PHARMACEUTICAL BULLETIN

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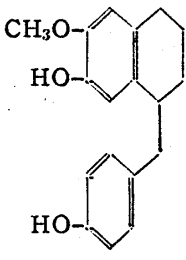
1. **Masao Tomita and Fuyuki Kusuda:** Studies on the Alkaloids of Menispermaceous Plants. CI[1]. Alkaloids of *Cocculus laurifolius* DC. (Suppl. III[2]).

(*Pharmaceutical Institute, Medical Faculty, University of Kyoto**)

The chemical structure of coclaurine, an alkaloid of *Cocculus laurifolius* DC. (Japanese name "Kohshu-uyaku"), has already been determined by H. Kondo and T. Kondo[3], M. Tomita, Nakaguchi, and Takagi[4], and by M. Tomita and Kusuda[5], by degradation and synthesis as being represented by formula (I) and this base is thought to be important as the parent base of biscoclaurine type bases.



(I)

Formerly, only coclaurine was known as the alkaloid of this plant, and no other secondary bases have been found as yet. This time, the authors made a systematic study of the alkaloids contained in this plant, and as a result, clarified the presence of several kinds of bases, quaternary as well as tertiary, besides coclaurine. The results obtained up to the present are reported in the following.

The bark and trunk of *Cocculus laurifolius* DC. collected in Kagoshima Prefecture were treated by the method described in the experimental section, and no less than six kinds of alkaloids were isolated, which are summarized in Table I, and the content of each base is given in Table II.

TABLE I

		No.	Free base	Derivative	Name
Tert. base	Phenolic base	I	m.p. 221°	HCl-salt m.p. 263～264°	Coclaurine
		II	m.p. 216～217°	HBr-salt m.p. 266°	New base
	Non-phenolic base	III	m.p. 237°	HBr-salt m.p. >300°	Trilobine
		IV		Picrate m.p. 172° (sint. 166°)	New base
Quat. base	Phenolic base	V		Chloride m.p. 253° (decomp.)	Laurifoline chloride (New base)
		VI		Picrate m.p. 88°	New base

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* Yoshida-konoe-cho, Sakyo-ku, Kyoto. (富田真雄, 楠田冬樹).

[1] Part C: J. Pharm. Soc. Japan, 72, 1232 (1952).

[2] Suppl. II: *Ibid.*, 27, 793, (1952).

[3] H. Kondo, T. Kondo: *Ibid.*, 45, 876 (1925); *ibid.*, 46, 1029 (1926); *ibid.*, 48, 324, 1156 (1928); *ibid.*, 50, 427 (1930); J. prakt. Chem., (2) 126, 24 (1930).

[4] M. Tomita, K. Nakaguchi, S. Takagi: J. Pharm. Soc. Japan, 71, 1046 (1951).

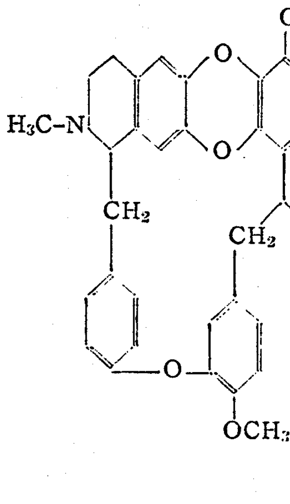
[5] M. Tomita, F. Kusuda: *Ibid.*, 72, 280, 793 (1952).

TABLE II

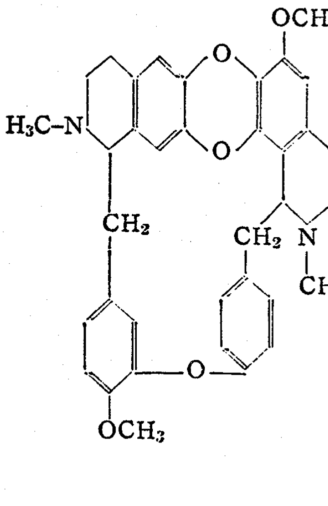
Base No.	Yield (g.) from 100 g. alc. ext.	% in bark and trunk
I	0.7～0.8	0.035～0.040
II	0.03～0.05	0.0015～0.0025
III	0.06～0.09	0.003～0.0045
IV	0.02～0.03	0.001～0.0015
V	1.3～1.5	0.065～0.075
VI	0.2～0.3	0.01～0.015

It is noteworthy that this plant furnishes a larger amount of quaternary bases than the tertiary. As a tertiary phenolic base, I-base (coclaurine) is the chief one, but during the purification of coclaurine hydrochloride sparingly soluble in water, a base forming a hydrochloride readily soluble in water was isolated in a small amount, which was named II-base. The free base forms colorless granular crystals, m.p. 216～217°, and its hydrobromide crystallizes in slightly orange pillars, m.p. 266°(decomp.), sparingly soluble in water, whereas the hydrochloride is readily soluble in water. Because of the small amount available, details of this base have not yet been examined, but it is a new one which has never been described in literature.

As a tertiary non-phenolic base, III-base, crystallizing in colorless short pillars, m.p. 237°, was obtained. The analytical values of this correspond to the composition of $C_{36}H_{36}O_5N_2$, and the base indicates $[\alpha]^{30}_{D}+281.37°$($CHCl_3$). The hydrobromide forms crystals of m.p. over 300°, and is sparingly soluble in water and in organic solvents. A concentrated sulfuric acid solution of this base gives a beautiful blue color on the addition of an oxidizing agent, such as nitric acid or potassium nitrate, which indicates that the diphenylene dioxide reaction[6] is positive. The data and the properties of the III-base agree perfectly with those given for trilobine[7], (II) or (III), an alkaloid of *Cocculus trilobus* DC.[8] (Japanese name "Aotsuzurafuji") and *Cocculus sarmetosus* Diels[9] (Japanese name "Hohzantsuzurafuji"). Eventually, the identity of III-base with trilobine, m.p. 237°, was confirmed by the mixed melting point determination.



(II)



(III)

Although the content of trilobine in this plant is very minute, being about one-tenth of that of coclaurine, this is the first instance that a biscoclaurine type base existed together with coclaurine, a parent substance of the biscoclaurine type bases. Moreover, the fact that

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[6] M. Tomita: J. Pharm. Soc. Japan, 52, 889 (1932); *ibid.*, 54, 893 (1934).
[7] M. Tomita, T. Tani: *Ibid.*, 62, 468, 476, 481 (1942); C. A., 45, 4728, 4729, 5146 (1951).
[8] H. Kondo, T. Nakazato: J. Pharm. Soc. Japan, 44, 691 (1924).
[9] H. Kondo, M. Tomita: *Ibid.*, 44, 267 (1927).

coclaurine occurs together with trilobine, possessing a diphenylene dioxide nucleus regarded as a special group even among the biscoclaurine type bases, may be considered to offer an important datum to the consideration of the biogenetic generation of biscoclaurine bases. It is also of interest from the taxonomical points of view that, by the discovery of trilobine from *Cocculus laurifolius*, the presence of trilobine could be proved throughout the genus Cocculus.

The mother liquor separated from trilobine was sirupy, and difficult to crystallize, but its picrate was obtained as a yellow crystalline powder (IV-base), m.p. 172°(sint. at 166°). The yield was very small.

Following the procedure employed in the isolation of quaternary bases from the plants of genus Magnolia[10], from the mother liquor left after separation of the above tertiary bases, two kinds of phenolic quaternary bases were obtained through the phosphotungstic acid and mercuric chloride process, which was described in the experimental part.

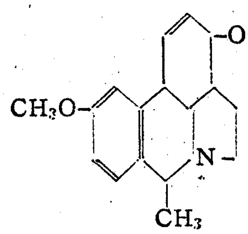
One was isolated as a chloride, crystallizing in colorless short pillars (V-base), m.p. 253°(decomp.). Its picrate forms yellow needles, m.p. 222°. The analytical values of V-base coincide with the composition of $C_{20}H_{24}O_4NCl$, and it contains two methoxyls and one dimethylimino group by Vieböck-Brecher method. The specific rotation is $[\alpha]_D^{24}+26.32°$ (H_2O). The Gaebel methylenedioxy and the Millon reactions are negative. Accordingly, since this base gives a green coloration with ferric chloride, it may be presumed that the remaining two oxygen atoms should be present as phenolic hydroxyl groups. The yield of V-base is about twice as much as that of coclaurine, and among the alkaloids contained in *Cocculus laurifolius* this base is the most predominant in amount, which is considered as the main alkaloid. This new base has been named laurifoline chloride.

The mother liquor separated from V-base (laurifoline chloride) was, as it stood, hard to crystallize, but its picrate was obtained as yellow needles, m.p. 88°. This is also a new base, but its amount is comparatively small.

Thus, it has been clarified that *Cocculus laurifolius* furnishes no less than five kinds of bases besides coclaurine. A neutral substance free from nitrogen was also obtained from this plant in considerably large quantities, but it has not yet been examined.

Recently, Yunusov[11] reported that from the leaves of *Cocculus laurifolius* growing in the region of Uzbeck, cocculidine, m.p. 86~87°, $C_{18}H_{23}O_2N$, as a non-phenolic base, and cocculine, m.p. 217~218°, $C_{17}H_{21}O_2N$, as a phenolic base, were isolated in good yield of 0.9%, and that cocculine yielded cocculidine on methylation. Subsequently, the same investigator[12] carried out the Hofmann degradation on cocculidine, and as a result, proposed for this base the formula (IV), having a phenanthridine nucleus peculiar to the Lycoris bases.

On the other hand, the authors also examined the alkaloids contained in the leaves of Japanese *Cocculus laurifolius* and identified a very small amount of a basic substance, but no such alkaloids as extracted by Yunusov could be detected. This seems due probably to the disagreement between the plant material used by Yunusov and the Japanese *Cocculus laurifolius* belonging to the Menispermaceae[13].



(IV)

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[10] M. Tomita, Y. Inubushi, M. Yamagata: J. Pharm. Soc. Japan, 71, 1069 (1951); M. Tomita, T. Nakano: *Ibid.*, 72, 197, 727, 766 (1952).

[11] S. Yunusov: J. Gen. Chem. U.S.S.R., 20, 368 (1950); C. A., 44, 6582 (1950).

[12] S. Yunusov: J. Gen. Chem. U.S.S.R., 20, 1514 (1950); C. A., 45, 2490 (1951).

[13] In 1951, M. Tomita sent a piece of the herbarium of Japanese *Cocculus laurifolius* DC. to Dr. S. Yunusov (Tashkent, Uzbeck S.S.R.) to compare the plant materials, but no communication has been received as yet.

The plant material of *Cocculus laurifolius* DC. used in this study was collected in the precincts of the Hirakiki Shrine in Kagoshima Prefecture. The authors wish to acknowledge their indebtedness to Mr. T. Tanigawa, Chief Shinto Priest, for his ready consent and assistance in the collection of the plant material ; to Dr. S. Kato, Mr. Hiyama, Mr. Kohri, and Mr. Tanno of the Osaka Factory of Dainippon Pharmaceutical Industries for according them facilities for the extraction of the material. The expenses for this research were defrayed by the Scientific Research Fund furnished by the Ministry of Education, to which the authors are also indebted.

Experimental[14]

Extraction and isolation of the alkaloids from *Cocculus laurifolius* DC.—The finely cut bark and trunk (2 kg.) of *Cocculus laurifolius* was extracted three times with boiling methanol. The extracts were combined and concentrated to a viscous residue (100 g.) under a diminished pressure. The residue was dissolved in 400 cc. of 2% sulfuric acid by warming, and filtered after cooling. The filtrate was shaken thoroughly with ether to remove acidic and neutral substances. The ether layer was washed with water and after drying, the solvent was distilled off, leaving a brownish black oil. This was dissolved in methanol and allowed to stand, yielding a neutral substance in the form of colorless pillars. Meanwhile, the sulfuric acid solution was made alkaline by the addition of sodium carbonate and the deposited basic substances were taken up in ether. The ether extract was washed with water, and after drying, the ether was removed, yielding the total crude tertiary bases (2.5 g.) as a reddish brown powder. This total base was again dissolved in approx. 50 cc. of 2% sulfuric acid, and after decoloration by charcoal, made alkaline with 10% aqueous sodium hydroxide. The deposited bases were taken up in ether, and the ether was distilled off, leaving slightly brownish amorphous non-phenolic bases (0.15 g.). They were again dissolved in 2% sulfuric acid, alkalized with aqueous sodium hydroxide, and extracted with ether. The ether extract was washed with water, dried, and the ether was removed. The residue was dissolved in a large amount of benzene and concentrated. On standing, colorless short pillars (trilobine, III-base), m.p. 237°, appeared. The yield was approx. 0.07 g.

The benzene mother liquor filtered from III-base was somewhat concentrated and extracted with 2% sulfuric acid. The sulfuric acid solution was treated with saturated sodium picrate solution, and the resulting yellow precipitate was recrystallized from hydrated acetone, yielding yellow crystalline powder (about 0.04 g.) (IV-base), m.p. 172°(sint. at 166°). The sodium hydroxide alkaline mother liquor separated from the non-phenolic bases was acidified with sulfuric acid, and after alkalization with sodium carbonate, shaken with ether. The ether solution was washed with water and evaporated to dryness. The residue was dissolved in 2% hydrochloric acid by warming, decolorized by charcoal, and the filtrate was concentrated. On standing, large quantities of needles deposited. They were recrystallized from methanol and melted at 264° (coclaurine hydrochloride, I-base) ; yield, approx. 1.0 g. The mother liquor separated from coclaurine hydrochloride, somewhat sparinly soluble in water, was again made alkaline with sodium carbonate, and shaken with ether. The ether layer was washed with water, and after drying, the ether was removed. The residue was dissolved in a small portion of methanol, and weakly acidified by addition of concentrated hydrobromic acid (48%). The solution was concentrated somewhat and allowed to stand, depositing slightly orange pillars. They were recrystallized from hot water to m.p. 266°. The crystals were dissolved in a large amount of water, alkalized with sodium carbonate, and the deposited base was taken up in ether. The ether solution was washed with water, and after drying, the solvent was removed. The residue was recrystallized from a mixture of acetone and chloroform to colorless granular crystals (II-base), m.p. 216~217°; yield, approx. 0.04 g.

The sodium carbonate alkaline mother liquor separated from the tertiary bases by ether extraction, as described above, was once filtered, and then neutralized by sulfuric acid. The solution was treated with sulfuric acid, so that the acidity could be of 5% sulfuric acid, followed by 25% phosphotungstic acid solution in 5% sulfuric acid, until no further precipitation occurred. The precipitate was decomposed with warm saturated baryta solution, and the alkaline filtrate from the barium sulfate and barium phosphotungstate was neutralized with 50% sulfuric acid to pH 3.0~4.0. The barium sulfate was again removed, and the filtrate decomposed with saturated barium chloride solution to convert the quaternary base sulfate to the chloride and at the same time, to precipitate excess sulfuric acid. This filtrate was concentrated at below 40° to approx. 50~100 cc. under reduced pressure, treated with aqueous sodium hydroxide, so that the acidity could be kept around pH 6.0, and saturated under stirring, with powdered mercuric chloride. The quaternary bases precipitated as the mercuric chloride salts (5 g.). They were dissolved in about 150 cc. of methanol, and decomposed with hydrogen sulfide. Mercuric sulfide was removed, and the filtrate concentrated under a reduced pressure, after the removal of deposited sodium chloride on the way, to approx. 5 cc. The solution was warmed on a water bath, and on addition of a few drops of

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14) All melting points are uncorrected. The authors wish to express their thanks to Mr. K. Hozumi and Mr. K. Imaeda for performing the microanalyses herein reported.

acetone, deposited laurifoline chloride (V-base) as short pillars. They were collected, dissolved in a small portion of methanol, and by the addition of acetone on warming, recrystallized. The mother liquor, after evaporating to dryness under a reduced pressure, dissolved in approx. 50 cc. of water, decolorized by a small quantity of charcoal, and submitted to the mercuric chloride process. The manipulation described above was repeated, and further amounts of laurifoline chloride were obtained as colorless short pillars, m.p. 253°(decomp.); total yield, 1.3~1.5 g. The mother liquor perfectly separated from laurifoline chloride, after being made free from organic solvents, was dissolved in approx. 30 cc. of water, and saturated sodium picrate solution added. The deposited yellow precipitate was recrystallized from hot water, yielding yellow needles, m.p. 88°; yield, 0.3 g. (VI-base).

Trilobine (III-Base)—This base was obtained from benzene as colorless short pillars, m.p. 237°, and when mixed with trilobine, m.p. 237°, obtained from *Cocculus trilobus*, no melting point depression was observed. It gives a persistent blue color with conc. sulfuric acid and nitric acid (or potassium nitrate). $[\alpha]_{D}^{30}=+281.37°$ (0.0184 g. in 7 cc. $CHCl_3$, $l=0.5$ dm.). *Anal.* Calcd. for $C_{36}H_{36}O_5N_2$: C, 74.96; H, 6.29; N, 4.86. Found: C, 74.72; H, 6.27; N, 4.92.

Laurifoline chloride (V-Base)—It crystallizes in colorless short pillars, m.p. 253°(decomp., with effervescence), and is readily soluble in water, soluble in methanol and ethanol on warming, and insoluble in other organic solvents. The Gaebel methylenedioxy reaction and the Millon reaction were both negative. It gives a green coloration with ferric chloride solution. $[\alpha]_{D}^{24}=+26.32°$ (0.0798 g. in 7 cc. H_2O, $l=0.5$ dm.). *Anal.* Calcd. for $C_{20}H_{24}O_4NCl$: C, 63.57; H, 6.36; N, 3.71; OCH_3, 16.42; $N(CH_3)_2$, 7.95. Found: C, 63.05; H, 6.58; N, 3.22; OCH_3, 14.12; $N(CH_3)_2$, 9.36.

Laurifoline chloride was dissolved in a little water, and saturated sodium picrate solution added. The yellow precipitate which resulted was collected and recrystallized from hot water, yielding the picrate of yellow needles, m.p. 222°(decomp.). *Anal.* Calcd. for $C_{20}H_{24}O_4N \cdot C_6H_2O_7N_3$: C, 54.73; H, 4.65; N, 9.82. Found: C, 54.56; H, 4.46; N, 9.63.

Summary

The authors made a systematic study of the alkaloids of *Cocculus laurifolius* DC., and as a result, clarified that besides coclaurine so far known, five other kinds of new bases were contained, as summarized in Table I.

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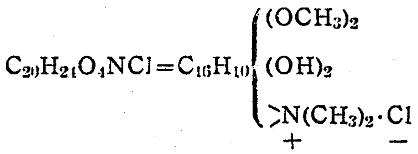
2. **Masao Tomita and Fuyuki Kusuda:** Studies on the Alkaloids of Menispermaceous Plants. CII(1). Alkaloids of *Cocculus laurifolius* DC. (Suppl. IV). Structure of Laurifoline Chloride.

(*Pharmaceutical Institute, Medical Faculty, University of Kyoto**)

In the previous paper(1), the authors reported having isolated from *Cocculus laurifolius* DC. a new phenolic quaternary base, which was named laurifoline chloride, as a crystalline chloride, m.p. 325°(decomp.), and as a result of further studies on its chemical properties, propose, for the representation of this base, the following rational formula:



$$C_{20}H_{24}O_4NCl=C_{16}H_{10}\begin{cases}(OCH_3)_2\\(OH)_2\\>\underset{+}{N}(CH_3)_2\cdot\underset{-}{Cl}\end{cases}$$

A concentrated sulfuric acid solution of laurifoline chloride is yellowish brown in color but on the addition of a drop of concentrated nitric acid, or a piece of potassium nitrate, a blue-green coloration develops which, on standing for a while, changes to a brown color. Apparently, this color

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* Yoshida-konoe-cho, Sakyo-ku, Kyoto. (富田眞雄, 楠田冬樹).

1) Part CI: This Bulletin, 1, 1 (1953).