Notes

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Polyphosphoric Acid-catalyzed Beckmann Rearrangement of 4-Methylcholest-4-en-3-one Oxime

Masaru Kobayashi and Hiroshi Mitsuhashi

Faculty of Pharmaceutical Sciences, Hokkaido University[1]

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In polyphosphoric acid, Beckmann rearrangement of α,β-unsaturated keto oximes takes place with migration of both alkyl and double bond sites.[2] Previously we reported that the polyphosphoric acid-catalyzed Beckmann rearrangement of cholest-1-en-3-one and cholest-4-en-3-one oximes gave the stable enamine lactams corresponding to *anti* oximes. In this condition, pure *anti* oxime also gave the α,β-unsaturated lactam, possively by partial isomerization to *syn* oxime.[3]

From the rigid *anti* oximes which resist to the thermal inversion, only enamine type lactams will be expected. 4-Methylcholest-4-en-3-one oxime (II) is a stable *anti* oxime by the steric repulsion of methyl and hydroxyl group and, in fact, its nuclear magnetic resonance (NMR) spectrum shows one broad doublet (δ 3.15, J=18 Hz) of 2α-equatorial proton deshielded by the hydroxyl group.

However, when the oxime (II) was treated with polyphosphoric acid at 100° for 1 hr, the products were not the postulated enamine lactam (III) but the hydrolyzed *seco*-amides, IV (46%) and V (14%). These structures were deduced from the spectral data as follows: IV, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3400 (NH), 1678, 1598 (CO and $CONH_2$); NMR δ: 0.65 (s, 18-Me), 0.97 (s, 19-Me), 2.12 (s, $COCH_3$), 5.4—6.2 (broad, 2H). V, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3525, 3410 (NH), 1700 (shoulder, CO), 1675, 1590 ($CONH_2$); NMR δ: 0.65 (18-Me), 2.11 (s, $COCH_3$), 5.6—6.1 (broad,

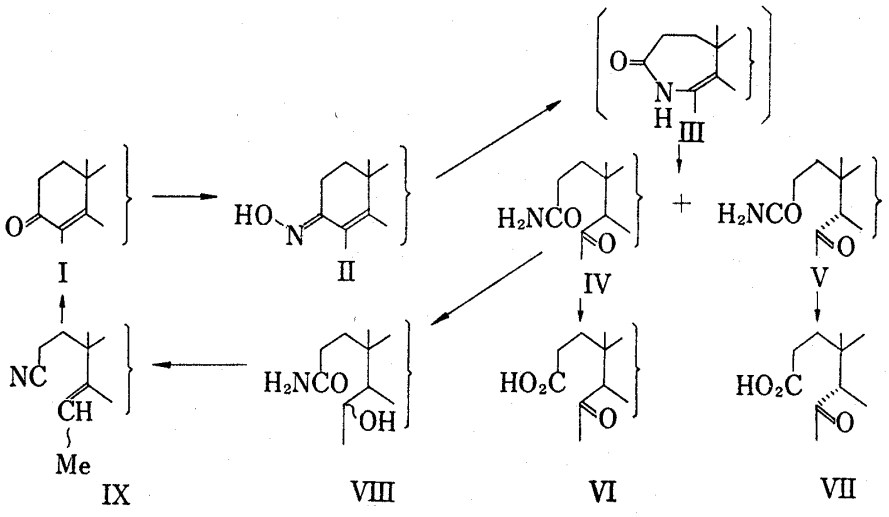

Chart 1

1) Location: *Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido.*
2) R.H. Mazur, *J. Org. Chem.*, **26**, 1289 (1961).
3) M. Kobayashi, Y. Shimizu, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 1255 (1969).

2H). The peaks at δ 5.4—6.2 and 5.6—6.1 disappear on treatment with D_2O. Both amides (IV and V) are convertible into the corresponding acids (VI and VII) respectively. When IV was treated with sulfuric acid, partial isomerization to V was observed on thin-layer chromatography.

The configuration of C-5 substituent of the major product (IV) was assigned as β since it should occupy more stable equatorial position and consequently, the minor product (V) has 5α-substituent.

The structure of IV was also confirmed by its conversion to the starting ketone (I). Sodium borohydride reduction of IV gave a mixture of alcohols (VIII) and treatment of VIII with *p*-toluensulfonyl chloride in pyridine gave an oily unsaturated nitrile (IX) in a high yield (92% from IV). Its NMR spectrum showed one vinylic proton multiplet centered at δ 5.1 and vinylic methyl at δ 1.56 (d, J=6 Hz).

In 1956, Hill and Conley reported that the polyphosphoric acid-catalyzed cyclization of certain unsaturated nitriles led not to ordinary lactams but to α,β-unsaturated ketones.[4] Although the reaction has disadvantage because of its low yield, it is applicable to the annelation of IX. On treatment with polyphosphoric acid at 140—145° for 5 min, the unsaturated nitrile (IX) gave about 50% of the starting material and 14% of an unsaturated ketone which was identified as 4-methyl-cholest-4-en-3-one in all respects.

Experimental[5]

Beckmann Rearrangement of 4-Methylcholestenone Oxime (II)——A mixture of 2 g of the oxime (II) and 30 g of polyphosphoric acid was heated in an oil bath (100°) for 1 hr with stirring and poured into ice water. The mixture was extracted with $CHCl_3$ and the extract was washed consecutively with 5% HCl, 5% $NaHCO_3$, H_2O, and saturated NaCl solution. The solvent was evaporated and the residue was chromatographed over 60 g of silica gel. The eluate with benzene–$CHCl_3$ (1: 2) gave 75 mg of 4-methylcholestenone (I) and 135 mg of the oxime (II). The eluate with $CHCl_3$ and $CHCl_3$–ether (9: 1) gave 975 mg of 5β-acetyl-3,5-*seco*-4-*nor*-cholestan-3-amide (IV), mp 166.5—170° (capillary), $[\alpha]_D$ +31.5° (c=0.37, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3400, 1678, 1598. NMR δ: 0.65 (18-Me), 0.97 (19-Me), 2.12 (s, 3H, $COCH_3$), 5.4—6.2 (broad, 2H, NH_2). *Anal.* Calcd. for $C_{28}H_{49}O_2N$: C, 77.90; H, 11.44; N, 3.25. Found: C, 77.96; H, 11.19; N, 3.29.

The eluate with $CHCl_3$–ether and $CHCl_3$–EtOH gave 290 mg of 5α-acetyl-3,5-*seco*-4-*nor*-cholestan-3-amide (V), mp 180.5—181.5° (capillary), $[\alpha]_D$ +3.2° (c=0.62, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3525, 3410, 1700, 1675, 1590. NMR δ: 0.65 (18-Me), 2.11 (s, 3H, $COCH_3$), 5.6—6.1 (broad, 2H, NH_2). *Anal.* Calcd. for $C_{28}H_{49}O_2N$: C, 77.90; H, 11.44; N, 3.25. Found: C, 77.68; H, 11.24; N, 3.27.

5β-Acetyl-3,5-*seco*-4-*nor*-cholestan-3-oic Acid (VI)——A mixture of the amide (IV, 300 mg) and 10 ml of AcOH–Ac_2O (1: 5) was chilled to −5° and 1 g of $NaNO_2$ was added during 1 hr. After stirring for 3 hr, the mixture was poured into water and the precipitate was collected, washed with H_2O, and recrystallized from ether–hexane to 250 mg of VI, mp 112—114°, $[\alpha]_D$ +4.3° (c=0.47, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1710. *Anal.* Calcd. for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18. Found: C, 77.75; H, 10.99.

5α-Acetyl-3,5-*seco*-4-*nor*-cholestan-3-oic Acid (VII)——A mixture of 300 mg of the amide (V) was treated by the same procedure as above. Recrystallization from hexane gave 270 mg of VII, mp 194—195°, $[\alpha]_D$ 0° (c=1.9, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1700. *Anal.* Calcd. for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18. Found: C, 77.46; H, 10.97.

4-Methyl-3,4-*seco*-cholest-4-eno-3-nitrile (IX)——A solution of 0.5 g of $NaBH_4$ in 12 ml of 50% MeOH was added to a solution of 1 g of the amide (IV) in 60 ml of MeOH and stirred at room temperature. After 1.5 hr, 0.5 g of $NaBH_4$ and 100 ml of EtOH were added and the mixture stirred for several hours. The solution was diluted with H_2O, extracted with ether, and the extract was washed thoroughly with water. The evaporation residue (VIII, *ca.* 1 g, mass: M$^+$ 433) was dissolved in 10 ml of pyridine and refluxed with 3.8 g of *p*-TsCl for 6 hr. The mixture was concentrated to a small volume in reduced pressure and extracted with ether. The extract was washed with dilute HCl and water, the solvent was evaporated, and the oily residue was passed through 10 g of silica gel column with hexane. Evaporation of the first fraction (30 ml) gave 850 mg of IX as a colorless oil (92% from IV). Mass Spectrum: M$^+$ 397. IR ν_{max}^{neat} cm^{-1}: 3045, 2250. NMR δ: 1.56 (d, J=6 Hz), 5.1 (m, 1H).

Cyclyzation of IX to 4-Methylcholestenone (I)——A mixture of 670 mg of the nitrile (IX) and 20 g of polyphosphoric acid was heated in an oil bath (145°) for 5 min with vigorous stirring and cooled in an ice

4) R.K. Hill and R.T. Conley, *Chem. Ind.* (London), **1956**, 1314; R.K. Hill and R.T. Conley, *J. Am. Chem. Soc.*, **82**, 645 (1960).

5) Melting points were determined on a Kofler's hot stage unless otherwise specified and are uncorrected. NMR spectra were measured in $CDCl_3$ solution with tetramethylsilane as internal standard.

bath immediately. The mixture was decomposed with ice water and extracted with $CHCl_3$. After washing with 5% $NaHCO_3$ and water, the extract solution was evaporated and the residue was chromatographed over silica gel. Elution with hexane gave *ca.* 350 mg of the unsaturated nitrile (IX) and further elution with hexane–benzene gave an oily residue which was crystallized from $CHCl_3$–MeOH to afford 93 mg of pure 4-methylcholestenone (I), mp 101.5—102°, mixed mp 101—101.5°, $[\alpha]_D$ +104.7° (c=0.43, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1670, 1607. UV λ_{max}^{EtOH} nm (log ε): 250 (4.22).

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Effect of Plasma pH on Stability and Capacity of Aggregation of Platelets

KIYOMI KIKUGAWA, KAZUHIRO IIZUKA, and MOTONOBU ICHINO

Research Laboratory, Division of Fermentation and Chemical Products, Kohjin Co., Ltd.[1]

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It has been known that blood platelets form aggregates in hemostasis and in thrombogenesis.[2] The key role of adenosine diphosphate (ADP) as an important initiator of platelet aggregation has been well documented.[3] Born[4] and O'Brien[5] have established an *in vitro* turbidimetric method for the estimation of ADP-induced platelet aggregation and its inhibition. Thus, platelet aggregation and its inhibition are estimated by measuring the change in the optical density of platelet-rich plasma.

It has been found in the course of our investigations of the platelet aggregation inhibitors[6,7] that the extent of ADP-induced platelet aggregation of platelet-rich citrated plasma (PRCP) fluctuated over the storage period of several hours. These fluctuations were then found due to the alterations in pH of the plasma. Although it has been described that aggregation capacity of platelet is low at pH 6.5,[8] effect of plasma pH on platelet aggregation has not yet been demonstrated. This paper describes that alterations in plasma pH greatly influenced the stability of platelet aggregating capacity and the extent of ADP-induced platelet aggregation.

Experimental[9]

Platelet-rich Citrated Plasma (PRCP)——Blood (pH 7.5) was collected from male rabbits anesthetized with ethyl ether and was mixed with 1/10 volume of 3.8% sodium citrate (pH 7.65). The mixture was then centrifuged at 1000 rpm at room temperature for 10 min and the supernatant (PRCP) was removed with a siliconized pipett. The PRCP thus prepared showed pH 7.7—7.9 and was stored at room temperature.

Bufferized PRCP——Tris(hydroxymethyl)aminomethane and sodium veronal were purchased from Sigma Chemical Company and Wako Pure Chemical Industries, Ltd. respectively. PRCP was bufferized with an equal volume of either of the following buffers; 0.1M Tris-HCl (0.1M Tris-HCl+0.08M NaCl), 0.1M

1) Location: *Higashihama, Saiki, Oita.*
2) J.F. Mustard and M.A. Packham, *Pharmacol. Rev.*, **22**, 97 (1970).
3) A. Gaarder, J. Jonsen, S. Laland, A. Hellem, and P.A. Owren, *Nature* (London), **192**, 531 (1961).
4) *a*) G.V.R. Born, *Nature* (London), **194**, 927 (1962); *b*) G.V.R. Born and M.J. Cross, *J. Physiol.*, **168**, 178 (1963).
5) J.R. O'Brien, *J. clin. Path.*, **15**, 452 (1963).
6) K. Iizuka and K. Kikugawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 614 (1972).
7) K. Kikugawa, K. Iizuka, Y. Higuchi, H. Hirayama, and M. Ichino, *J. Med. Chem.*, **15**, 387 (1972).
8) K. Yasunaga, *Ketsueki to Miyakukan*, **1**, 627 (1970) and the references cited therein.
9) Unless otherwise mentioned, materials and methods were same as those described in ref. **6** and **7**.