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Registry No. Canavanine, 543-38-4; arginine, 74-79-3.

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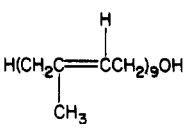
Direct Detection of Solanesol in Tobacco by ^{1}H and ^{13}C Magic Angle Spinning NMR

Jan B. Wooten

^{1}H and ^{13}C NMR have been used to detect solanesol directly in tobacco without destroying or modifying the sample. Magic angle sample spinning was employed to remove the resonance line broadening due to variations of magnetic susceptibility within the sample. ^{13}C line widths of ca. 10 Hz were obtained. The ^{1}H MAS spectrum of tobacco allows the solanesol signals to be resolved from the broad signal of exchangeable protons. ^{13}C spin-lattice relaxation times (T_1) and nuclear Overhauser enhancements (NOE) of solanesol in chloroform solution, in intact tobacco, and as neat oil indicate that the polyisoprene chain motion in tobacco is restricted relative to the motion in solution but still sufficient to average out the dipolar couplings between protons and carbons.

INTRODUCTION

Solanesol in Tobacco. Polyisoprenoid alcohols are found in diverse life forms including higher plants, mammalian tissues, amd microorganisms. These natural products are composed of isoprene units linked head-to-tail to form linear chains containing between 6 and 24 units depending on the source. Most of the polyprenols consist of both cis and trans units but solanesol is recognized to be composed of all trans isoprene units. Solanesol is a



common leaf constituent in plants which was first isolated from tobacco by Rowland et al. in 1956. In tobacco, solanesol occurs at levels as high as 4% of the dry leaf lamina weight, making solanesol the most abundant tobacco terpenoid. In other plant species solanesol occurs at significantly lower levels. In bacteria and mammalian tissues, phosphodiesters of polyprenols act as chemical carriers of saccharide units in the synthesis of complex polysaccharides (McCloskey and Troy, 1980; Morton, 1972). The physiological function of solanesol in green plants, however, remains undefined, and the significance of the greater solanesol content in tobacco relative to other plant species is not understood (Sheen et al., 1978).

Despite the high levels of solanesol in tobacco, there does not appear to be a direct relationship between solanesol and leaf quality (Davis, 1976). Solanesol is likely to influence cigarette smoke aroma, however, since pyrolysis at temperatures up to 550 °C yields mono- and diterpenes as well as isoprene (Grossman et al., 1962; Grossman et al., 1963). At temperatures above 650 °C, solanesol pyrolysis

Philip Morris Research Center, Richmond, Virginia 23261.

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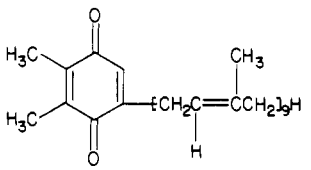
leads predominantly to aromatic compounds. Schlotzhauer et al. (1976) report that solanesol may contribute as much as 30% of the polynuclear aromatic hydrocarbons (PAH) in the pyrolyzate of the hexane extract of tobacco. The PAH levels in cigarette smoke condensate have also been correlated with the solanesol content in tobacco (Ellington et al., 1978a; Severson et al., 1979; Higman et al., 1979).

The importance of solanesol as the predominent terpenoid in tobacco and its significance as a precursor to smoke components has led to the development of several methods for its quantitation. The conventional modern approach usually involves extraction of tobacco samples with organic solvents, separation from leaf pigments by column chromatography, derivatization by methods such as hydrogenation or trimethylsilylation, followed by gas–liquid chromatography (Ellington et al., 1977; Severson et al., 1977; Sheen et al., 1978). Previously gravimetric (Rowland et al., 1956; Woollen et al., 1972) or TLC (Woollen and Jones, 1971) methods were employed; more recently DCI mass spectrometry has been shown to be effective (Einolf, 1984) as well as straight-phase (Keller et al., 1982) and reversed-phase (Smith et al., 1980; Prenzel and Lichtenthaler, 1982) HPLC. A substantial portion of the total solanesol in tobacco is not present as the free alcohol, but as esters of either low molecular weight or fatty acids. The predominant forms of these acids are palmitic, linoleic, linolenic, myristic, and oleic (Rowland and Latimer, 1959). To determine the total solanesol content, the "bound" (esterified) solanesol must first be liberated by alkaline hydrolysis. Solanesenes, bombiprenone (a C_{45} isoprenoid closely related to solanesol) (Irvine et al., 1972), and various other lipids can also be quantitated by either the GLC or HPLC techniques.

The solanesol content of tobacco depends upon a number of factors including the type and variety of tobacco, stalk position, duration of growth, and method of curing. With the development of the GLC methods for quantitating solanesol, several studies were inititated which demonstrate these effects. Early efforts compared free (Davis, 1976) and total (Severson et al., 1977) solanesol between different types of tobacco, but the validity of these early comparisons is doubtful since subsequent investigations showed significant solanesol content fluctuations with seasonal changes and between different varieties of tobacco of the same type. Ellington et al. (1978a) quantitated solanesol in two flue-cured varieties obtained at six intervals during growth, curing, and aging. The total solanesol content found in the newly cured leaf of NC95 was found to be as great as 2.6% of the dry leaf weight while only 1.5% in pale yellow (PY) tobacco, but these values fluctuated by as much as 50% depending on the year of cultivation. The solanesol content in flue-cured leaf reflects an increase of ca. 10–50% over ripe leaf at harvest depending on the variety and priming of the leaf (Schepartz et al., 1982). The increase in free solanesol closely corresponds to the decrease in solanesyl esters during curing (Sheen et al., 1978). Burton et al. (1984) made a similar study of Kentucky 14 burley and found that free solanesol increases monotonically during growth and continues to increase during air curing to a maximum of ca. 4% of the dry weight of the mid-stalk leaf laminae. Unlike the flue-cured tobacco, however, the bound solanesol content does not decrease with curing but remains essentially constant at ca. 1.2% after approximately 60 days of growth.

The distribution of solanesol among various plant parts has also been shown to vary significantly. It was recognized very early that the chloroplasts of green tobacco leaves contain high levels of solanesol (Stevenson et al., 1963). Evidence obtained subsequently supports the conclusion that solanesol synthesis and storage is confined largely to the chloroplasts. Plant leaves which have high numbers of chloroplasts are high in solanesol while plant parts with no chloroplasts such as stems, stalks, and flowers contain no detectable solanesol (Ellington et al., 1978b); neither is solanesol found in the cuticular waxes of the leaf (Davis, 1976; Sheen et al., 1978). Sheen et al. (1978) have also measured solanesol levels in isolated chloroplasts, mitochondria, and in mature leaf and found the highest levels of solanesol in chloroplasts. The low levels of solanesol found in mitochondria are probably not significant since it is difficult to isolate mitochondria completely from chloroplasts. Solanesol content also generally increases from lower to upper stalk positions, a trend which follows that of chlorophyll and carotenoids which are associated with chloroplasts (Sheen et al., 1978). The increase in free solanesol upon flue-curing is attributed to the disintegration of the chloroplasts and the concomitant degradation of the solanesyl esters. Solanesol is also known to be a precursor to plastoquinone-9, which acts as electron carrier between the two plant photosystems localized in the thylakoids, and is also a constituent of the chloroplast envelope membranes (Stevenson et al., 1963; Soll et al., 1980).



Magic Angle Spinning NMR. NMR spectroscopy has seldom been employed for quantitative determinations of the kind mentioned above for solanesol. In certain applications, however, NMR, especially ^{13}C NMR, can be highly effective for quantitative comparisons, as for example the determination of fatty acids in vegetable oils (Shoolery, 1977; Pfeffer et al., 1977). The presence of solanesol in cured tobacco at relatively high levels leads to the suspicion that NMR might be employed for the determination of solanesol in tobacco. This approach has previously been employed for the determination of oils in intact seeds (Schaefer and Stejskal, 1974, 1975) and isoprene rubber in guayule plants (Hayman et al., 1982). The ^{13}C or ^{1}H NMR spectra of tobacco samples obtained with a conventional high-resolution spectrometer, however, are disappointing, resulting only in broad, poorly resolved resonance lines. Recently we have employed a NMR probe designed for high-resolution NMR in the solid state to observe mobile components dispersed within solids. By combining the technique of magic angle sample spinning (MAS) with methods otherwise used for obtaining ^{13}C or ^{1}H spectra of liquids or solutions, well resolved, relatively narrow lines can be observed for solanesol and other components in intact tobacco. This use of MAS represents a new application of the technique and is based upon principles other than those usually associated with MAS NMR of solids.

Since 1976, when magic angle sample spinning was first combined with high power proton decoupling and ^{1}H–^{13}C cross-polarization, solution-like high-resolution ^{13}C NMR spectra have been routinely observed in many solids (Schaefer and Stejskal, 1976). MAS is employed to eliminate the chemical shielding anisotropy which is manifest in the solid state but not in solution since rapid, unrestricted molecular motion averages the chemical shifts to their isotropic values. Another virture of MAS, however, is that it removes the shielding resulting from the bulk

magnetic susceptibility of the material being observed (VanderHart et al., 1981). Within diamagnetic solids and liquids, the induced magnetic field is the sum of the static applied field, H_0, and a contribution from the bulk susceptibility as given by

$$\Delta H = \left(\frac{4\pi}{3} - \alpha\right)\chi_v H_0 \quad (1)$$

where χ_V is known as the volume susceptibility and α is a numerical factor which depends only on the shape of the sample. If a homogeneous sample conforms to a spherical shape, $\alpha = {}^4/_3$ and the induced susceptibility shift is zero. In the usual case for high-resolution solution NMR, the sample tubes are cylindrical and $\alpha = 0$ whenever the sample is oriented parallel to the magnetic field, as is the case for superconducting magnets (Rummens, 1975). Since typical values for χ_v of organic liquids fall in the range -0.4 to -0.7×10^{-6} (Pople et al., 1959), the susceptibility shift experienced by each nucleus is at most 1.7–2.9 ppm. Although it is well-known that MAS removes the susceptibility shift in solids, Garroway (1982) recently demonstrated that MAS can be employed to average out the difference in the susceptibility shift in liquids sequestered in similarly shaped chambers but having different χ_v values. The susceptibility shift for spinning samples is approximately given by

$$\delta_s = \frac{\Delta H}{H_0} = \frac{-4\pi\ \chi_v(3k - 1)}{3}\left(\frac{3\cos^2\theta - 1}{2}\right) \quad (2)$$

where k is a geometric factor. The average shift depends upon the angle, θ, that the spinning axis makes with the magnetic field. When $\theta = 54.7$ (the magic angle), the term $(3\cos^2\theta - 1) = 0$ and the susceptibility contribution to the chemical shift disappears.

Many naturally occuring and synthetic solid materials have liquid or mobile components dispersed within them. If the molecular motion of these components is sufficiently rapid, the resonance line broadening due to the dipolar and chemical shift anisotropy interactions is greatly reduced or eliminated altogether and NMR can be observed without resorting to solid-state methods. The fluid components, however, are still subject to the susceptibility shift which depends upon χ_v of the liquid, the geometry of the confining compartment within the solid bearing the liquid and its orientation relative to the applied field, and the shifts due to the local fields from surrounding compartments. This orientation dependent susceptibility shift has been demonstrated, for example, for water absorbed in cellulose ester membranes by Shporer et al. (1974) who showed that the position of the proton water resonance depends upon the angle that the membrane makes with respect to the magnetic field. In most solids, the liquid bearing compartments will have random orientations and irregular shapes giving rise to powder pattern type resonance line shapes (Drain, 1962). There may also be small distributions of shifts to variations in χ_v if the composition of the liquid varies within the sample. This resonance broadening resulting from spatial variations in the magnetic susceptibility within a solid substrate can be removed by MAS. The spinning speed need not be very fast since the shifts being averaged are only a few ppm in magnitude. It should be noted from eq 1 that the susceptibility shift is field dependent; thus the line broadening becomes more severe at higher magnetic field strengths and MAS becomes increasingly important for observing sharp lines. Also, because of the higher resonance frequency for protons, a higher spinning speed is required for protons than carbons to obtain spectra without spinning sidebands.

The MAS technique is likely to have widespread application to liquid bearing solids having commercial or agricultural significance. One such example, the determination of triglyceride fatty acids in intact seeds, was recently demonstrated by Shoolery (1983). Oils in seeds had previously been examined by ^{13}C NMR but with generally poor resolution. Application of MAS to remove susceptibility broadening resulted in significantly narrower lines, leading to better discrimination between different components and a substantial reduction in the time required to obtain a spectrum. In the present study, the application of MAS to observe the high-resolution ^{13}C and ^1H spectra of solanesol in cured tobacco is reported and comparisons are made between the spectra of solanesol in solution and in tobacco.

EXPERIMENTAL SECTION

The high-resolution ^{13}C and ^1H NMR spectra of solanesol in $CDCl_3$ solution were obtained at 75.4 MHz and 299.9 MHz, respectively, with a Varian XL-300 spectrometer. The magic angle spinning ^{13}C and ^1H spectra were obtained at 50.3 MHz and 200.1 MHz, respectively, with a Varian XL-200 spectrometer and a Doty Scientific magic angle spinning probe. Proton MAS spectra were observed through the decoupling channel of the Doty probe. Except for spinning at the magic angle, standard solution pulse techniques were employed, i.e., spinning speeds of typically <100 Hz, normal power broad-band proton decoupling when observing carbon, 90° rf pulses, and pulse repetition rates of 1–2.5 s. Adamantane was used as an external chemical shift reference for measuring the carbon shifts of solanesol in tobacco but shifts are reported relative to Me_4Si.

The ^{13}C spin-lattice relaxation times of solanesol in $CDCl_3$ solution and in tobacco were measured with the XL-200 by the inversion-recovery technique. An equilibration time equal to 5 times the longest T_1 was employed, excluding the non-protonated carbon in the solution spectra for which an accurate measurement of T_1 was not attempted. The ^1H–^{13}C NOE enhancements were measured by the gated-decoupler technique. For the NOE suppressed spectrum, the proton decoupling was gated on only during the short 0.5-s acquisition time. The equilibration time was set to 10 times the longest T_1 value. The T_1 values were computed by fitting the partially relaxed resonance intensities to exponential recovery curves; both T_1's and NOE's were computed with Varian software.

The tobacco employed for this study was a shredded, uncased, air-cured Burley obtained from a mid-stalk position; other tobacco types yielded similar ^1H and ^{13}C spectra. Pure solanesol was obtained from the Sigma Chemical Company.

RESULTS AND DISCUSSION

NMR Detection of Solanesol in Tobacco. The ^1H and ^{13}C MAS spectra of fully cured, shredded Burley tobacco observed under standard solution conditions are shown in Figures 1a and 2a, respectively. The corresponding spectra of solanesol in chloroform solution are shown in Figures 1b and 2b. Initially the origin of the sharp resonance peaks in the ^{13}C spectra of tobacco were unknown, but a proton-coupled ^{13}C MAS spectrum of tobacco indicated the presence of a methyl and two methylene resonances, and one protonated and one nonprotonated olefinic resonance. The large amount of solanesol known to be in tobacco led to the conjecture that the observed resonances were due to solanesol and comparison with the ^{13}C spectra of authentic solanesol confirmed the identification. A similar comparison was made

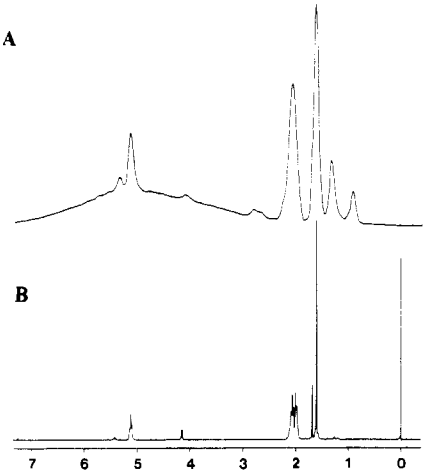


Figure 1. (A) 200-MHz ^1H MAS spectrum (resolution enhanced) of fully cured, shredded Burley tobacco. (B) 300-MHz ^1H high-resolution spectrum of solanesol in $CDCl_3$ solution. Chemical shifts are reported in Table I.

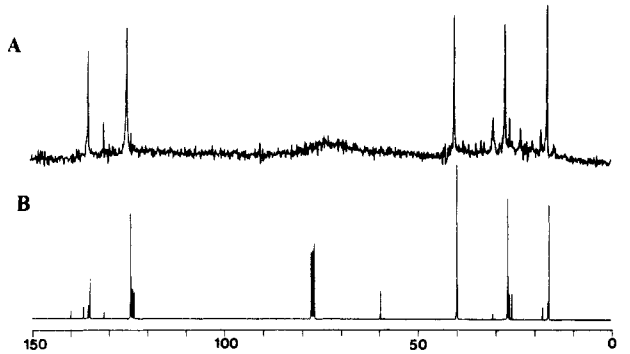


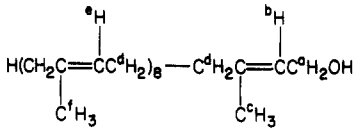
Figure 2. (A) 50-MHz ^{13}C MAS spectrum of fully cured Burley tobacco. (B) 75-MHz ^{13}C high-resolution spectrum of solanesol in $CDCl_3$ solution. Chemical shifts are reported in Table II.

between the proton spectra. Without MAS, only very broad signals bearing no detailed structure are observed in either the ^1H and ^{13}C spectra of tobacco samples. Other resonances besides those due to solanesol can be seen in both the ^1H and ^{13}C spectra of tobacco, but these components have not been identified. Of particular note is the very broad resonance in the ^1H MAS spectrum due to exchangeable protons including water which is observed in tobacco samples with high moisture content (Figure 1a). The extreme width of this resonance is likely to be due to broadening from rapid chemical exchange between sites with varying degrees of hydrogen bonding. The MAS approach may offer a significant advantage over conventional NMR for the study of tobacco–water interactions independently of the solanesol.

Only limited NMR data are available for solanesol; the ^1H chemical shifts at 60 MHz were previously published in conjunction with a synthetic method for solanesol (Sato et al., 1981), but the ^{13}C shifts have not been reported. The ^{13}C shifts of smaller chain polyprenols such as geranylgeraniol (Tanaka et al., 1982) and farnesol (Stothers, 1972) have been reported. The ^1H and ^{13}C chemical shifts from this work are presented in Tables I and II. The ^{13}C MAS spectrum of tobacco is dominated by five resonances corresponding to the central isoprene units in the nine unit chain of solanesol. The chemical shifts are very similar to the solution shifts but are less precise because the line widths are broader than in solution (even with MAS) and because external chemical shift referencing must be employed. Unlike the solution spectrum which exhibits numerous resolved single carbon resonances, the resolution is not sufficient to resolve individual carbon resonances. The resonance of the hydroxyl bearing carbon which should appear at 59.4 ppm and be well resolved even at 50 MHz has not been observed in any tobacco samples. The 300-MHz ^1H solution spectrum of solanesol exhibits resolved resonances only for the methyl, methylene, and olefinic protons of the terminal hydroxyl bearing isoprene unit. In some MAS spectra of tobacco, these resonances can also be seen (the terminal olefinic and methylene protons can be barely detected in Figure 1a).

Both the ^1H and ^{13}C line widths of solanesol in tobacco are significantly broader than the line widths in solution even with MAS elimination of susceptibility broadening. There is likely to be a number of contributing mechanisms for resonance line broadening within a heterogenous substrate such as tobacco. One likely source of line broadening is the effect of chemical shift dispersion. In isotropic solutions the diamagnetic shieldings of the solvent affect all atoms equally giving rise to uniform chemical shifts, but a liquid suspended or adsorbed within a solid substrate may experience non-uniform shielding due to irregular interactions at the solid–liquid interface. Furthermore, in tobacco, the large number of chemical substances present may make indeterminant contributions to the overall shifts. As discussed in the introduction, there are

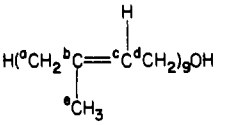
Table I. ^1H Chemical Shifts for Solanesol[a]



proton	shift	coupling
a	4.15 (d)	^3J(a,b) = 7.04 Hz
b	5.42 (m)	^4J(b,c) = 1.11 Hz
c	1.60 (s)	
d	2.04 (m)	
e	5.11 (t)	
f	1.68 (s)	

[a] Spectrum taken in $CDCl_3$ solution with Me_4Si as internal standard (shifts given in ppm).

Table II. ^{13}C Chemical Shifts for Solanesol in $CDCl_3$ Solution and in Shredded Burley Tobacco[a]



C	$CDCl_3$ solution	Burley tobacco	C	$CDCl_3$ solution	Burley tobacco
a	39.73[b]	40.3	d	26.74	
	39.55			26.68[b]	27.4
b	139.82			26.65	
	136.60			26.30	
	135.38			25.69	
	134.99		e	17.68	
	134.91[b]	134.9		16.28	
	131.23			16.02[b]	16.5
c	124.37		CH_2OH	59.39	
	124.33				
	124.23[b]	124.9			
	124.13				
	123.72				
	123.28				

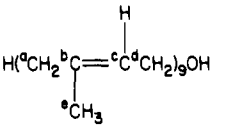
[a] Shifts given in ppm relative to internal Me_4Si. [b] Indicates principal resonances due to the central isoprene unit carbons. Remaining resonances are single carbon resonances due to the end isoprene unit carbons. No single carbon resonances were resolved in the tobacco spectra.

also likely to be numerous components present closely related to solanesol such as solanasenes, solanesyl esters, and neophytadiene which have very similar but inherently different shifts which may contribute to the total line width without exhibiting resolved resonances. Only in cases where the protons or carbons of the terminal hydroxyl bearing isoprene unit are observed can solanesol be unambiguously distinguished from these other components. The preponderence of solanesol known to be present, however, assures that much of the resonance intensity is due to solanesol.

Generally, homogeneous NMR line widths are related to the lifetime of the transverse component of the nuclear spin magnetization after the sample is pulsed with radio frequency radiation at the resonance frequency of the nucleus being observed. For nuclei which exhibit normal relaxation behavior as determined by a single exponential time constant, the line shapes are Lorentzian and the line width is given by $\Delta\nu = 1/\pi T_2$ where T_2 is the spin–spin or transverse relaxation time. T_2 is an inverse function of the degree of molecular mobility as described by correlation time τ_c. As τ_c becomes longer (decreasing mobility), T_2 becomes shorter and line widths increase. Usually ^{13}C line widths are more informative than ^1H line widths since no homonuclear scalar couplings are present to contribute to the line width. The ^{13}C line widths of solanesol in tobacco are ca. 10 Hz as compared to 1–2 Hz in solution. Solanesol in cured tobacco, however, is likely to reside as an oil or wax within the interstices of the disintegrated remnants of the tobacco lamina cells. Within such a restricted, solvent-free environment, lifetime broadening is likely to contribute significantly to the line width. A more valid comparison of line widths can be made between solanesol in tobacco and solanesol as a neat oil. The ^{13}C spectrum of neat solanesol oil just above its melting point (33–34 °C) (Grossman et al., 1963) was observed at 50 MHz yielding line widths very similar to the line widths of solanesol in tobacco. This result suggests that the observed line widths are largely determined by the degree of mobility of the solanesol in tobacco since the high viscosity of neat solanesol oil should greatly reduce molecular motion.

Indirect evidence obtained from ^{13}C dipolar-decoupled CP-MAS spectra of tobacco indicates that solanesol in tobacco exhibits liquid-like mobility. In the cross polarization experiment, nuclear spin polarization is transferred from protons to carbons via a Hartmann–Hahn matched spin–lock (Yannoni, 1982) pulse sequence. A process known as spin diffusion acts to render the entire ensemble of protons within the solid as a reservoir of nuclear magnetization which can be transferred to carbons. Since the proton reservoir is more highly polarized than the carbon reservoir, a net gain in carbon resonance intensity results. Spin diffusion and cross polarization are efficient only between nuclei which are dipolar coupled. For a static ^{13}C–^1H vector parallel to the magnetic field, a dipolar splitting of the carbon signal due to the attached proton of ca. 40 KHz (Yannoni, 1982) will be observed. If there is significant molecular motion present having frequency components significantly greater than the static dipolar couplings, the dipolar couplings will be reduced or eliminated altogether (as in the case of solutions and liquids). Since no solanesol resonances are observed in the CP-MAS spectra of tobacco (Wooten, 1984), the molecular motion is sufficient to eliminate the dipolar coupling and does not permit signal enhancement by cross-polarization. This places an upper limit on the value of rotational correlation time (τ_c) at roughly 10^{-6}–10^{-5} s.

Table III. ^{13}C Spin-Lattice Relaxation Times and NOE Enhancements for Solanesol



C	$CDCl_3$ solution		Burley tobacco		neat oil	
	T_1	NOE	T_1	NOE	T_1	NOE
a	0.90 ± 0.02	3.0	0.17 ± 0.03	2.5	0.22 ± 0.004	2.4
b	>35 ± 3	2.4	1.04 ± 0.02	1.8	2.53 ± 0.06	2.0
c	1.62 ± 0.03	2.8	0.24 ± 0.02	2.8	0.39 ± 0.01	2.4
d	0.85 ± 0.02	3.0	0.13 ± 0.01	2.2	0.23 ± 0.01	2.3
e	4.16 ● 0.2	2.8	1.00 ± 0.1	2.1	1.49 ± 0.09	2.2

^{13}C Spin-Lattice Relaxation and NOE's of Solanesol. A more informative indication of molecular motion can be obtained from ^{13}C spin-lattice relaxation times (T_1) and nuclear Overhauser effect enhancements (NOE). The spin-lattice relaxation time is a measure of the return to an equilibrium population of the nuclear spin energy levels following perturbation of the spin system by an rf pulse. Like T_2, T_1 is dependent upon the rotational correlation time τ_c, but T_1 is generally more experimentally accessible and frequently more accurate than T_2. The NOE enhancement is defined as the increase in the integrated carbon resonance intensity in the presence of a saturating proton rf field relative to the signal intensity in the absence of the rf field. The NOE depends solely on the rate of relaxation due to dipolar interactions between carbons and protons; other relaxation pathways make no contributions to NOE enhancements. For correlation times in the range of 10^{-11} to ca. 10^{-9} s, T_1 decreases monotonically with increasing molecular motion (longer τ_c) assuming simple isotropic motion. In this motional regime, known as the extreme narrowing region, both T_1 and NOE are independent of the magnetic field strength at which the measurements are made. Kuhlmann et al. (1970) have shown that the theoretical maximum for the NOE enhancement is 2.988 and independent of τ_c when molecular motions lie within the extreme narrowing region, provided that the ^{13}C relaxation mechanism is overwhelmingly dominated by ^{13}C–^1H dipolar interactions. For slower motions outside the extreme narrowing region, T_1, T_2, and the NOE depend both upon τ_c and the field strength (Doddrell et al., 1972). If a less than the theoretical maximum NOE is observed at any field strength for carbons known to be dominated by dipolar relaxation, it can immediately be concluded that the molecular motions lie outside the extreme narrowing region (τ_c > ca. 10^{-9} s).

The ^{13}C spin-lattice relaxation times and NOE enhancements for solanesol in chloroform solution, in Burley tobacco, and in the neat solanesol oil just above the melting point are given in Table III. In chloroform solution, the T_1 values are typical of T_1 values reported for other segmented hydrocarbons of similar chain length, as for example, phytol (Goodman et al., 1973). The two methylene groups exhibit T_1's which are one half the T_1 for the olefinic carbon (c). This is to be expected if the rotational motions for each of these groups is similar and the relaxation pathways are dominated by dipolar relaxation since the relaxation rate ($1/T_1$) is directly proportional to the number of attached protons. The longer T_1 observed for the methyl groups (e) results because of inefficient relaxation due to rapid rotation about the methyl symmetry axis. Both methylene groups exhibit full NOE's and the methine and methyl groups exhibit nearly full NOE, thus indicating the dominance of the dipolar relaxation mechanism. Plausible explanations for the small deviations

from the theoretical maximum NOE observed for these groups is the efficiency of competing relaxation mechanisms. Doubly bonded carbons may relax via the chemical shift anisotropy mechanism and rapidly rotating methyl groups may relax by the spin-rotation mechanism (Farrar and Becker, 1971). Because non-protonated carbons generally have long T_1's, the T_1 for the non-protonated olefinic carbon is likely to contain a significant systematic error since no attempt was made to measure this T_1 accurately.

The T_1's of solanesol in the Burley tobacco and in the neat oil are significantly reduced relative to the values in solution, indicating restricted molecular motion in these samples. Nonetheless, the ratio of the olefinic to methylene T_1 values is still two in the Burley tobacco sample. The NOE enhancements of solanesol in the Burley tobacco and in the neat oil are also significantly less than the theoretical maximum NOE. Since the dominance of dipolar relaxation was demonstrated by the full NOE enhancements observed in the solution spectra and by the ratio of the olefinic to methylene values both in solution and in tobacco, the decreased values for the NOE's must reflect the presence of molecular motions outside the extreme narrowing region. Comparison of the observed T_1 and NOE values with theoretical values calculated assuming simple isotropic motion indicates that the molecular motions in these samples are characterized by τ_c values in the range of 5×10^{-10} to 1×10^{-9} s. It is important to realize that the isotropic model of molecular reorientation is strictly valid only when molecular motion can be described by a single correlation time, whereas solanesol and other isoprenoid molecules are likely to exhibit segmental motions which may vary along the chain as demonstrated for phytol (Goodman et al., 1973) as well as overall chain tumbling. However, in a solid or highly viscous sample, the overall chain tumbling is likely to be very slow compared to the segmental motions and, in the present case, only the behavior of the central isoprene units is being observed. The actual motion is not likely to be significantly different from the motion predicted by a simple isotropic model (Schaefer, 1972) and the current results are believed to be accurate within the range of τ_c values given.

The Physical State of Solanesol in Tobacco. The observation of sharp resonance lines by MAS NMR and the relaxation data presented in this report raises two relevant questions: what is the physical state of solanesol in tobacco (is it present as a liquid oil or as a solid wax), and how is the solanesol distributed within the cured leaf after the disintegration of the chloroplasts? It is not a safe assumption that solanesol resides as a fluid oil even though narrow resonance lines are usually indicative of liquid samples. A valuable comparison can be made with *trans*-polyisoprene rubber, a high molecular weight polymer composed of the same *trans*-isoprene units as solanesol. Even though *trans*-polyisoprene is a solid, line widths in the range of 35–50 Hz have been observed in the ^{13}C spectrum even without MAS or other solid-state techniques (Schaefer, 1972). Narrow lines are observed in polymers if the temperature at which the experiment is performed is above the glass-transition temperature of the solid. In *trans*-polyisoprene, the sharp lines are attributed to the rapid segmental motion within the amorphous region of the polymer which effectively average the dipolar couplings and chemical shifts anisotropy to zero. (Susceptibility broadening is not severe in the reported example because the samples were observed at low field (22.6 MHz carbon); the samples were homogeneous in composition and were machined into uniform cylindrical shapes.) Similar motions in solanesol may give rise to sharp lines even if the solanesol is not fluid. The current results do not distinguish between free and bound solanesol. It is reasonable to expect that the motion of the bound solanesol is more restricted than the motion of the free solanesol, but unless the segmental motion of the polyisoprene chain is constrained within the chloroplasts or by interaction with other cell components, the end group isoprene units may be as mobile in the bound form as in the free. Additional NMR experiments may clarify the picture.

After curing and the putative disintegration of the chloroplasts, the solanesol may remain within the organelle phantom as a globule of wax or it may partially diffuse into the lamina. The degree of susceptibility broadening depends upon the shape of the confining compartments containing the solanesol and the local fields due to nearby compartments. If the compartment is nearly spherical, then the line broadening will be minimal and resonance lines can be observed without MAS. For example, oils in seeds such as oleic, palmitic, and linoleic acids can be detected without MAS but with poor resolution; observation with MAS allows individual carbon lines to be resolved (Shoolery, 1983). In the case of solanesol in tobacco, the situation is worse; without MAS the ^{13}C resonances are broadened nearly into the base line. It is likely that in seeds, the oil is present as globules with distorted spherical or oblong shapes which results in signal broadening but does not preclude NMR detection. In tobacco, however, solanesol probably forms a thin film on the surface of the plant cell structures, a geometry with the maximum distortion from spherical shape which results in the maximum possible susceptibility broadening due to random film orientation.

Analytical Applications of MAS NMR for Detecting Solanesol. MAS NMR may be a useful method for screening tobacco samples for solanesol and some of the other minor components which were detected but not identified. For quantitative comparisons using ^{13}C NMR, precautions should be taken to insure that the signal intensities are proportional to concentration. Generally this means that a pulse equilibration time equal to five times the longest T_1 value and NOE suppresion be employed. The suppresion of the NOE enhancement is required because the enhancements may vary from sample to sample or with temperature, or they may differ between different components due to the distribution of motional regimes present in tobacco. Alternatively, the NOE enhancements may be measured in each sample to confirm that the enhancements are the same for each observed carbon. This latter approach may be desirable because NOE suppressed ^{13}C spectra require significantly longer acquisition times. Comparisons between different tobacco samples are nonetheless difficult because of the lack of a convenient and accurate internal standard for intensity measurements. Furthermore, comparisons by weight are probably meaningless because many components, including water, will vary between samples and not just solanesol. However, if equal quantities of tobacco as measured by leaf surface area are employed and proper allowance is made for possible differential T_1's and NOE's between samples, ^{13}C MAS NMR may be used for relative solanesol determinations. Another practical application for ^{13}C MAS NMR might be to monitor the effects of solvent extraction without destroying the sample.

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Odor Thresholds of the Stereoisomers of Methyl Jasmonate

Terry E. Acree,* Ritsuo Nishida, and Hiroshi Fukami

Four stereoisomers of methyl jasmonate, methyl 3-oxo-2-(2-(*Z*)-pentenyl)cyclopentane-1-acetate, were isolated from a commercial sample of methyl jasmonate. Enantiomeric resolution was based on the separation of (−)-bornyl jasmonate diastereoisomers with liquid chromatography. Odor detection thresholds showed that methyl (+)-epijasmonate, (1*R*,2*R*)-(+)-methyl 3-oxo-2-(2-(*Z*)-pentenyl)cyclopentane-1-acetate, has the strongest odor–activity.

"Today jasmine flower oil and its synthetic substitutes are key ingredients universally employed in the manufacture of high grade perfumes···and methyl (−)-(*Z*)-jasmonate [is] said to be [one of] the specific carriers of the true natural jasmine fragrance." (Demole, 1982). However, an epimer of this compound, methyl epijasmonate is the sole form found in lemon peels (Nishida and Acree, 1984) where it seems to contribute significant odor. Furthermore, both epimers have been detected in the phermone glands of a moth (Nishida et al., 1982) but only the epijasmonate form showed biological activity for the insect (Baker et al., 1981). These results led us to question the notion that methyl (−)-(*Z*)-jasmonate, (1*R*,2*R*)-(−)-methyl 3-oxo-2-(2-*Z*-pentenyl)cyclopentane-1-acetate, is the isomer with the greatest odor activity.

Commercial methyl jasmonate is a racemic mixture of four *E* isomers and four *Z* isomers. Liquid chromatogra-

Cornell University, Food Research Department, New York State Agricultural Experimental Station, Geneva, New York 14456 (T.E.A.), and Kyoto University, Pesticide Research Institute, Sakyo-ku, Kyoto 606, Japan (R.N. and H.F.).

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