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BISBENZYLISOQUINOLINE ALKALOIDS FROM *COCCULUS PENDULUS**

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Key Word Index *Cocculus pendulus*; Menispermaceae; leaves; bisbenzylisoquinoline alkaloids; (+)-kohatamine; (+)-1,2-dehydrokohatine; (+)-1,2-dehydrokohatamine; (+)-5′-hydroxyapateline; (+)-5′-hydroxytelobine; (+)-1,2-dehydro-2′-nortelobine; (+)-siddiquine; (+)-siddiquamine.

Abstract Leaves of *Cocculus pendulus* have yielded eight new bisbenzylisoquinoline alkaloids, namely (+)-kohatamine, (+)-1,2-dehydrokohatine, (+)-1,2-dehydrokohatamine, (+)-5′-hydroxyapateline, (+)-5′-hydroxytelobine, (+)-1,2-dehydro-2′-nortelobine, (+)-siddiquine and (+)-siddiquamine.

INTRODUCTION

The climbing shrub *Cocculus pendulus* is generously rich in bisbenzylisoquinolines. Alkaloids of this type with three diaryl ether bridges which were originally known to be present in this plant are (+)-cocsuline and (+)-cocsoline [1]. In a study published in 1984, the alkaloidal content of *C. pendulus* which had been collected in northern Pakistan in the vicinity of Peshawar was studied. Two new bisbenzylisoquinolines with three bridges were obtained, namely (+)-kohatine and (+)-kurramine. Known compounds with three bridges which were also isolated are (+)-isotrilobine, (+)-tricordatine, (+)-*N*-methylapateline and (+)-1,2-dehydroapateline [2].

Following the above study, we came by a supply of the plant which had been gathered in southern Pakistan, in the Karachi area. It became of interest, therefore, to compare the alkaloidal content based on geographical origin. As it turned out, the bisbenzylisoquinolines present in the new sample, gathered in the Karachi area, were quite similar to those obtained from the northern part of the country with essentially the same alkaloids present as those listed above. However, in the present instance, since we started with a relatively large amount of plant material, we were able to obtain several new but minor alkaloids. This paper will thus be concerned solely with the novel bisbenzylisoquinolines found, all of which proved to incorporate three diaryl ether bridges as in the aforementioned alkaloids.

RESULTS AND DISCUSSION

Our first new alkaloid is the monophenolic (+)-kohatamine (**1**) $C_{35}H_{34}N_2O_6$, whose spectral characteristics point to a close structural relationship with the accompanying (+)-kohatine (**1a**). The mass spectrum indicates an M_r of 578, which is 14 more than that of kohatine (**1a**). The base peak, *m/z* 351, represents the upper portion of the dimer due to double benzylic cleavage, and in fact corresponds exactly to that encountered in kohatine (**1a**). Therefore it follows that the difference of 14 in M_r must be due to the lower part of the dimer. The NMR spectrum of (+)-kohatamine (**1**) parallels that of (+)-kohatine (**1a**), except for an additional *O*-methyl singlet at δ3.91. This indicates that C-12 bears a methoxyl rather than a phenolic function.

The next two new dimers are (+)-1,2-dehydrokohatine (**2**), $C_{34}H_{30}N_2O_6$, and (+)-1,2-dehydrokohatamine (**3**), $C_{35}H_{32}N_2O_6$. For each of these compounds, the mass spectral $[M]^+$ is intense (~ 75%), while the base peak is the $[M-1]^+$ ion. Significantly, no important fragmentation corresponding to double benzylic cleavage is observed. Such behaviour is typical of bisbenzylisoquinolines with one imine function. The NMR spectra for each of the above two imines include one *N*-methyl singlet at δ2.56. The absorption pattern present in the aromatic region is close to that for kohatine (**1a**) and kohatamine (**1**), except that H-8 falls further downfield, at *ca* δ6.55 instead of *ca* 6.20. Another important feature is the absence of the H-1 aliphatic absorption which is present in the δ3.60 region for kohatine (**1a**) and its 12-*O*-methyl analogue **1**. The main difference between the NMR spectra for **2** on the one hand, and **3** on the other, is the presence of an extra methoxyl singlet at δ3.90 in the spectrum of the latter.

Sodium borohydride treatment of (+)-1,2-dehydrokohatine (**2**) and (+)-1,2-dehydrokohatamine (**3**) resulted in reduction from the less hindered side with formation of (+)-5′-hydroxyapateline (**4**) and (+)-5′-hydroxytelobine (**5**), respectively. These two products are diastereomeric with (+)-kohatine (**1a**) and (+)-kohatamine (**1**), respectively, and possess the *R,S′* rather than the *S,S′* configuration.

Interestingly, both **4** and **5** were also found as new minor alkaloids of *C. pendulus* in the present study. The mass spectra of **4** and **5** are essentially identical with those

*This paper is dedicated to Professor Salimuzzaman Siddiqui on the occasion of his ninetieth birthday.

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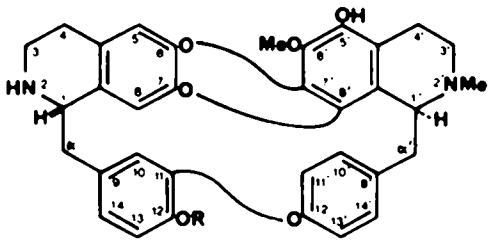

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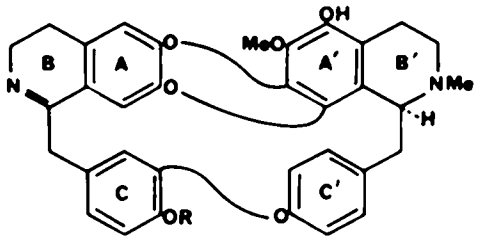
1a, R = H



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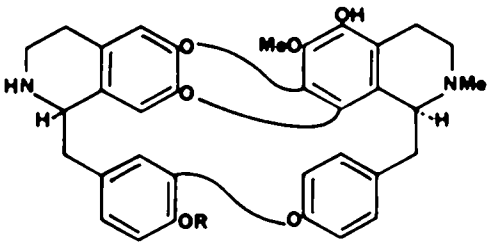

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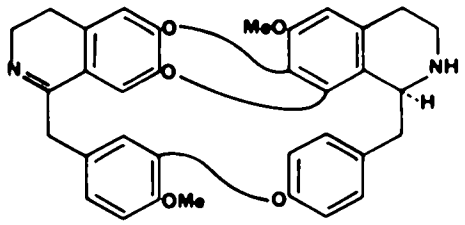
5, R = Me



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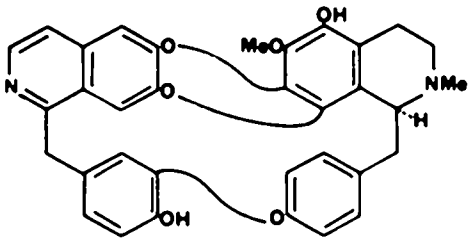

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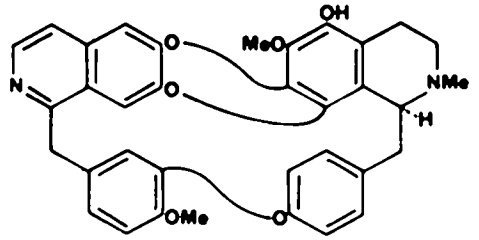

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for kohatine (**1a**) and kohatamine (**1**), respectively, even though diastereomeric compounds are being compared. It is in their NMR spectra that important differences come to the fore. H-10 is found at δ6.33 and 6.34 in dimers **4** and **5**, but the corresponding absorption is at *ca* δ6.60 for kohatine (**1a**) and kohatamine (**1**). Additionally, H-1 which is in evidence at *ca* δ3.65 in the spectra of kohatine and kohatamine, is situated near δ4.02 for **4** and **5**. The NMR spectra of 5'-hydroxyapateline (**4**) and 5'-hydroxytelobine (**5**) are very similar to that of apateline. The spectrum of **4**, however, does not include a singlet near δ6.40 because of substitution at C-5' with a hydroxyl group. The presence of this hydroxyl is also demonstrated by the downfield shift of the 6'-methoxyl of **5** which falls at δ3.96.

Our sixth new alkaloid is (+)-1,2-dehydro-2'-nortelobine (**6**), $C_{34}H_{30}N_2O_5$. The mass spectrum has an intense $[M]^+$ at *m/z* 546 (72%), and a base peak *m/z* 545 due to the $[M-1]^+$ ion. Again, the absence of a strong double benzylic cleavage should be noted, diagnostic of the presence of an imine function. The NMR spectrum of **6** is very similar to that of (+)-1,2-dehydrotelobine, a previously known dimer which we presently have re-isolated from *C. pendulus* [3]. One difference, however, is the absence of an *N*-methyl absorption at δ2.58, while another is the downfield shift of H-1' from δ4.02 in 1,2-dehydrotelobine to 4.43 in **6**. These changes are typical of the replacement of an *N*-methyl group by an NH function.

The remaining two new alkaloids are (+)-siddiquine (**7**), $C_{34}H_{28}N_2O_6$, and (+)-siddiquamine (**8**), $C_{35}H_{30}N_2O_6$. These are alkaloids of the cocsuline series (with three ether bridges at 6–7', 7–8', 11–12'), with a pyridinic ring B. Their mass spectra exhibit strong $[M]^+$ ions (~ 85%), accompanied by base peaks due to the $[M-1]^+$ ions. Doubly charged $[M]^{++}$ ions of *ca* 15% relative intensity were the only other readily identifiable feature of the mass spectra. The NMR spectra of both alkaloids presented the characteristic doublets of a pyridinic system close to δ7.22 and 8.23, with J_o = 5.2 Hz. In accordance with the presence of a pyridinic system, the two α protons were present as two doublets, in the vicinity of δ4.10 and 4.35, J_{gem} = 13 Hz. The remaining features of the two NMR spectra were essentially similar to those for the other compounds in this dimeric series. In particular, the *N*-methyl singlets appeared at δ2.61, which is typical for the 2'-*N*-methyl of cocsuline analogues. It follows that the pyridine system is located on the left hand side of the molecule as drawn. This conclusion is reinforced by the

Table 1. NMR spectral data of alkaloids 1–8*

Alkaloid	2-*N*-Me	2′-*N*-Me	H-1	H-1′	H-5	H-5′	H-8	H-10	H-13	H-14	H-10′	H-11′	H-13′	H-14′	MeO-6′	MeO-12
Kohatamine (1)	NH	δ2.60	3.63	4.02	6.60	OH	6.22	6.62	6.91	6.91	7.12	6.87	7.23	7.53	3.96	3.91
Kohatine (1a)	NH	2.59	3.67	4.00	6.61	OH	6.23	6.59	6.93	6.89	7.11	6.81	7.20	7.54	3.96	OH
5′-Hydroxytelobine (5)	NH	2.60	4.02	4.02	6.44	OH	6.37	6.34	6.82	6.82	6.80	6.64	7.03	7.36	3.96	3.90
5′-Hydroxyapateline (4)	NH	2.57	4.04	4.03	6.44	OH	6.32	6.33	6.84	6.76	6.80	6.70	7.05	7.36	3.96	OH
1,2-Dehydrokohatamine (3)		2.56		4.02	6.59	OH	6.57	6.61	6.84	6.97	6.92	6.74	7.20	7.38	3.94	3.90
1,2-Dehydrokohatine (2)		2.56		4.02	6.59	OH	6.53	6.55	6.84	6.91	6.94	6.72	7.21	7.41	3.95	OH
1,2-Dehydro-2′-nortelobine (6)		NH	-	4.43	6.64	6.41	6.56	6.57	6.85	6.98	6.88	6.77	7.19	7.47	3.89	3.91
Siddiquamine (8)†		2.61		4.10	7.17	OH	7.11	6.74	6.85	7.12	6.97	6.68	7.24	7.46	4.05	3.90
Siddiquine (7)‡		2.61	-	4.10	7.14	OH	7.06	6.70	6.86	7.05	7.00	6.65	7.24	7.46	4.02	OH

*For the compounds in the table, each of the H-10′, 11′, 13′ and 14′ signals is a doublet of doublets, $J_{ae} \simeq 2.2$ Hz and $J_a \simeq 8.2$ Hz. Also, H-10 *d*, $J_a \simeq 1.8$ Hz; H-13 *d*, $J_a \simeq 8.2$ Hz; H-14 *dd*, $J_{ae} \simeq 1.8$ Hz and $J_a \simeq 8.2$ Hz.

†H-3, δ8.24 *d*; H-4, δ7.24 *d*, $J_a = 5.5$ Hz, H-α$_1$, δ4.39 *d*; H-α$_2$, δ4.10 *d*, $J_{gem} = 13$ Hz.

‡H-3, δ8.23 *d*; H-4, δ7.22 *d*, $J_a = 5.6$ Hz, H-α$_1$, δ4.35 *d*; H-α$_2$, δ4.11 *d*, $J_{gem} = 13$ Hz.

downfield shift of H-8 and H-5 which are found at *ca* δ7.10 and 7.15, respectively. The difference between 7 and 8 resides in the substituent at C-12, which is hydroxyl in 7 and methoxyl in 8. Indeed, irradiation of the 12-methoxyl singlet at δ3.88 in 8 led to a 12% enhancement of the aromatic doublet at δ6.85 due to H-13, so that these protons must be proximate.

It should be noted in conclusion that the main bisbenzylisoquinoline alkaloids with three ether bridges of *C. pendulus* such as (+)-cocsuline, and (+)-cocsoline, possess the *S,S′* configuration and they are accompanied in the plant by other analogous *S,S′* dimers such as (+)-kohatamine (1). It is possible that some of these dimers could undergo *in vivo* oxidation at the less hindered C-1 site which is proximate to the unsubstituted C-8 position. Resulting imines such as 1,2-dehydroapateline or 1,2-dehydrokohatine (2) could then either suffer *in vivo* reduction from the less hindered side to produce dimers with the *R,S′* configuration, or alternatively could undergo further oxidation to afford alkaloids with a pyridinic system such as siddiquine (7) and siddiquamine (8).

EXPERIMENTAL

General. NMR spectra are at 360 MHz in $CDCl_3$. The NOE difference spectra were run in $CDCl_3$ after degassing the soln × 3 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 indicated; 18 sec were allowed between scans for nuclear relaxation.

Extraction and alkaloid isolation. Cocculus pendulus (Forsk.) Diels is synonymous with *Epibaterium pendulum, Cebatha pendula* and *Cocculus leaba*. The plant was identified by Prof. S. I. Ali of the Department of Botany, University of Karachi. A voucher specimen is deposited in the Department of Botany, University of Karachi. The leaves were collected in Karachi in April 1982, during the flowering season. Powdered leaves (30 kg) were extracted with cold EtOH (100 l.). The dried EtOH extracts were treated with 5% HCl, the acidic soln filtered and basified with NH_4OH. The aq. soln was extracted first with $CHCl_3$ and then with EtOAc. The organic extracts were combined, dried and the solvent evapd to afford the crude alkaloids (≃ 100 g). This material was placed on a silica gel (type 60, 70–230 mesh ASTM) column and eluted with $CHCl_3$ containing increasing percentages of MeOH. Final purification was achieved by TLC on silica gel. The major alkaloid was (+)-kohatine (1a), accompanied by its facile oxidation product (+)-1,2-dehydrokohatine (2). Minor alkaloids were (+)-kohatamine (1), (+)-1,2-dehydrokohatamine (3), (+)-5′-hydroxyapateline (4), (+)-5′-hydroxytelobine (5), (+)-1,2-dehydro-2′-nortelobine (6), (+)-siddiquine (7) and (+)-siddiquamine (8).

(+)-Kohatamine (1). EIMS *m/z* 578 ([M]$^+$, 58), 577 (48), 365 (17), 351 (100), 350 (22), 335 (24), 321 (13), 176 (24), 168 (11); UV λ_{max}^{MeOH} 237 sh, 267 sh nm (log ε 4.30, 3.98); $[\alpha]_D^{25}$ +99° (0.26, $CHCl_3$).

(+)-1,2-Dehydrokohatine (2). EIMS *m/z* 562 ([M]$^+$, 75), 561 (100), 547 (6), 545 (6), 281 (11); UV λ_{max}^{MeOH} 235 sh, 259, 287 sh, 347 nm (log ε 4.45, 4.29, 3.85, 3.47); $[\alpha]_D^{25}$ +53° (0.08, $CHCl_3$). A small sample was reduced with $NaBH_4$ in MeOH at room temp. Work-up afforded (+)-5′-hydroxyapateline (4).

(+)-1,2-Dehydrokohatamine (3). EIMS *m/z* 576 ([M]$^+$, 85), 575 (100), 561 (7), 559 (7), 288 (5); UV λ_{max}^{MeOH} 236, 274, 283, 305 nm (log ε 4.55, 4.14, 4.13, 4.08); $[\alpha]_D^{25}$ +100° (0.09, $CHCl_3$). A

small sample was reduced with $NaBH_4$ in MeOH at room temp. Work-up afforded (+)-5'-hydroxytelobine (5).

(+)-*5'-Hydroxyapateline* (4). $C_{34}H_{32}N_2O_6$; EIMS and UV spectra essentially identical to those for (+)-kohatine [1]; $[\alpha]_D^{25}$ + 185° (0.07, MeOH).

(+)-*5'-Hydroxytelobine* (5). $C_{35}H_{34}N_2O_6$; EIMS and UV spectra essentially identical with those for (+)-kohatamine (1); $[\alpha]_D^{25}$ + 154° (0.11, $CHCl_3$).

(+)-*1,2-Dehydro-2'-nortelobine* (6). EIMS *m/z* 546 ([M]$^+$, 73), 545 (100), 332 (2), 273 (16); UV λ_{max}^{MeOH} 229, 265 sh, 294 sh, 335 nm (log ε 4.45, 4.06, 3.73, 3.51); $[\alpha]_D^{25}$ + 100° (0.15, $CHCl_3$).

(+)-*Siddiquine* (7). EIMS *m/z* 560 ([M]$^+$, 79), 559 (100), 545 (10), 530 (18), 280 (13); UV λ_{max}^{MeOH} 232, 267 sh, 355 nm (log ε 4.70, 4.39, 3.68); $[\alpha]_D^{25}$ + 172° (0.12, $CHCl_3$); NMR NOE at 360 MHz in $CDCl_3$ H-4 to H-5 (11%), H-8 to H-α_b (6%), H-α_b to H-8 (12%), H-α_a to H-14 (4%), H-8 to H-10 (9%), H-10 to H-8 (12%), 2'-NMe to H-1' (12%), 2'-NMe to H-3'b (6%), H-10' to H-1' (8%).

(+)-*Siddiquamine* (8). EIMS 574 ([M]$^+$, 86), 573 (100), 55 (10), 544 (5), 287 (17), 279 (10); UV λ_{max}^{MeOH} 233, 267 sh, 355 nm (log ε 4.63, 4.32, 3.63); $[\alpha]_D^{25}$ + 113° (0.08, $CHCl_3$); NMR NOE at 360 MHz in $CDCl_3$ 12-OMe to H-13 (12%), H-13 to 12-OMe (9%).

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REFERENCES

1. Bhakuni, D. S. and Joshi, P. P. (1975) *Tetrahedron* **31**, 2575; Ikram, M., Shafi, N. and Abu Zarga, M. H. (1982) *Planta Med.* **45**, 253.
2. Hussain, S. F., Khan, L., Guinaudeau, H., Leet, J. E., Freyer, A. J. and Shamma, M. (1984) *Tetrahedron* **40**, 2513.
3. Schiff, P. L., Jr. (1983) *J. Nat. Prod.* **46**, 1; and references cited therein on bisbenzylisoquinoline alkaloids.