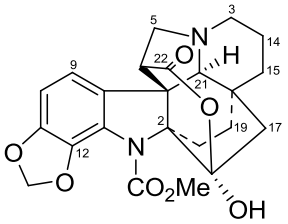
CHAPTER TWO

RESULTS AND DISCUSSION

2.1 Alkaloids from *Kopsia pauciflora*

The alkaloidal composition of *K. pauciflora* Hook f., a species found in Sabah was reinvestigated. Only a preliminary small-scale study of the stem-bark extract was carried out previously.[110] The present investigation of the alkaloidal content of the stem-bark of *K. pauciflora* provided a total of 40 alkaloids of which 12 are new. The new alkaloids isolated are made up of seven aspidofractinine alkaloids (**1**, **2**, **3**, **4**, **5**, **6**, **7**), and five eburnane alkaloids (**8**, **9**, **10**, **11**, **12**). The known alkaloids are tetrahydroalstonine (**13**), leuconoxine (**14**), *N*(1)-carbomethoxy-5,22-dioxokopsane (**15**), kopsanone (**16**), kopsifine (**17**), decarbomethoxykopsifine (**18**), paucidactine B (**19**), kopsamine (**20**), kopsamine *N*-oxide (**21**), kopsinine (**22**), *N*(1)-methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine (**23**), *N*(1)-methoxycarbonyl-12-hydroxy-$\Delta^{16,17}$-kopsinine (**24**), kopsinine *N*-oxide (**25**), *N*(1)-methoxycarbonyl-11,12-dimethoxykopsinaline (**26**), kopsilongine (**27**), pleiocarpine (**28**), 12-methoxypleiocarpine (**29**), pleiocarpine *N*-oxide (**30**), (+)-eburnamenine (**31**), (+)-eburnamonine (**32**), (–)-eburnamine (**33**), (+)-isoeburnamine (**34**), (+)-19-oxoeburnamine (**35**), (–)-19(*R*)-hydroxyisoeburnamine (**36**), (+)-19(*R*)-hydroxyeburnamine (**37**), (–)-norpleiomutine (**38**), (–)-demethylnorpleiomutine (**39**), and (+)-kopsoffinol (**40**). Kopsinine (**22**) is the major alkaloid present in the stem-bark. The alkaloidal composition is summarized in Table 2.1.



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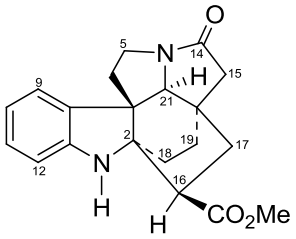

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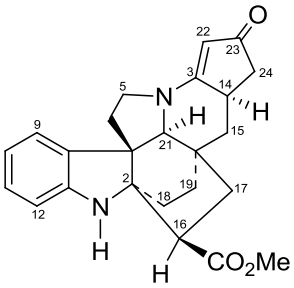

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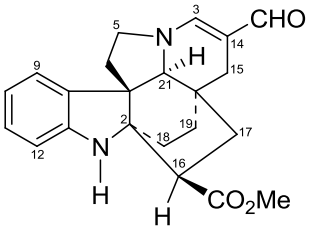

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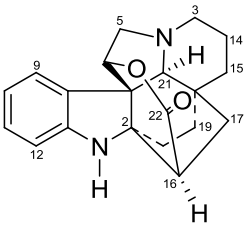

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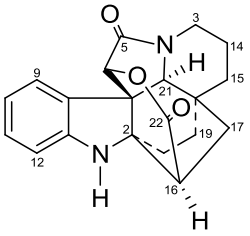

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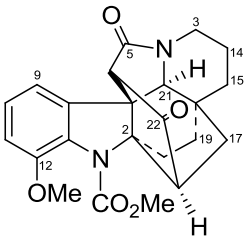

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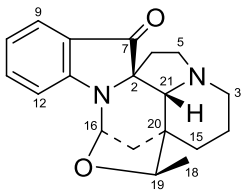

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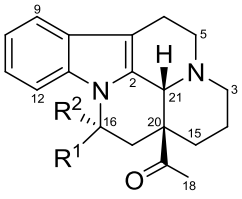
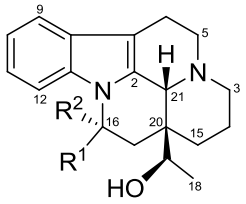
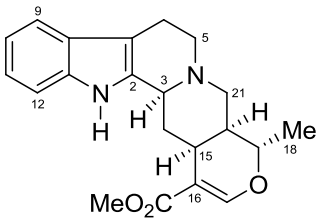


9 R^1, R^2 = O
10 R^1 = H, R^2 = OH



11 R^1 = H, R^2 = nil, $\Delta^{16,17}$
12 R^1 = H, R^2 = OEt



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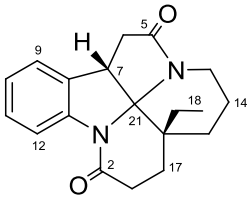

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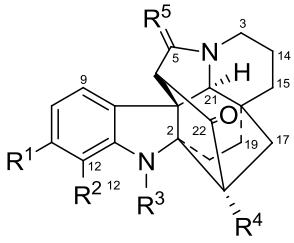
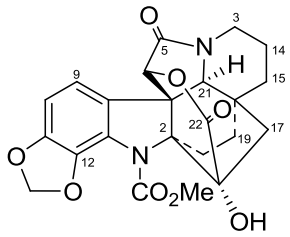


15 $R^1 = R^2 = R^4 = H$, $R^3 = CO_2Me$, $R^5 = O$
16 $R^1 = R^2 = R^3 = H$, $R^4 = OH$, $R^5 = H,H$
17 $R^1, R^2 = OCH_2O$, $R^3 = CO_2Me$, $R^4 = OH$, $R^5 = O$
18 $R^1, R^2 = OCH_2O$, $R^3 = H$, $R^4 = OH$, $R^5 = O$



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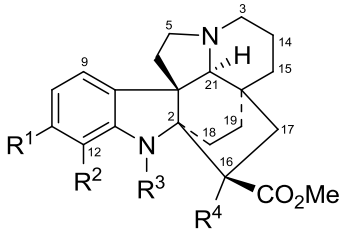
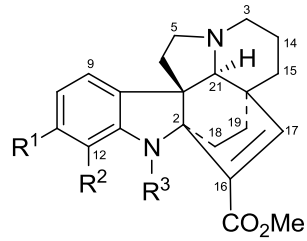
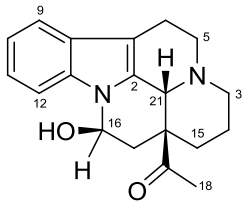


20 $R^1, R^2 = OCH_2O$, $R^3 = CO_2Me$, $R^4 = OH$
21 $R^1, R^2 = OCH_2O$, $R^3 = CO_2Me$, $R^4 = OH$, $N(4)\rightarrow O$
22 $R^1 = R^2 = R^3 = R^4 = H$
25 $R^1 = R^2 = R^3 = R^4 = H$, $N(4)\rightarrow O$
26 $R^1 = R^2 = OMe$, $R^3 = CO_2Me$, $R^4 = OH$
27 $R^1 = H$, $R^2 = OMe$, $R^3 = CO_2Me$, $R^4 = OH$
28 $R^1 = R^2 = R^4 = H$, $R^3 = CO_2Me$
29 $R^1 = R^4 = H$, $R^2 = OMe$, $R^3 = CO_2Me$
30 $R^1 = R^2 = R^4 = H$, $R^3 = CO_2Me$, $N(4)\rightarrow O$



23 $R^1 = H$, $R^2 = OMe$, $R^3 = CO_2Me$
24 $R^1 = H$, $R^2 = OH$, $R^3 = CO_2Me$



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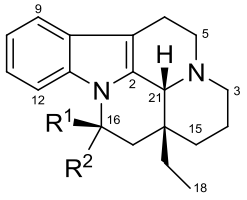
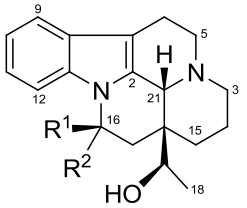
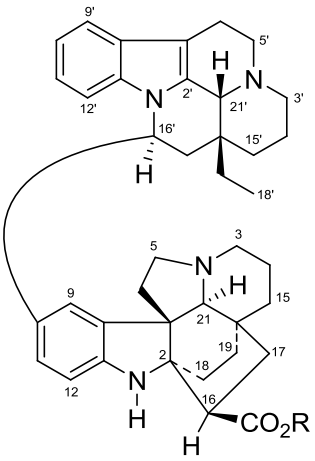


31 $R^1 = H$, $R^2 = nil$, $\Delta^{16,17}$
32 $R^1, R^2 = O$
33 $R^1 = OH$, $R^2 = H$
34 $R^1 = H$, $R^2 = OH$



36 $R^1 = H$, $R^2 = OH$
37 $R^1 = OH$, $R^2 = H$



38 R = Me
39 R = H

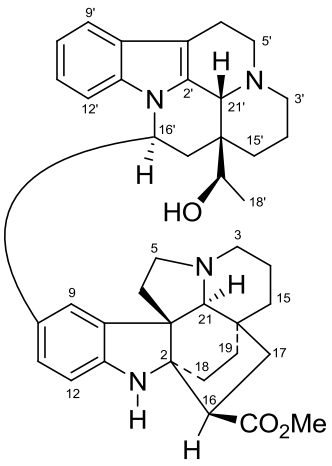


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Table 2.1 : Alkaloid composition of *K. pauciflora*

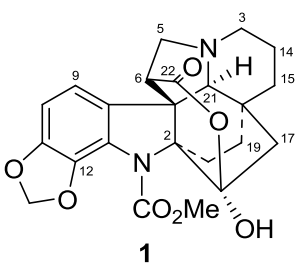
Plant part	Alkaloid	Yield (g Kg^{-1})
Stem-bark	Compound **1** [New]	0.0004
	Compound **2** [New]	0.0005
	Compound **3** [New]	0.0004
	Compound **4** [New]	0.0013
	Compound **5** [New]	0.0004
	Compound **6** [New]	0.0004
	Compound **7** [New]	0.0006
	Compound **8** [New]	0.0004
	Compound **9** [New]	0.0005
	Compound **10** [New]	0.0018
	Compound **11** [New]	0.0007
	Compound **12** [New]	0.0005
	Tetrahydroalstonine (**13**)	0.0030
	Leuconoxine (**14**)	0.0006
	N(1)-Carbomethoxy-5,22-dioxokopsane (**15**)	0.0013

Table 2.1, continued

Plant part	Alkaloid	Yield (g Kg^{-1})
	Kopsanone (**16**)	0.0005
	Kopsifine (**17**)	0.0017
	Decarbomethoxykopsifine (**18**)	0.0021
	Paucidactine B (**19**)	0.0008
	Kopsamine (**20**)	0.0747
	Kopsamine *N*-oxide (**21**)	0.0333
	Kopsinine (**22**)	0.4218
	N(1)-Methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine (**23**)	0.0799
	N(1)-Methoxycarbonyl-12-hydroxy-$\Delta^{16,17}$-kopsinine (**24**)	0.0006
	Kopsinine *N*-oxide (**25**)	0.0459
	N(1)-Methoxycarbonyl-11,12-dimethoxykopsinaline (**26**)	0.0031
	Kopsilongine (**27**)	0.0004
	Pleiocarpine (**28**)	0.0009
	12-Methoxypleiocarpine (**29**)	0.0029
	Pleiocarpine *N*-oxide (**30**)	0.0007
	(+)-Eburnamenine (**31**)	0.0028
	(+)-Eburnamonine (**32**)	0.0025
	(–)-Eburnamine (**33**)	0.0803
	(+)-Isoeburnamine (**34**)	0.0773
	(+)-19-oxoeburnamine (**35**)	0.0047
	(–)-19(*R*)-Hydroxyisoeburnamine (**36**)	0.0034
	(+)-19(*R*)-Hydroxyeburnamine (**37**)	0.0256
	(–)-Norpleiomutine (**38**)	0.1765
	(–)-Demethylnorpleiomutine (**39**)	0.0104
	(+)-Kopsoffinol (**40**)	0.0452

2.1.1 Compound 1

Compound **1** was isolated in minute amount as a light yellowish amorphous solid, and subsequently crystallized from CH_2Cl_2–hexanes as colorless block crystals, mp 206–208 ^oC, $[\alpha]^{25}_D$ +30 (*c* 0.15, $CHCl_3$). The UV spectrum showed absorption maxima at 210, 224, 250, and 289 nm typical of a dihydroindole chromophore. The IR spectrum showed bands at 3240, 1734, and 1685 cm^{-1} suggesting the presence of hydroxyl, lactone, and carbamate functions, respectively. The ESIMS of **1** showed a quasi molecular ion at *m/z* 441, and HRESIMS measurements established the molecular formula as $C_{23}H_{24}N_2O_7$ + H. The ^{13}C NMR spectrum showed a total of 23 resonances, comprising one methyl, eight methylene, four methine, and ten quaternary carbon atoms, in agreement with the molecular formula. The ^1H and ^{13}C NMR spectroscopic data (Table 2.2) showed the presence of a methylenedioxy substituent at C(11) and C(12), a hydroxyl group, a carbamate function at N(1), and a lactone group. In addition, analysis of the NMR spectral data indicated that **1** was an aspidofractinine-type alkaloid. The NMR data of **1** resembled that of paucidactine B (**19**)[107] which was also isolated from this plant, except for the absence of the lactam carbonyl at C(5), and the lactone carbonyl function, which was linked to C(6) instead of C(16). This was in accord with the observed carbon resonance of C(16) at δ 102.4, its downfield shift due to it being linked to both the two oxygen atoms. In addition, the observed carbon resonances of C(5) and C(6) at δ 54.4 and δ 51.2, respectively, confirmed the absence of the lactam carbonyl at C(5).



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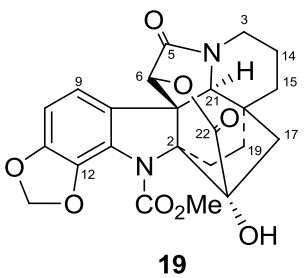

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This was further supported by a detailed analysis of the spectral data from COSY, HMQC, and HMBC experiments. The COSY and HMQC spectral data disclosed the presence of four partial structures *i.e.*, $NCH_2CH_2CH_2$, CHCH, NCH_2CH, and CH_2CH_2, corresponding to the N–C(3)–C(14)–C(15), C(9)–C(10), N–C(5)–C(6), and C(18)–C(19) units, respectively. In addition, the three-bond correlation from H(5) to C(22) confirmed the position of the lactone carbonyl function (Figure 2.1). The HMBC spectrum was in complete agreement with the proposed structure of **1** (Figure 2.1), which was also confirmed by X-ray diffraction analysis (Figure 2.2). A possible biogenetic pathway to **1** from 11,12-methylenedioxykopsine (**226**)[41] is shown in Scheme 2.1.

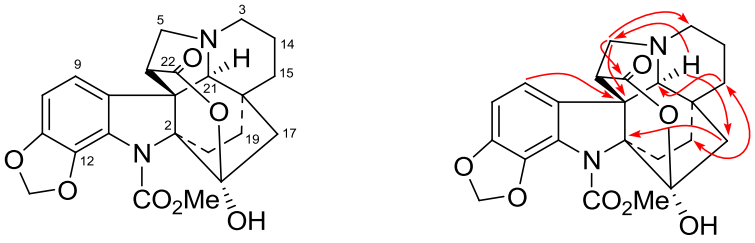


Figure 2.1 : Selected HMBCs of **1**

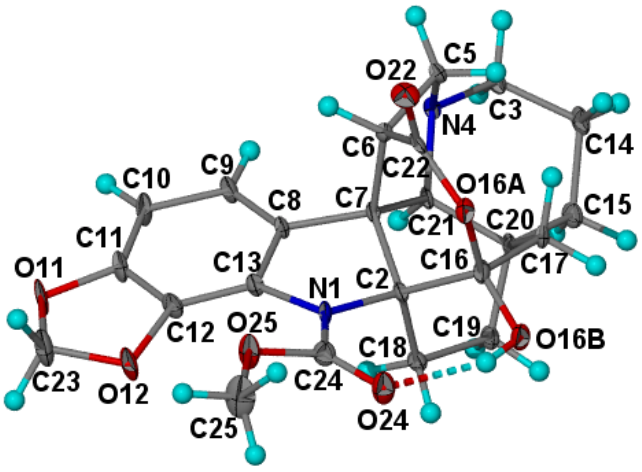
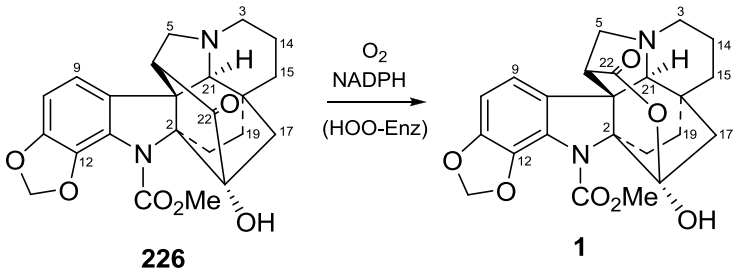


Figure 2.2 : X-ray crystal structure of **1**. Thermal ellipsoids are shown at the 50% probability level.



Scheme 2.1 : A possible biogenetic pathway to **1**

Table 2.2 : ^{1}H and ^{13}C NMR spectroscopic data of compound **1**[a]

Position	δ_H	δ_C
2	–	72.3
3	2.92 m	46.7
	3.01 m	
5	3.33 m	54.4
	3.55 t (10)	
6	3.16 m	51.2
7	–	56.2
8	–	132.8
9	6.82 d (7.5)	115.1
10	6.59 d (7.5)	105.3
11	–	149.1
12	–	135.7
13	–	122.6
14	1.26 m	15.9
	1.75 m	
15	1.19 m	32.5
	1.52 m	
16	–	102.4
17	1.89 d (14.5)	49.2
	2.50 dd (15.4, 3.6)	
18	1.61 m	22.3
	2.29 m	
19	1.33 m	34.4
	1.61 m	
20	–	33.1
21	3.03 s	68.3
22	–	171.0
N*C*O_2Me	–	154.9
NCO_2*Me*	3.83 s	53.9
16-OH	8.74 s	–
OCH_2O	5.90 d (1.4)	100.8
	5.93 d (1.4)	

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

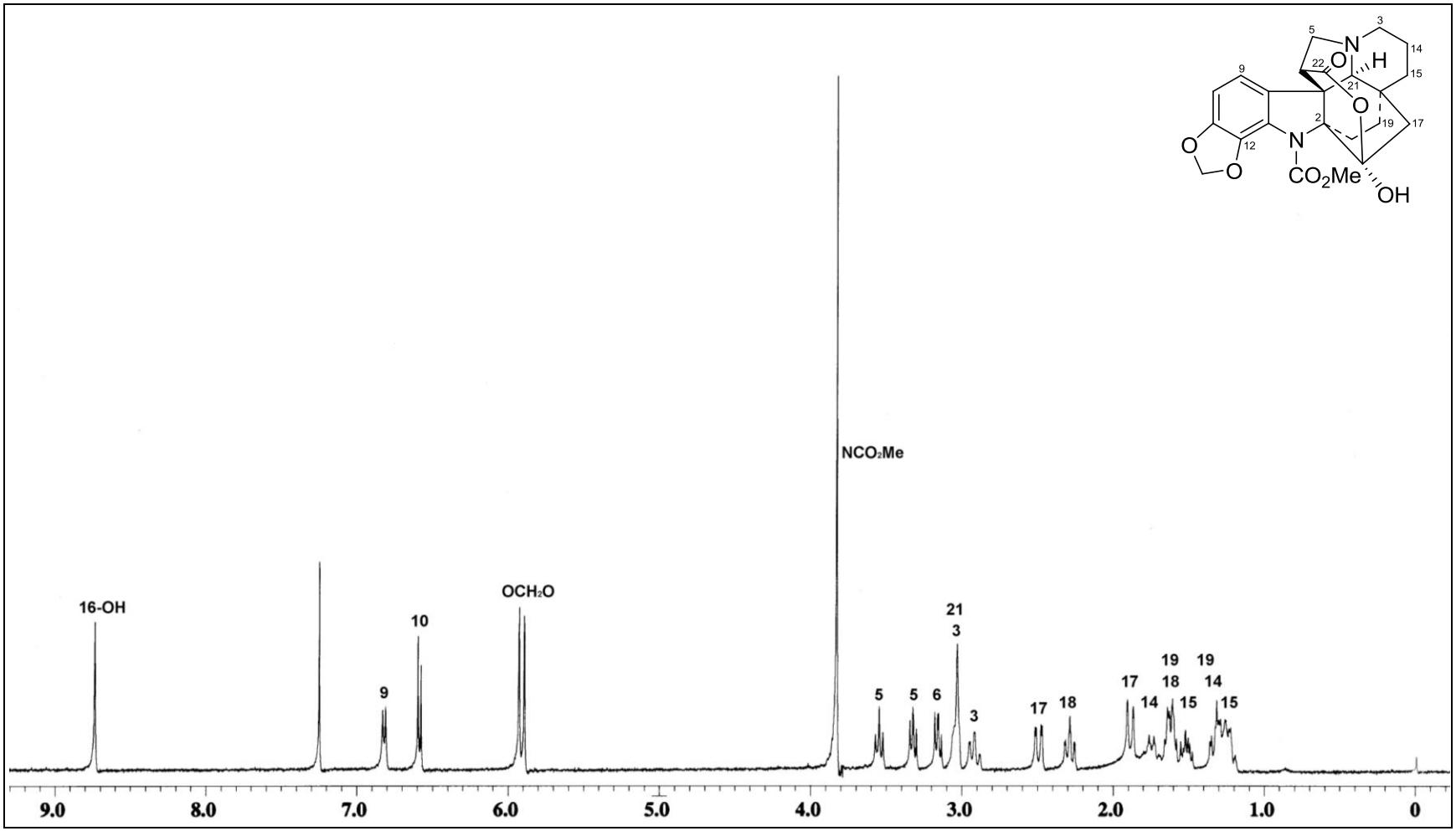
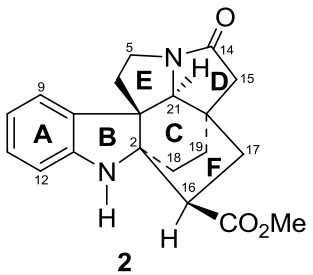


Figure 2.3 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **1**

2.1.2 Compound 2

Compound **2** was isolated as a light yellowish oil, and subsequently crystallized from CH_2Cl_2–MeOH as colorless block crystals, mp 180–182 °C, $[\alpha]^{25}_D$ –22 (*c* 0.13, $CHCl_3$). The UV spectrum showed absorption maxima at 209, 255, and 291 nm due to a dihydroindole chromophore, while the IR spectrum showed bands indicating the presence of NH (3344 cm^{-1}), ester (1729 cm^{-1}), and lactam (1694 cm^{-1}) functionalities. The ESIMS of **2** showed a quasi molecular ion at *m/z* 339, which analyzed for $C_{20}H_{22}N_2O_3$ + H. The ^1H and ^{13}C NMR spectral data of **2** (Table 2.3) showed features typical of aspidofractinine alkaloids. Notable features included the presence of an unsubstituted aromatic moiety (δ 6.70–7.08), absence of substitution on the indolic nitrogen from the observed NH signal at δ 3.91, an isolated methine singlet at δ 3.82, and a carbomethoxy group at δ 3.71 in the ^1H NMR data (Table 2.3). The ^{13}C NMR spectrum gave a total of 20 resonances (one methyl, six methylene, six methine, and seven quaternary carbons) in agreement with the molecular formula. The quaternary carbon resonance, which was observed at δ 173.2, was assigned to an ester carbonyl, in agreement with the IR spectrum. Comparison with the *Kopsia* alkaloid, kopsinine (**22**),[35] which was found in this plant, showed a close correspondence of the NMR spectral data. The main difference in **2** compared to **22** is the absence of a carbon resonance corresponding to C(3), indicating the presence of a five-membered lactam ring D in **2**. The presence of the lactam function was confirmed by the observed carbon resonance of C(14) at δ 178.7 in **2**. This was further supported by a detailed analysis of



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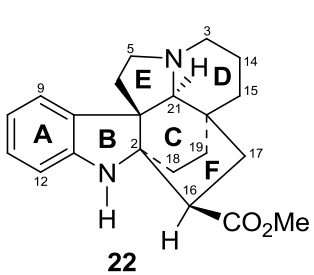

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the data from COSY, HMQC, and HMBC experiments. The COSY and HMQC spectral data of **2** revealed the following partial structures *i.e.*, CHCHCHCH, NCH_2CH_2, $CHCH_2$, and CH_2CH_2 (corresponding to the C(9)–C(10)–C(11)–C(12), N–C(5)–C(6), C(16)–C(17), and C(18)–C(19) units, respectively), in addition to an isolated aminomethine and two isolated methylenes. The observed three-bond correlations from H(5) to C(14) and from H(21) to C(14) and C(15) in the HMBC spectrum (Figure 2.4), not only confirmed the location of the lactam function, but also provide further support for the presence of the five-membered, lactam-containing ring D. Other correlations from the HMBC spectrum are shown in Figure 2.4, which are in complete accord with the proposed structure. Since, suitable crystals of **2** were obtained, an X-ray diffraction analysis was carried out (Figure 2.5) which confirmed the structure deduced based on the spectroscopic data. This is the first example of an aspidofractinine-type alkaloid which has lost one carbon in the piperidine ring D, resulting in a five-membered lactam ring D. A possible biogenetic pathway to **2** from kopsinine (**22**)[35] is shown in Scheme 2.2.

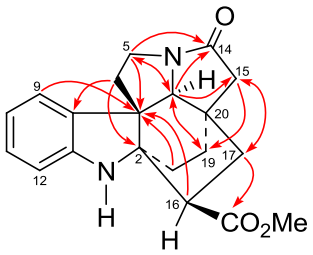


Figure 2.4 : Selected HMBCs of **2**

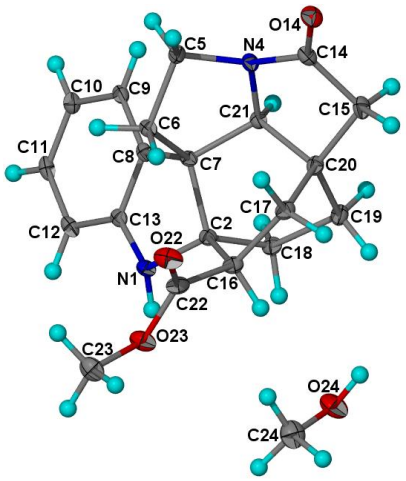
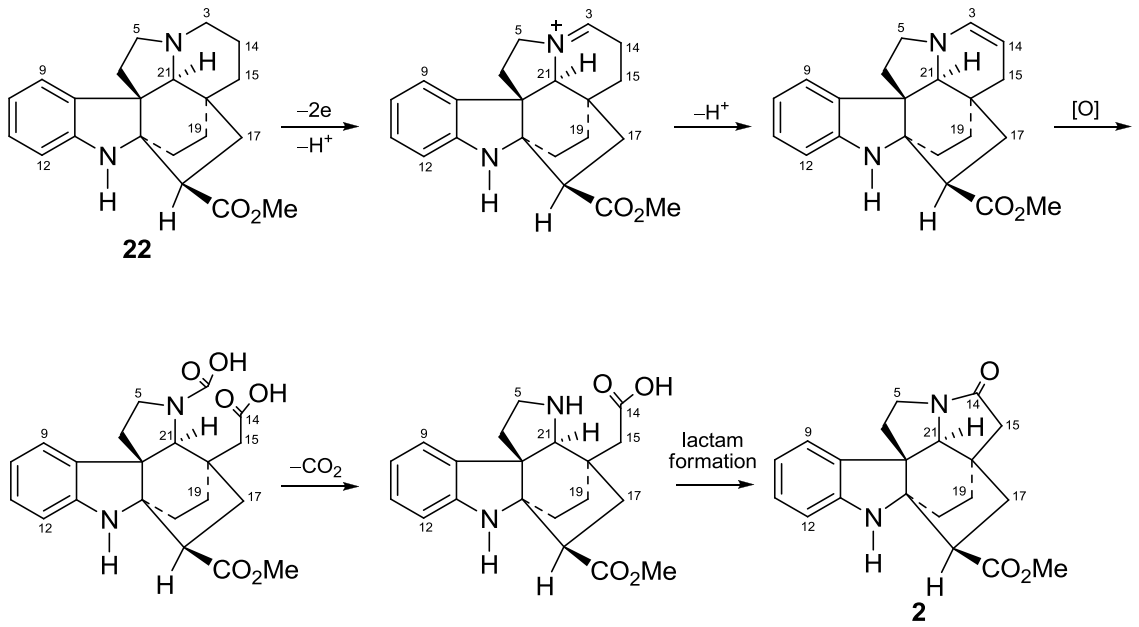


Figure 2.5 : X-ray crystal structure of **2**. Thermal ellipsoids are shown at the 50% probability level.



Scheme 2.2 : A possible biogenetic pathway to **2**

Table 2.3 : ^{1}H and ^{13}C NMR spectroscopic data of compound **2**[a]

Position	δ_H	δ_C
2	–	66.9
5	3.20 td (11.5, 6.3)	44.8
	4.01 dd (11.8, 8.1)	
6	1.43 dd (13.3, 6)	36.5
	2.74 m	
7	–	55.7
8	–	136.3
9	7.08 m	121.7
10	6.77 t (8)	120.1
11	7.08 m	128.2
12	6.70 d (8)	111.6
13	–	149.5
14	–	178.7
15	2.04 m	44.7
	2.51 d (15)	
16	2.94 t (10)	43.1
17	1.60 dd (14, 10.4)	30.6
	2.18 ddd (14, 10.4, 3.2)	
18	1.37 m	35.9
	2.05 m	
19	1.71 m	28.1
	1.86 m	
20	–	42.3
21	3.82 s	71.5
CO_2Me	–	173.2
CO_2*Me*	3.71 s	52.3
NH	3.91 br s	–

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

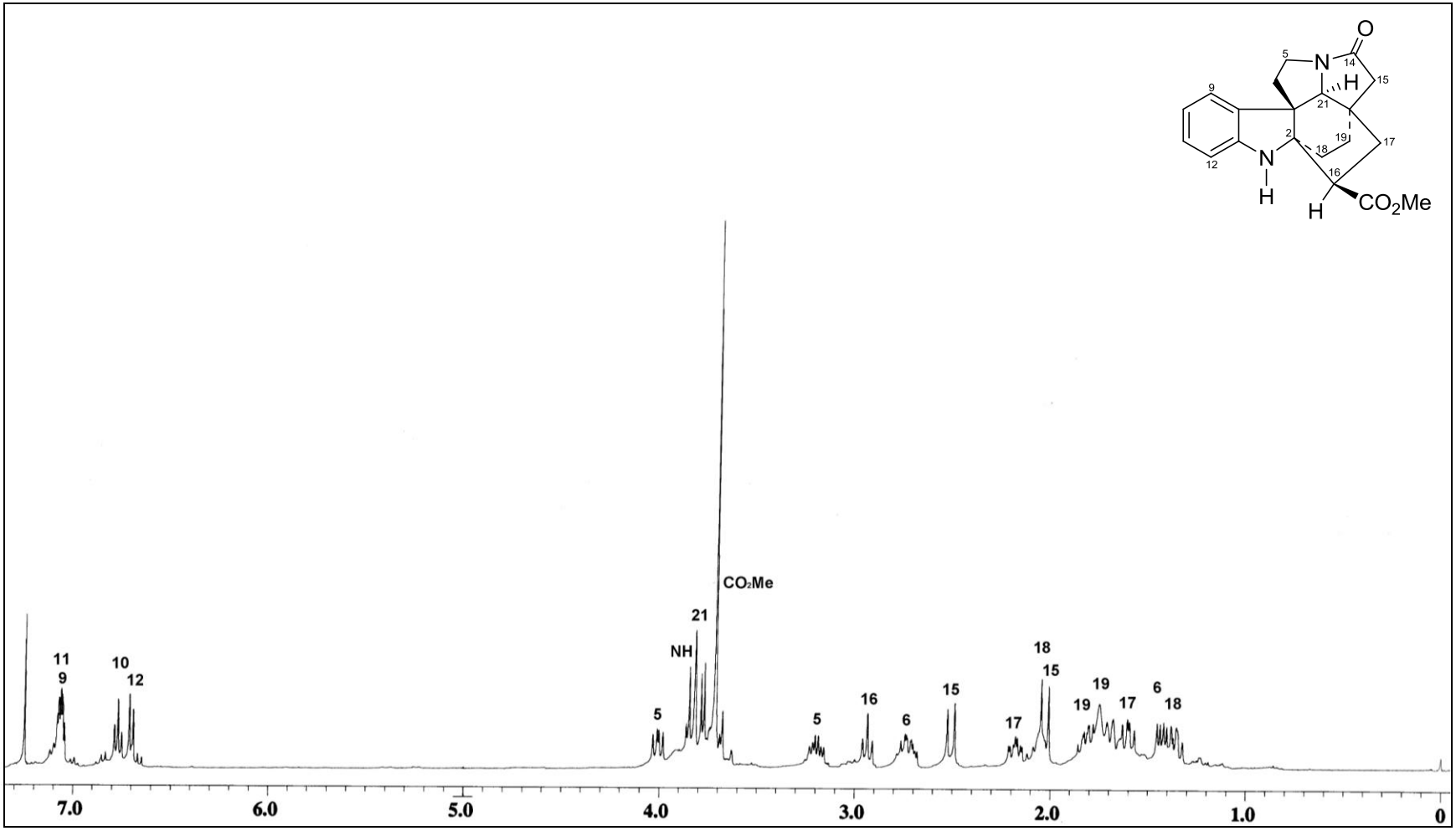
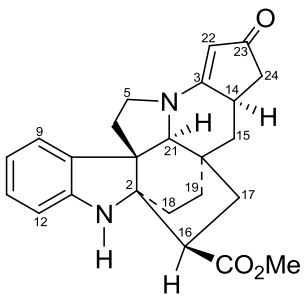


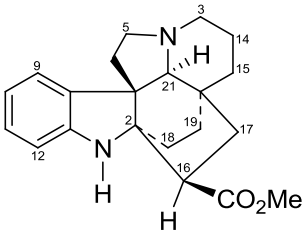
Figure 2.6 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **2**

2.1.3 Compound 3

Compound **3** was obtained as a light yellowish oil, and subsequently crystallized from CH_2Cl_2–hexanes as colorless needles, mp 268–270 ^oC, $[\alpha]^{25}_D$ +135 (*c* 0.11, $CHCl_3$). The UV spectrum showed absorption maxima at 205, 211, 229, and 279 nm, characteristic of a dihydroindole chromophore. The ESIMS of **3** showed an MH^+ ion at *m/z* 391, and HRESIMS measurements gave the molecular formula $C_{24}H_{26}N_2O_3$ + H. The IR spectrum showed bands due to NH (3349 cm^{-1}) and ketone/ester carbonyl (1720 cm^{-1}) functions. The ^{13}C NMR spectrum gave a total of 24 carbon resonances comprising one methyl, seven methylene, eight methine, and eight quaternary carbon atoms. The presence of a ketone carbonyl function was confirmed by the observed quaternary carbon shift at δ 202.2, while the quaternary carbon resonance at δ 173.3 was assigned to the ester carbonyl, in agreement with the IR spectrum. Detailed analysis of the ^1H and ^{13}C NMR spectroscopic data (Table 2.4) suggested that **3** also belong to the aspidofractinine group of alkaloids. The ^1H NMR spectrum of **3** (Figure 2.9) indicated the presence of an unsubstituted aromatic ring (δ 6.71–7.08), an isolated methine singlet (δ 3.63), a vinylic singlet (δ 4.96), an ester methoxy singlet (δ 3.74), and a broad NH singlet (δ 3.88). The NMR data of **3** resembled to those of kopsinine (**22**)[35] except for the incorporation of an additional five-membered ring linked from C(3) to C(14). This was supported by the COSY and HMQC spectral data, which revealed the presence of CHCHCHCH, NCH_2CH_2, CH_2CH_2, $CHCH_2$, and CH_2CHCH_2



3



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fragments, corresponding to the C(9)–C(10)–C(11)–C(12), N–C(5)–C(6), C(18)–C(19), C(16)–C(17), and C(15)–C(14)–C(24) units, respectively. The observed correlations from H(22) to C(3) and C(23), as well as from H(24) to C(3), C(14), and C(23), in the HMBC spectrum (Figure 2.7), allowed assembly of the five-membered ring incorporating a C(23) ketone carbonyl. The observed correlations from H(22) to C(23), and from H(24) to C(23), respectively confirmed the position of the ketone carbonyl function (Figure 2.7). The fusion of the five-membered ring to ring C via the quaternary C(3) and methine C(14), was indicated by the correlations from H(24) to C(3) and from H(15) to C(24). The complete structure assembled is in complete agreement with the remaining HMBC data (Figure 2.7). The structure is also consistent with the NOESY data, and the orientation of H(14) in **3** was assigned as α from the observed NOEs between H(14)/H(21), H(14)/H(15α), and H(14)/H(24α) (Figure 2.7). This assignment received additional confirmation from X-ray diffraction analysis (Figure 2.8). Alkaloid **3** is notable for having incorporated an additional five-membered ring fused to the piperidine ring D of an aspidofractinine carbon skeleton.

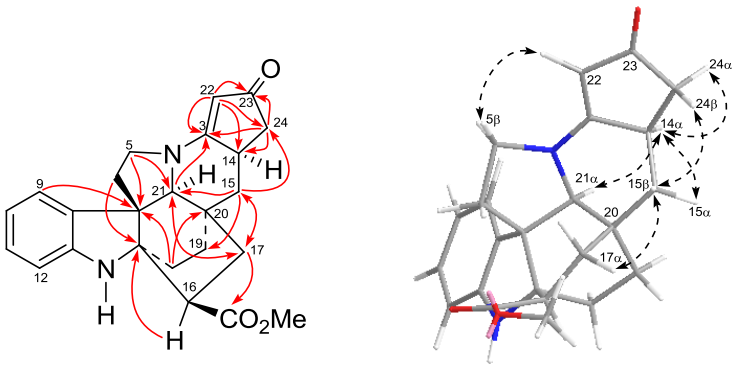


Figure 2.7 : Selected HMBCs and NOEs of **3** (= HMBC**;** = NOE)

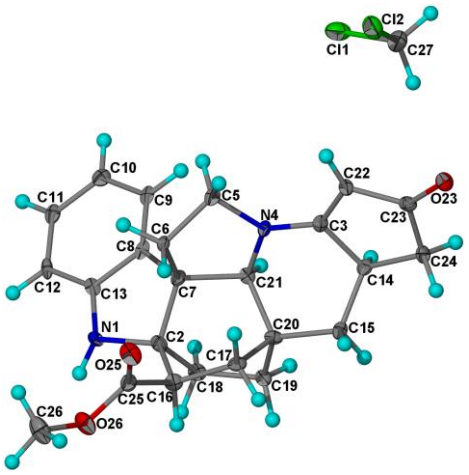


Figure 2.8 : X-ray crystal structure of **3**. Thermal ellipsoids are shown at the 50% probability level.

Table 2.4 : ^{1}H and ^{13}C NMR spectroscopic data of compound **3**[a]

Position	δ_H	δ_C
2	–	66.7
3	–	175.5
5α	3.48 dd (11.8, 6.3)	46.9
5β	3.58 dd (11.3, 6.3)	
6α	1.48 dd (13.1, 6.3)	34.4
6β	2.84 dd (12.2, 4)	
7	–	57.1
8	–	136.3
9	7.03 d (7.7)	121.1
10	6.77 t (7.7)	119.8
11	7.08 t (7.7)	128.2
12	6.71 d (7.7)	111.5
13	–	148.9
14	2.90 m	35.6
15β	1.08 t (13)	38.2
15α	2.02 m	
16	2.95 t (10)	43.9
17α	1.55 m	34.0
17β	2.23 ddd (10.4, 10.4, 1)	
18β	1.31 t (11.5)	33.5
18α	1.96 m	
19α	1.58 m	32.2
19β	1.77 m	
20	–	34.2
21	3.63 s	64.9
CO_2Me	–	173.3
CO_2*Me*	3.74 s	52.2
22	4.96 s	96.5
23	–	202.2
24β	1.96 m	41.3
24α	2.60 dd (17.5, 7)	
NH	3.88 br s	–

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, HMBC, and NOESY.

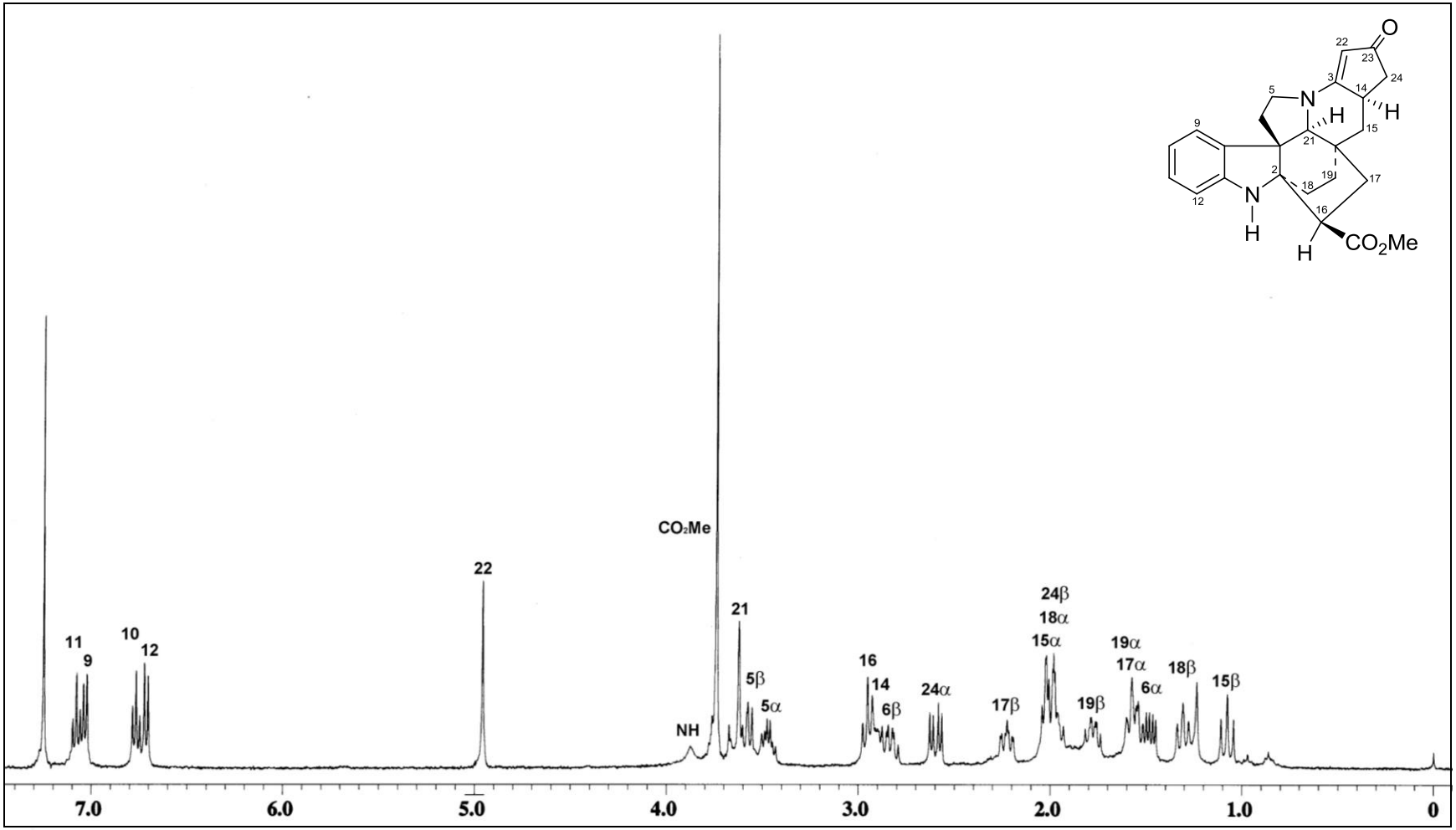


Figure 2.9 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **3**

2.1.4 Compound 4

Compound **4** was isolated as a light yellowish amorphous solid, mp 158–160 ^{o}C, $[\alpha]^{25}_{D}$ +156 (c 0.72, $CHCl_3$). The UV spectrum showed three absorption bands at 209, 245, and 303 nm, characteristic of a dihydroindole chromophore. The IR spectrum showed bands due to NH (3349 cm^{-1}) and ester/aldehyde carbonyl (1727 cm^{-1}) functionalities. The ESIMS of **4** showed a quasi molecular ion at *m/z* 365, which analyzed for $C_{22}H_{24}N_2O_3$ + H. The ^{13}C NMR spectroscopic data of **4** (Table 2.5) showed the presence of 22 carbon resonances (one methyl, six methylene, eight methine, and seven quaternary carbons) in agreement with the molecular formula. The ^{1}H NMR spectrum of **4** (Figure 2.11) showed the presence of an unsubstituted aromatic moiety (δ 6.73–7.06), a carbomethoxy group (δ 3.70), an isolated methine singlet (δ 3.49), and a broad NH singlet (δ 3.89). All these features are reminiscent of the aspidofractinine alkaloid, kopsinine (**22**),[35] except for the presence of an additional conjugated aldehyde group. The ^{1}H NMR spectrum of **4** also showed the presence of an aldehyde hydrogen at δ 8.94 (δ_C 187.0). The presence of a trisubstituted double bond was indicated from the lone vinylic signal at δ 7.25 (δ_C 153.0) while the other quaternary olefinic resonance was observed at δ 109.6. This was further supported by a detailed analysis of the 2-D NMR data. The COSY and HMQC spectral data revealed the presence of four partial structures *i.e.*, CHCHCHCH, NCH_2CH_2, $CHCH_2$, and CH_2CH_2 corresponding to the C(9)–C(10)–C(11)–C(12), N–C(5)–C(6), C(16)–C(17), and C(18)–C(19) units, respectively. The observed three-bond correlations from both H(3) and H(15) to CHO confirmed the position of the conjugated aldehyde group (Figure 2.10). The HMBC spectrum was in complete accord with the proposed structure of **4** (Figure 2.10). A possible biogenetic pathway to **4** from kopsinine (**22**)[35] is shown in Scheme 2.3.

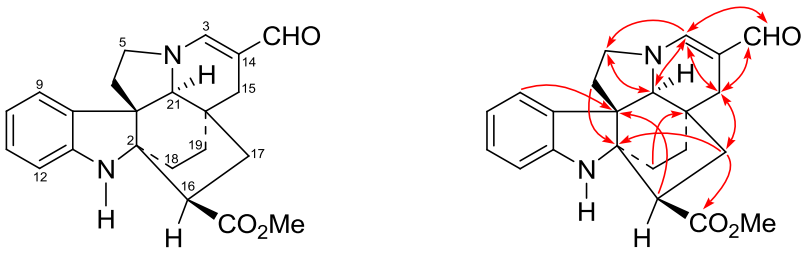
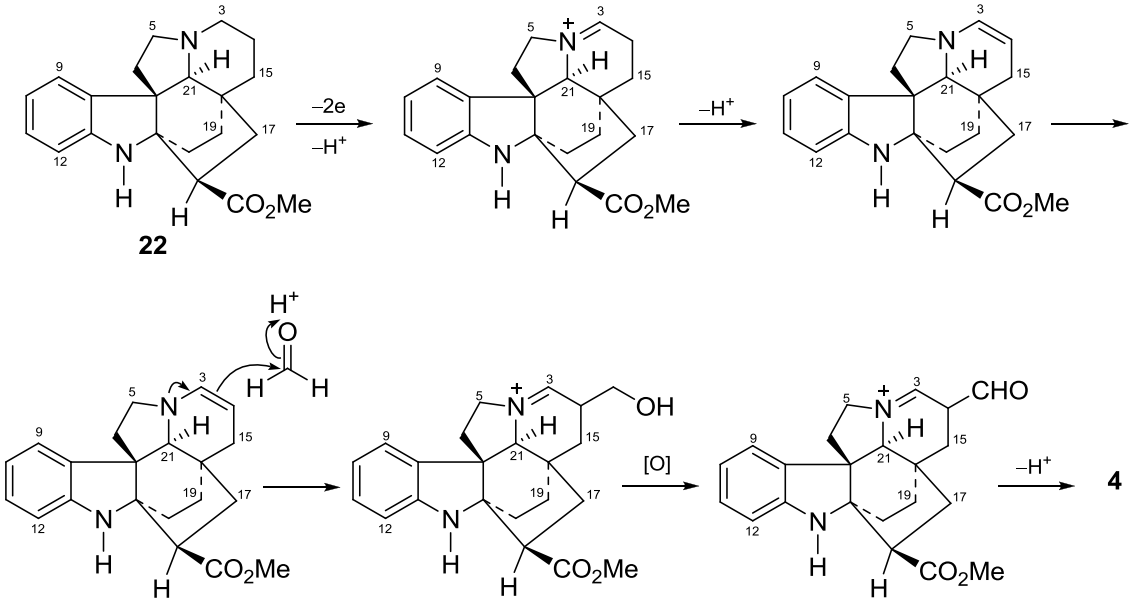


Figure 2.10 : Selected HMBCs of **4**



Scheme 2.3 : A possible biogenetic pathway to **4**

Table 2.5 : ^{1}H and ^{13}C NMR spectroscopic data of compound **4**[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
2	–	66.9	16	2.95 t (10)	43.6
3	7.25 s	153.0	17	1.34 m	29.1
5	3.44 dd (10, 4.7)	51.6		2.04 m	
	3.63 td (11.8, 4.9)		18	1.34 m	33.3
6	1.34 m	35.8		2.04 m	
	2.57 td (12.6, 7.2)		19	1.34 m	31.6
7	–	57.3		1.99 m	
8	–	137.0	20	–	28.5
9	7.06 m	121.0	21	3.49 s	64.4
10	6.73 m	119.5	*C*O_2Me	–	174.0
11	7.06 m	128.1	CO_2*Me*	3.70 s	52.3
12	6.73 m	111.5	CHO	8.94 s	187.0
13	–	149.3	NH	3.89 br s	–
14	–	109.6			
15	1.99 m	29.7			
	2.40 d (16.3)				

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

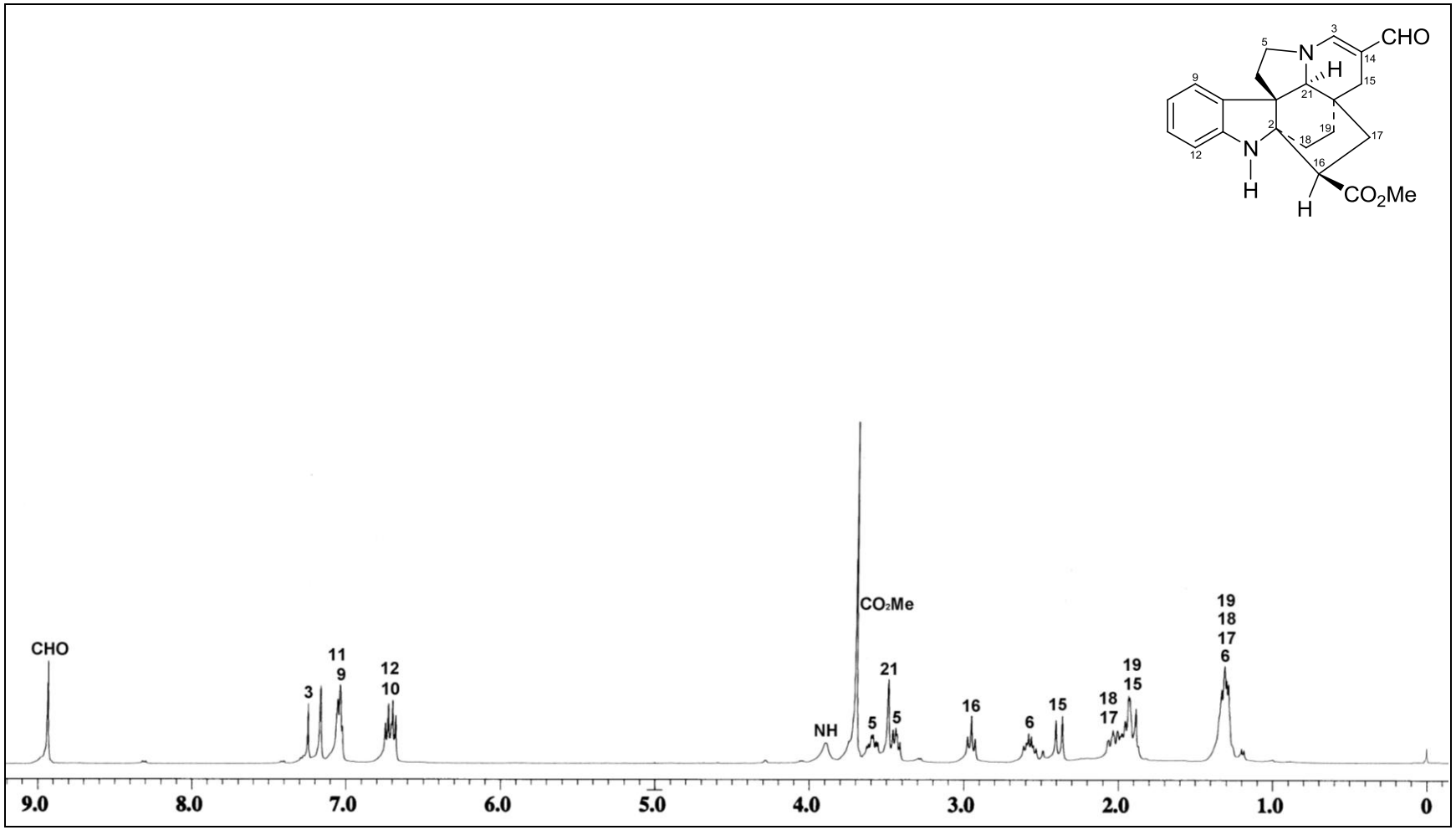


Figure 2.11 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **4**

2.1.5 Compound 5

Compound **5** was obtained as a light yellowish oil, $[\alpha]^{25}_{D}$ +123 (*c* 0.16, $CHCl_3$). The UV spectrum showed typical dihydroindole absorptions at 210, 242, 257, and 293 nm, while the IR spectrum showed bands at 3336 and 1736 cm^{-1}, corresponding to NH and lactone functionalities, respectively. The ESIMS of **5** showed an MH^+ ion at *m/z* 323, and HRESIMS measurements gave the molecular formula $C_{20}H_{22}N_2O_2$ + H. Analysis of the NMR spectroscopic data (Table 2.6) readily revealed **5** to be a derivative of the paucidactines.[41,107] The NMR spectroscopic data of **5** were essentially similar to those of paucidactine B (**19**),[107] except for the absence of the methylenedioxy substituent as well as the lack of the carbamate function. This was in complete accord with the presence of four aromatic hydrogens (δ 6.70–7.13) in **5** and the indolic NH, which was seen as a broad singlet at δ 3.54 in the ^{1}H NMR spectrum (Figure 2.13). In addition, the lactam carbonyl at C(5) and the hydroxyl group at C(16) in paucidactine B (**19**)[107] were also absent in **5**. This was in agreement with the observed carbon resonances due to C(5) and C(16), at δ 56.9 and 40.3, respectively, in the ^{13}C NMR spectrum of **5**. This was further confirmed by the observed three-bond correlations from H(16) to C(7) and H(3) to C(5) in the HMBC spectrum (Figure 2.12). The COSY spectrum showed fragments due to CHCHCHCH, $NCH_2CH_2CH_2$, NCH_2CH, $CHCH_2$, and CH_2CH_2, corresponding to the C(9)–C(10)–C(11)–C(12), N–C(3)–C(14)–C(15), N–C(5)–C(6), C(16)–C(17), and C(18)–C(19) units, respectively, which are in agreement with the proposed structure of **5**.

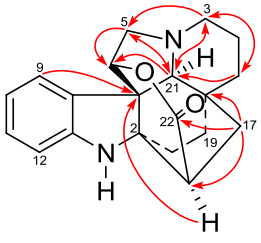


Figure 2.12 : Selected HMBCs of **5**

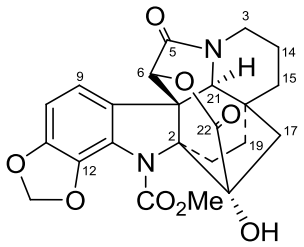


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Table 2.6 : ^{1}H and ^{13}C NMR spectroscopic data of compound **5**[a]

Position	δ_H	δ_C
2	–	66.1
3	2.79 dd (12.7, 3.2)	48.0
	3.06 dd (12, 4.5)	
5	3.10 dd (10, 3.2)	56.9
	3.20 dd (10, 5)	
6	4.57 dd (5, 3.2)	89.2
7	–	52.5
8	–	134.2
9	7.13 br d (7.2)	122.5
10	6.79 td (7.2, 1)	120.1
11	7.07 td (7.7, 1.4)	128.3
12	6.70 br d (7.7)	111.8
13	–	150.1
14	1.36 m	18.9
	1.81 m	
15	1.23 m	34.2
	1.50 m	
16	2.97 dd (12, 3.5)	40.3
17	1.66 m	32.6
	2.35 dt (14.5, 3.4)	
18	1.43 dd (11.8, 3.6)	29.2
	1.80 dd (11.8, 3.6)	
19	1.28 dd (13.6, 4.1)	34.1
	1.50 m	
20	–	31.8
21	2.83 s	68.7
22	–	172.9
NH	3.54 br s	–

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

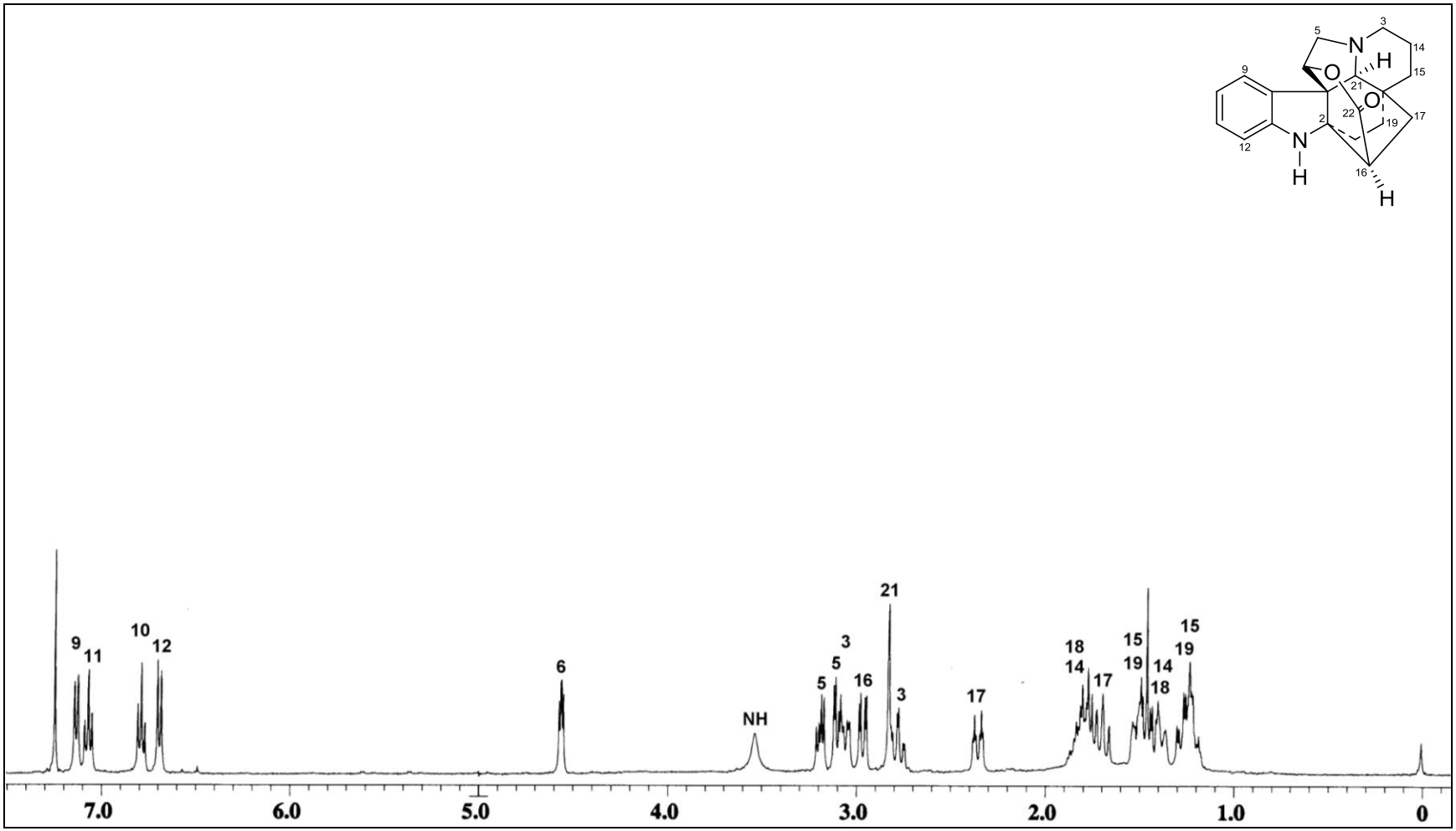


Figure 2.13 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **5**

2.1.6 Compound 6

Compound **6** was obtained as a light yellowish oil, $[\alpha]^{25}_{D}$ +208 (*c* 0.12, $CHCl_3$). The UV spectrum was similar to that of compound **5** with dihydroindole absorptions observed at 209, 243, 257, and 295 nm, while the IR spectrum showed bands due to NH (3332 cm^{-1}), lactone (1756 cm^{-1}), and lactam (1698 cm^{-1}) functionalities. The ESIMS of **6** showed a quasi molecular ion at *m/z* 337, and HRESIMS measurements established the molecular formula as $C_{20}H_{20}N_2O_3$ + H, indicating that **6** differed from **5** by the loss of two hydrogens and the addition of an oxygen atom. The ^{13}C NMR spectrum of **6** showed a total of 20 carbon resonances, comprising six methylene, seven methine, and seven quaternary carbon atoms. The ^{1}H NMR spectroscopic data (Table 2.7) of **6** was generally similar to that of **5**, except for the signals due to H(5) in **5** which was absent in **6**, having been replaced by a lactam carbonyl. This was in accord with the presence of an additional lactam carbonyl carbon resonance at δ 167.6 in the ^{13}C NMR spectrum of **6.** In addition, the observed three bond correlations from H(3) and H(21) to C(5), as well as from H(6) to C(5), further confirmed the position of the lactam carbonyl at C(5) (Figure 2.14). This was further supported by a detailed analysis of the 2-D NMR data. The COSY and HMQC spectral data revealed four partial structures *i.e.*, CHCHCHCH, $NCH_2CH_2CH_2$, $CHCH_2$, and CH_2CH_2, corresponding to the C(9)–C(10)–C(11)–C(12), N–C(3)–C(14)–C(15), C(16)–C(17), and C(18)–C(19) units, respectively.

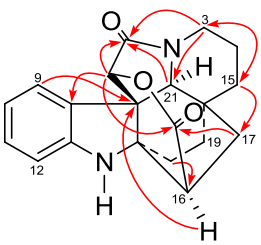
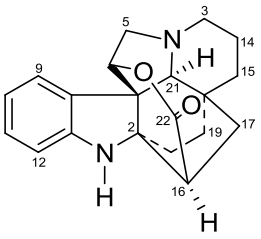


Figure 2.14 : Selected HMBCs of **6**



5

Table 2.7 : ^{1}H and ^{13}C NMR spectroscopic data of compound **6**[a]

Position	δ_H	δ_C
2	–	66.1
3	2.82 td (12.7, 3)	40.6
	4.24 dd (13.1, 4.5)	
5	–	167.6
6	4.62 s	86.0
7	–	45.7
8	–	132.3
9	7.13 d (7.7)	129.2
10	6.82 t (7.7)	120.7
11	7.07 t (7.7)	121.8
12	6.70 d (8.2)	112.1
13	–	141.4
14	1.55 m	20.1
	1.66 m	
15	1.48 dd (14, 4)	33.9
	1.59 m	
16	3.01 dd (11.8, 5)	40.5
17	1.50 m	27.7
	1.90 m	
18	1.40 m	32.0
	1.71 dd (12.4, 6)	
19	1.71 dd (12.4, 6)	28.3
	1.90 m	
20	–	32.9
21	3.72 s	65.1
22	–	170.0
NH	3.64 s	–

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

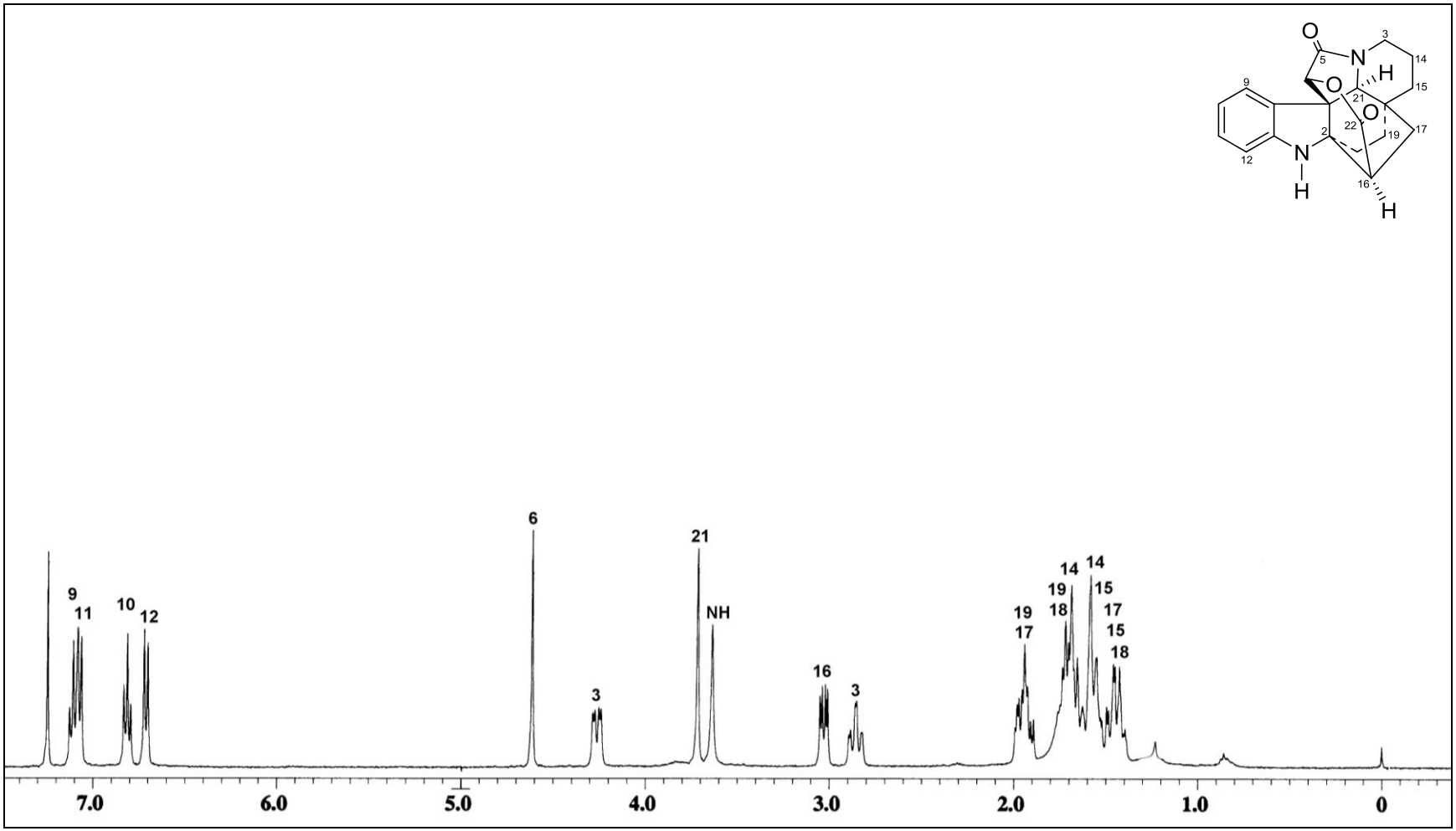


Figure 2.15 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **6**

2.1.7 Compound 7

Compound **7** was obtained as a light yellowish oil, $[\alpha]^{25}_{D}$ +88 (*c* 0.11, $CHCl_3$). The UV spectrum showed three absorption bands (212, 248, and 282 nm) characteristic of a dihydroindole chromophore. The IR spectrum showed bands at 1756 and 1693 cm^{-1} indicating the presence of ketone and carbamate/lactam functions. The ESIMS of **7** showed a quasi molecular ion at *m/z* 409, which analyzed for $C_{23}H_{24}N_2O_5$ + H. The ^{13}C NMR spectrum of **7** gave a total of 23 carbon resonances (two methyl, six methylene, seven methine, and eight quaternary carbons) in agreement with the molecular formula. The observed quaternary carbon resonances at δ 164.9 and 204.0 were consistent with the presence of a lactam and a ketone function, respectively, while the resonance at δ 152.5 was in agreement with the presence of a carbamate functionality. The NMR spectroscopic data of **7** (Table 2.8) showed features typical of a kopsine-type alkaloid and were essentially similar to those of *N*(1)-carbomethoxy-5,22-dioxokopsane (**15**),[76] except for the presence of OMe substitution at C(12) in **7**. This was confirmed by the observed three-bond correlation from 12-OMe to C(12) in the HMBC spectrum of **7**. Other correlations from the HMBC spectrum are shown in Figure 2.16, which are in complete accord with the proposed structure. Compound **7** is therefore the 12-methoxy derivative of *N*(1)-carbomethoxy-5,22-dioxokopsane (**15**).[76]

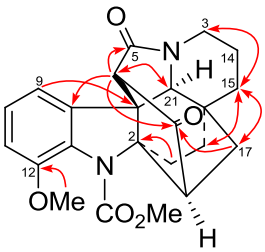


Figure 2.16 : Selected HMBCs of **7**

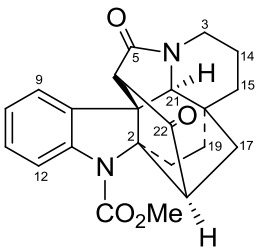


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Table 2.8 : ^{1}H and ^{13}C NMR spectroscopic data of compound **7**[a]

Position	δ_H	δ_C
2	–	72.6
3	2.88 td (10, 3)	40.8
	4.22 dd (13, 4.5)	
5	–	164.9
6	2.99 s	62.5
7	–	56.9
8	–	136.1
9	6.84 d (7.7)	114.5
10	7.08 t (7.7)	126.1
11	6.89 t (7.7)	113.5
12	–	149.7
13	–	131.0
14	1.54 m	19.6
	1.67 m	
15	1.42 dd (13, 4.5)	32.7
	1.61 dd (13, 4.5)	
16	3.61 d (9.1)	51.2
17	1.24 m	29.2
	1.76 d (9.5)	
18	1.70 m	21.6
	2.26 td (13, 4.5)	
19	1.42 dd (13, 4.5)	32.7
	1.61 dd (13, 4.5)	
20	–	33.3
21	3.66 s	65.7
22	–	204.0
NCO_2Me	–	152.5
$NCO_2\mathit{Me}$	3.75 s	53.0
12-OMe	3.84 s	56.3

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

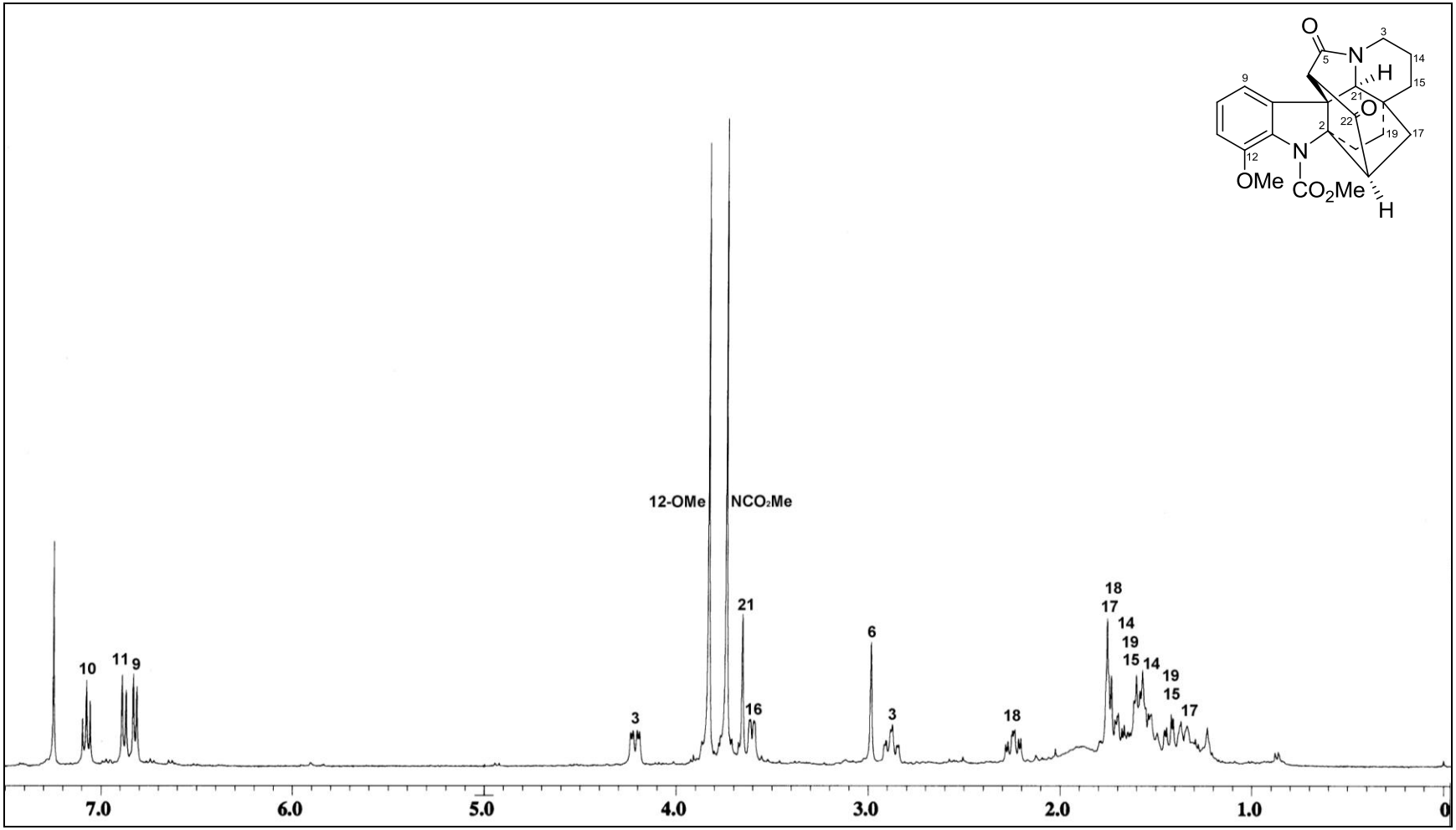


Figure 2.17 : ^{1}H NMR spectum ($CDCl_3$, 400 MHz) of compound **7**

2.1.8 Compound 8

Compound **8** was obtained as a light yellowish oil, which subsequently crystallized from CH_2Cl_2–MeOH as light orange block crystals, mp 190–192 ^oC, $[\alpha]_D$ +414 (*c* 0.26, $CHCl_3$). The mass spectrum showed a quasi molecular ion at *m/z* 311, and HRESIMS established the molecular formula as $C_{19}H_{22}N_2O_2$ + H. The UV spectrum showed absorption maxima at 210, 234, 257, 308, and 376 nm, indicating the presence of a pseudoindoxyl chromophore. The IR spectrum showed the presence of a ketone carbonyl function at 1610 cm^{-1} as well as Wenkert-Bohlmann bands at 2855 and 2799 cm^{-1} due to the presence of a *trans* C/D ring junction. The ^{13}C NMR spectrum gave a total of 19 carbon resonances comprising 1 methyl, 6 methylene, 7 methine, and 5 quaternary carbons. The presence of a ketone carbonyl function was confirmed by the observed quaternary carbon shift at δ 207.3. The ^1H NMR spectrum (Figure 2.20) indicated the presence of an unsubstituted aromatic ring (δ 7.03–7.65), an isolated methine (δ 2.63), and a methyl doublet (δ 0.85). Analysis of the NMR spectral data (Table 2.9) suggested the presence of an eburnane derivative, oxygenated at C(16), and differing from the other known eburnane alkaloids in that the C(20) ethyl side chain was missing. The presence of a methyl doublet and the carbon resonance of C(19) at δ 81.7, indicated the incorporation of an additional five-membered ring in **8**, in which an ether oxygen links C(19) to C(16). This was supported by a detailed analysis of the COSY, HMQC, and HMBC data. The COSY and HMQC spectral data revealed 5 partial structures *i.e.*,CHCHCHCH, $NCH_2CH_2CH_2$, NCH_2CH_2, $OCHCH_2$, and CH_3CH, corresponding to the C(9)–C(10)–C(11)–C(12), N–C(3)–C(14)–C(15), N–C(5)–C(6), O–C(16)–C(17), and C(18)–C(19) units, respectively. In addition, the observed three-bond correlation from H(9) to C(7) in the HMBC spectrum indicated the location of the pseudoindoxyl carbonyl at C(7) (Figure 2.18). This was also confirmed by the observed

three-bond correlations from both H(6) and H(21) to C(7) in the HMBC spectrum (Figure 2.18). The three-bond correlations from H(16) to C(2) and C(19), as well as from H(17) to C(15) and C(21), are also in complete accord with the proposed structure (Figure 2.18). The configurations at C(20) and C(21) are assumed to be similar to those in the other eburnane alkaloids found in this plant, on the grounds of a presumed biogenetic relationship. This being so, the stereochemistry of the C(16) ether oxygen has to be β to permit formation of the five-membered ring. The configuration of the H(18) methyl was assigned as β based on the observed NOE between H(18) and H(21). Since, suitable crystals of **8** were obtained, an X-ray diffraction analysis was carried out (Figure 2.19) which further confirmed the structure deduced based on the spectroscopic data. A possible biogenetic pathway to **8** is shown in Scheme 2.4.[77,101]

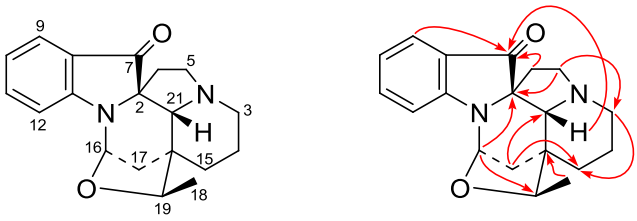


Figure 2.18 : Selected HMBCs of **8**

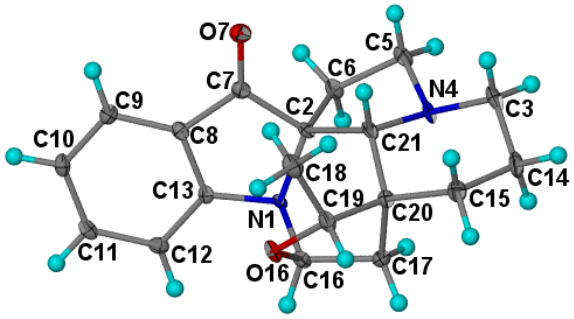
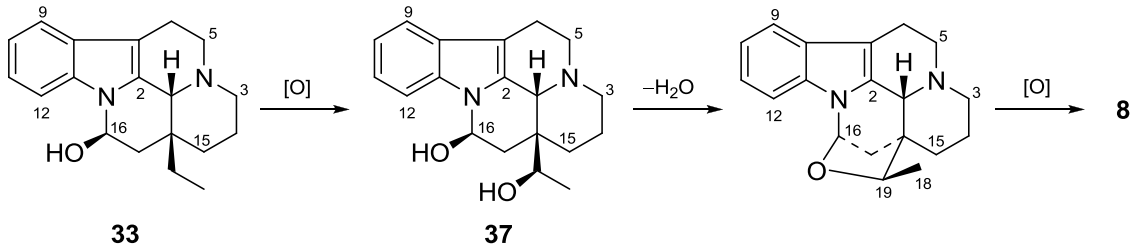


Figure 2.19 : X-ray crystal structure of **8**. Thermal ellipsoids are shown at the 50% probability level.



Scheme 2.4 : A possible biogenetic pathway to **8**

Table 2.9 : ^{1}H and ^{13}C NMR spectroscopic data of compound **8**[a]

Position	δ_H	δ_C
2	–	73.1
3β	2.25 m	51.0
3α	3.07 m	
5β	2.58 m	52.9
5α	3.03 m	
6	1.96 m	40.7
	1.96 m	
7	–	207.3
8	–	126.3
9	7.65 d (7.2)	124.6
10	7.03 t (7.2)	122.6
11	7.54 td (7.2, 1.4)	136.7
12	7.18 d (7.2)	117.0
13	–	162.3
14	1.68 m	23.6
	1.68 m	
15β	1.37 m	29.6
15α	1.47 br d (13.6)	
16	5.53 d (5.5)	89.3
17β	1.71 m	37.6
17α	2.91 d (11.3)	
18	0.85 d (6.8)	14.5
19	3.54 q (6.8)	81.7
20	–	43.8
21	2.63 s	66.4

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, HMBC, and NOESY.

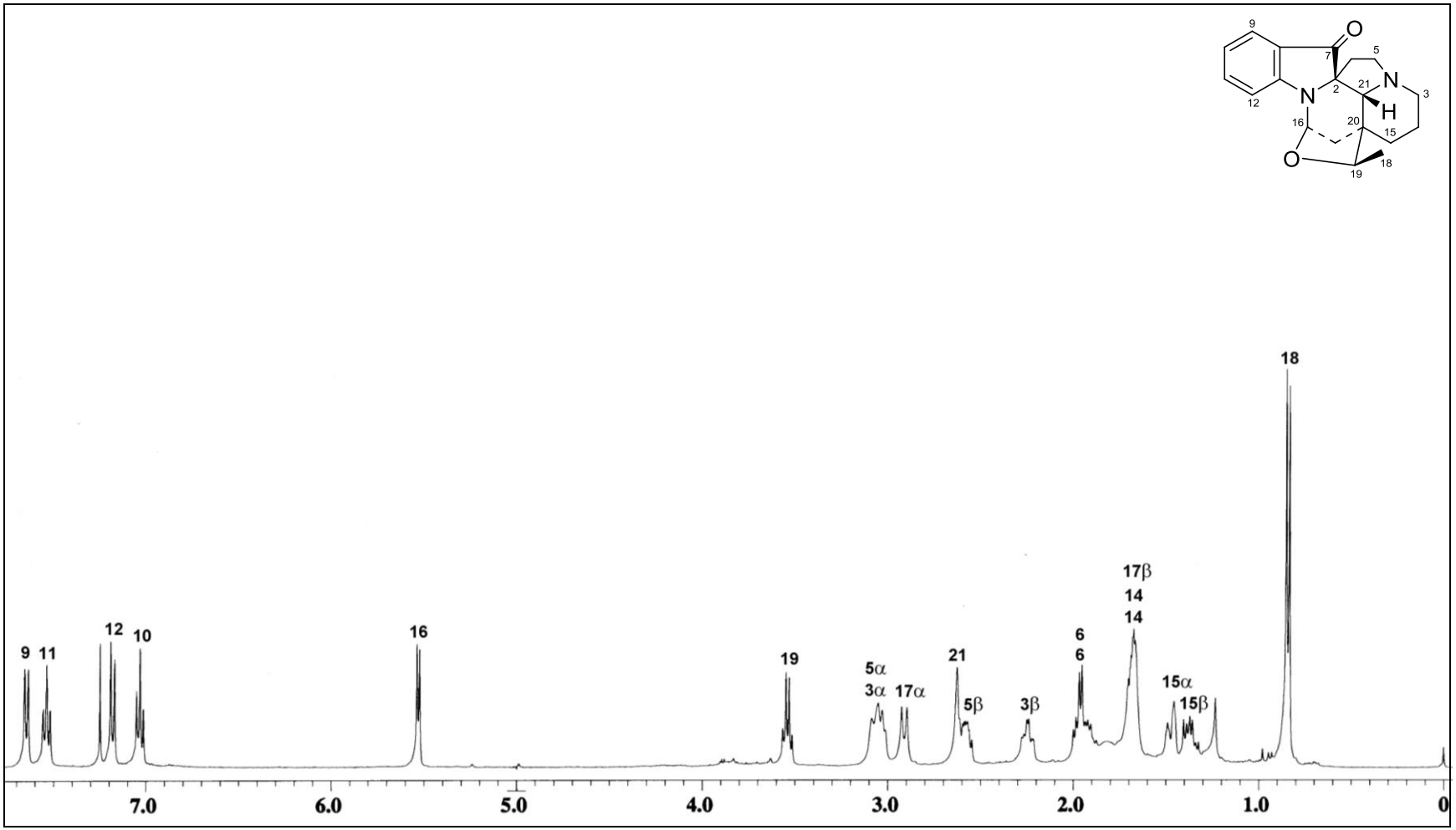


Figure 2.20 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **8**

2.1.9 Compound 9

Compound **9** was obtained in minute amount as a colorless oil, $[\alpha]^{25}_{D}$ −152 (*c* 0.11, $CHCl_3$). The UV spectrum showed absorption maxima at 209, 243, 282, and 299 nm, typical of an indole chromophore. The IR spectrum showed bands at 1707 and 1630 cm^{-1}, suggesting the presence of ketone and lactam functionalities, respectively. The ESIMS showed an MH^+ peak at *m/z* 309, and HRESIMS measurements established the molecular formula as $C_{19}H_{20}N_2O_2$ + H. The ^{13}C NMR spectrum showed a total of 19 carbon resonances, comprising one methyl, six methylene, five methine, and seven quaternary carbon atoms, in agreement with the molecular formula. The ^{1}H and ^{13}C NMR spectroscopic data (Table 2.10) showed features typical of eburnane alkaloids. Comparison with (+)-eburnamonine (**32**)[100] found in this plant, showed a close correspondence of the ^{1}H NMR spectral data, except for the absence of the H(19) methylene signals seen at δ 1.66 and 2.05 in **32**, and the downfield shift of the H(18) methyl signal in **9** to δ 2.38 from δ 0.93 in **32**. The same is true of the ^{13}C NMR spectrum of **9**, where the observed quaternary carbon resonance at δ 208.7 due to C(19) in **9**, has replaced the signal due to the C(19) methylene carbon (δ 28.3) in the spectrum of **32**, indicating oxygenation at C(19). Further comparison of the carbon chemical shifts between compounds **9** and **32**, showed that in the case of **9**, the C(18) methyl signal and the C(20) resonance have been shifted downfield to δ 25.8 and 52.5, respectively, whereas in the case of **32**, the C(18) and C(20) were seen at δ 7.6 and 38.5, respectively. In addition, the observed three-bond correlations from both H(15) and H(17) to C(19) in the HMBC spectrum (Figure 2.21) are also in agreement with the proposed structure. Compound **9** is therefore (–)-19-oxoeburnamonine.

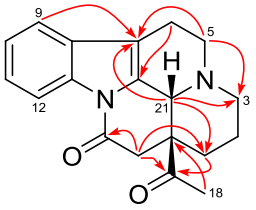


Figure 2.21 : Selected HMBCs of **9**

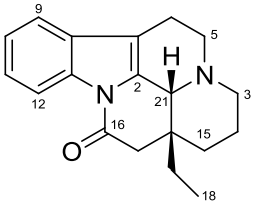


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Table 2.10 : ^{1}H and ^{13}C NMR spectroscopic data of compound **9**[a]

Position	δ_H	δ_C
2	–	130.9
3	2.47 dd (16.7, 3.5)	44.2
	2.63 d (16.7)	
5	3.32 dd (9, 3)	50.7
	3.32 dd (9, 3)	
6	2.58 m	16.6
	2.89 m	
7	–	113.9
8	–	130.3
9	7.43 dd (6, 1.8)	118.4
10	7.30 td (6, 1.8)	124.4
11	7.30 td (6, 1.8)	124.8
12	8.32 dd (6, 1.8)	116.4
13	–	134.5
14	1.54 m	22.8
	1.54 m	
15	1.14 td (13.6, 4)	26.9
	2.16 d (13.6)	
16	–	165.2
17	2.63 d (16.7)	42.6
	2.77 d (16.7)	
18	2.38 s	25.8
19	–	208.7
20	–	52.5
21	4.81 s	53.9

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

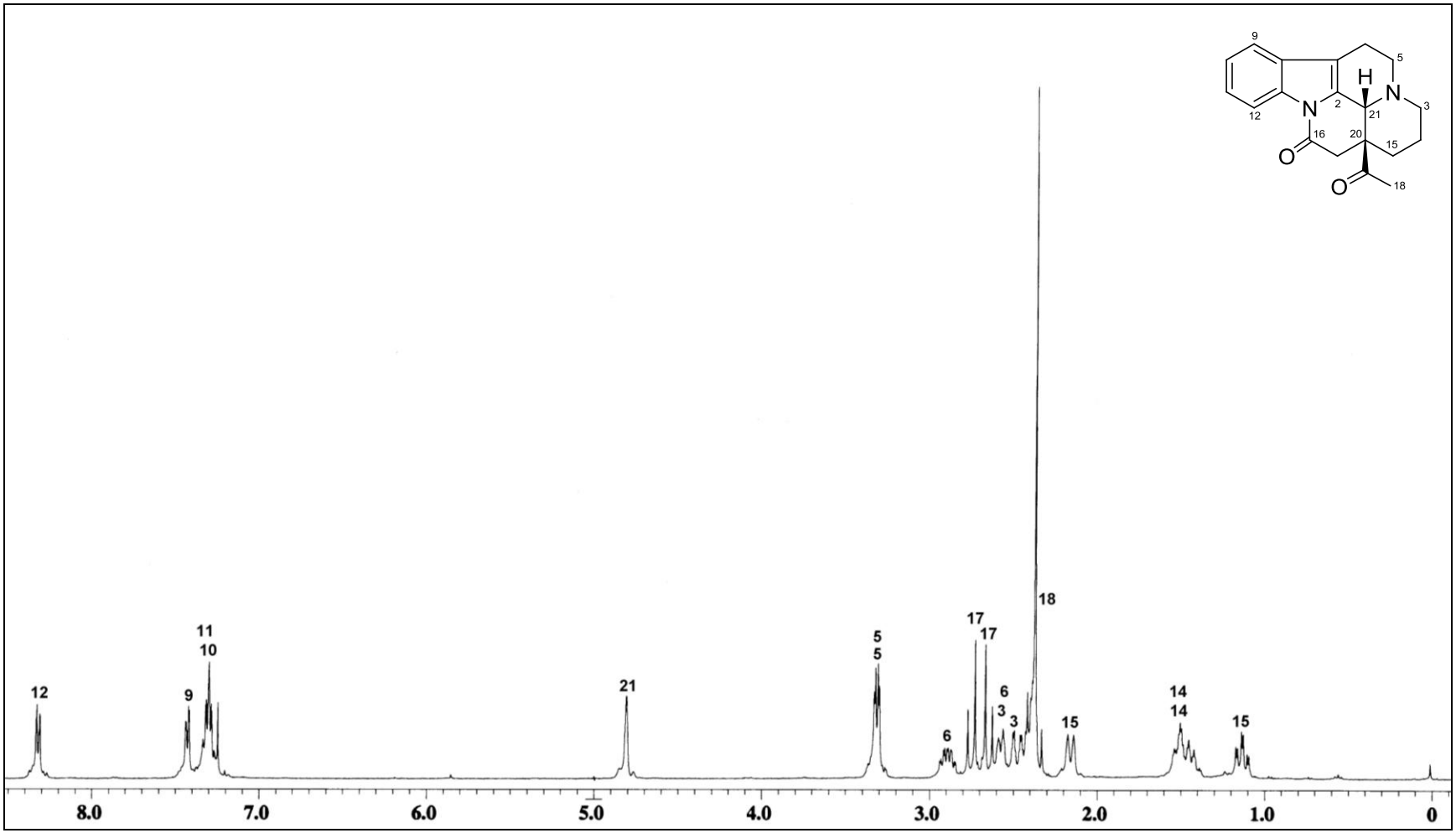
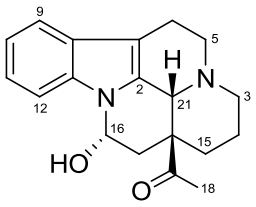


Figure 2.22 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **9**

2.1.10 Compound 10

Compound **10** was isolated as a light yellowish amorphous solid, mp 178−180 ^{o}C, $[\alpha]^{25}_{D}$ −17 (*c* 0.82, MeOH). The UV spectrum showed three absorption bands at 209, 227, and 277 nm, characteristic of an indole chromophore. The IR spectrum showed bands due to OH (3315 cm^{-1}) and ketone carbonyl (1704 cm^{-1}) functions. The ESIMS of **10** showed a quasi molecular ion at *m/z* 311, which analyzed for $C_{19}H_{22}N_2O_2$ + H. The ^{13}C NMR spectrum showed the presence of 19 carbon resonances (one methyl, six methylene, six methine, and six quaternary carbons) in agreement with the molecular formula. Detailed analysis of the NMR spectral data suggested that **10** also belong to the eburnane group of compounds. This was supported by both the ^{1}H and ^{13}C NMR spectroscopic data (Table 2.11), which showed the spectra of **10** to be essentially similar to those of (+)-isoeburnamine (**34**)[100] except for the replacement of the signals due to the 20-ethyl group by an acetyl group ($\delta_{H(18)}$ 2.32; $\delta_{C(18)}$ 25.3; $\delta_{C(19)}$ 211.5). Compound **10** is therefore, (−)-19-oxoisoeburnamine.



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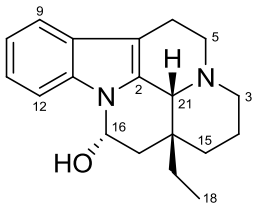

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Table 2.11 : ^{1}H and ^{13}C NMR spectroscopic data of compound **10**[a]

Position	δ_H	δ_C
2	–	129.8
3	2.56 m	44.4
	2.56 m	
5	3.27 dd (11.3, 5)	51.0
	3.27 dd (11.3, 5)	
6	2.54 m	16.8
	2.97 m	
7	–	105.8
8	–	128.7
9	7.43 d (7.7)	118.3
10	7.08 td (7.7, 1)	120.1
11	7.13 td (7.7, 1)	121.3
12	7.38 d (7.7)	110.3
13	–	135.2
14	1.36 m	23.1
	1.51 qt (13, 3)	
15	1.84 td (14, 3.6)	25.8
	2.16 t (14)	
16	5.99 br d (3.2)	73.6
17	2.06 dd (14.5, 4.5)	39.3
	2.16 d (14.5)	
18	2.32 s	25.3
19	–	211.5
20	–	50.0
21	4.69 s	54.6

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

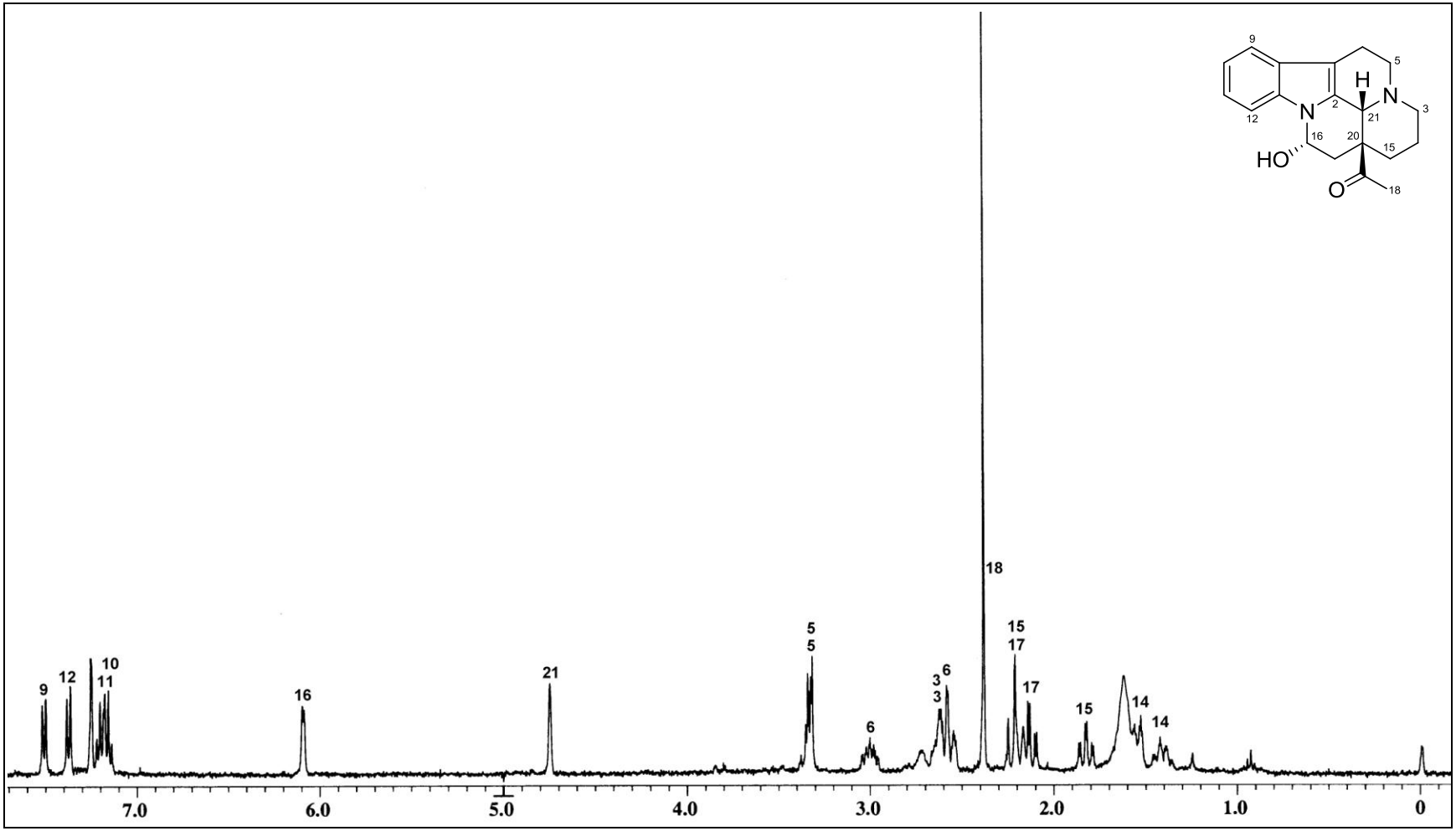


Figure 2.23 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **10**

2.1.11 Compound 11

Compound **11** was obtained as a light yellowish oil, and subsequently crystallized from CH_2Cl_2–MeOH as light orange block crystals, mp 162–164 ^oC, $[\alpha]^{25}_D$ –157 (*c* 0.07, $CHCl_3$). The UV spectrum showed absorption maxima at 225, 258, 302, and 309 nm, characteristic of an indole chromophore, while the IR spectrum showed the presence of a hydroxyl function (3364 cm^{-1}). The ESIMS of **11** showed the MH^+ ion at *m/z* 295, and HRMS measurements gave the molecular formula $C_{19}H_{22}N_2O$ + H. The ^1H and ^{13}C NMR spectroscopic data (Table 2.12) of **11** are reminiscent of those of (+)-eburnamenine (**31**)[146] except for the signals of the C(20) ethyl side chain which are replaced by a methyl doublet at δ 1.32 and an oxymethine quartet at δ 4.25, which indicated the presence of a hydroxyethyl group. In addition, the three-bond correlations from H(15), H(17) and H(21), to C(19), in the HMBC spectrum (Figure 2.24) are also in accord with the proposed structure. The configuration at C(20) and C(21) are assumed to be similar to those in the other eburnane alkaloids found in this plant, on the grounds of a presumed biogenetic relationship. This still left the configuration of C(19) to be established and since there were no suitable precedents, this was achieved by X-ray analysis (Figure 2.25) which yielded the structure as shown in **11**. Compound **11** is therefore, (–)-19(*R*)-hydroxyeburnamenine.

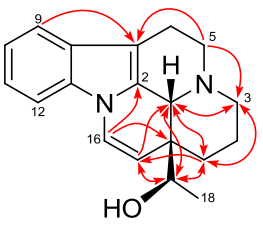
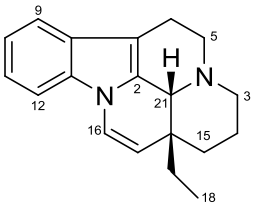


Figure 2.24 : Selected HMBCs of **11**



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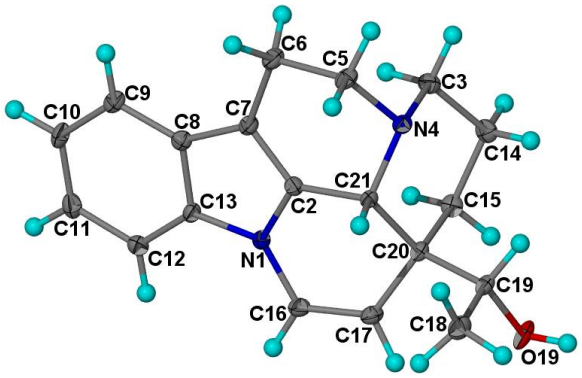


Figure 2.25 : X-ray crystal structure of **11**. Thermal ellipsoids are shown at the 50% probability level.

Table 2.12 : ^{1}H and ^{13}C NMR spectroscopic data of compound **11**[a]

Position	δ_H	δ_C
2	–	128.6
3	2.72 m	44.9
	2.83 td (12.5, 3.2)	
5	3.33 dd (8.6, 2.7)	51.9
	3.33 dd (8.6, 2.7)	
6	2.54 dd (15.4, 1.8)	16.5
	3.04 m	
7	–	107.0
8	–	128.3
9	7.47 br d (8)	118.7
10	7.12 td (8, 1)	120.3
11	7.19 td (8, 1)	122.0
12	7.33 br d (8)	108.7
13	–	133.7
14	1.43 d (13.6)	21.7
	2.10 qt (13.6, 3.6)	
15	1.07 td (14.1, 4.1)	26.5
	1.94 d (14.1)	
16	6.90 d (8.2)	120.1
17	5.02 d (8.2)	113.2
18	1.32 d (6.3)	18.0
19	4.25 q (6)	74.4
20	–	40.4
21	4.47 s	58.2

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

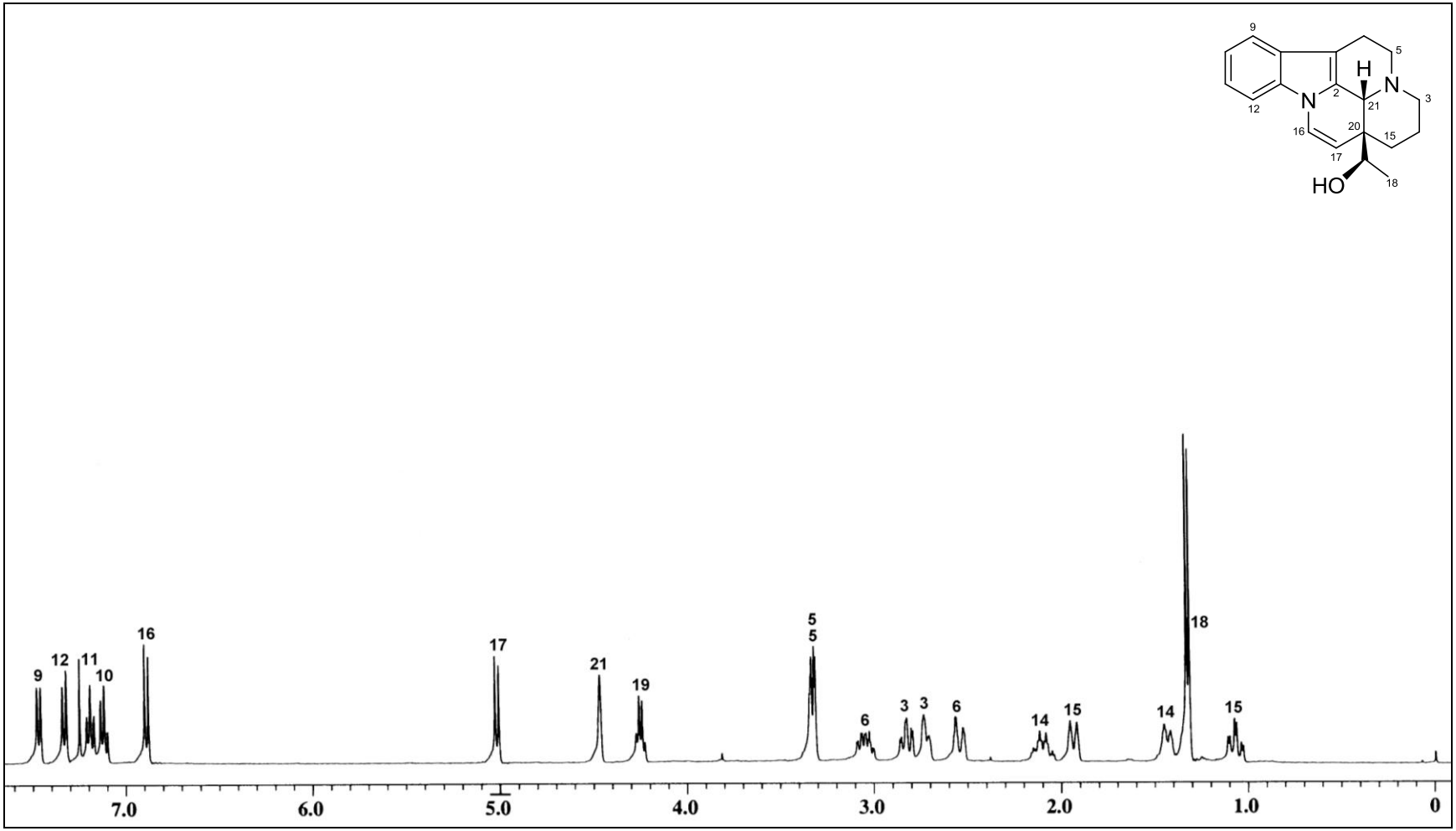


Figure 2.26 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **11**

2.1.12 Compound 12

Compound **12** was obtained in minute amount as a light yellowish oil, and subsequently crystallized from CH_2Cl_2–hexanes as colorless block crystals, mp 150−152 ^oC, $[\alpha]^{25}_D$ −47 (*c* 0.19, $CHCl_3$). The UV spectrum showed absorption maxima at 226 and 280 nm due to an indole chromophore. The IR spectrum showed a band at 3395 cm^{-1} suggesting the presence of a hydroxyl function. The mass spectrum showed an MH^+ peak at *m/z* 341, and HRESIMS established the molecular formula as $C_{21}H_{28}N_2O_2$ + H. The ^{13}C NMR spectrum gave a total of 21 carbon resonances comprising two methyl, seven methylene, seven methine, and five quaternary carbons. Analysis of the ^1H and ^{13}C NMR spectroscopic data (Table 2.13) of **12** showed a close resemblance to those of (–)-19(*R*)-hydroxyisoeburnamine (**36**)[77] except for the presence of an ethoxy group at C(16), as indicated by the observed signals of a methyl triplet at δ 1.23 and a methylene doublet of quartet at δ 3.70. In addition, the observed three-bond correlations from H(16) to C(22), and from H(22) to C(16), in the HMBC spectrum (Figure 2.27) also confirmed the substitution of the ethoxy group at C(16). The three-bond correlations from H(16) to C(20), as well as from H(17) to C(15), C(19) and C(21), are also in complete accord with the proposed structure (Figure 2.27). The configuration at C(20) and C(21) were assumed to follow that of the other eburnan-type compounds occurring in the plant, assuming that they share a common biogenetic origin. In any case, since we were able to obtain suitable crystals of **12**, an X-ray diffraction analysis was carried out (Figure 2.28) which provided further confirmation of the structure deduced based on the spectroscopic data. In addition, X-ray analysis revealed the configuration of C(19) as *R*. Compound **12** is therefore (–)-19(*R*)-hydroxy-*O*-ethyl-isoeburnamine.

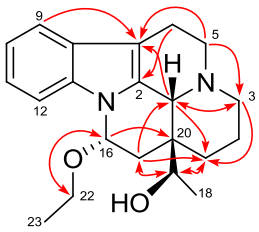
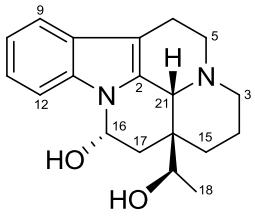


Figure 2.27 : Selected HMBCs of **12**



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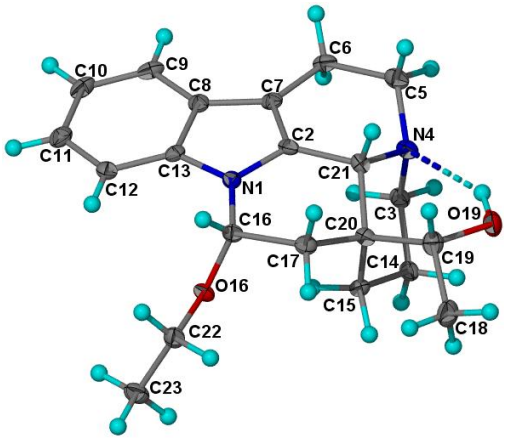


Figure 2.28 : X-ray crystal structure of **12**. Thermal ellipsoids are shown at the 50% probability level.

Table 2.13 : ^{1}H and ^{13}C NMR spectroscopic data of compound **12**[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
2	–	129.4	14	1.37 br d (14)	21.7
3	2.55 m	44.3		2.19 qt (14, 3)	
	2.64 td (14.9, 5)		15	1.67 dd (14.9, 4.5)	22.7
5	3.29 m	51.2		1.90 td (14.9, 4.5)	
	3.29 m		16	5.51 br d (3.2)	81.4
6	2.60 m	17.0	17	1.67 dd (14.9, 4.5)	35.4
	2.98 m			2.07 d (14.9)	
7	–	105.1	18	1.24 d (6)	17.8
8	–	128.8	19	3.93 q (6)	79.3
9	7.46 d (7)	118.5	20	–	37.2
10	7.12 t (7)	120.3	21	4.18 s	60.1
11	7.17 t (7)	121.5	22	3.70 dq (14, 7)	64.1
12	7.25 d (7)	110.8	23	1.23 t (7)	15.5
13	–	135.6			

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, HMBC, and NOESY.

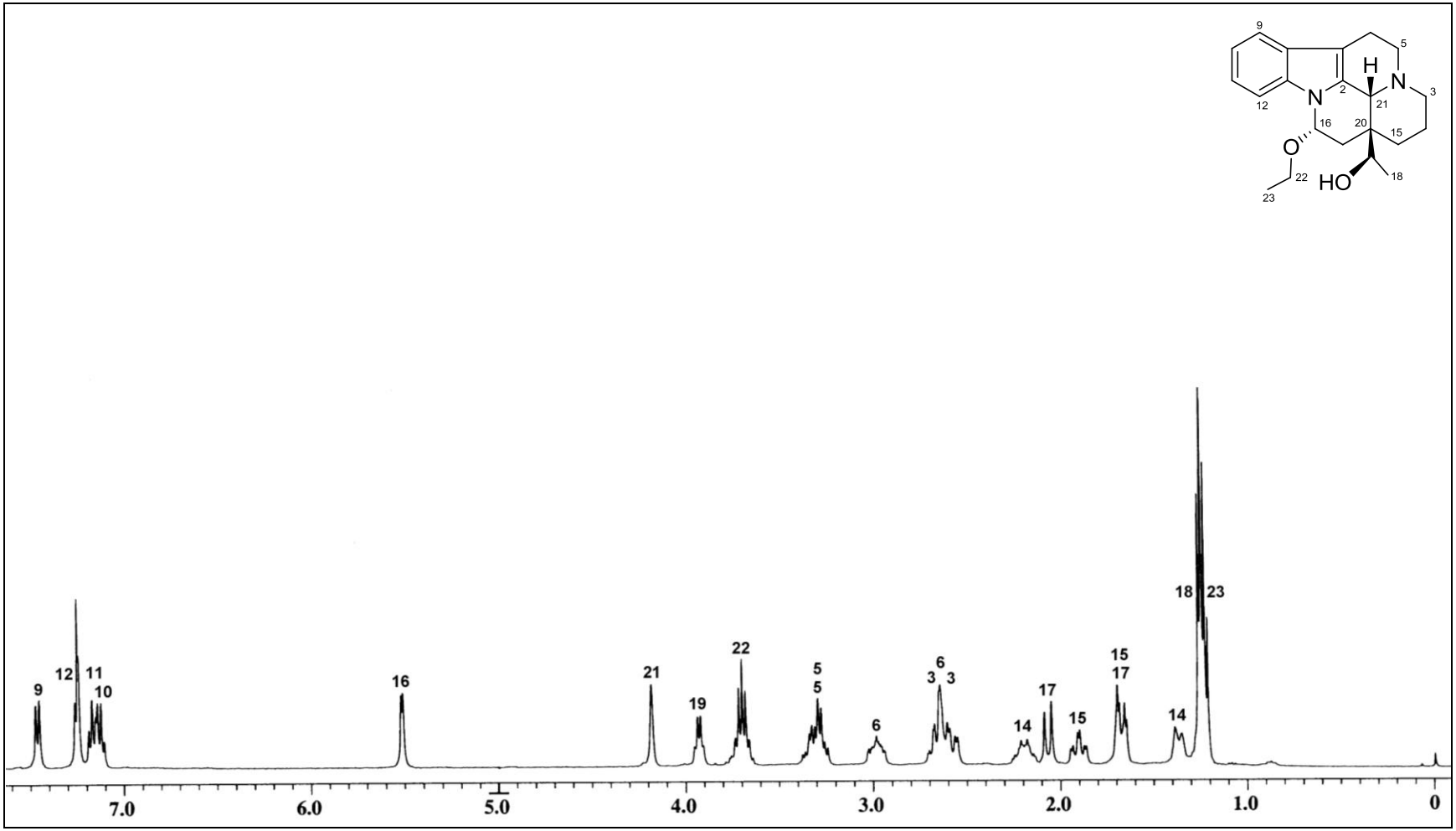


Figure 2.29 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **12**

In addition to the 12 new alkaloids discussed above, another 28 known alkaloids were also obtained from the stem-bark extract. The spectral data of these known alkaloids are presented in the following Tables and Figures.

Table 2.14 : Known alkaloids from the stem-bark of *K. pauciflora*

Alkaloids	Physical Appearance	$[\alpha]^{25}_{D}$ ($CHCl_3$)	UV/nm (EtOH) (log ε)	IR/cm^{-1}	NMR Spectral Data
Tetrahydroalstonine (**13**)	Light yellowish oil	–53 (*c* 0.10)	227 (4.31) 247 (3.77) 282 (3.64) 291 (3.49)	3370 (NH) 1703 (C=O, ester)	Table 2.15
Leuconoxine (**14**)	Colorless oil	–71 (*c* 0.08)	205 (4.42) 244 (3.82) 280 (3.16)	1695 and 1677 (C=O, lactam)	Table 2.16
N(1)-Carbomethoxy-5,22-dioxokopsane (**15**)	Colorless oil	+73 (*c* 0.12)	210 (4.38) 241 (4.11) 281 (3.46)	1757 (C=O, ketone) 1713 (C=O, carbamate) 1687 (C=O, lactam)	Tables 2.17 and 2.18
Kopsanone (**16**)	Colorless oil	+126 (*c* 0.20)	207 (4.38) 244 (3.84) 294 (3.50)	3336 (NH/OH) 1738 (C=O, ketone)	Tables 2.17 and 2.18
Kopsifine (**17**)	Colorless oil	+97 (*c* 0.04)	223 (4.41) 250 (3.94) 285 (3.16) 295 (3.10)	3295 (OH) 1765 (C=O, ketone) 1684 (C=O, lactam)	Tables 2.17 and 2.18
Decarbomethoxy-kopsifine (**18**)	Colorless oil	+52 (*c* 0.07)	220 (4.72) 243 (4.23) 288 (3.51)	3344 (NH/OH) 1761 (C=O, ketone) 1682 (C=O, lactam)	Table 2.19

Table 2.14, continued

Alkaloids	Physical Appearance	$[\alpha]^{25}_{D}$ ($CHCl_3$)	UV/nm (EtOH) (log ε)	IR/cm^{-1}	NMR Spectral Data
Paucidactine B (**19**)	Colorless oil	+2 (*c* 0.11)	224 (4.71) 244 (4.30) 285 (3.60) 295 (3.52)	3333 (OH) 1766 (C=O, lactone) 1703 (C=O, carbamate) 1691(C=O, lactam)	Table 2.19
Kopsamine (**20**)	Colorless crystals from $CHCl_3$–Et_2O; mp 200–201 ℃	–47 (*c* 0.21)	227 (4.36) 248 (3.92) 286 (2.92)	3315 (OH) 1736 (C=O, ester) 1684 (C=O, carbamate)	Table 2.20
Kopsamine *N*-oxide (**21**)	Light yellowish oil	–29 (*c* 0.36)	227 (4.15) 246 (3.69) 286 (2.15)	3292 (OH) 1732 (C=O, ester) 1684 (C=O, carbamate)	Table 2.20
Kopsinine (**22**)	Light yellowish oil	–68 (*c* 0.24)	205 (4.56) 246 (3.88) 296 (3.48)	3350 (NH) 1728 (C=O, ester)	Tables 2.21 and 2.22
N(1)-Methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine (**23**)	Light yellowish needles from EtOH; mp 150–152 ℃	–60 (*c* 0.10)	218 (4.50) 251 (4.01) 282 (3.45)	1717 (C=O, ester/ carbamate)	Tables 2.21 and 2.22
N(1)-Methoxycarbonyl-12-hydroxy-$\Delta^{16,17}$-kopsinine (**24**)	Light yellowish oil	–121 (*c* 0.04)	218 (3.98) 248 (3.61) 290 (3.13)	3400 (OH) 1716 (C=O, ester) 1674 (C=O, carbamate)	Tables 2.21 and 2.22
Kopsinine *N*-oxide (**25**)	Light yellowish oil	–56 (*c* 0.26)	204 (4.31) 243 (3.77) 294 (3.39)	3347 (NH) 1725 (C=O, ester)	Tables 2.23 and 2.24

Table 2.14, continued

Alkaloids	Physical Appearance	$[\alpha]^{25}_{D}$ ($CHCl_3$)	UV/nm (EtOH) (log ε)	IR/cm^{-1}	NMR Spectral Data
N(1)-Methoxycarbonyl-11,12-dimethoxy-kopsinaline (**26**)	Colorless prisms from Et_2O; mp 167–168 ℃	–21 (*c* 0.09)	223 (4.44) 251 (3.91) 286 (3.31) 292 (3.32)	3323 (OH) 1736 (C=O, ester) 1677 (C=O, carbamate)	Tables 2.23 and 2.24
Kopsilongine (**27**)	Light yellowish oil	–21 (*c* 0.09)	217 (4.34) 254 (3.92) 282 (3.22) 288 (3.20)	3318 (OH) 1737 (C=O, ester) 1675 (C=O, carbamate)	Tables 2.23 and 2.24
Pleiocarpine (**28**)	Colorless prisms from Et_2O; mp 149–150 ℃	–169 (*c* 0.08)	204 (4.45) 244 (3.88) 281 (3.99) 290 (3.51)	1736 (C=O, ester) 1674 (C=O, carbamate)	Tables 2.25 and 2.26
12-Methoxy-pleiocarpine (**29**)	Colorless oil	–82 (*c* 0.15)	217 (4.40) 253 (3.99) 283 (3.50)	1723 (C=O, ester) 1699 (C=O, carbamate)	Tables 2.25 and 2.26
Pleiocarpine *N*-oxide (**30**) [147]	Light yellowish oil	–103 (*c* 0.27)	208 (3.45) 244 (3.19) 256 (2.81) 284 (2.31)	1709 (C=O, ester/ carbamate)	Tables 2.25 and 2.26
(+)-Eburnamenine (**31**)	Colorless oil	+216 (*c* 0.07)	223 (3.95) 259 (3.98) 303 (3.42) 310 (3.44) 362 (2.48)	1638 (C=C)	Table 2.27
(+)-Eburnamonine (**32**)	Light yellowish oil	+108 (*c* 0.24)	207 (4.40) 246 (4.46) 270 (4.18) 302 (3.91)	1716 (C=O, lactam)	Table 2.27
(–)-Eburnamine (**33**)	Light yellowish oil	–77 (*c* 0.11)	205 (4.13) 229 (4.30) 282 (3.79) 292 (3.67)	3325 (OH)	Table 2.28

Table 2.14, continued

Alkaloids	Physical Appearance	$[\alpha]^{25}_{D}$ ($CHCl_3$)	UV/nm (EtOH) (log ε)	IR/cm^{-1}	NMR Spectral Data
(+)-Isoeburnamine (**34**)	Light yellowish oil	+93 (*c* 0.12)	207 (3.66) 230 (3.92) 283 (3.32) 290 (3.23)	3315 (OH)	Table 2.28
(+)-19-Oxoeburnamine (**35**)	Light yellowish oil	+83 (*c* 0.06)	202 (3.81) 229 (4.00) 282 (3.39) 292 (3.25)	3324 (OH) 1702 (C=O, ketone)	Tables 2.29 and 2.30
(–)-19(*R*)-Hydroxy-isoeburnamine (**36**)	Light yellowish oil	–16 (*c* 0.18)	203 (4.39) 229 (4.53) 282 (3.74) 292 (3.83)	3296 (OH)	Tables 2.29 and 2.30
(+)-19(*R*)-Hydroxy-eburnamine (**37**)	Colorless crystals from EtOH; mp 246–248 ℃	+111 (*c* 0.09)	201 (3.86) 229 (4.02) 283 (3.42) 291 (3.31)	3298 (OH)	Tables 2.29 and 2.30
(–)-Norpleiomutine (**38**)	Light yellowish oil	–49 (*c* 1.21)	208 (4.69) 229 (4.51) 255 (4.09) 287 (3.97) 293 (3.97)	3348 (NH) 1730 (C=O, ester)	Table 2.31
(–)-Demethyl-norpleiomutine (**39**)	Light Yellowish oil	–87 (*c* 1.21)	209 (4.48) 230 (4.34) 254 (3.99) 287 (3.89) 292 (3.84)	3341 (NH) 1720 (C=O, ester)	Table 2.32
(+)-Kopsoffinol (**40**)	Light yellowish oil	+22 (*c* 0.43)	211 (4.57) 231 (4.49) 254 (4.06) 287 (3.94) 293 (3.94)	3340 (OH) 1728 (C=O, ester)	Table 2.33

Table 2.15 : ^{1}H and ^{13}C NMR spectroscopic data of tetrahydroalstonine (**13**)[a]

Position	δ_H	δ_C
2	–	134.5
3	3.35 dd (12, 2)	59.8
5	2.55 m	53.5
	2.93 m	
6	2.69 m	21.7
	2.93 m	
7	–	108.0
8	–	127.2
9	7.27 d (7.5)	118.0
10	7.07 td (7.5, 1.5)	119.4
11	7.12 td (7.5, 1.5)	121.4
12	7.45 d (7.5)	110.8
13	–	136.0
14	2.50 m	34.2
	1.53 q (12)	
15	2.76 m	31.3
16	–	109.5
17	7.56 s	155.7
18	1.40 d (6.2)	18.5
19	4.50 dq (10.3, 6.2)	72.5
20	1.69 m	38.4
21	2.73 dd (12.3, 3.5)	36.3
	3.11 dd (12.3, 2)	
CO_2Me	–	168.0
CO_2*Me*	3.75 s	51.1
NH	7.87 br s	–

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

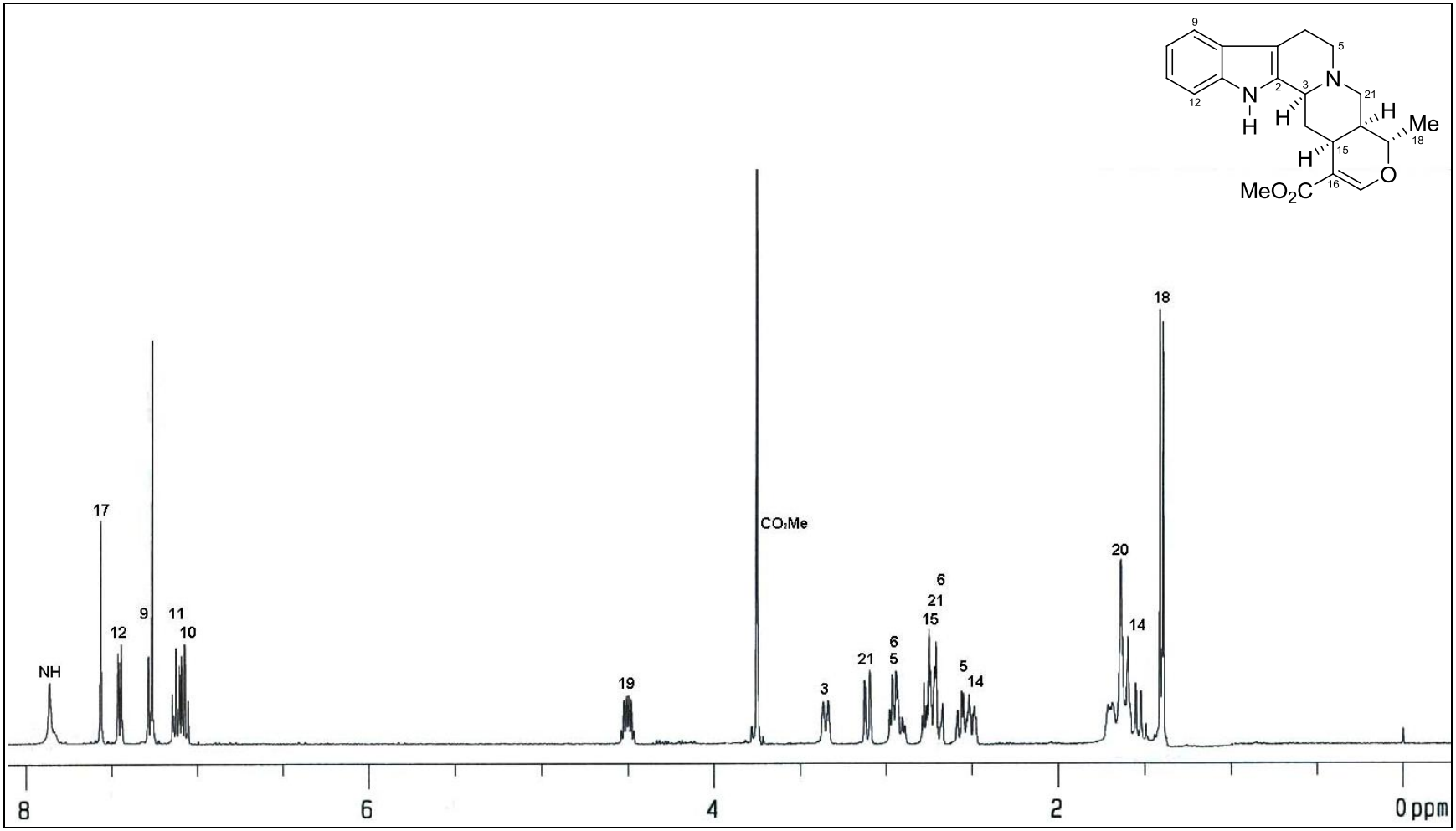


Figure 2.30 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of tetrahydroalstonine (**13**)

Table 2.16 : ^{1}H and ^{13}C NMR spectroscopic data of leuconoxine (**14**)[a]

Position	δ_H	δ_C
2	–	172.9
3	2.73 dd (13, 6.5)	36.8
	3.96 br d (13)	
5	–	170.8
6	2.69 d (17)	37.6
	2.87 dd (17, 7.5)	
7	3.82 d (7.5)	41.9
8	–	135.4
9	7.17 dd (7, 1)	123.8
10	7.14 td (7, 1)	125.5
11	7.26 td (7, 1)	128.0
12	7.77 br d (7)	120.1
13	–	142.1
14	1.65 m	20.1
	1.65 m	
15	1.68 m	26.2
	1.97 td (13, 4)	
16	2.49 ddd (19, 6, 1)	29.4
	2.80 m	
17	1.68 m	26.6
	1.86 ddd (14, 5, 1)	
18	0.93 t (7.4)	7.3
19	1.37 dq (13, 7.4)	26.9
	1.78 dq (13, 7.4)	
20	–	38.1
21	–	92.5

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

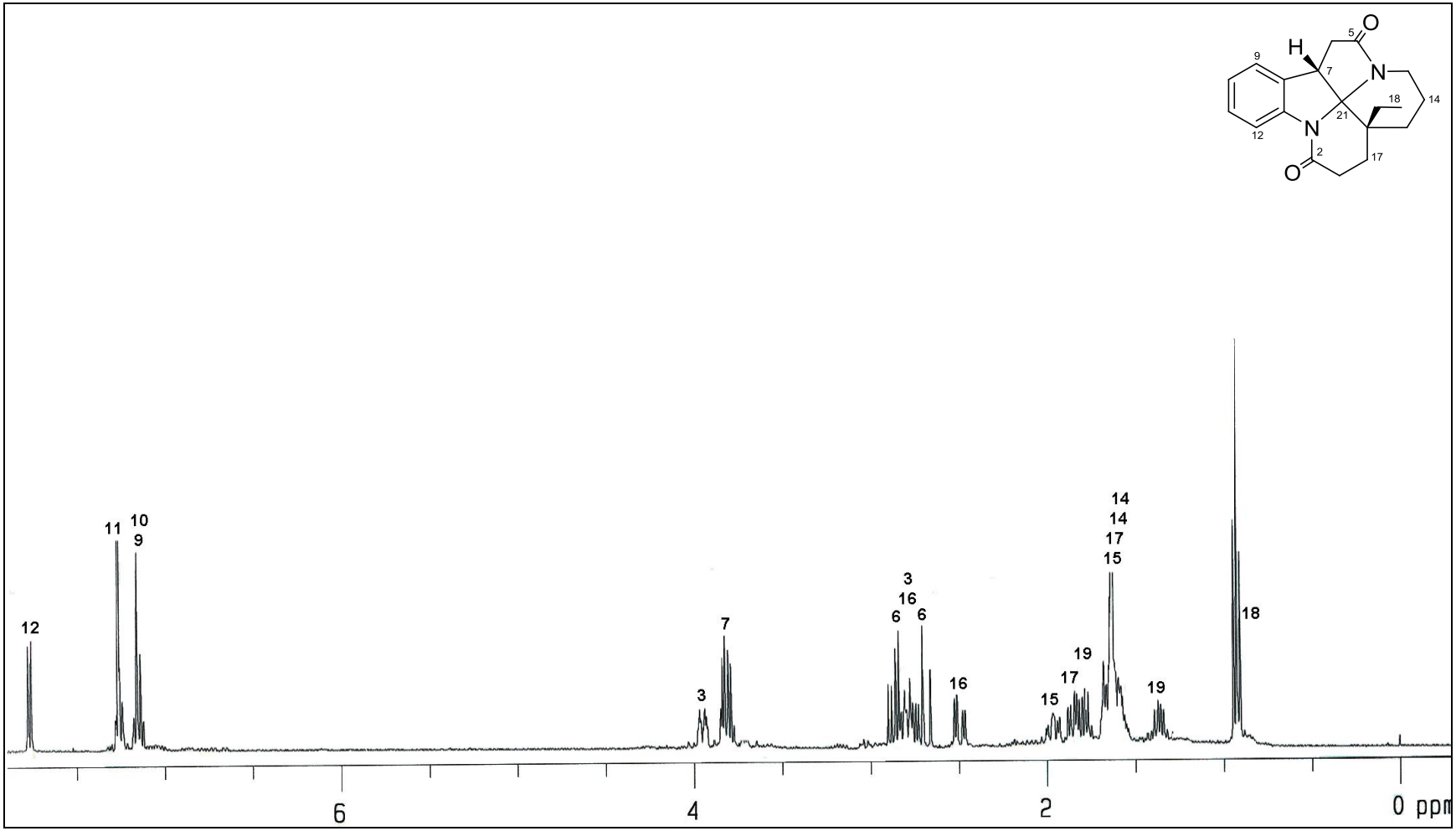


Figure 2.31 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of leuconoxine (**14**)

Table 2.17 : ^{1}H NMR spectroscopic data of *N*(1)-carbomethoxy-5,22-dioxokopsane (**15**), kopsanone (**16**), and kopsifine (**17**)[a]

H	**15**	**16**	**17**
3	2.93 td (13.5, 3.8)	3.03 m	2.91 td (13, 4)
	4.25 dd (13.5, 5)	3.03 m	4.22 dd (13, 5)
5	–	3.13 dd (10, 5)	–
	–	3.50 t (10)	–
6	2.87 s	2.57 ddd (10, 5, 2)	2.91 s
9	7.22 d (7.5)	7.30 br d (8)	6.72 d (8)
10	7.07 td (7.5, 1)	6.79 td (8, 1)	6.63 d (8)
11	7.28 m	7.06 td (8, 1)	–
12	7.28 m	6.67 br d (8)	–
14	1.52 m	1.80 m	1.51 m
	1.62 m	1.80 m	1.65 m
15	1.46 m	1.30 m	1.46 m
	1.67 m	1.30 m	1.65 m
16	2.35 m	2.69 br d (11)	–
17	1.64 m	1.62 ddd (14, 11, 1)	1.61 br d (15)
	1.64 m	2.03 d (14)	2.13 dd (15, 3)
18	1.47 m	1.30 m	1.70 m
	1.64 m	1.51 dt (14, 3)	2.56 ddd (13.5, 12, 4.5)
19	1.78 m	1.30 m	1.43 m
	1.78 m	1.30 m	1.80 td (12, 4.5)
21	3.78 br s	3.37 d (1)	3.62 d (2)
NCO_2*Me*	3.85 s	–	3.82 s
OCH_2O	–	–	5.94 d (1.5)
	–	–	5.96 d (1.5)
16-OH	–	–	7.09 br s
NH	–	3.60 br s	–

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC, and HMBC.

Table 2.18 : ^{13}C NMR spectroscopic data of *N*(1)-carbomethoxy-5,22-dioxokopsane (**15**), kopsanone (**16**), and kopsifine (**17**)[a]

C	**15**	**16**	**17**
2	71.3	69.5	74.9
3	40.7	46.7	40.7
5	164.9	54.3	164.3
6	63.3	57.3	59.0
7	55.5	63.1	52.6
8	132.0	133.1	128.6
9	122.0	122.8	115.1
10	123.8	119.6	105.2
11	129.1	127.7	149.9
12	116.6	110.9	136.1
13	143.7	150.8	123.9
14	19.5	24.2	19.3
15	32.7	15.4	32.6
16	51.4	52.3	83.1
17	33.2	33.6	39.3
18	20.3	33.9	18.8
19	29.3	36.3	31.8
20	32.6	31.2	32.8
21	65.6	70.5	65.4
22	204.6	218.2	202.5
N*C*O_2Me	153.7	–	155.3
NCO$_2$*Me*	52.9	–	53.8
OCH_2O	–	–	100.9

[a] $CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.

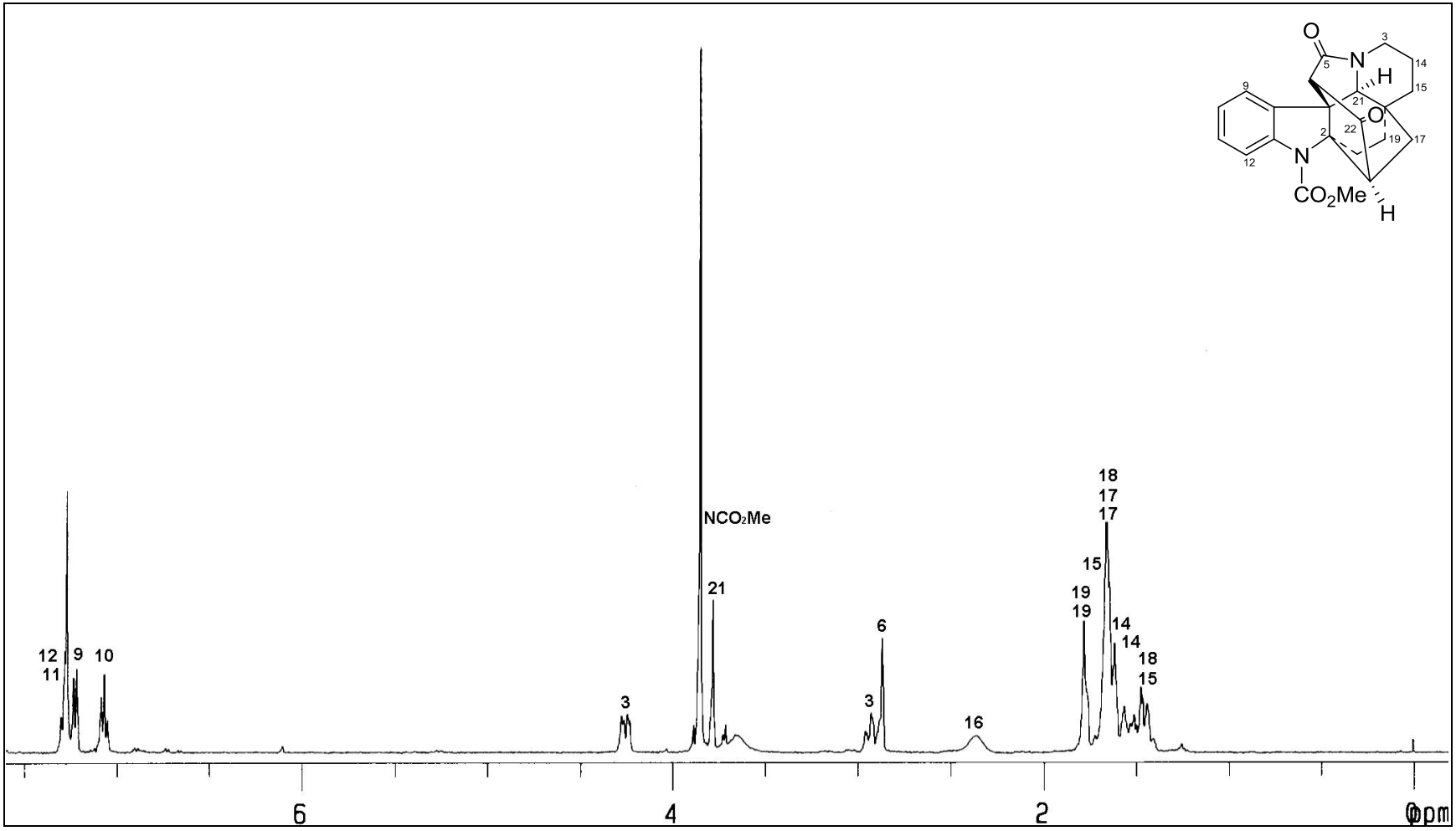


Figure 2.32 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of *N*(1)-carbomethoxy-5,22-dioxokopsane (**15**)

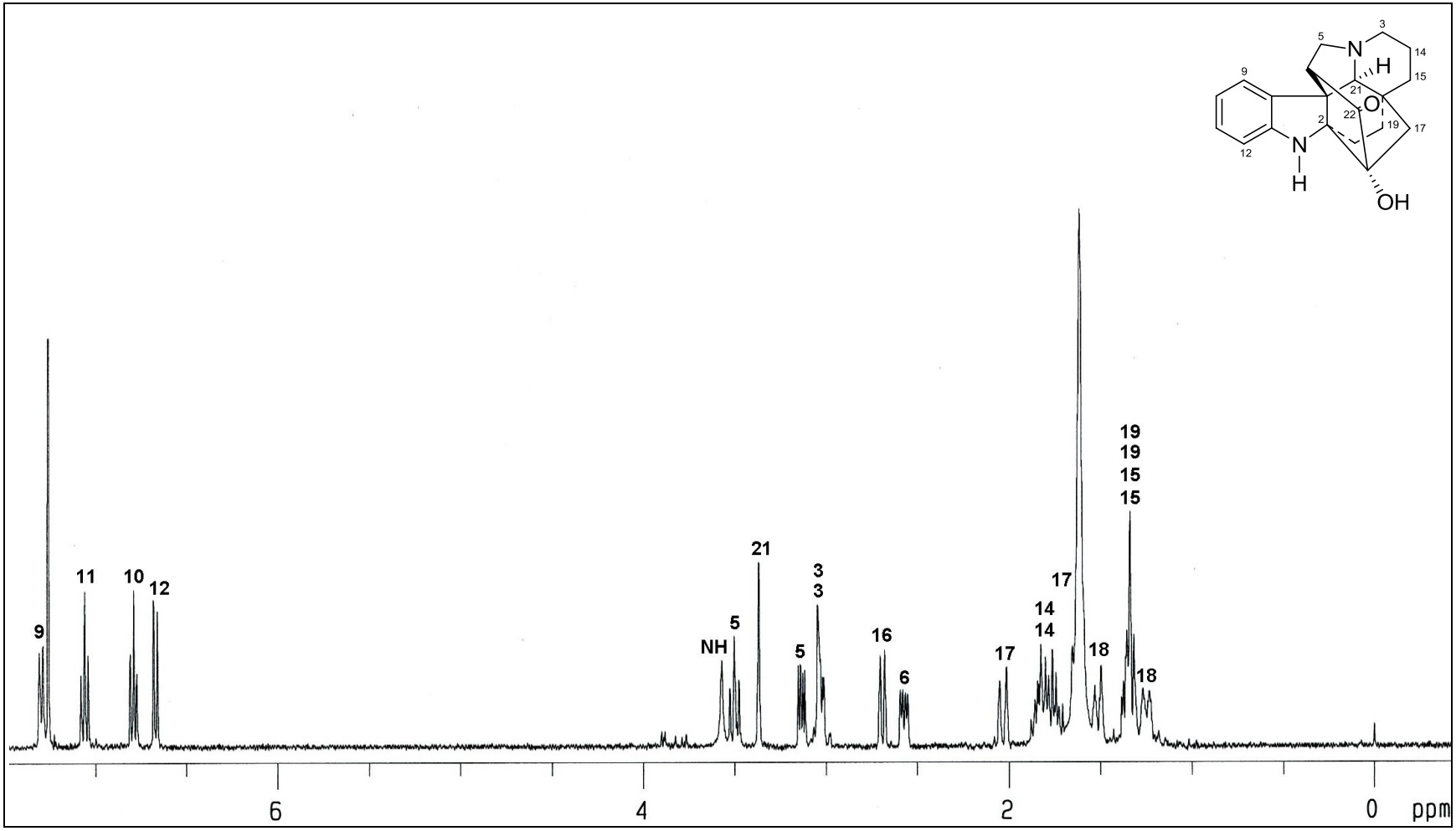


Figure 2.33 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of kopsanone (**16**)

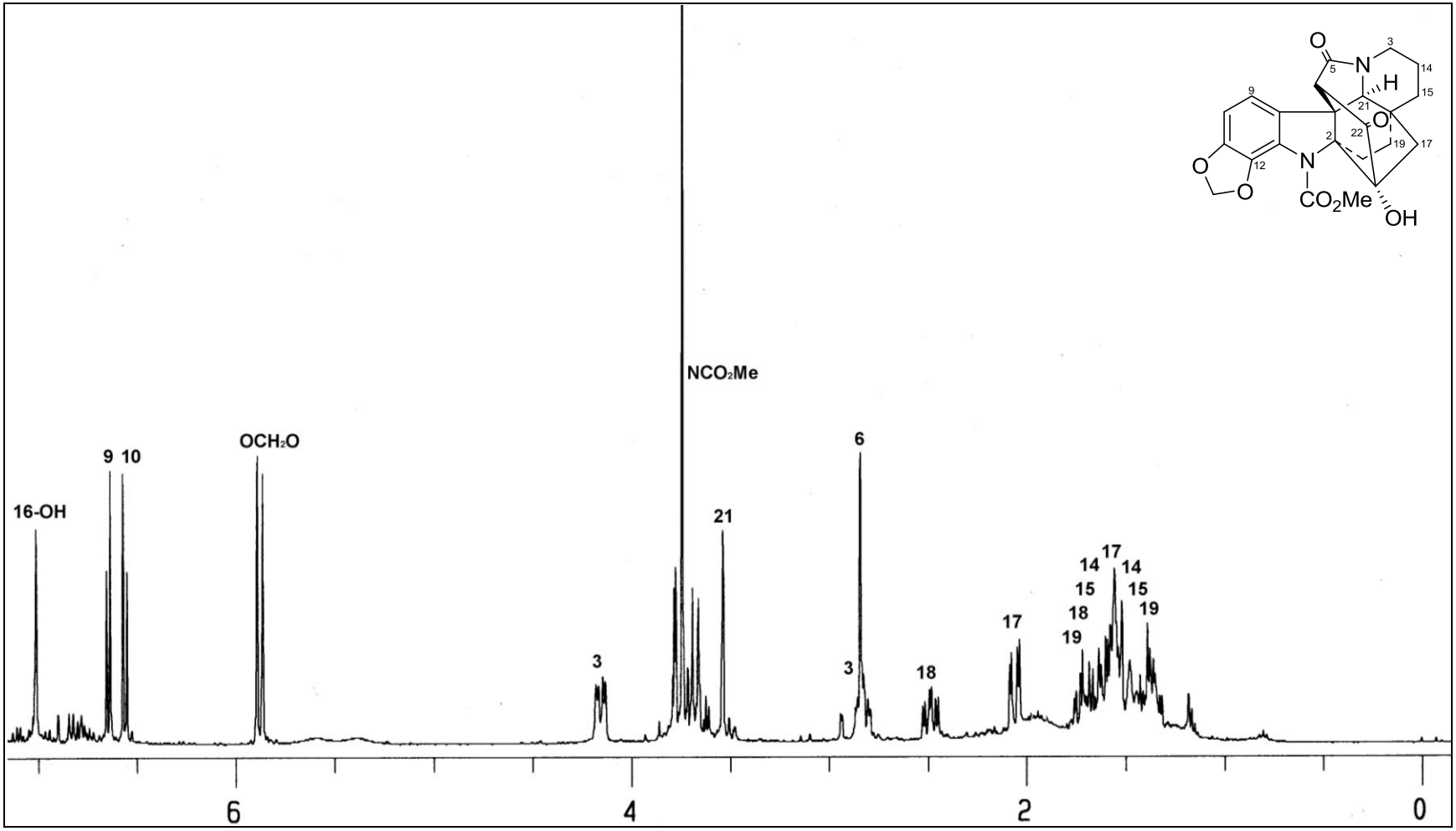


Figure 2.34 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of kopsifine (**17**)

Table 2.19 : ^{1}H and ^{13}C NMR spectroscopic data of decarbomethoxykopsifine (**18**) and paucidactine B (**19**)[a]

Position	**18**		**19**	
	δ_H	δ_C	δ_H	δ_C
2	–	71.9	–	71.0
3	2.91 td (13, 4)	40.8	2.87 td (13, 4)	40.6
	4.23 dd (13, 5)		4.27 dd (13, 4.5)	
5	–	164.7	–	166.9
	–		–	
6	2.92 s	59.6	4.63 s	83.3
7	–	53.6	–	48.7
8	–	127.1	–	129.7
9	6.67 d (8)	115.2	6.60 m	114.3
10	6.35 d (8)	100.5	6.60 m	105.1
11	–	148.9	–	150.1
12	–	132.6	–	136.2
13	–	132.4	–	123.0
14	1.53 m	19.5	1.60 m	19.7
	1.63 m		1.60 m	
15	1.46 m	33.0	1.48 m	33.5
	1.63 m		1.73 m	
16	–	82.3	–	74.0
17	1.50 br d (15)	37.6	1.69 m	39.4
	1.99 dd (15, 3)		2.34 dd (15, 2)	
18	1.75 m	20.1	1.67 m	21.0
	2.09 td (13, 4.5)		2.47 ddd (13, 12, 2)	
19	1.43 m	32.8	1.42 m	29.7
	1.73 m		1.97 ddd (13, 12, 7)	
20	–	34.7	–	33.7
21	3.66 br s	65.6	3.66 d (1.5)	64.5
22	–	204.6	–	169.5
NCO_2Me	–	–	–	153.8
$NCO_2\textit{Me}$	–	–	3.84 s	53.8
16-OH	–	–	6.61 s	–
OCH_2O	5.86 d (1.5)	101.1	5.96 d (1.5)	101.1
	5.91 d (1.5)		5.99 d (1.5)	

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

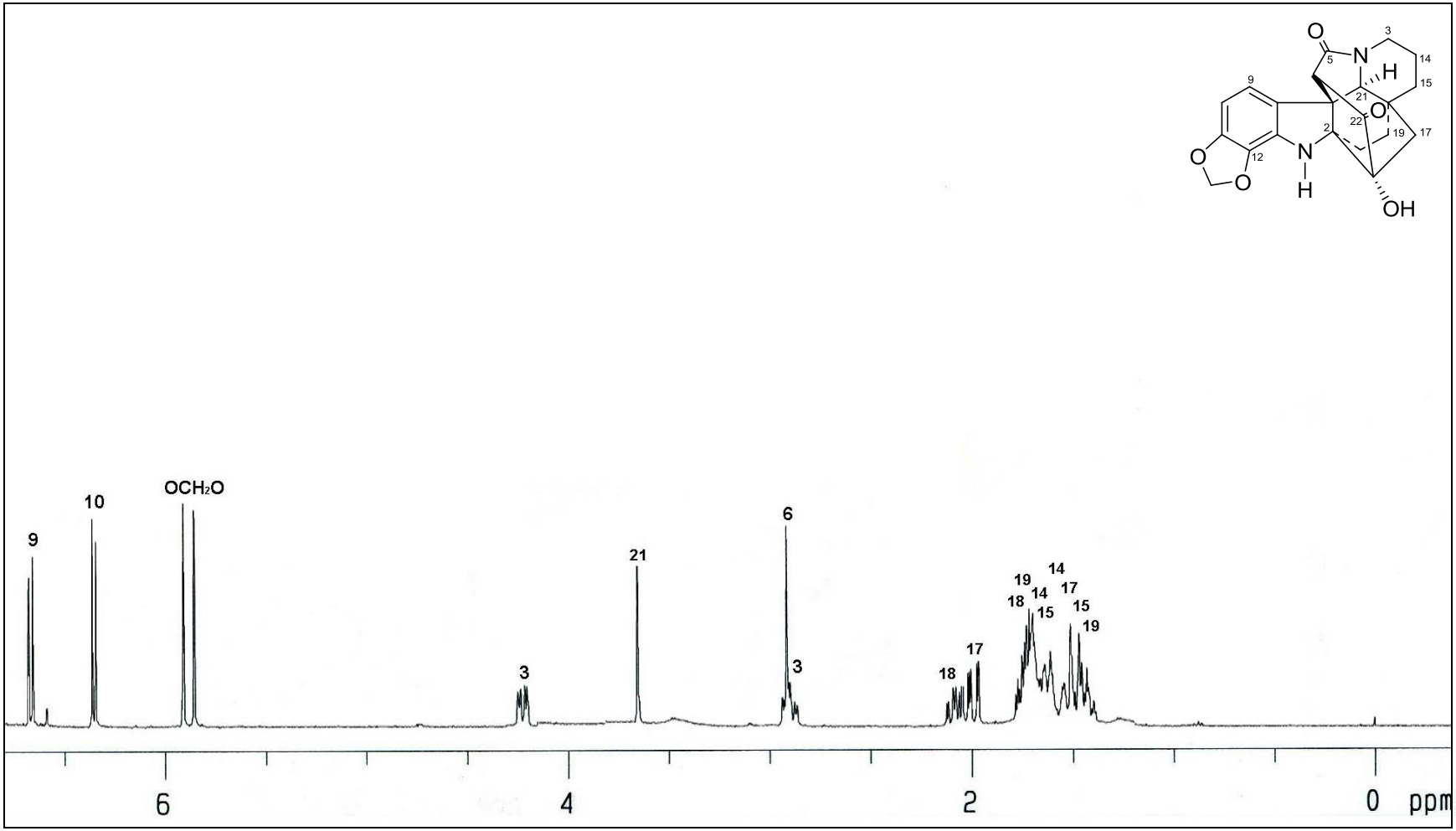


Figure 2.35 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of decarbomethoxykopsifine (**18**)

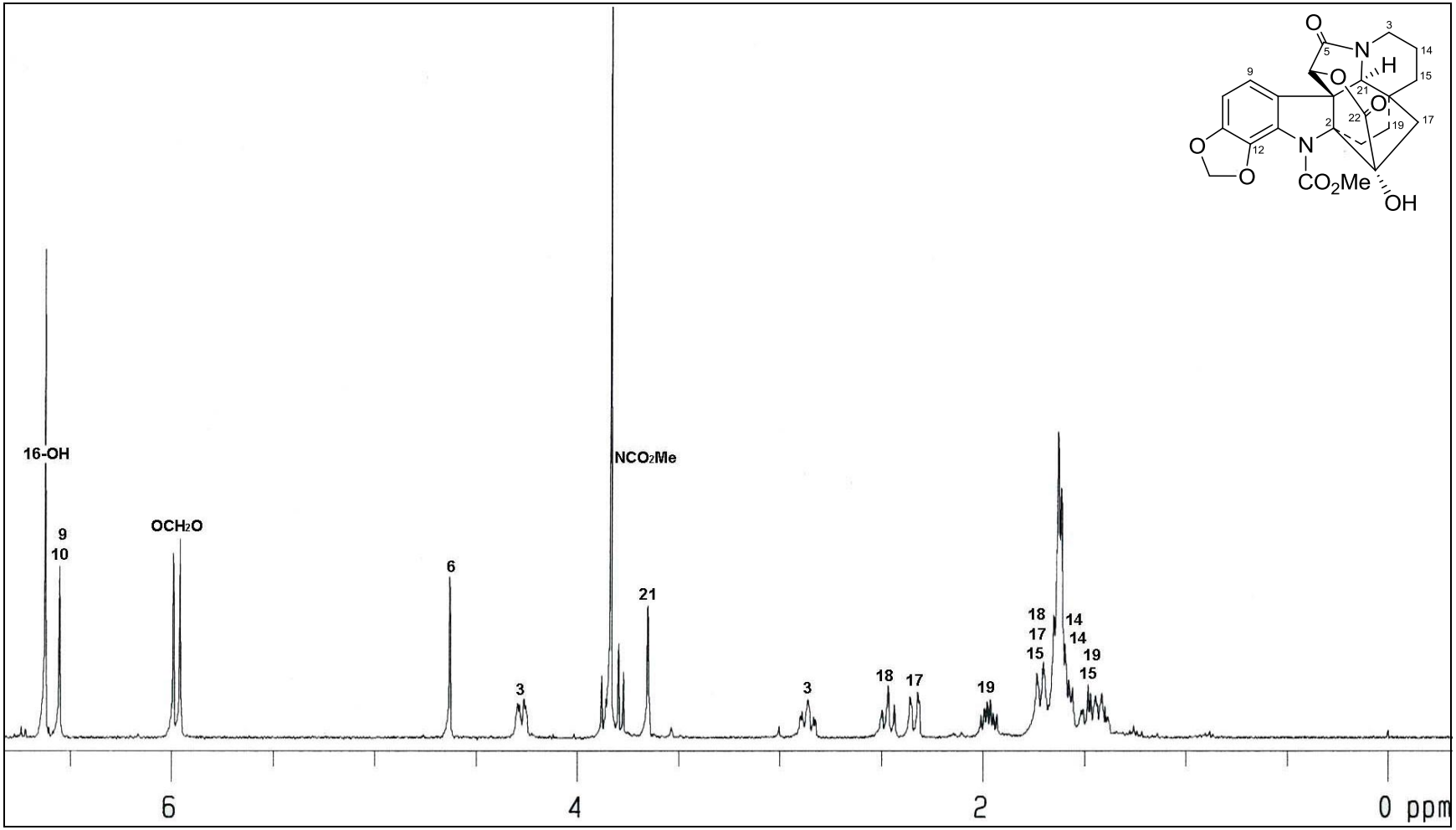


Figure 2.36 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of paucidactine B (**19**)

Table 2.20 : ^{1}H and ^{13}C NMR spectroscopic data of kopsamine (**20**) and kopsamine *N*-oxide (**21**)[a]

Position	**20**		**21**	
	δ_H	δ_C	δ_H	δ_C
2	–	74.3	–	73.7
3	2.87 td (13, 3) 3.05 m	47.5	3.55 td (13, 5) 3.84 m	65.6
5	2.94 td (8.5, 4) 3.09 td (8.5, 7)	50.2	3.38 br t (11) 3.84 m	65.4
6	1.63 m 2.06 ddd (14.5, 8.5, 4)	36.7	2.28 dd (15, 7.5) 2.46 m	34.1
7	–	57.5	–	58.7
8	–	134.1	–	133.4
9	6.76 d (7.8)	115.0	8.21 d (8)	119.2
10	6.52 d (7.8)	104.1	6.57 d (8)	104.3
11	–	148.2	–	148.5
12	–	136.2	–	133.0
13	–	123.1	–	123.0
14	1.25 m 1.81 m	17.0	1.88 m 1.88 m	19.3
15	1.27 td (14, 4) 1.65 m	35.0	1.45 m 1.68 m	33.3
16	–	74.5	–	73.6
17	1.40 dd (15, 1) 2.93 br d (15)	41.6	1.44 br d (15) 3.02 dd (15, 3)	40.5
18	1.50 ddd (13, 11, 8) 2.34 ddd (13, 11, 1.5)	23.7	1.59 ddd (13, 11, 8) 2.46 m	22.8
19	1.11 br t (11) 1.69 m	32.1	1.32 br t (11) 1.90 td (11, 8)	32.7
20	–	32.2	–	34.1
21	2.86 d (2)	67.9	3.62 br s	84.8
CO_2Me	–	173.0	–	172.7
CO_2*Me*	3.76 s	52.5	3.79 s	52.8
N*C*O_2Me	–	156.1	–	156.4
NCO_2*Me*	3.88 s	53.2	3.90 s	53.2
OCH_2O	5.88 d (1.5) 5.90 d (1.5)	100.2	5.88 d (1.5) 5.93 d (1.5)	100.2
16-OH	6.98 s	–	7.31 s	–

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

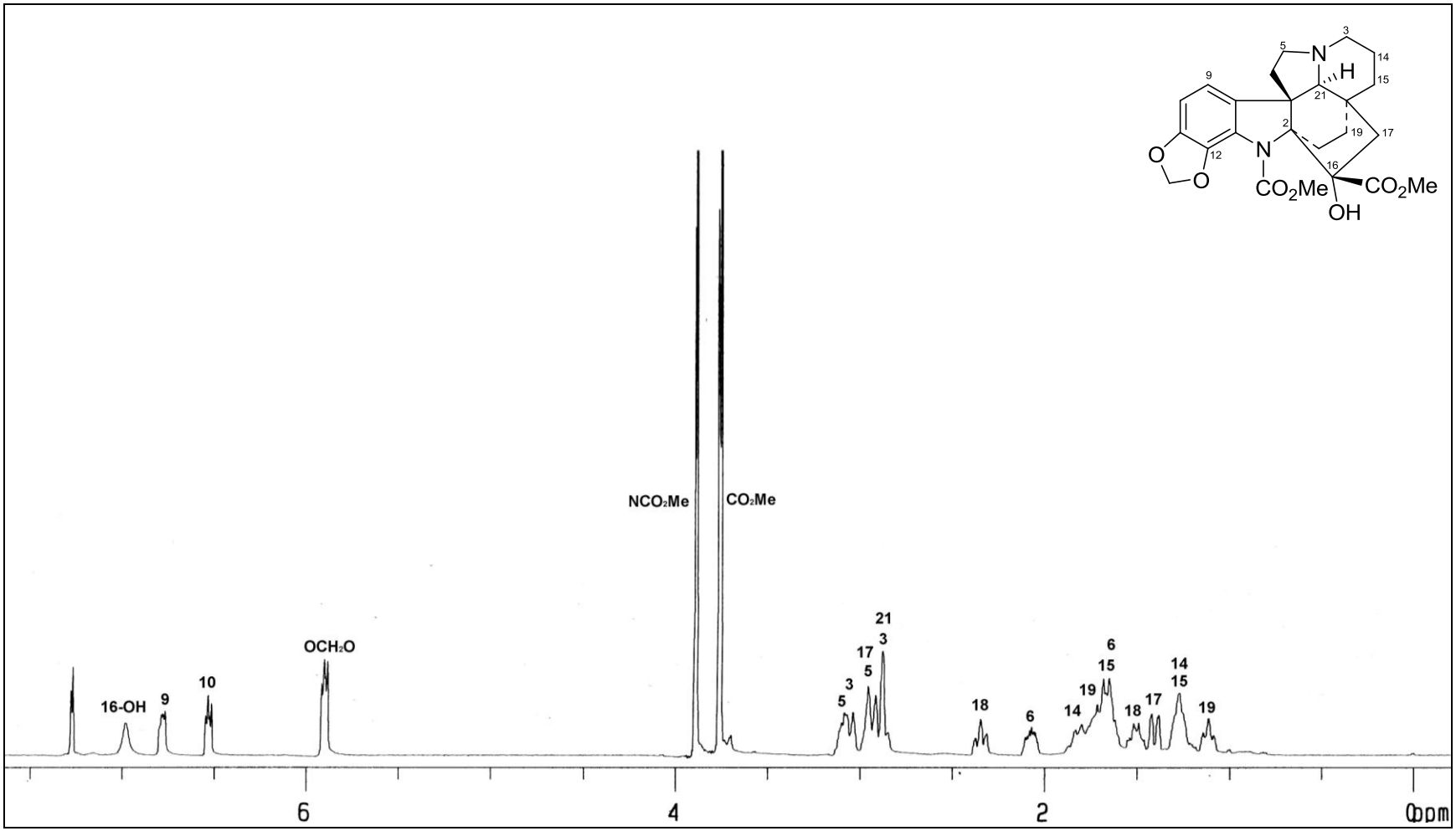


Figure 2.37 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of kopsamine (**20**)

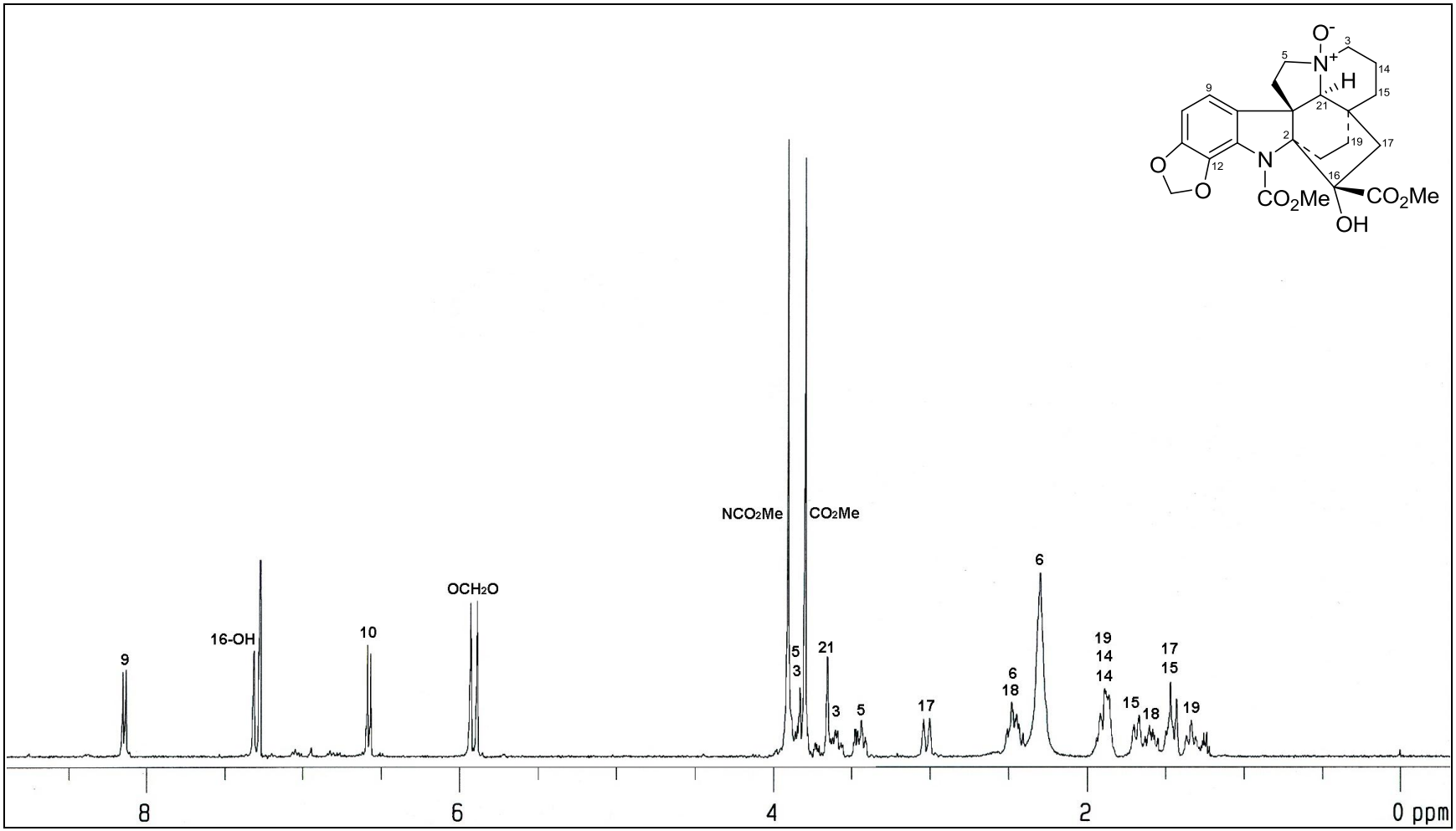


Figure 2.38 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of kopsamine *N*-oxide (**21**)

Table 2.21 : ^{1}H NMR spectroscopic data of kopsinine (**22**), *N*(1)-methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine (**23**), and *N*(1)-methoxycarbonyl-12-hydroxy-$\Delta^{16,17}$-kopsinine (**24**)[a]

H	**22**	**23**	**24**
3	3.00 m	2.94 m	3.05 m
	3.12 ddt (14, 4, 2)	2.99 m	3.05 m
5	2.96 m	2.51 m	2.50 m
	3.35 q (8.3)	2.62 t (8)	2.69 t (8)
6	1.56 ddd (14, 8.3, 7.6)	1.66 m	1.77 m
	2.64 ddd (14, 8.3, 3.4)	2.51 m	2.50 m
9	7.18 dd (7.5, 1.3)	6.85 dd (7.5, 1)	6.82 d (7)
10	6.75 td (7.5, 1)	6.92 t (7.5)	7.00 t (7)
11	6.99 td (7.5, 1.3)	6.74 dd (7.5, 1)	6.79 d (7)
12	6.66 br d (7.5)	–	–
14	1.23 m	1.24 m	1.22 m
	1.91 m	1.83 m	1.77 m
15	1.30 m	1.51 m	1.77 m
	1.61 m	1.81 m	1.77 m
16	2.89 td (9.3, 1)	–	–
17	1.37 m	6.76 s	6.86 s
	2.78 ddd (13.7, 9.3, 3)		
18	1.42 m	1.36 m	1.43 td (13, 3)
	1.91 m	1.96 dt (13, 3)	2.07 m
19	1.23 m	1.17 m	1.22 m
	1.41 m	1.17 m	1.22 m
21	3.01 d (1.5)	3.16 s	3.32 s
CO_2*Me*	3.76 s	3.68 s	3.75 s
NCO_2*Me*	–	3.72 s	3.82 s
12-OMe	–	3.78 s	–
12-OH	–	–	10.83 s
NH	3.75 br s	–	–

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC, and HMBC.

Table 2.22 : ^{13}C NMR spectroscopic data of kopsinine (**22**), *N*(1)-methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine (**23**), and *N*(1)-methoxycarbonyl-12-hydroxy-$\Delta^{16,17}$-kopsinine (**24**)[a]

C	**22**	**23**	**24**
2	66.4	72.3	71.4
3	47.4	47.2	47.1
5	50.5	50.2	50.0
6	34.5	37.8	38.2
7	57.7	63.4	62.9
8	140.4	140.7	140.1
9	121.4	114.9	117.7
10	119.5	124.5	126.7
11	126.4	112.8	113.3
12	110.6	148.7	145.5
13	148.9	134.9	134.8
14	16.9	16.1	16.0
15	36.3	25.8	26.0
16	43.6	130.8	126.1
17	31.9	143.2	143.6
18	33.6	33.8	33.7
19	33.7	31.9	32.0
20	31.5	37.9	38.7
21	68.1	69.4	68.9
CO_2Me	174.6	165.9	166.0
CO_2*Me*	51.7	51.8	51.9
N*C*O_2Me	–	153.8	156.0
NCO_2*Me*	–	52.2	53.3
12-OMe	–	56.5	–

[a] $CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.

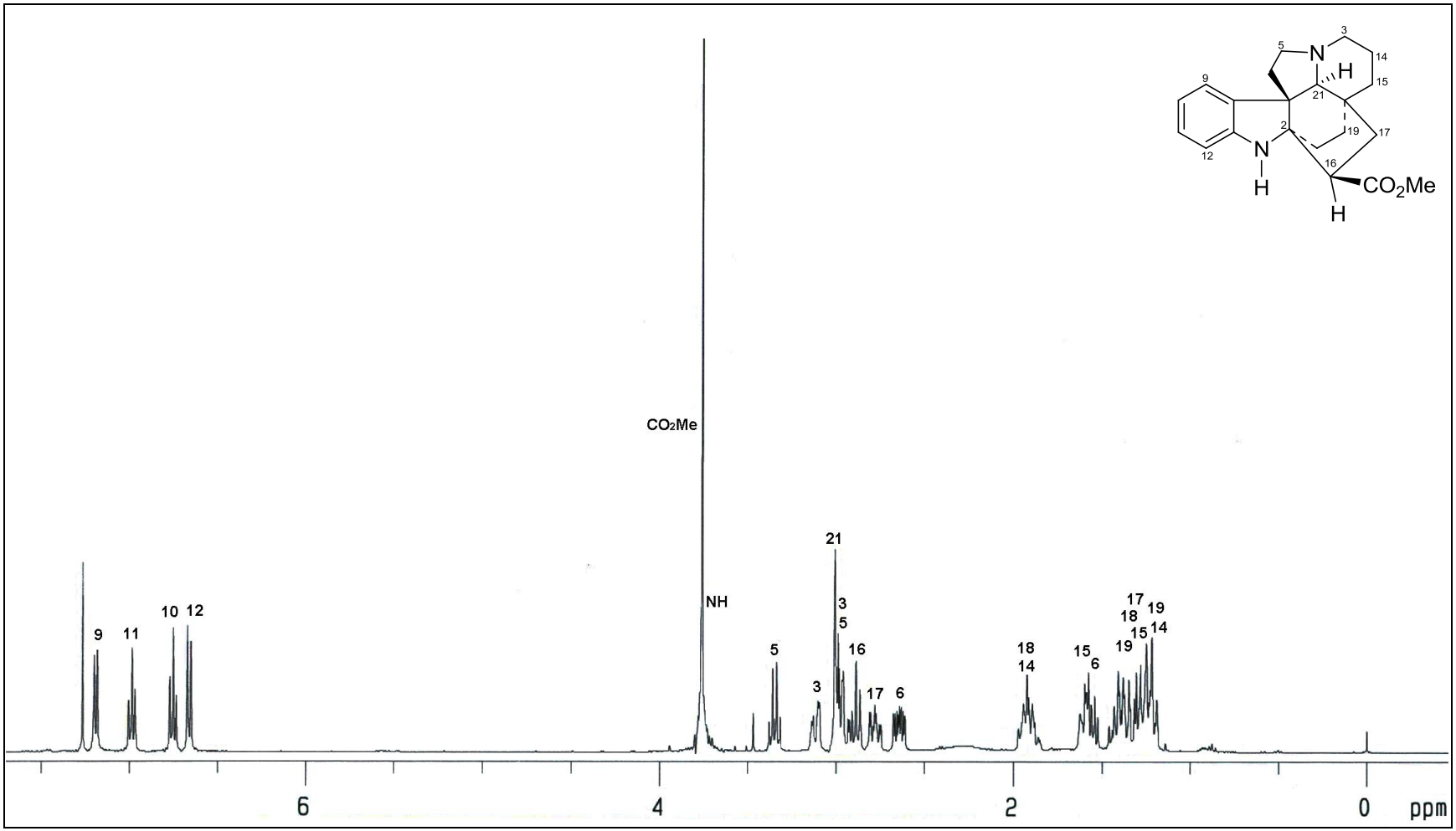


Figure 2.39 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of kopsinine (**22**)

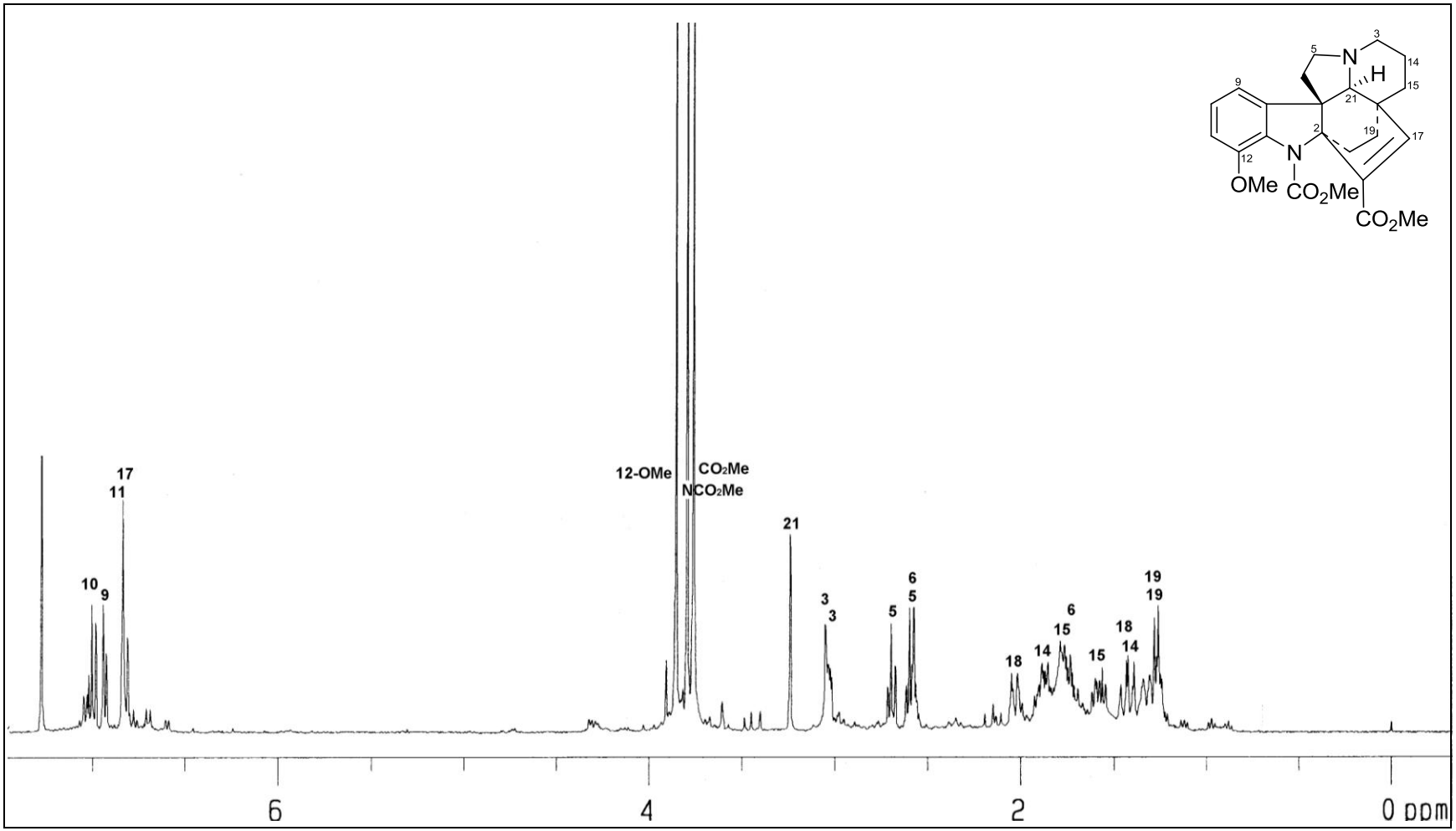


Figure 2.40 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of *N*(1)-methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine (**23**)

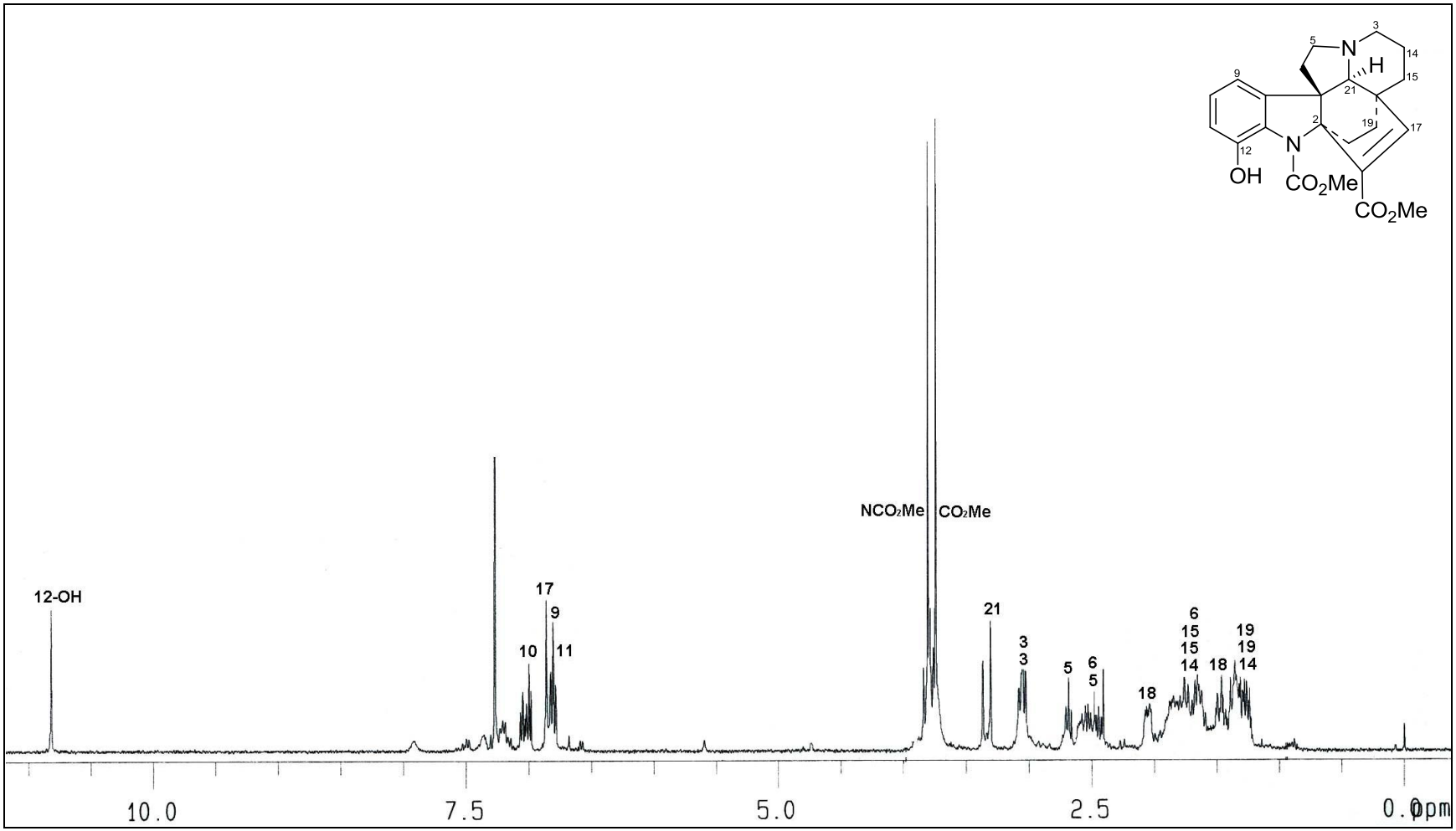


Figure 2.41 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of *N*(1)-methoxycarbonyl-12-hydroxy-$\Delta^{16,17}$-kopsinine (**24**)

Table 2.23 : ^{1}H NMR spectroscopic data of kopsinine *N*-oxide (**25**), *N*(1)-methoxycarbonyl-11,12-dimethoxykopsinaline (**26**), and kopsilongine (**27**)[a]

H	**25**	**26**	**27**
3	3.62 td (13.2, 5)	2.85 td (13, 3)	2.86 td (13, 3)
	3.93 br d (13.2)	3.07 m	3.05 m
5	3.48 dd (11, 9.5)	2.94 td (8.5, 4)	2.95 m
	4.04 td (11, 8.5)	3.07 m	3.08 m
6	2.21 ddd (14.5, 11, 9.5)	1.75 m	1.76 m
	2.79 dd (14.5, 8.5)	2.16 ddd (14.5, 8.5, 4)	2.21 ddd (14.5, 8.5, 4)
9	8.13 br d (7.5)	6.92 d (8)	6.91 dd (7.5, 1)
10	6.81 td (7.5, 1)	6.56 d (8)	6.99 t (7.5)
11	7.04 td (7.5, 1)	–	6.77 dd (7.5, 1)
12	6.65 br d (7.5)	–	–
14	1.90 m	1.27 m	1.25 m
	1.90 m	1.80 m	1.81 m
15	1.47 m	1.24 td (14, 4)	1.25 m
	1.47 m	1.65 m	1.64 m
16	2. 97 t (9.5)	–	–
17	1.64 br d (14)	1.41 br d (15)	1.41 dd (15, 1)
	2.72 ddd (14, 9.5, 3)	2.91 dd (15, 3)	2.94 br d (15)
18	1.29 ddd (13, 11.3, 1.8)	1.48 ddd (13, 11, 8)	1.47 ddd (13, 11, 8)
	2.04 td (13, 8)	2.37 ddd (13, 11, 1.5)	2.37 ddd (13, 11, 1.5)
19	1.44 m	1.06 br t (11)	1.06 br t (11)
	1.70 ddd (13, 11.3, 8)	1.67 m	1.66 m
21	3. 69 br s	2.78 d (2)	2.83 d (1.5)
CO_2*Me*	3.79 s	3.76 s	3.76 s
NCO_2*Me*	–	3.92 s	3.88 s
11-OMe	–	3.84 s	–
12-OMe	–	3.75 s	3.82 s
16-OH	–	–	6.66 s
NH	3.70 br s	–	–

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC, and HMBC.

Table 2.24 : ^{13}C NMR spectroscopic data of kopsinine *N*-oxide (**25**), *N*(1)-methoxycarbonyl-11,12-dimethoxykopsinaline (**26**), and kopsilongine (**27**)[a]

C	**25**	**26**	**27**
2	65.5	74.6	74.6
3	65.1	47.5	47.6
5	65.3	50.2	50.3
6	32.0	37.4	37.0
7	58.5	57.1	58.1
8	137.6	133.7	142.9
9	125.6	116.3	114.5
10	120.5	107.3	124.9
11	127.5	153.1	111.6
12	110.2	137.8	148.1
13	148.4	135.6	129.2
14	19.8	17.9	17.6
15	34.4	35.8	35.7
16	43.2	75.7	75.0
17	30.7	41.7	41.9
18	32.8	24.7	24.5
19	33.9	32.4	32.1
20	33.5	32.0	32.4
21	84.7	68.3	67.9
CO_2Me	174.4	173.2	173.2
CO_2*Me*	52.3	52.3	52.3
N*C*O_2Me	–	157.4	157.3
NCO_2*Me*	–	53.3	53.0
11-OMe	–	56.1	–
12-OMe	–	60.0	56.2

[a] $CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.

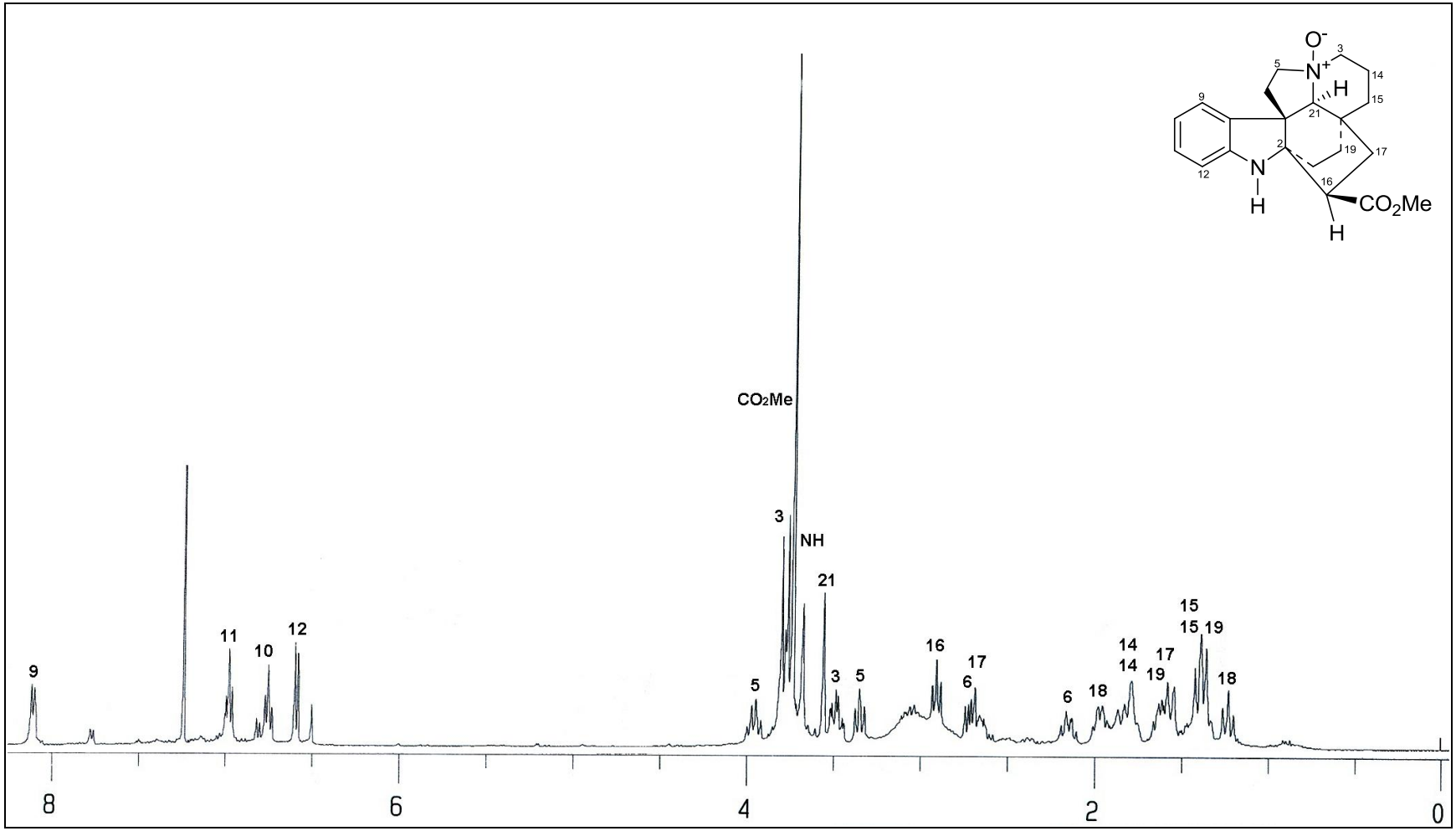


Figure 2.42 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of kopsinine *N*-oxide (**25**)

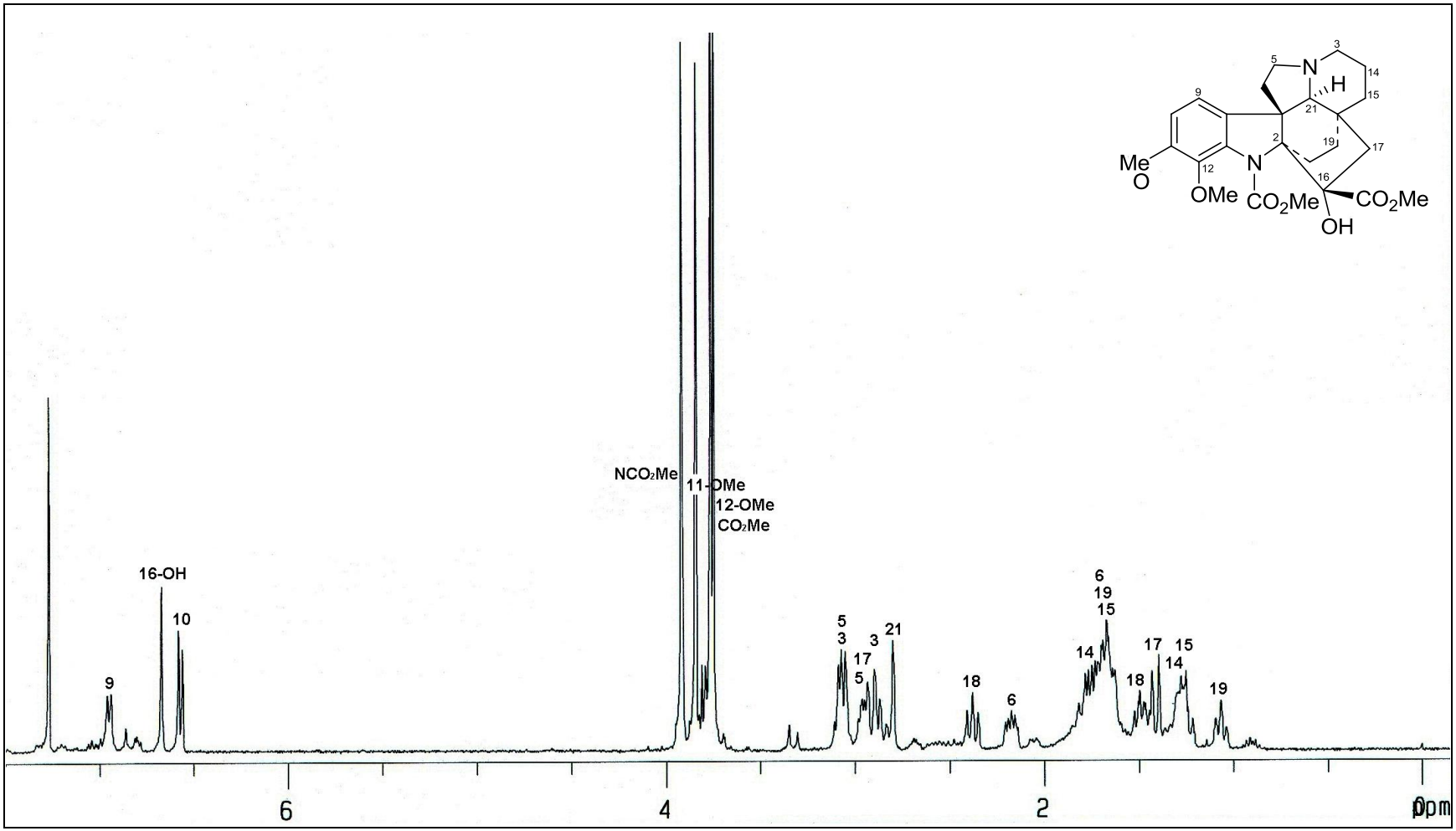


Figure 2.43 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of *N*(1)-methoxycarbonyl-11,12-dimethoxykopsinaline (**26**)

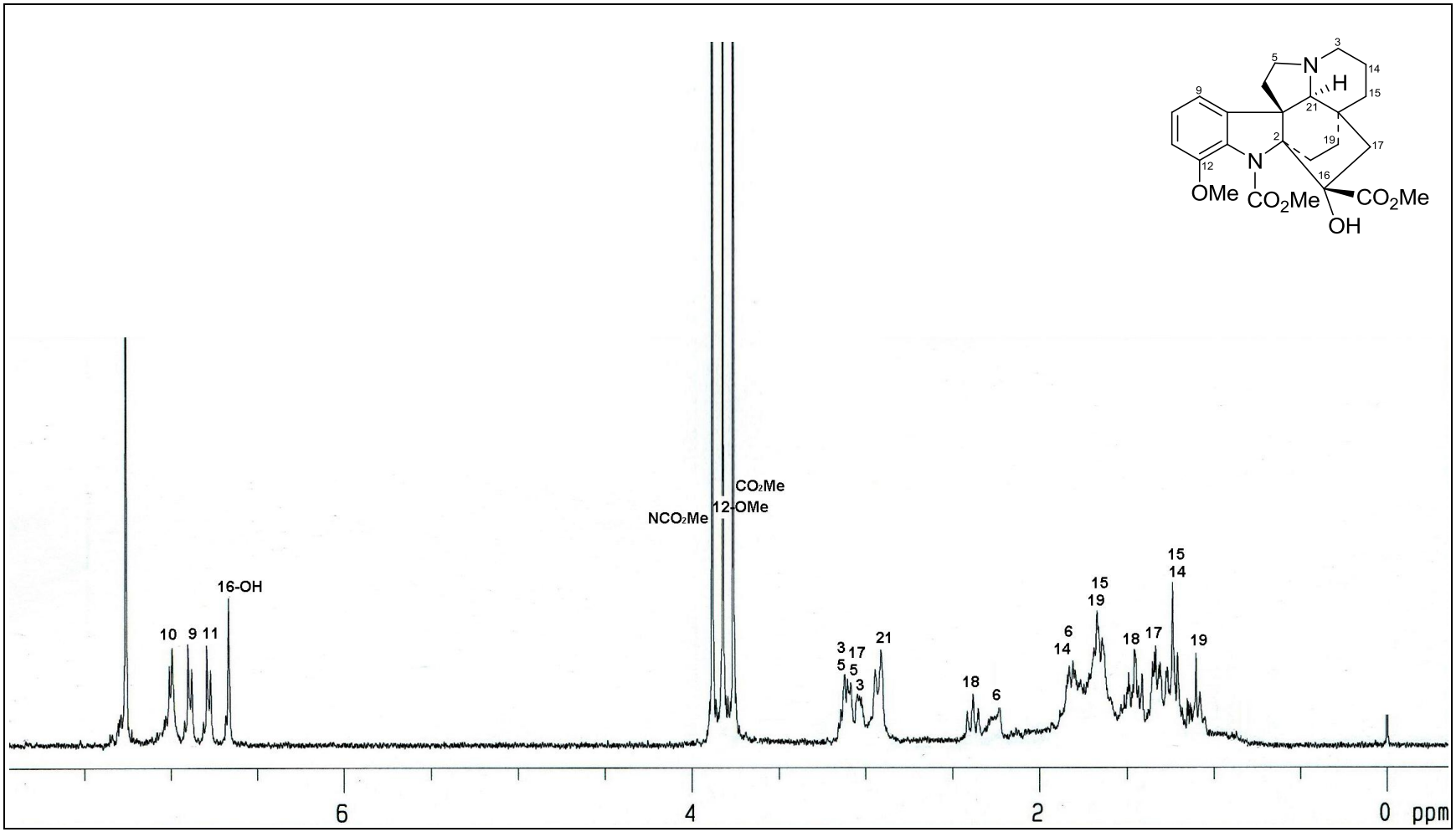


Figure 2.44 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of kopsilongine (**27**)

Table 2.25 : ^{1}H NMR spectroscopic data of pleiocarpine (**28**), 12-methoxypleiocarpine (**29**), and pleiocarpine *N*-oxide (**30**)[a]

H	**28**	**29**	**30**
3	2.91 td (13, 3)	2.89 td (13, 3)	3.35 t (10)
	3.07 ddt (13, 3.5, 1.8)	3.08 m	3.53 td (11.9, 5)
5	2.98 ddd (14, 8.5, 5)	2.67 ddd (14, 8.5, 4.5)	3.35 t (10)
	3.15 td (14, 8.5)	3.15 td (8.5, 7)	3.87 m
6	1.61 m	1.78 m	2.32 dd (9.5, 3.6)
	2.48 ddd (14, 8.5, 4.5)	2.99 td (8.5, 4.5)	2.55 m
9	7.25 dd (7.5, 1)	6.78 dd (8, 1)	8.44 d (7.2)
10	6.96 td (7.5, 1)	6.97 dd (8, 1)	7.00 t (7.2)
11	7.14 td (7.5, 1)	6.91 dd (7.3, 1)	7.16 t (7.2)
12	7.68 br s	–	7.63 br s
14	1.25 m	1.27 m	1.84 m
	1.85 tdd (13, 4, 3.5)	1.81 m	1.84 m
15	1.31 dd (13, 4)	1.46 ddd (13, 11, 7.5)	1.45 dd (13.6, 5)
	1.65 m	1.60 m	1.64 m
16	3.56 t (9)	3.56 dd (11, 9)	3.79 m
17	1.61 m	1.68 ddd (13.5, 11, 2)	1.60 m
	2.56 ddd (13, 10, 2)	2.51 ddd (13.5, 9, 3)	2.01 m
18	1.61 m	1.60 m	1.41 m
	1.80 ddd (12.8, 4.5, 2.5)	1.84 m	1.64 d (14.5)
19	1.19 dt (12, 2.5)	1.16 m	1.67 m
	1.49 m	1.27 m	2.55 m
21	2.97 br s	2.87 d (2)	3.65 s
CO_2*Me*	3.69 s	3.70 s	3.69 s
NCO_2*Me*	3.80 s	3.75 s	3.82 s
12-OMe	–	3.84 s	–

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC, and HMBC.

Table 2.26 : ^{13}C NMR spectroscopic data of pleiocarpine (**28**), 12-methoxypleiocarpine (**29**), and pleiocarpine *N*-oxide (**30**)[a]

C	**28**	**29**	**30**
2	69.0	69.9	68.1
3	47.6	47.7	65.6
5	50.3	50.4	65.3
6	37.6	36.1	34.7
7	57.4	58.0	58.5
8	139.3	129.4	137.3
9	121.6	112.1	126.2
10	123.0	124.4	124.1
11	127.1	114.4	128.1
12	115.1	148.2	114.2
13	140.3	143.3	141.7
14	17.6	17.5	19.9
15	36.3	37.1	34.0
16	42.4	41.8	40.9
17	29.3	30.1	27.7
18	33.3	33.6	34.2
19	33.1	33.1	31.7
20	32.4	32.2	34.0
21	68.7	68.6	85.3
CO_2Me	174.3	174.3	174.1
CO_2*Me*	51.7	51.8	52.7
N*C*O_2Me	154.2	153.6	154.1
NCO_2*Me*	52.0	52.2	52.7
12-OMe	–	56.3	–

[a]$CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.

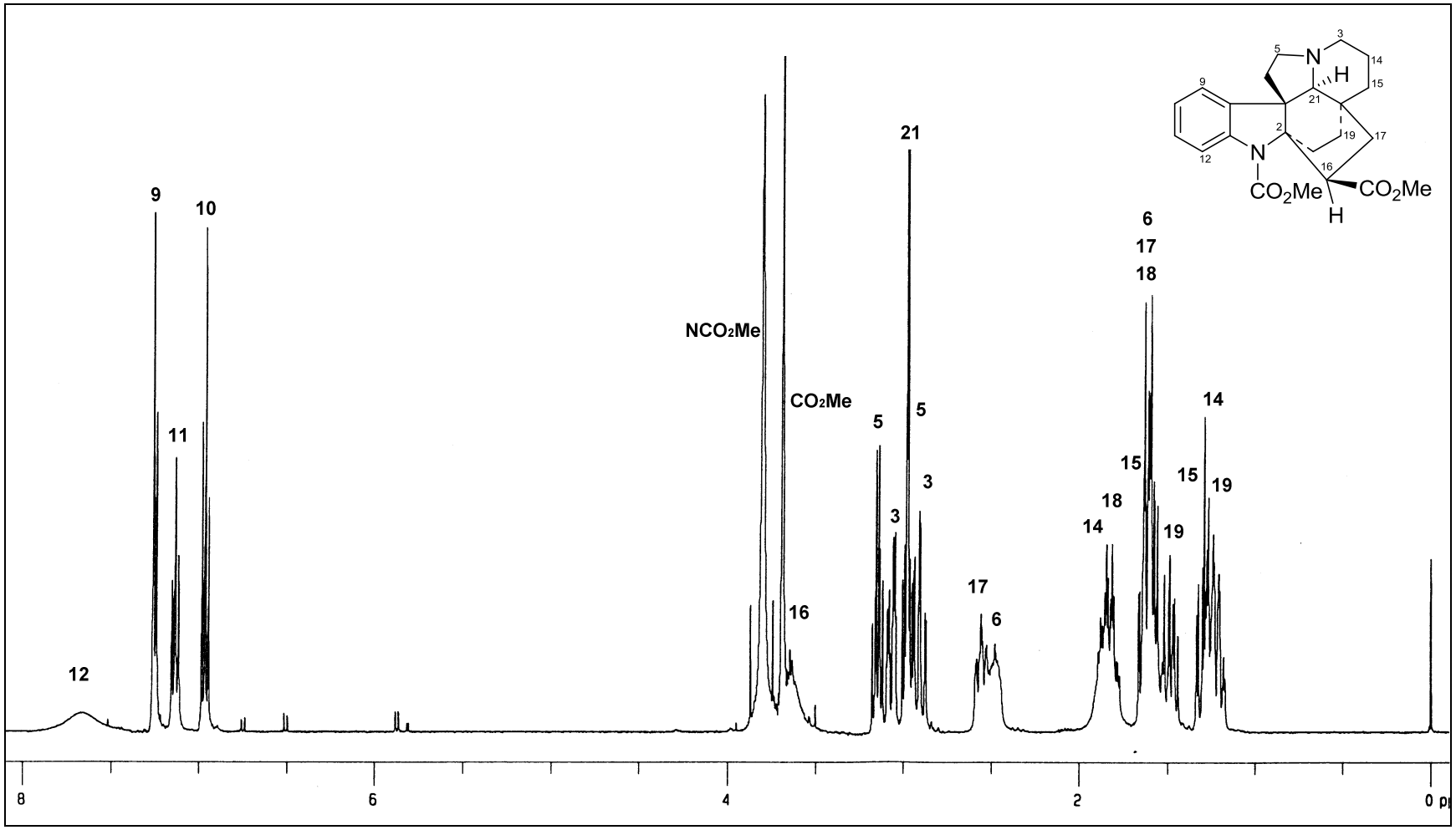


Figure 2.45 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of pleiocarpine (**28**)

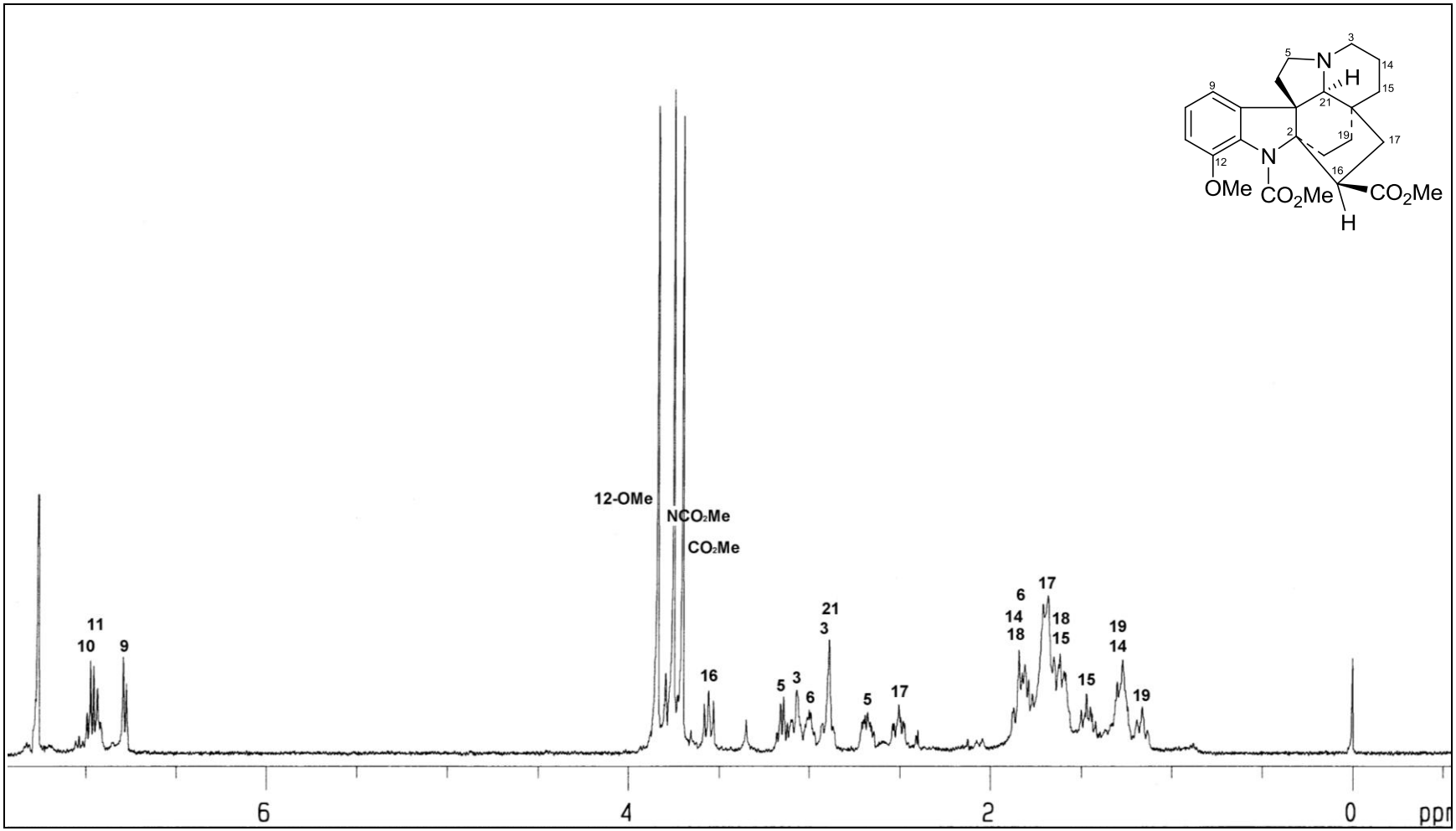


Figure 2.46 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of 12-methoxypleiocarpine (**29**)

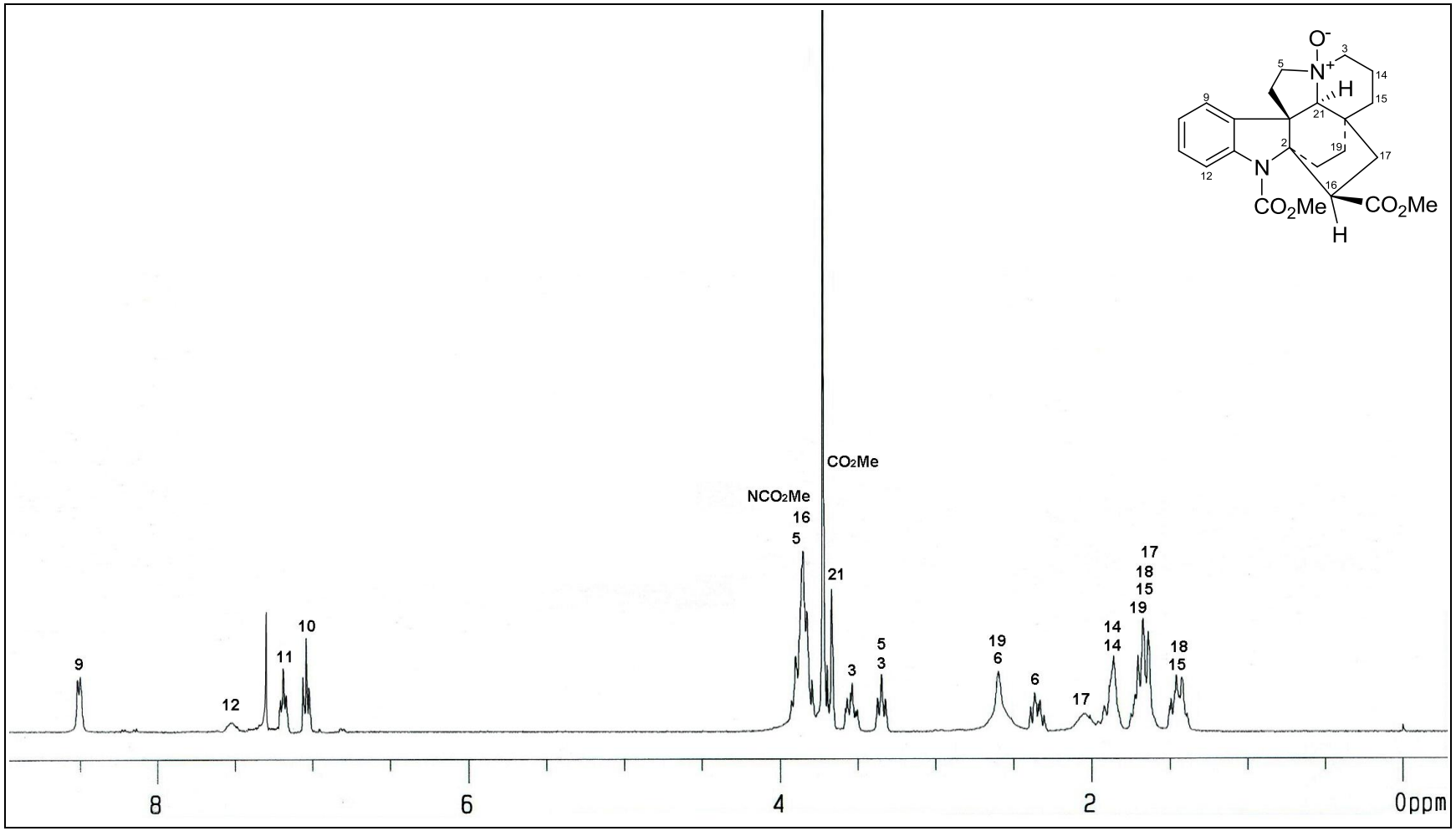


Figure 2.47 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of pleiocarpine *N*-oxide (**30**)

Table 2.27 : ^{1}H and ^{13}C NMR spectroscopic data of (+)-eburnamenine (**31**) and (+)-eburnamonine (**32**)[a]

Position	**31**		**32**	
	δ_H	δ_C	δ_H	δ_C
2	–	130.1	–	131.5
3	2.68 m	45.4	2.42 ddd (12.5, 11.3, 3)	44.3
	2.77 td (11, 4)		2.59 m	
5	3.26 ddd (14, 11, 5)	52.1	3.24 ddd (14, 11.3, 5.7)	50.7
	3.37 dd (14, 5)		3.33 dd (14, 6.3)	
6	2.51 br dd (15, 5)	16.5	2.49 ddd (16.8, 5.7, 2.5)	16.5
	3.02 m		2.91 dddd (16.8, 11.3, 6.3, 2.5)	
7	–	107.0	–	112.5
8	–	128.2	–	129.9
9	7.47 d (7)	118.4	7.44 dd (7, 1.5)	118.1
10	7.11 t (7)	119.8	7.29 td (7, 1.5)	123.9
11	7.19 t (7)	121.5	7.33 td (7, 1.5)	124.5
12	7.33 d (7)	108.5	8.37 dd (7, 1.5)	116.3
13	–	133.5	–	134.3
14	1.45 m	20.8	1.37 dqui (13.5, 3)	20.4
	1.72 m		1.76 m	
15	1.17 td (13, 4)	31.1	1.04 td (13.5, 3)	26.8
	1.48 br d (13)		1.49 dt (13.5, 3)	
16	6.91 d (8)	119.7	–	167.4
17	5.08 d (8)	116.7	2.58 d (16.7)	44.2
			2.67 d (16.7)	
18	0.99 t (7.5)	9.0	0.93 t (7.5)	7.6
19	1.72 m	27.5	1.66 dq (14.5, 7.5)	28.3
	1.98 dq (14, 7)		2.05 dq (14.5, 7.5)	
20	–	37.3	–	38.5
21	4.27 s	55.8	3.92 br s	57.7

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

Figure 2.48 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (+)-eburnamenine (**31**)

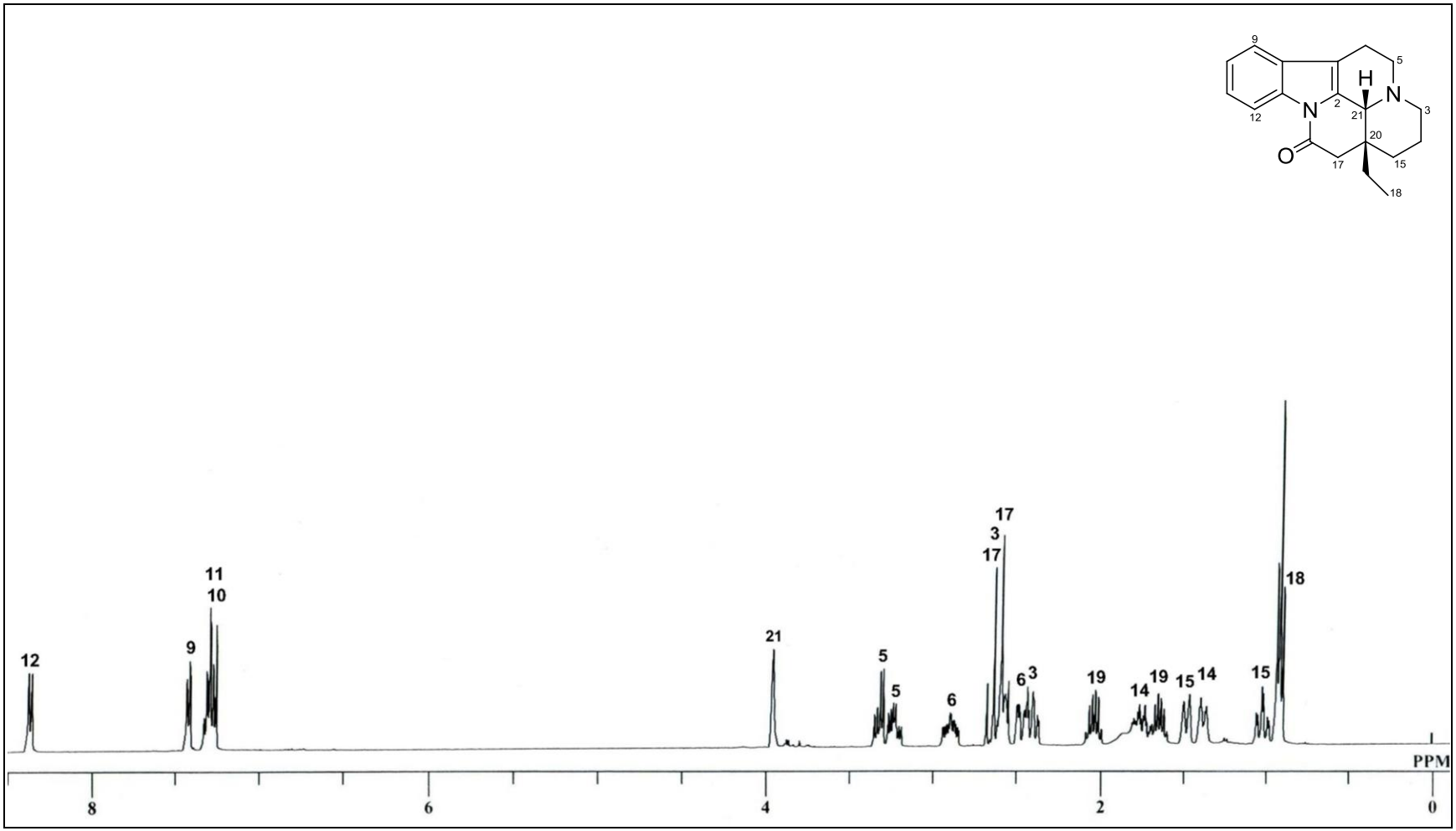


Figure 2.49 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (+)-eburnamonine (**32**)

Table 2.28 : ^{1}H and ^{13}C NMR spectroscopic data of (–)-eburnamine (**33**) and (+)-isoeburnamine (**34**)[a]

Position	**33**		**34**	
	δ_H	δ_C	δ_H	δ_C
2	–	132.4	–	131.1
3	2.29 m	44.2	2.59 td (13, 3)	44.9
	2.51 m		2.66 br d (13)	
5	3.17 ddd (14, 11, 6)	50.5	3.25 ddd (13.6, 11.5, 5.5)	51.3
	3.25 dd (14, 6)		3.33 dd (13.6, 6.5)	
6	2.48 m	16.7	2.54 ddd (16, 5.5, 1.5)	16.7
	2.94 m		2.91 dddd (16, 11.5, 6.5, 2.5)	
7	–	105.1	–	105.6
8	–	128.6	–	128.8
9	7.47 dd (6, 2)	117.9	7.50 br d (7.5)	118.5
10	7.16 m	120.0	7.15 td (7.5, 1.5)	120.2
11	7.16 m	121.1	7.20 td (7.5, 1.5)	121.2
12	7.73 dd (6, 2)	112.3	7.41 br d (7.5)	109.8
13	–	136.7	–	134.7
14	1.27 br d (13)	20.2	1.39 dt (13, 3)	21.0
	1.68 br qt (13, 3)		1.76 qt (13, 3)	
15	0.83 br td (13, 3)	24.6	1.55 dt (13, 3)	26.6
	1.34 br d (13)		1.64 td (13, 3)	
16	5.55 dd (9, 5)	76.6	6.06 dd (4.8, 1)	74.7
17	1.48 m	43.0	2.00 dd (15, 4.8)	39.9
	2.30 m		2.19 br d (15)	
18	0.89 t (7)	7.5	0.93 t (7.6)	7.6
19	1.43 m	28.4	1.46 dq (14.5, 7.6)	29.0
	2.04 dq (14, 7)		2.19 dq (14.5, 7.6)	
20	–	36.7	–	34.6
21	3.75 s	58.4	3.85 br s	59.3

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

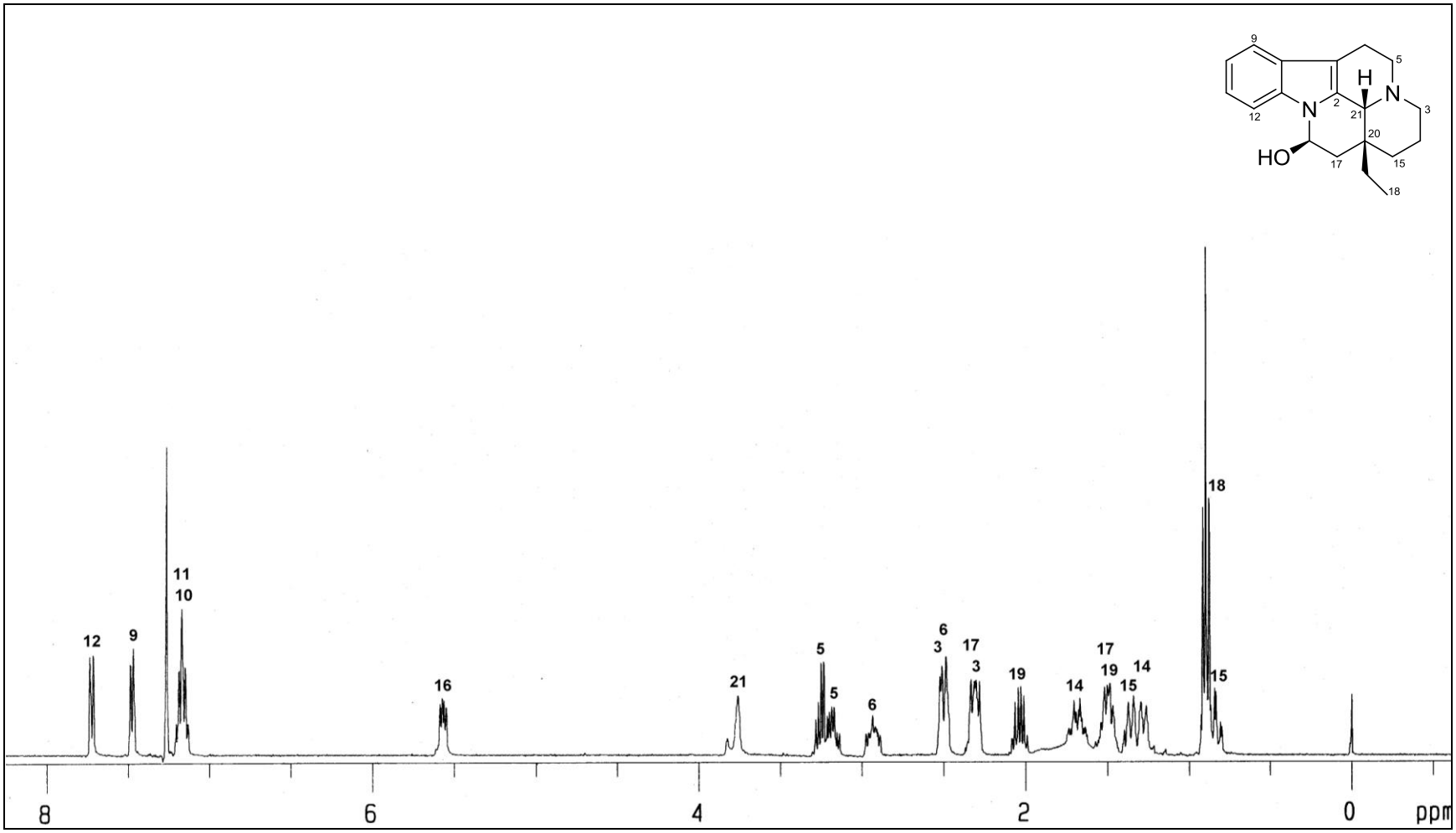


Figure 2.50 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (–)-eburnamine (**33**)

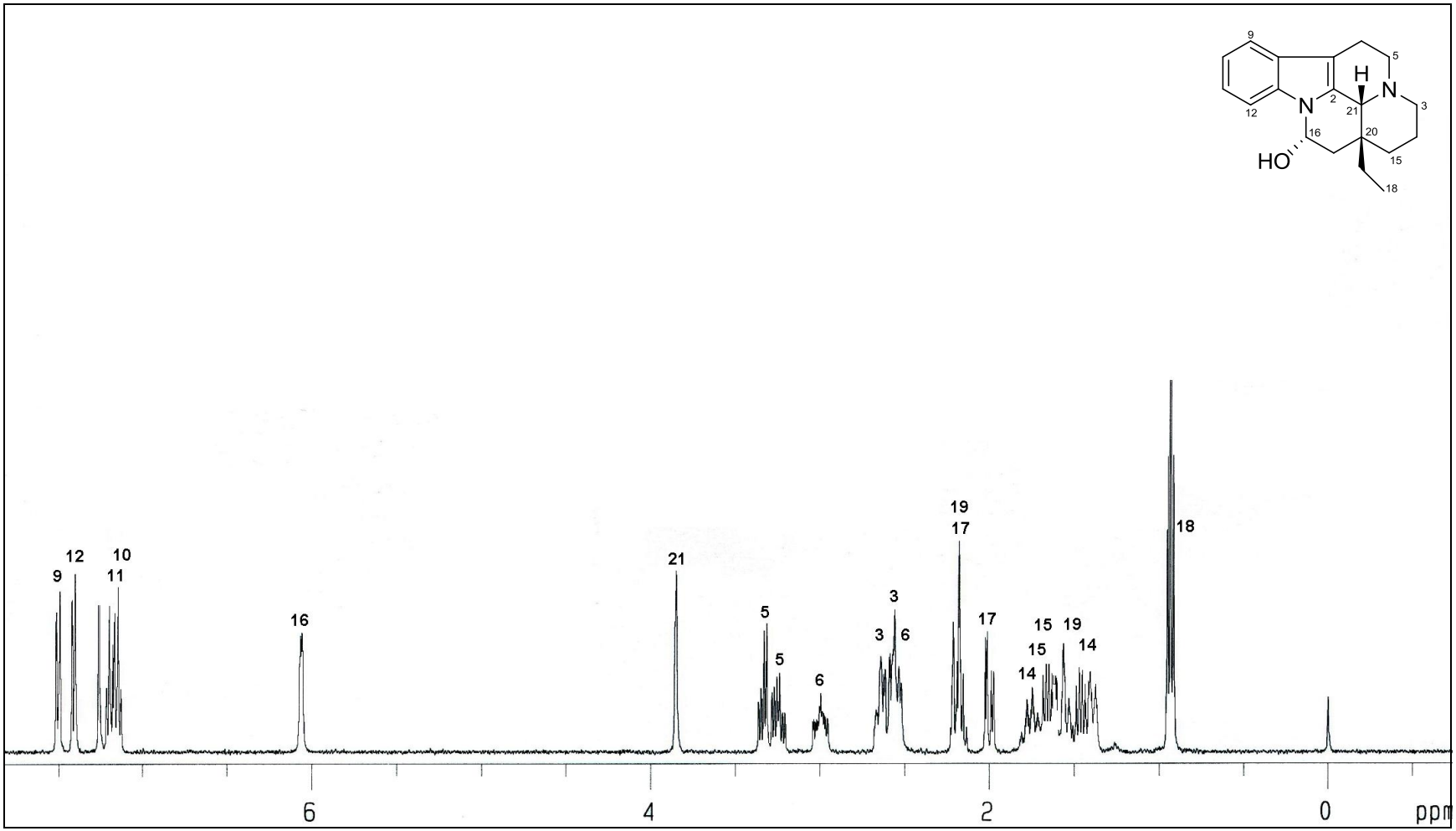


Figure 2.51 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (+)-isoeburnamine (**34**)

Table 2.29 : ^{1}H NMR spectroscopic data of (+)-19-oxoeburnamine (**35**), (–)-19(*R*)-hydroxyisoeburnamine (**36**), and (+)-19(*R*)-hydroxyeburnamine (**37**)[a]

H	**35**	**36**	**37**
3	2.36 m	2.66 m	2.42 td (13, 4)
	2.49 m	2.66 m	2.57 m
5	3.22 m	3.28 ddd (14, 6.5, 1.5)	3.28 ddd (13, 6.5, 1.5)
	3.22 m	3.33 ddd (13, 11.5, 5)	3.33 ddd (13, 11.5, 5)
6	2.49 m	2.59 ddt (16, 5, 1.5)	2.57 m
	2.95 m	3.00 dddd (16, 11.5, 6.5, 2.5)	2.97 dddd (16, 11.5, 6.5, 2.5)
9	7.49 dd (6, 2)	7.50 br d (7)	7.49 dd (7, 1.5)
10	7.16 m	7.15 td (7, 1.5)	7.17 td (7, 1.5)
11	7.19 m	7.20 td (7, 1.5)	7.21 td (7, 1.5)
12	7.71 dd (6, 2)	7.40 br d (7)	7.72 dd (7, 1.5)
14	1.43 m	1.38 br d (14)	1.32 br d (13)
	1.43 m	2.24 m	2.19 qt (13, 4)
15	0.93 br td (13, 5)	1.75 td (14, 4)	0.89 tdd (13, 4, 1)
	2.04 br d (13)	1.82 td (14, 4.5)	1.74 br d (13)
16	5.62 dd (9, 5)	6.02 dd (5, 1)	5.59 dd (10, 5)
17	1.70 dd (14, 9)	1.85 d (15, 5)	1.58 br d (15)
	2.30 m	2.02 dd (15, 1)	2.25 dd (14, 5)
18	2.36 s	1.25 d (6.5)	1.24 d (6.5)
19	–	3.91 qd (6.5, 1)	3.98 qd (6.5, 1)
21	4.67 s	4.18 br s	4.22 br s

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC, and HMBC.

Table 2.30 : ^{13}C NMR spectroscopic data of (+)-19-oxoeburnamine (**35**), (–)-19(*R*)-hydroxyisoeburnamine (**36**), and (+)-19(*R*)-hydroxyeburnamine (**37**)[a]

C	**35**	**36**	**37**
2	131.5	129.6	130.9
3	44.0	44.4	43.7
5	50.6	51.2	50.5
6	16.8	17.0	16.7
7	106.6	105.5	105.6
8	128.7	129.1	128.6
9	118.2	118.9	118.2
10	120.4	120.6	120.4
11	121.6	121.7	121.6
12	112.0	110.2	112.1
13	136.7	135.1	136.6
14	22.6	21.9	21.2
15	24.9	23.9	22.4
16	76.3	74.5	76.5
17	42.0	39.6	43.0
18	25.6	17.8	17.6
19	210.3	79.5	78.3
20	51.7	37.4	39.5
21	54.8	60.1	59.5

[a] $CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.

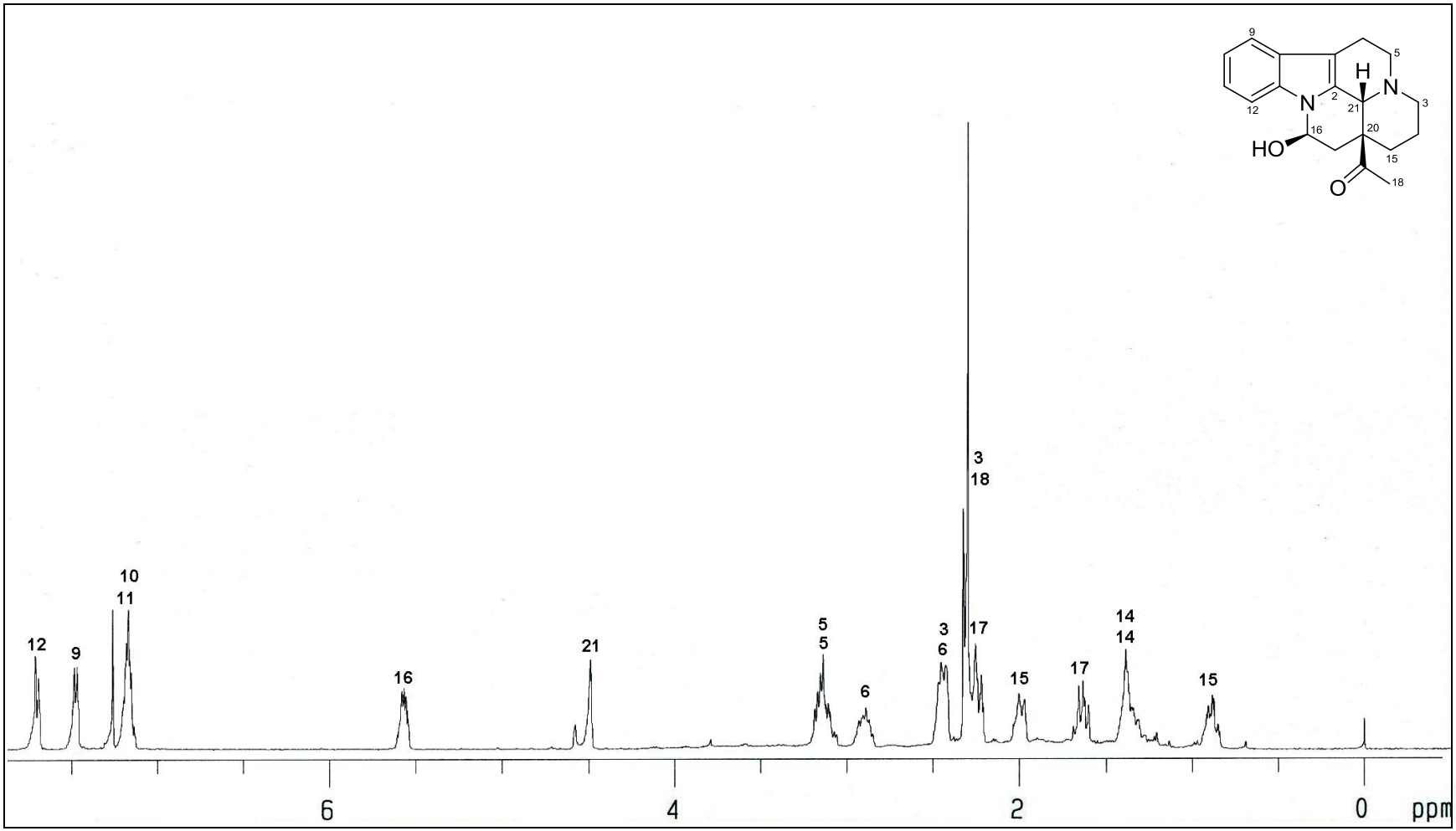


Figure 2.52 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (+)-19-oxoeburnamine (**35**)

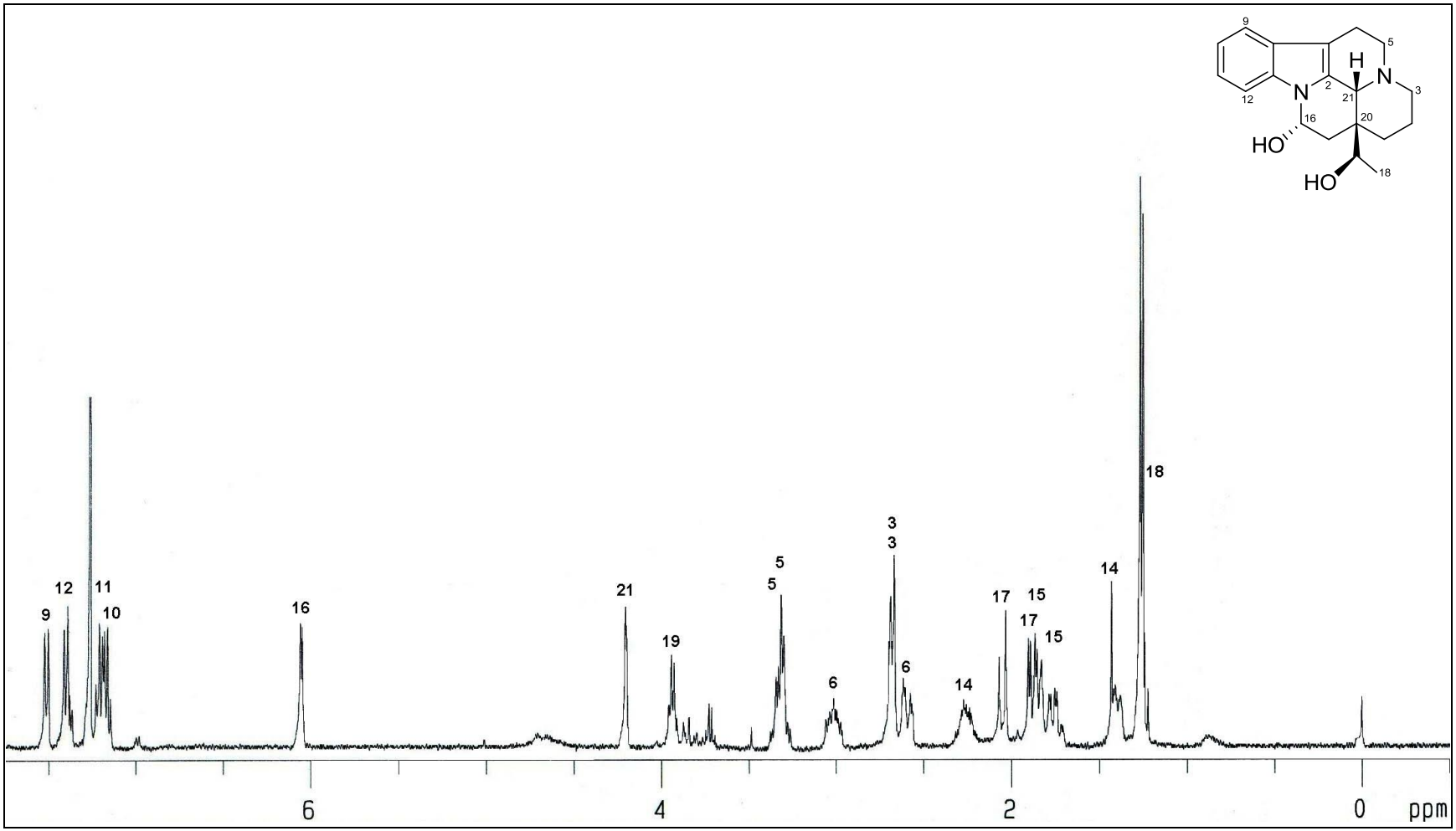


Figure 2.53 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (−)-19(*R*)-hydroxyisoeburnamine (**36**)

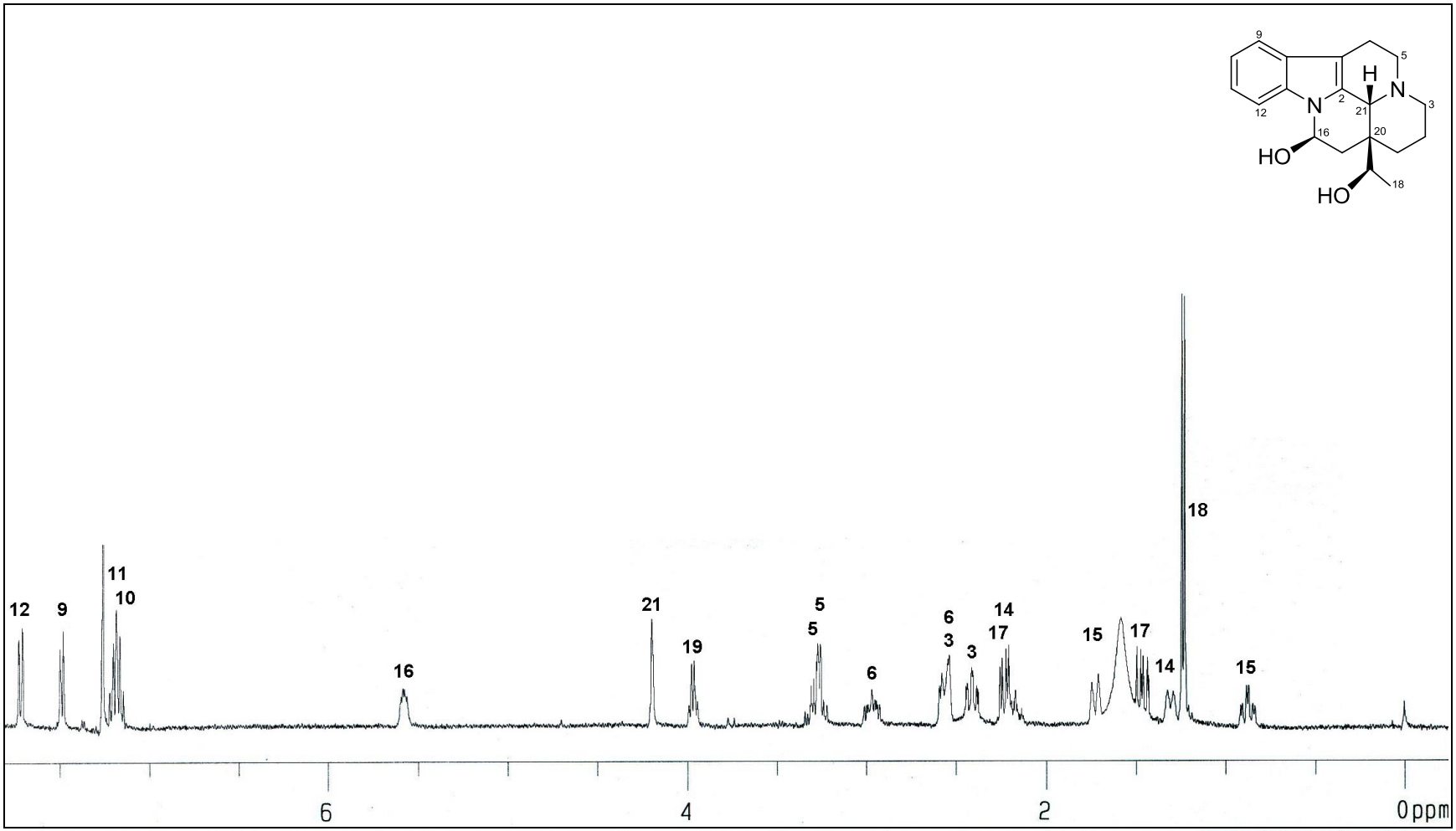


Figure 2.54 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (+)-19(*R*)-hydroxyeburnamine (**37**)

Table 2.31 : ^{1}H and ^{13}C NMR spectroscopic data of (–)-norpleiomutine (**38**)[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
2	–	66.8	2'	–	133.9
3	3.05 m	47.4	3'	2.49 br t (13)	44.4
	3.05 m			2.62 m	
5	3.05 m	50.8	5'	3.35 m	50.9
	3.35 m			3.35 m	
6	1.73 m	34.9	6'	2.62 m	17.0
	2.74 m			3.05 m	
7	–	58.1	7'	–	104.6
8	–	141.4	8'	–	128.4
9	7.17 s	120.0	9'	7.44 d (8)	117.6
10	–	134.5	10'	7.00 t (8)	118.9
11	6.90 d (8)	125.1	11'	6.83 t (8)	120.0
12	6.62 d (8)	111.0	12'	6.54 d (8)	112.1
13	–	148.5	13'	–	136.5
14	1.27 m	16.6	14'	1.38 m	20.7
	1.90 m			1.77 m	
15	1.30 m	36.3	15'	1.14 m	24.2
	1.62 br d (13)			1.38 m	
16	2.91 t (10)	43.7	16'	4.94 dd (11, 5)	55.9
17	1.38 m	33.0	17'	1.77 m	45.1
	2.76 m			2.11 m	
18	1.26 m	33.7	18'	0.87 t (7.5)	7.4
	1.92 m				
19	1.26 m	33.8	19'	1.49 m	28.7
	1.41 m			2.17 m	
20	–	31.6	20'	–	34.9
21	*ca* 3.02	67.9	21'	4.03 br s	59.5
CO_2Me	–	174.7			
CO_2*Me*	3.02 s	51.9			

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

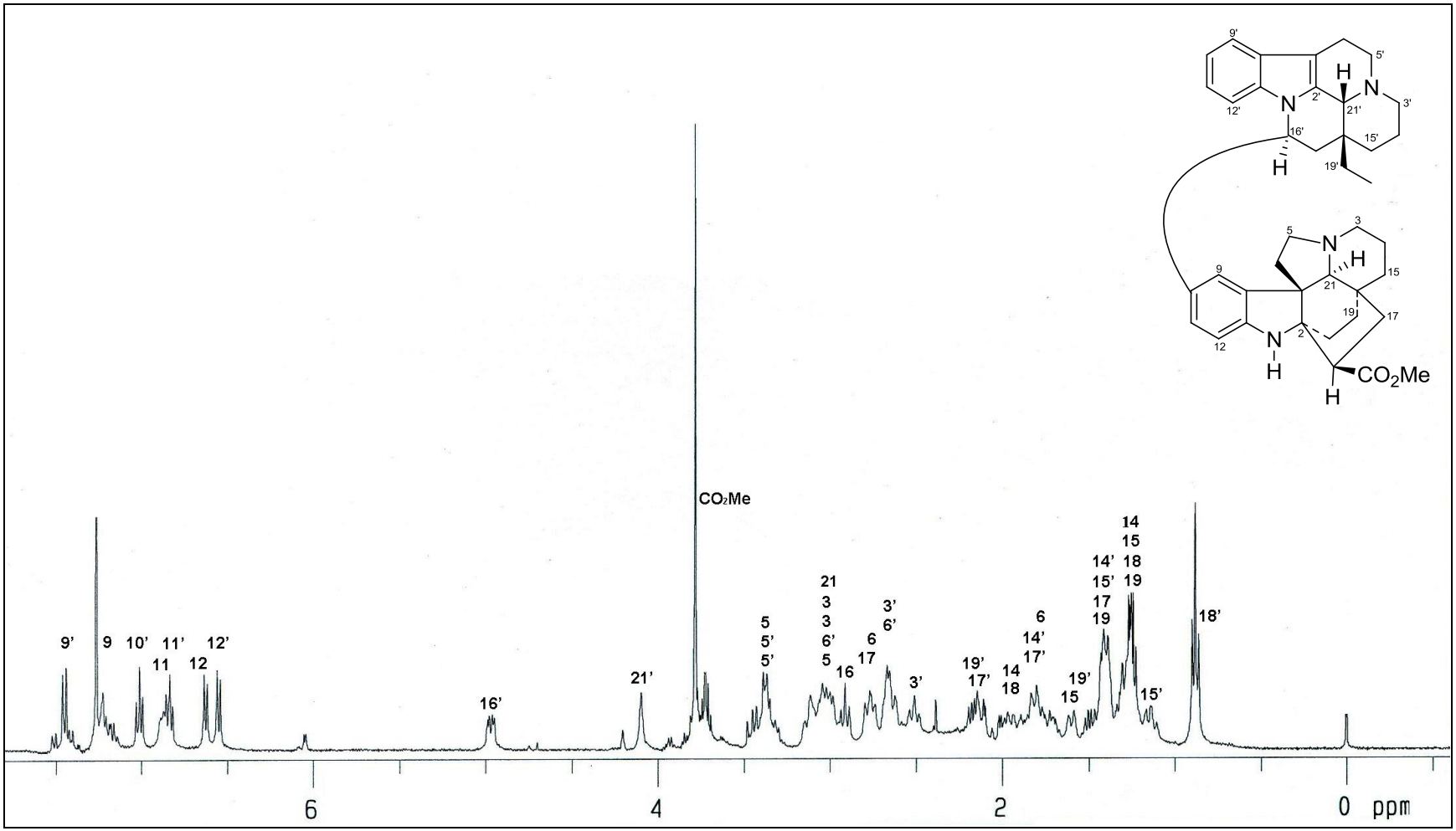


Figure 2.55 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (–)-norpleiomutine (**38**)

Table 2.32 : ^{1}H and ^{13}C NMR spectroscopic data of (–)-demethylnorpleiomutine (**39**)[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
2	–	66.5	2'	–	132.9
3	3.02 m	47.4	3'	2.52 br t (13)	44.2
	3.02 m			2.67 m	
5	3.02 m	50.4	5'	3.38 m	50.7
	3.38 m			3.38 m	
6	1.62 m	35.1	6'	2.67 m	16.9
	2.67 m			3.02 m	
7	–	58.4	7'	–	104.8
8	–	141.6	8'	–	128.4
9	7.27 s	120.7	9'	7.46 d (8)	117.9
10	–	136.6	10'	7.02 t (8)	119.3
11	6.95 d (8)	125.4	11'	6.86 t (8)	120.5
12	6.78 d (8)	114.3	12'	6.50 d (8)	112.0
13	–	146.7	13'	–	137.4
14	1.40 m	16.5	14'	1.43 m	20.3
	1.86 m			1.82 m	
15	1.32 m	36.0	15'	1.14 br t (13)	24.0
	1.62 m			1.43 m	
16	2.84 t (10)	42.4	16'	4.99 dd (11, 5)	56.0
17	1.62 m	33.4	17'	1.78 m	45.0
	2.67 m			2.18 m	
18	1.43 m	33.0	18'	0.86 t (7.5)	7.5
	2.01 m				
19	1.43 m	34.1	19'	1.46 m	28.6
	1.47 m			2.18 m	
20	–	32.3	20'	–	35.1
21	*ca* 3.22	68.5	21'	4.13 br s	59.4
COOH	–	177.4			

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

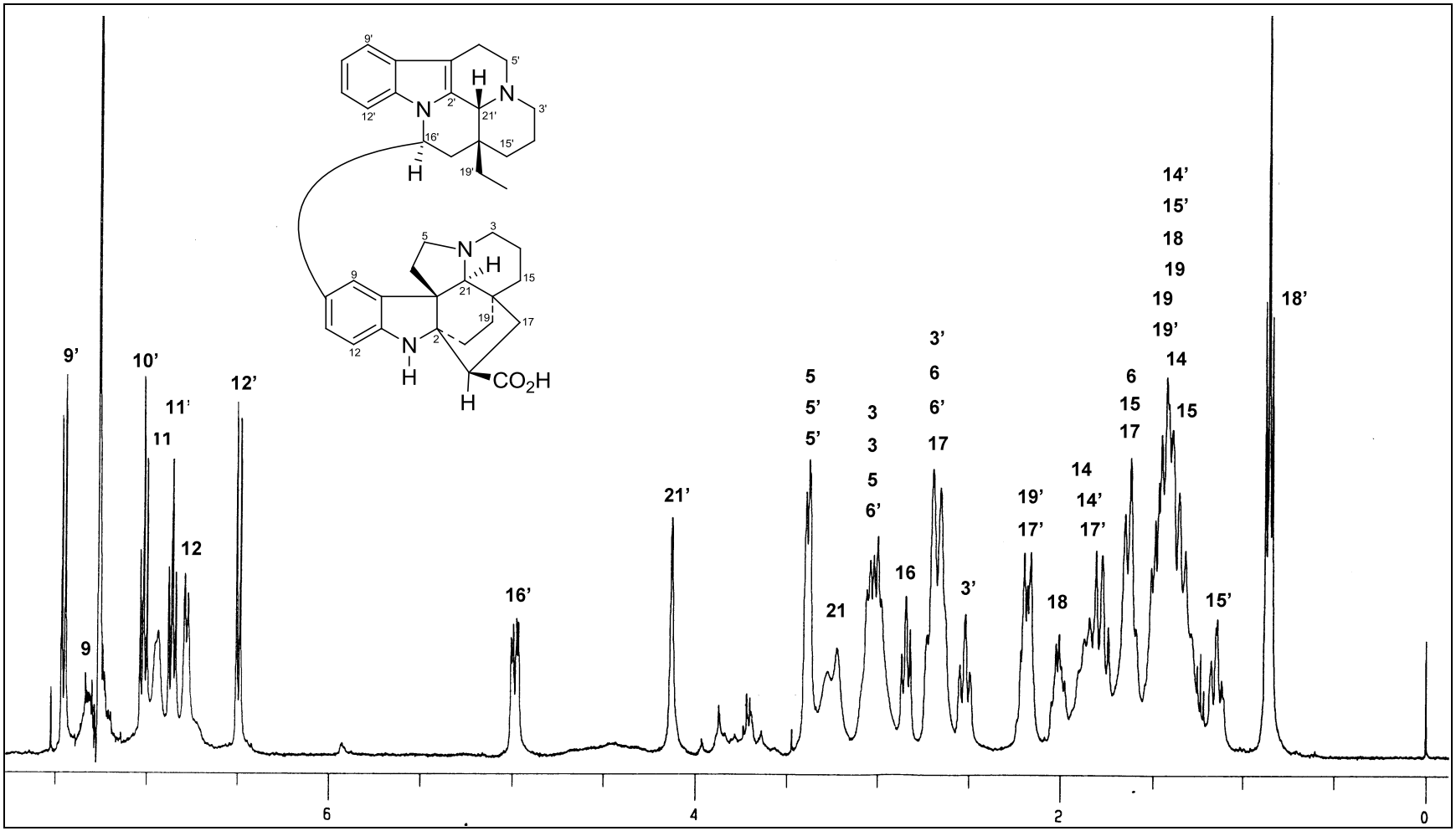


Figure 2.56 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (–)-demethylnorpleiomutine (**39**)

Table 2.33 : ^{1}H and ^{13}C NMR spectroscopic data of (+)-kopsoffinol (**40**)[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
2	–	66.9	2'	–	132.1
3	3.10 m	47.4	3'	2.54 td (13, 4)	43.8
	3.10 m			2.62 br d (13)	
5	3.10 m	50.9	5'	3.28 dd (14, 6.5)	50.5
	3.42 m			3.39 m	
6	1.69 m	34.9	6'	2.63 m	17.0
	2.76 m			3.01 m	
7	–	58.2	7'	–	104.4
8	–	141.5	8'	–	128.4
9	7.15 s	120.2	9'	7.45 br d (8)	117.7
10	–	133.8	10'	7.01 td (8, 1)	119.2
11	6.84 d (8)	125.2	11'	6.83 td (8, 1)	120.4
12	6.61 d (8)	111.1	12'	6.53 br d (8)	112.3
13	–	148.7	13'	–	136.6
14	1.31 m	16.5	14'	1.39 m	21.4
	1.92 m			2.26 qt (13, 4)	
15	1.31 m	36.4	15'	1.15 td (13, 4)	21.7
	1.59 br d (13)			1.71 br d (13)	
16	2.90 t (10)	43.7	16'	4.92 dd (11, 5)	55.9
17	1.39 m	32.2	17'	1.69 m	44.4
	2.76 m			1.92 dd (14, 4)	
18	1.25 m	33.8	18'	1.20 d (6.5)	17.5
	1.92 m				
19	1.25 m	33.9	19'	3.95 q (6.5)	78.7
	1.39 m				
20	–	31.7	20'	–	37.4
21	*ca* 3.03	68.0	21'	4.36 br s	60.0
CO_2Me	–	174.7			
CO_2Me	3.78 s	52.0			

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

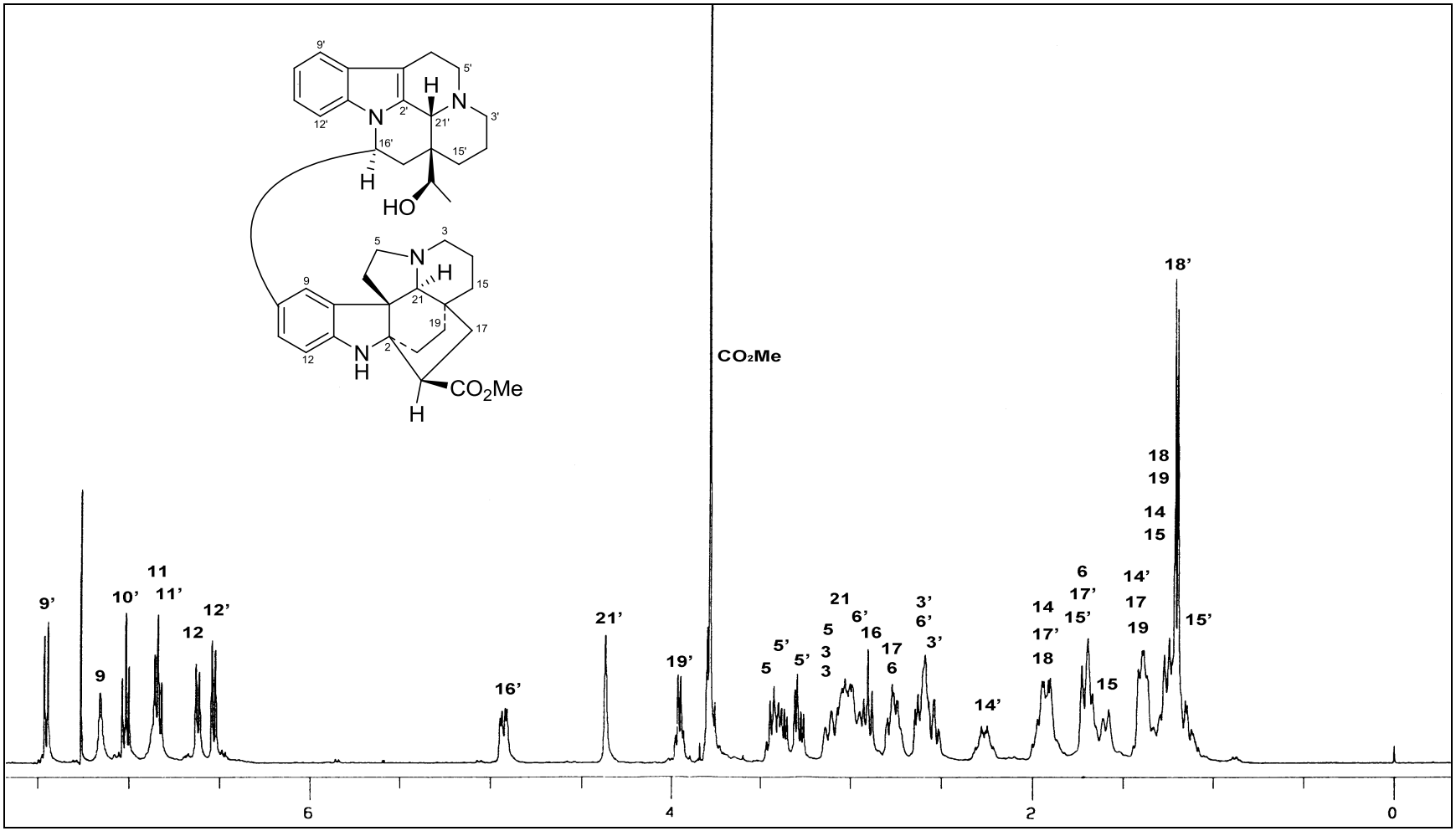
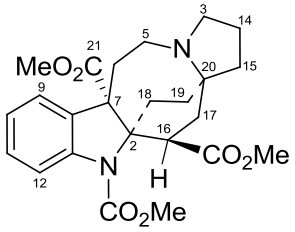


Figure 2.57 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of (+)-kopsoffinol (**40**)

2.2 Alkaloids from *Kopsia grandifolia*

A total of 8 alkaloids were isolated from the Malayan *K. grandifolia,* of which 3 are new. The new alkaloids are grandilodine A (**41**), grandilodine B (**42**), and grandilodine C (**43**).[91] The known alkaloids are lapidilectine A (**44**), isolapidilectine A (**45**), lapidilectam (**46**), lapidilectine B (**47**), and kopsinine (**22**). The structures were established using NMR and MS analysis and in the case of **41** and **42**, confirmed by X-ray diffraction analysis. Alkaloids **41**, **43**, and **47** were found to reverse multidrug resistance in vincristine-resistant KB cells. The alkaloidal composition is summarized in Table 2.34.



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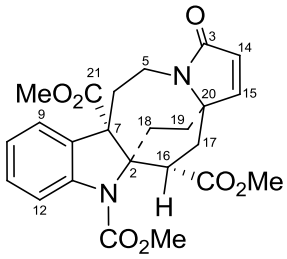

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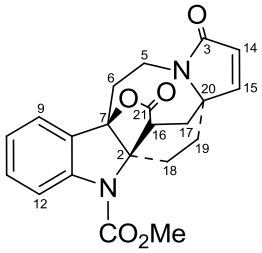

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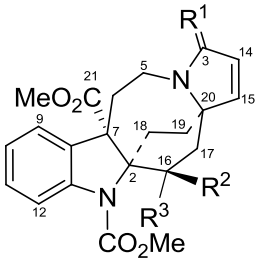
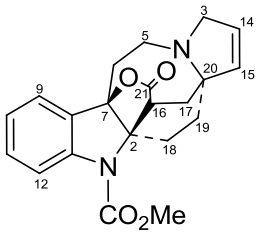


44 R^1 = H,H, R^2 = CO_2Me, R^3 = H
45 R^1 = H,H, R^2 = H, R^3 = CO_2Me
46 R^1 = O, R^2 = CO_2Me, R^3 = H



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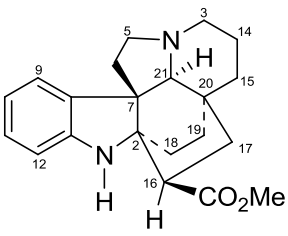


22

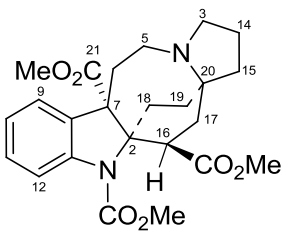
Table 2.34 : Alkaloid composition of *K. grandifolia*

Plant part	Alkaloid	Yield (g Kg^{-1})
Stem-bark	Grandilodine A (**41**) [New]	0.0021
	Grandilodine B (**42**) [New]	0.0007
	Lapidilectine A (**44**)	0.0318
	Isolapidilectine A (**45**)	0.0190
	Lapidilectam (**46**)	0.0002
	Kopsinine (**22**)	0.0010
Leaf	Grandilodine C (**43**) [New]	0.0160
	Lapidilectine B (**47**)	0.4040

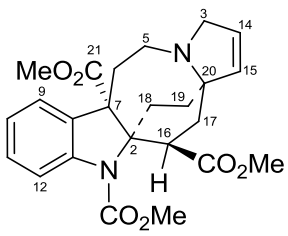
2.2.1 Grandilodine A (41)

Grandilodine A (**41**)[91] was isolated as a light yellowish oil, and subsequently as light yellowish block crystals from CH_2Cl_2–MeOH, mp 120–122 ^oC, $[\alpha]^{25}_D$ –76 (*c* 1.46, $CHCl_3$). The UV spectrum showed absorption maxima at 209, 253, and 289 nm, characteristic of a dihydroindole chromophore. The IR spectrum exhibited absorptions at 1731 cm^{-1} and 1704 cm^{-1}, corresponding to ester and carbamate functions, respectively. The ESIMS of **41** showed a quasi molecular ion at *m/z* 443, which analyzed for $C_{24}H_{30}N_2O_6$ + H. The ^{13}C NMR spectrum accounted for all of the 24 resonances, comprising three methyl, eight methylene, five methine, and eight quaternary carbon atoms, in agreement with the molecular formula. The presence of two ester groups and a carbamate functionality was supported by the observed quaternary carbon resonances at δ 173.0, 174.3, and 154.0, respectively. The ^1H NMR spectroscopic data (Table 2.35) showed the presence of an unsubstituted aromatic moiety (δ 6.88–7.51) and three methoxy singlets (δ 2.94, 3.51, 3.91) associated with the methyl ester and carbamate groups. The relatively shielded methoxy signal associated with one of the ester functions (δ 2.94) suggested the possibility of anisotropy from the aromatic ring of the indole moiety. The NMR signals, assigned with the aid of COSY and HMQC, indicated that **41** was a lapidilectine-type alkaloid. The ^1H NMR data resembled that of lapidilectine A (**44**), which was also isolated from the stem-bark extract of the same plant, except for the absence of the signals due to the 14,15-double bond seen at δ 5.49 and 5.71 in **44**. These were replaced in the spectrum of **41** by signals due to an ethylene (CH_2CH_2) group (Table 2.35). The same was true of the ^{13}C NMR spectrum, where the signals due to an ethylene fragment (δ 22.1, 39.8) replaced the signals due to the two olefinic carbons (δ 125.7, 138.4) in the spectrum of **44**. These observations were also consistent with the 2-D NMR data, which showed the presence

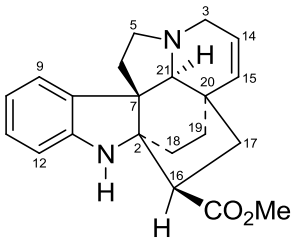
of a $NCH_2CH_2CH_2$ fragment in place of a $NCH_2CH{=}CH$. In the previous report,[92,93] the relative configurations at the various stereogenic centers in **44** were assigned on the grounds of biogenetic reasoning by reference to venalstonine (**142**), as well as on the NOESY spectrum. Since **41** is a congener of **44**, the relative configuration of **41** is assumed to be similar to that of **44** based on the similarity of the NMR data. However, the ^{1}H NMR spectra of this group of compounds tended to show broadened signals, which render interpretation of the NOESY spectrum difficult. Thus, in order to obtain unambiguous confirmation of the relative configuration of **44** (and hence **41**), suitable crystals of **41** were obtained, and X-ray diffraction analysis was carried out (Figure 2.58), which provided vindication of the original stereochemical assignment. In addition, catalytic hydrogenation of **44** gave a product identical ($[\alpha]^{25}_D$, ^{1}H and ^{13}C NMR, MS) to **41**.



41



44



142

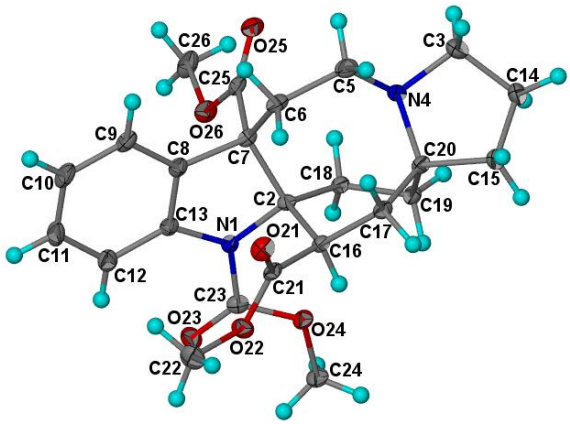


Figure 2.58 : X-ray crystal structure of **41**. Thermal ellipsoids are shown at the 50% probability level.

Table 2.35 : ^{1}H and ^{13}C NMR spectroscopic data of grandilodine A (**41**)[a]

Position	δ_H	δ_C
2	–	75.5
3	2.73 m	56.3
	2.99 m	
5	3.13 m	49.4
	3.13 m	
6β	2.78 t (5)	28.8
6α	2.85 dd (15.4, 7.7)	
7	–	62.4
8	–	131.7
9	7.00 d (7.7)	122.9
10	6.88 td (7.7, 1)	122.7
11	7.14 td (7.7, 1)	128.8
12	7.51 br s	115.4
13	–	144.0
14	1.73 m	22.1
	1.73 m	
15α	1.54 m	39.8
15β	1.70 m	
16	3.17 m	39.8
17β	1.85 dd (15, 10)	32.8
17α	2.40 m	
18α	1.54 m	31.5
18β	1.70 m	
19α	1.85 dd (15, 10)	32.8
19β	2.54 m	
20	–	60.3
21	–	173.0
21-OMe	3.51 s	51.9
CO_2Me	–	174.3
CO_2*Me*	2.94 s	51.7
N*C*O_2Me	–	154.0
NCO_2*Me*	3.91 s	52.4

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, HMBC, and NOESY.

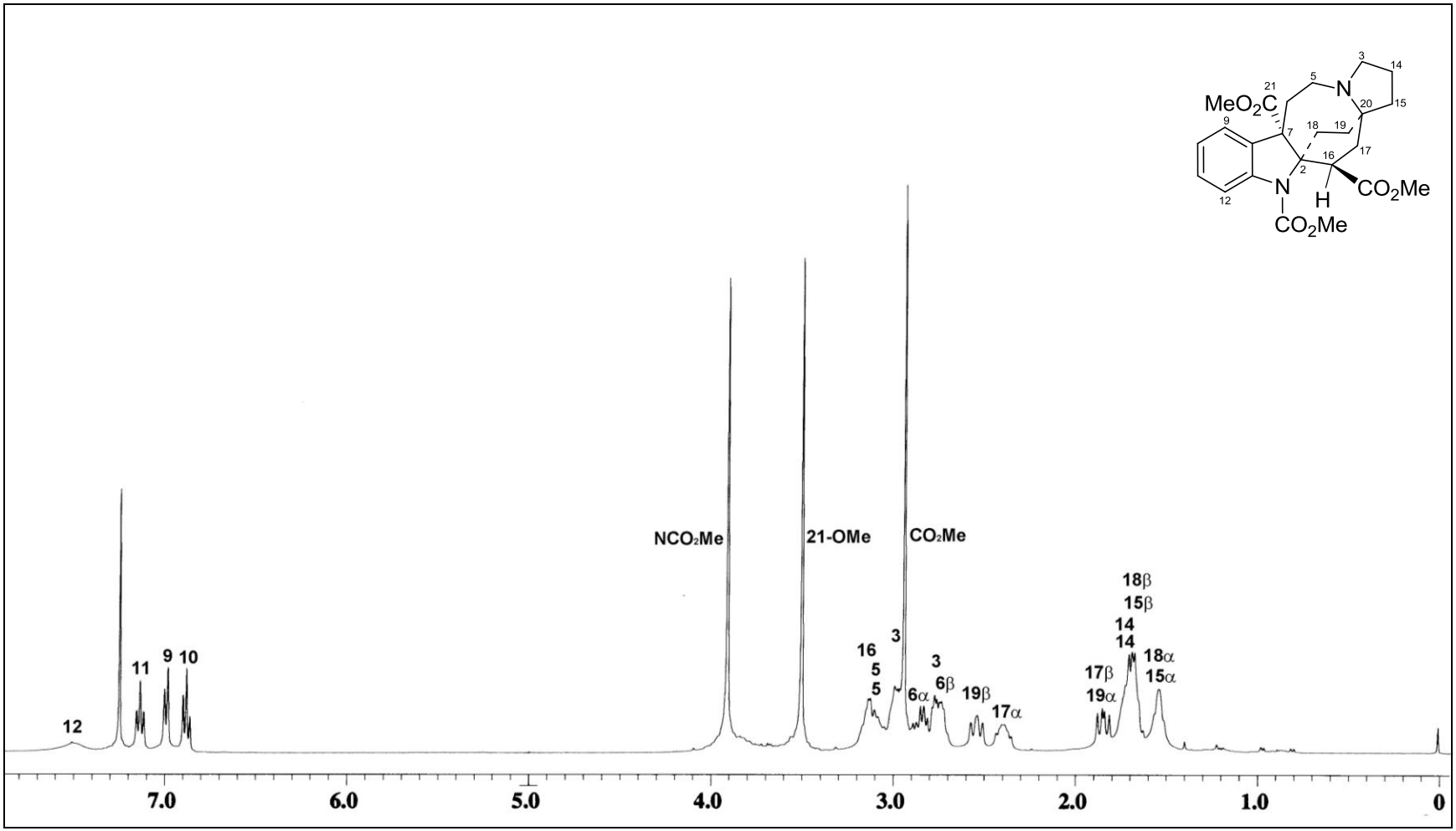
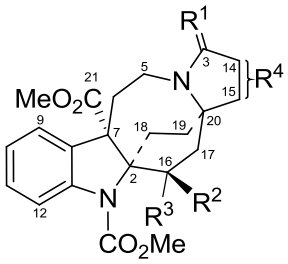


Figure 2.59 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of grandilodine A (**41**)

2.2.2 Grandilodine B (42)

Grandilodine B (**42**)[91] was initially obtained as a white amorphous solid, and subsequently crystallized from CH_2Cl_2–hexanes as colorless block crystals, mp 204–206 ^oC, $[\alpha]^{25}_D$ +66 (*c* 0.39, $CHCl_3$). The UV spectrum showed absorption maxima at 210, 252, and 286 nm due to a dihydroindole chromophore, while the IR spectrum showed carbonyl bands indicating the presence of ester (1734 cm^{-1}) and carbamate/lactam (1690 cm^{-1}) functionalities. The ESIMS of **42** showed a quasi molecular ion at *m/z* 455, consistent with the molecular formula $C_{24}H_{26}N_2O_7$ + H, indicating that **42** differed from **41** by the loss of 4 hydrogens and the addition of an oxygen atom. The ^{13}C NMR spectrum of **42** showed a total of 24 resonances, comprising three methyl, five methylene, seven methine, and nine quaternary carbon atoms. Notable differences in the ^{13}C NMR spectroscopic data (Table 2.36) of **42** from that of **41** included the presence of an additional lactam carbonyl at δ 172.8, in addition to the two esters and one carbamate signal at δ 171.7, 172.9, and 153.3, respectively. Comparison of the ^1H NMR spectrum of **42** with that of **41** revealed that these two compounds were generally similar except for the signals due to H(3), H(14), and H(15). The signals due to H(3) in **41**, were absent in **42**, having been replaced by a lactam carbonyl, while those due to the CH_2CH_2 unit corresponding to H(14) and H(15) were replaced by signals due to an olefinic CH=CH fragment (δ 6.00, 6.98; d, *J* = 5.9; δ_C 124.1, 156.3). Based on the NOESY spectrum, the configuration at C(16) in **42** was deduced to be *S* from the observed NOE interaction between H(16) and H(6β) (Figure 2.60). This assignment received additional support from consideration of the chemical shifts of the C(16) ester methyl signals. Comparison of the chemical shifts of the C(16) ester methyl group for compounds **41**, **42**, **44**, **45**, and **46**, showed that the C(16) ester methyl chemical shifts are of diagnostic significance for the assignment of C(16)

configuration. When the C(16) methyl ester group is β-oriented as in **41**, **44**, and **46**, the ester methyl signal was found at a somewhat shielded chemical shift value of *ca.* δ 3.0. In compounds **42** and **45**, with an α-oriented methyl ester group, the ester methyl signal was shifted downfield to the 'normal' value of *ca.* δ 3.5, since the ester function is presumably no longer within the anisotropic influence of the aromatic ring. The same behavior of the C(16) ester methyl signal was previously noted in the ^{1}H NMR spectrum of lapidilectine A (**44**) versus that of isolapidilectine A (**45**).[92] Based on these considerations, the C(16) ester methyl group in grandilodine B (**42**) was deduced to be α-oriented (C-16*S*). In any case, since we were able to obtain suitable crystals of grandilodine B (**42**), an X-ray diffraction analysis was carried out (Figure 2.61) which provided further confirmation of the structure deduced based on the spectroscopic data.



41 R^1 = H,H, R^2 = CO_2Me, R^3 = H, R^4 = nil
42 R^1 = O, R^2 = H, R^3 = CO_2Me, R^4 = $\Delta^{14,15}$
44 R^1 = H,H, R^2 = CO_2Me, R^3 = H, R^4 = $\Delta^{14,15}$
45 R^1 = H,H, R^2 = H, R^3 = CO_2Me, R^4 = $\Delta^{14,15}$
46 R^1 = O, R^2 = CO_2Me, R^3 = H, R^4 = $\Delta^{14,15}$

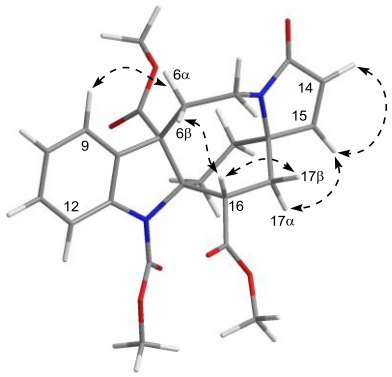


Figure 2.60 : Selected NOEs of **42**

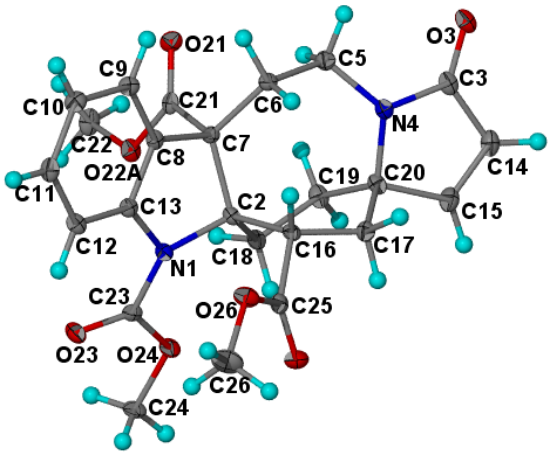


Figure 2.61 : X-ray crystal structure of **42**. Thermal ellipsoids are shown at the 50% probability level.

Table 2.36 : ^{1}H and ^{13}C NMR spectroscopic data of grandilodine B (**42**)[a]

Position	δ_H	δ_C
2	–	74.1
3	–	172.8
5	3.29 dd (15.4, 11.8)	36.6
	4.46 dd (15.5, 5.5)	
6α	3.15 dd (16, 6.5)	30.8
6β	1.91 dd (16, 12)	
7	–	61.5
8	–	131.8
9	7.18 dd (7.7, 1)	123.3
10	7.07 td (7.7, 1)	123.9
11	7.28 td (7.7, 1)	129.5
12	7.53 br d (7.7)	117.9
13	–	143.0
14	6.00 d (5.9)	124.1
15	6.98 d (5.9)	156.3
16	3.03 t (8.6)	42.9
17α	2.75 dd (14.9, 9)	33.6
17β	1.43 dd (14, 9)	
18	2.46 dd (14.9, 5.4)	23.6
	3.45 m	
19	1.67 m	23.5
	2.31 ddd (16, 8.6, 1.8)	
20	–	62.8
21	–	171.7
21-OMe	3.57 s	52.6
CO_2Me	–	172.9
CO_2*Me*	3.52 s	52.1
N*C*O_2Me	–	153.3
NCO_2*Me*	3.77 s	52.7

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, HMBC, and NOESY.

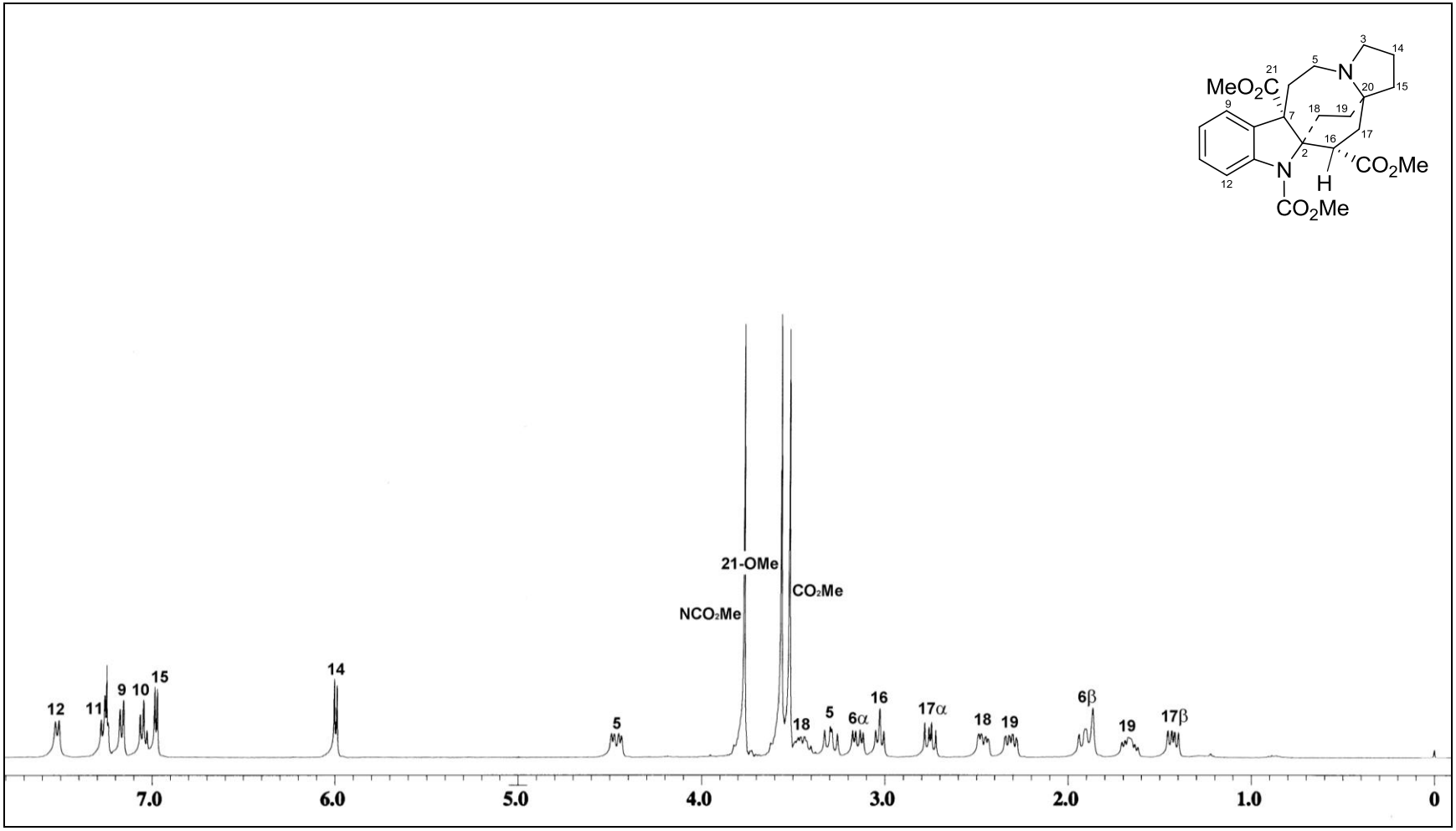
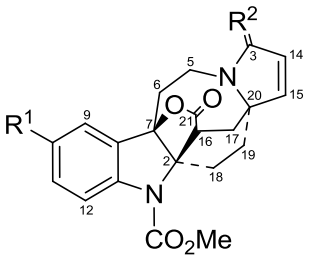


Figure 2.62 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of grandilodine B (**42**)

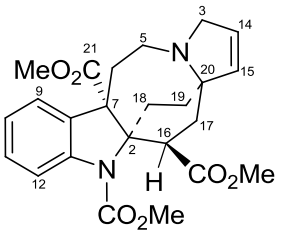
2.2.3 Grandilodine C (43)

Grandilodine C (**43**)[91] was obtained in minute amount as a light yellowish oil, $[\alpha]^{25}_{D}$ +61 (*c* 0.55, $CHCl_3$). The UV spectrum showed absorption maxima at 209, 241, and 286 nm, indicative of a dihydroindole chromophore. The IR spectrum showed carbonyl bands due to lactone (1772 cm^{-1}) and lactam/carbamate (1691 cm^{-1}) functions. The ESIMS of **43** showed a quasi molecular ion at *m/z* 381, which analyzed for $C_{21}H_{20}N_2O_5$ + H. The ^{13}C NMR spectroscopic data (Table 2.37) of **43** showed a total of 21 resonances, comprising one methyl, five methylene, seven methine, and eight quaternary carbon atoms, in agreement with the molecular formula. The observed quaternary carbon resonances at δ 178.2 and 171.6 were consistent with the presence of lactone and lactam functionalities, while the resonance at δ 153.1 was in agreement with the presence of a carbamate group (Table 2.37). The ^1H NMR spectrum (Figure 2.63) showed the presence of an unsubstituted aromatic moiety (δ 7.40–7.58), one methoxy singlet (δ 3.90) associated with the carbamate group, and two olefinic hydrogens (δ 6.07, 6.82, d, *J* = 5.9 Hz). The COSY spectrum revealed the following partial structures, NCH_2CH_2, CH_2CH_2, CH_2CH, and CH=CH. The NMR data of **43** showed a close resemblance to those of **44** except for the loss of two methyl ester signals, the appearance of lactone (δ 178.2) and lactam carbonyl (δ 171.6) resonances, and the downfield shift of the C(7) resonance to δ 91.5, indicating its attachment to the lactone oxygen. These features were generally similar to those of lapidilectine B (**47**)[93] or alkaloids of the tenuisine group,[131] which are characterized by a hexacyclic dihydroindole skeleton incorporating a five-membered lactone ring. The NMR data of **43** showed a striking resemblance to those of tenuisine C (**283**)[131] except for the aromatic region, where the 10-methoxy substituent of **283** was absent in compound **43**.

Grandilodine C (**43**) is therefore the 10-demethoxy derivative of tenuisine C (**283**) which is also in agreement with the HMBC data.



43 R^1 = H, R^2 = O
47 R^1 = H, R^2 = H,H
283 R^1 = OMe, R^2 = O



44

Table 2.37 : ^{1}H and ^{13}C NMR spectroscopic data of grandilodine C (**43**)[a]

Position	δ_H	δ_C
2	–	72.8
3	–	171.6
5	3.15 dd (16, 12)	34.9
	4.52 dd (16, 6)	
6	2.25 m	29.2
	2.91dd (16, 6)	
7	–	91.5
8	–	126.9
9	7.40 m	124.4
10	7.12 t (7.5)	123.8
11	7.40 m	132.0
12	7.58 br s	115.9
13	–	142.0
14	6.07 d (5.9)	124.9
15	6.82 d (5.9)	155.1
16	3.97 m	39.6
17	2.02 d (15.4)	31.4
	2.64 m	
18	1.91 m	21.2
	2.40 m	
19	1.60 m	28.7
	2.23 m	
20	–	64.2
21	–	178.2
NCO_2Me	–	153.1
$NCO_2\mathit{Me}$	3.90 s	52.8

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

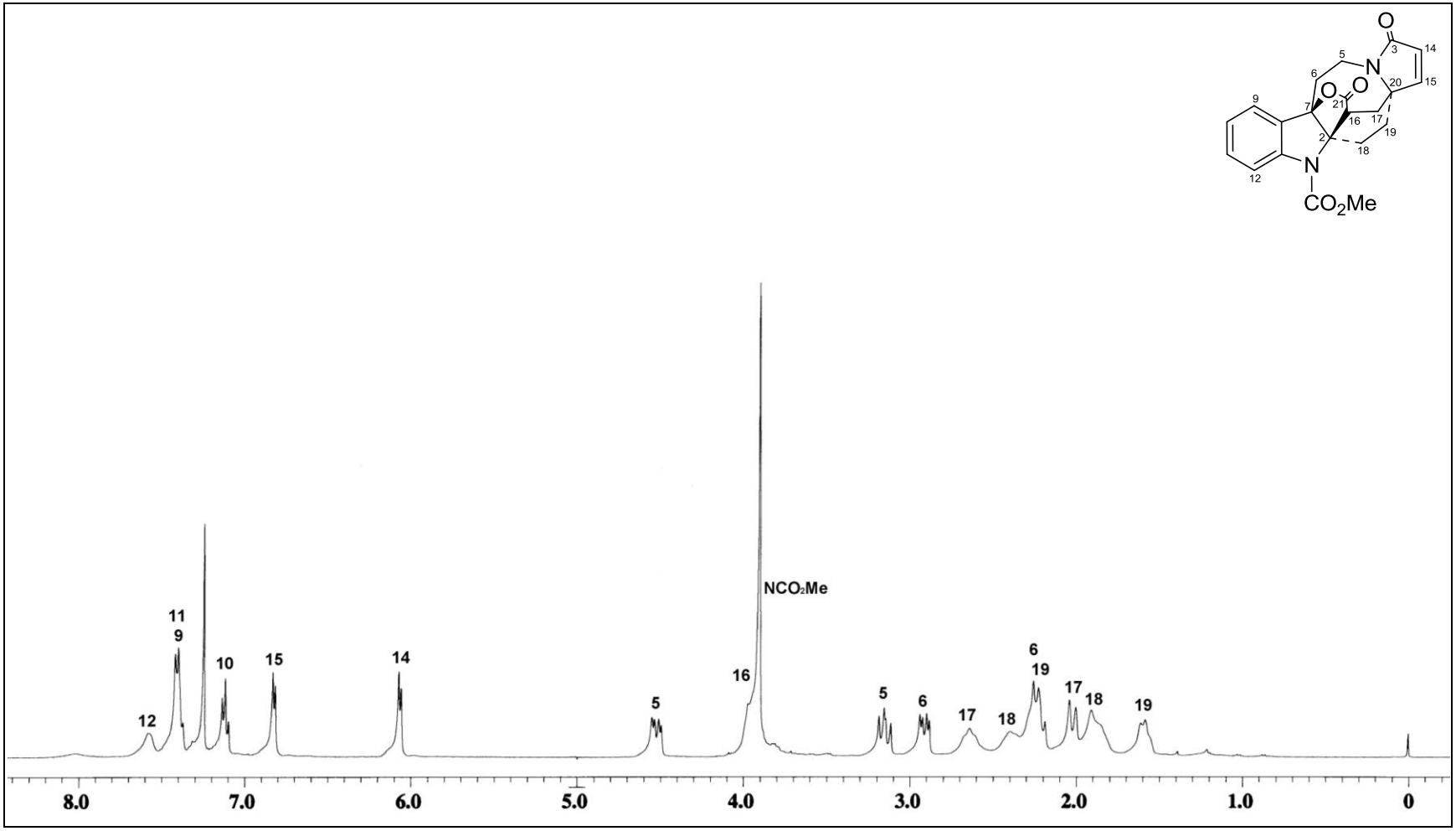


Figure 2.63 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of grandilodine C (**43**)

In addition to the alkaloids already mentioned, a further five known alkaloids were also obtained from the stem-bark and leaf extracts. The spectral data of these known alkaloids are presented in the following Tables and Figures.

Table 2.38 : Known alkaloids from the stem-bark and leaf of *K. grandifolia*

Alkaloids	Physical Appearance	$[\alpha]^{25}_{D}$ ($CHCl_3$)	UV/nm (EtOH) (log ε)	IR/cm^{-1}	NMR Spectral Data
Lapidilectine A (**44**)	White amorphous solid; mp 79–81 ℃	–34 (*c* 2.02)	209 (3.61) 225 (3.47) 253 (3.35) 289 (2.78)	1728 (C=O, ester) 1701 (C=O, carbamate)	Tables 2.39 and 2.40
Isolapidilectine A (**45**)	Light yellowish amorphous solid; mp 80–82 ℃	+89 (*c* 1.76)	211 (2.93) 228 (2.67) 252 (2.46) 283 (1.91)	1725 (C=O, ester) 1701 (C=O, carbamate)	Tables 2.39 and 2.40
Lapidilectam (**46**)	Light yellowish oil	+128 (*c* 0.47)	209 (4.02) 228 (3.84) 254 (3.69) 288 (2.99)	1728 (C=O, ester) 1694 (C=O, carbamate/ lactam)	Tables 2.39 and 2.40
Lapidilectine B (**47**)	Orange amorphous solid; mp 188–190 ℃	+23 (*c* 0.45)	209 (2.60) 242 (2.48) 282 (1.65)	1757 (C=O, lactone) 1705 (C=O, lactam/ carbamate)	Table 2.41
Kopsinine (**22**)	Light yellowish oil	–68 (*c* 0.24)	205 (4.56) 246 (3.88) 296 (3.48)	3350 (NH) 1728 (C=O, ester)	Tables 2.21 and 2.22

Table 2.39 : ^{1}H NMR spectroscopic data of lapidilectine A (**44**), isolapidilectine A (**45**), and lapidilectam (**46**)[a]

H	**44**	**45**	**46**
3	3.38 d (15)	3.29 d (12.5)	–
	3.69 d (15	3.80 d (14.2)	–
5	3.12 m	2.96 m	3.23 dd (15.6, 11)
	3.12 m	3.22 m	4.76 dd (15.9, 8)
6	2.76 m	1.97 m	2.80 dd (16, 11)
	2.76 m	2.92 m	3.13 dd (16, 8)
9	6.99 d (7.7)	7.06 d (7.6)	7.06 d (7.2)
10	6.87 ddd (7.7, 7.7, 1)	6.97 dd (7.6, 7.6)	6.95 t (7.2)
11	7.13 ddd (7.7, 7.7, 1)	7.18 dd (7.6, 7.6)	7.18 t (7.2)
12	7.55 br s	7.55 br s	7.46 br s
14	5.71 dt (6,1)	5.54 br d (5.8)	6.04 d (5.5)
15	5.49 br d (6)	5.46 br d (5.6)	6.75 d (5.9)
16	3.87 m	3.16 dd (11.5, 7.3)	3.84 m
17	1.99 dd (15.3, 10.6)	1.54 dd (16, 8)	2.28 m
	2.57 m	2.51 dd (13.9, 11.5)	2.28 m
18	2.57 m	2.13 dd (14.6, 6.4)	2.38 m
	3.02 m	3.26 m	3.45 m
19	1.53 m	1.54 m	1.62 m
	1.70 m	1.97 m	2.31 m
21-OMe	3.51 s	3.50 s	3.51 s
CO_2*Me*	2.94 s	3.42 s	2.89 s
NCO_2*Me*	3.90 s	3.71 s	3.94 s

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC, and HMBC.

Table 2.40 : ^{13}C NMR spectroscopic data of lapidilectine A (**44**), isolapidilectine A (**45**), and lapidilectam (**46**)[a]

C	**44**	**45**	**46**
2	75.7	74.3	73.6
3	62.3	63.5	170.8
5	49.1	46.9	39.8
6	29.8	33.1	25.2
7	62.3	60.9	64.5
8	131.9	132.8	129.7
9	123.0	122.8	123.0
10	122.8	123.4	123.7
11	129.0	128.9	129.5
12	115.4	117.5	116.0
13	142.8	143.4	141.0
14	125.7	123.7	124.2
15	138.4	138.9	154.5
16	40.5	42.9	40.6
17	32.2	37.0	33.6
18	29.3	24.6	27.1
19	31.1	22.1	29.7
20	67.0	64.4	65.1
21	173.0	172.6	172.4
21-OMe	51.8	52.3	52.8
CO_2Me	174.3	173.1	173.5
CO_2*Me*	52.1	51.5	52.2
N*C*O_2Me	154.1	153.4	154.0
NCO_2*Me*	52.5	52.3	52.9

[a] $CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.

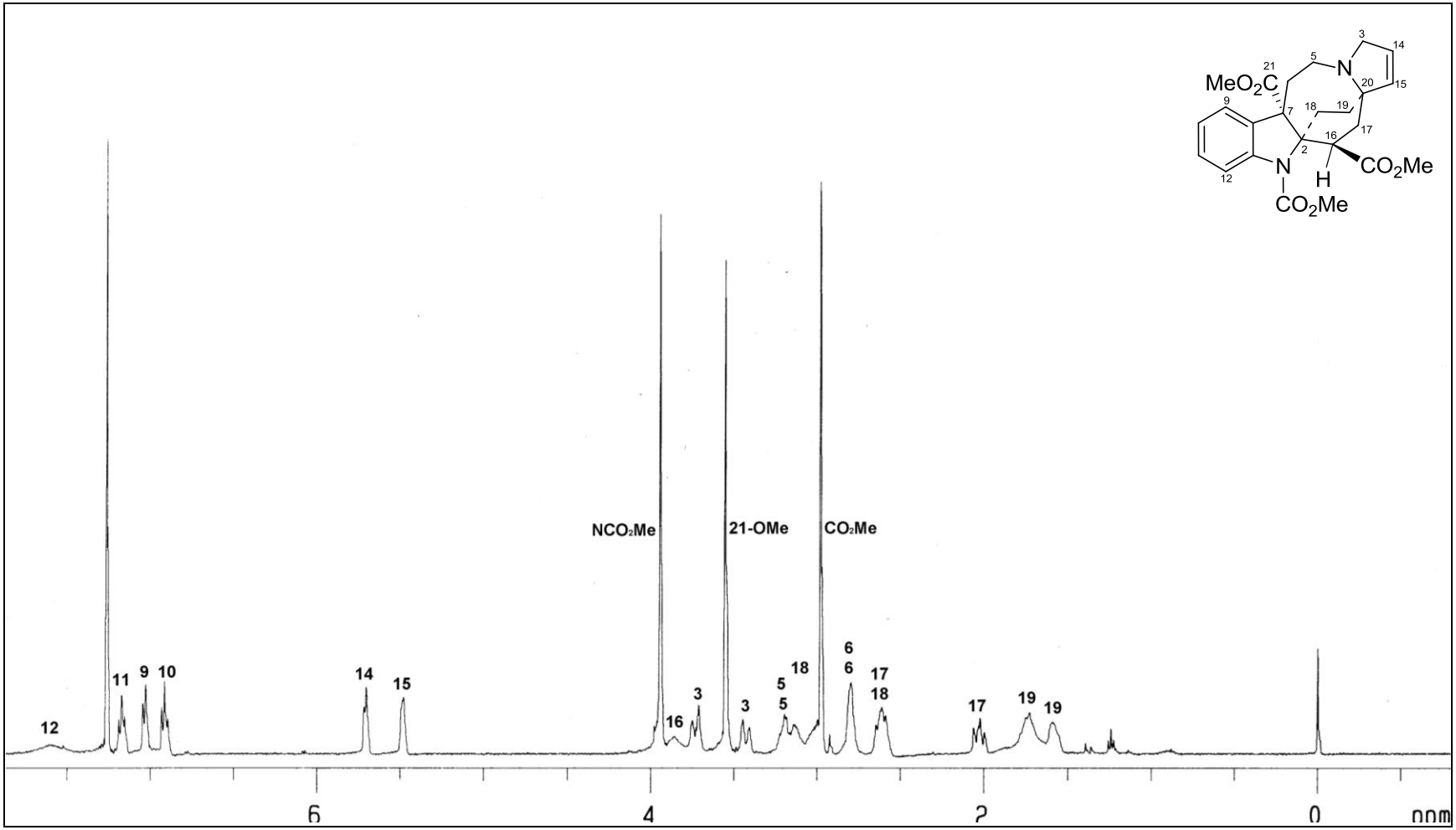


Figure 2.64 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of lapidilectine A (**44**)

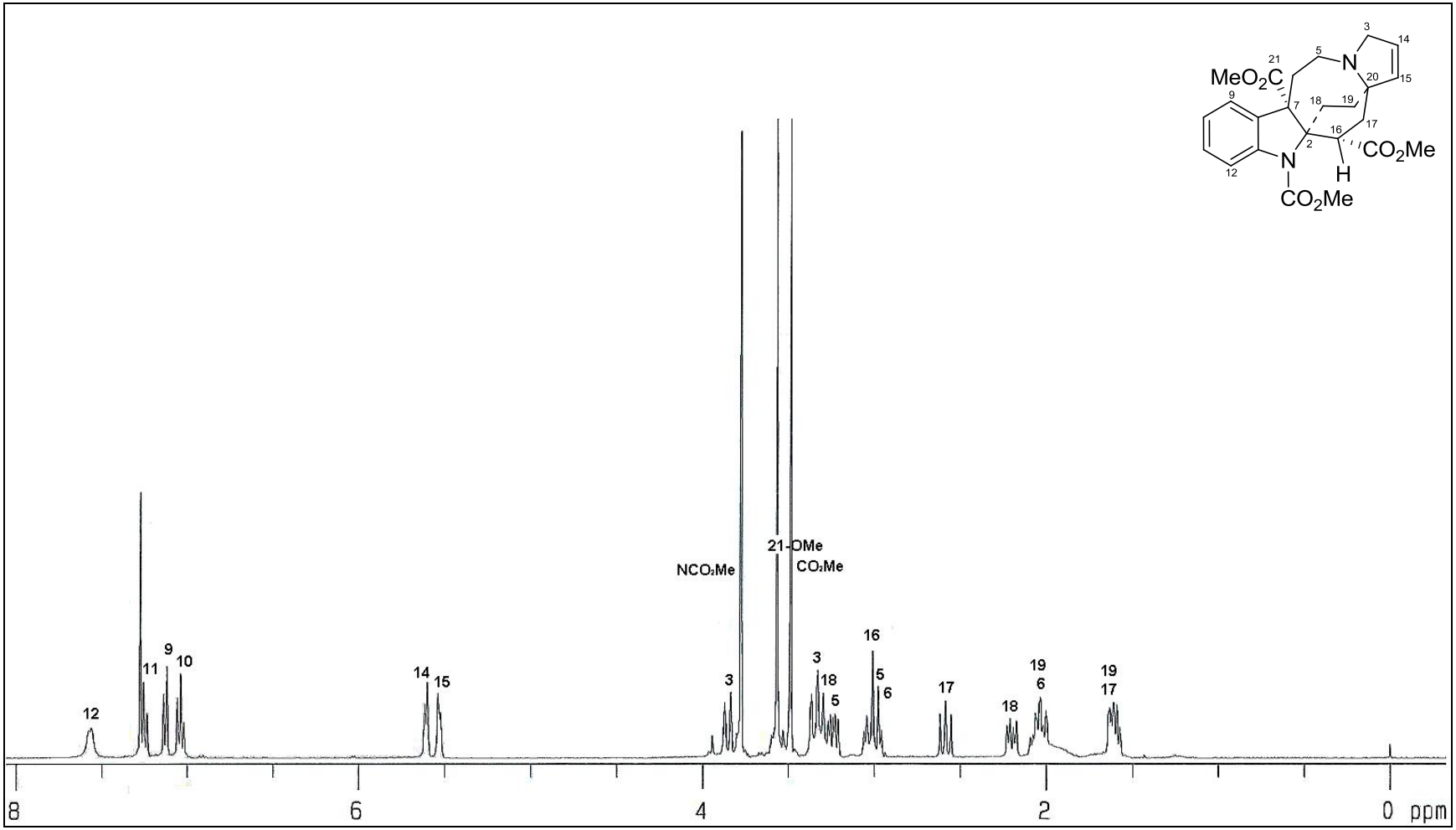


Figure 2.65 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of isolapidilectine A (**45**)

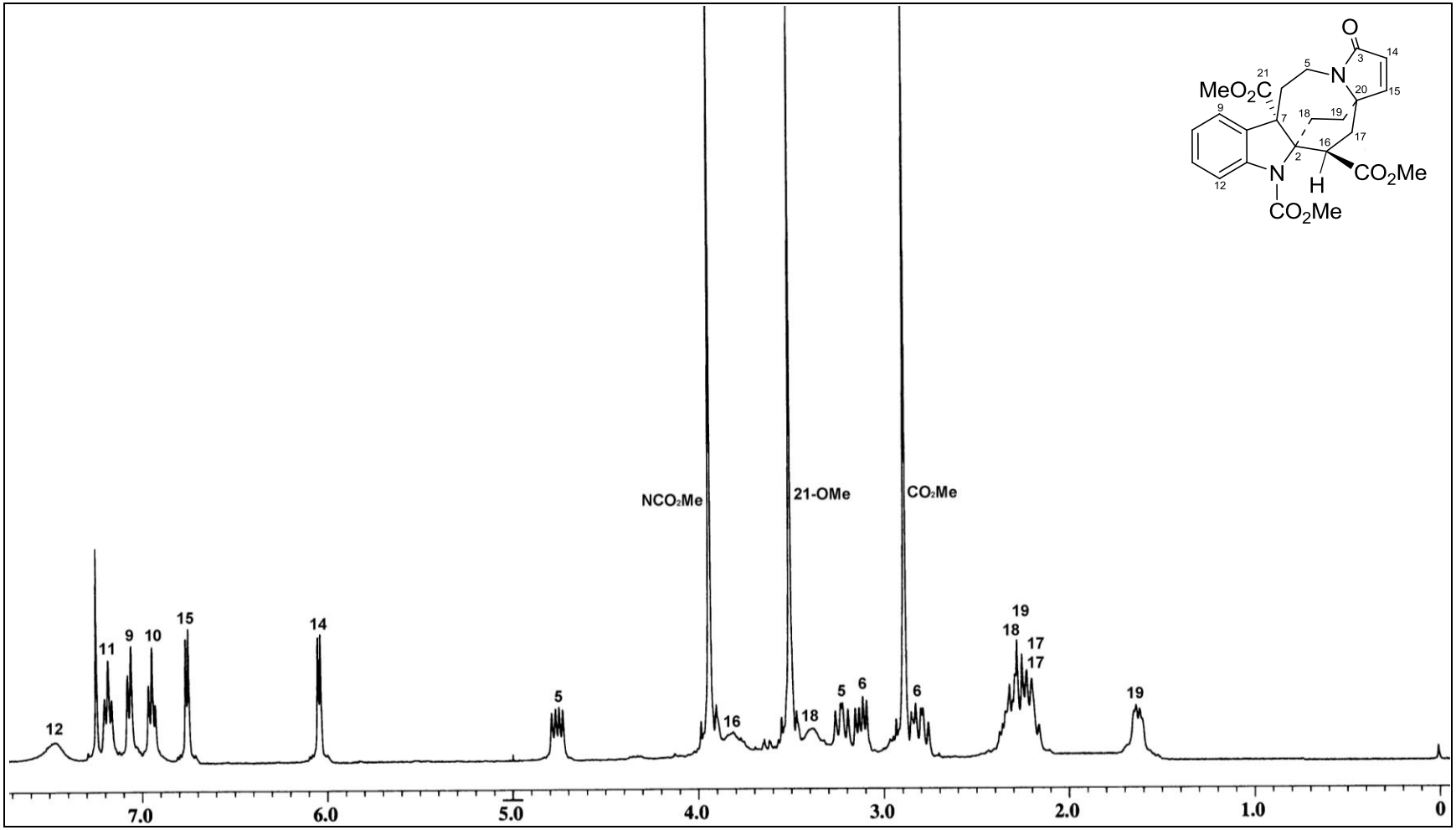


Figure 2.66 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of lapidilectam (**46**)

Table 2.41 : ^{1}H and ^{13}C NMR spectroscopic data of lapidilectine B (**47**)[a]

Position	δ_H	δ_C
2	–	74.0
3	3.20 m	61.7
	3.82 m	
5	2.90 m	47.5
	3.26 m	
6	2.13 m	29.9
	2.76 m	
7	–	90.5
8	–	126.9
9	7.39 m	124.8
10	7.09 t (7.4)	123.5
11	7.39 m	131.2
12	7.57 br s	115.8
13	–	140.9
14	5.73 d (5.2)	124.6
15	5.52 d (5.2)	136.0
16	3.42 m	44.9
17	2.00 m	38.8
	2.23 m	
18	2.01 m	24.9
	2.05 m	
19	1.70 m	21.8
	2.62 m	
20	–	67.3
21	–	177.6
NCO_2Me	–	152.9
$NCO_2\mathit{Me}$	3.90 s	52.9

[a] $CDCl_3$, 400 and 100 MHz, respectively; assignments based on COSY, HMQC, and HMBC.

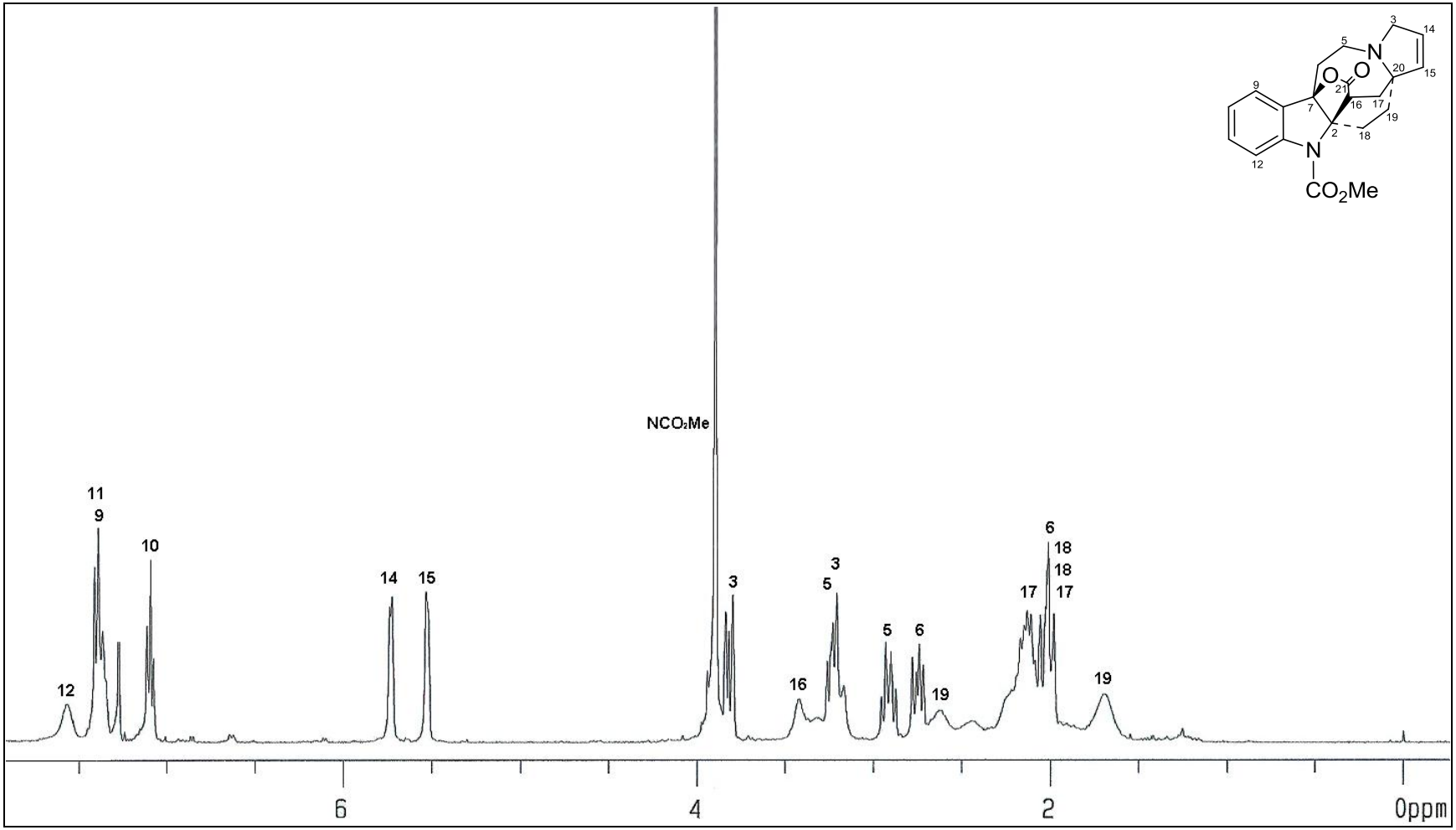


Figure 2.67 : ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of lapidilectine B (**47**)

2.3 Biological Activity

2.3.1 General

Alkaloids exhibit interesting biological activities. Indeed, the possibility of discovering new alkaloids with useful pharmacological activity still stimulate the investigations in this area as much as the intellectual rewards of structure elucidation and synthesis do. Hence, in addition to our systematic chemical investigations, alkaloids isolated from the present study were screened for their biological activity, in particular for their cytotoxic effects and their potential in reversing multidrug resistance (MDR) in drug-resistant tumor cells. This part of the work was carried out by Dr. K. Komiyama and his associates of The Kitasato Institute, Japan.

2.3.2 Cytotoxicity and Reversal of Multidrug Resistance (MDR)

Cancer is essentially a disease of the cells, where individual cells become abnormal and multiply out of control. Multidrug resistance (MDR) is one of the reasons for the failure in cancer treatment. MDR is characterized by the ability of a living cell to show resistance to a wide variety of structurally and functionally unrelated compounds. One of the underlying mechanisms of MDR is overproduction of the permeability-glycoprotein (P-gp) or multidrug resistance associated protein (MRP), which acts as a transmembrane drug efflux pump. Therefore, one of the approaches to counteract the mechanisms of drug resistance is to develop modulators of MDR, also known as chemosensitizers that are devoid of cytotoxicities but are capable of inhibiting the functions of these membrane-bound proteins in tumor cells. The Tsuruo group was the first to demonstrate that the sensitivity of P-gp-expressing multidrug-resistant cells was

enhanced by the presence of verapamil.[148] Subsequently many classes of chemicals, including steroids, cyclosporins, and other calcium channel blockers, have been found to enhance the intracellular accumulation and cytotoxic action of P-gp transported drugs. However, the mechanisms by which these drugs reverse MDR is not fully understood, and to date there are no MDR reversal agents available clinically due to their toxicity and low efficacy.[149-160]

As part of our investigation for new compounds with useful biological activity, alkaloids obtained from the present study were screened for cytotoxic effects against KB (human oral epidermoid carcinoma) cell line, including their potential in reversing multidrug resistance (MDR) in drug-resistant KB cells. The alkaloids were tested at an initial concentration of 25 μg/mL and the IC_{50} values were then determined for the more active compounds and the results are presented in Table 2.42. Grandilodine A (**41**), grandilodine C (**43**) and lapidilectine B (**47**) were found to reverse multidrug resistance in vincristine-resistant KB cells.

Table 2.42 : Cytotoxic effects of alkaloids isolated from *K. pauciflora* and *K. grandifolia*

Alkaloids	$IC_{50,}$ μg/mL (μM)		
	KB/S[a]	KB/VJ300[a]	KB/VJ300(+)[b]
Compound **1**	>25	>25	>25
Compound **8**	>25	>25	18.13 (58.48)
Grandilodine A (**41**)	>25	>25	4.35 (9.84)
Grandilodine B **(42**)	>25	>25	11.50 (25.33)
Grandilodine C (**43**)	>25	>25	4.11 (10.82)
Lapidilectine A (**44**)	>25	>25	>25
Isolapidilectine A (**45**)	>25	>25	>25
Lapidilectine B (**47**)	>25	>25	0.39 (1.07)

[a]KB/S and KB/VJ300 are vincristine-sensitive and vincristine resistant human oral epidermoid carcinoma cell lines, respectively. [b]With added vincristine, 0.1 μg/ml (0.121 μM), which did not affect the growth of the KB/VJ300 cells.