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SHORT REPORTS

^{1}H AND ^{13}C NMR ASSIGNMENTS OF ISOQUINOLINE ALKALOIDS*

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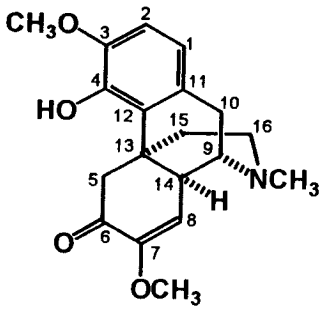
ABSTRACT

Use of two-dimensional NMR techniques, including the homonuclear COSY, NOESY, HETCOR and COLOC experiments, to unequivocally assign the ^{1}H and ^{13}C NMR data of the isoquinoline alkaloids (-)-salutaridine, (-)-dicentrine and (-)-tetrahydropalmatine is demonstrated.

INTRODUCTION

The isoquinoline alkaloids constitute an important group of tyrosine-derived natural products, possessing a diversity of structural type and functionality, and an amazingly wide range of biological activity.[2] Pharmacological properties of these naturally occurring bases can be illustrated, for instance, by the immunosuppressive activity of sinomenine (**1**),[3] an alkaloid structurally closely related to (-)-salutaridine (**2**), the anticancer potential of (-)-dicentrine (**3**),[4,5] and the sedative effect of (-)-tetrahydropalmatine (**4**).[6] These biologically significant compounds, being a promising source of natural product-based medicinal agents, have attracted interest from researchers in several disciplines. In the present investigation, compounds **2**, **3**, and **4** have been selected as representatives of the morphinandienone, aporphine, and tetrahydroprotoberberine alkaloids for detailed NMR studies. We demonstrate herein the utiltization of several NMR techniques, including the homonuclear COSY, NOESY, APT, HETCOR and COLOC, for the definitive NMR assignment of these alkaloids.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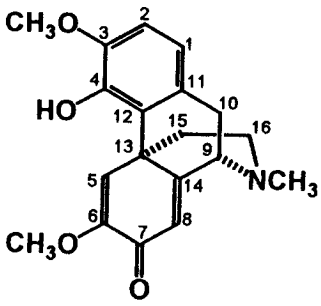
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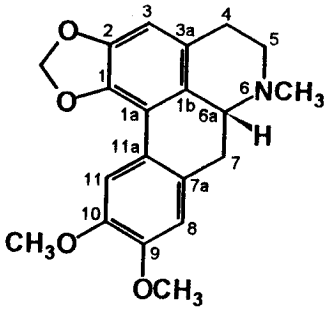
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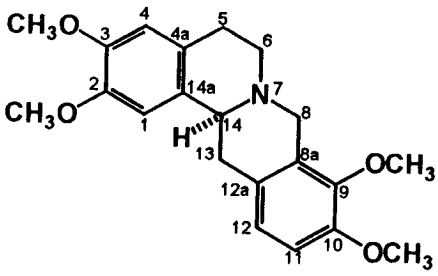
3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4

MATERIALS AND METHODS

Plant Material and Isolation: (-)-Salutaridine (**2**), (-)-dicentrine (**3**), and tetrahydropalmatine (**4**) were obtained from the tubers of *Stephania pierrei* Diels.[4,5]

(-)-Salutaridine, **2**. M.p. 191°C; $[\alpha]^{20}_D$ -65° (c 0.4, EtOH); UV λ_{max} (MeOH) 208 (log ε 4.34), 240 (4.09), 279 (3.61) nm; IR ν_{max} (film) 3349, 2934, 1669, 1643, 1485, 1283, 1213, 1067 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; MS EI m/z (rel. int.) 327 (M^+, 100), 313 (11), 312 (34), 299 (41), 285 (10), 284 (44), 268 (14), 256 (11), 242 (14), 226 (11).

(-)-Dicentrine, **3**. M.p. 170° C; $[\_]^{20}_D$ -60° (c 0.2, $CHCl_3$); UV λ_{max} (MeOH) 220 (log ε 4.57), 230 (4.52), 270 (4.12), 281 (4.25), 304 (4.28) nm; IR ν_{max} (KBr) 2928, 1605, 1520, 1458, 1271, 1100 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; MS EI m/z (rel. int.) 339 (M^+, 70), 338 (100), 324 (10), 307 (11), 296 (26), 265 (11).

(-)-Tetrahydropalmatine, **4**. M.p. 139° C; $[\alpha]^{20}_D$ -292° (c 0.2, EtOH); UV λ_{max} (MeOH) 208 (log ε 4.42), 227 (3.99), 282 (3.42) nm; IR ν_{max} (KBr) 3004, 2816, 1609, 1510, 1495, 1258, 1107, 1024 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; MS EI m/z (rel int.) 355 (M^+, 100), 354 (64), 324 (18), 190 (19), 165 (18), 164 (42), 149 (44).

Instrumentation: Melting points were determined on a Kofler hot plate and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Beckman DU-7 spectrometer and IR spectra measured on a Nicolet MX-1 FT-IR interferometer. ^1H NMR, homonuclear COSY, ^{13}C NMR, APT and HETCOR spectra were recorded in $CDCl_3$, with TMS as internal standard, employing a Varian XL-300 instrument. Standard Varian pulse sequences were used. The COLOC experiments were carried out at 75.6 MHz on a Varian XL-300 nmr spectrometer, using a relaxation delay 1.0 sec, acquisition time 0.112 sec, Δ_1 = 25 msec, Δ_2 = 30 msec. Mass spectra were obtained with a Varian MAT 112S instrument operating at 70 eV.

RESULTS AND DISCUSSION

The structure of (-)-salutaridine (**2**) (sinoacutine)[7], an alkaloid known to be a key intermediate for the biosynthesis of sinomenine (**1**),[8] has been well established through NMR studies and syntheses;[9-11] however, complete ^1H and ^{13}C NMR assignments of **2** have not been reported. In this study, a combination of the homonuclear COSY and NOESY experiments was used to obtain unequivocal ^1H NMR assignments of **2** (Table 1). Distinction between H-1 (δ 6.62, d, J = 8.3 Hz) and H-2 (δ 6.71, d, J = 8.3 Hz) was based on the NOE contour displayed between H-2 and 3-OCH_3 (δ 3.85) in the NOESY spectrum. Similarly, the downfield proton resonance at δ 7.62 (s) was assigned to H-5 according to its NOE with 6-OCH_3 (δ 3.72), leaving the signal at δ 6.29 (s) to be assigned to H-8. From the COSY spectrum, the aliphatic methine proton at δ 3.66 (d, J = 5.1 Hz) could be assigned to H-9, and this was corroborated by the NOE between H-8 and H-9. Assignment of the H_2-10, H_2-15, and H_2-16 methylene protons was performed by examination of the corresponding geminal and vicinal couplings in the COSY spectrum. The NOESY spectrum

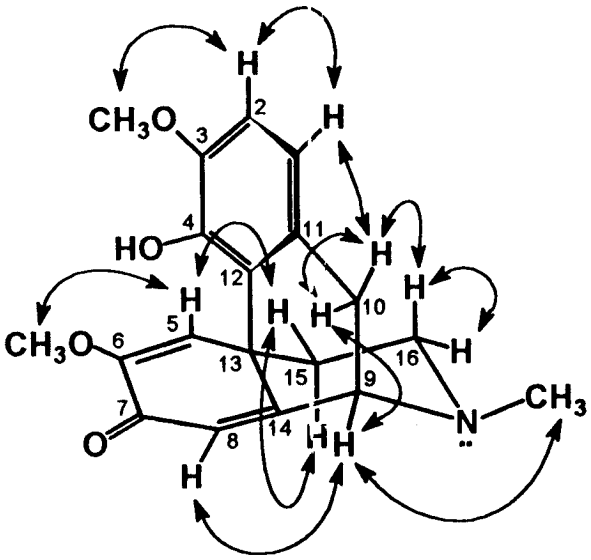

Fig.1. NOEs observed for (-)-saluaridine(**2**).

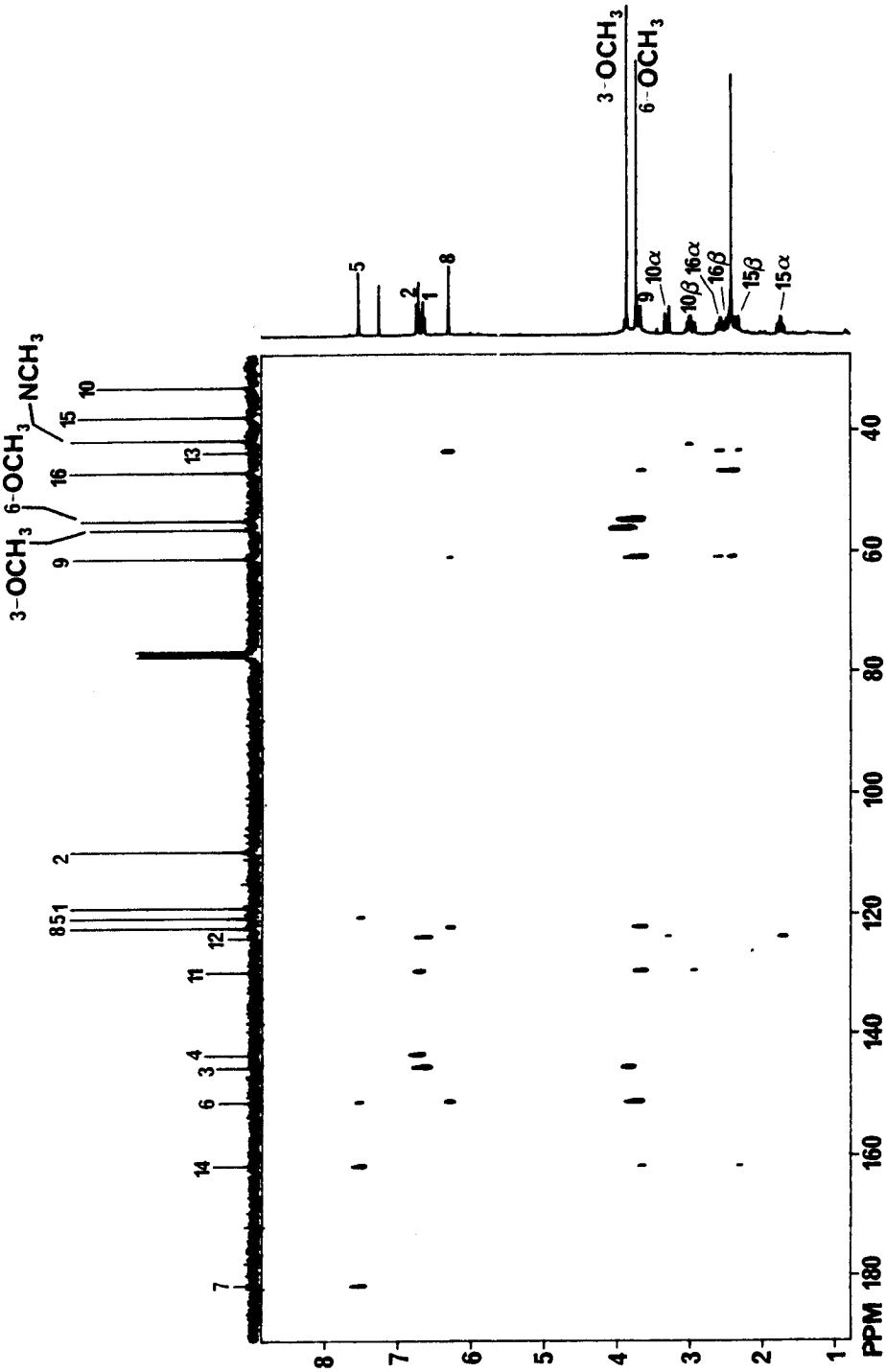

Fig.2. COLOC spectrum of (-)-salutaridine (**2**).

TABLE 1. ^{1}H and ^{13}C NMR Assignments of (-)-Salutaridine (**2**)[a].

Atom	^{1}H[b]	^{13}C
1	6.62 (d, 8.3)	119.66
2	6.71 (d, 8.3)	110.36
3		146.22
4		144.19
5	7.52 (s)	121.37
6		151.82
7		182.27
8	6.29 (s)	123.04
9	3.66 (d, 5.1)	61.88
10α	3.30 (d, 17.1)	33.55
β	2.97 (dd, 17.1, 5.1)	
11		130.58
12		124.81
13		44.50
14		162.36
15	1.71 (ddd, 12.7, 12.7, 4.6)	38.53
–	2.33 (ddd, 12.7, 2.7, 1.8)	
16	2.56 (ddd, 12.7, 4.6, 2.7)	47.82
–	2.45 (ddd, 12.7, 12.7, 1.8)	
3-OCH_3	3.85 (s)	57.13
6-OCH_3	3.72 (s)	55.66
NCH_3	2.41 (s)	42.42

[a] Recorded at 300 MHz for ^{1}H and 75.6 MHz for ^{13}C; chemical shifts are reported in ppm (_) from TMS in $CDCl_3$.

[b] Signal multiplicity and coupling constants are in parentheses.

TABLE 2. ^{1}H and ^{13}C NMR Assignments of (-)-Dicentrine (**3**)[a]

Atom	^{1}H[b]	^{13}C
1		141.70
1a		116.53
1b		126.45
2		146.53
3	6.48 (s)	106.68
3a		126.61
4 ax.	3.07 (m)	29.19
eq.	2.58 (m)	
5 ax.	2.45 (m)	53.53
eq.	3.01 (m)	
6a		3.10 (m) 62.34
7 ax.	2.63 (m)	34.22
eq.	3.08 (m)	
7a		128.37
8	6.76 (s)	111.29
9		148.24
10		147.67
11	7.64 (s)	110.61
11a		123.54
OCH_2O	5.89 (d, 1.4)	100.51
	6.04 (d, 1.4)	
9-OCH_3	3.88 (s)	55.82
10-OCH_3	3.89 (s)	56.06
NCH_3	2.52 (s)	43.87

[a] Recorded at 300 MHz for ^{1}H and 75.6 MHz for ^{13}C; chemical shifts are reported in ppm (_) from TMS in $CDCl_3$.

[b] Signal multiplicity and coupling constants are in parentheses.

TABLE 3. ^{1}H and ^{13}C NMR Assignments of (-)-Tetrahydropalmatine (**4**)[a]

Atom	^{1}H[b]	^{13}C
1	6.70 (s)	108.27
2		147.13
3		147.13
4	6.58 (s)	111.03
4a		126.51
5_	2.65 (m)	28.92
_	3.11 (m)	
6_	2.59 (m)	51.32
_	3.17 (m)	
8_	3.50 (d, 15.5)	53.80
_	4.21 (d, 15.5)	
8a		128.42
9		144.73
10		149.97
11	6.75 (d, 8.3)	110.61
12	6.84 (d, 8.3)	123.64
12a		127.45
13_	3.23 (dd, 15.5, 3.6)	36.16
_	2.79 (dd, 15.5, 11.5)	
14	3.49 (dd, 11.5, 3.6)	59.09
14a		129.43
2-OCH_3	3.85 (s)	55.82
3-OCH_3	3.83 (s)	55.60
9-OCH_3	3.82 (s)	59.93
10-OCH_3	3.80 (s)	55.60

[a] Recorded at 300 MHz for ^{1}H and 75.6 MHz for ^{13}C; chemical shifts are reported in ppm (δ) from TMS in $CDCl_3$.

[b] Signal multiplicity and coupling constants are in parentheses.

also displayed NOE contours between H-5 and H-15β, and H-9 and H-10β, thereby confirming the assignments of these protons. In addition, steric proximity between H-1 and H-10_, and between H-10β and H-16β, was reflected by their respective NOE contours in the NOESY spectrum. A summary of NOEs of **2** is shown in Figure 1.

Unambiguous assignment of the ^{13}C NMR resonances of **2** was accomplished by examination of the APT, HETCOR, and COLOC[12] spectra. The resonances of all of the protonated carbons were readily assigned through interpretation of the APT and HETCOR spectra. With the aid of the COLOC spectrum (Figure 2), all quaternary carbon signals could be unequivocally assigned. The resonance at δ 146.22 was assigned to C-3 based on its 3-bond coupling with H-1, whereas that at δ 144.19 was assigned to C-4 because of its 3-bond correlation with H-2. The assignment of C-3 was also supported by its 3-bond coupling with the 3-OCH_3 protons. On the other hand, the carbon at δ 151.82 displayed $^3J_{CH}$ correlation with the 6-OCH_3, and was therefore assigned to C-6. The resonance at δ 182.27 was assigned to C-7, and this assignment was supported by the 3-bond coupling between H-5 and C-7. The 3-bond coupling between the carbon at δ 130.58 and H-9 allowed this carbon to be assigned to C-11. The signal at δ 124.81 was assigned to C-12 from its 3-bond coupling with H-10_ and with H-15α. The resonance at δ 44.50, showing $^3J_{CH}$ couplings with H-8 and H-16α, was assigned to C-13. The last quaternary carbon was assigned to C-14 according to its 3-bond couplings with H-5 and H-15β, and this completed the ^{13}C NMR assignment of (-)-salutaridine (**2**) (Table 1).

The ^1H and ^{13}C NMR assignments of (-)-dicentrine (**3**) and (-)-tetrahydropalmatine (**4**) were obtained in a similar manner. The results from anaysis of the HETCOR spectrum in conjunction with the COLOC spectrum suggested a revision for the previous assignments of C-8 and C-11 of (-)-dicentrine (**3**)[13] (Table 2). In the case of (-)-tetrahydropalmatine (**4**), the earlier assignments of C-4a, C-8a, C-12a, C-9, and C-10[14] were revised (Table 3).

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