[Chem. Pharm. Bull. 23(10)2279—2283(1975)]

UDC 547.94.02.04 : 581.192

Studies on the Alkaloids of *Thalictrum Thunbergii* DC. XVIII.[1] Structure of Thalictine, the Tertiary Base in the Stems and Leaves

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(Received February 27, 1975)

The structure of thalictine (**1**), isolated from the stems and leaves of *Thalictrum thunbergii* DC., has been established as **1**, by nuclear magnetic resonance spectral data and chemical degradation.

Previous studies[3] dealing with the constituents of *Thalictrum thunbergii* DC. (Ranunclaceae) native of Tokushima have demonstrated the presence of four new alkaloids, thalicberine, O-methylthalicberine, thalicthuberine, and homoaromoline. The present paper describes the isolation and structural elucidation of an additional new alkaloid, thalictine, which was obtained from the stems and leaves of *T. thunbergii* DC. cultivated in the garden of medicinal herb.

Thalictine (**1**) was crystallized as its nitrate salt, colorless needles, mp 226—228°. The free base has a tendency to be oxidized rapidly in air and could not be crystallized. The molecular formula $C_{37}H_{40}O_6N_2$ of thalictine was established by elemental analysis together with a molecular weight determination by mass spectrometry. It is suggested by those studies that the base should most likely be a bisbenzylisoquinoline (biscoclaurine) alkaloid. The ultraviolet (UV) spectrum shows a peak at 284 mμ due to a benzenoid type. The base possesses $[\alpha]_D$ −15.8° ($CHCl_3$), −12.2° (MeOH). Amorphous thalictine was insoluble in dilute aqueous alkali, and it showed a positive ammonium phosphomolybdate color reaction.[4] The infrared (IR) spectrum shows the absorption band at 3550 cm^{-1}. Furthermore, methylation of thalictine with diazomethane under certain strict conditions gave O-methylthalictine (**2**), and similarly, O-ethylthalictine (**3**) was resulted from the reaction with diazoethane. These findings suggest that thalictine has a cryptophenolic hydroxyl group.

The nuclear magnetic resonance (NMR) spectrum of thalictine shows a characteristic pattern of a biscoclaurine alkaloid with two "head to head and tail to tail" diphenyl ether linkages.[5] It shows signals corresponding to two N-methyl groups at δ 2.19 and 2.62, three methoxyl groups at δ 3.62, 3.86, and 3.82, and ten aromatic protons in δ 5.84—6.90 regions. Out of the signals suggesting aromatic protons, two singlets at δ 5.84 and 6.01 are in higher fields than the rest of the aromatic protons. These singlets therefore can be ascribed to the protons at the 8 and the 8′ positions in both isoquinoline units as described later.

The mass spectrum of thalictine nitrate disclosses a molecular ion at *m/e* 608 ($C_{37}H_{40}O_6N_2$) and a base peak at *m/e* 198. The other significant peaks are at *m/e* 396, 395, and 175, as shown in Fig. 1. The base peak is a doubly charged ion, which suggests a double benzylic cleavage and loss of a diphenyl ether fragment. This fragmentation pattern has been shown to be

1) Part XVII: E. Fujita, A. Sumi and Y. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **20**, 368 (1972).
2) Location: *Sho-machi, Tokushima, 770, Japan.*
3) Part V—XI: E. Fujita and T. Tomimatsu, *Yakugaku Zasshi*, **79**, 1256, 1260, 1386 (1959); **80**, 1137 (1960); **82**, 311, 315, 320 (1962).
4) G.H. Stillson, D.W. Sawyer and C.K. Hunt, *J. Am. Chem. Soc.*, **67**, 303 (1945).
5) M. Tomita, T. Shingu, K. Fujitani and H. Furukawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 921 (1965).

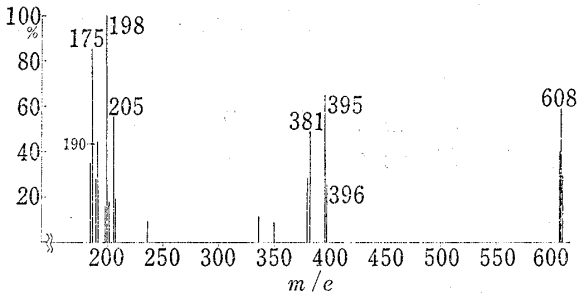
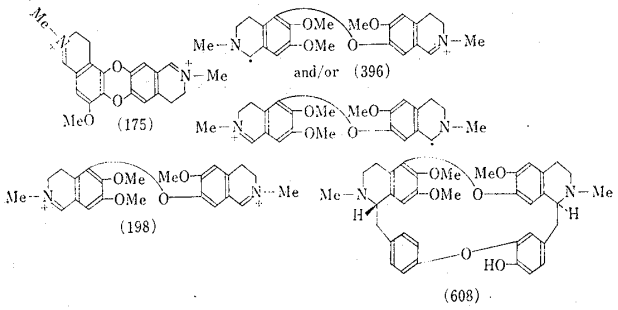


Fig. 1. Mass Spectrum of Thalictine



characteristic of oxyacanthine-berbamine type alkaloids.[6] Accordingly, these mass data indicate that three methoxyl groups are present in the isoquinoline units, and a cryptophenolic hydroxyl group is present in a benzyl moiety.

Permanganate oxidation of O-ethylthalictine methyl methine derived by the usual procedures yielded 4-ethoxy-3,4′-oxydibenzoic acid (**4**), mp 274—276°. The product was identified with an authentic sample by mixed melting point determination and comparisons of their IR and NMR spectra. From these evidences, it is concluded that the location of the cryptophenolic hydroxyl group of thalictine is fixed to the *ortho* position of the diphenyl ether linkage and the *para* position of the carboxylic acid, as shown in formula **5** of Chart 1.

The cleavage reaction of the diphenyl ether linkage with sodium in liquid ammonia[7] was applied to O-ethylthalictine (**3**). The reaction in the usual manner afforded a mixture of two species of phenolic cleavage products in an almost quantitative yield. One of the products was readily separated as its oxalate and identified as (*S*)-armepavine (**6**) by direct comparison of the free base and its oxalate with their authentic samples, respectively. Another phenolic product (**7**) was methylated by means of diazomethane to afford (*S*)-O-ethylarmepavine (**8**), which was identified by direct comparison with the authentic sample.

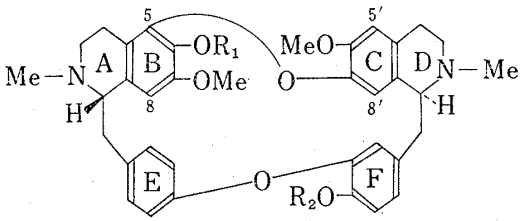
Similarly, the cleavage reaction of O-methylthalictine (**2**) with sodium in liquid ammonia gave a mixture of two phenolic cleavage products in an almost quantitative yield, which was separated as their oxalate salts. The crystalline oxalate was characterized as (*S*)-armepavine (**6**) oxalate, and identified with one of the cleavage products from O-ethylthalictine (**3**). For the rest (**9**) of the cleavage products, ethylation with diazoethane resulted in O-ethyl ether, and was characterized as (*S*)-1-(4-methoxybenzyl)-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydroisoquinoline (**10**) by direct comparison with the sample obtained from O-methyloxyacanthine.[8]

In view of the up-to-date informations on biscoclaurine type alkaloids and the foregoing chemical degradations, the formula **1** or **11** could be considered for the structure of thalictine. The formula **11** was, however, already assigned for the known alkaloid, repandine.[9] Thalictine was clearly distinguished from repandine by the present studies with physicochemical methods.

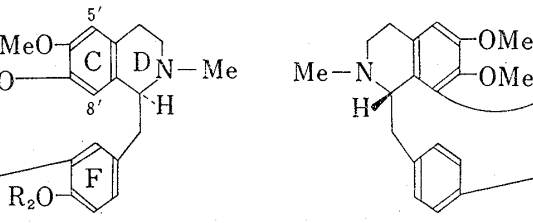
Recently, several biscoclaurines, such as thalmine (**12**),[10] lauberine (**13**),[11] dryadine

6) M. Shamma, B.S. Dudock, M.P. Cava, K.V. Rao, D.R. Dalton, D.C. DeJongh and S.R. Shrader, *Chem. Commun.*, **1966**, 7; M. Tomita, T. Kikuchi, K. Fujitani, H. Kato, H. Furukawa, Y. Aoyagi, M. Kitano and T. Ibuka, *Tetrahedron Letters*, **1966**, 857; D.C. DeJongh, S.R. Shrader and M.P. Cava, *J. Am. Soc.*, **88**, 1052 (1966).
7) M. Tomita, *Progr. Chem. Org. Nat. Prod.*, **9**, 175 (1952).
8) T. Tomimatsu and Y. Kano, *Yakugaku Zasshi*, **82**, 320 (1962).
9) I.R.C. Bick and A.R. Todd, *J. Chem. Soc.*, **1948**, 2170.
10) S. Yu. Yunusov and N.N. Progressov, *Zh. obsch. Kh.*, **20**, 1151 (1950); S. Yu. Yunusov and Z.F. Ismailov, *Doklady Akad. Nauk Uz. SSR*, **10**, 17 (1956).
11) M.R. Falco, J.X. de Vries, A.G. de Brovetto, Z. Maccio, S. Rebuffo and I.R.C. Bick, *Tetrahedron Letters*, **1968**, 1953.

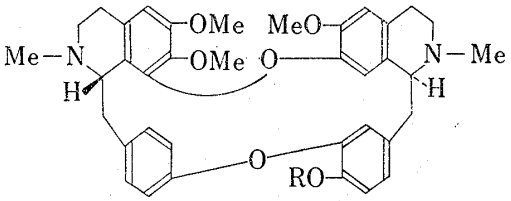
(**14**),[12] and dryadodaphnine (**15**)[12] have been shown to have the structure with a 5—7′ ether link between the isoquinoline units and a 21 membered heterocyclic ring. While, the other well-known types of biscoclaurines, like oxyacanthine-berbamine type alkaloids, have the structure with an 8—7′ ether link between the isoquinoline units and an 18 membered heterocyclic ring. The distinction between these types is observable in their NMR spectra



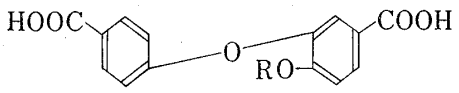
1 : R_1=Me, R_2=H (thalictine)
2 : R_1=R_2=Me
3 : R_1=Me, R_2=Et



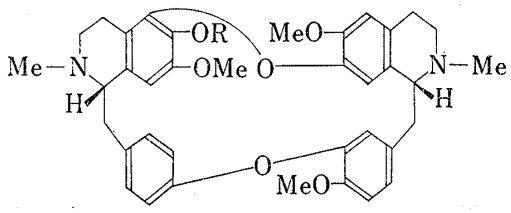
12 : R_1=H, R_2=Me (thalmine)
16=**2**



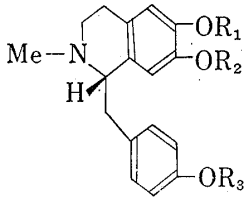
11: R=H, (repandine)
17: R=Me



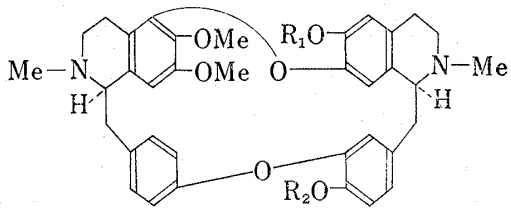
4 : R=Et
5 : R=H



13 : R=H (lauberine)
18 : R=Me



6 : R_1=R_2=Me, R_3=H
7 : R_1=Me, R_2=H, R_3=Et
8 : R_1=R_2=Me, R_3=Et
9 : R_1=R_3=Me, R_2=H
10 : R_1=R_3=Me, R_2=Et



14 : R_1=H, R_2=Me (dryadine)
19 : R_1=R_2=Me
15 : R_1=R_2=H (dryadodaphnine)

Chart 1

TABLE I. Chemical Shifts Data of Thalictine and Related Alkaloids in δ Values

	N-CH_3		OCH_3				Aromatic H		Absol. Conf.		Ref.
	2	2′	4″	6	6′	7	8 and 8′				
Thalictine (**1**)	2.19	2.62	—	3.62	3.86	3.82	6.01	5.84	*S*	*S*	*a*)
O-Methylthalictine (**2**)	2.16	2.64	3.90	3.68	3.89	3.86	6.08	5.86	*S*	*S*	*a*)
Repandine (**11**)	2.47	2.49	—	3.72	3.37	3.01	—		*S*	*S*	*a*)
O-Methylrepandine (**17**)	2.51	2.54	3.94	3.74	3.40	3.01	—		*S*	*S*	*a*)
Thalmine (**12**)	2.22	2.64	3.93	—	3.93	3.93	6.06	6.06	*S*	*S*	10)
O-Methylthalmine (**16**)	2.16	2.65	3.91	3.69	3.90	3.87	6.09	5.88	*S*	*S*	10)
Lauberine (**13**)	2.30	2.65	3.95	—	3.92	3.92	6.12	6.06	*S*	*R*	10)
O-Methyllauberine (**18**)	2.25	2.67	3.93	3.64	3.93	3.93	6.20	6.03	*S*	*R*	10)
Dryadine (**14**)	2.30	2.65	3.93	3.48	—	3.93	6.28	6.00	*R*	*S*	10)
O-Methyldryadine (**19**)	2.23	2.65	3.92	3.62	3.92	3.92	6.17	6.00	*R*	*S*	10)
Dryadodaphnine (**15**)	2.25	2.66	—	3.44	—	3.90	6.25	6.02	*R*	*S*	10)

a) this work

12) I.R.C. Bick, G.K. Douglas and W.I. Taylor, *J. Chem. Soc.*, **1969**, 1627.

as pointed out by Bick, *et al.*[13] According to them, the methoxyl at the 6 position in the 5—7′ linked biscoclaurines appears to be subjected to the influence of ring C and/or F, but the remaining methoxyls are not subjected to any particular shielding effect from other rings, whereas, the protons at the 8 and the 8′ position are evidently shielded by other aromatic rings. The chemical shifts in the NMR spectra of thalictine (**1**) and O-methylthalictine (**2**) are listed in Table I together with those of thalmine (**12**) and the related alkaloids. The information from the data indicates that thalictine should be one of the 5—7′ linked biscoclaurines, and particularly O-methylthalictine (**2**) have the chemical shifts identical with those of O-methylthalmine (**16**).

Consequently, it is concluded that the structure **1** should be assigned for thalictine. The mass spectral data and the molecular models also support the structure **1** for thalictine. Thalictine is the second example of a cryptophenolic hydroxyl group in the benzyl moiety next to dryadodaphnine (**15**). A 5—7′ ether link between the isoquinoline units and a 21 membered heterocyclic ring have so far been reported only in limited number of alkaloids,[10,11,12] and thalictine is an additional example. Besides, the stereostructure of thalictine is established as (*S*) and (*S*) type, and this conclusive evidence can be extended the structure of thalmine.

Experimental

Melting points were determined on a Yanako Micro Melting Point Apparatus and are uncorrected. UV spectrum was obtained on a Hitachi Model 124 spectrophotometer. IR spectra were taken by a Hitachi EPI-G-2 spectrometer. NMR spectra were recorded on a JEOL PS-100 spectrometer, using approximately 5% (M/V) solutions of compounds in $CDCl_3$ with Me_4Si as internal standard, unless otherwise stated. Mass spectrum was measured with a Hitachi Model RMU-6E spectrometer.

Isolation of Thalictine (**1**)——Stems and leaves of *T. thunbergii* DC. were obtained from the garden of medicinal herb of the Faculty. The raw material (44.2 kg) were chopped, air-dried, and followed by extraction with boilling MeOH for 3 days. The MeOH extract was concentrated under reduced pressure to dark semi-solid concentrate, and poured into warm 5% AcOH with stirring. The acid solution was freed from acidic and neutral substances by extraction with ether, made alkaline with NH_4OH soln, and extracted exhaustively with ether. The ether solution was extracted with 5% KOH to remove phenolic bases, then dried over anhyd. K_2CO_3, and evaporated to dryness on a steam bath. A solution of the residue in 5% AcOH was treated with conc. aqueous KNO_3, and allowed to stand until precipitation was completed. Recrystallization of the precipitate from MeOH yielded 3.15 g of colorless needles, mp 226—228°. *Anal.* Calcd. for $C_{37}H_{40}O_6N_2 \cdot 2HNO_3 \cdot 1\frac{1}{2}H_2O$ (thalictine nitrate): C, 58.33; H, 5.95; N, 7.36. Found: C, 58.40; H, 6.02; N, 7.18. Mass spectrum, see Fig. 1.

Thalictine (**1**)——The free base was unable to be crystallized. $[\alpha]_D^{28}$ −15.8° (c=1.203, $CHCl_3$); −12.2° (c=1.639, MeOH). UV λ_{max}^{EtOH} mμ: 284. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH). NMR, see text and Table I. Thalictine was insoluble in dilute aqueous alkali, and gave a positive ammonium phosphomolybdate color reaction for a cryptophenolic hydroxyl group (*cf.* O-methylthalictine (**2**) and O-ethylthalictine (**3**): negative). It was oxidized rapidly in air and the IR spectrum of the oxidized base showed $\nu_{max}^{CHCl_3}$ cm^{-1}: 1690 (C=O).

Hofmann Degradation of O-Ethylthalictine (**3**)——A solution of thalictine (**1**) (516 mg) in MeOH was treated with an excess of an ethereal solution of diazoethane, and the solution was allowed to stand for 6 days at room temperature. The solvent was evaporated and the residue was dissolved in 2% AcOH, and extracted with ether. After the acidic solution was ajusted to alkali with dilute KOH soln, the alkaline solution was extracted with ether, and then the ethereal solution was dried over anhyd. K_2CO_3. The residue obtained upon evaporation of the ether was characterized by NMR spectrum as O-ethylthalictine (**3**). NMR δ: 1.44 (3H, t, $-CH_2\underline{CH_3}$), 4.09 (2H, q, $-\underline{CH_2}CH_3$), 2.14 and 2.64 (2×N–CH_3), 3.68 (C_6–OCH_3), 3.89 ($C_{6'}$–OCH_3), 3.85 (C_7–OCH_3), 5.81—6.94 (aromatic H).

A solution of O-ethylthalictine (**3**) in MeOH was heated with methyl iodide for 3 hr under reflux, and the solvent was evaporated to give O-ethylthalictine dimethiodide. The dimethiodide was converted into the dimethochloride by the action of fresh AgCl, and then dimethochloride in 30% aqueous KOH was heated for 3 hr. The alkaline solution was exhaustively extracted with ether. The combined ethereal solution was dried over anhyd. K_2CO_3, and evaporated to get O-ethylthalictine methyl methine (435 mg).

13) J. Baldas, Q.N. Poter, I.R.C. Bick, G.K. Douglas, M.P. Falco, J.X. de Vries and S. Yu. Yunusov, *Tetrahedron Letters*, **1968**, 6315.

Permanganate Oxidation of O-Ethylthalictine Methyl Methine——To a solution of O-ethylthalictine methyl methine (430 mg) in acetone (60 ml), 2% $KMnO_4$ soln (300 ml) was gradually added with vigorous agitation at room temperature for 4 hr, and then heated in a water bath at 60° for 40 min. The reaction mixture was treated with SO_2 to destroy MnO_2. The aqueous solution was exhaustively extracted with ethyl acetate, and the latter phase was extracted with 10% Na_2CO_3. Acidification with dilute HCl, and extraction with ether, drying over anhyd. Na_2SO_4, and evaporation yielded colorless prisms of an acid. Recrystallization from MeOH yielded 4-ethoxy-3,4′-oxydibenzoic acid (4) (100 mg), mp 274—276°. *Anal.* Calcd. for $C_{16}H_{14}O_6$ (4-ethoxy-3,4′-oxydibenzoic acid): C, 63.57; H, 4.67. Found: C, 63.11; H, 4.77. NMR (in DMSO) δ: 1.16 (3H, t, $-CH_2C\underline{H}_3$), 4.10 (2H, q, $-C\underline{H}_2CH_3$), 6.94 and 7.90 (2×2H, AB type C=C), 7.26 and 7.82 (2H, AB type C=C), 7.57 (1H, s), —3.80 (broad OH). The melting point was not depressed by admixture with an authentic sample, and the IR(KBr) and NMR spectra were superimposable upon those of the reference sample.

Sodium in Liquid Ammonia Cleavage of O-Ethylthalictine (3)——A solution of O-ethylthalictine (3) (400 mg) in 30 ml of tetrahydrofuran and metallic Na(1 g) were alternately added drop by drop to 400 ml of dry liquid NH_3 over 4 hr, which was collected in 1 liter three necked flask. The blue colored solution was allowed to stand with vigorous agitation for 40 min at −70°, and then followed by standing overnight in a hood to evaporate the NH_3. After the residue was obtained, 10% HCl and ether were added, and extracted. The aqueous solution was made alkaline with NH_4OH and extracted with ether. The ethereal solution was dried over anhyd. $MgSO_4$ and evaporated to dry a mixture of cleavage phenolic bases.

(*S*)-Armepavine (6)——A solution of cleavage phenolic bases in EtOH was treated with a saturated solution of oxalic acid in EtOH, and allowed to stand until precipitation was completed. Recrystallization of the precipitate from EtOH gave colorless needles (217 mg), mp 201—202°. *Anal.* Calcd. for $C_{19}H_{23}O_3N\cdot(COOH)_2\cdot1/4\,H_2O$ ((*S*)-armepavine oxalate): C, 61.83; H, 6.30; N, 3.43. Found: C, 61.75; H, 6.24; N, 3.25. The oxalate salt was identified by mixed melting point determination, and IR spectral comparison with an authentic sample. The colorless needles having mp 144—145° of the free base was obtained from benzene. *Anal.* Calcd. for $C_{19}H_{23}O_3N$ ((*S*)-armepavine): C, 72.82; H, 7.40; N, 4.47. Found: C, 72.45; H, 7.45; N, 4.07. $[\alpha]_D^{22}$ +86.9° (c=0.115, $CHCl_3$). The compound was characterized by mixed melting point, IR(KBr) and NMR spectral studies with an authentic sample.

(*S*)-O-Ethylarmepavine (8) from (*S*)-1-(4-Ethoxybenzyl)-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (7)——The filtrate from the above-mentioned oxalate salt was treated to liberate the free base of **7**. A solution of the free base in MeOH was added an ethereal solution of diazomethane and left for 24 hr at room temperature. An amorphous solid was resulted from the following evaporation. The solid base was converted to its oxalate, and recrystallized from EtOH to yield colorless needles (170 mg), mp 159—161°. *Anal.* Calcd. for $C_{21}H_{27}O_3N\cdot(COOH)_2$ ((*S*)-O-ethylarmepavine oxalate): C, 64.02; H, 6.77; N, 3.25. Found: C, 64.09; H, 7.24; N, 2.92. The oxalate salt was characterized by mixed melting point and IR spectral comparison with an authentic sample. Liberation of the free base from the oxalate gave amorphous (*S*)-O-ethylarmepavine (**8**). $[\alpha]_D^{20}$ +58.7° (c=0.205, $CHCl_3$). TLC, IR, and NMR studies were held to identify the product with authentic (*S*)-O-ethylarmepavine.

Sodium in Liquid Ammonia Cleavage of O-Methylthalictine (2)——A solution of O-methylthalictine (2) (400 mg) in 30 ml of ether was treated with metallic Na(1 g) in liquid NH_3 according to the method described in the case of O-ethylthalictine (3). After the reaction, the NH_3 removed, and the residue was obtained. The residual fraction was acidified with 10% HCl, and washed with ether. The aqueous solution was made alkaline with NH_4OH, and then exhaustively extracted with ether. The combined ethereal solution was dried over anhyd. $MgSO_4$, and evaporated to dryness to give a mixture of phenolic bases.

(*S*)-Armepavine (6) and (*S*)-1-(4-Methoxybenzyl)-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydroisoquinoline (10)——One of the cleavage products was crystallized as its oxalate, colorless needles (205 mg), mp 200—201°, and liberation of the free base gave (*S*)-armepavine (**6**), colorless needles, mp 144—145°. $[\alpha]_D^{22}$ +83.5° (c=0.136, $CHCl_3$). The base was identified by mixed melting point, IR (KBr), and NMR spectral comparisons with authentic (*S*)-armepavine (**6**).

The filtrate from the oxalate salt was treated to liberate free base (**9**). NMR δ: 2.46 ($N-CH_3$), 3.80 (C_6-OCH_3), 3.75 ($C_{4'}-OCH_3$), —5.06 (broad OH), 6.29 (C_8-H), 6.50 (C_5-H), 6.71, 6.80, 6.97, and 7.05 (aromatic H). The free base was ethylated with an ethereal solution of diazoethane in the usual method. The O-ethyl ether was crystallized as its oxalate salt, colorless needles (185 mg), mp 169—170°. *Anal.* Calcd. for $C_{21}H_{27}O_3N\cdot(COOH)_2\cdot H_2O$ ((*S*)-1-(4-methoxybenzyl)-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydroisoquinoline oxalate): C, 61.45; H, 6.95; N, 3.12. Found: C, 61.52; H, 6.60; N, 3.03. The oxalate salt was identified by mixed melting point, and IR(KBr) spectral comparison with an authentic sample. The free base from the oxalate was unable to be crystallized. $[\alpha]_D^{22}$ +65.7° (c=0.153, $CHCl_3$). The compound also identified by TLC, IR, and NMR spectral comparisons with authentic (*S*)-1-(4-methoxybenzyl)-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydroisoquinoline (**10**).

Acknowledgements We are grateful to Emeritus Prof. M. Tomita and Prof. E. Fujita, Kyoto University, for their kind encouragement. Thanks are also due to Drs. K. Hirai and Y. Nakagawa, Shionogi & Co., Ltd., for mass spectral measurement, and to the staffs of the Elemental Analysis Centre of this Faculty.