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128. Masao Shimizu and Fumihiko Uchimaru : The Isolation of Alkaloids from *Vinca (Lochnera) rosea* (L.) REICHB.*2

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Vinca rosea (L.) REICHB. (Japanese name "Nichinichi-so") is widely cultivated in Japan as an ornamental plant. Since the isolation of reserpine from *Rauwolfia serpentina*, studies on the alkaloidal constituents of various Apocynaceae such as Vinca, Iboga, and Alstonia have been carried out all over the world. This paper presents the survey of alkaloidal constituent of the root of domestic *Vinca rosea* grown in the Narita Farm (Chiba Prefecture) of this Company. The plant material used was collected in October, dried, and milled. The extraction was carried out as that of reserpine from *Rauwolfia serpentina*.[1]

From the weakly basic alkaloidal fraction, two crystalline alkaloids were obtained by chromatography with aluminium oxide (Activity III). The first eluted alkaloid crystallized from methanol in the form of colorless plates, m.p. 225~227°, and was proved to be tetrahydroalstonine which had been found in *Rauwolfia sellowii*.[2] This alkaloid produced a brownish violet color in the Keller reaction and exhibited ultraviolet absorptions of λ_{max}^{EtOH} 226 mμ (log ε 4.54) and 280(3.84), and λ_{min}^{EtOH} 265 mμ (log ε 3.80). The infrared spectrum showed the presence of -NH and ROOC-$\overset{|}{C}$=$\overset{|}{C}$-O-R groups. The hydrochloride, crystallized from methanol, melted at 277~280°(decomp.), and showed no depression on admixture with the authentic specimen.*3

The second eluted alkaloid crystallized from methanol in colorless prisms, m.p. 253~255°, and was found to be identical with δ-yohimbine (ajmalicine) which had been found in Rauwolfia species[3] and also in *Vinca rosea*.[4] The Keller reaction also showed brownish violet color. The UV and IR spectra were similar to those of tetrahydroalstonine, but the IR absorption in the range of 6~10 μ showed a difference between two bases. A mixture of authentic δ-yohimbine*4 and the free base showed no depression of the melting point.

These two alkaloids are the stereoisomers arising from the difference of D/E ring juncture.[5] In the paper partition chromatography, the Rf values of these bases were

TABLE I. Rf Values in Paper Partition Chromatography

Solvent	Tetrahydroalstonine	δ-Yohimbine
I	0.45	0.46
II	0.89	0.90
III	0.53	0.19

Solvent I : Ether-saturated 0.2% tartaric acid soln.
II : *n*-BuOH:AcOH:H_2O (4:1:5)
III : Stationary phase : Formamide:MeOH (7:3)
Mobile phase : Benzene: cyclohexane: formamide (1:1:saturated)

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*2 Preliminary communication : This Bulletin, **6**, 324(1958).
*3 The sample was kindly provided by Prof. R. C. Elderfield, University of Michigan.
*4 We wish to thank Dr. E. Schlittler, Ciba Pharmaceutical Products, Inc., for his kind donation of this sample.

1) E. Schlittler, *et al.* : Helv. Chim. Acta, **37**, 59(1954).
2) S. C. Pakrashi, C. Djerassi, R. Wasicky, N. Neuss : J. Am. Chem. Soc., **77**, 6687(1955).
3) A. Hofmann : Helv. Chim. Acta, **37**, 849(1954).
4) C. Djerassi, *et al.* : Chem. & Ind. (London), **1956**, 173.
5) E. Wenkert, D. K. Roychaudhuri : J. Am. Chem. Soc., **80**, 1613(1958).

essentially the same in most cases, but they were different on using the solvent of formamide and methanol (7:3) as a stationary phase, as shown in Table I.

On the other hand, a colored alkaloid was obtained from the strongly basic fraction by chromatography of reineckate, which formed yellow crystalline nitrate of m.p. 190~200°(decomp.). The ultraviolet spectrum of this alkaloid was typical of those found in anhydronium base of indole alkaloids. The ultraviolet spectrum of this alkaloid was compared with that of the pure serpentine obtained by palladium-maleic acid dehydrogenation from δ-yohimbine according to Wenkert's method,[5] and the two were found to be almost the same. In addition, the reduction of this base with sodium borohydride in methanol gave a good yield of δ-yohimbine (ca. 90%) which showed no depression on mixed fusion. On the basis of these data, it seemed reasonable to assume that above nitrate is an almost pure serpentine nitrate. The dilute alcoholic solution of these nitrates showed strong blue fluorescence usually found in anhydronium bases.

A strongly basic crude fraction similarly obtained in another batch was reduced with sodium borohydride in methanol, tetrahydroalstonine and δ-yohimbine were formed in the ratio of about 2 to 3, and the presence of alstonine in the strongly basic alkaloids was also presumed. Serpentine and alstonine are stereoisomers corresponding to δ-yohimbine and tetrahydroalstonine, and because of the instability of the free bases it seemed fairly difficult to isolate these two in the form of strong bases.

From *Vinca rosea*, five alkaloids, δ-yohimbine,[6,7,4] serpentine,[4] lochnerine,[4] akuammine,[8] and reserpine,[9] have so far been isolated. Tetrahydroalstonine and also alstonine have now been added.

After the present work had been completed, it was learned that Svoboda[10] also reported the isolation of tetrahydroalstonine from *Vinca rosea*.

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Experimental[*5]

Isolation of Tetrahydroalstonine and δ-Yohimbine from *Vinca rosea* (L.) Reichb.—The isolation of alkaloids was carried out according to the procedure employed by Schlittler, *et al.*[1] The dried and milled root of *Vinca rosea* (5 kg.) was extracted with two 20-L. portions of MeOH. The residue after removal of water-soluble fraction was subjected six times to counter-current distribution using 50% MeOH and $CHCl_3$. The data of resulting 12 fractions are given in Table II. Among these fractions, lower layer of fraction No. 6, considered to be the most rich in alkaloid, was evaporated to dryness *in vacuo* and chromatographed on alumina (Activity III) using benzene as the developing solvent. With benzene alone or benzene:ether (1:1) as a solvent, first tetrahydroalstonine, then δ-yohimbine were obtained in the form of crude crystals.

Tetrahydroalstonine—The above crude base, m.p. 220~223°, after recrystallization from MeOH, formed colorless plates, m.p. 225~227°. Yield of the crude base was 0.037% based on the dried roots. *Anal.* Calcd. for $C_{21}H_{24}O_3N_2$: C, 71.57; H, 6.87; N, 7.95; OCH_3, 8.81; C-CH_3, 4.27. Found : C, 71.35; H, 6.72; N, 7.77; OCH_3, 8.66; C-CH_3, 5.09. $[\alpha]_D^{21}$ −86°(c=0.46, pyridine), $[\alpha]_D^{22}$ −120°(c=0.44, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε) : 226(4.54), 280(3.84); λ_{min}^{EtOH} mμ (log ε) : 265(3.80). IR λ_{max}^{Nujol} μ : 2.94(-NH), 5.89, 6.17 ($CH_3OOC-\overset{|}{C}=\overset{|}{C}-O-R$), 9.19 (C-O-C), 13.33 (*o*-disub. benzene).

Hydrochloride : m.p. 277~280° (decomp.). *Anal.* Calcd. for $C_{21}H_{24}O_3N_2 \cdot HCl$: C, 64.85; H, 6.49;

[*5] All melting points are uncorrected.

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Table II. Counter-current Distribution of Crude Methanol Extract

Fraction No.	Layer	Color	Residue (g.)	Alkaloid (Meyer's reag.)
1	upper	deep red	32.9	—
	lower	yellow	0.5	—
2	upper	deep red	7.2	—
	lower	yellow	1.5	+
3	upper	deep red	6.2	+
	lower	brown	2.7	+
4	upper	deep red	2.3	+
	lower	deep red	3.2	+
5	upper	deep red	2.9	+
	lower	deep red	6.9	—
6	upper	deep red	1.5	+
	lower	deep red	81.8	+

Upper layer : 50% MeOH Lower layer : $CHCl_3$

N, 7.20. Found : C, 64.90; H, 7.06; N, 7.31.

δ-Yohimbine—The above crude base, m.p. 245~250°, after recrystallization from MeOH or MeOH-$CHCl_3$, formed colorless prisms, m.p. 253~255°. The yields of crude base ranged from 0.035% to 0.065% based on the dried roots depending on the extracting procedure. *Anal.* Calcd. for $C_{21}H_{24}O_3N_2$: C, 71.57; H, 6.87; N, 7.95; OCH_3, 8.81; C-CH_3, 4.27. Found : C, 71.29; H, 6.65; N, 7.86; OCH_3, 9.43; C-CH_3, 3.36. $[\alpha]_D^{23}$ −54°(c=0.52, pyridine); $[\alpha]_D^{28}$ −63°(c=0.51, $CHCl_3$). UV λ_{max}^{EtOH} mμ (log ε) : 228(4.34), 280(3.65); λ_{min}^{EtOH} 261 mμ (lor ε : 3.49). IR λ_{max}^{Nujol} μ : 2.96 (-NH), 5.93, 6.27 (CH_3OOC-$\overset{|}{C}$=$\overset{|}{C}$-O-R), 9.03 (C-O-C), 13.55 (*o*-disubst. benzene).

Hydrochloride : m.p. 272~275°(decomp.). *Anal.* Calcd. for $C_{21}H_{24}O_3N_2 \cdot HCl$: C, 64.85; H, 6.49; N, 7.20. Found : C, 64.44; H, 6.03; N, 7.12.

Paper Partition Chromatography of Tetrahydroalstonine and δ-Yohimbine—Toyo Roshi No. 50 was used as the filter paper and development was effected by ascending technique. For the detection of alkaloidal spots, fluorescence under ultraviolet rays was employed. The solvent mixture used and Rf values are given in Table I.

Isolation of Serpentine—MeOH extract of the dried root (10 kg.) was treated with water at room temperature, evaporated to dryness *in vacuo*, dissolved in 10% AcOH, and filtered. After extracting with ether the aqueous layer was made alkaline (pH 11.0) with NaOH solution and extracted with ether containing 10% MeOH. The aqueous layer thus obtained was again acidified (pH 2.0) with conc. HCl and ammonium reineckate solution added. The resulting crude reineckate was collected by suctional filtration and dried in a vacuum desiccator. The crude reineckate was chromatographed twice on alumina and two eluates were obtained after elution with Me_2CO and MeOH. The combined first eluted fraction, after removal of solvent below 50° *in vacuo*, was converted to chloride solution by the usual method and the crude chloride was obtained by evaporation of the solvent again below 50°. This crude chloride foamed at 167~175° and decomposed at 230~240°, but the Beilstein test was not obvious. The yield of the crude chloride was 0.023% based on the dried roots.

Nitrate : Prepared by dissolving the chloride in 10% AcOH, saturated NH_4NO_3 solution added, and recrystallized from hot water (containing a small amount of HNO_3) to yellow needles, m.p. 190~200° (foaming)/254~257°(decomp.). *Anal.* Calcd. for $C_{21}H_{20}O_3N_2 \cdot HNO_3 \cdot 1\frac{1}{2}H_2O$: C, 57.53; H, 5.52; N, 9.59. Found : C, 57.46; H, 5.69; N, 9.79. UV λ_{max}^{EtOH} mμ (log ε) : 250 (4.48), 305 (4.30), 365 (3.63).

Reduction of Serpentine with $NaBH_4$—The above nitrate (100 mg.) was converted to free base with NaOH solution, dissolved in 10 cc. of MeOH, and reduced with 100 mg. of $NaBH_4$. After refluxing for 1 hr., the solution was concentrated to one-half the original volume. A crude crystalline residue, m.p. 234~237°, was obtained (90 mg., 93%). After chromatography on alumina the crude base was recrystallized from MeOH to prisms, m.p. 250~252°, which showed no depression on mixed fusion with the authentic δ-yohimbine.

Reduction of Crude Strong Base with $NaBH_4$—The crude strong base (1.2 g.) after chromatography of the reineckate as mentioned above was immediately reduced with $NaBH_4$ in MeOH in the same manner. A crude reduced base (860 mg., 71% yield) was chromatographed on alumina and recrystallization from MeOH gave tetrahydroalstonine and δ-yohimbine in the ratio of about 2:3. Both of them showed no depression on fusion with authentic samples.

Summary

From the root of *Vinca rosea* (L.) Reichb. (Apocynaceae), three alkaloids, tetrahydroalstonine, δ-yohimbine, and serpentine were isolated, and the presence of alstonine was also presumed.

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