KCl solution, and homogenized by a Teflon-glass Potter-Elvehjem homogenizer. The homogenate was centrifuged at 9000 × *g* for 30 min and the pellet was removed. The supernatant was again centrifuged at 105000 × *g* for 60 min by a Hitachi Model 40P ultracentrifuge. The supernatant was separated and used for the incubation study (supernatant fraction). The microsomal pellet was resuspended in a 1.15% KCl solution in such a way that 1 ml was equivalent to 200 mg wet weight of rat liver (microsomal fraction).

Incubation Study——i) 16α-Chloroestrone-6,7-^{3}H or estrone-6,7-^{3}H (1 μCi, 0.25 μmole) in propylene glycol (0.1 ml), NADH (5 μmoles), NADPH (5 μmoles), and the microsomal or supernatant fraction (equivalent to 200 mg wet weight of rat liver) were brought to 5 ml with 0.05M Tris–HCl buffer (pH 7.4) and incubated at 37° for 30 min. The incubation mixture was extracted with AcOEt (5 ml × 3). The organic layer was combined, dried over anhydrous Na_2SO_4, and evaporated. The residue was submitted to TLC using benzene–AcOEt (5: 1) as developing solvent. The adsorbent was scraped from the plate in each 1 cm width and submitted to radioactivity counting (Fig. 1).

ii) 16α-Chloroestrone or estrone (0.5 μmole), NADH or NADPH (5 μmoles), and the microsomal or supernatant fraction (equivalent to 200 mg wet weight of rat liver) were brought to 3 ml with 0.05M Tris–HCl buffer (pH 7.4) and incubated at 37° for 30 min. After addition of AcOEt to stop the reaction, estrone methyl ether (25 μg) was added and extracted with AcOEt (3 ml × 3). The organic layer was combined, dried over anhydrous Na_2SO_4, and evaporated. The residue was submitted to preparative TLC using benzene–AcOEt (5: 1) as developing solvent. Elution of the adsorbent corresponding to 16α-chloroestradiol (*Rf* 0.39) or estradiol (*Rf* 0.21) and estrone methyl ether (internal standard) (*Rf* 0.61) with AcOEt and the eluate was submitted to gas chromatographic determination by condition GC—I (Fig. 2).

iii) 16α-Chloroestrone (0.06, 0.075, 0.15, 0.3 μmole), estrone (0, 0.05, 0.1, 0.2 μmole), NADH (2 μmoles), and the microsomal fraction (equivalent to 200 mg wet weight of rat liver) were brought to 3 ml with 0.05M Tris–HCl buffer (pH 7.4) and incubated at 37° for 10 min. The incubation mixture was processed and 16α-chloroestradiol was determined by GLC in the manner as described in ii) (Fig. 3).

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Reaction of Vinylogous Esters with Grignard Reagent[1)]

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With Grignard reagent, five-membered vinylogous esters gave 1,2-addition products in a similar fashion to the cases for six-membered vinylogous esters. On the other hand, the six-membered vinylogous ester substituted with *t*-butyl group at the α-position of carbonyl function also gave only 1,2-addition product. However the five-membered vinylogous ester substituted with hydroxy group at the α-position of methoxy group gave 1,2-addition product and 1,4-addition products.

In these Grignard reactions, the catalytic effect of cuprous chloride was scarcely observed.

In our previous papers, the reactions of some vinylogous esters with lithium aluminum hydride were examined,[3)] that is, six-membered vinylogous esters were reduced to β-alkoxy-α,β-unsaturated alcohols (due to 1,2-addition), on the other hand, five-membered vinylogous esters were reduced to α,β-unsaturated alcohols (due to 1,4-addition followed by 1,2-addition). The vinylogous esters substituted with *t*-butyl or hydroxy groups were reduced to characteteristic products respectively.

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1) A part of this work was presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April, 1975.
2) Location: *Gofuku, Toyama.*
3) K. Matoba and T. Yamazaki, *Yakugaku Zasshi*, **93**, 1406 (1973) and references therein.

In connection with those interesting results, the reactions of those vinylogous esters and methyl magnesium iodide (the same nucleophilic reagent as hydrides) are reported in this paper. The reaction of vinylogous esters and phenyl magnesium bromide has already been reported for the simple six-membered vinylogous esters[4] and only one five-membered vinylogous ester.[5] In these cases, only 1,2-addition products were detected and the effect of cuprous chloride has not hitherto been reported.

At first, from the viewpoint of the reaction conditions, 3-ethoxy-5,5-dimethyl-2-cyclohexenone (Ia) was used, because the examinations for Ia had been carried out moderately in detail.[4] From this examination, it was found that the general conditions mentioned in the experimental section were the best ones. The structure of isophorone (IIa) obtained from Ia was confirmed by its infra red (IR) and nuclear magnetic resonance (NMR) spectra, and identified with the authentic sample as its 2,4-dinitrophenyl hydrazone (2,4-DNPH).

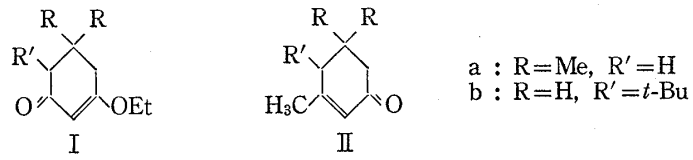
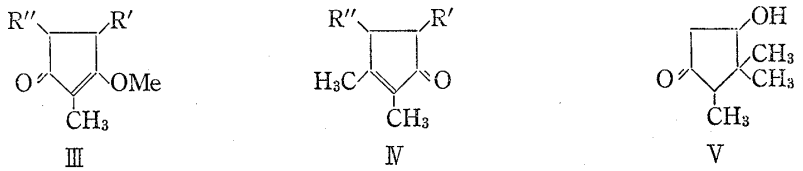


Chart 1

From 6-*t*-butyl-3-ethoxy-2-cyclohexenone (Ib), 1,4-addition product was expected because of the steric effect of the bulky substituent, *t*-butyl group. Contrary to our expectation, it was revealed from the examination of gas–liquid chromatography (GLC), IR and NMR that the reaction product was only 1,2-addition product, 4-*t*-butyl-3-methyl-2-cyclohexenone (IIb). IIb gave quantitatively 2,4-DNPH derivative[6] in contrast to 6-*t*-butyl-2-cyclohexenone.[3] Thus it was elucidated that six-membered vinylogous ester substituted with *t*-butyl group even at the α-position of carbonyl group selectively gave 1,2-addition product, that is, no effect due to *t*-butyl substituent was observed.

Next, from the point of view that the result of hydride reduction of five membered vinylogous ester was in contrast to that of six-membered one.[3] Grignard reaction of 3-methoxy-2-methyl-2-cyclopentenone (IIIa) was examined. However, the result obtained in this case was also fairly parallel with that obtained for Ia. GLC pattern of product from IIIa was single peak, and its IR and NMR spectra showed that the product was 2,3-dimethyl-2-cyclopentenone (IVa). Thus the effect due to five-membered ring was not observed.

In order to examine the effect of hydroxy group as a substituent, the following experiments were carried out. Model compounds selected were following two isomers,[3] 5-hydroxy-3-methoxy-2-methyl-2-cyclopentenone (IIIb) and 4-hydroxy-3-methoxy-2-methyl-2-cyclopentenone (IIIc). From IIIb, 4-hydroxy-2,3-dimethyl-2-cyclopentenone (IVb) was obtained as



a: R′=R″=H b: R′=H, R″=OH c: R′=OH, R″=H

Chart 2

4) G.F. Wood, *J. Am. Chem. Soc.*, **69**, 2549 (1947).
5) V.I. Sladkov, V.F. Shner, O.S. Anisimova, V.P. Pakhomov, N.S. Kuryatov, and N.N. Suvorov, *Zh. Org. Khim.*, **9**, 810 (1973) [*C. A.*, **79**, 42230*m* (1973)].
6) G. Stork and W.N. White, *J. Am. Chem. Soc.*, **78**, 4604 (1956).

expected, which was supported by NMR spectrum of IVb whose 2-methyl group coupled with C_4-proton.[7]

But products from IIIc were complicated. Three spots were observed on thin-layer chromatography (TLC) plate. These products purified by means of preparative TLC were revealed from their physical data and the elementary analyses of their *p*-nitrobenzoate (PNB) to be two isomers of 4-hydroxy-2,3,3-trimethyl-cyclopentanone (V), and 5-hydroxy-2,3-dimethyl-2-cyclopentenone (IVc), in the decreasing order of *Rf* value. IVb was converted quantitatively to V with methyl magnesium iodide. Among these products from IIIc, two isomers of V are 1,4-addition products and IVc is 1,2-addition product. The yields of 1,4-addition products and 1,2-addition product were nearly equal. The hydroxy group in IIIc exhibited such a characteristic effect differing from that in IIIb in the reaction with Grignard reagent.

Finally, the catalytic effect of cuprous chloride was examined for Ib and IIIa under the same conditions as that used for IIa.[8] However no remarkable difference was observed and only a small amount of unidentifiable products were obtained (see also experimental section).

Experimentals

All the melting points taken on a Kofler block and boiling points are uncorrected. The following equipments were used: IR spectra, Hitachi Grating Infrared 215 spectrophotometer; NMR spectra, JEOL C-60H spectrometer with tetramethylsilane as an internal reference; GLC, Shimazu gas chromatograph Model GC-3AF (5% SE-30 column, N_2-gas, 40 ml/min). The TLC values were obtained with Kiesel gel G nach Stahl (Merck) as adsorbent. The spots were detected by spraying with 1% ceric sulfate-10% sulfuric acid and heating. For column chromatography and preparative TLC, Wakogel C-200 and Kieselgel 60 PF (Merck) were used respectively. The chemical shifts and coupling constants in NMR were described in τ-value and cps respectively. The abbreviations used to demonstrate coupling pattern are as follows; singlet-s, doublet-d, triplet-t, quartet-q, multiplet-m, broad-br. Unless otherwise stated, all the solvents were evaporated under reduced pressure.

General Conditions of Grignard Reaction——A similar reaction procedure was adopted for all experiments, one full description is given. Methyl magnesium iodide was prepared by dropwise addition of methyl iodide (1.6 ml, 0.21 mole) in dry ether (20 ml) to stirred magnesium turning (0.51 g, 0.21 mole). When the magnesium had been consumed, the solution was heated under reflux for 10 min, then cooled to 20° (when CuCl is necessary, 0.2 g (2.1 mmoles) of it was added in this step). To the Grignard reagent thus prepared, the vinylogous ester (70 mmoles) in ether (20 ml) except otherwise mentioned was added during 2 hr. The reaction mixture was refluxed for further 1 hr. Then the excess of reagent was destroyed by cautious addition of saturated NH_4Cl solution. The organic layer was separated and aqueous layer was extracted several times with ether. The combined organic solution was washed with saturated NaCl solution and dried over anhyd. $MgSO_4$ then concentrated. The residue was analyzed with GLC and TLC, and separated through silicagel column chromatography or preparative TLC.

Grignard Reaction Products from 3-Ethoxy-5,5-dimethyl-2-cyclohexenone (Ia) in the Presence of Catalyst——GLC (150°) min: t_R 3.6 (3-ethoxy-1,5,5-trimethyl-2-cyclohexenol, *ca.* 45%), 4.2 (3,5,5-trimethyl-2-cyclohexenone (IIa), *ca.* 45%), and 8.2 (Ia, *ca.* 10%). When this mixture was stood overnight, only two peaks, t_R 4.2 min (ca. 90%) and 8.2 min (*ca.* 10%) were observed. Purified through silicagel column chromatography, IIa eluted with benzene and starting material eluted with ether. IIa-2,4-DNPH: mp 143—145° (red plates from EtOH, lit.[9] mp 146°). *Anal.* Calcd. for $C_{15}H_{18}O_4N_4$: C, 56.59; H, 5.70; N, 17.60. Found: C, 56.46; H, 5.70; N, 17.35.

From 6-*t*-Butyl-3-ethoxy-2-cyclohexenone (Ib)[3]——GLC (150°) min: t_R 6.3 (4-*t*-butyl-3-methyl-2-cyclohexenone (IIb) *ca.* 50%), 8.3 (Ib, *ca.* 50%). Treated with a few drops of 10% HCl in acetone Ib was converted into 4-*t*-butyl-cyclohexane-1,3-dione,[3] which was excluded by washing with alkali. IIb thus obtained was distilled. bp 113—117° (1.5 mmHg). IR (film) cm^{-1}: $\nu_{C=O}$ 1680, $\nu_{C=C}$ 1620. NMR (CCl_4): 4.18 (1H, s, vinylic H), 7.96 (3H, s, vinylic CH_3), 8.94 (9H, s, *t*-Bu). IIb-2,4-DNPH: mp 155—157° (red plates from EtOH). *Anal.* Calcd. for $C_{17}H_{22}O_4N_4$: C, 58.94; H, 6.40; N, 16.18. Found: C, 58.74; H, 6.33; N, 16.15.

7) C.M. Cimarusti and J. Wolinsky, *J. Org. Chem.*, **31**, 4118 (1966); M. Anteunis and F. Compernolle, *Bull. Soc. Chim. Belges*, **76**, 482 (1967).
8) H.O. House, W.L. Respess, and G.M. Whitesides, *J. Org. Chem.*, **31**, 3128 (1966).
9) E.A. Braude and E.A. Evans, *J. Chem. Soc.*, **1954**, 607.

2,3-Dimethyl-2-cyclopentenone (IVa)——bp 100—104° (1.0 mmHg). IR (film) cm^{-1}: $\nu_{C=O}$ 1700, $\nu_{C=C}$ 1650.[10] NMR (CCl_4): 7.60 (4H, br.s, $2\times CH_2$), 7.96 (3H, s, β-CH_3), 8.32 (3H, br.s, α-CH_3). IVa-2,4-DNPH: mp 232—234° (red plates from EtOH, lit.[11] mp 233°). *Anal.* Calcd. for $C_{13}H_{14}O_4N_4$: C, 53.79; H, 4.86; N, 19.30. Found: C, 53.51; H, 4.62; N, 19.34.

From Ib in the Presence of CuCl——GLC (150°) t_R min: 3.6, 4.5 (each unidentified products, *ca.* each 5%), 6.3 (IIb, *ca.* 60%), 8.3 (Ib, *ca.* 30%). The mixture was fractionated through column chromatography to unidentified products (eluted with *n*-hexane), IIb (eluted with benzene), and Ib (eluted with ether). IIb was identified with the authentic sample mentioned above.

From IIIa in the Presence of CuCl——GLC (120°) t_R min: 1.1 (unidentified product, *ca.* 2%), 3.0 (IVa, *ca.* 90%), 7.2 (IIIa, *ca.* 8%). The mixture was fractionated through silicagel column chromatography to unidentified product (eluted with *n*-hexane), IVa (eluted with benzene), and IIIa (eluted with ether). IVa and IVa-DNPH were identified with each authentic sample by comparison with their physical data.

From 5-Hydroxy-3-methoxy-2-methyl-2-cyclopentenone (IIIb)——TLC (acetone: $CHCl_3$=1: 1) *Rf*: 0.53 (4-hydroxy-2,3-dimethyl-2-cyclopentenone (IVb)), 0.36 (IIIb). GLC (170°) t_R min: 1.0 (IVb), 1.3 (IIIb). IVb was isolated by means of micro-distillation. IVb: bp<120° (7 mmHg). IR (film) cm^{-1}: ν_{OH} 3400, $\nu_{C=O}$ 1685, $\nu_{C=C}$ 1640. NMR ($CDCl_3$); 5.30 (1H, m, >C$\underline{H}_X$–OH), 6.45 (1H, br.s, –OH), 7.22 (1H, d.d, J=18, 6, >C$\underline{H}_A$H$_B$), 7.78 (1H, d.d, J=18, 2.5, >CH$_A$$\underline{H}_B$), 7.91 (3H, s, β-CH_3), 8.30 (3H, nearly s, α-CH_3). IVb-PNB: mp 124° (from ether-*n*-hexane). IR ($CHCl_3$) cm^{-1}: $\nu_{C=O}$ 1722, 1705, ν_{NO} 1528, 1342. NMR ($CDCl_3$): 1.80 (4H, s, aromatic H), 4.05 (1H, m, >CH$_X$–O–), 6.98 (1H, d.d, J=18, 6, >C$\underline{H}_A$H$_B$), 7.63 (1H, d.d, J=18, 2.5, >CH$_A$$\underline{H}_B$), 7.91 (3H, s, β-CH_3), 8.20 (3H, d, J=1,[7]) α-CH_3). *Anal.* Calcd. for $C_{13}H_{14}O_5N$: C, 61.09; H, 4.76; N, 5.09. Found: C, 61.20; H, 4.65; N, 5.11.

From 4-Hydroxy-3-methoxy-2-methyl-2-cyclopentenone (IIIc)——TLC (AcOEt: *n*-hexane=2: 1) *Rf*: 0.48, 0.38 (4-hydroxy-2,3,3-trimethyl-cyclopentanone (V)), 0.25 (5-hydroxy-2,3-dimethyl-2-cyclopentenone (IVc)), 0.12 (IIIc). GLC[12] (130°) t_R min: 5.7 (IVc), 7.6, 7.8 (V). *cf*) IVb: 13.2 min. GLC (170°) t_R min: 1.7 (IVc), 2.1 (V), 6.7 (IIIc). These products were isolated by means of preparative TLC (benzene: AcOEt= 1: 2). IVc: mp <30°. IR (film) cm^{-1}: ν_{OH} 3400, $\nu_{C=O}$ 1703, $\nu_{C=C}$ 1640. NMR ($CDCl_3$): 5.75 (1H, d.d, J=6, 3, >C$\underline{H}$–OH), 6.87 (1H, br.s, –OH), 6.90—7.75 (2H, m, >CH_2), 7.93 (3H, s, β-CH_3), 8.27 (3H, nearly s, α-CH_3). V: oil. bp <130° (3 mmHg). IR (film) cm^{-1}; ν_{OH} 3440, $\nu_{C=O}$ 1732. NMR ($CDCl_3$): 5.90 (1H, d.d, J=5, 1.5, >C$\underline{H}$–OH), 7.76 (1H, br.s, –OH), 8.81 (3H, s, >C(C$\underline{H}_3$)CH_3), 9.01 (3H, d, J=8, >CH–C$\underline{H}_3$), 9.21 (3H, s, >C(CH_3)-C$\underline{H}_3$), When IIIc in benzene (30 ml) was added to Grignard reagent in ether (10 ml), starting material, IIIc, was not recovered.

IVc-PNB——mp 145—147° (from acetone–ether). IR ($CHCl_3$) cm^{-1}; $\nu_{C=O}$ 1725, 1708, $\nu_{C=C}$ 1638. NMR ($CDCl_3$): 1.76 (4H, s, aromatic H), 4.63 (1H, d.d, J=6, 3, >CH–O–), 7.88 (3H, s, β-CH_3), 8.21 (3H, br.s, α-CH_3). *Anal.* Calcd. for $C_{13}H_{14}O_5N$: C, 61.09; H, 4.79; N, 5.09. Found: C, 60.94; H, 4.46; N, 4.86.

V-PNB——mp 110°, 177—178° (without recrystallization). IR ($CHCl_3$), cm^{-1}: $\nu_{C=O}$ 1742, 1730, 1722. NMR ($CDCl_3$): 1.78 (4H, s, aromatic H), 4.63 (1H, m, >CH–O–), 8.77 (3H, s, >C(C$\underline{H}_3$)CH_3), 8.97 (3H, d, J=11, >CH–C$\underline{H}_3$), 9.02 (3H, s, >C(CH_3)C$\underline{H}_3$). *Anal.* Calcd. for $C_{15}H_{17}O_5N$: C, 61.85; H, 5.88; N, 4.81. Found: C, 62.06; H, 6.12; N, 5.10.

10) E.S. Waight, *J. Chem. Soc.*, **1966** (**B**), 73.
11) H. Strickler, G. Ohloff, and E. sz. Kovats, *Tetrahedron Letters*, 1964, 649.
12) 5% OV-17 was used as packing reagent of column in Shimazu Gas Chromatograph Model GC-6A.