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NINE BISBENZYLISOQUINOLINE ALKALOIDS FROM *THALICTRUM CULTRATUM*

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ABSTRACT.—*Thalictrum cultratum* collected in northern Pakistan has yielded, besides several known alkaloids, the two norbisbenzylisoquinolines (+)-2′-noroxyacanthine [**1**] and (+)-2′-northaliphylline [**2**], the diphenolic imine (+)-cultithalminine [**3**], and the six *N*-oxides (+)-neothalibrine-2′-α-*N*-oxide [**7**], (−)-thalrugosaminine-2-α-*N*-oxide [**8**], (−)-thaligosine-2-α-*N*-oxide [**9**], (+)-thaliphylline-2′-β-*N*-oxide [**10**], (+)-thalidasine-2-α-*N*-oxide [**11**], and (−)-5-hydroxythalidasine-2-α-*N*-oxide [**12**].

We have found nine new bisbenzylisoquinolines in our continuing study of *Thalictrum cultratum* Wall. (Ranunculaceae) that had been collected in northern Pakistan. All of these alkaloids are structurally related to those previously reported in the plant (1,2). Of the nine alkaloids, two were nor bases, one was an imine, while the remaining six were *N*-oxides.

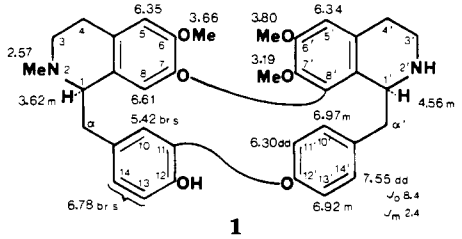
(+)-2′-Noroxyacanthine [**1**] showed a mass spectral molecular ion *m/z* 594 (67%) with base peak *m/z* 381. These important ions are 14 a.m.u. less than in oxyacanthine, indicating one less methyl group for the top part of the dimer.

The ^{1}H-nmr spectrum, while definitely similar to that of (+)-oxyacanthine, displayed only one *N*-methyl singlet (δ 2.57), suggesting that a secondary amine was present in the molecule (3). This finding corroborated the absence of a methyl group observed in the mass spectrum. The downfield shift of H-1′ from δ 4.19 in oxyacanthine to 4.56 in **1** indicated that the secondary amine was on the right-hand side of the molecule. The positive specific rotation pointed to the 1*R*,1′*S* chirality, as in (+)-oxyacanthine.

The second norbisbenzylisoquinoline is (+)-2′-northaliphylline [**2**]. This compound had previously been obtained by $NaBH_4$ reduction of the dimer (+)-thalsivasine and has been fully described (2).

The diphenolic imine (+)-cultithalminine [**3**] exhibited mass spectral molecular ion and base peak *m/z* 608, which was accompanied by a strong *m/z* 607 peak. Also present was a doubly charged molecular ion, *m/z* 304 (9%). Such a pattern is usually observed for bisbenzylisoquinoline imines (3).

The nmr spectrum was very close to that for (+)-thalmiculimine [**4**] (2); however,



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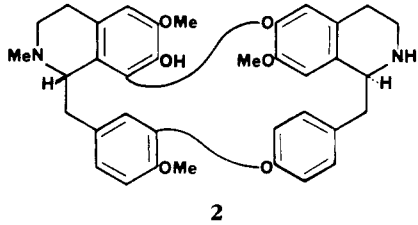

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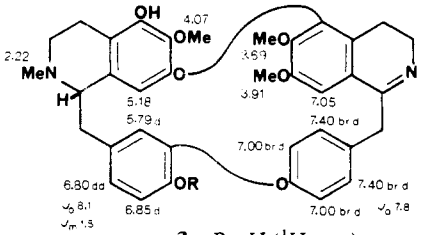
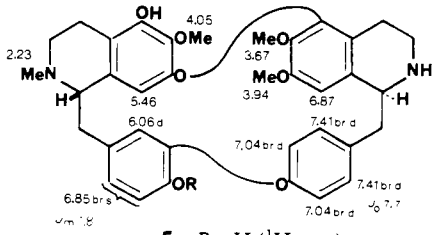
only one rather than two methoxyl singlet absorptions was present at δ 3.91. This suggested that a phenolic function rather than a methoxyl was present at either C-7′ or C-12. In order to resolve this question, the alkaloid was reduced with $NaBH_4$. The resulting (−)-2′-norcultithalmine [**5**] presented an nmr spectrum close to that for the known (−)-2′-northalmiculine [**6**] obtained by similar reduction of (+)-thalmiculimine [**4**] (2). Again, only one methoxyl singlet could be observed at δ 3.94.

The mass spectrum of (−)-2′-norcultithalmine was the key factor in the assignment of the position of the lone methoxyl absorbing at δ 3.94. The molecular ion, *m/z* 610 (65%), for (−)-2′-norcultithalmine [**5**] is accompanied by base peak *m/z* 397, which corresponds to the upper half of the dimer. This *m/z* 397 ion is also observed in the mass spectrum of (−)-2′-northalmiculine [**6**], so that both secondary amines bear the identical substitution pattern at the top. It follows that a phenolic function is present at C-12, and a methoxyl is at C-7′ in both species **3** and **5**.



3 R=H (¹H nmr)
4 R=Me



5 R=H (¹H nmr)
6 R=Me

Turning now to the *N*-oxide dimers, the phenolic (+)-neothalibrine-2′-α-*N*-oxide [**7**] presented a mass spectral cleavage pattern characteristic of singly bridged bisbenzylisoquinolines (3). No molecular ion could be observed. Instead, two strong peaks, *m/z* 192 (100) and 206 (52), were present. These suggested the presence of two tetrahydroisoquinoline moieties, one bearing a methoxyl and a phenol and the other two methoxyls.

The nmr spectrum of (+)-neothalibrine-2′-α-*N*-oxide [**7**] showed features characteristic of an 11-12′ monobridged dimer and was, in fact, very close to that of (+)-neothalibrine (3,4). An *N*-methyl absorption relatively downfield at δ 3.24 pointed to an *N*-oxide functionality. The complete structure elucidation then followed from an nmr nOe study. No nOe's could be observed between H-1′ (δ 4.75) and the *N*-oxide methyl (δ 3.24), testifying to an *anti* relationship between these two entities. On the other hand, irradiation of H-1′ (δ 4.75) induced enhancements of H-8′ (δ 6.48) and H-14′ (δ 7.25). The H-8′ signal could also be enhanced through irradiation of the 7′-methoxyl (δ 3.62).

The five remaining new *N*-oxides are bisbenzylisoquinolines with two oxygenated bridges. Their mass spectra presented closely related patterns which are typical of such *N*-oxides. In each instance, the molecular ion is weak (≃7-8%) and is accompanied by an $(M-1)^+$ ion (10-15%) and an $(M-2)^+$ ion (≃20%). The $(M-16)^+$ ion is an important fragment (60-70%), and the base peak corresponds either to the top half of the molecule minus oxygen or to the corresponding doubly charged ion (3).

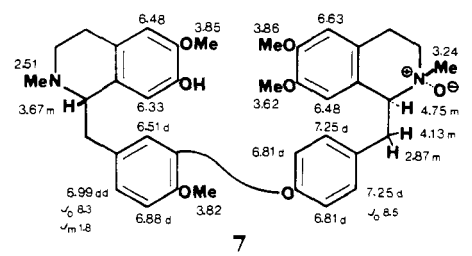
(−)-Thalrugosaminine-2-α-*N*-oxide [**8**] and the phenolic (−)-thaligosine-2-α-*N*-oxide [**9**] are doubly bridged and belong to the 7-8′, 11-12′ series. They differ only in the presence of a 5′-methoxyl in **8** and a 5′-hydroxyl in **9**.

Appropriately, the nmr spectra for *N*-oxides **8** and **9** bore direct similarities but with one methoxyl singlet less in the spectrum of (−)-thaligosine-2-α-*N*-oxide [**9**]. The strong downfield shift of H-1 to ≃ δ 4.82, while H-1′ was found only slightly further downfield (≃ δ 4.45) than in the parent free base (≃ δ 4.25), pointed to the fact

that in both instances the *N*-oxide function was located on the left hand side of the molecule (3). An nOe analysis confirmed the site of the *N*-oxide function as well as the relative configuration. In particular, reciprocating nOe's were recorded between H-1 ($\simeq \delta$ 4.82) and the 2-*N*-methyl group ($\simeq \delta$ 3.45) in accord with a *syn*-relationship between these hydrogens. Additionally, the H-1′ signal ($\simeq \delta$ 4.45) was enhanced through irradiation of the 2′-*N*-methyl ($\simeq \delta$ 2.60); and H-14′ ($\simeq \delta$ 7.50) is affected by irradiation of H-1′.

While an nOe could be observed between the C-4′ methylene protons and the 5′-methoxyl in the case of (−)-thalrugosaminine-2-α-*N*-oxide [**8**], no enhancement of the methylene protons in base **9** could be detected from irradiation of a methoxyl group of (−)-thaligosine-2-α-*N*-oxide [**9**]. Conversely, irradiation of the C-4′ methylene protons did not affect either a methoxyl or an aromatic absorption. The conclusion is that C-5′ in base **9** must bear the phenolic hydroxyl.

The phenolic (+)-thaliphylline-2′-β-*N*-oxide [**10**] has a mass spectral molecular ion 16 a.m.u. higher than that for (+)-thaliphylline. The nmr spectrum is close to that of the free base except for the protons in the vicinity of the *N*-oxide function (2). Significantly, the 2-*N*-methyl signal was at δ 2.07 as in (+)-thaliphylline itself. On the other hand, the 2′-*N*-methyl singlet had shifted from δ 2.57 in (+)-thaliphylline to δ 3.26 in *N*-oxide **10**, clearly denoting oxidation at the 2′ center. Furthermore, the downfield shift of H-1′ from δ 3.95 to 4.63 in our *N*-oxide and the upfield shift of H-8′ and H-10′ in *N*-oxide **10** confirm the structural assignment. A complete nOe study then led to a conclusive assignment of each of the chemical shifts, as well as to the *syn*-relationship between H-1′ (δ 4.63) and the 2′-*N*-methyl (δ 3.26).



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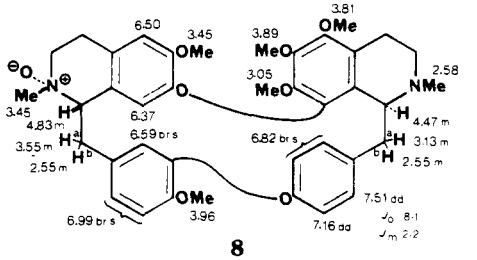

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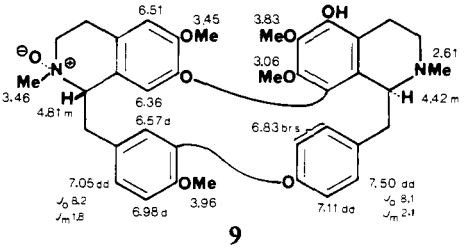

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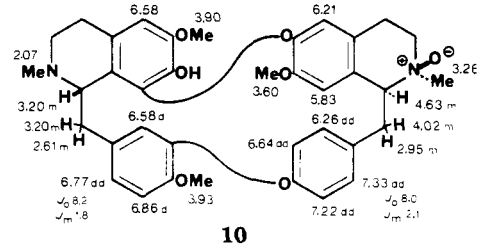

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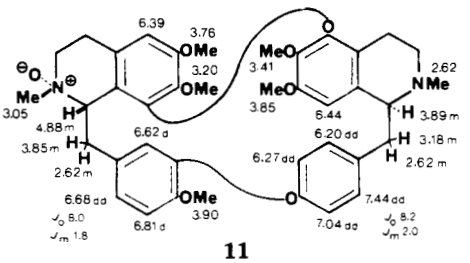
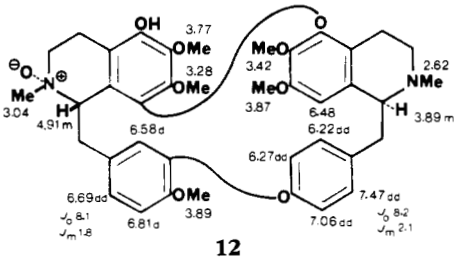
The last two dimeric *N*-oxides, namely (+)-thalidasine-2-α-*N*-oxide [**11**] and (−)-5-hydroxythalidasine-2-α-*N*-oxide [**12**], are structurally related and belong to the 8-5′, 11-12′ subgroup (1,2). The only difference between the two compounds resides in the presence of an extra 5-hydroxyl in species **12**. In both cases the nmr spectra are related to the parent free bases, viz., (−)-thalidasine and (−)-5-hydroxythalidasine (1,2). An nOe study on (+)-thalidasine-2-α-*N*-oxide [**11**] led to the assignment of the *N*-oxide function to the left portion of the dimer with a *syn*-relationship between the 2-*N*-methyl (δ 3.05) and H-1 (δ 4.88). By analogy the identical relation must prevail in (−)-5-hydroxythalidasine-2-α-*N*-oxide [**12**]. The assignment of the phenolic function to C-5 in dimer **12** was also substantiated by the absence of the H-5 aromatic singlet ab-





sorption that falls at δ 6.39 in the spectrum of (+)-thalidasine-2-α-*N*-oxide [**11**].

The nmr spectra of the doubly bridged dimers **2**, **5**, and **8-12**, are characteristic of either the 1*R*,1′*R*, or the 1*S*,1′*S* configurations (1-3). For each of these compounds, the sign of the specific rotation points to the 1*S*,1′*S* chirality (3). These stereochemical assignments are in accord with the rules correlating the structures of *Thalictrum* bisbenzylisoquinoline alkaloids with their absolute configurations (5).

Known bisbenzylisoquinolines also found in the plant include (+)-thaligosine, (+)-thalirugine, (+)-neothalibrine, (+)-aromoline, (+)-obaberine, and (+)-oxyacanthine. It should be noted that for each *N*-oxide isolated, its corresponding free base was also present (1,2); additionally, the possibility always exists that at least some of the *N*-oxides could have been formed through oxidation of the dimeric bases during the isolation-purification process.

EXPERIMENTAL

For the isolation procedure, see Hussain *et al.* (1). All the new alkaloids are amorphous and were obtained in relatively small amounts (3-9 mg).

(+)-2′-NOROXYACANTHINE [**1**].—$C_{36}H_{38}N_2O_6$; *m/z* 594 (M^+, 67), 593 (55), 382 (26), 381 (100), 367 (21), 192 (30), 191 (76), 174 (27); $[\alpha]_D$+125° (*c* 0.1, MeOH).

(+)-CULTITHALMININE [**3**].—$C_{36}H_{36}N_2O_7$; *m/z* 608 (M^+, 100), 607 (94), 593 (10), 304 (9), 192 (20), 191 (10), 190 (18), 164 (11); $[\alpha]_D$+7° (*c* 0.17, MeOH). Imine **3** (2 mg) was dissolved in MeOH (1 ml) and $NaBH_4$ (100 mg) added. Work-up furnished **5** (1 mg).

(−)-2′-NORCULTITHALMINE [**5**].—$C_{36}H_{38}N_2O_7$; *m/z* 610 (M^+, 65), 609 (47), 411 (15), 397 (100), 383 (26), 199 (72), 192 (31), 191 (39), 190 (44), 176 (21); $[\alpha]_D$−39° (0.07, MeOH).

(+)-NEOTHALIBRINE-2′-α-*N*-OXIDE [**7**].—$C_{38}H_{44}N_2O_7$; *m/z* 222 (0.5), 208 (0.7), 206 (52), 192 (100), 190 (5), 177 (6); $[\alpha]_D$+74° (*c* 0.2, MeOH). Principal nmr nOe's are 2-NMe to H-1 (13%), H-1 to 2-NMe (14%), H-1 to H-10 (9%), H-10 to H-1 (7%), H-8 to H-1 (8%), H-1 to H-8 (9%), H-1′ to H-8′ (8%), H-8′ to H-1′ (14%), H-1′ to H-10′ and H-14′ (19%), H-5 to MeO-6 (18%), MeO-6 to H-5 (27%), H-5′ to MeO-6′ (25%), MeO-6′ to H-5′ (32%), MeO-7′ to H-8′ (21%), H-8′ to MeO-7′ (13%).

(+)-NEOTHALIBRINE.—Nmr (200 MHz, $CDCl_3$) δ 7.02 d (*J*=8.5 Hz, H-10′ and H-14′), 6.80 d (*J*=8.5 Hz, H-11′ and H-13′), 6.91 dd (J_o=8.2 Hz, J_m=1.8 Hz, H-14), 6.86 d (J_o=8.2 Hz, H-13), 6.62 d (J_m=1.8 Hz, H-10), 6.57 (H-5′), 6.47 (H-5), 6.38 (H-8), 6.08 (H-8′), 3.60 (MeO-7′), 3.81 and 3.82 (MeO-6 and MeO-6′), 3.84 (MeO-12), 2.47 (2-NMe), 2.55 (2′-NMe).

(−)-THALRUGOSAMININE-2-α-*N*-OXIDE [**8**].—$C_{39}H_{44}N_2O_8$; *m/z* 668 (M^+, 6), 667 (10), 666 (22), 652 (82), 651 (60), 637 (35), 608 (18), 607 (19), 441 (1), 425 (63), 412 (26), 411 (92), 397 (15), 213 (100), 212 (13), 206 (51), 174 (42); $[\alpha]_D$−33° (*c* 0.2, MeOH). Principal nOe's are H-1 to 2-NMe (9.6%), 2-NMe to H-1 (13%), H-1 to H-14 (5%), H-8 to H-10 (6%), H-10 to H-8 (6%), H-8 to H-α_a (7%), H-1′ to 2′-NMe (4%), 2′-NMe to H-1′ (7%), H-1′ to H-14′ (7%), H-1′ to H-α'_b (3%), H-14′ to H-α'_a (4%), H-5 to MeO-6 (15%), MeO-6 to H-5 (5%), MeO-5′ to H-4′ (2%), H-13 to MeO-12 (19%), MeO-12 to H-13 (14%).

(−)-THALRUGOSAMININE.—Nmr (200 MHz, $CDCl_3$) δ 7.32 dd (J_o=8.2 Hz, J_m=2.0 Hz, H-14′), 7.13 dd (J_o=8.2 Hz, J_m=2.0 Hz, H-13′), 6.85 (br. s, H-10′ and H-11′), 6.91 dd (J_o=8.3 Hz, J_m=1.9 Hz, H-14), 6.98 d (J_o=8.3 Hz, H-13), 6.64 (*J*=1.9 Hz, H-10), 6.48 (H-8), 6.41 (H-5), 4.24 (H-1′), 3.49 (H-1), 2.57 (2′-NMe), 2.52 (2-NMe), 3.96 (MeO-12), 3.85 (MeO-6′), 3.81 (MeO-5′), 3.46 (MeO-6), 3.07 (MeO-7′).

(−)-THALIGOSINE-2-α-*N*-OXIDE [**9**].—$C_{38}H_{42}N_2O_8$; *m/z* 654 (M^+, 2), 653 (8), 652 (18), 651 (21), 638 (47), 637 (39), 624 (13), 623 (21), 425 (5), 411 (100), 397 (79), 206 (57), 192 (54), 191 (15), 190 (21), 176 (20); $[\alpha]_D$ −59° (*c* 0.13, MeOH). Principal nOe's are H-1 to 2-NMe (7%), 2-NMe to H-1 (11%), H-1 to H-8 (5%), H-8 to H-10 (7%), H-10 to H-8 (8%), 2′-NMe to H-1′ (13%), H-1′ to H-14′ (3%), H-5 to MeO-6 (5%), MeO-6 to H-5 (14%), H-13 to MeO-12 (16%), MeO-12 to H-13 (16%).

(−)-THALIGOSINE.—Nmr (200 MHz, $CDCl_3$) δ 7.33 dd (J_o=8.2 Hz, J_m=1.8 Hz, H-14′), 7.13 dd (J_o=8.2 Hz, J_m=1.8 Hz, H-13′), 6.85 br. s (H-10′ and H-11′), 6.97 d (J_o=8.3 Hz, H-13), 6.90 dd (J_o=8.3 Hz, J_m=1.8 Hz, H-14), 6.65 d (J_m=1.8 Hz, H-10), 6.47 (H-8), 6.40 (H-5), 4.23 m (H-1′), 3.50 m (H-1), 2.58 (2′-NMe), 2.52 (2-NMe), 3.96 (MeO-12), 3.80 (MeO-6′), 3.41 (MeO-6), 3.07 (MeO-7′).

(+)-THALIPHYLLINE-2′-β-*N*-OXIDE [**10**].—$C_{37}H_{40}N_2O_7$; *m/z* 624 (M^+, 3), 623 (9), 622 (22), 608 (43), 607 (31), 594 (6), 381 (87), 367 (20), 192 (24), 191 (100), 190 (33), 176 (28), 174 (39); $[\alpha]_D$ +257° (*c* 0.7, MeOH). Principal nOe's are 2-NMe to H-1 (4%), H-1 to 2-NMe (4%), H-1 to H-10 (4%), H-10 to H-1 (2%), H-1 to H-5′ (3%), H-5′ to H-1 (4%), H-10 to H-α_a (1.5%), H-α_a to H-10 (10%), H-α_b to H-14 (10%), H-14 to H-α_b (2%), H-1′ to 2′-NMe (3%), 2′-NMe to H-1′ (7%), H-1′ to H-8′ (7%), H-8′ to H-1′ (6%), H-8′ to MeO-7′ (9%), MeO-7′ to H-8′ (11%), H-4 to H-5 (6%), H-5 to MeO-6 (10%), MeO-6 to H-5 (11%).

(+)-THALIDASINE-2-α-*N*-OXIDE [**11**].—$C_{39}H_{44}N_2O_8$; *m/z* 668 (M^+, 3), 667 (6), 666 (12), 652 (70), 637 (25), 425 (69), 411 (86), 213 (100), 206 (58), 204 (59), 190 (89), 176 (18), 174 (21); $[\alpha]_D$ +6° (*c* 0.15, MeOH). Principal nOe's are H-1 to 2-NMe (4%), 2-NMe to H-1 (6%), H-1 to H-10 (16%), H-10 to H-1 (11%), 2-NMe to H-14 (6%), H-1′ to 2′-NMe (15%), 2′-NMe to H-1′ (10%), H-1′ to H-8′ (5%), H-8′ to H-1′ (4%), H-α'_b to H-10′ (10%), H-8′ to MeO-7′ (25%), MeO-7′ to H-8′ (22%), H-5 to MeO-6 (20%), MeO-6 to H-5 (20%), H-4 to H-5 (9%), H-13 to MeO-12 (13%), MeO-12 to H-13 (16%).

(−)-5-HYDROXYTHALIDASINE-2-α-*N*-OXIDE [**12**].—$C_{39}H_{44}N_2O_9$; *m/z* 684 (M^+, 0.4), 683 (0.6), 682 (2), 668 (68), 667 (27), 653 (23), 457 (2), 411 (86), 227 (58), 222 (57), 221 (100), 206 (22), 204 (34), 198 (35), 190 (35), 176 (15); $[\alpha]_D$ −11° (*c* 0.4, MeOH).

ACKNOWLEDGMENTS

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