

Facile Synthesis of N-Permethylspermine and N-Permethylspermidine from their Unmethylated Precursors

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A rapid synthesis of the title compounds employing formaldehyde and sodium borohydride in acidic aqueous solution in combination with the unmethylated amines is described. High yields and the characteristics of the methylated amines allow the application of the method to gas chromatographic and mass spectrometric analyses.

Although a few N-permethylated α,ω -diamines showed some neuromuscular blocking¹ and hypotensive² activity, it is surprising to find that no published report is yet available about the N-permethyl derivatives of the biologically important polyamines spermine (**1**) and spermidine (**2**). Moreover, only one synthesis was described for the N-permethylspermine (**3**), but the compound has received a scant characterization³. N-Permethylspermidine (**4**) is not known from the literature.

We wish to report here a straightforward and unexpensive method of preparation of both **3** and **4** from the unmethylated precursors in excellent yields. Our synthesis follows an early suggestion made by BIEMANN⁴ for the N-methylation of protein free amino groups.

A typical preparative procedure is as follows: Sulphuric acid⁵ (13.2 mmol, *ca.* 3 M) was added to the amine⁶ to be methylated (2.23 mmol). Formaldehyde (40% in water, 2.5 ml, *ca.* 33 mmol) was added at room temperature to the solution kept in Erlenmeyer flask (50 ml) equipped with a 2 cm magnetic bar to provide adequate stirring. Solid sodium borohydride⁷ (21 mf) was added slowly portionwise during 20 min with temperature control (10–20 °C), then the reaction mixture was made strongly acidic with 3 M sulphuric acid^{5, 8}, extracted with ether (2 × 3 ml), made strongly basic with solid potassium hydroxide and reextracted with ether (5 × 10 ml). Average yields are above 80%

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for **3** and above 90% for **4**, as determined by gas-chromatography⁹. If pure starting materials were used, the reaction products are chromatographically homogeneous. Pure amines (**3**) and (**4**) may be isolated from their dried (Na₂SO₄) ether solutions after evaporation of the solvent by vacuum distillation. In the described small preparation, we distilled the amines from a microapparatus on an oil bath at controlled temperature¹⁰.

The infrared spectra of **3** and **4** are practically identical (Fig. 1), as well as the proton magnetic resonance spectra with the obvious exclusion of the integral values. All the methyl protons locations coincide in deuteriochloroform, but the protons of the lone methyls may be separated from those of the geminal methyls in the spectrum by addition of excess trifluoroacetic acid, as shown in Fig. 2. The mass spectra of **3** and **4** exhibit weak, but distinct parent ions, with a complex fragmentation pattern dominated by the ion at *m/e* 58 at 70 eV. At lower ionization energies (15–20 eV) several transitions are enhanced and the parent ions are relatively intense (Fig. 3).

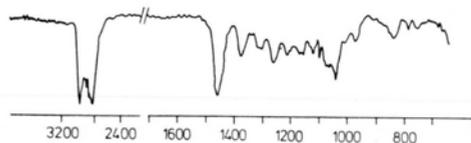


Fig. 1. The infrared spectrum of N-permethylspermine (**3**), recorded as a neat liquid between NaCl windows.

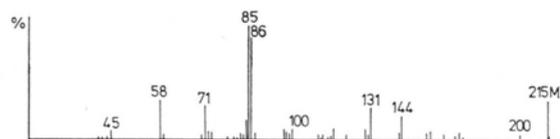


Fig. 2. Mass spectrum of N-permethylspermidine (**4**). Electron energy 15 eV. Chamber temp. 270 °C.

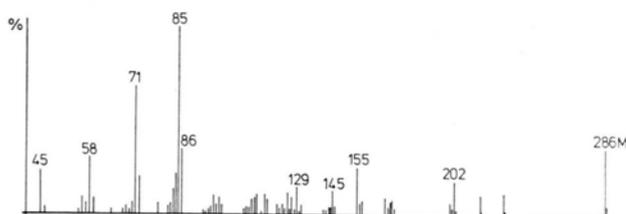


Fig. 3. Mass spectrum of N-permethylspermine (**3**). Electron energy 15 eV. Chamber temp. 270 °C.

The fact that the N-permethyl derivatives (**3**) and (**4**) of spermine (**1**) and spermidine (**2**) can be easily prepared in near to quantitative yields in aqueous solution, from which a standard acid-base separa-

tion works beautifully, coupled with the excellent gaschromatographic and mass spectrometric properties they exhibit not only in comparison with the familiar *N*-perfluoroacetyl derivatives¹¹, but also with the free amines¹², and the stability against water and oxygen, makes them superior candidates

for an accurate and fast qualitative and quantitative method of determination of **1** and **2** in biological fluids, a need strongly felt nowadays in biological laboratories¹².

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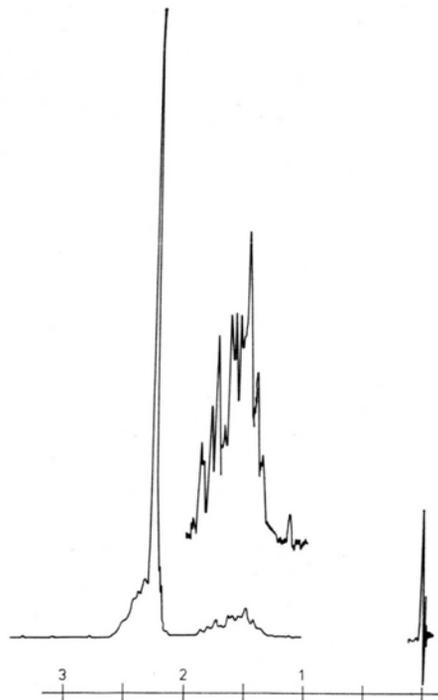


Fig. 4. The proton magnetic resonance spectrum of *N*-permethylspermine in deuteriochloroform at 60 MHz. The large singlet is due to the methyl protons resonances, which are partially superimposed on the *N*-methylene protons resonances. Different methylene protons are indistinguishable. The peaks for the C-methylene protons are also shown at higher gain. The peak to the right is due to tetramethylsilane, an internal standard.

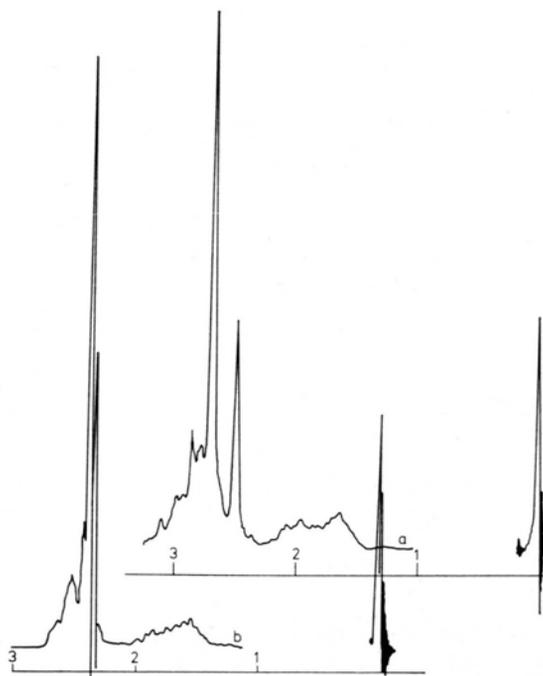


Fig. 5. Curve **a** shows the effect of the addition of excess trifluoroacetic acid to a deuteriochloroform solution of *N*-permethylspermine (**3**) on the proton magnetic resonance spectrum, where the two types of methyl groups are neatly distinct. A lesser amount of the acid (curve **b**) causes a smaller separation of the methyl resonances.

¹ C. J. CAVALLITO, A. P. GRAY, and E. E. SPRINNER, *J. Amer. Chem. Soc.* **76**, 1862 [1954].

² A. P. PHILLIPS, *J. Amer. Chem. Soc.* **77**, 1693 [1955].

³ H. W. DUDLEY, O. ROSENHEIM, and W. W. STARLING, *Biochem. Journ.* **20**, 1086 [1926].

⁴ K. BIEMANN, "Applications to Natural Products and Other Problems in Organic Chemistry" in "Mass Spectrometry of Organic Ions" by F. W. McLAFERTY, Academic Press, New York 1963. No experimental details are given.

⁵ Hydrochloric acid should be avoided owing to the potential formation of the extremely toxic and volatile *α,α'*-dichlorodimethylether.

⁶ The free unmethylated amines absorb water and carbon dioxide from air very quickly.

⁷ This reagent is moderately unstable in acidic water solutions giving off hydrogen: the reaction must therefore be performed away from flames and sparks in a well ventilated hood.

⁸ Acidification and ether extraction at this point of the work-up is necessary to get rid of a volatile boron compound which codistilled with the amine, infrared bands at 2220–2500 cm^{-1} (B–H bond stretching).

⁹ The choice of the column packing is not at all critical for the methylated derivatives. We used *e.g.* a 1 m by 0.25 cm copper column packed with Carbowax 20 M (10%)–KOH (5%) on 80–100 mesh Chromosorb W, operating at 140–170 °C, with a flow rate of 15 ml/min (nitrogen). The injector temperature was kept at 300 °C.

¹⁰ The pressure was *ca.* 0.15 torr; **3** distilled with the oil at *ca.* 140 °C, **4** at *ca.* 70 °C.

¹¹ T. WALLE, in "Polyamines in Normal and Neoplastic Growth", edited by D. H. RUSSELL, p. 355, Raven Press, New York 1973.

¹² See the book quoted in ref. 11 and S. S. COHEN, "Introduction to the Polyamines", Prentice-Hall, New Jersey 1971.