生 薬 学 雑 誌
Shoyakugaku Zasshi
47(3), 305~310 (1993)

Quantitative Studies on Terpenes of Japanese and European Valerians[1)]

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(Received April 1, 1993)

In order to identify the sedative principles in valerians, dried roots and rhizomes of cultivated Japanese and European valerians were extracted with dichloromethane, and the extracts were assayed by gas chromatography. Japanese valerians were different from European ones in that panese valerians contained varieties of mono- and sesquiterpenes and that their total terpene contents were about 2 to 25 times those of European ones. Kessyl glycol diacetate (trace to 1.89%) and α-kessyl acetate (trace to 2.25%) were characteristic terpenes of Japanese ones, not detect in European ones. Therefore, the sedative action of European valerian must be due to some components other than mono- and sesquiterpenes.

Keywords——*Valeriana fauriei*; *Valeriana officinalis*; valeri an; terpene; GC

Japanese valerian, (Japanese name, "Kanokoso"), is defined as the root and rhizome of *Valeriana fauriei* BRIQ. or the allied plants (*Valerianaceae*), in the Pharmacopoeia of Japan.[2)] In European Pharmacopoeia, *Valeriana officinalis* L. is listed as the original plant of Valerian Root.[3)] The drugs are used as a sedative both in Europe and in Japan, and Japanese valerian was once exported to Europe as the drug. Many studies have been reported on its sedative and other pharmacological activities. In Europe, valepotriates, an iridoid mixture from Europea n valerians, has been shown to prolong pentobarbital-induced sleeping time, a dose-dependently to have an effect on changes in electroencephalogram pattern, and to have a spasmolytic effect *etc.*[4)] In Japan Takamura *et al.* studied pharmacological activities of a dichloromethane extract of "Kanokoso" and its main component, kessyl glycol diacetate (KGD), and reported that they prolonged hexobarbital-induced sleeping time.[5)] The sedative action of "Kanokoso" and its chemical components was studied by Hikino *et al.*[6)] They observed that the active principles of "Kanokoso" were KGD and its deacetoxy derivatives, because each of the compounds showed a fairly strong sedative action and because the valepotriates content was very low in "Kanokoso."

It is interesting that in Europe and in Japan different components were considered to be responsible for the sedative action of the crude drugs. Therefore, in the present work, chemical constituents of valerians in both regions were compared by means of gas chromatography (GC).

Experimentals and Methods

Instruments——Proton and carbon nuclear magnetic resonance (^{1}H-NMR: 300 MHz and ^{13}C-NMR: 75.5 MHz) spectra were recorded on a Varian XL-GFM 300 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were measured with a JMS-DX 300 mass spectrometer (JEOL) with an ionization voltage of 70 eV in positive mode. Gas chromatogram was recorded on a Gas chromatograph model 163 (Hitachi Ltd.). Samples were pulverized with a high-speedy grinder TI-200 (Heiko Seisakusho, Ltd., Japan). Extraction with sonication was carried out with an Ultrasonic-wave cleaner (150 W, volume: 6000 ml, Bransonsonic 32, USA).

Operation conditions of GC——Detector: flame ionization detector. Carrier gas: nitrogen. Flow rate: 30 ml/min. Column: 5% Silicone SE-30/Chromosorb WHP (80/100 mesh) in a glass column, 3 mm $\phi \times 2$ m. Column temperature: programmed at a rate of 4°C/min from 110 to 230°C. Detector temperature: 250°C. Injection volume: 1 μl. The parameter of detector was set so that 1 ng of *n*-docosane was detected.

TABLE I. Plant Materials

Valerian	Japanese name	Abbr.	Collected from
V. fauriei BRIQ.	Hokkai-kisso*	A	Hokkaido, Japan
	Kanagawa-kisso	B	Ibaraki, Japan
	Kanagawa-kisso (Kameba type)	C	Hokkaido, Japan
	Kanokoso	D	Ohita, Japan
V. officinalis L. ssp. *officinalis*		E	Hamburg, Germany
		F	Tubingen, Germany
		G	Saitama, Japan
		H	Poznan, Poland
		I	Moscow, USSR
		J	Helsinki, Finland
V. officinalis L. ssp. *sambucifolia*		K	Liberec, Czechoslovakia
		L	Helsinki, Finland

* Japanese valerian is mostly cultivated in Hokkaido (the northern area of Japan), and called "Hokkai-kisso" in the market.

Plant materials and preparation——Four kinds of Japanese valerian plants and 8 European ones were grown in the Tsukuba Medicinal Plant Research Station of our institute (TABLE I). Two ridges (growing districts 1 and 2), which were separated by a space of 60 cm from each other, were prepared to examine the content variation of constituents according to district. Each of the stumps was divided into parts, which were planted with a spacing 30 cm in November. In the next November roots were harvested, dried in an aerated room for a month and pulverized (less than 300 μm). The valerians grown in the grown district 1 were Valerian No. 1 and those in the district 2, Valerian No. 2. Details of the cultivation procedure will be published later.

Reference standards: Bornyl acetate (**1**) was purchased from Wako Pure Chemical Industries Ltd. (1st grade) and terpinyl acetate (**3**), from Tokyo Kasei Kogyo Co., Ltd. (1st grade).

α-Kessyl acetate (KA)[8] (7) and kessyl glycol diacetate (KGD)[11] (10): Dichloromethane extract of "Hokkai-kisso" was applied to repeated chromatography over silica gel using a series of *n*-hexane and ethyl acetate mixtures (10:0 to 10:2) as eluents, to give KAA and then KGD.

KA——Oil. Purity by GC: 99.9%. MS *m/z* (%): 280 (M^+), 126 (100), 108 (46), 81 (29). IR ($CHCl_3$) ν cm^{-1}: 1715, 1240 (acetate). ^{1}H-NMR ($CDCl_3$): 0.84 (3H, d, J=7.0, H_3-14), 1.21 (3H, s), 1.28 (3H, s), 1.30 (3H, s), 2.06 (3H, s, OAc), 3.49 (1H, septet-like, H-5), 5.03 (1H, t-like, H-2). ^{13}C-NMR ($CDCl_3$): 18.42 (q), 21.91 (q), 24.32 (t), 27.71 (q), 28.29 (q), 30.58 (q), 31.19 (t), 32.76 (t), 35.65 (d), 36.06 (d), 36.63 (d), 42.22 (t), 53.69 (d), 73.32 (s), 74.90 (s), 77.76 (d), 171.28 (s).

KGD——Colorless needles (chloroform-methanol), mp 118–118.5°C. Purity by GC: 99.8%. Identified by direct comparison with an authentic specimen[13] (mp, TLC, IR, ^{1}H-NMR, GC).

Kessyl glycol 2-acetate: Prepared by partial saponification of KGD according to the method described by Takamura *et al.*[12] Purity by GC: 99.5%. MS *m/z* (%): 296 (M^+), 236 (44), 142 (100). The ^{1}H-NMR data were in good accordance with the reported ones.[12]

Internal standard solution——One hundred mg of *n*-docosane (Nakarai Chemicals, Ltd., purity: 99%) was dissolved in 100 ml of dichloromethane.

Preparation of standard solutions——Fifty mg each of bornyl acetate, terpinyl acetate, KA and KG was weighed accurately. To the mixed standard, dichloromethane was added to exactly 100 ml. Into 0-ml, 3-ml, 5-ml, 7-ml and 10-ml portions of this solution, 10 ml, 7 ml, 5 ml, 3 ml and 0 ml of dichloromethane were added, respectively, and to each mixture, 1 ml of the internal standard solution was added.

Preparation of sample solutions——Each pulverized sample was dried in a desiccator (silica gel) at room temperature for 24 h. About 0.5 g was, accurately weighted into a 100-ml flask, to which 20 ml of dichloromethane was added. The flask was subjected to supersonic extraction for 20 min. After filtration, the residue was re-extracted in the same manner. The combined filtrate was concentrated to less than 10 ml under reduced pressure at a temperature not higher than 50°C and made to 10 ml with dichloromethane. To the solution, 1 ml of the internal standard solution was added to make the sample solution.

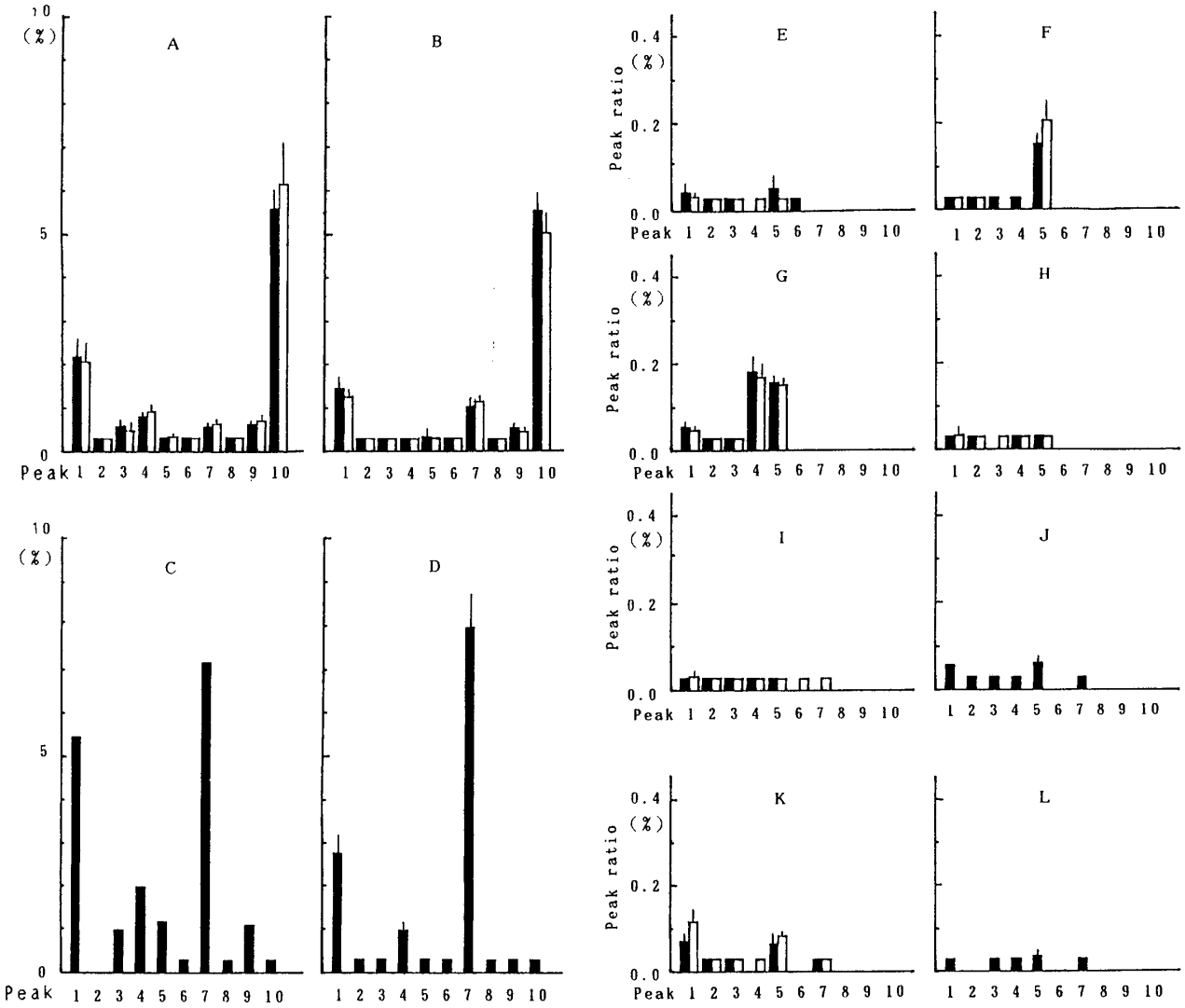

Fig. 1. Chromatographic Patterns of Valerians

The ratios of the peak area of each constituent to that of the internal standard. A to D: Japanese valerians, E to L: European valerians. ■ : samples from the growing district 1, □ : samples from the growing district 2. Sample numbers are in bracket: A-1 (6); A-2 (8); B-1 (8); B-2 (9); C-1 (1); D-1 (4); E-1 (8); E-2 (8); F-1 (9); F-2 (10); G-1 (10); G-2 (9); H-1 (9); H-2 (7); I-1 (9); I-2 (7); J-1 (5); K-1 (4); K-2 (4); L-1 (5). The compounds giving the peaks **1–9** are identified in Fig. 2. Each value represents the mean ± S.D.

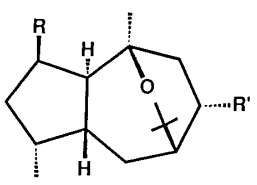

Chart 1.

R=H	R′=H	Kessane (**4**)
R=OH	R′=H	α-Kessyl alcohol (**6**)
R=OAc	R′=H	α-Kessyl acetate (KA) (**7**)
R=OH	R′=OH	Kessyl glycol (KG) (**8**)
R=OAc	R′=OH	Kessyl glycol 2-acetate
R=OAc	R′=OAc	Kessyl glycol diacetate (KGD) (**10**)

Results and Discussion

1. GC column

SE-30, OV-1 and OV-17 columns were tested for the efficiency in peak separations. A packed column of 5% Silicone SE-30/Chromosorb WHP was found to be the most satisfactory.

2. Internal standard

Of the paraffins tested C_{18-22}, *n*-docosan was found to be the most suitable internal standard (Fig. 2).

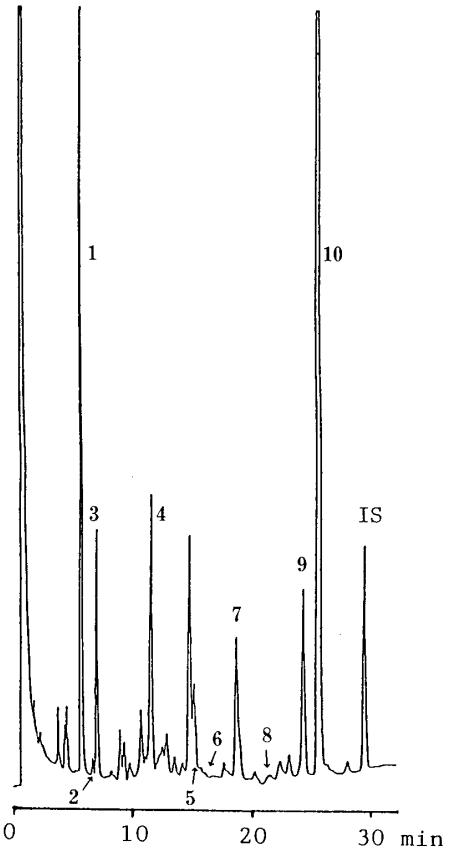

Fig. 2. GC Profile of Japanese Valerian

1: bornyl acetate; **2**: unknown; **3**: tepinyl acetate; **4**: kessane; **5**: valeranone; **6**: KA; **7**: α-kessyl acetate; **8**: KG; **9**: kanokonyl acetate (?)*; **10**: KGD; IS: *n*-docosane. Compounds for the peaks **4**, **5** and **9** were identified from the elution order[7-10] and their MS. * The MS at the peak **9** was different from that of kessyl glycol 12-acetate which was derived from KGD.[11]

3. Calibration curves

The standard curves for bornyl acetate, terpinyl acetate, KA and KGD were obtained by the internal standard method. When the ratio of the peak area of each standard to that of the internal standard was determined, the injected amount was found to be proportional to the ratio and the coefficient of correlation was more than 0.998 in all reference standards.

4. Extraction

Hikino *et al.* used distillation method to prepare the sample solution for GC. The method is not suitable for analysis of terpenes such as KA and KGD having high boiling temperatures. Therefore, a supersonic extraction, which has an advantage that 5 or more samples may be treated at a time, was performed by using 0.5 g of sample with two 20-ml portions of dichrolomethane, as directed previously. Complete extraction was performed by this procedure, which was confirmed by the fact that the third extract with dichloromethane gave no peak in GC.

5. Reproducibility of analysis

The analysis of a valerian (A-1) by this extraction and GC method was repeated, and the peak areas of the major constituents were measured. The coefficient of variation for each constituent was as follows: 5.78% for bornyl acetate, 5.56% for terpenyl acetate, 1.46% for KA, 0.39% for KGD. Therefore, the combination of extraction and GC was proved to be satisfactory for the determination.

6. Comparison of major peaks

The major terpenes contents are shown in Fig. 1 as the ratio of the ratio of the peak area of each sample to that of *n*-docosane. The analytical results of the plants in the districts 1 and 2 showed that the mean and the standard deviation of each valerian was very similar. Therefore, the difference of plant

TABLE II. Terpene Contents of Valerians

Valerians	No. of samples	Terpenes (wt% of dry sample)			
		Bornyl acetate	Terpinyl acetate	α-Kessyl acetate (KA)	α-Kessyl glycol diacetate (KGD)
A-1	6	0.53±0.09	0.19±0.06	0.13±0.06	1.71±0.15
B-1	8	0.34±0.07	Trace	0.27±0.05	1.70±0.15
C-1	1	1.32	0.36	2.03	Trace
D-1	4	0.67±0.11	Trace	2.25±0.22	Trace
E-1	8	0.11±0.02	Trace	ND	ND
F-1	9	Trace	Trace	ND	ND
G-1	10	0.11±0.03	Trace	ND	ND
H-1	9	Trace	ND	ND	ND
I-1	9	Trace	Trace	ND	ND
J-1	5	0.12±0.03	Trace	Trace	ND
K-1	4	0.16±0.04	Trace	ND	ND
L-1	5	Trace	Trace	ND	ND

Trace: less than 0.10%; ND: not detectable. Each value represents the mean±S.D.

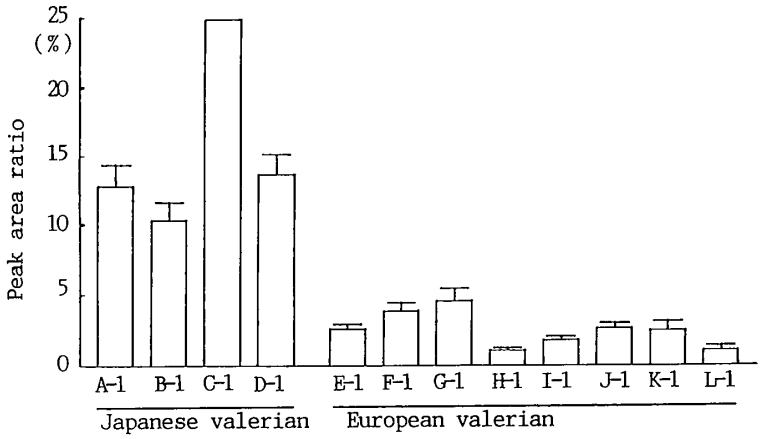

Fig. 3. Total Terpene Contents

Total terpene contents are presented as the ratio of the sum of the peak areas of terpenes (from peak of bornyl acetate to that just before *n*-docosane) to the peak area of internal standard. Each value represents the mean±S.D.

growth between the districts was not significant, and all the analytical results are characteristic of the valerians themselves.

On the gas chromatograms of Japanese valerians, a number of peaks appeared of which, the peaks **1**, **3**, **7**, **9** and **10** were major ones. Terpenes of kessane type were rich in Japanese valerians, whereas scarcely detected in European ones (Fig. 1). The amounts of terpenes giving major peaks in GC are given in percentage scale in TABLE II. KA and KGD were the major terpenes of Japanese valerians. The contents were as follows: trace to 1.89% for KA; trace to 2.25% for KGD. The two terpene contents are in an inverse relation: when one peak was high, the other was low.

7. Comparison of total amount of terpenes

The essential oil content in crude drugs is usually determined by measuring the distilled oil volume. However, this method does not give reliable results when the oil contents in the drug samples are low because some part dissolves in water and some part adheres the glass wall of the apparatus. Then, by measuring the areas of GC peaks, the terpene content can be accurately estimated within 2 h. The sum of the peak areas of the peaks No. **1** (bornyl acetate) to No. **10** (the peak appearing just before *n*-doco-

sane) was compared with the peak area of the internal standard (Fig. 3). The present analytical method consisting of extraction and GC provides an easy and efficient method for the estimating essential oil content in crude drugs. Consequently, the total mono- and sesquiterpene contents in Japanese valerians were about 2 to 25 times those in European ones.

Conclusion

GC method was used to compare the terpene contents of Japanese and European valerians cultivated in the same place. Although the terpene compositions of individual valerians varied, Japanese valerians always contained KGD and the related compounds, which was reported to have a sedative action, whereas European ones contained a small quantity of terpenes. The results strongly suggested that sedative action by European valerian was to be attributed to the components other than mono- and sesquiterpenes.

Acknowledgements: The authors express their thanks to Dr. T. Yamagishi for providing the authentic samples of kessyl glycol diacetate and kessyl glycol. This research was supported in part by a grant of Japan Health Sciences Foundation (1987 to 1989). One of us, B.C. Zhang, owed Sasagawa Medical Scholarship to Japan-China Medicinal Association (1987).

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