Bisbenzylisoquinoline Alkaloids from Cyclea barbata

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BISBENZYLISOQUINOLINE ALKALOIDS FROM *CYCLEA BARBATA*[1]

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ABSTRACT.—Continuing studies of the alkaloidal fraction from the roots of *Cyclea barbata* afforded two new bisbenzylisoquinoline alkaloids, namely, (−)-2′-norlimacine [**1**] and (+)-cycleabarbatine [**2**]. The known (+)-tetrandrine-2′-β-*N*-oxide [**3**], for which the configuration of the *N*-oxide function is now assigned, was identified, as were (+)-berbamine, (−)-repandine, (+)-cycleanorine, (+)-daphnandrine, (−)-curine, (+)-coclaurine, and (−)-*N*-methylcoclaurine.

The alkaloid extract from the roots of *Cyclea barbata* (Wall) Miers (Menispermaceae) shows in vitro cytotoxic and antimalarial activities. In an initial analysis, five major alkaloids were isolated and identified: (+)-tetrandrine, (−)-limacine, (+)-thalrugosine, (+)-homoaromoline, and (−)-cycleapeltine (2).

Continuing studies of the alkaloidal fraction afforded two new bisbenzylisoquinoline alkaloids: (−)-2′-norlimacine [**1**], and (+)-cycleabarbatine [**2**]. (+)-Tetrandrine-2′-β-*N*-oxide [**3**] was also found in the extract. This alkaloid was isolated previously from this plant as the first bisbenzylisoquinoline *N*-oxide, but its structure was not fully determined (3). The known alkaloids (+)-berbamine, (−)-repandine, (+)-cycleanorine, (+)-daphnandrine, (−)-curine, (+)-coclaurine, and (−)-*N*-methylcoclaurine were also identified.

The mass spectrum of (−)-2′-norlimacine [**1**] is characteristic of a doubly bridged, tail-to-tail dimer with a molecular peak at *m/z* 594 (100%), corresponding to $C_{36}H_{38}N_2O_6$. A strong fragment ion at *m/z* 367 corresponds to the upper part of the molecule. These two fragments are 14 daltons less than the corresponding ions in the mass spectrum of limacine.

The ^{1}H-nmr spectrum presents only one *N*-methyl group signal at δ 2.32 ppm, which should be assigned to the left hand side isoquinoline unit (4). Compared to the ^{1}H-nmr spectrum of (−)-limacine, the absence of a three-proton singlet around 2.55 ppm indicates the presence of a secondary amine on the right hand side of the molecule. As expected, signals due to the protons situated near N-2′ are shifted further downfield: H-1′ at δ 4.17 ppm, instead of 3.87 ppm for limacine (2), the methylene protons at C-3′ at 3.20 ppm and 3.51 ppm instead of 2.83 and 3.49 ppm, and the protons at C-α′ at 3.20 ppm and 2.98 ppm instead of 2.76 ppm and 3.22 ppm (4). The other resonances in the spectrum are similar to those observed for (−)-limacine (2,4).

A ROESY experiment showed a spatial relationship between the 2-*N*-methyl signal (2.32 ppm) and H-1 (3.74 ppm), as well as between the 2-*N*Me and H-4 (3.51 ppm). An effect between H-1′ (4.17 ppm) and H-8′ (6.07 ppm) was also visible. These results confirmed the presence of an N-2′ secondary amine. Homonuclear COSY, HMQC and HMBC experiments permitted the complete assignment of the ^{1}H and ^{13}C spectra of (−)-2′-norlimacine [**1**] as presented in Figure 1.

[1]Paper 25 in the series "Traditional Medicinal Plants of Thailand." For paper 24, see L.-J. Lin *et al.* (1).

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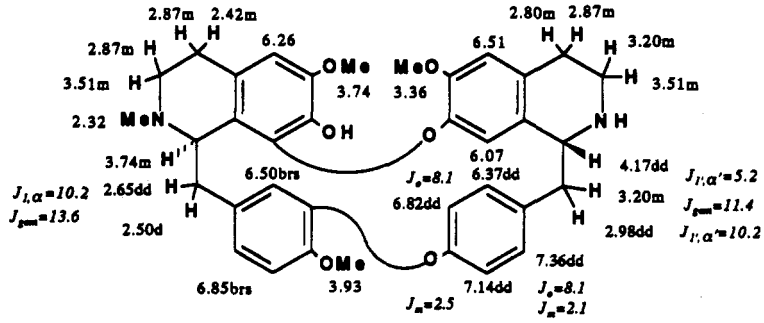

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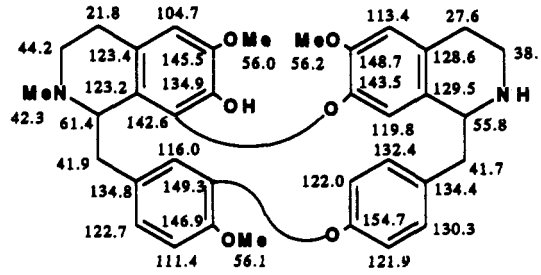

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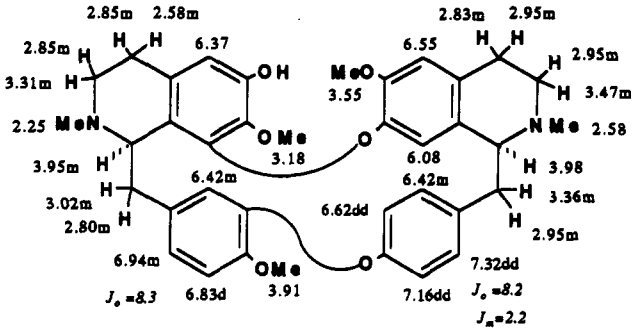
FIGURE 1. ^{1}H and ^{13}C assignments of norlimacine [**1**].

The second new bisbenzylisoquinoline, (+)-cycleabarbatine [**2**], afforded a mass spectrum showing a molecular ion peak at *m/z* 608 ($C_{37}H_{40}N_2O_6$) accompanied by a base peak at *m/z* 381. The mass of the latter fragment corresponds to $C_{22}H_{26}N_2O_4$ and indicates that the upper part of the molecule is substituted by two methoxyl groups and one hydroxyl group, with two methylated tertiary amines, or by three methoxyl groups and an *N*-methyl group and a secondary amine. The presence in the ^{1}H-nmr spectrum of two three-proton singlets at 2.25 ppm and 2.58 ppm due to two *N*-methyl groups indicated the former structural hypothesis to be correct. Except for the absence of a three-proton singlet near 3.75 ppm, the signals observed in the spectrum are similar to those observed in the spectrum of (−)-isotetrandrine (4). This similarity suggested that compound **2** was identical to *6-O*-demethylisotetrandrine.

The small absolute value of the positive specific rotation of **2** confirmed that (+)-cycleabarbatine belongs to the tetrandrine subgroup and incorporates the 1*R*,1′*S* configuration (5).

A ROESY experiment confirmed these assignments. In particular, effects were observed between H-5′ (6.55 ppm) and 6′-OMe (3.55 ppm), H-1′ (3.98 ppm) and H-8′ (6.08 ppm), 2′-NMe (2.58 ppm) and H-1′ (3.98 ppm), and 2-NMe (2.25 ppm) and H-1 (3.95 ppm). The ^{1}H-nmr data are summarized on structure **2**.

The mass spectrum of (+)-tetrandrine-2′-β-*N*-oxide [**3**] displays a weak molecular ion at *m/z* 638 (16%), which corresponds to $C_{38}H_{42}N_2O_7$. The base peak at *m/z* 622 is due to the loss of an oxygen atom and suggests the presence of an *N*-oxide function. The other fragment ions are similar to those observed in the mass spectrum of (+)-tetrandrine, with a strong peak at *m/z* 395 (45%) due to the bistetrahydroisoquinoline fragment following facile cleavage of the benzylic bonds (4).



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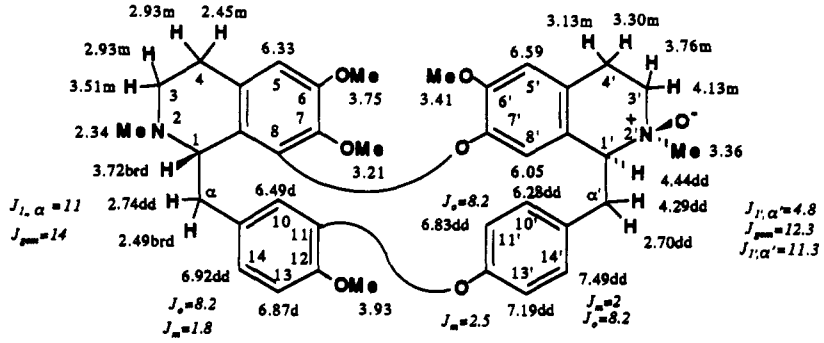
The ^{1}H-nmr spectrum (in $CDCl_3$, 500 MHz) (Figure 2) indicated a three-proton singlet due to a 2-*N*-Me at 2.34 ppm, while the 2′-*N*-methyl group resonates further downfield than usual at 3.36 ppm. The doublet of doublets due to H-1′ at 4.44 ppm, as well as the two multiplets corresponding to the C-3′ methylene group at 3.76 and 4.13 ppm, are also more downfield than in the spectrum of (+)-tetrandrine (respectively at 3.84, 2.83, and 3.39 ppm). These features confirmed the presence of an *N*-oxide function at N-2′. Other resonances, especially those of the aromatic protons, were very similar to those observed for (+)-tetrandrine (2).

The downfield shifts of 0.8 ppm for the 2′-*N*-methyl singlet and of 0.6 ppm for the H-1′ doublet of doublets suggested that H-



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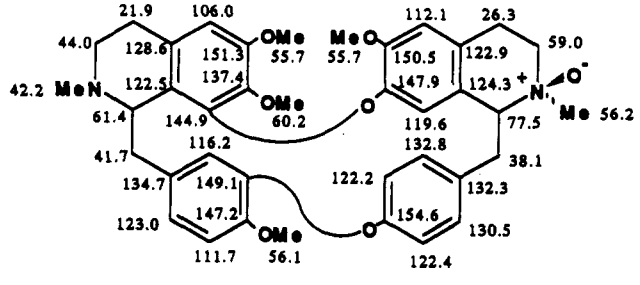

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FIGURE 2. ^{1}H and ^{13}C assignments of (+)-tetrandrine-2′-β-*N*-oxide [3].

1′ is on the side opposite for the *N*-oxide (3). The positive optical rotation indicates that **3** possesses the 1*S*,1′*S* configuration (3). Therefore the *N*-oxide function occupies the β orientation.

This configuration of the *N*-oxide function was confirmed by a ROESY experiment; a significant correlation was observed between H-1′ (4.44 ppm) and the 2′-*N*Me (3.36 ppm), while no effects were detected between H-3′ and the 2′-*N*Me. Therefore, the NMe group is on the same side of the molecule as H-1′, and the oxygen atom on the opposite side.

The complete assignments of ^{1}H and ^{13}C spectra have been confirmed by homonuclear COSY, HMBC and HMQC experiments and are presented in Figure 2.

The bisbenzylisoquinoline alkaloids isolated during the course of this work have been submitted for cytotoxicity and antimalarial activity evaluation. The results will be presented subsequently together with the activity of a number of other alkaloids from the same series.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The optical rotations were determined on a Perkin-Elmer 241 polarimeter. All nmr spectra were recorded in $CDCl_3$ at 500 MHz on a General Electric OMEGA 500 instrument. HMQC and HMBC experiments were obtained at 500.12/125.76 MHz. Eims (70 eV) were recorded on a Varian MAT-112S spectrometer, and hrms were recorded on a Finnigan MAT-90 instrument.

PLANT MATERIAL, EXTRACTION AND SEPARATION.—Plant material, extraction process and separation are the same as described by Lin *et al.* (2).

(−)-2′-Norlimacine [**1**].—$C_{36}H_{38}N_2O_6$; hrms 594.2724 (calcd 594.2730); eims 594 (100), 593 (80), 592 (11), 579 (19), 416 (13), 368 (12), 367 (40), 353 (18), 192 (11); $[\alpha]_D$ −125° ($CHCl_3$, c=0.13).

(+)-Cycleabarbatine [**2**].—$C_{37}H_{40}N_2O_6$; hrms 608.2889 (calcd 608.2886); eims 608 (48), 607 (26), 594 (4), 593 (7), 382 (25), 381 (100), 367 (14), 192 (15), 191 (16), 174 (7), 168 (6); $[\alpha]_D$ +20° ($CHCl_3$, c=0.1).

(+)-Tetrandrine-2′-β-N-oxide [**3**].—$C_{38}H_{42}N_2O_7$; hrms 638.2983 (calcd 638.2992), 622.3042 (calcd 622.3042); eims 638 (16), 637 (16), 636 (15), 622 (100), 621 (67), 608 (28), 607 (34), 396 (22), 395 (42), 381 (25), 198 (27), 174 (10); $[\alpha]_D$ +157° ($CHCl_3$, c=0.24).

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