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FIVE NEW BISBENZYLISOQUINOLINE ALKALOIDS FROM *THALICTRUM CULTRATUM*

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ABSTRACT.—*Thalictrum cultratum* (Ranunculaceae), of Pakistani origin, has supplied the new bisbenzylisoquinoline alkaloids: (−)-thalmiculine (**1**), (−)-5-hydroxythalmine (**4**), (+)-thalmiculatimine (**5**), (−)-thalmiculimine (**6**), and (−)-5-hydroxythalidasine (**10**).

Thalictrum cultratum Wall. (Ranunculaceae) is a small plant with relatively large roots growing in the northern regions of Pakistan. An initial investigation of the whole plant in our laboratory had revealed the presence of several bisbenzylisoquinolines of the thalmine and the thalidasine series (1). Presently, a continuing investigation of the extracts has supplied five new bisbenzylisoquinolines together with the known dimers (−)-thalictine, (−)-thalrugosidine, (+)-*O*-methylthalicberine, (+)-thaliphylline, (−)-thalrugosaminine, (−)-thalisopine [≡ (−)-thaligosine] (2), (+)-thalsivasine.

Four of the five new alkaloids, namely, (−)-thalmiculine (**1**), (−)-5-hydroxythalmine (**4**), (+)-thalmiculatimine (**5**), and (−)-thalimiculimine (**6**), belong to the (−)-thalmine series and incorporate in the upper moiety an ether bridge between C-7 and C-5′.

The mass spectrum of (−)-thalmiculine (**1**) shows molecular ion *m/z* 638, which is also the base peak. This value is 16 mass units greater than that for the known (−)-*O*-methylthalmine (**2**) (3) and indicates the molecular composition $C_{38}H_{42}N_2O_7$. The major fragment *m/z* 411 corresponds to the upper half of the molecule and is also 16 mass units larger than the corresponding ion for (−)-*O*-methylthalmine (**2**). These data point to the presence of an extra oxygen in the top half of the molecule.

The nmr spectrum of (−)-thalmiculine (**1**) shows the features characteristic of a dimer of the (−)-thalmine series. In particular, the presence of two three-proton singlets at δ 2.18 and 2.67 is due to the 2-*N*-methyl and the 2′-*N*-methyl groups, respectively. A poorly defined hump around δ 7.30 and a broad singlet at δ 6.95 were sharpened by an increase in temperature to 60°. These two signals may be assigned to H-10′,14′ and to H-11′,13′, respectively. Significantly, the absence of a one-proton singlet near δ 6.63 indicates substitution at C-5 by a phenolic function (1).

Diazomethane *O*-methylation of (−)-thalmiculine (**1**) afforded (−)-*O*-methylthalmiculine (**3**), $C_{39}H_{44}N_2O_7$. A complete nmr nOeds study of this derivative led to a full assignment of chemical shifts. It should be noted that in (−)-*O*-methylthalmine (**2**) itself (3), H-8 appears at δ 5.88 (4). This absorption shifts upfield by ≃ 0.2 ppm when C-5 bears a methoxyl substituent as in *O*-methylthalmiculine (**3**), which shows an absorption at δ 5.70. The upfield shift is even more pronounced in (−)-thalmiculine (**1**) where H-8 is found at δ 5.45, and whose C-5 substituent is now a phenolic group.

The chemical shift of the C-6 methoxyl is also diagnostic of the substitution at C-5. It falls at δ 3.92 in (−)-*O*-methylthalmine (**2**), but is found at δ 4.00 in *O*-methylthalmiculine (**3**) and at δ 4.05 in (−)-thalmiculine (**1**).

Our second new alkaloid, (−)-5-hydroxythalmine (**4**), $C_{37}H_{40}N_2O_7$, exhibited a spectral data set very close to that for (−)-thalmiculine (**1**). The mass spectrum showed

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TABLE 1. ^{1}H-nmr Spectra for Dimers of the Thalmine Series[a]

Alkaloid	2-NMe	2′-NMe	H-1	H-1′	H-5	H-8	H-8′	H-10	H-13	H-14	H-10′	H-11′	H-13′	H-14′	MeO-6	MeO-6′	MeO-7′	MeO-12
O-Methylthalmine (**2**) .	δ2.20	2.66	3.27	3.60	6.63	5.88	6.79	6.08	6.79	6.79	7.30	6.94	6.96	7.30	3.92	3.70	3.89	3.93
Thalictine (**8**)	2.19	2.66	3.28	3.63	6.63	5.89	6.76	6.05	6.82	6.72	7.36	6.92	6.92	7.36	3.91	3.68	3.88	OH
Thalmiculine (**1**) . . .	2.18	2.67	3.22	3.64	OH	5.45	6.81	6.09	6.80	6.75	7.30	6.95	6.95	7.30	4.05	3.63	3.90	3.93
O-Methylthalmiculine (**3**)	2.18	2.66	3.27	3.63	3.93 (MeO)	5.70	6.79	6.12	6.79	6.76	7.37	6.93	6.93	7.37	4.00	3.65	3.88	3.93
5-Hydroxythalmine (**4**)	2.19	2.66	3.23	3.63	OH	5.54	6.79	6.11	6.80	6.75	7.35	6.95	6.95	7.35	4.04	OH	3.93	3.93
2′-Northalictine (**7**) . .	2.19	NH		4.46	6.63	5.85	6.84	6.04	6.84	6.74	7.37	6.95	6.95	7.37	3.91[b]	3.73	3.92[b]	OH
2′-Northalmiculine (**9**)	2.26	NH	3.30	4.50	—	5.46	6.90	6.12	6.85	6.81	7.35	6.99	6.99	7.35	4.05	3.69	3.93	3.95
Thalmiculatimine (**5**) .	2.17	—	3.09	—	6.64	5.58	7.02	5.81	6.79	6.73	7.40	7.02	7.02	7.40	3.94	3.76	3.90	OH
Thalmiculimine (**6**) . .	2.16	—	3.04	—	OH	5.17	7.06	5.84	6.81	6.75	7.38	7.04	7.04	7.38	4.07	3.69	3.92	3.92

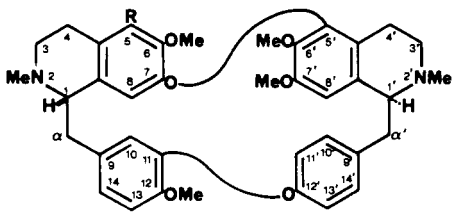
[a]For each compound above, H-10 d, J_m 2 Hz;H-13 d, J_o ~8.2 Hz; H-14 dd, J_m ~2 Hz, J_o ~8.2 Hz. For dimers **5** and **6**, H-11′,13′ and H-10′,14′ appear as doublets, with apparent J 8 Hz.
[b]Chemical shifts are interchangeable.

a decrease of 14 mass units from that of species **1** in the molecular and the base peaks, indicating substitution of a methoxyl group by hydroxyl. In accord with this observation, the nmr spectrum was devoid of a methoxyl singlet near δ 3.60, pointing to the presence of a phenolic function at C-6′ (1). As expected, *O*-methylation of (−)-5-hydroxythalmine (**4**) led to (−)-*O*-methylthalmiculine (**3**).

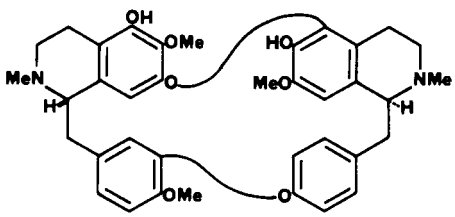
The remaining two new alkaloids of the (−)-thalmine series, namely, (+)-thalmiculatimine (**5**) and (−)-thalmiculimine (**6**), incorporate an imine function, and their uv spectra show significant bathochromic shifts in acid. The singlet *N*-methyl absorption present is upfield at δ 2.16-2.17, diagnostic of a 2-*N*-methyl function. It follows that the imine function must be anchored at C-1′.

Characteristic of a bisbenzylisoquinoline imines, the mass spectral molecular ion for (+)-thalmiculatimine (**5**), *m/z* 592 ($C_{36}H_{36}N_2O_6$), was very intense and was flanked by an $(M-1)^+$ base peak, *m/z* 591 (4). $NaBH_4$ reduction of the imine supplied the new compound (−)-2′-northalictine (**7**) whose *N*-methylation then afforded the known (−)-thalictine (**8**) (2), which we also found in the plant.

A conspicuous aspect of the nmr spectrum of (+)-thalmiculatimine (**5**) is the well-defined pair of doublets at δ 7.02 and 7.40 representing H-11′,13′ and H-10′,14′, respectively. Additionally, H-8 and H-10 which appear at δ 5.58 and 5.81, respectively, have each undergone an upfield shift of about 0.3 ppm in relation to the corresponding values in (−)-thalictine (**8**).



1 R=OH
2 R=H
3 R=OMe



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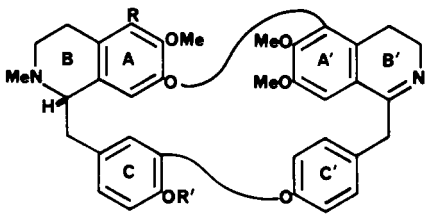
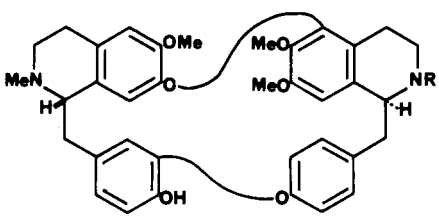


5 R=R′=H
6 R=OH,R′=Me



7 R=H
8 R=Me

Turning now to (−)-thalmiculimine (**6**), its mass spectrum displays a strong molecular ion *m/z* 622 ($C_{37}H_{38}N_2O_7$), accompanied by base peak *m/z* 621. $NaBH_4$ reduction gave the new derivative (−)-2′-northalmiculine (**9**) whose *N*-methylation generated (−)-thalmiculine (**1**).

The nmr spectra of (+)-thalmiculatimine (**5**) and (−)-thalmiculimine (**6**) were generally similar. But the presence of the C-5 phenolic function in **6** produces an upfield shift of ≃0.4 ppm for the H-8 absorption.

A comment should be added here concerning the specific rotations of imines **5** and **6**. (+)-Thalmiculatimine (**5**) is slightly positive, $[\alpha]_D$ +7.5°, while (−)-thalmiculimine (**6**) is weakly negative, $[\alpha]_D$ −5°. Nevertheless, their cd spectra are similar (see Experimental section), pointing to the identical absolute configuration at C-1.

Our fifth new alkaloid belonged to the (−)—thalidasine series, meaning that C-8

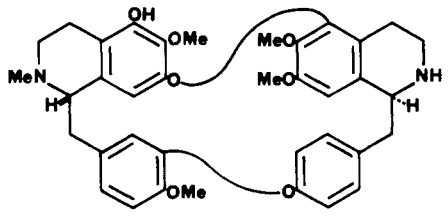
and C-5′, as well as C-11 and C-12′, are linked through ether bridges. The compound was identified as (−)-5-hydroxythalidasine (**10**), $C_{39}H_{44}N_2O_8$. In the mass spectrum, the molecular ion, *m/z* 668, and the ion representing the upper portion of the dimer, *m/z* 441, were 16 mass units larger than in the spectrum (−)-thalidasine, suggesting the presence of an additional oxygen in the top half of the molecule. The nmr spectrum was almost identical to that of (−)-thalidasine (1), except that the singlet at δ 6.30, corresponding to H-5, was absent due to substitution.

In order to establish the exact position of the phenolic function in (−)-5-hydroxythalidasine (**10**), an nmr nOeds study was carried out. No reciprocating nOe's could be observed between the C-4 or C-4′ protons and any of the methoxyl signals, except for one of the C-4′ protons (δ 2.32) and the C-7 methoxyl (δ 3.35). This last nOe is typical of C-8 to C-5′ bridging. A parallel situation prevails with (−)-thalidasine. It follows that in (−)-5-hydroxythalidasine (**10**) the phenolic group must be situated at C-5.

One of the known dimers we have presently isolated is the recently characterized (+)-thalsivasine (**13**) (5), $C_{36}H_{36}N_2O_6$, which has a mass spectrum with a strong molecular ion, *m/z* 592, and base peak *m/z* 591, suggesting an imine grouping. This was corroborated by a uv bathochromic shift in acid. The nmr spectrum of (+)-thalsivasine (**13**) showed only one *N*-methyl singlet, relatively upfield at δ 1.96, so that this function is located on the left-hand portion of the dimer. A right-hand *N*′-methyl group, if present, would have absorbed downfield near δ 2.58.

$NaBH_4$ reduction of (+)-thalsivasine (**13**) led to the hitherto unknown (+)-2′-northaliphylline (**14**), whose *N*-methylation produced (+)-thaliphylline (**15**) (6), also present in the extracts.

It should be remarked in conclusion that all five new alkaloids are structurally in accord with the biogenetic rules relating to *Thalictrum* bisbenzylisoquinolines (6).



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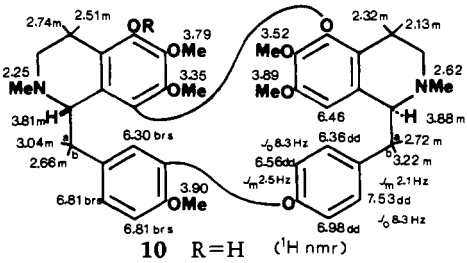
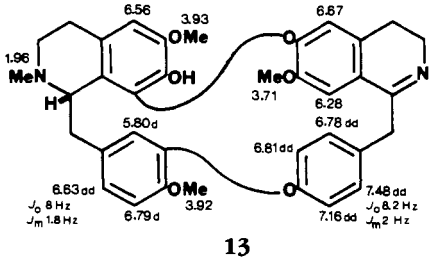


10 R=H (¹H nmr)
11 R=Me
12 R=Ac



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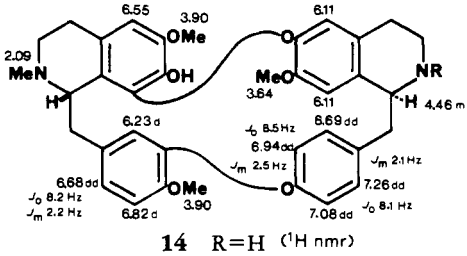

14 R=H (¹H nmr)
15 R=Me

EXPERIMENTAL

GENERAL PROCEDURES AND ISOLATION.—Details of the plant extraction and the silica gel column chromatography have been given earlier (1). The alkaloids described here were obtained from the later chromatographic fractions. The tlc was on silica gel glass plates using the systems C_6H_6-MeOH-NH_4OH

(95:5:trace) or (90:10:trace). ^{1}H-nmr spectra are at 360 MHz in $CDCl_3$. Uv and cd spectra are in MeOH. Ir spectra are in $CHCl_3$. All compounds obtained are amorphous.

From 11 kg of dried plant, the crude alkaloid fraction (176 g) furnished the following new alkaloids given as percentages of the crude alkaloid fraction: thalmiculine (**1**), 0.1%; 5-hydroxythalidasine (**10**), 0.03%; 5-hydroxythalmine (**4**), 0.001%; thalmiculimine (**6**), 0.001%; and thalmiculatimine (**5**), 0.001%. Known alkaloids obtained were thaliphylline 3%, thalrugosaminine 0.3%, thalisopine 0.3%, *O*-methylthalicberine 0.01%, thalrugosidine 0.01%, thalictine 0.01%, and thalsivasine (0.001%).

(−)-THALMICULINE (**1**).—*M/z* 638 $(M)^+$ ($C_{38}H_{42}N_2O_7$) (100), 637 (62), 411 (67), 397 (41), 206 (73), 183 (38); λ max 235 sh, 281 nm (log ε 4.28, 3.58); Δε (nm) 0 (300), −3.2 (280), −0.8 sh (268), 0 (260), +11.2 (238), negative tail below 225 nm; $[\alpha]_D$ −35° (c 2.2, MeOH).

(−)-*O*-METHYLTHALMICULINE (**3**).—*M/z* 652 $(M)^+$ ($C_{39}H_{44}N_2O_7$) (91), 651 (12), 638 (18), 622 (19), 426 (82), 412 (79), 410 (31), 213.5 (100), 206.5 (16), 204 (41), 190.5 (73); $[\alpha]_D$ −38° (c 0.2, MeOH). Important nmr nOe's were 2-NMe to H-1, 10%; H-1 to 2-NMe, 11%; H-1 to H-8, 11%; H-8 to H-1, 8%; H-αa to H-10, 6%; H-αa to H-8, 12%; H-αb to H-14, 9%; H-8′ to MeO-7′, 13%; MeO-7′ to H-8′, 20%; H-8′ to H-α′a, 5%; H-α′a to H-8′, 11%; MeO-5 to H-4a, 10%; H-4a to MeO-5, 7%; MeO-12 to H-13, 16%; H-13 to MeO-12, 18%; H-11′,13′ to H-10, 5%; H-1′to 2′-NMe, 10%.

(−)-5-HYDROXYTHALMINE (**4**).—*M/z* 624 $(M)^+$ ($C_{37}H_{40}N_2O_7$) (50), 623 (41), 397 (100), 383 (37), 199 (57), 190 (57), 176 (23); λ max 236 sh, 281 nm (log ε 4.43, 3.78); Δε (nm) 0 (300), −3.5 (280), 0 (272), +1.6 (262), +1.1 (258), +12.1 (238), negative tail below 235 nm; $[\alpha]_D$ −69° (c 0.08, MeOH).

(+)-THALMICULATIMINE (**5**).—*M/z* 592 $(M)^+$ ($C_{38}H_{36}N_2O_6$) (72), 591 (100), 397 (5), 296 (11), 273 (4); λ max 280 nm (log ε 4.02); λ max (MeOH + H^+) 237 sh, 285 nm (log ε 4.38, 4.01); Δε (nm) 0 (330), −6.5 (302), 0 (294), +7.5 (278), 0 (217), −10.5 (245), positive tail below 238 nm; $[\alpha]_D$ +7.5°(c 0.093, MeOH).

(−)-THALMICULIMINE (**6**).—*M/z* 622 $(M)^+$ ($C_{37}H_{38}N_2O_7$) (86), 621 (100), 607 (44), 591 (16), 561 (18), 311 (18), 288 (10); λ max 274 nm (log ε 3.97); λ max (MeOH+H^+) 237 sh, 284 nm (log ε 4.40, 3.93); Δε (nm) 0 (330), −5.4 (302), 0 (289), +7.4 (278), 0 (269), −12 (245), positive tail below 235 nm; $[\alpha]_D$ −5° (c 0.09, MeOH).

(−)-2′-NORTHALICTINE (**7**).—*M/z* 594 $(M)^+$ ($C_{36}H_{38}N_2O_6$) (52), 593 (77), 592 (78), 591 (100), 395 (17), 381 (47), 365 (24), 191 (38); λ max 237 sh, 284 nm (log ε 4.33, 3.90); Δε (nm) 0 (300), +2.4 (291), 0 (288), −3.3 (281), 0 (268), 0 (258), +14.5 (240), negative tail below 230 nm; $[\alpha]_D$ −54° (c 0.1, MeOH).

(−)-2′-NORTHALMICULINE (**9**).—*M/z* 624 $(M)^+$ ($C_{37}H_{40}N_2O_7$) (72), 623 (100), 397 (57), 383 (49), 199 (71), 176 (64); λ max 236 sh, 281 nm (log ε 4.33, 3.63); Δε (nm) 0 (300), −7 (281), 0 (268), 0 (255), +23.5 (238), negative tail below 225 nm; $[\alpha]_D$ −44° (c 0.1, MeOH.

(−)-5-HYDROXYTHALIDASINE (**10**).—*M/z* 668 $(M)^+$ ($C_{39}H_{44}N_2O_8$) (8), 667 (31), 666 (75), 651 (21), 441 (65), 427 (60), 411 (36), 221 (100), 206 (28), 204 (30), 190 (23); λ max 237 sh, 282 nm (log ε 4.20, 3.54); 0 (300), −6.8 (282), 0 (269), 0 (255), +12.0 (242), negative tail below 230 nm; $[\alpha]_D$ −51° (c 0.1, MeOH). Important nmr nOe's are 2-NMe to H-1, 14%; H-1 to 2-NMe, 6%; H-1 to H-10, 11%; H-1 to H-αa, 3%; H-αa to H-1, 5%;H-αb to H-14, 10%; H-8′ to OMe-7′, 20%; OMe-7′ to H-8′, 18%; H-8′ to H-1′, 10%; H-1′ to H-8′, 3%; H-8′ to H-αa, 7%; H-α′a to H-8′, 4%; H-1′ to H-α′a, 3%; H-α′a to H-1′, 8%; H-α′b to H-14′, 7%; 2′-NMe to H-1′, 10%; H-1′ to 2′-NMe, 6%; OMe-7 to H-4′a, 2%; H-4′a to OMe-7, 5%; OMe-7 to OMe-6, 5%.

(−)-5-METHOXYTHALIDASINE (**11**).—*M/z* 682 $(M)^+$ ($C_{40}H_{46}N_2O_8$) (70), 681 (30), 455 (41), 441 (42), 424 (14), 228 (100), 205 (23); λ max 237 sh, 281 nm (log ε 4.40, 3.67); Δε 0 (300), −6.8 (282), 0 (269), 0 (255), +12 (242), negative tail below 230 nm; δ 2.24 (s, 2-NMe), 2.62 (s, 2′-NMe), 3.35 (s, MeO-7), 3.49 (s, MeO-6′), 3.80 (s, MeO-5 and MeO-6), 3.89 (s, MeO-7′), 3.90 (s, MeO-12), 6.31 (br s, H-10), 6.35 (dd, J_o 8.3 Hz, J_m 2 Hz, H-10′), 6.46 (s, H-8′), 6.54 (dd, J_o 8.3 Hz, J_m 2.5 Hz, H-11′), 6.81 (br s, H-13,14), 6.99 (dd, J_o 8.3 Hz , J_m 2.5 Hz, H-13′), 7.52 (dd, J_o 8.3 Hz, J_m 2 Hz, H-14′).

(−)-5-ACETOXYTHALIDASINE (**12**).—*M/z* 710 $(M)^+$ ($C_{41}H_{46}N_2O_9$) (100), 709 (23), 695 (23), 484 (20), 483 (62), 469 (49), 242 (57); ν max 1710 cm^{-1}; δ2.22 (s, 2-NMe), 2.32 (s, OAc-5), 2.62 (s, 2′-NMe), 3.36 (s, MeO-7), 3.49 (s, MeO-6′), 3.71 (s, MeO-6), 3.88 (s, MeO-7′), 3.90 (s, MeO-12), 6.27 (br s, H-10), 6.35 (dd, J_o 8.2 Hz, J_m 2.5 Hz, H-10′), 6.46 (s, H-8′), 6.52 (dd, J_o 8.2 Hz, J_m 2.5 Hz, H-11′), 6.80 (br s, H-13,14), 6.98 (dd, J_o 8.5 Hz, J_m 2.5 Hz, H-13′), 7.53 (dd, J_o 8.5 Hz, J_m 2.5 Hz, H-14′).

(+)-THALSIVASINE (**13**).—*M/z* 592 $(M)^+$ ($C_{36}H_{36}N_2O_6$) (94), 591 (100), 578 (25), 577 (62), 561 (38), 296 (28), 204 (13), 191 (17), 190 (12); λ max 234 sh, 281, 313 nm (log ε 4.39, 4.05, 3.78); λ max (MeOH+H^+) 239, 285, 308, 354 nm (log ε 4.36, 3.99, 3.69, 3.82); Δε (nm) 0 (320), +14 (280), 0 (271), −9.6 (265), −11.0 (255), −14.4 (245), negative tail below 225 nm; $[\alpha]_D$ +196° (c 0.2, MeOH).

(+)-2′-NORTHALIPHYLLINE (**14**).—*M/z* 594 $(M)^+$ ($C_{36}H_{38}N_2O_6$) (89), 593 (84), 592 (25), 367 (100), 353 (10), 208 (31), 192 (22), 191 (39), 190 (20), 184 (84); λ max 237 sh, 285 nm (log ε 4.16, 3.83);Δε (nm) 0 (300), +7 (285), 0 (270), −1 (250), positive tail below 220 nm; $[\alpha]_D$ +197° (c 0.15, MeOH).

(−)-THALRUGOSIDINE.—Δε (nm) 0 (300), −4.6 (281), 0 (270), 0 (253), +11.5 (240), negative tail below 228 nm.

(−)-THALICTINE (**8**).—Δε 0 (300), +5 (291), −2 (275, 0 (251)), +3.5 (240), negative tail below 230 nm; $[\alpha]_D$ −33° (c 0.2, MeOH).

O-METHYLATION PROCEDURE.—The compound was dissolved in MeOH, and ethereal CH_2N_2 was added. The mixture was allowed to stand overnight.

REDUCTION OF IMINES.— $NaBH_4$ in MeOH was used.

N-METHYLATION.—To the secondary amine in MeOH was added aqueous HCHO. The mixture was stirred for 1 h. $NaBH_4$ was added in small portions, and stirring continued for 1 h.

ACKNOWLEDGMENTS

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