# (+)-Angchibangkine, a New Type of Bisbenzylisoquinoline Alkaloid, and Other Dimers from *Pachygone dasycarpa*

Hélène Guinaudeau,*[,†,‡] Mark Böhlke,[†] Long-Ze Lin,[‡,§] Cindy K. Angerhofer,[†] Geoffrey A. Cordell,[†] and Nijsiri Ruangrungsi[||]

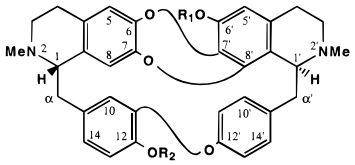
*Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, and Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand*

*Received July 26, 1996*[⊗]

Analysis of the alkaloidal fraction of the stem bark extract of *Pachygone dasycarpa* (Menispermaceae) resulted in the isolation of 10 known bisbenzylisoquinolines, (+)-tetrandrine, (+)-penduline, (+)-fangchinoline, (+)-atherospermoline, (+)-*N*-methyl-7-*O*-demethylpeinamine, (+)-daphnoline, (+)-isotrilobine (**1**), (+)-cocsuline (**2**), (+)-tricordatine (**3**), (+)-2′-norcocsuline, and the new alkaloid (+)-12-*O*-methyltricordatine (**4**). The last bisbenzylisoquinoline alkaloid isolated, (+)-angchibangkine (**5**), is the first member of this alkaloid class found to possess three diphenyl ether bridges in the 7−6′, 8−7′, and 11−12′ positions. Structure elucidation of these alkaloids and of (+)-*O*-methylangchibangkine (**6**) was achieved by analysis of spectral data. Compounds **4**−**6** show antiplasmodial activity against *Plasmodium falciparum*.

Bisbenzylisoquinoline alkaloids continue to show interesting antiplasmodial activity.[1,2] In the course of a systematic study of this group of alkaloids and their antiplasmodial activity, we became interested in *Pachygone* species. Earlier studies of *P. ovata*[3−5] and *P. loyaltiensis*[6] have led to the isolation of several bisbenzylisoquinolines with two or three diphenyl ether bridges. *Pachygone dasycarpa* Kurz (Menispermaceae), which grows in Thailand, has not been studied previously. *P. dasycarpa* is a woody climber, known locally in Thai as "pok phaai" or "Yaa naang channg". The stem is used as a diuretic for nephritis, as an antipyretic, and as a treatment for edema.[7]

The alkaloid extract of the stem bark of *P. dasycarpa* displayed antiplasmodial activity, which suggested that bisbenzylisoquinoline alkaloids might be constituents of this fraction. A study of the alkaloid content has resulted in the isolation of 12 bisbenzylisoquinolines. Among them, five belong to the tetrandrine group, with diphenyl ether linkages at the 8−7′ and 11−12′ positions. (+)-Tetrandrine, (+)-penduline, (+)-fangchinoline, and (+)-atherospermoline have the 1*S*,1′*S* configuration, while (+)-*N*-methyl-7-*O*-demethylpeinamine possesses the 1*S*,1′*R* configuration.[8−11] One of the isolated dimers was (+)-daphnoline with two diphenyl ether bridges (7−8′/11−12′), possessing the 1*R*,1′*S* absolute configuration.[8−11] Five of the bisbenzylisoquinolines contained three diphenyl ether bridges (7−8′/6−7′/11−12′). (+)-Isotrilobine (**1**), (+)-cocsuline (**2**), (+)-tricordatine (**3**), and (+)-2′-norcocsuline have the 1*S*,1′*S* configuration.[8−11] These alkaloids were identified by comparison of their spectral data with those of authentic samples. The last bisbenzylisoquinoline member of this subgroup is the new alkaloid (+)-12-*O*-methyltricordatine (**4**).




**1** $R_1$ = Me, $R_2$ = Me
**2** $R_1$ = Me, $R_2$ = H
**3** $R_1$ = H, $R_2$ = H
**4** $R_1$ = H, $R_2$ = Me

The $^1$H-NMR spectrum of alkaloid **4** is very similar to that given by (+)-isotrilobine (**1**) (see Table 1). The main difference is the absence of a three-proton singlet around 3.85 ppm due to the methoxyl group at C-7′ in the spectrum of (+)-isotrilobine (**1**). The mass spectrum of **4** presents a molecular ion at *m*/*z* 562 ($C_{35}H_{34}O_5N_2$), smaller by 14 Da than the molecular ion given by (+)-isotrilobine (**1**). This difference is also reflected in the base peak at *m*/*z* 335, due to the upper part of the molecule after the facile cleavage of the two benzylic bonds. The difference between **4** and **1** must therefore be the presence of a hydroxyl group at C-6′ in **4** instead of a methoxyl group, as in (+)-isotrilobine (**1**). This hypothesis was further supported by 2D-NMR experiments, in particular by the ROESY data, showing that the proton at C-13 ($\delta$ 6.89 ppm) correlates with the three-proton singlet attributed to 12-OMe ($\delta$ 3.98 ppm). The optical rotation of **4** is positive as in (+)-isotrilobine (**1**), and both alkaloids present very similar CD curves.[9] Therefore, the absolute configuration of (+)-12-*O*-methyltricordatine (**4**) is 1*S*,1′*S*.

(+)-Angchibangkine (**5**) belongs to a new subgroup of bisbenzylisoquinoline alkaloids containing three diaryl ether linkages. The mass spectrum of **5** displayed an abundant molecular ion at *m*/*z* 562 (94%) corresponding to the formula $C_{35}H_{34}O_5N_2$. This molecular ion is similar to the molecular ion of (+)-cocsuline (**2**), although more abundant, and the base peak is at *m*/*z* 349, identical to the base peak observed in the mass spectrum of **2**. These results suggested that the type

* To whom correspondence should be addressed. Phone: 33 (0) 2 41 48 66 63. Fax: 33 (0) 2 41 22 67 33. E-mail: helene.guinaudeau@univ.angers.fr.
† University of Illinois at Chicago.
‡ Alternative address: Faculty of Pharmacy, 16 Boulevard Daviers, 49045 Angers Cedex, France.
§ Present address: East Earth Herb, Inc., 4091 W. 11th Ave., Eugene, OR 97402.
|| Chulalongkorn University.
⊗ Abstract published in *Advance ACS Abstracts,* March 1, 1997.

S0163-3864(96)00568-X CCC: $14.00 

**Table 1.** $^1$H-NMR Spectral Data of Bisbenzylisoquinoline Alkaloids **1**, **2**, **4**, and **5**[a]

| | (+)-isotrilobine (**1**) | (+)-12-*O*-methyltricordatine (**4**) | (+)-cocsuline (**2**) | (+)-angchibangkine (**5**) | (+)-*O*-methylangchibangkine (**6**) |
|---|---|---|---|---|---|
| 1 | 3.24 br s | 3.13 m | 3.30 br s | 3.48 br d (10.4) | 3.47 br d (10.3) |
| 1′ | 4.04 br s | 4.06 br s | 4.02 dd | 3.68 d (11.4) | 3.70 d (10.4) |
| α | | 2.91 m | 2.98 m | 2.66 t (11.5) | 2.68 m |
| | | 2.53 dd | 2.76 dd | 2.52 dd (10.5, 13.3) | 2.52 dd (10.3, 12.5) |
| α′ | | 3.36 br d | 3.36 dd | 3.54 d (12.7) | 3.58 d (13.9) |
| | | 2.69 dd | 2.69 dd | 2.67 t (11.5) | 2.68 m |
| 5 | 6.62 | 6.54 | 6.62 | 6.28 | 6.31 |
| 5′ | 6.32 | 6.36 | 6.33 | 6.68 | 6.71 |
| 8 | 6.13 | 6.18 | 6.15 | | |
| 8′ | | | | 5.33 | 5.44 |
| 10 | 6.58 s | 6.59 s | 6.53 d | 6.54 d (1.7) | 6.60 d (1.8) |
| 13 | 6.87 s | 6.89 s | 6.90 d | 6.88 d (8.1) | 6.91 d (8.2) |
| 14 | 6.87 s | 6.89 s | 6.92 dd | 6.76 dd (8.1, 1.7) | 6.82 dd (8.1, 1.8) |
| 10′ | 7.17 dd | 7.15 dd | 7.17 dd | 7.15 dd (8.1, 2.1) | 7.17 br d (8.1) |
| 11′ | 7.00 dd | 6.98 dd | 6.96 dd | 7.21 dd (8.1, 2.1) | 7.23 br d (8.9) |
| 13′ | 7.22 dd | 7.22 dd | 7.17 dd (8.5, 2.5) | 7.14 dd (8.1, 2.1) | 7.23 br d (8.1) |
| 14′ | 7.59 dd | 7.59 dd | 7.59 dd (8.5, 2.0) | 7.49 dd (8.1, 2.1) | 7.53 br d (8.1) |
| 2-NMe | 2.41 | 2.41 | 2.40 | 2.16 | 2.17 |
| 2-NMe | 2.60 | 2.61 | 2.59 | 2.65 | 2.68 |
| 6′-OMe | 3.86 | OH | 3.86 | | |
| 6-OMe | | | | 3.84 | 3.88 |
| 12-OMe | 3.98 | 3.98 | OH | OH | 4.01 |

[a] Obtained at 500 MHz in $CDCl_3$, TMS as internal standard.

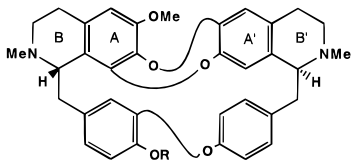
and number of substituents are identical to those of cocsuline (**2**).

The $^1$H-NMR spectrum of **5**, presented in Table 1, indicated the presence of two *N*-methyl singlets ($\delta$ 2.16 and 2.66 ppm), somewhat more separated than those observed for **2** ($\delta$ 2.40 and 2.58 ppm). A one-proton singlet, which could be assigned to H-8 or H-8′, is present at $\delta$ 5.33 ppm, while H-8 resonates at $\delta$ 6.14 ppm in the spectrum of (+)-cocsuline (**2**). The other aromatic signals are similar to those of **2**. Finally, no doublet of doublets around 4.00 ppm corresponding to H-1 and H-1′ was observed, as in the spectrum of **2**. Therefore, compound **5** is a bisbenzylisoquinoline possessing one diaryl ether bridge on the lower part of the molecule, and, as in **2**, a hydroxyl group at C-12, and, in the upper part of the molecule, two diphenyl ether bridges and a methoxyl group, although the positions of attachment for the bridges must be different.

In order to establish the termini of the linkages and the locations of the substituents, a ROESY experiment was carried out subsequent to a $^1$H−$^1$H COSY experiment. Reciprocating effects could be observed between H-10 and H-1, H-10 and the methylene proton at C-α, and H-1 and the 2-*N*-methyl, which also correlates with the methylene proton at C-3. Reciprocating effects were also noted between the two protons at C-4 and the proton at C-5, while the latter also correlates with the 6-OMe. These results along with the absence of correlation between the broad doublet due to H-1 and any of the aromatic proton singlets, indicate that the two upper bridges originate at C-8 and C-7. The one-proton singlet at $\delta$ 5.33 ppm is assigned to H-8′. Concerning the right-hand moiety of the molecule, correlations were observed between H-1′ and H-14′, H-10′, and H-8′, as well as between H-10′ and H-8′. The 2′-*N*-methyl signal correlates with the H-1′ broad doublet and the H-3′ multiplet at d 3.09 ppm. The one-proton singlet assigned to H-5′ correlates with the multiplet due to H-4′. Consequently, the two diphenyl ether bridges are located at C-8/C-7′ and C-7/C-6′.

The CD curves of (+)-cocsuline and (+)-angchibangkine are very similar, and the optical rotation is positive, as in (+)-cocsuline (**2**). These data suggested that the absolute configuration of (+)-angchibangkine might be 1*S*,1′*S* as in (+)-cocsuline. Due to the small amount of (+)-angchibangkine available, Na−liquid ammonia cleavage of the diphenyl ether bridges could not be carried out, and thus the absolute configuration at 1 and 1′ could not be confirmed directly.

Treatment of (+)-angchibangkine (**5**) with $CH_2N_2$ led to a methylated derivative, (+)-*O*-methylangchibangkine (**6**), which presents a mass spectrum identical to the spectrum of (+)-isotrilobine (**1**). The $^1$H-NMR spectrum of **6** is very similar to the spectrum of **5** except for the three proton singlet at $\delta$ 4.01 ppm attributed to the methoxyl group at C-12.




**5** R = H
**6** R = Me

(+)-Angchibangkine is thus the first representative of a new subgroup of bisbenzylisoquinoline alkaloids. It is interesting that among dimers isolated from the stem bark of *P. dasycarpa*, only one dimer with two diaryl linkages, (+)-daphnoline, possesses a diphenyl ether bridge in the 7,8′ position, as in the four known dimers from the (+)-cocsuline subgroup with three bridges, while the five other bisbenzylisoquinolines with two linkages belong to the (+)-tetrandrine subgroup and possess a diphenyl ether bridge in the 8,7′ position as in (+)-angchibangkine. A similar situation has been described for *Cocculus pendulus*,[12] another Menispermaceous species, in that no dimer from the angchibangkine type had been isolated from that plant. The assumption was made that additional head-to-head coupling in a dimer of the tetrandrine type was precluded because of the preferred conformation of the molecule in which rings A and A′ are appreciably distant from each other, as shown by models. The isolation of

(+)-angchibangkine contradicts this hypothesis and indicates how little is known of the different conformations that can be assumed by bisbenzylisoquinoline alkaloids.

In the antiplasmodial assay measuring incorporation of $^3$H-labeled hypoxanthine by *Plasmodium falciparum*, (+)-angchibangkine produced $IC_{50}$ values of 306 ng/mL and 265 ng/mL vs the chloroquine-sensitive D6 and the chloroquine-resistant W2 clones, respectively. (+)-*O*-Methylangchibangkine exhibits the same activity, producing $IC_{50}$ values of 326 ng/mL vs clone D6 and 204 ng/mL vs clone W2. Cytotoxicity assays with mammalian KB cells yielded $IC_{50}$ values of 12 000 ng/mL for (+)-angchibangkine and 5900 for its *O*-methylated derivative; thus, (+)-angchibangkine appears to have slightly better antiplasmodial selectivity.

These results are very similar to those obtained with the other bisbenzylisoquinoline alkaloids isolated during this work. The results will be presented subsequently together with the activity of a number of other alkaloids with related structure.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured with a Perkin-Elmer 241 polarimeter. CD spectra were obtained with a JASCO J-710 spectropolarimeter. NMR spectra were recorded with General Electric GE Omega 500, Nicolet NMC-360, and Varian XL-300 spectrometers in $CDCl_3$ solution with TMS as an internal standard. EIMS and HRMS were recorded on a Finnegan MAT-90 instrument.

**Plant Material.** Stem bark of *P. dasycarpa* was collected in May−June 1993, from the Erawan waterfall, Kanchanaburi Province, Thailand. Authentication was achieved by comparison with a herbarium specimen at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok, Thailand. The herbarium specimen has been deposited in the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

**Extraction and Isolation.** The dried, powdered stem bark of *P. dasycarpa* (5 kg) was macerated twice for 3-day periods in EtOH and filtered. The combined filtrates were evaporated under reduced pressure to give a syrupy mass (220 g). The residue was treated with aqueous HCl (5%), and the acidic solution was basified with $NH_4OH$ to pH 9−10 and extracted with $CHCl_3$. Evaporation of the organic solvent under reduced pressure led to the crude nonquaternary alkaloids (14.3 g). This alkaloid extract was passed through a column of silica gel 60 (70−230 mesh ASTM). Elution was conducted initially with $CHCl_3$ gradually enriched with MeOH and yielded 20 fractions. Further purification of fractions 4, 5, 7, 9, and 15 was performed to obtain a small amount of the most representative bisbenzylisoquinoline alkaloids present in the extract. They were obtained by column chromatography using Si gel 60 with different solvent systems followed by preparative TLC. The solvent system used for column chromatography of fractions 4, 5, and 9 was $CHCl_3$−$C_6H_{12}$−MeOH−$NH_4OH$ (70:20:8:1). The solvent system used for column chromatography of fraction 15 was $CHCl_3$−$C_6H_6$−EtOAc−MeOH−$NH_4OH$ (8:60:16:18:2). A small part of fraction 7 was analyzed through preparative TLC. The solvent system used for all preparative TLC was $CHCl_3$−$C_6H_{12}$−MeOH (70:20:10) with 0.5 to 1 volume of $NH_4OH$.

**(+)-12-*O*-Methyltricordatine (4):** $[\alpha]_D$ +55° (MeOH, *c* 0.196); CD MeOH, $\Delta\epsilon$ ($\lambda$ nm) 0 (300), +3.6 (293), +2 (261), +13.3 (233), 0 (210), negative tail; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 206 (4.82), 236 sh (4.29), 286 (3.73); HREIMS *m/z* 562.2472; calcd for $C_{35}H_{34}O_5N_2$ 562.2467; EIMS *m/z* 562 (21), 561 (10), 336 (25), 335 (100), 321 (27), 168 (27); $^1$H NMR, see Table 1.

**(+)-Angchibangkine (5):** $[\alpha]_D$ +450° ($CHCl_3$, *c* 0.1); CD MeOH, $\Delta\epsilon$ ($\lambda$ nm) 0 (321), +20 (294), 0 (289), +7.3 (261), +90 (238), +16.5 sh (217), 0 (211), negative tail; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 206 (4.90), 234 sh (4.67), 290 (3.71); HREIMS *m/z* 562.2459; calcd for $C_{35}H_{34}O_5N_2$ 562.2467; EIMS *m/z* 562 (94), 561 (43), 400 (43), 386 (51), 372 (50), 356 (34), 358 (34), 350 (33), 349 (100), 335 (44), 224 (30), 197 (45), 191 (300, 175 (66); $^1$H NMR ($CDCl_3$, 500 MHz), see Table 1.

**(+)-12-*O*-Methylangchibangkine (6):** $[\alpha]_D$ +392° ($CHCl_3$, *c* 0.05), +356° (MeOH, *c* 0.05); CD MeOH, $\Delta\epsilon$ ($\lambda$ nm) 0 (321), +9.6 (292), +3.7 (260), +49 (239), 0 (211), negative tail; EIMS *m/z* 576 (47), 561 (2), 350 (27), 349 (100), 335 (41), 175 (54); $^1$H NMR ($CDCl_3$, 300 Hz), see Table 1.

**Antiplasmodial and Cytotoxic Activity.** Antiplasmodial and cytotoxic activities were determined as previously described.[2] Alkaloidal fraction: KB cells $IC_{50}$, 3600 ng/mL; *P. falciparum* Clone $D_6$ $IC_{50}$, 13.8 ng/mL; *P. falciparum* Clone $W_2$ $IC_{50}$, 56.4 ng/mL. Compound **4**: KB cells $IC_{50}$, 3400 ng/mL; *P. falciparum* Clone $D_6$ $IC_{50}$, 17.1 ng/mL; *P. falciparum* Clone $W_2$ $IC_{50}$, 63.0 ng/mL. Compound **5**: KB cells $IC_{50}$, 12 000 ng/mL; *P. falciparum* Clone $D_6$ $IC_{50}$, 306 ng/mL; *P. falciparum* Clone $W_2$ $IC_{50}$, 265 ng/mL. Compound **6**: KB cells $IC_{50}$, 5900 ng/mL; *P. falciparum* Clone $D_6$ $IC_{50}$, 326 ng/mL; *P. falciparum* Clone $W_2$ $IC_{50}$, 204 ng/mL.

**Acknowledgments.** The authors would like to thank the Research Resources Center, UIC, for the provision of NMR spectroscopic facilities.

## References and Notes

(1) Lin, L.-Z.; Xue, L.; Shieh, H.-L.; Angerhofer, C. K.; Pezzuto, J. M.; Johnson, M.E.; Cordell, G. A. *J. Nat. Prod.* **1993**, *56*, 22−29.
(2) Likhitwitayawuid, K.; Angerhofer, C. K.; Ruangrungsi, N.; Cordell, G. A.; Pezzuto, J. M. *J. Nat. Prod.* **1993**, *56*, 30−38.
(3) Sultanbawa, M.; Sotheeswaran, S.; Balusubramaniam, S.; El-Kawi, M. A.; Slatkin, D. J.; Schiff, P. L., Jr. *Heterocycles* **1983**, *20*, 1927−1932.
(4) El-Kawi, M. A.; Slatkin, D. J.; Schiff, P. L., Jr.; Dasgupta, S.; Chattopadhyay, S.K.; Ray, A. B. *J. Nat. Prod.* **1984**, *47*, 459−464.
(5) Sultanbawa, M.; Sotheeswaran, S.; Balusubramaniam, S.; El-Kawi, M. A.; Slatkin, D. J.; Schiff, P. L., Jr. *Phytochemistry* **1985**, *24*, 589−592.
(6) Leboeuf, M.; Abouchacra, M. L.; Cavé, A.; Debray, M. *Plantes Med. Phytother.* **1987**, *21*, 106−115.
(7) Pongboonrod, S. *Mai Thet Muang Thai*; Kasembunnakich Press: Bangkok, 1979; pp 423−424.
(8) Guha, K. P.; Mukherjee, B.; Mukherjee, R. *J. Nat. Prod.* **1979**, *42*, 1−84.
(9) Schiff, P. L., Jr. *J. Nat. Prod.* **1983**, *46*, 1−43.
(10) Schiff, P. L., Jr. *J. Nat. Prod.* **1987**, *50*, 529−599.
(11) Schiff, P. L., Jr. *J. Nat. Prod.* **1991**, *54*, 645−749.
(12) Hussain, S. F.; Khan, L.; Guinaudeau, H.; Leet, J. E.; Shamma, M. *Tetrahedron* **1984**, *40*, 2513−2517

NP960568E