Notes

Oxidation of α-Amino Acids and α-Hydroxy Acids by Fremy's Salt. A Model for Oxidases?

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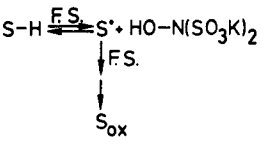
Enzymatic dehydrogenation of biological substrates such as α-amino and α-hydroxy acids by flavoenzymes has attracted considerable attention in recent years. In fact, the search for a precise understanding of the mechanism for these and other flavoenzyme-controlled biological redox reactions has led to a lively debate and controversy.[1]

On the other hand, all attempts to mimic the flavin-catalyzed oxidation of α-amino acids to the corresponding α-keto acids,[2] by nonenzymatic means, have invariably produced the corresponding nor-aldehydes (oxidative decarboxylation).[3] Oxidative decarboxylation is also a main route in the chemical oxidation of α-hydroxy acids.[4] In spite of this, oxidative methods for the preparation of α-keto acids and derivatives have been developed, which avoid or at least reduce the magnitude of the undesirable decarboxylation.[5]

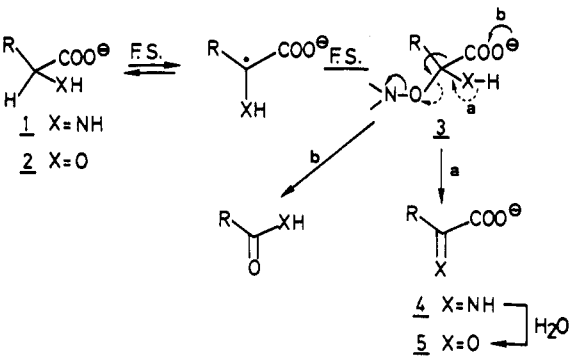
As part of a research program directed to fully evaluate the oxidizing power of a well-known one-electron oxidant such as Fremy's salt (F.S.),[6] we report here recent findings on the F.S. oxidation of some α-amino and α-hydroxy acids to the corresponding α-keto acids.[2] To the best of our knowledge, this represents the first succesful nonenzymatic attempt to mimic the action of flavoproteins in generating α-keto acids from these important biological substrates.

Recent work[7] from our laboratories has led us to view F.S. as a smooth oxidant capable of generating radicals stabilized by captodative substituents[8] which further react with F.S.,[9] thus yielding oxidized substrates (Scheme I).

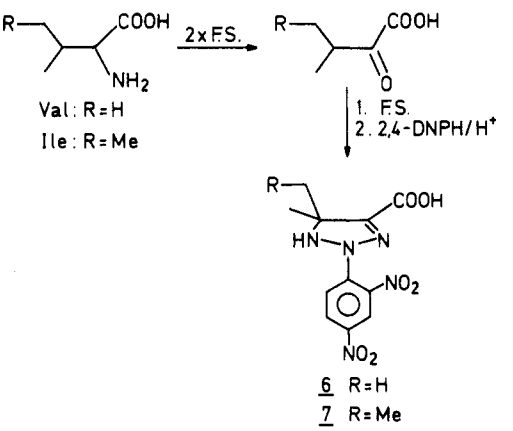
Scheme I



Scheme II



Scheme III



According to this general scheme, it seemed to us that α-amino and α-hydroxy acids should be ideal substrates for oxidation with F.S. since they should give rise, under appropriate conditions, to the corresponding α-carbon radicals.[10] These, according to Viehe et al.[8] are stabilized radicals due to the synergistic effect of capto dative substituents.

Following this rationale, we decided to submit typical α-amino **1** and α-hydroxy acids **2** to the action of F.S. We expected that the α-carbon radical, if generated, would combine with excess F.S. giving rise to intermediate **3** (Scheme II). Furthermore, at the outset of the work, two possible outcomes were envisioned, leading to either net

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(4) *Methoden der Organischen Chemie (Houben-Weyl)*, Georg Thieme Verlag: Stuttgart, 1983; Bd. E-3, p 527. See also: Pocker, Y.; Davis, B. C. *J. Am. Chem. Soc.* **1973**, *93*, 6216. Barakat, M. Z.; Abdel Wahab, M. F.; El-Sadr, M. M. *J. Chem. Soc. C* **1956**, 4685. Toussaint, O.; Capdevielle, P.; Mansuy, M. *Tetrahedron Lett.* **1984**, *25*, 3819.

(5) Baer, E.; Kates, M. *J. Am. Chem. Soc.* **1945**, *67*, 1482. Hassan, F.; Roceck, J. *J. Am. Chem. Soc.* **1975**, *97*, 1444. Banerji, K. B. *Indian J. Chem.* **1979**, *17A*, 300. Samal, P. C.; Pattnaik, B. B.; Darma Rao, S. C.; Mahapatro, S. N. *Tetrahedron* **1983**, *39*, 143. Anatol, J.; Medele, A. *C. R. Hebd. Seances Acad. Sci.* **1971**, *272*, 1157. *Synthesis* **1971**, 538; *Bull. Soc. Chim, Fr.* **1972**, 189. Haddadin, M. J.; Kattan, A. M.; Freeman, J. P. *J. Org. Chem.* **1982**, *47*, 723. Tanaka, M.; Kobayashi, T.; Sakakura, T. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 518.

(6) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

(7) Morey, J.; Dzielendziak, A.; Saá, J. M. *Chem. Lett.* **1985**, 263.

(8) Viehe, H. G.; Merenyi, R.; Stella, L.; Janousek, Z. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 917.

(9) Analogous trapping techniques have been used. See, for example: Moriya, F.; Makino, K.; Suzuki, I. R. K.; Rokushika, S.; Hatano, H. *J. Am. Chem. Soc.* **1982**, *104*, 830. Turro, N. J.; Zimmt, M. B.; Gould, I. R. *J. Am. Chem. Soc.* **1983**, *105*, 6347.

(10) These as well as α-aminocarboxamido and α-aminocyano radicals have been detected. See ref 8, as well as: Poupko, R.; Loewenstein, A.; Silver, B. L. *J. Am. Chem. Soc.* **1971**, *93*, 580. Kleyer, D. L.; Haltiwanger, R. C.; Koch, T. H. *J. Org. Chem.* **1983**, *48*, 147. De Vries, L. *J. Am. Chem. Soc.* **1978**, *100*, 926.

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Table I. Fremy's Salt Oxidation of α-Amino 1 and α-Hydroxy Acids 2

entry	substrate RCH(XH)COOH R	X	time (h)	product RC(O)COOH[a] R	yield (%)	mp of 2,4-DNP (°C)[b]
1	H	NH	20	H	0–22[c]	200–202
2	CH_3	NH	30	CH_3	37	214–216
3	$(CH_3)_2CH$	NH	24	$(CH_3)_2CH$	40	195–196
4	$CH_3CH_2CH(CH_3)$	NH	60	$CH_3CH_2CH(CH_3)$	47	167–169
5	$HOOCCH_2CH_2$	NH	24	$HOOCCH_2CH$	21	217–219
6	Ph	NH	60	Ph	82	190–192
7	H	PhCONH	240	N.R.[d]	83[e]	
8	CH_3	PhCONH	96	N.R.[d]	78[e]	
9	CH_3	O	65	CH_3	32	214–216
10	Ph	O	168	Ph	83	190–192

[a] Isolated and characterized as (2,4-dinitrophenyl)hydrazones. [b] DNP = (dinitrophenyl)hydrazone. Melting points were found to be identical with those indicated in ref 2. [c] This reaction has proved to be capricious, perhaps due to further oxidation of glyoxylic acid. [d] N.R. = no reaction. [e] Recovered starting material.

dehydrogenation and/or oxidative decarboxylation (paths a and b, respectively, Scheme II).

Oxidation of α-amino 1 and α-hydroxy acids 2 (1 mmol), in a buffered (pH 10) aqueous solution, with an excess of F.S. (3 mmol), at room temperature, for several hours (see Table I), led only to the corresponding α-keto acids in moderate yield (isolated as the corresponding (2,4-dinitrophenyl)hydrazones) (Table I). Most important of all, no degradation products from oxidative decarboxylation (path b, Scheme II) were detected (^{13}C NMR monitoring).

^{13}C NMR monitoring of several reactions revealed that the final α-keto acids exist in the reaction mixture, in part as enolates (Ile, Val) and/or aldol oligomers[11] (Ala, lactic acid). This together with the fact that enolates undergo slow oxidation by F.S. (vide infra) allow for a simple explanation of the low yields of α-keto acids obtained. Thus, only in those cases where enolization is not posible (phenylglycine, mandelic acid) the corresponding α-keto acid (detected by ^{13}C NMR at very short reaction times) was customarily obtained in 80–82% yield (entries 6 and 10, Table I). On the other hand α-keto acids having just one β-hydrogen were slowly oxidized by F.S. Thus, Val and its corresponding α-keto acid (α-ketoisovaleric acid) when allowed to react with a large excess of F.S. under the usual conditions (see Experimental Section); followed by treatment with 2,4-dinitrophenylhydrazine in strong acid (10 N HCl) yielded triazoline 6 (41% and 58% yield, respectively). Similarly triazoline 7 was obtained in 38% from Ile (Scheme III). Finally F.S. oxidation of α-keto acids with more than one β-hydrogen (phenylpyruvic acid) gives rise to complex mixtures which were not further studied.

From a mechanistic point of view several observations are worthy of note. First is the high recovery (80%) of starting material (entries 7 and 8) where the electron pair of the amino group is delocalized over the neighboring amide carbonyl.[12] Second is that the optimized conditions (see Table II) for the oxidation of 1 require operation at a pH close to the pK_2 of α-amino acids (pH 9–10). Since the redox potential[13] of F.S. at this pH value is +0.24 V (vs. SCE), the above observations tentatively suggest that the first step for the oxidation of α-amino acids might be that of the generation of the corresponding amminium radicals,[14] followed by the loss of the α-carbon proton, thus providing a stabilized α-carbon radical.[10] Further combination of this radical[15] with excess F.S. leads to intermediate 3 which eliminates hydroxylamino disulfonate yielding the final α-keto acids 5, via the easily hydrolizable α-imino acids[16] 4.

Table II. Fremy's Salt Oxidation of Alanine to Pyruvic Acid. pH Dependence

pH	yield[a] (%)
6.0	0
7.2	0
8.1	0
9.0	20
10.0	37

[a] Reaction time: 24 h.

In contrast to that observed with α-amino acids, F.S. oxidation of α-hydroxy acids 2 takes place at pH 6[17] (PhCHOHCOOH → PhCOCOOH, 80%, 48 h) more rapidly than at pH 10 (entry 10, Table I) in line with its higher redox potential at pH 6.[13] Both this observation and the higher oxidation potential associated with oxygen vs. nitrogen compounds suggest that F.S. oxidizes α-hydroxy acids 2 directly[18] to the corresponding α-carbon radical,[19] which, as shown in Scheme II, provides 5 via intermediate 3.

In summary, Fremy's salt, which might be viewed as a very simple model[20] for oxidases, provides a useful alternative to the enzymatic[21] methods for the direct preparation of some α-keto acids from the corresponding α-amino acids.[22]

(11) Waldmann, E.; Prey, V.; Jelinek, F. *Monatsh. Chem.* **1954**, *85*, 873.

(12) For some succesful oxidations of these type of compounds, see: Obata, N.; Niimura, K. *J. Chem. Soc., Chem. Commun.* **1977**, 238. Yijima, C.; Himo, F.; Suda, K. *Synthesis* **1981**, 610.

(13) Balasubramanian, P. N.; Gould, E. S. *Inorg. Chem.* **1983**, *22*, 1100 and references therein.

(14) Chow, Y. L.; Danen, W. C.; Nelsen, S. F.; Rosenblatt, D. H. *Chem. Rev.* **1978**, *78*, 243.

(15) α-Amino acid radicals are much stronger reductants than α-hydroxy acid radicals. See: Rao, P. S.; Hayon, E. *J. Am. Chem. Soc.* **1974**, *96*, 1287.

(16) See Tamaguchi et al. [Tamaguchi, M.; Saburi, M.; Toshikawa, S. *J. Am. Chem. Soc.* **1984**, *106*, 8293.] for the oxidative dehydrogenation of cobalt(III) α-amino acidate complexes leading to isolable α-imino acid complexes.

(17) At this pH benzoic acid was detected (GLC) as a minor product.

(18) Lactic and amino acid oxidases have been shown to catalyze reactions that involve ionization of the α-CH bond prior to its reaction with enzyme-bound Fl_{ox}. See ref 1 and Williams, R. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 7752.

(19) See: Guengerich, F. P.; McDonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9 for a related discussion.

(20) A Flavin N^5-nitroxyl radical has been proposed as a model for the flavin-dependant monooxygenases. See: Wagner, W. R.; Spero, D. M.; Rastetter, W. H. *J. Am. Chem. Soc.* **1984**, *106*, 1476.

(21) Meister, A. *Methods Enzymol.* **1957**, *3*, 404.

(22) We have not fully explored the synthetic applicability of our method to all natural α-amino acids. However, no doubt exists as its main limitations will derive from the well-known reactivity of the enolates formed, namely, racemization, polymerization, and further oxidation. Fortunately a large number of excellent synthetic protocols leading to α-keto acids are now available (see ref 2), and, most important, some of them allow for the preparation of optically active α-keto acids.

Experimental Section

Melting points are uncorrected. ^1H NMR and ^{13}C NMR spectra were run with a Varian CFT-80 instrument using tetramethylsilane and dioxane as internal standard, respectively. Mass spectra were recorded with a Kratos-25 instrument as well as with a Hewlett-Packard 5985 B at 70 eV. IR spectra were recorded with a Hitachi 260-10 instrument. Elemental analyses were carried out at the Instituto de Química Bioorgánica del C.S.I.C. (Barcelona).

All commercial α-amino acids and α-hydroxy acids were directly used as received. Fremy's salt was prepared, recrystallized, and stored as reported.[6]

Two buffered solutions[23] were used: sodium carbonate buffer (pH 10) and phosphate buffer (pH 7.2). The phosphate buffer was then appropriately adjusted to the pH needed by adding 0.2 M NaOH.

Oxidation of α-Amino and α-Hydroxy Acids. General Procedure. To a stirred solution of 1 mmol of **1** or **2** in 12 mL of buffer (pH 10), 3 mmol of Fremy's salt were added all at once. Stirring was continued for the time shown. The resulting solution, after acidification with 2 N HCl, was added to a solution of 2,4-dinitrophenylhydrazine (1.2 mmol) in 25 mL of 12 N HCl, previously filtered. The (2,4-dinitrophenyl)hydrazones obtained were recrystallized from ethanol. Their spectroscopic data (IR, NMR, MS) were identical with those of authentic samples.

Preparation of Triazolines 6 and 7. To a stirred solution of Val (1 mmol) in 12 mL of sodium carbonate buffer (pH 10) was added a large excess of Fremy's salt (3 mmol). After 5 days of stirring, a second batch of Fremy's salt (3 mmol) was added. Stirring was continued for another 5 days. The resulting solution was then treated with a filtered solution of 2,4-dinitrophenylhydrazine (2.2 mmol) in 50 mL of 12 N HCl. A precipitate slowly appeared (24 h). Filtration and final recrystallization (twice) from ethanol yielded triazoline **6** in 41% yield as yellow needles, mp 202–203 °C: IR (KBr) 3275, 3100, 2980, 1690, 1610, 1590, 1500, 1410, 1335, 1305, 1260, 1140, 1110, 1075, 915, 860, 830, 740, 710 cm^{-1}; ^1H NMR (Me_2SO-d_6, Me_4Si) 1.60 (6 H, s), 8.05 (1 H, d, J = 9.4 Hz), 8.47 (1 H, dd, J = 9.4 Hz, J = 2.5 Hz), 8.95 (1 H, d, J = 2.5 Hz), 15.50 (1 H, s) ppm; ^{13}C NMR (Me_2SO-d_6, Me_4Si) 25.55, 82.50, 115.60, 122.66, 129.69, 129.96, 138.00, 143.80, 149.50, 158.27 ppm; mass spectrum, m/e (relative intensity) 309 (M^+, 60), 294 (100), 273 (10), 259 (15), 195 (20), 183 (25). Anal. Calcd for $C_{11}H_{11}N_5O_6$: C, 42.72; H, 3.56; N, 22.65. Found: C, 42.64; H, 3.58; N, 22.37.

Identical treatment of Ile (1 mmol) as above yielded triazoline **7** in 38% yield, as yellow prisms: mp 194–195.5 °C; IR (KBr) 3275, 3100, 2975, 1680, 1605, 1595, 1500, 1420, 1330, 1310, 1135, 1110, 1090, 1045, 920, 880, 830, 740, 700 cm^{-1}; ^1H NMR (Me_2SO-d_6, Me_4Si) 1.00 (3 H, t, J = 7.3 Hz), 1.54 (3 H, s), 1.90 (2 H, q, J = 7.3 Hz), 8.05 (1 H, d, J = 9.6 Hz), 8.38 (1 H, dd, J = 9.6 Hz, J = 2.4 Hz), 9.08 (1 H, d, J = 2.4 H), 14.09 (1 H, s) ppm; ^{13}C NMR (Me_2SO-d_6, Me_4Si) 7.38, 23.72, 31.93, 85.56, 115.57, 122.60, 130.02, 130.14, 138.29, 143.82, 149.00, 158.22 ppm; mass spectrum, m/e (relative intensity) 323 (M^+, 19), 294 (100), 273 (24), 183 (21). Anal. Calcd for $C_{12}H_{13}N_5O_6$: C, 44.59; H, 4.05; N, 21.66. Found: C, 44.66; H, 3.86; N, 21.34.

Treatment of commercial (Sigma) α-keto isovaleric acid (1 mmol) with Fremy's salt (30 mmol) as indicated for Val yielded triazoline **6** in 58% yield.

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Registry No. **6**, 104197-46-8; **7**, 104197-47-9; $H_2NCH_2CO_2H$, 56-40-6; $H_2NCH(CH_3)CO_2H$, 56-41-7; $(CH_3)_2CHCH(NH_2)CO_2H$, 72-18-4; $CH_3CH_2CH(CH_3)CH(NH_2)CO_2H$, 73-32-5; $HO_2CCH_2CH_2CH(NH_2)CO_2H$, 56-86-0; $H_2NCH(Ph)CO_2H$, 69-91-0; $PhCONHCH_2CO_2H$, 495-69-2; $PhCONHCH(CH_3)CO_2H$, 2198-64-3; $CH_3CH(OH)CO_2H$, 50-21-5; $PhCH(OH)CO_2H$, 90-64-2; $OHCCO_2H$, 298-12-4; CH_3COCO_2H, 127-17-3; $(CH_3)_2CHCOCO_2H$, 759-05-7; $CH_3CH_2CH(CH_3)COCO_2H$, 1460-34-0; $HO_2CCH_2CH_2COCO_2H$, 328-50-7; $PhCOCO_2H$, 611-73-4; DNP-NHN=CHCO_2H, 3158-42-7; DNP-NHN=C$(CH_3)CO_2H$, 790-12-5; DNP-NHN=C(Pr-i)CO_2H, 6064-65-9; DNP-NH-N=C(Bu-*sec*)CO_2H, 1459-83-2; DNP-NH-N=C$(CH_2CH_2CO_2H)CO_2H$, 1237-47-4; DNP-NH-N=C$(Ph)CO_2H$, 31334-72-2; Fremy's salt, 14293-70-0; oxidase, 9035-73-8; (2,4-dinitrophenyl)hydrazine, 119-26-6.

(23) Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ion Control*, Chapman and Hall Laboratory Manual: London, 1974.

An Investigation of the Mode of Formation of the 10-P-5 Protonated Phosphatrane $HP(OCH_2CH_2)_3N^+$

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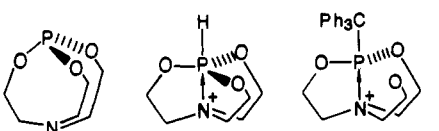
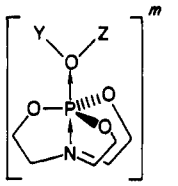
In 1977 it was reported that the unstable phosphite ester amine **1** reacted with Me_3O^+ and Ph_3C^+ to give the first 10-P-5 cations **2** and **3**, respectively.[1] Cation **2** (1-



1 2 3

hydro-2,8,9-trioxa-1-phospha-5-azatricyclor[3.3.3.0]undecane) was shown to possess a TBP structure by X-ray crystallographic means.[2] In contrast to the novel 10-P-5 TBP species such as **4–10** which have been reported from



	Y	Z	m	ref
4:	1p	BF_3	O	3
5:	1p	Et	1+	5
6:	Et	Et	2+	4
7:	1p	Et_3Si	1+	3
8:	Et_3Si	Et_3Si	2+	3
9:	1p	H	1+	4
10:	H	H	2+	4

our laboratories recently, **2** and **3** feature the relatively nonapicophilic proton and triphenylmethyl group, respectively, in the apical position. It is also curious that the hydride–phosphorane cation **2** is the only nonpolymeric product isolated from the reaction of **1** with Me_3OBF_4 (Scheme I) rather than the methyl analogue. In contrast, **3** was reported to form in the analogous reaction with Ph_3CBF_4.[1] Here we show that the unreacted triethanolamine is the source of the protons in the reaction involving Me_3OBF_4. Moreover, we now find that **2** as well as **3** can be formed in the reaction of **1** with Ph_3CBF_4 (Scheme I).

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(2) Clardy, J. C.; Milbrath, D. S.; Springer, J. P.; Verkade, J. G. *J. Am. Chem. Soc.* **1976** *98*, 623.
(3) Carpenter, L. E.; Verkade, J. G. *J. Am. Chem. Soc.* **1985**, *107*, 7084.
(4) Carpenter, L. E.; de Ruiter, B.; van Aken, D.; Buck, H. M.; Verkade, J. G. *J. Am. Chem. Soc.*, in press.
(5) van Aken, D.; Merkelbach, I. I.; Koster, A. S.; Buck, H. M. *J. Chem. Soc., Chem. Commun.* **1980**, 1045.

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