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BISBENZYLISOQUINOLINE ALKALOIDS FROM *THALICTRUM CULTRATUM*. THE STRUCTURES OF THALRUGOSINONE AND THALPINDIONE

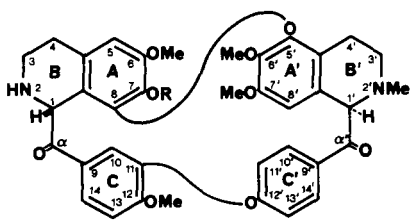
S. FAZAL HUSSAIN,[1] HÉLÈNE GUINAUDEAU,[2] ALAN J. FREYER, and MAURICE SHAMMA*

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

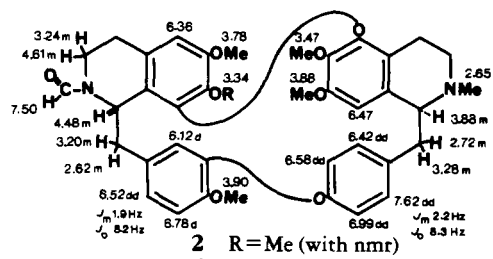
ABSTRACT.—*Thalictrum cultratum* of Pakistani origin has yielded the new bisbenzylisoquinoline (−)-2-northalmine (**13**). Also found was the known dimer (−)-thalrugosinone, whose structure is now represented as compound **2**. The structurally related alkaloid (−)-thalpindione is consequently assigned structure **6**.

Although the literature on the bisbenzylisoquinoline alkaloids of the botanical genus *Thalictrum* is extensive, no systematic study of *Thalictrum cultratum* Wall. (Ranunculaceae) had been carried out. This plant was collected by one of us in the Neelam Valley of Azad Kashmir in August 1984, and its investigation has presently yielded the new bisbenzylisoquinoline (−)-2-northalmine (**13**). Additionally, five known dimers were obtained, namely, (−)-*O*-methylthalmine, (−)-thalmine (**12**), (−)-thalidasine (**4**), (−)-2-northalidasine (**3**) [(−)-*N*-desmethylthalidasine], and (−)-thalrugosinone (**2**).

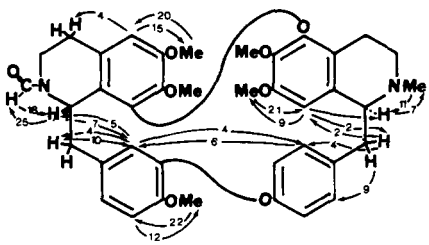
The original structure assignment of (−)-thalrugosinone, which had initially been



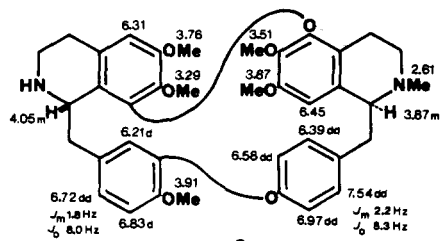
1 R=Me
5 R=H



2 R=Me (with nmr)
6 R=H



2-nOe



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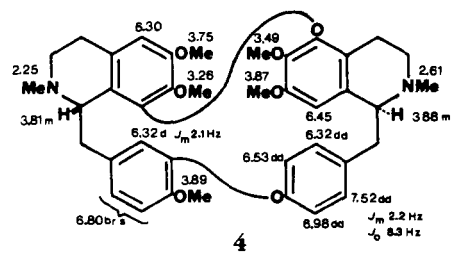

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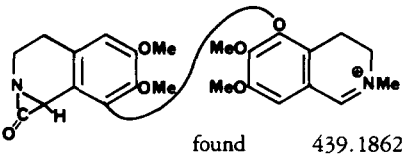
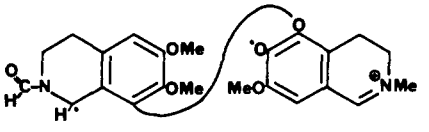
[1]Permanent address: PCSIR Laboratories, Pershawar, Pakistan.

[2]Permanent address: Faculté de Pharmacie, 87025 Limoges, France; or CNRS, UA 496, Centre d'Etudes Pharmaceutiques, 99290 Chatenay-Malabry, France.



found 439.1862
7 $C_{24}H_{27}N_2O_6$ calculated 439.1866



found 425.1704
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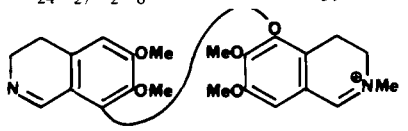

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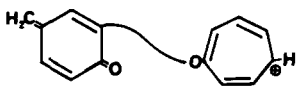

found 211.0762
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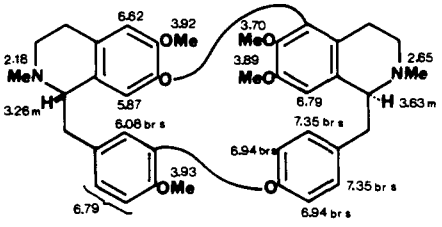

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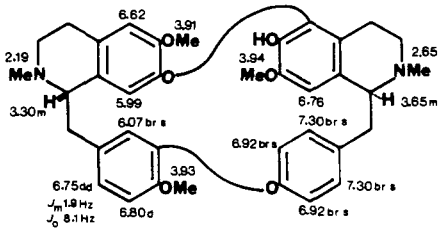

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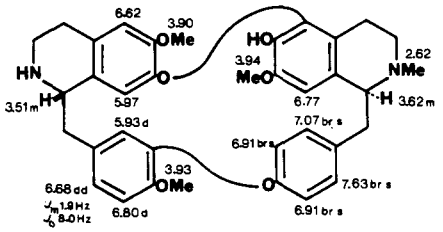

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found in *Thalictrum rugosum*, had been severely handicapped by the limited quantities of material available for chemical characterization (1). Furthermore, only a 90 MHz nmr spectrometer was available at the time. The alkaloid was believed to analyze for $C_{38}H_{38}N_2O_9$, and showed a very intense ir carbonyl band at 1660 cm^{-1}. Based on nmr and mass spectral data, structure **1** was assigned to the compound (1).

We were fortunate in isolating large amounts of thalrugosinone from *T. cultratum*, and so we were first interested in ascertaining the correspondence of our material with that formerly obtained from *T. rugosum* (1). No original and authentic sample of thalrugosinone was available, but comparison of the nmr and mass spectra showed the two materials to be the same compound. The originally recorded (1) specific rotation was $[\alpha]^{22}D$ −46.6° (*c* 0.125 MeOH), and the value we registered was in the same range: $[\alpha]^{22}D$ −42° (*c* 0.30 MeOH). All of our nmr measurements were carried out using a 360 MHz spectrometer.

The fact that we had sufficient quantities of (−)-thalrugosinone on hand prompted us to study some of its chemistry. Repeated treatment with $NaBH_4$ in MeOH failed to give a reduction product, even though the alkaloid was assumed to incorporate two ketonic functions. However, $LiAlH_4$ reduction readily furnished the known alkaloid (−)-thalidasine (**4**) (2), which we also found as a natural product in *T. cultratum*. Furthermore, acid hydrolysis of (−)-thalrugosinone afforded the known (−)-2-northalidasine (**3**) (3), also present as an alkaloid in our plant.

The above chemical transformations led us to modify the structural assignment for

thalrugosinone so that it can be represented by expression **2**. Thalrugosinone (**2**) thus corresponds to 2-formyl-2-northalidasine. Further support for structure **2** for thalrugosinone was provided by ^{1}H nmr, ^{13}C nmr, and hrms as detailed below.

Ten aromatic proton absorptions were distinctly present in the 360 MHz ($CDCl_3$) nmr spectrum of thalrugosinone, and the chemical shifts have been indicated around structure **2**. All assignments were confirmed by a complete nOe study. In particular, irradiation at δ 7.50 produced an 18% increase in the area of the H-1 multiplet absorption at δ 4.48. Conversely, irradiation at δ 4.48 led to a 25% increase in the area of the δ 7.50 peak (**2**-nOe).

Some remarks are in order concerning the influence of the *N*-formyl function on the shifts of the adjacent protons. By comparison with the spectrum of the related compound 2-northalidasine (**3**), two protons have been displaced appreciably downfield. These are H-1, which has shifted from δ 4.05 to 4.48, and one of the C-3 protons, which lies proximate to the oxygen of the formyl group and shifts from δ 3.36 to 4.61. The formyl proton itself falls at δ 7.50, which is further upfield than usually observed (δ 8.5-9.0) for such a proton. This is because the conformation of the dimer is such that the formyl group comes under the shielding influence of the aromatic rings.

The presence of an amidic carbonyl carbon in thalrugosinone (**2**) was also indicated by a ^{13}C-nmr study in $CDCl_3$, which showed a characteristic downfield absorption at δ 161.4. That the carbonyl carbon was bonded to a hydrogen atom was further confirmed by a seFt (spin echo Fourier transform) analysis (4). No ketonic absorption was in evidence in the region around δ 199 where such carbons usually absorb.

The high resolution mass spectrum of thalrugosinone provided further evidence in favor of structure **2**. The molecular ion is *m/z* 666.2882 ($C_{39}H_{42}N_2O_8$), calcd 666.2940. There is a distinct $(M-1)^+$ ion, *m/z* 665.2821, calcd 665.2861. Other important mass fragments *m/z* 439, 425, 411, and 211 may be represented by expressions **7-10**.

Thalrugosinone (**2**) is thus the first bisbenzylisoquinoline alkaloid possessing an *N*-formyl group. Its biogenesis probably proceeds by oxidation of the left-hand *N*-methyl group of the accompanying thalidasine (**4**) to furnish the hydroxymethyl analog (HO-CH_2-N<), which is further oxidized to thalrugosinone. Thalrugosinone (**2**) is a stable compound, in keeping with its nature as both a tertiary amine and an amide. The original structure assignment **1** would have meant that the alkaloid incorporated two α-aminoketone moieties, each of which would have imparted a degree of instability by making the material sensitive to air oxidation.

With the present structural revision of thalrugosinone, the nature of the earlier reported bisbenzylisoquinoline (−)-thalpindione, obtained from *Thalictrum alpinum* (5), should also be reconsidered, even though no thalpindione was in our possession. The alkaloid is monophenolic and was originally assigned structure **5**, inasmuch as its *O*-methylation with CH_2N_2 supplied (−)-thalrugosinone. It now follows that (−)-thalpindione must be represented by expression **6**.

A complete nmr nOe study of the known (−)-thalmine (**12**) and (−)-*O*-methylthalmine (**11**) (7) has allowed the specific assignment of chemical shifts. For both species, at room temperature, the 11′ and 13′ protons on ring C′ appear as a broad hump close to δ 6.92, while no signal corresponding to the 10′ and 14′ protons is noticeable. However, at 60°, the signal around δ 6.92 becomes a clean two-proton doublet, and a hump centered around δ 7.35 appears which corresponds to H-10′ and H-14′. This assignment was confirmed by spin decoupling experiments at that temperature.

The new bisbenzylisoquinoline alkaloid also belonged to the (−)-thalmine series and was identified as (−)-2-northalmine (**13**), $C_{36}H_{38}N_2O_6$. The ^{1}H-nmr spectrum of **13** is close to that of thalmine (**12**), with the difference that the upfield *N*-methyl

singlet at δ 2.19 in thalmine is absent. All nmr assignments were confirmed by nOes. Noteworthy among the nOe results is the finding that H-1 (δ 3.51) can be interconnected with the H-8 singlet (δ 5.97) as well as the H-10 doublet (δ 5.93). Alternatively, the right-hand *N*-methyl signal (δ 2.62) can be connected with H-1′ (δ 3.62), which is interrelated in turn with the H-8′ singlet (δ 6.77), which is then connected with the 7′-methoxyl singlet (δ 3.94). The ring C′ aromatic protons are more distinguishable in this instance than in thalmine or *O*-methylthalmine. H-11′ and H-13′ appear as a broad singlet at δ 6.91; H-10′ is represented by a hump at δ 7.07, and H-14′ by a broad absorption centered at δ 7.63.

Significantly, the mass spectral molecular ion for 2-northalmine (**13**), *m/z* 594, as well as the base peak *m/z* 367, are each 14 mass units less than the corresponding peaks in thalmine (**12**).

The cd spectrum of (−)-2-northalmine (**13**) is similar to that of (−)-thalmine (**12**), and *N*-methylation of the former using formaldehyde and $NaBH_4$ provided the latter which was characterized by identical tlc Rf values as well as an nmr spectrum corresponding to that of natural (−)-thalmine (**12**).

It is interesting to point out, in conclusion, that the structures of thalrugosinone, thalpindione, *O*-methylthalmine, and 2-northalmine are all in accordance with the general biogenetic rules relating to *Thalictrum* bisbenzylisoquinoline alkaloids (9).

EXPERIMENTAL

GENERAL PROCEDURES.—All ir spectra were recorded in $CHCl_3$, while uv and cd spectra are in MeOH. ^{1}H-nmr spectra were obtained in $CDCl_3$ on a 360 MHz Bruker instrument. The nOe difference spectra were run in $CDCl_3$ after degassing the solution three times by evacuation during freeze-thaw cycles. All nOe spectra were obtained at steady-state by discarding the first two scans of each frequency file before data addition and then cycling through all the frequency files repeatedly. For the purpose of simplification, only the more important nOe interactions are shown in diagram **2**-nOe. Between scans for nuclear relaxation 18 seconds were allowed. The ^{13}C-nmr spectrum of thalrugosinone (**2**) was obtained on a Bruker WP-200 at 50.3 MHz, using a seFt (GASPE) sequence with τ set at 7 millisec to phase CH and CH_3 spectral lines below the base line (such as the *N*-formyl carbon of thalrugosinone), and CH_2 and quaternary carbons above the base line. Preparative tlc was on Merck silica gel glass plates, 0.5 mm thick; analytical tlc was on 0.25 mm thick plates.

PLANT MATERIAL AND EXTRACTION.—*T. cultratum* was identified by Dr. Eugene Nasir, Director, National Herbarium, Islamabad, Pakistan, where a specimen has been deposited. The dried whole plant (11 kg) was extracted several times with EtOH at room temperature and the solvent removed under reduced pressure at 40-50°. The dried alcoholic extract was taken up in 5% HCl, filtered, and the filtrate basified with NH_4OH. The mixture was then exhaustively extracted with $CHCl_3$ to provide 176 g of crude alkaloidal extract. This material was passed through a column of silica gel 60 (70-230 mesh ASTM). Elution was with $CHCl_3$ gradually enriched with MeOH.

(−)-THALRUGOSINONE (**2**).—Preparative tlc of a small portion (390 mg) from the combined fractions of the first group (3.0 g) collected, using C_6H_6-MeOH (9:1), provided thalrugosinone (79 mg), Rf 0.43 (C_6H_6-MeOH-NH_4OH) (95:5:trace); uv λ max 241 sh, 280 nm (log ε 4.34, 3.62); ir ν max 1660 cm^{-1}; ms *m/z* 666 (M^+) (74), 651 (54), 635 (100), 439 (72), 425 (18), 411 (32), 409 (36), 204 (49), 190 (30); cd Δε (nm) 0 (300), −3.9 (282), 0 (270), −0.2 (255), 0 (252), +11.8 (239), negative tail below 230 nm.

$LiAlH_4$ REDUCTION OF (−)-THALRUGOSINONE (**2**).—Thalrugosinone (8 mg) in dry ether was stirred with excess $LiAlH_4$ for 4 h. Workup and purification by tlc afforded thalidasine (**4**) (4 mg), identified by direct comparison with the natural product (nmr, tlc).

ACID HYDROLYSIS OF (−)-THALRUGOSINONE (**2**).—Thalrugosinone (9 mg) was refluxed with 18% HCl (5 ml) for 5 h. The mixture was cooled, basified with NH_4OH, and extracted with $CHCl_3$. Purification by tlc afforded as the major compound 2-northalidasine (**3**) (3.5 mg), identified by comparison with an authentic sample (nmr, tlc).

(−)-*O*-METHYLTHALMINE (**11**).—Preparative tlc in C_6H_6-MeOH (80:20) of a subsequent combined fraction (70 mg) obtained by elution of the column with 1% MeOH in $CHCl_3$ yielded **11** (12 mg), Rf 0.58

in C_6H_6-MeOH-NH_4OH (95:5:trace); $[\alpha]^{25}D$ −25° (*c* 0.26 MeOH); uv λ max 237 sh, 283 nm (log ε 4.32, 3.84); ms *m/z* 622 (M^+) (88), 621 (88), 395 (100), 381 (93), 198 (72), 175 (46); cd Δε (nm) 0 (300), +4.1 (290), 0 (283), −2.9 (268), 0 (250), +5.7 (240), negative tail below 232 nm.

(−)-THALIDASINE (**4**).—From a subsequent combined fraction (3.2 g) from the column, a portion (250 mg) was fractionated by preparative tlc as above to provide a major fraction (207 mg) identified as **4** by comparison of spectral data (nmr, uv, ms, specific rotation); cd Δε (nm) 0 (300), −5.6 (283), 0 (270), +0.5 (265), 0 (255), +9.3 (242), negative tail below 230 nm.

(−)-THALMINE (**12**).—The minor fraction (10 mg) from the above tlc plate was **12** by comparison of spectral data; $[\alpha]^{25}D$ −30.8° (*c* 0.37 MeOH); uv λ max 237 sh, 282 nm (log ε 4.23, 3.79); ms *m/z* 608 (M^+) (53), 607 (53), 381 (100), 366 (45), 191 (77), 174 (34); cd Δε (nm) +4.1 (289), 0 (276), −1.2 (268), 0 (257), 239 (+5.1), negative tail below 225 nm.

(+)-NORTHALIDASINE (**3**).—From the subsequent group of column chromatographic fractions (3.09 g), a portion (190 mg) was fractionated by tlc in C_6H_6-MeOH-NH_4OH (95:5:trace) to obtain **4** (75 mg), **12** (7 mg), and **3** (36 mg). The last was identified by comparison of spectral data and specific rotation; cd Δε (nmr) −10.5 (282), 0 (277), +0.6 (262), 0 (257), +18.2 (239), negative tail below 230 nm.

(−)-2-NORTHALMINE (**13**).—The subsequent group (1.18 g) of fractions from the chromatographic column was fractionated by tlc as above to yield besides **4** (163 mg), **3** (558 mg), and **12** (5 mg), the base **13** (14 mg), Rf 0.18 in C_6H_6-MeOH-NH_4OH (95:5:trace); $[\alpha]^{25}D$ −31.8° (*c* 0.43 MeOH); uv λ max 234 sh, 283 nm (log ε 4.45, 3.97); ms *m/z* 594 (M^+) (22), 367 (100), cd Δε (nm) 0 (300), +7.6 (289), 0 (276), −2.1 (260), 0 (257), +6.3 (238), negative tail below 230 nm.

N-METHYLATION OF (−)-2-NORTHALMINE (**13**).—Compound **13** (5 mg) in MeOH (1 ml) was stirred near 25° with aqueous formaldehyde (1 ml) for 45 min. $NaBH_4$ (2 g) was added in very small portions, and stirring continued for 1 h. Two ml of H_2O/MeOH was added, and the solvent evaporated. The residue was acidified with dilute HCl and basified with NH_4OH. The product was extracted with $CHCl_3$ and purified by tlc to afford **12** (3 mg).

ACKNOWLEDGMENTS

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