Phytochemistry, Vol 28, No 10, pp 2833–2839, 1989
Printed in Great Britain

0031 9422/89 $3.00+0.00

ASSIGNMENT OF ^{1}H AND ^{13}C NMR RESONANCES OF SOME ISOQUINOLINE ALKALOIDS

RICHARD H. A. M. JANSSEN,* ROBERT J. J. CH. LOUSBERG,† PETER WIJKENS, COR KRUK‡ and HUBERT G THEUNS

Organic Chemical Laboratory, State University of Utrecht, Transitorium 3, Padualaan 8, 3584 CH Utrecht, The Netherlands, †Ministry of Welfare, Health and Culture, Leidschendam, The Netherlands; ‡University of Amsterdam, Department of Organic Chemistry, Nwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands

(*Received* 15 *November* 1988)

Key Word Index—Alkaloids, ^{1}H and ^{13}C NMR chemical shift assignments; lanthanide-induced chemical shifts, ^{13}C–^{1}H shift correlated 2D NMR, backebergine, isobackebergine; 6,7-dimethoxyisoquinoline, 7,8-dimethoxyisoquinoline; isoquinoline, papaverine, 6,7-dimethoxy-2-methylisoquinolinium iodide, 7,8-dimethoxy-2-methylisoquinolinium iodide, berberine chloride

Abstract—Aided by model compounds and lanthanide-induced chemical shifts (LIS), the assignment of ^{1}H and ^{13}C NMR resonances of some isoquinoline alkaloids is discussed and demonstrated. Two-dimensional shift correlated NMR and ^{1}H NOE difference experiments are used in order to assign all proton and carbon chemical shifts in an unambiguous way. Due to solubility problems LIS experiments are impossible for the quaternary compounds investigated These compounds are studied using models and 2D NMR

INTRODUCTION

The lanthanide shift reagent Pr(fod)$_3$ proved to be a valuable tool for discriminating between methoxyl resonances in ^{1}H NMR of the dibenz[*d*, *f*]azonine alkaloids neodihydrothebaine and bractazonine from *Papaver bracteatum* [1] It was also valuable in assigning the ^{1}H NMR methoxyl resonances of the aporphine alkaloid isothebaine [2]. The usefulness of lanthanide-induced shifts (LIS) in ^{13}C NMR was shown in the assignment of the ^{13}C NMR resonances of the latter alkaloid and some phenanthrenes [2]. Other literature on the use of LIS in ^{13}C NMR of related alkaloids is scarce Levin *et al.* [3] used Pr(fod)$_3$ induced shifts for the ^{13}C NMR assignments of reserpine, showing that an isolated methoxyl group is virtually uninfluenced, and that in the attached 3,4,5-trimethoxybenzoate the 4-methoxyl group and the C-4 carbon are influenced to a larger extent than the 3- and 5-methoxyl groups and the C-3 and C-5 carbons.

In this study some aspects of the use of lanthanide shift reagents in ^{1}H and ^{13}C NMR are reported on a few representatives of the vast group of isoquinoline alkaloids. Properly applied and interpreted this technique can be very helpful in assigning NMR resonances. One must be alert, however, for various other effects, some of which are demonstrated and discussed.

The use of model compounds in the assignment of ^{13}C NMR resonances is well-accepted. Calculation of ^{13}C NMR chemical shifts using the well-known incremental values of standard chemical shift theory gives for polysubstituted compounds results which are inconsistent with their actual chemical shifts. Two-dimensional ^{13}C–^{1}H shift correlated NMR [4], together with ^{13}C–^{1}H long range correlation experiments, are used as a reliable means for obtaining absolute assignments of all signals

RESULTS AND DISCUSSION

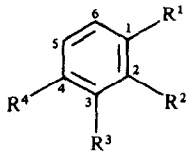
Structural features common to many isoquinoline alkaloids are oxygen-substituted phenyl nuclei. In our earlier work on *P. bracteatum* constituents [1, 2], the lanthanide shift reagent Pr(fod)$_3$ was found to chelate specifically with the oxygens of *ortho*-dimethoxy and *ortho*-hydroxy methoxy substituents, and to exhibit virtually no interaction with isolated methoxyl groups. The interaction with an *N*-methyl group was found to be weak.

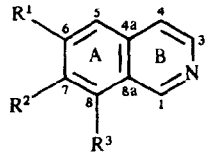
In order to assess these effects in ^{13}C NMR, we studied first some substituted benzenes serving as model compounds for substitution patterns commonly encountered in natural products A study of 2-methoxyphenol was included in our report on isothebaine [2]. The exact geometry of the complex of substrate and chelating agent was reflected in secondary effects observed These effects, mainly observed in the peak heights of the resonances [2], are so dependent upon the exact geometry, that their discussion is beyond the scope of the present paper.

Lanthanide-induced effects for some substituted benzenes

For 1,2-dimethoxybenzene (**1**) the preferential coordination of the shift reagent Pr(fod)$_3$ with the oxygen atoms is reflected in the induced shifts dδ, in ^{1}H NMR as well as in ^{13}C NMR (Table 1). A polysubstitution effect is noted in the data obtained for 2,3-dimethoxytoluene (**2**). In the ^{1}H NMR shift experiment, the C-2 methoxyl substituent, found at δ3.81, is most influenced. The already hindered 2-OMe methyl group of **2** is further

*Present address Bureau Post-Graduate Medical Education (PAOG), Faculty of Medicine, State University of Utrecht, Bijlhouwerstraat 6, 3511 ZC Utrecht, The Netherlands.





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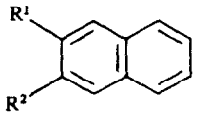
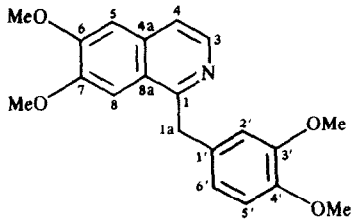
2 $R^1 = Me$, $R^2 = R^3 = OMe$, $R^4 = H$
3 $R^1 = Me$, $R^2 = H$, $R^3 = R^4 = OMe$
4 $R^1 = R^2 = OMe$, $R^3 = H$
5 $R^1 = R^2 = R^3 = H$
9 $R^1 = H$, $R^2 = R^3 = OMe$
6 $R^1 = R^2 = R^3 = R^4 = H$
10 $R^1 = Me$, $R^2 = R^3 = R^4 = H$
11 $R^1 = Me$, $R^2 = OAc$, $R^3 = OMe$, $R^4 = H$
12 $R^1 = OAc$, $R^2 = OMe$, $R^3 = R^4 = H$



7 $R^1 = R^2 = OMe$
8 $R^1 = R^2 = H$



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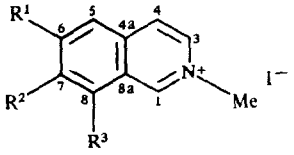

14 $R^1 = R^2 = OMe$, $R^3 = H$
15 $R^1 = R^2 = R^3 = H$
16 $R^1 = H$, $R^2 = R^3 = OMe$

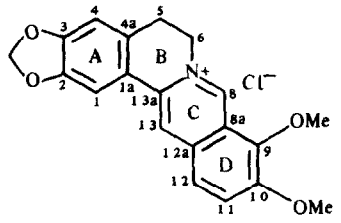

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Table 1 ^1H and ^{13}C NMR chemical shifts (ppm) and lanthanide-induced chemical shifts (normalized shielding gradients) for model compounds **1–3** in $CDCl_3$

Identification of nucleus		1				2				3
^1H	^{13}C	δ_H	$d\delta_H$	δ_C	$d\delta_C$	δ_H	$d\delta_H$	δ_C	$d\delta_C$	δ_C
	C-1	—	—	148 5	58	—	—	131.6	16	130 5
	C-2	—	—	148 5	58	—	—	147 1	66	113 0
H-3	C-3	6 90	16	110 9	17	—	—	152 4	59	149 2
H-4	C-4	6 90	5 4	120.3	9 4	6 96	13	109 8	14	147.3
H-5	C-5	6 90	5 4	120 3	9 4	6 78	4 7	123 3	5 3	111.9
H-6	C-6	6 90	16	110 9	17	6 74	4 5	122 5	5 3	121 1
Me	Me	—	—	—	—	2 26	8 7	15 4	11	20 9
1-OMe	1-OMe	3.89	30	55.1	45	—	—	—	—	—
2-OMe	2-OMe	3.89	30	55 1	45	3 81	26	59 6	46	—
3-OMe	3-OMe	—	—	—	—	3 86	17 8	55 3	32	55 8*
	4-OMe	—	—	—	—	—	—	—	—	56.0*

*Interchangeable assignments.

forced out of plane by the presence of the C-1 methyl substituent on chelation with the shift reagent. These results are in accordance with the ^{13}C NMR experiment (Table 1). Consequently, the induced shifts are asymmetrically distributed over the aromatic nucleus of **2**. In Table 1 the ^{13}C NMR data on 3,4-dimethoxytoluene (**3**) are included. The assignments of compounds **2** and **3** were mainly based on examination of their ^{1}H-coupled ^{13}C NMR spectra

Backebergine

Hughes *et al.* [5] published calculated ^{13}C NMR spectral parameters for 6,7-dimethoxyisoquinoline (**4**), recently found as a natural alkaloid, and named backebergine [6]. These calculated values were indiscriminately adopted as representing measured values by several authors [7–9]. These shifts, however, differ considerably from the actual values, obtained for this alkaloid (see Table 2).

In a 50.32 MHz ^{13}C–^{1}H shift correlated 2D experiment, optimized for polarization via $^{1}J_{CH}$ of 140 Hz, the ^{13}C NMR resonances at δ 103.7, 104.4, 118 4, 141.1 and 149.1 were shown to be connected with the ^{1}H NMR resonances at δ7.00 (*d*, J=0 8 Hz), 7 15 (*d*, J=0.8 Hz), 7.50 (*ddd*, J=5 7, 0.9 and 0.8 Hz, H-4), 8.47 (*d*, J=5.7 Hz, H-3) and 9.09 (*dd*, J=0.9 and 0.8 Hz, H-1), respectively. In a NOE-difference experiment, irradiation at δ9.09 (H-1) resulted in a clear NOE-effect [10] on the resonance at δ7.15. Consequently, the latter resonance must be ascribed to H-8.

In the ^{13}C–^{1}H correlated spectrum, the ^{13}C NMR resonances at δ 55.12 and 55.17 were found to be connected with the ^{1}H NMR singlets at δ 4.024 and 4.015, respectively. A NOE experiment in ^{1}H NMR was performed by irradiation of the methoxyl resonances using low decoupling power and increasing the irradiation frequency stepwise while observing the intensities of the H-5 and H-8 resonances. This experiment showed that the 7-OMe resonance is to be assigned at δ4.024 and the 6-OMe at δ4.015 (see Table 2).

Hughes *et al.* [5] calculated the ^{13}C NMR resonance positions of the benzenoid ring of **4** by applying shift parameters for two *ortho*-methoxy groups to the literature data on isoquinoline **5** [11]. Those shift parameters were obtained by comparison of the spectra of benzene **6** (δ128 5) and 1,2-dimethoxybenzene **1** (δ_1–δ_6) From the data in Table 2 it can be derived that the latter parameters have a bad fit with experimental data 2,3-Dimethoxynaphthalene **7** as expected will serve as a better model for assessing the effects of the *ortho*-methoxy groups in the ^{13}C NMR spectrum of **4**. Comparison of the chemical shifts of **7** [12] with those of naphthalene **8** [12] affords the incremental values (δ_7–δ_8), which indeed have a better fit with the experimental data than the increments used in ref. [5]. These data show the necessity of using models having structural features most comparable to the ones of the compounds being studied.

In sharp contrast to the induced chemical shift behaviour of model compound **1**, the corresponding data on **4** reveal a substantially different interaction of the shift reagent with the substrate. A very strong coordination with the nitrogen atom of **4** is observed, whereas the methoxyl resonances remain almost uninfluenced (see Table 2).

Isobackebergine

For isobackebergine **9** [6] (7,8-dimethoxyisoquinoline) a ^{13}C–^{1}H correlated 2D experiment showed that the ^{13}C NMR resonances at δ146.2, 140.2, 121.7, 119.1 and 118.9 were connected with the ^{1}H NMR resonances at δ9.49 (*dd*, J=1.0 and 0.9 Hz, H-1), 8.36 (*d*, J=5.8 Hz, H-3), 7.49 (*dd*, J=9.0 and 0.9 Hz, H-5), 7.41 (*d*, J=9 0 Hz, H-6) and 7 48 (*dd*, J=5.8 and 1 0 Hz, H-4), respectively The methoxyl resonances were correlated as follows. δ60.5–4.01 and δ55.9–3.93 A NOE difference experiment in ^{1}H NMR showed upon irradiation at δ 9.49 (H-1) a clear effect on the methoxyl resonance at δ4 01, which therefore is to be assigned to the 8-OMe group, whereas irradiation at δ3.93 showed a corresponding effect on the H-6 resonance at δ7 41. These assignments were verified

Table 2. ^{1}H and ^{13}C NMR chemical shifts and Pr(fod)$_3$ induced chemical shifts of 6,7-dimethoxyisoquinoline (backebergine) **4** and isoquinoline **5** in $CDCl_3$; some incremental values, based on comparison of model compounds

Identification of nucleus		4				5				increments in ^{13}C NMR observed		calculated
^{1}H	^{13}C	δ_H	dδ_H	δ_C	dδ_C	δ_H	dδ_H	δ_C	dδ_C	δ_4–δ_5	δ_1–δ_6†	δ_7–δ_8
H-1	C-1	9.09	56 4	149 1	111	9 27	98	152 3	116	−3 2	—	−1 5
H-3	C-3	8.47	56 2	141 1	104	8 55	98	142 8	112	−1 7	—	−1 6
H-4	C-4	7.50	18 9	118 4	23 6	7 63	33	120 2	28	−1 8	-	−1 5
	C-4a	—	—	131 7	30.4	—	—	135 5	34	−3 8	− 7 6	−4 3
H-5	C-5	7 00	10 1	103 7	10 8	n d *	n d	126.2	12	−22 5	−16 9	−21 5
H-6	C-6	—	—	152 2	9 2	n d.	n d	130 1	11	+22 1	+20 8	+23 8
H-7	C-7	—	—	149.5	5 6	n d	n d	127 0	13	+22 5	+20 8	+23 8
H-8	C-8	7 15	11 6	104.4	15 0	n d	n d	127 4	11	−23 0	−16 9	−21 5
	C-8a	—	—	124 0	21 4	—	—	128 4	24	−4 4	−7 6	−4 3
6-OMe	6-OMe	4 015	4 44	55 2	4.2	—	—	—	—	—	—	—
7-OMe	7-OMe	4 024	2.87	55 1	4 2	—	—	—	—	—	—	—

*n d. = not determined

†Used in ref. [5]

Table 3 ^1H and ^{13}C NMR chemical shifts and $Pr(fod)_3$ induced chemical shifts of isobackebergine **9** in $CDCl_3$

Identification of nucleus		**9**				**9***		Increments in ^{13}C NMR observed	calculated		
^1H	^{13}C	δ_H	$d\delta_H$	δ_C	$d\delta_C$	δ_H†	δ_C	$\delta_9-\delta_5$	$\delta_2-\delta_{10}$	$\delta_{12}-\delta_6$	$\delta_{11}-\delta_{10}$
H-1	C-1	9 49	51 2	146 2	105	9 91	147 6	−6 1			
H-3	C-3	8 36	51 2	140 2	109	8 55	141 9	−2 6			
H-4	C-4	7 48	17 3	118 9	26 3	7 18	119 9	−1 3			
	C-4a	—	—	130.5	30 5	—	131 8	−5 0	−6 1	−9 0	−7 1
H-5	C-5	7 49	9 1	121 7	11 5	7 11	122 6	−4 5	−4 6	−2 9	−2 7
H-6	C-6	7 41	6 4	119 1	8 9	6 94	120 5	−1 1 0	−15 1	−16 6	−16 2
	C-7	—	—	147 8	5 5		149 0	+20 8	+24 6	+21 6	+22 5
	C-8	—	—	142 6	15 0	—	144 1	+11 2	+18 6	+10 3	+9 0
	C-8a	—	—	123 2	20 4	—	124 6	−5 2	−5 9	−6 9	−6 9
7-OMe	7-OMe	3 93	4 2	55 9	3 6	3 33	56 6	—	-		-
8-OMe	8-OMe	4 01	5 3	60 5	6.0	3 70	61 0	—	-		-

*In C_6D_6

†$J_{1,4}=1.03$ Hz, $J_{1,5}=0.78$ Hz, $J_{3,4}=5.74$ Hz, $J_{5,6}=8.97$ Hz

by recording a similar ^{13}C–^1H correlated 2D experiment in C_6D_6. In this solvent the ^1H NMR resonances of H-4, H-5, and H-6 were better separated (Table 3). The resonances of the quaternary carbons were identified on account of their multiplicities in the fully proton coupled spectrum (in C_6D_6).

Examination of the figures for $\delta_9-\delta_5$ (the increments observed for compound **9** with respect to isoquinoline **5**; Table 3) indicates profound differences from the additivity parameters observed for *ortho*-dimethoxy substitution in the pairs **4/5**, **1/6** and **7/8** (Table 2). Bearing in mind the limitations set by the absence of the second aromatic ring in the models, the $\delta_9-\delta_5$ figures for C-7 and C-8 roughly resemble the parameters observed for the pairs **2/10**, **11/10** and **12/6** (Table 3), and consequently indicate that the mesomeric interaction of the C-8 methoxyl group with the aromatic π-system is largely inhibited. The 8-OMe methyl group preferentially occupies an out of plane position, as a result of steric crowding. A similar effect was noted earlier in the ^{13}C NMR spectra of some phenanthrene derivatives [2]. The *ortho*-effect exerted by the two methoxyl groups on the methyl bearing carbon in the model **2** amounts to −5.9 ppm, in good agreement with the chemical shift observed for C-8a in **9**

The induced chemical shifts indicate that the bulky shift reagent is slightly hindered in its coordination with the nitrogen function of **9**, when compared with compound **4** The induced chemical shift for C-3 now just surpasses the one of C-1 The distribution of the induced shifts over the isoquinoline nucleus otherwise is completely in line with the results obtained for **4** Some coordination with the C-7 and C-8 methoxyl oxygens is responsible for the small induced shifts of the methoxyl resonances of compound **9** The 8-OMe methyl group will be forced further out of plane, which explains the larger shift observed for the C-8 methoxyl resonance than for the unhindered C-7 methoxyl group This result is fully in line with the data for the model 2,3-dimethoxytoluene **2**

Papaverine

Papaverine **13** combines a 6,7-dimethoxyisoquinoline (**4**) moiety with a 3,4-dimethoxytoluene (**3**) part The ^{13}C NMR spectrum of **13** was assigned by Marsaioli *et al.* [8] by comparison with calculated spectral parameters of **4** [5], which, as already stipulated, differ considerably from the actual values (Table 2) Comparison of the chemical shifts of **13** (Table 4) with those of **4** shows that the benzylic C-1 substituent does not have an appreciable γ-effect on the resonance position of C-8, as was supposed by Marsaioli [8] in order to explain the differences observed with the calculated figures for **4** The actual shifts of **4** fit very well with the spectral parameters of **13**, and, as anticipated, 3,4-dimethoxytoluene is a good model for the benzylic part of papaverine **13**

The $Pr(fod)_3$ induced shift experiment in ^{13}C NMR of **13** gave most surprising results. In sharp contrast to the induced shift behaviour of backebergine **4**, for **13** no coordination of the shift reagent with the nitrogen atom is observed (Table 4) Instead, the oxygens of the benzylic part of the molecule function as ligands, while, again surprisingly, the oxygens of the isoquinoline moiety remain virtually uninfluenced Consequently, it is concluded that the coordination of the shift reagent depends on the structure of the substrate and displays a remarkable selectivity It is likely that the nitrogen function in **13** cannot be approached by the bulky shift reagent, because of the vicinity of the benzylic C-1 substituent

6,7-Dimethoxy-2-methylisoquinolinium iodide

The assignments of H-1 and H-3 of **14** were obtained in a NOE difference experiment upon irradiation of the proton resonance at δ4 35 (*N*-methyl). A ^{13}C–^1H correlated 2D spectrum then provided assignment of the proton-bearing carbons

Application of this technique via long-range J_{CH} [13] proved particularly useful for these aromatic ring containing alkaloids. Cross-section plots of the individual

Table 4. ^1H and ^{13}C NMR chemical shifts and $Pr(fod)_3$ induced chemical shifts for papaverine **13** in $CDCl_3$

Identification of nucleus		**13**			
^1H	^{13}C	δ_H	$d\delta_H$	δ_C	$d\delta_C$
	C-1	—	—	157 5	7 5
H-3	C-3	8 38	3 8	140 7	7.1
H-4	C-4	7 40	1 9	118 4	2 5
	C-4a	—	—	133 1	2 8
H-5	C-5	7 03	2 6	105.0	2.8
	C-6	—	—	152 1	7 2
	C-7	—	—	149 5	7.9
H-8	C-8	7.35	5 7	103.9	4.6
	C-8a	—	—	122 6	4 1
H-1a	C-1a	4 55	5 7	41 9	8 0
	C-1′	—	—	132 0	9.4
H-2′	C-2′	6 74	14 5	111 7	15 5
	C-3′	—	—	148 8	48 4
	C-4′	—	—	147.3	48 6
H-5′	C-5′	6.78	13 6	111 0	15 0
H-6′	C-6′	6 81	7 1	120.2	8 8
6-OMe	6-OMe	3 99	3 3	55 5	4.8
7-OMe	7-OMe	3 91	4 8	55 5	6.5
3′-OMe	3′-OMe	3 78*	25 2	55 5	37 6
4′-OMe	4′-OMe	3 82*	25 7	55 5	37 6

*Interchangeable assignments

columns through each carbon shift showed which protons are involved in long-range couplings. Under the chosen experimental conditions $^3J_{CH}$- and some $^2J_{CH}$-correlations were observed, whereas $^1J_{CH}$-correlations were suppressed.

The C-1 resonance at δ 144.2 proves to be coupled with proton resonances at δ 4.35 (*N*-methyl), 7.56 (evidently H-8), and 8.12 (H-3). The carbon resonance at δ 157 6 showed long-range correlations with proton resonances at δ 3.93 and 7.56 (H-8) Therefore the former resonances represent C-6 and the proton signal of 6-OMe. The signal at δ 135.1 was correlated with δ 7.56 (H-8), 8.12 (H-3) and 9 41 (H-1), and consequently is due to C-4a. Herewith all resonances in ^1H as well as ^{13}C NMR of **14** are fully identified (Table 5).

The C-3/C-4 double bond of backebergine **4** is highly polarized upon *N*-quaternization (C-3 −8.2 ppm, and C-4 +5.0 ppm). With respect to the methoxyl group bearing carbons, C-6 is found most downfield as a result of charge delocalization, in which the *quasi-para* position of C-6 is prominent [14]. The effect of *N*-quaternization on the resonance position of C-6 in **4** is virtually identical with the effect observed for C-6 in isoquinoline methiodide **15**.

7,8-*Dimethoxy-2-methylisoquinolinium iodide*

Irradiation of the ^1H methoxyl signal of compound **16** at δ 4.03 in a NOE difference experiment gave a clear

Table 5 ^1H and ^{13}C NMR chemical shifts 6,7- and 7,8-dimethoxy-2-methylisoquinolinium iodide, **14** and **16**, respectively, in $CDCl_3$/CF_3COOD (5 1)

Identification of nucleus		**14**		**15***	**16**	
^1H	^{13}C	δ_H†	δ_C	δ_C	δ_H‡	δ_C
H-1	C-1	9 41	144 2	150 5	9 61	144.8
H-3	C-3	8 12	132 9	135 7	8 18	132.5
H-4	C-4	8 03	123 4	126 9	8 19	126 1
	C-4a	—	135 1	137 7	—	131 5
H-5	C-5	7 33	104 9	128 0	7.86	122 9
H-6	C-6	—	157 6	137 7	7 91	126 8
H-7	C-7	—	152 6	130 6	—	151.3
H-8	C-8	7.56	106 3	132 1	—	144 6
	C-8a	—	123 8	127 9	—	123 6
6-OMe	6-OMe	3.93	56 7	—	—	—
7-OMe	7-OMe	3 86	56 3	—	4.03	57.0
8-OMe	8-OMe	—	—	—	4 15	62 2
N-Me	N-Me	4.35	47.5	49.0	4.54	49 1

*The data on isoquinoline methiodide **15** (in D_2O/H_2O, 1 : 1) are taken from ref [14], in ref [15] the assignments of C-7 and C-8 are erroneous and should be interchanged

†$J_{1,3}$ = 1 44 Hz, $J_{3,4}$ = 6 74 Hz.

‡In dilute solution $J_{1,3}$ = 1 45 Hz; $J_{3,4}$ = 6 80 Hz, $J_{5,6}$ = 9.10 Hz, the signal of H-5 was broadened due to coupling with H-1

effect on the resonance at δ 7.91. These resonances consequently are due to 7-OMe and H-6, respectively.

For compound **16** ^{13}C–^{1}H correlated 2D spectra were recorded, in separate experiments optimized for couplings of 140 Hz ($^{1}J_{CH}$) and 12 Hz (long-range J_{CH}), respectively. The ^{13}C NMR resonance at δ 144.8 was coupled with ^{1}H NMR signals at δ 7.91 (H-6), 4.15 (8-OMe) and 9.61 (H-1). Consequently this ^{13}C resonance is due to C-8. The ^{13}C NMR resonance at δ 131.5 shows long-range couplings with ^{1}H NMR signals at δ 7.91 (H-6), 8.18 and 9.61 (H-1), and therefore is to be assigned to C-4a, whereas H-3 is found at δ 8.18. The practically coinciding H-3 and H-4 resonances could be distinguished in spectra of **16** in dilute solution, the H-3 resonance then showing a $^{3}J_{1,3}$ of 1.45 Hz (verified by irradiation at δ 9.61). C-3 was observed at δ 132.5, showing $^{3}J_{CH}$ with δ 4.54 (*N*-Me) and 9.61 (H-1).

In agreement with the observations on isoquinoline methiodide **15**, the unsubstituted C-6 in compound **16** is still capable of donating to the electron deficiency at nitrogen, whereas the contribution of an *o*-quinonoidal structure involving the C-8 methoxyl group is virtually nil. The latter effect may be explained from the out-of-plane position of the C-8 methoxyl group, which consequently cannot contribute to the electron deficiency in the isoquinolinium moiety.

Berberine chloride

Though compound **16** serves as a good model for the assignment of berberine chloride **17**, some resonances yet had to be considered as interchangeable. To overcome this, ^{13}C–^{1}H correlated 2D spectra were recorded similarly as for compound **16**.

Carbon C-6 (δ 56.3) showed long-range coupling with the signal at δ 9.54, which consequently is to be assigned to H-8. The carbon resonance at δ 144.1 (C-8) coincided with the C-9 resonance. This was confirmed by using the APT (Attached Proton Test) pulse sequence in ^{13}C NMR [16].

The ^{1}H NMR data obtained (Table 6) show that the literature data on berberine (in DMSO-d_6) reported in ref [17] are erroneous as far as H-8 and H-13 are concerned, these assignments are to be interchanged. Among the few assigned carbon resonances of **17** in ref [7], those of C-11, C-12 and C-13 are erroneous. The correct assignments are given in Table 6.

Table 6. ^{1}H and ^{13}C NMR chemical shifts of berberine chloride **17** in $CDCl_3/CF_3COOD$ (5:1)

Identification of nucleus		**17**	
^{1}H	^{13}C	δ_H*	δ_C
H-1	C-1	7.39	105.1
	C-1a	–	119.8
	C-2		148.6
	C-3	–	151.0
H-4	C-4	6.83	108.5
	C-4a	—	129.8
H-5	C-5	3.24	27.2
H-6	C-6	4.88	56.3
H-8	C-8	9.54	144.1
	C-8a	-	121.8
	C-9	–	144.1
	C-10	—	150.5
H-11	C-11	7.90	126.9
H-12	C-12	7.88	123.1
	C-12a	–	133.5
H-13	C-13	8.34	120.2
	C-13a	—	138.2
OCH_2O	OCH_2O	6.07	102.3
9-OMe	9-OMe	4.19	61.9
10-OMe	10-OMe	4.07	56.7

*$J_{5,6}$ = *ca* 6 Hz, $J_{11,12}$ = 9.09 Hz.

CONCLUSIONS

The use of proper model compounds and correct use of the incremental values of standard chemical shift theory can be helpful in the assignment of much more complicated structures. Yet, in some cases certain resonances cannot be discriminated on account of such comparison alone. The use of lanthanide shift reagents may then provide additional information. The provisions for such use of LIS are the presence of chelating groups, and the use of a non-interfering solvent, such as $CDCl_3$. The presence of an electric charge in the molecule requires the use of highly polar solvents, like DMSO, alcohols, acids, etc., which preclude successful use of lanthanide shift reagents. The latter provision prevented the use of LIS for the assignments of the quaternary alkaloids studied above. One must be alert for specific effects, dependent on structural features of the compound studied. This is exemplified by the chelating effect of a tertiary nitrogen (weak) [1, 2], versus the effect of an aromatic nitrogen (strong), and the observation that even a benzylic substituent at an α-carbon may prevent successful chelation with an otherwise strongly chelating function. 2D Experiments enable full assignment of all resonances. Such experiments, however, are more time and money consuming than the more conventional techniques. Therefore, the data presented here may serve as a guidance for assignments of related molecules.

EXPERIMENTAL

^{1}H and ^{13} NMR spectra were recorded from 0.2 mol/l $CDCl_3$ solns unless noted otherwise. The ^{1}H and ^{13}C NMR spectra of compounds **1**–**3**, **5**, and **11**–**13** were recorded at 90 and 20 MHz, respectively. ^{13}C NMR spectra of some of these compounds were also recorded at 50.32 MHz. Compound **9** was studied in C_6D_6 soln at 250.13 MHz (^{1}H) and 62.89 MHz (^{13}C). The spectra of the other compounds and the 2D ^{13}C–^{1}H shift correlation spectra were obtained at 200.13 (^{1}H) and 50.32 MHz (^{13}C). Solvents are mentioned in the Tables. Chemical shifts (δ) in ppm were determined relative to the solvent signal and converted to the TMS scale.

Lanthanide-induced shift expts were performed in $CDCl_3$ using $Pr(fod)_3$ as shift reagent, as reported in ref [2]. The resulting induced chemical shifts are expressed as normalized shielding gradients $d\delta$ (calculated induced shifts in ppm for equimolar complexes).

Synthesis of compounds **4**, **9**, **14** *and* **16**. These syntheses were performed according to ref [18].

Acknowledgements—The authors are indebted to Mr D. Seijkens, Mr A. V. E. George, Drs J. C. Roos-Venekamp and Dr H. W. A. Biessels (Organic Chemical Laboratory, State University of Utrecht, The Netherlands), for their contributions to this research, and to Ir. E Buurman (Diosynth B. V, Apeldoorn, The Netherlands) for a generous gift of papaverine.

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