filtered and dried under vacuum.

The ^{17}O-enriched benzoic acid (0.70 g) and 0.20 mL of thionyl chloride were mixed and refluxed for 3 h under nitrogen. The excess thionyl chloride and hydrogen chloride were completely removed under vacuum. The resulting residue (oil) was dissolved in 10 mL of dry benzene, and the resulting solution was added to a stirred benzene solution (ca. 150 mL) containing a large excess of ammonia at 0–5 °C. After filtration, the mixture was evaporated under reduced pressure to leave a crystalline mass, which was crystallized from benzene: colorless needles, mp 128.5–129.5 °C; yield 79% (0.55 g, 4.5 mmol). The ^{17}O atom percent of the amide was determined from its mass spectrum to be 5.9.

^{17}O-enriched **1e** was obtained in 23% yield (0.10 g, 0.48 mmol) from 0.25 g (2.1 mmol) of the ^{17}O-enriched benzamide by the same procedure as described above.

ESR Measurements. A 5–20-mg portion of amide **1** or **3** and 0.40 mL of 1:4 (v/v) di-*tert*-butyl peroxide–benzene (or toluene) were placed in an ESR cell. The cell was degassed by three freeze–pump–thaw cycles using a high-vacuum line and sealed off. It was then set in the cavity of an ESR instrument and irradiated directly with an 100-W high-pressure mercury (JEOL JES-UV-1) or an 1-kW xenon lamp (Wacom Xenon UV-10X). ESR spectra were recorded during irradiation or in the dark after irradiation of a few minutes with a JEOL JES-ME-3X or JEOL JES-FE-2XG spectrometer equipped with an X-band microwave unit and 100-kHz field modulation. Hyperfine splitting constants and g values were determined by comparison with Fremy's salt in K_2CO_3 aqueous solution (a_N 13.09 G,[24] g 2.0057[25]). Estimated accuracy was as follows: ±0.1 G for a_N, a_H, and $a_{^{17}O}$, ±0.2 G for $a_{^{33}S}$, and ±0.0002 for g.

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Registry No. **1a**, 100682-56-2; **1b**, 100682-58-4; **1c**, 100682-60-8; **1d**, 100682-62-0; **1e**, 100682-64-2; **2a**, 100682-69-7; **2b**, 100682-70-0; **2c**, 100682-71-1; **2d**, 100682-72-2; **2e**, 100682-73-3; **3a**, 100682-57-3; **3b**, 100682-59-5; **3c**, 100682-61-9; **3d**, 100682-63-1; **3e**, 100682-65-3; **4e**, 100682-74-4; *z*-BuSCl, 52322-55-1; ^{17}O-enriched benzoic acid, 100682-66-4; ^{17}O-enriched benzamide, 100682-67-5; ^{17}-enriched *N*-(*tert*-butylthio)benzamide, 100682-68-6; acetamide, 60-35-5; propionamide, 79-05-0; isobutyramide, 563-83-7; pivalamide, 754-10-9; benzamide, 55-21-0; benzoic acid, 65-85-0.

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Use of Proton Spin–Lattice Relaxation and Nuclear Overhauser Effect Data in Structure Analysis of Alkaloids

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The diagnostic potential of ^1H spin–lattice relaxation measurements has been evaluated for seven morphine, four cinchona, and four tropane alkaloids. Using an approach that stresses examination of specific relaxation pathways, analyzed with the aid of calculations on model structures, it has been shown that ^1H spin–lattice relaxation can provide detailed information on molecular structure and relative stereochemistry of alkaloids.

Analysis of ^1H spin–lattice relaxation is now routinely used to gain insight into the primary structure of organic molecules in solution. The most commonly used methods involve spin–lattice relaxation rate (R_1) measurements,[1] determination of nuclear Overhauser effect[2] (NOE) enhancements, or combinations of these. It has been shown previously that qualitative analysis of spin–lattice relaxation can be useful for determination of certain details of the three-dimensional solution structure (e.g., ref 3 and 4), whereas precise, highly accurate data are required for three-dimensional structures at the angstrom level (e.g., ref 5, 6, and 7). The approach used in our studies involves detailed calculation of relaxation pathways from model structures and qualitative experimental measurement of nonselective R_1 values and NOE enhancements, from which it is possible to determine solution structures at a level sufficient to characterize primary structure and relative stereochemistry.[8,9] Experimental procedures have been examined in detail,[10,11] and the diagnostic potential has been surveyed for carbohydrates[12] and steroids.[4] In this report, our investigations of the potential of ^1H R_1 values for the determination of the structure and stereochemistry of natural products are extended, by examining spin–lattice relaxation in some typical alkaloids, and are refined by the addition of NOE experiments to examine specific relaxation pathways. A summary of studies on some strychnos alkaloids has been described elsewhere.[9]

With appropriate sample preparation,[10,11] spin–lattice relaxation of protons in organic molecules is dominated by intramolecular dipolar interactions with other protons. For a molecule tumbling isotropically in the extreme narrowing limit (molecular weight up to about 1000 for field strengths in common use today), the rate (R_1) whereby a proton, j, relaxes is given by (1), where K is the

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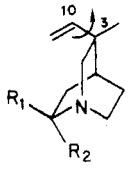
$$R_{1_j} = 1/T_{1_j} = K\sum_i (1/r_{ij})^6 \tau_{c_{ij}} \qquad (i \neq j) \qquad (1)$$

product of constants, r_{ij} is the internuclear distance, and $\tau_{c_{ij}}$ is the rotational correlation time for the ij vector. The reduced expression (1) demonstrates that R_1 values can be used for structural and/or motional analysis. Each term in the summation constitutes a specific dipolar relaxation pathway which can be evaluated experimentally by measuring either selective R_1 values[13] or signal enhancements due to the NOE.[2] The latter has proven to be a more practical method, particularly when carried out in the difference mode.[14]

The dependence on motional correlation time in (1) implies that ^{1}H R_1 values can also give an indication of additional degrees of motional freedom which may be available to certain parts of a molecule. The specific case of methyl rotation has been taken elsewhere.[15] In addition to methyl rotation, there are various types of segmental motion possible in side chains of the molecule. An increase in the relative mobility of a particular portion of the molecule will have the effect of reducing τ_c and, correspondingly, the R_1 value. Uncharacteristically small R_1 values are an indication of additional motional freedom. For example, in the quinuclidine moiety of cinchona alkaloids, e.g., I, the 11c and 11t methylene protons relax



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considerably slower than the relative rates predicted by calculations assuming isotropic motion. The rates are much slower (1.1–1.5 s^{-1}) than those observed for all other methylene protons (4.5–7.3 s^{-1}) and even slower than the aliphatic *methine* protons (2.2–4.0 s^{-1}). These very low R_1 values reflect the relatively greater motional freedom of the vinyl group, due to rotation about the C-3–C-10 bond.

The inverse sixth power dependence on interproton distance in (1) determines that relaxation is mediated almost exclusively by near-neighbor protons. Due to this strong dependence on distance, it is possible to make a fast and reliable characterization of protons on the basis of the efficiency of their spin–lattice relaxation. We have observed that three separate groups of protons can usually be distinguished by their ^{1}H R_1 values. (1) Methylene protons: relax rapidly due to the close proximity of the geminal partner. (2) Aliphatic methine protons: relax in an intermediate range. (3) Aromatic and other isolated protons: relax more slowly than aliphatic methines, because they have fewer vicinal neighbors and no trans-diaxial interactions. We note that, in certain molecules, specific structural features may substantially raise or lower certain R_1 values, such that the ranges of R_1 values would overlap. This classification may also break down when there is a significant degree of anisotropic motion. ^{1}H R_1 values can also allow for the identification of two or more overlapped resonances with differing R_1 values.[16]

In any R_1 analysis of a complex molecule, some form of modelling is required to aid in interpretation of the data. The following method has been used here. When a molecule tumbles isotropically, all values of $\tau_{c_{ij}}$ are equivalent, and a very simple relationship between R_1 values for any two protons can be derived from (1) to give (2).

$$\frac{R_{1_j}}{R_{1_k}} = \frac{\sum_i (1/r_{ij})^6}{\sum_i (1/r_{ik})^6} \qquad (2)$$

$$(i \neq j,\ i \neq k)$$

Relative R_1 values were calculated by using interproton distances measured from Dreiding molecular models or from crystal structure coordinates determined by X-ray or neutron diffraction.[17] Specific relaxation pathways were also evaluated to aid in the interpretation of NOE data. An indication of the quality of the modelling scheme can be obtained by comparing the fit of calculated and observed R_1 values. The comparison is particularly sensitive when the molecule has a considerable range of structural features and R_1 values. Some comparative data for two morphine alkaloids are presented in Figure 1. Since there is excellent agreement between calculated and observed data, isotropic tumbling is implied, and calculated relaxation pathways could be used with confidence for the analysis of experimental data. All modelling schemes used in this study were verified in a similar manner.

Our initial approach involved structural analysis based solely on R_1 data and calculation of relaxation pathways; such an approach worked well for analysis of some strychnos alkaloids.[9] Experience has shown, however, that the usefulness of this method can be greatly extended by the addition of a selected number of NOE measurements, giving corroboration of certain key results obtained from the R_1 data.

Though, in some cases, it is clear that NOE data are more effective for determination of a specific structural feature, recent reports[18,19] have demonstrated that analyzing NOE data without the knowledge of relative R_1 values can often be misleading. Since R_1 data can be determined with a substantially smaller investment of time, we propose that R_1 analysis is more useful for a general approach and serves to guide the selection of relevant NOE (or selective R_1) experiments when information on specific relaxation pathways is required.

In our approach, the interpretation of ^{1}H spin–lattice relaxation of a specific proton is not based upon a single R_1 value but upon satisfactory fitting of all experimental data. The use of several observations to obtain information on a single site compensates for inaccuracies associated with calculations based on a model and for experimental errors. This experimental approach, with inherent simplicity and efficiency, affords a straightforward entry to detailed structural analysis of organic molecules in solution.

Results and Discussion

Morphine Alkaloids. Relaxation studies have been carried out on the following morphine alkaloids: morphine (**1**), three simple morphine derivatives—codeine (**2**), heroin (diacetyl morphine) (**3**), and nalorphine (**4**), and three structurally modified derivatives—thebaine (**5**), naloxone

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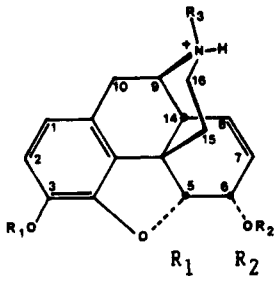
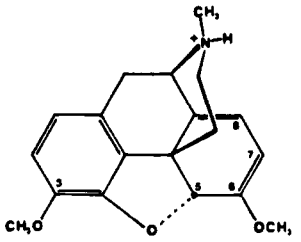
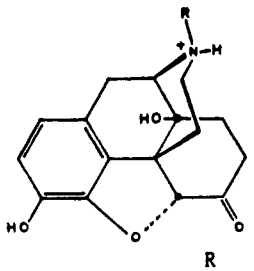
(6), and naltrexone (7). These molecules are all rigid and

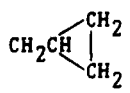


		R_1	R_2	R_3
1	Morphine	H	H	CH_3
2	Codeine	CH_3	H	CH_3
3	Heroin	Ac	Ac	CH_3
4	Nalorphine	H	H	$CH_2CH{=}CH_2$



5 Thebaine



		R
6	Naloxone	$CH_2CH{=}CH_2$
7	Naltrexone	

have a T-shaped structure. They tumble approximately isotropically in solution, hence relative ^1H R_1 values were calculated by using (2). The interproton distances used were derived from crystal structure coordinates[20-23] and molecular models.

Partial assignments for morphine and some derivatives have been reported.[24-27] A complete set of chemical shift assignments for all seven compounds has been made, largely based upon an analysis of relaxation pathways. The 400-MHz ^1H spectrum of codeine (2) (Figure 2) and the corresponding chemical shift and coupling constant data (Table I) are given as an example. Chemical shift assignments for the other morphine alkaloids are also given in Table I.

The 400-MHz ^1H spectra of most of the morphine alkaloids (as their salts) showed the presence of a second component. The presence of major and minor signals for the C-15, C-16, and C-17 carbon atoms and protons in an acidified sample of morphine in a high field ^{13}C and ^1H NMR study has been reported previously.[28] In other studies, morphine sulfate[29] and both morphine hydrochloride and nalorphine hydrochloride[30] were found to be configurationally heterogeneous in solution, due to the presence of epimers at the quaternary nitrogen, the major isomer having the N-CH_3 group equatorial. Evidence for heterogeneity has also been found in the ^{13}C CPMAS spectrum of crystalline morphine sulfate.[31] Data are reported here for only the major component, assumed to be the epimer (with the N-CH_3 group equatorial) identified in the X-ray investigations. Under the conditions of the experiments, proton exchange at the quaternary nitrogen is slow on the NMR time scale, so that calculations of relaxation contributions due to chemical exchange were not required.

The procedure used for assignment will be summarized by using morphine (1) as an example. The 3-OH, 6-OH, NH, and methyl resonances were assigned by standard methods. The low field AB quartet near 6.5 ppm could be assigned to the aromatic protons, H-1 and H-2, on the basis of chemical shift and magnetic isolation—only one major coupling constant and very slow relaxation (1.05 and 1.02 s^{-1}). Observation of a small benzylic coupling to H-1 in a resolution-enhanced[32] spectrum indicated that the previous assignments[27] were incorrect.

The proton assignments in the more crowded region of the spectrum (1.5–6.0 ppm) of 1 were classified according to their relaxation rates (Table II). Methine protons relaxed in the range 1.0–3.2 s^{-1}, whereas methylene protons relaxed in the range 4.2–5.1 s^{-1}. The methine resonances between 3.5 and 5.8 ppm were assigned to H-5, H-6, H-7, H-8, and H-9 on the basis of chemical shift; thus, H-14 was the high field methine proton (3.1 ppm). C-10 and C-16 are adjacent to the aromatic ring and the nitrogen atom, respectively, so the high field methylene protons (1.9 and 2.3 ppm) were assigned to C-15. These assignments were further refined by decoupling experiments and spectral simulation.

Using these tentative assignments, relaxation pathways and R_1 values were calculated and compared to the observed values. The agreement (Figure 1) was excellent, supporting the assignments. R_1 values allow for a clear differentiation between H-7 and H-8 (R_1H-7 $\ll$ R_1H-8) and H-15a and H-15e (R_1H-15a > R_1H-15e). This analysis, based solely on ^1H R_1 values, allowed unambiguous assignment for all but three pairs of signals—H-1 could not be distinguished from H-2, nor could the diastereotopic methylene pairs, H-10a from H-10b and H-16a from H-16e. It was necessary, therefore, to characterize experimentally specific relaxation pathways through the use of NOE experiments. The results of a selected number of NOE difference experiments on both morphine and codeine are given in Table III.

These experiments confirmed all previously made as-

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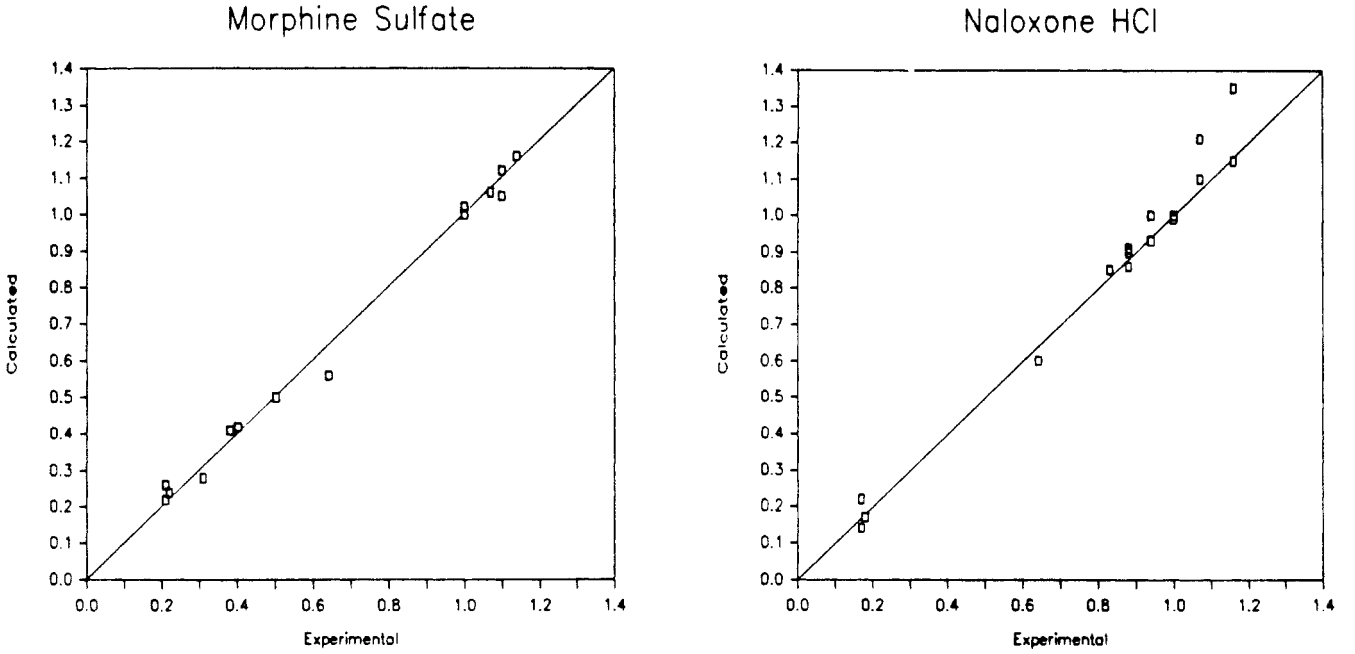


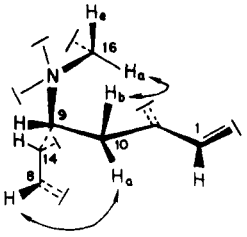
Figure 1. Plot of calculated vs. normalized experimental ^1H R_1 values for (a) morphine sulfate and (b) naloxone hydrochloride.

Table I. ^1H NMR Spectral Parameters of Some Morphine Alkaloids[a]

H	morphine (1)	codeine (2)	J (Hz)			heroin (3)	nalorphine (4)	thebaine (5)	naloxone (6)	naltrexone (7)
1	6.45	6.57 (6.52)	1, 2 = 8.0	1, 10a = 0.2	1, 10b = 0.2	6.71	6.44	6.70	6.63	6.61
2	6.58	6.66 (6.67)				6.86	6.57	6.83	6.72	6.71
3-OR	9.0 (H)	3.84 (3.72) (Me)				2.24 (Ac)	9.04 (H)	3.58 (Me)	9.54 (H)	9.55 (H)
5	5.12	4.90 (4.76)	5, 6 = 6.5	5, 7 = 1.0		5.20	4.82	5.44	5.04	5.05
6	4.82	4.19 (4.14)	6, 7 = 3	6, 8 = 1.7		5.17	4.12			
6-OR	4.11 (H)	3.0 (OH)				2.05 (Ac)	5.1 (H)	3.74 (Me)		
7	5.65	5.71 (5.59)	7, 8 = 10.0	7, 14 = 2.2		5.65	5.64	5.24		
7ax									3.01	3.04
7eq									2.11	2.12
8	5.27	5.30 (5.25)	8, 14 = 3.7			5.52	5.30	5.92		
8ax									1.48	1.51
8eq									2.00	2.04
9	4.06	3.35 (3.69)	10a = 6.3	9, 10b < 0.2	9, 14 = 3.5	4.14	4.09	4.43	3.67	4.02
10a	2.71	2.30 (2.52)	10a, 10b = 19.0			2.78	2.68	3.65	2.92	3.01
10b	3.18	3.07 (3.08)				3.30	3.19	3.65	3.37	3.34
14-R	3.07 (H)	2.67 (2.87)				2.99 (H)	3.25 (H)		6.99 (OH)	7.11 (OH)
15ax	2.35	2.07 (2.22)	15a, 15e = 13.0	15a, 16a = 12.1	15a, 16e = 1.4	2.41	2.35	2.42	2.69	2.70
15eq	1.90	1.88 (1.74)	15e, 16a = 3.6	15e, 16e = 5.2		1.87	1.92	1.80	1.51	1.48
16ax	2.8	2.40 (2.55)	16a, 16e = 12.3			3.38	2.77	3.18	2.50	2.46
16eq	3.1	2.60 (2.85)				3.24	3.25	3.18	3.14	3.08
N-CH_3	2.81	2.45 (2.62)				2.82		2.79		
17							3.88		4.20	3.34
17							3.88		3.79	2.92
18							6.13		5.95	1.01
19							5.50 (t)		5.52 (t)	0.68
19							5.57 (c)		5.63 (c)	0.54
20										0.60
20										0.40

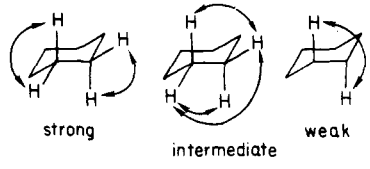
[a] Chemical shifts in ppm from internal Me_4Si, with a precision of ±0.01 ppm, unless indicated otherwise by the number of significant figures. All values measured from 0.1 M Me_2SO-d_6 solutions of the HCl salts, except codeine, measured from 0.1 M solutions of the phosphate salt in Me_2SO-d_6 and the free base in $CDCl_3$ (given in parentheses). Coupling constants measured from $CDCl_3$ solution with a precision of ±0.2 Hz. The signs of the coupling constants have not been determined. Cis (c) and trans (t) indicated in parentheses.

signments and differentiated the diastereotopic methylene protons at C-10 and C-16. In addition, proof that the tentative assignments of H-1 and H-2 were correct was obtained from the observation of an enhancement of the H-1 signal upon saturation of H-10a or H-10b. H-10a was identified by its relaxation pathway with H-8, whereas H-10b could be distinguished on the basis of an NOE enhancement from H-16a. The H-16a ↔ H-10b relaxation pathway served as an entry into the C-15/C-16 system of protons. Another key enhancement was observed from H-15a to H-14. Observation of these two relaxation pathways was actually sufficient to assign all four reso-



nances, although further corroborative evidence was obtained from other experiments.

Relaxation pathways among the four methylene protons at C-15 and C-16 are characteristic and demonstrate a general relationship that can be employed to distinguish diastereotopic methylene pairs. The spatial relationship between the methylene protons on two adjacent carbon atoms in the cyclohexane ring (standard chair conformation) is given in the following diagram, with arrows indicating the relaxation pathways. It is clear that axial



protons can be distinguished from equatorial protons by the number of intermediate vicinal pathways. Experimentally, this implies that when a proton of one of the two diastereotopic methylene pairs is saturated, the observation of NOE enhancement of both vicinal proton signals identifies the saturated resonance as that of the equatorial methylene proton. Upon saturation of the axial proton resonance, only one of the vicinal proton signals will be significantly enhanced, since the axial/axial interproton distance on adjacent carbon atoms is too great to give rise to efficient cross-relaxation. This method of differentiating diastereotopic methylene protons is based upon extensive experimental observation in a variety of alkaloids and other natural products.[4,9,10,33] From the enhancements of both H-15a and H-15e, it was possible to identify the H-16e resonance in morphine alkaloids. The lack of enhancement of H-15e in the {H-16a} experiment probably indicates that the spatial geometry in ring E is not exactly the same as in cyclohexane, in agreement with the spin–spin coupling constant data (Table I). Nonetheless, the assignment of H-16e through its relaxation pathways is unambiguous.

A second relaxation concept is also made clear by the relaxation pathway approach and is demonstrated by the NOE experiments involving H-1, H-10a, and H-10b. Recall, from (1), that the rate of dipolar relaxation of any proton, j, is dependent on the sum total of all available dipolar interactions. Since the total of all pathways for proton k (R_{1_k}) is not necessarily the same as the total for j (R_{1_j}), the *relative* contribution of the j↔k dipolar interaction to the relaxation of k is not necessarily the same as that for j. As an example, consider the relaxation pathways between H-10b and H-1 and H-10a and H-1. Since H-10b has several efficient relaxation pathways available (e.g., H-10b↔H-10a, H-10b↔H-16a), but H-1 has only one additional efficient relaxation pathway (to H-2), there is a sharp contrast in the relative relaxation contributions[34] from H-10b to H-1 (13%) vs. from H-1 to H-10b (3%). The corresponding values for H-10a and H-1 are 19% and 4%, respectively. These data are manifested experimentally by the observation of a significant enhancement of the H-1 resonance upon presaturation of

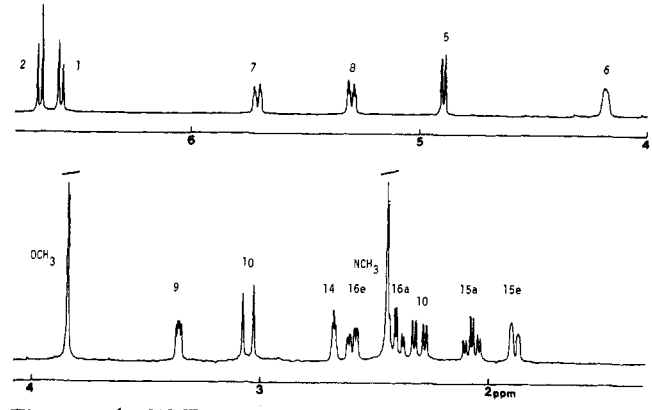
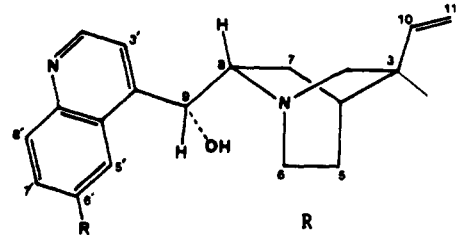


Figure 2. ^{1}H NMR spectrum (400 MHz) of codeine, 0.1 M $CDCl_3$ solution.

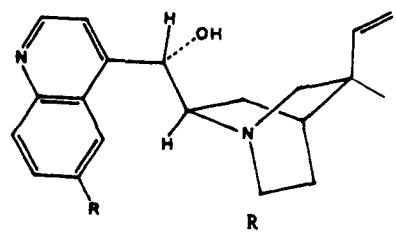
H-10a or H-10b, yet no enhancement of H10a or H-10b when H-1 is presaturated under corresponding conditions (Table III). This asymmetry in the NOE clearly demonstrates the necessity for making a complete analysis of all available relaxation pathways when attempting to interpret NOE enhancements.[19]

Finally, from NOE characterization of specific relaxation pathways in morphine and codeine, it was possible to establish certain important features of the molecular geometry. These are summarized in the following. (1) The relaxation pathway between H-6 and H-14 indicates that ring D is in a boat conformation. (2) Experimentally observed relaxation pathways among H-5, H-6, H-14, and H-9 prove that these protons are all situated on the same face of the molecule, allowing for the determination of relative stereochemistry at all four positions. (3) Interring NOE enhancements (e.g., H-10↔H-1 and H-8↔H-10a) provide information on the spatial proximity of rings and on ring conformations. (4) Conformational preferences for O-methyl and O-acetyl ethers in these compounds have been determined from relaxation pathway analysis. This topic will be discussed in detail elsewhere.[35]

Cinchona Alkaloids. Four representative examples from the cinchona alkaloid family, cinchonidine (**8**), quinine (**9**), cinchonine (**10**), and quinidine (**11**), were selected for relaxation studies. This set of compounds provided



8 Cinchonidine H
9 Quinine OCH_3



10 Cinchonine H
11 Quinidine OCH_3

(33) Chazin, W. J.; Colebrook, L. D., unpublished data.
(34) From calculations using X-ray structure coordinates.[20]
(35) Chazin, W. J.; Colebrook, L. D., in preparation.

Table II. Normalized Experimental R_1 Values of Morphine Alkaloids[a]

H	morphine (1)	codeine (2)	heroin (3)	nalorphine (4)	thebaine (5)	naloxone (6)	naltrexone (7)
1	0.22 (1.05)	0.18 (0.83)	0.17 (0.91)	0.17 (0.90)	0.16 (0.96)	0.18 (0.94)	0.16 (0.94)
2	0.21 (1.02)	0.18 (0.83)	0.10 (0.59)	0.17 (0.91)	0.17 (1.03)	0.17 (0.96)	0.17 (0.96)
3-OR		0.26 (0.94) (Me)	0.22 (1.14) (Ac)	0.20 (1.03) (H)	0.13 (1.22) (Me)	0.23 (1.20) (H)	0.22 (1.22) (H)
5	0.41 (1.82)	0.37 (1.51)	0.30 (1.47)	0.34 (1.65)	0.15 (0.94)	0.17 (0.90)	0.17 (0.95)
6	0.43 (1.87)	0.40 (1.65)	0.27 (1.36)	1.07 (4.95)			
6-OR			0.22 (1.16) (Ac)	0.25 (1.26) (H)	0.40 (2.24) (Me)		
7	0.21 (1.02)	0.18 (0.82)	0.17 (0.89)	0.18 (0.94)	0.23 (1.36)		
7ax						0.88 (4.08)	1.00 (4.95)
7eq						0.83 (3.85)	0.77 (3.85)
8	0.33 (1.47)	0.28 (1.20)	0.28 (1.39)	0.26 (1.33)	0.24 (1.39)		
8ax						0.88 (4.08)	0.82 (4.08)
8eq						1.00 (4.62)	0.93 (4.62)
9	0.74 (3.15)	0.68 (2.67)	0.64 (3.01)	0.52 (2.48)	0.68 (3.65)	0.64 (3.01)	0.69 (3.47)
10a	1.00 (4.20)	1.00 (3.85)	1.00 (4.62)	1.00 (4.62)	1.00 (5.33)	1.00 (4.62)	1.00 (4.95)
10b	1.10 (4.62)	1.13 (4.33)	1.00 (4.62)	1.21 (5.55)	1.09 (5.78)	1.07 (4.95)	1.08 (5.33)
14-R	0.74 (3.15)[b] (H)	0.61 (2.39) (H)	0.64 (3.01)[b] (H)	0.40 (1.93) (H)		0.26 (1.36) (OH)	0.26 (1.44) (OH)
15ax	1.23 (5.13)	1.21 (4.62)	1.07 (4.95)	1.07 (4.95)	1.00 (5.33)	0.94 (4.33)	0.93 (4.62)
15eq	1.10 (4.62)	0.97 (3.75)	1.00 (4.62)	0.94 (4.33)	0.86 (4.62)	0.88 (4.08)	0.87 (4.33)
16ax	1.10 (4.62)[b]	1.13 (4.33)	1.00 (4.62)[b]	1.07 (4.95)	1.00 (5.33)	1.16 (5.33)	0.93 (4.62)
16eq	1.10 (4.62)	1.21 (4.62)	1.07 (4.95)	1.07 (4.95)	1.09 (5.78)	0.94 (4.33)	0.87 (4.33)
N-CH_3	0.78 (3.30)	0.79 (3.08)	0.64 (3.01)		0.62 (3.38)		
17				1.11 (5.13)[c]		1.07 (4.95)	1.08 (5.33)
17				1.11 (5.13)[c]		1.16 (5.33)	1.00 (4.95)
18				0.28 (1.41)		0.29 (1.44)	0.47 (2.39)
19				0.40 (1.93) (t)		0.47 (2.24) (t)	0.69 (3.47)[c]
19				0.45 (2.17) (c)		0.42 (2.04) (c)	0.69 (3.47)[c]
20							0.73 (3.65)
20							0.77 (3.85)

[a] All values measured from 0.1 M Me_2SO-d_6 solutions of the HCl salts, except for codeine, as the phosphate. The observed R_1 values (s^{-1}), given in parentheses ( ), were corrected for contributions from dissolved oxygen and normalized to the R_1 of H-10a. Cis (c) and trans (t) indicated in parentheses. [b] Overlap problem. [c] Tight coupling.

Table III. NOE Enhancements[a] in Morphine and Codeine

{ }[b]	key enhancements[c,d]	other enhancements[d]
1 (m)		2
2 (m)		1
5	14 (m), 15a, 15e	6, 7 (m)
6	14	5, 7
8	6 (m), 10a	5, 7, 9, 14
10a (c)	1, 8	10b, 9
10b	1	10a, N-CH_3 (c)
14	5,6	8,9
15a	5, 14, 16e	15e
15e	5, 16e (m)	15a
16a (c)	10b	16e
16e	15a, 15e	16a

[a] Experiments run at ambient temperature on 0.1 M solutions of morphine sulfate in Me_2SO-d_6 and codeine (free base) in $CDCl_3$. Those experiments carried out on only one of the compounds are indicated by "m" for morphine or "c" for codeine, in parentheses. [b] Proton irradiated. [c] A key enhancement is defined as an NOE enhancement which serves to identify either the transitions irradiated or the transitions enhanced, or which identifies a unique relaxation pathway having implications concerning overall molecular structure. [d] Enhancements observed for only one of the compounds are indicated by "m" or "c", in parentheses.

an opportunity to probe the reliability and sensitivity of the proposed methods of analysis, since they have only very subtle differences in structure. Even though the molecules are composed of two separate, rigid ring systems, only limited motional freedom is expected about the bonds in the single atom (C-9) bridge between the two substructures due to adverse steric interactions. The experimental relaxation data were fitted by a motional model which assumes isotropic overall tumbling but with different correlation times for the quinuclidine and the quinoline rings and the C-3 vinyl group.

Assignments of the ^1H spectrum were made on the basis of standard approaches (decoupling experiments, etc.) and from consideration of the relaxation data. The 400-MHz ^1H spectrum of quinine hydrochloride is shown in Figure 3, and corresponding chemical shift and coupling constant data are given in Table IV. Complete chemical shift assignments for **8**, **10**, and **11** are also given in this table.

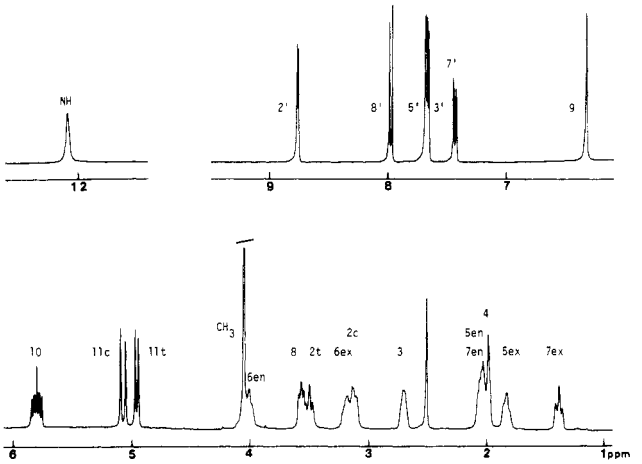


Figure 3. ^1H NMR spectrum (400 MHz) of quinine hydrochloride, 0.1 M Me_2SO-d_6 solution.

The similarity in primary and secondary structure of these four molecules is evidenced experimentally in the remarkable consistency of the measured ^1H R_1 values (Table V). Analysis of relaxation pathways by calculation (using proton coordinates[36] generated from X-ray diffraction data) indicated that the effective environments of protons at some distance from the site of structural differences (positions 2', 8', 2t, 3, 4, 5ex, 6ex, 10, 11c, and 11t) should be the same in all four compounds, so these ^1H R_1 values were expected to be equivalent.

(36) (a) Doherty, R.; Benson, N. R.; Maienthal, M.; Stewart, J. *J. Pharm. Sci.* **1978**, *67*, 1698. (b) Oleksyn, B.; Lebioda, L.; Ciechanowicz-Rutkowska, M. *Acta Crystallogr., Sect B: Struct. Crystallogr. Cryst. Chem.* **1979**, *5*, 440.

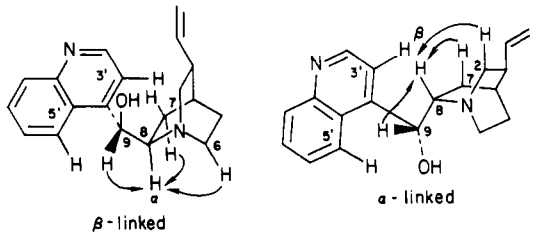
Table IV. ^{1}H NMR Spectral Parameters of Cinchona Alkaloids[a]

H	cinchonidine (8)	quinine (9)	J (Hz)			cinchonine (10)	quinidine (11)
2′	8.92	8.758	2′,3′ = 4.5			8.93	8.75
3′	7.69	7.659				7.69	7.64
5′	8.65	7.681	5′,7′ = 2.7			8.54	7.66
6′	7.66					7.69	
6′-OCH_3		4.044					4.03
7′	7.80	7.432	7′,8′ = 9.0			7.81	7.42
8′	8.07	7.975				8.09	7.97
2c	3.09	3.108	2c, 2t = 13.5	2c, 3 = 6	2c, 6en = 2	3.98	3.99
2t	3.49	3.489	2t, 3 = 10.5			3.42	3.41
3	2.69	2.695	3, 4 = 7 3, 11t = 0.9	3, 10 = 7	3, 11c = 0.9	2.65	2.65
4	1.98	1.973	4, 5ex = 2 4, 7en = 1	4, 5en = 2.5	4, 7ex = 2	1.91	1.91
5ex	1.82	1.824	5ex, 5en = 12 5ex, 7ex = 3.6	5ex, 6ex = 11.6	5ex, 6en = 2.5		
5en	2.05[c]	2.03	5en, 6ex = 5.7	5en, 6en = 11.4		1.77[b]	1.77[b]
6ex	3.19	3.172	6ex, 6en = 11.6			3.19	3.16
6en	3.97	4.000				3.34	3.38
7ex	1.41	1.378	7ex, 7en = 13.5	7ex, 8 = 10.5		2.32	2.32
7en	2.05[c]	2.03	7en, 8 = 7.0			1.14	1.11
8	3.55	3.558	8, 9 = 1.0			3.49	3.52
9	6.29	6.32				6.49	6.51
10	5.79	5.824	10, 11c = 17.0	10, 11t = 10.5		6.06	6.06
11c	5.07	5.071	11c, 11t = 1.2			5.22	5.22
11t	4.95	4.954				5.21	5.21
NH	11.83	12.09				11.75	12.07

[a] Chemical shifts in ppm from 0.1% internal Me_4Si, with a precision of ±0.01 ppm, except for quinine, ±0.003 ppm, and ±0.2 Hz in coupling constant (J) unless otherwise indicated by a smaller number of significant figures. The signs of J values have not been determined. All measurements from 0.1 M Me_2SO-d_6 solutions of the HCl salts at ambient temperature. [b] Tight coupling. [c] Severe overlap.

Calculations led to predictions of several key *interring* relaxation pathways, which were also characterized by NOE experiments. Detailed relaxation pathway analysis, using NOE data, enabled the determination of stereochemistry at C-8. The determination of the conformation of the 6′-OCH_3 group in **8** and **9** was possible by ^{1}H R_1 analysis alone and will be described in another context elsewhere.[35]

The determination of C-8 configuration was carried out as follows. Initially, significant relaxation pathways and the projected differences in R_1 values for the α- and β-linked isomers were calculated. When H-8 is in the α-



position (β-linkage), relaxation of H-8 is dominated by interactions with H-9, H-7en, and H-6en. When H-8 is in the β-position (α-linkage), relaxation is dominated by interaction with H-9, H-7ex, and H-2c. The sum totals of contributions to H-8 relaxation were found to be nearly equivalent for the two configurational isomers, which meant that appreciable differences in ^{1}H R_1 values of H-8 were not expected. The lack of significant differences in the experimental R_1 values (Table V) was consistent with this analysis.

A second approach would be to differentiate C-8 isomers on the basis of differences in relaxation contributions from H-8 to nearby protons. For methylene protons (positions 2, 6, and 7), the effects would be too small to result in any significant differences in R_1 values. This left only H-9, which does have a substantial contribution *from* H-8. Unfortunately, the interproton distances (and relaxation contributions) are essentially the same in both isomers, as the result of the differences in C-9 configuration which accompany the differences at C-8. In summary, the determination of C-8 configuration is not possible solely from ^{1}H R_1 values.

Table V. Normalized Experimental R_1 Values of Cinchona Alkaloids[a]

H	cinchonidine (8)	quinine (9)	cinchonine (10)	quinidine (11)
2′	0.11 (0.64)	0.15 (0.84)	0.13 (0.82)	0.10 (0.65)
3′	0.26 (1.33)	0.25 (1.31)	0.23 (1.31)	0.23 (1.30)
5′	0.48 (2.39)	0.52 (2.57)	0.49 (2.62)	0.53 (2.77)
6′	0.20 (1.08)		0.23 (1.31)	
6′-OCH_3		0.37 (1.87)		0.36 (1.93)
7′	0.18 (0.96)	0.10 (0.60)	0.16 (0.96)	0.09 (0.62)
8′	0.10 (0.61)	0.11 (0.64)	0.09 (0.60)	0.09 (0.59)
2c	1.08 (5.13)	1.08 (5.13)	1.00 (5.13)	0.96 (4.95)
2t	1.12 (5.33)	1.33 (6.30)	1.29 (6.60)	1.04 (5.33)
3	0.45 (2.24)	0.47 (2.31)	0.48 (2.52)	0.43 (2.30)
4	0.52 (2.57)	0.57 (2.77)	0.46 (2.43)	0.45 (2.38)
5ex	1.00 (4.78)	1.00 (4.78)	1.00 (5.13)[b]	1.00 (5.13)[b]
5en	1.00 (4.78)[c]	0.93 (4.47)[c]	1.00 (5.13)[b]	0.96 (4.95)[b]
6ex	1.22 (5.78)	1.12 (5.33)	1.18 (6.03)	1.10 (5.66)
6en	1.12 (5.33)	1.54 (7.30)	1.23 (6.30)	1.08 (5.55)
7ex	1.00 (4.78)	0.97 (4.62)	0.93 (4.78)	1.00 (5.13)
7en	1.00 (4.78)[c]	0.93 (4.47)[c]	1.00 (5.13)	0.94 (4.86)
8	0.72 (3.47)	0.70 (3.38)	0.72 (3.75)	0.67 (3.47)
9	0.76 (3.65)	0.74 (3.55)	0.76 (3.96)	0.65 (3.38)
10	0.22 (1.16)	0.21 (1.14)	0.21 (1.17)	0.20 (1.16)
11 (c)	0.29 (1.51)	0.28 (1.44)	0.23 (1.31)	0.24 (1.36)
11 (t)	0.25 (1.31)	0.23 (1.24)	0.18 (1.07)	0.21 (1.19)

[a] All values measured at ambient temperature from 0.1 M Me_2SO-d_6 solutions of the HCl salts. The observed R_1 values (s^{-1}), given in parentheses ( ), were corrected for the contribution from dissolved oxygen and normalized to the R_1 of H-5ex. Cis (c) and trans (t) indicated in parentheses. [b] Tight coupling. [c] Overlap problem.

The specific relaxation pathways mentioned above can be characterized by NOE enhancements (Table VI), thereby allowing for determination of C-8 configuration. The large enhancements of the H-8 signal upon presatu-

Table VI. NOE Enhancements of Cinchona Alkaloids[a]

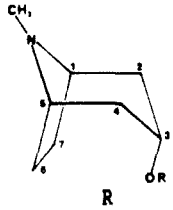
{}[b]	key enhancements[c]	other enhancements
3′	7en, 9	2′
5′	8, 9	6′
6′-CH_3	5′ > 7′	
2c	3′	2t, 7ex, 10, 11c, 11t
6en	8	6ex, 5en
7ex	3′, 8b	4, 7en, 10
7en	3′, 8a	4, 7ex
8a	5′, 7en	9
8b	5′, 7ex	9
9	3′, 5′	8

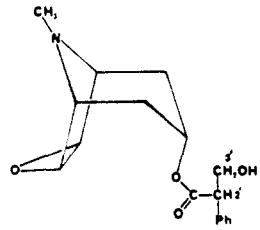
[a] Cumulative results obtained at ambient temperature on 0.1 M $CDCl_3$ or Me_2SO-d_6 solutions of the four cinchona alkaloids. The choice of free base or HCl salt and of solvent was made on the basis of maximizing chemical shift separations. [b] Proton irradiated. [c] See footnote c to Table III.

ration of one of the C-7 proton transitions clearly distinguishes between H-8α and H-8β. Other key NOE enhancements which were identified were those from H-8 to the corresponding C-7 proton and a NOE observed in quinidine from H-6en to H-8.

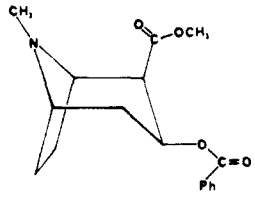
Insights into the relative orientations of the quinoline and quinuclidine rings were obtained from detection, by NOE experiments, of interring relaxation pathways between H-7ex, H-7en, and H-8 on the quinuclidine ring and H-3′ and H-5′, respectively, on the quinoline ring (Table VI). Although there is a possibility for a number of widely differing relative orientations of the quinoline and the quinuclidine rings, steric factors prohibit most orientations, as had been predicted by inspection of the molecular models of these compounds. These data serve to identify the relative orientations of the two rings, as that shown in the diagram.

Tropane Alkaloids. ^1H spin–lattice relaxation has been studied in four tropane alkaloids, tropine (**12**), atropine (**13**), scopolamine (**14**), and cocaine (**15**). ^1H





12 Tropine H
13 Atropine $OCOCH(C_6H_5)CH_2OH$
14 Scopolamine



15 Cocaine

NMR studies at 400 MHz offer a significant improvement in spectral dispersion over the previous NMR studies (250 and 270 MHz) of tropane alkaloids.[37,38] The 400-MHz ^1H NMR spectrum of cocaine (**15**) is shown in Figure 4. Complete chemical shift assignments have been made for all four compounds and are given in Table VII. The

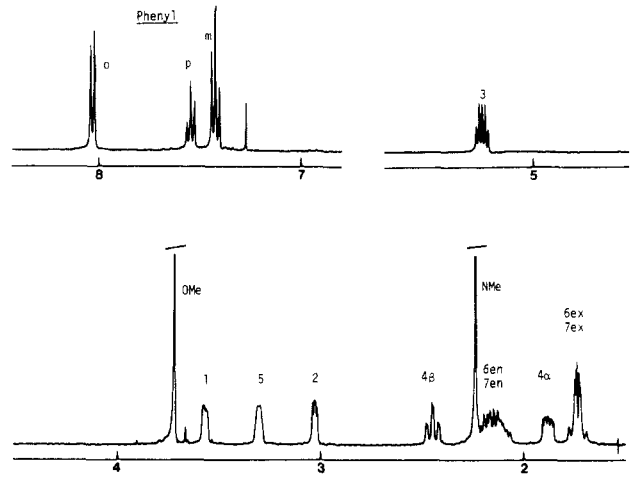


Figure 4. ^1H NMR spectrum (400 MHz) of cocaine, 0.1 M $CDCl_3$ solution.

Table VII. Chemical Shifts of Tropane Alkaloids[a]

H	tropine (12)	atropine (13)	scopolamine (14)	cocaine (15)
1	3.08	2.91	2.97	3.56
2ax	2.10	2.02	2.02	
2eq	1.67	1.47	1.33	3.02
3	4.03	5.02	5.02	5.25
4ax	2.10	2.10	2.11	2.44
4eq	1.67	1.68	1.58	1.87
5	3.08	3.03	3.11	3.30
6ex	2.0	1.86		1.72[b]
6en	2.0	1.74	3.38	2.14[b]
7ex	2.0	1.68		1.72[b]
7en	2.0	1.17	2.66	2.14[b]
N-CH_3	2.26	2.19	2.45	2.23
2′		3.82	3.75	3.72 (Ac)
3′a		3.79	3.81	
3′b		4.16	4.17	
o-Ph		7.29	6.72	8.03
m-Ph		7.37	7.35	7.42
p-Ph		7.35	7.30	7.54

[a] All values in ppm, measured from 0.1 M $CDCl_3$ solutions of free base at ambient probe temperature. The precision of measurement is ±0.01 ppm or better, except where indicated by a smaller number of significant figures. [b] The assignment of protons in the ABCD spin system of C-6 and C-7 is only tentative due to extremely tight coupling.

assignments and measured J values are in agreement with previous studies,[37,38] except for the relative assignments of the axial and equatorial diastereotopic methylene protons at C-2 and C-4 of scopolamine.[38] As will be shown below, the relaxation data were instrumental in making these and other key assignments. There are no reports of ^1H R_1 data from these compounds in the literature, though some NOE data have been reported.[38]

^1H R_1 values (Table VIII) were initially employed to distinguish the three previously mentioned groups of protons for assignment purposes. Methylene protons relaxed in the range 0.6–2.1 s^{-1}, aliphatic methine protons in the range 0.4–1.0 s^{-1}, and aromatic protons in the range 0.34–0.47 s^{-1}. Detailed analysis of relaxation rates was based on calculations using interproton distances measured on molecular models, with the aid of NOE experiments on scopolamine (Table IX). This analysis allowed for verification of all assignments and determination of certain details of solution structure.

^1H relaxation was first analyzed for the simple, achiral molecule tropine (**12**). The agreement between calculated and observed R_1 values (Figure 5) indicated that the molecule had been correctly modelled by using molecular

(37) Carroll, F. I.; Coleman, M. L.; Lewin, A. H. *J. Org. Chem.* **1982**, *47*, 13.
(38) Feeney, J.; Foster, R.; Piper, E. A. *J. Chem. Soc., Perkin Trans. 2* **1977**, 2016.

Table VIII. Normalized Experimental R_1 Values of Some Tropane Alkaloids[a]

H	tropine (12)	atropine (13)	scopolamine (14)	cocaine (15)
1	0.46 (0.39)	0.40 (0.78)	0.39 (0.68)	0.48 (0.82)
2ax	1.04 (0.69)	1.06 (1.82)	1.08 (1.85)	
2eq	1.00 (0.67)	1.00 (1.73)	0.97 (1.69)	0.37 (0.66)
3	0.50 (0.41)	0.28 (0.60)	0.36 (0.72)	0.60 (0.99)
4ax	1.04 (0.69)	1.06 (1.82)	1.11 (1.90)	0.81 (1.28)
4eq	1.00 (0.67)	1.00 (1.73)	1.00 (1.73)	1.00 (1.54)
5	0.46 (0.39)	0.40 (0.78)	0.34 (0.68)	0.52 (0.87)
6ex	1.02 (0.68)	0.94 (1.63)		1.40 (2.10)[b]
6en	0.94 (0.64)	0.91 (1.58)	0.19 (0.45)	1.40 (2.10)[b]
7ex	1.02 (0.68)	0.94 (1.63)		1.40 (2.10)[b]
7en	0.94 (0.64)	0.91 (1.58)	0.19 (0.45)	1.40 (2.10)[b]
N-CH_3	0.89 (0.61)	0.68 (1.22)	0.55 (1.02)	0.89 (1.39)
2′		0.35 (0.70)	0.26 (0.56)	0.29 (0.55) (OCH_3)
3′a		0.61 (1.12)	0.65 (1.17)	
3′b		0.73 (1.31)	0.69 (1.24)	
o-Ph		0.20 (0.46)	0.20 (0.47)	0.14 (0.34)
m-Ph		0.16 (0.41)	0.16 (0.40)	0.22 (0.45)
p-Ph		0.16 (0.41)	0.14 (0.37)	0.19 (0.42)

[a] All values measured from 0.1 M $CDCl_3$ solutions of free base at ambient probe temperature. The observed R_1 values (s^{-1}), given in parentheses ( ), were corrected for relaxation to dissolved oxygen and normalized to the R_1 value of H-4eq. [b] The assignment of protons in the ABCD spin system of C-6 and C-7 is only tentative due to extremely tight coupling.

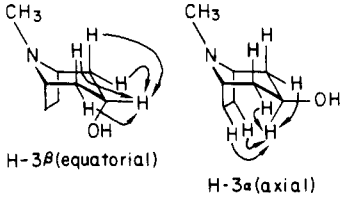
Table IX. NOE Enhancements in Scopolamine[a]

{ }[b]	key enhancements[c]	other enhancements
1	7	
2a	1	2e, 3
2e	1, 7	2a, 3
3	2a > 2e, 4a > 4e	
4a	5	4e, 3
4e	5, 6	4a, 3
5	6	
6	5	7
7	1	6
2′	*o*-Ph[d]	3′b
3′a	*o*-Ph[d]	3′b
3′b	*o*-Ph[d]	2′, 3′a
CH_3		1, 5

[a] Measurements on a 0.1 M solution in $CDCl_3$ at ambient temperature. [b] Irradiated proton. [c] See footnote c to Table III. [d] Ortho phenyl protons.

model interproton distances and assuming isotropic tumbling.[39]

The calculations predicted that the C-3 configuration could be determined on the basis of R_1 data alone (see Figure 5). A significantly higher R_1 value for H-3 is expected for H-3α (axial, down) as opposed to H-3β (equatorial), because with H-3α (C-3β configuration) efficient relaxation pathways to H-6en and H-7en are present.



For example, the relaxation of H-3 is appreciably faster in **15** than in **13** or **14**, even though one of the strong (H-3) relaxation pathways has been lost in **15** due to the substitution of a carbomethoxy group at C-2. The higher R_1 value for H-3 in **15** could only be accounted for by a difference in the C-3 stereochemistry. There is good agreement between the calculated and observed R_1 values of H-3, when both H-3 and the C-2 substituent are in axial positions, confirming both C-2 (β) and C-3 (β) stereochemistry in cocaine.[37,40]

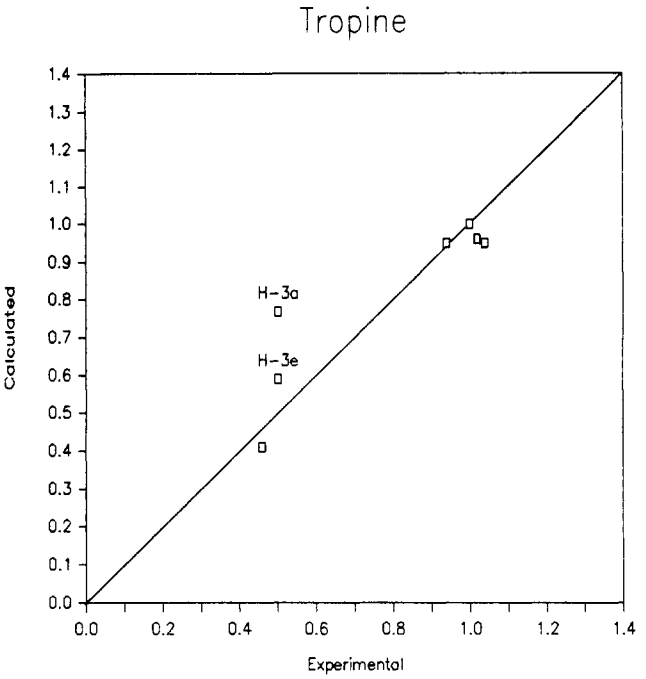


Figure 5. Plot of calculated vs. normalized experimental ^1H R_1 values for tropine. Calculated values for both the equatorial and axial configurations of H-3 are shown.

It was also possible to differentiate the diastereotopic protons at C-2 and C-4 on the basis of R_1 values alone. Calculations predicted that, in **12**, the axial proton will relax faster than the equatorial proton, and this was observed experimentally. The major portion of this differential arises from the flattening of the tropane ring in the C-2, C-3, and C-4 region[38,41] such that the H-2a↔H-3 and the H-4a↔H-3 relaxation pathways are more efficient than the corresponding pathways if the ring were in a standard chair conformation, as found in cyclohexane. There is a second, much smaller, contribution from the 1,3-diaxial interaction between H-2a and H-4a. This trans-diaxial pathway is well-characterized in carbohydrates[12] and steroids[4] but is much weaker in the tropane ring, due to the ring flattening.[38,41]

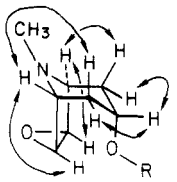
The R_1 of H-4a is smaller than that of H-4e in **15**, the opposite trend from that observed in the other tropane alkaloids. This reduction is attributed to the loss of the strong relaxation pathway to H-3, which is axial (down) in **15** but equatorial in the other tropane alkaloids studied. The lower R_1 value for H-1 relative to H-5 in **15** is a result of the absence of one of the protons at C-2. Since calculations showed that the H-1 relaxation pathway to the C-2 axial proton is much more efficient than the pathway to the equatorial proton, the significant reduction in the R_1 of H-1 verifies the C-2 stereochemistry—the remaining H-2 is equatorial; the $COOCH_3$ is axial.

Note that all of the deductions made above have been based solely on R_1 data. To corroborate and further identify structural details in **13**–**15**, NOE experiments on scopolamine (**14**) were carried out. In the tropane ring, relaxation pathways can be used to trace sequential connectivities, as summarized in the following diagram.

(39) Uusvuori, R.; Lounasmaa, M. *Org. Magn. Reson.* **1984**, *22*, 286.

(40) Gabe, E. J.; Barnes, W. H. *Acta Crystallogr.* **1963**, *16*, 796.

(41) Bishop, R. J.; Fodor, G.; Katritzky, A. R.; Soti, F.; Sutton, L. E.; Swinbourne, E. J. *J. Chem. Soc. A* **1966**, 74.

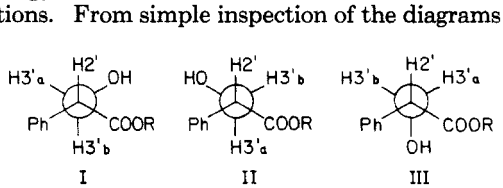


Such sequential information is particularly critical for **14**, where the vicinal coupling from H-5 to H-6 and H-1 to H-7 is very small, and, therefore, difficult to identify by decoupling experiments. Corroborative evidence for the H-6 and H-7 structural assignment was also available from observation of NOE enhancements of H-6 and H-7 resonances in the {H-4e} and {H-2e} experiments, respectively. The presence of significant relaxation pathways between protons at C-2 and C-7 and C-4 and C-6, respectively, is only possible when H-6 and H-7 are endo and H-2 and H-4 are equatorial. These experiments provide, therefore, an unambiguous assignment of the C-6 and C-7 stereochemistry (H-6 and H-7 are both endo, the epoxide is exo[42]) and the assignment of the diastereotopic methylene protons at C-2 and C-4, initially made on the basis of the R_1 data.

The differentiation of the C-2 and C-4 methylene protons was also possible from the {H-3} NOE experiment. As mentioned previously, calculations predicted that for H-3 equatorial in a partially flattened tropane ring conformation, the relaxation pathway to the axial C-2 and C-4 protons should be more effective than the corresponding pathways to the equatorial protons. This was observed experimentally, the enhancements of H-2a and H-4a upon presaturation of H-3 being 3 times as large as the H-2e and the H-4e enhancements.[43] The accumulated evidence left no doubt that the previous assignments of these diastereotopic methylene protons[38] were incorrect.

The final topic to be discussed involves investigations of ^{1}H relaxation in the tropic acid moieties of **13** and **14**, for which the appropriate motional model is extremely complex. These studies demonstrated that useful information and valid qualitative insights can be obtained even when relaxation within a particular system is clearly beyond the scope of the simplified model described by (2).

The motional model for tropic acid derivatives involves a superposition of overall tumbling, phenyl ring rotation, and rotameric equilibrium about the C-2′–C-3′ bond. Phenyl ring rotation was modelled assuming 180° flips between two indistinguishable ring orientations. The phenyl ring rotation is not hindered *on the NMR time scale*, so that only single, time-averaged relaxation pathways can be observed for the magnetically equivalent ortho and meta protons, even if, at any instant, the relaxation pathways are very different for two "equivalent" protons. The rotameric equilibrium was accounted for by the generally accepted model of rapid jumps between the three low energy conformations, as shown below in Newman projections. From simple inspection of the diagrams II–IV it is evident that the rotameric equilibrium will have an effect on spin–lattice relaxation, so the weighted average of the relaxation rate found in the model for each conformational isomer was used for calculations. The required fractional populations were calculated from an analysis of spin–spin coupling constants[38,44] and were found to be 0.67, 0.30, and 0.03 for scopolamine and 0.58, 0.22, and 0.19 for atropine, for II, III, and IV, respectively.



To analyze the R_1 values for the tropic acid moiety of **13** and **14**, calculations were made for each of the three standard conformers and were then weighted according to the relative populations. While these calculations predicted that H-3′a should relax faster than H-3′b, the observed R_1 values followed an opposite trend (Table VIII). This discrepancy may be attributable to cross-correlation[45] between the slowly relaxing H-2′ and the fast relaxing H-3′a. It arises as a consequence of mixing of spin states due to tight spin coupling and has the effect of reducing the sensitivity of the R_1 values to the molecular environment. Though a more detailed examination of the relaxation of H-2′, H-3′a, and H-3′b is beyond the scope of this report, we note that R_1 values were useful for the assignment of this ABX spin system (computer simulation required); H-2′ and H-3′a had nearly identical chemical shifts (Table VII) but the methine proton (H-2′) had a considerably slower relaxation rate (Table VIII).

Finally, we examine the relaxation of the phenyl ring protons. In **15**, the relative R_1 values of ring protons (Table VIII) follow the usual trend observed in monosubstituted benzene derivatives, $o < m \simeq p$. In **13** and **14**, H-2′, H-3′a, and H-3′b make strong contributions to the relaxation of the ortho protons, thus, the ortho proton R_1 values are considerably larger than the corresponding values in **15** and even larger than the R_1 values for meta and para protons. These relaxation pathways were experimentally characterized by observation of a large NOE enhancement of the ortho proton signal in **14** upon presaturation of H-2′ and smaller enhancements upon presaturation of H-3′a and H-3′b. The existence of relaxation pathways from both H-3′a and H-3′b to the ortho protons in **13** and **14** is consistent with the relative populations of II–IV calculated from J values.

Despite differences in the proportions of the three conformational isomers, a significant difference in the relaxation rate of the ortho protons of **13** and **14** was not expected or observed, since (1) only the small contributions from H-3′a and H-3′b vary among the three conformational isomers and (2) there is a preference for II in both molecules.

Conclusions

The application of spin–lattice relaxation parameters to the assignment of high field ^{1}H spectra, the determination of primary and secondary structure, and the relative stereochemistry of alkaloids have been examined.

^{1}H R_1 values provide an efficient method for the classification of the spectral resonances into three groups: methylene protons relax rapidly; aromatic methine protons relax slowly; and aliphatic methine protons relax in an intermediate range. When combined with mathematical modelling of relaxation pathways, qualitative measurement of relaxation parameters (R_1 and NOE enhancements) can be used to determine solution structures at a level sufficient to characterize primary structure and relative ster-

(42) (a) Pauling, P. J.; Pechter, T. J. *J. Chem. Soc. D* **1969**, 1001. (b) Pauling, P. J.; Pechter, T. J. *Nature (London)* **1970**, *228*, 673.

(43) In our studies, quantitative comparison of enhancements is only possible within a single experiment, and only if relaxation rates for the protons being compared are similar. Comparison of enhancements observed in separate experiments is not possible due to the use of subsaturating power levels, short irradiating periods, etc.

(44) Dimitrov, V. S.; Spassov, S. L.; Radeva, T. Z.; Ladd, J. A. *J. Mol. Struct.* **1975**, *27*, 167.

(45) (a) Werbelow, L. G.; Grant, D. M. *J. Chem. Phys.* **1975**, *63*, 544. (b) Werbelow, L. G.; Grant, D. M. *Adv. Magn. Reson.* **1977**, *9*, 189.

eochemistry. The accuracy of a model can be determined from the quality of the fit of calculated and observed values. For the alkaloids used in these studies, the solution and the solid-state structures appeared to be closely similar, since the relaxation data determined in solution could be accurately modelled using previously reported crystal structure coordinates. These studies serve to demonstrate that the approach is valid, and that it can provide insights into the solution structure of alkaloids.

Experimental Section

All spectra were measured at 400 MHz at ambient temperature (about 20 °C) on a Bruker WH-400 spectrometer at the Montreal Regional High Field NMR Laboratory. Solutions were 0.1 M in deuterated solvent and were not degassed, since highly accurate experimental R_1 values were not required. All relaxation data were, however, corrected for contributions from dissolved oxygen, as described elsewhere.[11,35]

^1H R_1 values were measured by nonlinear regression analysis or by the null point method,[10] by using the standard inversion-recovery technique[46] with careful calibration of pulse lengths. NOE enhancements were measured by the difference technique,[14] as described elsewhere.[9] Interproton distances for calculation of R_1 values were obtained from crystal structure coordinates of morphine,[20] codeine,[21] heroin,[22] cinchonine, and quinidine[36] and from molecular models. R_1 values were calculated by using a Hewlett-Packard 1000 computer.

Samples used in this study were obtained from the following suppliers: Aldrich, tropine; Baker, quinine; BDH, cocaine and codeine; F. E. Cornell, heroin and morphine; Endo, naloxone and naltrexone; Fisher, cinchonine; Frosst, nalorphine; Sigma, cinchonidine, quinidine, and scopolamine; and T. and H. Smith, thebaine. The samples were dried before use.

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Registry No. 1·HCl, 52-26-6; 2·H_3PO_4, 52-28-8; 3·HCl, 1502-95-0; 4·HCl, 57-29-4; 5·HCl, 850-57-7; 6·HCl, 357-08-4; 7·HCl, 16676-29-2; 8·HCl, 524-57-2; 9·HCl, 130-89-2; 10·HCl, 5949-11-1; 11·HCl, 1668-99-1; 12, 120-29-6; 13, 51-55-8; 14, 51-34-3; 15, 50-36-2.

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A Chemoenzymatic Synthesis of Leukotriene B_4

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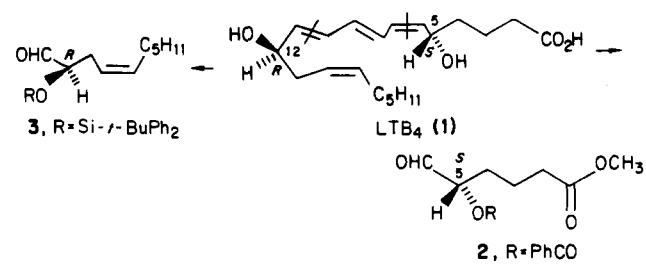
A total synthesis of leukotriene B_4 (1) is accomplished by the assembly of the key chirons 2 and 3, which are prepared via enzymatic methods.

The dihydroxy acid leukotriene B_4 (LTB_4, 1) is biosynthesized from arachidonic acid via the 5-lipoxygenase pathway.[1] Studies on the biological properties of LTB_4 have shown it to be one of the most potent chemotactic factors known for neutrophils.[2] The perceived importance of LTB_4 in allergic and inflammatory states[3] and the difficulty in isolating LTB_4 in quantity from biological sources prompted several groups to embark on the synthesis of this compound. Several elegant total syntheses of LTB_4 have now appeared.[4] With one exception,[4b] LTB_4 was constructed by the successive assembly of prefabricated chirons of carbohydrate origin that possess the stereochemical configuration corresponding to C-5 and C-12 of LTB_4. In this paper, we wish to report an alternative strategy of LTB_4 synthesis that utilizes enzymatic methods for the preparation of the key chiral fragments 2 and 3. The experimental details leading to a convergent synthesis of LTB_4 are described below.

Retrosynthetic analysis of the LTB_4 molecule (Scheme I) reveals that it could be conveniently constructed from two chirons, 2 and 3, each of which possesses the key chiral centers (C-5 and C-12) of the target molecule as shown.

Scheme I



We envisaged that 2 may be conveniently prepared by an enantioselective yeast reduction of the ketone 4 to yield the (5*S*)-carbinol 7, which may then be transformed into 2 using conventional methods.

4, R = CH_3
5, R = *t*-Bu
6, R = H

7, $R_1 = CH_3$; $R_2 = H$
8, R_1 = *t*-Bu; $R_2 = H$
9, $R_1 = R_2 = H$
10, R_1 = *t*-Bu; R_2 = PhCO
11, $R_1 = CH_3$; R_2 = PhCO

Reaction of the sodium salt of monomethyl glutarate with 2 equiv of 2-lithio-1,3-dithiane[5] afforded the acid 6 (78% yield), which was refluxed with 2,2-dimethoxy-

(1) Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 2643.
(2) For reviews, see: (a) Borgeat, P.; Sirois, P. *J. Med. Chem.* **1981**, *24*, 121. (b) Ford Hutchinson, A. W. J. *R. Soc. Med.* **1981**, *74*, 831. (c) Bailey, D. M.; Casey, F. B. *Annu. Rep. Med. Chem.* **1982**, *17*, 203.
(3) Goetzl, E. J. *New Engl. J. Med.* **1980**, *303*, 822.
(4) See: (a) Rokach, J.; Adams, J. *Acc. Chem. Res.* **1985**, *18*, 87 and references cited therein. (b) Nicolaou, K. C.; Zipkin, R. E.; Dolle, R. E.; Harris, B. D. *J. Am. Chem. Soc.* **1984**, *106*, 3548.
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