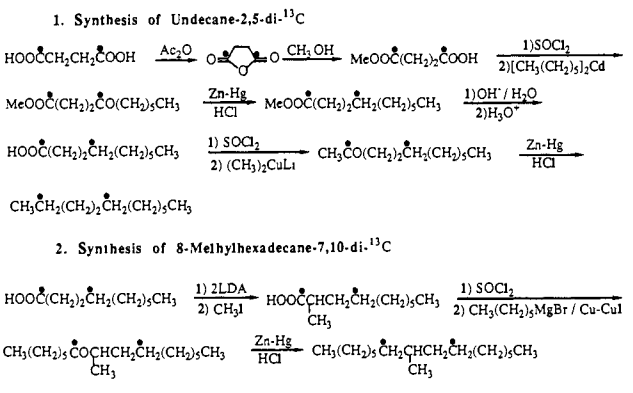
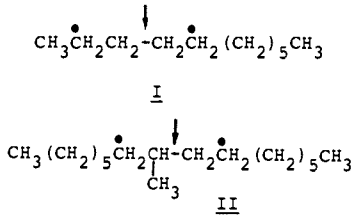
Scheme I



to predominate. With regard to solvent effects on chain conformation, Rosenthal et al.[10] find, via Raman spectroscopy, that trans/gauche energy differences vary little when *n*-butane is transferred from pure liquid to CH_2Cl_2 or CCl_4. In contrast, IR work of Casal et al.[11] on *n*-tridecane-7,7-d_2 shows that the average gauche fraction increases from 35% to 60% when the solvent is changed from *n*-heptane to hexadecane. To compound the uncertainties, trans/gauche ratios have never been determined in polar solvents. No one knows how flexible chains acquiesce to the constraints (if such exist) imposed by various solvent shells.[12]

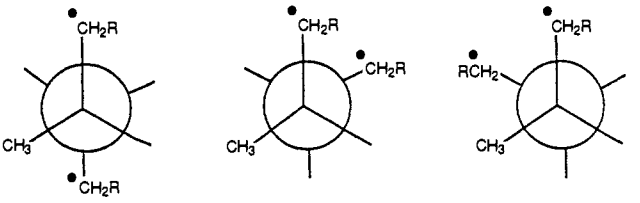
We have exploited the di-^{13}C-labeling method (already applied to several chemical and biological systems[13-15]) to conformational changes in hydrocarbons I and II dissolved in diverse solvents.



The method is based on the Karplus-like responses of $^3J_{CC}$ to the relative disposition of the two ^{13}C atoms about the central bond. Thus, when trans rotates to gauche, $^3J_{CC}$ decreases from 4 to 2 Hz. Once the labeled compounds were in hand (Scheme I),[17] securing the couplings, and hence the conformational preferences, was trivial; we merely had to measure separation between members of the two doublets looming above the natural abundance peaks.

NMR runs on hydrocarbon I were performed at 25 °C with 0.5 and 2.0 M solutions of 10% dilabeled material with 400 aquisitions at a sweep-width of 1600 Hz on an IBM 200 MHz instrument. A $^3J_{CC}$(obsd) of 3.6 ± 0.1 Hz was obtained consistently in four solvents of widely differing polarity: chloroform, ether, ethanol, and 13% water-in-ethanol. The coupling of 3.6 Hz corresponds to 24% gauche and 76% trans (calculated from J_g = 2.0 Hz and J_t = 4.1 Hz[16] and by assuming that $^3J_{CC}$(obsd) is a weighted average of the two). Since the couplings in the four solvents are constant (within the precision of the experiments that allowed detection of $a \geq 10\%$ change in gauche value), it is clear that the conformation of I about the C_3–C_4 bond is unaffected by solvation phenomena. Note that only one of the four distinct C/C dihedral angles in undecane has been probed. There is no reason to suspect, however, that the C_3–C_4 linkage behaves atypically among its neighbors although further work on this point is required.

Dilabeled hydrocarbon II was investigated because, according to molecular mechanics calculations (MODEL), two of its major rotamers (trans T and gauche G_1) possess virtually identical steric



T G_1 G_2

energies (12.7 kcal/mol). Since interconversion costs little or no "conformational energy", solvation effects could conceivably dictate the equilibria in the solution phase. Such is not the case however. Long-range couplings of 2.8 ± 0.1 Hz were observed with neat II and with material dissolved in hexane, cyclohexane (spectra traced normally and with an INADEQUATE pulse sequence), xylene, chloroform, acetone, ethanol, and 14% water-in-ethanol. The remarkable insensitivity of chain conformation to surroundings was further demonstrated by MM2 dihedral driver computations from which we deduced populations of 62% (G_1 + G_2) and 38% T. These numbers, along with J_g = 2.0 Hz and J_t = 4.1 Hz, gave a calculated $^3J_{CC}$ = 2.8 Hz in the gas phase—the *identical* value found in various solvents including aqueous ethanol.

Hydrocarbon folding in 100% water remains an unknown (insolubility prevented the experiment). Extrapolating our results to water would, of course, be reckless despite the wide range of solvent polarities employed. Water's unique behavior, like its natural purity, must be respected.

Acknowledgment. This work was supported by the National Institutes of Health.

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Structure of Aranorosin, a New Antibiotic of a Novel Skeletal Type†

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A novel antibiotic, designated aranorosin (**1**) has been isolated from a fungal strain, *Pseudoarachniotus roseus*. The compound is active against Gram-positive bacteria and fungi and also shows antitumor properties.[1] The antibiotic, present in both the mycelium and culture filtrate, is extractable and purified by repeated chromatography over silica gel.[2] In this paper we report

† Dedicated to Prof. N. S. Narasimhan of the University of Poona on the occasion of his 60th birthday.

(1) Minimum inhibitory concentration (μg/mL) of aranorosin against a few selected test organisms: *Staphylococcus aureus* 209P, 1.5; *Streptococcus faecalis*, 3.0; *Bacillus subtilis*, 1.5; *Pseudomonas aeruginosa* ATCC 9027, >100; *Candida albicans*, 30; *Aspergillus niger*, 7.5; *Penicillium italicum*, 30; *Microsporum gypseum*, 3.0.
(2) Roy, K.; Mukhopadhyay, T.; Reddy, G. C. S.; Desikan, K. R.; Rupp, R. H.; Ganguli, B. N., manuscript submitted to *J. Antibiotics*.

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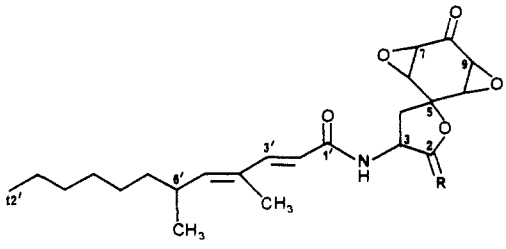
on the structure elucidation of aranorosin [mp 150 °C dec; $[\alpha]^{20}_D$ −2.42° (*c* 2.58, $CHCl_3$); HRMS *m/z* 419.2281 (M^+), $C_{23}H_{33}NO_6$; UV (MeOH) λ_{max} 264 nm] which has a hitherto unreported cyclohexanone bisoxirane moiety as part of the novel 1-oxaspiro[4.5]decane ring system.

One of the two D_2O exchangeable hydrogens of **1** undergoes acetylation with acetic anhydride–pyridine to give aranorosin acetate (**2**). The $\Delta\delta$ of the α hydrogens upon acetylation (δ 5.63



1: R = H, OH
2: R = H, $OCOCH_3$
3: R = O

to δ 6.51) and their integration indicate the presence of the hydroxymethinyl group[3] which is part of a hemiacetal functionality (methine proton δ 5.63/d, J = 4.3 Hz and hemiacetal carbon δ 96.58/d). As expected these signals are absent in derivative **3**, obtained by Jones oxidation of **1**. The other D_2O exchangeable hydrogen (δ 6.09/d, J = 8.2 Hz) is a secondary amide hydrogen (α-H δ 4.80/m). The presence of a —C—CH_2CH(NH)CH(OH)O— fragment in **1**, as an isolated five proton spin system, was established by the ^{1}H–^{1}H COSY spectrum[4] of **1**.

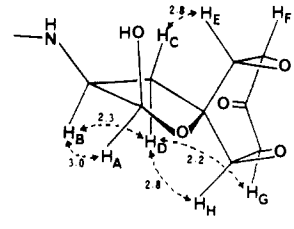
Another set of an isolated four proton spin system, identified by the COSY experiments resulted in an intriguing situation. Each of the four methinyloxy-like protons (δ 3.68 (E), 3.57 (H), 3.46 (G), and 3.44 (F)) is coupled to two of the remaining three protons (J_{EH} = 3.9 Hz, J_{GF} = 2.9 Hz, and J_{HG} = J_{FE} = 3.5 Hz). While the hemiacetal group accounted for two oxygens—one OH and one tertiary alkoxy-like group (δ 78.83/s), the carbonyl groups (δ 198.44/s and 166.97/s) accounted for two more oxygens. Thus four out of six oxygens present in **1** could be accounted for. The remaining two oxygens must be linked to the four methinyloxy-like carbons. This led to an impossible proposition of a cyclobutane bisoxirane moiety without any point of linkage to the rest of the molecule.

This ambiguity was resolved by 2D INADEQUATE spectra[5] of **1** which established the carbon–carbon connectivity pattern. The attachment of heteroatoms to the appropriate carbons of the skeleton was made according to the carbon chemical shifts. This led to the assignment of a spiro ring system, 1-oxaspiro[4.5]decane, with the spiro carbon (δ 78.83) attached to three carbons (δ 64.36/d, 63.01/d, and 35.85/t) and an oxygen. The 1,4-related spiro carbon and the carbonyl carbon (δ 198.44) are interspaced on both sides by carbons attached to oxygen. The fully coupled ^{13}C NMR spectrum of **1** unequivocally established that these four carbons were part of two oxirane rings—the direct bond J_{CH} values (183.0 and 184.2 Hz) being in the typical range for oxiranes and readily distinguished from typical methinyloxy carbon direct bond J_{CH} values (130–140 Hz).[6]

The PND and SFORD spectra of **1** indicated the presence of an amide carbonyl (δ 166.97) and four olefinic carbons. Three out of the four olefinic carbons are monosubstituted (δ 148.45/d, 147.36/d, and 117.00/d), and the remaining one is disubstituted (δ 130.78/s). While ^{1}H–^{1}H COSY experiments established the presence of a —$CH(CH_3)$CH=$C(CH_3)$CH=CH— moiety in **1**, the 2D INADEQUATE spectrum showed that this group forms part of an $\alpha,\beta,\gamma,\delta$ unsaturated carbonyl system. In conjunction with the HETCOSY[7] data this enabled the complete spectral assignment and structure of the acyclic fatty acid portion. Mass spectral fragmentation[8] of **1** was in conformity with the presence of such a group. Further, this acidic fragment was actually isolated during an attempted reductive acetylation. The mutually coupled α (δ 5.77) and β (δ 7.25) protons having a coupling constant of 15.3 Hz is typical of an *E* configurated olefin. NOE[9] studies indicated the *E* configuration for the γ,δ (δ 5.67) double bond also. Thus, irradiation at δ 5.67 resulted in 10.9% enhancement in the signal intensity at δ 7.25.

The acyclic acidic and the tetracyclic spiro amino alcohol portions are joined through an amide bond. This is evidenced by the ^{13}C–^{1}H long range coupling[10] (observed with an optimized J_{CH} value of 8 Hz) between the carbonyl carbon at δ 166.97 and the amide hydrogen and further supported by mass spectral fragmentation.[8] This completed the assignment of the constitutional formula of aranorosin (**1**). As may be assumed considering the structure of **1**, it is a mixture of two isomers epimeric at C-2. This resulted in two sets of signals for most protons and carbons. The structure elucidation is based on the spectroscopic data of the major isomer which is estimated to constitute ~75% of the mixture.

The distant constraints deduced from NOE experiments and the angular constraints derived from J_{HH} values collectively defined the relative configuration of the amine part (I) of **1**. The vicinally



I (% NOE)

disposed OH and NH groups are cis with θ(A–B) ≤ 40° (J_{AB} = 4.3 Hz). The dihedral angles θ(B–D) ≤ 20° (J_{BD} = 8.6 Hz) and the θ(B–C) ≥ 160° (J_{BC} = 10.6 Hz) indicated the conformation of the five-membered ring. While the long range couplings, J_{EH} and J_{FG}, necessitate that the cyclohexanone ring exists in a boat conformation, the spatial proximity of H_C, H_E and H_D, H_H are possible only if the two oxiranes and the hemiacetal group are on the same face of the molecule.

The carbon skeleton of the amine part of **1** has not been reported in the literature. The unique structural features of aranorosin combined with its interesting bioactivity make it an interesting lead compound. Structure-activity relationship studies are in progress in our laboratories.

Acknowledgment. We thank Dr. K. Roy and K. R. Desikan for providing us with the fermented broth from which aranorosin was isolated. We also thank Dr. P. K. Inamdar for some of the spectra.

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(8) *m/z* (EIMS): 419 (M^+), 401 (M − H_2O), 330 (M − H_2O–C_5H_{11}), 316 (M − H_2O–C_6H_{13}), 306 (M − C_8H_{17}), 288 (M − C_8H_{17}–H_2O), 250 ($C_{16}H_{28}NO$), 224 ($C_{14}H_{26}NO$), 207 ($C_{14}H_{23}O$), 179 ($C_{13}H_{23}$).

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Supplementary Material Available: Mass spectrum, 400 MHz ^1H NMR, and 100 MHz ^{13}C NMR spectra with assignments and ^1H-^1H and ^1H-^{13}C COSY and ^{13}C-^{13}C INADEQUATE 2D spectra of **1** (7 pages). Ordering information is given on any current masthead page.

A Stable, Simple Enol: Ketonization of 2-Methylprop-1-en-1-ol in Nonaqueous Solvents

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Simple aliphatic enols generated by various methods are mostly unstable under the conditions (mostly aqueous solution) under which they are produced,[1,2] while sterically crowded mesityl-substituted enols are so stable that they can be purely isolated.[3] Detailed kinetic studies have been carried out for the ketonization of simple enols in aqueous solution in the presence of acid and base,[1,2a,4,5] while no report has been made on the kinetics in nonaqueous solvents.

In this communication we report the production of a simple aliphatic enol, 2-methylprop-1-en-1-ol, which is quite stable in the absence of solvents as well as in aprotic solvents and kinetic data for the ketonization of the enol in nonaqueous solvents.

Addition of $[Rh(CO)(PPh_3)_3]ClO_4$ (**1**) (0.02 mmol) into neat 2-methylprop-2-en-1-ol (**2**) (12 mmol) under nitrogen at room temperature immediately initiated the exothermic isomerization (eq 1). (Using a greater amount of **1** resulted in boiling the

2 3 (1)

reaction mixture to produce some unknown decomposition products.) The warm-hot reaction mixture was kept at 0 °C for 1 h until all of **2** disappeared in the reaction mixture. Volatile materials, separated from the catalyst (**1**) by dry ice/acetone trap, contained ca. 95% of **3** and 5% of 2-methylpropanal (**4**) according to ^1H NMR spectra.[6] Almost pure enol ($(CH_3)_2C{=}CHOD$, **3D**) could be obtained when the deuteriated reactant ($(CH_2{=}C(CH_3)CH_2OD$, **2D**) was used (^1H NMR spectrum of the fresh product showed no signals of the corresponding aldehyde ($(CH_3)_2CDCHO$, **4D**)). The ^1H NMR spectra of **3** in CD_3COCD_3 (a sharp doublet at δ ca. 7 (O*H*) with J (O*H*-C*H*) being ca. 5.5 Hz[7] and a multiplet at δ 6.12 (C*H*OH) with equal integrals) indicates that **3** exists mainly in anti conformation.[8]

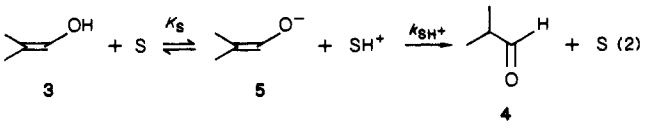
Table I. Observed First-Order Rate Constants for the Ketonization of 2-Methylprop-1-en-1-ol (**3**) in Various Organic Solvents at 27 °C

solvent	k_{obsd}, s^{-1}
CD_3COCD_3	$(1.5 \pm 0.2) \times 10^{-6}$
C_6D_6	$(2.2 \pm 0.3) \times 10^{-5}$
CH_3OH	$(1.3 \pm 0.2) \times 10^{-4}$
CD_3OD	$(5.3 \pm 0.5) \times 10^{-6}$
H_2O	$(4.6 \pm 0.3) \times 10^{-4}$

The enol **3** is quite stable in the absence of a solvent, e.g., only a half of **3** disappears to give **4** and unknown products[9] after 24 h at 25 °C and after more than 10 days at −10 °C, and it shows no sign of reaction for several days at −78 °C where it freezes. Disappearance of **3** in the absence of a solvent was followed by measuring the ^1H NMR spectral changes,[6] and the kinetic data could not be fitted into any simple equation of first or second order in **3**.

Ketonization of **3** to give **4** was followed by measuring the absorbance at 205 nm in H_2O and 210 nm in CH_3OH and ^1H NMR signals of **3**[6] in CD_3COCD_3, C_6D_6, and CD_3OD.[10] The rate data conform well to first-order kinetics. The observed first-order rate constants in H_2O is in good agreement with the previously reported value (4.2×10^{-4} s^{-1} at 25 °C) by Kresge.[4]

The rate data in Table I may well be understood according to the mechanism involving a fast equilibrium (K_S) between enol and enolate ion followed by protonation of carbon (k_{SH^+}) by protonated solvent (SH^+) (eq 2), which is identical with the one suggested



3 5 4

for the water-catalyzed (or uncatalyzed) ketonization of enols in aqueous solution.[4,5] Observed rate constants (k_{obsd} are then products of K_S and k_{SH^+}. The larger k_{obsd} values in protic solvents (H_2O, CH_3OH) than in aprotic solvents (CD_3COCD_3, C_6D_6) may be due to large K_S values for protic solvents (pK_a values for H_3O^+, $CH_3OH_2^+$, and $CH_3COHCH_3^+$ are −1.74, −2.0, and −7.0, respectively; no pK_a value has been reported for $C_6H_7^+$).[11] Larger k_{obsd} value in C_6D_6 than that in CD_3COCD_3 is somewhat unexpected and may be interpreted by larger k_{SH^+} in C_6D_6 than in CD_3COCD_3. The large value of $k_{obsd(CH_3OH)}/k_{obsd(CD_3OD)} = 24.5$ is a supporting evidence for the proposed mechanism (eq 2) since it could be considered as the product of the equilibrium isotope effect for the first step (K_S) and the kinetic isotope effect for the second step (k_{SH^+}). A relatively large value of $k_{H_2O}/k_{D_2O} = 12$ was reported for the ketonization of vinyl alcohol and understood as a product of the equilibrium and kinetic isotope effect.[5] It was found that as the concentration of **4** increases in the reaction mixture, formation of acetal and H_2O from the reaction of **4** with solvent (CH_3OH or CD_3OD) becomes significant, and the rate of the ketonization is accelerated by H_2O. Therefore, k_{obsd} was obtained in CH_3OH and CD_3OD at the early stage of the ketonization.

Finally it might be said that the ketonization catalyzed by the solvent molecule playing the role of acid, whether through the concerted[2a] or stepwise mechanism,[4,5] should be negligible since

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(9) It has been found by means of TLC separation and ^1H NMR analyses that unknown products contain a significant amount of aldol condensation products from the reaction of **3** and **4**.

(10) Ketonization of **3** in $CDCl_3$ is quite rapid.[6] Careful experiments revealed that $CDCl_3$ (Fluka 31330; $H_2O + D_2O < 0.01\%$) contains trace amounts of impurities that rapidly catalyze the ketonization. The ketonization rate is considerably slow ($t_{1/2} = 3$ min) in $CDCl_3$ treated with molecular sieves (Aldrich 3A) for 24 h. Molecular sieve treatment seems to remove the impurities only in part.

(11) Values of pK_a are approximate and relative to water. See: March, J. *Advanced Organic Chemistry*; Wiley-Interscience: New York, 1985; pp 220-222.

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