Tetrahedron Vol. 40, No. 11, pp. 2133 to 2139, 1984
Printed in Great Britain.

0040-4020/84 $3.00 + .00
Pergamon Press Ltd.

NEW DIMERIC APORPHINE–BENZYLISOQUINOLINE ALKALOIDS FROM *THALICTRUM FABERI*

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(*Received in Germany* 23 *June* 1983)

Abstract—From *Thalictrum faberi* ten new aporphine–benzylisoquinoline dimers were isolated and the complete structures assigned by spectral methods. Of them, thalifaberine (**1**), thalifabine (**2**), thalifarapine (**3**), thalifabatine (**4**), thalifasine (**5**) and dehydrothalifaberine (**6**) constitute a new type of aporphine–benzylisoquinoline dimers. Huangshanine (**7**), faberonine (**8**), faberidine (**9**) and dehydrohuangshanine (**10**) are fetidine-type alkaloids.

In a previous communication[1] we reported on the preliminary characterization of three new alkaloids, thalifaberine (**1**), thalifabine (**2**) and huangshanine (**7**) from *Thalictrum faberi* Ulbr. (Ranunculaceae), a perennial herb indigenous to China, which is used in Chinese folk medicine as an antiphlogistic and recently in the treatment of stomach cancer. A continuing study led to the isolation and characterization of additional seven new dimers from the root of the plant. Of them, thalifarapine (**3**), thalifabatine (**4**), thalifasine (**5**) and dehydrothalifaberine (**6**) belong to thalifaberine-(**1**)-type alkaloids. The other three, faberonine (**8**), faberidine (**9**) and dehydrohuangshanine (**10**) belong to fetidine-type alkaloids. We report herewith the isolation and structural elucidation of all the ten alkaloids.

Thalifaberine (**1**) was obtained from the nonphenolic base fraction of the roots of the plant as an amorphous solid. The physical properties (UV, IR, NMR and MS) were in accord with a nonphenolic aporphine–benzylisoquinoline dimeric structure and isomeric with thalicarpine (**12**) and thalirevolutine (**13**).[3] The NMR spectrum (table) shows a characteristic AA′BB′ quartet (J = 8.5 Hz) at δ 6.75 and δ 6.96, typical for the four symmetric protons of the C-ring of the benzylisoquinoline moiety, so the remaining C-12′ position should be the terminus of the diaryl ether bridge in the benzylisoquinoline moiety.

In order to determine the ether linkage position in the aporphine moiety of thalifaberine (**1**), its permanganate oxidation product (**11**) was subjected to NMR nuclear Overhauser effect (nOe) difference spectroscopy study on 200 MHz (FT). The result, namely, clear nOe between H-7 and H-11′ as well as no nOe between H-7 and one of the methoxyls suggested that the ether linkage position was the C-8 of the aporphine moiety.

A recently performed nOe difference study on 400 MHz (FT) allowed the assignment of all the methoxyl and aromatic proton signals (see Table). Separate irradiations of each of the seven methoxyls also confirm that all the aromatic protons except H-11 (δ 7.88) belong to the benzylisoquinoline moiety. One part of the AA′BB′ quartet at δ 6.76 for H-11′ and H-13′ showed enhancement (see Figure) upon the irradiation of one of the seven methoxyls (δ 3.78), which suggested that the methoxyl was attached to C-9. Similarly, two of the methoxyls (at C-1 δ 3.78 and C-10 δ 3.92) showed enhancements upon irradiation of H-11. These observations were supportive for C-8 as the terminus position of the ether linkage in the aporphine moiety.

Since the CD curve showed similarity with that of the alkaloids belonging to thalicarpine series, the same absolute configuration as in thalifaberine (**1**)[1,3] can be deduced. Recently, one oxidation product of **1**, dehydrothalifaberine (**6**) with only one asymmetric center at C-1′ of the benzylisoquinoline moiety in S-configuration, has been obtained from the plant, as well as by chemical conversion of **1**. The reaction gave evidence to support the deduced structure for **6**, as well as the S-configuration for the benzylisoquinoline moiety of **1**.

Thalifabine (**2**), $C_{41}H_{46}O_9N_2$, showed nearly identical spectral characters (UV, MS, NMR and CD) as thalifaberine (**1**), indicating that **2** is an analog of **1**. Analysis of the MS base peak (*m/z* 220) and NMR spectrum (Table) of **2** revealed that **2** contained an isoquinoline unit with a methoxyl (δ 3.64) and one methylenedioxy group (δ 5.95). So far, thalifabine (**2**) is the second aporphine–benzylisoquinoline dimer with a methylenedioxy group, the first one is thalmelatidine.[3]

Thalfaberine (**1**) and thalifabine (**2**) are the first naturally occurring aporphine–benzylisoquinoline dimers with an exceptional C-1,2,3,8,9,10-hexasubstituted aporphine moiety and one C-12′-O–C-8 ether linkage.[2] On the contrary, most of aporphine–benzylisoquinoline dimers (thalicarpine-type and fetidine-type) possess one C-10′-O-C-9 ether linkage.[3]

Thalifabatine (**4**), $C_{41}H_{48}O_9N_2$, a very minor alkaloid of the plant, shows a IR spectrum with a hydroxyl absorption at 3530 cm^{-1}, UV spectrum with absorptions at 281 and 311 nm, and major MS-fragments at *m/z* 712 (M^+), 490 ($M^+ - a$) and 222 (a, base peak), indicating that **4** is a thalifaberine-(**1**)-type dimer with an additional phenolic group at C-5′ of the isoquinoline unit, which was also found in thalmineline,[3] a dimer of that carpine-type. The

Table 1. NMR spectral data (δ value) of alkaloids of thalifaberine type

	thalifaberine (1)	thalifabine (2)	thalifarapine (3)	thalifabatine (4)	thalifasine (5)
N-6-Me	2.30	2.30	2.30	2.32	2.30
N-2'-Me	2.50	2.50	2.50	2.51	2.50
C-7'-OMe	3.54	----	3.55	3.53	3.55
C-1-OMe	3.78	3.78	3.76	3.79	3.77
C-9-OMe	3.78	3.78	3.87*	3.79	3.88*
C-6'-OMe	3.82	----	3.81*	3.82	3.83*
C-3-OMe	3.89	3.89	----	3.87	----
C-2-OMe	3.95	3.95*	3.94	3.90*	3.95
C-10-OMe	3.92	3.92*	3.94	3.93*	3.95
H-8'	6.02	5.73	5.98	5.64	5.67
H-5'	6.53	----	6.51	----	----
H-11',13'	6.76 (d)	6.76 (d)	6.77 (d)	6.74 (d)	6.81 (d)
H-10',14'	6.96 (d)	6.96 (d)	6.93 (d)	6.95 (d)	6.97 (d)
H-11	7.88	7.88	7.84	7.85	7.87

1) Chemical shifts of each alkaloid with superscripts are interchangeable.

2) For compound 2, C-5'-OMe singlet at δ 3.64, methylenedioxy singlet at δ 5.95.

3) $J_{10', 11'}$ and $J_{13', 14'}$ are equal to about 8.5 Hz for each compound in the table.

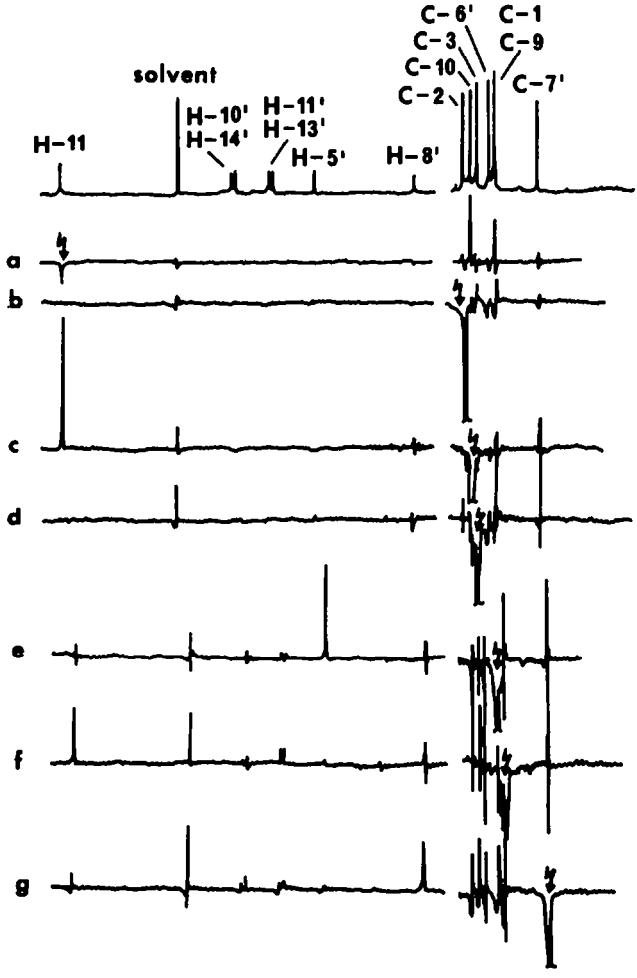


Fig. 1. Normal and nOe difference spectra of 1 at 400 MHz in $CDCl_3$. Each nOe difference spectrum (under seven traces) is identified using the normal spectral peak which was pre-irradiated in the corresponding experiment.

NMR spectrum (Table 1) showed the presence of two N-methyl, seven methoxyl and two aromatic proton singlets, as well as the characteristic AA'BB' quartet, which also indicates that **4** is a phenolic analogue of **1**. The H-8 signal (δ 5.64) appears rather upfield, supporting the presence of C-5'-hydroxyl instead of aromatic proton.

The CD curve resembled that of thalifaberine (**1**) and indicated same absolute configuration as **1**.

Thalifasine (**5**), $C_{40}H_{46}O_9N_2$, was obtained as a yellow solid. The IR spectrum showed phenolic absorption at 3528 cm^{-1}. The major fragments of the mass spectrum m/z 698 (M^+, < 0.1%), 476 ($M^+ - a$) and 222 (a, base peak) led to the assumption that **5** was a phenolic analogue of thalifaberine (**1**) with two phenolic groups, one of them should be on the aporphine unit, the other one on C-5' of the benzylisoquinoline moiety and isomeric with iznikine, bursanine.[4] The UV spectrum showed an absorption at 310 nm, as well as the expected bathochromic shift with hyperchromism in strong base, indicating that one phenolic function should be located on C-3 or C-9 position of the aporphine moiety.[4]

In order to determine the position for the phenolic function of the aporphine moiety, its diacetate (**14**) was prepared by usual way. Acetylation of the diphenolic groups led only to downfield shift (about 0.3 ppm) of the H-8', but no downfield shift from H-11 signal was observed, which indicated that one methoxyl rather than a phenolic group was attached to C-9 position of the same aromatic ring as H-11. This was strong evidence for the phenolic group on C-3 position.

The CD curve of thalifasine (**5**) is very close to that of thalifaberine (**1**), and supportive of the identical absolute configuration for **5**.

Thalifarapine (**3**), $C_{40}H_{46}O_8N_2$, a very minor alkaloid of the plant, was obtained as an amorphous solid. The mass spectrum m/z 682 (M^+), 476 ($M^+ - a$) and 206 (a, base peak), indicated that **3** was an O-desmethyl analog of thalifaberine (**1**) with a phenolic group located on the aporphine moiety. In analogy with the UV spectrum of thalifasine (**5**), the UV spectrum of **3** showed a bathochromic shift of about 20 nm, as well as hyperchromism at 328 nm in strong base, supporting the presence of a C-3 or C-9 phenolic function.[5] Comparison of the NMR spectrum with that of thalifasine (**5**), indicated that both the spectra showed all the six methoxyls with corresponding chemical shifts (Table 1), respectively. This fact suggested that the methoxyls of **3** were located on the same positions as those of **5**, therefore, the phenolic group of **3** should be on C-3 of the aporphine unit as that of **5**. Biogenetic considerations also supported this position for the phenolic function. Besides **3** and **5**, thalilutine[3] also possesses a C-3-hydroxyl group.

The CD curve showed again close similarity to that of thalifaberine (**1**) and gave evidence that it possessed the identical absolute configuration. **3** is isomeric with about eight dimers of thalicarpine series, such as thalictropine, thalilutidine, thalmelatine, thalidoxine, pennsylvanine, fetidine, thalirevoline and N-northalicarpine.[3]

Dehydrothalifaberine (**6**), a minute alkaloid of the plant, was obtained as an amorphous solid. The UV spectrum exhibited the absorption at 256, 272 and 332 nm which are indicative of the 6a,7-dihydroaporphine system.[1,6,7] The mass spectrum showed the major fragments at m/z 694 (M^+), 488 ($M^+ - a$) and 206 (a, base peak), indicating that dehydrothalifaberine (**6**) is a dehydro derivative of thalifaberine (**1**) and isomeric with dehydrothalicarpine.[3] The NMR spectrum showed the presence of two N-methyl, seven methoxyl and four aromatic proton singlets, as well as the characteristic AA'BB' quartet which also suggested that **6** should be placed into the thalifaberine-type series. The exceptional downfield shifts for H-11 (δ 9.05) and N-methyl signal (δ 2.92), as well as the presence of H-7 aromatic proton signal (δ 6.80), were characteristic for **6a**, 7-dehydroaporphine.[6]

The CD curve of **6** shows positive maxima at 231 and 212 nm, indicating the S-configuration at C-1' which is the only asymmetric centre in the molecule.

Dehydrothalifaberine (**6**) might be considered to be a biosynthetic *in vivo* oxidation product of **1**, obtainable also from **1** by chemical conversion with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone as dehydrothalicarpine (**15**) from thalicarpine (**12**).[5]

Since **6** coexists with thalifaberine (**1**) in the same fraction, it was of importance to rule out an artificial origin for **6** by air oxidation on handling. Stirring of **1** at room temperature in ethanol for four days and then refluxing for 24 h, however, did not change the alkaloid.

Among the more than thirty naturally occurring aporphine–benzylisoquinoline dimers so far reported in the literature,[3] only dehydrothalicarpine (**15**) has a dehydroaporphine moiety. Therefore, dehydrothalifaberine would be the second one.

Huangshanine (**7**), $C_{42}H_{50}O_9N_2$, showed a UV spectrum with absorptions at 281, 302 and 312 nm, and major fragments in the mass spectrum at m/z 725 ($M^+ - H$), 520 ($M^+ - a$), 370 (b), 354 (c) and 206 (a, base peak), indicating that **7** is an aporphine–benzylisoquinoline dimer with two methoxyls on the isoquinoline, two methoxyls on the benzyl ring, and four methoxyls on the aporphine unit and isomeric with adiantifoline.[3] The NMR spectrum exhibited the presence of two N-methyl-, eight methoxyl- and four aromatic proton singlets. An AB quartet (J = 8.5 Hz) with δ_A 6.73 and δ_B 6.76 was supportive of the ortho aromatic protons H-13' and H-14', and gave the suggestion that **7** could be placed into the fetidine-class.[8] The CD curve showed close similarity with that of thalifaberine (**1**) and suggested an identical absolute configuration for **7**.[11]

Faberonine (**8**), $C_{41}H_{48}O_9N_2$, was obtained in small amount from the plant. The IR spectrum showed phenolic absorption at 3540 cm^{-1}. The UV spectrum with absorptions at 281, 301 and 312 nm, was reminiscent of that for huangshanine (**7**). Comparison of the mass spectrum m/z 712 (M^+), 520 ($M^+ - a$), 370 (b), 354 (c) and 192 (a, base peak) with that of **7**, indicated that **8** should be an analog of **7** with one phenolic group located at C-7' or C-6' of the isoquinoline B-ring. The NMR spectrum contained singlets for two N-methyls, seven methoxyls and four aromatic protons.

The H-8' signal at δ 6.39 instead of near δ 6.20, and the absence of the signal near δ 3.60 assigned to C-7'-OMe, was diagnostic of the presence of C-7'-OH.[9] As in **7**, the most significant feature of the

NMR spectrum is the AB quartet (J = 8.5 Hz) at δ_A 6.72 and δ_B 6.89 due to the pair of adjacent aromatic hydrogens H-13′ and H-14′, which also suggested that **8** must be a member of the fetidine type,[8] and therefore C-7′-desmethylhuangshanine.

Since the CD spectrum of **8** is much like that of **7**, an identical absolute configuration for **8** is probable. **8** is an isomer of **4** and four dimers of thalicarpine-type: thalilutine, thaliadanine, O-desmethyladiantifoline and N-noradiantifoline.[3]

Faberidine (**9**), $C_{40}H_{46}O_8N_2$, an amorphous base, exhibited hydroxyl absorption in the IR spectrum at 3540 cm^{-1}. The UV spectrum showed absorptions at 281, 302 and 313 nm in accord with an aporphine–benzylisoquinoline structure. A comparison of the MS-fragmentation of **9** (*m/z* 682 (M^+), 490 ($M^+ - a$), 340 (b), 324 (c) and 192 (a, base peak)) with those of **7** and **8**, showed only three methoxyls in the aporphine moiety of **9**. The NMR spectrum exhibited two N-methyl singlets, six O-methyl singlets and five aromatic proton singlets. Like faberonine (**8**), its H-8′ signal at δ 6.39, as well as the absence of the methoxyl signal near δ 3.60, suggested the phenolic function at C-7′.[9] Similarly, the H-13′ and H-14′ signals as an AB quartet (J = 8.5 Hz) with δ_A 6.72 and δ_B 6.86 is the most important feature for the NMR spectra of fetidine dimers.[8]

Since the CD spectra of huangshanine (**7**), faberonine (**8**) and faberidine (**9**) with two negative maxima near 300 and 277 nm and one positive maximum at about 242 nm are nearly alike, they must have identical absolute configuration.[11]

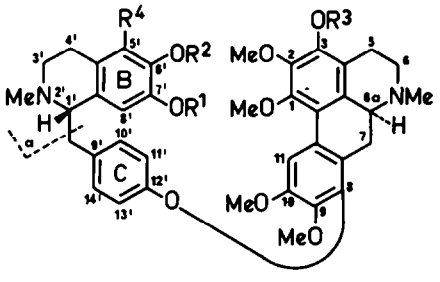
Faberidine (**9**) is an isomer of the other two dimers of fetidine-type, fetidine (with C-1-hydroxyl) and thalirevoline (with C-12′-hydroxyl).[3]

A comparison of the H-11 signals between **8** (at δ 7.99) and **9** (at δ 8.11), **7** (at δ 8.03) and thalrevolutine (**12**) (at δ 8.16),[8] revealed that the C-3-oxygenated group (such as OH, OMe) caused an about 0.1 ppm upfield shift of H-11 signal.

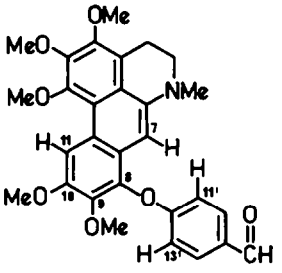
Dehydrohuangshanine (**10**), $C_{42}H_{48}O_9N_2$, is another dimer with dehydroaporphine moiety from the plant. The UV spectrum also exhibited an absorption at 332 nm diagnostic for a 6a,7-dehydroaporphine system. The mass spectrum with the major fragments at *m/z* 724 (M^+), 519 ($M^+ - a + H$), 206 (a, base peak) suggests that **10** is the dehydroderivative of huangshanine (**7**). The NMR spectrum also exhibited the H-13′ and H-14′ AB quartet characteristic for fetidine-type dimers. Furthermore the H-11 signal obtained at δ 9.10, as well as the N-methyl signal at δ 2.95, was typical for a 6a,7-dehydroaporphine structure.[6]

The CD curve, very close to that for dehydrothalifaberine (**6**), gave evidence for the benzylisoquinoline moiety with S-configuration at the C-1′ asymmetric centre.

Thalifaberine-(1)-type dimers may be considered to



1. $R^1 = R^2 = R^3 = CH_3$, $R^4 = H$
2. $R^1 - R^2 = CH_2$, $R^3 = CH_3$, $R^4 = OCH_3$
3. $R^1 = R^2 = CH_3$, $R^3 = R^4 = H$
4. $R^1 = R^2 = R^3 = CH_3$, $R^4 = OH$
5. $R^1 = R^2 = CH_3$, $R^3 = H$, $R^4 = OH$
6. $R^1 = R^2 = R^3 = CH_3$, $R^4 = H$, 6a,7-dehydro
14. $R^1 = R^2 = CH_3$, $R^3 = CH_3CO$, $R^4 = CH_3COO$



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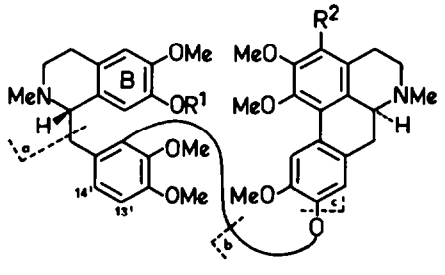
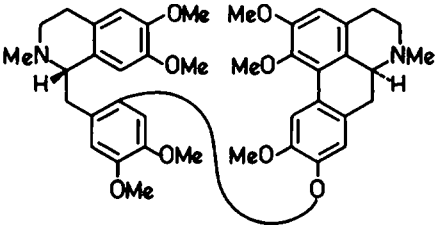


7. $R^1 = CH_3$, $R^2 = OCH_3$
8. $R^1 = H$, $R^2 = OCH_3$
9. $R^1 = R^2 = H$
10. $R^1 = CH_3$, $R^2 = OCH_3$, 6a,7-dehydro
13. $R^1 = CH_3$, $R^2 = H$



12

15. 6a,7-dehydro

be biogenitically formed from a (+)-N-methylcoclaurine- and an (+)-aporphine moiety derived from (+)-N-reticuline by intermolecular phenolic oxidative coupling between the C-12′–OH of (+)-N-methylcoclaurine and H-8 of the aporphine. A general feature of the six dimers reported in this paper is an additional exceptional C-9-methoxyl group, besides the usual C-10-methoxyl.

EXPERIMENTAL

All m.ps determined on a Kofler block are uncorrected. UV spectra were taken in MeOH on a Beckman DK-2A spectrophotometer, IR spectra were recorded in KBr pellet or $CHCl_3$ on a Beckman IR-8 instrument, NMR spectra were run in $CDCl_3$ with TMS as an internal standard using Brucker WP-80 instrument at 80 MHz unless stated otherwise, and with chemical shifts (δ) reported in ppm. EI mass spectra were obtained on AEI MS 30 spectrometer by direct inlet probe at 70 eV. Specific rotation were measured on Polarimeter LEPA 2 (Zeiss).

CD spectra were taken in MeOH on ISA-Jobin-Yvon Dichrograph III or Jobin-Yvon Auto-Dichrograph Mark V instrument.

Chromatography was performed as follows unless otherwise noted: silica gel F-254 plate (0.25 mm thickness for TLC; 0.5 mm thickness for preparative TLC) with the solvent system: toluene–acetone–ammonium hydroxide (A: 50:50:1; B: 50:100:1; C: 100:140:1; D: 60:90:1); silica gel 60 PF-254 (Merck) or alumina (Merck, No. 1077, active grade 1, 70–230 mesh) with stated solvents for column chromatography. Detection on TLC was with Dragendorff's spray reagent.

Extraction and initial fractionation. The powered roots (10 kg) of *Thalictrum faberi* Ulbr. which was collected from Huangshan Mountain, China, in 1979, were percolated at room temperature with ethanol (120 L). Evaporation of solvent at reduced pressure and 50° left a thick residue which was repeatedly triturated with 2% HCl (approximately 10 L). The acidic soln, after extraction with $CHCl_3$ (5 L for each time, two times), was basified to pH 9 with concentrated NH_4OH soln and extracted four times with $CHCl_3$ (5 L × 4). The combined $CHCl_3$-extract was washed twice with water, dried over Na_2SO_4, and evaporated to dryness to yield a crude base (70 g). The solution of the base in 2 L of $CHCl_3$, after extraction with 5% NaOH-solution (1 L × 4) to remove the phenolic base, was washed (water), dried (Na_2SO_4), and evaporated to give the non-phenolic base (60 g, A).

The combined NaOH-solution was treated with solid NH_4Cl, extracted with $CHCl_3$ (2 L × 4). The $CHCl_3$-solution, after washing with a small quantity of water and drying over Na_2SO_4, was evaporated to give the phenolic base (7 g, B).

Chromatography of Fraction A. 20 g of the tertiary non-phenolic base (A) was chromatographed on 1 kg of silica gel 60 PF-254 (E. Merck, column 9 × 39 cm). The column was continuously eluted using pure $CHCl_3$ (6 L, fractions No. 1–12), the following increasingly polar mixtures of MeOH in $CHCl_3$: 1% (10 L, fractions No. 13–32), 2% (15 L, fractions No. 33–62), 5% (15 L, fractions No. 63–92), 10% (10 L, fractions No. 93–112), 20% (8 L, fractions No. 113–128), and finally MeOH (6 L, fractions No. 129–140), taking 0.5 L fractions. The eluted fractions were evaporated, examined by TLC, and then purified by preparative TLC or rechromatography, as indicated below, to yield pure alkaloids.

Isolation of thalifaberine (**1**) *dehydrothalifaberine* (**6**), *thalifabatine* (**4**) *and faberonine* (**8**). The residue (2 g) from fractions No. 76–82 contained four alkaloids, **1**, **6**, **4** and **8** (R_f values: silica gel F-254 plate with solvent system C, R_f (6) = 0.50, R_f (1) = 0.30, R_f (4) = 0.29, R_f (8) = 0.31; alumina 60 F-254 (Merck, No. 5713) with solvent system $CHCl_3$, R_f (1) = 0.87, R_f (4, 8) = 0.20; alumina 60 F-254 plate with $CHCl_3$–MeOH 99:1, R_f (4) = 0.55, R_f (8) = 0.60, R_f (1) = 0.92). They were separated by column chromatography and preparative TLC.

Dehydrothalifaberine (**6**). The above residue (2 g) was subjected to preparative TLC (silica gel 60 F-254, 2 mm thick, 10 plates, with solvent system C, development three times), the first band was taken from the plates, washed with Me_2CO and gave a yellow solid which was purified by the same TLC and yielded **6** as a yellow amorphous base (20 mg), $[\alpha]_D^{24}$ + 95.9° (c 0.143, MeOH), UV λ_{max} 256, 272 and 332 nm (log ϵ 4.56, 4.56 and 4.06); NMR δ 2.57, 2.92 (2s, each 3H, 2 × NMe) 3.46, 3.85, 3.94, 3.99, 4.04, 4.07 and 4.11 (7s, each 3H, 7 × OMe), 5.97 (s, 1H, H-8′), 6.59 (s, 1H, H-5′), 6.80 (s, 1H, H-7), 6.90 and 6.99 (AA′BB′ quartet, J = 8.5 Hz, 4H, H-11′, H-13′, H-10′ and H-14′), 9.05 (s, 1H, H-11); MS, *m/z* 694 (M^+, 0.2%, for $C_{41}H_{46}O_8N_2$), 488 ($M^+ - a$), 487, 206 (a, base peak); CD $\Delta\epsilon$ (nm) + 0.170 (385.0), +0.840 (334.4), +4.689 (290.0), +13.000 (231.4), +12.733 (212.6), −26(196).

Oxidation of thalifaberine (**1**) *with DDQ to dehydrothalifaberine* (**6**). To a stirred soln of **1** (50 mg, 0.0718 mMol) in C_6H_6 (3.25 ml) was added a soln of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (16 mg, 0.0705 mMol) in C_6H_6 (0.5 ml), and the mixture was heated at 62°, and stirred for 5 h. After removing the black powdery solid by filtration, the C_6H_6 soln was extracted with 2% HCl. The acidic solution was made alkaline with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract, after drying (Na_2SO_4) and evaporation of solvent, was separated by preparative TLC with solvent system A. The first band gave dehydrothalifaberine (10 mg), identified by comparison with an authentic natural sample: they showed the same R_f value, CD curve, IR, UV, NMR and MS.

Thalifaberine (**1**). The residue (1 g) from the second band of the above preparative TLC was rechromatographed on 100 g of neutral alumina (Merck, active grade 1, 70–230 mesh, column 24 × 1 cm). Elution with $CHCl_3$ gave crude **1** (0.6 g), which was purified by preparative TLC to give pure **1** (300 mg) as a yellowish solid, m.p. 80–85°C; $[\alpha]_D^{25}$ + 94.6° (c 0.38, MeOH); UV λ_{max} 282 and 310 sh nm (log ϵ 4.36 and 3.98); NMR: see Table; MS (EI) *m/z* 696 (M^+, 0.1%, $C_{41}H_{48}O_8N_2$ requires 696), 490 ($M^+ - a$), 489, 206 (a, base peak). MS (CI, isobutane as the reactant gas) *m/z* 697 (MH^+, 6.5%), 206 (100%); CD $\Delta\epsilon$ (nm) −2.520 (299.0), −3.173 (278.4), +29.933 (241.6).

Oxidation of thalifaberine (**1**) *with $KMnO_4$.* To a solution of **1** (100 mg, 0.1435 mM) in Me_2CO (20 ml) was added portionwise 200 mg of $KMNO_4$ (1.2655 mM), over a period of 2 h while the solution was stirred. After further stirring for additional 3 h, excess MeOH (10 ml) was added to consume the unreacted reagent, and the precipitate was removed by filtration. The filtrate was evaporated to give a residue which was separated by preparative TLC with toluene–Me_2CO–NH_4OH (90:10:1, v/v) and the first band (R_f = 0.62) gave an oxidation product of **1**, **11** (20 mg, 0.0397 mM), as a yellow amorphous solid, IR (KBr) $\bar{\nu}$ 1692 cm^{-1} (conjugated aldehyde carbonyl); UV λ_{max} 257, 270 and 335 nm (log ϵ 5.13, 5.13 and 4.49); NMR δ 2.90 (s, NMe), 3.26 (br. s, four aliphatic protons), 3.92, 3.99, 4.05, 4.09 and 4.12 (5 s, 5 × OMe), 6.60 (s, H-7), 7.10 and 7.80 (AA′BB′ quartet, J = 8.5 Hz, confirmed by double irradiation experiment, 4H, H-11′, H-13′, H-10′ and H-14′), 9.05 (s, H-11), 9.87 (s, aldehydic proton); MS *m/z* 501 (M^+, 100%, $C_{29}H_{29}O_7N$ requires 503).

NMR nOe difference study on **11** (10 mg of **11** in 0.5 ml of $CDCl_3$, 200 MHz). The singlets at δ 4.05 (C-1-OMe) and 4.09 (C-10-OMe) showed enhancements on irradiation of H-11 signal (δ 9.05). Similarly, the doublet at δ 7.10 (H-11′ and H-13′) and singlet at δ 2.90 (NMe) showed enhancements on irradiation of H-7 signal (δ 6.60), but, none of the methoxyl signals showed any enhancement.

Thalifabatine (**4**). The residue (100 mg) from the $CHCl_3$–MeOH-fraction of the alumina column was sepa-

rated by preparative TLC (alumina 60 F-254, 0.25 mm thickness, five plates, $CHCl_3$–MeOH 99 : 1, two times development). The first band gave faberonine (**8**) (20 mg), and the second band gave **4** (15 mg) as a yellow solid, $[\alpha]_D^{22} + 60.5°$ (c 0.154, MeOH); IR ($CHCl_3$) $\tilde{\nu}$ 3530 cm^{-1} (OH); UV λ_{max} 281 and 311 sh nm (log ϵ 4.26 and 3.93), no change in strong base; NMR: see Table 1; MS *m/z* 712 (M^+, 0.1%, for $C_{41}H_{48}O_9N_2$ requires 712), 490 ($M^+ - a$), 489, 222 (a, base peak); CD $\Delta\epsilon$ (nm) −6.033 (305.2), −11.000 (281.2), +51.800 (242.0), −24.333 (211.6).

Faberonine (**8**). The first band from the alumina plates gave 20 mg of **8** as a yellow solid, $[\alpha]_D^{24} + 82.8°$ (c 0.498, MeOH); IR ($CHCl_3$) $\tilde{\nu}$ 3540 cm^{-1} (OH); UV λ_{max} 281, 301 and 312 sh nm (log ϵ 4.33, 4.18 and 4.12), no change in strong base; NMR δ 2.30, 2.43 (2s, 2 × NMe), 3.70, 3.78 (double intensity), 3.86, 3.88, 3.96 and 4.01 (6s, 7 × OMe), 6.39 (double intensity), 6.45 and 7.99 (3s, 4 × ArH), 6.72 and 6.89 (AB quartet, J = 8.5 Hz, 2H, H-13′, H-14′); MS *m/z* 712 (M^+, about 0.1%, for $C_{41}H_{48}O_9N_2$), 520 ($M^+ - a$), 519, 370 (b), 354 (c), 192 (a, base peak); CD $\Delta\epsilon$ (nm) −4.900 (306.6), −5.833 (277.8), +47.000 (242.6), +6.200 (213.4), −38.967 (198.2).

Thalifabine (**2**). The residue (0.3 g) from column fractions No. 56–61 contained the following alkaloids: N-methylthalidasine ($R_f = 0.56$), **2** ($R_f = 0.45$) and T-18-B ($R_f = 0.64$, with solvent system C), which were separated by preparative TLC (six plates) with solvent system C. The third band gave **2** (after purification with the same TLC, weighting 30 mg) as a yellow amorphous solid, $[\alpha]_D^{25} + 78.3°$ (c 0.53, MeOH); UV λ_{max} 282 and 310 sh nm (log ϵ 4.30 and 4.03); NMR: see Table 1; MS (EI) *m/z* 710 (M^+, 0.1%, $C_{41}H_{46}O_9N_2$ requires 710), 490 ($M^+ - a$), 489, 220 (a, base peak) MS (CI, isobutane) *m/z* 711 (MH^+, 11.7%), 220 (100%); CD $\Delta\epsilon$ (nm) −8.400 (294.6), −11.267 (280.0), +87.933 (242.2), −28.733 (212.8).

Isolation of huangshanine (**7**), *dehydrohuangshanine* (**10**) *and thalfinine*. The residue (0.6 g) from column fractions No. 68 and 69 contained the three alkaloids: **7**, **10** and thalfinine, which were separated by preparative TLC (six plates) with solvent system C (three times development). The first band gave **10** ($R_f = 0.63$), second band gave thalfinine ($R_f = 0.46$) and third band gave **7** ($R_f = 0.36$ on TLC with the same solvent system).

Huangshanine (**7**). The sample from the third band was purified three times by the same TLC and gave 30 mg of **7** as a yellowish powder, m.p. 70–75°, $[\alpha]_D^{25} + 120.6°$ (c 0.43, MeOH); UV λ_{max} 281, 302 and 312 sh nm (log ϵ 4.41, 4.20 and 4.13); NMR ($CDCl_3$, 200 MHz) δ 2.36, 2.45 (2s, 2 × NMe), 3.61, 3.75, 3.81, 3.83, 3.88 (double intensity), 3.97 and 4.00 (7s, 8 × OMe), 6.16 (H-8′), 6.41, 6.53 and 8.03 (H-11) (4s, 4 × ArH), 6.73 and 6.76 (AB quartet, J = 8.5 Hz, H-13′ and H-14′) MS (EI) *m/z* 725 ($M^+ - H$), 520 ($M^+ - a$), 519, 370 (b), 354 (c) and 206 (a, base peak), MS (CI, isobutane) *m/z* 727 (MH^+, 27%, $C_{42}H_{50}O_9N_2$ requires 726), 206 (100%); CD $\Delta\epsilon$ (nm) −4.767 (300.6), −4.900 (276.8), +29.200 (243.4), +8.067 (211.0), −23.867 (199.2).

Dehydrohuangshanine (**10**). The first band of the above TLC gave **10**, after purification by the same TLC, as yellow solid (10 mg), UV λ_{max} 257, 267 sh, 275 sh and 332 nm (log ϵ 4.54, 4.51, 4.49 and 3.85); NMR ($CDCl_3$, 200 MHz) δ 2.39, 2.95 (2 s, 2 × NMe), 3.55, 3.78, 3.79, 3.90, 3.93, 3.99, 4.06 and 4.09 (8s, 8 × OMe), 6.06 (H-8′), 6.38, 6.49, 6.72 and 9.10 (H-11) (5s, 5 × ArH), 6.75 and 6.82 (AB quartet, J = 8.5 Hz, H-13′ and H-14′); MS *m/z* 724 (M^+, 0.2%, $C_{42}H_{48}O_9N_2$ requires 724), 519 ($M^+ - a + H$, 4%), 518 ($M^+ - a$, 2%), 517 ($M^+ - a - H$, 4%), 411 (6%), 206 (a, 100%); $[\alpha]_D^{23} + 42.2°$ (c 0.17, MeOH); CD $\Delta\epsilon$ (nm) + 1.61 (290), +7.04 (233), +18.4 (213), −21.3 (202).

Isolation of thalifarapine (**3**), *faberidine* (**9**) *and thalifabomine*.[10] The residue (5 g) from column fractions No. 99 through 106 was rechromatographed (column 77 × 4.2 cm) on 500 g of silica gel, collecting 100 ml fractions for TLC analysis. The eluting solvents were the following mixtures of MeOH in $CHCl_3$: 2.5% (3 L, fractions No. 1–30), 5% (6 L, fractions No. 31–90), 7.5% (5 L, fractions No. 91–140), 10% (5 L, fractions No. 141–190), 20% (3 L, fractions No. 191–220), and the following alkaloids were obtained by preparative TLC.

Thalifarapine (**3**). The residue (80 mg) from the fractions No. 44 and 45 was twice purified by preparative TLC with solvent system D (development three times), and gave 15 mg of **3** as an amorphous base, $[\alpha]_D^{24} + 98.6°$ (c 0.422, MeOH); UV λ_{max} 283, 310 sh nm (log ϵ 4.33 and 4.10), UV (MeOH + OH^-) λ_{max} 293, 320 and 328 nm (log ϵ 4.19, 4.26 and 4.26); NMR: see Table 1; MS *m/z* 682 (M^+, $C_{40}H_{46}O_8N_2$ requires 682), 476 ($M^+ - a$), 475, 206 (a, base peak); CD $\Delta\epsilon$ (nm) −7.733 (304.8), −8.400 (277.6), +63.933 (243.0), −17.167 (216.6).

Faberidine (**9**). The residue (160 mg) from fractions No. 49–52 contained two alkaloids (**9** and thalifaromine) which were separated by preparative TLC with solvent system B (two times development). The first band gave **9** which was purified by the same TLC, as an amorphous solid (25 mg), $[\alpha]_D^{22} + 105.5°$ (c 0.675, MeOH); IR ($CHCl_3$) 3540 cm^{-1} (OH); UV λ_{max} 281, 302 and 313 sh nm (log ϵ 4.30, 4.14 and 4.06), UV (MeOH + OH^-) λ_{max} 281, 302 and 313 sh nm (log ϵ 4.30, 4.17 and 4.07); NMR δ 2.28, 2.44 (2s, 2 × NMe), 3.66, 3.78 (double intensity), 3.86, 3.88 and 3.99 (5s, 6 × OMe), 6.39 (double intensity), 6.45, 6.53 and 8.11 (H-11) (4s, 5 × ArH), 6.72 and 6.86 (AB quartet, J = 8.5 Hz, H-13′ and H-14′); MS *m/z* 682 (M^+, $C_{40}H_{46}O_8N_2$) requires 682), 490 ($M^+ - a$). 489, 340 (b), 324 (c), 192 (a, base peak); CD $\Delta\epsilon$ (nm) −3.700 (302.2), −6.333 (277.8), +45.833 (241.0), +7.800 (210.8), −44.133 (198.6).

Chromatography of Fraction B. 7 g of the phenolic base fraction was chromatographed on 500 g of silica gel PF-254 (column 77 × 4.2 cm) with the following mixtures of MeOH in $CHCl_3$: 2.5% (6 L), 5% (6 L), 7.5% (8 L), 10% (6 L), 20% (4 L) and MeOH (3 L), collecting 0.5 liter fraction. The fractions No. 42–45 contained thalifasine (**5**).

Thalifasine (**5**). The residue (250 mg) from fractions No. 42–45 of the phenolic base column was separated by preparative TLC with solvent system B (three times development), and gave **5** (50 mg) as a yellow solid $[\alpha]_D^{25} + 67.9°$ (c 0.80, MeOH); IR ($CHCl_3$) $\tilde{\nu}$ 3528 cm^{-1}; UV λ_{max} 282 and 310 sh nm (log ϵ 4.29 and 4.05), UV (MeOH + OH^-) λ_{max} 283, 312 and 330 (log ϵ 4.12, 4.17 and 4.15); NMR: see Table; MS(EI) *m/z* 698 (M^+, about 0.1%, $C_{40}H_{46}O_9N_2$ requires 698), 476 ($M^+ - a$), 475 ($M^+ - a - H$), 222 (a, base peak), MS (FD) *m/z* 699 (MH^+, 69%), 698 (M^+, 68%), 476 (5%), 475 (12%), 222 (a, 100%); CD $\Delta\epsilon$ (nm) −6.600 (298.4), −7.833 (285.0), +61.167 (243.2), −21.933 (216.4).

Diacetate of thalifasine (**5**). 10 mg of **5** was acetylated with acetic anhydride (0.5 ml) and pyridine (5 drops) at room temperature for 24 h and processed in the usual way. After purification by preparative TLC, the diacetate (**14**) was obtained as an amorphous solid (10 mg), UV λ_{max} 279 and 308 sh nm (log ϵ 4.23 and 3.94); NMR δ 2.08, 2.26 (2s, 2 × $OCOCH_3$), 2.26, 2.43 (2s, 2 × NMe), 3.51, 3.70, 3.77, 3.83 (double intensity) and 3.90 (5s, 6 × OMe), 5.96, 7.83 (2s, 2 × ArH), 6.79 and 6.90 (AA′BB′ quartet, J = 8.5 Hz, 4 × ArH); MS (CI, isobutane) *m/z* 783 (MH^+, 14.03%, $C_{44}H_{52}O_{11}N_2$ requires 782).

Acknowledgements—We thank Prof. H. Achenbach (Freiburg) for the CI mass spectra of **1**,**2** and **7**, Prof. G. Snatzke (Bochum) and Prof. H. Scheer (Munich) for the CD spectra, Prof. F. Bohlmann (Berlin) for the nOe difference spectra of **1**, Dr. A. Neszmelyi (Budapest) for some NMR spectra, Dr. H. R. Schulten (Bonn) for the FD mass spectrum of **5**, Prof. R. W. Doskotch (Columbus) for some of the mass and NMR spectra of **1**, Prof. H. Guinaudeau (Limoges) for her valuable discussions and some of the mass and NMR spectra of **7**, and Dr. J. Stöckigt of our Institute for the CI mass spectrum of **14**. L.-Z. Lin is supported by a grant from the Alexander von Humboldt-Stiftung (Bonn), on leave from Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

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[10]Thalifabomine, another new alkaloid from the plant, with formula $C_{39}H_{44}O_7N_2$, NMR spectrum δ 2.29, 2.54 (s, 2 × NMe), 3.51, 3.67, 3.79, 3.85, 3.90 (s, 5 × OMe), 5.92, 6.50, 6.54, 7.94 (s, 4 × ArH), 6.77 and 6.91 (dd, J = 8.5 Hz, for 4 H), CD $\Delta\epsilon$ (nm) −2.25 (304), −3.37 (276), +36.4 (243), −11.0 (219), UV λ_{max} 283 and 310 sh nm (log ϵ 4.20 and 3.87), UV (MeOH + OH^-) λ_{max} 290 and 325 nm (log ϵ 4.02 and 4.12), $[\alpha]_D^{26}$ + 67.5° (c 0.16, MeOH), MS *m/z* 652 (M^+), 446, 206 (base peak), might represent a new type of aporphine–benzylisoquinoline dimer, and the characterization will be reported in a separate paper.

[11]About 26 aporphine–benzylisoquinoline dimers from *Thalictrum* genus so far reported in the literature possess identical absolute configuration. Most of them show the CD spectra with two small negative troughs near 275 and 305 nm, one positive maximum near 240 nm, and one negative tail near 210 nm,[5] however, some of them, for example, thalirevoline, as **7**, **8** and **9** show the CD spectrum with the same shape only in the 230–320 nm region, but with an additional small positive band near 210 nm, and then with the negative tail near 200 nm.