as those for the analogous carbons in compounds **3** and **1**. Thus for the methine carbon $(1 - x)(50.7\ \text{Hz}) = x(-8.2\ \text{Hz}) = 38.5$ Hz, and for the methyl carbon $(1 - x)(-9.2\ \text{Hz}) + x(50\ \text{Hz}) = 0$ Hz, giving estimates of 0.2 and 0.16 for the mole fraction (x) of the minor form.
(9) M. Witanowski and G. A. Webb, "Nitrogen NMR", Plenum Press, New York, 1973.
(10) I. G. Csizmadia, A. H. Cowley, M. W. Taylor, and S. Wolfe, *J. Chem. Soc., Chem. Commun.*, 432 (1974); A. H. Cowley, M. W. Taylor, M.-H. Whangbo, and S. Wolfe, *ibid.*, 838 (1976).
(11) E. D. Morris, Jr., and C. E. Nordman, *Inorg. Chem.*, **8**, 1672 (1969).
(12) A. Grand, J. B. Robert, and A. Filhol, *Acta Crystallogr., Sect. B*, **33**, 1526 (1977), and references cited therein.
(13) K. Paxton and T. A. Hamor, *J. Chem. Soc., Dalton Trans.*, 647 (1978), and references therein.
(14) W. B. Jennings, D. R. Boyd, C. G. Watson, E. D. Becker, R. B. Bradley, and D. M. Jerina, *J. Am. Chem. Soc.*, **94**, 8501 (1972), and references cited therein.
(15) M.-P. Simonnin, C. Charrier, and R. Burgada, *Org. Magn. Reson.*, **4**, 113 (1972).
(16) H. Boudjebel, H. Goncalves, and F. Mathis, *Bull. Soc. Chim. Fr.*, 628 (1975).
(17) J. Burdon, J. C. Hotchkiss, and W. B. Jennings, *J. Chem. Soc., Perkin Trans. 2*, 1052 (1976).
(18) For a detailed discussion of the possible conformations of phospholane ring systems see ref 4g.
(19) P. Haake, J. P. McNeal, and E. J. Goldsmith, *J. Am. Chem. Soc.*, **90**, 715 (1968).
(20) J.-P. Albrand, D. Gagnaire, J. Martin, and J.-B. Robert, *Org. Magn. Reson.*, **5**, 33 (1973).
(21) G. Y. Schultz, I. Hargittai, J. Martin, and J.-B. Robert, *Tetrahedron*, **30**, 2365 (1974).
(22) M. Revel, J. Roussel, J. Navech, and F. Mathis, *Org. Magn. Reson.*, **8**, 406 (1976).
(23) One must be a little cautious here since the cyclic methylene carbon can also couple to the phosphorus by a three-bond route, and this additional pathway could contribute to the $^2J_{PNC}$. However, the observation that the coupling constant is precisely equal to that for the exocyclic NC suggests that the three-bond route is not important.
(24) G. A. Gray and T. A. Albright, *J. Am. Chem. Soc.*, **98**, 3857 (1976).
(25) J.-P. Albrand, H. Faucher, D. Gagnaire, and J.-B. Robert, *Chem. Phys. Lett.*, **38**, 521 (1976); J.-P. Albrand, A. Cogne, and J.-B. Robert, *ibid.*, **42**, 498 (1976); J.-P. Albrand, J.-B. Robert, and H. Goldwhite, *Tetrahedron Lett.*, 949 (1976).
(26) A. H. Cowley, J. H. Schweiger, and S. L. Manatt, *Chem. Commun.*, 1491 (1970).
(27) D. W. W. Anderson, J. E. Bentham, and D. W. H. Rankin, *J. Chem. Soc., Dalton Trans.*, 1215 (1973).
(28) F. Ramirez, A. U. Patwardham, H. J. Kugler, and C. P. Smith, *J. Am. Chem. Soc.*, **89**, 6276 (1967).
(29) J. H. Hargis and W. D. Alley, *J. Am. Chem. Soc.*, **96**, 5927 (1974).

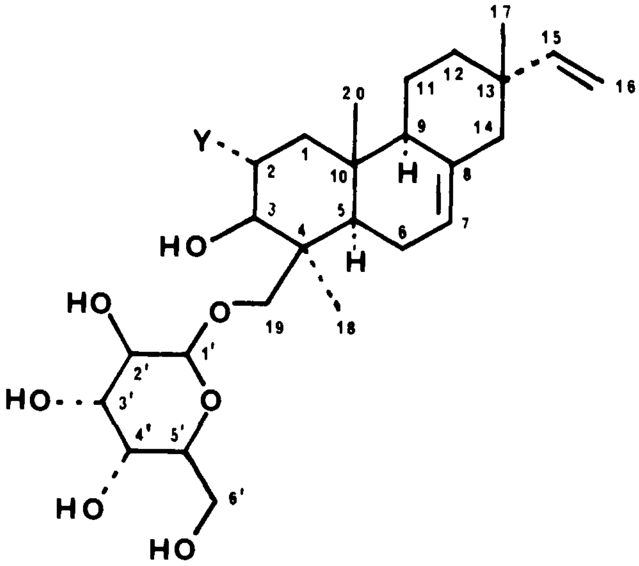
An Unusual Solvent Dependence of the Carbon-13 Nuclear Magnetic Resonance Spectral Features of Some Glycosides as Studied by Relaxation-Time Measurements[1]

Nera Bellavita,[2a] Jean-Marie Bernassau,[2b] Paolo Ceccherelli,[2a] Muppala S. Raju,[2b] and Ernest Wenkert*[2b]

Contribution from the Istituto di Chimica Organica, Facolta di Farmacia, Università degli Studi, 06100 Perugia, Italy, and the Department of Chemistry, Rice University, Houston, Texas 77001. *Received November 6, 1978*

Abstract: The relaxation times of the carbon centers of virescenoside A and B, glycoside metabolites of the mushroom *Oospora virescens* (Link) Wallr., have been determined for different deuteriochloroform-methanol solvent mixtures. In the absence of methanol the ^{13}C NMR spectra show broad lines, corresponding to long correlation times and the presence of hydrogen-bonded intermolecular complexes. The methyl groups show a relationship between relaxation time and steric constraint, and the vinyl side chain reveals a connection of the T_1 values of its two carbons with its unique geometry.

The metabolites of the mushroom *Oospora virescens* (Link) Wallr. are isopimaradienic altrosides, the ^{13}C NMR spectral analysis of some aglycones of which have been reported.[3] Even though thus the carbon shifts of the aglycones virescenol A and B are on record,[3] their glycosides virescenoside A (**1a**) and B (**1b**) had to be ignored heretofore in view of their unusual spectral behavior. As Figure 1 indicates, the proton-decoupled ^{13}C NMR spectrum of a deuteriochloroform solution of virescenoside A is characterized by many broad, diffuse signals which sharpen into the customary singlets of narrow line width upon the addition of ca. 20 molar equiv of methanol. In order to gain insight into this phenomenon, it was decided to carry out a systematic study of the solvent dependence of the ^{13}C NMR spectral characteristics. Since the carbon relaxation times appeared to be a good NMR parameter for the assessment of the mechanism of the unusual effect, their measurement was initiated.



1a, Y = OH
b, Y = H

Experimental Section

The spectra of solutions of 500 mg of each virescenoside in 3 mL of deuteriochloroform-methanol mixtures, the deuteriochloroform having been filtered through basic alumina to avoid the presence of acid impurities, were recorded on a Varian XL-100-15 NMR spectrometer operating at 25.2 MHz in the Fourier transform mode. The methanol was added to the deuteriochloroform solutions in multiples of 1 molar equiv. The spectrometer probe temperature of 25, 35, or 50 °C was kept constant throughout any experiment. The T_1 values were obtained by the inversion-recovery method[4] and the pulse width

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Table I. Experimental δ and T_1 Values and Computed τ_r Values of Virescenoside A (**1a**)[a]

		molar equiv of methanol							
	δ	0	1	2	3	5	7	9	11
C(1)	45.6	0.043	0.043	0.044	0.040	0.049	0.058	0.068	0.080
C(2)	68.0		0.060	0.057	0.075	0.081	0.088	0.11	0.13
C(3)	83.8	0.11	0.071	0.064	0.068	0.085	0.096	0.11	0.13
C(4)	42.6		0.40	0.45	0.52	0.43	0.45	0.43	0.72
C(5)	51.9		0.075	0.073	0.078	0.074	0.10	0.12	0.14
C(6)	23.4		0.044	0.046	0.046	0.053	0.061	0.070	0.080
C(7)	120.7	0.13	0.072	0.070	0.078	0.088	0.10	0.12	0.14
C(8)	135.2	0.22	0.38	0.39	0.51	0.60	0.95	1.3	0.95
C(9)	51.1	0.094	0.077	0.072	0.070	0.068	0.10	0.12	0.14
C(10)	36.6	0.45	0.54	0.34	0.57	0.26	1.0	0.64	0.40
C(11)	20.3		0.045	0.043	0.050	0.053	0.062	0.070	0.079
C(12)	35.9		0.36	0.20	0.36	0.21	0.24	0.19	0.20
C(13)	35.9								
C(14)	45.6	0.043	0.043	0.044	0.040	0.049	0.058	0.068	0.080
C(15)	149.7	0.30	0.32	0.45	0.46	0.41	0.51	0.65	0.79
C(16)	109.0	0.072	0.082	0.066	0.078	0.089	0.098	0.12	0.12
C(17)	21.3	0.19	0.20	0.22	0.22	0.21	0.27	0.29	0.30
C(18)	24.6	0.17	0.19	0.19	0.21	0.20	0.25	0.26	0.29
C(19)	71.0		0.048	0.045	0.050	0.054	0.067	0.072	0.089
C(20)	15.9	0.30	0.27	0.29	0.31	0.25	0.33	0.33	0.53
C(1′)	99.1	0.075	0.077	0.064	0.064	0.080	0.092	0.12	0.14
C(2′)	70.2	0.073	0.067	0.060	0.064	0.080	0.093	0.11	0.12
C(3′)	70.0							0.11	0.12
C(4′)	64.7	0.059	0.063	0.058	0.074	0.079	0.096	0.11	0.13
C(5′)	75.0	0.095	0.068	0.067	0.070	0.091	0.10	0.12	0.14
C(6′)	61.8		0.040	0.039	0.046	0.047	0.063	0.074	0.087
τ_r		0.83 18.0	1.4 12.0	1.5 12.0	1.4 13.0	1.1	0.85	0.63	0.53

[a] δ values in parts per million downfield from Me_4Si; $\delta(Me_4Si) = \delta(CDCl_3) + 76.9$ ppm. T_1 values are in seconds, τ_r values in nanoseconds. τ_r values are given when the extreme narrowing condition does not apply.

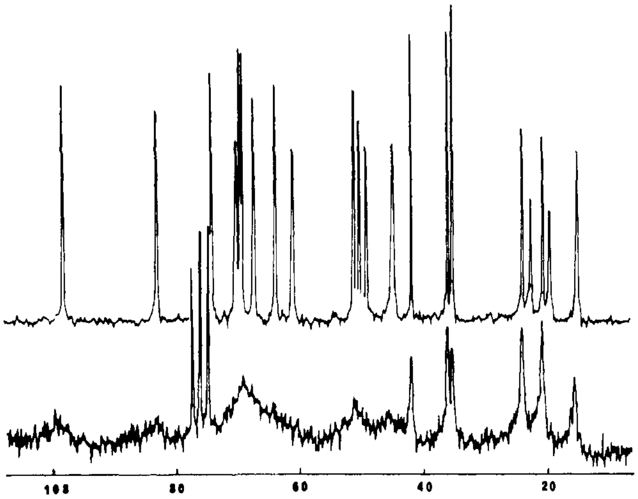


Figure 1. Proton-decoupled ^{13}C NMR spectra of virescenoside A (**1a**) in deuteriochloroform solution (bottom picture) and in solution of deuteriochloroform and 50 molar equiv of methanol (top picture) at 25 °C, recorded in parts per million downfield from Me_4Si.

was calibrated occasionally to check the stability of the instrument. In view of the large differences in the T_1 values measurements with two different sets of τ values were performed for each concentration of the solutions. The use of the short acquisition time of 0.1 s gave good signal-to-noise ratios of the broad lines, i.e., those corresponding to carbons with short T_1 values. In order to obtain fully relaxed spectra, the T_1 measurements always included one made with a delay time of five times the largest T_1 value.[4] The peak heights were measured by hand and fitted to an exponential curve $a + be^{-t/c}$, via a three-parameter minimization. The standard deviation for most lines was ca. 5%, reaching up to 13% in some instances. Approximate T_2 values were obtained for solutions of low methanol concentration by the measurement of the line width of some signals.

Discussion

The spectrum of a deuteriochloroform solution of virescenoside A (**1a**) at 25 °C, presented in the bottom part of Figure 1, reveals sharp peaks for only methyl groups and quaternary carbon centers. A temperature increase causes broad peaks of ca. 50-Hz line width to emerge from the noisy background. The addition of methanol to the solution leads to the appearance of all signals (cf. upper part of Figure 1). Since virescenoside A (**1a**) is more soluble in deuteriochloroform than virescenoside B (**1b**), the former could be studied at lower temperature (35 °C) than the latter (50 °C). Tables I and II present the carbon longitudinal relaxation times of the two substances and computed[5] overall reorientation correlation times.

The 26-carbon systems can be divided into four groups of carbons with related relaxation-time characteristics. The altrose carbons and the nuclear methines and methylenes of the diterpene unit exhibit the following behavior. The T_1 values decrease or remain constant for solutions containing 0–3 molar equiv of methanol and then increase steadily with increasing concentration of methanol, remaining constant or increasing slowly from 22 equiv upward. These effects are visualized easily in Figure 2, wherein the T_1 and T_2 values for some of the signals are plotted against the methanol concentration. The T_2 values of **1a** correspond to line widths of 50, 25, 26, and 10 Hz for 0, 1, 2, and 3 methanol equiv, respectively, and those of **1b** 15, 8, 6, and 4 Hz for 0, 2, 4, and 6 methanol equiv, respectively.

A NOE measurement on a solution containing 20 methanol equiv showed the relaxation mode to be only dipolar. If a decrease in correlation time is assumed to be related to an increase in methanol concentration, the T_1 variations of some carbons in solutions of low methanol concentration show the relaxation times to follow the theoretically predicted U-shaped curve,[5] at the bottom of which the correlation time is ca. 10^{-8} s. In the absence of methanol natural products containing five

Table II. Experimental δ and T_1 Values and Computed τ_r Values of Virescenoside B (**1b**)[a]

		molar equiv of methanol										
	δ	0	2	4	6	8	10	12	14	18	22	26
C(1)	37.8	0.039	0.043	0.055	0.064	0.076	0.090	0.10	0.11	0.13	0.14	0.14
C(2)	27.6	0.037	0.039	0.052	0.062	0.075	0.085	0.095	0.10	0.12	0.13	0.13
C(3)	79.8	0.063	0.068	0.085	0.10	0.12	0.14	0.15	0.16	0.20	0.22	0.22
C(4)	41.5	0.38	0.35	0.37	0.39	0.41	0.46	0.45	0.48	0.67	0.77	0.72
C(5)	51.7	0.031	0.078	0.10	0.12	0.14	0.16	0.18	0.18	0.23	0.25	0.25
C(6)	23.1		0.058	0.066	0.083	0.094	0.11	0.12	0.12	0.15	0.17	0.17
C(7)	120.7	0.062	0.072	0.096	0.11	0.13	0.15	0.16	0.17	0.21	0.22	0.22
C(8)	135.2	0.28	0.38	0.41	0.39	0.38	0.40	0.38	0.29	0.60	0.52	0.57
C(9)	51.2	0.068	0.075	0.092	0.11	0.13	0.15	0.16	0.17	0.22	0.23	0.23
C(10)	34.8	0.32	0.40	0.32	0.37	0.37	0.40	0.42	0.40	0.62	0.69	0.65
C(11)	20.1	0.045	0.045	0.057	0.072	0.086	0.096	0.11	0.12	0.14	0.15	0.15
C(12)	35.8	0.045	0.050	0.063	0.073	0.088	0.098	0.11	0.12	0.13	0.15	0.15
C(13)	36.5	0.28	0.37	0.53	0.36	0.37	0.31	0.42	0.45	0.67	0.58	0.54
C(14)	45.6	0.039	0.046	0.058	0.071	0.085	0.095	0.11	0.12	0.14	0.15	0.16
C(15)	149.7	0.27	0.40	0.38	0.30	0.31	0.32	0.36	0.36	0.47	0.49	0.42
C(16)	108.9	0.070	0.072	0.090	0.096	0.12	0.14	0.13	0.14	0.16	0.17	0.17
C(17)	21.2	0.16	0.19	0.20	0.22	0.23	0.24	0.25	0.25	0.33	0.33	0.34
C(18)	23.3	0.10	0.16	0.16	0.18	0.21	0.22	0.23	0.24	0.32	0.32	0.32
C(19)	71.3	0.039	0.040	0.055	0.065	0.079	0.090	0.10	0.11	0.13	0.14	0.14
C(20)	15.5	0.23	0.25	0.25	0.27	0.29	0.31	0.31	0.33	0.45	0.47	0.47
C(1′)	99.2	0.058	0.061	0.080	0.095	0.12	0.13	0.15	0.16	0.21	0.22	0.22
C(2′)	69.7	0.054	0.059	0.074	0.090	0.11	0.13	0.15	0.15	0.19	0.21	0.21
C(3′)	69.8								0.15	0.20	0.21	0.22
C(4′)	65.1	0.054	0.061	0.077	0.095	0.11	0.13	0.15	0.16	0.19	0.23	0.23
C(5′)	75.2	0.056	0.067	0.080	0.096	0.12	0.13	0.15	0.16	0.21	0.23	0.23
C(6′)	62.0	0.052	0.038	0.053	0.066	0.080	0.092	0.11	0.11	0.13	0.14	0.15
τ_r		2.5 8.6	1.5	0.93	0.70	0.56	0.47	0.43	0.39	0.33	0.29	0.29

[a] δ values in parts per million downfield from Me_4Si; $\delta(Me_4Si) = \delta(CDCl_3) + 76.9$ ppm. T_1 values are in seconds, τ_r values in nanoseconds. τ_r values are given when the extreme narrowing condition does not apply.

to six hydroxy groups can be assumed to be associated through hydrogen bonds, which are broken by the added methanol thereby increasing the mobility of the monomeric structures and leading to longer relaxation times. The difference in temperature of the experiments with the two virescenosides results in slight differences of relaxation-time behavior.

The methyl groups display a T_1 variation different from that of the aforementioned carbons. This effect, due to the internal motion whose rate may be assumed to be independent of the methanol concentration, reveals itself in the form of the T_1 values being nearly constant at low methanol concentrations and increasing steadily after the overall reorientation correlation time becomes shorter than the internal rotation correlation time.[6] In the case of long correlation times the differences in the T_1 values are related to rotation rate differences, which, in turn, have qualitatively an inverse relationship with the rotation barriers. Thus the decreasing order of T_1 values for C(20), C(17), and C(18) reflects an increasing order of rotation barriers for these methyl groups. By the use of published equations[5] the correlation time for internal rotation, τ_g, can be computed for each of the methyl groups at each methanol concentration. The τ_g value ranges were 0.2-0.4 $\times 10^{-9}$, 0.5-0.7 $\times 10^{-9}$, and 0.6-0.9 $\times 10^{-9}$ s for C(20), C(17), and C(18), respectively, in quantitative support of the above discussion. These observations are in agreement with the view of the barrier of rotation of a methyl group within a rigid molecular framework depending on the number of its 1,3-diaxial interactions with hydrogens.[7] The angular methyl group at C(10) interacts in this manner with H(2β), H(6β), and H(11β), the 13-methyl group with H(11β) and and H(16) (in one of the side-chain conformations), and the 4α-methyl function with H(6α).

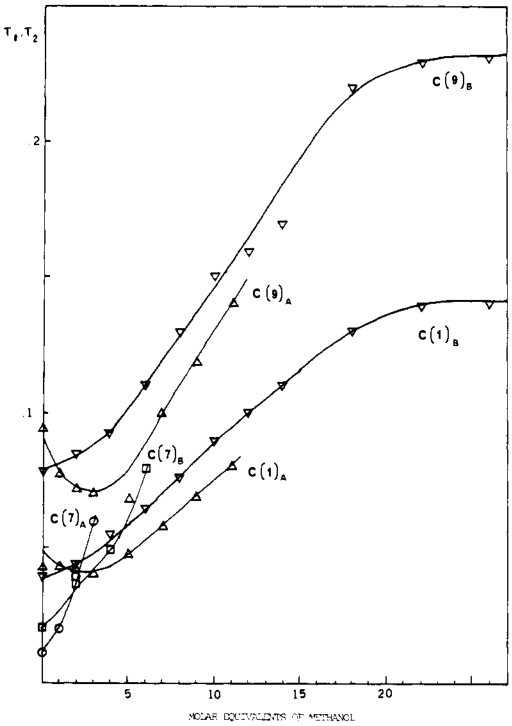


Figure 2. The longitudinal relaxation times (T_1 values) of C(1) and C(9) of the virescenosides A (**1a**) (△) and B (**1b**) (▽) as a function of the methanol concentration and the transverse relaxation times (T_2 values) of C(7) of **1a** (○) and **1b** (□) as a function of the methanol concentration.

The T_1 values of the methine and methylene centers of the vinyl side chain do not show the expected 2:1 relationship. Whereas anisotropic rotation, noticeable from the relaxation times of the protonated, skeletal carbons, can be invoked as an explanation for this anomaly, the latter can be interpreted more readily by the difference of orientation of the individual car-

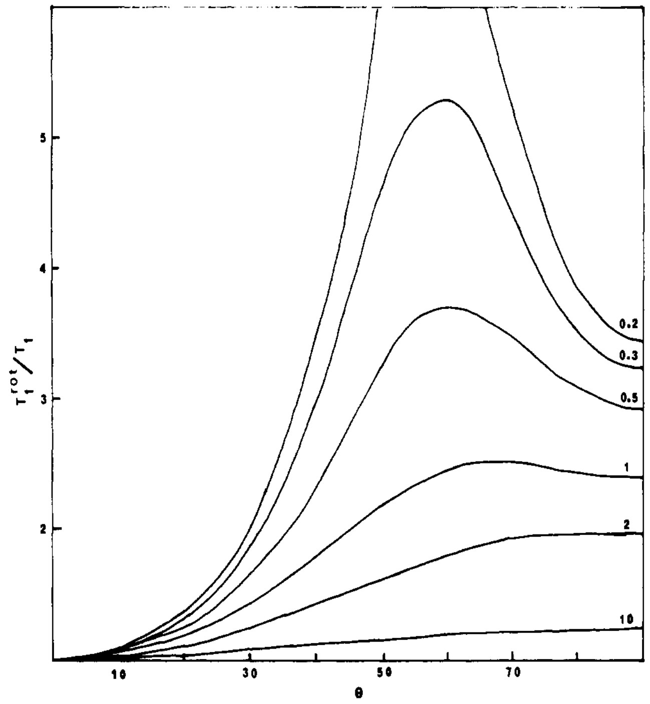
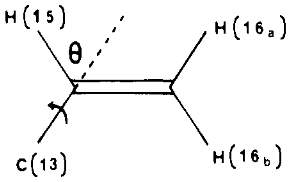


Figure 3. The ratio of T_1^{rot} (longitudinal relaxation time of a methine reorienting in the stochastic diffusion model with a correlation time τ_g on an isotropically reorienting substance with a correlation time $\tau_r = 5 \times 10^{-10}$ s) to T_1 (longitudinal relaxation time of a methine on the isotropically reorienting substance) as a function of θ (angle between the carbon-hydrogen internuclear vector and the axis of rotation) for six τ_g/τ_r values.

bon-hydrogen bonds with respect to the C(13)-C(15) bond axis. In order to assess the importance of the orientation difference, the relationship of the ratio of T_1^{rot} (the spin-lattice relaxation time of a methine undergoing internal rotation in an isotropically reorienting substance) to T_1 (the relaxation time of a methine in the substance) with θ (the angle of the carbon-hydrogen internuclear vector with the axis of rotation) requires analysis. As Figure 3[8] indicates, τ_g-τ_r ratios (ratios of the internal rotation to the overall reorientation correlation times) smaller than 0.1 lead to large T_1^{rot}-T_1 ratios for angles close to 60°. Since the geometry of C(15) satisfies this condition, the carbon center possesses an anomalously long relaxation time. Whereas the C(16)-H(16b) orientation also satisfies the above criterion, yielding a minimal contribution to the C(16) T_1 value, the C(16)-H(16a) vector is collinear with the C(13)-C(15) rotation axis, giving a T_1^{rot}-T_1 ratio of 1. Hence the C(16) T_1 value is close to that of a nuclear methine.



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In view of the long relaxation times of the nonprotonated carbons the accuracy of their measured T_1 values is low. Nevertheless, a trend can be perceived, indicating an increase of the relaxation times with increasing methanol concentration.

As the above discussion has indicated, the unusual line broadening observed in the ^{13}C NMR spectra of deuteriochloroform solutions of the virescenosides is due to the long correlation times of certain carbons caused by intermolecular association through hydrogen bonds. On the other hand, the few signals with narrow line width correspond to methyl groups, i.e., rapidly rotating functions, and to nonprotonated carbon sites. The rotation barriers of the methyl groups are linked inversely to the methyl relaxation times. The unusual relationship of the T_1 values for the carbons of the vinyl side chain is a consequence of its unique geometry.

References and Notes

(1) Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances. 68. For part 67 see T. D. J. Halls, M. S. Raju, E. Wenkert, M. Zuber, P. Lefrancier, and E. Lederer, *Carbohyd. Res.*, in press.
(2) (a) Università di Perugia; (b) Rice University.
(3) J. Polonsky, Z. Baskevitch, N. Cagnoli-Bellavita, P. Ceccherelli, B. L. Buckwalter, and E. Wenkert, *J. Am. Chem. Soc.*, **94**, 4369 (1972).
(4) D. Canet, G. C. Levy, and I. R. Peat, *J. Magn. Reson.*, **18**, 199 (1975).
(5) D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, **56**, 3683 (1972).
(6) Cf. Figure 6 in ref 5.
(7) J. W. Apsimon, H. Beierbeck, and J. K. Saunders, *Can. J. Chem.*, **53**, 338 (1975).
(8) This figure, computed from eq 24 of ref 5, is based on the values of virescenoside A and 11 molar equiv of methanol (Table I), yielding τ_r = ca. 5×10^{-10} s. The ratio of the T_1(C-15) value to the average of the skeletal carbon T_1 values is ca. 5, making τ_g:τ_r = 0.3.