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Study on the Alkaloids from *Thalictrum fauriei*

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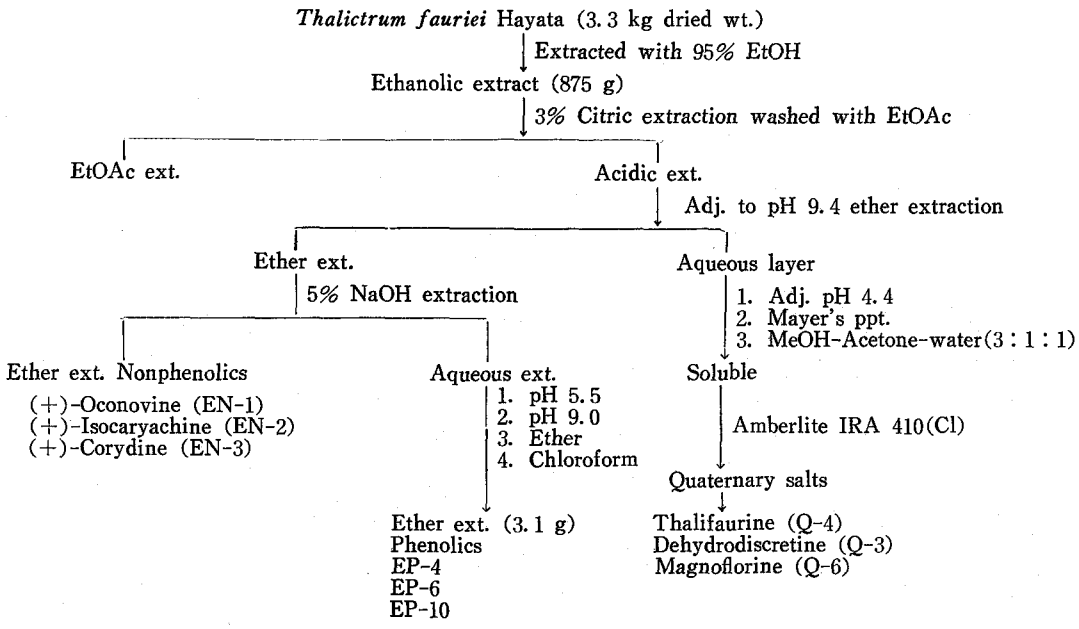
Abstract—To explore the biological activities of Thalictrum alkaloids, a study was initiated to investigate the alkaloidal constituents from *T. fauriei*, a species indigenous to Taiwan. Extensive fractionation of the ethanolic extract provided three known aporphines, (+)-oconovine (Ia), (+)-isocorydine (Ib), and (+)-corydine (Ic) from nonphenolic tertiary base fraction. From quaternary base fraction two new protoberberinium salts, thalifaurine (IIa), dehydrodiscretine (IIb), in addition to magnoflorine (III), were isolated. The structure of both new compounds were confirmed by total syntheses. Fractionation of phenolic bases yielded a dihydromorphinandienone, (−)-ocobotrine (IV), as well as a novel aporphine-pavine dimer, tentatively named EP-10. The structure of EP-10 was established by means of 2D NMR studies. Preliminary study indicated a weak activity of lyzing Hela cells, *in vitro*, for EP-10.

Keywords—Thalictrum alkaloids • thalifaurine • dehydrodiscretine • aporphine-pavine dimer • EP-10

The genus *Thalictrum* has been shown to be a rich source of alkaloids with wide structural varieties, for which various biological activities have been associated with, such as antimicrobial,[1] antitumor,[2] hypotensive,[3] and other activities. In view of the increasing interest in the biological activity of these alkaloids, this study was initiated to investigate these constituents from *Thalictrum fauriei* Hayata (Ranunculaceae). This is a perennial herb distributed widely over the mountainous area of Taiwan, especially in the wet places.[4] To our knowledge no study has been carried out on the constituents of this species. The ethanolic extract of the whole plant material was processed by the usual acid-base treatment and solvent partitioning as shown in Scheme I, resulting into three main fractions, the nonphenolic and phenolic tertairy bases, and the quaternary bases. Each fraction was separated by extensive silica gel column chromatography with various solvent systems.

Fractionation of the ether-soluble nonphenolic fraction provided three constituents, EN-1, EN-2, and EN-3. Base EN-1 was amorphous with phenolic absorption in IR spectrum. The UV absorption indicates characteristic absorptions for 1, 2, 3, 10, 11-substituted aporphines. The mass spectrum was also indicative of the aporphine structure by relatively high absorption at M^+, M-15, and M-31 ions. NMR spectrum suggested one N-methyl and three O-methyl groups, in addition to the singlet of two protons at C-8 and C-9. All these data are in good agreement with those of (+)-oconovine (I).[5] Methylation of I provided O-methyl oconovine which was then N-methylated to the methiodide, m.p.

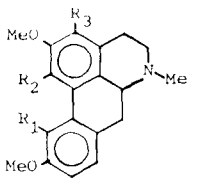


Scheme I. Separation and fractionation of alkaloids from *T. fauriei*

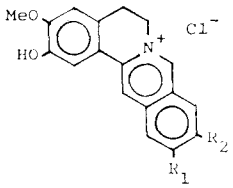
208~211°, with IR spectrum comparable with those of authentic sample.

Both bases EN-2 and EN-3 were crystalline with m.p. 188° and 185°, respectively. The phenolic function was obvious from IR absorption and the bathochromic shift in UV on addition of base. The absorption pattern suggested 1, 2, 10, 11-substituted aporphines, which is also reflected by the M-15, M-31 ions in its mass spectrum. In the NMR spectra both possess N-methyl, two O-methyl, an aromatic proton at C-3, in addition to the AB quartet for C-8 and C-9 protons. All these data agreed well with those of (+)-isocorydine (Ib),[6] and (+)-corydine (Ic),[7] respectively. The identities of both compounds were established by comparisons with authentic samples.

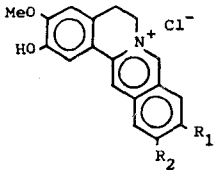
The quanternary fraction was separated as the chloride salt on silica gel column to yield three crystalline compounds, designated as Q-3, Q-4, and Q-6. Alkaloid Q-4, mp 258~260°, was optically inactive. The UV absorption showed characteristics for a 2, 3, 10, 11-substituted pseudo-protoberberinium salt. Bathochromic shift in the presence of base and the IR spectrum suggested the phenolic nature of Q-4. The NMR spectrum revealed one methoxy, one methylenedioxy, and six aromatic proton singlets. The data were similar to those of pseudopalmatinium (IIe) and pseudoepiberberinium salt (IIf), consistent with the 2, 3, 10, 11-substitution pattern. The chemical ionization mass spectrum indicated the fragments a-g, with fragment g serving to locate the methylenedioxy group on ring D. Up to this point two structures are compatible with the evidence: 3-hydroxy-2-methoxy-10, 11-methylenedioxyberberinium chloride (IIa) and the 2-hydroxy-3-methoxy-isomer, dehydropseudocheilanthifoline (IIc), a naturally occuring compound isolated from *Isopyrum thalictroides.*[8] As the authentic of this latter compound was completely different from Q-4, structure IIa was assigned to Q-4. On reduction with sodium borohydride provided a tetrahydroprotoberberine Q-4-H, mp 142~144°. The spectral properties of this compound were identical to those of an authentic of 3-hydroxy-2-methoxy-10, 11-methylenedioxyberberine.[9] Thus str-



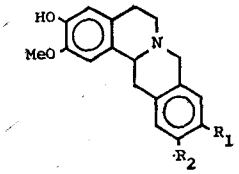
	R_1	R_2	R_3
Ia	OH	OMe	OMe
Ib	OH	OMe	H
Ic	OMe	OH	H
III	OH	OH	H



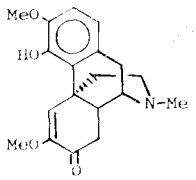
	R_1	R_2
IIa	OMe	OMe
IIb	OCH_2O	



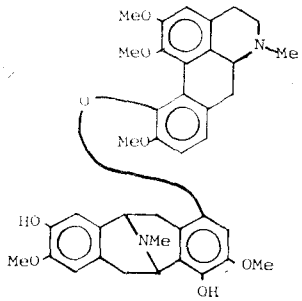
IIc $R_1 = R_2 = OCH_2O$
IId $R_1 = R_2 = OMe$



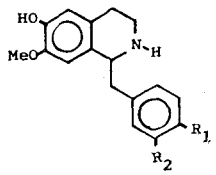
VIIa $R_1 = R_2 = OCH_2O$
VIIb $R_1 = R_2 = OMe$



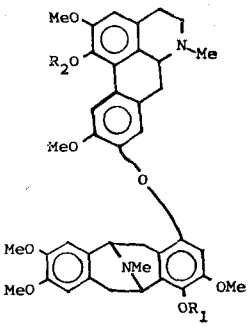
IV



V



VIa $R_1 = R_2 = OCH_2O$
VIb $R_1 = R_2 = OMe$



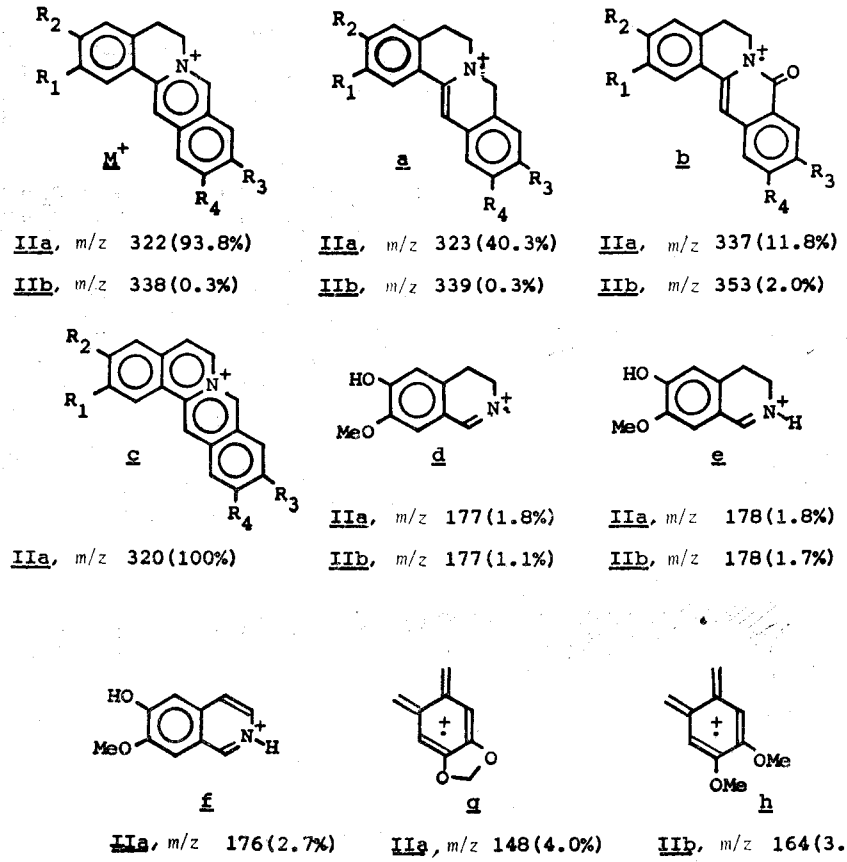
IXa $R_1 = H$, $R_2 = Me$
IXb $R_1 = R_2 = H$

ucture IIa can be assigned to Q-4. To confirm this structural assignment, the tetrahydroisoquinoline (VIa) was synthesized and condensed with formaldehyde to give VIIa, which was identical with Q-4-H. Further oxidation with mercuric acetate provided the pseudoberberinium salt IIa, identical in every aspect with Q-4. Since this is a new protoberberine from nature it was named thalifaurine.

Alkaloid Q-3, m.p. 230~234°, was also optically inactive. The UV spectrum, being similar to that of Q-4, suggested a phenolic function by base treatment and supported by a corresponding IR absorption as well. However, the NMR spectrum showed three methoxy groups in addition to six protons in the aromatic region. Striking similarity in these data indicated that Q-3 is another 2, 3, 10, 11-substituted pseudoprotoberberinium salt. The mass spectrum revealed characteristic ions (a-f, h, Scheme II) which was in agreement with the presence of three methoxy groups and located the phenolic function on A-ring. Thus two alternative structures were compatible with these data, IIb and II d. Structure II d is the known alkaloid, pseudocolumbamine, isolated from *I. thalictroides.*[8] As an authentic of IId was entirely different from Q-3, which necessitated structure IIb to be assigned to Q-4. Sodium borohydride of Q-4 provided the tetrahydroprotoberberine, Q-4-H, mp 180~182°, the spectral data of which were in good agreement with those of discretine (VII b) isolated from *Xylopia discreta.*[10] To confirm the suggested structure the 1-benzyl-1, 2, 3, 4-tetrahydroisoquinoline (VIb) was synthesized and condensed with formaldehyde to yield the racemic discretine (VIIb). Oxidation of the latter with mercuric oxide produced IIb that is identical in every aspect with Q-3. Since this is a dehydro form of discretine, it was named dehydrodiscretine.

Alkaloid Q-6, mp 250-252°, is the major alkaloid in the quaternary base fraction. It was identical with magnoflorine (III) chloride by spectral comparison with authentic sample.

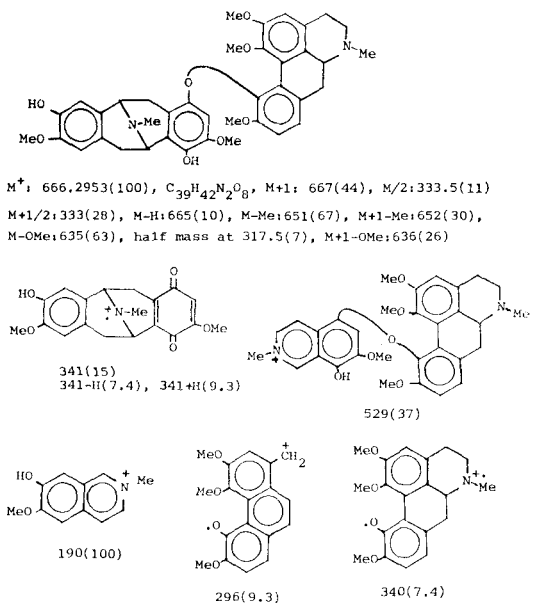
Fractionation of the ether soluble phenolic bases provided three bases. EP-4, EP-6, and EP-



Scheme II. Isobutane chemical ionization mass spectral fragmentation of Q-4 (IIa), R_1=OMe, R_2=OH, R_3=R_4=—OCH_2O—, and Q-3(IIb), R_1=R_3=R_4=OMe, R_2=OH

10. The alkaloid EP-4, mp 100-101°, showed a positive Cotton effect in its CD curve. The phenolic function was implicated in its IR and UV spectra with bathochromic shift on base treatment. The absorption pattern is indicative of a 8, 14-dihydromorphinandienone skeleton. This is also supported by the NMR spectrum, where one N-methyl, two methoxyl singlets, and an exchangeable phenolic broad singlet were observed. In the aromatic region an AB quartet served to implicate a vicinal C-1 and C-2 protons. The characteristic vinylic proton on C-5 was also obvious. Two separated ABX spin systems were observed in the aliphatic region that accounts for the protons at C-9, C-10, and those at C-14, and C-8. In CMR spectrum a ketonic carbon was observed that accounts for the C-7 keto function, all other signals were in good agreement with the 8, 14-dihydromorphinandienone structure. A perusal of the literatures revealed the identity of this compound as ocobotrine (IV) that was isolated from *Ocotea brachyobotra*[11].

The second phenolic alkaloid from this fraction is EP-10, mp 205-210°, which has a composition of $C_{39}H_{42}N_2O_8$ by high resolution mass spectrometry. The UV spectrum indicated a composite pattern of 1, 2, 3, 4- tetrasubstituted aporphine chromophore at 210, 270, and 300 nm, and that of pavine chromophore at 294 nm. This is strikingly similar to those of pensylpavine (IXa) and pensylpavoline (IXb) isolated from



Scheme III. Electron impact mass spectrum of EP-10

Thalictrum polygamum[12], both being a composite of pavine and aporphine. The mass spectum also indicated fragments that can account for the aporphine and the pavine skeletons as illustrated in Scheme III. In the PMR spectrum there existed two N-methyl, and six O-methyl signals. The O-methyl-EP-10 indicated one additional methoxyl group which would reflect the phenolic function in EP-10. In the aromatic region the AB quartet at δ6.75 serves to accomodate the protons at C-8 and C-9 of aporphine part, a singlet at δ6.68 could be assigned to C-3 proton. As the C-11 low field singlet is missing from EP-10, an ether linkage at this point is implicated. The high field singlet at δ5.62 is quite unusual in having great shielding effect, which is delegated to the proton at C-9′ of pavine part. In the aliphatic region two doublets of doublet, originated from protons on C-6′ and C-12′, were observed that constituted the X part of ABX system characteristics for the bridgehead protons of pavine skeleton. In the DEPT spectrum various carbon numbers counted are quaternary 18, methyl 7, methylene 5, and methine 9. Among these carbon atoms those with proton attached can be correlated with proton chemical shifts by H-C correlation spectroscopy. From COSY study the supporting evidences for the ABX systems for the pavine bridgehead protons on C-5′, C-6′ and C-11′, C-12′ can be clearly observed. The ABX system from C-a6 and C-7 protons can also be discerned. While all these data can be explained quite satisfactory by structure V, due to the complexity of this mole-

Table I. NMR data of EP-10 in comparison with pennsylpavine and pennsylpavoline

	N-Me	N′-Me	OMe					Ar-H				
EP-10($CDCl_3$)	2.55	2.55	3.41	3.62	3.62	3.62	3.74	5.62	6.39	6.62	6.68	6.75(ABq)
($CDCl_3$+D_2O)	2.51	2.54	3.42	3.61	3.61	3.65	3.74	5.64	6.39	6.61	6.68	6.78(ABq)
O-methyl-EP-10	2.54	2.60	3.41	3.60	3.60	3.64	3.77	5.63	6.44	6.59	6.68	6.79(ABq)
($CDCl_3$)							3.84					
Pennsylpavine	2.50	2.57	3.76	3.76	3.78	3.88	3.71	6.48	6.48	6.52	6.60	
($CDCl_3$)			3.91	(C-10)			(C-1)	6.23	(C-9′)	8.15	(C-11)	
Pennsylpavoline	2.48	2.55	3.75	3.78	3.78	3.91		6.45	6.49	6.55	6.55	
($CDCl_3$)			3.91	(C-10)				6.26	(C-9′)	8.14	(C-11)	

CMR DEPT study of EP-10

Quaternary carbons—124.7, 125.6, 128.4, 130.3, 130.7, 132.2, 132.4, 132.7, 138.5, 144.6, 147.2, 147.4, 147.7, 149.6, 153.0, 154.2, 156.3, 157.3

Methyl carbons— 41.1, 45.2, 58.0, 58.1, 58.6, 59.1, 63.5

Methylene carbons—25.9, 27.3, 30.2, 35.3, 53.0

Methine carbons—121.9, 113.3, 112.1, 111.4, 96.9, 64.0, 55.4, 51.6

cule, the exclusion of other alternative structures requires further studies which is currently under investigation. The presence of the novel pavine-aporphine linked dimer like EP-10 in this plant is quite unusual. The biological activities of this novel structure remains to be explored. Prelinminary *in vitro* tests indicated weak activity of lyzing Hela cells compared with thalicarpine.

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