Autodxation of Cholesterol in Aqueous Colloidal Dispersions with Different Detergents

MICHIIYA KIMURA, MEIJI KAWADA, AND TAKUI SAWAYA

Faculty of Pharmaceutical Sciences, Hokkaido University

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Metal-free autodxation of cholesterol (I) was examined at 70°C for twenty hours in an aqueous colloidal dispersion which was made by sodium cholesteryl sulfate as a solubilizer and entirely freed of organic solvent. The products isolated and/or identified were cholest-5-ene-3β,7α-diol (II), cholest-5-ene-3β,7β-diol (III) (16.7% as the diols), 5α-cholestane-3β,5,6β-triol (IV) (8.3%), 5β-hydroxycholesterol-5-en-7-one (V) (14.5%), cholesta-5,7-dien-7-one (VI) (5.2%), and the unidentified compounds A (3.0%), B (1.4%), and C (0.7%) as shown in Table 1.

Autodxation of I was also observed in the colloidal systems dispersed by four kinds of solubilizer, sodium stearate, sodium taurocholate, sodium cholesteryl hemisuccinate, and the sulfate as shown in Fig. 1. Effects of the detergent in higher concentration were noticeable in the hemisuccinate dispersions.

It has been well recognized that the air oxidation of cholesterol (I) is greatly complicated. In the presence of air, I is also known to be unstable towards light, heat, and other radiation. The autodxation of I in aqueous colloidal dispersion has thus been the subject of a number of papers. Although monomolecular films of I are quite sensitive to air oxidation, all the dispersed forms are, in crystalline state, highly time-consuming to be autodxized at room temperature. Rapid oxidation, however, occurs when the colloidal dispersion of I is aerated at 85°C or on irradiation with ultra violet light. Much interest has been shown in the products and some of them have been identified as cholest-5-ene-3β,7α-diol (II), cholest-5-ene-3β,7β-diol (III), 5α-cholestane-3β,5,6β-triol (IV), 5β-hydroxycholesterol-5-en-7-one (V), cholestan-5,7-dien-7-one (VI), 3β,5-dihydroxy-5α-cholestan-6-one, and cholest-5-ene-3β,25-diol. Interest has also been shown in their possible physiological, pathological significance in relation to cell, atherosclerosis, toxins, to liver-microsomal oxidation, and to disordered conditions such as carcinoma, atherosclerosis, cholelithiasis,


2) Location: Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.


cerebrotenodinous xanthomatosi,109 and Wolman's disease,108 respectively. Antiatherogenic, hypcholesterolemic, and antilipemic action of the triol (IV) was found in a screening survey of cholesterol-related compounds which may modify lipid metabolism.106

During the course of studies of this series, it was found that the sulfate as well as hemisuccinate of I can be employed as solubilizing agent for the aqueous colloidal dispersion of I. The present paper deals with the metal-free autoxidation of cholesterol (I) in such aqueous dispersions which, contrary to the most of those reported, were entirely freed of organic solvent as in vivo.

Results and Discussion

Autoxidation of cholesterol (I) in a preparative scale was examined under the conditions employing cholesteryl sulfate as a solubilizer and giving the unknown product A described below, at 70° for twenty hours. The reaction mixture was submitted to column chromatography on silica gel and about half amounts of substrate (I) was found to be consumed as shown in Table I. The main products were the diols (II and III; 16.7% yield) and the ketone (V; 14.5%), though it was reported that autoxidation of I in sodium stearate-dispersion at 88° gave them in the yields of 20% and 44%, respectively.51 Although the presence of numerous products in minor amounts was noticed in thin-layer chromatography (TLC) of the fractions, the other products isolated were the triol (IV; 8.3%), the diene (VI; 5.2%), the unidentified compounds A (3.0%), B (1.4%), and C (0.7%). Chemical structure of the compound A is under investigation at present and will be reported in a subsequent paper.

The aqueous dispersions of I (5 x 10^{-6} M) were made by using four kinds of solubilizer—sodium stearate,5,7 sodium taurocholate,11 sodium cholesteryl hemisuccinate, and sodium cholesteryl sulfate—in different molar ratios to the substrate (I). After each dispersion was treated with molecular oxygen at 70° for twenty hours, the reaction mixture was submitted to TLC. Except the case of taurocholate, in which no autoxidation of I occurred in contrast to the results reported,89 the oxidation products detected were the diols (II and III), the triol (IV), the ketone (V), and the unidentified compound A which was formed solely under the conditions employing sodium cholesteryl sulfate in a 1:4 molar ratio to the substrate. Some of the reaction profiles are presented in Fig. 1, which were observed with reference to the effects of these different solubilizers in different concentrations on the formation of V and the consumption of I. The ratios of the product (V) to the remained substrate (I) were obtained from their peak areas in TLC and the amounts of the product(s) showing the light absorption at 240 nm were calculated tentatively as those of V. It seemed that the autoxidation of I is rather accelerated in the stearate-dispersion and is little dependent on the concentrations of stearate and cholesteryl sulfate. Effects of the solubilizer in higher concentration were noticeable in the cholesteryl hemisuccinate-dispensions as shown in Fig. 1 (b). It was reported that the saturated and monoenoic fatty acyl esters of I were almost completely resistant15,13 and the dienic as well as other polyunsaturated fatty acyl esters of I were more vulnerable to autoxidation in aqueous colloidal suspensions.13,14 Cholesteryl hemisuccinate remained almost intact under the conditions of the present experiments, though a few per cent of this solubilizer was found to be hydrolyzed to free I. Such a remarkable autoxidation of I dispersed by the hemisuccinate as described above is worth consideration and further studies are now in progress. Cholesteryl sulfate, several per cent of which was also found to be hydrolyzed into I, was not particularly accelerative and sodium taurocholate was inhibitory for the

TABLE I. Column Chromatography of Autoxidation Products from Cholesterol

<table>
<thead>
<tr>
<th>Solvent system (ml)</th>
<th>Fraction No.</th>
<th>Rf (TLC)</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene (3000)</td>
<td>1</td>
<td>0.69 (C)</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.64 (VI)</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.59 (B)</td>
<td>65</td>
</tr>
<tr>
<td>Benzene-ethyl acetate (1400:600)</td>
<td>4</td>
<td>0.55 (cholesterol)</td>
<td>2520</td>
</tr>
<tr>
<td>Benzene-ethyl acetate (800:1200)</td>
<td>5</td>
<td>0.51 (II and III)</td>
<td>771</td>
</tr>
<tr>
<td>Ethyl acetate (2000)</td>
<td>6</td>
<td>0.47 (V)</td>
<td>667</td>
</tr>
<tr>
<td>Ethyl acetate-ethanol (95:50)</td>
<td>7</td>
<td>0.25 (IV)</td>
<td>386</td>
</tr>
<tr>
<td>Ethyl acetate-ethanol (700:300)</td>
<td>8</td>
<td>0.13 (A)</td>
<td>140</td>
</tr>
<tr>
<td>Methanol (3000)</td>
<td>9</td>
<td>0.00</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.00</td>
<td>269</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.00</td>
<td>437</td>
</tr>
</tbody>
</table>


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Fig. 1. Effects of Detergent-Concentration

- a) $\text{O}$: sodium cholesterol sulfate
- \(\text{O}^{-}\): sodium cholesteryl hemisuccinate
- \(\text{O}^{-}\): sodium stearate
- b) $\text{O}$: sodium cholesterol sulfate
- $\text{O}^{-}\$: sodium cholesteryl hemisuccinate
- $\text{O}^{-}\$: sodium stearate

Autoxidation of I as mentioned above. Another detergents from biological origin, lecithins which compose the lipid of cholesterol-containing cell membranes, are also known to be effective inhibitor of cholesterol oxidation.\textsuperscript{15} Taurocholate micelles are enlarged by adding I to form the aggregate in a molar ratio of one guest compound to twenty five hosts.\textsuperscript{11} These results obtained in the present study might thus reflect the difference in the micellar structures formed in the different dispersions of I.

Although details of the fashion of autoxidation in these systems are not clear at present, it is of interest that the corresponding ketonic products such as cholestenones and V were, similarly to the results reported,\textsuperscript{5} not obtainable when the substrates were the secondary alcohols such as the 3-hydroxy- (I) and 7-hydroxy-steroids (II and III), respectively.

Experimental\textsuperscript{10}

Materials and Authentic Specimens—Cholesterol (I) and sodium stearate were of commercial sources and purified by recrystallization. Sodium taurocholate (mp 225—235\textdegree),\textsuperscript{15} sodium cholesteryl sulfate (mp 177—181\textdegree, lit.\textsuperscript{10} mp 165—166\textdegree), and sodium cholesteryl hemisuccinate (mp 145—147\textdegree),\textsuperscript{18} were prepared by the modified method. The authentic specimens of cholest-5-ene-3\textbeta,7\alpha-diol (II; mp 157—160\textdegree, lit.\textsuperscript{19} mp 157—160\textdegree).

\textsuperscript{16} Melting points were taken on a micro hot-stage apparatus and are uncorrected. Ultraviolet (UV) and infrared (IR) spectral measurements were run on Hitachi Model 3T recording and JASCO Model IR-S spectrometers, respectively. Nuclear magnetic resonance (NMR) and mass (MS) spectra were obtained by Hitachi Model R-20-B and Hitachi Model RMU-6R spectrometers, respectively. The peak areas in TLC were determined on IATRON Model TFG-10 Thinograph by using the solvent system of AcOEt-cyclohexane (1:3) and developing twice.
\textsuperscript{17} A. Norman, Arkiv för Chem., 8, 331 (1955).
188—189°, cholest-5-ene-3β,7β,7β-diol (III; mp 172—175°, lit.20 mp 172—176°/180—181°), 5α-cholestane-3β,5β,6β-triol (IV; mp 237—239°, lit.21 mp 236—238°), 3β-hydroxycholest-5-en-7-one (V; mp 164—165°, lit.22 mp 159—161°), and cholesta-3,5-dien-7-one (VI; mp 112—114°; lit.23 mp 111—112.5°) were prepared and purified as reported.

Preparation of Aqueous Dispersion——An ethanol solution (50 ml) of cholesterol (I; 1 × 10⁻⁸ mole, 387 mg) and the solubilizer (1 × 10⁻⁵, 2.5 × 10⁻⁴, 1 × 10⁻⁴, or 5 × 10⁻⁴ mole)—sodium stearate, sodium taurocholate, sodium cholesteryl sulfate, or sodium cholesteryl hemisuccinate—was mixed with water (20 ml) at 70° under stirring. After EtOH was evaporated out from the mixture in vacuo, water was added to the aqueous residue so that the whole volume was brought to 200 ml.

Autoxidation Reaction (Fig. 1)——Aqueous dispersion of I was thermostated at 70° ± 2° in a flask (500 ml), in which was installed an oxygen inlet tube ending in a bulb with fine holes. A slow stream of oxygen gas was allowed to pass for 20 hr through the tube into the contents of the flask, which were vigorously stirred. A portion (10 ml) of the reaction mixture was evaporated to dryness in vacuo and the residue was dissolved in EtOH (2 ml). A definite increment of volume of the EtOH solution was submitted to TLC which was developed three times with the same solvent system after drying at each end of run.

Autoxidation of I in a Preparative Scale (Table I)——The aqueous dispersion containing I (356 mg), sodium cholesteryl sulfate (100 mg), and water (200 ml) was made and aerated as described above. In the same manner, I in total amounts of 4.63 g was autoxidized in the dispersion containing 1.30 g of the solubilizer. The combined reaction mixture was evaporated into dryness in vacuo and the residue was dissolved in the mixture of MeOH (50 ml) and acetone (50 ml). Small amounts of silica gel was added to this solution which was then evaporated again into dryness in vacuo. After the residue was put on the top of the column (3.5 × 50 cm) which was packed with silica gel (180 g) and n-hexane, it was chromatographed by using the solvent system as shown in Table I. The diols (771 mg) from the fraction No. 5 were rechromatographed on silica gel (48 g) by developing with the solvent system of AcOEt—benzene (15: 85) and separated into II (80 mg) and III (317 mg). The other products, IV, V, and VI, were isolated from the fractions Nos. 7, 6, and 2, respectively and purified by the ordinary manner. Their melting points and spectral data such as UV, IR, NMR, and/or MS were in fair agreement with those of the authentic specimens.

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