Table II. Contributions of Chemical Shift Anisotropy to the Relaxation of sp^2 Carbons at 67.9 MHz

	Carbon	T_1CSA[a] (sec)	$\Delta\sigma$[b] (exptl) (ppm)
I. Indole[c]	C-8	109	220
	C-9	94	230
II. Me-OMe-Podocarpate[d]	C-7	33	220
	C-8	42	200
	C-9	33	220
	C-12	49	180
	C-16	54	...[e]
III. Cholesteryl chloride[f]	C-5	5.3	340

[a] For the calculation of τ_{eff} the following values were used: γ_C = 6720, $\hbar = 1.05 \times 10^{-27}$, $r_{CH} = 1.09 \times 10^{-8}$ cm, γ_H = 26,700. [b] Estimated accuracy ±10%; maximum error ±20% (est) due to the relative low accuracy of the NOE's. [c] $\tau_{eff} \approx 8.1 \times 10^{-12}$ sec/rad using $NT_1 = 5.8$. [d] $\tau_{eff} \approx 2.6 \times 10^{-11}$ sec/rad using $NT_1 = 1.8$. [e] Correlation time unknown; see text. [f] $\tau_{eff} \approx 6.6 \times 10^{-11}$ sec/rad using $NT_1 = 0.72$.

tion in liquids,[3] only a very few studies *at high magnetic field and low temperatures* have been performed where important or dominant ^{13}C CSA relaxation has been established (at higher temperatures SR relaxation replaced the CSA contribution).[4] A variable field study of the acetylenic carbons in diphenyldiacetylene (DPDA) is the only reported example at room temperature where the CSA mechanism has been found to be important.[2b,5]

Table I summarizes the results of the present study. Accurate T_1 and NOE data for the three compounds shown in Table I clearly show that CSA relaxation contributes strongly to the relaxation *of all nonprotonated sp^2 carbons* at 67.9 MHz, with minor contributions also indicated at 22.6 MHz. This can be seen from the field dependence of the T_1's and NOE's[6] given in Table I. In the case of nonprotonated sp^3 carbons there is no observed field dependence whereas for nonprotonated aromatic and olefinic sp^2 carbons, the T_1 values at 67.9 MHz are ca. half those observed at 22.63 MHz, and the NOE's simultaneously drop from 1.5–1.9 to 0.8–1.1(η). This results from a field independent R_1^{DD} term[6] being augmented at high field by a comparable R_1^{CSA} term. Because of the large threefold magnetic field ratio it is possible to accurately calculate R_1^{CSA} contributions of 2–10% at 22.6 MHz. These results indicate that quantitative ^{13}C studies performed at moderate or high magnetic fields may be less accurate than expected due to variable NOE for nonprotonated carbons that can have anisotropic chemical shift tensors.

Evaluation of the anisotropy of the shift tensor, $\Delta\sigma$, from eq 3 by measurements of ^{13}C T_1's and NOE's is limited because of several reasons. Most importantly, accuracy is limited due to the uncertainty in the experimental T_1 and NOE values. Secondly, if the molecular motion is anisotropic the uncertain orientation of the principal axis system for the carbon chemical shift tensor places in doubt the validity of the calculated τ_{eff} for relaxation through this mechanism.

Although the protonated carbon T_1 data for I and II indicate limited motional anisotropy, all three compounds in Table I can be considered to undergo largely rigid, isotropic molecular reorientation. Thus it is possible to calculate approximate chemical shift tensor anisotropies for the sp^2 carbons in I–III; these are given in Table II. In Table II no $\Delta\sigma$ value is given for the carbonyl carbon C-16 of II. The ester group can undergo group segmental motion and thus τ_{eff} for C-16 is unknown. The very large $\Delta\sigma$ for C-5 in cholesteryl chloride (340 ppm) is somewhat surprising. We have no explanation at this time for this fact.

The earlier study[5] of DPDA yielded an anisotropy of ca. 270 ppm for the inner acetylenic carbons. For toluene, a thoroughly studied molecule both in liquid and solid state, an upper limit for $\Delta\sigma$ of 295 ppm was reported[4b] using the effective correlation time obtained from the dipolar relaxation rate. Another investigation[7] reported the anisotropic shielding tensors for the unsaturated carbons in acetonitrile and acetone as 460 and 390 ppm, respectively. However, quite recently a double resonance study of acetonitrile determined $\Delta\sigma$ to be 307 ± 4 ppm.[8]

Where it is possible experimentally the method of proton-enhanced nuclear induction spectroscopy for ^{13}C observation of solids is superior in accuracy and furthermore the cross polarization experiment gives directly the principal elements (σ_{ij}) of the chemical shift tensor.[9] In these studies benzene has been found to have an anisotropy of 180 ± 2 ppm.[9b]

In spite of the shortcomings of the liquid phase high resolution technique for determining $\Delta\sigma$, it is the only method useful for examination of molecules of even moderate complexity.

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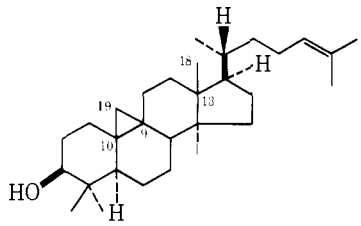
On the Role of Cycloartenol in the Formation of Phytosterols. Biosynthesis of [19-^{2}H]Sitosterol in Deuterium Oxide Germinated Peas

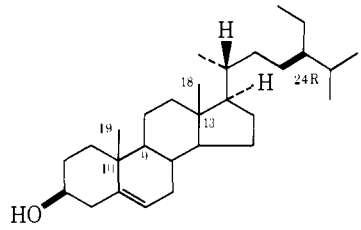
Sir

The hypothesis that cycloartenol rather than lanosterol is the key precursor of phytosterols in higher plants rests on two sets of evidence. Firstly it was noted that cycloartenol was present in many plants, while lanosterol was found only rarely (latex of **Euphorbia** family) and then in minor amounts.[1] Also, [1-^{14}C]acetate[2] and [2-^{14}C]mevalonic acid[3] (MVA) were efficiently incorporated into cycloarten-

ol by plants and plant tissues. Secondly it was observed that radioactive cycloartenol was a considerably more efficient precursor of phytosterols than lanosterol.[4] The conversion of cycloartenol to lanosterol by a plant tissue was reported.[5]



cycloartenol



sitosterol

These and other[6] observations though plausible do not establish a biosynthetic interdependence of cycloartenol and phytosterols. In a search for a more immediate correlation we focused our attention on the enzymatic transformations of the cyclopropane ring. We reasoned that should cycloartenol be a key precursor of phytosterols, then at a certain stage of the anabolic transformations the cyclopropane ring must be cleaved between C-9 and -19. In analogy to other enzymatic reactions of polyprenoids biosynthesis it is very likely that the bond cleavage will be "ionic" in nature.[7,8] The mechanism of the enzymatic opening of the cyclopropane ring could be concerted, involve a C-9 cation[7] or a transiently stabilized C-9 cation.[8,9] However, irrespective of the actual mechanism it may be assumed with considerable certainty that the "cationic terminus" will be located at C-9 and the "anionic terminus"[9] at C-19. Stabilization will then occur through the formation of a double bond[9] (e.g., 8(9), 9(11) etc.) and the acquisition of a proton at C-19. The proton acquired at C-19 will most likely originate from the water of the medium. Should this be the case, by carrying out the biosynthesis of phytosterols in vivo in deuterium oxide, deuterium should be incorporated at C-19. Optimally one ^2H atom should be located at C-19. In practice, however, due to the presence of endogenous phytosterols and ^1H_2O in the system, statistically less than one atom of deuterium will be incorporated. The presence of deuterium at C-19 will result in a decrease of the intensity of the C-10 methyl (C^1H_3) signal in the ^1H NMR spectrum of the biosynthesized phytosterols. This hypothesis will remain valid only as long as *no detectable amounts* of endogenously produced [^2H]acetyl CoA will be incorporated into phytosterols.

Peas (120; Burpees Blue Bantam Variety) were sterilized by immersing for 5 min in a dilute Clorox solution (9:1), then rinsing with sterile water and finally with ^2H_2O (×3). The seeds were placed in a sterilized Petri dish which was located in a sterilized desiccator. The cover of the desiccator was equipped with two sterilized air filters (glass tubes ca. 2 × 50 cm filled with cotton), and a sterilized separatory funnel containing deuterium oxide (99.8% ^2H_2O). The assembly was kept at ambient temperature and was exposed to daily light. Deuterium oxide was supplied to the peas and was replenished as needed. The air was changed once daily

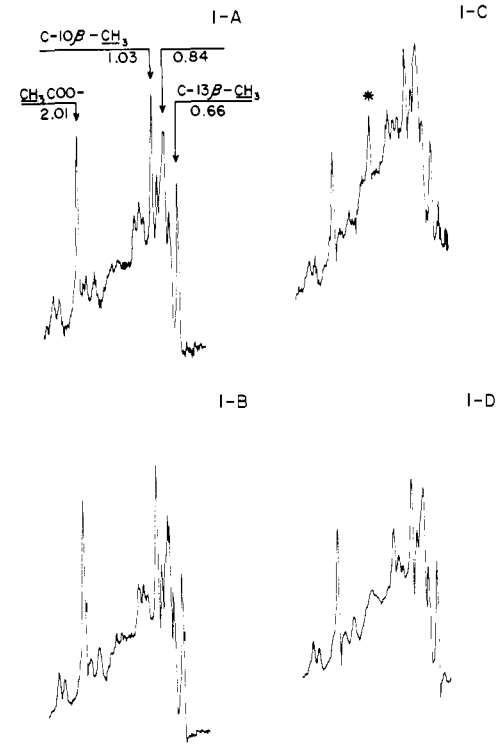


Figure 1. Spectra of samples of sitosterol acetate. The spectra were recorded in microcells in alcohol-free C^2HCl_3 (35 μl) at 100 MHz. Integration of the methyl peaks was carried out on expanded spectra (4:1): 1-A, multiply recrystallized sample (1 scan); 1-B, sample (3) from embryos-^1H (5 scans); 1-C, sample (1) from embryos-^2H (337 scans) (The peak indicated with an * is probably due to a trace of H_2O); 1-D, sample (2) from cotyledons-^2H (240 scans).

by applying slight suction for 5–10 min through the same air filter. The seeds germinated very slowly[10] and after 15 days when 79 peas (66%) had developed shoots the experiment was terminated. The sterile conditions were necessary to avoid fungal infections during the long germination period.

Only the germinated peas (79) were processed, and their embryos (2.58 g) were separated from the remaining tissues (cotyledons) (76.5 g). The embryos and the cotyledons were saponified and the recovered nonsaponifiable residues were fractionated twice by TLC (silica gel; hexane:$CHCl_3$:MeOH (20:10:1)). The sterol fractions from the embryos (1.1 mg) and the cotyledons (4 mg) were acetylated. The acetates were first fractionated by TLC (silica gel; hexane:ethyl acetate (19:1)) and then by argentation TLC (silica gel; 10% $AgNO_3$; $CHCl_3$ alcohol free). The obtained phytosterol fractions were then resolved by preparative GLC to yield two samples of homogeneous sitosterol acetate.

A control experiment with peas (192) and water was carried out and was terminated after 5 days (92% germination). The germinated peas were processed as above to yield two additional samples of sitosterol acetate.

The four samples of sitosterol acetate, (a) from embryos-^2H (1), (b) from cotyledons-^2H (2), (c) from embryos-^1H (3), and (d) from cotyledons-^1H (4), were dissolved in purified C^2HCl_3 (35 μl) and their spectra recorded on Varian HA100 D-15 spectrometer equipped with a Varian C 1024 computer. Also the spectrum of authentic sitosterol acetate (homogeneous by argentation TLC, GLC, MS, and recrystallized five times) was recorded.

Inspection of the NMR spectrum of the extensively purified and crystallized sitosterol acetate (Figure 1A) clearly shows that the singlet for the C-19 hydrogen atoms at 1.03 ppm is significantly higher than the multiplet at 0.84 ppm.

The same holds for the spectra of sitosterol acetate (3) (Figure 1B) and (4) (not shown in the figure) isolated from peas germinated in ^1H_2O. These spectra were recorded on several samples at different concentrations and the differences in the peak heights were always observed. The signals of the acetate, C-10 and C-13 methyls were integrated by weighing the cut-out traces of these peaks. Throughout, all integrations were carried out on expanded spectra (4:1). Since the acetate methyl most certainly contains three ^1H atoms it was accepted as an internal standard. The ratios of the C-10 and C-13 methyls to the acetate methyl were 1:1, respectively.

The spectra of the samples of sitosterol acetate (1) and (2) biosynthesized in deuterium oxide are given in (Figure 1C) and (Figure 1D). It is clear that in both cases the signals for the C-10 methyls at 1.03 ppm relative to those at 0.84 ppm are significantly smaller. Although a slight decrease of the methyl multiplets at 0.84 ppm due to deuteration at C-26 (etc.) was anticipated, it is obvious that the singlets for the C-19 hydrogen atoms decreased to a much greater extent. Integration of the area for the C-10 methyl of (1) (Figure 1C) revealed that it corresponds to 85% of the area of the acetate peak. This would indicate the incorporation of 0.45 atom of deuterium at C-19. Similarly the C-10 peak of (2) (Figure 1D) corresponded to 87% of the acetate peak and this is equivalent to the incorporation of 0.40 atom of deuterium at C-19. In contrast deuterium was not incorporated into the C-13 methyls of (1) and (2) as evidenced by the fact that the ratios of the integrated peaks for the C-13 methyls of (1) and (2) to the acetate methyls remained unchanged (1:1). This constitutes proof that in the germination experiment of 15 days duration, deuterated acetyl CoA, if formed endogenously, was not incorporated in detectable amounts into the sitosterol.

Our results show that a proton from the medium was incorporated at C-19 of (1) and (2). This is consistent with the presence of an "anionic terminus" at this carbon in a precursor of this phytosterol. This electron-rich center could be formed through the cleavage of the C-9(19) bond of the cyclopropane ring as discussed above. The relatively high incorporation of deuterium at C-19 (0.4–0.45 atom) is in accord with the view that cycloartenol is an important key intermediate in the biosynthesis of sitosterol in the pea.

The absence of deuterium at C-18 of sitosterol is of added importance. It may be recalled that the formation of cycloartenol involves a backbone rearrangement of the C-20 protosterol cation.[11] During the rearrangement the 13α and 17β hydrogen atoms of the protosterol cation migrate to the 17α and 20 positions and the 14β and 8α methyls migrate to the 13β and 14α positions, respectively. Then, following the shift of the 9β hydrogen to 8β position and the loss of a C-19 hydrogen, cycloartenol is formed.[11] Our results show that in the course of the methyl migration to the 13β position, the hydrogens of this methyl were not exchanged. The same can be inferred for the backbone rearrangement leading to lanosterol in rat livers.[12,13] This therefore poses the interesting question of whether the migration of the methyls in the elaboration of cycloartenol and lanosterol proceeds with retention, inversion, or racemization of the hydrogens of the migrating carbons. According to the Woodward-Hoffman rules, if applicable to biological systems, the migration should occur with retention of configuration of the hydrogens.[14]

Note Added in Proof. Clifford and Phillips[15] have now reported that in the biosynthesis of lanosterol in rat livers, the methyl migrating to C-13, indeed retained its chirality. This was proven by the degradation of the biosynthesized cholesterol and isolation of the C-13 methyl as acetic acid ($C^{18}H_3C^{13}OOH$).

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Synthesis and Structural Characterization of a New Type of Homonuclear Metal Carbonyl, $[Ni_5(CO)_9(\mu_2\text{-}CO)_3]^{2-}$. A Trigonal Bipyramidal Metal Cluster System[1]

Sir:

We wish to report the isolation and stereochemical analysis of the $[Ni_5(CO)_9(\mu_2\text{-}CO)_3]^{2-}$ dianion which represents the first known example of a pentanuclear metal carbonyl with 12 ligands and of a homonuclear metal cluster possessing a bonding trigonal bipyramidal architecture.[2-4] Unlike the vast majority of nontransient polynuclear metal carbonyls which comply to the conceptually localized electron-pair mode of metal–metal bonding, this pentanickel carbonyl dianion necessitates a delocalized metal cluster bonding description which thereby provides a possible analogy between discrete metal clusters and one-dimensional metallic materials. This work was a direct result of continuing research effort in the area of the nickel and platinum carbonyl anions[5,6] for the purpose of unequivocally establishing their highly unusual structures coupled with an elucidation of their physicochemical properties and intriguing bonding behavior. The results further support our premise that the $Ni_3(CO)_3(\mu_2\text{-}CO)_3$ fragment, which was also found[6] to be the structural basis for the $[Ni_3(CO)_3(\mu_2\text{-}CO)_3]_2^{2-}$ dianion, is a fundamental building block for the nickel carbonyl anions.

Previously it has been reported[7] that the reduction of $Ni(CO)_4$ with lithium or sodium amalgam gives rise to the formation of nickel carbonyl dianions of formulas $[Ni_3(CO)_8]^{2-}$ and $[Ni_4(CO)_9]^{2-}$. We have found, however, that $Ni(CO)_4$ is reduced by alkali metals or alkali amal-