Fractions 11~17 (eluted with hexane and with hexane-benzene (2:1)) yielded 90 mg. of O-methyllophocerine (Ⅲb). The picrate was recrystallized from EtOH to give 145 mg. of yellow plates, m.p. 184~185°. *Anal.* Calcd. for $C_{16}H_{25}O_2N \cdot C_6H_3O_7N_3$: C, 53.65; H, 5.73. Found : C, 53.71; H, 5.92.

From the following hexane-benzene eluate (Fract. 19~22), 15 mg. of unchanged O-methylpilocereine was recovered as colorless pillars, m.p. 152~153° (100° sinter), and identified by mixed melting point and IR comparison (in $CHCl_3$). Fract. 23~25 (eluted with benzene and with benzene-Et_2O (4:1)) yielded 30 mg. of a base. Two recrystallizations from hexane gave 15 mg. of colorless octahedrons, m.p. 130~131°. This compound showed blue color on treatment with Gibbs reagent and did not depress the melting point on admixture with the bisected phenolic base, m.p. 130~131° (see above). Fract. 27~34 (eluted with benzene-Et_2O (4:1~1:1) and with Et_2O) was converted into the picrate and recrystallized from Me_2CO-EtOH to yield 38 mg. of yellow plates, m.p. 234~235°. This picrate was identified with isopilocereine dipicrate by mixed melting point and IR comparison (in Nujol). *Anal.* Calcd. for $C_{30}H_{44}O_4N_2 \cdot 2C_6H_3O_7N_3$: C, 52.82; H, 5.28. Found : C, 52.64; H, 5.52.

Summary

The potassium-liq. ammonia cleavage of O-methylpilocereine was carried out in order to re-examine the structure (Ia) of pilocereine. In addition to the expected bases (Ⅱ, Ⅲb, Ⅳa, and Ⅲa), isopilocereine (Ia) and a phenolic base which is considered to be Ⅴ were obtained. This result and new molecular formula $C_{45}H_{65}O_6N_3$ proposed lately by Djerassi, *et al.* pointed out the structure of pilocereine to be Ⅵa. The formation of isopilocereine on treatment of pilocereine with potassium-liq. ammonia was illustrated as a normal cleavage reaction.

(Received June 25, 1963)

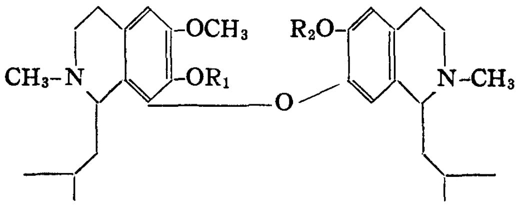
[Chem. Pharm. Bull. 11 (12) 1507 ~ 1510]

UDC 547.94 : 582.852

233. Kiyoshi Bessho[*1,*2] : Studies on Pilocereine and Related Compounds. VIII.[*3] Structures of Isopilocereine and Desmethylisopilocereine.

(*Faculty of Pharmaceutical Sciences, Kyoto University*[*1])

In the preceding papers, the structure of O-methylisopilocereine (Ia) was confirmed by potassium-liq. ammonia cleavage reaction and synthesis. This paper deals with the locations of the phenolic hydroxyl groups of isopilocereine and desmethylisopilocereine.



Ia : $R_1=R_2=CH_3$

Ib : $R_1=H$, $R_2=CH_3$

Ic : $R_1=R_2=H$

Id : $R_1=C_2H_5$, $R_2=CH_3$

Ie : $R_1=R_2=C_2H_5$

[*1] Yoshida-konoe-cho, Sakyo-ku, Kyoto (別所 清).

[*2] Present address : Institute for Chemical Research, Kyoto University, Takatsuki-shi, Osaka.

[*3] Part Ⅶ. This Bulletin, **11**, 1504 (1963).

A negative Gibbs reaction of isopilocereine suggested that the hydroxyl group would locate at the 7-position of the tetrahydroisoquinoline nucleus, *i e.*, the structure of isopilocereine would be Ib. The potassium-liq. ammonia reaction of O-ethylisopilocereine, which gave O-ethyllophocerine (II) and lophocerine (III) in quantitative yields, confirmed the above assumption.

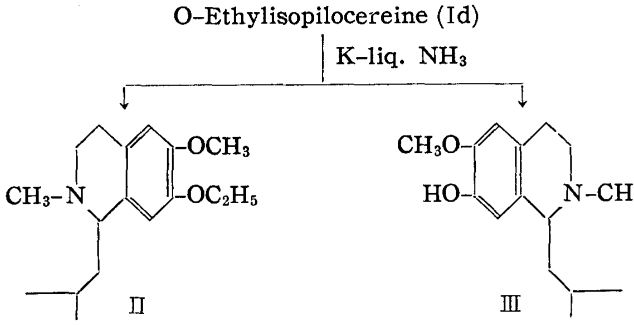
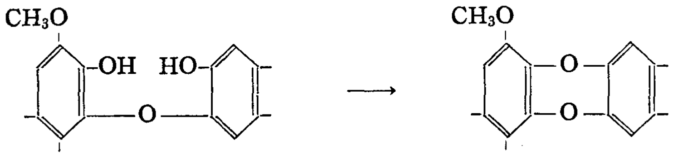


Chart 1.

Desmethylisopilocereine is supposed to be produced by demethylation of one of the methoxyl groups of isopilocereine during the potassium-liq. ammonia procedure. A partial methylation of this substance was attempted by Djerassi, *et al.*[1] who succeeded to isolate a small amount of isopilocereine picarate. They also attempted a complete methylation and obtained an oily product, infrared spectrum of which was identical with that of O-methylisopilocereine. In the experiment carried out in our laboratory, methylation of desmethylisopilocereine with diazomethane was completed on standing for 6 hours at 6° to yield O-methylisopilocereine quantitatively, which crystallized as needles melting at 91~92°.

These result suggested that desmethylisopilocereine has the structure (I) in which R_1 is H and 6-position of one of the isoquinoline nuclei bears a hydroxyl group. Of these two possibilities, the catechol type structure seemed improbable because the base resisted mild oxidation,[1] and therefore the second hydroxyl group was suppoosed to locate on the other ring, *i.e.*, $R_1=R_2=H$ (Ic). This assumption was further supported by the observation that a deep bluish-green color appeared when a piece of potassium nitrate was added to desmethylisopilocereine dissolved in concentrated sulfuric acid.*4 This color reaction has been used to detect dibenzo-*p*-dioxin nuclei. In the present case, the positive reaction means the formation of dibenzo-*p*-dioxin structure by the dehydration between two hydroxyl groups at *o,o'*-positions of the diphenyl ether linkage with concentrated sulfuric acid, as described earlier by Tomita and Inubushi[3] for demethylisotetrandrine.



In addition, the potassium-liq. ammonia cleavage of O,O-diethyldesmethylisopilocereine yielded O-ethyllophocerine (II) and 6-ethoxy-7-hydroxy compound (IV) as the bisected bases. The latter was characterized as the oxalate, m.p. 187~189°, and as the picrate, m.p. 170~171°. Furthermore, picrate of its O-methyl ether was identified with

---

*4 Pilocereine and isopilocereine are negative on this color test.

1) C. Djerassi, S.K. Figdor, J.M. Bobbitt, F.X. Markley : J. Am. Chem. Soc., **79**, 2203 (1957).

2) M. Tomita : Yakugaku Zasshi, 52, 889 (1932).

3) M. Tomita, Y. Inubushi : This Bulletin, **1**, 360 (1953).

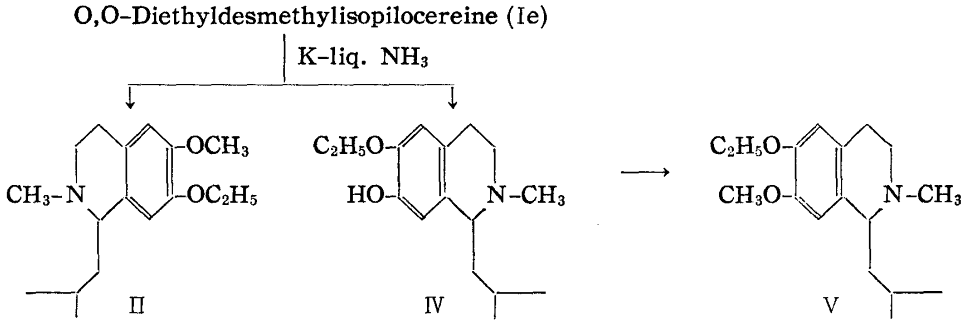


Chart 2.

Djerassi's synthetic sample of 1-isobutyl-2-methyl-6-ethoxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (V) picrate.[4]

As the results, the structure of isopilocereine (Ib) and desmethylisopilocereine (Ic) were unequivocally established.

Experimental[*5]

O-Methyltion of Desmethylisopilocereine——To a solution of 300 mg. of desmethylisopilocereine in 150 ml. of $MeOH-Et_2O$ (1:3) mixture was added an ethereal solution of diazomethane (prepared from 5 g. of nitrosomethylurea), and the mixture was kept in a refrigerator at 6° for 6 hr. The solvent was removed on evaporation and the residue was dissolved in Et_2O, washed with 10% KOH, dried over anhyd. K_2CO_3, and evaporated. The oily residue was chromatographed on Al_2O_3 (0.8 × 5 cm.) with hexane and Et_2O, and all fractions crystallized on treatment with hexane. Recrystallizations from hexane gave 310 mg. of colorless needles, m.p. 91∼92°. Identification of this compound with O-methylisopilocereine (Ia) was achieved by mixed melting point determination and IR comparison (KBr disk).

The alkaline washings and $Et_2O-MeOH$ eluate were further examined but any other base was not found.

O-Ethylisopilocereine (Id)——An ethereal solution of diazoethane (prepared from 10 g. of nitrosoethylurea) was added to an ethereal solution of isopilocereine, regenerated from 510 mg. of the dipicrate, and the solution was allowed to stand at room temperature for 4 days. Then a half quantity of diazoethane was added and the reaction was continued for an additional day. The reaction mixture showed a single spot on TLC.[*6] After usual acid-alkali treatment, the product was chromatographed on Al_2O_3 (1 × 10 cm.) with hexane-Et_2O (19:1) to give 263 mg. of Id as a colorless oil.

Cleavage of Id by Potassium in Liquid Ammonia——O-Ethylisopilocereine (Id) (180 mg.) was cleaved by the usual method with K (150 mg.) in liq. NH_3 (300 ml.) at $-58\sim-55°$ to give 90 g. of a non-phenolic base and 82 mg. of a phenolic base.

The picrate of the non-phenolic base, which was prepared in EtOH solution, was recrystallized from $Me_2CO-EtOH$ to give 160 mg. of yellow plates, m.p. 149∼150°. This was identified with O-ethyllophocerine (Ⅱ) picrate by mixed melting point and IR comparison (in Nujol). *Anal.* Calcd. for $C_{17}H_{27}O_2N \cdot C_6H_3O_7N_3$: C, 54.54; H, 5.97. Found : C, 54.41; H, 6.08.

The phenolic base was also converted into the picrate in EtOH solution and recrystallized from the same solvent to give 155 mg. of lophocerine (Ⅲ) picrate, m.p. 194∼195°. Identification was achieved by mixed melting point and IR comparison (in Nujol). *Anal.* Calcd. for $C_{15}H_{23}O_2N \cdot C_6H_3O_7N_3$: C, 52.71; H, 5.48. Found : C, 53.00; H, 5.50.

Further examination of the mother liquors of these picrates did not give any other base.

O,O-Diethyldesmethylisopilocereine (Ie)——A $MeOH-Et_2O$ (1:1) solution of 500 mg. of desmethylisopilocereine was treated with an ethereal solution of diazoethane (prepared from 10 g. of nitrosoethylurea). After 3 days, a half quantity of diazoethane was added and the mixture was allowed to react further for 2 days. The product, which showed a single spot on TLC, was purified through acid-alkali treatments and chromatographed on Al_2O_3 (1 × 10 cm.) with hexane-Et_2O (19:1) to yield 510 mg. of Ie as a colorless oil.

[*5] All melting points are uncorrected.

[*6] TLC : thin layer chromatography. Cf. the footnote in Part Ⅱ (this Bulletin, **11**, 1477 (1963)).

4) C. Djerassi, F.X. Markley, R. Ehrlich : J. Org. Chem., **21**. 975 (1956).

Cleavage of Ie by Potassium in Liquid Ammonia——The treatment of Ie (470 mg.) by the usual method with K (200 mg.) in liq. NH_3 (300 ml.) at $-63\sim-61°$ afforded 275 mg. of a non-phenolic base and 160 mg. of a phenolic base.

The non-phenolic base was converted into the picrate, Recrystallizations from EtOH yielded 250 mg. of yellow plates, m.p. $149\sim150°$, which were identified with O-ethyllophocerine (II) picrate by mixed melting point and IR comparison (in Nujol). *Anal.* Calcd. for $C_{17}H_{27}O_2N \cdot C_6H_3O_7N_3$: C, 54.54; H, 5.97. Found : C, 54.53; H, 6.20.

The free base, regenerated from the mother liquor, was distilled in vacuum (0.1 mm. Hg, at 80°) to give additional 10 mg. of II which was characterized as its picrate. The light-yellow oily residue on distillation (110 mg.) was identified with the starting material (Ie) by IR comparison (in $CHCl_3$).

The phenolic base was dissolved in EtOH and treated with 40 mg. of oxalic acid to form fine crystals. Two recrystallizations from EtOH yielded 120 mg. of colorless pillars, m.p. $187\sim189°$. IV oxalate. *Anal.* Calcd. for $C_{16}H_{25}O_2N \cdot \frac{1}{2}(COOH)_2$: C, 66.21; H, 8.50. Found : C, 66.36; H, 8.65.

The mother liquor of the phenolic base oxalate was converted to the picrate and recrystallized from EtOH to give 40 mg. of IV picrate as yellow plates, m.p. $170\sim171°$. *Anal.* Calcd. for $C_{16}H_{25}O_2N \cdot C_6H_3O_7N_3$: C, 53.65; H, 5.73. Found : C, 53.74; H, 5.90.

The free base derived from 60 mg. of the oxalate was treated with an ethereal solution of diazomethane. Chromatography of the non-phenolic product on Al_2O_3 (0.8×10 cm.) with hexane-Et_2O yielded a colorless oily base (35 mg.). This was converted into the picrate and recrystallized from Me_2CO-EtOH to give 60 mg. of yellow plates, m.p. $157\sim158°$, which were identified with 1-isobutyl-2-methyl-6-ethoxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (V) picrate by mixed melting point and IR comparison (in Nujol). *Anal.* Calcd. for $C_{17}H_{27}O_2N \cdot C_6H_3O_7N_3$: C, 54.54; H, 5.97. Found : C, 54.54; H, 6.21.

The author expresses his deep gratitude to Prof. M. Tomita and Dr. T. Kikuchi of this University and to Prof. Y. Inubushi of Osaka University for their guidances and hearty encouragements throughout these studies.

The author is also indebted to Prof. C. Djerassi of Stanford University for his valuable advices and for providing samples of several compounds. Thanks are also due to the members of Microanalytical Center of this Faculty for elemental analyses and to Dr. K. Machida, Mr. K. Hanai, Mrs. I. Hamanaka, and Mrs. M. Miyajima of this Faculty and to Mr. M. Narisada of Shionogi & Co. for measurements of IR spectra.

Summary

The structure of isopilocereine (Ib) and desmethylisopilocereine (Ic) were established by potassium-liq. ammonia cleavage reactions of their O-ethyl ethers, Id and Ie.

(Received June 25, 1963)