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AN NMR STUDY OF FOUR BENZOPHENANTHRIDINE ALKALOIDS

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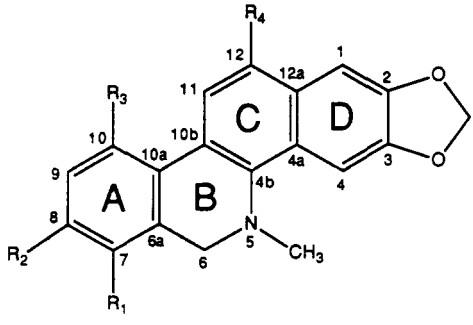
ABSTRACT.—A new dihydrobenzo[*c*]phenanthridine alkaloid, 12-methoxydihydrochelerythrine [**1**], has been isolated from a lipophilic extract of the leaves of *Bocconia integrifolia* together with three known compounds, dihydrochelerythrine [**2**], dihydrosanguinarine [**3**], and dihydrochelirubine [**4**]. The structure of **1** was established by analysis of its spectral data (ir, uv, eims, ^{1}H- and ^{13}C-nmr). Extensive ^{1}H-, ^{13}C-, and nOe 1D and 2D nmr experiments allowed ^{1}H and ^{13}C assignments for **1–4**. This is the first report of ^{13}C-nmr assignments for compounds **2–4**.

Plants of the genus *Bocconia* Plum., which is native to tropical and subtropical areas of Central and South America, are known to biosynthesize protoberberine, protopine, rhoeadine, and benzophenanthridine alkaloids (1). In this first phytochemical investigation on *Bocconia integrifolia* Humb. & Bonpl. (Papaveraceae), we report one novel and three known dihydrobenzo[*c*]phenanthridine alkaloids. Alkaloids of this structural type have been shown to possess antimicrobial (2–5), anti-inflammatory (4), cytotoxic (6), antitumor (6–9), and antiviral (10) activity. Extracts containing benzophenanthridine alkaloids are also known to be active against bacteria that cause periodontal disease, and due to their low toxicity, they may be safely used clinically as antiplaque agents (5,11).

The structures of the vast majority of benzophenanthridine alkaloids have in the past been proposed on the basis of their ^{1}H-nmr data and from nOe measurements (12). In this report we describe the structure elucidation of the new compound, 12-methoxydihydrochelerythrine [**1**], as well as detailed ^{1}H- and ^{13}C-nmr assignments for compounds **1–4**.



1 $R_1=R_2=R_4=OMe$, $R_3=H$
2 $R_1=R_2=OMe$, $R_3=R_4=H$
3 $R_1,R_2=-OCH_2O-$; $R_3=R_4=H$
4 $R_1,R_2=-OCH_2O-$; $R_3=OMe$; $R_4=H$

[1]Guest professor at the Department of Pharmacy ETH Zurich, 1 April–31 August 1990.

RESULTS AND DISCUSSION

Air-dried leaves of *B. integrifolia*, collected in Bolivia, were extracted with petroleum ether. From this extract four dihydrobenzo[*c*]phenanthridine alkaloids **1–4** were isolated using a combination of chromatographic methods.

Compound **1** was obtained as a slightly yellow powder. The molecular formula of $C_{22}H_{21}NO_5$ was deduced from ms (*m/z* $[M]^+$ 379) and ^{13}C-nmr spectroscopy. The presence of sixteen sp^2 carbon resonances in the ^{13}C-nmr spectrum of **1**, representing eight carbon-carbon double bonds, dictated the molecule to be pentacyclic. Uv maxima at 323 and 288 nm suggested the presence of an extended aromatic system, as did absorbances in the ir spectrum at 1625 and 1605 cm^{-1}. The ^1H-nmr spectrum of **1** contained resonances for three aromatic methoxy groups (δ 3.88, 3.93, 4.03), a methylenedioxy function (δ 6.05), an *N*-methyl group (δ 2.53), and a methylene adjacent to nitrogen (δ 4.27), as well as resonances for five aromatic protons consisting of three singlets (δ 7.05, 7.55, 7.65) and two doublets from a pair of ortho-coupled protons (δ 6.94, 7.48, $J = 8.5$ Hz). Comparison of these spectroscopic data with those for dihydrobenzo[*c*]phenanthridine alkaloids (12) revealed that **1** was structurally similar to dihydrochelerythrine [**2**]. The virtually identical ^1H- and ^{13}C-nmr data for rings A and B of **1** and **2** showed them to have the same substitution pattern in this region. The only difference between **1** and **2** was the presence of an additional methoxy group (δ 4.03, 55.7) in **1**. The coupling pattern for the aromatic protons in the ^1H-nmr spectrum of **1** indicated the methoxy group to be at either the C-11 or C-12 position. That the methoxy group was affixed at C-12 was deduced on the basis of the results of a 2D NOESY experiment performed on **1**. From this experiment (Figure 1) nOe's were evident between the proton at C-11 and both the three protons of the methoxy function at C-12 (δ 4.03) and the proton at C-10 (δ 7.48). Thus the methoxy function must reside at C-12, and compound **1** was therefore assigned as 12-methoxydihydrochelerythrine.

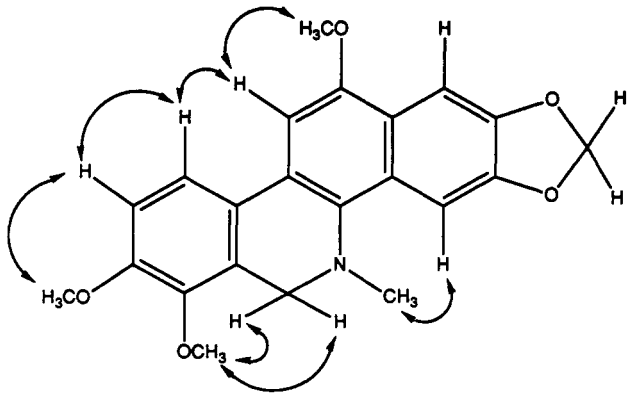


FIGURE 1. Observed nOe interactions for compound **1**.

The three remaining isolates were dihydrochelerythrine [**2**] (13,14,16,17), dihydrosanguinarine [**3**] (13–18), and dihydrochelirubine [**4**] (13,15, 18–20), all of which have been previously reported as natural products. The identification of these compounds was, however, not possible from the reported physical and spectroscopic data (12). Also, for compounds **2–4** there are no reported ^{13}C-nmr data. In an attempt to resolve these questions and to provide ^1H- and ^{13}C-nmr data for all four isolates, a series of 1D and 2D nmr experiments were undertaken. The 2D experiments included NOESY for compound **1** and ^{13}C-^1H correlation experiments for compounds **1–4**. Short-range ^{13}C-^1H 2D correlation experiments with carbon detection or in the inverse

TABLE 1. ^{1}H- and ^{13}C-nmr Data of Compounds **1–4**.

Carbon	Compound							
	1		**2**		**3**		**4**	
	δ ^{1}H[a] mult; (*J*, Hz)	^{13}C[b]	δ ^{1}H[a] mult; (*J*, Hz)	^{13}C[b]	δ ^{1}H[a] mult; (*J*, Hz)	^{13}C[b]	δ ^{1}H[a] mult; (*J*, Hz)	^{13}C[b]
C-1	7.55 s	99.5 d	7.11 s	104.3 d	7.11 s	104.4 d	7.10 s	104.1 d
C-2		148.5 s[c]		148.0 s[c]		148.1 s[c]		147.8 s[c]
C-3		147.2 s[c]		147.4 s[c]		147.5 s[c]		147.5 s[c]
C-4	7.65 s	100.7 d	7.67 s	100.7 d	7.68 s	100.8 d	7.69 s	100.6 d
C-4a		127.5 s[d]		126.3 s		126.5 s		126.4 s
C-4b		136.3 s		142.7 s		142.5 s		143.0 s
C-6	4.27 s	49.0 t	4.29 s	48.7 t	4.20 s	48.5 t	4.10 s	48.9 t
C-6a		126.6 s[e]		126.2 s		113.6 s		115.7 s
C-7		146.3 s		146.1 s		144.6 s		138.7 s
C-8		152.4 s		152.2 s		147.1 s		147.2 s
C-9	6.94 d (8.5)	110.9 d	6.94 d (8.5)	111.0 d	6.85 d (8.1)	107.2 d	6.61 s	94.4 d
C-10	7.48 d (8.5)	118.5 d	7.51 d (8.5)	118.6 d	7.30 d (8.1)	116.2 d		152.2 s
C-10a		126.5 s[e]		126.2 s		127.3 s		114.3 s
C-10b		124.2 s[d]		124.2 s		124.4 s		123.8 s
C-11	7.05 s	98.7 d	7.70 d (8.6)	120.1 d	7.69 d (8.5)	120.4 d	8.30 d (8.8)	124.7 d
C-12		152.4 s	7.48 d (8.6)	123.7 d	7.49 d (8.5)	124.0 d	7.46 d (8.8)	122.9 d
C-12a		122.4 s		130.8 s		130.8 s		130.3 s
-OCH_2O- (2,3)	6.05 s	101.0 t	6.04 s	101.0 t	6.05 s	101.0 t	6.04 s	100.9 t
-OCH_2O- (7,8)					6.03 s	101.3 t	6.00 s	101.4 t
N-Me	2.53 s	41.3 q	2.59 s	41.2 q	2.62 s	41.6 q	2.59 s	40.7 q
7-OMe	3.87 s	61.0 q	3.87 s	61.0 q				
8-OMe	3.93 s	55.9 q	3.92 s	55.8 q				
10-OMe							3.87 s	56.6 q
12-OMe	4.03 s	55.7 q						

[a] ^{1}H nmr recorded in $CDCl_3$ at 300 MHz; shifts in ppm.

[b] ^{13}C nmr recorded in $CDCl_3$ at 75.5 MHz; shifts in ppm; multiplicities by DEPT.

[c–e] Values in the same column with the same superscript may be interchanged.

mode permitted the association of all protons with the carbon atoms to which they were directly attached. The long-range ^{13}C-^{1}H 2D correlation experiments revealed connectivities between protons and carbons separated by one, two, or three bonds. Thus, we were able to assign unambiguously all ^{13}C- and ^{1}H-nmr resonances for the protonated carbons in compounds **1–4**. The quaternary carbons in **1–4** occur as either ring-junction carbons (C-4a, C-4b, C-6a, C-10a, C-10b, and C-12a) or sp^2 carbons substituted with oxygen (C-2, C-3, C-7, C-8, C-10, and C-12). Results obtained from the long-range ^{13}C-^{1}H correlation experiments allowed us to assign unambiguously all resonances of ring-junction carbons for compounds **2–4** and all resonances of oxygenated carbons in **1–4**, except for those of C-2 and C-3, which resonate in close proximity (Δ 0.3–0.6 ppm). The relevant ^{13}C-^{1}H correlations that allowed the assignments of quaternary carbon resonances for compound **3** are: H-4 (δ 7.68) correlates to C-12a (130.8 ppm), H-12 (δ 7.49) to C-4a (126.5 ppm) and C-10b (124.4 ppm), H-1 (δ 7.11) to C-4a (126.5 ppm), H-11 (δ 7.69) to C-4b (142.5 ppm) and C-12a (130.8 ppm), H-6 (δ 4.20) and *N*-methyl (δ 2.62 ppm) to C-4b (142.5 ppm). These correlations permit a clear distinction between the resonances of C-4a, C-4b, C-10b, and C-12a. Similarly the long-range ^{13}C-^{1}H correlations from H-6 (δ 4.20) to C-6a (113.6 ppm), C-7 (144.6 ppm), and C-10a (127.3 ppm); H-9 (δ 6.85) to C-7 (144.6 ppm) and C-10a (127.3 ppm); H-10 (δ 7.30) to C-6a (113.6 ppm) and C-8 (147.1 ppm); as well as H-11 (δ 7.69) to C-10a (127.3 ppm) allow the unambiguous assignment of the resonances for C-6a, C-7, C-8, and C-10a. Table 1 shows ^{1}H- and ^{13}C-nmr assignments for compounds **1–4**.

B. integrifolia is used in Bolivia by the Kallawayan Indians as a soporific and against infections (21). Therefore, we investigated antifungal and antibacterial activities of compounds **1–4** by tlc-bioautographic tests using *Penicillium oxalicum* (CBS 219.30), *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), and *Escherichia coli* (ATCC 25922) as test organisms (22). None of the test compounds exhibited antibacterial or antifungal activity at the tested concentration (25 μg).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a Perkin-Elmer 141 polarimeter using $CHCl_3$ as solvent. Ir spectra were recorded on a Perkin-Elmer 781 infrared spectrometer. Uv spectra were recorded on a Perkin-Elmer Lambda 3 uv/vis spectrophotometer. Eims were recorded on a Hitachi-Perkin-Elmer RMU-6M mass spectrometer. All nmr measurements were performed with a Bruker AMX-300 spectrometer. All samples prepared for nmr measurement were made as $CDCl_3$ solutions with TMS (δ 0) as the internal standard. Short-range ^{13}C-^{1}H 2D correlation experiments with carbon detection (Bruker hxco) or in the inverse mode (Bruker invbtp with GARP decoupling) were performed with the relevant delays optimized for J_{XH} 136 Hz, while long-range ^{13}C-^{1}H 2D correlation experiments (Bruker hxco) were performed with the relevant delays optimized for J_{XH} 10 and 15 Hz. Hplc was carried out with a Waters 6000A solvent delivery system connected to a Rheodyne hplc injector and a Perkin-Elmer LC-55 uv/vis spectrometer (detection at 285 nm). Hplc columns were from Knauer (250 mm × 4 mm and 250 mm × 16 mm, LiChrosorb Si60, 5 μm). Vacuum liquid chromatography (vlc) was carried out with a vlc column (200 mm × 65 mm) and 250 g silica (tlc-Silica 60HF 15 μm, Merck). Medium-pressure liquid chromatography (mplc) was performed with columns from Büchi (800 mm × 36 mm), 550 g silica (tlc-Silica 60HF 15 μm, Merck), and a Lewa pump (Lab M5). Solvent systems for mplc were optimized by over pressure liquid chromatography (oplc) with Chrompres 10 (23). Sephadex LH-20 (Pharmacia) 250 g was used for gel chromatography (column 800 mm × 36 mm). Aluminium-backed plates coated with Si gel 60 F_{254} (0.2 mm thick, Merck) were used for tlc.

PLANT MATERIAL.—Leaves of *B. integrifolia* were collected in September 1989 in the Yungas valleys of Bolivia and air-dried at an altitude of 4000 m in the dark, at room temperature (22°) and an atmospheric humidty of 30%. Voucher specimens are deposited at the National Herbarium of Bolivia in La Paz and at the Department of Pharmacy, ETH Zurich.

EXTRACTION AND ISOLATION.—The dried leaves (1 kg) were extracted successively with petroleum ether (5 liters), CH_2Cl_2 (5 liters), EtOAc (5 liters), and MeOH (5 liters). The petroleum ether extract

(41 g) was fractionated by vlc (24) over Si gel using hexane–methyl *t*-butyl ether (95:5) (2 liters), (80:20) (1 liter), (70:30) (1 liter), (50:50) (1 liter), and (0:100) (1 liter). Ten fractions, each 500 ml, were collected and examined by tlc. Fractions 2–7 contained alkaloids, as indicated by a positive Dragendorff reaction. The combined fractions 6 and 7 from the vlc were separated on Sephadex LH-20 using MeOH as eluent. The alkaloid-containing fractions were combined and purified by hplc using CH_2Cl_2–methyl *t*-butyl ether–hexane (16.5:1.5:60). This afforded 22 mg of **1** and 10 mg of **2** as slightly yellow powders. Separation of combined fractions 4 and 5 from the vlc using Sephadex LH-20 and hplc with the same eluents mentioned above afforded a further 64 mg of **2**. Mplc separation of combined fractions 2 and 3 from the vlc, using Si gel as adsorbent with CH_2Cl_2-hexane (75:25) as eluent, yielded compound **3** (40 mg) and compound **4** (18 mg) as white amorphous powders. Separation of other mplc fractions containing both **3** and **4** using hplc on Si gel with methyl *t*-butyl ether–hexane (2.5:97.5) as eluent yielded a further 52 mg of **3** and 24 mg of **4**.

12-Methoxydihydrochelerythrine [**1**].—Compound **1** (22 mg, 0.002% w/w): $[\alpha]^{20}D -15°$ ($c = 0.2$, $CHCl_3$); ir ν max (KBr) cm^{-1} 2920, 2846, 1625, 1605, 1495, 1464, 1272, 1247, 1220, 1072, 1039; uv λ max (EtOH) nm (log ϵ) 219 (4.68), 288 (4.70), 323 (4.24), 355 sh; ^1H and ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 379 (100), 378 (23), $[M-Me]^+$ 364 (71), 363 (15), 349 (8), 348 (9), 334 (7), 320 (10), 306 (9), 190 (8), 182 (9), 175 (6), 160 (6), 152 (6).

Dihydrochelerythrine [**2**].—Compound **2** (74 mg, 0.007% w/w): $[\alpha]^{20}D -10°$ ($c = 0.2$, $CHCl_3$); ir ν max (KBr) cm^{-1} 2920, 2850, 1598, 1463, 1270, 1247, 1218, 1078, 1040; uv λ max (EtOH) nm (log ϵ) 228 (4.64), 282 (4.75), 318 (4.26), 348 sh; ^1H and ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 349 (100), 348 (85), $[M-Me]^+$ 334 (17), 333 (14), 332 (12), 319 (15), 318 (15), 304 (11), 290 (19), 276 (9), 174 (8).

Dihydrosanguinarine [**3**].—Compound **3** (92 mg, 0.009% w/w): $[\alpha]^{20}D -2°$ ($c = 0.2$, $CHCl_3$); ir ν max (KBr) cm^{-1} 2883, 1462, 1253, 1038; uv λ max (EtOH) nm (log ϵ) 236 (4.65), 285 (4.67), 322 (4.28), 334 sh, 347 sh; ^1H and ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 333 (100), 332 (70), $[M-Me]^+$ 318 (9), 317 (8), $[M-CH{=}O]^+$ 304 (3), 274 (4), 260 (4).

Dihydrochelirubine [**4**].—Compound **4** (42 mg, 0.004% w/w): $[\alpha]^{20}D -20°$ ($c = 0.2$, $CHCl_3$); ir ν max (KBr) cm^{-1} 2884, 1640, 1615, 1462, 1242, 1060, 1040; uv λ max (EtOH) nm (log ϵ) 231 (4.64), 278 (4.55), 336 (4.28), 350 sh; ^1H and ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 363 (100), 362 (34), $[M-Me]^+$ 348 (40), 332 (7), 320 (4), 181 (10).

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