetranone from the flower buds of *Tussilago farfara* L. (Compositae). The structures of new compounds were elucidated on the basis of spectroscopic evidence.

Keywords *Tussilago farfara*; Compositae; sesquiterpenoid; notonipetranone derivative

The flower buds of *Tussilago farfara* L. (Compositae), called "Kan-to-ka," have been widely used in China, Northern Africa, Siberia and Europe as an antitussive herbal medicine. It is also used for the treatment of some minor bronchitic and asthmatic conditions.[2] A number of chemical constituents have been isolated from this plant, *e.g.* faradiol, phytosterol,[3,4] rutin,[5] tussilagone (I) and 14-acetoxy-7β-(3-ethyl *cis*-crotonoyloxy)-1α-(2-methylbutyryloxy)-notonipetranone[6–8] (II). The absolute configuration of I has been determined by the X-ray diffraction method.[8] In the previous paper,[1] we reported the isolation and structural characterization of principal volatile constituents of the flower buds of *T. farfara*. In further studies on the flower buds of this plant, we have isolated four new compounds belonging to the notonipetranone group and we now report on the structures of new sesquiterpenoids.

The dried flower buds were extracted with ether, and the ether extract was subjected to steam distillation.[1,9] From the steam distillation residue, four new compounds (III—VI) were isolated, together with compounds I and II, 7β-(3-ethyl *cis*-crotonoyloxy)-14-hydroxy-1α-(2-methylbutyryloxy)-notonipetranone (VII)[7] and 7β-(3-ethyl *cis*-crotonoyloxy)-1α-(2-methylbutyryloxy)-3,14-dehydro-*Z*-notonipetranone (VIII).[7]

Compound III was isolated as a colourless gum, $[\alpha]_D^{22}$ −102.4°. The molecular formula of III was determined to be $C_{21}H_{32}O_4$ by high resolution mass spectrometry (HR-MS). The infrared (IR) spectrum exhibited absorption bands due to the presence of hydroxyl (3519 cm^{-1}), ester (1727 cm^{-1}), α,β-unsaturated ester (1708 cm^{-1}), and double bond (1649 cm^{-1}). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of III was very similar to that of I, except that the acetoxyl proton signal was absent and the H-14 signal was clearly shifted up-field (Table I). In the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectrum, the H-7 signal showed a cross peak due to long-range coupling with the C-1′ signal at δ 165.9, so that the 3-ethyl *cis*-crotonoyloxyl group was localized at the C-7 position. On acetylation with acetic anhydride and pyridine, III afforded a monoacetate which was identical with I. Thus, the structure of III was determined to be 7β-(3-ethyl *cis*-crotonoyloxy)-14-hydroxy-notonipetranone.

Compound IV was isolated as a colourless gum, $[\alpha]_D^{22}$ −48.8°. The molecular formula of IV was determined to be $C_{22}H_{32}O_5$ by HR-MS. The IR spectrum exhibited absorption bands due to ester, α,β-unsaturated ester, and double bond. The ^{1}H-NMR spectrum of IV was very similar to that of I except for the signal of unsaturated acid moiety (Table I). The ^{1}H-NMR showed signals at 1.87, 1.97 and 6.07 ppm, indicating that it possesses an angeloyloxyl group. The ^{13}C-NMR spectrum of IV also confirmed the presence of a notonipetrone skeleton, angeloyloxyl and acetoxyl groups. The position of angeloyloxyl and acetoxyl linkages were confirmed by an analysis of the HMBC spectrum of IV. In the HMBC spectrum of IV, cross peaks due to long-range coupling were observed between the H-7 signal and C-1′ signal at δ 167.0, and between the H-14 signal and acetoxyl carbonyl carbon at δ 171.0. Therefore, angeloyloxyl and acetoxyl groups attached to the C-7 and C-14 positions, respectively. Thus, the structure of IV was determined to be 14-acetoxy-7β-angeloyloxy-notonipetranone.

Compound V was isolated as a colourless gum, $[\alpha]_D^{22}$ −45.8°. The molecular formula of V was determined to be $C_{22}H_{32}O_5$ by HR-MS. The IR spectrum exhibited absorption bands due to an ester, α,β-unsaturated ester, and a double bond. The ^{1}H (Table I) and ^{13}C-NMR spectra of V indicated the presence of a notonipetrone

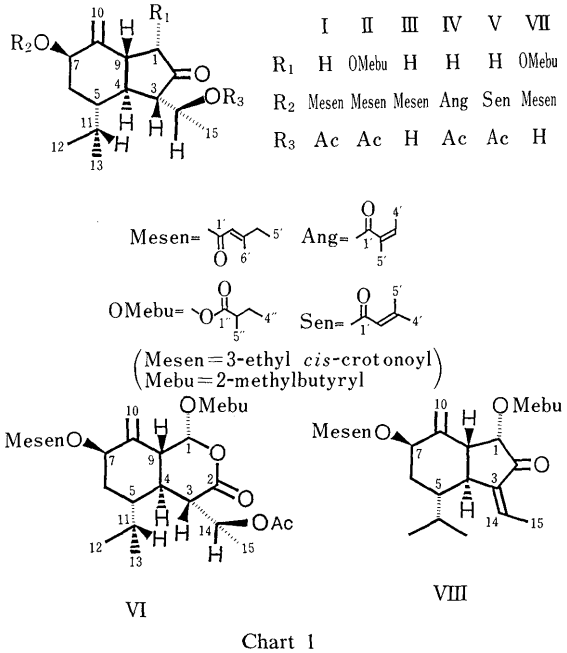

Chart 1

TABLE I. ^{1}H-NMR Chemical Shifts of Compounds I—VI ($CDCl_3$, 400 MHz)

Proton	I	II	III	IV	V	VI
1α	2.14[a]	—	2.42[a]	2.16 dd (14.3, 16.5)	2.14[a]	—
1β	2.37 dd (4.8, 16.5)	5.46 d (4.0)	2.46 ddd (1.1, 1.5, 17.2)	2.38 ddd (1.1, 5.9, 16.5)	2.37 ddd (1.1, 5.7, 16.9)	6.71 d (1.5)
3β	2.50 dd (3.3, 10.6)	2.60[a]	2.50 dd (4.0, 11.0)	2.48 dd (2.4, 11.7)	2.47 dd (2.9, 10.6)	3.06 t (5.9)
4α	1.45[a]	2.04[a]	1.39[a]	1.47 dd (11.0)	1.46[a]	1.84 dt (5.9, 11.4)
5β	1.97 m	1.91[a]	1.98 m	1.92 m	1.98[a]	1.73 q (7.3)
7α	5.58 br t (2.9)	5.54 br s	5.58 t (2.9)	5.65 t (2.9)	5.57 t (2.9)	5.46 d (2.9)
9β	2.59 m	2.58[a]	2.64 m	2.55 m	2.57 m	2.73 br d (11.4)
10	4.79 s	4.78 s	4.79 d (1.1)	4.81 d (1.1)	4.78 d (1.1)	4.59 d (1.5)
10′	5.14 s	5.18 s	5.15 s	5.17 s	5.14 s	5.19 m
11	2.31 m	2.37 m	2.02 m	2.31 m	2.30 m	2.24 t (7.0)
12	0.98 d (6.6)	1.00 d (7.0)	0.95 d (6.6)	0.98 d (6.9)	0.98 d (6.9)	0.98 d (7.0)
13	0.78 d (6.6)	0.82 d (7.0)	0.73 d (6.9)	0.78 d (6.9)	0.78 d (6.9)	0.81 d (6.6)
15	1.22 d (6.6)	1.23 d (6.6)	1.19 d (6.2)	1.23 d (6.6)	1.23 d (6.6)	1.33 d (6.2)
OAc	2.10 s	2.14 s	—	2.10 s	2.10 s	2.15 s
OCOR						
2′	5.63 d (1.1)	5.61 s	5.65 d (1.1)	—	5.66 t (1.3)	5.62 s
3′	—	—	—	6.07 m	—	—
4′	2.18[a]	2.16 d (7.7)	2.18 d (1.1)	1.97 dd (7.3, 1.5)	1.89 d (1.1)	2.18 d (7.3)
5′	1.07 t (7.5)	1.07 t (7.3)	1.08 t (7.5)	1.87 t (1.5)	2.15 d (1.1)	1.09 t (7.3)
6′	2.15 d (1.1)	2.10 d (0.7)	2.15 d (1.1)			2.10 d (3.3)
2″		2.37 m				2.45 m
3″		—				1.50 m
4″		0.89 t (7.5)				0.93 t (7.3)
5″		1.15 d (6.6)				1.19 d (7.0)

Coupling constants (J in Hz) are given in parentheses. *a*) Overlapping.

TABLE II. ^{13}C-NMR Chemical Shifts of Compounds I—VI ($CDCl_3$, 100 MHz)

Carbon	I	II	III	IV	V	VI
1	42.6	72.5	42.2	42.6	42.6	91.3
2	214.8	208.1	221.4	214.7	214.8	168.9
3	57.2	56.5	60.2	57.2	57.2	50.8
4	49.1	44.0	49.3	49.1	49.1	37.3
5	43.9	44.1	44.2	44.4	44.0	43.8
6	31.2	30.3	31.3	31.4	31.2	30.3
7	73.0	73.4	73.0	73.6	72.9	73.0
8	146.1	140.6	146.0	145.7	146.1	141.8
9	42.3	47.0	42.6	42.5	42.3	42.9
10	110.1	113.4	110.1	110.5	110.1	112.1
11	27.6	27.6	28.3	27.6	27.6	25.1
12	21.6	21.5	21.4	21.6	21.6	21.9
13	15.4	15.4	15.3	15.4	15.4	16.1
14	69.6	69.6	68.5	69.5	69.6	70.8
15	15.2	15.8	18.9	15.2	15.2	17.6
$\underline{C}H_3CO$	21.4	21.3		21.4	21.4	19.0
$CH_3\underline{C}O$	170.9	170.9		171.0	170.9	170.6
1′	166.0	165.8	165.9	167.0	165.6	165.7
2′	114.6	114.5	114.6	128.1	116.3	114.2
3′	162.0	162.2	162.1	137.8	156.9	162.8
4′	33.8	33.8	33.8	15.9	27.4	33.8
5′	11.9	11.6	11.9	20.7	20.3	11.9
6′	18.9	18.9	18.9			21.5
1″		174.9				174.6
2″		44.2				41.1
3″		26.7				26.3
4″		11.9				11.6
5″		16.8				17.0

skeleton, senecioyloxyl and acetoxyl groups. The position of senecioyloxyl and acetoxyl linkages were determined by an analysis of the HMBC spectrum of V. Thus, the structure of V was determined to be 14-acetoxy-7β-senecioyloxy-notonipetranone.

Compound VI was isolated as a colourless gum, $[\alpha]_D^{22}$ −51.5°. The molecular formula of VI was determined to be $C_{28}H_{42}O_8$ by HR-MS, which has one more oxygen atom than II. The IR spectrum exhibited absorption bands due to an ester, α,β-unsaturated ester, and a double bond. The ^{1}H-NMR spectrum of VI showed the presence of 3-ethyl *cis*-crotonoyloxyl and 2-methylbutyryloxyl groups. In comparison with the data of II, the signals of H-1 and H-3 were shifted down-field by 1.25 and 0.44 ppm, respectively. The ^{13}C-NMR spectrum of VI also suggested the presence of 3-ethyl *cis*-crotonoyloxyl, 2-methylbutyryloxyl and acetoxyl groups. Comparison of the ^{13}C-NMR chemical shifts of VI with those of II showed that C-1 signal of VI was shifted down-field by 18.8 ppm, and C-2, C-3, C-4 and C-9 signals were shifted up-field by 39.2, 5.7, 6.7 and 4.1 ppm, respectively. The above data indicate that C-1 (δ 91.3, d) is clearly an acetal carbon, and a lactone carbon of C-2 was exchanged with a ketone carbon of II. In the HMBC spectrum of VI, the C-2 signal at δ 168.9 showed a cross peak with H-1 and H-14, so that the extra oxygen atom was linked to the C-1 and C-2 position. The signal of H-7 showed a cross peak with a C′-1 signal at δ 165.7. The carbonyl carbon of an acetoxyl group at δ 170.6 showed a correlation with H-14. Therefore, 3-ethyl *cis*-crotonoyloxyl and acetoxyl groups were attached at C-7 and C-14 positions, respectively. In the two dimensional (2D) nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum, each of the following signals: H-3 and H-5, H-5 and H-9, H-9 and H-3, showed the correlation. The signal of H-1 (6.71, d, J=1.5 Hz) showed a strong cross peak with H-9. However, a correlation of H-1 and H-4 was not observed. Thus, the structure of VI, named tussilagolactone, could be represented by formula VI.

Compounds III, IV and V have not been reported in

Nature; compound VI is new type sesquiterpenoid.

Experimental

Optical rotations were determined with a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Parkin Elmer FT-IR 1725X infrared spectrophotometer and UV spectra with a Beckman DU-64 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JOEL JMN-GSX 400 (400 and 100 MHz, respectively) spectrometer. Chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet). The electron impact (EI)- and high resolution (HR)-MS were recorded on a JEOL JMS-DX 300 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh). Thin layer chromatography (TLC) was carried out with pre-coated Kiesel gel 60 plates (Merck) and detection was achieved by spraying with 50% H_2SO_4 followed by heating. Preparative HPLC was carried out on a Tosoh HPLC system (pump; CCPD, detector; UV-8011, column; TSKgel ODS-120T, 7.8 mm i.d. × 30 cm, mobile phase; MeOH–H_2O (2 : 1), flow rate; 1.5 ml/min).

Isolation The dry buds of *Tussilago farfara* (5.0 kg) on the market were extracted with ether at room temperature for one week. The ether extract was separated by steam distillation to give the essential oil (7.7 g)[1,9] and the residue (65.4 g). A part of this residue (23.5 g) was chromatographed on a silica gel column developed with benzene–AcOEt (9 : 1), and the eluate was separated into 17 fractions (frs. A—Q). Fraction E was re-chromatographed on a silica gel column using benzene–AcOEt (19 : 1) to give fraction E-1 (145 mg). Fraction E-1 was separated into four peaks at t_R (min) 24 (VI, 60 mg), 27 (II, 30 mg) and 42 (VIII, 25 mg) by HPLC. Fraction J was re-chromatographed on a silica gel column using benzene–AcOEt (9 : 1) to give fraction J-1 (210 mg). Fraction J-1 was separated into four peaks at t_R (min) 42 (I, 100 mg) and 63 (VII, 50 mg) by HPLC. Fraction R was re-chromatographed on a silica gel column using $CHCl_3$–MeOH (29 : 1) to give fraction R-1 (160 mg). Fraction R-1 was separated into three peaks at t_R (min) 74 (III, 70 mg), 92 (IV, 40 mg) and 88 (V, 35 mg) by HPLC.

7β-(3-Ethyl *cis*-crotonoyloxy)-14-hydroxy-notonipetranone (III) Colourless gum. $[\alpha]_D^{22}$ −102.4° (c=0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3519, 1727, 1708, 1649. UV λ_{max}^{MeOH} nm (log ε): 218 (3.65). HR-MS m/z: 348.2284 (M^+, Calcd for $C_{21}H_{32}O_4$: 348.2301). ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

14-Acetoxy-7β-angeloyloxy-notonipetranone (IV) Colourless gum. $[\alpha]_D^{22}$ −48.8° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1744, 1714, 1661. UV λ_{max}^{MeOH} nm (log ε): 216 (2.98). HR-MS m/z: 376.2235 (M^+, Calcd for $C_{22}H_{32}O_5$: 376.2250). ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

14-Acetoxy-7β-senecioyloxy-notonipetranone (V) Colourless gum. $[\alpha]_D^{22}$ −45.8° (c=0.4, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1743, 1717, 1653. UV λ_{max}^{MeOH} nm (log ε): 217 (2.98). HR-MS m/z: 376.2261 (M^+, Calcd for $C_{22}H_{32}O_5$: 376.2250). ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

Tussilagolactone (VI) Colourless gum. $[\alpha]_D^{22}$ −51.5° (c=0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$: 1756, 1711, 1647, 1252. UV λ_{max}^{MeOH} nm (log ε): 218 (3.65). HR-MS m/z: 506.2892 (M^+, Calcd for $C_{28}H_{42}O_8$: 506.2880). ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

7β-(3-Ethyl *cis*-crotonoyloxy)-14-hydroxy-1α-(2-methylbutyryloxy)-notonipetranone (VII) Colourless gum. $[\alpha]_D^{22}$ −106.4° (c=0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3683, 1746, 1710, 1648. HR-MS m/z: 448.2835 (M^+, Calcd for $C_{26}H_{40}O_6$: 448.2825). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.78 (3H, d, J=6.6 Hz, 13-H), 0.89 (3H, t, J=7.5 Hz, 4″-H), 0.96 (3H, d, J=6.6 Hz, 12-H), 1.08 (3H, t, J=7.3 Hz, 5′-H), 1.15 (3H, d, J=7.0 Hz, 5″-H), 1.20 (3H, d, J=6.6 Hz, 15-H), 1.46 (1H, m, 6α-H), 1.89 (1H, m, 4α-H), 1.92 (1H, m, 6β-H), 2.03 (1H, m, 11-H), 2.15 (3H, d, J=1.1 Hz, 6′-H), 2.17 (2H, br d, J=7.3 Hz, 4′-H), 2.39 (1H, m, 2″-H), 2.58 (1H, dd, J=4.4, 9.5 Hz, 3β-H), 4.10 (1H, q, J=6.6 Hz, 14-H), 4.77 (1H, d, J=1.1 Hz, 10-H), 5.20 (1H, s, 10′-H), 5.53 (1H, d, J=4.4 Hz, 1β-H), 5.55 (1H, t, J=2.9 Hz, 7α-H), 5.63 (1H, d, J=1.5 Hz, 2′-H).

7β-(3-Ethyl *cis*-crotonoyloxy)-1α-(2-methylbutyryloxy)-3,14-dehydro-*Z*-notonipetranone (VIII) Colourless gum. $[\alpha]_D^{22}$ −56.2° (c=0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1648. HR-MS m/z: 430.2736 (M^+, Calcd for $C_{26}H_{38}O_5$: 430.2719). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.87 (3H, t, J=7.3 Hz, 4″-H), 0.90 (3H, t, J=7.0 Hz, 13-H), 0.98 (3H, d, J=6.6 Hz, 12-H), 1.07 (3H, t, J=7.3 Hz, 5′-H), 1.13 (3H, d, J=7.0 Hz, 5″-H), 2.02 (1H, m, 11-H), 2.15 (3H, s, 6′-H), 2.18 (3H, d, J=7.3 Hz, 15-H), 2.40 (1H, m, 2″-H), 4.18 (1H, s, 10-H), 5.17 (1H, s, 10′-H), 5.51 (1H, d, J=3.3 Hz, 7α-H), 5.56 (1H, d, J=4.0 Hz, 1β-H), 5.64 (1H, s, 2-H), 6.39 (1H, q, J=7.3 Hz, 14-H).

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References and Notes

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