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Studies on the Constituents of *Desmodium caudatum* DC.[1]

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A new flavone derivative named desmodol was isolated together with N,N-dimethyltryptamine, bufotenine and bufotenine N-oxide from the roots and stems of *Desmodium caudatum* DC. The structure of desmodol was presented to be 5-hydroxy-6-methyl-8,8-dimethyl-2-(3,4-dihydroxyphenyl)-4*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-4-one (5,3',4'-trihydroxy-6-methyl-8,8-dimethylpyrano (2,3-*h*)-flavone) by the spectral and chemical data.

Keywords——desmodol; dimethylpyranoflavone; alkaloid; N,N-dimethyltryptamine; bufotenine; bufotenine N-oxide; *Desmodium caudatum*

Desmodium caudatum DC. (Leguminosae; japanese name: Misonaoshi) is distributed in the west region of Japan and is known as a chinese drug "Moh-Ts'ao". On the components

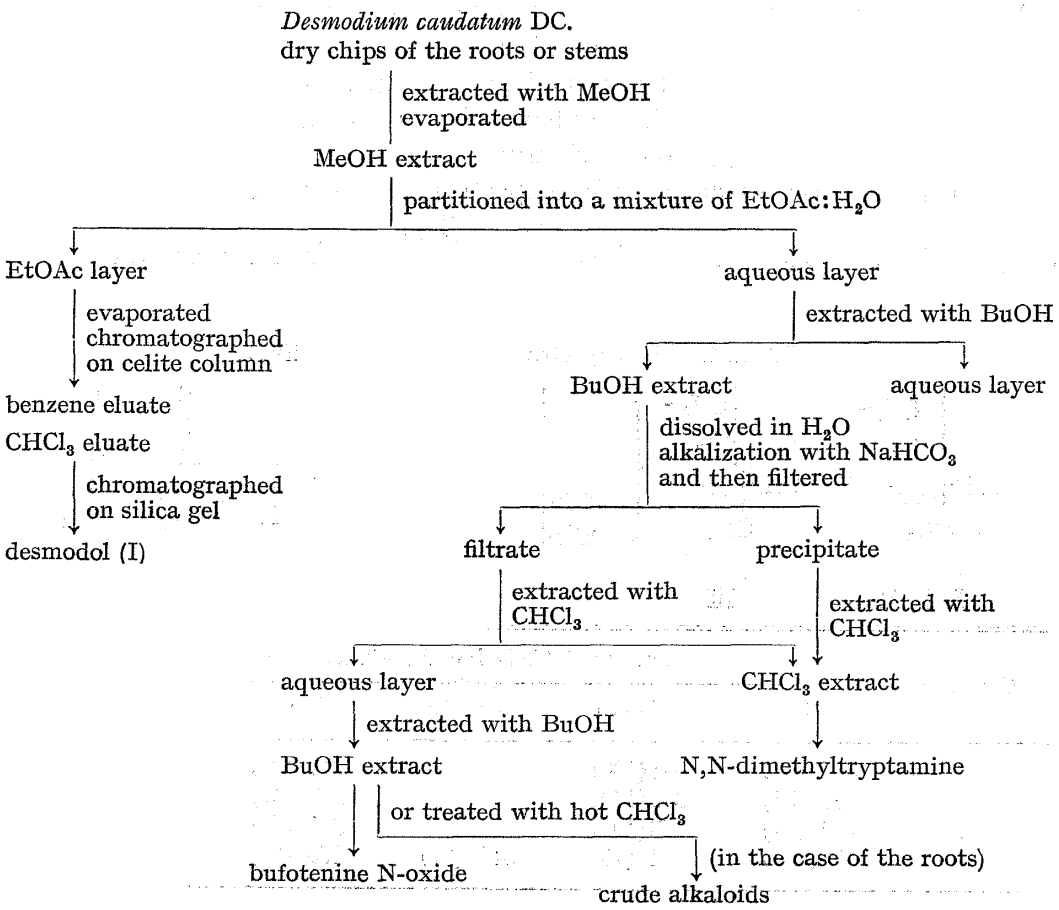


Chart 1. Isolation of Desmodol and Alkaloids

1) This work was presented at 93th (Sendai, April, 1974) Annual Meeting of Pharmaceutical Society of Japan, Abstract of Paper II-247 (1974).

2) Location: *a) 2–2–1, Oshika, Shizuoka*; *b)* Present address: *Tsumura Laboratory, 1421, Izumi, Komae-shi, Tokyo*; *c) Hiromachi 1–2–58, Shinagawaku, Tokyo.*

of this plant, swertisin[3] and two kinds[4] of unidentified alkaloids have been isolated. In our further studying on the constituents of this plant, a new flavone named desmodol, N,N-dimethyltryptamine, bufotenine and bufotenine-N-oxide were isolated.

The dried roots and stems were separately extracted with boiling methanol, and the extracts were treated as shown on Chart 1. Desmodol (I) was obtained in the parts soluble in ethyl acetate from the roots and stems as yellow prisms, mp 281—282°. Its molecular formula was decided by the elemental analysis and the mass spectrum (MS) (M^+=366). The ethanolic solution of I showed a green color by ferric chloride and a red color by the magnesium hydrochloric acid reaction. The infrared (IR) spectrum of I showed the presence of hydroxyl at 3400 cm^{-1} and carbonyl group due to flavone[5] at 1645 cm^{-1}.

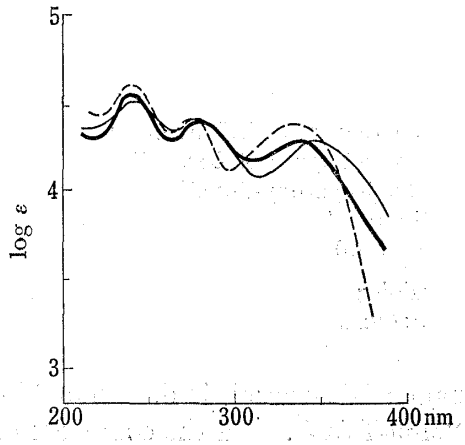


Fig. 1. UV Spectra of I (——), II (——) and IV (-----) in EtOH

The methylation of I with diazomethane gave desmodoldimethyl ether (II), which afforded dihydrodesmodoldimethyl ether (III) by the catalytic hydrogenation with 5% palladium on charcoal. Both II and III maintained a positive ferric chloride reaction. On the other hand, the methylation of II with dimethyl sulfate and potassium carbonate gave desmodoltrimethyl ether (IV) which showed negative ferric chloride reaction.

The ultraviolet (UV) spectra of I, II and IV as shown on Figure 1, resemble to those[6] of flavones, and were different from those of flavonols. These chemical and spectral properties of I suggest that I is a hydroxyflavone derivative.

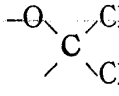
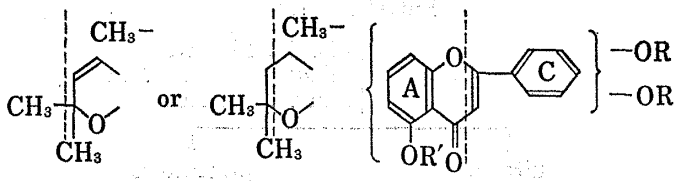
The nuclear magnetic resonance (NMR) spectrum of I showed following signals: singlet at δ 2.29 (3H, s, arom-CH_3), δ 1.53 (6H, s, ), a pair of doublet at δ 5.65 and δ 6.85

Table I. Mass Spectra of I and Its Derivatives



	R	R'
I	H	H
II	CH_3	H
III	CH_3	H
IV	CH_3	CH_3

I, II, IV III

Compound	Fragment (Relative abundunce; %)				
	M^+	M^+-15	M^+-55	Side A	Side B
I	366 (33)	351 (100)		217 (27)	134 (8)
II	394 (34)	379 (100)		217 (37)	
IV	408 (87)	393 (100)		231 (67)	
III	396 (100)	381 (13)	341 (67)	179 (32)	162 (15)

3) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1842 (1968).
4) P.-Y. Yen, L.-C. Lin, and S.-S. Yang, *J. Chinese Chem. Soc.* (Taiwan), **12** (1—2), 61 (1965).
5) *a*) W. Baker, A.C.M. Finch, W.D. Ollis, and K.W. Robinson, *J. Chem. Soc.*, **1963**, 1477; *b*) S.K. Mukerjee, S.C. Sarkar, and T.R. Seshadri, *Tetrahedron*, **25**, 1063 (1969).
6) S.K. Kupchan, C.W. Sigel, R.J. Hemingway, J.R. Knox, and M.S. Udayamurthy, *Tetrahedron*, **25**, 1603 (1969); J.J. Chirikjam and W. Bleier, *Sci. Pharm.*, **39**, 65 (1971).

(each 1H, d, J=10 Hz, –CH=CH–, cis coupling to each other). On the NMR spectra of I and II, the signals at δ 5.65 and δ 6.85 in I replaced by two difused triplets at δ 1.87 and δ 2.86 ($-CH_2-CH_2-$) in coupling to each other, which was confirmed by means of decoupling. In the MS of I and its derivatives as shown in Table I, the fragment of M^+-15 in I, II and IV is the base peak, and M^+-55 is strong in III. These are characteristic[7,8] in the MS of the derivatives of 2,2-dimethylchromone and 2,2-dimethylchromane respectively. These finding of NMR and MS suggest the presence of a 2,2-dimethylchromene ring[5b,7] in the structure of I. In Table I, the fragments of side A (217=flavone: $120+CH_2+C_4H_3O+O$, $179=120+CH_2+CH+O_2$ and $231=120+CH_2+C_4H_3O+OCH_2$) and side C ($134$=flavone: $102+O_2$ and $162=102+2\ OCH_2$) due to retro Diels-Alder type, inform that a 2,2-dimethylchromene, methyl and chelated hydroxyl groups are located to A ring of the flavone nucleus and two hydroxyl groups are attached to C ring of the flavone.

The signal of a chelated hydroxyl proton at δ 12.94 in the NMR spectrum of II, disappears in that of IV. In the IR spectra of II and IV, the absorption of a carbonyl group at 1640 cm^{-1} in II shifted to 1630 cm^{-1} in IV. The similar shift of the carbonyl absorption has been noted in the derivatives[5a,7,9] of 5-hydroxychromone and 5-hydroxyflavone, therefore the chelated hydroxyl group in II exists at 5 position of the flavone. As is shown in Table II and Chart 2, nuclear Overhauser effect (NOE) in the NMR spectrum of IV was

Table II. NOE (%) and Chemical Shift (PPM) in the NMR Spectrum of IV

Saturated signals		Observed signals						
		C6′-H 7.49	C2′-H 7.31	C5′-H 6.96	C3-H 6.58	C10-H 6.85	C9-H 5.67	C6-CH_3 2.14
C5-OCH_3	3.85	—	—	—	—	0	0	0
C3′-OCH_3 C4′-OCH_3	3.94, 3.95	0	11	10	0	—	—	—
C3-H	6.58	7	11	0	—	—	—	—
C6-CH_3	2.14	—	—	—	—	0	0	—

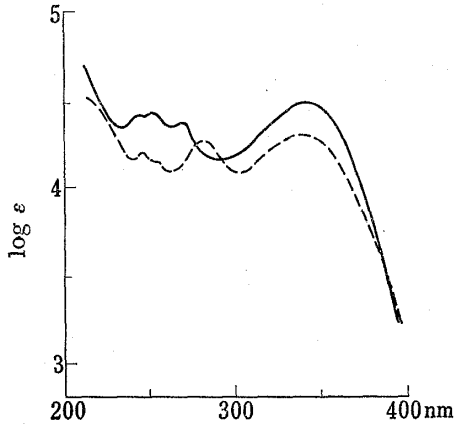


Fig. 2. UV Spectra III (----) and 5-Hydroxy-7,3′,4′-trimethoxyflavone (——) in EtOH

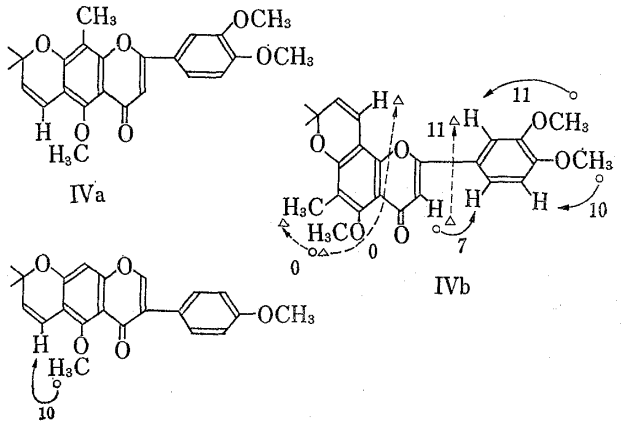


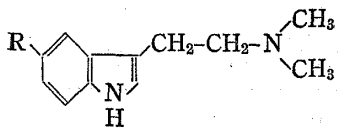
Chart 2. Structure of IV and NOE (%) in the NMR Spectrum of IV

7) B.F. Burrows, W.D. Ollis, and L.M. Jackson, *Proc. Chem. Soc.*, **1960**, 177; B. Jackson, H.D. Locksley, and F. Scheimann, *J. Chem. Soc.* (*C*), **1967**, 2500.
8) *a*) A. Pelter, P. Stainton, and M. Barber, *J. Heterocycl. Chem.*, **2**, 262 (1965); *b*) B. Willham, A.F. Thomas, and F. Gantshi, *Tetrahedron*, **20**, 1185 (1964).
9) A.J. East, W.D. Ollis, and R.E. Wheeler, *J. Chem. Soc.* (*C*), **1969**, 365.
10) B. Jackson, P.J. Owen, and F. Scheinmann, *J. Chem. Soc.* (*C*), **1971**, 3389.

observed between proton signals of two methoxyl groups (δ 3.94 and 3.95) and C2′–H or C5′–H of aromatic proton signals on the C ring of the flavone. Furthermore, NOE was found between C3–H and C2′–H or C6′–H, and so two methoxyl groups are located on C3′ and C4′ position of the flavone in the structure of IV. The UV spectrum of III is similar to that of 5-hydroxy-7,3′,4′-trimethoxyflavone as shown in Figure 2 and shows that A ring of the flavone nucleus in III is to be phloroglucinol type.[5b]

On the basis of the foregoing facts, the structure of IV is formulated as IVa or IVb (Chart 2). If IV has the structure of IVa, NOE between C5–OCH_3 and C6–H could be observed as in the case of alpiniumisoflavone-5,4′-dimethyl ether,[10] but no NOE was observed between C10–H (δ 6.85) and C5–OCH_3 (δ 3.85) or C6–CH_3 (δ 2.14). This finding of NOE favors IVb for the structure of IV. However, NOE between C6–CH_3 and C5–OCH_3 could not be observed. This fact may be explained by the following assumption: the proton signal at δ 3.85 of C5–OCH_3 exists in higher magnetic field in comparison with other methoxyl proton signals, and the some bond angle between the aromatic ring plane and C6–CH_3 or C5–OCH_3 may be formed because of the steric hindrance by the bulky neighbor groups like C6–CH_3, C5–OCH_3 and C4–CO of the flavone, therefore no NOE between C5–OCH_3 and C6–CH_3 is reasonable. Consequently, the structure of I is proposed to be 5-hydroxy-6-methyl-8,8-dimethyl-2-(3,4-dihydroxyphenyl)-4*H*,8*H*-benzo[1,2-*b*: 3,4-*b*′]dipyran-4-one (5,3′4′-trihydroxy-6-methyl-8,8-dimethylpyrano (2,3-*h*)flavone).

On the chromatographical separation, N,N-dimethyltryptamine, bufotenine and bufotenine N-oxide were isolated from the butanolic extracts of the stems and roots. These alkaloids were identified by the melting point, UV, MS and NMR spectra. The major alkaloid of the roots was N,N-dimethyltryptamine and that of the stems was bufotenine, and indole-3-alkylamines like N,N-dimethyltryptamine and bufotenine originated tryptophan have been known to distribute in the some species[11] of *Demodium*. This paper elucidated that *D. caudatum* belonged to the group which produced the alkaloids.



R=H tryptamine
R=OH bufotenine

Chart 3

Experimental

All melting points are uncorrected. UV spectra were measured using a Hitachi recording spectrometer EPS-032 type. IR spectra were recorded for KBr tablet with JASCO IRA-2 spectrometer. NMR spectra were taken at 60 MHz or 100 MHz with TMS as an internal standard using JNM-C-60 or Hitachi R 24 or Varian HA-100 high resolution NMR spectrometer. The signals were given on chemical shifts in δ and following abbreviation: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. Mass spectra were determined at 80 eV with Hitachi RMU-7 spectrometer. TLC was carried out using the plates prepared by Kiesel gel nach Stahl and column chromatography was performed on Wakogel-C200.

Isolation of Desmodol (I)——The dried roots and stems of *Desmodium caudatum* DC. which was collected at Senbon, Numazu-shi, Shizuoka in May, 1973, were extracted four times with MeOH under refluxing for 18 hr separately. The each extract was concentrated and partitioned into a mixture of EtOAc and H_2O. The water layer was washed with EtOAc and extracted with BuOH. In this experiment, EtOAc-extracts (48 g from the roots and 92 g from the stems) and BuOH-extracts (63 g from the roots and 92 g from the stems) were obtained. The EtOAc-extracts from the roots and stems were combined, because the TLC of both extract was similar to each other. This was chromatographed on celite column and the following eluates were obtained: hexane 71 g, hexane: benzene (3: 1) 3.5 g, hexane: benzene (1: 1) 5 g, benzene 11 g, CH_2Cl_2 17.5 g, ether 4.7 g, EtOAc 4.3 g, EtOAc: MeOH (4: 1) 14 g, MeOH 12.4 g. The benzene and CH_2Cl_2 eluates were combined and chromatographed repeatedly on silica gel column, and the eluate with benzene: EtOAc (3: 7) was recrystallized from benzene: acetone to give yellow needles, mp 281—282° (dec.), yield 79 mg. *Anal.* Calcd. for $C_{21}H_{18}O_6$: C, 68.84; H, 4.95. Found: C, 68.66; H, 4.91. This ethanolic solution

11) S. Ghosal and P.K. Banerjee, *Aust. J. Chem.*, **22**, 2029 (1969); S. Ghosal and B. Mukkerjee, *Chem. Ind.*, **1964**, 1800; *idem, ibid.*, **1965**, 793; *idem, J. Org. Chem.*, **31**, 2284 (1966); S. Ghosal and R.S. Srivastava, *Phytochemistry*, **12** (1), 193 (1973); S. Ghosal, R.S. Srivastava, P.K. Banerjee, and S.K. Dutta, *Phytochemistry*, **10**, 3312 (1971).

showed a red color by Mg+HCl and a green color by $FeCl_3$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1645 (C=O). UV λ_{max}^{EtOH} nm (log ε): 240 (4.49), 277 (4.37), 284 (4.35), 347 (4.26). MS *m/e* (%): 366 (M^+, 33), 351 (100), 217 (27), 134 (8). NMR (60 MHz, in C_5D_5N): δ 1.53 (6H, s), 2.29 (3H, s), 5.65 (1H, d, J=10 Hz), 6.99 (3H, s), 6.85 (1H, d, J=10 Hz).

Desmodoldimethyl Ether (II)——To a solution of I (51 mg) in MeOH (1 ml) was added an ethereal solution of CH_2N_2. After standing overnight, the mixture was concentrated and the residue was spoted on TLC. The TLC was developed by benzene: EtOAc (4: 1) and the resulting adsorbent band was collected, eluted with MeOH, and the obtained product was recrystallized from MeOH–H_2O to give 28 mg of yellow needles, mp 185—186°. The methanolic solution showed a green color by $FeCl_3$. IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O), UV λ_{max}^{EtOH} nm (log ε): 240 (4.55), 279 (4.39), 318 (4.19), 338 (4.27). MS *m/e*: (%) 394 (M^+, 34), 379 (100), 217 (37). NMR (60 MHz, in $CDCl_3$): δ 1.45 (6H, s), 2.04 (3H, s), 5.49 (1H, d, J=10 Hz), 6.40 (3H, s), 6.64 (1H, d, J=10 Hz), 6.83 (1H, d, J=9 Hz), 7.15 (1H, d, J=2.5 Hz), 7.35 (1H, dd, J=9 and 2.5 Hz), 3.87 (6H, s, 2×OCH_3), 12.94 (1H, s).

Dihydrodesmodoldimethyl Ether (III)——II (20 mg) was hydrogenated with 5% Pd–C (40 mg) in EtOH (10 ml) at room temperature for 2 hr. The catalyst was filtered off and the filtrate was concentrated. The residue was purified by TLC (benzene: EtOAc=4: 1) and recrystallized from MeOH to give 16 mg of yellow needles, mp 214—215°. *Anal.* Calcd. for $C_{23}H_{24}O_6$: C, 69.68; H, 6.10. Found: C, 68.59; H, 6.06. UV λ_{max}^{EtOH} nm (log ε): 245 (4.19), 255 (4.13), 282 (4.26), 339 (4.29). MS *m/e* (%); 396 (M^+, 100), 381 (13), 341 (67), 179 (32), 162 (15). NMR (60 MHz, in $CDCl_3$): δ 1.40 (6H, s), 1.87 (2H, t, J=7 Hz), 2.04 (3H, s), 2.86 (2H, t, J=7 Hz), 3.92 (6H, s), 6.45 (1H, s), 6.87 (1H, d, J=9 Hz), 7.20 (1H, d, J=2.5 Hz), 7.42 (1H, dd, J=9 and 2.5 Hz), 12.50 (1H, s).

Desmodoltrimethyl Ether (IV)——A mixture of II (32 mg), K_2CO_3 (0.5 g), Me_2SO_4 (0.35 ml) and dry acetone (10 ml) was refluxed for 6.5 hr and then filtered. The filtrate was concentrated and the residue was purified by the TLC (benzene: EtOAc=3: 7) and recrystallized from MeOH to give 8 mg of pale yellow needles, mp 177—179°. *Anal.* Calcd. for $C_{24}H_{24}O_6$: C, 70.57; H, 5.92. Found: C, 70.69; H, 5.85. IR ν_{max}^{KBr} cm^{-1}: 1630 (C=O). UV λ_{max}^{EtOH} nm (log ε): 214 (4.43), 240 (4.60), 272 (4.37), 278 (4.36), 335 (4.36). MS *m/e* (%): 408 (M^+, 87), 393 (100), 231 (67). NMR (100 MHz, in $CDCl_3$): δ 1.49 (6H, s), 2.14 (3H, s), 3.85 (3H, s), 3.94 (3H, s), 3.95 (3H, s), 5.67 (1H, d, J=10 Hz), 6.58 (1H, s), 6.85 (1H, d, J=10 Hz), 6.96 (1H, d, J=9 Hz), 7.31 (1H, d, J=2.5 Hz), 7.49 (1H, dd, J=9 and 2.5 Hz).

Isolation of Alkaloids from the Roots——The filtered solution of BuOH–extract (48 g) of the roots in H_2O (200 ml) was alkalized with $NaHCO_3$ (11 g), and the resulting precipitate was collected by filtration and then extracted with $CHCl_3$ ($CHCl_3$–fraction). The filtrate was extracted with $CHCl_3$ and BuOH successively and the $CHCl_3$–extract was combined with the foregoing $CHCl_3$–fraction. The each extract was concentrated to give $CHCl_3$–fraction (11.1 g) and BuOH–fraction (22.8 g).

N,N-Dimethyltryptamine Picrate: To a suspension of the $CHCl_3$–fraction (3.39 g) in 5% aq. HCl (45 ml), was added a solution saturated with picric acid, and the resulting precipitate was collected, washed with benzene and chromatographed on a column of silica gel. The eluate with benzene: EtOAc (13: 7) was recrystallized from MeOH: H_2O (1: 4) to give 1.32 g of yellow needles, mp 167—168°. *Anal.* Calcd. for $C_{18}H_{19}N_5O_7$: C, 51.80; H, 4.59; N, 16.78. Found: C, 52.09; H, 4.55; N, 16.53. MS *m/e* (%): 229 (100, picric acid), 188 (51), 144 (18), 143 (18), 130 (41), 115 (20), 103 (73.5).

N,N-Dimethyltryptamine: The picrate (1.147 g) was dissolved in a solution of K_2CO_3 (1 g) in H_2O (100 ml) under warming and the solution was extracted with $CHCl_3$. After removal of the solvent, the $CHCl_3$ extract was recrystallized from petroleum ether to give 0.388 g of colorless needles, mp 48.5—49°. *Anal.* Calcd. for $C_{12}H_{16}N_2$: C, 76.55; H, 8.57; N, 14.88. Found: C, 76.59; H, 8.49; N, 15.16. UV λ_{max}^{EtOH} nm (log ε): 223 (4.39), 283 (3.76), 291 (3.69). MS *m/e* (%): 188 (100), 142 (28), 143 (26), 130 (67), 115 (31), 103 (27), 58 (strongest). The total yield: 0.087% of the dry roots.

Bufotenine N-Oxide: The BuOH–fraction (22.8 g) was treated by the same method with $CHCl_3$–fraction to afford picrate (3.3 g). This was chromatographed on a column of silica gel and the eluate with EtOAc, was recrystallized from 95% EtOH to give 0.98 g of bufotenine N-oxide picrate. The picrate (0.836 g) was treated with aq. K_2CO_3 and the resulting free base was purified on TLC (Et_2O: *t*-BuOH: H_2O: Et_2NH= 10: 10: 4: 1 and MeCOEt: BuOH: H_2O=4: 6: 3). The eluate from the TLC was recrystallized from EtOH: MeOH to give 14 mg of colorless prisms, mp 212—214° (dec.). *Anal.* Calcd. for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.21; H, 7.30; N, 12.23. UV λ_{max}^{EtOH} nm (log ε): 223 (4.52), 278 (3.99), 302 (3.85), 314 (3.71). MS *m/e* (%): 204 (M^+, 77), 160 (40), 159 (70), 146 (100), 58 (strongest).

Isolation of Alkaloids from the Stems——The filtered solution of the BuOH–extract (75 g) of the stems in H_2O (300 ml) was alkalized with $NaHCO_3$ (20 g) and then extracted with BuOH. After removal of the BuOH, the residue was extracted with hot $CHCl_3$ and the $CHCl_3$–soluble portion was chromatographed on a column of silica gel to divided fraction 1—9.

N,N-Dimethyltryptamine: The fraction 3 (380 mg) eluted with $CHCl_3$: MeOH (49: 1) containing 1% Et_2NH, was recrystallized from *n*-hexane to give colorless prisms, mp 48—49°, which was identical with the sample of N,N-dimethyltryptamine obtained from the roots by the mixed melting point and their spectral data.

Bufotenine N-Oxide: The fraction 9 (447 mg) eluted with $CHCl_3$: MeOH (3: 1) containing 1% $(C_2H_5)_2NH$, was purified on TLC $(C_2H_5)_2O$: *t*-BuOH: $(C_2H_5)_2NH$=10: 10: 4: 1) to give colorless prisms (from MeOH), mp 214—217° (dec.). UV λ_{max}^{EtOH} nm (log ε): 220 (4.14), 277 (3.73), 302 (3.62), 312 (3.52). This was identical with the sample of bufotenine N-oxide obtained from the roots by the mixed melting point and their spectral data.

Bufotenine Monopicrate: The fraction 6 (6.9 g) eluted with $CHCl_3$: MeOH (47: 3) containing 1% $(C_2H_5)_2NH$, was dissolved in 5% aq. NaOH (40 ml) and the solution was saturated with CO_2 and then extracted with BuOH. After removal of the solvent, the BuOH-extract was treated with picric acid to afford 8.5 g of the picrate. This picrate (1.1 g) was dissolved in Et_2NH (3 ml) and chromatographed in silica gel column using a mixture of $CHCl_3$, MeOH and 1% Et_2NH as the eluent to give brown gum (557 mg). This (220 mg) was converted to the picrate and the product was recrystallized from $(C_2H_5)_2OAc$: acetone and MeOH to give red needles (270 mg), mp 177—178°. The yield as bufotenine was added up to 0.04% of the dry stems. *Anal.* Calcd. for $C_{12}H_{14}N_2O$: $C_6H_3N_3O_7$: C, 49.88; H, 4.42; N, 16.16. Found: C, 49.89; H, 4.52; N, 16.22. MS *m/e* (%): 229 (64, picric acid), 204 (62), 160 (34), 159 (31), 146 (94), 132 (32), 130 (31), 117 (51), 105 (73), 62 (100), 58 (strongest).

Bufotenine Dipicrate: The monopicrate was treated with excess picric acid and the product was recrystallized from MeOH to give dark red needles, mp 173—174°.

Bufotenine Oxalate: To a solution of crude bufotenine (60 mg) in $(C_2H_5)_2O$: MeOH (1: 1) was added an ethereal solution saturated with oxalic acid, and the resulting precipitate was recrystallized from Et_2O: MeOH to give 37 mg of colorless prisms, mp 86—89°.

Bufotenine: The monopicrate was purified on TLC and the collected band was eluted with a mixture of $CHCl_3$ and MeOH containing NH_3 to afford colorless sirup. UV λ_{max}^{EtOH} nm (log ε): 222 (4.21), 278 (3.68), 302 (3.55), 313 (3.43).

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