General Procedure for Synthesis of 2-Aryl-3-alkyl-3-methoxyacrilonitrile——A mixture of 0.1 mole of acylarylacetonitrile, 30 cc. of $(CH_3)_2SO_4$, 33 g. of $NaHCO_3$, 8 cc. of H_2O, and 72 cc. of dioxane was heated with stirring at 87° for 2 hr. After washing the benzene layer with H_2O and drying over anhyd. Na_2SO_4, benzene was distilled off. The residue was dissolved in 50 cc. of EtOH and 10.6 g. of guanidine hydrochloride, EtONa solution (2.6 g. of Na, 30 cc. of EtOH) were added, and the solution was refluxed for 3~4 hr. After allowing to stand overnight and removal of EtOH, 30% NaOH solution was added to the residue. The insoluble product was collected on a filter, dissolved in glacial AcOH, and the solution was made alkaline with 2*N* NaOH. The precipitate was collected and recrystallized from EtOH.

General Procedure for Synthesis of 2,4-Diamino-5-alkyl-6-hydroxypyrimidine——To a solution of 4.6 g. of Na dissolved in 60 cc. of dehyd. EtOH, 13.9 g. of guanidine hydrochloride and then 0.15 mole of ethyl alkylcyanoacetate were added slowly. After refluxing for 5 hr., EtOH was evaporated in a reduced pressure and the residue was diluted with 100 cc. of H_2O with stirring. The solution was filtered, the filtrate was neutralized to pH 6.2 using Bromothymol Blue paper. Isolated product was recrystallized from hydr. EtOH. Five compounds synthesized are listed in Table VI.

Summary

To find chemotherapeutic agents for influenza and polio virus and adeno virus, alkylbiguanide, arylbiguanide, 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine, 4-amino-6-arylamino-2-alkyl-1,3,5-triazine, 2,4-diamino-5-alkyl-6-hydroxypyrimidine, and 2,4-diamino-5-aryl-6-alkylpyrimidine were synthesized. In process of the synthesis of these compounds, it was found that the reaction of arylbiguanide with ethyl ester of carboxylic acid afforded, without the presence of alkaline catalyst, 4-amino-6-arylamino-2-alkyl-1,3,5-triazine. None of the compounds of this series showed activity on any of the viruses. Among these compounds, however, butyl- aned hexyl-biguanide and 1-(*p*-tolyl)-, 1-(*o*-methoxyphenyl)-, 1-(*p*-methoxyphenyl)-, 1-(*o*-ethoxyphenyl)-, and 1-(*p*-ethoxyphenyl)-4,6-diamino-2,2-dimethyl-1,2-dihydro-1,3,5-triazine had inhibitory action on the growth HeLa cells.

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142. Yukio Akahori : Principles of *Peucedanum japonicum* Thunb. I. Isolation of New Compounds; Peucedalactone, Iso-peucedalactone, and Peucin.

(*Shizuoka College of Pharmacy*[*1])

The root of *Peucedanum japonicum* Thunb. (Umbeliferae) was used as a substitute for Ginseng (*Panax Ginseng* Nees) for tonics and therapeutics for many kinds of diseases during the Tokugawa Era (17~19th Century), because the root has a similar outward appearances as Ginseng. However, chemical studies on this plant have not yet been reported and following three new compounds were isolated in the present series of work.

The neutral portion of ether extract from the roots collected in Izu peninsula was separated by fractional distillation in vacuum as shown in Chart 1. From the fraction II, one crystalline compound, m.p. 187.0~187.5°, $C_{12}H_8O_4$, was obtained and was identified as bergapten by ultraviolet spectrum,[1] paper chromatography[2] (Table I), infrared spect-

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1) T. Yoshida : Kôryô, No. 50, 4 (1958).

2) R. Fujita, T. Furuya : Yakugaku Zasshi, **76**, 535 (1956).

Table I. Paper Chromatography[a]

		Method A		Method B
Method	Paper	Toyo Roshi No. 50 (2×40 cm.)		Same as A, except that paper was soaked in propylene glycol-water (2:8) and dried
	Eluting solvent	Petr. benzine (60~70°)-benzene-H_2O (5:4:2)		Petr. benzine (60~70°)
	Temp.	25~27°		25~27°
	Eluting time	5~6 hr.		2~5 hr.
	Detection	UV irradiation		UV irradiation
Rf value	Bergapten (from the plant)		0.85	0.27
	Bergapten (authentic sample)		0.85	0.26
	Compound A		0.86	0.03
			0.86	0.28
	Peucedalactone		0.66	0.03
	Isopeucedalactone		0.89[b]	0.30[b]
	Peucin		0.92	0.00
	Acetyl compound of (A)		0.94	
	Acetyl peucedalactone		0.93	

a) The method was essentially the same as that reported by Fujita and Furuya.[1]
b) Temperature was 7°.

rum,[1] and mixed melting point. Ultraviolet spectrum and mixed melting point of the nitro compound[3] were also used for further identification.

Fraction III was a slightly yellow resin from which no crystalline compound was obtained. At least two carbonyl absorptions were seen in its infrared spectrum (Fig. 2). Ester or lactone group was detected by color reaction and the absence of carbonyl group was presumed from test with carbonyl reagents. Ultraviolet spectrum of this fraction (Fig. 1) and pale blue fluorescence after saponification suggested skeleton like coumarins. From its saponification product, acid portion was separated, from which two fractions, b.p_1 50° and b.p_1 210°, were obtained by vacuum distillation. The first fraction was a carboxylic acid and was named tentatively as "p-acid," which will be discussed in the

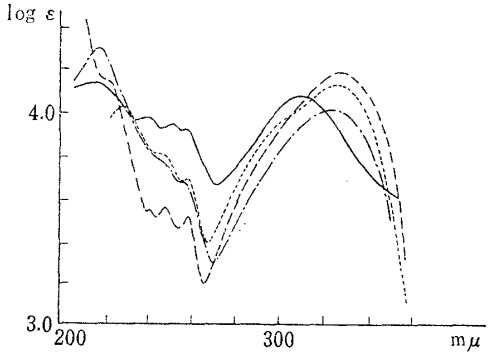


Fig. 1. Ultraviolet Spectra

——— Nitrobergapten
····· Fraction III
– – – Peucedalactone, Isopeucedalactone, Compound A, Acetylpeucedalactone[a]
—·—· Peucin

a) All these spectra were almost overlapped.

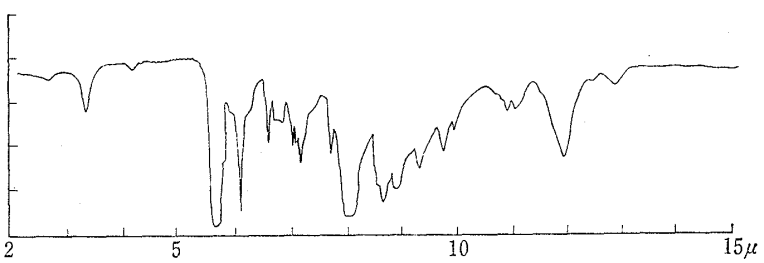
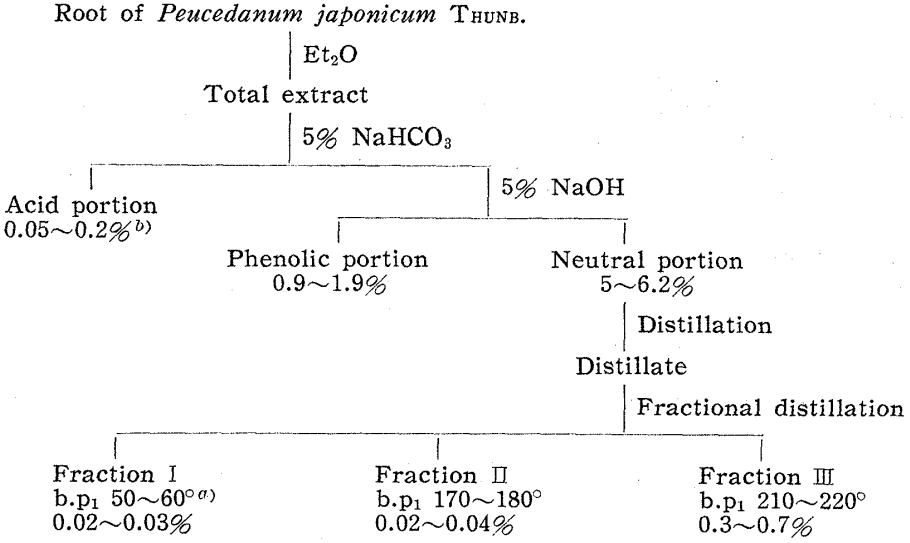


Fig. 2. Infrared Spectrum of Fraction III (melted film)

3) M. Pomeranz : Monatsh., **14**, 28 (1891).

following paper. Two crystalline compounds, compound (A), m.p. 144.0°, $C_{16}H_{18}O_5$, and compound (B), m.p. 159.0°, $C_{16}H_{18}O_5$, were obtained from the second fraction. Careful examination, including paper chromatography (Table I), of compound (A) and its acetate proved that it is a mixture of compound (B) and another compound (C), m.p. 122.5°, $C_{16}H_{18}O_5$, which were separated from each other by alumina chromatography. These compounds (B) and (C) no longer had acidic property, which suggested that lactonization had occurred during a distillation. Their infrared spectra indicated the presence of a hydroxyl group (3480 cm^{-1}) and a lactone ring (1728 cm^{-1}). Their ultraviolet spectra (Fig. 1) and blue fluorescence after saponification suggested the presence of a skeleton like coumarins. Acetylation of compound (B) gave monoacetyl compound, $C_{18}H_{20}O_6$, m.p. 167.0~167.5°, which suggested, together with disappearance of infrared absorption at 3480 cm^{-1}, that the compound (B) had only one hydroxyl group. Ultraviolet spectrum of this acetate (Fig. 1) indicated that this acetate group had no spectral effect on the conjugated system. The compounds (B) and (C) were respectively named "peucedalactone" and "isopeucedalactone."

In the same plant growing in Fukuoka Prefecture, the components were proved to be different from those of the plant from Izu peninsula. After single distillation of the neutral portion, a crystalline compound of m.p. 291.5~292.0°, $C_{15}H_{14}O_4$, deposited from the distillate. Although the hydroxamic acid-ferric chloride reaction showed a negative result, its ultraviolet spectrum (Fig. 1), infrared spectrum (1728 cm^{-1}), and saponification test of this crystalline compound indicated that it should contain one lactone group and a very similar skeleton as those of peucedalactone and isopeucedalactone. It had no methoxyl nor methylenedioxy group. The data of paper chromatopraphy are shown in Table I. This compound was named "peucin." The mother liquor after separation of peucin was fractionated into three parts by the same method as illustrated in Chart 1. From fraction II, a crystalline compound, m.p. 62.5~63.2°, $C_{16}H_{32}O_2$, was obtained instead of bergapten and was identified as palmitic acid,*2 which was further confirmed as its *p*-bromophenacyl ester.



a) All b.p.s were measured at 1 mm. Hg.
b) All yields were calculated on the weight of dried root.

Chart 1. Isolation Method[7]

*2 It is reasonable to obtain palmitic acid from the neutral portion because salts of long-chain fatty acid can be somewhat soluble in ether.

Experimental

Extraction and Isolation of Each Fraction——The crushed root of a plant from Izu peninsula was continuously extracted with Et_2O. The heating period was limited to 2 days to avoid thermal decomposition. Separation into acidic, phenolic, and neutral fractions was carried out as shown in Chart 1. The acid portion was slightly brown viscous oil, which showed pale violet fluorescence in an alkaline solution. The phenolic portion was a brown semi-solid having a specific odor. The neutral portion was a yellow fragrant paste and it was positive to hydroxamic acid-ferric chloride reaction (lactone-ester test). This neutral portion was distilled at 1 mm Hg. The first distillation was made rapidly without fractionation, because prolonged heating tended to decrease the yield and the distillate was redistilled fractionally.

Bergapten——0.8 g. of fraction II was allowed to stand at room temperature and the deposited white crystals were triturated with cold Et_2O, filtered, and recrystallized repeatedly from Et_2O. Colorless needles, m.p. 187.0~187.5°. Mixed melting point with bergapten did not show any depression. *Anal.* Calcd. for $C_{12}H_8O_4$: C, 66.67; H, 3.73; 1 OCH_3, 14.35; mol. wt., 216.18. Found: C, 66.66; H, 3.66; OCH_3, 14.41; mol. wt. (micro rast), 206.51.

Nitrobergapten——This was prepared from the above sample by the method of Pomeranz.[3] The mixed melting point with the authentic sample did not show any depression and its ultraviolet spectrum (Fig. 1) was also used for identification.

Saponification of Fraction III——15 g. of fraction III was gently refluxed with 10% EtOH-KOH in N_2 current for 1 hr. After cool, most of the solvent was removed in vacuum and 50 cc. of cold water was added to the residue. The resulting yellow solution was extracted with Et_2O to remove neutral compounds. Evaporation of the Et_2O solution gave 800 mg. of slightly brown paste. The aqueous layer was acidified with dil. HCl with cooling and extracted with ten 20-cc. portions of Et_2O. Ether was evaporated from the extract in a reduced pressure and the oily residue (14.0 g.), which had a slightly brown color and very strong odor, was distilled in vacuum. The first fraction (50°/1 mm. Hg; 2.3 g.) was a colorless liquid (p-acid) having very strong odor like butyric acid. The second fraction (210°/1 mm. Hg; 6.1 g.) was a slightly yellow resin.

Compound (A)——By treatment of the above second fraction with Et_2O, white crystals deposited. The crystals were collected, washed with cold Et_2O, and recrystallized from dil. EtOH and then from EtOH to 1.27 g. of colorless crystals (A), m.p. 144.0°, $[\alpha]_D$ −39.8°; UV λ_{max}^{EtOH} mμ (log ε): 220 (4.13, shoulder), 248 (3.55), 259 (3.52), 327 (4.18). IR: λ_{max}^{Nujol} 1782, 3480 cm^{-1}. *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.30; H, 6.35.

Acetylation of Compound (A)——50 mg. of compound (A) was acetylated by the usual way with 1 cc. of Ac_2O and 100 mg. of AcONa by refluxing for 3 hr. Four recrystallizations from EtOH gave 19 mg. of colorless crystals, m.p. 167.0~167.5°, $[\alpha]_D$ +33.1°(MeOH). UV: λ_{max}^{EtOH} mμ (log ε): 220 (4.17, shoulder), 247 (3.59), 259 (3.55), 329 (4.17). IR: λ_{max}^{KBr} 1726 cm^{-1}. *Anal.* Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.81; H, 6.14.

Compound (B) (Peucedalactone)——Ethereal mother liquor of compound (A) was concentrated and the deposited crystals were recrystallized from Et_2O to 850 mg. of colorless needles, m.p. 159.0°. *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25; mol. wt., 290.3. Found.: C, 66.13; H, 6.44; mol. wt., 288.6. $[\alpha]_D$ +70.8°(MeOH); UV λ_{max}^{EtOH} mμ (log ε): 220 (4.17, shoulder), 247 (3.59), 259 (3.55), 329 (4.17). IR: λ_{max}^{KBr} 1732, 3485 cm^{-1}.

Acetylpeucedalactone——30 mg. of peucedalactone was acetylated by the same way as for compound (A) and 21 mg. of colorless crystals, m.p. 167.5°, were obtained. Mixed melting point with acetyl compound (A) did not show any depression. *Anal.* Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 65.00; H, 6.12. $[\alpha]_D$ +32.8° (MeOH). UV λ_{max}^{EtOH} mμ (log ε): 220 (4.16, shoulder), 247 (3.58), 259 (3.54), 329 (4.16). IR: λ_{max}^{KBr} 1732 cm^{-1}.

Compound (C) (Isopeucedalactone)——A solution of 100 mg. of compound (A) dissolved in 5 cc. of petr. ether-benzene (1:1) was chromatographed on acid Al_2O_3. By elution with 6 cc. of the solvent, 5 mg. of compound (C) was obtained after recrystallization from Et_2O. Colorless prism, m.p. 122.5°. *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.16; H, 6.12. $[\alpha]_D$ −145.6°(MeOH). UV λ_{max}^{EtOH} mμ (log ε): 220 (4.12), 248 (3.54), 259 (3.52), 327 (4.17).

Further elution of above column with benzene and MeOH gave peucedalactone, m.p. 157.5°.

Peucin——From a single distillation of the neutral portion from the Fukuoka product, white crystals separated and recrystallized from $CHCl_3$-MeOH to colorless needles, m.p. 291.5~292.0°. Soluble in H_2SO_4 with pale yellow fluorescence. Tetranitromethane test gave yellow color in $CHCl_3$, but the bromine test was negative. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46; ester number (as monolactone); mol. wt., 258.26. Found: C, 69.84; H, 5.45; ester number, 222.3; mol. wt., 262.1. $[\alpha]_D$ −163.5°(MeOH). UV λ_{max}^{EtOH} mμ (log ε): 214 (4.29), 246 (3.77, inflexion), 256 (3.67), 325 (4.02). IR: λ_{max}^{KBr} 1732 cm^{-1}.

Palmitic Acid——The slightly yellow crystals deposited from fraction II of Fukuoka product were recrystallized from MeOH to colorless lustrous scales, m.p. 62.5~63.2°. The mixed melting point with palmitic acid did not show any depression. *Anal.* Calcd. for $C_{16}H_{32}O_2$: C, 74.94; H, 12.58; mol. wt., 256.4. Found : C, 75.02; H, 12.63; mol. wt. (titration), 253.9.

Its *p*-Bromophenacyl ester : m.p. 85.3~86.0°. *Anal.* Calcd. for $C_{24}H_{37}O_3Br$: C, 63.57; H, 8.23. Found : C, 63.71; H, 8.32. Mixed melting point with the authentic sample did not show any depression and their UV and IR spectra agreed completely.

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Summary

Three new compounds, peucedalactone, $C_{16}H_{18}O_5$, m.p. 159.0°, isopeucedalactone, $C_{16}H_{18}O_5$, m.p. 122.5°, and peucin, $C_{15}H_{14}O_4$, m.p. 291.5~292.0°, were isolated from the ether extract of *Peucedanum japonicum* THUNB. Peucedalactone and isopeucedalactone were obtained, together with a short-chain fatty acid (p-acid), after saponification of a resinous distillate, $b.p_1$ 210~220°, of the extract. All these three new compounds were optically active and seemed to have coumarin-like skeleton. Some of their properties were also described. Bergapten and palmitic acid were isolated besides above compounds.

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143. Kyosuke Tsuda, Hiroshi Iizuka, Yoshihiro Sato, Atsushi Naito, und Mitsugi Kato : Untersuchungen auf dem Gebiet der mikrobiologischen Umsetzung. XIV.[1] C_1-Dehydrierung von Reichsteins Substanz S Hydrocortison, Pregnenolon und Dehydroepiandrosteron durch *Bacillus pulvifaciens*. (1).

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Betreffs der mikrobiologischen C_1-Dehydrierung des 3-Oxo-4-en-steroids sind von anderen Autoren schon die folgenden Mikroben als nutzbar angegeben worden : *Alternaria* Arten,[2] *Calonectria decora*,[2] *Didymella lycopersici* KLEB.,[3] *Fusarium solani*,[4] *Bacillus sphäricus* ATCC No. 7055,[5] *Corynebacterium simplex*[6] und *Pseudomonus testosteroni* ATCC No. 11996.[7]

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