SYNTHESIS OF N-(2,5-[2-^{13}C]DIMETHYL-1H-PYRROL-1-YL)-6-(4-MORPHOLINYL) -3-PYRIDAZINAMINE HYDROCHLORIDE

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SUMMARY

N-(2,5-[2-^{13}C]dimethyl-1H-pyrrol-1-yl)-6-(4-morpholinyl)-3-pyridazinamine hydrochloride ([2-^{13}C]methyl MDL-899), a new antihypertensive agent, was synthesized for metabolic studies, in five steps via 2,5-[1-^{13}C]hexanedione, and characterized by ^{1}H- and ^{13}C-NMR, IR, and MS.

Key words: ^{13}C, MDL-899, pyridazinamine, ^{13}C-NMR, synthesis, MS.

INTRODUCTION

The recent increase of applications of stable isotopes in biomedical research is due to the improved technologies which allow their production and measurement. The main advantages of the compounds labelled with stable isotopes are: i) the administration of labelled drugs to humans in preliminary

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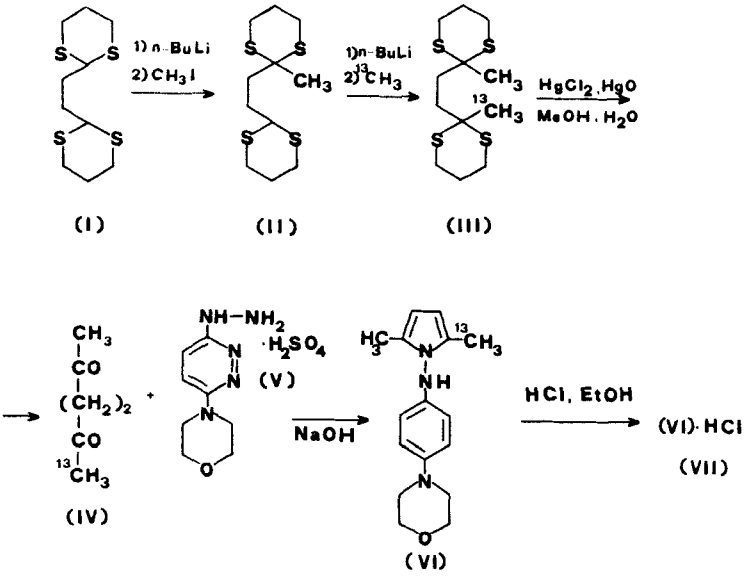
metabolism studies; ii) the absence of radioactivity; iii) the ease of measurement by spectroscopic techniques such as NMR and MS, which can also contribute to the structural analysis of the bio transformation products (1).
For these reasons ^{13}C-labelled MDL-899 (VII, see Scheme 1) was prepared to study the metabolic pathways of this new antihypertensive agent (2,3), especially to assess whether or to which extent its metabolism occurs through the formation of an intermediate containing a hydrazino group (4-6).
The procedure described here is easy and inexpensive. The final yield of ^{13}C-MDL-899 (VII) is good and the enrichment in ^{13}C obtained is 94%, as determined by NMR.

SCHEME 1



EXPERIMENTAL

The IR spectra were obtained as nujol mull with a P.E. 157 spectrophotometer and the IR data are reported in cm^{-1}.
The ^{1}H NMR spectra were obtained at 270 MHz in DMSO-D_2O or $CDCl_3$ with TMS as internal standard or in D_2O with TSP as internal standard.
The FT ^{13}C spectrum of VII was obtained, under conditions of complete proton noise decoupling, at 67.88 MHz with TMS as internal standard.

The Bruker WH-270 FT NMR criospectrometer equipped with the 36 K BNC-12 computer and disk unit was used.

The mass spectrum was run on Varian-MAT 112 instrument, at 70 eV by direct inlet system (DIS) at 120°C, ion source temperature 200°C.

2-[2-(1,3-Dithian-2-yl)ethyl]-2-methyl-1,3-dithiane (II) (M.W. 280.54)

2,2'-(1,2-Ethanediyl)bis-1,3-dithiane(I) (34,86 g, 0.1308 mol) dissolved in THF (730 mL) was placed under dry nitrogen atmosphere in a stirred reaction flask and cooled to -30°C with a dry ice-isopropyl alcohol bath. THF was purified prior to use by distillation over sodium and then over lithium aluminum hydride. n-Butyl lithium (100 mL, 1.6 M solution in hexane, 0.16 mol) was added dropwise over a 15 min period. Stirring was continued for 3.5 h at -20° to -30°C, during which time a deep orange solution was formed.

The temperature was then allowed to rise to -5°C and methyl iodide (22.8 g, 0.16 mol) dissolved in THF (70 mL) was added dropwise at that temperature, during which time the solution color changed to light yellow.

After allowing the reaction mixture to stand at 0°C in a refrigerator for 15 h, it was quenched with water (50 mL), concentrated to about 300 mL, diluted with water (200 mL) and extracted with ether (4 X 500 mL). The combined extracts were washed with saturated aqueous NaCl (300 mL) dried over anhydrous K_2CO_3 and evaporated to give a slowly crystallizing oil. The residue was triturated with hexane (100 mL), filtered and recrystallized from ligroin 75°-120°C (250 mL) giving 19.8 g (54%) of pure product: mp 52-54°C

IR (nujol mull): 1410 (δ CH_2); 790 (ν C-S)

^{1}H-NMR ($CDCl_3$): $1CH_3$(s,1.55 δ); $4CH_2$(m,1.8-2,2 δ); $4CH_2$-s(m,2.7-3.0 δ); 1CH-S (t,4.08 δ).

Elemental analysis for $C_{11}H_{20}S_4$: Calc.: C, 47.09% ; H, 7.18%. Found: C, 47.22% ; H, 7.24%.

2-([^{13}C]methyl)-2[2-(2-methyl-1,3-dithian-2-yl)ethyl]-1,3-dithiane.(III)(M.W. 295.57)

Compound II (8.047 g,0.02868 mol) was alkylated with $^{13}CH_3I$(5 g, 0.035 mol) following the same procedure described above.

The crude product (9.1 g) obtained after working up was purified by flash column chromatography over 1000 g of silica gel 60 (230-400 mesh) Merck using

hexane: Et_2O 9:1 as eluent. Crystallization from ligroin 75°-120°C gave 4.35g (52.6%) of pure compound: mp 106-107°C.

IR (nujol mull): 1410(δ CH_2); 770(ν C-S)

^{1}H NMR ($CDCl_3$):2CH_3(s,1.55 ; d,1.55 , ^{1}JC-H= 129 Hz)

2(CH_2)-CH_2-(CH_2)(m,1.8-2.1 δ); CH_2-CH_2(s,2.20 δ); 4CH_2-s(m,2.7-3.0 δ)

2,5-[1-^{13}C]Hexanedione (IV) (M.W. 115.14)

A mixture of III (4.35 g, 0.0147 mol), mercuric chloride (17.13 g, 0.0631 mol), red mercuric oxide (5.31 g, 0.0245 mol), water (22.15 mL), methanol (335 mL) and acetone (37 mL) was heated at reflux for 4.5 h with rapid stirring under nitrogen.

The cold mixture was filtered and the residue washed with methylene chloride (40 mL). The filtrate was concentrated to 40 mL under reduced pressure, diluted with a threefold volume of water and extracted with methylene chloride (3x150 mL).

The combined extracts were washed with saturated ammonium chloride solution (2 x 40 mL) and dried over magnesium sulfate. Evaporation of the solvent at 40°C (160 mm) gave 2.5 g of crude product.

Distillation furnished 1.61 g (95%) of pure diketone (IV) : bp 96-100°C (35 mm).

IR (nujol mull): 1700 (ν C=O); 1350(δ CH_3); 1150 [ν $\overset{C}{\underset{C}{|}}$C(=O)].

N-(2,5-[2-^{13}C]dimethyl-1H-pyrrol-1-yl)-6-(4-morpholinyl)-3-pyridazinamine (VI) (M.W. 274.34)

To a stirred solution of V (4.88 g, 0.01664 mol) in water (9 mL), 1 N sodium hydroxide (16.65 mL, 0.01665 mol) was slowly added under nitrogen at 20-25°C; 2,5-|1-^{13}C|hexanedione (1.81 g, 0.01572 mol) was then added dropwise at the same temperature over a few min. The solution was heated to 70°C and after 0.5 h a precipitate formed.

After stirring at 70°C for 4 h the mixture was cooled and 1 N sodium hydroxide (16.65 mL, 0.01665 mol) was slowly added at 20°C in order to complete the precipitation of the reaction product. The insoluble material was collected by suction filtration, washed with water and dried in vacuo at 50°C over phosphorous pentoxide.

The crude product (3.2 g) was then purified by flash column chromatography on 320 g of silicagel 60(230-400 mesh) Merck eluting with CH_2Cl_2: EtOAc 6:4. Yield 2.85 g (66%) of pure (VI).

N-(2,5-[2-^{13}C]dimethyl-1H-pyrrol-1-yl)-6-(4-morpholinyl)-3-pyridazinamine hydrochloride (VII) (M.W. 310.79)

A mixture of N-(2,5-[2-^{13}C]dimethyl-1H-pyrrol-1-yl)-6-(4-morpholinyl)-3-pyridazinamine (VI)(2.85 g) and ethanol (45 mL) was heated at 50°C under nitrogen with magnetic stirring until complete solution occurred; 37% hydrochloric acid (1.05 mL) in ethanol (2.5 mL) was slowly added and the solution was cooled at 0°C for 1 h. The precipitate was collected by suction filtration, washed with ethanol (2 x 5 mL) and dried in vacuo at 30°C. The salt (3 g) was recrystallized from EtOH 50% (13 mL) yielding 2.32 g (72%) of pure VII m.p. 230-240°C dec.Elemental analysis for $^{13}CC_{13}H_{19}N_5O.HCl$. Calc. : C, 54.42%; H, 6.48%; N, 22.53%; Cl^- ,11.40%. Found : C, 53.41%; H, 6.66%; N, 22.10%; Cl^-, 11.28%.

Spectral data of VII

IR(nujol mull, Fig.1): 3160, 3140(ν NH); 3200-2500 (νNH^+); 1660, 1600, 1560, (ν C=N, ν C=C); 1280, 1120, 935 (ν C-O, ν C-N); 820, 760 (arom γ CH)
UV absorption (λmax, nm): 254 ($\pi \rightarrow \pi^*$); 350 ($n \rightarrow \pi^*$).

^{1}H NMR (D_2O, Fig.2) 1 CH_3(s,2.08 δ); 1 CH_3(d,2.08 δ, ^{1}J C-H=129 Hz), 2 CH_2N (t,3.62 δ); 2 CH_2O(t,3.89 δ); H-8,9(s,5.93 δ); H-4(d,7.51 δ); H-5(d,7.84 δ). From the integration values of the $^{13}CH_3$ doublet and CH_3 singlet, the ^{13}C enrichment concerning one methyl group is calculated at 94%.

^{13}C NMR [(^{12}C-DMSO-d_6:D_2O(5:1),Fig.3]: 2 CH_3(12.34 δ); 2 CH_2-N(47.60 δ); 2 CH_2O(66.97 δ); C-8, C-9(125.76 δ); C-4, C-5(126.76 δ); C-7, C-10(s,129.31 δ; d,129.31 δ, ^{1}J C-C= 51 Hz); C3, C6(153.18 δ, 153.65 δ).

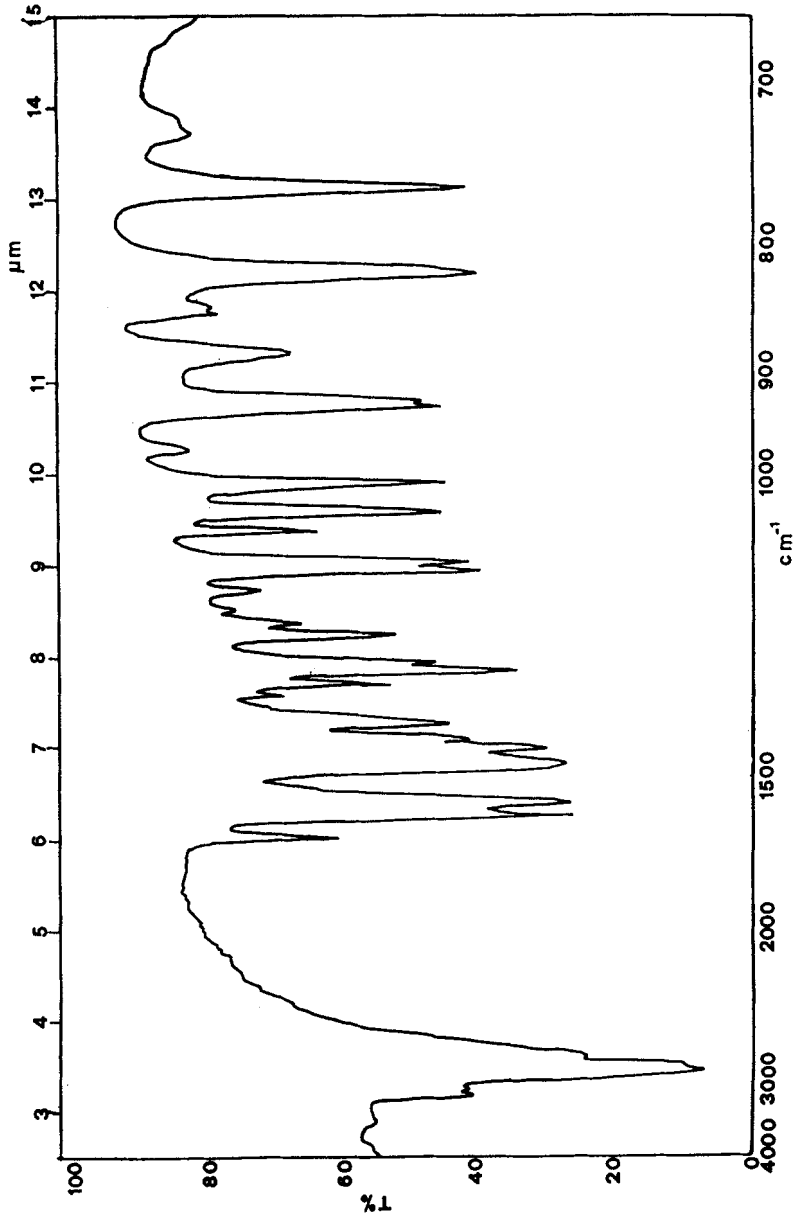


Fig. 1 - IR spectrum of (VII) in mineral oil.

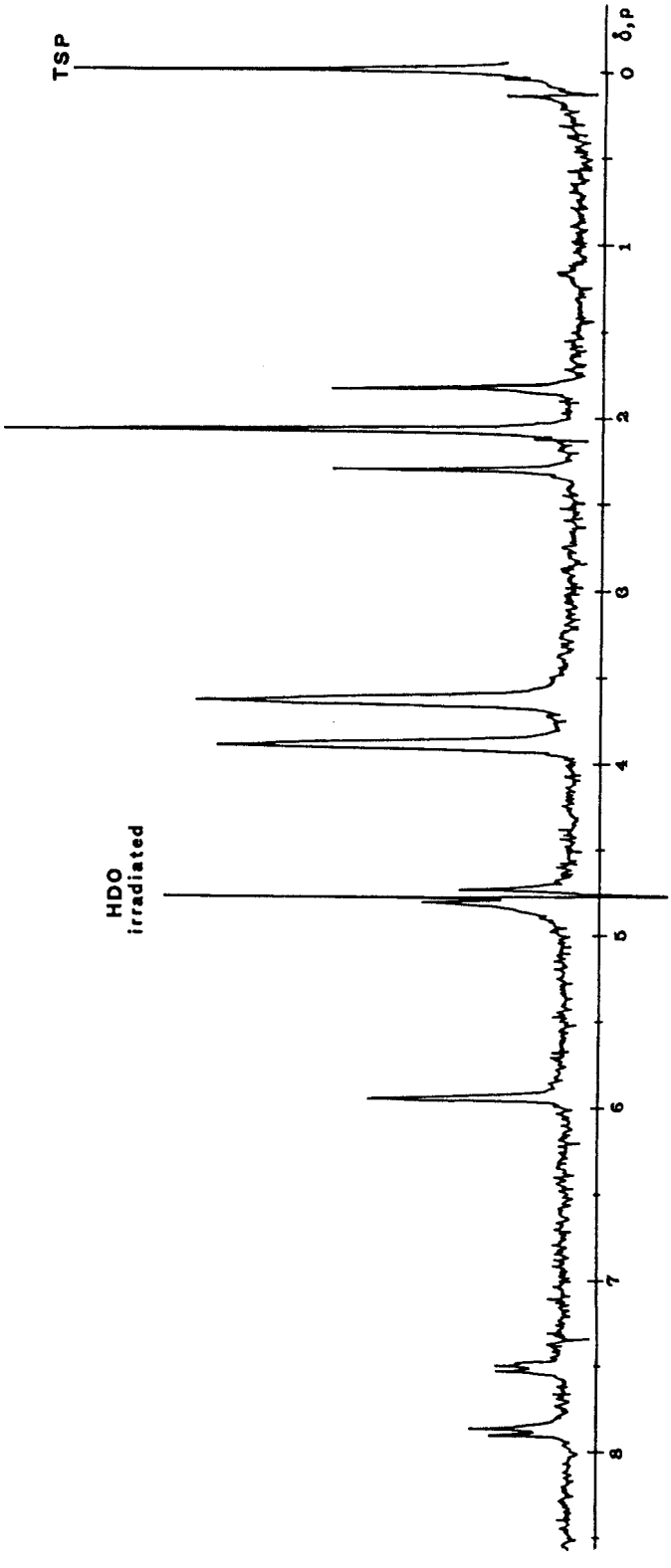


Fig. 2 - ^{1}H NMR spectrum at 270 MHz of (VII) in D_2O.

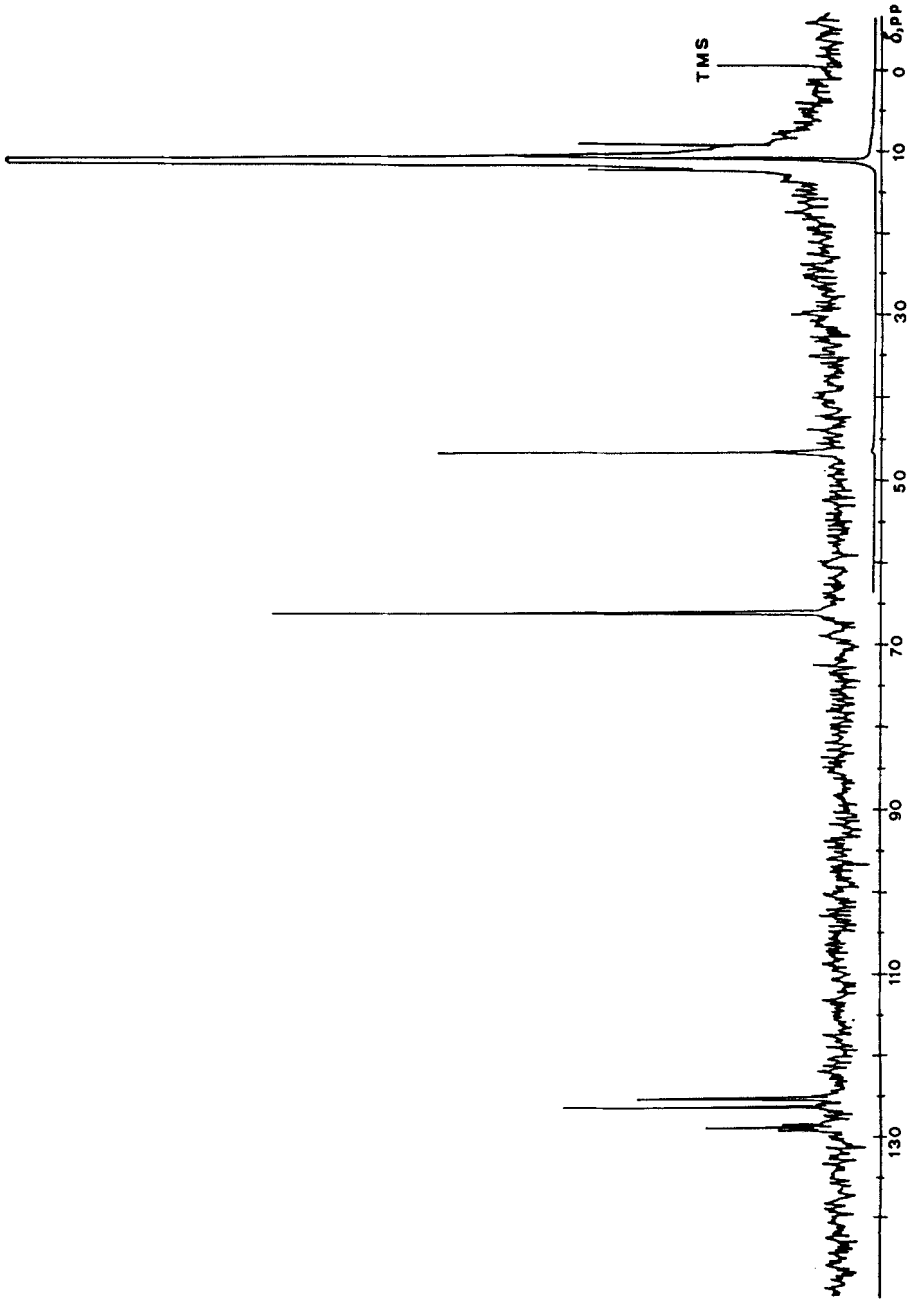


Fig. 3 - ^{13}C NMR spectrum at 67.88 MHz of (VII) in ^{12}C-DMSO-d_6: D_2O (5:1).

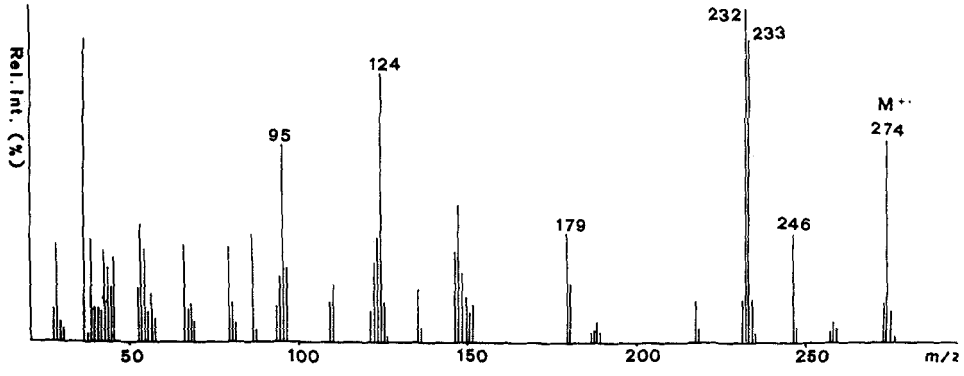


Fig. 4 - Mass spectrum of (VII) at 70 eV in D.I.S. at 120°C.

MS (EI,DIS,Fig.4),m/z (rel.int.)[attribution]: 274(60)$[M]^{+\cdot}$, 246(32)$[M-N_2]^{+\cdot}$, 233(90)$[M-CH_3CN]^{+\cdot}$, 232(100)$[M-{}^{13}CH_3CN]^{+\cdot}$, 179(32)$[M-{}^{13}CC_5H_8N]^{+\cdot}$, 124(80)$[C_7H_{10}NO]^{+\cdot}$, 95(55)$[{}^{13}CC_5H_8N]^{+}$.

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