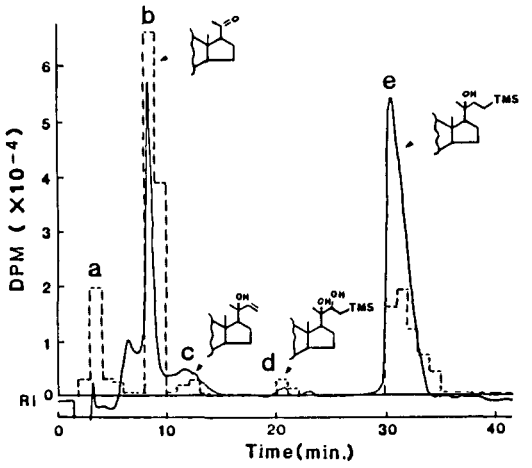
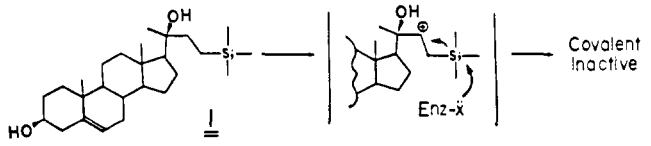


Figure 2. HPLC product analysis of P-450$_{scc}$ reaction with steroid **1** on a Whatman Partisil PXS: ODS-2 column. Methanol: water, 85:15, v/v; 1.5 mL/min. The solid line indicates refractive index response, and bars indicte radioactivity in dpm. The radioactive steroid **1** (tritium at C-7) was synthesized as in ref 10 except [7-^{3}H]pregnenolone (20300 dpm/nmol) was used. The incubation contained 83 nmol P-450$_{scc}$, 50 nmol adrenodoxin, and 1.5 nmol adrenodoxin reductase. After a 4-h incubation, the reaction mixture was extracted exhaustively with ethyl acetate. The isolated steroids were dissolved in methanol before HPLC injection.

Scheme II



timated as earlier described,[13] was 0.78 μM. Incubation of **1** with P-450$_{scc}$ in the presence of an NADPH-generating system led to a time-dependent absorbance decrease in the Soret region ($t_{1/2} \sim 2$ min, data not shown). A 10-fold molar excess of **1** was sufficient to titrate the system (Figure 1a). The partition ratio (r),[7] calculated from the final ΔA at each value of [steroid]/[P-450], is 5.5 ± 0.5 (Figure 1b).

Preincubation studies[14] with steroid **1** showed a time-dependent loss of enzyme activity ($t_{1/2} \sim 2$ min) in the presence of NADPH and O_2 (Figure 1c). No inactivation was observed in the absence of NADPH and/or O_2, suggesting that inactivation is catalysed by P-450$_{scc}$. The irreversibility of the inhibition was suggested by the inability of inactivated enzyme to regain its activity after gel filtration. Furthermore, incubations were carried out in the presence of 2–10 mM β-mercaptoethanol, dithiothreitol, or NaF as a scavenger for reactive electrophiles. There was no protection from the inactivation, by these reagents, which suggested that the enzyme-generated inactivator does not become accessibile to other solutes prior to inactivation.

Incubation of C-7 tritium-labeled steroid **1** with P-450$_{scc}$ in the presence of NADPH and O_2 produced at least four steroid products. Figure 2 shows an HPLC analysis of the extracted steroids after a 4-h incubation. No products were found in the absence of NADPH. The assignments for the structure of materials in peaks b, c, and e were made on the basis of comigration during HPLC with authentic steroids under several solvent conditions and on GC-MS analyses. The peak d was assigned to be the diol because periodate treatment of the material in d produced pregnenolone. The partition ratio (r) estimated from the total products, assuming complete inactivation, was 5.8, agreeing with the titration study. While the products are consistent with the cationic mechanism shown in Scheme II, an alternative mechanism would involve oxidative cleavage to a Me_3Si radical plus ethylene as observed by Trahanovsky.[15] Ethylene is produced (0.6 mol/mol enzyme) in the turnover of steroid **1** with kinetics similar to those for inactivation.[16] In each alternative, trimethylsilylation of some group in the holoenzyme is predicted, a point under current investigation.

In summary, we have described a novel mechanism-based inhibition of P-450$_{scc}$ by steroid **1**. The rate of the Soret absorbasnce decrease, the enzyme activity loss, and ethylene formation are indistinguishable with a $t_{1/2}$ of 2 min. The partition ratio is estimated to be approximately 5.5 from the product analysis and the Soret absorbance titration. Detailed mechanistic studies of the inactivation process and in vivo inhibition studies are being conducted.[17] We are also investigating whether Me_3Si compounds will be generally useful as a new class of monoxygenase mechanism-based inhibitors.

Acknowledgment. This work was supported by NIH Grants GM28358 (W.H.O.-J.) and GM31482 (S.R.W.).

(13) Orme-Johnson, N. R.; Light, D. R.; White-Stevens, R. W.; Orme-Johnson, W. Y. *J. Biol. Chem.* **1979**, *254*, 2103–2111.

(14) The final turnover mixture contained 2.5 μM adrenodoxin, 150 nM adrenodoxin reductase, 50 mM K$^+$MOPS (pH 7.2), 10 mM $MgCl_2$, 0.2% Tween 20, 1 unit/mL catalase, a NADPH-generating system of 16 μM NADPH, 3 mM G6P, and 1 unit/mL G6P dehydrogenase. Activity was assayed essentially according to the method of Takikawa et al. Takikawa, O.; Gomi, T.; Suhara, K.; Itagaki, E.; Takemori, S.; Katagiri, M. *Arch. Biochem. Biophys.* **1978**, *190*, 300–306.

(15) Trahanovsky, W. S.; Himstedt, A. L. *J. Am. Chem. Soc.* **1974**, *96*, 7974–7976.

(16) The incubation was carried out as in ref 14 except the reaction was done in a small vial fitted with a rubber septum. Ethylene production was assayed by using a gas chromatograph (GC) equipped with a Porapak N (Waters) column and a flame ionization detector. At various times, an aliquot of the atmosphere above the incubation mixture was removed and injected into the GC with gas-tight syringe.

(17) We (R. J. Krueger, A. Nagahisa, and W. H. Orme-Johnson) find that **1** inhibits corticoids formation by ACTH-stimulated adrenal cortical cells, work in progress.

On the Detailed Pathway of Methyl Loss from Ionized Methyl Isobutyrate in the Gas Phase

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There is now ample evidence,[1] both experimental and theoretical, that the unimolecular loss of alkyl radicals R· from gaseous cation radicals having the general structure **1** cannot be described in terms of a direct, radical-induced carbon–carbon bond cleavage (Scheme I, **1** → **2**). Instead, energetically more favored is the multistep reaction **1** → **3** → **5** → **6**, despite the fact that this sequence contains such unusual steps as consecutive [1,2] migrations of a protonated carboxyl group (**1** → **3**)[2] and hydrogen.[3] For R^3 = H the eventually generated product ion **6** differs from **2** in that in the former the protonated carboxyl group is attached

(1) (a) Schwarz, H.; Weiske, T.; Levsen, K.; Maquestiau, A.; Flammang, R. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *45*, 367. (b) Weiske, T.; Schwarz, H. *Chem. Ber.* **1983**, *116*, 323; (c) 31st Annual Conference on Mass Spectrometry and Allied Topics, Boston, MA, May 8–13, 1983; Abstract TOC5, p 265.

(2) [1,2] migrations of cationic groups in radical cations have been analyzed in great detail by: (a) Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1976**, *98*, 6331. (b) Bouma, W. J.; Nobes, R. H.; Radom, L. *Ibid.* **1983**, *105*, 1743. (c) Holmes, J. L.; Burgers, P. C.; Terlouw, J. K.; Schwarz, H.; Ciommer, B.; Halim, H. *Org. Mass. Spectrom.* **1983**, *18*, 208. (d) For experimental work, see also: Terlouw, J. K.; Heerma, W.; Dijkstra, G. *Ibid.* **1981**, *16*, 326.

(3) Although there are no authentic [1,2]-hydrogen migrations in monoradicals in solution (see, for review: Beckwith, A. L. J.; Ingold, K. U. In "Rearrangements in Ground and Excited States"; de Mayo, P., Ed.; Academic Press: New York, **1980**; Vol. 42–1, p 161) evidence in favor of such a process in the gas phase has been presented: Gordon, A. S.; Tardy, D. C.; Ireton, R. *J. Phys. Chem.* **1976**, *80*, 1400.

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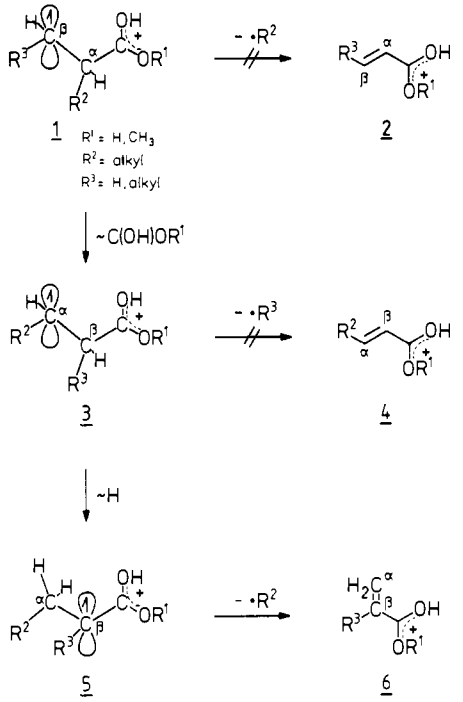
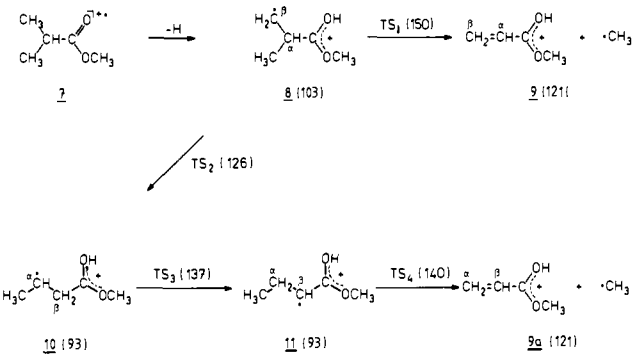
Scheme I



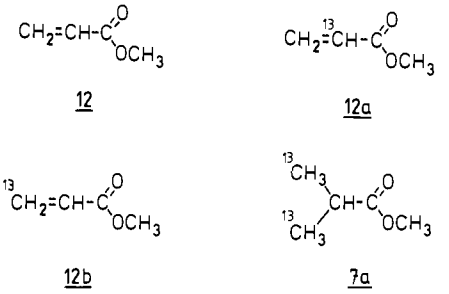
Scheme II



to C(β), whereas in the latter the original carbon skeleton sequence C(α)–C(β)–C(OH)OR remains unchanged. In fact, all evidence accumulated[4] clearly indicates that alkyl radical elimination occurs only from the *enol* ion **5**; R· losses from "unconjugated" cation radicals **1** and **3** have transition states (TS) that lie substantially higher in energy than any of the transition states involved in the overall process **1** → **3** → **5** → **6**.[5]

In distinct contrast with this result is the explanation put forward some time ago[6] for the unimolecular equilibration and decomposition of ionized methyl isobutyrate (**7**), a case that is now widely accepted as *the* prototype for the operation of "hidden"

Chart I



hydrogen rearrangements.[7] By combination of experimental techniques as different as ion cyclotron resonance, field ionization kinetics, collisional activation mass spectrometry, kinetic energy release measurements, and investigation of labeled precursors, it was suggested that **7** first undergoes a rate-determining hydrogen migration (Scheme II, **7** → **8**) followed by a fast C–C cleavage (**8** → **9**), which generates protonated methyl acrylate (**9**). Whereas there is neither doubt concerning the structure of the $[M-CH_3]^+$ ion of **7**, i.e., ion **9**, nor the operation of a rate-determining hydrogen migration **7** → **8**, the sequence **7** → **8** → **9** conflicts with the results outlined in Scheme I, in that **8** is not an *enol* cation radical and, thus, should not act as the actual precursor for the generation of protonated methyl acrylate (**9**).

We present here unambiguous experimental evidence that methyl elimination from **8** is indeed preceded by a combination of rearrangement processes that include (i) the migration of $C(OH)OCH_3^+$ via TS_2, (ii) [1,2]-hydrogen migration via TS_3, and (iii) dissociation of **11** via TS_4 to **9a** and CH_3· (the numbers given in parentheses in Scheme *II* refer to MNDO-calculated energies (kcal/mol) of completely geometry-optimized species and rigorously characterized transition states[8]). The proof for the interchange of C(α)/C(β) comes from the analysis of the collision-induced dissociations of ions generated from the precursors shown in Chart I. Among the many collision-induced fragments of the $[MH]^+$ ion of **12** (generated by protonation of methyl acrylate with $C_4H_9^+$) and the $[M-CH_3]^+$ ions from **7** it is the weak (3% relative intensity) but highly indicative signal corresponding to the loss of CH_2 that provides an answer to the problem outlined above. The $[MH]^+$ ions from **12** as well as from **12a** undergo collision-induced loss of $^{12}CH_2$; protonated **12b**, however, gives rise only to the loss of $^{13}CH_2$ (this signal coincides with that for the elimination of $^{12}CH_3$; important is that there is *no* signal corresponding to the loss of $^{12}CH_2$). The collisional activation mass spectrum of the $[M-{}^{13}CH_3]^+$ ion of **7a**, isobaric with the $[MH]^+$ ions of **12a** and **12b**, contains a signal for $^{12}CH_2$ loss only with a relative intensity comparable to that for the elimination of $^{12}CH_2$ from protonated **12** and **12a**. Thus, we have to conclude that methyl loss from the cation radical **8** is preceded by the rearrangements depicted in Scheme II. In conclusion, the rearrangement/dissociation features of ionized methyl isobutyrate do *not* constitute an exception; in contrast, its behavior is perfectly in line with the one observed for many other structurally quite different systems.[4] *The central intermediate from which eventual dissociation takes place is, in all cases studied so far, an enol cation radical of the general structure* **5**.

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(4) Weiske, T.; Halim, H.; Schwarz, H. *Chem. Ber.*, submitted for publication.

(5) The detailed investigation[4] of metastable molecular ions of 2-methylbutanoic acid clearly shows that methyl loss from **1** ($R^3 = R^2 = CH_3$; R^1 = H) does not only proceed via pathway **1** → **3** → **5** (Scheme I) but also involves loss of R^3 following hydrogen migration from C(α) to C(β). Such a migration, which, however, is energetically more demainding than the [1,2] rearrangement of the protonated C(OH)OR group, constitutes an additional pathway to generate the enol cation radical analogous to **5** with C(α)–R^2 and C(β)–R^3 interchanged.

(6) (a) Weisz, A.; Mandelbaum, A. *J. Chem. Soc., Chem. Commun.* **1978**, 521. (b) Wesdemiotis, C.; Schwarz, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 678. (c) Hemberger, P. H.; Kleingeld, J. C.; Levsen, K.; Mainzer, N.; Mandelbaum, A.; Nibbering, N. M. M.; Schwarz, H.; Weber, R.; Weisz, A.; Wesdemiotis, C. *J. Am. Chem. Soc.* **1980**, *102*, 3736. (d) Frenking, G.; Schmidt, J.; Schwarz, H. *Z. Naturforsch., B* **1982**, *37B*, 355. (e) See also: Burgers, P. C.; Holmes, J. L.; Lossing, F. P.; Povel, F. R.; Terlouw, J. K. *Org. Mass Spectrom.* **1983**, *18*, 335.

(7) (a) Schwarz, H. *Nachr. Chem., Tech. Lab.* **1980**, *28*, 158. (b) Bar-Shai, R.; Bortinger, A.; Sharvit, J.; Mandelbaum, A. *Isr. J. Chem.* **1980**, *137*, 20. (c) Schwarz, H. *Org. Mass Spectrom.* **1980**, *15*, 491; (d) *Top. Curr. Chem.* **1981**, *97*, 1.

(8) Complete geometries are available on request from the authors.