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SHORT COMMUNICATION

ALKALOIDS AND TRITERPENOIDS OF *SYMPHYTUM OFFICINALE**

TSUTOMU FURUYA and MANABU HIKICHI

School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan

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Abstract—Alkaloids, symphytine and echimidine, triterpenoids, isobauerenol, and phytosterols, β-sitosterol, have been isolated from the roots of *Symphytum officinale* (Boraginaceae). The structure of symphytine was also determined as 7-tiglylretronecine viridiflorate (I), and is the first isolated pyrrolizidine alkaloids with tiglic acid.

IN OUR earlier paper,[1] we reported the isolation of alkaloids, symphytine and echimidine, from the roots of *Symphytum officinale* Linn. At the same time, it was presumed that symphytine was 7-angelylretronecine viridiflorate. However, a question remained whether angelic acid or tiglic acid is attached to the C-7 atom in symphytine.

The present paper deals with studies on the acid at C-7 in symphytine, by NMR double irradiation experiments and careful hydrolysis, and the isolation of triterpenoids, isobauerenol, and phytosterols, β-sitosterol.

ALKALOIDS

The alkaloids were obtained according to the previous procedure. Symphytine (I) was shown to be a diester alkaloid, $C_{20}H_{31}O_6N$, and its IR and NMR spectra were the same as that reported previously.

As shown in Table 1 and Fig. 1, the peak at δ 6·69 ppm (1H, q, $J = 6{\cdot}0$ cps) had been assigned to be a signal of the olefinic proton of the angelic ester and coupled with the peak at 2·05 (3H, d, $J = 6{\cdot}0$). However, by irradiation at 2·05 (H_2-a), no influence was observed on the peak at 6·69, but the broad singlet at 5·35 became a sharp doublet. Irradiating at 6·69 (H_2-b), the peak at 1·77 became a clean singlet and furthermore at 1·77 (H_2-c), the peak at 6·69 became a sharp singlet.

From the above results and the chemical shift,[2] it was assumed that the peak at 2·05 is due to the protons at C-6 and that at 5·34 to the proton at C-7 in pyrrolizidine nucleus, respectively and that at 6·69 to the olefinic proton of angelic or tiglic acids. The NMR spectra of methyl angelate and methyl tiglate were reported that the chemical shifts of β-olefinic and β-methyl protons in methly angelate exhibit 5·98 and 1·97, respectively and those of methyl tiglate 6·73 and 1·77, respectively.[3] Ordinarily, the chemical shifts of the olefinic protons in angelic esters exhibit 5·60–6·20, whilst those of tiglic esters 6·50–7·30.[4] It was concluded that the peak at 6·69 is due to the olefinic proton of tiglic ester and the peaks at 1·77 and 1·72 to α- and β-methyl protons, respectively.

* Part II in the series "Studies on Constituents of Crude Drugs". For Part I see Ref. 1.

[1] T. FURUYA and K. ARAKI, *Chem. Pharm. Bull.* **16,** 2512 (1968).
[2] C. C. J. CULVENOR, M. L. HEFFERNAN and W. G. WOOD, *Aust. J. Chem.* **18,** 1605 (1965).
[3] L. A. JACKMAN and R. H. WILEY, *J. Chem. Soc.* 2886 (1960).
[4] M. D. NAIR and R. ADAMS, *J. Amer. Chem. Soc.* **82,** 3786 (1960).

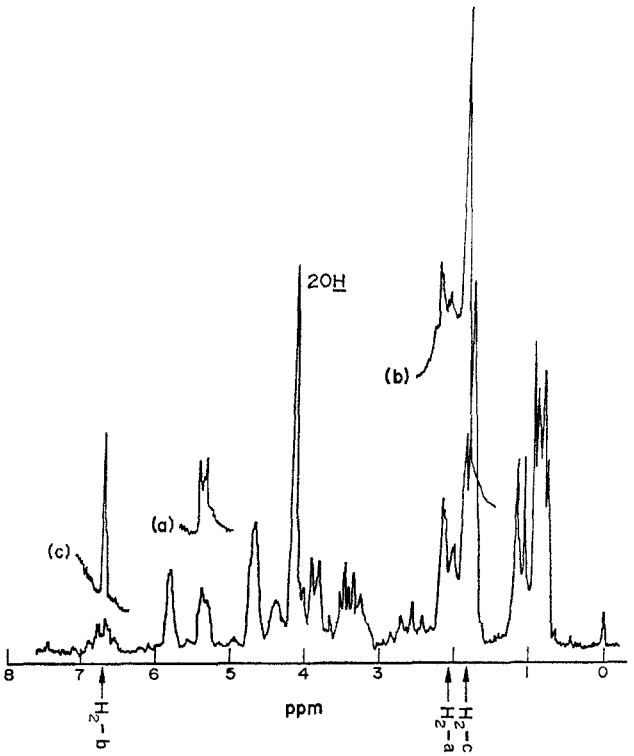


FIG. 1. NMR SPECTRUM OF SYMPHYTINE.

TABLE 1. NMR SPECTRAL DATA OF SYMPHYTINE AT 60 Mc (IN $CDCl_3$)

δ (ppm)	J (counts/sec)	Integral	Assignment
0·81	*d*, 6·1	3H	v-4,5
0·96	*d*, 6·6	3H	
1·14	*d*, 6·3	3H	v-2
1·72		3H	t-1
1·77	*d*, 6·0	3H	t-2
1·92–3·55		7H	C_3–H_2, C_5–H_2, C_6–H_2, v-3
3·84	*q*, 6·3	1H	v-1
4·35		1H	C_8–H
4·65		2H	C_9–H_2
5·34	broad singlet	1H	C_7–H
5·78		1H	C_2–H
6·69	*q*, 6·0	1H	t-3

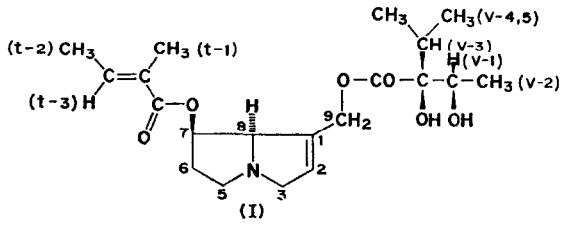
Spectrum was measured by Hitachi–Perkin–Elmer R-20.
d = doublet, *q* = quartet.

On the other hand, it was also confirmed that symphytine gave tiglic acid and retronecine by careful hydrolysis with 2N–NaOH in EtOH. The other esterifying acid in symphytine, (−)-viridifloric acid whose absolute configuration was shown by Kochetkov,[5] was

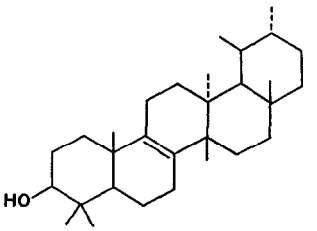
[5] N. K. KOCHETKOV, *Tetrahedron* **25,** 2313 (1969).

obtained by hydrogenolysis with PtO_2 in acidic solution. The obtained material was identical with the authentic sample of (−)-viridifloric acid by admixture and spectroscopic data.

The structure of symphytine was therefore determined as (I). Symphytine, which was the first demonstrated pyrrolizidine alkaloids with tiglic acid, was also isolated from the fresh plant.



(I)



(II)

ISOBAUERENOL AND β-SITOSTEROL

Isobauerenol and β-sitosterol[6] were separated from ethereal extract by silica gel column chromatography. Colorless needles (II), m.p. 165–166°, was obtained from fraction No. 180–231 and easily acetylated to give colorless needles, m.p. 213–214·5° and oxidized by Kiliani's reagent to give colorless needles, m.p. 184–185°. The crystal (II) was the same retention time as the authentic sample of isobauerenol by GLC, and the m.p. was unchanged on admixture with isobauerenol. Although it was known that bauerenol is easily isomerized to isobauerenol with acid,[7] the presence of isobauerenol without acid treatment was confirmed by GLC using the fresh and dried roots of *Symphytum officinale*.

Recrystallization of fraction No. 240–282 gave white crystals, m.p. 138–140°, which was identical with β-sitosterol together with a small amount of stigmasterol by GLC.

EXPERIMENTAL

Extraction of Alkaloids

The dried, powdered roots (4·0 kg) of *S. officinale* were extracted with hot MeOH (25:1). MeOH was removed under reduced pressure and a crude allantoin appeared from the concentrated MeOH soln. The allantin was separated by filtration. After drying the filtrate *in vacuo*, the residue was extracted with 0·5 N HCl and separated into HCl soluble (Fraction I) and an insoluble (Fraction II) fractions. Into Fraction I, under stirring at 15°, 50 ml of conc. HCl and 30 g of Zn dust were gradually added. After 5 hr, unreacted Zn was removed and the filtrate was made alkaline to phenolphthalein with NH_4OH, and a crude alkaloid (Fraction-IA, 14·5 g) was obtained by extraction with $CHCl_3$.

Purification of Symphytine

The crude base (Fraction IA) (13·0 g) was submitted to SiO_2 (1·4 kg) column chromatography. On elution with 4% MeOH in $CHCl_3$, symphytine (2·4 g) was present in fractions No. 210–276 and echimidine (1·8 g) in fractions No. 391–426. The eluants (each fraction 100 ml) were as follows: fraction No. 1–15, $CHCl_3$; 16–27, 1% MeOH in $CHCl_3$; 28–39, 2% MeOH in $CHCl_3$; 40–500, 4% MeOH in $CHCl_3$.

Hydrolysis of Symphytine

Symphytine (0·2 g) in EtOH (10 ml) was treated with 2 N NaOH (1·0 ml) over 20 min below at 20°. After 15 hr, 2 N HCl (1·0 ml) was added and EtOH was evaporated under reduced pressure. After addition of HCl to Congo red, the reaction mixture was extracted 3× light petroleum, the extracts collected and dried

[6] T. TAKEMOTO and F. KITAME, reported at *Meeting of Tohoku Branch, Pharmaceutical Society of Japan* (February 1966).

[7] S. NATORI, private communication.

(Na_2SO_4). Evaporation of the solvent gave colorless needles. Recrystallization from H_2O gave m.p. 63·5–64·5°, unchanged on admixture with the authentic sample of tiglic acid.

The acidic layer was basified with NaOH (2·0 g) and extracted with $CHCl_3$ many times. Recrystallization of the $CHCl_3$ residue from Me_2CO gave retronecine as colorless prisms m p. 115·0–116·5°.

Isolation of Viridifloric Acid

Symphytine (0·5 g) was shaken with H_2 and PtO_2 in dil. HCl, until 134 ml of H_2 was absorbed. The acidic soln. was filtered and extracted with $CHCl_3$. Evaporation of the $CHCl_3$, gave a white residue. Recrystallization from C_6H_6 gave m.p. 122–123°, $[\alpha]_D$ −1 8° (EtOH). The m.p. was unchanged on admixture with the authentic sample of viridifloric acid from Dr. C. C. J Culvenor.

Separation of Isobauerenol and β-Sitosterol

To Fraction II, in 70% MeOH (3·5:1), was added a saturated aq. soln. of $Pb(AcO)_2$ (450 ml) The precipitate was removed by filtration, the filtrate concentrated *in vacuo* and a gummy material remained. The gummy substance was more extracted with Et_2O, the Et_2O extract was washed with 5% NH_4OH, and dried (Na_2SO_4). The solvent was removed and a dark brownish material (Fraction IIA, 9·0 g) was obtained. This was separated by SiO_2 column chromatography and eluted with C_6H_6 as follows· fraction No. (each fraction 100 ml) solvents 1–40, C_6H_6; 41–100, 1% Et_2O in C_6H_6; 101–283, 2% Et_2O in C_6H_6; 284–350, 4% Et_2O in C_6H_6.

Colorless needles (2·1 g) was obtained from fraction No. 180–231. Recrystallization from 90% EtOH gave m.p. 165–166·5° (lit.[8] 168–170°), M^+426·391, $C_{30}H_{50}O$ requires 426·386, TLC (silica gel G) R_f 0·58 (C_6H_6/Et_2O : 9/1), $[\alpha]_D$ +38° (dioxane). The m.p., IR and NMR spectra of the substance was the same as the authentic sample of isobauerenol.

Recrystallization of fraction No. 240–282 gave colorless needles (1·4 g), m.p. 138–140° (from 90% EtOH) and was identical with β-sitosterol. However, it was shown to contain a small amount of stigmasterol by GLC.

GLC was carried out with Shimazu GC-1C gas chromatograph fitted with flame ionization detector. Silanized glass column (0·4 cm 1 8 m) was packed with 1% SE-30 on demineralized Gas-Chrom Q (80–100 mesh). Bauerenol and isobauerenol have retention times (min) of 8 7 and 7 2, respectively (column temp. 270°, carrier gas H_2 84 ml/min). β-Sitosterol and stigmasterol had retention times of 12·4 and 10·8, respectively (column temp. 250°, N_2 84 ml/min).

Isobauerenol Acetate

A solution of isobauerenol (0·1 g) in Ac_2O (1·0 ml) and pyridine (1·0 ml) was left at room temp. for 18 hr and worked up as usual giving m.p 213–214·5° (lit [8] 212–213°) (0·08 g), $[\alpha]_D$ +37° (dioxane).

Isobauerenone

Isobauerenol (0·1 g) in Me_2CO (10 ml), was treated with 2·0 ml of Kiliani's reagent (1·3 g of CrO_3 in 10 ml of 25% H_2SO_4) over 10 min at 0°. The excess reagent was destroyed with MeOH and the solution was poured into ice H_2O. The precipitate was filtered and washed with H_2O. Recrystallization from Me_2CO gave m.p. 184–185° (lit.[8] 184–185°) (0 07 g).

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[8] D. F THEUMAN and J. COMIN, *Phytochem.* **8**, 781 (1969).