Notes

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Studies on Constituents of Crude Drugs. I. Alkaloids of *Symphytum officinale* Linn.

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Two pyrrolizidine alkaloids, symphytine (I), a new compound, and echimidine (II) have been isolated from the dried roots of *Symphytum officinale*. Both have a retronecine nucleus esterified on the 7-hydroxyl group with angelic acid. The other esterifying acid of symphytine is *l*-viridifloric acid (III), identical with the authentic sample. The structure of symphytine, a diastereoisomer of echiumine (retronecine esterified with angelic acid and trachelanthic acid), has been confirmed. A large amount of allantoin has also been isolated from the dried roots and leaves.

Symphytum officinale Linn.[2] is widely distributed in Europe and now cultivated in Japan. It is called comfrey or Russian comfrey, which indicates generally the dried roots, and in Japan the fresh or dried leaves or occasionally the dried roots. Comfrey is used as a demulcent in chronic catarrhs and certain mucous membrane affections of the gastrointestinal tracts in domestic medicine of Europe and as a tonic in Japan.

On the chemical constituents the presence of allantoin,[3] alkaloids[3e,4] such as consolicine, consolidine, and symphytocynoglossine, polyphenols,[5] amino acids,[6] phytosterols,[3e,6a] triterpenoids,[6a] and saccharides[6b,7] *etc.* have already been reported. However there are now little informations on the chemical structures and biological activities of these alkaloids. The present report describes on the isolation of two alkaloids from methanol extracts of the dried roots, their chemical structure, and the presence of a large amount of allantoin in the leaves and roots.

Symphytum officinale belongs to the family Boraginaceae, and consequently the presence of pyrrolizidine alkaloids was strongly suggested. Preliminary investigation disclosed that the alkaloids were present mainly in the form of N–oxides. After reduction of the aqueous

1) Location: *Shiba, Minato-ku, Tokyo.*

2) G. Madaus, "Lehrbuch der Biologischen Heilmittel," Abt. 1, Heilpflanzen Bd. III, Georg Thieme Verlag, Leipzig, 1938, pp. 2648—2654; H.W. Youngken, "A Textbook of Pharmacognosy," 6 ed., The Blakiston Company, Philadelphia, 1950, pp. 701—703; F. Berger, "Handbuch der Drogenkunde," Bd. V, Wilhelm Maudrich Verlag, Wien, 1960, pp. 147—151.

3) *a*) K. Mothes and L. Engelbrecht, *Hoppe Seylers Z.*, **295**, 387 (1953); *b*) F. Kaczmarek and A. Walicka, *Biul. Inst. Roślin Leczniczych*, **4**, 273 (1958) [*C.A.*, **53**, 15487*h* (1959)]; *c*) M. Repta, *Farmacia* (Bucharest), **10**, 645 (1962) [*C.A.*, **58**, 13713*f* (1963)]; *d*) M.V. Tracey, "Modern methods of plant analysis," ed. by K. Paech and M.V. Tracey, Springer–Verlag, Berlin, 1955, pp. 119—141. *e*) T. Furuya and H. Kojima, reported at Pharmacognostical Society of Japan, Toyama, April 1966.

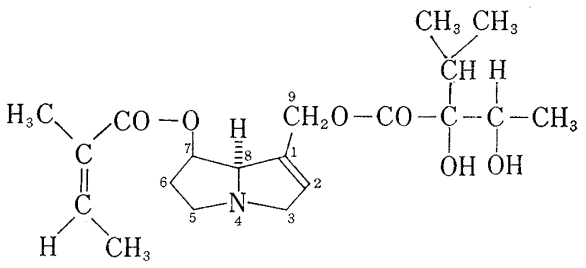
4) *a*) K. Greimer, *Arch. Pharm.*, **238**, 505 (1900); *b*) F.L. Warren, "Fortschritte der Chemie organischer Naturstoffe," Vol. 24, Springer–Verlag, Wien, 1966, pp. 329—406.

5) F. Kaczmarek and A. Walicka, *Biul. Inst. Roślin Leczniczych*, **5**, 89 (1959) [*C.A.*, **54**, 3606 *e* (1960)].

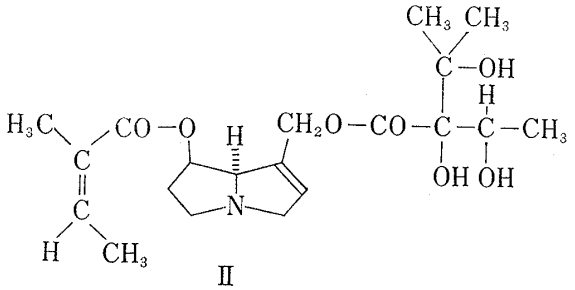
6) *a*) T. Takemoto and F. Kitame, reported at Meeting of Tohoku Branch, Pharmaceutical Society of Japan, Sendai, February 1966; *b*) T. Iida, N. Hoshino and T. Murakami, *Syoyakugaku Zasshi*, **21**, 131 (1967).

7) R. Bourdu, *Compt. Rend.*, **239**, 1524 (1954) [*C.A.*, **49**, 6387*f* (1955)]; R. Bourdu, *Rev. Gén. Botan.*, **64**, 153, 197 (1957) [*C.A.*, **51**, 16751*g* (1957)]; R. Bourdu, *Compt. Rend.*, **246**, 973 (1958) [*C.A.*, **52**, 11196*d* (1958)]; V. Plouvier, *Compt. Rend.*, **247**, 2190 (1958) [*C.A.*, **53**, 13295*d* (1959)].

acid solution of total base, the reduced bases were investigated. Thin-layer chromatogram showed the crude base consists of two major components. The reduced bases were separated on silica gel column in chloroform–methanol. Base-1(I) was eluted rapidly with chloroform and base-2(II) of lower *Rf* value on thin-layer chromatogram very slowly.



I



II

Base–1 was obtained only as a clear liquid glass but did not give any crystalline derivatives. The empirical formula, $C_{20}H_{31}O_6N$, indicated for base-1, was confirmed by the degradation results.

The IR spectrum in KBr showed the presence of a ester (1720, 1705, 1250, 1160 cm^{-1}), a hydroxyl function (3470 cm^{-1}) and a double bond (1650 cm^{-1}). The NMR spectrum[8] (Fig. 1) gave valuable implications. Five methyl signals were observed as doublets at δ 0.87, 0.91, 1.18, 1.77, 2.05. The former two methyl groups are expected to be isopropyl, the third to be linked with secondary alcohol, and the latter two signals to be linked with a double bond, presumably with 2- and 3- carbons of angelic acid. Viridifloric acid[9] esters tend to have the isopropyl methyl groups magnetically non-equivalent,[10] and the chemical shift of the methyl proton in CH_3-CH-OH is near 1.20 ppm. The presence of viridifloric acid is therefore presumed.

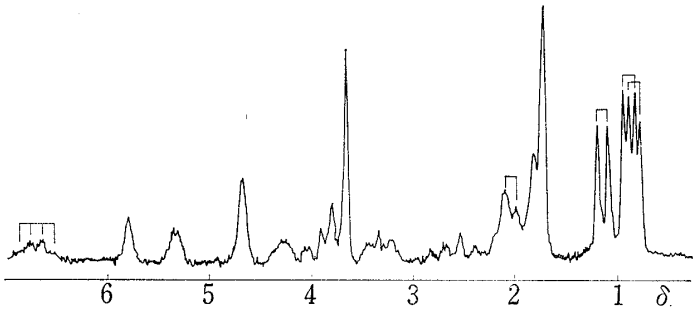


Fig. 1. NMR Spectrum of Symphytine

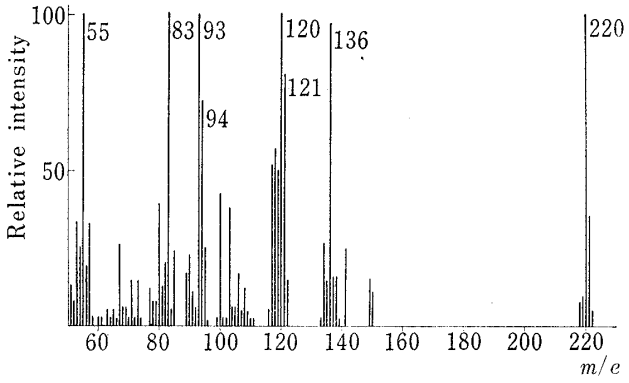


Fig. 2. Mass Spectrum of Symphytine

The mass spectrum of base-1 (Fig. 2) exhibited the characteristic fragmentations[11] (Chart 1). The molecular ion was not confirmed, but the ion at *m/e* 220 is possibly to be 7-angelyl-dehydroxyretronecine and therefore angelicacid may be possibly esterified on the position of 7-hydroxyl group.

Hydrolysis of base-1 gave rise to retronecine, angelic acid, and the second esterifying acid. The second esterifying acid showed mp 122—123°, $[\alpha]_D^{25}$ −1.4° (c=2.34, EtOH). The mass spectrum

8) C.C.J. Culvenor, M.L. Heffernan, and W.G. Woods, *Aust. J. Chem.*, **18**, 1605 (1965); C.C.J. Culvenor and W.G. Woods, *Aust. J. Chem.*, **18**, 1625 (1965).
9) H.C. Crowley and C.C.J. Culvenor, *Aust. J. Chem.*, **12**, 694 (1959).
10) H.C. Crowley and C.C.J. Culvenor, *Aust. J. Chem.*, **15**, 139 (1962); C.C.J. Culvenor and L.W. Smith, *Aust. J. Chem.*, **19**, 1955 (1966).
11) C.K. Atal, K.K. Kapur, C.C.J. Culvenor, and L.W. Smith, *Tetrahedron Letters*, **1966**, 537.

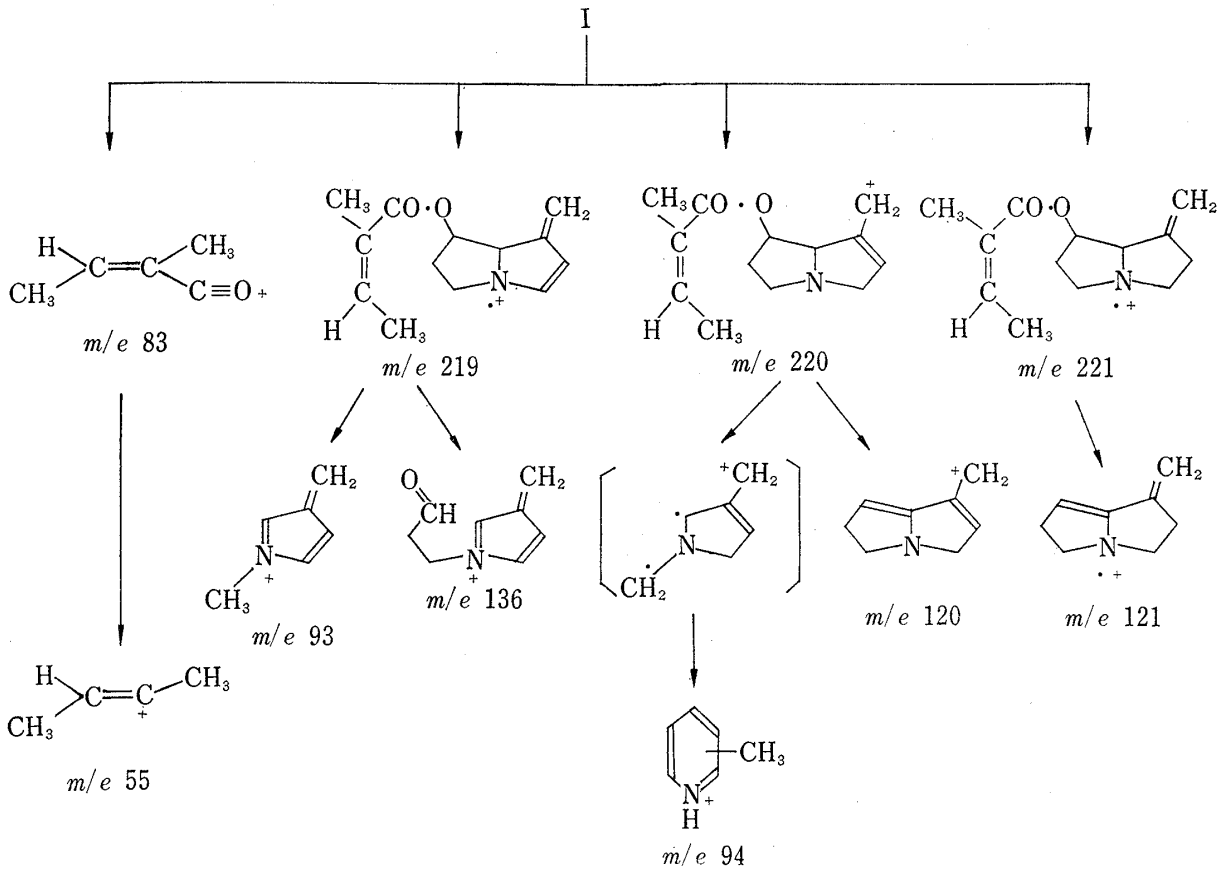


Chart 1. Proposed Fragmentation of Symphytine

of the acid is shown in Fig. 3, and the compositions of the molecular ion and major fragments were determined by high resolution measurements (Chart 2). From the mass spectrometry and the mixed melting point mp 122—123° by Culvenor, the acid was shown to be identical with *l*-viridifloric acid (III). On hydrogenation with a platinum catalyst, 3 moles of hydrogen were consumed, the basic product was an oil, whose picrate gave mp 202—203°, unchanged on mixing with the picrate of 7-(2′-methylbutyryl)retronecanol[12] by Culvenor. It follows from these results, NMR, and Mass spectra that chemical structure of base-1, named symphytine, was shown to be I. Symphytine is therefore a diasteroisomer of echiumine[12], diester of retronecine and two different monobasic acids, angelic acid and trachelanthic acid.

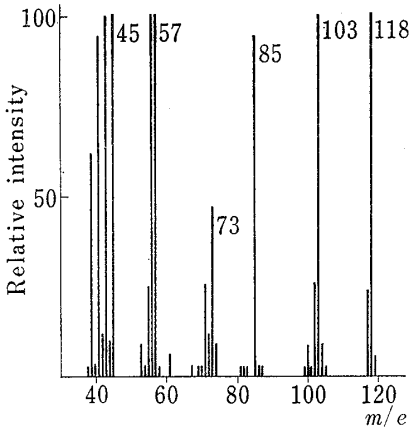


Fig. 3. Mass Spectrum of *l*-viridifloric acid

On alkaline hydrolysis, base-2 yielded retronecine, angelic acid, and an esterifying acid. The brucine salt of an esterifying acid showed mp 207—210°, no depression on admixture with the salt of echimidic acid. Hydrogenolysis of base-2 with a platinum gave a base, $C_{13}H_{23}O_2N$, identical with 7-(2′-methylbutyryl)retronecanol obtained from echimidine. From these chemical results, IR, NMR, and, Mass spectral data base-2 was the same compound as echimidine, which was isolated from *Echium plantagineum*[12] (Boraginaceae).

12) C.C.J. Culvenor, *Aust. J. Chem.*, **9**, 512 (1956).

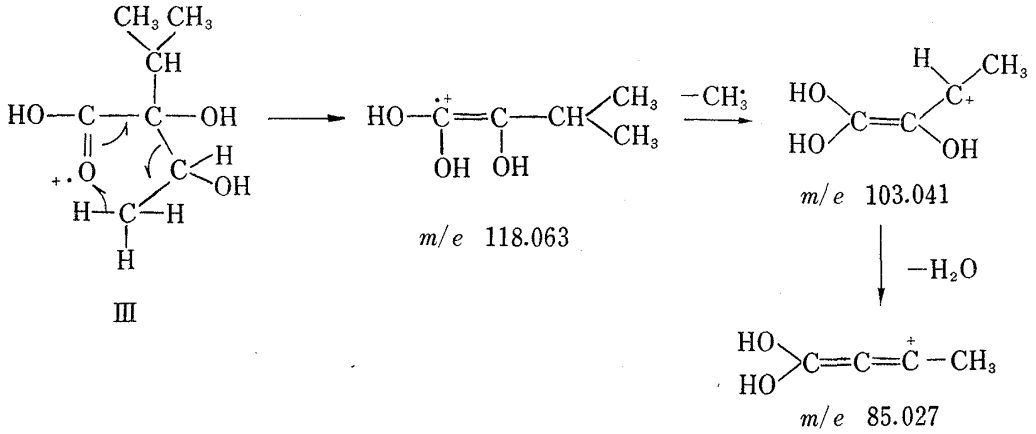


Chart 2. Proposed Fragmention of *l*-Viridifloric Acid

The presence of symphytine and echimidine, diester of pyrrolizidine alkaloids having retronecine nucleus, is of interest from chemotaxonomical point of view.

From the results of pharmacological test, symphytine showed LD_{50} of about 300 mg/kg, did not show cytotoxical effect, and depressed 30 mmHg of blood pressure at dose level of 5 mg/kg rat. Symphytine, echimidine, and methanol extract were slightly effective against Ehrlich ascites tumor and S-180, although methanol extract of whole plant showed considerably protective effect on mice bearing spontaneous and transplant tumors.[13]

Experimental[14]

Isolation of Crude Alkaloids and Allantoin from *S. officinale*——The dried, milled root (5 kg) of *S. officinale* was extracted with methanol. Methanol was removed from the extract under reduced pressure. From the concentrated methanol solution, crude allantoin was separated. After recrystallization from water, pure allantoin (32 g) of mp 230—231° (decomp.), $[\alpha]_D$ ±0°, was obtained, no depression with authentic sample. *Anal.* Calcd. for $C_4H_6O_3N_4$: C, 30.39; H, 3.83; N, 35.43. Found: C, 30.43; H, 3.87; N, 35.86. By the same treatment of the leaves (1 kg), pure allantoin (3 g) was obtained.

The obtained residue was extracted with dilute hydrochloric acid. The aqueous acid solution was reduced by stirring with zinc dust and additional 30% hydrochloric acid to make the solution 1N with respect to acid. The crude alkaloid was obtained by making alkaline to phenolphthalein with ammonia, and extracting with chloroform. The chloroform solution contained symphytine of Rf=0.60 and echimidine of Rf=0.51 in TLC using $CHCl_3$–MeOH–NH_4OH (60:10:1). In TLC of alkaloid fraction from the leaves, the same two spots were detected in a small amount. Crude base (11.3 g) was submitted to SiO_2 column chromatography. After elution by 4% methanol in $CHCl_3$, base-1, symphytine, (2.8 g) was present in fraction No. 55—79 and base-2, echimidine, (2.7 g) in fraction No. 100—140. Mixed base (5.3 g) was recovered from fraction No. 80—100.

Purification of Symphytine and Echimidine——The fractions containing symphytine and echimidine could not be induced to crystallize even after keeping for 6 months in a refrigerator.

Symphytine: An almost colorless oil, $[\alpha]_D^{24}$ +3.65° (c=4.28, EtOH), IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 1720, 1705, 1250, 1160 (ester), 1650 (double bond). NMR (10.5% solution in CCl_4) δ: 0.87 (3H, doublet, J=6.1 cps $-CH\langle^{CH_3}_{CH_3}$), 0.91 (3H, doublet, J=6.6 cps, $-CH\langle^{CH_3}_{CH_3}$), 1.18 (3H, doublet, J=6.3 cps, $-\underset{OH}{\overset{H}{C}}-CH_3$), 1.77 (3H, singlet, $^{CH_3}_{H}\rangle C=C\langle^{CO^-}_{CH_3}$), 2.05 (3H, doublet, J=6.5 cps, $^{CH_3}_{H}\rangle C=C\langle^{CO-}_{CH_3}$), 2.3—3.0 (2H, multiplet), 3.0—3.5 (2H, multiplet), 3.68 (2H, singlet, $-\underset{HO}{C}-\underset{OH}{C}-$), 3.85 (H), 4.05 H, $-\underset{OH}{CH}-CH_3$), 4.30 (H, C_8–H), 4.68 (2H, singlet,

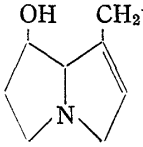
13) A. Taylor and N.C. Taylor, *Proc. Soc. Exper. Biol. Med.*, **114**, 772 (1963).

14) Melting points are uncorrected. $[\alpha]_D$ were measured with a JASCO DIP-SL. NMR spectra were measured with Hitachi H-60 at 60 Mc. Chemical shifts were recorded in δ values, using tetramethylsilane as the internal reference. Mass spectra were measured with JMS-OlS Mass Spectrometer.

C_9–H), 5.35 (H, singlet, C_7–H), 5.80 (H, singlet, C_2–H), 6.70 (H, quartet, J=6.5 cps, $=C\langle^{CH_3}_{H}$). Mass Spectrum *m/e*: 220, 136, 121, 120, 118, 103, 94, 93, 83, 55.

Echimidine: $[\alpha]_D^{24}$ +0.51° (c=4.31, EtOH), IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1720, 1708, 1245, 1165 (ester), 1648 (double bond). NMR (17.6% solution in CCl_4) δ: 1.14 (3H, doublet, J=5.7 cps, $-\underset{OH}{CH}-CH_3$), 1.15 (3H, singlet, $-C\langle^{CH_3}_{CH_3}$ OH), 1.19 (3H, singlet, $-C\langle^{CH_3}_{CH_3}$ OH), 1.80 (3H, singlet, $^{CH_3}_{H}\rangle C=C\langle^{CO^-}_{CH_3}$), 1.91 (3H, doublet, J=6.8 cps, $^{CH_3}_{H}\rangle C=C\langle^{CO^-}_{CH_3}$), 2.55, 3.20—4.00, 4.20 (3H, singlet, $-\underset{OH}{CH}-CH_3$), 4.31 (2H, singlet, C_8–H), 4.72 (2H, singlet, C_9–H), 5.48 (H, singlet, C_7–H), 5.83 (H, singlet, C_2–H), 6.07 (H, quartet, J=6.8 cps, $=C\langle^{CH_3}_{H}$). Mass spectrum *m/e*: 220, 136, 121, 120, 119, 118, 106, 94, 93, 83, 55.

Hydrolysis of Symphytine——Symphytine (500 mg) was boiled for 2 hr in 2 N sodium hydroxide (20 ml) under partial reflux. The reaction mixture was acidified to Congo red, filtered, and extracted three times with light petroleum. The light petroleum extracts gave an oil (100 mg) which crystallized readily and separated from water in long needles, mp 43—45°, unchanged on admixture with angelic acid. *Anal.* Calcd. for $C_5H_8O_2$: C, 59.98; H, 8.05. Found: C, 60.02; H, 8.21.

The light petroleum–insoluble acid was not able to obtain in crystalline from. The aqueous layer was concentrated to 5—10 ml. Basic solution obtained by the addition of solid sodium hydroxide (5 g) was extracted many times with chloroform. Recrystallization of the chloroform residue substance (40 mg) from acetone gave retronecine as colorless prisms, mp 116—117°, mixed mp 117—119°. *Anal.* Calcd. for $C_8H_{13}O_2$–N: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.90; H, 8.51; N, 9.30. Mass Spectrum *m/e*: 155.095 (M^+) (Calcd. 155.095 for $C_8H_{13}O_2N$), 138.094 (M^+-OH)  (Calcd. 138.092 for $C_8H_{12}ON$), 94.065 (Calcd. 94.066 for C_6H_8N).

Hydrogenolysis of Symphytine——Symphytine (574 mg) absorbed 124 ml (3.0 moles) for 3.5 hr, when it was shaken with hydrogen and reduced platinum oxide catalyst in dilute hydrochloric acid. The solution was made alkaline, and extracted with chloroform to give an oil (200 mg), which formed a picrate, yellow needles, mp 202—203° (from EtOH) (145 mg), unchanged on admixture with the picrate of 7-(2′-methylbutyryl)retronecanol obtained by hydrolysis of echimidine by Culvenor. *Anal.* Calcd. for $C_{19}H_{26}O_9N_4$: C, 50.22; H, 5.77; N, 12.33. Found: C, 49.88; H, 5.85; N, 11.84.

The residual aqueous solution was acidified to Congo red and extracted continuously with chloroform. The chloroform extracts gave a gum (31 mg) which crystallized completely. Recrystallization from benzene–light petroleum gave *l*-viridifloric acid, mp and mixed mp 122—123° by Culvenor, $[\alpha]_D^{25}$ −1.4° (c=2.34, EtOH). *Anal.* Calcd. for $C_7H_{14}O_4$: C, 51.84; H, 8.70. Found: C, 52.00; H, 8.66. Mass Spectrum *m/e*: 118.063 (Calcd. 118.063 for $C_5H_{10}O_3$), 103.041 (Calcd. 103.040 for $C_4H_7O_3$), 85.027 (Calcd. 85.029 for $C_4H_5O_2$).

Hydrolysis and Hydrogenolysis of Echimidine——Hydrolysis and hydrogenolysis of echimidine were carried out by the same method used for symphytine. Both retronecine and angelic acid were obtained by hydrolysis. By hydrogenolysis 7-(2-methylbutyryl) retronecanol and echimidinic acid, $[\alpha]_D^{20.7}$ +15.4° (c=1.58, EtOH) (its brucine salt, mp 207—210° (decomp.). *Anal.* Calcd. for $C_{30}H_{40}O_9N_2$: C, 62.92; H, 7.04; N, 4.89. Found: C, 62.44; H, 6.86; N, 4.52) were confirmed.

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