

mination with an authentic sample, I was confirmed to be identical with decursin. Similarly, the compound II was identified as decursinol,<sup>2)</sup> a hydrolysis product of decursin.

During this investigation nodakenetin a known coumarin from the root of *Angelica decursiva* FR. et SAV., was not isolated. However, from the fact that decursin is common to *Angelica gigas* NAKAI and *A. decursiva* FR. et SAV., it can be considered that the both species are closely related taxonomically to each other, although the latter was formerly regarded as a species of genus *Peucedanum*.

### Experimental

**Extraction and Isolation of the Compounds**—The dried and crushed root (1.7 kg) of the plant cultivated in Kang won (江原) Province, Korea, was extracted with ether (15 liter) at a room temperature for 3 weeks. The ether solution was concentrated to yield brown colored viscid oil (50 g). The isolation of the compounds from the ether extract was carried out as shown in Chart 1.

**Decursin (I)**—Recrystallized from EtOH to colorless prisms, mp 111°, yield 0.8 g. *Anal.* Calcd. for  $C_{19}H_{20}O_5$ : C, 69.50; H, 6.14. Found: C, 69.75; H, 6.26.  $[\alpha]_D^{25} +142^\circ$  ( $c=1.3$ ,  $CHCl_3$ ), IR  $cm^{-1}$ : 1730, 1690 (C=O); 1650 (C=C); 1630, 1565, 1495 (aromatic C=C). NMR ( $\tau$ )<sup>3)</sup>: 2.55, 3.98 (2H, doublet  $J=9.5$  cps,  $CH=CH$ ); 2.93, 3.39 (2H,  $2 \times$  aromatic H); 4.41 (1H, multiplet,  $(CH_3)_2C=CH$ ); 5.0, 7.0 (ABX pattern,  $CH-CH_2$ ); 7.87, 8.12 (6H, doublet  $J=1.0$  cps,  $(CH_3)_2C=CH$ ); 8.65 (6H,  $2 \times C-CH_3$ ). Melting point was not depressed on admixture with an authentic sample of decursin.

**Decursinol (II)**—Recrystallized from  $n-C_6H_{14}$  to colorless needles, mp 178°, yield 0.5 g. *Anal.* Calcd. for  $C_{14}H_{14}O_4$ : C, 68.28; H, 5.73. Found: C, 68.23; H, 5.78.  $[\alpha]_D^{25} +18^\circ$  ( $c=1.0$ ,  $CHCl_3$ ). IR  $cm^{-1}$ : 3350 (OH); 1725 (C=O); 1630, 1565, 1495 (aromatic C=C). NMR ( $\tau$ )<sup>3)</sup>: 2.43, 3.83 (2H, doublet  $J=9.5$  cps,  $CH=CH$ ); 2.82, 3.15 (2H,  $2 \times$  aromatic H); 6.12, 7.03 (3H, ABX pattern,  $CH-CH_2$ ); 7.35 (1H, singlet  $HO-CH$ ); 8.60 (6H,  $2 \times C-CH_3$ ). Melting point was not depressed on admixture with an authentic sample of decursinol.

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3) Measured in  $CDCl_3$  solution by Varian Associates Recording Spectrometer A 60, TMS was used as inner standard.

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### Gas Liquid Chromatography of Steroids of Ch'an Su. II.<sup>1)</sup> Reinvestigation on the Determination of Bufadienolides<sup>2)</sup>

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In the previous paper,<sup>1)</sup> the authors presented a gas chromatographic method for the determination of cinobufagin which is generally a major bufadienolide in Ch'an Su (Senso).<sup>4)</sup>

1) Part I: K. Sakurai, E. Yoshii, and K. Kubo, *Yakugaku Zasshi*, **84**, 1166 (1964).

2) Presented at the 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April, 1967.

3) Location: a) Gofuku, Toyama; b) Umezawa-cho, Toyama.

4) J.P. Ruckstuhl and K. Meyer, *Helv. Chim. Acta*, **38**, 883 (1955).

The next abundant and pharmacologically significant principles such as resibufogenin<sup>5)</sup> and bufalin,<sup>6)</sup> however, could not have been analyzed since they showed overlapped peaks from serious tailings. It has been thought that this unfavorable result might have come from the partial decomposition of these compounds in either preheater or stainless steel column or in both.

Recently the use of a glass column and on-column injection technique have been suggested for the gas chromatography of compounds sensitive to catalytic decomposition on metal surface. The present paper describes a successful application of this technique for the solution of the due problem by which a systematic analysis of Senso bufadienolides was established.

### Experimental

**Apparatus**—Gas chromatographic data were obtained with a Shimadzu Model GC-1C equipped with a hydrogen flame ionization detector and an on-column injection set-up. A glass column of 4 mm in diameter and 187.5 cm in length packed with 1.5% SE-30 on silanized Chromosorb W 60/80 mesh was used. The column was maintained at 230° with a nitrogen flow rate of 37 ml per minute. For on-column injection the top part of the column was maintained between 235 and 240° by means of a tape heater. The recorder settings were generally as follows: sensitivity, 1000 mΩ; recorder range, 3.2 V; chart speed, 5 mm/min.

**Calibration Curves**—Calibration curves of five bufadienolides were obtained as trimethylsilyl ethers according to the previous paper. Progesterone was used as an internal standard.

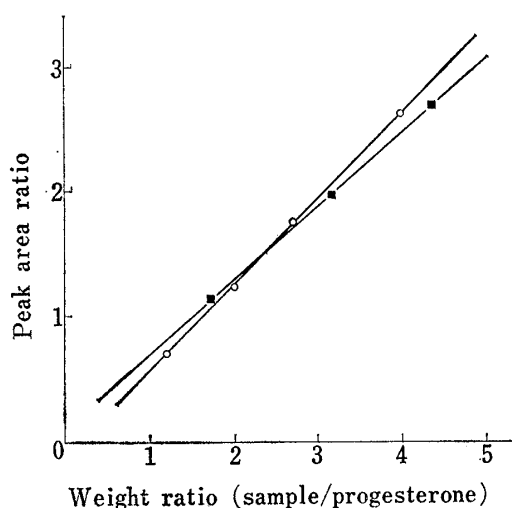


Fig. 1. Calibration Curves

Cinobufagin —■—  
Resibufogenin —○—

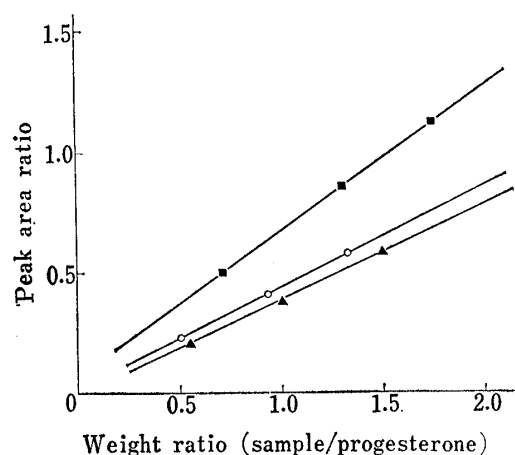


Fig. 2. Calibration Curves

Bufalin —■—  
Bufotalin —○—  
Cinobufotalin —▲—

**Determination of Bufadienolides in Ch'an Su**—About 15 g of crashed and pulverized Ch'an Su was thoroughly mixed with sea sand (30/40 mesh) of the same weight and extracted with a Soxhlet apparatus with 70 ml of chloroform containing 1% ethanol. The extraction was repeated twice, each for two hours. The extracts were combined and evaporated under a reduced pressure to dryness. The residue was dissolved in fresh chloroform and brought exactly to 25 ml in a measuring flask. Five milliliters of the stock solution was pipetted and adsorbed on a column (1 cm in diameter) of neutral alumina (8 g, Woelm, Activity 1). Elution of the column was made with a mixture of chloroform and methanol (97:3) until cinobufotalin was completely removed from the column. Generally 80 ml of the solvent was required for this procedure as determined by thin-layer chromatography.<sup>7)</sup> The dried eluate was dissolved in 5 ml of anhydrous tetrahydrofuran and 1 ml of it was subjected to trimethylsilylation by the usual method. The amounts of bufadienolides were gas chromatographically determined by the use of calibration curves.

5) M. Okada and F. Sakai, *Itsum Kenkyusho Nempo*, **11**, 67 (1960).

6) M. Okada and T. Ishihara, *Itsum Kenkyusho Nempo*, **11**, 67 (1960).

7) M. Komatsu, T. Kamano, and M. Suzuki, *Bunseki Kagaku*, **14**, 1049 (1965).

## Result and Discussion

In Fig. 3 was presented a gas chromatogram of a mixture of five authentic bufadienolides which are most frequently found in Ch'an Su. Each compound showed well-defined symmetrical peak without any notable tailing or decomposition. Retention times obtained in the present investigation are recorded in Table I. For obtaining the best chromatogram the temperature for vaporization of the samples was maintained at 235–240°. Above 240° some decomposition was observed with resibufogenin and cinobufotalin. At the temperatures lower than 235° significant tailings were noted with resibufogenin and bufotalin giving overlapped peaks. Most noteworthy here is the success in the separation between resibufogenin and bufalin. Under the conditions described in the previous paper (with stainless steel column at 235° and with the temperature for vaporization at 290°)<sup>1)</sup> shapes and separation of their peaks were far from practical use for quantitative analyses.

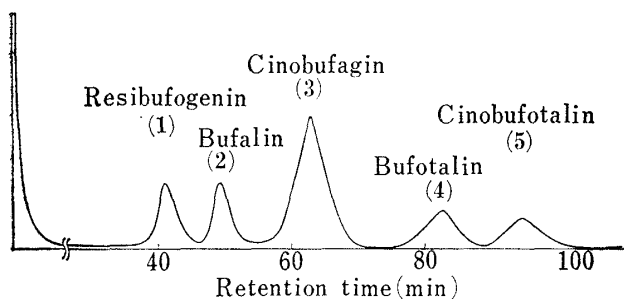


Fig. 3. Gas Chromatogram of a Mixture of Trimethylsilyl Ethers of Toad Bufadienolides

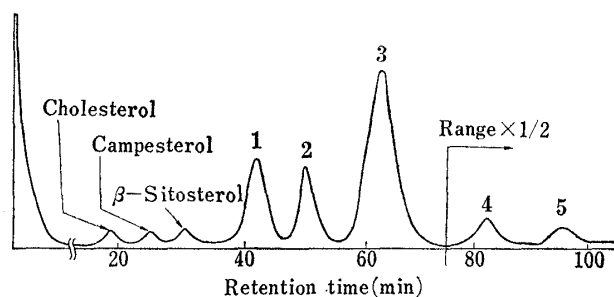


Fig. 4. Gas Chromatogram of Trimethylsilyl Ethers of Steroids in a Large Disk Ch'an Su

TABLE I. Retention Times of Trimethylsilyl Ethers of Steroids in Ch'an Su

	Retention time (min)	Relative retention time
Cholesterol	19.3	1.00
Campesterol	24.8	1.23
$\beta$ -Sitosterol	30.6	1.59
Resibufogenin	42.3	2.19
Bufalin	50.0	2.59
Cinobufagin	63.8	3.30
Bufotalin	82.0	4.24
Cinobufotalin	93.6	4.84
Progesterone <sup>a)</sup>	8.0	0.41

a) Used as an internal standard for calibration.

A sample of Ch'an Su was thus processed as described in the experimental section. A chromatogram obtained was shown in Fig. 4 and revealed that it is quite satisfactory for the determination of representative steroids in Ch'an Su. Based on the calibration curves two different products of Ch'an Su—large disk<sup>8)</sup> and small disk<sup>9)</sup> were analyzed for their bufadienolides. The result was summarized in Table II.

It should be emphasized that there was a remarkable difference in the relative amounts of bufadienolides depending on the shapes or origins. Cinobufagine was the most abundant bufadienolide in large disk Senso, whereas in small disk the amount of resibufogenin exceeds

8) Approximate size was 7 cm in diameter and 1.5 cm thick weighing 120 g.

9) Approximate size was 3 cm in diameter and 0.5 cm thick weighing 8 g.

TABLE II. Determination of Bufadienolides in Ch'an Su

Sample		Cinobufagin	Resibufogenin	Bufalin	Bufotalin	Cinobufotalin
Large Disk <sup>8)</sup>	a)	6.0	2.3	1.6	0.73	0.68
	b)	6.9	2.8	1.7	0.76	1.1
Small Disk <sup>9)</sup>	a)	5.9	7.1	3.3	1.1	—
	b)	4.4	5.6	2.2	0.99	—

a) Purchased January, 1967.

b) Purchased May, 1965.

that of cinobufagin. Also noted was that even with the same type of Ch'an Su some difference in the relative amount of bufadienolides was observed depending when purchased.

Komatsu and Okano have separated all bufadienolides in Ch'an Su on a thin-layer of alumina and densitometrically analyzed seven of them.<sup>10)</sup> For the characterization of bufadienolides thin-layer chromatography could be most conveniently carried out. However, when applied to a quantitative analysis of such complex mixture as Senso bufadienolides, it is difficult to expect reliable and reproducible result, being governed by factors which should be strictly controlled. The present authors believe that the gas chromatographic method presented here should be recommended for the determination of bufadienolides in Ch'an Su from the stand point of reliability and simplicity.

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10) M. Komatsu and S. Okano, *Bunseki Kagaku*, **15**, 1115 (1966).