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NMR SPECTRA OF TRIMETHYLSILYL ETHERS OF FLAVONOID GLYCOSIDES*

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Abstract—NMR spectra including signal assignments are presented for the trimethylsilyl ethers of five flavonoid glycosides; quercitrin, rutin, hesperidin, naringin and tectoridin, three flavonoid aglycones: luteolin, morin and hesperetin and two sugars: glucose and rhamnose.

INTRODUCTION

WE WISH to present the first NMR spectra of trimethylsilyl ethers of flavonoid glycosides. Since most flavonoids occur naturally as glycosides it was of interest to investigate the potential of NMR spectroscopy for structure analysis in this class of compounds. Although Waiss, Lundin and Stern[1] reported preliminary NMR data for trimethylsilyl ethers of some flavonoid aglycones, we came independently to the domain and now describe our results.

In addition to the NMR spectra of the trimethylsilyl ethers of glycosides, spectra are presented for three trimethylsilyl derivatives of aglycones not previously reported. For comparison, rhamnose and glucose trimethylsilyl derivatives are included since these sugars often occur in flavonoid glycosides.

The low solubility of most flavonoid glycosides in the organic solvents commonly used has limited the application of NMR spectroscopy, in most instances, to methoxy and acetyl derivatives[2] neither of which is suitable for all members of this class of natural products. Previously trimethylsilyl ethers of numerous substances including sugars,[3–5] phenolserinols,[6]

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[1] A. C. WAISS, Jr., R. E. LUNDIN and D. J. STERN, *Tetrahedron Letters* No. 10, 513 (1964).

[2] Leading references: (a) J. MASSICOT, J.-P. MARTHE and S. HEITZ, *Bull. Soc. Chim. France* 2712 (1963); J. MASSICOT and J.-P. MARTHE, *Bull. Soc. Chim. France* 1962 (1962); (b) J. W. CLARK-LEWIS and L. M. JACKMAN, *Proc. Chem. Soc.* 165 (1961); J. W. CLARK-LEWIS, T. M. SPOTSWOOD and L. R. WILLIAMS, *Proc. Chem. Soc.* 20 (1963); J. W. CLARK-LEWIS, L. M. JACKMAN, and L. R. WILLIAMS, *J. Chem. Soc.* 3858 (1962); J. W. CLARK-LEWIS and G. F. KATEKAR, *J. Chem. Soc.* 4502 (1962); J. W. CLARK-LEWIS, T. M. SPOTSWOOD and L. R. WILLIAMS, *Australian J. Chem.* **16**, 107 (1963); (c) C. P. LILLYA, S. E. DREWES and D. G. ROUX, *Chem. & Ind. (London)* 783 (1963); (d) D. H. S. HORN and J. A. LAMBERTON, *Chem. & Ind. (London)* 691 (1963); (e) L. CROMBIE and D. A. WHITING, *J. Chem. Soc.* 775 (1962); (f) S. F. DYKE, W. D. OLLIS and M. F. SAINSBURY *Proc. Chem. Soc.* 179 (1963); (g) E. J. COREY, E. M. PHILBIN and T. S. WHEELER, *Tetrahedron Letters* No. 13, 429 (1961).

[3] (a) C. C. SWEELEY, R. BENTLEY, M. MAKITA and W. W. WELLS, *J. Am. Chem. Soc.* **85**, 2497 (1963); (b) C. C. SWEELEY, R. BENTLEY, M. MAKITA and W. W. WELLS, *Biochem. Biophys. Res. Comm.* **11**, 14 (1963).

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[5] R. J. FERRIER and M. F. SINGLETON, *Tetrahedron* **18**, 1143 (1962).

[6] P. D. SHAW, *Anal. Chem.* **35**, 1580 (1963).

sterols,[7–9] aromatic amines[10] and flavonoids[11] were described but were used mostly for gas chromatographic analysis. We used, in modified form, the trimethylsilylation procedure described for the preparation of volatile carbohydrate derivatives[3,4] which employs simpler and milder reaction conditions than those used by the above-mentioned workers.

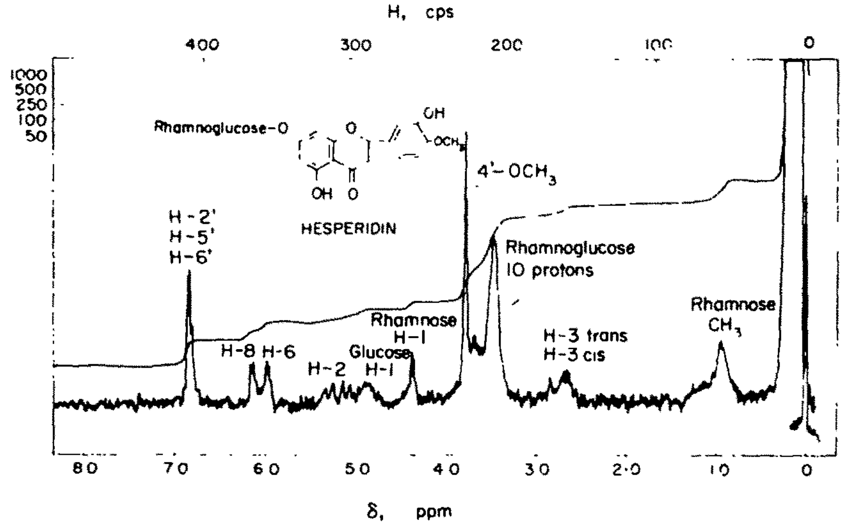

FIG. 1. NMR SPECTRUM OF SILYLATED HESPERIDIN.

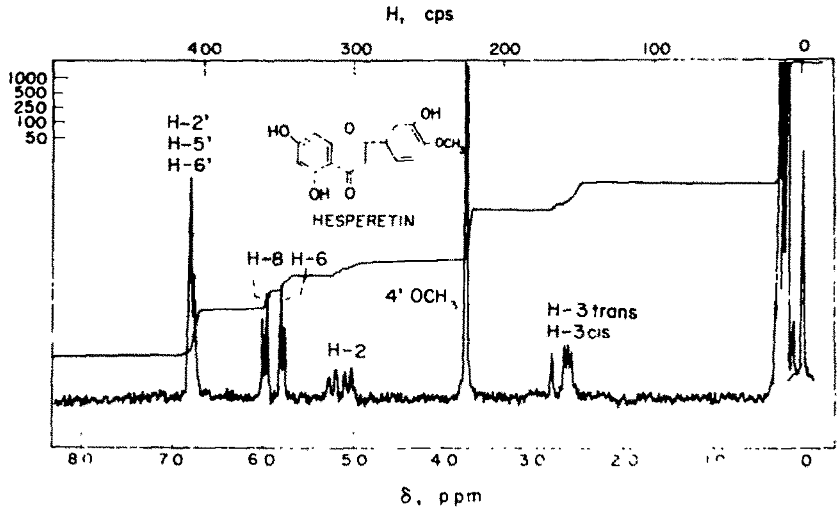

FIG. 2. NMR SPECTRUM OF SILYLATED HESPERETIN.

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[7] T. LUUKKAINEN, W. J. A. VANDENHEUVEL, E. O. A. HAAHTI and E. C. HORNING, *Biochim. et Biophys. Acta* **52**, 599 (1961).

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[10] N. P. SEN and P. L. MCGEER, *Biochem. Biophys. Res. Commun.* **13**, 390 (1963).

[11] (a) F. A. HENGLEIN and J. KRÄMER, *Chem. Ber.* **92**, 2585 (1959); (b) E. VON RUDLOFF, *J. of G.C.* **2**, 89 (1964).

RESULTS AND DISCUSSION

A typical flavonoid can be converted to a trimethylsilyl ether and prepared for NMR analysis in about 20 min; all the ethers thus far examined were soluble in carbon tetrachloride and the flavonoid could readily be regenerated under mild conditions. In contrast to methoxy

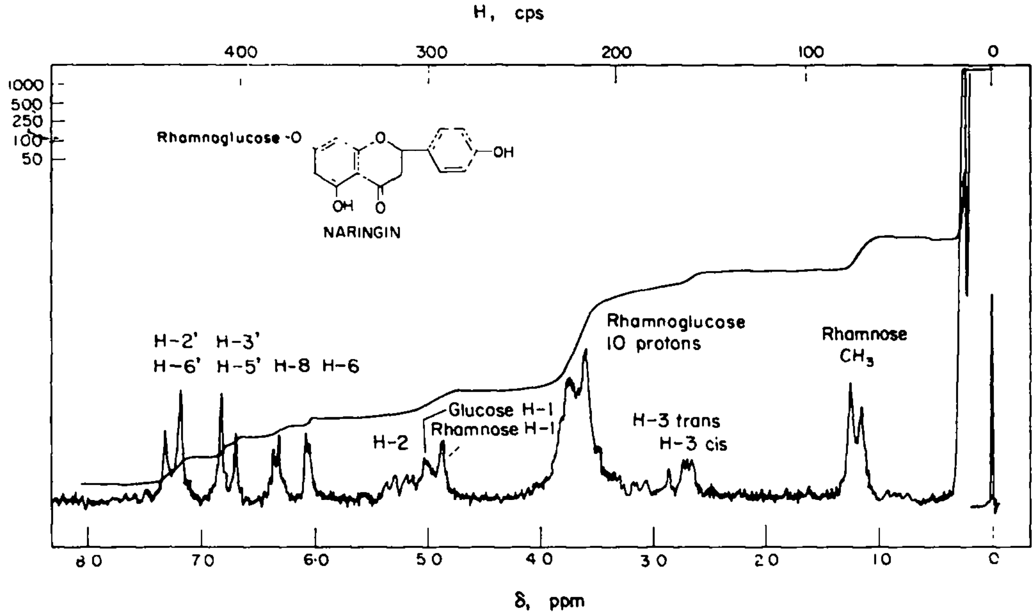


FIG. 3. NMR SPECTRUM OF SILYLATED NARINGIN.

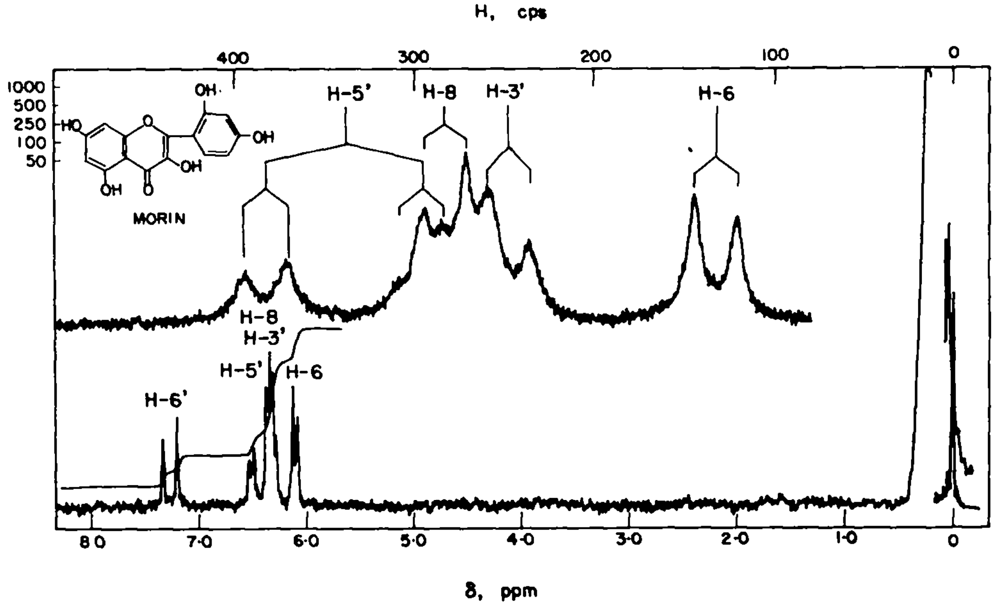


FIG. 4. NMR SPECTRUM OF SILYLATED MORIN.

and acetyl derivatives of flavonoids, the NMR signals arising from the protons of the trimethylsilyl groups occur out of the absorption region of protons in flavonoids, coming at about ±0·2 ppm from an external reference sample of TMS in CCl_4. The trimethylsilyl groups, in one sense, serve as an internal standard.

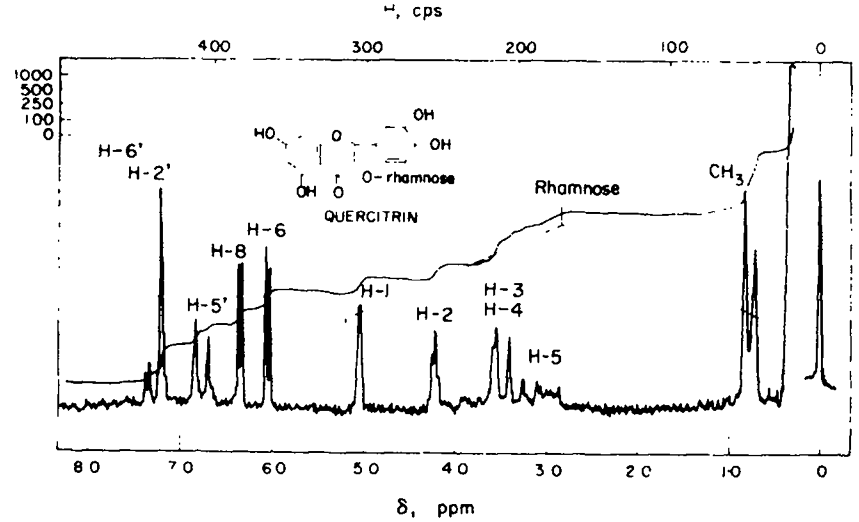


Fig. 5. NMR spectrum of silylated quercitrin.

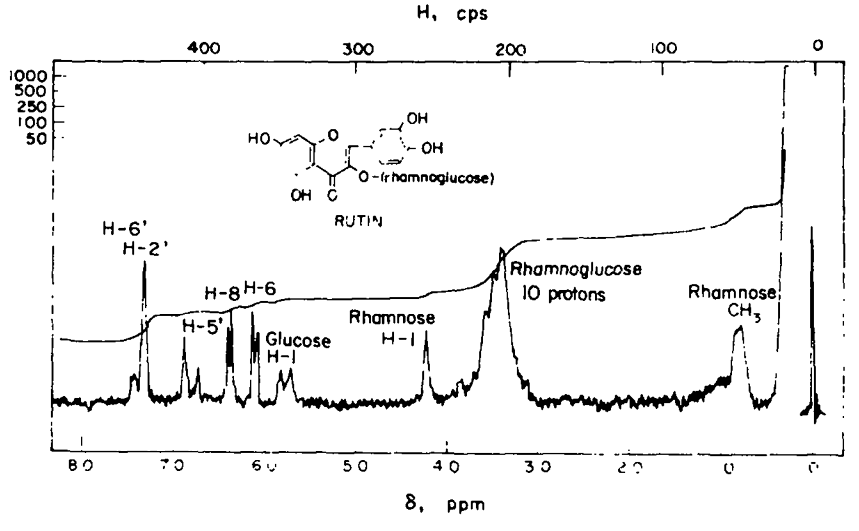


Fig. 6. NMR spectrum of silylated rutin.

The integration of the NMR spectra of the trimethylsilyl ethers of flavonoids indicated that all hydroxyl groups in both the aglycones and glycosides had reacted. If the sirup obtained on drying the derivative was allowed to stand exposed to the atmosphere, occasionally the silyl group at C-5 was hydrolyzed. The C-5 hydroxyl group was readily detectable by NMR since the proton, which is hydrogen-bonded to the C-4 keto group, gives a singlet near 13 ppm.

As previously observed[1,2] the C-6 and C-8 protons of the *A*-ring of flavonoids give rise to two doublets ($J = 2{\cdot}5$ c.p.s. for the meta spin–spin interaction) usually between 6 to 6·5 ppm when, as is often the case, the other positions of ring *A* are substituted. The signals from the *B*-ring protons normally absorb downfield around 6·7 to 7·5 ppm.

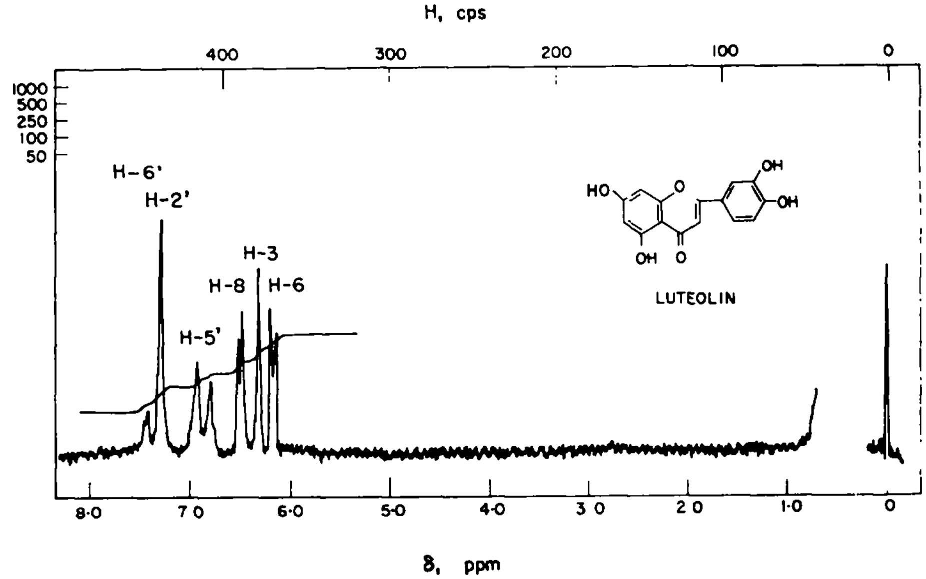


FIG. 7. NMR SPECTRUM OF SILYLATED LUTEOLIN.

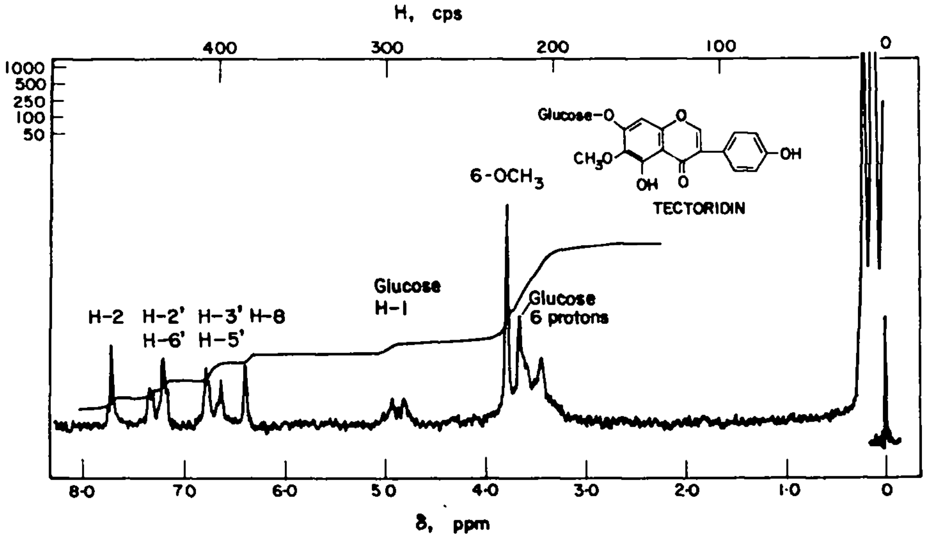


FIG. 8. NMR SPECTRUM OF SILYLATED TECTORIDIN.

Flavonoid classes are differentiated according to the oxidation level and substitution of ring *C*, and considerable variation is generally found for the chemical shifts of the ring *C* protons among the several flavonoid classes. For example, the C-3 proton in flavones gives a sharp singlet near 6·3 ppm while the C-2 proton of isoflavones is observed downfield at about 7·7 ppm. The C-2 proton in flavanones, in which ring C is, in part, saturated, is split by the C-3 protons into a quartet ($J_{cis} \cong 5$ c.p.s., $J_{trans} \cong 11$ c.p.s.) and occurs near 5·2 ppm. The C-3 protons occur as two doublets around 2·6 ppm.

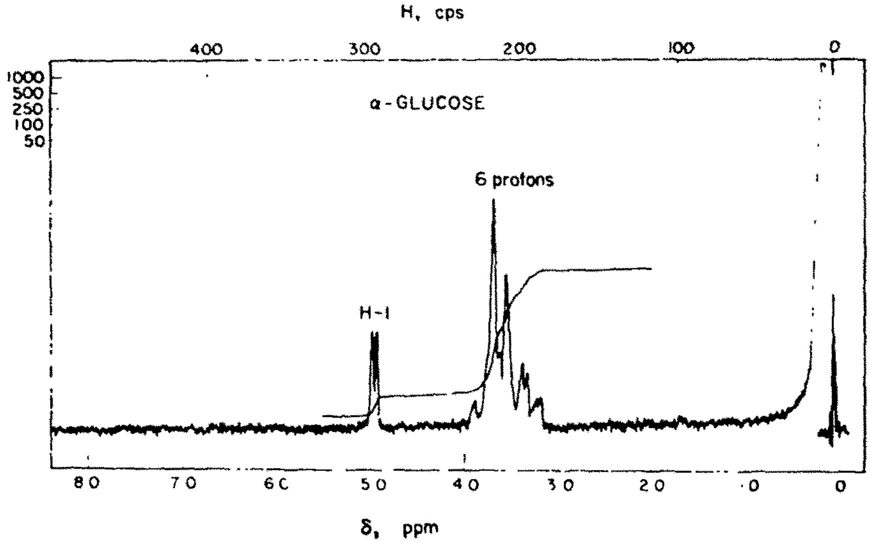


FIG. 9. NMR SPECTRUM OF SILYLATED GLUCOSE.

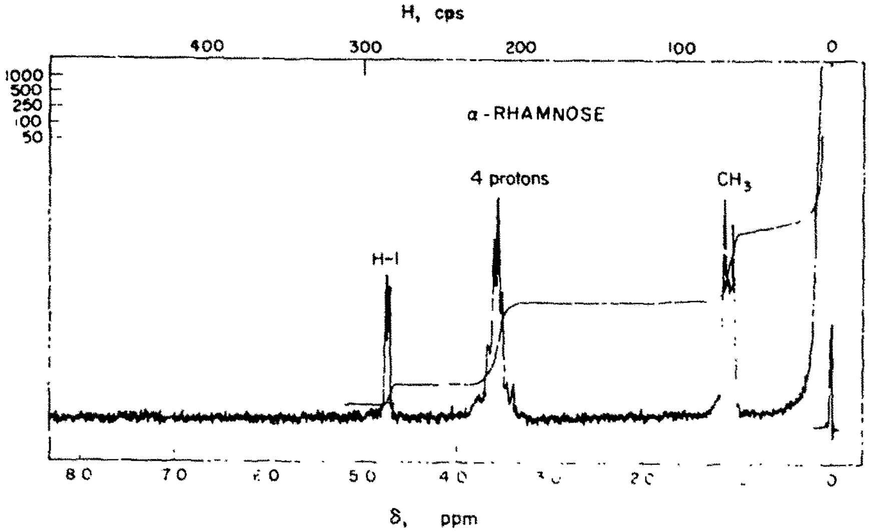
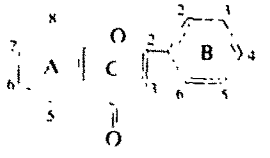


FIG. 10. NMR SPECTRUM OF SILYLATED RHAMNOSE.

The NMR spectra of the trimethylsilyl derivatives of flavonoids are presented with signal assignments for the protons using the numbering scheme shown for flavone (I) in Figs. 1–18.



(I)

EXPERIMENTAL

In a typical experiment with a flavonoid glycoside, rutin, the following conditions were employed: Fifty mg of rutin was dissolved in 3 ml of pyridine and treated successively with 0·5 ml of hexamethyldisilazane and 0·5 ml of trimethylchlorosilane. Excess of the solvent and reagents was immediately removed under vacuum and the residue extracted with carbon tetrachloride. A clear carbon tetrachloride solution was obtained by filtering off the salts. The flavonoid glycoside could be regenerated unaltered by allowing the trimethylsilyl ether to stand overnight in 50 ml of 20% aqueous methanol. Often the flavonoid crystallized directly; otherwise the substance was recovered by removing the solvent under vacuum.

All the NMR spectra were obtained in CCl_4, and were recorded versus an external reference sample of the tetramethylsilane in CCl_4. Similar results were observed with internal tetramethylsilane.The sweep time, sweep offset and sweep width were 500 sec, 0 c/s and 500 c/s respectively in all cases. The R.F. field was 0·16 mG for Figs. 1, 3, 4, 6, 9, and 10; 0·14 mG for Fig. 2; and 0·12 mG for the rest. The filter bandwidth was 0·4 c/s for all but Figs. 2, 9 and 10 where 1·0 c/s was used.

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