

FOUR AMIDIC BISBENZYLISOQUINOLINE ALKALOIDS FROM *CYCLEA ATJEHENSIS*

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ABSTRACT.—Four amidic bisbenzylisoquinolines have been isolated from *Cyclea atjehensis*. These are (–)-curicycleatjenine [1], (–)-curicycleatjine [2], (–)-isocuricycleatjenine [3], and (–)-isocuricycleatjine [4]. ¹H-nmr spectroscopy at 500 MHz in CDCl₃ solution can clearly differentiate between the two geometric isomers for each of the four alkaloids.

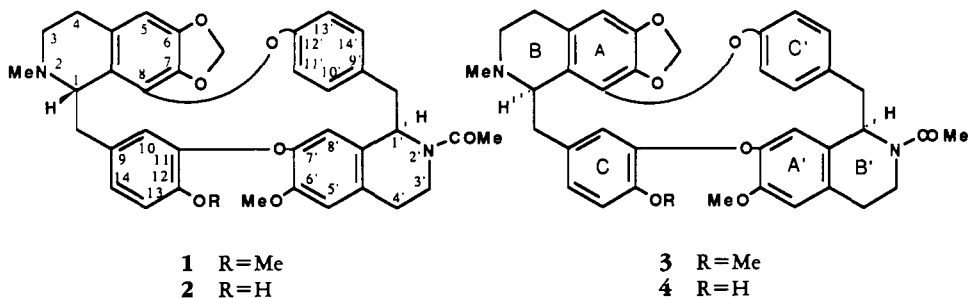
Cyclea species (Menispermaceae) are found in southeastern Asia, and *Cyclea atjehensis* Forman, the plant of particular interest in the present study, is available in Thailand.

We have found that *C. atjehensis* is rich in bisbenzylisoquinolines of the head-to-tail curine type (8-12', 11-7'). Four of these alkaloids were actually obtained, and it is interesting to note that all four possess a methylenedioxy group—an unusual substituent among the bisbenzylisoquinolines in general (1–3)—as well as an *N*-acetyl function.

The four alkaloids could be clearly divided into subgroups based upon their absolute configuration. The first subgroup included the non-phenolic (–)-curicycleatjenine [1] and the phenolic (–)-curicycleatjine [2], which possess the 1*S*, 1'*R* configuration. The second subgroup consists of the non-phenolic (–)-isocuricycleatjenine [3] and the phenolic (–)-isocuricycleatjine [4] which incorporate the 1*R*, 1'*R* configuration.

Alkaloids 1 and 3 present nearly identical mass spectra, with a strong molecular ion at *m/z* 634 (C₃₈H₃₈N₂O₇), and a base peak at *m/z* 189 corresponding to rings A and B of the molecules. Other important peaks were found at *m/z* 340 and 295 due to facile cleavage of the doubly benzylic bonds and corresponded to rings C, A', and B' and rings A, B, and C', respectively.

Similarly, phenolic alkaloids 2 and 4 showed closely related mass spectral patterns with a very strong molecular ion at *m/z* 620, which was also the base peak in 4. The molecular ion was flanked by an equally strong [M – 1]⁺ ion, *m/z* 619, which was the base peak in the spectrum of 2. The fact that the molecular ions were 14 daltons less than those for alkaloids 1 and 3 pointed to the presence of a phenolic function in lieu of a methoxyl group. Additionally, because the strong *m/z* 340 peak in the spectra of alkaloids 1 and 3 is replaced by a peak at *m/z* 326 in 2 and 4, it was logical to conclude



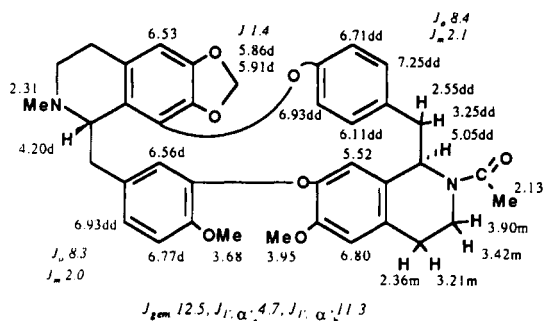
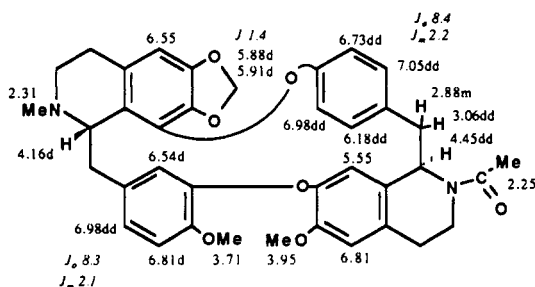
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that the phenolic function in **2** and **4** was located on ring C or A'. Buttressing this argument was the fact that the m/z 295 and 189 peaks observed in the spectra of **1** and **3**, which are descriptive of the top half of the dimers, were also present in the spectra of **2** and **4** with the same intensities.

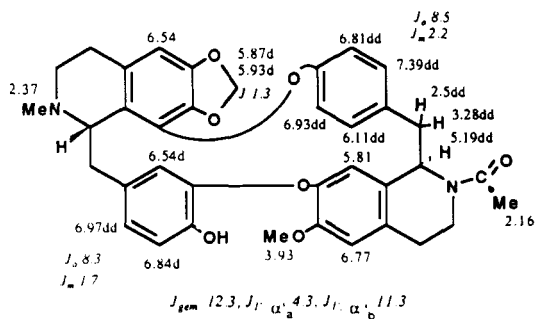
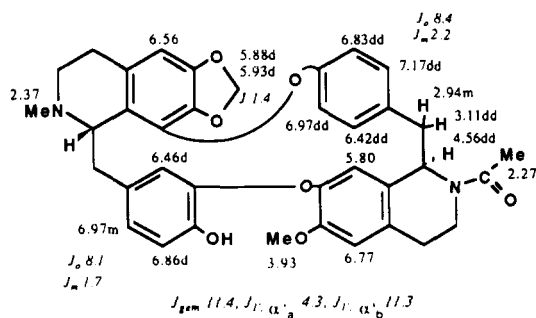
The ^1H -nmr spectra at 500 MHz in CDCl_3 revealed that two isomeric species were present in solution in each instance due to geometric isomerism about the amidic bond. The two isomeric species could be clearly distinguished and were present in each instance in a ratio of ca. 3.5:1. Furthermore, through nmr nOe measurements, it was possible to define the geometric isomerism in each case.

The ^1H -nmr spectrum of the major isomer for (-)-curicycleatjenine is summarized around expression **1a**, and that for the minor isomer around **1b**. The protons of the methylenedioxy group resonate as two close doublets at δ 5.86 and 5.91 in species **1a** and at δ 5.88 and 5.91 in **1b**. The two methoxyl singlets were at δ 3.68 and 3.95 in one instance and at δ 3.71 and 3.95 in the other. The *N*-methyl signal remained constant at δ 2.31, and the H-1 broad doublet was evident at δ 4.20 for **1a** and at δ 4.16 for **1b**, while the two *N*-acetyl singlets were appreciably different, appearing at δ 2.13 in **1a** and at 2.25 in **1b**. The non-equivalence of the two acetyl signals is a reflection of the prevailing geometrical isomerism. This difference is also emphasized by the chemical shifts of H-1'. This proton appears as a doublet of doublets centered at δ 5.05 for the major isomer **1a** and at δ 4.45 for **1b**. A small but significant difference was also noted for the shifts for H-10' and H-14' with signals at δ 6.11 and 7.25 for **1a** and δ 6.18 and 7.05 for **1b**.

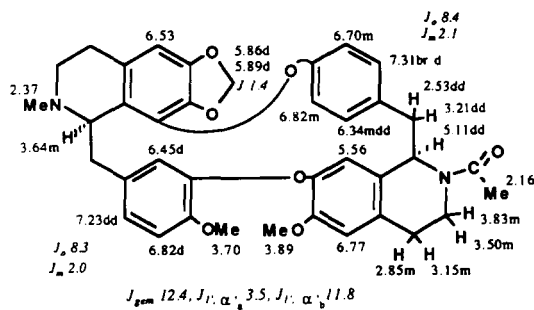
**1a****1b**

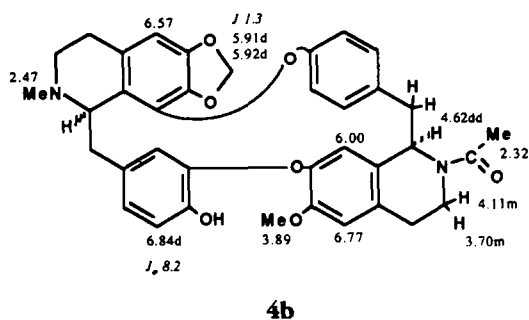
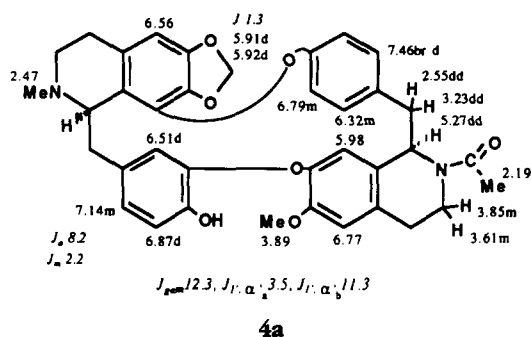
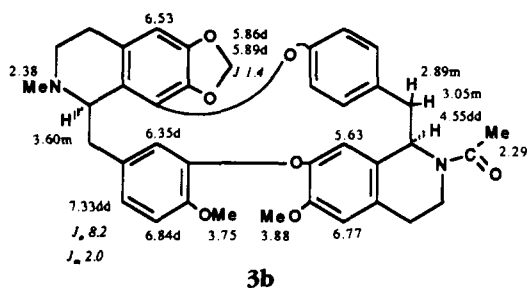
A complete nmr nOe study (see Experimental) confirmed each of the above assignments. In particular, irradiation of the *N*-acetyl singlet of **1a** (δ 2.13) led to an enhancement of the H-3' signals (δ 3.42 and 3.90) with no effect upon the H-1' signal (δ 5.05). On the other hand, irradiation of the **1b** *N*-acetyl (δ 2.25) produced an enhancement of the H-1' signal (δ 4.45), while H-3' was unaffected.

The nmr spectrum of the phenolic (–)-curicycleatjine [2] showed the obvious lack of a methoxyl singlet near δ 3.70, which led us to place the phenolic hydroxyl function at C-12. Species **2a** was the major isomer, and **2b** was the minor. It will be noted that for both isomers the signals for H-8' are shifted further downfield by about 0.3 ppm in relation to the corresponding signals for **1a** and **1b**. As expected, *O*-methylation of **2** led to (–)-curicycleatjenine [1].

**2a****2b**

Our second subgroup, consisting of (–)-isocuricycleatjenine [3] and (–)-isocuricycleatjine [4], again showed the same type of geometric isomerism in the nmr spectra with the **a** isomers predominating. Differences in chemical shifts, parallel to those mentioned in the cases of **1a** and **1b** above, could be observed between **3a** and **3b** and also between **4a** and **4b**. The H-1 signal was now more upfield, appearing at δ 3.64 for **3a** and at δ 3.60 for **3b**. Additionally, H-14 was shifted downfield to δ 7.23 for **3a** and 7.33 for **3b**. Again, as in compounds **1** and **2**, a complete nmr nOe analysis served to confirm the assignments of the chemical shifts.

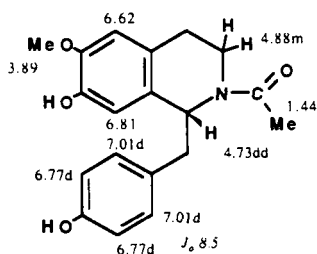
**3a**



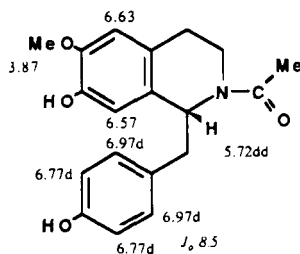
The nmr spectrum of the phenolic (-)-isocuricyclatejine [4] indicated the lack of a C-12 methoxyl. There was also a downfield shift of H-8', which is near δ 6.00 as compared to around δ 5.56 in 3. CH_2N_2 O-methylation of 4 furnished (-)-isocuricyclatejine [3].

Some differences in specific rotations could be observed between our four alkaloids, and we had to resort to sodium in liquid NH_3 cleavage to establish the absolute configurations. Reduction of (-)-curicyclatejine [1] supplied a complex mixture of products from which small yields of (-)-N-acetylcoclaurine [5] and (+)-N-methyl-7-hydroxy-12-methoxytetrahydrobenzylisoquinoline [6] were obtained. Alternatively, reduction of (-)-isocuricyclatejine [3] led to a mixture from which we could identify (-)-N-acetylcoclaurine [5] and (-)-N-methyl-7-hydroxy-12-methoxytetrahydrobenzylisoquinoline which is enantiomeric with 6. It follows that (-)-curicyclatejine [1] and by extension (-)-curicyclatejine [2] partake of the $1S, 1'R$ configuration, while (-)-isocuricyclatejine [3] and (-)-isocuricyclatejine [4] belong to the $1R, 1'R$ series.

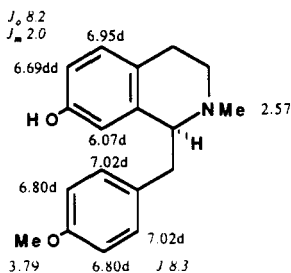
It is interesting to note that, just like alkaloids 1-4, the cleavage product (-)-N-acetylcoclaurine [5] exists in CHCl_3 solution as two isomers, 5a and 5b, in a ratio of approximately 7:3, which can be readily differentiated by nmr spectroscopy (4).



5a



5b



6

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr data presented were obtained on either a Bruker AM-500 or WM-360 spectrometer in CDCl_3 . Mass spectral data were collected on a Kratos MS-50 high resolution mass spectrometer.

PLANT COLLECTION AND EXTRACTION AND ALKALOID ISOLATION.—*C. atjebensis* was collected in Somchitra tin mine, Kanchanaburi Province, near the Burmese border, in January 1987. A voucher specimen was deposited in the herbarium of the Faculty of Pharmacy, Chulalongkorn University. The dried leaves (2.5 kg) were extracted first with petroleum ether and then with EtOH. After evaporation of the solvent, the EtOH extract was mixed with 10% HOAc in H_2O and filtered. Following basification with NH_4OH , the aqueous solution was extracted with CHCl_3 , which furnished, after evaporation, 55.4 g of crude alkaloids. A rough separation was obtained by Si gel cc using a $\text{CHCl}_3/\text{MeOH}$ gradient. Further purification of the alkaloids was achieved by cc using Si gel for tlc and through the use of Si gel tlc glass plates. Amorphous (–)-curicycleatjenine [1] (190 mg), (–)-curicycleatjine [2] (31 mg), (–)-isocuricycleatjenine [3] (95 mg), and (–)-isocuricycleatjine [4] (68 mg) were thus obtained. The same alkaloids were obtained when the isolation procedure was repeated using phosphoric acid in lieu of HOAc.

(–)-CURICYCLEATJENINE [1].— $\text{C}_{38}\text{H}_{38}\text{N}_2\text{O}_7$; eims m/z [M]⁺ 634 (81), 633 (76), 620 (19), 619 (36), 591 (3), 341 (15), 340 (68), 339 (2), 298 (12), 297 (14), 295 (49), 294 (8), 282 (20), 190 (14), 189 (100), 187 (14), 159 (48); hreims 634.2545 for $\text{C}_{38}\text{H}_{38}\text{N}_2\text{O}_7$ (calcd 634.2669), 591.2486 for $\text{C}_{36}\text{H}_{35}\text{N}_2\text{O}_6$ (calcd 591.2486), 340.1533 for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_4$ (calcd 340.1543), 295.1207 for $\text{C}_{18}\text{H}_{17}\text{NO}_3$ (calcd 295.1204), 189.0787 for $\text{C}_{11}\text{H}_{11}\text{NO}_2$ (calcd 189.0787); uv λ max (MeOH) 232 sh, 283 nm (log ϵ 4.62, 4.08); ir ν max (CHCl_3) 3025, 2995, 1625, 1605, 1495 cm^{-1} ; $[\alpha]_D -120^\circ$ ($c = 0.3$, CHCl_3), $[\alpha]_D -101^\circ$ ($c = 0.3$, MeOH); cd $\Delta\epsilon$ (nm) (MeOH) 0 (307), -2.6 sh (294), -4.7 (286), -3.2 (281), -3.4 (278), -0.9 (254), -9.8 (240), 0 (235), positive tail below 230 nm.

Principal nOe's.—For 1a: H-1 to NMe-2 (6%), NMe-2 to H-1 (13%), H-1 to H-10 (10%), H-10 to H-1 (8%), H-13 to 12-OMe (10%), 12-OMe to H-13 (18%), H-10 to H-8' (6%), H-8' to H-10 (6%), 6'-OMe to H-5' (17%), H-5' to 6'-OMe (14%), H-5' to H-4' (6%), H-4' to H-5' (7%), H-8' to H-1' (10%), H-1' to H-8' (12%), H-1' to H- α' (δ 3.25) (8%), H- α' (δ 3.25) to H-1' (3%), CO-Me to H-3' (δ 3.90) (6%), H-3' (δ 3.90) to CO-Me (6%), H-3' (δ 3.42) to CO-Me (5%), CO-Me to H-3' (δ 3.42) (3%). For 1b: H-1 to NMe-2 (6%), NMe-2 to H-1 (10%), H-1 to H-10 (10%), H-10 to H-1 (8%), H-13 to 12-OMe (10%), 12-OMe to H-13 (13%), H-10 to H-8' (6%), H-8' to H-10 (6%), 6'-OMe to H-5' (15%), H-5' to 6'-OMe (10%), H-8' to H-1' (9%), H-1' to H-8' (10%), CO-Me to H-1' (11%), H-1' to CO-Me (11%).

(-)-CURICYCLEATJINE [2].— $C_{37}H_{36}N_2O_7$; eims m/z $[M]^+$ 620 (94), 619 (100), 577 (3), 326 (65), 296 (10), 295 (30), 284 (35), 189 (96), 187 (35), 159 (65); uv λ max (MeOH) 230 sh, 283 nm (log ϵ 4.62, 411); λ max (OH⁻, MeOH) 233 sh, 284, 309 sh; $[\alpha]_D -193^\circ$ ($c=0.27$, CHCl₃), $[\alpha]_D -122^\circ$ ($c=0.27$, MeOH); cd $\Delta\epsilon$ (nm) (MeOH) 0 (312), -2.4 sh (296), -3.9 (285), -2.6 (282), -3.1 (279), 0 (253), -9.4 (241), 0 (235).

(-)-ISOCURICYCLEATJENINE [3].— $C_{38}H_{38}N_2O_7$; eims m/z $[M]^+$ 634 (86), 633 (81), 620 (16), 619 (36), 591 (3), 341 (13), 340 (57), 339 (2), 298 (12), 296 (14), 295 (47), 294 (7), 282 (24), 190 (13), 189 (100), 187 (10), 159 (52); hreims 634.2669 for $C_{38}H_{38}N_2O_7$ (calcd 634.2669), 591.2542 for $C_{36}H_{35}N_2O_6$ (calcd 591.2486), 340.1536 for $C_{20}H_{22}N_2O_4$ (calcd 340.1543), 295.1108 for $C_{18}H_{17}NO_3$ (calcd 295.1204), 189.0787 for $C_{11}H_{11}NO_2$ (calcd 189.0787); uv λ max (MeOH) 232 sh, 283 nm (log ϵ 4.58, 4.04); $[\alpha]_D -238^\circ$ ($c=0.18$, CHCl₃), $[\alpha]_D -191^\circ$ ($c=0.18$, MeOH); cd $\Delta\epsilon$ (nm) (MeOH) 0 (308), -9.4 (289), -1.2 sh (282), 0 (279), +4.0 (275), 0 (258), -20.7 (241), 0 (232), positive tail below 230 nm.

Principal nOe's.—For **3a**: H-1 to MNe-2 (3%), NMe-2 to H-1 (2%), H- α (δ 2.90) to H-1 (3%), H-10 to H- α (δ 2.62) (3%), H- α (δ 2.62) to H-10 (8%), H-13 to 12-OMe (15%), 12-OMe to H-13 (17%), H-10 to H-8' (4%), H-8' to H-10 (4%), 6'-OMe to H-5' (22%), H-5' to 6'-OMe (19%), H-8' to H-1' (12%), H-1' to H-8' (10%), H-1' to H-14' (8%), H-14' to H-1' (10%), H-1' to H- α' (δ 3.21) (6%), H- α' (δ 3.21) to H-1' (5%), H-1' to H-10' (2%), H-10' to H-1' (6%), CO-Me to H-3' (δ 3.83) (2%), H-3' (δ 3.83) to CO-Me (5%). For **3b**: H-1' to H-8' (4%), CO-Me to H-1' (6%), H-1' to CO-Me (8%).

(-)-ISOCURICYCLEATJINE [4].— $C_{37}H_{36}N_2O_7$; eims m/z $[M]^+$ 620 (100), 619 (97), 577 (3), 326 (57), 296 (9), 295 (12), 293 (28), 284 (28), 268 (2), 190 (11), 189 (81), 159 (47); uv λ max (MeOH) 231 sh, 283 nm (log ϵ 4.80, 4.25); λ max (OH⁻, MeOH) 235 sh, 284, 305 sh; $[\alpha]_D -173^\circ$ ($c=0.13$, CHCl₃), $[\alpha]_D -149^\circ$ ($c=0.13$, MeOH); cd $\Delta\epsilon$ (nm) (MeOH) 0 (309), -5.5 (290), 0 (287), +0.3 sh (282), +3.8 (276), +3.1 (270), 0 (256), -16.5 (244), -17 (235).

O-METHYLATION OF **2** AND **4**.—In a 10-ml flask, the alkaloid (5 mg) was dissolved in MeOH (5 ml) and ethereal CH₂N₂ (5 ml) was added. After 15 h in the refrigerator, workup supplied 4 mg of the *O*-ethylated derivative.

SODIUM LIQUID AMMONIA REDUCTION.—The procedure described by Bruneton *et al.* (5) was followed.

(-)-*N*-ACETYLCOCLAURINE [5].— $C_{19}H_{21}NO_4$; eims m/z $[M-2]^+$ 325 (0.3), 297 (0.25), 284 (0.24), 220 (61), 178 (100), 163 (28), 162 (44); $[\alpha]_D -15^\circ$ ($c=0.2$, CHCl₃), $[\alpha]_D -20^\circ$ ($c=0.2$, MeOH).

(+)-*N*-METHYL-7-HYDROXY-12-METHOXYTETRAHYDROBENZYLISOQUINOLINE [6].— $C_{18}H_{21}NO_2$; eims m/z 162 (100); $[\alpha]_D +11^\circ$ ($c=0.2$, CHCl₃), $[\alpha]_D +22^\circ$ ($c=0.2$, MeOH).

(+)-*N*-METHYL-7-HYDROXY-12-METHOXYTETRAHYDROBENZYLISOQUINOLINE.— $C_{18}H_{21}NO_2$; $[\alpha]_D -25^\circ$ ($c=0.08$, MeOH).

ACKNOWLEDGMENTS

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