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44. Fuyuki Kusuda: Studies on the Alkaloids of Menispermaceous Plants. CX.[1] Alkaloids of *Cocculus laurifolius* DC. (Suppl. VI)[2]. Structure of Coclanoline.*

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In the previous paper[3], it was shown that M. Tomita and Kusuda isolated from *Cocculus laurifolius* DC. a phenolic tertiary base, m.p. 216~217° (hydrobromide, m.p. 266°), in a small quantity, besides coclaurine, but the amount available was so small that no further investigation was made. Afterwards, by treatment of a larger amount of the extract (approx. 1 kg.), a new phenolic base obtainable through its crystalline oxalate other than the above-mentioned base, was identified in small quantities (approx. 0.7 g.) from the same fraction of the phenolic tertiary bases. The present paper describes the study on the chemical constitution of this new base.

As described in detail in the experimental section, coclaurine was first removed as its hydrochloride from the fraction of the phenolic tertiary bases. By the acidification of the mother liquor with hydrobromic acid, a small amount of the above hydrobromide of m.p. 226° separated. By treatment of its mother liquor, a new phenolic tertiary base was obtained as its crystalline oxalate.

The oxalate of this base crystallizes in colorless microscopic needles, m.p. 120~122°. The analytical values correspond to $C_{19}H_{23}O_4N\cdot(COOH)_2.H_2O$, containing one methylimino and two methoxyl groups by the Vieböck-Brechner method.

The free base did not crystallize by various treatment and came as a colorless amorphous powder, m.p. 90~92° (sint. at 83°), $[\alpha]_D^{18}$:+132.20° (in methanol). The Gaebel's methylenedioxy and the Liebermann's nitroso reactions are both negative. It gives a weak Millon reaction, and produces no coloration with concentrated sulfuric acid when cold, but a rose-red color gradually develops on heating. With sulfuric acid-nitric acid it gives at first a green color, which immediately changes to brown. Since this base gives a weak green coloration with ferric chloride, it is assumed that the two remaining oxygen atoms, other than in the methoxyl groups, are present as the phenolic hydroxyls. From these results, formula $C_{16}H_{12}(OCH_3)_2\cdot(OH)_2\cdot N(CH_3)$ may well be assigned to this base. This base is a new one, to which the name coclanoline has been proposed. The ultraviolet absorption spectrum of this base agrees reasonably well with that of the benzylisoquinoline type of alkaloids, as shown in Fig. 1.

The Hofmann degradation was carried out on its methyl ether methiodide. Coclanoline (I) was converted by methyl iodide, in the presence of alkali, into O,O-dimethylcoclanoline methiodide (II), which in turn was subjected to the first stage of the Hofmann degradation and led to O,O-dimethylcoclanoline methylmethine (III). This substance crystallized in the form of colorless needles, m.p. 95~96°, has the composition of $C_{22}H_{29}O_4N$, and is optically in-

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1) Part CIX: Ann. Repts ITSUU Lab. (Japan), 4, 12 (1953).
2) Suppl. V.: This Bulletin, 1, 55 (1953).
3) M. Tomita, F. Kusuda: This Bulletin, 1, 1 (1953).

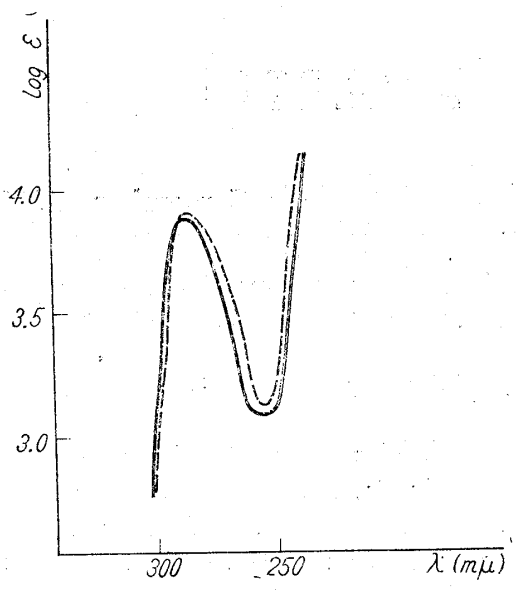


Fig. 1
—— Coclanoline (C_2H_5OH)
------ Laudanosine (C_2H_5OH)

active. Following the same procedure as described in the case of coclaurine[4] and magnocurarine[5], the methine base (III) was oxidized by potassium permanganate at a low temperature, and through the process shown in Fig. 2, arrived at the acidic substances (IV) and (VIII).

The substance (IV) crystallizes in the form of needles, m.p. 179°. On admixture with a sample of the synthetic veratric acid, m.p. 179°, no depression was observed, and for the sake of confirmation, with a sample of anisic acid, m.p. 182~184°, an evident depression occurred, melting at 140~155°. These results reveal that the substance (IV) is identical with veratric acid.

The mother liquor left after separation of the substance (IV), containing the amino acid (V), was treated with dimethyl sulfate, and yielded the methyl methosulfate (VI). The substance (VI), when subjected to the second stage of the Hofmann degradation, furnished an acid (VII) crystallizing in colorless microscopic needles, m.p. 184~184.5°, which is identical with the data of 3,4-dimethoxy-6-vinylbenzoic acid, m.p. 184°, derived from coclaurine. The acid (VII),

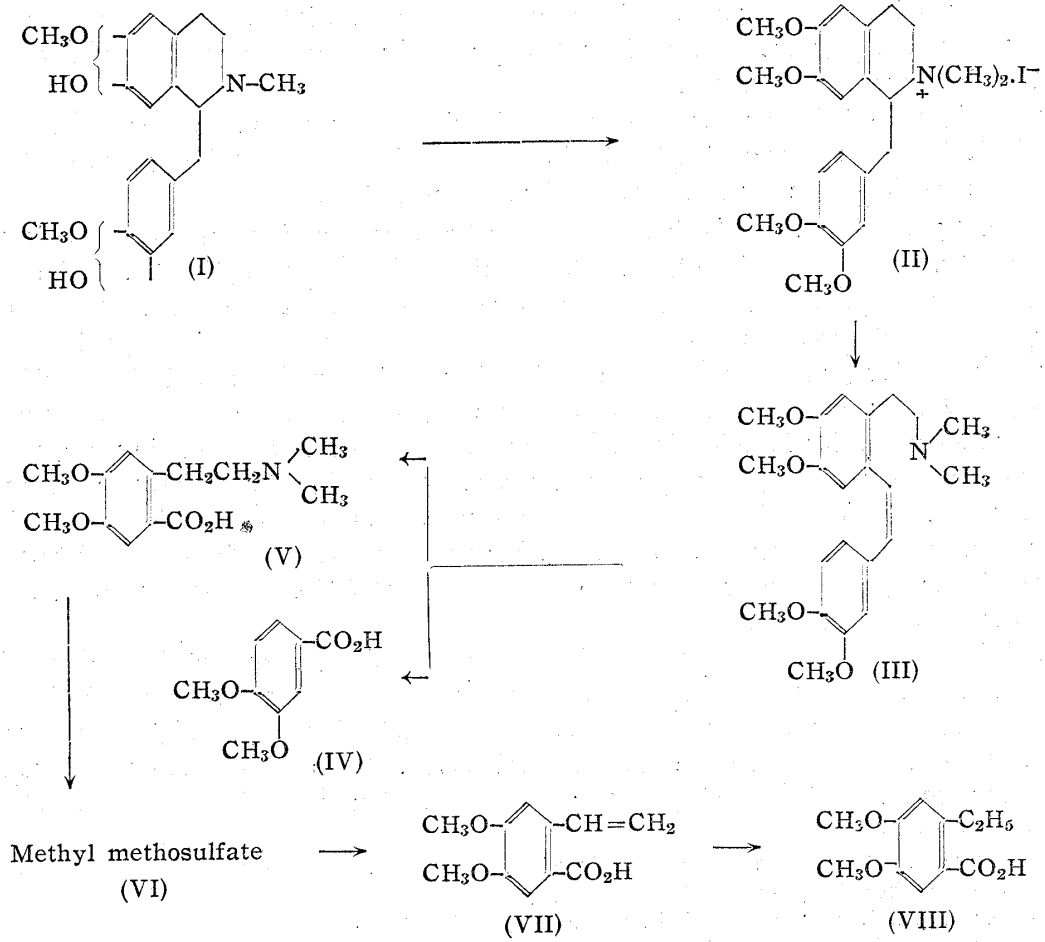


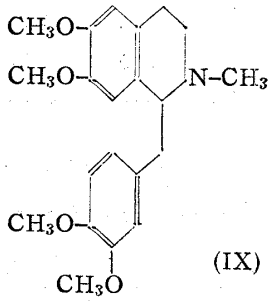
Fig. 2

on hydrogenation with Pd-charcoal as a catalyst, gave an acid (VIII) crystallizing in slightly yellowish pillars, m.p. 142°, the identity of which was confirmed by mixing with 3,4-dimethoxy-6-ethylbenzoic acid, m.p. 142~143°, derived from coclaurine.

Meanwhile, *dl*-laudanosine, m.p. 115°, was converted into the methiodide, which was then submitted to the first stage of the Hofmann degradation and led to laudanosine methylmethine[6], m.p. 96°. Thus, the identification of O,O-dimethylcoclanoline methyl methine (III), m.p. 95~96°, was established by admixture with the above authenticated methyl methine.

The foregoing experimental results indicate that O,O-dimethylcoclanoline is identical with *d*-laudanosine, its structure being represented by formula (IX).

Concerning the location of the two hydroxyl groups, a direct confirmation should be obtained by applying the same degradation reaction as described above to the O,O-diethyl derivative, but the amount of the material available was so small that this reaction could not be repeated again.



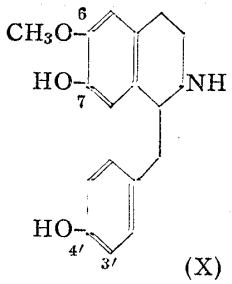
(IX)

Such being the case, it became necessary to determine their locations by comparison of the results obtained by examining the color reactions by the Gibbs[7] and the Millon reagents[8] with those given for analogous alkaloids.

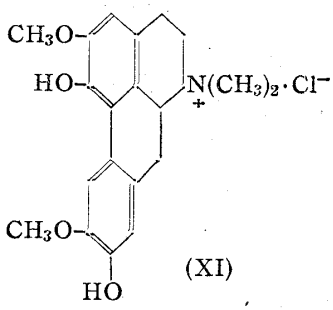
As already described, this base gives a weak green coloration with ferric chloride, but on being alkalized it causes no peculier coloration. It follows, therefore, that the two hydroxyl groups are not present in the vicinal form of the same benzene nucleus. The Gibbs reagent produces a distinct indigo blue color, which shows that this reaction is positive. The color reactions of the other related alkaloids were investigated, with the results shown in Table I.

TABLE I

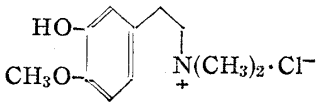
Compound	Gibbs reaction	Color
Coclaurine (X)	−	Greenish yellow
Laurifoline chloride (XI)	−	Green
Salicifoline chloride (XII)	+	Indigo blue
Coclanoline	+	Indigo blue



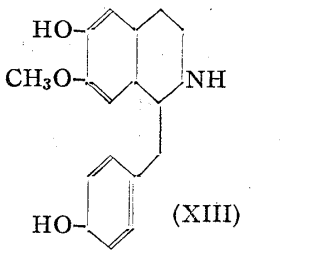
(X)



(XI)



(XII)



(XIII)

The foregoing results reveal that the para-position of the hydroxyl group in the benzyl residue is vacant, and consequently, its hydroxyl group is not present at the 4'-position as that of coclaurine, but at the 3'-position.

Concerning the position of the hydroxyl group in the benzene portion constituting the tetrahydroisoquinoline nucleus, Table II in which the Millon reaction of coclaurine (natural) is compared with that of isococlaurine (synthetic), answers the purpose.

4) H. Kondo, T. Kondo: J. Pharm. Soc. Japan, 48, 324 (1928).
5) M. Tomita, Y. Inubushi, M. Yamagata: *Ibid.*, 71, 1069 (1951).
6) H. Decker, L. Galatty: Ber., 42, 1179 (1909).
7) M. Tomita, S. Uyeo: J. Pharm. Soc. Japan, 61, 144 (1941); M. Satomi: *Ibid.*, 72, 834 (1952).
8) H. King: J. Chem. Soc., 1940, 737.

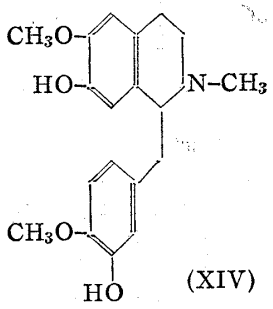
TABLE II

Compound	Millon reaction	Color
Coclaurine (X)	+	Red→Yellowish brown
Isococlaurine (XIII)	++	Blood red→Deep violet
Coclanoline	+	Red→Yellowish brown

Isococlaurine gives a typical Millon reaction. Namely, it produces on the addition of the reagent a red color even when cold, and on warming a blood red color develops, which on cooling gradually changes to a deep violet. On the other hand, in the case of coclaurine and coclanoline, a red color appears first by warming, and on cooling becomes a yellowish brown, which fact shows a weak Millon reaction. The nature of the color reaction of coclanoline is identical with those of coclaurine.

The above facts lead to the conclusion that the hydroxyl group on the tetrahydorisoquinoline nucleus in the coclanoline molecule is present at the 7-position as that in coclaurine.

From these experimental results, it appears reasonable that formula (XIV) should be put forward for coclanoline.

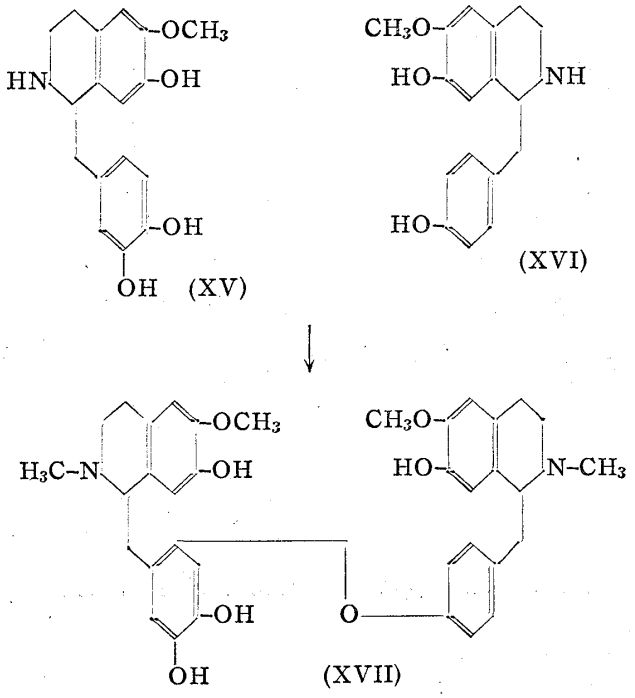


(XIV)

Although a large number of the laudanosine type alkaloids have hitherto been found in opium, this is the first instance of the discovery of this type of alkaloid from the Menispermaceous plants.

The fact that in *Cocculus laurifolius* DC. coclanoline (XIV) is present together with coclaurine (X) and laurifoline (XI) stimulates much interest on the biogensis of alkaloids in plants. Coclanoline (XIV), discovered by the present study, is a kind of hydroxycoclaurine-type alkaloids, from which laurifoline may form by undergoing dehydrogenation within the same molecule.

Formerly, M. Tomita[9] and his co-workers discussed on the constitution of magnolamine, an alkaloid of Caucasian *Magnolia fuscata* studied by Proskournina, *et al.*, and rectified the formula proposed by them, assigning formula (XVII) to this alkaloid. At that time they pointed out that in the plant body O_7-base, such as magnolamine, particularly in the biscoclaurine-type of alkaloids, might be formed by dehydrogenation between the two molecules of a hydroxycoclaurine-type base (XV) and the coclaurine-type base (XVI).



Coclanoline (XIV), which has been proved to occur naturally by the present investigation, is no doubt one of the hydroxycoclaurine-type bases (XV), and it may be said that the finding of this base in *Cocculus laurifolius* DC., which is most closely related to the Magnoliaceous plants among the Menispermaceae, has given a positive evidence to the

9) M. Tomita, E. Fujita: J. Pharm. Sco. Japan, **70**, 411 (1950); M. Tomita, E. Fujita, T. Nakamura: *Ibid.*, **71**, 1075 (1951).

above consideration of the mode of O_7-base in the biscoclaurine type.

The author is indebted to Prof. Dr. M. Tomita for his unfailing guidance during the course of this study. The author is also grateful to Prof. Dr. S. Sugasawa of Tokyo University, who provided him with the material of *dl*-laudanosine used in this study. The expenses for this study were defrayed by the Scientific Research Fund provided by the Ministry of Education, to whcih many thanks are due.

Experimental[10]

Isolation of Coclanoline—From the total phenolic bases isolated from the methanol extract of *Cocculus laurifolius* DC., coclaurine and a small quantity of (II)-base, m.p. 216~217°, were removed by the procedure described in the previous paper[3]. The mother liquor which remained was alkalized with sodium carbonate, and the depositing base was taken up in ether. The ether solution was dried over anhydrous potassium carbonate, and the solvent removed, yielding a slightly yellowish, amorphous powder. This was suspended in a small amount of water, an approximately equivalent amount of oxalic acid added, and the mixture was dissolved by warming in a water bath. After decolorization with charcoal, the filtrate was concentrated in vacuo and kept standing, depositing colorless microscopic needles. Recrystallization was effected from a mixture of methanol and acetone, m.p. 120~122°. This oxalate was dissolved in a small amount of warm water, made alkaline with sodium carbonate, and the depositing base was taken up in ether. The ether solution was dried over anhydrous potassium carbonate and the solvent distilled off. Various attempts to crystallize the residue failed. The free base thus obtained was a colorless amorphous powder.

Coclanoline (XIV)—Colorless amorphous powder, m.p. 90~92° (sint. 83°). It is insoluble in water, soluble in methanol, acetone, and chloroform, and very sparingly soluble in petroleum ether and benzene. $[\alpha]_D^{18}$: +132.20° (in methanol, l=0.5 dm., c=1.18).

Coclanoline forms an oxalate which crystallizes in the form of colorless microscopic needles, m.p. 120~122°. It is soluble in methanol, ethanol, and acetone, when warm, and hot water, but very sparingly soluble in other organic solvents. *Anal.* Calcd. for $C_{19}H_{21}O_4N\cdot(COOH)_2\cdot H_2O$: C, 57.70; H, 6.23; OCH_3, 14.18; NCH_3, 3.43. Found: C, 58.27; H, 6.52; OCH_3, 13.74; NCH_3, 3.21.

Hofmann degradation of coclanoline (XIV) (1) O,O-Dimethylcoclanoline methiodide (II)—0.6 g. of coclanoline was added to 7.5 cc. of 0.5*N* methanolic potash, followed by 4 cc. of methyl iodide. The mixture was refluxed on a water bath for 2 hrs. After the completion of the reaction, the deposited inorganic material was removed. Distillation of methanol and the excess of methyl iodide left a yellowish brown resinous product. This was recrystallized from 20% hydrated methanol and yielded yollowish brown pillars. Recrystallization was effected from methanol-acetone to slightly yellowish pillars, m.p. 200°; yield, 0.7 g. *Anal.* Calcd. for $C_{22}H_{30}O_4NI$: C, 52.91; H, 6.06. Found: C, 53.00, 53.04; H, 6.29, 6.22.

(2) O,O-Dimethylcoclanoline methyl methine (III)—0.6 g. of O,O-dimethylcoclanoline methiodide (II) was added to a solution of 6 g. of potassium hydroxide dissolved in 25 cc. of water. After heating in a water bath for 2.5 hrs., the depositing oily product was extracted with ether. The aqueous layer was again heated in a water bath, this manipulation being repeated three times. The combined ether extracts were concentrated, and extracted with 5% hydrochloric acid solution. The aqueous extract was again alkalized with potassium hydroxide and extracted with ether. The ether solution was washed with water, dried over anhydrous potassium carbonate, and the solvent removed, leaving a slightly yellowish oily substance. To this were added approx. 10 cc. of water and subsequently 0.2 cc. of concentrated hydrobromic acid. On standing, the methine base crystallized as the hydrobromide. This was collected and recrystallized from hot water to colorless needles, m.p. 210~211°; yield, 0.3 g. *Anal.* Calcd. for $C_{22}H_{29}O_4N\cdot HBr\cdot H_2O$: C, 56.17; H, 6.86. Found: C, 56.39; H, 6.88. 0.25 g. of the above hydrobromide was suspended in approx. 10 cc. of water, and alkalized by the addition of 2% aqueous potassium hydroxide. The depositing methine base was taken up in ether, and the ether solution, after being washed with water, was dried over anhydrous potassium carbonate. The residue left after removal of the solvent was dissolved in anhydrous benzene, and purified through an alumina column. Distillation of benzene left a colorless solid. Recrystallization was effected from petroleum benzine to colorless microscopic needles, m.p. 95~96°; yield, 0.17 g. *Anal.* Calcd. for $C_{22}H_{29}O_4N$: C, 71.16; H, 7.88; OCH_3, 33.42. Found: C, 71.46, 70.88; H, 7.95, 7.99; OCH_3, 32.56. $[\alpha]_D^{19}$: ±0° (in benzene, l=0.5 dm., c=1.01).

(3) Oxidation of methine base (III)—To a solution of 0.08 g. of methine base (III) dissolved in 3 cc. of acetone were added 3 cc. of water and 2.5 cc. of 0.5% sulfuric acid solution. The mixture was chilled with a freezing agent to between 0° and 5°, and 14 cc. of 5% potassium permanganate

10) All melting points are uncorrected. The author is indebted to Mr. K. Hozumi and Mr. K. Imaeda in the Microanalytical Laboratory of this Institute for the microanalyses reported herein.

solution added dropwise with stirring during the course of about 1.5 hrs. After the completion of the addition, stirring was continued for a further 30 mins., the temperture being kept between 0° and 5°. The deposited manganese dioxide was dissolved by the passage of sulfur dioxide gas, and the mixture was extracted with ether. The ether layer (A) and the aqueous layer (B) were separated.

The ether layer (A) was dried over anhydrous sodium sulfate and the solvent removed, leaving yellowish brown needles. Recrystallization from methanol yielded slightly yellowish needles, m.p. 179°; yield, 0.015 g. They were undepressed by a sample of synthetic veratric acid, m.p. 179°. For the sake of confirmation, when mixed with a sample of anisic acid, m.p. 182~184°, obvious melting point depression occurred, melting at 140~155°.

(4) **Hofmann degradation of the amino acid (V)**—The above aqueous layer (B) was made alkaline with sodium carbonate, and the resulting manganese carbonate was filtered off. The filtrate was concentrated in vacuo to approximately 10 cc., to which 1.3 cc. of dimethyl sulfate was added. The mixture was shaken for about 1 hr., and the excess dimethyl sulfate was removed with ether. To this solution was added 7 g. of potassium hydroxide and the mixture was heated in a water bath for 6 hrs. After the reaction was completed, the content was diluted to twice its volume by the addition of water, and after acidification with hydrochloric acid, extracted with chloroform while warm. The chloroform solution was washed with water, dried over anhydrous sodium sulfate, and the solvent removed. The residue was recrystallized form chlorofrom to colorless microscopic needles, m.p. 184~185.5°; yield, 0.025 g. The melting point of this substance agreed with that of 3,4-dimethoxy-6-vinylbenzoic acid (VII), m.p. 184°, derived form coclaurine.

(5) **3,4-Dimethoxy-6-ethylbenzoic acid (VIII)**—0.015 g. of 3,4-dimethoxy-6-vinylbenzoic acid (VIII) was dissolved in 10 cc. of acetone, and reduced with Pd-charcoal (1% $PdCl_2$ 0.1 cc.+charcoal 0.01 g.) as a catalyst. After the lapse of 20 mins., the absorption of hydrogen ceased. The catalyst was removed by filtration, and on removal of the solvent, yellowish brown crystals appeared. They were recrystallized from hot water to slightly yellowish pillars, m.p. 142°; yield, 0.01 g. The identity of this substance was confirmed by mixing with a sample of 3,4-dimethoxy-6-ethylbenzoic acid, m.p. 142~143°.

Hofmann degradation of *dl*-laudanosine (1) Laudanosine methiodide—0.5 g. of *dl*-laudanosine was dissolved in 4 cc. of methanol, followed by 1 cc. of methyl iodide. After gentle digestion for 2 hrs., the excess of methyl iodide and the methanol were removed, and the residue was recrystallized from methanol to almost colorless pillars, m.p. 212~213°; yield, 0.65 g.

(2) **Laudanosine methyl methine**—0.56 g. of laudanosine methiodide was added to a solution of 6 g. of potassium hydroxide in 25 cc. of water. The Hofmann degradation was effected by the same procedure as described in the case of O,O-dimethylcoclanoline methyl methine (III), and gave the free base, m.p. 96°, through its hydrobromide crystallizing in colorless needles, m.p. 211°. When the above two substances were fused with the substance (III) and its hydrobromide, respectively, no melting point depressions occurred.

Summary

A new phenolic tertiary base was discovered from *Cocculus laurifolius* DC., and was named coclanoline. The Hofmann degradation of its methyl ether methiodide gave veratric acid and 3,4-dimethoxy-6-ethylbenzoic acid. From the color reaction by the Gibbs and the Millon reagents, it was determinted that this base is a laudanosine type, the structure of which should be represented by formula (XIV).

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