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Studies on the Lupin Alkaloids. VII.[1] Isolation and Structure of (−)-Δ^7-Dehydrosophoramine[2]

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The alkaloids of the epigeal part of Japanese *Sophora flavescens* Aiton were investigated for the first time, and (−)-Δ^7-dehydrosophoramine (XII), the most highly unsaturated compound among the known lupin alkaloids of matrine type, was isolated and the structure was determined, and (+)-sophocarpine-N-oxide (XI) was isolated from this plant at first time.

Keywords——Leguminosae; *Sophora flavescens* Aiton; alkaloids; quinolizidine; hexadehydro-matrine

In the course of our studies on the antitumor activity of Leguminosae plants constituents, we found (+)-matrine demonstrates antitumor activity against Ehrlich ascites tumor of mice *in vivo* as well as *in vitro*[4] and that (+)-isomatrine[1,5] shows antitumor activity against Sarcoma-180 solid type. The alkaloids of the root of *Sophora flavescens* Aiton have been investigated by Japanese[6] and German[7] research groups independently. Thus several lupin alkaloids, (+)-matrine (I), (+)-matrine-N-oxide (II), (+)-allomatrine (III),[8] (+)-isomatrine (IV), (+)-sophoranol (V), (+)-sophoranol-N-oxide (VI),[8] (−)-sophocarpine (VII), (−)-sophoramine (VIII),[8] (−)-N-methylcytisine (IX), (−)-anagyrine (X) and (−)-baptifoline (XI) were isolated. However no investigation on the alkaloids of epigeal part of this plant had been made until we started the present work.

The alkaloidal fraction (112 g) from 26 kg of fresh epigeal part of this plant, harvested in May 1972, at Awagatake in Kakegawa city, Shizuoka prefeture, was subjected to the repeated column chromatography on celite No. 535 and and alumina (*cf.* Chart 1). (−)-N-Methylcytisine (IX), (−)-sophocarpine (VII), (−)-sophoramine (VIII), and two new alkaloids A and B were isolated. Matrine (I), matrine-N-oxide (II), sophoranol (V), anagyrine (X) and baptifoline (XI) were detected by thin-layer chromatography (TLC) and also gas liquid chromatography (GLC) except the N-oxide. Table I shows *Rf* and t_R values of the above new alkaloids comparing with those of the other lupine alkaloids, hitherto isolated from the root of this plant.

The new alkaloid A, colorless prisms (benzene), mp 200° (dec.), $[\alpha]_D^{18}=+21°$ ($c=2.0$, EtOH), possesses an empirical formula $C_{15}H_{22}N_2O_2$, determined by elemental analysis. The ultraviolet (UV) spectrum of this alkaloid shows the presence of α,β-unsaturated lactam system, λ_{max}^{EtOH} nm (log ε): 260 (3.38), and infrared (IR) spectrum shows the presence of

1) Part VI: A. Ueno, K. Morinaga, S. Fukushima, Y. Iitaka, Y. Koiso, S. Okuda, *Chem. Pharm. Bull.* (Tokyo), **23**, 2560 (1975).
2) This work was presented at the meeting of Tokai branch of Pharmaceutical Society of Japan (June, 1973, Nagoya).
3) Location: *a) 2–2–1, Oshika, Shizuoka*; *b) 1–1–1, Yayoi, Bunkyo-ku, Tokyo.*
4) R. Kojima, S. Fukushima, A. Ueno, and Y. Saiki, *Chem. Pharm. Bull.* (Tokyo), **18**, 2555 (1970).
5) This alkaloid is cited as a new base (III) in literature 4.
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7) F. Bohlmann, D. Rahts, and C. Arndt, *Chem. Ber.*, **91**, 2189 (1958).
8) The isolation of these alkaloids: Private communication from S. Okuda, M. Yoshimoto, and K. Tsuda.

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α,β-unsaturated lactam carbonyl group, $\nu_{max}^{CHCl_3}$ cm^{-1}: 1662, 1600, This alkaloid was expected to be an N-oxide, because of its low mobility on TLC, and its characteristics are similar to those of (+)-sophocarpidine (sophocarpine-N-oxide)[9] (XI). Treatment of this alkaloid with saturated sulfur dioxide solution in methanol gave a deoxidized product which was identified with the authentic sample of (−)-sophocarpine (VII) (monohydrate) by mixed melting point test and by the comparison of their UV, IR and nuclear magnetic resonance (NMR) spectra and optical rotation. Therefore the alkaloid A is confirmed to be (+)-sophocarpine-N-oxide, and this is the first case that the N-oxide of unsaturated matrine type alkaloid was isolated from this plant.

The new alkaloid B, colorless prisms (ether–methylene chloride), mp 146—148°, $[\alpha]_D^{17}$= −110° (c=1.0, H_2O), possesses a molecular formula $C_{15}H_{18}N_2O$, determined by the elemental

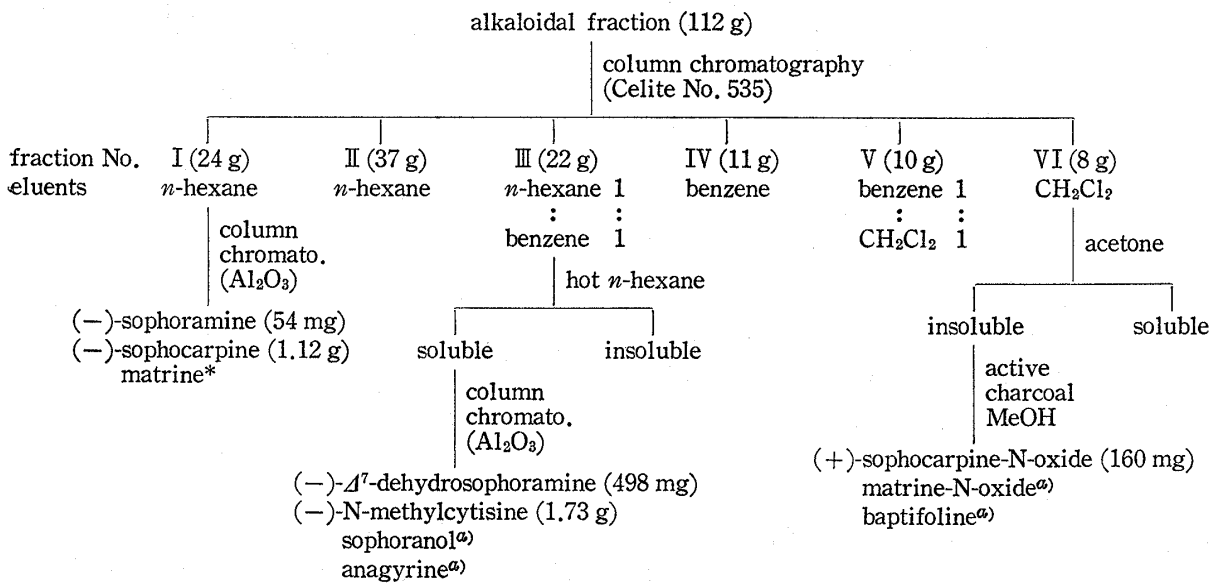

Chart 1. Separation Schema of Alkaloidal Fraction of Epigeal Part of *Sophora flavescens* Aiton

a) Detected by TLC and/or GLC.

Table I. *Rf* and t_R Value of the Two New Alkaloids and the Other Alkaloids hitherto isolated from the Root of *Sophora flavescens* Aiton

	TLC (*Rf*)	GLC (t_R: min)
(−)-Sophoramine (VIII)	0.65$^+$	18.4[a]
(−)-Sophocarpine (VII)	0.59^{++}	6.3[a]
(+)-Matrine	0.57	5.9[a]
(+)-Sophoranol (V)	0.54	10.0[a]
(−)-Anagyrine (X)	0.52$^+$	16.8[a]
(−)-N-Methylcytisine	0.50$^+$	3.7[a]
New base B, (−)-Δ^7-dehydro-sophoramine (XII)	0.44^{++}	15.3[a]
(+)-Allomatrine (III)	0.41	5.1[a]
(−)-Baptifoline (XI)	0.35$^+$	27.6[b]
(+)-Isomatrine (IV)	0.33	9.0[a]
(+)-Matrine-N-oxide (II)	0.20	dec.[c]
New base A, (+)-sophocarpine-N-oxide	0.17^{++}	dec.[c]
(+)-Sophoranol-N-oxide (VI)	0.13	dec.[c]

TLC: Silicagel HF_{254}: Avical SF=25: 3, $CHCl_3$: MeOH=4: 1, ascending development, visualized by H_2PtCl_6–KI solution, $^+$ faint dark spot under UV light (3650 Å) before staining, $^{++}$ dark spot under UV light (3650 Å) before staining.

GLC: Silicon GE XE-60 2% on Gas-Chrom Q (80—100 mesh), 4 mm×1m (spiral glass column). *a*) column temp.: 190°, *b*) column temp.: 220°, *c*) In each case, t_R of main peak was identical with that of correspoding deoxidized compound.

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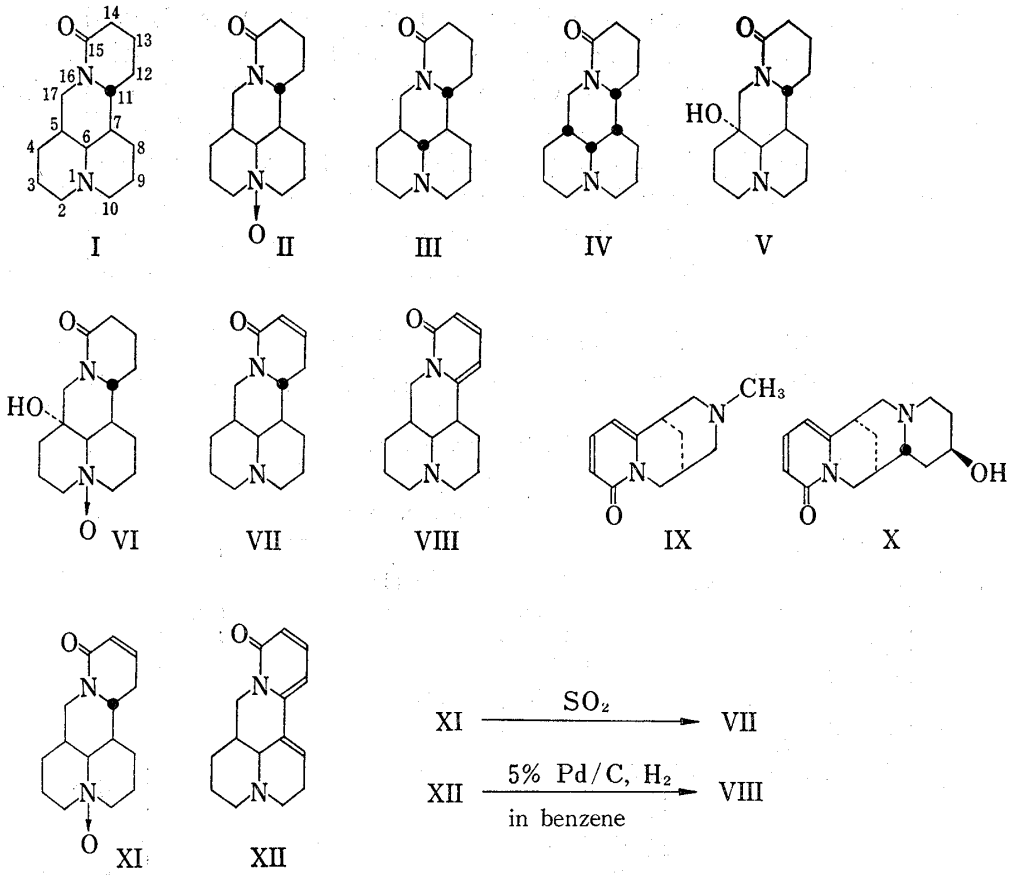

Chart 2. Alkaloids of *Sophora flavescens* Aiton

analysis and mass (MS) spectral data (M^+=*m/e* 242). The IR spectrum of alkaloid B shows the presence of α-pyridone ring, $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650, 1570, 1535, and no trans quinolizidine band. The catalytic hydrogenation of this alkaloid with 5% Pd/C in benzene gave the corresponding dihydro derivative, colorless prisms, mp 164° (ether), $C_{15}H_{20}N_2O$, $[\alpha]_D^{18}=-83.7°$ (c=0.37, EtOH), M^+=*m/e* 244, λ_{max}^{EtOH} nm (log ε): 235 (3.63), 310 (3.21), $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650, 1570, 1540 (α-pyridone). This compound was identified with the authentic sample of (−)-sophoramine (VIII) by mixed melting point test and comparison of their IR, UV, NMR, optical rotation, TLC and GLC data. Accordingly the new alkaloid B is a didehydrosophoramine, in which the double bond conjugates to α-pyridone ring. In its NMR spectrum, C_{12}–, C_{13}– and C_{14}–H of α-pyridone ring exhibit the signals at δ 6.02 (quartet, $J_{12,14}$=1.4, $J_{12,13}$=7.0 Hz), δ 6.85 (quartet, $J_{12,13}$=7.0, $J_{13,14}$=9.0 Hz), δ 6.57 (quartet, $J_{13,14}$=9.0, $J_{12,14}$=1.4 Hz). The signal at about δ 6.0 (1H, multiplet), partly overlapped on that of C_{12}–H, is assigned as that of proton on the double bond conjugate to α-pyridone ring. As no other signal due to the olefinic proton is observed, this conjugated double bond must be tri-substituted. Accordingly the position of the conjugated double bond must be C_7=C_8 or C_5=C_{17}. In the NMR spectrum of the new alkaloid B, the signals of C_{17}-methylene protons were observed at δ 3.80 (1H, quartet) and δ 4.06 (1H, quartet). Consequently the new alkaloid B is (−)-Δ^7-dehydrosophoramine (XII).

The Chart 2 shows the alkaloids which are contained in *S. flavescens*, with two new alkaloids isolated in this work, and all the naturally occurring unsaturated alkaloids of matrine type, hitherto isolated, are listed in Chart 3. It is interesting that (−)-Δ^7-dehydrosophoramine the most highly unsaturated alkaloid, has been isolated only from the epigeal part of *S. flavescens* but not from the root.

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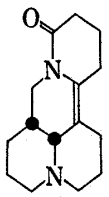
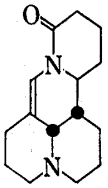
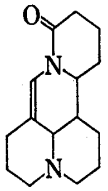


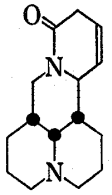
leontalbinine[a]
(*Leontice albertii*)[10]
mp 107–8°
$[\alpha]_D$ −135.5°
Bohlmann band (+)



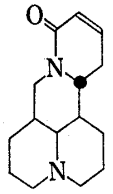
leontalbine[a]
(*L. albertii*)[10]
mp 180–5°/5mm
$[\alpha]_D$ −167°
Bohlmann band (+)



darvasine[a]
(*L. darvasica*)[11]
mp 145°
$[\alpha]_D$ −183°
Bohlmann band (+)

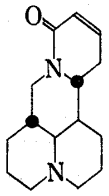


lehmannine[a]
(*Ammothamnus lehmanni*)
mp 93–4°
$[\alpha]_D$ +37.03°
Bohlmann band (+)

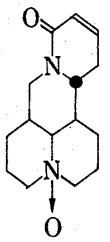


(VII)

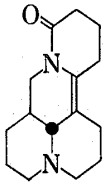
sophocarpine[a,b]
(*Sophora sp.*)[6]
mp 81–2°
$[\alpha]_D$ −29.44°
Bohlmann band (+)



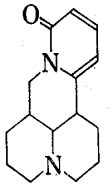
Δ^{13}-dehydrosophoridine[a]
(*S. flavescens*)[13]
mp 89–90°
$[\alpha]_D$ −88°
Bohlmann band (+)



sophocarpidine[a,b]
(sophocarpine-N-oxide)[9]
(*S. pachycarpa*)
mp 202–4°, $[\alpha]_D$ +32.4°

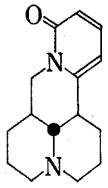


albertine[a]
(*L. albertii*)[15]
mp 161°
$[\alpha]_D$ −101.5°
Bohlmann band (+)

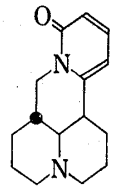


(VIII)

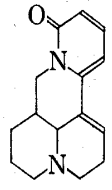
sophoramine[a,b]
(*Sophora sp.*)[14]
mp 164–5°
$[\alpha]_D$ −90.85°
Bohlmann band (+)



isosophoramine[a]
(*S. pachycarpa*)[16]
mp 143–4°
$[\alpha]_D$ +52.64°
Bohlmann band (+)



neosophoramine[a]
(*S. alopeculoides*)[17]
mp 124–5°
$[\alpha]_D$ −29.4°
Bohlmann band (−)



(XII)

Δ^{7}-dehydrosophoramine[a]
(*S. flavescens*)[2]
mp 146–8°
$[\alpha]_D$ −110°
Bohlmann band (−)

Chart 3. Naturally Occurring Unsaturated Alkaloids of Matrine Type

a) Obtained from epigeal part of the plant.
b) Obtained from under ground part of the plant.

Experimental

All the melting points were taken in a H_2SO_4 bath and were uncorrected. IR, UV, MS and NMR spectral measurements were run on Hitachi EPI G_{21} spectrometer, Hitachi EPS 3T spectrometer, Hitachi RMU 7 mass spectrometer and JEOL 4H-100 spectrometer using TMS as an internal standard. Optical rotations

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were measured with Yanaco OR-50 automatic polarimeter and GLC was carried out with Hitachi K-53 gas chromatograph.

Extraction and Separation——Fresh epigeal part (leaves and stems: 26 kg) of *Sophora flavescens* Aiton, collected at Awagatake in Kakegawa city Shizuoka prefecture, was chopped in small pieces and extracted with *ca.* 200 l of MeOH at room temperature. The MeOH extract was concentrated to *ca.* 6 l under reduced pressure, which contained water as a main solvent and was slightly acidic. The concentrated solution was extracted with ether (2 l) and EtOAc (2 l) to obtain the nonalkaloidal components. After allowed to stand in an ice box (refrigerator) for three days, the precipitated nonalkaloidal solid was filterd off and the aqueous layer was passed through a column of Amberlite IRC-50 (H^+ type, 300 ml). The adsorbed alkaloidal fraction was eluted with 2 N aq. NH_4OH (2 l), the eluate was made slightly acidic (pH: 5—6) with conc. HCl under cooling with ice and concentrated *in vacuo* to a paste. This paste was made strongly alkaline with a large excess of K_2CO_3 under cooling with ice and extracted with $CHCl_3$ repeatedly After dried over Na_2SO_4, the combined $CHCl_3$ extract was evaporated *in vacuo* to give 112 g of alkaloidal fraction.

Column chromatography of this alkaloidal fraction on celite (No. 535, 510 g) gave following six fractions. (*cf.* Chart 1). Fraction I: 24 g (eluted with *n*-hexane), Fraction II: 37 g (eluted with *n*-hexane), Fraction III: 22 g (eluted with *n*-hexane: benzene=1: 1), Fraction IV: 11 g (eluted with benzene), Fraction V: 10 g (eluted with benzene: CH_2Cl_2=1: 1), Fraction VI: 8 g (eluted with CH_2Cl_2).

In this study, Fraction I, III and VI were investigated.

Isolation of (−)-Sophocarpine (VII) and (−)-Sophoramine (VIII) from Fraction I——Fraction I (12 g) was chromatographed on neutral alumina (active grade I) and the fraction eluted with *n*-hexane: benzene (=1: 1) was made into HI-salt, and the salt was recrystallized from EtOH. The HI-salt was changed into a free base with usual method and the free base was recrystallized from *n*-hexane, colorless needles (sophocarpine) were obtained, mp 80°, $[\alpha]_D^{18}=-32°$ (c=1.0, EtOH), 1.12 g. In this column chromatography, benzene eluate gave colorless solid, the recrystalization of this solid from ether gave colorless needles (sophoramine), mp 164°, $[\alpha]_D^{18}=-88°$ (c=0.5, EtOH). Isolated sophocarpine and sophoramine were identified by mixed melting point tests with the authentic samples of (−)-sophocarpine and (−)-sophoramine and comparison of IR, UV, and NMR spectra. On the TLC and GLC of this fraction, matrine was detected, Rf=0.57 ($CHCl_3$: MeOH=4: 1; Silica gel HF_{254}: Avicel SF=25: 3), t_R=5.9 (column: 2% silicon GE XE-60 on Gas-Chrom Q, 1 m, column bath temp. 190°, carrier gas N_2 60 ml/min).

Isolation of (−)-N-Methylcytisine (IX) and the New Alkaloid B (XII) from Fraction III——Fraction III was treated with boiling *n*-hexane, and 9 g of a soluble fraction in hot *n*-hexane was obtained. The soluble fraction was chromatographed on neutral alumina (active grade I) and the fraction eluted with benzene: *n*-hexane: EtOH=100: 100: 1 gave a solid. Recrystalization of the solid gave colorless needles (N-methylcytisine, mp 136°, $[\alpha]_D^{17}=-224°$ (c=1.0, H_2O), 1.73 g. From the next fraction of this column chromatography a brown solid was obtained, this brown solid was recrystallized from ether–CH_2Cl_2 to give colorless prisms (the new alkaloid B), mp 146—148°, $[\alpha]_D^{17}=-110°$ (c=1.0, H_2O), 498 mg. *Anal.* Calcd. for $C_{15}H_{18}N_2O$, C: 74.35; H, 7.49; N, 11.56. Found: C, 74.53, H, 7.65; N, 11.58. UV λ_{max}^{EtOH} nm (log ε): 246 (3.71), 340 (3.98). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650, 1570, 1535 (α-pyridone), no transquinolizidine band. On the TLC and GLC of Fraction III, sophoranol (Rf=0.54, t_R=10.1) and anagyrine (Rf=0.52, t_R=16.8) were detected in the above conditions.

Isolation of (+)-Sophocarpine-N-oxide (Alkaloid A) (XI) from Fraction VI——Fraction VI was treated with acetone and the insoluble solid was obtained, recrystallization of this solid from benzene gave colorless prisms (sophocarpine-N-oxide), mp 200° (dec.), $[\alpha]_D^{17}=+21°$ (c=2.0, EtOH) 160 mg. On the TLC of this fraction matrine-N-oxide (Rf=0.2) and baptifoline (Rf=0.35) were detected.

Identification of (−)-Sophocarpine-N-oxide (Alkaloid A)——Alkaloid A (50 mg) was dissolved in 2 ml of MeOH, and 2 ml of MeOH saturated with SO_2 was added into the solution and stored for 16 hr at room temperature. The reaction product was isolated by the usual method. Recrystallization of this product from *n*-hexane gave colorless needles (sophocarpine monohydrate), mp 53°, $[\alpha]_D^{18}=-31°$ (c=1.0, EtOH). This compound was identified with authentic (−)-sophocarpine by IR, UV and NMR spectra, and mixed melting point test.

Selective Reduction of the New Alkaloid B——At room temperature, 25.5 mg of the new alkaloid B in 2.5 ml of benzene was hydrogenated with 10 mg of 5% Pd/C under atmospheric pressure of H_2 for three hours with stirring. The reaction product (25.0 mg) was recrystallized from ether to give 8.4 mg of colorless needles, mp 164°, $[\alpha]_D^{18}=-38.7°$ (c=0.37, EtOH), $M^+=m/e$ 244, UV λ_{max}^{EtOH} nm (log ε): 235 (3.63), 310 (3.21). IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1650, 1570, 1530 (α-pyridone). This compound was identified with authentic (−)-sophoramine by mixed melting point test and comparison of IR, UV, NMR, TLC and GLC data.

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