

Communications to the Editor

[Chem. Pharm. Bull.]
36(5)1926—1929(1988)

OXYGENATION OF METHYL CYCLOHEXYLPHENYLGlyCOLATE, A CONSTITUTIONAL
UNIT OF DRUGS, CATALYSED BY IRON(II) ACETONITRILE SOLVATE

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Oxygenation of methyl O-acetyl- α -cyclohexyl- α -phenylglycolate (4c) with the reagent system $\text{Fe}(\text{MeCN})_6^{2+}$ - H_2O_2 - Ac_2O , a model enzyme system for mono-oxygenase, was investigated in connection with the metabolism of the drug oxybutynin hydrochloride (1) in humans and dogs. Among the oxygenation products 5d, 7, 8, and 9, 5d, 7, and 8 correspond to the mammalian metabolites of 1 except for the stereochemistry in 7 and 8. This suggests implications regarding the mechanism of the mammalian metabolism of the drug.

KEYWORDS — oxygenation; methyl cyclohexylphenylglycolate; oxybutynin; iron(II) acetonitrile solvate; hydrogen peroxide; model enzyme; mono-oxygenase

α -Cyclohexyl- α -phenylglycolic acid (4a) has frequently been used as a unit along with α -diphenylglycolic acid to constitute drugs, such as oxybutynin hydrochloride (1), oxypyronium bromide (2), and oxyphencyclimine hydrochloride (3), etc. Among these, 1 is a newly developed drug used as an agent to treat pollakisuria nervosa.¹⁾ The metabolites of 1 in humans and dogs are 4a, 5a, 5b, 5c, 6a, 6b, and 6c as shown in Chart 1.²⁾ We have investigated the oxygenation reaction of 4c with the reagent system $\text{Fe}(\text{AN})_6^{2+}$ - H_2O_2 - Ac_2O (AN= MeCN), a model reagent system for mono-oxygenase,³⁾ with a view to preparing the metabolites in large quantities for toxicological investigations and as a laboratory model for studying bio-oxygenation mechanism.

The treatment of 4c with the reagent system proceeded as follows. To a solution of $\text{Fe}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ and 4c in AN and Ac_2O (sufficient amounts of Ac_2O to remove water of the iron salt and H_2O_2), a solution of 30% H_2O_2 in AN was added dropwise, keeping the temperature at 25–30°C, in appropriate molar ratios of the substrate : H_2O_2 : Fe^{2+} (Table I). The resulting solution was worked up as reported previously³⁾ followed by hydrolysis with alcoholic KOH and then methylation with CH_2N_2 . GLC of the residue showed four main peaks. Then, the residue was subjected to HPLC using a LiChrosorb RP-18 (Merck) column and AN : H_2O (3 : 7) as an eluent to give 4d, 5d, and a mixture of 7 and 8, and 9 in the

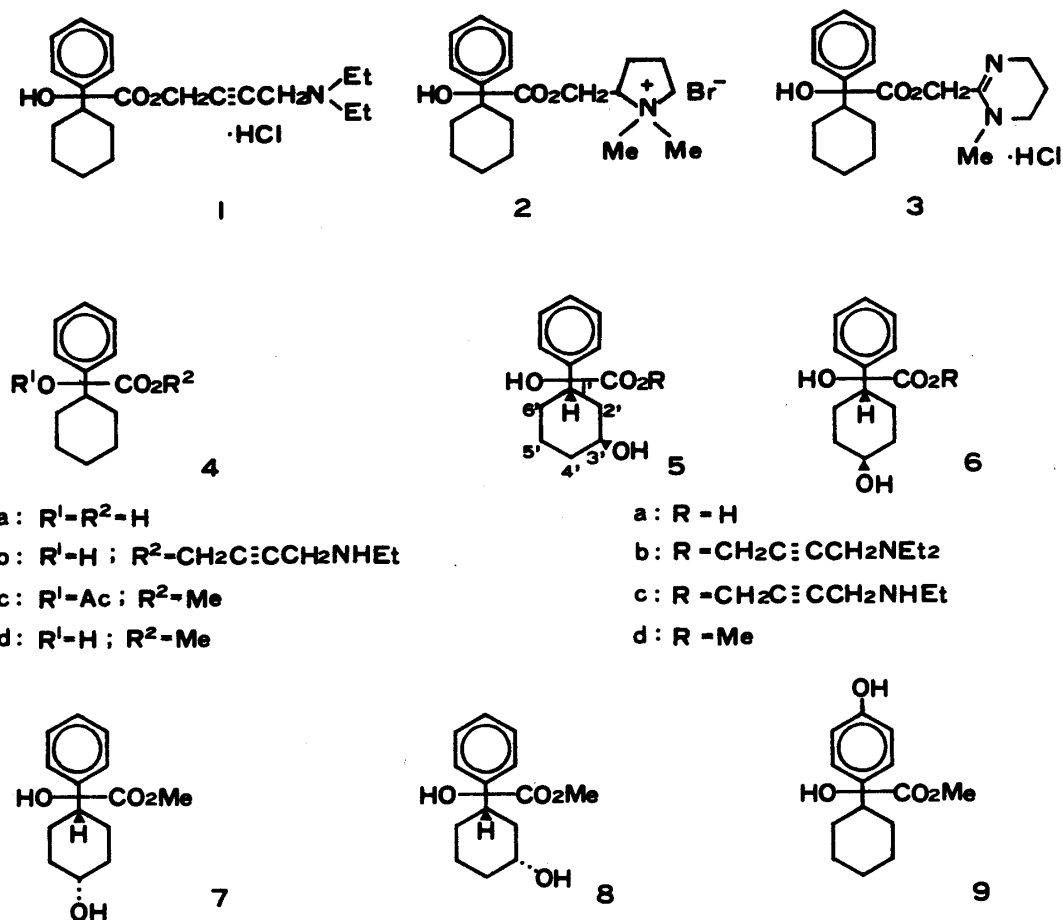


Chart 1

yields shown in Table I. The structures of 5d and the mixture of 7 and 8 were determined by analyzing the spectra of proton magnetic resonance (^1H -NMR) in which the assignments were aided by off-resonance decoupling; and 9 was identified by comparison with an authentic sample. The position of the hydroxy group in 5d was determined by a stepwise assignment of the protons bound to alicyclic carbon atoms, based on the signal of C-1' βH at 2.713 ppm (tt, $J=12.1$ and 3.4 Hz). The configuration of the hydroxy group in 5d was assigned according to the coupling pattern of C-3' αH at 4.238 ppm (quintet, $J=2.9$ Hz). The ^1H -NMR and the GLC of oxygenation product 5d were identical with those of the corresponding metabolite. The ^1H -NMR spectrum of the inseparable mixture of 7 and 8 showed that they are a 1 : 1 mixture, and the structures of 7 and 8 were determined by an analysis similar to that for 5d. That is, the C-4' βH of 7, one of the constituents of the mixture, was centered at 4.027 ppm (quintet, $J=2.5-2.8$ Hz), the C-3' βH of another constituent, 8, showed a signal at 3.667 ppm (tt, $J=10.4$ and 4.4 Hz), and there was a sharp signal of C-2' αH at 1.44 ppm (q, $J=12$ Hz).

The results of Run 4 and 5 in Table I to give significant amounts of oxygenation products at alicyclic carbon atoms was particularly interesting because the oxidation potential of benzene is lower than that of cyclohexane.

Table I. Reaction of Methyl Cyclohexylphenylglycolate (4c) with the Reagent System $\text{Fe}(\text{MeCN})_6^{2+}\text{-H}_2\text{O}_2\text{-Ac}_2\text{O}$

Run	Molar ratio of reagent		Product (%) ^{a)}		
	H_2O_2	$\text{Fe}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$	5d	7 + 8	9
1	1.5	2.0	4.56	4.91	28.18
2	1.5	1.0	6.08	6.65	38.80
3	1.5	0.5	8.80	9.08	23.43
4	1.5	0.1	16.84	19.02	6.08
5	1.5	0.05	10.46	14.52	4.49

a) Yields were estimated by the GLC analysis.

Table II. Reactions of Cyclohexane, Benzene, Chlorobenzene, and Methyl 2-Acetoxy-2-phenylpropanoate and Their Mixed Substances with the Reagent System $\text{Fe}(\text{MeCN})_6^{2+}\text{-H}_2\text{O}_2\text{-Ac}_2\text{O}$

Run	Molar ratio of substrate : H ₂ O ₂ : Fe ²⁺	Product: acetate (mol %) ^{a)}				Reaction efficiency ^{b)} (%)
		cyclohexane		aromatic		
		mono-	di-	mono-	di-	
Cyclohexane						
1	1 : 1 : 0.01	6.7				6.7
2	1 : 1 : 0.1	34.0	2.4			38.8
3	1 : 1 : 0.2	48.2	3.7			55.6
4	1 : 1 : 0.3	40.0	2.5			45.0
5	1 : 1 : 1	26.4				31.4
Benzene						
6	1 : 1 : 0.01			23.3	2.3	27.9
7	1 : 1 : 0.1			19.0	2.0 ^{c)}	23.0
8	1 : 1 : 1			5.7	1.0	7.7
Chlorobenzene						
9	1 : 1 : 0.1			21.4 ^{d)}		21.4
Methyl 2-Acetoxy-2-phenylpropanoate						
10	1 : 1 : 0.1			15.8		15.8
Cyclohexane + Benzene						
11	(1 + 1): 1 : 0.1	23.0		20.0		43.0
Cyclohexane + Chlorobenzene						
12	(1 + 1): 1 : 0.1	33.5	1.6	2.6		39.3
Cyclohexane + Methyl 2-Acetoxy-2-phenylpropanoate						
13	(1 + 1): 1 : 0.1	43.0	1.3	2.5		48.1

a) Determined by GLC analysis. b) Estimated based on H_2O_2 used.

c) Ratio of the o- and p-diacetates was 1 : 1.86.

d) Ratio of the o- and p-acetates was 1 : 0.8.

Thus, the following experiments to search for such unique phenomena were undertaken.

A few interesting phenomena were observed in the reactions of cyclohexane, benzene, chlorobenzene, and methyl 2-acetoxy-2-phenylpropanoate and their mixed substances with the oxygenation reagent system as follows (Table II). (a) The best conditions to afford the highest yield of oxygenation products are different with substrates and independent of their oxidation potentials (Run 1-9). (b) In the reactions of mixed substrates, namely, cyclohexane and chlorobenzene (Run 12) and cyclohexane and methyl 2-acetoxy-2-phenylpropanoate (Run 13), the yield of cyclohexyl acetate was increased in the case of Run 13, but yields of aromatic acetates were decreased significantly in both cases. These results suggest the presence of a certain kind of interaction between cyclohexane and chlorobenzene or the methyl acetoxyphenylpropanoate, and between the methyl acetoxyphenylpropanoate and the reagent, particularly the reactive species of the reagent. The latter interaction may participate in the production of 5d, 7, and 8 in the oxygenation reaction of 4c (Run 4 and 5 in Table I).

In the reaction of 4c, there was no oxygenation product at C-1' or C-2' of the cyclohexane ring. Therefore, the regioselective oxygenation at C-3' and C-4' of 4c may be attributable to a chelate formation between the acetoxy and carbomethoxy groups of 4c and the higher valent iron complexes such as $\text{Fe}^{\text{IV}}=\text{O}$ or $\text{Fe}^{\text{V}}=\text{O}$, a hypothetically active species. And the predominant formation of the alicyclic acetates 5d, 7, and 8 in the presence of small amounts of Fe^{2+} (Run 4 and 5 in Table I) may be caused, for the same reason, by the reaction of the mixed substrate cyclohexane and methyl 2-acetoxy-2-phenylpropanoate (Run 13 in Table II).

Further studies are now underway regarding the nature of the interaction between cyclohexane and chlorobenzene or methyl 2-acetoxy-2-phenylpropanoate observed in these experiments.

ACKNOWLEDGMENT The authors expresses his grateful thanks to the director of the institute Dr. Hideyo Shindo and to Dr. Harumitsu Kuwano, Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., for ^1H -NMR analysis.

REFERENCES

- 1) A. G. Diokno and J. Lapidés, *J. Urology*, **1972**, 307; K. Mayuzumi, K. Watanabe, K. Tamura, and T. Kasama, *Folia Pharmacol. japon.*, **87**, 557 (1986).
- 2) Y. Akimoto, H. Kobayashi, Y. Shinozaki, and G. Urakubo, *Iyakuin Kenkyu*, **17**, 1070 (1986); Y. Shinozaki, R. Monden, A. Manaka, H. Hisa, S. Naito, T. Igarashi, H. Sakai, Y. Iwata, T. Kasama, Y. Akimoto, and G. Urakubo, *Xenobiotic Metabolism and Disposition*, **1**, 341, (1986).
- 3) E. Kotani, S. Kobayashi, Y. Ishii, and S. Tobinaga, *Chem. Pharm. Bull.*, **33**, 4671 (1985).

(Received February 8, 1988)