Transformations of Cyclopropanol Derivatives. 4. Alkyl Substituent Effects in Ring-Opening Reactions of 6-Methyl-5-hydroxytricyclo[4.4.0.0^{1,5}]decan-9-one

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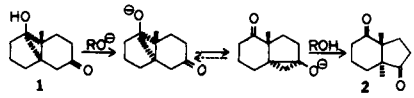
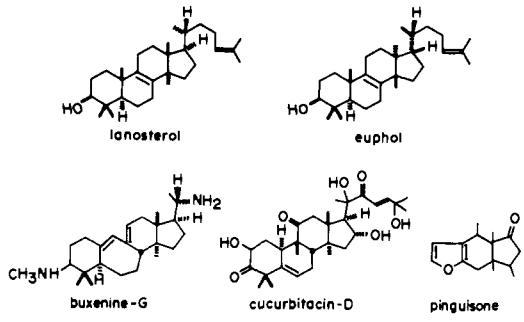
Abstract: Acid- and base-catalyzed rearrangements of the fused-ring cyclopropanol derivatives **1, 3, 4,** and **6** in methanol solution have been studied. Two distinct modes of reaction are observed. Acid-catalyzed reactions of **1, 3,** and **5** give methyl ether derivatives of the initial or rearranged cyclopropanols as major products. Base-catalyzed reactions of the same three cyclopropanols give perhydroindene diketones, resulting from ring opening of the rearranged cyclopropanols. Compounds **4** and **6** give *cis*-decalindiones or derived intramolecular aldol condensation products as the chief products of both acid- and base-catalyzed reactions. A stereoelectronic factor, favoring inversion of configuration in corner interactions of the cyclopropanol ring with the carbonyl group has been pointed out. This factor permits rearrangement of A_1 type conformers to isomeric cyclopropanols, having configuration B_1. Perhydroindene products from B_1 intermediates are always trans fused, and the corresponding cis isomers are not formed. The substituent effects noted here can be rationalized by conformational analysis of the A_1–A_2 equilibrium.

Our previous work[1] has demonstrated that keto cyclopropanol **1** undergoes a variety of rearrangements, the products from which depend on the specific reaction conditions. Of these reactions, the most interesting and potentially useful is the conversion of **1** to the perhydroindene **2** on treatment with alkoxide bases. A mechanism for this unusual rearrangement has been proposed (eq 1); however, the remarkable stereo-



selectivity displayed in the formation of **2** requires additional comment.

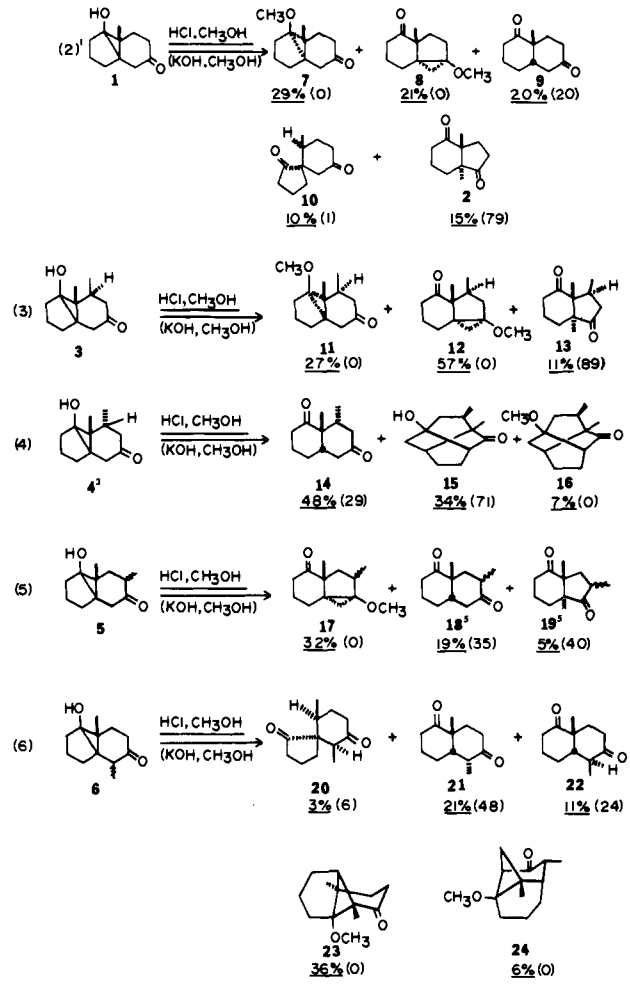
Our interest in **2** was further stimulated by its possible role as an intermediate in natural product synthesis. Indeed the *trans*-1,6-dimethylbicyclo[4.3.0]nonane skeleton of **2** is found in a variety of natural substances, including triterpenes such as lanosterol and euphol, the buxus alkaloids, the cucurbitacins, and a novel sesquiterpene, pinguisone.[2]



In this paper we report the results of a study of acid- and base-catalyzed rearrangements of methyl-substituted derivatives of **1**, in which all the substitutions are on the cyclohexanone ring. Such a study provides a necessary foundation for the planning and execution of syntheses using derivatives of **2.**

Results and Discussion

Rearrangement of cyclopropanols **1, 3, 4, 5,** and **6** was effected by the action of methanolic acid (HCl) and base (KOH). The chief products from these reactions are shown in eq 2 through 6 (yields of acid-catalyzed products are underlined, base-catalyzed products are in parentheses).

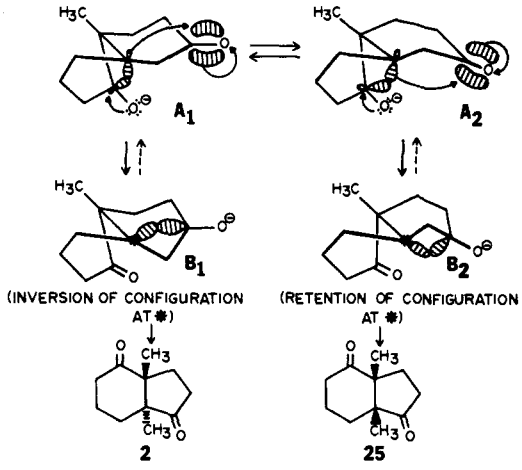


Before discussing these results, it is important to recognize that tricyclic compounds **15, 16, 23,** and **24** are derived from the *cis*-decalindiones **14** and **21** (or **22**) by intramolecular aldol cyclizations.[6] Therefore, from the standpoint of our interest in the rearrangements of these cyclopropanols we should regard the above products as equivalent to the *cis*-decalins. With this in mind, reactions 2 through 6 can be divided into two distinct classes. The first, which includes the transformations of cyclopropanols **1, 3,** and **5,** is characterized by rapid acid-cata-

lyzed formation of cyclopropanol methyl ethers—particularly rearranged methyl ethers such as **12** and **17**. Ring-opened products appear to be formed more slowly. Base catalysis, on the other hand, leads predominantly to perhydroindenes such as **2**, **13**, and **19**, accompanied by lesser amounts of *cis*-decalins. The second class (reactions 4 and 6) yields *cis*-decalin products under both acid and base catalysis, and in neither case have we been able to detect any perhydroindene derivatives. This dichotomy is especially striking for cyclopropanols **3** and **4**, which are simple epimeric methyl derivatives of **1**.

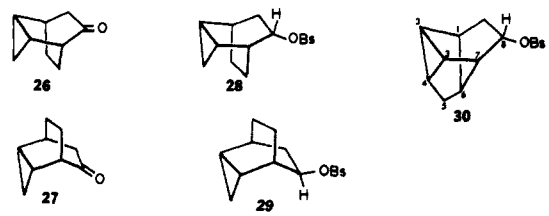
We believe that the remarkable substituent effects noted above and the stereoselectivity of the **1** → **2** rearrangement both reflect an important stereoelectronic factor in the cyclopropanol interconversion mechanism presented in eq 1. To facilitate our discussion of this factor, we have identified certain conformationally plausible interactions in the conjugate base of **1** (Scheme I). A parallel set of equations may be written for the corresponding conjugate acid.

Scheme I



The conjugate base of **1** can assume two boat-like conformations, A_1 and A_2, which are formally capable of undergoing bond reorganization to the corresponding isomeric bases B_1 and B_2. These rearrangements differ stereochemically in that B_1 is formed with inversion of configuration and B_2 with retention of configuration at the common bridgehead carbon atom (*). Since we know that B_1 suffers rapid ring cleavage to **2**,[4] it is reasonable to expect that B_2 would yield the cis isomer (**25**) of this perhydroindene. All our efforts to detect and identify such a compound among the products of these reactions have been fruitless,[7] and we can only conclude that this rearrangement pathway ($A_2 \rightarrow B_2$) has a prohibitive activation energy. In fact, approximate measurements made with the aid of Dreiding models suggest that the orbital overlap in the inversion rearrangement ($A_1 \rightarrow B_1$) is more favorable than the overlap in the retention rearrangement.

The remarkable conformational discrimination displayed by this cyclopropanol rearrangement is similar to certain previously noted configurational effects involving neighboring cyclopropane moieties.[8] For example, the enhanced rotational strength of **26** compared with **27**,[9] the observation of direct anchimeric assistance by the cyclopropane ring during solvolysis of **28** but not **29** or the other two diastereoisomers,[10] and



the compelling evidence put forth for participation of the C3:C4 bond in solvolysis of **30**[11] all require a corner interaction of the three-membered ring with a nearby electrophilic carbon atom. The configurations of these rigid compounds are consistent only with an inversion equivalent to that shown in A_1.

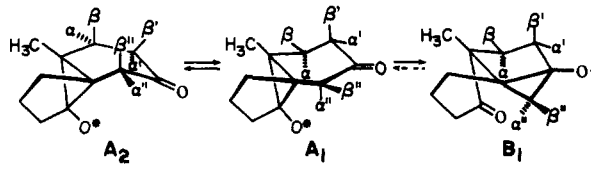
The substituent effects noted in reactions 2 through 6 still require discussion, and it will be helpful to preface this with a summary of two important conclusions drawn from our present studies.

(1) A bonding interaction between the cyclopropanol ring and the carbonyl function in **1** occurs in conformation A_1 but not A_2. This interaction often culminates in rearrangement to the isomeric cyclopropanol B_1. Indeed, the formation of rearranged methyl ethers such as **8**, **12**, and **17** under acid-catalyzed conditions, or perhydroindene products such as **2**, **13**, and **19** under base-catalyzed conditions requires such a rearrangement.

(2) The base-catalyzed formation of *cis*-decalin products such as **9**, **14**, **18**, **21**, and **22** (or any products derived from these) takes place by ring cleavage of cyclopropanol intermediates of type A and not from B_1. This has been demonstrated experimentally for the parent system (**1**).

Acid- and base-catalyzed reactions of **1** clearly show that the conformational equilibrium between A_1 and A_2 (eq 7)

(7)



* = Conjugate base or acid of the corresponding cyclopropanol

favors the former, since products derived from the rearranged isomer B_1 predominate. This preference for conformer A_1 may be the result of steric interactions between the cyclopropanol oxygen atom and the α'-hydrogen in A_2. Steric hindrance is expected to shift the conformational equilibrium to the left (in favor of A_2) if alkyl substituents occupy α,β' or α'' positions, but should enhance the stability of A_1 in the alternative β,α' and β'' locations. This conformational analysis accounts nicely for the products obtained from **3** and **4**, but is complicated by the possibility of acid- or base-induced epimerization in those cases having a substituent α to the carbonyl function (e.g., **5** and **6**). The products from reactions of **5** and **6** suggest that mixtures of epimers may indeed be present, with the quasi-equatorial α' or α'' isomer predominating. Finally, an α'' or β'' substituent on B_1 would be expected to retard the rate of base-catalyzed ring opening to a perhydroindene product, thereby favoring transformation of intermediate A to *cis*-decalin products.

Experimental Section

Melting points were determined in capillaries or on a Kofler hot stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 237B grating spectrophotometer. Proton magnetic resonance spectra were obtained with either a Varian T-60 or a Varian HA-100 spectrometer, using tetramethylsilane as an internal standard. Mass spectra were obtained with an Hitachi RMU-6 mass spectrometer. All reactions involving alkaline conditions were carried out under dry N_2 or Ar, using solvents freshly purified by distillation from suitable drying agents. Microanalyses were performed by Spang Microanalytical Labs, Ann Arbor, Mich.

The preparation and identification of cyclopropanols **1**, **3**, **4**, **5**, and **6** are described in an earlier paper.[4] Base-catalyzed rearrangement of these cyclopropanols was effected in aqueous methanol solution (50:50) containing a modest excess of potassium hydroxide (ca. 1.2 equiv). These reactions proceeded for 5–8 h at room temperature.

Acid-catalyzed rearrangement of the cyclopropanols was also effected in aqueous methanol solution to which sufficient hydrochloric acid was added to achieve a pH <1 (usually 3–6 drops of concentrated HCl/mmol of substrate).

Base-Catalyzed Transformation of (1*R,5α,6β,7β)-5-Hydroxy-6,7-dimethyltricyclo[4.4.0.0^{1,5}]decan-9-one (3).** Reaction of **3** (128 mg, 0.66 mmol) with aqueous methanolic potassium hydroxide yielded 128 mg of a crystalline residue, which GLC analysis showed to be largely (ca. 90%) a single component. Purification by preparative GLC yielded an analytical sample of (1α,6β,9α)-1,6,9-trimethylbicyclo[4.3.0]nonane-2,7-dione (**13**): mp 96–100 °C; IR (CCl_4) 1739, 1710, 1458, 1378, 1092, 1020 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.98 (s, 3 H), 1.10 (d, J = 6.0 Hz, 3 H), 1.12 (s, 3 H), 2.2–3.1 (m, 9 H); mass spectrum (70 eV) *m/e* (rel abundance) 194 (27), 179 (9), 153 (9), 137 (13), 124 (100), 109 (28), 96 (51).

Anal. ($C_{12}H_{18}O_2$) C, H.

Base-Catalyzed Transformation of (1*R,5α,6β,7α)-5-Hydroxy-6,7-dimethyltricyclo[4.4.0.0^{1,5}]decan-9-one (4).** Reaction of 200 mg (1.0 mmol) of a 3:1 mixture of cyclopropanol **4** and the isomeric *trans*-decalin which accompanies it[4] with aqueous methanolic potassium hydroxide yielded 200 mg of a light yellow oil. Analysis of this material by GLC (4% QF-1) showed it to be a mixture of the *trans*-decalin contaminant (ca. 25%), the twistane ketol **15**[6] (57%), and (1α,6α,10β)-1,10-dimethylbicyclo[4.4.0]decane-2,8-dione (**14**, 16%). The latter compounds were identified by direct comparison with authentic materials prepared by catalytic reduction of *trans*-5,6-dimethylbicyclo[4.4.0]dec-1-ene-3,7-dione,[4] followed by intramolecular aldolization.[6]

Base-Catalyzed Transformation of (1*R,5α,6β,8α)-5-Hydroxy-6,8-dimethyltricyclo[4.4.0.0^{1,5}]decan-9-one (5).** Reaction of cyclopropanol **5** (200 mg, 1.0 mmol) with aqueous methanolic potassium hydroxide yielded 200 mg of an oil, which GLC analysis (4% QF-1, 185 °C) showed to be chiefly two components. These products were isolated by preparative GLC and identified by their characteristic properties.

(A) (1β,6α,8ξ)-1,6,8-Trimethylbicyclo[4.3.0]nonane-2,7-dione (**19**), mp 90–95 °C, was obtained in 40% yield and proved to be homogeneous in GLC and TLC. The spectroscopic properties of this substance: IR (CCl_4) 1739, 1713, 1455, 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$) two pair of singlets δ 0.86 and 0.96 (3 H total), δ 1.11 and 1.16 (3 H total), and a 3 H doublet (δ 1.24, J = 7.0 Hz) which separated into two doublets (J' = 7.0 Hz, J'' = 7.2 Hz) in C_6D_6 solution at 100 MHz; mass spectrum (70 eV) *m/e* (rel abundance) 194 (59), 179 (48), 151 (44), 137 (38), 125 (59), 124 (57), 110 (98), 95 (100), confirm the structural assignment and show it to be mixture (roughly 3:1) of 8-methyl epimers.

Anal. ($C_{12}H_{18}O_2$) C, H.

(B) (1α,6α,9ξ)-1,9-Dimethylbicyclo[4.4.0]decane-2,8-dione (**18**) was obtained in 35% yield as an oil which appeared to be homogeneous by GLC and TLC. The spectroscopic properties of this substance: IR (film) 1708, 1450, 1423, 1375, 1152, 1095 cm^{-1}; ^{1}H NMR ($CDCl_3$) a pair of singlets δ 1.31 and 1.47 (3 H total) and a pair of doublets δ 0.95 and 1.01 (J' = 6.0 Hz, J'' = 6.7 Hz, 3 H total); mass spectrum (70 eV) *m/e* (rel abundance) 194 (51), 124 (59), 111 (100), 95 (44), 81 (32), 69 (56), confirm the structural assignment and show it to be a mixture of 9-methyl epimers.

Anal. ($C_{12}H_{18}O_2$) C, H.

Base-Catalyzed Transformations of (1*R,5α,6β,10ξ)-5-Hydroxy-6,10-dimethyltricyclo[4.4.0.0^{1,5}]decan-9-one (6).** Reaction of cyclopropanol **6** (100 mg, 0.52 mmol) with aqueous methanolic potassium hydroxide yielded 95 mg of an oil, which GLC analysis (4% QF-1) showed to be chiefly three components. These products were isolated by preparative GLC and identified by their characteristic properties.

(A) (1*R**,6β,10β)-6,10-Dimethylspiro[4.5]decane-2,7-dione (**20**), mp 64–66 °C, was obtained in 6% yield and shown to be identical with the major product from heterogeneous protonation of the conjugate base of **6**.

(B) (1α,6α,7β)-1,7-Dimethylbicyclo[4.4.0]decane-2,8-dione (**21**), mp 98–100 °C, was obtained in 48% yield. The spectroscopic properties of **21**: IR (CCl_4) 1708 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.98 (d, J = 6.5 Hz, 3 H), 1.33 (s, 3 H), 1.5–3.0 (m, 12 H); mass spectrum (70 eV) parent ion at *m/e* 194, are consistent with the assigned structure. Furthermore, **21**, proved identical with the chief product from the catalytic hydrogenation of 2,6-dimethylbicyclo[4.4.0]dec-1-ene-3,7-dione.[12] Treatment of pure **21** in aqueous methanol solution with either potassium hydroxide or hydrochloric acid gave a 2:1 mixture of **21** and **22**.

(C) (1α,6α,7α)-1,7-Dimethylbicyclo[4.4.0]decane-2,8-dione (**22**) was obtained in 24% yield. Infrared absorption at 1708 cm^{-1} and a ^{1}H NMR spectrum displaying peaks at δ 1.01 (d, J = 7.0 Hz, 3 H) and 1.51 (s, 3 H) support the assigned structure.

Heterogeneous Quenching of the Sodium Salt of 6. To a vigorously stirred suspension of sodium hydride (0.34 g, 13.5 mmol) in 150 mL of dry benzene was added dropwise a solution of cyclopropanol **6** (2.2 g, 11.2 mmol) in 50 mL of benzene. Following 4.5 h of stirring at ambient temperature, the salt suspension was cooled and carefully decomposed by the addition of excess methanol (25 mL) followed by 50 mL of ice water. The organic layer was separated, and the aqueous portion was extracted with ether. The combined organic extracts were then washed, dried, and concentrated. The crude product (2.03 g) was sublimed at 75 °C (0.1 Torr), yielding 1.82 g (83.7%) of pure spirodione **20**, mp 64–66 °C. Spectroscopic evidence supporting the assigned structure includes IR ($CHCl_3$) 1735, 1705 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (br d, J = 6.5 Hz, 6 H), 1.7–2.5 (m, 11 H), 2.85 (q, J = 6.5 Hz, 1 H); ^{1}H NMR (C_6D_6) δ 0.51 (d, J = 6.5 Hz, 3 H), 0.79 (d, J = 6.5 Hz, 3 H), 1.1–2.3 (m, 11 H), 2.53 (q, J = 6.5 Hz, 1 H); mass spectrum (70 eV) parent ion at *m/e* 194.

Anal. ($C_{12}H_{18}O_2$) C, H.

Acid-Catalyzed Transformation of Cyclopropanol 3. Treatment of **3** (130 mg, 0.67 mmol) with aqueous methanolic hydrochloric acid yielded 124 mg of an oily product. Analysis of this substance by GLC (4% QF-1) showed that it consisted of three components, the perhydroindene **13** (11%), obtained as the major product of base-catalyzed rearrangement of **3**, and two methoxycyclopropanes **11** (27%) and **12** (57%), identified as follows.

11, (1*R**,5β,6α,7α)-5-methoxy-6,7-dimethyltricyclo-[4.4.0.0^{1,5}]decan-9-one: IR (Film) 1708 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3 H), 1.06 (d, J = 6.5 Hz, 3 H), 1.20–2.85 (m, 13 H), 3.32 (s, 3 H); mass spectrum (70 eV) *m/e* (rel abundance) 208 (19), 193 (26), 177 (17), 165 (35), 151 (41), 138 (100), 123 (39), 105 (28), 93 (44), 91 (37).

12, (1*S**,3α,5α,6α)-3-methoxy-5,6-dimethyltricyclo-[4.4.0.0^{1,3}]decan-7-one: IR (film) 1708, 1325, 1234, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.32 (d, J = 5.5 Hz, 1 H), 0.80–0.99 (overlapping d, J' = 5.5 Hz, 1 H, and J'' = 6.0 Hz, 3 H), 1.3–2.8 (m, 11 H), 3.30 (s, 3 H); mass spectrum (70 eV) *m/e* (rel abundance) 208 (16), 193 (54), 165 (80), 137 (70), 123 (100), 105 (36), 91 (40).

Acid-Catalyzed Transformation of Cyclopropanol 4. Treatment of a 3:1 mixture of **4** and the accompanying *trans*-decalin isomer[4] (230 mg, 1.1 mmol) with aqueous methanolic hydrochloric acid yielded 230 mg of an oil, which GLC analysis showed to be a mixture of the *trans*-decalin contaminant (23%), the *cis*-decalin **14** (36%), the twistane ketol **15** (26%), and the corresponding twistane methyl ether **16** (5%). Identification of the latter compounds was achieved by direct comparison with authentic samples prepared by us in an earlier study.[6]

Acid-Catalyzed Transformation of Cyclopropanol 5. Treatment of **5** (300 mg, 1.5 mmol) with aqueous methanolic hydrochloric acid yielded 237 mg of an oily product from which three products were separated by preparative GLC (4% QF-1). Two of these were the previously identified *cis*-decalin **18** (19%) and the perhydroindene **19** (5%). The chief product, (1*S**,3α,4β,6α)-3-methoxy-4,6-dimethyltricyclo[4.4.0.0^{1,3}]decan-7-one (**17**), was isolated in 32% yield and identified by its characteristic spectra: IR (film) 1708, 1445, 1232, 1050, 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.24 (d, J = 5.5 Hz, 1 H), 0.73 (d, J = 5.5 Hz, 1 H), 0.98 (d, J = 6.5 Hz, 3 H), 1.32 (s, 3 H), 1.4–2.8 (m, 9 H), 3.33 (s, 3 H); mass spectrum (70 eV) *m/e* (rel abundance) 208 (10), 193 (100), 176 (8), 165 (30), 152 (18), 137 (57), 123 (54), 105 (33), 91 (33).

Acid-Catalyzed Transformation of Cyclopropanol 6. Treatment of **6** (250 mg, 1.2 mmol) with aqueous methanolic hydrochloric acid yielded 248 mg of an oily product. Analysis of this material by GLC (4% QF-1) showed that it consisted of five components, three being identical with the products of the base-catalyzed rearrangement of **6**: **20**, (3%), **21** (21%), **22** (11%). The other two components were identified as the tricyclic methoxy ketones **23** (36%) and **24** (6%) as follows.

23, (1*R**,2*S**,6*R**,7*R**)-2-methoxy-1,7-dimethyltricyclo-[4.4.0.0^{2,7}]decan-8-one: IR (film) 1708, 1318, 1231, 1065, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (s, 3 H), 1.30 (s, 3 H), 1.3–2.9 (m, 11 H), 3.28 (s, 3 H); mass spectrum (70 eV) *m/e* (rel abundance) 208 (22),

193 (100), 175 (18), 165 (20), 161 (16), 151 (23), 137 (52), 124 (38), 105 (53), 91 (44).

Anal. ($C_{13}H_{20}O_2$) C, H.

24, (1*R**,2*S**,4*S**,6*S**,7*R**)-2-methoxy-1,7-dimethyltricyclo-[4.4.0.0^{2,9}]decan-8-one: IR (film) 1710, 1450, 1150, 1014 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 3 H), 1.05 (d, J = 7.0 Hz, 3 H), 1.2–2.7 (m, 11 H), 3.26 (s, 3 H); mass spectrum (70 eV) *m/e* (rel abundance) 208 (18), 151 (100), 137 (32), 121 (26), 105 (26), 91 (33).

Anal. ($C_{13}H_{20}O_2$) C, H.

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References and Notes

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(3) As noted in our first paper of this series,[4] cyclopropanol **4** is contaminated with a small amount (ca. 10%) of the corresponding trans epimer of **14**. The yields given in eq 4 are corrected for this impurity.
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(7) These have included GLC and TLC analysis of reaction mixtures as well as careful examination of the angular methyl signals in the ^{1}H NMR spectra of the reaction products. Miss J. Huang Shau has recently prepared the cis isomer **21** by an independent route, and it is clear that this compound would have been detected had it been present in greater than 1%.
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Electron Transfer Reactivity of Spinach Ferredoxin

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Abstract: The oxidation of reduced spinach ferredoxin by Fe(EDTA)$^-$, Fe(HEDTA), horse heart ferricytochrome *c*, and horse metmyoglobin has been investigated. Each reaction follows second-order kinetic behavior (rate = k_{12}[ferredoxin][oxidant]). Rate parameters are: Fe(EDTA)$^-$, $k_{12} = 2.9 \times 10^5$ M^{-1} s^{-1} (12.5 °C, μ = 0.1 M, pH 7.8), $\Delta H^{\ddagger}$ = 0.7 kcal/mol, $\Delta S^{\ddagger} = -31$ cal/mol-deg; Fe(HEDTA), $k_{12} = 2.5 \times 10^4$ M^{-1} s^{-1} (26 °C, μ = 0.1 M, pH 7.8), $\Delta H^{\ddagger}$ = 0.3 kcal/mol, $\Delta S^{\ddagger} = -37$ cal/mol-deg; ferricytochrome *c*, $k_{12} = 8.1 \times 10^4$ M^{-1} s^{-1} (25 °C, μ = 0.1 M, pH 7.0), $\Delta H^{\ddagger}$ = 9.6 kcal/mol, $\Delta S^{\ddagger} = -4$ cal/mol-deg; metmyoglobin, $k_{12} = 2.3 \times 10^6$ M^{-1} s^{-1} (25 °C, μ = 0.1 M, pH 7.0), $\Delta H^{\ddagger} - 0.9$ kcal/mol, $\Delta S^{\ddagger} = -32$ cal/mol-deg. The electrostatics-corrected self-exchange rate constant calculated for ferredoxin based on Fe(EDTA)$^-$ is 1.7×10^{-3} M^{-1} s^{-1}, which indicates an extremely inaccessible protein redox center. Related Marcus-type analysis also suggests that electron transfer from ferredoxin to ferricytochrome *c* is particularly inefficient. The pH dependences of the ferredoxin-Fe(EDTA)$^-$ and ferredoxin-metmyoglobin reactions, and the ionic strength dependence of the ferredoxin-Fe(EDTA)$^-$ reaction, have been analyzed. At 25.8 °C and pH 7.8, the best fit to the Marcus ionic strength equation yields a charge of −9.4 on ferredoxin. Oxidation of ferredoxin by metmyoglobin fluoride has also been studied. The oxidation apparently is rate limited by fluoride dissociation (k = 0.4 s^{-1}; 25 °C, μ = 0.8 M, pH 7.7).

As part of our continuing study of the reactivity of electron transfer proteins with small molecule probe reagents and with each other, we have studied the reactions of reduced spinach ferredoxin with Fe(EDTA)$^-$, Fe(HEDTA), horse heart ferricytochrome *c*, and horse metmyoglobin. It is generally accepted that the oxidized form of spinach ferredoxin contains a (Cys-S)$_2$Fe^{III}(S^{2-})$_2$Fe^{III}(Cys-S)$_2$ redox center.[1,2] Further, electronic spectroscopic studies have indicated that the two Fe(III) sites are nonequivalent, with the more tetrahedral one being involved in the Fe(III)-Fe(II) redox shuttle.[3]

In previous papers[4,5] we have presented a model for interpreting protein electron transfer reactions within the framework of the Marcus outer sphere theory. In this model, the contributions to the kinetic activation energy due to the reagent, general electrostatic influences, and the thermodynamic driving force for the overall reaction, are factored out to leave a quantity that is characteristic of the protein and the particular mechanism by which it engages in electron transfer with the reagent. This quantity is the electrostatics-corrected self-exchange rate constant for the protein, or k_{11}^{corr}. Such a Marcus-type analysis of the available data on the reactions of cytochromes *c*, blue copper proteins, and HiPIP has led to the definition of a "kinetic accessibility" scale,[5] and to identification of the factors that control small molecule-protein and protein-protein reactivities. The data reported in this paper allow extension of this analysis to spinach ferredoxin.

Experimental Section

The protein was prepared by variations on published procedures.[6,7] Approximately 20 kg of fresh spinach leaves, 10 mL of 1 M Tris base, and 500 "mL" of ice were ground for 5 min in a Waring blender. All subsequent operations were carried out at 4 °C. The extract was squeezed through cheese cloth, and the ionic strength of the solution was made 0.15 M in NaCl. Approximately 500 mL of DEAE-cellulose (Whatman Type 52) was added to the 15 L of filtrate; this was stirred for 2 h, and then allowed to stand for 1.5 h. Most of the filtrate was siphoned off, and the DEAE cellulose was collected by filtration. This was washed with 2 L of 0.15 M NaCl, 0.01 M Tris (pH 7.5), and eluted with 0.15 M Tris (pH 7.5), 0.8 M NaCl. Ammonium sulfate was then added to 90% saturation and the solution was centrifuged