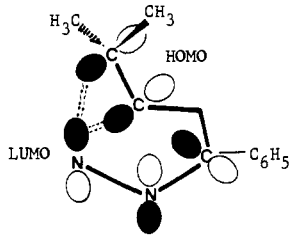

Figure 1.

satisfactory linear log K vs. $1/T$ plots were obtained. The calculated equlibrium constants at 50 °C were listed in Table I, from which the Hammett relation, log $K^X/K^H = 1.12\sigma_p^+$ ($r = 0.975$), was derived. The magnitude of the equlibrium constant increases as the electron-withdrawing nature of the substituent increases. This coincides with the difference in the calculated free energy change (ΔG) for the six derivatives and implies that the more electron-withdrawing substituent destabilizes **4** but stabilizes **5**. Thus, when **4a** was dissolved in carbon tetrachloride, yellow orange color due to **5a** spontaneously developed and a mixture containing 31% of **4a** and 69% of **5a** was obtained even at 25 °C. In contrast, **4f** was stable at 25 °C. Destablization of **4** and stabilization of **5** by the more electron-withdrawing group reflects on the rate acceleration of the retro-1,1-cycloaddition by the more electron-withdrawing substituent as shown in the table. The same substituent effect on the first-order rate constants was also observed in the 1,1-cycloaddition, but the rate acceleration by a strong electron-withdrawing group such as the nitro group is not significant as compared with that of the retro-1,1-cycloaddition as the Hammett relation, log $k_2^X/k_2^H = 0.38\ \sigma_p$ ($r = 0.996$), shows. The observed rate acceleration of the 1,1-cycloaddition by the more electron-withdrawing substituent contradicts the conclusion reached by Padwa and Fukunaga that allyldiazomethane substituted with an electron-withdrawing group cannot be expected to undergo 1,1-cycloaddition.[11] Their conclusion unfortunately was based on a single experimental result that prolonged heating of *trans*-1-phenyl-4-(*p*-nitrophenyl)-4-diazobut-1-ene did not afford any clear product but not on a rate analysis based on the reverisibility of this reaction.

It should be noted that the substituent effect on the rate of the 1,1-cycloaddition is opposite to that of the intermolecular 1,3-dipolar cycloaddition of the substituted phenyldiazomethanes with dipolarophile[7] but resembles that of the amino–nitrene 1,1-cycloaddition to the C=C double bonds.[12] This observation indicates the electrophilic nature of the terminal nitrogen of diazomethane and provides an intriguing mechanistic rationale for the 1,1-cycloaddition reaction of allyldiazomethane. When the terminal nitrogen approaches the C=C double bond in a manner that resembles the nonlinear nitrene 1,1-cycloaddition, as shown in Figure 1, the highest stabilization should be gained by interaction between the LUMO(diazomethane)[13] and the HOMO(olefin). This interaction is strongly reinforced by the cyclic geometry which prevents the HOMO(diazomethane)–LUMO(olefin) controlled parallel-plane approach.[14] Thus, the substituent effect on the rate of the 1,1-cycloaddition reaction of allyldiazomethane is a result of the change of the electrophilic LUMO energy level of diazomethane.[15] Further mechanistic investigations of the intramolecular 1,1-cycloaddition reaction of diazoalkenes are in progress and will be soon reported elsewhere.

Registry No. (*E*)-**1b**, 87013-62-5; (*E*)-**1c**, 87013-63-6; (*E*)-**1d**, 87013-64-7; **3c**, 87013-65-8; **3d**, 87039-24-5; **4a**, 87013-66-9; **4b**, 87013-67-0; **4c**, 87013-68-1; **4d**, 76620-31-0; **4e**, 87013-69-2; **4f**, 87013-70-5; **5a**, 87013-71-6; **5b**, 87013-72-7; **5c**, 87013-73-8; **5d**, 76620-33-2; **5e**, 87013-74-9; **5f**, 87013-75-0.

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Stereospecificity in the Biosynthesis of Phytosterol Side Chains: ^{13}C NMR Signal Assignments of C-26 and C-27

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The biosynthetic mechanism of phytosterol side-chain formation has been postulated to include transalkylation at C-24 from the methyl group of methionine (Scheme I).[1,2] The hydrogen migration from C-24 to C-25 was demonstrated to be stereospecific by examining isofucosterol (**3**-I). Thus, the *pro-R* methyl group (C-26) in **3**-I originates from the *E* methyl group in lanosterol (**1**).[3] Double-bond migration (**3** → **4** or **5**) followed by reduction was suggested to produce sitosterol.[2,4] From the stereochemical viewpoint, each migration and hydrogenation reaction occurs in two ways, leading to opposite configurations at C-25 (**6** or **7**). Recently, we demonstrated that the sitosterol side chain (**6**) is formed stereospecifically from [1,2-^{13}C]acetate, although ^{13}C NMR signals of C-26 and C-27 (*pro-R* and *pro-S* methyl group at C-25, respectively) could not be unambiguously assigned.[5]

Here we report the assignments of these signals and the biosynthetic stereospecificity of phytosterol side chains. We established cell cultures of *Physalis peruviana* from which isofucosterol as well as 24-methylenecholesterol, sitosterol, and stigmasterol, but not withanolide,[6] were isolated. [1,2-^{13}C]Acetate was incorporated into the sterols, which were isolated as their acetates.

Of the ^{13}C-labeling patterns of the phytosterols obtained from [1,2-^{13}C]acetate, the singlet at δ 20.93 and the doublet at δ 21.01 of [^{13}C]isofucosteryl acetate (**3**-II) can be assigned to C-26 and C-27, respectively, according to Nicotra et al.[3]

On catalytic deuteration, **3**-II was converted into an acetate mixture of [^{13}C,24,28-^{2}H]sitosterol (**10**-I) and [^{13}C,24,28-^{2}H]-clionasterol (**11**-I) (M^+ of **10**-II and **11**-II, m/z 456:457:458 = 6:7:5). As no signals arisen from 25-^{2}H could be observed, the labeling patterns of C-26 and C-27 should be almost the same as those of **3**-II.

To analyze the labeling patterns of the methyl groups at C-25 in the mixture, we applied the "INADEQUATE" pulse sequence, which was originally proposed for observing naturally abundant ^{13}C–^{13}C couplings.[8] ^{13}C–^{13}C coupled signals due to doubly enriched carbons from [1,2-^{13}C]acetate were observed selectively. Carbon-13 NMR spectral analysis of the **10**-II and **11**-II mixture was done at 50.309 MHz with a Varian XL-200 NMR spectrometer. The sample was dissolved in [^{2}H]chloroform (40.7 mg in 0.5 mL) in a 10-mm spherical cell. The "INADEQUATE" pulse sequence was optimized for $^{1}J_{CC} = 37$ Hz {$\tau = 1/(4J)$}: 32K data points were acquired with a frequency range of 3200 Hz giving a digital resolution of 0.2 Hz/point. To suppress the single ^{13}C signals, an optimal 90° pulse was set for the sample and steady-state condition was employed before data accumulation (number of transients, 29K). Results are shown in Figure 1,[9] and

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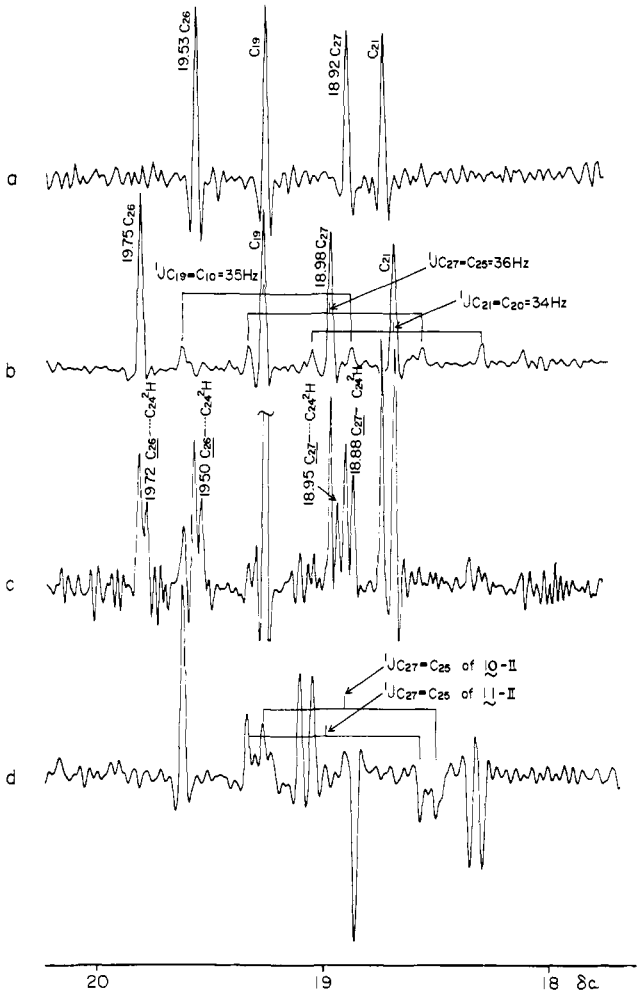
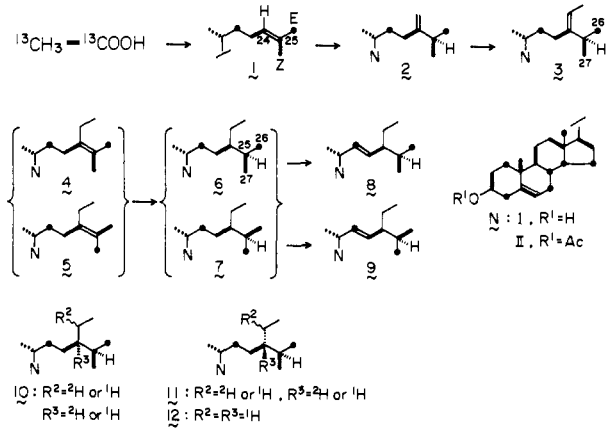


Figure 1. ^{1}H-complete-decoupled ^{13}C NMR spectra, region of C-26 and C-27 of (a) clionasteryl acetate (**12**-II), (b) sitosteryl acetate (**6**-II) from [1,2-^{13}C]acetate in tissue cultures of *P. peruviana*, (c) a mixture of [^{13}C,24,28-^{2}H]sitosteryl acetate (**10**-II) and [^{13}C,24,28-^{2}H]clionasteryl acetate (**11**-II) derived from **3**-II which was biosynthesized from [1,2-^{13}C]acetate in *P. peruviana*, and (d) the same sample determined using Freeman's "INADEQUATE" pulse sequence (ref 8). The following ^{13}C-^{13}C coupled signals are observed in d: C_{19} (δ_C 19.24, J_{CC} = 35), C_{21} (δ_C 18.77, J_{CC} = 34), and C_{27} (δ_C 18.92, J_{CC} = 36) coupled to C_{10} (δ_C 36.46), C_{20} (δ_C 36.15), and C_{25} (δ_C 28.84), respectively, for **11**-II; C_{19} (δ_C 19.24 J_{CC} = 35), C_{21} (δ_C 18.72, J_{CC} = 34), and C_{27} (δ_C 18.98, J_{CC} = 36) coupled to C_{10} (δ_C 36.46), C_{20} (δ_C 36.03), and C_{25} (δ_C 29.06), respectively, for **10**-II. The singly labeled C_{26} signals (δ_C 19.53 for **11**-II, δ_C 19.75 for **10**-II) were suppressed in d.

Scheme I



the signal assignments of C-26 and C-27 of sitosteryl and clionasteryl acetates are shown in Table I.

Table I. ^{13}C NMR Spectral Data for C-26 and C-27 of Phytosterols from [1,2-^{13}C]Acetate in Tissue Cultures of Some Higher Plants[a] [10]

	3-II[7]	6-II	12-II	8-II	8A-II	2-II
C-26 δ_C	20.93, s	19.75, s	19.53[b]	21.01, s	21.02, s	21.79, s
C-27 δ_C	21.01,	18.98,	18.92[b]	18.92,	18.93,	21.93,
($^1J_{CC}$, Hz)	d (36)	d (36)		d (36)	d (36)	d (36)

[a] 3-II and 2-II: Isofucosteryl acetate and 24-methylenecholesteryl acetate from *Physalis peruviana*. 6-II: Sitosteryl acetate from *Physalis peruviana*, *Dioscorea tokoro*,[4] and *Isodon japonicus*.[5] 8-II: Stigmasteryl acetate from *Physalis peruviana*, *Bupleurum falcatum*, and *Dioscorea tokoro*. 12-II: Clionasteryl acetate. 8A-II: α-Spinasteryl acetate (Δ^7 isomer of 8-II) from *Bupleurum falcatum*. [b] These assignments were reversed in ref 4, 11, 12, and 13.

We examined the labeling patterns of C-26 and C-27 of several typical sterols, sitosterol (**6**-I), stigmasterol (**8**-I), α-spinasterol (**8A**-I), and 24-methylenecholesterol (**2**-I), biosynthesized from [1,2-^{13}C]acetate in cell cultures of some higher plants (see Table I[10]). In all cases, C-26 (*pro-R* methyl group at C-25) predominantly originated from C-2 of MVA and C-27 (*pro-S* methyl group) from C-6.

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Registry No. 2-I, 474-63-5; **3**-I, 481-14-1; **6**-I, 83-46-5; **8**-I, 83-48-7; **8A**-I, 481-18-5; **12**-I, 83-47-6.

(10) Carbon-13 NMR spectra were recorded on a Varian XL-200 NMR spectrometer in a 10-mm spherical cell at 23 °C at 0.02–0.2 M in $CDCl_3$. Typical FT measurement conditions: spectral width, 9058 Hz; pulse width, 6 μs (45°); acquisition time, 1.766 s; number of transients, 70K. Accuracies of δ_C and J_{CC} are within 0.02 ppm and 1 Hz, respectively.

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Novel Silicon-Promoted Cyclialkylation of Alkenylmetal Derivatives

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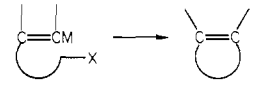
Cyclization of alkenylmetals via cyclialkylation (eq 1) is a



(1)

potentially useful but largely untested methodology.[2] We disclose herein two such reactions in which silicon plays subtle but critical roles. A particularly noteworthy feature of these reactions is that the cis arrangement of the two cyclizing groups, i.e., M and X, that might seem a requisite, either is unimportant or can readily be attained under the cyclization conditions.

In our recent study of the effects of hetero substituents on the Zr-catalyzed carbometalation of alkynes,[3] 1-(trimethylsilyl)-4-bromo-1-butyne (**1b**) was treated with Me_3Al (2 equiv) in the

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(2) For cyclialkylation of aryllithiums, see: Parham, W. E.; Bradsher, C. K. *Acc. Chem. Res.* **1982**, *15*, 300.
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