Chem. Pharm. Bull. 16(10)2070—2073(1968)

UDC 547.94.05 : 581.19 : 582.675.1

Alkaloids of *Thalictrum*. VIII.[1] Isolation of Thalidasine from *Thalictrum rugosum*

Toshiaki Tomimatsu, Mitsuko Hashimoto,[2a] and Jack L. Beal[2b]

Faculty of Pharmaceutical Sciences, Tokushima University[2a]

(Received February 16, 1968)

In the previous paper,[3] the presence of magnoflorine, berberine, and obamegine in the roots of *Thalictrum rugosum* Ait. (*T. glaucum* Desf.) was reported. The present report deals with the isolation and identification of a nonphenolic tertiary base from the roots of *T. rugosum*. The free base (Base A) was isolated from the crude nonphenolic base fraction. Although the base was only obtained as an amorphous solid, it was crystallized as its oxalate, $C_{39}H_{44}O_7N_2 \cdot 2\,(COOH)_2 \cdot C_2H_5OH$, mp 144—145°.

The ultraviolet (UV) spectrum of the Base A in methanol suggested a benzenoid ring, (λ_{max} 275 and 282 mμ), while the nuclear magnetic resonance (NMR) spectrum in deutero-chloroform indicated the presence of two N-methyl and five methoxyl groups, at τ 7.75, 7.38 (6H, $2NCH_3$) and 6.73, 6.50, 6.25, 6.13, 6.09 (15H, $5OCH_3$), respectively, as shown in Fig. 1. In addition, the NMR spectrum showed the presence of nine aromatic protons in τ 2.46—3.70 region.

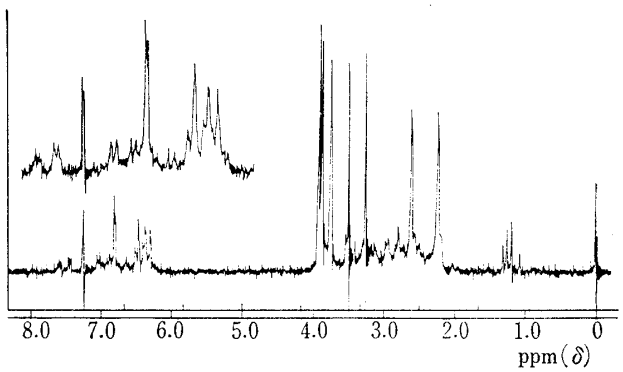

Fig. 1. Nuclear Magnetic Resonance Spectrum of Base A in $CDCl_3$

The mass spectrum of this oxalate did not show a molecular ion peak, and the most intense peak was a doubly charged ion of *m/e* 213 ($C_{24}H_{30}O_5N_2$). The main peaks of the re-

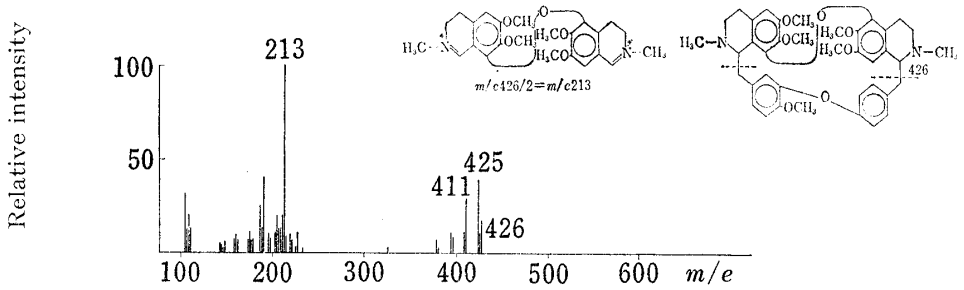

Fig. 2. Mass Spectrum of Base A Oxalate

1) *a*) Part VII: H. H-S. Fong, J. L. Beal and M. P. Cava, *Lloydia*, **29**, 94 (1966); *b*) A part of this paper was presented at the Annual Meeting of Chu-Shikoku Branch of the Pharmaceutical Society of Japan, Kochi, October 21, 1967.

2) Location: *a*) *Sho-machi, Tokushima*; *b*) Present address: *College of Pharmacy, The Ohio State University, Columbus, Ohio, U.S.A.*

3) T. Tomimatsu, C.R. Gharbo and J.L. Beal, *J. Pharm. Sci.*, **54**, 1390 (1965); T. Tomimatsu and J.L. Beal, *J. Pharm. Sci.*, **55**, 208 (1966).

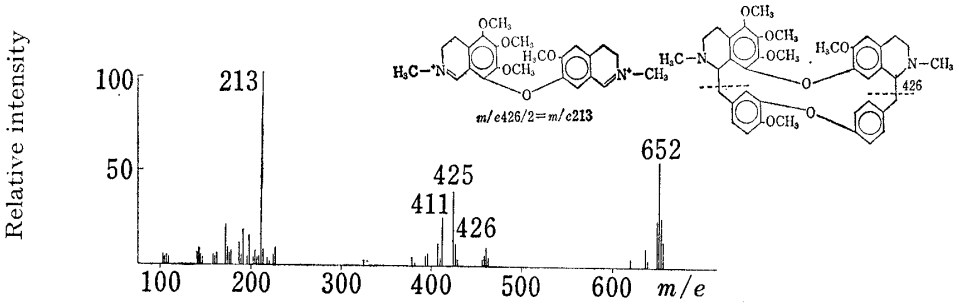

Fig. 3. Mass Spectrum of Hernandezine

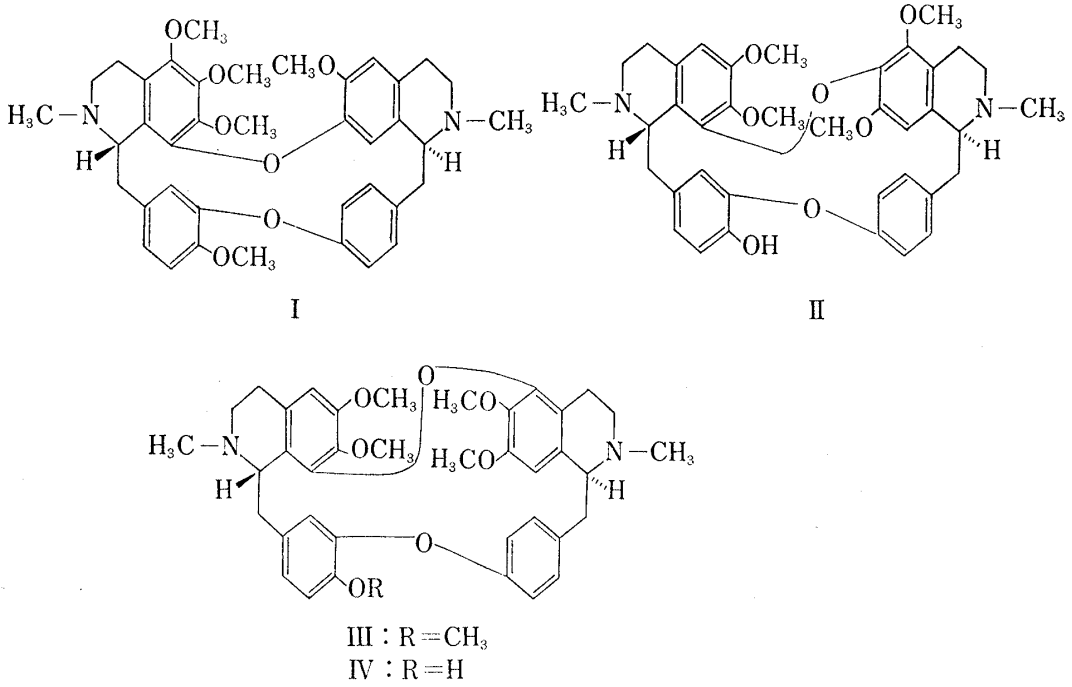

Chart 1

mainder were at *m/e* 425 and 411. The mass spectra of Base A oxalate and hernandezine (I) are shown in Fig. 2 and 3.

The foregoing data suggest the Base A to be a bisbenzylisoquinoline-type alkaloid possessing four methoxyl groups in the tetrahydroisoquinoline moiety. A nonphenolic base of a bisbenzylisoquinoline–type possessing four methoxyls in the tetrahydroisoquinoline moiety reported to date is only hernandezine[4] (I). Consequently, IR spectra (in chloroform) of Base A and hernandezine were compared but they were clearly different and their identity was excluded. Thalfoetidine[5,7] (II) is also a bisbenzylisoquinoline–type alkaloid with four methoxyls in the tetrahydroisoquinoline moiety but it is a phenolic base. It follows, therefore, that Base A is a new base of a berbamine–type alkaloids possessing four methoxyls in the tetrahydroisoquinoline moiety. Accordingly, the remaining problem was the determi-

4) J. Padilla and J. Herran, *Tetrahedron*, **18**, 427 (1962); M. Shamma, B.S. Dudock, M.P. Cava, K.V. Rao, D.R. Dalton, D.C. DeJongh and S.R. Shrader, *Chem. Commn.*, **1966**, 7.

5) It was reported[6] that direct comparison of IR and NMR spectra established the identity of O–methylthalfoetidine with thalidasine and that thalifoetidine possesses structure (IV).

6) S.M. Kupchan, T.H. Yang, G.S. Vasilikiotis, M.H. Barnes, and M.L. King, *J. Am. Chem. Soc.*, **89**, 3075 (1967).

7) N. Mollov and V. St. Georgiev, *Chem. Ind.* (London), **1966**, 1178.

Table I. Physical Data of Base A and Several Related Alkaloids

Alkaloid	mp (°C)	$[\alpha]_D$ ($CHCl_3$)	Occurrence
Base A	amorphous solid	−71.6	*T. rugosum* Ait.
Hernandezine (I)	192—193	+250	*T. hernandezii* Taush
Thalfoetidine (II)	168—170	−88.6	*T. foetidum* L.
(O-Methyl derivative)	108—109		
Thalidasine (III)	amorphous solid	−70	*T. dasyarpum*

Table II. Chemical Shift in NMR Spectra of Base A and Several Related Alkaloids in τ-Value

Alkaloid	N-Methyl group	O-Methyl group
Base A	7.78, 7.39	6.75, 6.51, 6.27, 6.15, 6.12
Hernandezine (I)	7.70, 7.37	6.76, 6.66, 6.21, 6.17, 6.09
Thalfoetidine (II)	7.68, 7.30	6.68, 6.49, 6.23, 6.11, —
Thalidasine (III)	7.75, 7.38	6.73, 6.50, 6.25, 6.13, 6.09

nation of the exact position of the methoxyl groups and ether linkage in Base A. In order to clarify this problem, Base A was reduced with sodium in liquid ammonia at −65°.

During the course of the present study, the isolation and elucidation of the structure of thalidasine (III), a novel bisbenzylisoquinoline alkaloid, were recently reported by Kupchan and his co-workers.[6] Since the data of Base A seemed very similarly to those of thalidasine, as shown in Tables I and II, Base A oxalate was directly compared with an authentic sample of thalidasine oxalate. As its result, the nonphenolic base (Base A) oxalate was found to be identical with thalidasine oxalate by comparison of IR (KBr) spectra and a mixed melting point determination.

This is the first example of the isolation of thalidasine from *T. rugosum* Ait. and its presence may account in part for the antitumor activity exhibited by the aqueous and ethanolic extracts of the root of *T. rugosum*.[8]

Experimental[9]

Extraction of Alkaloids from *Thalictrum rugosum* Ait.——Roots of *T. rugosum* were obtained from Wayside Gardens, Mentor, Ohio. Milled roots, 8.5 kg, were extracted with hot MeOH for several days until a negative test of the extract for alkaloid was obtained with the Meyer reagent. The MeOH extract was concentrated under reduced pressure to a dark semi-solid concentrate, 0.95 kg, and poured under stirring into warm 5% AcOH. The acidic solution was freed from acidic and neutral substances by extraction with ether, made alkaline with NH_4OH solution, and extracted exhaustively with ether to remove ether soluble bases.

Isolation of Tertiary Nonphenolic Bases——The ether solution obtained as above was extracted with 5% KOH to remove phenolic bases. The ether solution was dried over anhyd. K_2CO_3 and evaporated to dryness on a steam bath. Thus, 30.4 g of the residue which consists of crude nonphenolic base was obtained. The crude substance was dissolved in benzene, and the solution was chromatographed on an alumina column.

8) Kupchan, *et al.*[6] reported that thalidasine has antitumor activity. The extracts of *T. rugosum* roots exhibited antitumor activity against sarcoma-180 in mice. Obamegine, which was isolated from *T. rugosum*,[3] may also contribute in part to the antitumor activity since it was demonstrated to have significant activity in the 9 KB cell culture assay. These assays were made under the auspices of the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Maryland, U.S.A.

9) Melting points were uncorrected. The NMR spectrum is run in $CDCl_3$ soln. with TMS as internal standard, using a Varian A-60 Spectrometer. The mass spectrum is determined with a Hitachi R.M.U.-6E Spectrometer, ionizing potential 70 eV, ionizing current 38 μA.

Crystallization was induced in those fractions containing Base A by converting the base into its oxalate. Recrystallization from EtOH yielded 3.5 g of colorless prisms, mp 144—145°. The compound was analyzed after drying at room temperature for 3 days in an improved Abderhalden pistol. *Anal.*[10] Calcd. for $C_{39}H_{44}O_7N_2 \cdot 2(COOH)_2 \cdot C_2H_5OH$: C, 61.49; H, 6.19; N, 3.19. Found: C, 60.95; H, 6.25; N, 3.19. The mass spectrum is shown in Fig. 2. The IR spectrum (KBr) was identical with that of the authentic sample of thalidasine (III) oxalate. The melting point was not depressed on admixture with thalidasine oxalate.[11]

The oxalate was converted into its free base by the usual method but could not be crystallized from any ordinary solvent. Also, it was not possible to crystallize the hydrochloride and hydrobromide of the base. Physical data for the base are as follows: $[\alpha]_D^{30}$ −71.6° (c=1.10, $CHCl_3$). UV λ_{max}^{MeOH} mμ: 275, 282. NMR: τ 7.78, 7.39 (2N–CH_3), 6.75, 6.51, 6.27, 6.15, 6.12 (5OCH_3) (Fig. 1, Table II). The IR spectrum in $CHCl_3$ differs clearly from that of hernandezine (I).

Acknowledgement The authors express their gratitude to Prof. S.M. Kupchan, University of Wisconsin, for the gift of thalidasine oxalate, and to Prof. M.P. Cava, Wayne State University, for the gift of the copy of the mass spectral chart of hernandezine. The authors are indebted to Dr. T. Shingu and Mr. A. Kato, Kyoto University, for the NMR and mass determination. Financial support from the National Institutes of Health, U.S. Public Health Service (Grant HE-07502 and Grant CA-6028), is gratefully acknowledged.

10) The crystals, mp 144—145°, absorb moisture slightly from the air when they are weighed by a micro-balance for its elementary analysis.

11) The melting point of thalidasine oxalate, mp 146—148°, was determined with Yanagimoto micro-melting point apparatus.