Synthesis and Configuration of *trans*-1-Amino-4-benzyl-2,6-dimethylpiperazine as an Intermediate of Semi-synthetic Rifamycins

Giorgio Cignarella (1)

Instituto di Chimica Farmaceutica, Università di Sassari

and

Gian Gualberto Gallo

Laboratori Ricerche Lepetit S.p.A., Milano, Italy

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The synthesis of *trans*-1-amino-4-benzyl-2,6-dimethylpiperazine (Ib), *trans*-4-benzyl-2,6-dimethylpiperazine (VIIb) and *trans*-2,6-dimethylpiperazine (IIb) are described, by condensation of *N*-benzyl-1,2-propanediamine with α-bromopropionate and successive thermal cyclization and reduction with lithium aluminum hydride.

The assignment of the *cis*, *trans* configuration to the isomers was based on a thorough examination of the ring proton nmr signals of the two isomers of 4-benzyl-2,6-dimethylpiperazine (VIIa and VIIb) interpreted in terms of conformational considerations.

Among the various derivatives obtained by chemical modification of the aminopiperazine side chain of rifampicin one of the most interesting appears 2′,6′-dimethyl-4′-benzyl-4′-desmethylrifampicin (DMB) because of its activity on the reverse transcriptase of cancerogenic viruses (2,3). It also seems to delay the onset of chemically induced mamary tumors in rats (4). However, DMB has poor selectivity as it is active against other transcriptases (5). The hydrazone side-chain of DMB is formed by 1-amino-4-benzyl-2,6-dimethylpiperazine (I) (6) which, itself, is claimed to inhibit the growth of vaccinia virus and of herpex simplex viruses (7).

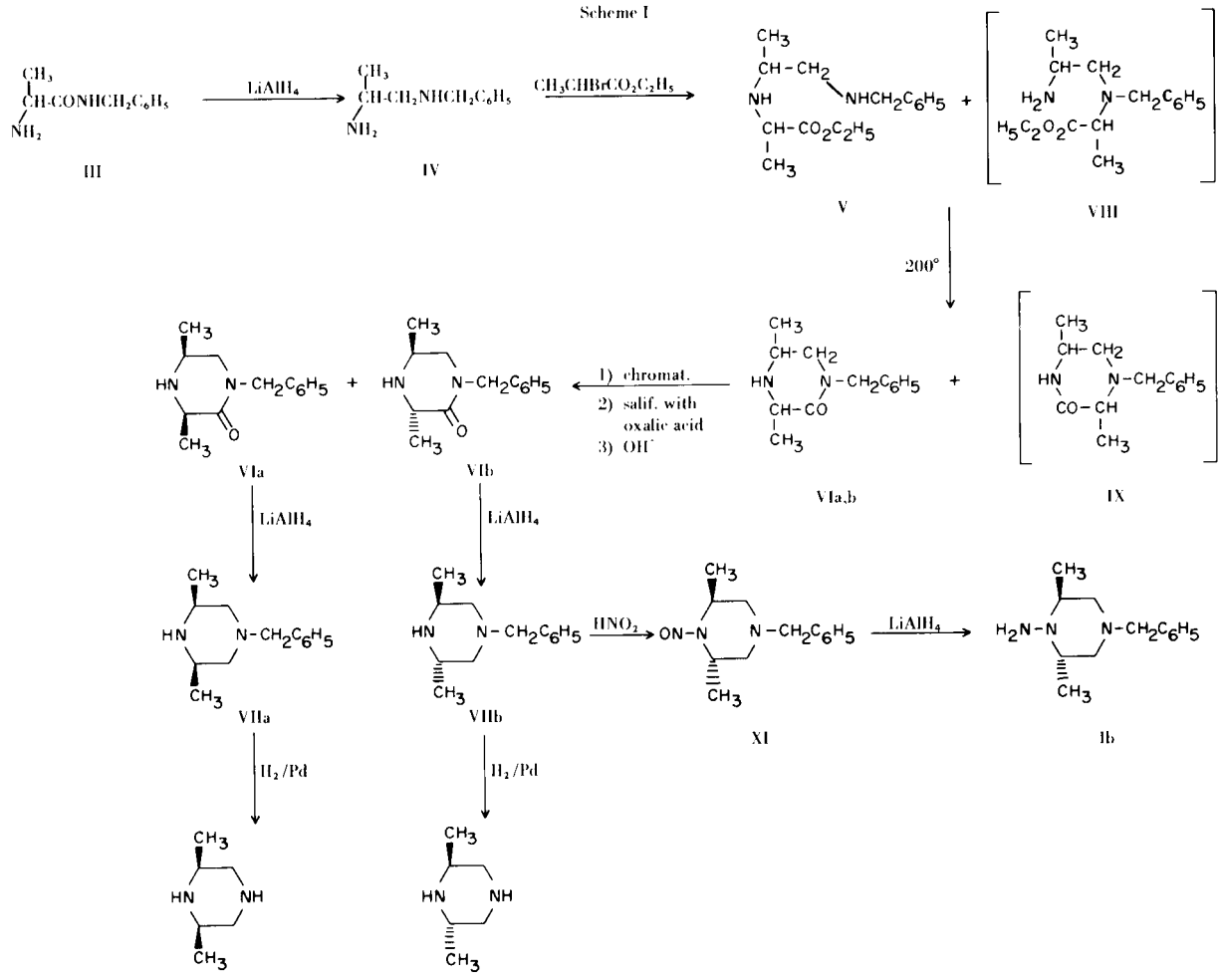
On observing that in Ia and consequently in DMB the 2,6-dimethylpiperazine moiety must have the *cis* configuration because their syntheses arise from *cis*-4-benzyl-2,6-dimethylpiperazine (VIIa) (8), we were attracted by the hypothesis that the replacement of the *cis* with the *trans* 2,6-dimethylpiperazine moiety in the structures of DMB and of Ia could modify their inhibitory activity.

Since Ib is unknown as well as *trans*-2,6-dimethylpiperazine (IIb), we wish now to report its synthesis through *trans*-4-benzyl-2,6-dimethylpiperazine (VIIb) which is also the key intermediate for IIb (9). Accordingly, 2-amino-*N*-benzylpropanamide (III) was reduced with lithium aluminum hydride to N^1-benzyl-1,2-propanediamine (IV), which was allowed to react with ethyl α-bromopropionate to give the ethyl ester of *N*-(2-benzylamino)isopropyl alanine (V) besides minor amounts of the isomer VIII. The heating at 200° of crude V *via* an intramolecular loss of ethanol yielded a mixture of *cis* and *trans*-4-benzyl-2,6-dimethylpiperazin-3-ones (VIa, VIb) and of 4-benzyl-3,6-dimethylpiperazin-2-one (IX) (11). The isomers were separated by silica gel chromatography, eluting with benzene containing increasing amounts of acetone (IX and VIa), then with 9:1 benzene-methanol (VIb). Compounds VIa and VIb were further purified by salification with oxalic acid which transformed VIa into an oxalate (Xa), m.p. 186-188°, while VIb gave a hemi-oxalate (Xb), m.p. 167-169°. These salts when treated with 6*N* sodium hydroxide liberated the pure bases and their configurations were derived by that of their reduced products. Lithium aluminum hydride reduction of VIa and VIb led to *cis* (VIIa) and *trans*-4-benzyl-2,6-dimethylpiperazine (VIIb), respectively; their configurations were assigned on the basis of their nmr spectra as described afterwards.

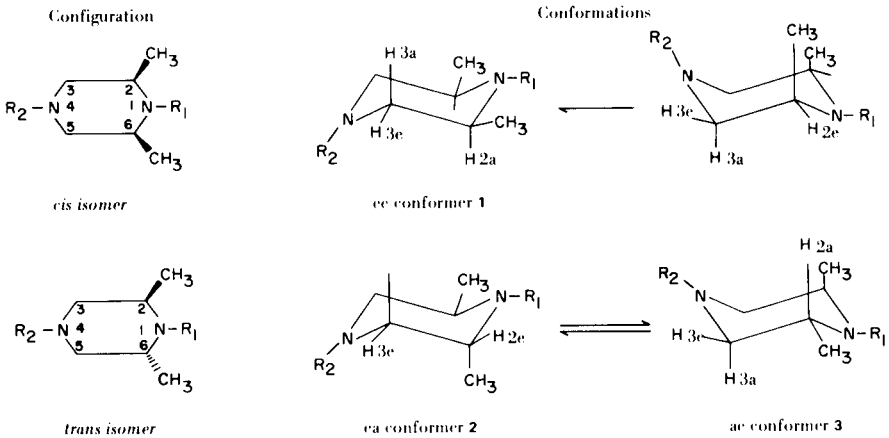
Standard procedures involving nitrosation followed by lithium aluminum hydride reduction allowed VIIb to be transformed into the desired *trans*-1-amino-4-benzyl-2,6-dimethylpiperazine (Ib), whose configuration is supported by the similarity of the nmr pattern with that of the parent VIIb. Finally, the removal of the benzyl group of VIIb by hydrogenolysis afforded *trans*-2,6-dimethylpiperazine (IIb), as an oil b.p. 125-130°, which rapidly absorbs water and carbon dioxide from air.

The assignment of the *cis*, *trans* configuration is based on the assumption that the *cis* isomer (compounds VIIa, Ia and IIa) exists, at room temperature, only in the more stable diequatorial conformation, **1**, while the *trans* isomer

Scheme I



Scheme II



(compounds VIIb, Ib and IIb) exists in both axial-equatorial, **2**, and equatorial-axial, **3**, energetically equivalent conformations, which interconvert at a rate fast on the nmr time scale (scheme II). Consequently, the *cis* isomer will show signals corresponding to the fixed conformer **1**, while the *trans* isomer will show signals corresponding to the average of the two conformers **2** and **3**. Moreover, the nmr spectra of the *cis* isomer will not change by lowering the temperature; this approach was used by one of us for the assignment of the *cis*, *trans* configurations in 4-phenyl-2,6-

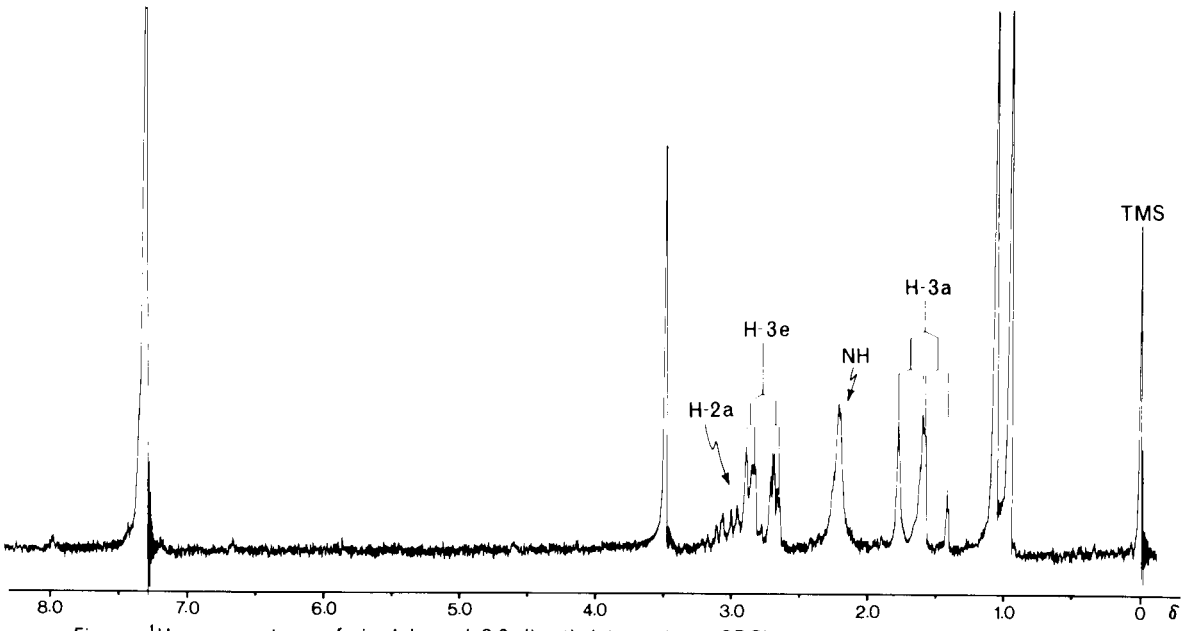


Fig.1 ^{1}H nmr spectrum of cis 4-benzyl-2,6-dimethylpiperazine in $CDCl_3$

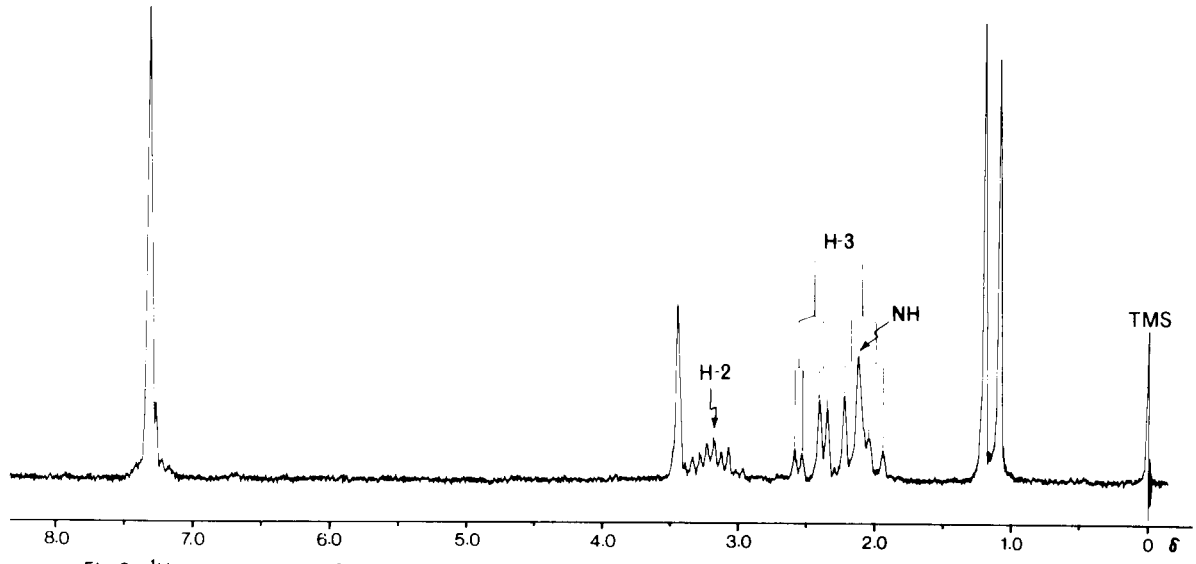


Fig.2 ^{1}H nmr spectrum of trans 4-benzyl-2,6-dimethylpiperazine in $CDCl_3$

dimethylpiperazines (12). In the present case we confined our investigation to room temperature spectra because we could apply a first order analysis, as it was reported for 2,6-dimethylpiperidines (13).

In fact, by comparing, the spectra of the two 4-benzyl-2,6-dimethylpiperazines (VIIa and VIIb), Figure 1 and 2, it can be observed that the chemical shift difference $\Delta\nu$ between the geminal axial and equatorial protons is larger in the *cis* (1.18 ppm) than in the *trans* (0.38 ppm) isomer. The same trend is observed for coupling constants, *i.e.* for the *cis* isomer $J_{2a3a} = 10.5$ and $J_{2a3e} = 3.0$ Hz whereas for the *trans* isomer.

$\frac{1}{2}(J_{2e3a} + J_{2a3e}) = 4.0$ and $\frac{1}{2}(J_{2e3e} + J_{2a3a}) = 6.5$ Hz (14).

As reported in Table I, the ν and the J values for *cis* isomers are in agreement with the existence of the fixed conformer **1**, for which H-3a and H-3e show the highest difference in magnetic shielding; in the *trans* isomers the two H-3 protons have the lowest difference in magnetic shielding which, however, cannot average to zero.

Although in *N*-benzyl derivatives of identically α,α' disubstituted cyclic amines (15) the assignment of the *cis*, *trans* configuration was generally obtained by the magnetic non-equivalence of the benzyl protons displayed by the *trans* isomer contrary to the *cis* isomer, this approach does

not appear definitely convincing in our case. In fact, as apparent in Figure 2 there is only slight evidence for the non-equivalence of the *trans* isomer.

EXPERIMENTAL

Melting points are uncorrected. The ir spectra were recorded with a Perkin-Elmer model 157 spectrophotometer. The nmr spectra were determined in deuteriochloroform on a Varian T-60 and A-60 D spectrometers with TMS as internal reference.

2-Amino-*N*-benzylpropanamide (III).

A solution of 26 g. of bromopropionylbenzylamide (7), 450 ml. of ethanol and 500 ml. of 6 *N* aqueous ammonia was saturated with gaseous ammonia, then was kept at room temperature for a week in a 2000 ml. flask fitted with a rubber stopper. The solvent was then removed *in vacuo* and the concentrated solution (200 ml.) was saturated with ammonium chloride and extracted with 5 x 50 ml. of chloroform. After drying over sodium sulphate the solvent was evaporated to give 18.3 g. (94%) of crude III as a yellowish oil which was employed as such for the next step.

The analytical sample was distilled *in vacuo*, b.p. 130-140° at 0.1 mm, m.p. 23-25°; nmr (δ): 7.9 deuterium oxide - exchangeable broad signal, 1H, CONH), 7.0-7.4 (m, 5H, C_6H_5), 4.35 (d, J = 6 Hz, deuterium oxide - collapsing to s, 2H, CH_2Ph), 3.50 (q, J = 6.5 Hz, 1H, CH), 1.52 (deuterium oxide - exchangeable s, 2H, NH_2), 1.20 (d, J = 6.5 Hz, 3H, CH_3).

Anal. Calcd. for $C_{10}H_{14}N_2O$: C, 67.38; H, 7.92; N, 15.72. Found: C, 67.02; H, 7.96; N, 15.57.

N'-Benzyl-1,2-propanediamine (IV).

A suspension of 9 g. of lithium aluminum hydride, 18 g. of III and 300 ml. of anhydrous tetrahydrofuran was refluxed with stirring for 8 hours. On cooling, the reaction mixture was decomposed with 20 ml. of 10% sodium hydroxide and 35 ml. of water, filtered and the filtrate dried over sodium sulphate and evaporated. The oily residue was distilled twice to remove traces of amide compounds, to give 9.7 g. (57%) of IV, b.p. 90-95° at 0.1 mm; nmr (δ): 7.1-7.4 (m, 5H, C_6H_5), 3.72 (s, 2H, CH_2Ph), 2.15-3.10 (m, 3H, CH-CH_2), 1.54 (deuterium oxide - exchangeable s, 3H, NH and NH_2), 1.00 (d, J = 6.5 Hz, 3H, CH_3).

Anal. Calcd. for $C_{10}H_{16}N_2$: C, 73.12; H, 9.82; N, 17.06. Found: C, 72.87; H, 9.84; N, 16.89.

Reaction of IV with Ethyl α-Bromopropionate. Cyclization of the Resulting V to 4-Benzyl-2,6-dimethylpiperazin-3-one (VI). Separation of the *cis* (VIa) and *trans* (VIb) Isomers.

A solution of 8.4 g. of IV, 9.1 g. of ethyl α-bromopropionate and 5 g. of triethylamine in 100 ml. of toluene was refluxed for 20 hours. Triethylamine hydrobromide was filtered off and the filtrate was evaporated. The ir spectrum of the oily residue showed ester (1710 cm^{-1}) and amide (1650 cm^{-1}) absorptions which indicated a partial cyclization of the amino ester V. The product (12 g.) as such was kept at 200-210° for 1 hour, under slightly reduced pressure to eliminate the formation of volatile material (mainly ethanol). The residue was distilled collecting 7.5 g. (73%) of an oil, b.p. 120-125° at 0.1 mm. whose ir spectrum lacked ester absorption. Chromatography on 75 g. of silica gel in a 27 mm diameter column eluting with 9:1 benzene-acetone (12 x 50 ml.) separated an unidentified red oil (1 g.), then, eluting with 8:2 benzene-acetone (6 x 50 ml.), a fraction (0.5 g.) identified by nmr as a 1:1 mixture of *cis* and *trans* isomers of 4-benzyl-3,6-dimethylpiperazin-2-one (IX).

The following fraction (2 g.) eluted with 7:3 benzene:acetone (0.5 l.) was identified as VIa. Eventually, the column was eluted with 9:1 benzene-methanol (12 x 50 ml.) to give 2.4 g. of a fraction enriched in VIb, which was re-chromatographed and yielded 0.3 g. of VIa, 0.35 g. of a mixture of both isomers and 1.55 g. of VIb. Both VIa and VIb are slightly contaminated with the other isomer (tlc on silica gel; 7:3 benzene-acetone as mobile phase), thus requiring a further purification which was accomplished by salification with oxalic acid. Accordingly, 1.9 g. of crude VIa was treated with equimolar oxalic acid dihydrate in ethanol to give 2.1 g. of *oxalate* (Xa) which melted at 186-188° (ethanol).

Anal. Calcd. for $C_{28}H_{38}N_4O_6$: C, 63.86; H, 7.27; N, 10.64. Found: C, 63.76; H, 7.47; N, 10.13.

The free base VIa was liberated from Xa with 6 *N* sodium hydroxide, extracted with ether and distilled, b.p. 120-125° at 0.1 mm; ir (chloroform) cm^{-1}: 3420 and 3300 (NH), 1650 (CON<); nmr (δ): 7.2-7.4 (m, 5H, C_6H_5), 4.45 and 4.74 (2d, J = 15 Hz, 2H, CH_2Ph), 3.60 (q, J = 7 Hz, 1H, CO-CH), 2.9-3.15 (m, 3H, CH-CH_2), 1.70 (deuterium oxide - exchangeable s, 1H, NH), 1.45 (d, J = 7 Hz, 3H, CH_3), 1.10 (d, J = 6 Hz, 3H, CH_3).

Crude VIb (1.45 g.) gave 1.8 g. of ***hemi-oxalate*** (Xb) which after two crystallizations from ethanol melted at 167-169°.

Anal. Calcd. for $C_{15}H_{20}N_2O_5$: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.01; H, 6.46; N, 9.26.

Free VIb distilled at 120-125°/0.1 mm; ir (chloroform) cm^{-1}: 3400 and 3300 (NH), 1650 (CON<); nmr (δ): 7.2-7.4 (broad s, 5H, C_6H_5), 4.52 and 4.62 (2d, J = 15 Hz, 2H, CH_2Ph), 3.70 (q, J = 7 Hz, 1H, COCH), 2.8-3.5 (m, 3H, CH-CH_2), 2.10 (deuterium oxide - exchangeable s, 1H, NH), 1.45 (d, J = 7 Hz, 3H, CH_3), 1.10 (d, J = 6 Hz, 3H, CH_3).

cis-4-Benzyl-2,6-dimethylpiperazine (VIIa).

A suspension of 0.8 g. of lithium aluminum hydride and 1.6 g. of VIa in 50 ml. of anhydrous tetrahydrofuran was refluxed under stirring for 10 hours. The mixture was worked up in the usual manner and the oily reaction product was distilled collecting 1.04 g. (70%) of the fraction boiling at 70°/0.1 mm which on standing solidified and melted at 48-50° (lit. (7) b.p. 85-86° at 0.6 mm); nmr (δ): 7.35 (broad singlet, 5H, C_6H_5), 3.52 (s, 2H, CH_2Ph), 2.95 (centered) (m, 2H, CH 2 and 6), 2.24 (deuterium oxide - exchangeable broad s, 1H, NH) 1.60 and 2.78 (2dd, J_{gem} = 11 Hz, J_{vic} = 10.5 and 3 Hz, 4H, CH_2, 3 and 5), 0.98 (d, J = 6.5 Hz, 6H, $2CH_3$).

trans-4-Benzyl-2,6-dimethylpiperazine (VIIb).

By reducing 1.7 g. of VIb with the procedure reported for VIa, 1.3 g. (82%) of VIIb was obtained an an oil distilling at 85-90°/0.5 mm; nmr (δ): 7.27 (broad s, 5H, C_6H_5), 3.43 (s, 2H, CH_2Ph), 3.17 (centered) (m, 2H, CH 2 and 6), 2.10 and 2.43 (2dd, J_{gem} = 11 Hz, J_{vic} = 6.5 and 4 Hz, 4H, CH_2 3 and 5), 2.10 (deuterium-exchangeable broad s, 1H, NH), 1.15 (d, J = 6.5 Hz, 6H, $2CH_3$).

Anal. Calcd. for $C_{13}H_{20}N_2$: C, 76.42; H, 9.86; N, 13.71. Found: C, 76.26; H, 9.70; N, 13.63.

trans-1-Amino-4-benzyl-2,6-dimethylpiperazine (Ib).

To a solution at 0° of 0.8 g. of VIIb in 10 ml. of 2*N* hydrochloric acid, an aqueous solution of 0.30 g. of sodium nitrite was added dropwise. After stirring for 2 hours at room temperature the mixture was made basic with 20% hydroxide and extracted with ether. The yellowish oily residue was distilled to give 0.72 g. of the 1-nitroso derivative (XI) b.p. 130-135° at 0.6 mm.

Anal. Calcd. for $C_{13}H_{19}N_3O$: C, 66.92; H, 8.21; N, 18.01. Found: C, 66.72; H, 8.04; N, 17.84.

Table I

Nmr Data of *cis* (Conformer **1**) and of *trans* 2,6-Dimethylpiperazine Derivatives (Conformer **2** and **3**)

Compound	Chemical shifts δ (ppm)			Coupling constants J (Hz)	
	H-3 ax	H-3 eq	H-2 ax	J_{2a3e}	J_{2a3a}
VIIa	1.60	2.78	2.7 - 3.2	3.0	10.5
Ia	1.93	2.78	2.1 - 2.6	3.0	10.0
IIa	2.22	2.88	2.5 - 3.0	3.0	11.0
	H-3 (ax ⇌ eq)	H-3 (eq ⇌ ax)	H-2 (eq ⇌ ax)	$\frac{1}{2}(J_{2e3a} + J_{2a3e})$	$\frac{1}{2}(J_{2e3e} + J_{2a3a})$
VIIb	2.10	2.43	2.95- 3.45	4.0	6.5
Ib	2.19	2.47	2.7 - 3.2	4.0	6.5
IIb	2.50	2.92	2.9 - 3.3	4.0	6.5

A solution of 0.6 g. of XI in 5 ml. of anhydrous tetrahydrofuran was added dropwise with stirring to a suspension of 0.3 g. of lithium aluminum hydride in 5 ml. of tetrahydrofuran at such a rate that the inner temperature did not rise over 40-45°. After additional stirring and refluxing for 3 hours, the reaction mixture was worked out by the usual procedure to give 0.32 g. of Ib which distilled at 95-100°/0.6 mm; nmr (δ): 7.24 (broad s, 5H, C_6H_5), 3.42 (s, 2H, CH_2Ph), 2.92 (centered) (m, 2H, CH 2 and 6), 2.70 (deuterium oxide-exchangeable broad s, 2H, NH_2), 2.19 and 2.47 (2dd, J_{gem} = 11 Hz, J_{vic} = 6.5 and 4.4 Hz, CH_2 3 and 5), 1.08 (d, J = 6.5, 6H, $2CH_3$).

Anal. Calcd. for $C_{13}H_{21}N_3$: C, 71.19; H, 9.65; N, 19.16. Found: C, 71.00; H, 9.88; N, 18.93.

trans-2,6-Dimethylpiperazine (IIb).

A solution of 0.8 g. of VIIb in 10 ml. of ethanol was hydrogenated at 3 atmospheres over 0.25 g. of 10% palladium-on-carbon. After 3 hours the catalyst was filtered and the filtrate was distilled at atmospheric pressure collecting 0.38 g. of IIb boiling at 130-135°; nmr (δ): 3.03 (centered) (m, 2H, CH 2 and 6), 2.50 and 2.92 (2dd, J_{gem} = 12 Hz, J_{vic} = 6.5 and 4Hz, 4H, CH_2 3 and 5), 2.10 (deuterium oxide-exchangeable s, 2H, 2NH), 1.12 (d, J = 6.5 Hz, 6H, 2 CH_3).

Anal. Calcd. for $C_6H_{14}N_2$: N, 24.53. Found: N, 24.31.

When exposed to air IIb is rapidly transformed into *the carbonate* $(C_6H_{14}N_2)_2 \cdot H_2O$, m.p. 83-86°.

Anal. Calcd. for $C_{13}H_{32}N_4O_4$: C, 50.62; H, 10.36. Found: C, 50.95; H, 10.18.

The dihydrochloride is hygroscopic. After drying at 80° and 0.1 mm over phosphorus pentoxide it decomposed at 222-225°.

Anal. Calcd. for $C_6H_{16}Cl_2N_2$: C, 38.54; H, 8.62; N, 14.97; Cl, 37.90. Found: C, 38.31; H, 8.75; N, 14.81; Cl, 37.87.

For the sake of comparison we also recorded the nmr spectrum of the now commerically available *cis*-2,6-dimethylpiperazine, m.p. 115-116° (ether) (IIa), never before reported in full detail (7); nmr (δ): 2.75 (centered) (m, 2H, CH 2 and 6), 2.22 and 2.88 (2dd, J_{gem} = 12 Hz, J_{vic} = 11 and 3 Hz, CH_2 3 and 5), 1.40 (deuterium oxide-exchangeable s, 2H, 2HN), 0.95 (d, J = 6 Hz, 6H, $2CH_3$).

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