Tritium Nuclear Magnetic Resonance Spectroscopy of [*Pyrrolidine*-^{3}H]Bepridil

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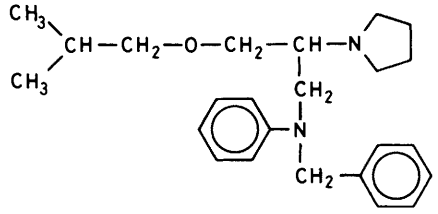
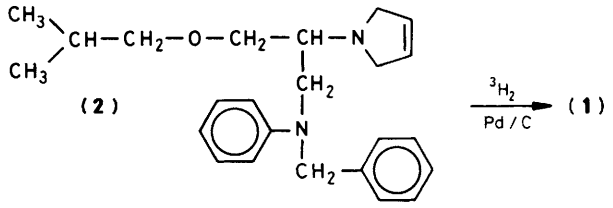
Bepridil [*N*-benzyl-3-isobutoxy-*N*-phenyl-2-(pyrrolidin-1-yl)propylamine] tritiated in the pyrrolidine ring by reduction of the 2,5-dihydropyrrol-1-yl analogue with tritium shows a complicated ^{1}H-decoupled ^{3}H n.m.r. spectrum. With the aid of isotopic fractionation by h.p.l.c. this spectrum could be interpreted as a sum of the spectra of various mono-, di-, and tri-tritiated diastereoisomers. In the case of the (+)-(*R*)-isomer of bepridil the optical purity could be determined by ^{3}H n.m.r. in the presence of Pirkle's alcohol.

Tritium n.m.r. is the method of choice for the determination of the position and distribution of the tritium in labelled compounds.[1,2] The ^{1}H decoupling which is usually applied simplifies the ^{3}H signals to singlets; for vicinal or geminal ditritiated compounds doublets are obtained which usually appear 0.05—0.1 p.p.m. upfield of the corresponding signals for the monotritiated compounds because of the isotope effect.[3]

Bepridil [*N*-benzyl-3-isobutoxy-*N*-phenyl-2-(pyrrolidin-1-yl)propylamine] (**1a**) as its monohydrochloride monohydrate is a new anti-anginal drug with calcium antagonistic properties.[4] For biological and metabolical studies we synthesized this compound and (+)-(*R*)-bepridil (**1b**) tritiated in the pyrrolidine ring. The ^{3}H n.m.r. spectra obtained for these compounds show very complex multiplets. The analysis of these spectra is described in this paper.



(**1**) a; racemate
b; (+)-(*R*)-isomer



a; racemate
b; (+)-(*R*)-isomer

Experimental

N.m.r. spectra (^{1}H, ^{2}H, and ^{3}H) were recorded with a Bruker AM360 spectrometer operating at 360.13, 55.28, and 384.13 MHz, respectively. The ^{1}H n.m.r. spectra in C^2HCl_3 or C^2HCl_3 containing 20% (by volume) Pirkle's alcohol were referred to internal Me_4Si; multiplication of the ^{1}H frequency thus obtained by 1.066 6397 5 yielded a ghost reference for the ^{3}H spectra.[1] The ^{3}H spectra were recorded with broad-band ^{1}H decoupling. Electron impact mass spectra were measured with a Varian MAT 311-A spectrometer; field desorption mass spectra were recorded as described elsewhere[5] by Dr. W. D. Lehmann, University Hospital Eppendorf, Hamburg, Federal Republic of Germany.

Tritiation reactions were carried out at Amersham International p.l.c., U.K., by their labelling service.

Pirkle's alcohol (L-2,2,2-trifluoro-1-phenylethanol) was obtained from Burdick and Jackson Labs Inc. (USA).

For h.p.l.c. fractionations a column of LiChrosorb (7 μ) was used, with n-hexane–ethanol–propan-2-ol–aq. ammonia (24%) (95:2.5:2.5:0.005 v/v) as mobile phase.

[pyrrolidine-^{3}H]*Bepridil*.—The 2,5-dihydropyrrolyl analogue (**2**) of bepridil (10 mg) was dissolved in benzene (2 ml); Pd–C (10%; 10 mg) was added and the mixture was stirred for 30 min with tritium gas (370 GBq). The catalyst was removed by filtration and washed with benzene (2 ml). The filtrate was concentrated *in vacuo* and labile tritium was removed by dissolving the residue in methanol followed by lyophilization. The product was purified by h.p.l.c. [μBondapak C18 with aqueous 0.01M-ammonium acetate (pH 4.8)–acetonitrile (6:4 v/v) as mobile phase]. The tritiated product was desalted by chromatography over Seppak C18.

[pyrrolidine-^{2}H]*Bepridil*.—This was prepared analogously. The product was purified by preparative t.l.c. (silica gel; ethyl acetate); δ(^{2}H) (C^1HCl_3) 1.73 (m, 90%) and 2.85 (m, 10%); *m/z* (e.i.) 367 (0.2%), 368 (0.7%), and 369 (0.2%).

[pyrrolidine-2,2-$^2H_2$]*Bepridil*.—A mixture of the 2-pyrrolidone analogue of bepridil (300 mg) and $LiAl^2H_4$ (200 mg) in freshly distilled tetrahydrofuran (5 ml) was refluxed under nitrogen for 3 h, then cooled to 0 °C. Ethyl acetate and aq. NaOH were added slowly and the mixture was stirred for 30 min. The precipitate was filtered off and washed with dioxane. The filtrate was evaporated to dryness and the residue was chromatographed over silica gel with $CHCl_3$–methanol (9:1 v/v) as eluant; δ(^{2}H) (C^1HCl_3) 2.72 (m); *m/z* (e.i.) 368 (0.8%) and 369 (0.2%).

Results and Discussion

Synthesis of Tritiated Bepridils.—The [*pyrrolidine*-^{3}H]bepridils were synthesized by reduction of the 2,5-dihydropyrrolyl analogues (**2a**) with $^3H_2$, catalysed by Pd–C; the products were purified by reversed-phase h.p.l.c. Complicated ^{3}H n.m.r. (^{1}H-decoupled) spectra were obtained. The spectrum of (+)-(*R*)-bepridil is shown in Figure 1, with the ^{1}H spectrum of bepridil for comparison. In addition to labelling at positions 3 and 4 of

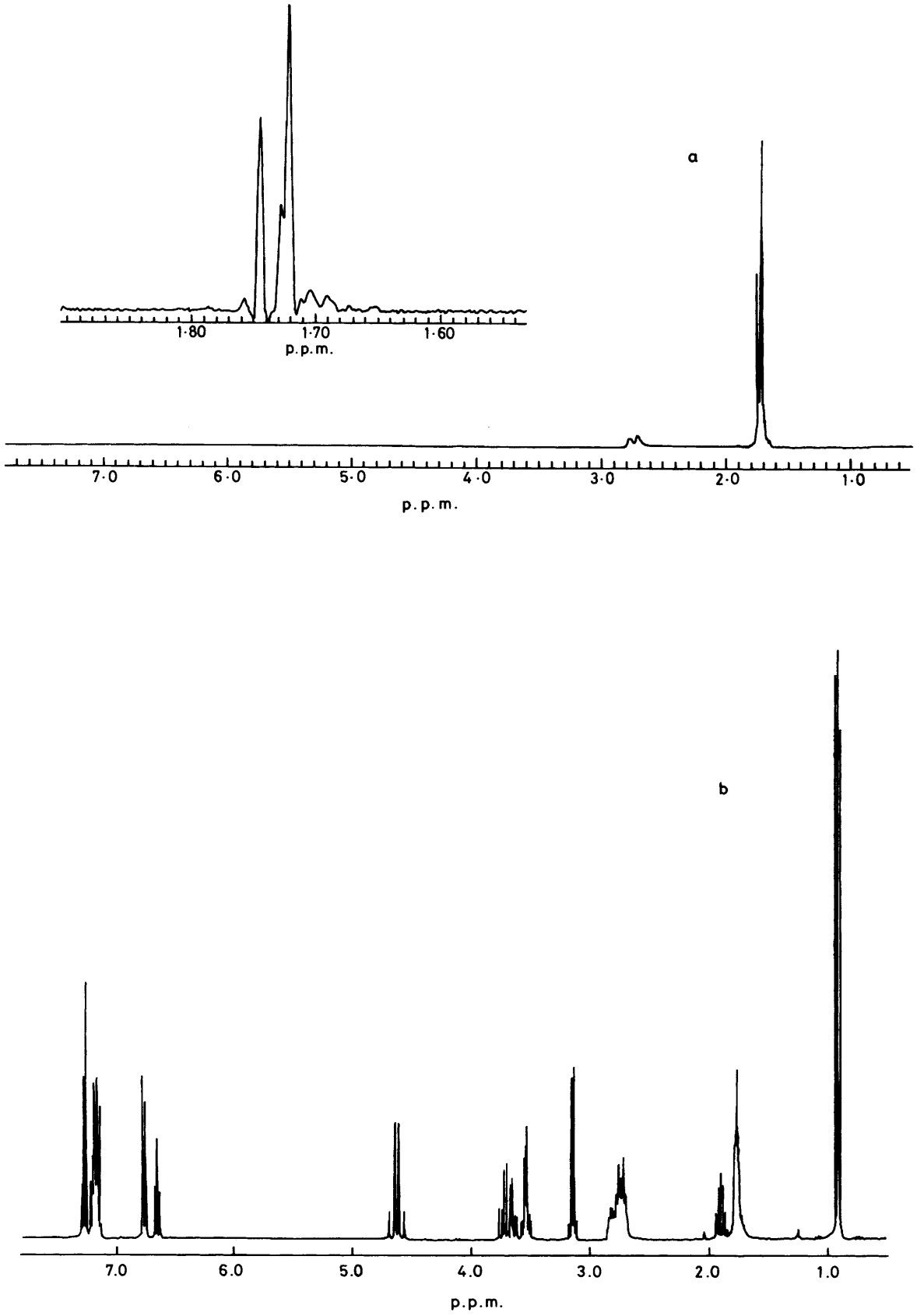


Figure 1. N.m.r. spectra of bepridil: (a) ^{1}H-decoupled ^{3}H spectrum of (+)-[^{3}H]bepridil; upper curve resolution enhanced; (b) ^{1}H spectrum of unlabelled bepridil

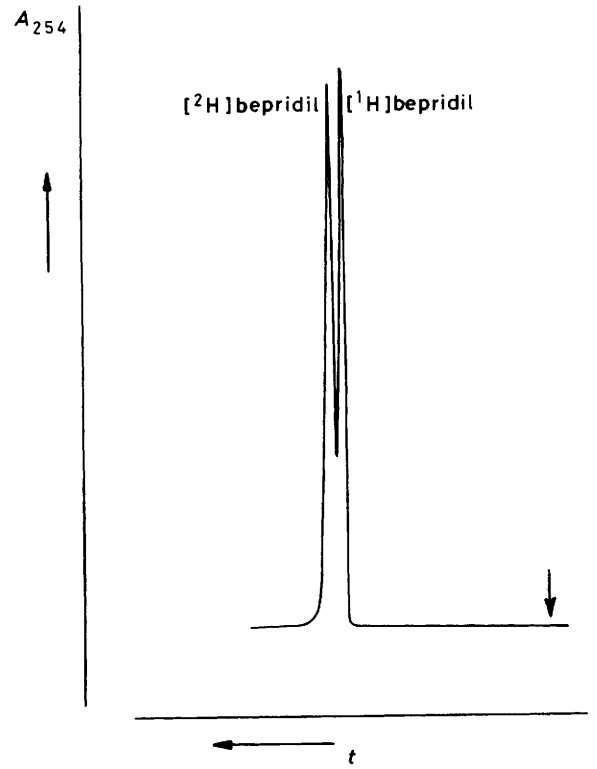


Figure 2. H.p.l.c. of a mixture of bepridil and [*pyrrolidine*-2-$^2H_2$]bepridil column Lichrosorb 7 μ; mobile phase n-hexane–ethanol–propan-2-ol–aq. ammonia; detection λ 254 nm

the pyrrolidine ring (signals around 1.75 p.p.m.), labelling at positions 2 and 5 also occurred (signals around 2.70 p.p.m.) probably through allylic exchange. An unambiguous assignment of the signals is not possible since a mixture of mono-, di-, and tri-tritiated material was present. The ^{3}H spectrum is further complicated by the interaction of the asymmetrically substituted C-2 of the propyl chain with the carbon atoms in the pyrrolidine ring which become asymmetric upon introduction of the tritium (*see later*). However, an interpretation of the complicated spectra was possible by using the following techniques.

(1) Measurement of the relative abundances of mono-, di-, and tri-tritiated material in [^{3}H]bepridils by field desorption mass spectrometry. For [^{3}H]bepridil a ratio of monotritiated to ditritiated to tritritiated materials of 10:10:1 was found, and for (+)-[^{3}H]bepridil a ratio of monotritiated to ditritiated material of about 5:1 was observed.

(2) Fractionation of the tritiated material by h.p.l.c. and measurement of the ^{3}H n.m.r. spectra of the fractions.

Isotopic Fractionation.—It is well established[6–10] that tritiation or deuteriation of aliphatic and alicyclic amines in the vicinity of the nitrogen atom results in enhanced retention on h.p.l.c. By using [*pyrrolidine*-2,2-$^2H_2$]bepridil and [*pyrrolidine*-3,4-^{2}H]bepridil as model compounds, we demonstrated a similar isotopic fractionation for bepridil, at least on straight-phase h.p.l.c.: for [*pyrrolidine*-3,4-^{2}H]bepridil the ^{2}H content increased with increasing retention time, while [*pyrrolidine*-2-$^2H_2$]bepridil could be separated completely from unlabelled bepridil (see Figure 2).

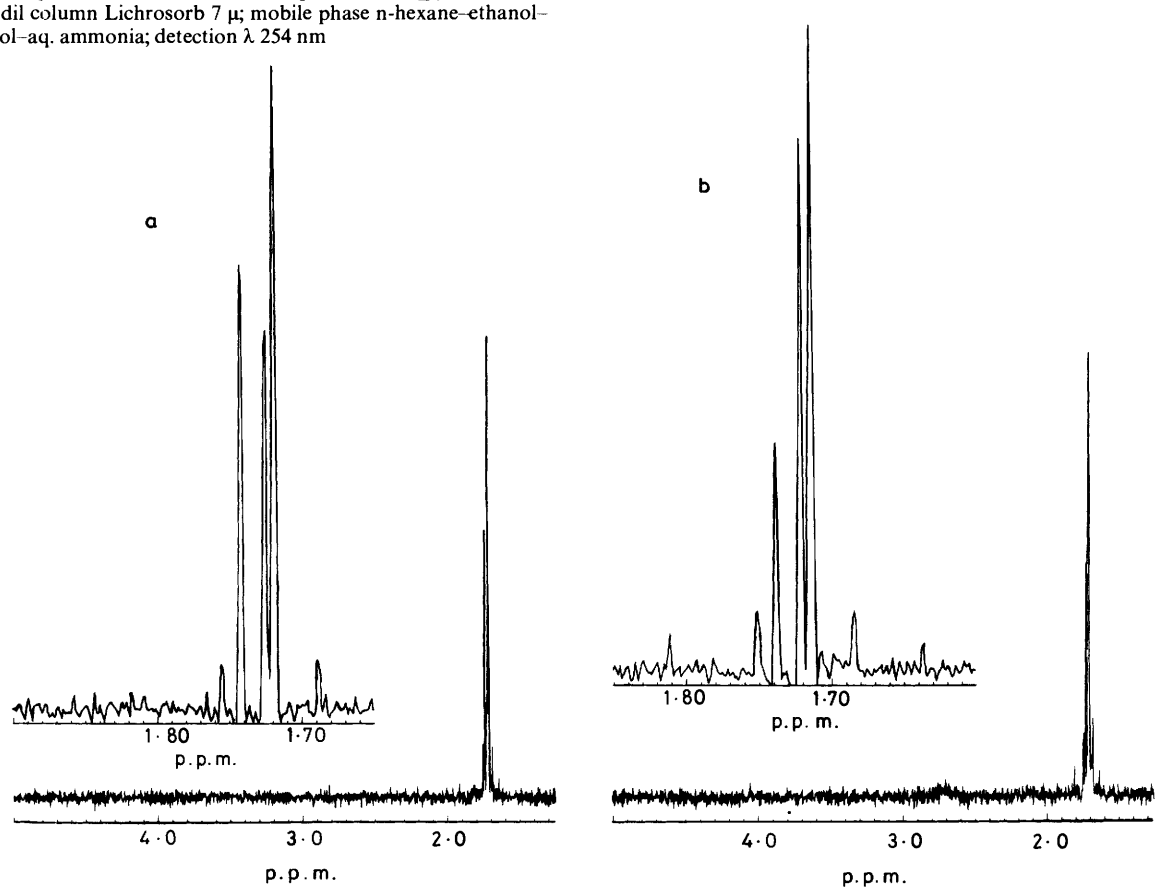


Figure 3. ^{1}H-Decoupled ^{3}H n.m.r. spectra of fractionated bepridil; the upper traces are resolution enhanced. The chemical shifts of the pyrrolidine protons and tritons are very sensitive to the acidity of the solvent. In the ^{1}H-decoupled ^{3}H n.m.r. spectra the temperature of the sample is higher than in the case of ^{1}H n.m.r. measurements, resulting in small differences in chemical shifts. For simplicity we fixed the chemical shift of H-3 in one of the monotritiated species at 1.72 p.p.m.

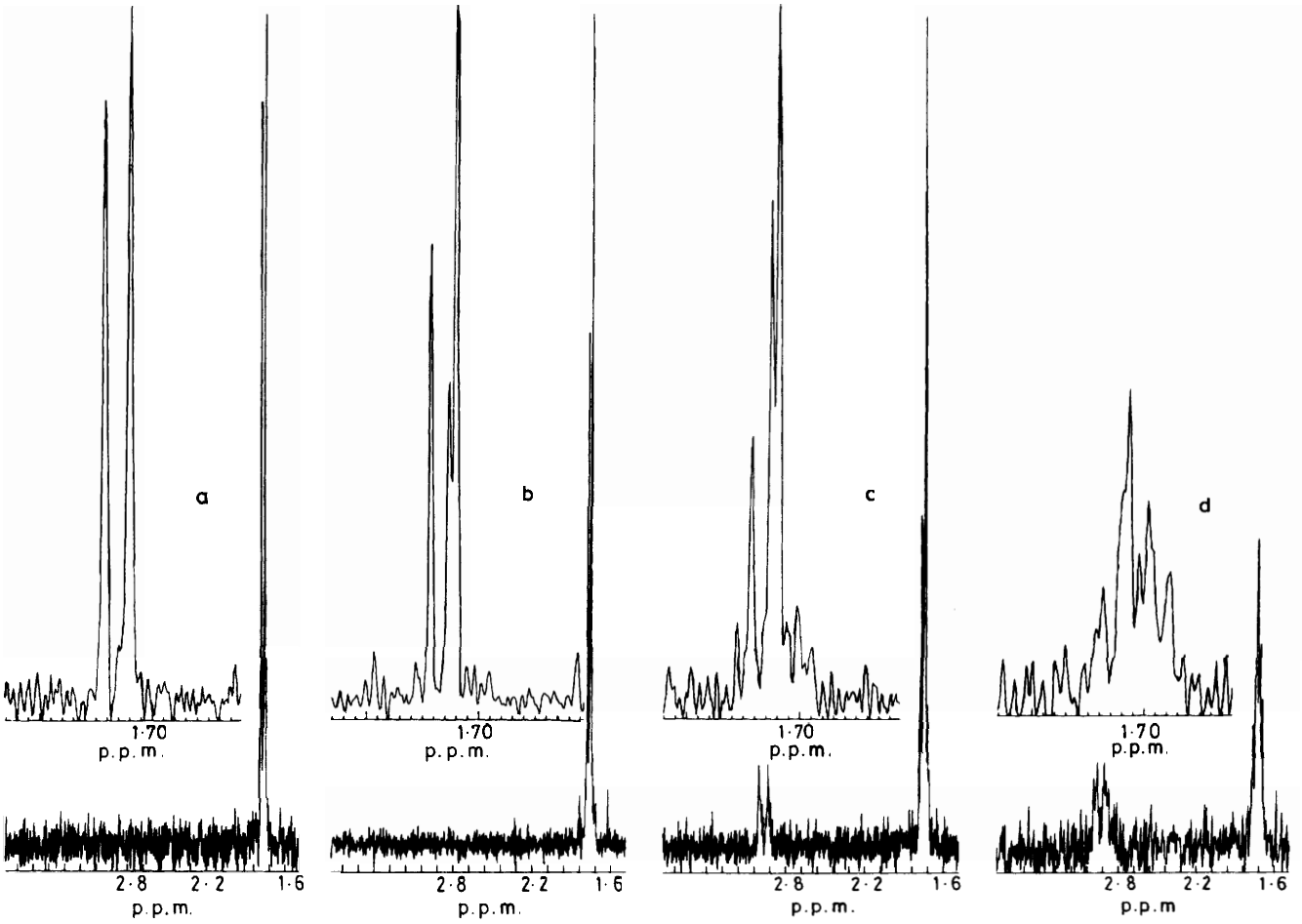


Figure 4. ^{1}H-Decoupled ^{3}H n.m.r. spectra in C^2HCl_3 of fractionated (+)-(*R*)-bepridil; the upper traces are resolution enhanced

A similar fractionation was carried out for the tritiated bepridils. The ^{3}H n.m.r. spectra of these fractions are shown in Figures 3 and 4. The tritiated compounds show the same trend as the deuteriated bepridils: molecules tritiated at positions 2 and/or 5 have longer retention times than molecules tritiated at positions 3 and/or 4.

Interpretation of the ^{3}H *N.m.r. Spectra.*—If we assume an average flat conformation for the pyrrolidine ring (Figure 5), H-3/H-4′ and H-3′/H-4 form homotopic pairs (C_2 relations)[11] and are isochronous in n.m.r. The pairs H-3/H-3′, H-4/H-4′, H-3/H-4, and H-3′/H-4′ are diastereoisotopic (σ-relation); the chiral perturbation by the rest of the bepridil molecule makes these pairs anisochronous in n.m.r.

With the ratio of mono- to di-tritiated material of the starting material in mind it is possible to interpret the ^{3}H n.m.r. spectra of the tritiated bepridils; the following labelled species are present.

(1) [pyrrolidine-3-$^3H_1$]*Bepridil.* Two diastereoisomers are possible: 3-^{3}H(≡4′-^{3}H) and 3′-^{3}H(≡4-^{3}H) (see Figure 5), giving rise to two singlets of about equal intensity. This situation is best shown in Figure 4a; the chemical shift difference is about 0.024 p.p.m.

(2) cis-[pyrrolidine-3,4-$^3H_2$]*Bepridil.* Only one diastereoisomer is possible since the fast rotation of the pyrrolidine ring interconverts homotopic positions and thus makes 3,4-$^3H_2$ and 3′,4′-$^3H_2$ equivalent. Since H-3 and H-4 are enantiotopic and thus anisochronous in bepridil, their signals are split to an AB pattern. This situation is best observed in Figures 3b and 4b; unfortunately the singlet of one $^3H_1$-diastereoisomer overlaps with the central right line of the AB pattern. The chemical shift difference is about 0.002 p.p.m., and the vicinal coupling constant about 11 Hz. The centre of the AB pattern is shifted about 0.08 p.p.m. upfield as compared with the centre of both 3-$^3H_1$ singlets, in agreement with the tritium isotope effect in ^{3}H n.m.r.[3]

(3) trans-[pyrrolidine-3,4-$^3H_2$]*Bepridil.* Two diastereoisomers, *viz.* 3,4′-$^3H_2$ and 3′,4-$^3H_2$, are possible. Since H-3/H-4′ and H-3′/H-4 are both isochronous pairs, these species give rise

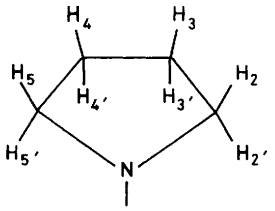


Figure 5. Local symmetry of the pyrrolidine ring

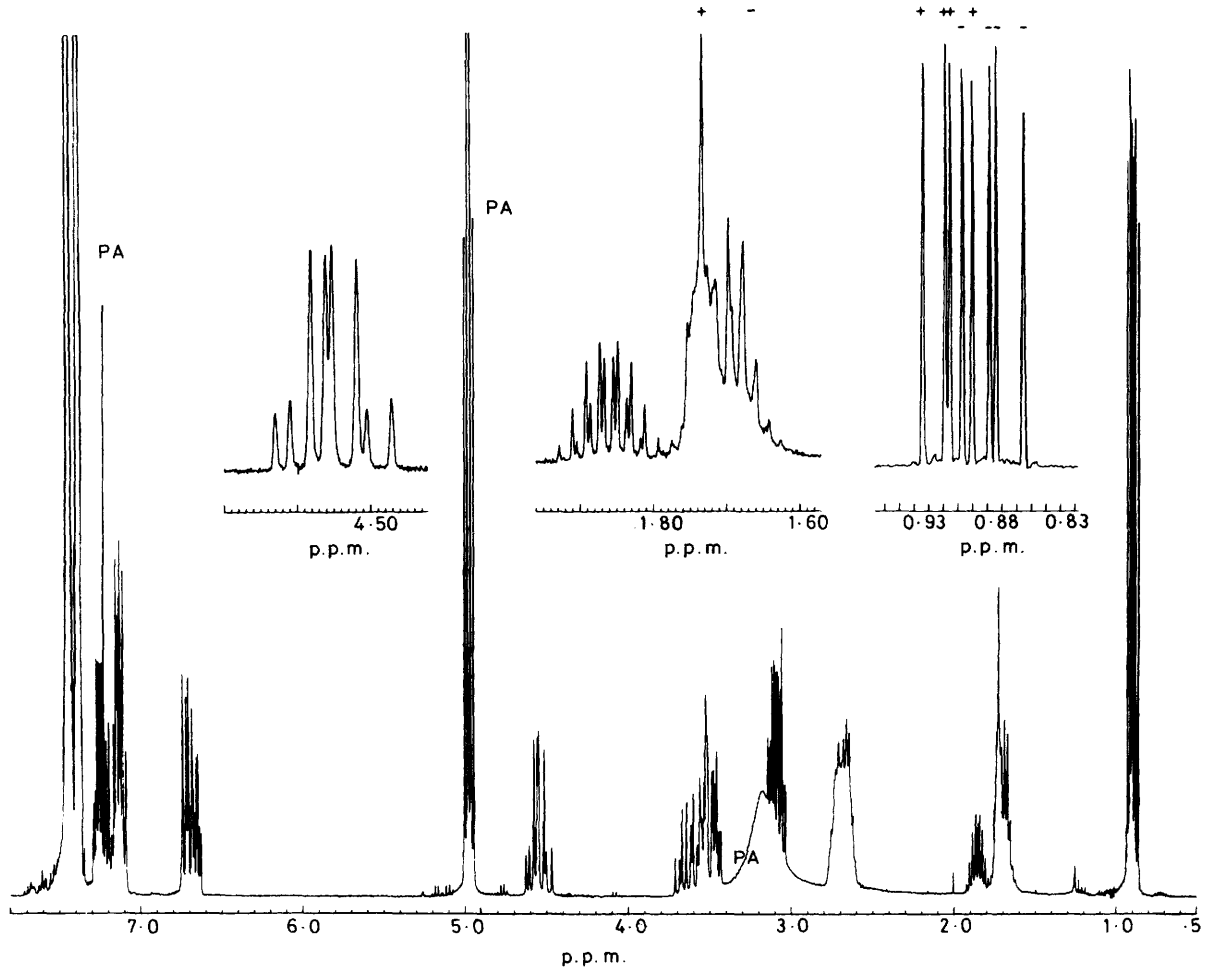


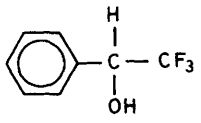
Figure 6. ^{1}H N.m.r. spectrum of bepridil in the presence of Pirkle's alcohol (PA)

to two singlets which are in fact observed (Figures 3b and 4b) as low-intensity lines at the extremes of the other signals; the chemical shift difference is about 0.17 p.p.m. The *cis*:*trans* ratio is about 25:1, in agreement with expectation that reduction with $^3H_2$ will be mainly *cis*.

(4) [pyrrolidine-2-$^3H_1$]*Bepridil*. As in the case of the material monotritiated at position 3, two diastereoisomers are present. Figure 4c shows the corresponding two singlets at about 2.9 p.p.m., separated by 0.055 p.p.m. The line-broadening of these signals is also found in the ^{1}H n.m.r. spectrum (Figure 1b). The fraction shown in Figure 4c probably consists of a mixture of this material with [3-$^3H_1$]- and [3,4-$^3H_2$]-material.

(5) [pyrrolidine-2,3-$^3H_2$]*Bepridil and* [pyrrolidine-2,3,4-$^3H_3$]*bepridil*. These species give very complicated ^{3}H n.m.r. spectra because of the presence of four diastereoisomers in the former and eight in the latter case. As can be seen in Figure 4d, the signals are shifted to lower field in comparison with the mono- and di-tritiated material, in agreement with the n.m.r. isotope effect.

^{3}H *N.m.r. Studies in the Presence of Pirkle's Alcohol*.—Enantiomeric purity can be determined by n.m.r. spectroscopy using chiral additives such as Pirkle's alcohol (**3**). For bepridil



(**3**)

(see Figure 6), splitting of several signals is observed and the enantiomeric purity of the (unlabelled) compound can be determined with good precision from the methyl signals of the isobutyl group. For the pyrrolidine signals (1.7 and 2.7 p.p.m.) also, differences between the racemic mixture and the (+)-(*R*)-enantiomer are observed, but because of broadening and the more complex splitting pattern the enantiomeric purity cannot be derived easily from these signals. We also recorded ^{3}H n.m.r. spectra in the presence of Pirkle's alcohol (Figure 7); although differences are observed no conclusions can be drawn about the optical purity of (+)-[^{3}H]bepridil.

A more simple picture was obtained with fractionated bepridil. The resonances of both the monotritiated diastereoisomers and the (+)-*cis*-[*pyrrolidine*-3,4-$^3H_2$]bepridil collapse

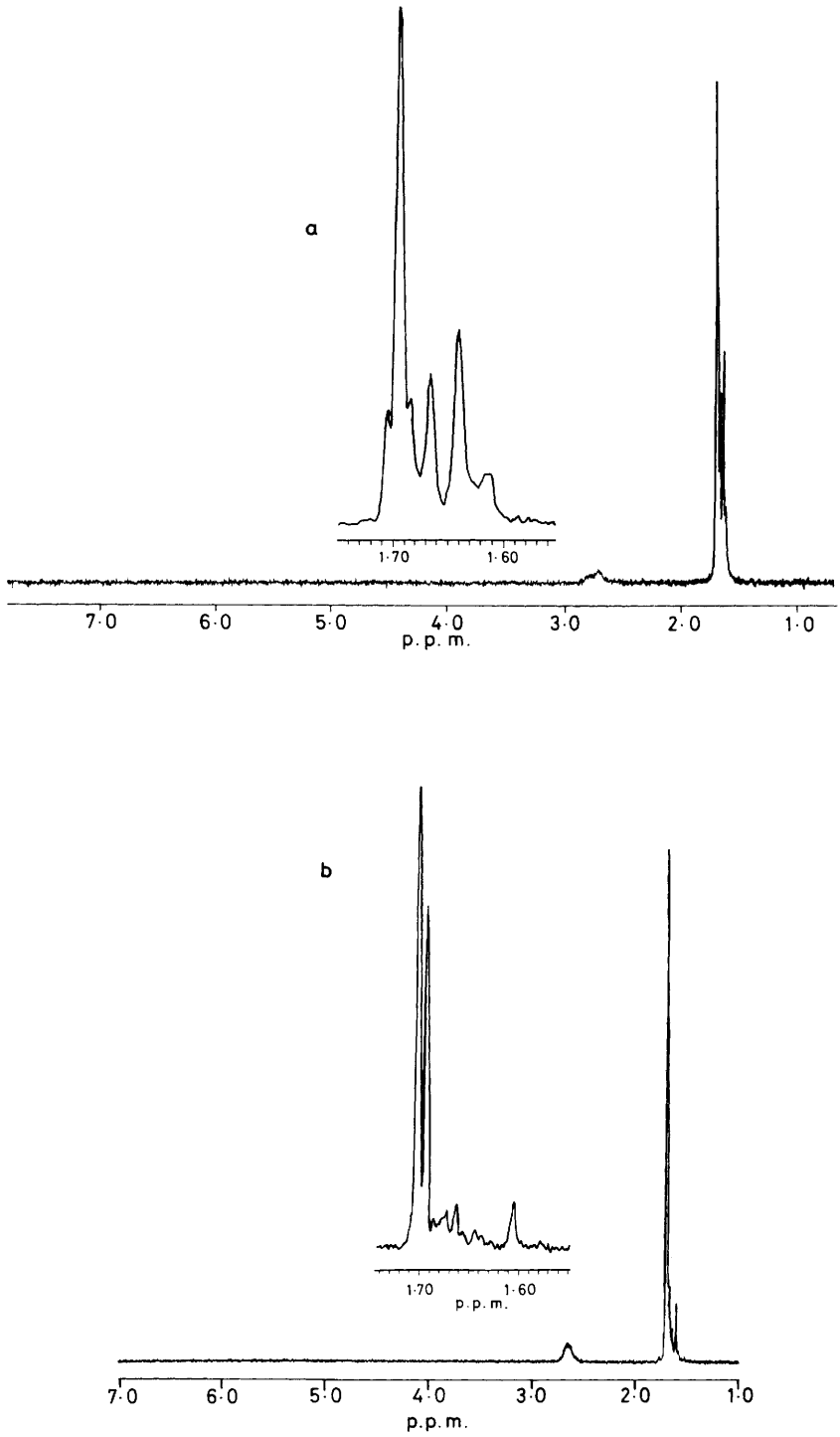


Figure 7. ^{3}H N.m.r. spectra (^{1}H-decoupled) in the presence of Pirkle's alcohol of (a) [*pyrrolidine*-^{3}H]bepridil and (b) (+)-[*pyrrolidine*-^{3}H]bepridil; the upper traces are resolution enhanced. Pirkle's alcohol induces also a concentration-dependent shift of the resonances; to be able to compare all spectra we fixed the chemical shift of the singlet of monotritiated (+)-bepridil at 1.70 p.p.m.

to a broad signal at 1.70 p.p.m., while the signals of the corresponding (−)-enantiomers appear at higher field, in agreement with the observations in ^{1}H n.m.r. for unlabelled bepridil. An example, together with the assignment of the signals, is given in Figure 8.

On the basis of these spectra, an optical purity for (+)-[^{3}H]bepridil of >95% can be estimated. This value is in agreement with the value (>95%) obtained for the optical purity of the starting material.*

* With $^{2}H_{2}$ no racemization was observed on reduction of compound (**2b**).

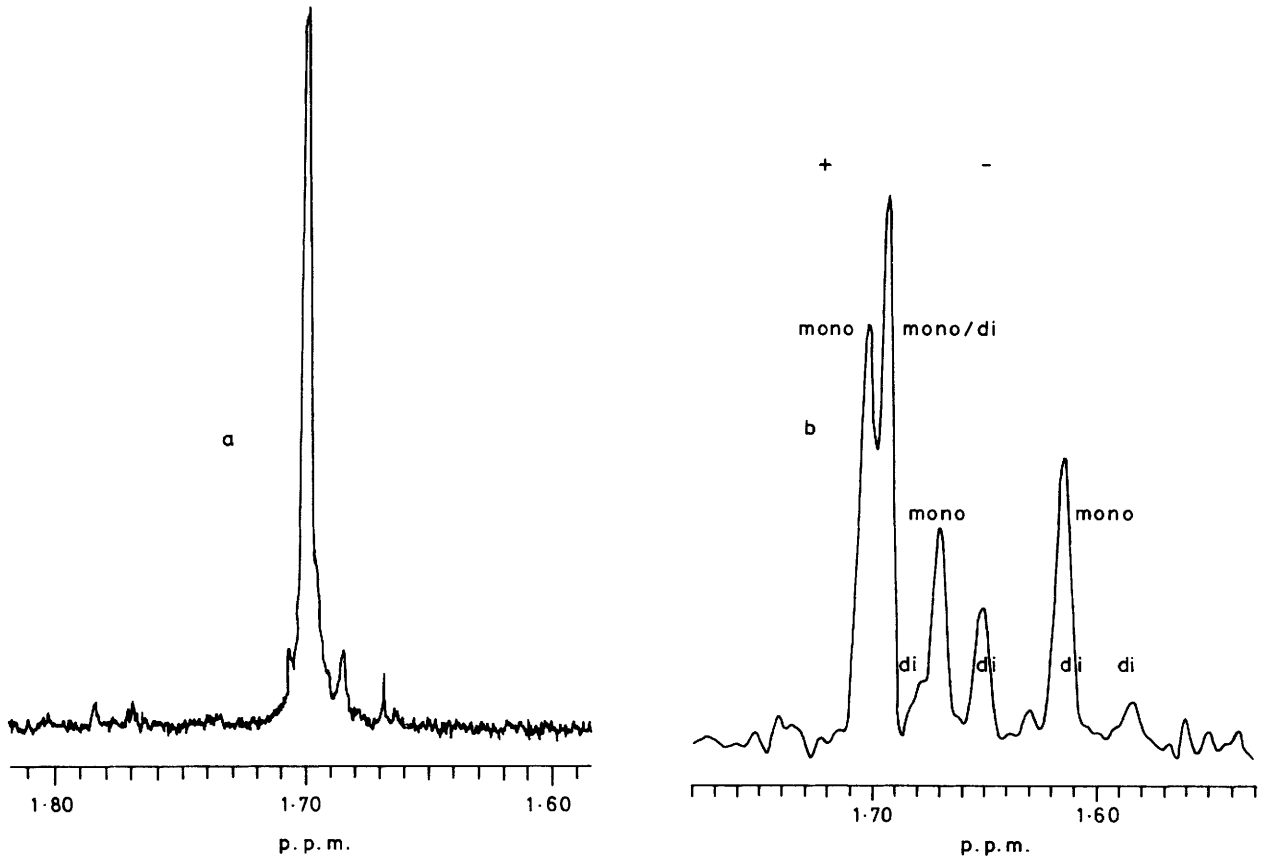


Figure 8. ^{3}H N.m.r. spectra (^{1}H-decoupled), in the presence of Pirkle's alcohol, of fractionated (a) (+)-[^{3}H]bepridil and (b) [^{3}H]bepridil: (a) fraction containing 54% (+)-[*pyrrolidine*-3-^{3}H]bepridil and 46% (+)-*cis*-[*pyrrolidine*-3,4-$^3H_2$]bepridil; (b) fraction containing 66% [*pyrrolidine*-3-^{3}H]bepridil and 33% *cis*-[*pyrrolidine*-3,4-$^3H_2$]bepridil

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Received 1*st July* 1985; *Paper* 5/1103