Non-enzymatic Oxygenation of (+)-Camphor Catalyzed by Iron(II) Acetonitrile Solvate

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The oxygenation reaction of (+)-camphor with a simple model reagent, $Fe(MeCN)_6^{2+}$–H_2O_2–Ac_2O, for mono-oxygenase was investigated in connection with its bio-oxygenation. The products 1b, 2b, 3b, 5, 10, 11, and 12 were obtained.

Keywords oxygenation; (+)-camphor; iron(II) acetonitrile solvate; hydrogen peroxide; model enzyme; mono-oxygenase; bio-oxygenation

Camphor has been used as a probe for studies on bio-oxygenation in mammals and microorganism[1] and on the mechanism of mono-oxygenases[2,3] in a century. Several metabolites have been isolated, namely, compounds **1a**, **2a**, **3a**, and **4** from the urine of dogs[4] and **1a**, **2a**, **3a**, and **6** from the urine of rabbits[5] after feeding of camphor, and **1a**, **2a**, **5**, **7**, **8**, and **9** from the camphor-containing culture medium of *Pseudomonas putida*.[6,7]

We investigated the oxygenation reaction of (+)-camphor catalyzed by the reagent system $Fe(MeCN)_6^{2+}$–H_2O_2–Ac_2O,[8] a non-heme enzyme model for mono-oxygenase having a high reaction efficiency, in connection with its bio-transformation. Oxygenation was carried out at the molar ratio of $Fe(MeCN)_6(ClO_4)_2$[9]:(+)-camphor:H_2O_2=0.5:1:3 in MeCN and the resulting solution was worked up in the manner reported previously.[8] Oxygenation products **1b**, **2b**, **3b**, **5**, **10**, **11**, and **12** were isolated by repeated column chromatography of the reactant solution in yields of 7.3% (**1b**+**2b**+**3b**), 2.7% (**10**), 2.1% (**5**), 1.8% (**11**), and 7.2% (**12**) (estimated by gas-liquid chromatographic analysis).

The products **5**[5] and **10**[10] were identified by direct comparison with authentic samples and **1b**, **2b**, and **3b** by comparison of the physical properties of their hydrolysis products **1a**,[6] **2a**,[5] and **3a**[5] with the reported values. The identities of the latter three were also confirmed by the Jones' oxidation of the alcohols to give the corresponding diketones **5** and **13**.[11]

The structures of the lactones **11** and **12** were deduced by analyses of the proton and carbon-13 nuclear magnetic resonance spectra (^{1}H- and ^{13}C-NMR), in which the assignments were aided by off-resonance decoupling. The absorptions centered at 1780 and 1740 cm^{-1} in the infrared (IR) spectra of **11** and **12** suggested the presence of lactone rings and acetoxy groups in both compounds. The position of the oxygen atom of the lactone in **12** was assigned from the fact that the C(1) signal was observed at δ 87.74 (lower field than the corresponding signal in camphor (δ 57)) while the C(4) signal (δ 45.93) was observed at almost the same position as that of camphor (δ 43.2; C(3) in camphor) in the ^{13}C-NMR spectra. The positions and configuration of the two acetoxy groups of **12** were deduced by the stepwise assignment of all proton signals as shown in Chart 2. The structure of the lactone **11** was deduced analogously (Chart 2). The structures of **11** and **12** were supported by the fact that oxygenation of the lactone **7** with the same reagent system gave the lactones **11** and **12**.

There are two other oxygenation reagent systems related to the present one, namely, Groves' system[12] $Fe(ClO_4)_2 \cdot 6H_2O$–MeCN–AcO_2H (a system containing a small amount of water) and Sawyer's system[13] $Fe(MeCN)_6(ClO_4)_2$–MeCN–anhydrous H_2O_2 (an anhydrous system). Although the reaction of (+)-camphor with Sawyer's reagent system was not investigated because that system was reported to have no reactivity to insert oxygen into an aliphatic C–H bond, the reaction of (+)-camphor with Groves' reagent system afforded only a diketone **5**[14] in 4.8% yield but no Baeyer–Villiger type reaction products such as **7**, **11**, and **12**.

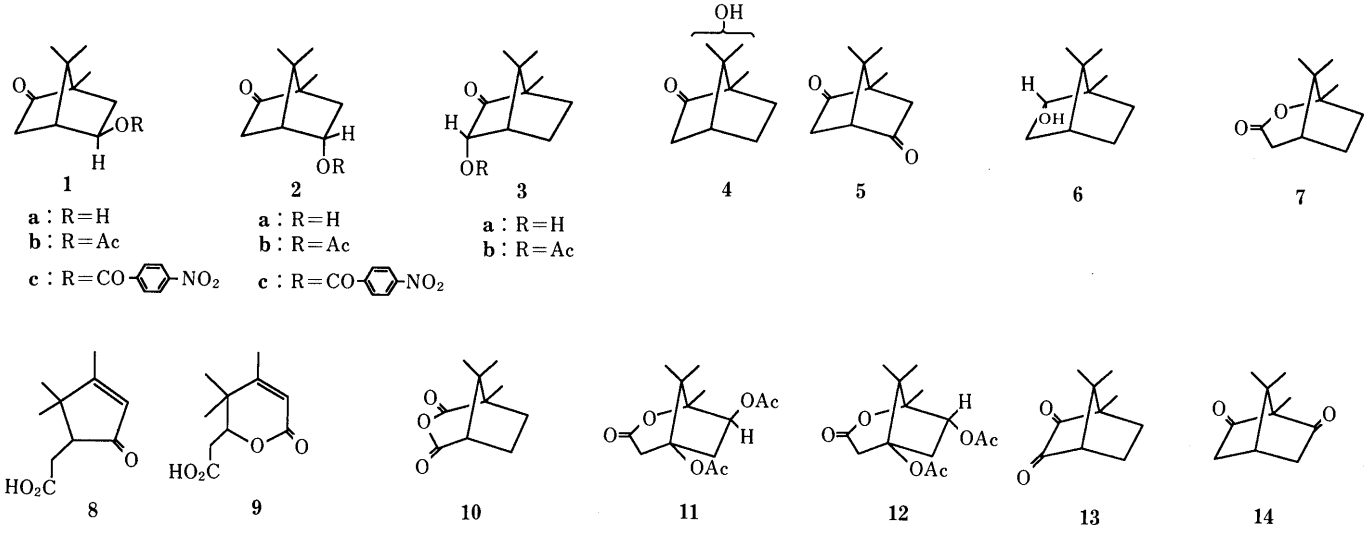

Chart 1

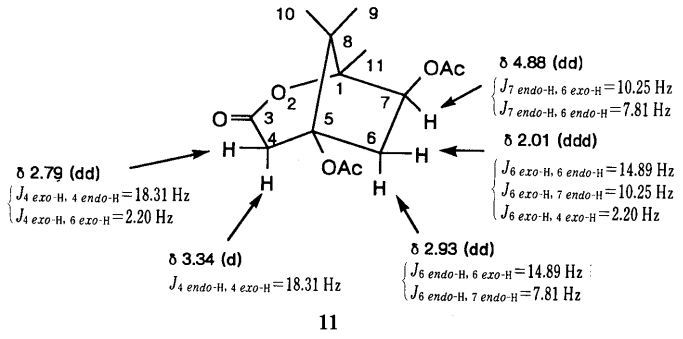

Chart 2

Compounds **1b**, **2b**, and **3b** may be formed by reaction with the active species of this reagent, Fe^{III}–O· or Fe^{IV}–O· (Fe^{IV}=O or Fe^{V}=O).[15] Formation of **5** also suggests that the initial oxygenation products of these mono-acetates may be the corresponding hydroxy compounds **1a** and **2a**. In fact, the reaction of **1a** with this reagent system afforded **5**. Further, the reaction of the 1,2-diketone **13** with the oxygenation reagent gave the anhydride **10**, which suggested that the diketone may be a precursor of the anhydride. Formation of the Baeyer–Villiger type reaction products **11** and **12** shows a clear difference from the reaction in Groves' system though the reason for this is unclear. The positions of acetoxy groups of **11** and **12** differ from that of the mono-acetates. This may suggest that the precursor for **11** and **12** is the camphor lactone **7** rather than the mono-acetates **1b** and **2b**. This hypothesis is supported by the fact that the oxygenation reaction of **7** gave the lactones **11** and **12** as described above.

Although the mammalian metabolite **4** is lacking, and the lactones **11** and **12** have not been isolated as metabolites, the present results provide some chemical analogy with the bio-oxygenation of (+)-camphor by cytochrome P-450 enzyme and flavin-dependent cyclohexanone mono-oxygenase.[3]

Experimental

All melting points are uncorrected. IR spectra were recorded with a Hitachi 260-10 spectrometer, ^{13}C- and ^{1}H-NMR spectra with JEOL JNM-GX 100 and 270 spectrometers with tetramethylsilane as an internal standard ($CDCl_3$ solution), mass spectra (MS) with a JEOL JMS-D 300 spectrometer, and $[\alpha]_D$ with a JASCO DIP-140 digital polarimeter. Wako Silica gel C-200 (200 mesh) and Merck Kieselgel 60 F-254 were used for column and thin-layer chromatographies (TLC), respectively. Gas-liquid chromatographic analyses (GLC) were performed on a Hitachi 163 analyzer equipped with an FID detecter and a 10% Chromosorb W, SE-30, 2 m column (programmed temperature 100—250 °C at 5 °C/min, inlet pressure of nitrogen used as a carrier gas 1.0 fkg/cm^2). Elementary analyses were done by Mr. K. Yoda, Kissei Pharmaceutical Company, Matsumoto, Japan.

Oxygenation of (+)-Camphor Catalyzed by $Fe(MeCN)_6(ClO_4)_2$ A solution of 30% H_2O_2 (9.9 ml, 90 mmol) in MeCN (60 ml) was added to a solution of $Fe(ClO_4)_2 \cdot 6H_2O$ (5.43 g, 15 mmol), Ac_2O (75 ml), and (+)-camphor (4.56 g, 30 mmol) in MeCN (180 ml) with vigorous stirring at 35—40 °C for 5 min. Ice-water and diluted HCl were added to the solution, and the whole was extracted with ether–CH_2Cl_2 (3:1). The organic layer was washed with aqueous Na_2SO_3, saturated aqueous $NaHCO_3$, and brine, dried on Na_2SO_4, and then evaporated at 60 °C to give an oily residue, which showed five spots on TLC (*Rf*: 0.17, 0.21, 0.41, 0.48, 0.50; eluated with hexane:AcOEt=3:1). A GLC analysis of the residue showed 10 peaks as follows; retention times (t_R) (yields): 11.6 min (65.7%), 14.1 min (2.1%), 18.4 min (1.2%), 18.8 min (2.7%), 19.5 min (7.3%), 24.0 min (2.3%), 25.2 min (1.0%), 25.9 min (1.8%), 27.6 min (7.2%), 28.3 min (1.8%).

Isolation of 10, 11, and 12 The residue obtained from the above oxygenation reaction was subjected to silica gel column chromatography with pentane as an eluent followed by gradual addition of ethyl formate to yield three fractions. The first fraction yielded (+)-camphor (t_R: 11.6 min). The second fraction was further subjected to silica gel column chromatography with pentane–ethyl formate (10:1) to give **10** (*Rf*: 0.41; t_R: 18.8 min) and a mixture of **1b** (*Rf*: 0.48; t_R: 19.5 min), **2b** (*Rf*: 0.48; t_R: 19.5 min), and **3b** (*Rf*: 0.50; t_R: 19.5 min). A similar chromatographic purification of the third fraction gave **11** (*Rf*: 0.21; t_R: 28.3 min) and **12** (*Rf*: 0.17; t_R: 27.6 min).

Camphoric Anhydride (10) Colorless crystals (ether), mp 214—216 °C (lit.[10] mp 219—220 °C). IR (KBr) cm^{-1}: 1800, 1750. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, s, –Me), 1.10 (3H, s, –Me), 1.27 (3H, s, –Me), 1.90—2.33 (4H, m, C(6)-H and C(7)-H), 2.83 (1H, d, J=6.34 Hz, C(5)-H). CI-MS *m/z*: 183 ($M^+ + 1$). Compound **10** was also obtained by the oxygenation of **13** in the same manner as described for the reaction of (+)-camphor catalyzed by $Fe(MeCN)_6(ClO_4)_2$.

5,7-*exo*-Diacetoxy-1,8,8-trimethyl-2-oxabicyclo[3.2.1]octan-3-one (11) Colorless oil. $[\alpha]_D^{25}$ +53.84 (c=0.145, EtOH). IR (CH_2Cl_2) cm^{-1}: 1780, 1740. ^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, s, –Me), 1.09 (3H, s, –Me), 1.38 (3H, s, –Me), 2.01 (1H, ddd, J=14.89, 10.25, 2.20 Hz, C(6) *exo*-H), 2.06 (3H, s, –OCOMe), 2.09 (3H, s, –OCOMe), 2.79 (1H, dd, J=18.31, 2.20 Hz, C(4) *exo*-H), 2.93 (1H, dd, J=14.89, 7.81 Hz, C(6) *endo*-H), 3.34 (1H, d, J=18.31 Hz, C(4) *endo*-H), 4.88 (1H, dd, J=10.25, 7.81 Hz, C(7) *endo*-H). CI-MS *m/z*: 285 ($M^+ + 1$).

5,7-*endo*-Diacetoxy-1,8,8-trimethyl-2-oxabicyclo[3.2.1]octan-3-one (12) Colorless crystals (ether), mp 105—106 °C. $[\alpha]_D^{25}$ −13.26 (c=0.200, EtOH). IR (KBr) cm^{-1}: 1780, 1740. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, s, –Me), 1.08 (3H, s, –Me), 1.39 (3H, s, –Me), 2.03 (3H, s, –OCOMe), 2.09 (3H, s, OCOMe), 2.44 (1H, ddd, J=15.87, 5.25, 1.95 Hz, C(6) *exo*-H), 2.58 (1H, d, J=15.87 Hz, C(6) *endo*-H), 2.88 (1H, dd, J=18.55, 1.95 H, C(4) *exo*-H), 3.29 (1H, d, J=18.55 Hz, C(4) *endo*-H), 5.03 (1H, d, J=5.25 Hz, C(7) *exo*-H). ^{13}C-NMR ($CDCl_3$) δ: 16.71 (q, –Me), 17.65 (q, –Me), 20.92 (q, –Me), 21.18 (q, –Me), 23.78 (q, –Me), 44.09 (t, C(6)), 45.93 (t, C(4)), 48.45 (s, C(8)), 81.03 (d, C(7)), 87.74 (s, C(1)), 97.70 (s, C(5)), 169.43 (s, C=O), 170.22 (s, C=O), 172.44 (s, C=O). CI-MS *m/z*: 285 ($M^+ + 1$). *Anal.* Calcd for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 58.85; H, 7.16.

Oxygenation of 7 Compound **7**[16] was oxygenated in the same manner as described for the reaction of (+)-camphor catalyzed by $Fe(MeCN)_6(ClO_4)_2$. GLC and TLC analyses of the residue showed the presence of **11** and **12**.

Separation of 1b, 2b, and 3b A solution of the mixture of **1b**, **2b**, and **3b** in 10% methanolic KOH was heated at 60 °C for 1 h. The reaction mixture was poured into water and extracted with ether. The organic layer was washed with water and dried on Na_2SO_4. After concentration, the residue was subjected to silica gel column chromatography using petroleum ether–ether (5:1) as an eluent to yield a mixture of **1a** and **2a** and **3a**.

3-*endo*-Hydroxycamphor (3a) Colorless crystals (ligroin), mp 188—190 °C, (lit.[5] mp 196—197 °C). IR (CH_2Cl_2) cm^{-1}: 3450, 1740. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, –Me), 0.93 (3H, s, –Me), 1.01 (3H, s, –Me), 1.31—2.01 (4H, m, C(5)-H and C(6)-H), 2.27 (1H, t, J=4.64 Hz, C(4)-H), 4.21 (1H, d, J=4.64 Hz, C(3) *exo*-H). CI-MS *m/z*: 169 ($M^+ + 1$). The Jones' oxidation of **3a** gave camphorquinone (**13**), which gave the same IR spectra and behavior on TLC as those of an authentic sample.[11]

Separation of 1a and 2a A mixture of **1a** and **2a** obtained from the above chromatography (168 mg, 1 mmol) in dry pyridine (85 ml) was added to a solution of *p*-nitrobenzoyl chloride (278 mg, 1.5 mmol) in dry dioxane (10 ml) with ice cooling, and the whole was heated under reflux overnight. The reaction mixture was poured into ice-water and extracted with ether.

The organic layer was washed with diluted HCl, aqueous $NaHCO_3$, and brine, dried on Na_2SO_4, and then concentrated. The residue was subjected to silica gel column chromatography using CH_2Cl_2–hexane as an eluent to yield **1c** (25 mg) and **2c** (30 mg). The products (**1c** and **2c**) were hydrolyzed in the same way as described for **3b** to give **1a** (10 mg) and **2a** (15 mg), respectively.

5-*exo*-Hydroxycamphoryl *p*-Nitrobenzoate (1c) Colorless crystals (hexane), mp 158—159 °C, (lit.[17] mp 158—159 °C).

5-*endo*-Hydroxycamphoryl *p*-Nitrobenzoate (2c) Colorless crystals (hexane), mp 147—148 °C, (lit.[6] 147—148 °C).

5-*exo*-Hydroxycamphor (1a) Colorless crystals (petroleum ether), mp 219—220 °C, (lit.[6] mp 220.5—221.5 °C). IR (CH_2Cl_2) cm^{-1}: 3450, 1740. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, –Me), 0.94 (3H, s, –Me), 1.25 (3H, s, –Me), 1.69 (1H, d, J=18.30 Hz, C(3) *endo*-H), 1.74—1.84 (2H, m, C(6) *exo* and *endo*-H), 2.15 (1H, d, J=5.40 Hz, C(4)-H), 2.33 (1H, dd, J=18.30, 5.40 Hz, C(3) *exo*-H), 4.03 (1H, dd, J=6.89, 4.19 Hz, C(5) *endo*-H). ^{13}C-NMR ($CDCl_3$) δ: 8.96 (q, C(10)), 19.85 (q, C(9)), 21.00 (q, C(8)), 36.64 (t, C(6)), 40.01 (t, C(3)), 46.51 (s, C(7)), 50.84 (d, C(4)), 58.73 (s, C(1)), 74.59 (d, C(5)), 218.49 (s, C(2)). CI-MS m/z: 169 ($M^+ + 1$).

5-*endo*-Hydroxycamphor (2a) Colorless crystals (petroleum ether), mp 214—216 °C (lit.[5] mp 212—214 °C). IR (CH_2Cl_2) cm^{-1}: 3450, 1740. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s, –Me), 0.86 (3H, s, –Me), 1.00 (3H, s, –Me), 1.24 (1H, dd, J=14.4, 5.40 Hz, C(6) *endo*-H), 2.12—2.24 (3H, m, C(3) *endo*-H, C(4)-H), C(6) *exo*-H), 2.70 (1H, dd, J=19.79, 1.50 Hz, C(3) *exo*-H), 4.64 (1H, dddd, J=7.50, 5.40, 4.90, 1.50 Hz, C(5) *exo*-H). ^{13}C-NMR ($CDCl_3$) δ: 9.34 (q, C(10)), 19.31 (q, C(9), 20.31 (q, C(8)), 40.45 (t, C(3)), 40.96 (t, C(6)), 47.60 (s, C(7)), 48.85 (d, (C(4)), 59.09 (s, C(1)), 69.54 (d, C(5)), 218.44 (s, C(2)). CI-MS m/z: 169 ($M^+ + 1$).

Camphane-2,5-dione (5) Compound **5** was identified by comparison of its retention time on GLC, t_R: 14.1 min, with that of an authentic sample which was obtained by the Jones' oxidation of **1a** and **2a**. The oxygenation of **1a** in the same manner as described for (+)-camphor also gave **5**.

Oxygenation of (+)-Camphor by Groves' Method Peracetic acid (0.3 ml, 1 mmol) in MeCN (1 ml) was added to a solution of $Fe(ClO_4)_2 \cdot 6H_2O$ (362 mg, 1 mmol) and (+)-camphor (152 mg, 1 mmol) in MeCN (6 ml) with vigorous stirring at −10 °C for 10 min. The mixture was stirred for an additional 1 h at room temperature. The whole was poured into diluted HCl and extracted with ether. The organic layer was washed with aqueous Na_2SO_3 and saturated aqueous $NaHCO_3$, dried on Na_2SO_4 and then concentrated at 60 °C. GLC and TLC analyses of the residue showed the presence of camphane-2,5-dione (**5**) or camphane-2,6-dione (**14**).

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