[Chem. Pharm. Bull. 29(12)3561—3564(1981)]

Studies on the Furanoid Diterpenes from *Teucrium japonicum* Houtt.

Toshio Miyase,*,[a] Hiroko Kawasaki,[a] Tadataka Noro,[a] Akira Ueno,[a] Seigo Fukushima,[a] and Tsunematsu Takemoto[b]

Shizuoka College of Pharmacy,[a] 2-2-1, Oshika, Shizuoka, 422, Japan and Institute of Pharmacognocy, Tokushima-Bunri University,[b] Yamashiro-cho, Tokushima, 770, Japan

(Received June 9, 1981)

Two new furanoid diterpenes, teucjaponin A (II) and teucjaponin B (V), have been isolated from *Teucrium japonicum* Houtt. together with teucvin (I) and their structures were determined on the basis of chemical and spectral data. Teucjaponin A showed antifeedant activity for *Prodenia litura*.

Keywords——*Teucrium japonicum*; teucjaponin A; teucjaponin B; teucvin; antifeedant activity

Furanoid diterpenes of the clerodane and nor-clerodane type are common in *Teucrium* species (Labiatae).[1-4] Teucvin (I) and teucvidin (nor-clerodane type diterpenes) were isolated from *Teucrium viscidum* Blume var. *miquelianum* Hara by Fujita *et al.*[1] Now we report the structures of two new clerodane type diterpenes, teucjaponin A and B, isolated from *Teucrium japonicum* Houtt. Teucjaponin A was shown to be an antifeedant for *Prodenia litura*.

Teucvin (I), $C_{19}H_{20}O_5$ (*m/e* 328, M^+), mp 204—206°C, $[\alpha]_D^{14}$ +82.6°, gave a positive Ehrlich test. The infrared (IR) spectrum suggested the presence of two γ-lactones (1760, 1740 cm^{-1}) and a furan ring (875 cm^{-1}). These functional groups account for all of the five oxygens. The nuclear magnetic resonance (NMR) spectrum revealed a secondary methyl group [δ 1.06 (d, J=6 Hz, 3H)], two protons attached to a carbon bearing a lactonic oxygen atom [δ 4.78 (br t, J=9 Hz, $W_{1/2}$=18 Hz, 1H) and 5.47 (t, J=9 Hz, 1H)] and three protons on a β-substituted furan ring [δ 6.41 (m, 1H) and 7.48 (m, 2H)]. From these data this compound was assumed to be a furanoid diterpene having two γ-lactones . In view of the previously reported clerodane type diterpenes from *Teucrium* species, it was concluded to be identical with teucvin (I).[1]

Teucjaponin A (II), $C_{22}H_{28}O_7$ (*m/e* 404, M^+), mp 145—148°C, $[\alpha]_D^{20}$ +38.8°, the main diterpene, gave a positive Ehrlich test. The IR spectrum suggested the presence of a hydroxyl group (3450 cm^{-1}), a γ-lactone (1755 cm^{-1}), an ester (1730 cm^{-1}) and a furan ring (880 cm^{-1}). These functional groups account for six of the seven oxygens. Therefore the seventh oxygen is present as an ether. The NMR spectrum of II revealed a secondary methyl group [δ 1.00 (d, J=6.5 Hz, 3H)], an acetoxyl group [δ 2.08 (s, 3H)], a carbinol proton [δ 4.17 (br d, J=4 Hz, $W_{1/2}$=7.5 Hz, 1H)], two epoxide protons [δ 2.26 (d, J=6 Hz, 1H) and 3.78 (dd, J=6 and 2 Hz, 1H)], two protons attached to a carbon bearing an oxygen atom as an AB quartet [δ 4.90 and 4.94 (J=13 Hz)], a proton attached to a carbon bearing a lactonic oxygen atom [δ 5.37 (t, J=9 Hz, 1H)] and three protons on a β-substituted furan ring [δ 6.41 (m, 1H) and 7.44 (m, 2H)]. Comparing these data with those of I, we concluded that II is a furanoid clerodane type diterpene having a γ-spirolactone, an axial hydroxyl group, a primary acetoxyl group and an epoxide ring.

Acetylation of II with acetic anhydride in pyridine afforded the acetate (III), $C_{24}H_{30}O_8$, mp 159—161°C. The NMR spectrum of III revealed two acetoxyl groups [δ 2.07 (s, 6H)], an epoxide proton [δ 2.96 (dd, J=5 and 2 Hz, 1H); 0.82 ppm upper field shift] and a proton attached to a carbon bearing an acetoxyl group [δ 5.12 (m, $W_{1/2}$=7.5 Hz, 1H); 0.95 ppm

downfield shift]. These acetylation shifts indicate that the hydroxyl group and the epoxide proton lie close to each other. It can be presumed that II has a 6-axial-hydroxyl group and a 4,18-epoxide from a comparison of the above spectral data with those of gnaphalin,[2] a furanoid clerodane type diterpene having a primary hydroxyl group at C_5 and a 4,18-epoxide.

Oxidation of II with chromium trioxide–pyridine complex afforded the ketone (IV), $C_{22}H_{26}O_7$, mp 227—230°C, ν_{max}^{KBr} 1745, 1730, 1710 cm^{-1}. The circular dichroism (CD) spectrum of IV showed a negative Cotton effect $\Delta\varepsilon_{302}$ −0.42. This compound was identical with 19-acetylgnaphalin.[2,5] Therefore we concluded the structure of II to be that of teucjaponin A.

Teucjaponin B (V), $C_{22}H_{28}O_7$, mp 255—258°C, $[\alpha]_D^{19}$ +45.5°, the minor diterpene, gave a positive Ehrlich test. The IR spectrum suggested the presence of a hydroxyl group (3520 cm^{-1}), a γ-lactone (1760 cm^{-1}), an ester (1720 cm^{-1}) and a furan ring (878 cm^{-1}). The NMR spectrum is very similar to that of II, except that a double doublet signal at δ 3.78 due to an epoxide proton is shifted to δ 3.23 and a carbinol proton signal ($W_{1/2}$=7.5 Hz) at δ 4.17 is shifted to δ 3.66 with $W_{1/2}$=18 Hz.

Reduction of IV with sodium borohydride afforded an alcohol, which was identical with V. Thus, V is a 6-epimer of II, and can be assigned as teucjaponin B.

Teucjaponin A showed antifeedant activity towords the larvae of *Prodenia litura*. Its threshold concentration for inhibitory activity was about 400 ppm in the leaf disk test.[6]

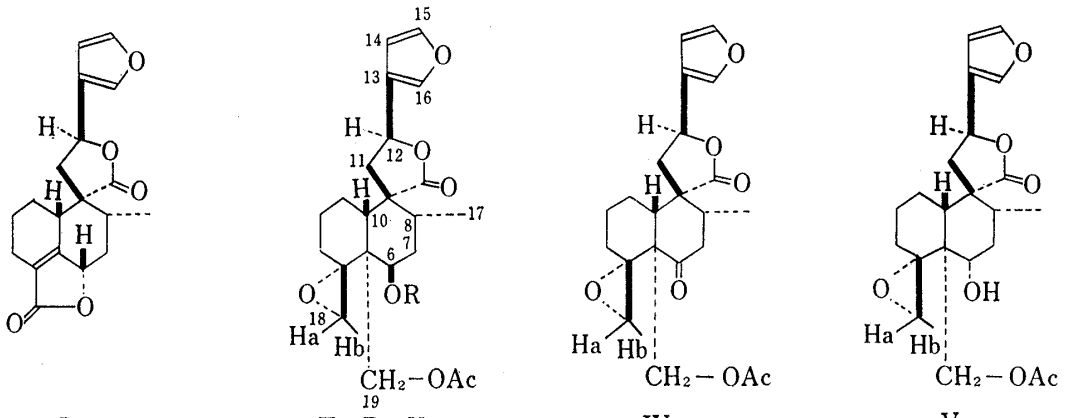

Chart 1

TABLE I. NMR Chemical Shifts and Coupling Constants

Proton	II	III	IV	V
6	4.17 (br d, J=4 Hz, $W^1/_2$=7.5 Hz)	5.12 (m, $W^1/_2$=7.5 Hz)		3.66 (dd, J=11, 4Hz, $W^1/_2$=18 Hz)
7α			3.53 (t, J=14 Hz)	
8	2.04		1.93	1.56
11	2.45 (dd, J=9, 3 Hz)	2.46 (dd, J=9, 2 Hz)	2.45 (d, J=8.5 Hz)	2.35 (d, J=9 Hz)
12	5.37 (t, J=9 Hz)	5.38 (t, J=9 Hz)	5.46 (t, J=8.5 Hz)	5.35 (t, J=9 Hz)
14	6.41 (m)	6.37 (m)	6.40 (m)	6.39 (m)
15	7.44 (m)	7.43 (m)	7.47 (m)	7.44 (m)
16	7.44 (m)	7.43 (m)	7.47 (m)	7.44 (m)
17	1.00 (d, J=6.5 Hz)	0.96 (d, J=6 Hz)	1.07 (d, J=6.5 Hz)	1.03 (d, J=6.5 Hz)
18a	2.26 (d, J=6 Hz)	2.26 (d, J=5 Hz)	2.24 (d, J=6 Hz)	2.45 (d, J=4 Hz)
18b	3.78 dd, J=6, 2 Hz)	2.96 (dd J=5, 2 Hz)	3.56 (dd, J=6, 2 Hz)	3.23 (dd, J=4, 2 Hz)
19	{4.90 (d, J=13 Hz) 4.94 (d, J=13 Hz)	4.97 (s)	{5.04 (d, J=12.5 Hz) 5.48 (d, J=12.5 Hz)	{4.76 (d, J=12.5 Hz) 5.02 (d, J=12.5 Hz)
OAc	2.08 (s)	2.07 (s) × 2	2.06 (s)	2.09 (s)

Experimental

Melting points were determined on Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical rotations were determined with a Yanaco automatic polarimeter. IR spectra were run on a Jasco IRA-2 grating infrared spectrophotometer and mass spectrum (MS) on a Hitachi RMU-7 mass spectrometer. NMR spectra were recorded on a Varian XL-100A spectrometer; chemical shifts are given in δ (ppm) with tetramethyl-silane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad).

Isolation——Air-dried and coarsely powdered *T. japonicum* (aerial part; 3.1 kg) was extracted twice with acetone (30 l) at room temperature. Removal of the acetone *in vacuo* afforded a gum (130 g), which was chromatographed on a silica gel (1 kg) column using hexane–acetone [(85: 15)—(60: 40)] as an eluent to give teucvin (I), teucjaponin A (II) and teucjaponin B (V).

Teucvin (I)——Recrystallization from methanol gave colorless crystals (1.4 g), mp 204—206°C. *Anal.* Calcd for $C_{19}H_{20}O_5 \cdot 1/2H_2O$: C, 67.64; H, 6.27. Found: C, 67.83; H, 6.47. IR ν_{max}^{KBr} cm^{-1}: 1760, 1740, 1685, 1170, 1160, 1145, 1020, 875. MS *m/e*: 328 (M^+, 86), 327 (53), 309 (93), 298 (68), 104 (54), 94 (100), 93 (58), 90 (68), 80 (53). NMR ($CDCl_3$) δ: 1.06 (3H, d, $J=6$ Hz, CH_3), 2.58 (2H, d, $J=9$ Hz, C_{11}–H_2), 4.78 (1H, br t, $J=9$ Hz, $W^1/_2=18$ Hz, C_6–H), 5.47 (1H, t, $J=9$ Hz, C_{12}–H), 6.41 (1H, m, C_{14}–H), 7.48 (2H, m, C_{15}–H, C_{16}–H). This was identified by direct comparison (mp, IR, thin layer chromatography (TLC), NMR) with an authentic sample (lit. mp 207—208°C).

Teucjaponin A (II)——Recrystallization from acetone–benzene gave colorless crystals (3.6 g), mp 145—148°C, $[\alpha]_D^{20}$ +38.8° ($c=3.84$, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{28}O_7 \cdot 1/2C_6H_6$: C, 67.70; H, 7.05. Found: C, 67.71; H, 7.08. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1755, 1730, 1255, 1175, 1153, 1028, 880. NMR ($CDCl_3$) δ: Table I. MS *m/e*: 404 (M^+).

Teucjaponin B (V)——Recrystallization from chloroform–methanol gave colorless needles (39 mg), mp 255—258°C, $[\alpha]_D^{19}$ +45.5° ($c=0.67$, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.11; H, 6.92. IR ν_{max}^{KBr} cm^{-1}: 3520, 1760, 1720, 1260, 875. NMR ($CDCl_3$) δ: Table I.

Acetylation of Teucjaponin A——Teucjaponin A (120 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml) and left at room temperature overnight. The reaction mixture was diluted with water (20 ml) and extracted with ethyl acetate (5×10 ml). The extract was washed with water, dried over sodium sulfate and concentrated to give the residue. Recrystallization from methanol gave the acetate (III) (52 mg) as colorless needles, mp 159—161°C. *Anal.* Calcd for $C_{24}H_{30}O_8$: C, 64.56; H, 6.77. Found: C, 64.46; H, 6.70. IR ν_{max}^{KBr} cm^{-1}: 1760, 1730, 1720, 1245, 1035, 875. NMR ($CDCl_3$) δ: Table I.

Oxidation of Teucjaponin A——Teucjaponin A (130 mg) was treated with chromium trioxide (80 mg) and pyridine (2 ml) and left in an ice-box overnight. The reaction mixture was diluted with water (20 ml) and extracted with ethyl acetate (3×10 ml). The extract was washed with water, dried over sodium sulfate and concentrated to give the residue. Recrystallization from methanol gave the ketone (IV) (100 mg) as colorless prisms, mp 227—230°C. *Anal.* Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51. Found: C, 65.60; H, 6.56. IR ν_{max}^{KBr} cm^{-1}: 1745, 1730, 1710, 1185, 1020, 880. CD ($c=0.09$, methanol) $\Delta\varepsilon$: −0.42 (302) (negative maximum). NMR ($CDCl_3$) δ: Table I. This was identified by direct comparison (mp, IR, TLC) with authentic 19-acetylgnaphalin (lit. mp 227—229°C).

Reduction of the Ketone——The ketone (IV) (85 mg) was reduced with sodium borohydride (25 mg) in methanol (3.5 ml) at room temperature for 5 min. The reaction mixture was acidified with acetic acid (3 drops), diluted with water (30 ml) and extracted with chloroform (4×10 ml). The extract was washed with water, dried over sodium sulfate and concentrated to give the residue. Recrystallization from chloroform–methanol gave an alcohol as colorless needles, mp 255—259°C. *Anal.* Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.34; H, 6.93. IR ν_{max}^{KBr} cm^{-1}: 3520, 1760, 1720, 1260, 875. NMR ($CDCl_3$) δ: identical with the NMR of V. This was found to be identical with V by direct comparison (mp, IR, TLC, NMR).

Biological Assay——Antifeedant activity of II against the larvae of *Prodenia litura* was tested according to the method reported by Munakata *et al.*[6]: 10×3 larvae of *P. litura* were used. The leaf disks (cabbage, 15 mm in diameter) were immersed in an acetone solution of the compound (1×10^4, 2×10^3, 4×10^2, 0 ppm) for 2 min. The feeding inhibitory activity was measured after 5 h. The threshold concentration of II was about 400 ppm.

Acknowledgement This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan, for which the authors are indebted. Thanks are due to the staff of the Central Research Division, Takeda Chemical Industries, Ltd. for the measurements of NMR and CD spectra and to the staff of the Life Science Research Institute, Kumiai Chemical Industry, Ltd. for biological assay. The authors also thank Dr. G. Savona (University of Palermo, Italy) and Dr. S. Valverde (C.S.I.C., Spain) for providing 19-acetylgnaphalin and Prof. E. Fujita (Kyoto University) for teucvin.

References and Notes

1) E. Fujita, I. Uchida, and T. Fujita, *Chem. Commun.*, **1973**, 793; I. Uchida, T. Fujita, and E. Fujita, *Tetrahedron*, **31**, 841 (1975).

2) G. Savona, M. Paternostro, F. Piozzi, and B. Rodriguez, *Tetrahedron Lett.*, **1979**, 379.
3) E. Gácb-Baitz, L. Radics, G.B. Oganessian, and V.A. Mnatsakanian, *Phytochemistry*, **17**, 1967 (1978).
4) G. Savona, S. Passnanti, M.P. Paternostro, and F. Piozzi, *J. Chem. Soc.* (*I*), **1978**, 356.
5) C. Marquez and S. Valverde, *J. Chem. Soc.* (*I*), **1979**, 2526.
6) K. Wada and K. Munakata, *J. Agr. Food Chem.* **16**, 471 (1968).