A chemical study on “Yakatsu (治葛)” stored in Shosoin repository  
Isolation and characterization of four indole alkaloids from  
a 1250 year-old sample of the Chinese toxic medicine

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Abstract: On the occasion of the second scientific investigation on ancient medicines stored in Shosoin repository, a chemical study of the constituents of a medicine with the code name N-127, Uyaku-no-zoku (北第127号、烏薬之属), was carried out. We isolated four indole alkaloids, gelsevirine, koumine, gelsemine, and sempervirine, in pure states from the toxic medicine of the 8th century. This result indicates that the medicine is obviously Yakatsu (治葛) listed in the dedicatory document, Shuju-yaku-cho (種々薬物), and the original plant is Gelsemium elegans Benth. endemic to southern part of China.

Key words: Shosoin repository; Yakatsu (治葛); Ancient toxic medicine; Gelsemium elegans; gelsevirine; koumine; gelsemine; sempervirine.

Introduction. The treasures owned by the Emperor Shomu (聖武天皇) (A.D. 701-756) were dedicated to the Great Buddha of Todaiji temple by the Empress Dowager Komyo (光明皇太后) on June 21, Tempyo-shoho 8th year (天平勝宝8年、A.D. 756) on the occasion of the seven-times-seventh day (七七忌) of the late Emperor.

Sixty kinds of medicines along with an accompanying dedicatory records, Shuju-yaku-cho (種々薬物), were also donated on the same day. All those treasures and medicines were stored in the old wooden store house of Shosoin for 1200 years until 1963, when they were housed in Nishi-hoko (西宝庫), a newly built steel framed concrete house in the same campus, equipped with modern air-conditioning.

As mentioned above, sixty kinds of herbal and some mineral crude drugs were stored in Shosoin in A.D. 756. Thus Shosoin is highly reputed to be the only place in the world where such old herbal medicines have been kept indoor (not buried in tomb) with an accurate historical record. The repository had been kept closed for a long time under the Imperial Seal (秘封), and only quite limited number of officials in charge could examine the items and put them in orders. The first scientific investigation on the stored ancient herbal medicines was performed in 1948–1949 soon after the World War II. Yasuhiko Asahina, Professor emeritus of University of Tokyo, organized a team of 10 scientists under the special permission of the Imperial Household Agency. The results were published as a monograph of 520 pages entitled “Shosoin Yakubutsu” (正倉院薬物).1) In 1994–1995, 50 years after the first investigation, Prof. Shoji Shibata was requested to conduct the second scientific investigation by the Shosoin Office, Imperial Household Agency.2) Prof. Shibata, who himself participated in the first investigation3) as one of the members of the team, organized a group of 6 specialists of pharmacognosy and phytochemistry including one of the present authors (N.A.).

Historical review and previous survey on Yakatsu (治葛). We were deeply interested in a particular crude drug, Yakatsu, listed in the dedicatory document, Shuju-yaku-cho, as the last item (No. 60) with the quantity of ca. 7 kg. It is known that the name of Yakatsu is also found in the oldest Chinese law document, To-ritsu-so-gi (唐律疏義, the Tang Code), written in A.D. 653, Tang Dynasty in China. This law system influenced not only descending Chinese dynasties but also neighboring countries such as Japan or Korea. In fact Japan quickly introduced this system and modified it to two Japanese codes, Taiho-ritsu-ryo (大宝律令, A.D. 701) and Yororitsu-ryo (養老律令, A.D. 757). In a section of the Tang Code, Zoku-to-ritsu (諸葛十律, Law of thieves and violence), a provision to control the abuse of toxic medicines is

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described. The text states that a wrongdoer who poisons
the others or sells toxins with criminal intention is
condemned to death by strangulation. If a wrongdoer sells
or purchases toxins even if he does not use it, he must be
exiled to an isolated island. Following these statements,
actual names of 4 lethal toxic medicines are cited. They are
Chin poison (鴨毒), Yakatsu (治葛), Uzu (烏薙), and Bushi
(附子). The same text and the same names of toxic
medicines are found in the Japanese Code, Yoro-ritsu-ryo,
formalized in almost the same age of building Shosoin. Of
these 4 toxic medicines, Uzu and Bushi have been known
as to be the root of Ranunculaceous medicinal plant,
Aconitum species, but we have only limited information
about the other two, Chin and Yakatsu. Chin is a
mysterious legendary bird in China, whose feathers are
said to contain some extremely toxic substance used for
assassination by dipping it in wine, but it is doubtful now
that this bird really existed. The fourth toxic drug,
Yakatsu, really exists as indicated in the list of Shosoin
drugs. It is mysterious why such an extremely poisonous
herb was included in the dedicated drugs. Of medicines
stored in Shosoin repository, some were supplied through
a clinic, Seyaku-in (施薬院), to ordinary people suffering
from diseases. The merciful intention of the Empress
dowager Komyo to utilize the stored drugs for such
purposes is clearly stated at the end of the dedicatory
document. Apart from such obvious consumption, some
medicines were spoiled by the invasion of worms and fungi,
and some partly disappeared without particular notes of
release or were completely lost during the long period of
times. Thus 38 kinds of drugs actually remain at present
out of original 60 recorded on the dedicatory list in the year
of A.D. 756.

Before the first scientific investigation in 1948,
Yakatsu had been thought to be scattered or lost leaving
only the container, a pottery with a lid on which a script of
Yakatsu remained. During the first investigation, however,
the late Prof. Konoshima pointed out a possibility that a
bundle of woody roots with the code name N-127, Uyaku-
no-zoku (附第127号、準備之属), a variety of Uyaku
(Lindera spp. Lauraceae), might be Yakatsu. By the
careful examination of the external and internal morpholo-
gy of the drug, he concluded that the plant material in
question was not a plant of Lauraceae. He found several
characteristic structures of Gelsemium spp. (Loganiaceae)
by the microscopical observation of the drug material.\[1b\]

Fig. 1. A part of Shuju-yaku-cho (種々薬帳). The name
Yakatsu (治葛) is seen in the fourth vertical line from the right.

Fig. 2. N127 Uyaku-no-zoku (附第127号、烏薨之屬). Photograph offered from the Office of the Shosoin Treasure House,
the Imperial Household Agency, Japan.
In China the name of Yakatsu had not appeared in old literatures since the Tang Code, and there were no sign that the herbal medicine with this name were on market. In Honzo-komoku (本草綱目, Ben cao-gangmu) written in the 16th century in China, the name Yakatsu is shown as one of the synonyms of Ko-fun (鶴問, Gou-wen). The source plant of Yakatsu was thought to be a species of Gelsemium. Therefore, Prof. Konoshima concluded that N-127 could be Yakatsu.

If it really was a plant of genus Gelsemium, occurrence of alkaloids was expected. By the preliminary coloring test for alkaloids he observed the positive indication of their existence, but further identification of alkaloids was not made due to the methodological difficulty at that time. Fifty years have passed since the first investigation, and on the second investigation we undertook experiments using advanced modern methods to isolate and identify alkaloidal constituents from the 1250 year old specimen of Yakatsu.

A chemical study on the herbal medicine coded N-127 Uyaku-no-zoku (北第127号, 烏薬之属). A small piece of the sample N-127 Uyaku-no-zoku (北第127号, 烏薬之屬) was cut into small pieces and pulverized. Extraction of the sample (2.8 g) with hot MeOH gave the MeOH extract as a syrupy residue (264 mg). The extract (129 mg) was shaken with a mixture of dil aq. HCl (1N) and AcOEt, and the acid layer was taken. Basification with Na2CO3 followed by extraction of the liberated basic constituents with 5% MeOH-CHCl3 afforded a crude base mixture (23 mg). The obtained syrup was dissolved in CHCl3 and the solution was filtrated through a short column of SiO2 with MeOH-CHCl3 (20-50%). The eluted fractions were combined and the whole was submitted to MPLC (Medium pressure liquid chromatography) separation over a column of SiO2 with McOH-CHCl3 (20-50%). Fractions eluted with 8% MeOH-CHCl3 gave crude compound 1, elution with 10% MeOH-CHCl3 crude compound 2, and elution with 15% MeOH-CHCl3 crude compound 3. Fractions eluted with 3% AcOH-MeOH were combined and the whole was submitted to a short SiO2 (70-230 Mesh) column. Elution with 10% MeOH-CHCl3 gave crude compound 4.

The fraction consisting of crude compound 1 was submitted to an Al2O3 column (Al2O3 200 mg, column φ =0.4 cm, l=1.4 cm) and elution with CHCl3 gave compound 1 as a syrupy residue (3.1 mg). The fractions containing compound 2 and compound 3 as the main components were also purified by use of Al2O3 column respectively and pure compound 2 (4.2 mg) and compound 3 (1.7 mg) were obtained. The fraction containing compound 4 as the main component was applied to a SiO2 column (SiO2 (230-400 Mesh) 200 mg, φ =0.4 cm, l=2.7 cm). Elution with 2% MeOH in ammonical CHCl3 gave pure compound 4 (1.4 mg).

The UV spectrum of compound 1 possessing the absorption maxima at 208, 255.5, and 280 (inf.) nm, was characteristic to an oxindole alkaloid. The 1H-NMR spectrum (500 MHz, CDCl3) showed signals due to N=CH3 (δ 2.25 (3H, s)), N-OCH3 (δ 3.97 (3H, s)), and a vinyl group standing on a quaternary carbon (δ 4.96 (1H, dd, J=17.8, 1.3 Hz), δ 5.15 (1H, dd, J=11.0, 1.3 Hz), δ 6.23 (1H, dd, J=17.9, 11.0 Hz)). The aromatic protons at δ 6.95 (1H, d, J=7.8 Hz), δ 7.06 (1H, dd, J=7.6, 7.6, 1.1 Hz), δ 7.29 (1H, ddd, J=7.8, 7.8, 1.2 Hz), and δ 7.46 (1H, d, J=7.6 Hz) revealed the presence of the expected aromatic part structure of oxindole ring system. The other signals were also observed to be well-resolved, and careful analysis by use of 2D HH-COSY measurement indicated compound 1 to be gelsevirine (1). (Chart 1).

Direct comparison of the spectrum with that of the authentic sample of 1 obtained from Gelsemium elegans from Thailand unambiguously proved that they were the same compound.

Compound 2 showed the UV absorption maxima at 214, 220, and 259 (plateau) nm; these peaks indicated the compound 2 has the indolenine-type chromophore. The 1H-NMR spectrum showed characteristic signals due to N=CH3 (δ 2.61 (3H, s)), a vinyl group standing on a quaternary carbon (δ 4.68 (1H, dd, J=17.6, 11.2 Hz), δ 4.79 (1H, dd, J=11.2, 1.2 Hz), and δ 4.83 (1H, dd, J=17.6, 1.2 Hz)). A proton on a carbon carrying an ethereal oxygen was observed as a broad signal at δ 5.02. Careful assignment of these and other signals with the help of 2D HH-COSY measurement revealed the compound to be koumine (2). The identity was confirmed by direct comparison of the spectrum with the authentic one we had in our laboratory.

Compound 3 was shown to be an oxindole alkaloid as was evidenced by the UV spectrum possessing the absorption maxima at 208, 251, and 279 (inf.) nm. The 1H-NMR spectrum revealed the presence of the signals due to N=CH3 (δ 2.25 (3H, s)), a vinyl group on a quaternary carbon (δ 4.94 (1H, d, J=17.8 Hz), δ 5.10 (1H, d, J=11.0 Hz), and δ 6.24 (1H, dd, J=17.9, 11.0 Hz)). The aromatic protons at δ 6.95 (1H, d, J=7.8 Hz), δ 7.06 (1H, dd, J=7.6, 7.6, 1.1 Hz), δ 7.29 (1H, ddd, J=7.8, 7.8, 1.2 Hz), and δ 7.43 (1H, d, J=7.6 Hz) revealed the presence of a quaternary carbon and a quaternary nitrogen and a quaternary carbon was evidenced by an AB-
type protons at $\delta$ 2.31 (1H, d, $J=10.2$ Hz) and $\delta$ 2.78 (1H, d, $J=10.2$ Hz). Further detailed examination of the spectrum clearly indicated that compound 3 was gelsemine (3). Direct comparison of the $^1$H-NMR spectrum and TLC and HPLC behaviors fully verified this conclusion.5~

The UV spectrum of compound 4 possessing the absorption maxima at 229, 242, 248, 294, 341, and 386 nm strongly indicated that this alkaloid has the chromophore of indolo[2,3-a]quinolindium derivative. The high polarity of compound 4 as evidenced by the slow running on TLC plates supported the amphoteric molecular structure of compound 4. The $^1$H-NMR spectrum (CD$_3$OD) showed the signals due to the expected 8 aromatic protons ($\delta$ 7.46 (1H, d, $J=7.7$, 7.7 Hz), $\delta$ 7.70 (1H, d, $J=7.7$, 7.7 Hz), $\delta$ 7.79 (1H, d, $J=8.3$ Hz), $\delta$ 8.32 (1H, d, $J=8.1$ Hz), $\delta$ 8.52 (1H, br s), $\delta$ 8.54 (1H, d, $J=7.0$ Hz), $\delta$ 8.71 (1H, d, $J=7.0$ Hz), $\delta$ 9.05 (1H, s). In addition to the above signals we observed signals due to 8 aliphatic protons at $\delta$ 3.24 (2H, br dd, $J=6.6$, 6.6 Hz), $\delta$ 3.09 (2H, br dd, $J=5.8$, 5.8 Hz), and $\delta$ 2.01 (4H, m). All the above observation indicated the structure of compound 4 to be sempervirine (4). This structural deduction was confirmed to be correct by comparison of the obtained $^1$H-NMR spectral data with those reported for sempervirine (4).5~

As described thus far we succeeded in isolating four alkaloids from a medicine stored in Shosoin. The molecular structures of the obtained alkaloids definitely indicated that the original plant of the medicine was a species of *Gelsemium* (Loganiaceae). Three species of *Gelsemium* are known in the world, one Asian (*G. elegans* Benth.) and two North American (*G. sempervirens* Ait., and *G. rankinii* Small).7~ The fact that koumine (2), an alkaloid characteristic to the Asian species, was isolated indicated that the species to be *G. elegans*. Comparison of the pattern of HPLC also supported this view. It is needless to say that in the 7th or 8th Century there was no chance for American species of *Gelsemium* could arrive at Shosoin in Nara, Japan.

It was truly amazing to find that four *Gelsemium* alkaloids have been isolated from the more than 1250 year old drug material in such high purity. Though the name of toxic crude drug Yakatsu (治鬱) was known in old Chinese literatures, the real object was not known even in China not saying about Japan. In Honzo-komoku (本草綱目), an old Chinese encyclopedia of herbal medicines originally written by Li Shi-zhen (李時珍) in 1590 in the era of Ming Dynasty, there is the heading for a herbal medicine Ko-Fun (Gou Wen). Under this heading the author referred several
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Chart 2. Structures of four monoterpenoid indole alkaloids isolated from Yakatsu stored in Shosoin repository.

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