gave 1-methyl-2,3,5,6,7,8-hexahydro-4(1*H*)-quinolone (Ⅲ) accompanied with a small amount of 1-methyl-3,4,5,6,7,8-hexahydro-2(1*H*)-quinolone (Ⅳ), while the same condensation of 2-tetralone and methyl 2-methyl-3-methylaminopropionate gave 2,4-dimethyl-1,2,5,6-tetrahydrobenzo[*f*]quinolin-3(4*H*)-one (Ⅷ) as a major product, and 2,4-dimethyl-3,4,5,6-tetrahydrobenzo[*f*]quinolin-1(2*H*)-one (Ⅸ) and 1,3-dimethyl-1,2,5,10-tetrahydrobenzo[*g*]-quinolin-4(3*H*)-one (Ⅹ) as minor products. Unequivocal synthetic proofs were given to these reaction products. The results enabled us to develop some discussion on the course of this type of the reaction. Revisions on the structures of the products in the previous paper were also described.

(Received August 3, 1964)

Chem. Pharm. Bull. 12(12)1415~1418(1964)

UDC 543.8-4 : 547.922

192. Tsutomu Momose, Yo Ueda, Mitsuyoshi Kageura, Toshiko Masumura, and Kiyoko Ohta : Color Reaction Mechanism of Cholesterol with Perchloric Acid, Phosphoric Acid and Ferric Chloride Reagent. I. (Organic Analysis. LVIII.*1)

(*Faculty of Pharmaceutical Sciences, Kyushu University**2)

In a previous paper of this series, a new method of determination of cholesterol in blood serum was presented with a mixture of perchloric acid, phosphoric acid and ferric chloride as the coloring reagent.[1] The developed color was so stable that fitted for measuring absorption intensities of a large number of samples in a clinical laboratory. A cholesterol solution in acetic acid was mixed with the color reagent and 0.2% ferric chloride solution in acetic acid, and the mixture was heated in a boiling water-bath for 20 minutes. During this period the color changed with the time of heating from

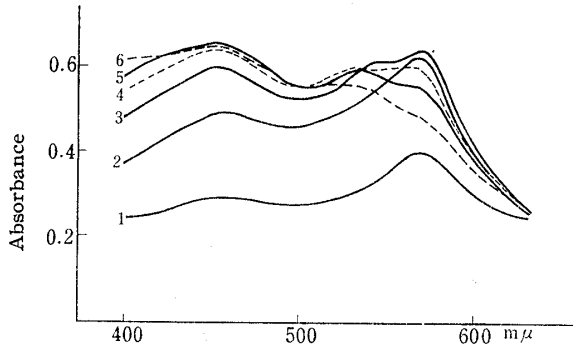

Fig. 1. Change of Absorption Spectrum of the Reaction Mixture of Cholesterol, Ferric Chloride, and the Color Reagent with the Time of Heating

1 : 3 min., 2 : 5 min., 3 : 10 min.,
4 : 15 min., 5 : 20 min., 6 : 30 min.,

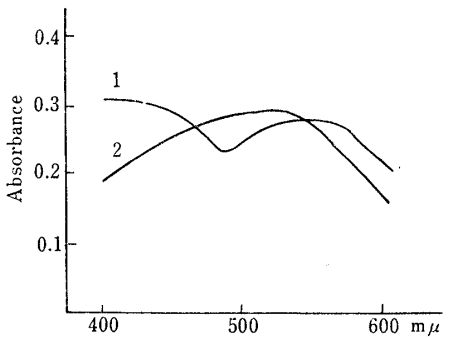

Fig. 2. Absorption Spectra of (1) Chloroform, and (2) Acid Layer of the Reaction Mixture of Cholesterol, Ferric Chloride, and the Color Reagent

*1 Part LVII. This Bulletin, **12**, 1500 (1964).
*2 Katakasu, Fukuoka (百瀬 勉, 上田 陽, 影浦光義, 益邑紀子, 太田紀代子).
1) T. Momose, Y. Ueda, K. Yamamoto, T. Masumura, K. Ohta : Anal. Chem., **35**, 1751 (1963).

purple red to orange red, and transitions of the absorption spectra were shown in Fig. 1. This change in color indicated that some of the primary reaction products of cholesterol were converted by a prolonged heating in the reagent to the secondary products which were rather stable against the strong acidic reagent.

The orange red color of cholesterol developed by the reagent could be partially extracted with chloroform from the acid mixture, and both chloroform and acid layer showed a similar color. Their absorption spectra are shown in Fig. 2. The chloroform layer held its color only in the presence of strong acid, and an addition of a small amount of water discolored to faint yellow. This fact indicates that the remarkable red shift is due to the action of strong acids, just as samely as in the other color reactions of cholesterol. Therefore, our main purpose of investigation was directed to the isolation of reaction products which might be obtained in almost colorless forms and might give colorations with strong acids.

A separation of chloroform extract of the color reaction mixture by the solubilities and chromatography on alumina yielded in turn, 3,3′-bi[cholesta-2,4-diene], oily substances, cholesta-3,5-diene, cholesteryl chloride, cholesteryl acetate, and other resinous products. The oily substances were a mixture of several compounds and obtained with a rather good yield. They develop an intense color with the color reagent, and therefore, this fraction should take the major responsibility for the coloration. However, it is very difficult to separate to their components by usual methods, and an information about them will be published later. 3,3′-Bi[cholesta-2,4-diene] is colorless plates, and gives an orange red color with the acid mixture in the presence of ferric chloride (Fig. 3). This compound was already isolated as one of the main coloring matter of cholesterol in the Liebermann-Burchard reaction,[2] the Tschugaeff reaction,[3] the Salkowski reaction,[4] and the Zak-Henly reaction.[5] Cholesta-3,5-diene is colorless prisms, but its chloroform solution develops a weak orange red color by shaking with the color reagent, and its absorption spectrum is shown in Fig. 4. This fact shows that cholesta-3,5-diene is also one of the main reaction product responsible for the coloration.

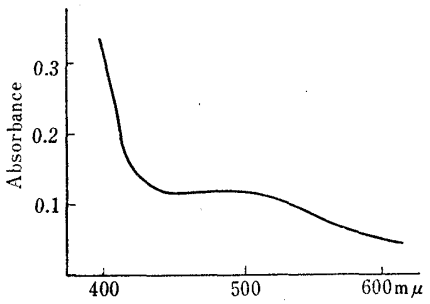

Fig. 3. Absorption Spectrum of the Color developed by 3,3′-Bi[cholesta-2,4-diene] with the Reagent Blank

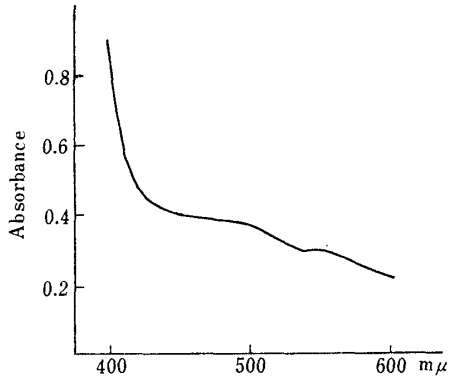

Fig. 4. Absorption Spectrum of the Color developed by Cholesta-3,5-diene with the Color Reagent

In a previous paper[5] we noticed that ferric chloride played a considerably important role on the red shift in the coloration of 3,3′-bi[cholesta-2,4-diene] in a mixture of sulfuric acid and acetic acid.

2) T. Watanabe : Eisei Shikenjo Hokoku, **77**, 87 (1959).
3) *Idem* : Yakugaku Zasshi, **80**, 442, 445 (1960).
4) R. Dulou, J. Chopin, Y. Raoul : Bull. soc. chim. France, **1951**, 616; J. Chopin : *Ibid.*, **1956**, 258.
5) T. Momose, Y. Ueda, M. Kageura, T. Masumura, K. Ohta : This Bulletin, **12**, 1500 (1964).

In the present case the remarkable fact to be mentioned is that neither a solution of chlesta-3,5-diene nor a solution of 3,3′-bi[cholesta-2,4-diene] in chloroform shows an appreciable visible color with a mixture of perchloric acid and phosphoric acid in the absence of ferric chloride. This fact means that the contribution of ferric chloride on the red shift should be much greater than that of strong acids.

Experimental*3

Color Reaction——Cholesterol, 4.0 g., was dissolved in 270 ml. of glac. AcOH by warming in a water-bath. To this solution were added 270 ml. of 0.2% $FeCl_3$ solution in AcOH and 270 ml. of the color reagent,[1] and the mixture was heated in a boiling water-bath with continuous stirring for 25 min.

Extraction of the Reaction Product——The reaction mixture was diluted with H_2O to about 2.5 L. and the reaction product was extracted with $CHCl_3$ three times. The combined $CHCl_3$ layer, about 500 ml., was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*.

The color reaction and the extraction were repeated five times. The total yield of the extracts was 19.5 g.

Separation of Reaction Product——Isolation of 3,3′-bi[cholesta-2,4-diene]: The whole extract was dissolved in a small amount of $CHCl_3$. Insoluble yellowish brown substances, 0.5 g., were collected by filtration, and purified by chromatography on alumina and recrystallization from benzene to give a faint yellow plates. A further purification was difficult by the amount obtained. M.p. (in evacuated capillary) 341~342°. No depression was observed on admixture with an authentic sample.[6] $[\alpha]_D$ −50 (c=0.10), UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 271 (4.50), 281 (4.55), 292 (4.43). Its IR spectrum was identical with that of an authentic sample.

Isolation of oily mixtures, cholesta-3,5-diene and cholesteryl chloride: The above filtrate was evaporated *in vacuo* and redissolved in petr. ether. This solution was poured on an alumina column (I), and the column was eluted with petr. ether. The solvent was distilled off from a few initial fractions leaving yellow oily residue. The combined residue was separated by repeated chromatography on alumina to give faint yellow oily substances, about 4.5 g.

The next several fractions eluted from the column (I) with petr. ether left colorless crystalline residues by evaporation of the solvent. The combined residue, about 2 g., was separated by a chromatography on alumina column (II). The petr. ether elute left colorless crystals, which was further purified by repeated chromatography on alumina, yielding about 200 mg. of colorless prisms. The analytically pure sample was obtained by recrystallization from benzene-EtOH, and drying over P_2O_5 *in vacuo* for 20 hr., m.p. 79~80.5°, no depression was observed on admixture with an authentic sample of cholesta-3,5-diene.[7] $[\alpha]_D$ −109 (c=0.55), UV λ_{max}^{hexane} mμ (log ε): 228.5 (4.39), 235.5 (4.42), 244 (4.24). *Anal.* Calcd. for $C_{27}H_{44}$: C, 87.95; H, 12.03. Found: C, 87.64; H, 11.95. Its IR spectrum was identical with that of an authentic sample.

The successive elution of the column (II) with benzene gave another colorless crystals. It was further purified by chromatography on alumina leaving cholesteryl chloride. The analytically pure cholesteryl chloride was obtained by recrystallization from benzene-EtOH, and drying over P_2O_5 *in vacuo* for 20 hr., m.p. 96°, no depression was observed on admixture with an authentic sample.[8] $[\alpha]_D$ −26.8 (c=1.5), *Anal.* Calcd. for $C_{27}H_{45}Cl$: C, 80.01; H, 11.20. Found: C, 80.05; H, 11.20. Its IR spectrum was identical with that of an authentic sample.

Isolation of cholesteryl acetate: Successive elution of the column (I) with benzene yielded about 6 g. of yellowish brown resinous substance. A repeated chromatography of it on alumina gave cholesteryl acetate as colorless prisms. A further purification by chromatography on alumina and recrystallization from Et_2O-EtOH gave an analytically pure cholesteryl acetate. Dried over P_2O_5 *in vacuo* for 20 hr., m.p. 115~116°. No depression was observed on admixture with an authentic cholesteryl acetate. $[\alpha]_D$ −45.1 (c=0.13). *Anal.* Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.47; H, 11.39. Its IR spectrum was identical with that of cholesteryl acetate.

Absorption Spectra——All spectra were measured in a glass cell of 10 mm. optical path length with a Shimadzu SV-50A Recording Spectrophotometer.

Fig. 1: A mixture of 2.00 ml. of cholesterol solution in glac. AcOH, 0.33 mg./2 ml., 2.00 ml. of 0.2% $FeCl_3$ solution in aq. AcOH, and 2.00 ml. of color reagent[1] was heated in a boiling water bath for 3, 5,

*3 All melting points were uncorrected. The measurement of optical rotation was carried out in chloroform solution.

6) J. L. Owades, A. E. Sobel: J. Am. Chem. Soc., **73**, 4223 (1951).

7) A. E. Sobel, M. J. Rosen: *Ibid.*, **63**, 3536 (1941).

8) O. Diels, P. Blumberg: Ber., **44**, 2847 (1911).

10, 15, 20, and 30 min. Absorption spectra of them were drawn against the reagent blank which was prepared by substituting glac. AcOH for the cholesterol solution.

Fig. 2 : The same mixture as described above was heated similarly for 20 min., and the color developed was extracted with 5.00 ml. of $CHCl_3$. Both the $CHCl_3$ and acid layer were submitted to the spectral measurements. The spectra were drawn against the corresponding layers prepared similarly from the reagent blank.

Fig. 3 : A solution of 0.406 mg. of 3,3′-bi[cholesta-2.4-diene] in 5.00 ml. of $CHCl_3$ was shaken with the reagent blank, and the orange red $CHCl_3$ layer was submitted to the spectral measurement. The spectrum was drawn against the corresponding $CHCl_3$ layer prepared from the reagent blank.

Fig. 4 : A solution of a small amount of cholesta-3,5-diene in about 1 ml. of $CHCl_3$ was shaken with about 1 ml. of the color reagent. The absorption spectrum of the orange red $CHCl_3$ layer was drawn against the corresponding $CHCl_3$ layer prepared similarly from the color reagent.

The authors expess their gratitudes to Mr. M. Shidō and Mrs. S. Matsuba for micro analyses and to Mr. H. Matsui and Miss Y. Soeda for spectral measurements.

Part of the expenses of this work was defrayed by a Grant-in-Aid of Scientific Research for 1964 from the Ministry of Education, which is gratefully acknowledged.

Summary

3,3′-Bi[cholesta-2,4-diene], an oily mixture, cholesta-3,5-diene, cholesteryl chloride, and cholesteryl acetate were isolated from the reaction mixture of cholesterol with perchloric acid, phosphoric acid, and ferric chloride. The first three substances were shown to be responsible for the coloration. Ferric chloride played a considerably important role in the coloration.

(Received August 22, 1964)

[Chem. Pharm. Bull. 12(12) 1418~1424 (1964)]

UDC 547.853.07

193. Akira Takamizawa and Kentaro Hirai : Studies on the Pyrimidine Derivatives. XXXII.*1 Syntheses of N-Substituted Pyrimidine and Related Compounds. (1).*2

*(Shionogi Research Laboratory, Shionogi & Co., Ltd.*3)*

In previous papers*1,1) of this series, we have reported about the acid catalyzed condensation reaction of urea, N-methylurea, and N-phenylurea with enol ether ester (ethyl 2-methoxymethylene-3-ethoxypropionate (I)) or enol ether nitrile(2-methoxymethylene-3-ethoxypropionitrile (II)).

In order to extend the scope of this reaction, N-benzylurea and N-allylurea were made to react with I or II.

Reaction of I with N-benzylurea in ethanol solution in the presence of hydrochloric acid afforded a product of m.p. 117~118°, $C_{14}H_{16}O_3N_2$, which showed two spots on a thin-layer chromatogram (TLC).*4 The proton magnetic resonance (NMR)*5 spectrum of the

*1 Part XXXI. A. Takamizawa, K. Hirai : This Bulletin, **12**, 804 (1964).

*2 A part of this paper was presented at the 84th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1964.

*3 Sagisu, Fukushima-ku, Osaka (高見沢 映, 平井健太郎).

*4 TLC : Alumina plate, AcOEt solvent, detected by I_2 vapor.

*5 All NMR spectra were taken with a Varian A-60 spectrometer on about 10% solution in deuteriochloroform containing about 1% tetramethylsilane (TMS) as an internal reference.

1) A. Takamizawa, K. Hirai, Y. Sato, K. Tori : J. Org. Chem., **29**, 1740 (1964).