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THE ALKALOIDAL PROFILE OF *COCCULUS PENDULUS*

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Abstract—(+)-Kohatine (**5**), (+)-kurramine (**7**), (+)-norpenduline (**11**), and (+)-cheratamine (**12**), are four new bisbenzylisoquinolines obtained from *Cocculus pendulus* (Forsk) Diels (Menispermaceae). Ten known bisbenzylisoquinolines are also present. All fourteen alkaloids possess the *S* chirality at C-1′. The biogenetic sequence involves initial condensation of two coclaurine-type units to form a dimer such as **16**. Species **16** may in turn lead to dimers **17** or **18** with two diaryl ether linkages. Only dimer **17** may undergo further oxidative coupling to bisbenzylisoquinolines with three diaryl ether bridges.

Cocculus pendulus (Forsk) Diels (Menispermaceae) is a climbing shrub growing along rocks in the dry, mountainous areas of northern Pakistan. The roots are sometimes used in the treatment of intermittent fevers and as a tonic. Some hypotensive and anticancer activity has also been asssociated with the alkaloidal fractions of the leaves and stems. A few bisbenzylisoquinolines have been reported to be present in the plant, including (+)-cocsuline (**1**), (+)-cocsoline (**2**), (+)-penduline (**10**) and (+)-cocsulinine (**15**).[1]

We became interested in *C. pendulus* when preliminary TLC we carried on some of the plant extracts indicated that more alkaloids were present than originally reported. Eight kilograms of the dried stems were, therefore, collected near Kohat. This material was powdered and extracted with cold ethanol. The alkaloidal portion was fractionated first over an alumina column, and then by TLC or column chromatography on silica gel. Fourteen bisbenzylisoquinolines were thus separated and characterized, of which four proved to be new.

The initial group of alkaloids we studied belonged to the (+)-cocsuline (**1**) and (+)-cocsoline (**2**) series. Besides these two bases, it was possible to separate and characterize the known alkaloids (+)-isotrilobine (**3**) and (+)-tricordatine (**4**).[2]

The first new alkaloid encountered was the diphenolic (+)-kohatine (**5**), whose spectral characteristics pointed to a close relationship with the accompanying (+)-cocsoline (**2**). The mass spectrum indicated a molecular weight of 564, meaning that kohatine has one oxygen more than (+)-cocsoline (**2**). The base peak, *m/z* 351, representing the upper portion of the dimer (rings A, B and A′, B′), and resulting from double benzylic cleavage, was again 16 mass units higher than the corresponding peak for (+)-cocsoline. The extra oxygen was, therefore, located in the upper half of the molecule.

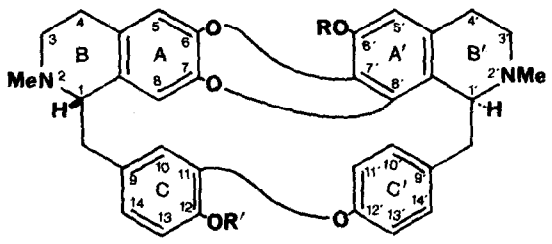
The NMR spectrum of (+)-kohatine (**5**) bears distinct similarities to that for (+)-cocsoline (**2**), and the respective chemical shifts have been summarized around expressions **5** and **2**. An immediately noticeable difference, however, was the aromatic H-5′ singlet absorption at δ 6.33 in the latter spectrum, which was absent in the former. The conclusion was that one of the two phenolic functions of kohatine (**5**) was located at C-5′, while the other had to be situated at the usual C-12 site as in species **1**, **2** and **4**.

Consonant with the placement of a phenolic function at C-5′ for (+)-kohatine (**5**) was the fact that an NOEDS (NOE difference study)[3] of the alkaloid revealed that irradiation of the C-6′ OMe singlet absorption at δ 3.96 had no effect whatever on the aliphatic protons. Additionally, the relatively downfield chemical shift (δ 3.96) of the C-6′ OMe found analogy with the monomeric aporphines possessing a related substitution pattern, i.e. those bearing a OMe at C-2 of the aporphine system, and a OH at C-3, as in 3-hydroxynuciferine where the OMe absorption in question falls at δ 3.98.[4]

Additional useful information could be derived from NOEDS. Irradiation of the H-10 aromatic doublet absorption at δ 6.59 led to a 7.5% NOE of the H-8 singlet (δ 6.23). Alternatively, irradiation of the H-1′ broad doublet at δ 4.00 produced a 3% NOE of the N-Me singlet (δ 2.59), as well as a 3% NOE of the δ 7.54 aromatic doublet of doublets representing H-14′. It follows that the N-Me group is situated on the r.h.s. of the molecule as drawn, rather than on the left.

Kohatine (**5**) incorporates the 1*S*–1′*S* absolute configuration since it is dextrorotatory like cocsuline (**1**), and its CD curve is close to that of **1**.

It is apparently possible for a dimer such as cocsoline (**2**) to suffer oxidation at C-1 since we have presently determined that the known imine (+)-1,2-dehydroapateline (**6**) (≡(+)-1,2-dehydrococsoline) is also present in the alkaloidal pool. Alternatively, it is possible that **6** is formed from C-1 oxidation of cocsuline (**1**) followed by N-demethylation. Analogous oxidation of (+)-tricordatine (**4**)



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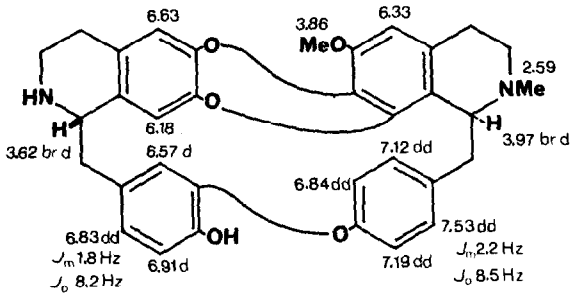

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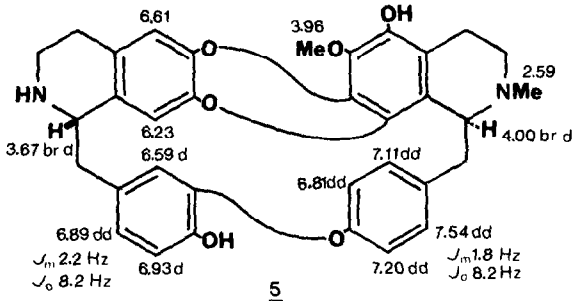

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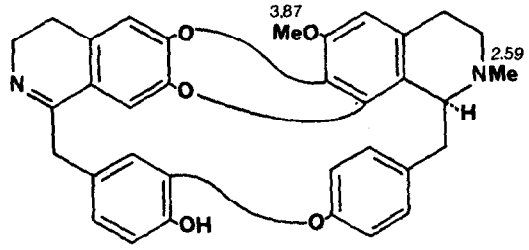

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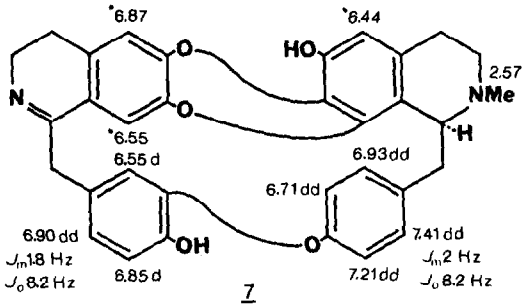

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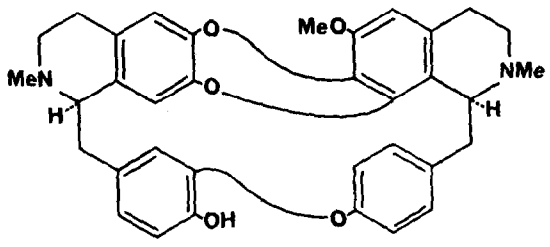

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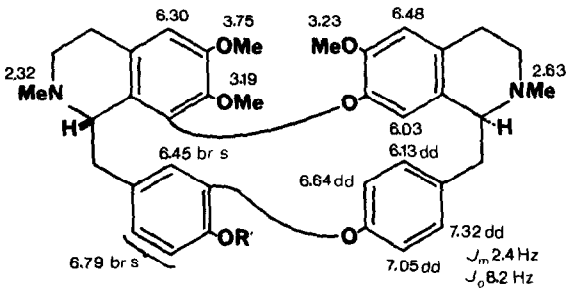
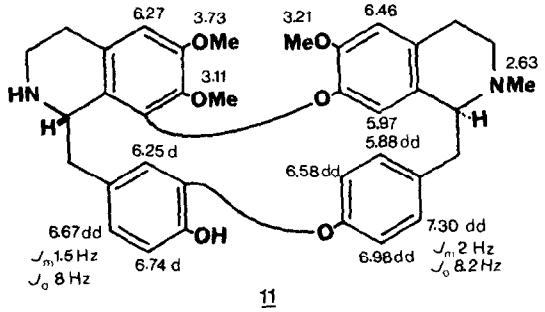


9 R = Me
10 R = H

NMR values are for 10



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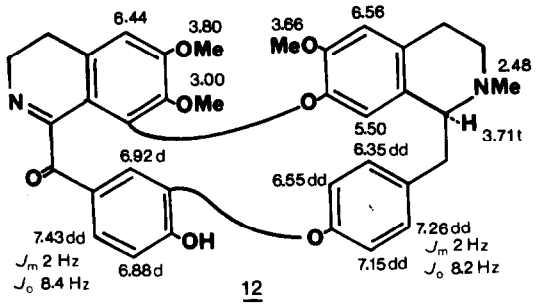
may explain the formation of our second new bisbenzylisoquinoline, the imine (+)-kurramine (7).

The mass spectrum of 7 displays a strong molecular ion m/z 532, and a base peak m/z 531. All the other peaks are minor and the alkaloid does not tend to cleave into two moieties upon electron impact.

The NMR spectrum of kurramine is summarized around expression 7, and is close to that of dehydroapateline (6). The main difference is that since kurramine is diphenolic, its spectrum lacks the OMe singlet at δ 3.87 present in the spectrum of 6. The N-Me singlet absorption for kurramine (7) falls at δ 2.57, close to the corresponding signal for kohatine (5) which is at δ 2.59.

The absolute configuration of kurramine (7) at C-1′ is forthcoming from the similarity of the CD curve of this imine with that of (+)-1,2-dehydroapateline (6) of known (1′*S*) configuration.

Although no formal proof exists, it is nevertheless a convenient working hypothesis to assume that the



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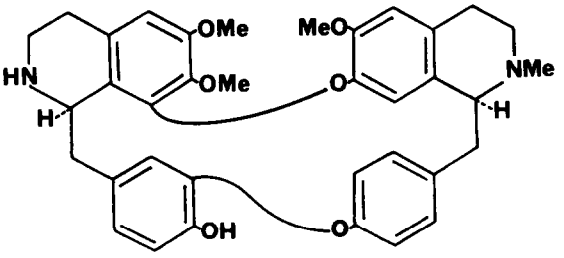

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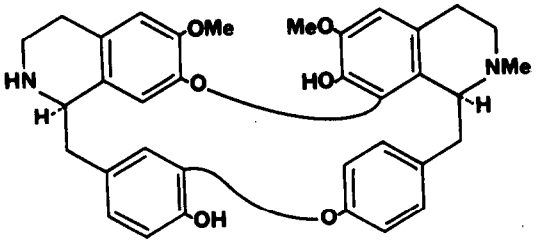

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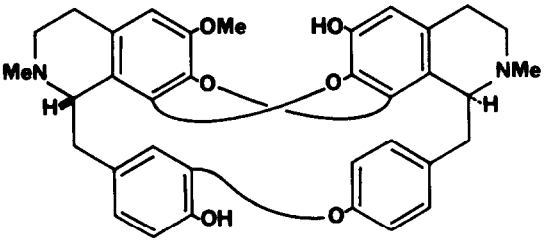

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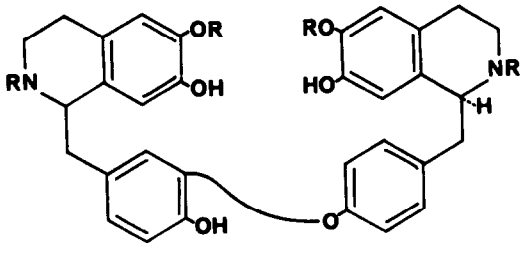

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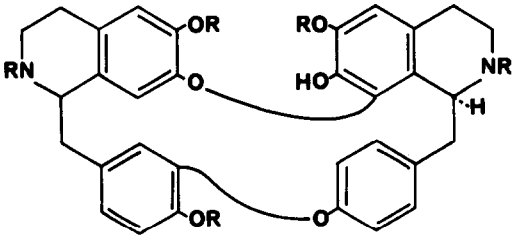

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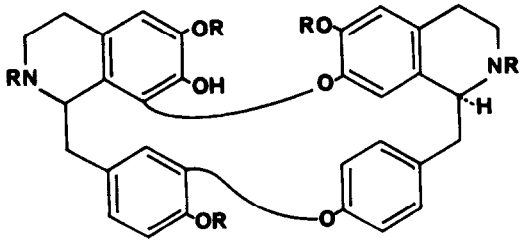

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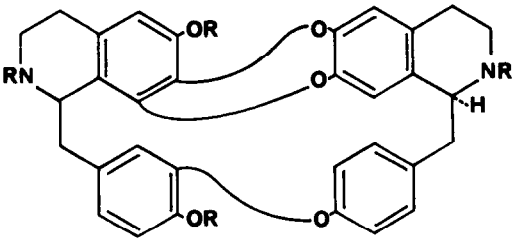

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imine function of 1,2-dehydroapateline (**6**) or of kurramine (**7**) (or alternatively the corresponding N-metho iminium salts) can undergo *in vivo* reduction to provide dimers of the 1*R*–1′*S* configuration as exemplified by the known alkaloid (+)-N-methylapateline (**8**) which we have now found to be present in *C. pendulus*. Significantly, of the eight dimers with three diaryl ether bridges characterized above, six possess the 1*S*–1′*S* configuration, two are imines of the 1′*S* configuration, and only one incorporates the 1*R*–1′*S* stereochemical arrangement.

The generality of the 1′*S* chirality for the bisbenzylisoquinolines of *C. pendulus* extends itself to the next group of alkaloids characterized and comprised of (+)-tetrandrine (**9**), (+)-penduline (**10**), and (+)-norpenduline (**11**). The last named compound is the third new alkaloid reported in the present study. These three dimers incorporate only two diaryl linkages, and all three possess the 1*S*–1′*S* stereochemistry and have related CD curves.

The NMR chemical shift assignments for (+)-penduline (**10**), displayed around its structural formula, was confirmed by NOEDS. (+)-Norpenduline (**11**) has an NMR spectrum very close to that of (+)-penduline (**10**), the main difference being the absence of the N-Me singlet near δ 2.32. In

accord with the structural assignment, the mass spectrum of norpenduline (**11**) includes molecular ion *m/z* 594 and base peak 381, whereas penduline has molecular ion *m/z* 608 and base peak 395.

As with bisbenzylisoquinolines possessing three diaryl ether bridges, oxidation at C-1 is possible in dimers with only two ether bridges. (+)-Norpenduline (**11**) could thus act as biogenetic precursor to the fourth of our new alkaloids, the monophenolic (+)-cheratamine (**12**) which possesses an imino ketone moiety.

The IR spectrum of (+)-cheratamine (**12**) displays a conjugated ketone absorption at 1675 cm^{-1}, as well as an imine at 1600 cm^{-1}. The UV spectrum is somewhat unusual in that a bathochromic shift was recorded in basic as well as in acidic solution. The shift in base was pronounced, from λ_{max} 289 nm in neutral solution to 349 nm, suggesting a *para* relationship between the ketonic function and the phenol.[5] The bathochromic shift in acid, on the other hand, is due to nitrogen protonation at the conjugated imine grouping.

The mass spectrum of cheratamine (**12**) shows an intense *m/z* 606 molecular ion, as well as an *m/z* 605 base peak. Another significant ion is *m/z* 379 representing the top portion of the dimer resulting from double benzylic cleavage.

The chemical shift assignments for the NMR spectrum of cheratamine are presented around expression **12**. These assignments were confirmed by NOEDS. Irradiation of H-5′ (δ 6.56) produced a 15% enhancement of the C-6′ OMe signal at δ 3.66. In turn, irradiation at δ 3.66 resulted in a 15% increase of H-5′. Additionally, irradiation of H-5 (δ 6.44) produced a 9% enhancement of the C-6 OMe signal at δ 3.80, and irradiation at δ 3.80 resulted in a 13% increase of the H-5 signal.

The absolute configuration of cheratamine (**12**) can be derived from its positive rotation, from the similarity of its structure with that of the accompanying (+)1,2-dehydroapateline (**6**), and on biogenetic grounds since all bisbenzylisoquinolines from *C. pendulus* incorporate within their right hand moieties N-methylcoclaurine units of the *S* configuration.

Finally, two additional known bisbenzylisoquinolines were found in the extracts. These are (+)-norberbamine (**13**) which is related to (+)-cheratamine (**12**), and (+)-daphnoline (**14**) which in the present context stands structurally by itself, since it is an analog of (+)-oxyacanthine.

With the present determination of the alkaloidal landscape of *C. pendulus*, a few remarks are in order concerning the biogenetic pathways followed in this plant. Two coclaurine or N-methylcoclaurine units may be subjected to phenolic oxidative coupling to furnish a dimer of type **16**. This dimer can undergo additional head to head bonding to provide bisbenzylisoquinolines **17** or **18**.

The sole alkaloid of type **17** found in *C. pendulus* is (+)-daphnoline (**14**). Its isolation, however, is of more than passing interest since it is dimers of type **17** that can undergo further and facile coupling *in vivo* to generate dimers with three diaryl bridges, *viz* (+)-cocsuline (**1**), (+)-cocsoline (**2**), (+)-isotrilobine (**3**), (+)-tricordatine (**4**), (+)-kohatine (**5**), (+)-1,2-dehydroapateline (**6**), (+)-kurramine (**7**), and (+)-N-methylapateline (**8**).

In contradistinction, additional head to head coupling in alternate dimer **18** is precluded, probably because, and as shown by models, the molecule exists in a preferred conformation in which rings A and A′ are appreciably distant from each other. The net result is that alkaloids of type **19** are not formed, and are in fact unknown in the plant kingdom; while several dimers of type **18** have been isolated, e.g. (+)-tetrandrine (**9**), (+)-penduline (**10**), (+)-norpenduline (**11**), (+)-cheratamine (**12**) and (+)-norberbamine (**13**).

The detailed study of the alkaloidal profile of *C. pendulus* has thus supplied us with an insight into the biogenetic processes available in that plant.

EXPERIMENTAL

General procedures. All NMR spectra were recorded on a Bruker 360 MHz spectrometer, in $CDCl_3$ soln and with TMS as internal standard. The numerical values quoted for the NMR NOEDS represent the apparent NOE percentages actually observed.

Isolation. Eight kg of the dried stems were extracted with cold EtOH. The dried ethanolic extract was treated with 5% HCl. The acid soln was basified with NH_4OH, and the mixture extracted with $CHCl_3$ to give about 20 g (dry weight) of crude alkaloids. Initial separation was by column chromatography on alumina using a $CHCl_3$–MeOH gradient. The cuts were consolidated into five fractions: First fraction: non-alkaloidal. Second fraction: cocsuline (**1**), tetrandrine (**9**), 1,2-dehydroapateline (**6**), and isotrilobine (**3**). Third fraction: cocsuline (**1**), penduline (**10**), cheratamine (**12**), and N-methylapateline (**8**). Fourth fraction: cocsoline (**2**), tricordatine (**4**), kurramine (**7**), kohatine (**5**), norberbamine (**13**), and norpenduline (**11**). Fifth fraction: daphnoline (**14**).

Following further purification by column and TLC on silica gel, the weights obtained were: (**1**) 1.2 g; this is the main alkaloid in the plant; (**2**) 30 mg; (**3**) 10 mg; (**4**) 3 mg; (**5**) 9 mg; (**6**) 5 mg; (**7**) 9 mg; (**8**) 9 mg; (**9**) 17 mg; (**10**) 78 mg; (**11**) 15 mg; (**12**) 8 mg; (**13**) 10 mg; (**14**) 3 mg. None of these weights can be considered as absolute since substantial quantities of alkaloids were lost, of necessity, during the chromatographic processes.

Superior TLC chromatographic solvent combinations were: $CHCl_3$–MeOH–NH_4OH 90:10:1 v/v, and $CHCl_3$–$(Et)_2NH$ 90:10 v/v.

(+)-*Cocsuline* (**1**). $C_{35}H_{34}N_2O_5$. CD $\Delta\epsilon_{nm}$ (MeOH) 0_{320}, $+7.3_{292}$, $+2_{250}$, $+27_{232}$, 0_{214}, negative tail.[6]

(+)-*Cocsoline* (**2**). $C_{34}H_{32}N_2O_5$. MS *m/z* 548 $(M)^+$ (57), 547 (57), 349 (4), 335 (100), 334 (27), 321 (26), 305 (13), 168 (61), 107 (4). CD $\Delta\epsilon_{nm}$ (MeOH) 0_{315}, $+9_{290}$, $+1_{252}$, 0_{251}, $+32_{229}$, 0_{215}, negative tail.

(+)-*Isotrilobine* (**3**). $C_{36}H_{36}N_2O_5$. MS *m/z* 576 $(M)^+$ (22), 575 (15), 349 (100), 335 (44), 319 (7), 175 (59). CD $\Delta\epsilon_{nm}$ (MeOH) 0_{320}, $+8_{290}$, $+2_{251}$, $+31_{234}$, 0_{213}, negative tail. UV λ_{max} (MeOH) 234, 287 nm ($\log\epsilon$ 4.56, 3.65).

(+)-*Tricordatine* (**4**): $C_{34}H_{32}N_2O_5$. CD $\Delta\epsilon_{nm}$ (MeOH) 0_{310}, $+2.5_{290}$, $+2.3$ sh_{270}, 0_{255}, $+9_{234}$, 0_{218}, negative tail.

(+)-*Kohatine* (**5**). $C_{34}H_{32}N_2O_6$. MS *m/z* 564 $(M)^+$ (49), 365 (30), 352 (39), 351 (100), 337 (32), 321 (15), 214 (8), 176 (39), 168 (29), 107 (7). CD $\Delta\epsilon_{nm}$ (MeOH) O_{323}, $+8_{284}$, 0_{251}, $+30_{234}$, 0_{215}, negative tail. $[\alpha]_D^{25}+183°$ (0.2, MeOH). UV λ_{max} (MeOH) 233, 288 nm ($\log\epsilon$ 4.59, 3.77).

(+)-1,2-*Dehydroapateline* (**6**). $C_{34}H_{30}N_2O_5$. MS *m/z* 546 $(M)^+$ (70), 545 (100), 349 (1.6), 335 (2), 333 (8), 273 (9). CD $\Delta\epsilon_{nm}$ (MeOH) 0_{350}, $+4_{305}$, $+1.6_{290}$, $+7_{272}$, 0_{263}, -2_{260}, 0_{255}, $+38_{225}$, 0_{217}, negative tail.

(+)-*Kurramine* (**7**). $C_{33}H_{28}N_2O_5$. MS *m/z* 532 $(M)^+$ (64), 531 (100), 327 (5), 326 (7), 319 (13), 107 (16). CD $\Delta\epsilon_{nm}$ (MeOH) 0_{325}, $+6_{305}$, $+2_{291}$, $+9_{272}$, 0_{265}, -2.4_{258}, 0_{255}, $+47_{226}$, 0_{215}, negative tail. $[\alpha]_D^{25}+83°$ (0.13, MeOH). UV λ_{max} (MeOH) 224 sh, 261 sh, 290, 338 nm ($\log\epsilon$ 4.71, 4.25,

3.86, 3.77); λ_{max} (MeOH–H^+) 222 sh, 263 sh, 329, 389 nm (log ϵ 4.67, 4.26, 3.80, 3.85).

(+)-*N-Methylapateline* (**8**). $C_{35}H_{34}N_2O_5$. CD $\Delta\epsilon_{nm}$ (MeOH) 0_{320}, $+9_{272}$, 0_{250}, -2_{245}, 0_{243}, $+51_{228}$, 0_{210}, negative tail.

(+)-*Tetrandrine* (**9**). $C_{38}H_{42}N_2O_6$. CD $\Delta\epsilon_{nm}$ (MeOH) 0_{310}, $+5.7_{280}$, 0_{253}, -5_{245}, 0_{242}, $+70_{223}$, 0_{210}.

(+)-*Penduline* (**10**). $C_{37}H_{40}N_2O_6$. MS *m/z* 608 $(M)^+$ (100), 607 (76), 416 (11), 396 (29), 395 (96), 381 (49), 364 (10), 349 (6), 198 (92), 175 (26), 174 (52). CD $\Delta\epsilon_{nm}$ (MeOH) 0_{310}, $+5_{285}$, 0_{251}, -4_{246}, 0_{242}, $+54_{225}$, 0_{209}, negative tail.

(+)-*Norpenduline* (**11**). $C_{36}H_{38}N_2O_6$. MS *m/z* 594 $(M)^+$ (35), 381 (100), 367 (11), 351 (13), 335 (7), 191 (57), 174 (15). CD $\Delta\epsilon_{nm}$ (MeOH) $+3_{282}$, 0_{257}, -11_{244}, 0_{238}, $+20_{229}$, 0_{222}, -24_{212}. $[\alpha]_D^{25}+260°$ (0.09, MeOH). UV λ_{max} (MeOH) 239 sh, 283, 293 sh, 311 nm (log ϵ 4.36, 3.95, 3.83, 3.44).

(+)-*Cheratamine* (**12**). $C_{36}H_{34}N_2O_7$. MS *m/z* 606 $(M)^+$ (73), 605 (100), 589 (4), 379 (8), 363 (2), 347 (3.5), 333 (2), 190 (9), 174 (7). CD $\Delta\epsilon_{nm}$ (MeOH) 0_{365}, $+2_{335}$, 0_{317}, -2_{303}, 0_{290}, $+1_{284}$, $+2_{260}$, $+20_{232}$, 0_{223}, -13_{216}, positive tail. $[\alpha]_D^{25}+190°$ (0.33, MeOH). UV λ_{max} (MeOH) 227, 289 nm (log ϵ 4.28, 3.99); λ_{max} (MeOH + OH^-) 288, 349 nm (log ϵ 3.95, 3.91); λ_{max} (MeOH + H^+) 287, 337 nm (log ϵ 3.87, 3.95). IR ν_{max} ($CHCl_3$) 1675, 1600 cm^{-1}.

(+)-*Norberbamine* (**13**). $C_{36}H_{38}N_2O_6$. MS *m/z* 594 $(M)^+$ (41), 593 (42), 382 (21), 381 (100), 367 (14), 192 (17), 191 (68), 190 (21), 174 (22), 168 (18). CD $\Delta\epsilon_{nm}$ (MeOH) 0_{302}, $+4_{284}$, 0_{275}, $-1.5\,sh_{258}$, -14_{246}, 0_{240}, $+27_{238}$, 0_{220}, -5_{215}, positive tail.

(+)-*Daphnoline* (**14**). $C_{35}H_{36}N_2O_6$. MS *m/z* 581 $(M+1)^+$ (10), 579 (100), 473 (3), 368 (20), 367 (32), 353 (19), 335 (3), 192 (16), 184 (17), 162 (5). $[\alpha]_D^{25}+273°$ (0.15, $CHCl_3$).

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[2]For complete listings of the bisbenzylisoquinolines, see K. P. Guha, B. Mukherjee and R. Mukherjee, *J. Nat. Prod.* **42**, 1 (1979); P. L. Schiff, Jr., *Ibid.* **46**, 1 (1983).

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[6]Known compounds were identified spectrally or by comparison with authentic samples. The spectral data quoted here for the known compounds are those not readily available, especially in Ref. 2 above.