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Synthesis and Properties of Trimethylsilyl Derivatives of Vitamin B_6 (1)

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In our studies on the biosynthesis of vitamin B_6 in yeast (2), we are confronted with the problem of a fairly rapid assay of the different forms (known and unknown) of this nutrient that are excreted into the culture medium. The existing assay procedures for vitamin B_6 are subject to at least one of several restrictions. They are time consuming, incapable of distinguishing between the three forms of the vitamin (pyridoxol, pyridoxal, and pyridoxamine), or preclude the regeneration and reisolation of the material after analysis.

The advent of gas-liquid chromatography has provided a powerful technique that potentially could be as valuable for the detection and quantitative determination of the various forms of vitamin B_6 as it has proved to be for other biochemical substances. However, due to their low volatility, the various forms of vitamin B_6 cannot be chromatographed directly. Very recently Prosser and Sheppard (3) reported the successful separation of the acetates of the three forms of vitamin B_6 by gas-liquid chromatography. In an attempt to find derivatives more suitable for subjection to gas chromatography with subsequent regeneration and isolation, we prepared the trimethylsilyl ethers of pyridoxol (I), pyridoxal (II), and pyridoxamine (III) (4). These are abbreviated pyridoxol-TMS, pyridoxal-TMS, and pyridoxamine-TMS respectively (Fig. 1).

Preliminary experiments indicate that the above derivatives can be readily separated by gas-liquid chromatography and should lend themselves to determinations of vitamin B_6 in pharmaceuticals and possibly in foodstuffs. When subjected to gas-liquid chromatography, each trimethylsilyl ether gave a single symmetrical peak. Retention times of 2.5, 4.0, and 4.5 minutes from the air peak were observed for pyridoxal-TMS, pyridoxol-TMS, and pyridoxamine-TMS respectively. After each sample was injected, it was collected at the exit port of the gas chromatograph. Its infrared and ultraviolet spectra were run and compared with infrared and ultraviolet spectra of the injected material. In all cases, spectra of material collected from the instrument were identical to those of material originally injected.

Trimethylsilyl ethers were prepared in a manner similar to that reported by Langer *et al.* (5). Similar yields of product were obtained when either the hydrochloride or free base of pyridoxol was used as the starting material.

The structures of the trimethylsilyl ethers were confirmed with the aid of their proton magnetic resonance spectra (Table I). The assignments of the 2-CH_3, 4-CH_2, 5-CH_2, and 6-H peaks were made on the basis of literature values (6-8). The eighteen proton peak at 0.13 p.p.m. in the trimethylsilyl ether of pyridoxol was taken to be due to the α^4-*O*-trimethylsilyl and α^5-*O*-trimethylsilyl protons having the same chemical shift. The nine proton peak at 0.27 p.p.m. would then be due to the 3-*O*-trimethylsilyl protons. Using this compound as a basis of comparison, it was possible to make all the assignments of TMS protons shown in Table I, except those of the α^4-*N* and α^5-*O* trimethylsilyl protons of pyridoxamine-TMS. These last two assignments were made by comparing the spectrum of the trimethylsilyl ether of pyridoxamine with those of similar derivatives (9). It was noted that the two protons of the 5-CH_2 group of pyridoxal-TMS comprised an AB system due to the magnetic nonequivalence of the two hydrogens when the ring is formed (10).

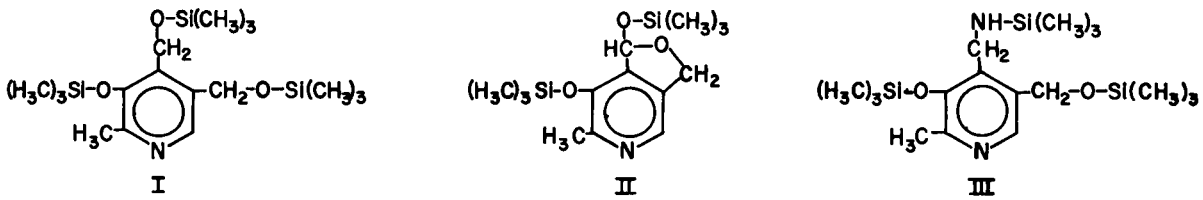

Figure 1. Structures of trimethylsilyl derivatives of vitamin B_6.

TABLE I

Proton Magnetic Resonance Spectra of Trimethylsilyl Ethers of Vitamin B_6 in $CDCl_3$ (a)

Compound	2-CH_3	4-CH_2	5-CH_2	6-H	α^4-*O*-TMS	α^5-*O*-TMS	3-*O*-TMS	Other
Pyridoxol-TMS	2.47	4.85	4.72	8.25	0.13	0.13	0.27	
Pyridoxamine-TMS	2.47	3.93 (b)	4.80	8.03		0.15	0.30	0.07 (α^4-*N*-TMS)
Pyridoxal-TMS	2.48		4.97	8.12			0.30	6.43 (d) (Acetal-H)
			5.18 (c)					0.22 (Acetal-*O*-TMS)

(a) Expressed in p.p.m. (delta) units with tetramethylsilane internal standard. (b) Doublet (J = 8 c.p.s.) which collapsed to singlet upon being shaken with deuterium oxide, therefore, split by exchangeable hydrogen on α^4-nitrogen. (c) AB doublets (J = 12.7 c.p.s.). (d) Doublet (J = 1.4 c.p.s.).

TABLE II

Analyses and Ultraviolet Spectral Properties of Trimethylsilyl Ethers of Vitamin B_6 (a)

Compound	Formula	Boiling Range at 0.01 mm Hg	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found	λ max (mμ)	E max
Pyridoxol-TMS	$C_{17}H_{35}NO_3Si_3$	95-96	52.93	53.20	9.15	9.39	3.63	3.69	280	4.9×10^3
Pyridoxamine-TMS	$C_{17}H_{36}N_2O_2Si_3$	110-112	52.72	53.07	9.67	9.43	7.63	7.28	275	4.5×10^3
									306(s)	3.8×10^2
Pyridoxal-TMS	$C_{14}H_{35}NO_3Si_2$	93-95	53.45	53.72	8.97	8.81	4.45	4.58	273	4.1×10^3

(a) Ultraviolet spectra measured in spectrograde 2,2,4-trimethylpentane.

Integration of spectra tended to confirm all assignments.

The observations made in this study indicate that when elucidating the structures of compounds similar to vitamin B_6, it might prove worthwhile to convert them to their trimethylsilyl derivatives and identify hydroxyl and amino groups by the locations of their trimethylsilyl protons in a p.m.r. spectrum.

EXPERIMENTAL

Ultraviolet spectra were determined with a Cary recording spectrometer, model 11M. Spectrograde 2,2,4-trimethylpentane, obtained from Distillation Products Industries was used as the solvent for all ultraviolet analyses. Proton magnetic resonance spectra were obtained using a Varian A-60 instrument at 60 Mc.p.s. Compounds were used as 30% solutions in deuterated chloroform. Results are expressed in delta units from tetramethylsilane as internal standard. Microanalyses were performed by the Clark Microanalytical Laboratories, Urbana, Illinois. Boiling points were measured as the materials were being distilled and are uncorrected.

Gas chromatography was performed on a Barber-Colman model 5340 gas chromatograph equipped with a thermal conductivity detector. A 6 foot x 1/4 inch copper column packed with 15% Dow-Corning high vacuum grease on 60-80 mesh Gas Chrom Q was used. Excellent results were obtained with a column temperature of 200°, a detector temperature of 220°, and an injector temperature of 220°. Carrier gas (helium) was used at a flow rate of 80 ml./min.

Trimethylsilyl Ethers:

In a typical experiment, 20 ml. of hexamethyldisilazane were added to 3 g. of the appropriate form of vitamin B_6 hydrochloride or free base. A few drops of trimethylchlorosilane were added, and the reaction flask fitted with a condenser. A drying tube containing indicating Drierite was placed thereon and the mixture subjected to heating at a bath temperature of about 135°. Although the free base of pyridoxol was sufficiently soluble in the hexamethyldisilazane to form a solution at the outset, none of the parent hydrochlorides were soluble. They dissolved as they reacted. An additional white precipitate of ammonium chloride soon formed, but slowly sublimed from the reaction mixture onto the condenser. Heating was continued for about one hour past the time the solution appeared clear. This was taken to be the end of the reaction (about 3 hours). The flask was then transferred to a distillation apparatus and the unreacted hexamethyldisilazane removed at atmospheric pressure. The residue was distilled at 0.01 mm. Hg. The trimethylsilyl ethers of pyridoxal and pyridoxamine were isolated in 85% yields; that of pyridoxol in 97% yield. The pyridoxamine derivative was completely colorless, while the other two were slightly yellow. All three were clear liquids which started to hydrolyze to bright yellow solids with about one minute exposure to atmospheric moisture.

Pyridoxol Free Base:

Ten grams of pyridoxol hydrochloride were suspended in 300 ml. of anhydrous diethyl ether. Anhydrous ammonia was bubbled through the mixture for about five minutes, allowing the ammonia to agitate the suspension, insuring complete reaction between the gas and the pyridoxol hydrochloride. It was noted that the volume of the white insoluble material had about doubled. The reaction mixture was then filtered. The residue was placed in a soxhlet extractor and extracted for 96 hours with 500 ml. of anhydrous diethyl ether. Due to its

limited solubility in the ether, the pyridoxol free base precipitated from solution as it was extracted. At the end of 24, 48, and 72 hours of extraction, the contents of the extraction thimble were removed, spread out on a glass sheet to dry, mixed, returned to the thimble, and the extraction continued. This procedure appeared to increase the rate of extraction, thus increasing the yield of final product. At the end of 96 hours, the precipitate of pyridoxol free base, in the form of colorless needles, was filtered and dried. Analysis showed it to be free of contaminating ammonium chloride and pyridoxol hydrochloride; m.p. 159-160°; (lit. 160°); yield 7.6 g. (92.5%).

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