

Structural and Conformational Study of Repanduline Using Long-Range Nuclear Overhauser Effect Difference Spectroscopy

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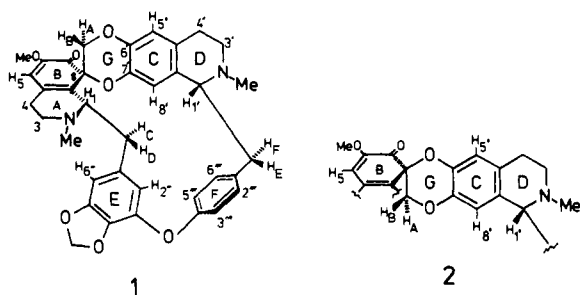
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Abstract: The ¹H NMR spectrum of repanduline (**1**) was assigned by using the results of an extensive series of NOE difference experiments. These and further enhancement data were used to establish the regioisomerism and relative stereochemistry of the molecule. One important enhancement of only 0.18% was measured by prolonged accumulation, demonstrating the high sensitivity of difference NOE spectroscopy. Finally, a detailed analysis of all the data revealed the solution conformation of repanduline.

Nuclear Overhauser effect (NOE) difference spectroscopy² is one of a number of recent pulsed NMR techniques that are revolutionizing the ways in which NMR spectroscopy can be applied to structural problems. In this paper we illustrate the very great power of NOE difference spectroscopy by describing its application to a study of the bis(benzylisoquinoline) alkaloid repanduline (**1**).^{3,4}

We particularly wish to emphasize the increase in sensitivity offered by the difference method, which makes possible for the first time reliable measurement of long-range enhancements. While such experiments are no less prone to problems of interpretation than were previous NOE studies, we suggest that this ability to "see" protons over longer distances, in the present case as far as 4.5 Å, will allow many previously intractable problems to be solved. The technique has another important feature that we have put to use, namely its ability, under certain circumstances, to render visible specific multiplets from within a complex envelope. This clearly offers considerable possibilities for analysis of crowded spectral regions, particularly since the multiplets so revealed can often be assigned by using a knowledge of the spatial relationships of the protons involved.

The aim of the present study was to resolve ambiguities left by the original structural work on repanduline.³ In particular, it was hoped to distinguish between the subtly related possible regioisomers **1** and **2**, to determine the relative stereochemistry



at all three chiral centers, and to establish, as far as possible, the solution conformation.

Although most of the underlying concepts necessary for understanding and interpreting NOE difference experiments are established, we feel that such material is often not conveniently accessible. The next two sections, therefore, are intended to provide the nonspecialist reader with a short summary of this background before the results are discussed in detail.

(1) Department of Chemistry, University of Tasmania, Hobart, Tasmania.

(2) Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703 and references therein.

(3) Harley-Mason, J.; Howard, A. S.; Taylor, W. I.; Vernengo, M. J.; Bick, I. R. C.; Clezy, P. S. *J. Chem. Soc. C* **1967**, 1948. Bick, I. R. C.; Bowie, J. H.; Harley-Mason, J.; Williams, D. H. *Ibid.* **1967**, 1951.

(4) No assignment of absolute configuration was made; see also Figure 4.

Background

The NOE is defined, in the interproton case, as the change in intensity of the multiplet due to one proton or group of protons (I) that occurs when another proton or group of protons (S) is saturated. Theoretical treatments are available elsewhere.⁵ The effect is caused by dipole-dipole cross-relaxation between the I and S protons, and it is the relative contribution which this particular relaxation mechanism makes to the *total* relaxation of the I proton(s) that determines the magnitude of the effect. This contribution depends in turn on the inverse sixth power of the distance between the I and S protons, (r_{IS})⁻⁶, so that the relative magnitudes of enhancements reflect the spatial relationships of the protons involved. Measurements of NOE enhancements have therefore been much used as aids to spectral assignment and structural elucidation and in conformational studies.⁵

Until recently, NOE enhancements could only be measured by careful integration of the I multiplet both with and without saturation of the S multiplet; this method resulted in a lower detection limit of ~5%. NOE difference spectroscopy avoids the problems of inaccurate integration by directly subtracting spectra within the spectrometer's computer. Thus, a control spectrum in which no NOE enhancements are present is subtracted from a spectrum obtained while the S multiplet is saturated. The result is a difference spectrum that, ideally, consists entirely of changes caused by saturation of the S multiplet. The detection limit of this method depends only on the signal to noise ratio and the efficiency of subtraction (i.e., the extent to which unchanged signals are correctly nulled to give a flat base line in the difference spectrum).

An important feature of the difference method is that the saturation field (decoupler) is switched off a few milliseconds before data acquisition begins, with the result that only population effects, such as the NOE, persist at the moment of the observe pulse. This is because population effects build up and decay over several seconds at rates governed by spin-lattice relaxation processes, whereas decoupling effects and Bloch-Siegert shifts disappear essentially at the instant the decoupler is switched off.⁶ Interpretation of the difference spectrum is thereby greatly simplified, since the spectra subtracted differ only in the intensities of lines and not in their positions. A description of the method (due to Hall and Sanders²) we used to acquire the difference spectra is given in the Experimental Section.

Interpretation

The main feature of each NOE difference spectrum is a large negative signal, shown truncated in the figures, corresponding to the preirradiated multiplet (S). The intensity of this signal depends

(5) Noggle, J. H.; Shirmer, R. E. "The Nuclear Overhauser Effect; Chemical Applications"; Academic Press: New York, 1971.

(6) We used a delay of 50 ms between preirradiation and the observe pulse. Shorter delays sometimes result in poor nulling.

Table I. ¹H Assignments for Repanduline in (I) CDCl₃ and (II) [²H₆]Benzene (20%) in CDCl₃

proton	assignment (I) δ	assignment (II) δ	proton	assignment (I) δ	assignment (II) δ
H ₁	2.9-3.0	2.80-2.88	H ₂ ''	7.37	7.23
H ₃ eq }	{2.9-3.05		H ₃ ''	7.12	7.10
H ₃ ax }	{2.4-2.6		H ₅ ''	6.92	7.00
H ₄ eq	1.95-2.15	1.78-1.92	H ₆ ''	6.73	6.77
H ₄ ax	2.3-2.5		H _A	4.15	4.08
H ₅	5.54	4.29	H _B	3.88	3.78
H ₁ '	3.6-3.7	3.6-3.7	H _C	2.95-3.10	2.98-3.08
H ₃ 'eq }	{2.85-3.0		H _D	2.90-3.05	2.85-2.95
H ₃ 'ax }	{3.2-3.35	3.1-3.25	H _E	3.30-3.38	3.25-3.35
H ₄ 'eq	2.5-2.7		H _F	2.63-2.73	2.57-2.67
H ₄ 'ax	2.8-3.0		N ₂ Me	2.40	2.30
H ₅ '	6.49	6.50	N ₂ 'Me	2.65	2.57
H ₈ '	5.11	5.13	OMe	3.62	3.32
H ₂ ''	6.08	6.13	OCH ₂ O	5.92 + 5.97	5.71 + 5.75
H ₆ ''	6.77	6.83			

on the extent of saturation of the S multiplet in the preirradiated spectrum. Generally this is less than 100% because (i) subsaturating power levels were used to achieve adequate frequency selectivity and (ii) some slight recovery occurs during the short delay between preirradiation and the observe pulse. In certain cases described below, only ~50% saturation took place since only one component of a doublet was being irradiated at a time. Such partial saturation only excites approximately the corresponding fraction of any possible NOE enhancement; the absolute magnitudes of enhancements measured by difference thus have no particular quantitative significance. This is in contrast to the older method of comparing integrals, which yields such magnitudes as its only result (and therefore requires complete saturation of the S multiplet during measurement).

Other peaks in the difference spectrum arise through population disturbances induced by saturation of the S proton. These can be of three types.

(i) **NOE Enhancements** (transmitted through space to protons close to the presaturated proton, S): In previous NOE studies a quantitative interpretation of enhancement data has often been used in order to derive internuclear distances. This approach, which is fully discussed by Noggle and Shirmer,⁵ relies heavily on comparisons of enhancements of the same proton(s) that occur when various neighboring protons are separately irradiated. The results of NOE difference experiments are not well suited to such interpretation, mainly because the required comparisons cannot easily be quantified. Instead, we adopted a qualitative, or at most semiquantitative, approach, discounting structures or conformations if they could not account for the approximate relative magnitudes of particular observed enhancements. Comparisons of the experimental data with enhancements calculated from distances measured on molecular models were made in a few cases, but only to reinforce qualitative conclusions.⁷

In addition to the direct enhancements, transmitted enhancements were also observed. These occur when a strongly enhanced proton (I) is itself sufficiently close in space to another proton (X) for their mutual cross-relaxation to transmit population disturbance to X; for such an indirect enhancement to outweigh direct enhancement of X by S, the distance r_{SX} should be large relative to r_{SI} and r_{IX} .⁵ It is an important feature of difference spectra from rapidly tumbling molecules that transmitted enhancements build up only slowly, are weak, and show a sign alternation for each intermediate cross-relaxation step: three spin effects (one intermediate spin) are negative (approximately -2-0%, depending on the length of preirradiation), while four spin effects (two intermediate spins) are positive, but so weak as to be rarely seen. This enables relaxation pathways (e.g., S → I → X) to be identified easily and analyzed, as is illustrated in the section on

long range NOE difference spectroscopy.

In total contrast, indirect enhancements in *slowly* tumbling molecules quickly build up to levels comparable to those of direct enhancements, and both are negative; relaxation pathways therefore cannot be identified, and the structural significance of enhancements is lost. Spin diffusion, as this process of rapid propagation of population disturbance is called, must therefore be avoided by using short preirradiation times when working with such molecules.⁸

(ii) **SPT (Selective Population Transfer) Effects** (transmitted through bonds to protons coupled to the presaturated proton, S):⁹ These effects, which are the basis of the INDOR and FTINDOR experiments, occur whenever the component lines of a preirradiated multiplet (S) are partially saturated to differing extents. SPT then results in a *redistribution* of intensity between the component lines of other multiplets that share a scalar coupling with the S multiplet. Unlike the NOE this effect (in isolation) produces no overall change in the intensity of the affected multiplet as a whole. Multiplets due to protons coupled to the S proton but spatially remote from it therefore appear in the difference spectrum as a series of positive and negative lines whose summed intensities balance. If, however, a proton is both coupled to and close to the S proton, the NOE also operates and a complex intensity pattern results. The relative contributions of the two effects then depend on (i) the magnitude of the NOE enhancement and (ii) the extent and manner in which the partial saturation of the S multiplet differs between its components. In practice, we have found that substantial SPT effects commonly do occur as a result of the low preirradiation powers employed. This is useful in some ways, since it reveals coupling connectivities, but it can also complicate the detection of NOE enhancements.

(iii) **Saturation Transfer** (transmitted via chemical exchange involving the S proton itself): Since repanduline possesses no exchangeable protons, saturation transfer is impossible in the present work.

Results and Discussion

Assignments (summarized in Table I). The normal spectrum of repanduline in CDCl₃ is shown at the base of Figures 1 and 2. Assignments in the high-field region (δ 1.8-3.5) clearly represent a considerable challenge, while the low-field region (δ 3.5-7.5) consists of simple, well-separated, first-order patterns. The low-field region will be considered first since its assignment is a prerequisite for further discussion.

(i) **Low-Field Region (δ 3.5-7.5).** A number of assignments were made immediately. The three proton singlet at δ 3.62 was assigned to the *O*-methyl group, the doublets at δ 3.88 and 4.15 to the *O*-methylene group of ring G, and the four double doublets

(7) Accurate Dreiding models were used throughout this work. Less accurate models did not allow conformational energy minima to be recognized, and showed significantly different interproton distances in some cases.

(8) Kalk, A.; Berendsen, H. J. C. *J. Magn. Reson.* 1976, 24, 343.

(9) Although the term SPT has more commonly been used to describe heteronuclear effects, it is equally correct here and we feel the alternative term, generalized Overhauser effect, is unnecessarily confusing in this context.

Table II. NOE Enhancements Observed for Repanduline in CDCl_3 ^a

pre-irradiate	observe
H_6''	H_2'' (spt only), H_D (w), $\text{H}_{4\text{ax}}$ (w), N_2Me (w)
H_5'	$\text{H}_4'\text{ax}$ (w), $\text{H}_4'\text{eq}$ (m)
H_2''	H_3''' (m), H_2''' (m), H_6'' (spt only), H_6' (s), H_C (m)
H_5	H_A (w), OMe (w), $\text{H}_{4\text{ax}}$ (w), $\text{H}_{4\text{eq}}$ (m)
H_5'	H_2''' (m), H_3''' (w), H_5''' (w), H_6''' (m), H_2'' (s), H_1' (s)
H_A	H_5' (w), H_5 (w), H_B (s, spt), H_1 (w, negtv)
H_B	H_5' (w), H_6' (w), H_A (s, spt), H_1 (m)
H_1'	H_2''' (m), H_3''' (w, negtv), H_2'' (w, negtv), H_5 (w), ^b H_5' (s), H_E (m, spt), H_F (spt only), N_2Me (m)
H_E	H_2''' (m), H_3''' (w, negtv), H_6''' (w, negtv), H_1' (w, spt), H_F (s, spt), N_2Me (w)

^a Abbreviations: s, strong NOE (>5%); m, medium NOE (1–5%); w, weak NOE (<1%); negtv, negative NOE (three-spin effect); spt, selective population transfer. ^b Small enhancement due to slight saturation of nearby OMe signal.

at δ 6.73, 6.92, 7.12, and 7.37 to the aromatic protons of ring F. Small couplings (more clearly resolved in other spectra) were used to assign the peaks at δ 5.92 and 5.97 to the methylenedioxy group and those at δ 6.08 and 6.77 to the two aromatic protons of ring E ($J_m = 1.2$ Hz). It then remained to assign the singlets at δ 5.11, 5.54, and 6.49 to H_5 , H_8 , and H_5 , and also to make relative assignments within the sets of signals already allocated to the protons of rings E, F, and G.

To resolve these ambiguities, a series of NOE difference experiments was carried out, the results of which are summarized in Table II. The simplest assignment was that of the peak at δ 5.54 (H_5), preirradiation of which caused clear enhancement of the *O*-methyl signal. Protons H_8 and H_2'' were assigned on the basis of the strong enhancements observed between them; molecular models very clearly showed that no other pair of protons on rings C and E could be sufficiently close to each other. Assignments of H_5' and H_6' followed automatically. The doublet at δ 3.66 enhanced on preirradiation of H_8 was assigned to H_1' and the doublet enhanced on preirradiation of H_1' was assigned to H_2''' . Coupling connectivities from H_2''' then led automatically to assignment of the remaining protons of ring F. Confirmatory evidence for these was provided by the strong enhancements of H_3''' and H_5''' on preirradiation of H_2'' and by the enhancement of H_6''' on preirradiation of H_F (next section).

The relative assignments of H_A and H_B (the methylene protons of ring G) followed from the observation of an enhanced signal at δ 3.0 on preirradiation at δ 3.87 and 3.91.¹⁰ The enhanced signal can only be due to one or more of the protons in the isolated three-spin system comprised of H_1 , H_C , and H_D , implying that H_B was the proton preirradiated. A detailed analysis of enhancements in this portion of the molecule must include consideration of the relative stereochemistries at C_1 and C_8 , and such an analysis is given later; the relative assignments of H_A and H_B , however, are independent of these considerations.

(ii) **High-Field Region (δ 1.8–3.5).** The high-field region of the repanduline spectrum is obviously complicated and crowded. Nonetheless, one may assume that it consists, in addition to the *N*-methyl singlets, of four superimposed subspectra arising from isolated portions of the molecule. Of these subspectra, two are three-spin systems (one consisting of H_1 , H_C , and H_D , the other of H_1' , H_E , and H_F) while the remaining protons of rings A and D comprise two four-spin systems. Although full spectral parameters of the three-spin systems were required for the stereochemical assignments, it was considered sufficient to characterize the four-spin systems only in terms of chemical shift.

Several assignments were already obvious from the NOE difference experiments discussed in the previous section. Preirradiation of H_1' revealed the multiplets due to H_E (δ 3.33) and

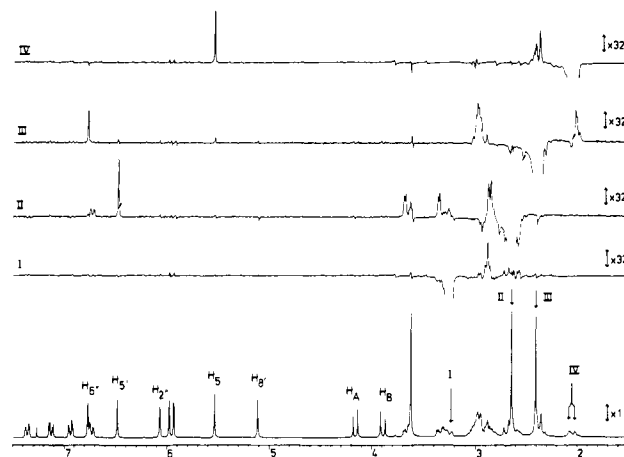


Figure 1. Normal (lower) and NOE difference (upper) spectra of repanduline in CDCl_3 . Preirradiation sites (I, II, III, and IV) are referenced in the text.

H_F (δ 2.68) through large SPT effects. A simultaneous enhancement of the singlet at δ 2.65 suggested that this be assigned to the *N*₂-methyl group. Assignments for $\text{H}_{4\text{eq}}$ (δ 1.95–2.15) and $\text{H}_{4'\text{eq}}$ (δ 2.5–2.7) were made on the basis of the expected enhancements of these protons caused by preirradiation of H_5 and H_5' , respectively. The latter experiment also revealed a weak enhancement of $\text{H}_{4'\text{ax}}$ (δ 2.8–3.0).

To make the remaining assignments, more NOE difference experiments were required, and the results of these appear in Figure 1. The preirradiation frequencies used are indicated in the normal spectrum; since three of these are in regions of spectral overlap, it is important to consider how many protons are likely to be saturated by each preirradiation. Integration of the normal spectrum clearly shows that there are two protons resonating in the region δ 3.2–3.4, six in the region δ 2.8–3.1, two each in the regions δ 2.55–2.75 and 2.3–2.5 (excluding the two *N*-methyl singlets), and one at δ 1.95–2.15. Thus preirradiation at site I corresponds to a single proton partially overlapped with H_E , site II to an *N*-methyl group overlapped only with H_F and $\text{H}_{4'\text{eq}}$, site III to an *N*-methyl group overlapped with two protons, and site IV to $\text{H}_{4\text{eq}}$ only.

Preirradiation of $\text{H}_{4\text{eq}}$ (site IV) led only to one major enhancement, that of $\text{H}_{4\text{ax}}$ (δ 2.3–2.5). The assignments for $\text{H}_{4\text{ax}}$ and $\text{H}_{4'\text{ax}}$ and also the relative assignments of the *N*-methyl singlets were confirmed by experiments II and III. Preirradiation at site II caused the expected strong enhancement of $\text{H}_{4'\text{ax}}$ (saturation of $\text{H}_{4'\text{eq}}$) together with others of H_6''' (saturation of H_F), H_5' (saturation of $\text{H}_{4'\text{eq}}$), H_1 , and H_E (saturation of the *N*₂-methyl group and H_F) and SPT effects. Preirradiation at site III caused the expected enhancement of $\text{H}_{4\text{eq}}$ among others.

A coupling from $\text{H}_{4\text{eq}}$ to a multiplet at δ 2.9–3.05 was revealed by the SPT effect observed in experiment IV and was confirmed by difference decoupling. Since $\text{H}_{4\text{ax}}$ had already been located, this multiplet was assigned to one of $\text{H}_{3\text{eq}}$ or $\text{H}_{3\text{ax}}$. The multiplet corresponding to site I was assigned to one of $\text{H}_{3'\text{eq}}$ or $\text{H}_{3'\text{ax}}$ since (i) it was too complex (eight lines) to form part of a three-spin system and (ii) preirradiation of the multiplet caused strong enhancement of another multiplet at δ 2.85–3.0 (presumably the geminal partner), which was *not* that previously shown to be coupled to $\text{H}_{4\text{eq}}$.

Assignments were now lacking only for H_1 , H_C , H_D , and one of $\text{H}_{3\text{ax}}$ or $\text{H}_{3\text{eq}}$. The integral data discussed earlier, in conjunction with those assignments already made, showed that one of these resonances was overlapped with $\text{H}_{4\text{ax}}$ and the *N*₂-methyl group at δ 2.4–2.6, while the other three were virtually coincident near δ 3. This coincidence, and the still unknown relative stereochemistry at C_1 and C_8 , precluded any further assignments using the existing data. A further experiment, however, avoided these difficulties by using [²H₆]benzene to induce differential solvent shifts, which removed the coincidences. The stereochemistry and the remaining assignments were all deduced from this experiment,

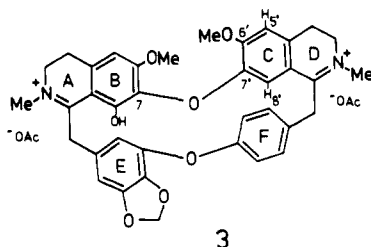
(10) Data from two separate preirradiations were summed in this case (as described for the long-range experiment), so as to minimize SPT effects.

which is discussed in the section on stereochemistry and conformation.

Long-Range NOE Difference Spectroscopy

One of the principal aims of the present work was to establish the substitution pattern in rings B and C of repanduline. In particular, it was hoped to distinguish between the two most likely regioisomers, **1** and **2**, which differ only in the relative positions of the spiro link and the methylene group in ring G. A choice of **1** was made on purely biogenetic grounds in the original papers.³

A possible approach to this problem was suggested by recent work on the closely related alkaloid, dihydrodaphnine diacetate (**3**),¹¹ in which the position of the ring C methoxy group was



established by measuring its enhancements on separate preirradiation of $H_{5'}$ and $H_{8'}$. While the relative proximity of $H_{5'}$ and the protons of the 6'-methoxy group of daphnine made this experiment relatively simple, conformational constraints imposed by the presence of ring G obviously make the analogous experiment for repanduline much more difficult. Examination of models of structures **1** and **2** clearly showed that H_A was always significantly closer to one of the aromatic protons of ring C than to the other, but that even the shorter distance was in excess of 4 Å. Nonetheless, it was hoped that preirradiation of H_A might cause a detectable enhancement of either $H_{5'}$ or $H_{8'}$ and hence show which was the closer to the *O*-methylene group.

The result of this crucial experiment is shown in Figure 2; there is a clear enhancement of $H_{5'}$ (0.18%) but essentially none of $H_{8'}$ (<0.04%). This very strongly suggests that $H_{5'}$ is the closer to H_A , and therefore that **1**, rather than **2**, is the correct structure of repanduline. To be completely sure of this, however, it is necessary to rule out the possibility that structure **2** could give rise to the observed enhancements. For it to do so, $H_{5'}$ would have to receive its 0.18% enhancement from H_A across 6 Å, while $H_{8'}$, which is only 4.5 Å from H_A in structure **2**, would have to be massively relaxed by other pathways to avoid showing a similar (or larger) enhancement itself. Such a drastic difference in relaxation behavior between $H_{5'}$ and $H_{8'}$ is completely unreasonable.¹²

Other peaks in the difference spectrum are enhancements of H_5 (0.42%) and H_B (14%, shown truncated) and a three-spin effect at δ 2.97 (due to H_1 ; see section on stereochemistry and conformation). Enhancements of H_A on preirradiation of either $H_{5'}$ or $H_{8'}$ were undetectably small, presumably due to efficient relaxation of H_A by H_B . When a degassed solution of repanduline was used, the enhancement of $H_{5'}$ on preirradiation of H_A , measured identically, increased to 0.26%, so providing independent evidence that the observed effect was a genuine NOE.

(11) Neuhaus, D.; Rzepa, H. S.; Sheppard, R. N.; Bick, I. R. C. *Tetrahedron Lett.* **1981**, 2933.

(12) Further confirmation was provided by calculating the approximate enhancements expected for structure **1**, assuming the conformation shown in Figure 4. Distances measured directly on the Dreiding model were used in the following formula, due to Noggle and Shirmer,³ for the fractional enhancement, $f_d(s)$, expected for a proton d on saturation of a proton s:

$$f_d(s) = \frac{1}{2} \frac{\rho_{ds}}{R_d} - \frac{1}{2} \sum_n \frac{\rho_{dn}f_n(s)}{R_d}$$

where ρ_{ds} is the direct dipole-dipole relaxation between d and s, proportional to $(r_{ds})^{-6}$, R_d is the total direct relaxation rate of d, proportional to $(r_{ds})^{-6} + \sum_n (r_{dn})^{-6}$, and \sum_n represents a summation over all protons other than d and s. The calculated results are in reasonable agreement with the experiment (allowing for the effects of partial saturation and dissolved oxygen): $f_B(A) = +0.45$ (calcd), +0.14 (exptl); $f_{5'}(A) = +0.0047$ (calcd), +0.0018 (exptl); $f_{8'}(A) = -0.0001$ (calcd), <0.0004 (exptl).

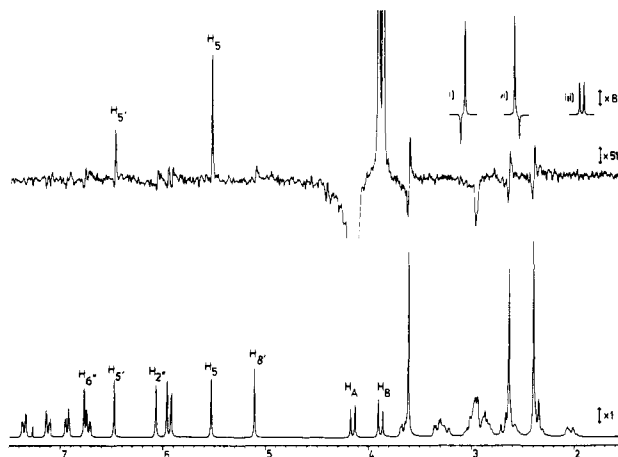


Figure 2. Long-range NOE difference experiment in which H_A of repanduline was preirradiated. The insets show the pattern of H_B on preirradiation of (i) the downfield component of H_A and (ii) the upfield component of H_A , while (iii) shows the sum of (i) and (ii). The solvent was $CDCl_3$.

The possibility that the enhancement of $H_{5'}$ was a transmitted effect was also considered. Since the enhancement is positive it cannot be a three-spin effect, but the four-spin pathway $H_A \rightarrow H_B (+14\%) \rightarrow H_1 (\sim -2\%) \rightarrow H_{5'} (+0.18\%)$ at first appears consistent with the data in Figure 2. This interpretation, aside from the structural improbability of H_1 and $H_{5'}$ being close enough for appreciable mutual cross-relaxation, was, however, ruled out by the observation that preirradiation of H_B again caused a positive enhancement of $H_{5'}$, rather than the negative enhancement (three-spin effect) that the above pathway would require.

The very small size of the enhancement of $H_{5'}$ on preirradiation of H_A calls for some comment since, as far as we are aware, there is no precedent for using such small enhancements in structural elucidation. The principal requirements for detection of any NOE enhancement by difference spectroscopy are that unenhanced peaks be nulled to a sufficiently low level and that the residual signal due to the NOE be clear of the base-line noise. It is clear from Figure 2 that these conditions were both met in the present experiment. Nulling was effective to within $\pm 0.04\%$ at worst, and the signal to base-line noise ratio was about 5:1 for $H_{5'}$. This was achieved by prolonged accumulation; a total of 32 000 transients was collected over one weekend (60 h).¹³ For comparison, some other experiments in this paper used only 500–2000 transients from each preirradiation site and yielded a detection limit of ~ 0.2 –0.1% for most protons.

Satisfactory nulling of unenhanced signals is clearly a vital consideration in NOE difference experiments, particularly since, unlike signal to noise ratio, nulling is not improved on raising the sample concentration. As we have shown, it is most important that a sufficiently large number of transients be collected. Prolonged repetition of the experimental cycle always progressively improves the nulling quality, although the rate at which it does so depends in some complex way on the relative values of the carrier and decoupler frequencies. Individual difference spectra from an automated multiple experiment thus commonly show significantly different degrees of nulling, despite the fact that all the data are effectively averaged over the same period. This observation, together with the characteristic dispersion-like shape of the signals that result from incomplete nulling, suggests that its main cause is instrumental frequency instability.¹⁴ Other factors that we find to be influential are use of a sufficiently low preirradiation power¹⁵ and an adequate delay between preirradiation and the observe pulse.⁶

(13) Four files, each containing 8000 transients, were summed to yield the difference spectrum in Figure 2.

(14) "Aspect 2000 Software Manual 1981"; Bruker Spectrospin: Part 6, p 24. Sanders, J. K. M.; Mersh, J. D. *J. Magn. Reson.* **1982**, *50*, 289.

(15) The decoupler attenuation was set at 30L (HD mode). The actual decoupler power output was not calibrated.

Table III. (¹H, ¹³C) Long-Range Couplings Involving C₆' and C₇'

$ J $ (Hz)	H ₅ '	H ₈ '	H _A	H _B
C ₆ ' (δ 140.2)	3.7	6.7	6.7	
C ₇ ' (δ 139.6)	7.5	4.3		

Another feature of this experiment that requires comment is the method used to preirradiate H_A. When the decoupler was placed centrally between the two components of the H_A doublet, high power levels were required to produce adequate saturation. This was unsatisfactory since it caused poor nulling and, more significantly, an unwanted enhancement of H₈' via slight saturation of H₁'. When the decoupler was placed on one of the components of the H_A doublet, however, lower power levels were sufficient to saturate that component, and both problems were avoided.¹⁵ Although H_A was only approximately 50% saturated, the resulting partially excited enhancements could still be detected more clearly than in the higher power experiments.

In order to suppress the very large SPT effect at H_B caused by the unsymmetrical partial saturation of H_A, two such difference spectra were accumulated, in each of which a different component of the H_A doublet was preirradiated. The asymmetry of the saturation (and hence of the SPT) was approximately equal and opposite between these spectra, so that addition of the two during data processing resulted in cancellation of the asymmetry, giving a combined spectrum in which the SPT effect was suppressed (Figure 2).¹⁶

Long-Range ¹³C{¹H} Specific Decoupling

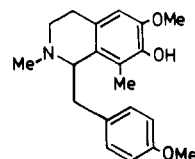
The long-range NOE experiment described in the previous section establishes quite definitely that **1**, not **2**, is the correct substitution pattern for ring C of repanduline. Nonetheless, since an important objective of the experiment was to set a precedent for the use of very small enhancements in structural work, we felt an independent proof of this conclusion was still desirable. This was obtained from ¹³C{¹H} specific decoupling experiments.

Although specific decoupling of ¹³C from directly bound protons is commonplace, relatively little use has been made of specific long-range ¹³C{¹H} decoupling;¹⁷ this is principally due to the difficulty of interpreting fully coupled ¹³C spectra, which usually requires computer analysis.¹⁸ For carbon atoms weakly coupled only to well-separated protons, however, the relevant ¹³C satellites in the ¹H spectrum are first order, and a simple interpretation based on line separations in the carbon spectrum is appropriate. These conditions are met by C₆', C₇', and C₇ of repanduline, which couple only with H₅', H₈', H_A, H_B, and H₅. In order to assign the couplings involving these atoms, ¹³C spectra were accumulated in which H₅', H₅, and H₈' were separately decoupled at very low power. The results of these experiments are summarized in Table III.

Consideration of model compounds¹⁹ [6,7-dimethoxy-1-(4-methoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline and 1-(4-methoxybenzyl)-2-methyl-6,7-(methylenedioxy)-1,2,3,4-tetrahydroisoquinoline], multiplicities, and the changes caused by decoupling H₅', H₅, and H₈' led straightforwardly to identification of the multiplets due to C₆', C₇', and C₇.²⁰ The relative assignment of C₆' and C₇' was made by comparing the couplings involving these atoms and H₅' and H₈'. It is known that three-bond couplings, ³J(C,H), are generally larger than two-bond couplings, ²J(C,H), in substituted benzenes.¹⁸ The multiplet which loses the larger coupling on irradiation of H₅' is thus due to C₇', and that which loses the larger coupling on irradiation of H₈' to C₆'.²¹ The

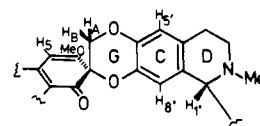
remaining coupling from C₆' is to H_A, and for this to be as large as 6.7 Hz, it must follow a three-bond rather than a four-bond path. The conclusion from this must again be that **1**, not **2**, is the correct structure of repanduline.

No other specific ambiguities of regioisomerism were left open by the original chemical and spectroscopic work on repanduline.³ It is interesting to note, however, that the entire substitution pattern can be independently deduced from the ¹H NOE difference spectra presented in this paper, assuming only the presence of two tetrahydrobenzylisoquinoline skeletons. Without describing this process in detail one point may be mentioned, namely the relative positions of the spiro link and the carbonyl group in ring B. This problem was originally resolved by chemical degradation; treatment of repanduline with potassium in liquid ammonia produced hemirepanduline (**4**), the structure of which was confirmed by

**4**

synthesis.²² In view of the recent discovery of an unprecedented 7-7' ether link in the very closely related alkaloid daphnine (**3**),²³ however, we wished to obtain direct physical evidence from repanduline itself.

A further ¹³C experiment in which H₅ was selectively decoupled provided this. The relatively large coupling ($|J| = 8.5$ Hz) found between H₅ and the carbonyl carbon (δ 194.1) must follow a three-bond path,¹⁸ thus discounting structures such as **5**. Also,

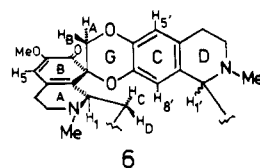
**5**

models⁷ clearly showed that only in structures having the spiro link at C₈ could H₂' and H₈' or H_B and H₁ be sufficiently close to explain the strong ¹H NOE enhancements observed between them.

Stereochemistry and Conformation

With the regioisomerism of repanduline fully defined, the remaining structural possibilities now comprised only the four diastereomers differing in relative configurations at C₁, C₈ and C₁'. Clearly, the stereochemistry at C₁' is the least accessible to investigation by NMR, being relatively remote from the other chiral centers. We therefore first tackled the problem of the relative configuration of C₁ and C₈.

A distinction between diastereomers **1** and **6** requires mainly

**6**

a knowledge of the spatial relationships of H₁, H_C, and H_D with their neighbors H_B, H₂', and H₆'. In the original experiments, preirradiation of H_B, H₂', and H₆' all caused enhancements at virtually the same shift; these coincidences prevented assignment of the enhanced multiplets, and no structural conclusions could be drawn. When the experiment was repeated after addition of [²H₆]benzene (20%) to the solution, however, the coincidences were removed (Figure 3) and assignments became obvious.

(16) See also: Neuhaus, D.; *J. Magn. Reson.* **1983**, *53*, 109.

(17) Fujimoto, Y.; Tsunoda, H.; Uzawa, J.; Tatsuno, T. *J. Chem. Soc., Chem. Commun.* **1982**, 83 and references therein.

(18) Hansen, P. E. *Prog. Nucl. Magn. Reson. Spectros.* **1981**, *14*, 175, and references therein.

(19) Marsaioli, A. J.; Ruveda, E. M.; de A.M. Reis, F. *Phytochemistry*, **1978**, *17*, 1655.

(20) A complete assignment of the ¹³C spectrum of repanduline is in preparation (F. de A.M. Reis, unpublished work).

(21) Although (¹³C, ¹H) couplings of 4.3 and 3.7 Hz are not so small as to be inconsistent with a three-bond coupling path, values of 6.7 and 7.5 Hz are too large to be consistent with a two-bond coupling path.

(22) Aoki, K.; Harley-Mason, J. *J. Chem. Soc. C* **1967**, 1957.

(23) Guilhem, J.; Bick, I. R. C. *J. Chem. Soc., Chem. Commun.* **1981**, 1007.

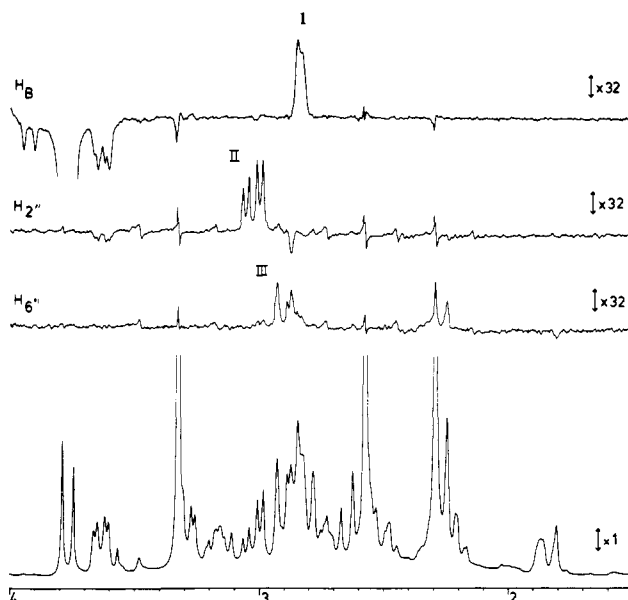


Figure 3. Normal (lower) and NOE difference (upper) spectra of repanduline in CDCl_3 containing $[\text{2H}_6]$ benzene (20%). Preirradiation sites were $\text{H}_{6''}$, $\text{H}_{2''}$, and H_B .

Multiplet II, which is enhanced by $\text{H}_{2''}$ and possesses a geminal coupling ($|J| = 14$ Hz) to multiplet III, must be due to H_C . Multiplet III, by similar reasoning, must be due to H_D . Multiplet I, since it is the only proton within the three-spin system to lack a geminal coupling, can only correspond to H_1 , and its enhancement on preirradiation of H_B necessarily implies that diastereomer 1 is the correct one. Preirradiation of $\text{H}_{6''}$ also caused enhancements of the N_2 methyl group and $\text{H}_{4\text{ax}}$.

The more difficult problem of the stereochemistry at C_1 could not be so directly solved. Diastereomers 1 and 7 would only be expected to show relatively subtle differences in NMR properties arising from their different overall conformations. Distinguishing between them was thus unavoidably linked with the final part of this work, elucidation of the solution conformation of repanduline. Our strategy was to use the large amount of qualitative data already obtained to specify as many individual details of the conformation as possible, and then to incorporate these into a model of the whole molecule.⁷ In the event, sufficient detail was available not only to establish almost completely the conformation of the macro ring but also to rule out diastereomer 7, since it could not simultaneously accommodate all the conformational requirements described below.

The result of this strategy was the proposed conformation shown in Figure 4. The evidence on which this proposal is based is given below, describing each flexible portion of the macro ring in turn.

(1) **Ring G** is evidently fairly rigid, with H_A pseudo-equatorial and H_B pseudo-axial. This may be deduced from the following: (i) H_A gives an enhancement to H_5 , but not to H_8 , (ii) preirradiation of H_B enhances H_5 and H_8 to roughly equal extents, (iii) H_A gives a modest enhancement to H_5 (0.42%), while H_B gives none, and (iv) the proton-carbon coupling data suggest that C_6 is anti-periplanar with H_A while C_7 is anti-periplanar with H_B .²⁴ If ring G were not rigid, H_B would spend some time in a pseudo-equatorial position and H_A in a pseudo-axial one, so that these clear distinctions would be lost.

(2) **Ring B** spends most time in the conformation shown, in which the H_5 -C bond is roughly parallel to the H_A -C bond ($r_{\text{H}_5\text{H}_A} \approx 3.8$ Å) and the carbonyl oxygen is "down" relative to the ring G methylene group. The only alternative arrangement for ring B suggested by the model would be obtained by "depressing" C_{4a} , C_5 , and C_6 until the H_5 -C bond is roughly perpendicular to the H_A -C bond, with the carbonyl oxygen now "up" relative to the

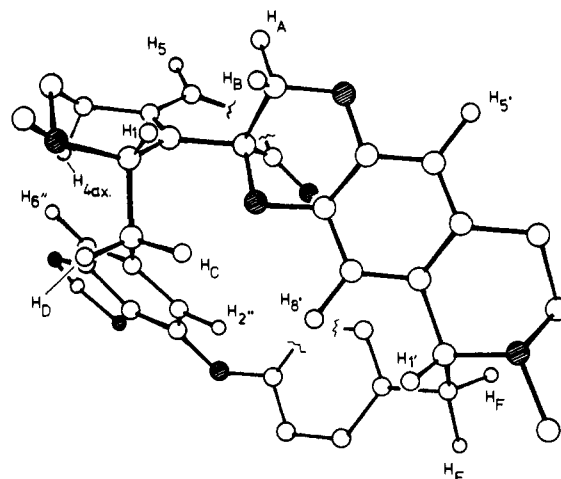


Figure 4. Proposed solution conformation of repanduline. This drawing was traced from a photograph of a Dreiding model (see text). The enantiomer shown was chosen only because it most resembles the conventional planar representation of repanduline.³ No assignment of absolute configuration was made. Atoms C_6 , $\text{C}_{5''}$, and the methoxy group are omitted for clarity; selected protons are shown.

ring G methylene group. In this conformation $r_{\text{H}_5\text{H}_A}$ would be ~ 5.8 Å, which is clearly inconsistent with the observed enhancement (0.42%) of H_5 on preirradiation of H_A .

(3) **Rings A and E and the C_α Link.** The positions of H_1 , H_C , and H_D may be specified in some detail. H_1 must be relatively close to H_B but distant from $\text{H}_{2''}$ and $\text{H}_{6''}$, while H_C and H_D are remote from H_B but relatively close to $\text{H}_{2''}$ and $\text{H}_{6''}$, respectively (Figures 3 and 4). Most significantly, the couplings involved reveal that the torsion angle ($\text{H}_1, \text{C}_1, \text{C}_\alpha, \text{H}_D$) must be close to 90° ($J_{\text{H}_1\text{H}_D} \approx 0$ Hz), while the angle ($\text{H}_1, \text{C}_1, \text{C}_\alpha, \text{H}_C$) is close to 30° ($J_{\text{H}_1\text{H}_C} \approx 5$ Hz). The position of ring E is further evidenced by the large enhancement of $\text{H}_{2''}$ on preirradiation of H_8 (and vice versa). Our proposed conformation accounts for these data and is also consistent with the observed enhancement of the N_2 -methyl group and $\text{H}_{4\text{ax}}$ on preirradiation of $\text{H}_{6''}$.

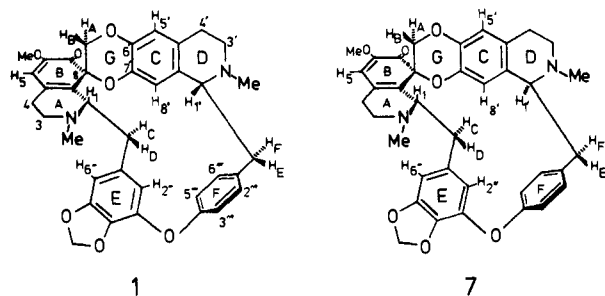
(4) **Rings C, D, and F and the C_α Link.** Several observations lead to the conclusion that $\text{H}_{1'}$ is pseudo-equatorial: (i) preirradiation of H_8 strongly enhances $\text{H}_{1'}$ but does not affect either H_E or H_F , (ii) preirradiation of $\text{H}_{1'}$ does not affect $\text{H}_{3'\text{ax}}$, and (iii) preirradiation of either $\text{H}_{1'}$ or H_E causes strong enhancement of $\text{H}_{2''}$. The large coupling between $\text{H}_{1'}$ and H_F ($J = 12$ Hz) suggests that these protons spend most time in an antiperiplanar arrangement, while the chemical shift of H_F (δ 2.68 as opposed to δ 3.33 for H_E) suggests this proton is shielded by ring C. The proposed conformation is also consistent with the unexpectedly high shifts of H_8 (strongly shielded by ring F), $\text{H}_{2''}$ (shielded by rings C and F), $\text{H}_{5''}$ and $\text{H}_{6''}$ (shielded by ring C), and perhaps H_5 (slightly shielded by ring E).²⁵

Exhaustive examination of a molecular model⁷ showed that imposition of all these details specified the overall conformation of repanduline quite closely. The most demanding constraint was that the torsion angle ($\text{H}_1, \text{C}_1, \text{C}_\alpha, \text{H}_D$) be close to 90° ; in practice, this meant that the plane of ring E had to be "bent back" towards that of rings A and B, as in Figure 4. When the relative positions of H_A and H_5 were also constrained as described, this left very little option for the remainder of the molecule. In particular, these two requirements could not be simultaneously met by diastereomer 7.

In conjunction, the model and the enhancement data suggested that there is not much flexibility in the repanduline molecule. Apart from the (presumably) mobile methoxy group, the only significant conformational motions seemed to be a restricted ro-

(24) A Karplus dependence of $^3J(\text{C},\text{H})$ on the dihedral angle has been established.¹⁸

(25) Additivity rules predict the following shifts (assuming all alkyl linkages equivalent to Me and all oxygen linkages equivalent to OMe): H_8 , δ 6.49; $\text{H}_{2''}$, δ 6.21; $\text{H}_{6''}$, δ 6.75; $\text{H}_{6''}$, δ 7.01; H_5 , δ 6.43. Data taken from: Jackman, L. M.; Sternhell, S. "Applications of N.M.R. Spectroscopy in Organic Chemistry", 2nd ed.; Pergamon Press: Elmsford, NY, 1969.



tation of ring F about its $C_{1'''}-C_{4''}$ axis and a "flexing" of ring E involving mainly movement about the $C_{\alpha}-C_{1''}$ and $C_{3''}-O$ bonds, with some concomitant movement of ring F and the C_{α} link.

Finally, it must be emphasized that Figure 4 is merely a representation of one conformation of repanduline that fits the available evidence. It is in no way intended to be a map of the actual average atomic positions, but may instead be taken to represent one point in the "conformational space" occupied by the real molecule. Strictly, the results apply only to the conformation in $CDCl_3$ containing $[^2H_6]$ benzene (20%), since the solvent shifts imparted by the benzene may be due in part to slight conformational changes (critical experiments originally run in $CDCl_3$ alone were repeated in the mixed solvent where necessary). Nonetheless, the similarity of the enhancement data obtained in the two solvent systems suggests that the proposed conformation is valid in $CDCl_3$ also, at least within the level of approximation of our qualitative interpretation.

Conclusion

We believe these experiments show that the potential of NOE difference spectroscopy is still largely unrealized. In each of the traditional areas of application of interproton NOE measurements, problems that would have been completely beyond the scope of older methods succumbed to the difference technique. We were able to measure reproducibly enhancements near the 0.1% level and to show that they provide reliable structural information. Enhancements induced within regions of spectral overlap revealed vital and otherwise inaccessible spectral details. At the end of the study the regioisomerism and relative stereochemistry of repanduline had been established and a detailed picture of the molecule had emerged (the only major omission being its absolute chirality).

Although repanduline is in some respects particularly suited to this type of study, we feel that such an approach would be applicable to many other molecules. The information gained may not be as direct or accurate as that from X-ray crystallography, but it is nonetheless comparable in many respects and has the considerable advantage that it is obtained from solution.

Experimental Section

Repanduline³ was purified by six recrystallizations from methanol and was dissolved in $CDCl_3$ containing Me_4Si as internal standard. For some

experiments, $[^2H_6]$ benzene (20%) was also added. A moderately concentrated solution (0.26 M) was used in a high-quality 5-mm o.d. tube. On one occasion only, the sample was degassed by using a repeated freeze-pump-thaw cycle and then closed under nitrogen (subseal). Spectra were obtained at 250 MHz (1H) and 62.9 MHz (^{13}C), without temperature regulation, on a Bruker WM250 spectrometer operating under ASPECT 2000 control. For the 1H experiments a spectral width of 3 kHz was used with 8K data points (for convenience in data processing), resulting in an acquisition time of 1.36 s and a digital resolution of 0.73 Hz per point.²⁶ Single-phase detection was employed with phase cycling.

NOE difference experiments were run automatically by using a microprogram based on the method of Hall and Sanders.² This works by repetition of the basic sequence preirradiation¹⁵ (5 s), delay (50 ms), pulse, and acquisition of one transient. When four transients had been so collected using one preirradiation frequency, the summed data were stored, the preirradiation frequency was changed, and four transients were collected using the new frequency. In this way the preirradiation frequency was cycled through all the required positions, always adding the four new transients to the data accumulated using the same preirradiation frequency on previous passes around the cycle. An off-resonance preirradiation frequency was included to provide the control spectrum. After N passes around the full cycle, each data file (corresponding to one particular preirradiation frequency) contained $4N$ transients; for large N , long-term variations in temperature, field, etc., affected each file identically. Typically, N was set to reach 100–500, but in the long-range experiments a value of 2000 was used (see text).¹³

After the experiment, difference spectra were generated by subtracting the control file from each individual preirradiation file in turn. Data were subtracted in the form of FID's and the resulting difference spectra phased identically by using constants derived for the control spectrum. No line broadening was used.

The $^{13}C\{^1H\}$ specific decoupling experiments were run with low-power single-frequency irradiation during acquisition and high-power noise irradiation during the relaxation delay (2s).²⁷ In this way, some of the extra sensitivity due to the $^{13}C\{^1H\}$ NOE was retained. A sweep width of 15.15 kHz was used with 32K data points, giving an acquisition time of 1.08 s and a digital resolution of 0.92 Hz per point. Quadrature detection was employed with phase cycling.

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Registry No. Repanduline, 20398-02-1.

(26) For the long-range experiment, a sweepwidth of 4.5 kHz was used, giving an acquisition time of 0.91 s and a digital resolution of 1.1 Hz per point.

(27) The decoupler attenuation was set at 28 H (single frequency) and 4 H (noise). The actual decoupler power output was not calibrated.

(28) **Note Added in Proof:** Since the completion of this work, a single crystal X-ray structure of repanduline has become available (I. R. C. Bick, D. Neuhaus, and D. J. Williams, to be published). This revealed a conformation essentially identical with that shown in Figure 4, but with the opposite absolute stereochemistry.