5-chloro-*N*-methylisatoic anhydride, 338, 405, 1.21; 5-sulfo-*N*-methylisatoic anhydride, 330, 395, 0.92; 3-azaisatoic anhydride, 350, 435, 3.55.

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Registry No.—**1**, 6579-46-0; **2**, 118-48-9; 5-Cl-**2**, 20829-96-3; 5-NO_2-**2**, 20829-97-4; *N*-Me-**2**, 10328-92-4; 5-Cl-*N*-Me-**2**, 40707-01-5; 5-sulfo-*N*-Me-**2**, 63016-84-2; 5-aza-**2**, 63016-85-3; **7**, 63016-86-4; **8**, 630160-87-5; anthranibic acid, 118-92-3.

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Stereochemistry of Valerenane Sesquiterpenoids. Crystal Structure of Valerenolic Acid[1]

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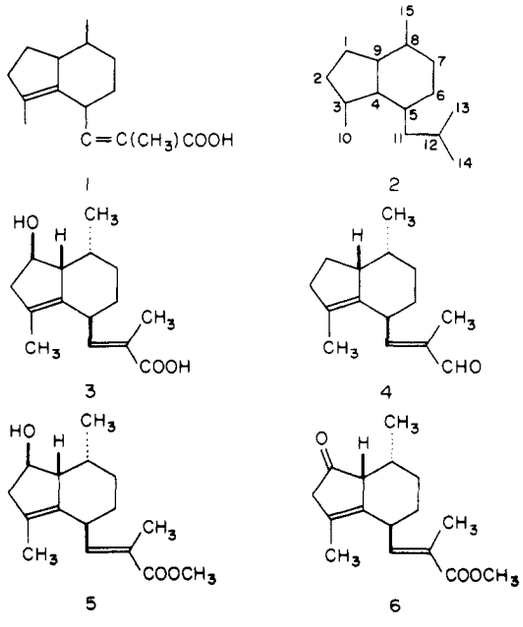
The three-dimensional structure of valerenolic acid, $C_{15}H_{22}O_3$, was determined by x-ray crystallography. The substance crystallizes in the orthorhombic space group $P2_12_12_1$ and the unit-cell dimensions are a = 12.705 (2), b = 14.476 (3), c = 15.477 (1) Å. Intensity data were measured with Cu radiation on a four-circle diffractometer. The structure was solved by direct methods and refined to R = 3.6% for 2543 reflections. The hydroxyl group attached to the five-membered ring is cis to the hydrogen atom at the adjacent ring junction, and both are trans to the axial methyl substituent on the six-membered ring. The latter is trans to the methacrylic acid side chain, in which the methyl group is cis to the ring carbon atom. Chemical and spectroscopic data indicate that the same stereochemistry also occurs in valerenic acid and in valerenal. The absolute configuration was established on the basis of the CD spectrum of methyl 1-ketovalerenate.

Valeriana officinalis L. has been used for centuries in popular medicine as a mild sedative or tranquilizing agent in the form of aqueous or alcoholic extracts of its roots and rhizomes, and it has been included in pharmacopeias of many countries.[3] The search for its active principle took more than a century and it was shown relatively recently that, while the main active principles are undoubtedly esters and glucosides of terpenoids possessing an iridoid skeleton,[4,5] some of its sesquiterpenoid constituents such as valeranone[6] (a mild sedative) and valerenic acid[7] (a spasmolytic) may well contribute to the overall effect of the drug.

Valerenic acid and the closely related acetylvalerenolic acid were first isolated from the drug in Sandoz laboratories,[7] where their pharmacological profiles were investigated as well. It was Büchi and co-workers[8] who showed that valerenic acid possesses the unique structure **1**. It represents the first example of a quite unusual skeletal type **2** (valerenane) in terpenoid chemistry, and since its discovery only valerenolic acid (**3**)[9] and valerenal (**4**)[10] have been confirmed as belonging to the same family. However, the stereochemistry of all chiral centers and the geometry around the double bond in the side chain have remained unknown.



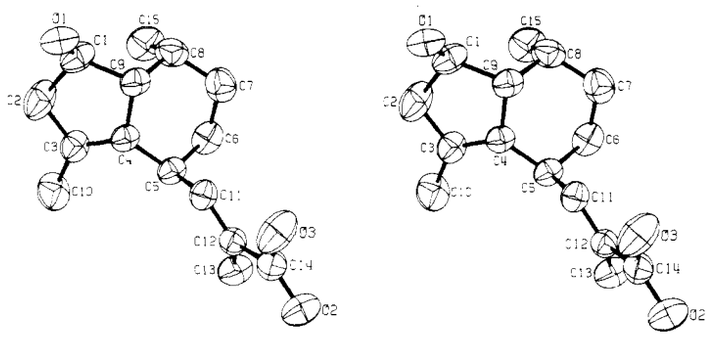


Figure 1. Stereoscopic view of valerenolic acid (mol. A). The thermal ellipsoids correspond to 50% probability.

Table I. Torsion Angles (in Degrees)

	mol. A	mol. B
C(9)-C(1)-C(2)-C(3)	6.6	15.9
C(1)-C(2)-C(3)-C(4)	−3.5	−10.9
C(2)-C(3)-C(4)-C(9)	−1.2	1.1
C(3)-C(4)-C(9)-C(1)	5.4	9.1
C(4)-C(9)-C(1)-C(2)	−7.1	−15.2
C(9)-C(4)-C(5)-C(6)	−48.7	−51.3
C(4)-C(5)-C(6)-C(7)	48.4	49.6
C(5)-C(6)-C(7)-C(8)	−57.1	−57.1
C(6)-C(7)-C(8)-C(9)	58.8	57.4
C(7)-C(8)-C(9)-C(4)	−54.9	−53.8
C(8)-C(9)-C(4)-C(5)	53.8	55.5
C(11)-C(12)-C(14)-O(3)	−3.8	2.1
C(13)-C(12)-C(14)-O(2)	−5.2	2.5

Because of the uniqueness of the structure and the physiological activity of at least one of these three compounds, we considered the determination of the relative and absolute stereochemistry of this group of compounds to be desirable. An x-ray analysis appeared worthwhile and valerenolic acid (3) was the best candidate, as it contains one additional chiral center at C-1.

Results and Discussion

X-Ray Analysis. A stereoscopic view of valerenolic acid is shown in Figure 1. The x-ray analysis revealed the configuration at the four chiral centers as well as the isomerism of the exocyclic double bond. The hydroxyl group is cis to the hydrogen atom at the adjacent ring junction and both are trans to the methyl substituent on the six-membered ring; the latter is trans to the methacrylic acid side chain. Both of these substituents are in axial orientation. The six-membered ring is chair shaped and the torsion angles (Table I) indicate that the conformation in the two crystallographically independent molecules is very similar. The exocyclic double bond and the axial substituents[11] cause the torsion angles to deviate from the normal values of ±55.9° in a cyclohexane chair.[12] The decreased average torsion angle (54°) expresses the expected flattening of the ring. Very similar torsion angles were observed in capsidiol[13] in which there also is a double bond exocyclic to a six-membered chair with axial substituents. These distortions are also manifested by bond angles: the angles are smaller at the carbon atoms bearing the axial substituents than the normal value of 111.1° [12] and larger at C-6 and C-7. The same pattern was observed in capsidiol.[13]

The presence of two trigonal atoms in a five-membered ring normally causes that ring to adopt a flattened envelope conformation.[14] A calculation of Δ, the phase angle of pseudorotation,[15] reveals that in molecule B its value deviates by only 7° from that in a perfect envelope. However, in molecule A the conformation is just halfway between an envelope and half-chair. The maximum torsion angle, ϕ_m, is 7.5° in molecule A and 16.7° in molecule B. The decreased pucker (in cyclopentene the angle of pucker was found to be 29° [16]) is presumably caused by the fusion to the six-membered ring. Small deviations of C-1 from the mean plane through the four other atoms in the ring, 0.114 and 0.257 Å in molecules A and B, respectively, are additional indications of the ring's unusual flatness.

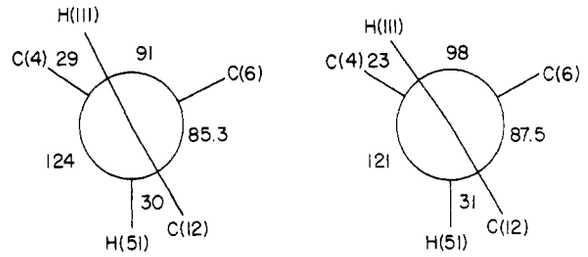


Figure 2. Newman projection along the C(11)-C(5) bond for molecules A (left) and B (right).

The Newman projections in Figure 2 show the attachment of the methacrylic acid side chain to the six-membered ring. From the remarkable similarity between the conformations in the two independent molecules one may conclude that this conformation represents an energy minimum. It is also of interest to examine the isomerism and conformation within the side chain. Firstly, it should be pointed out that, contrary to previous publications,[8,9] the carboxyl group is trans to C-5. Secondly, the O=C—O—H groups were found to be in the more stable synplanar conformation[17] in both independent molecules. Finally, the conformation of the C=C—C=O groups is antiplanar; this conformation is rather rare and requires some comment.

A recent review of carboxylic acids[18] reveals that in saturated acids the C—C—C=O group is invariably synplanar. Leiserowitz and Schmidt[19] attributed this phenomenon to nonbonded interactions. In unsaturated carboxylic acids the effect of such interactions is reduced. On the other hand, a bent-bond description of carbonyl double bonds favors an antiplanar conformation of C=C—C=O because it corresponds to the energetically preferable staggering of bonds about the central single bond.[20] Einspahr and Donohue predicted that in α,β-unsaturated acids for which nonbonded interactions are ambivalent, the antiplanar conformation should be preferred; they found their prediction confirmed in the structure of dimethyl *trans,trans*-2,5-dichloromuconate.[21] Bulky substituents in the α position are likely to cause a reversal in the conformational preference attributable to nonbonded interactions; i.e., they reinforce the preference for an antiplanar conformation due to bond staggering. With the α substituent in our structure being bulkier than Cl, it is not surprising to find an antiplanar conformation and the C(13)-C(12)-C(14) bond angle (115.9°) over 2° larger than the corresponding angle in the chloromuconate.[21]

All bond lengths, including those involving hydrogen atoms,

Table II. Distances and Angles for Hydrogen Bonds

	Distances, Å		Angles, deg	
	O···O	O···H	OH···O	HO···O
O(3A)–H···O(1A)[a]	2.645	1.89	162	13
O(3B)–H···O(1B)[b]	2.577	1.76	168	8
O(1A)–H···O(2B)[c]	2.797	1.96	171	6
O(1B)–H···O(2A)[d]	2.777	1.90	179	1

[a] At $\bar{x}, -1/2 + y, 1/2 - z$. [b] At $1 - x, -1/2 + y, 3/2 - z$. [c] At $1/2 - x, 1 - y, -1/2 + z$. [d] At $1/2 - x, 1 - y, 1/2 + z$.

Table III. Carbon Chemical Shifts (in ppm) in the NMR Spectra of Valerenic (1) and Valerenolic (3) Acids

Carbon	1	3
1	24.6	73.4
2	37.5	47.8
3	131.3	128.6
4	133.2	131.7
5	34.7	34.7
6	25.4	25.4
7	28.8	28.8
8	33.1	31.4
9	47.5	57.7
10	12.0[a]	12.9[a]
11	146.2	145.0
12	125.4	125.9
13	13.5	13.4
14	174.3	172.8
15	12.1[a]	12.1[a]

[a] The assignments can be interchanged, even if the slight downfield shift of C-10 can be expected from the homoannular hydroxyl effect.

were found to have expected values and require no further comment. The highly satisfactory agreement between equivalent bonds in molecules A and B, in most cases within 2σ, indicates that the relatively low estimated standard deviations (0.003–0.004 Å, 0.2°) are realistic.

All A molecules in the crystal are joined by a strong hydrogen bond in which the carboxyl group donates its proton to the hydroxyl group in a symmetry-related molecule. The same scheme, with an even stronger hydrogen bond, join all the B molecules. The two chains are cross-linked by hydrogen bonds in which the hydroxyl groups in one chain act as proton donors and the C=O portions of the carboxyl in the other chain act as acceptors. Geometrical details are given in Table II.

Correlation of 3 with 1 and 4. Having determined the structure of **3**, we wished to correlate its stereochemistry with that in **1** and **4**. On the basis of chemical and spectroscopic data, we can demonstrate that the steric relationships of all chiral centers and the geometry of the double bond in the side chain are identical in all three compounds. Valerenic acid (**1**) was transformed unambiguously into **4**,[10] and **3** gave the saturated hydrocarbon **2**, identical with that prepared from **1**.[9] The identity of configurations at C-5, -8, and -9 is further confirmed by the ^{1}H NMR spectra of **1** and **3** which exhibit signals of principal proton groupings with the same chemical shifts and coupling constants.[9]

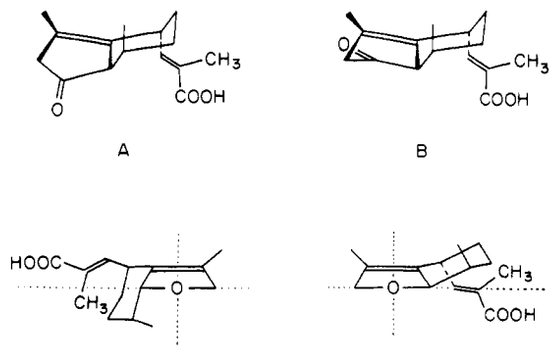
It is well known[22] that carbon chemical shifts are influenced by chiral centers to such an extent that, even if such a center is removed by four bonds from the carbons considered, the shielding differences reflected in differences in chemical shifts for various configurations are significant. As these configurational differences are reflected more strongly on carbons than on protons, we expected that, should the configurations of chiral centers of **1** and **3** be different, the ^{13}C NMR spectra would show considerable differences in carbon chemical shifts, while similar effects in the ^{1}H NMR spectrum might be overlooked. Table III indicates, however, that most carbons exhibit the same chemical shifts. The carbons forming the five-membered ring are an exception, as the hydroxyl located on C-1 in **3** causes, as expected, a profound difference in shielding of these carbons. As can be seen, the actual values of chemical shifts of carbons[23] in both **1** and **3** agree well with the assumption that **3** is a hydroxylated derivative of **1** (with conservation of configuration at all chiral centers). C-1 is shifted to considerably lower fields in **3** because of the α-effect of the hydroxyl substitution, and similarly both C-2 and C-9 experience a strong β-effect (deshielded by 10 ppm), while both C-3 and C-4 are shielded by 2.7 and 1.5 ppm, respectively, and C-8 by 1.7 ppm (expected γ-effects) relative to the values for **1**. Otherwise, all remaining signals retain their chemical shifts in both **1** and **3**.

Table IV. Circular Dichroism Data (in Methanol) for Valerenic and Valerenolic Acids

Valerenic acid λ, nm (Δε)	Valerenolic acid λ, nm (Δε)
	272 (−0.81)
263 (−1.89)	260 (−1.75)
237 (+4.03)	236 (+3.7)
217 (−11.1)	216 (−9.9)
	198 (+7.2)

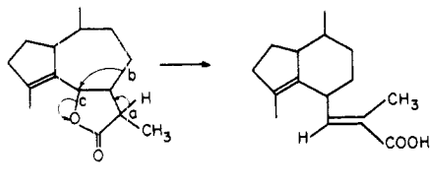
These comparisons show convincingly enough that relative configurations at all chiral centers in **1**, **3**, and **4** are the same, but they do not exclude the possibility that we are dealing with antipodes. In order to prove that the absolute configurations of all three compounds are also identical, we compared the results of two chiroptical methods, i.e., optical rotatory dispersion and circular dichroism. As CD curves have a less complex appearance than ORD curves when several Cotton effects are superimposed to shape the resulting curve, we felt that CD might support earlier reports, based on ORD, on configurational identity of chromophores in **1** and **3**.[9,24] In fact, the overall similarity of the CD curves over the whole spectral range[25] (Table IV) makes this identity practically certain.

Absolute Configuration. We expected the determination of the absolute configuration of the valerenane group to be possible by measurement of the Cotton effect of the carbonyl group resulting from an oxidation of the C-1 hydroxyl in methyl valerenolate. The presence of the Δ^3 double bond forces the five-membered ring to adopt a conformation in which C-2, -3, -4, -9 are approximately coplanar with C-1 displaced from that plane (Table II). This displacement can be either below (A) or above (B) the plane. Both conformations, however, have the same contribution to the Cotton effect (cf. the projections with right-handed Cartesian coordi-



nates), i.e., octant consignate. While recent literature[26-30] deals with six-membered ring systems containing carbonyls, there is a lack of comparative material for five-membered rings. Consequently, we felt at the beginning that any assignment of absolute configuration on this basis would not be devoid of certain ambiguity. We prepared methyl valerenolate (**5**) by treatment of **3** with diazomethane, and it was subsequently transformed to a mixture of oxidation products from which methyl 1-ketovalerenate (**6**) was isolated by chromatography on a silica gel column. It showed only a weak Cotton effect in its CD spectrum at 325 nm ($\Delta\epsilon$ −0.49) in addition to the absorption at 238 nm ($\Delta\epsilon$ +3.20), comparable with similar values in both **1** and **3**. The absolute configuration of valerenolic acid should be therefore as it is portrayed in **3**, since the antipodal structure would exhibit a positive sign of the Cotton effect. The correct absolute configuration was used in the refinement of atomic parameters and is shown in Figure 2.

Biosynthesis. A working hypothesis which helped Büchi and co-workers[8] to determine the structure of **1** was based on



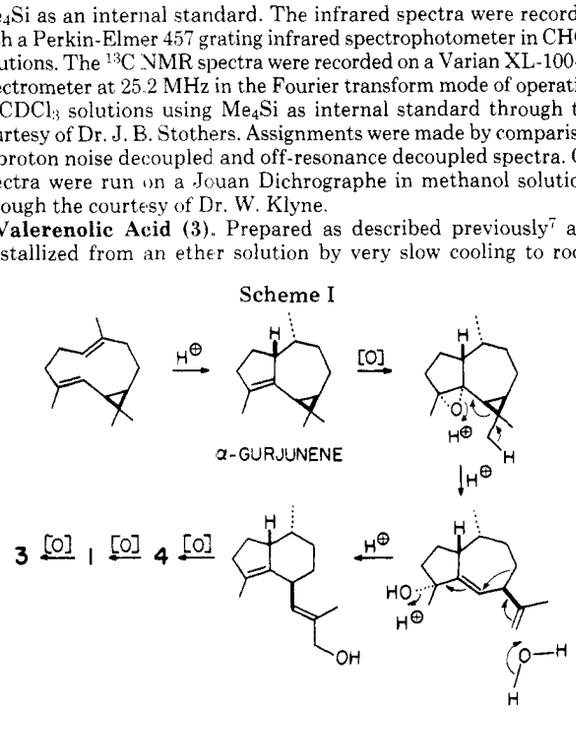
its possible origin from a guaianolide via a rearrangement. Considering that an elimination reaction can proceed only if bonds a and b are coplanar, it is clear that CH_3 and H must eventually appear on the same side of the double bond. However, the configuration determined by the x-ray analysis is the opposite one. On the grounds that the oxidation of an aldehyde to the corresponding acid is more likely than the reverse process, Bates and Paknikar[10] suggested that the immediate precursor of **1** is **4**, which, in turn, may be derived from a guaiane. They also postulated that the valerenanes are biogenetically related to α-gurjunene. Accordingly, Scheme I may now be proposed.

Experimental Section

Melting points were determined on a Kofler hot stage apparatus and are not corrected. Mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6D spectrometer at 70 eV. The ^{1}H NMR spectra were recorded in $CDCl_3$ solutions with a Varian T-60 instrument using Me_4Si as an internal standard. The infrared spectra were recorded with a Perkin-Elmer 457 grating infrared spectrophotometer in $CHCl_3$ solutions. The ^{13}C NMR spectra were recorded on a Varian XL-100-15 spectrometer at 25.2 MHz in the Fourier transform mode of operation in $CDCl_3$ solutions using Me_4Si as internal standard through the courtesy of Dr. J. B. Stothers. Assignments were made by comparison of proton noise decoupled and off-resonance decoupled spectra. CD spectra were run on a Jouan Dichrographe in methanol solutions through the courtesy of Dr. W. Klyne.

Valerenolic Acid (3). Prepared as described previously[7] and crystallized from an ether solution by very slow cooling to room temperature, mp 169.0–169.5 °C. Valerenic acid (**1**), mp 136–137 °C, was crystallized similarly from an ether solution.

Scheme I



Methyl Valerenolate (5). A solution of **3** (16 mg) in 1 mL of purified ether was treated with an excess of ethereal CH_2N_2 for 5 min. The solution was evaporated to dryness and yielded a colorless thick oil: IR ν_{max} 1708 (CO), 1636 (C=C), 3550 cm^{-1} (OH); MS *m/e* 264 (calcd 264). It was used without purification in the following step.

Methyl 1-Ketovalerenate (6). Chromic acid[31] (0.15 mL) was added with stirring to a 17-mg sample of **5** dissolved in dry ether (purified by distillation with $KMnO_4$) and cooled by an ice–water bath. The course of oxidation was monitored by TLC on silica gel and the reaction was stopped when no more **5** was detectable. After a customary workup,[31] the mixture was subjected to chromatography on a column of 15 g of silica gel (Merck, G) in CH_2Cl_2–1 to 5% ether mixtures, and the fractions exhibiting absorptions at 1638, 1708, and 1739 cm^{-1}, but no OH bands, were collected. The solvent was evaporated to give an oily product (5 mg) which showed *m/e* 262 (calcd 262); it was used for CD measurement without further purification.

X-Ray Analysis of Valerenolic Acid (3). Precession photographs indicated orthohombic symmetry with systematic absences of reflections $h00$ for h odd, $0k0$ for k odd, and $00l$ for l odd. The space group was thus uniquely determined to be $P2_12_12_1$. A crystal fragment with dimensions 0.25 × 0.35 × 0.50 mm was mounted along the b axis on a card-controlled Picker four-circle diffractometer equipped with a Cu target. Cell dimensions were determined from angular settings of ten high-angle reflections and both Cu $K\alpha_1$ (λ 1.54051 Å) and Cu $K\alpha_2$ (λ 1.54433 Å) radiations were used. The following crystal data were obtained: a = 12.705 (2), b = 14.476 (3), c = 15.477 (1) Å; V = 2846.5 Å^3; D_x = 1.17, D_m = 1.18 (1) g cm^{-3} (flotation in chlorobenzene/bromobenzene); Z = 8; $F(000)$ = 1088; μ (Cu Kα) = 6.1 cm^{-1}.

The moving-crystal/moving-counter technique (θ–2θ scan) was used to collect the intensity data, and monochromatization was achieved by the use of a nickel filter and a pulse-height analyzer. A net count of 70 or 10% of the background, whichever was higher, was determined as threshold intensity below which reflections were considered unobserved. There were 2726 unique reflections accessible to the diffractometer ($2\theta \leq 130°$) of which 2506 (92%) had intensities above threshold values. The intensities were corrected for Lorentz and polarization factors. Absorption effects were considered insignificant and corrections were not applied.

The structure was determined by direct methods with a multisolution method similar to that described by Kennard et al.[32] With α_{min} = 2.33 and t_{min} = 0.3, one of the 32 permutations yielded R_E = 0.22 for 381 reflections with $E \geq 1.40$ after a tangent refinement carried out in four steps. The E map revealed the positions of all 36 nonhydrogen atoms (two independent molecules) in the asymmetric unit. Atomic parameters were refined by block-diagonal least squares. All scattering factors were taken from the "International Tables for X-Ray Crystallography"[33] and the oxygen curve was corrected for anomalous dispersion ($\Delta f'' = 0.032$). However, the anomalous scattering power of oxygen was not sufficient for a reliable determination of the absolute configuration.[34] The absolute configuration indicated by the CD spectrum is the one reported here. All hydrogen atoms were located on difference Fourier maps and their parameters were refined isotropically. Throughout the refinement, the function $\Sigma w(|F_o| - |F_c|)^2$ was minimized and a factor of 0.8 was applied to all shifts. The following weighting scheme was used during the final stages: $w = w_1w_2$, where $w_1 = 1$ for $|F_o| \leq 10.0$, $w_1 = 10.0/|F_o|$ for $|F_o| > 10.0$; and $w_2 = \sin^2\theta/0.40$ for $\sin^2\theta < 0.40$, $w_2 = 1$ for $\sin^2\theta \geq 0.40$. After the final cycle, the average parameter shift equaled 0.1 σ, and the largest one equaled 0.8 σ. The agreement index $R(\Sigma|\Delta F|/\Sigma|F_o|)$ is 0.036 and the weighted index $R'(\Sigma w\Delta F^2/\Sigma wF_o^2)$ is 0.042 for 2543 reflections, including 40 unobserved ones for which $|F_o| < |F_c|$. Three strong reflections appeared to suffer from extinction effects and were given zero weights. A final difference Fourier map was featureless. A listing of observed and calculated structure factors may be obtained from G.I.B.

Registry No.—**1**, 64130-69-4; **3**, 1619-16-5; **5**, 64130-70-7; **6**, 64130-71-8.

Supplementary Material Available. Atomic coordinates and temperature factors, bond lengths, bond angles, and mean planes (6 pages). Ordering information is given on any current masthead page.

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Steroid Conformations in Solid and Solution: Stereoselectivity of Grignard Addition to 20-Keto Steroids

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Stereoselectivity of the Grignard addition to pregnenolone was studied by use of regiospecific isotope labeling in order to reconcile conflicting concepts for the conformational isomerism of the steroid side chain. (20*S*)- and (20*R*)-[20-methyl-labeled]-20-methyl-5-pregnene-3β,20-diols (**4a** and **4b**) were synthesized by addition of (a) CD_3MgI to pregnenolone acetate, (b) CH_3MgI to pregnenolone-17α,21,21,21-d_4, and (c) $^{13}CH_3MgI$ to pregnenolone acetate. Stereoselectivity of the Grignard addition was analyzed by proton NMR at 60 MHz in $CDCl_3$ [20(*pro-S*)-CH_3 at 72 Hz, 20(*pro-R*)-CH_3 at 79 Hz, $J_{^{13}C-H}$ = 126 Hz], and the ratio of 20*S* to 20*R* was observed in all cases to be 9:1. Ethyl-Grignard addition to pregnenolone also gave ca. 9:1 for the 20*S*/20*R* ratio. The results indicate that the rotational isomerism around the C(17)–C(20) bond of pregnenolone in solution highly favors the above-*D*-ring conformation of the carbonyl group, opposing the recent claim that pregnenolone exists in a 6:4 equilibrium of "cis" and "trans" conformers. The assignment of the 20*S* configuration to the major product of the Grignard addition to pregnenolone was confirmed by x-ray crystallography for the first time.

Conformational isomerism in steroid chemistry still remains enigmatic. Nes and Varkey[2] recently reported that pregnenolone exists in benzene/ether as a 6:4 equilibrium of cis and trans conformers, directly based on their observed ratio of 20-hydroxycholesterol to 20-hydroxyisocholesterol which was obtained by isohexyl-Grignard addition to pregnenolone acetate. The same reaction has been previously reported by Petrow and Stuart-Webb[3] and Mijares et al.[4] as giving only one product which was assigned to be 20*S*.[4,5] These two groups reported that in no case was there any evidence for the formation of more than one C(20) stereoisomer,[3] and the isolated compound was the only product formed during the condensation, although a careful search was made to isolate the 20*R* epimer.[4] The hypothesis of Rakhit and Engel[6] that there exists four preferred conformations of 20-keto steroids, A_1, A_2, B_1, and B_2 as designated, has been used[2,5] for rationalization of their conclusions, but without specific evidence.

We have recently found[7] a high stereoselectivity for methyl-Grignard addition to 17α-hydroxypregnenolone (99% 20*S* addition) and 16α,17α-epoxypregnenolone (93% 20*R* addition), which indicates that these 20-keto steroids exist in solution highly selectively in the preferred conformation found in the solid state, as determined by x-ray crystallography. Therefore, in this paper we decided to measure the stereoselectivity of methyl-Grignard addition to pregnenolone in order to clarify the conformational preference of 20-keto steroids in solution. Examination of the side-chain conformation in the solid state of 35 steroid structures[8] shows that the carbonyl oxygen is located above the D ring as depicted in **1a** (Figure 1) whether or not the structure has a hydroxy substituent at the neighboring 17α and/or 21 positions. An unusual 20-carbonyl conformation eclipsed with the C(13)–C(17) bond (**1b**) has been observed in 16β-bromo,[9] 16α,17α-epoxy,[10] and 16β-methyl[11] substituted structures. The preferred *si*-face attack by the Grignard reagent on conformation **1a** and the *re*-face attack on conformation **1b** are predicted to occur for steric reasons,[7] therefore giving a 20*S* configuration (**2a**, Figure 1, the incoming alkyl (R) being C(17) > R > C(21)) for the major product from the pregnenolone reaction. To distinguish and quantitatively assess the chemically like, paired methyl groups at C(20) of the methyl-Grignard reaction product, we have chosen three regiospecific labeling sets, (a) deuterated Grignard reagent and pregnenolone acetate, (b) Grignard reagent and deuterated pregnenolone, and (c) ^{13}C-labeled Grignard reagent and pregnenolone acetate, and ^1H NMR at 60 MHz for analysis of the stereoselectivity of the reaction. Ethyl-Grignard addition to pregnenolone was also analyzed.

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