[Chem. Pharm. Bull. 26(8)2386—2390(1978)]

UDC 547.932.04 : 547.924.04

Synthesis of 1α,24(*R*)- and 1α,24(*S*)-Dihydroxycholesterol from Cholic Acid Derivatives[1]

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(Received January 18, 1978)

From 5α-cholestane-3α,24(*R*)- or -3α,24(*S*)-diol (**2a** and **2b**) obtained from lithocholic acid in the previous work, both 24-epimers of 1α,24-dihydroxycholesterol (**6a** and **6b**) were synthesized. Thus, syntheses of these compounds were accomplished by dehydrogenation of the diols with dichlorodicyanobenzoquinone followed by the 1α-hydroxylation of the resulted 1,4-dien-3-ones (**3a** and **3b**) by the three-step procedure originally uncovered by one of the present authors (C. K.). The last step in the above transformation, the modified procedure reported recently by Ikekawa's group: oxymercuration–demercuration, was used.

An alternative preparation of 1α,24ξ-dihydroxycholesterol (**6**) either from lithocholic acid or from hyodeoxycholic acid was also described.

Keywords——1α,24-dihydroxycholesterol; 1α,24-dihydroxycholecalciferol; vitamin D_3; lithocholic acid; hyodeoxycholic acid; oxymercuration–demercuration

1α,24(*R*)- and 1α,24(*S*)-Dihydroxycholecalciferols are the interesting artificial polar analogues of active vitamin D_3, having significant biological activity. These two compounds were synthesized for the first time by Morisaki *et al.*[3] from 24-oxocholesterol obtained from fucosterol *via* 1α,24-dihydroxycholesterol as a key intermediate, utilizing the 1α-hydroxylation procedure of Barton *et al.*[4] Separation of the two 24-isomers of the dihydroxycholesterol was successfully carried out by chromatography of its 3,24-dibenzoate. In the previous paper of this series,[5] we also synthesized these two 1α,24-dihydroxycholecalciferol epimers from the corresponding 24-hydroxycholesta-1,4,6-trien-3-one epimers by the use of the 1α-hydroxylation procedure of Kaneko, *et al.* reported in 1974,[6] and found that the effective separation of the two 24-isomers could be achieved by chromatographic separation of a diastereomeric mixture of 5β-cholestane-3α,24-diol (readily available from lithocholic acid).[7] However, since the preparation of the trienones used in our syntheses from two C-24 epimers of 5β-cholestane-3α,24-diol required two successive dehydrogenation procedures in which the yields of the trienones from dienones were not satisfactory (*ca.* 30%), some alternative

1) This forms Part IV of "Synthetic Studies of Vitamin D_3 Analogues from Bile Acids." For Part III, see ref. 5). A part of this work was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April, 1976.

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3) *a*) M. Morisaki, N. Koizumi, N. Ikekawa, T. Takeshita, and S. Ishimoto, *J. Chem. Soc. Perkin I*, **1975**, 1421; *b*) N. Koizumi, M. Morisaki, N. Ikekawa, A. Suzuki, and T. Takeshita, *Tetrahedron Lett.*, **1975**, 2203; *c*) H. Kawashima, K. Hoshina, S. Ishizuka, M. Morisaki, and H. Orimo, The Third Workshop on Vitamin D, Abstract California, 1977, p. 143.

4) *a*) D.H.R. Barton, R.H. Hesse, M.M. Pechet, and E. Rizzardo, *J. Am. Chem. Soc.*, **95**, 2748 (1973); *b*) *J. Chem. Soc. Chem. Commun.*, **1974**, 203.

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6) C. Kaneko, A. Sugimoto, Y. Eguchi, S. Yamada, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron*, **30**, 2701 (1974).

7) K. Ochi, I. Matsunaga, M. Shindo, and C. Kaneko, *J. Chem. Soc. Perkin I*, **1978**, in press.

routes which skip out the trienones in the reaction sequence seem to be attractive. The efforts along this line now led us find a new preparation method of 1α,24-dihydroxycholesterol epimers, the key intermediates in the synthesis of two 24-epimers of 1α,24-dihydroxycholecalciferol.

As described in the previous paper, the dehydrogenation of 5β-cholestane-3α,24(*R*)- or -3α,24(*S*)-diol (**2a,2b**) derived from lithocholic acid afforded the corresponding 24-hydroxycholesta-1,4-dien-3-ones (**3a,3b**) in *ca.* 40% yields. The conversion of these dienes to the respective 1α,24-dihydroxycholesterol epimers (**6a** and **6b**) was achieved essentially in the same manner with the 1α-hydroxylation procedure of Kaneko, *et al.* reported in 1973.[8] Their procedure consists of three steps starting from 3-oxo-$\Delta^{1,4}$-steroids available readily from 3-oxygenated steroids: 1) deconjugation to 3-oxo-$\Delta^{1,5}$-steroids, 2) reduction to 3β-hydroxy-$\Delta^{1,5}$-steroids, and 3) hydration to 1α,3β-dihydroxy-Δ^{5}-steroids. Quite recently, Morisaki, *et al.* synthesized 1α-hydroxycholesterol essentially by this hydroxylation procedure using oxymercuration–demercuration in the final step[9] and noted that not only 2β-hydroxycholesterol was formed as the major by-product,[10] but also an inertness of the Δ^{5}-function to this procedure. Originally, Kaneko, *et al.*[8] employed hydroboration-oxidation in the final step in this general procedure and noted that under the use of *ca.* 0.8 mol equivalent of diborane 2α-hydroxylated products were formed, together with the recovered material, as the major by-products whose amounts exceeded slightly those of the 1α-hydroxylated products, and the double bond at the 5-position was also hydroxylated when an excess amount of di-

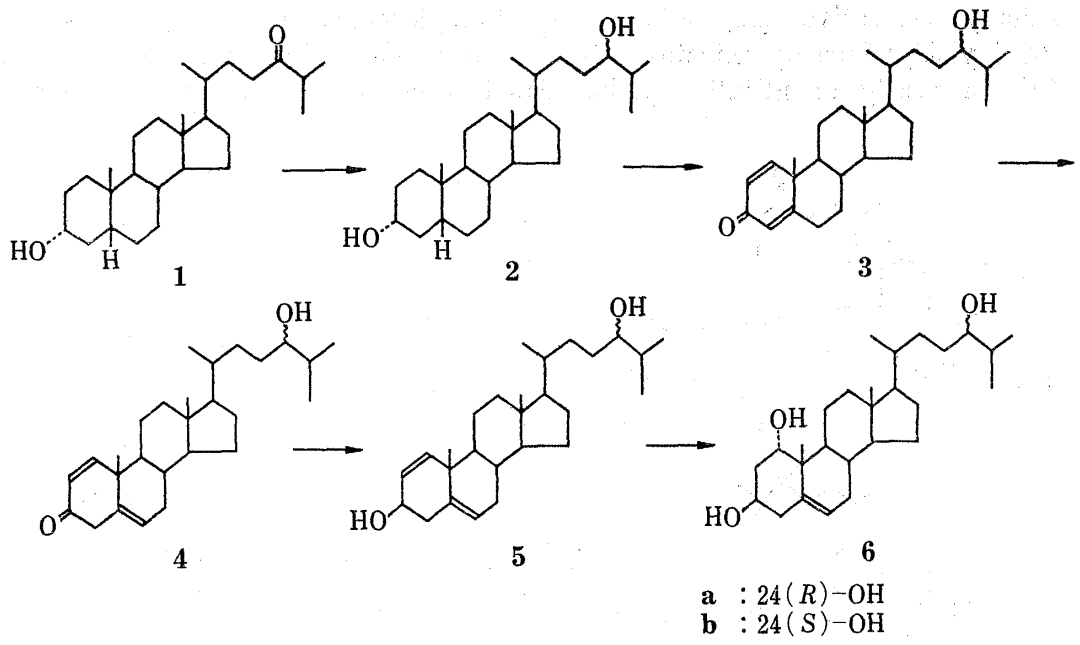

Chart 1

8) *a*) C. Kaneko, S. Yamada, A. Sugimoto, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron Lett.*, **1973**, 2339; *b*) C. Kaneko, A. Sugimoto, S. Yamada, M. Ishikawa, S. Sasaki, and T. Suda, *Chem. Pharm. Bull.* (Tokyo), **22**, 2101 (1974).

9) M. Morisaki, K. Bannai, and N. Ikekawa, *Chem. Pharm. Bull.* (Tokyo), **24**, 1948 (1976).

10) Though more thorough experiments are needed to clarify this regio-selective 2β-hydroxylation, one reasonable explanation is to assume a complex formation (at least partly) of mercuric trifluoroacetate with the 3β-hydroxy (or 3β-acetoxy[9]) group. In accordance with this tentative explanation, the methylenation of 1,5-diene-3β-hydroxy-steroids with iodomethylzinc iodide and the related reagents proceed through preferential β-face attack of the reagent to give 1β,2β-methylene compounds. The complex formation of the reagents with the hydroxy group followed by intramolecular transfer of methylene has been suggested; *a*) E.P. Blanchard and H.E. Simmons, *J. Am. Chem. Soc.*, **86**, 1337 (1964); *b*) H.E. Simmons, E.P. Blanchard, and R.D. Smith, *ibid.*, **86**, 1347 (1964).

borane was used. Since we also noticed that a double bond at the 5-position was intact to mercuric trifluoroacetate,[7] we employed this hydration reaction in the last step in the present synthesis.

Thus, these dienones (**3a** and **3b**) were deconjugated by treatment with potassium *t*-butoxide in dimethylsulfoxide followed by addition into ice water to give 24(*R*)- and 24(*S*)-hydroxycholesta-1,5-dien-3-ones (**4a**; mp 113—116° and **4b**; mp 96—97°) in *ca.* 80% yields, respectively. The deconjugated ketones were then reduced with calcium borohydride to give 24(*R*)- and 24(*S*)-hydroxycholesta-1,5-dien-3β-ols (**5a**; mp 179—181° and **5b**; mp 197—200°) in almost quantitative yields, respectively. As the final step, oxymercuration–demercuration to these dienediols led the desired 1α-hydroxylated products (**6a**; mp 93—94.5° and **6b**: mp 89—91°) in *ca.* 25% yields.

The syntheses of 1α,24(*R*)- and 1α,24(*S*)-dihydroxycholesterols described as above constitute the formal synthesis of 1α,24-dihydroxycholesterol (**6**). We have also accomplished an alternative synthesis of this compound from both lithocholic acid or hyodeoxycholic acid. Thus, 3α-hydroxy-5β-cholestane-24-one (**1**), the precursor of **2** used in the above study (obtainable readily from lithocholic acid) was dehydrogenated with dichlorodicyanobenzoquinone (DDQ) in refluxed dioxane to give the 1,4-dien-3-one (**9**), mp 142—144°. The same dienone was also obtained from 24-oxocholesterol derived from hyodeoxycholic acid by Oppenauer oxidation followed by dehydrogenation with DDQ in refluxed benzene. Since the deconjugation procedure necessarily use a strong basic condition, the ketone function in **9** was protected by its conversion to the ethylene ketal function. Deconjugation of the 24,24-ethylenedioxy derivative (**10**) afforded 24,24-ethylenedioxycholesta-1,5-diene-3,24-dione (**11**) in 60% yield, which by reduction with sodium borohydride to give the 3β-ol (**12**), mp 151—154°, in nearly quantitative yield. Application of oxymercuration–demercuration

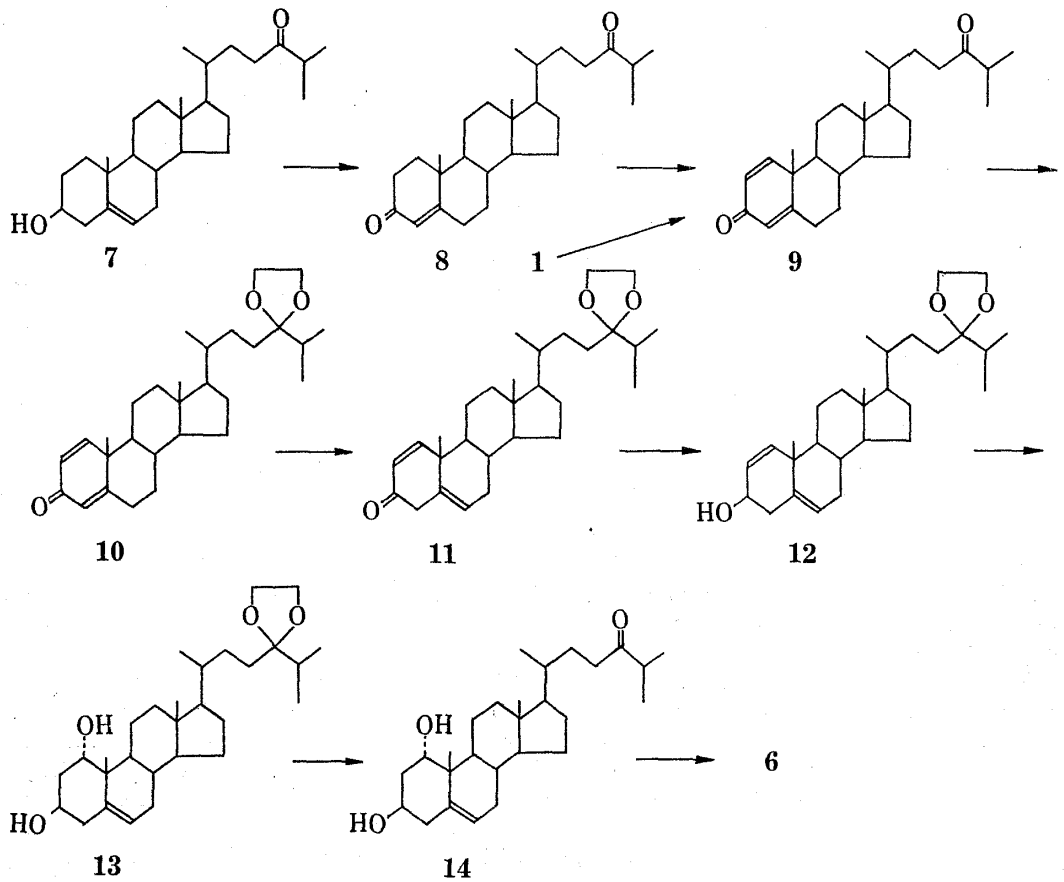

Chart 2

procedure as above[11] afforded 24,24-ethylenedioxycholest-5-ene-1α,3β-diol (**13**), mp 167—169°, as the major hydroxylation product,[12] which by acid-catalyzed hydrolysis to give cholest-5-ene-1α,3β-diol-24-one (**14**), mp 148—149°. Reduction with sodium borohydride then afforded a diastereomeric mixture of **6**, mp 97—104°.

Thus, it is now shown that 24(*R*)-, 24(*S*)-, and 24(ξ)-hydroxylated 1α-hydroxycholesterols can be prepared efficiently from either lithocholic acid or from hyodeoxycholic acid. The ready availability of these bile acids makes possible the preparation of these and the related analogues in quantity. The two C-24 epimers of 1α,24-dihydroxycholesterols were successfully converted to the corresponding 1α,24-dihydroxy-7-dehydrocholesterols *via* the usual four step procedures: 1) acetylation, 2) bromination, 3) dehydrobromination, and 4) hydrolysis, and each of 5,7-dienes was identified by mixture melting point determination with the sample obtained in our previous work.

Experimental[13]

Preparation of Cholesta-1,4-diene-3,24-dione (9)——a) To the solution of **1** (9.29 g) in dry dioxane (173 ml), DDQ (17.3 g) was added and the mixture was refluxed for 20 hr. After removal of the precipitate by filtration, the filtrate was evaporated and the residue was chromatographed on the short column of alumina. Elution with $CHCl_3$ afforded 3.39 g of **9**, mp 142—144° (from EtOH), IR ν_{max}^{KBr} cm^{-1}: 1711 (C=O at C-24), 1663 (C=O at C-3), 1622, 1601 (C=C). UV λ_{max}^{EtOH} nm: 243. NMR ($CDCl_3$) δ: 0.73, 1.02, 1.13, 1.22 (3H each, s), 6.09 (1H, m), 6.18 (1H, d, J=10), 7.05 (1H, d, J=10).

b) To the refluxed solution of cholest-5-en-3β-ol-24-one (**7**) (1.0 g) in dry toluene (100 ml), aluminum isopropoxide (1 g) in dry toluene (20 ml) was added dropwise through a dropping funnel for 30 min. Refluxing was continued for 4 hr while evaporating 80 ml of toluene. The reaction mixture was extracted with ether. The extract was washed with dil. HCl and then H_2O, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel ($CHCl_3$) to afford 763.9 mg of cholest-4-ene-3,24-dione (**8**). NMR ($CDCl_3$) δ: 0.72, 1.02, 1.13, 1.19 (3H each, s), 5.74 (1H, br. s). To the solution of **8** (321.5 mg), DDQ (220 mg) was added and the mixture was refluxed for 17 hr. After removal of the precipitate by filtration, the filtrate was evaporated and the residue was purified by column chromatography using a short column of alumina. Elution with $CHCl_3$ afforded 247.3 mg of **9**, identical (IR, NMR, UV, and mixed mp) with the foregoing sample.

24,24-Ethylenedioxycholesta-1,4-diene-3,24-dione (10)——To the solution of **9** (1.73 g) in dry benzene (50 ml), ethylene glycol (0.71 ml) and *p*-toluenesulfonic acid (28 mg) was added and the mixture was refluxed for 5 hr while removing H_2O resulted as an azeotropic mixture with water separator. The reaction mixture was washed with aq. $NaHCO_3$ and then H_2O, dried over $MgSO_4$, and evaporated, yielding 1.7 g of **10** as an oil. NMR ($CDCl_3$) δ: 0.72, 0.85, 0.96, 1.21 (3H each, s), 3.91 (4H, s, –O–C$\underline{H}_2$–C$\underline{H}_2$–O–), 6.08 (1H, m), 6.21 (1H, d, J=10), 7.05 (1H, d, J=10).

24,24-Ethylenedioxycholesta-1,5-diene-3,24-dione (11)——To the solution of **10** (1.7 g) in dry DMSO (30 ml), *t*-BuOK (1.0 g) (prepared freshly from K metal and *t*-BuOH) was added and the mixture was stirred vigorously for 1 hr at 20° under argon atmosphere. The reaction mixture was poured into ice water contained acetic acid 9 ml) and the whole was extracted with ethyl acetate. The extract was washed with H_2O, dried, and evaporated. The residue was chromatographed on silica gel to give a colorless solid. Recrystallization from MeOH afforded 1.015 g of pure **11**, mp 136—138°. IR ν_{max}^{KBr} cm^{-1}: 1690 (C=O at C-3). NMR ($CDCl_3$) δ: 0.72, 0.88, 0.98, 1.22 (3H each, s), 3.94 (4H, s, –O–C$\underline{H}_2$–C$\underline{H}_2$–O–), 5.45(1H, m), 5.89 (1H, d, J=10), 7.01 (1H, d, J=10).

24,24-Ethylenedioxycholesta-1,5-dien-3β-ol-24-one (12)——To the solution of **11** (2.45 g) in MeOH (120 ml), $NaBH_4$ (1.15 g) was added portionwise at 0°. After 1 hr, the excess reagent was decomposed with aq. acetic acid and the mixture was evaporated and the residue was extracted with ether. The extract was washed with H_2O, dried, and evaporated to give a colorless solid. Recrystallization from MeOH afforded

11) While Morisaki *et al.* used 3-acetate for this reaction, we used the unprotected 3-ols (**5a**, **5b**, and **12**). It should be noted that excess of mercuric oxide (yellow) must be used as an acceptor of the generated trifluoroacetic acid in the oxymercuration of **12**. Otherwise, a hydrolytic removal of the ethylenedioxy function resulted.

12) In this case, 2β-hydroxy derivative; mp 147—150°, was formed as the major by-product.

13) Melting points are uncorrected. IR spectra were obtained with a Hitachi 285 spectrophotometer and NMR spectra with a Hitachi Perkin-Elmer R-20A spectrometer using TMS as internal standard. UV spectra were taken with a Hitachi 124 spectrophotometer.

2.1 g of **12**, mp 151—154°. NMR ($CDCl_3$) δ: 0.70, 0.87, 0.98, 1.09 (3H each, s), 3.92 (4H, s), 4.22 (1H, s), 5.42 (1H, m), 5.54 (1H, d, $J=10$), 5.79 (1H, d, $J=10$).

24,24-Ethylenedioxycholest-5-ene-1α,3β-diol-24-one (13)——To the solution of **12** (447.9 mg) in THF (1.4 ml) and DMF (1.4 ml), mercuric trifluoroacetate (227.8 mg) in H_2O (1.4 ml) and yellow HgO (140.9 mg) were added. The mixture was stirred for 7 hr at 20°. To the reaction mixture, 3 N NaOH (2 ml) and followed $NaBH_4$ (280 mg) were added and the whole was stirred for 1 hr. After removal of the precipitate by filtration, the filtrate was extracted with ethyl acetate. The extract was washed with dil. HCl, aq. $NaHCO_3$, and then H_2O, dried, and evaporated. The residue was chromatographed on alumina. Elution with $CHCl_3$ afforded 112 mg of **13**, mp 167—169° (from MeOH). NMR ($CDCl_3$) δ: 0.69, 0.89, 0.98, 1.01 (3H each, s), 3.7—4.1 (2H, m), 3.90 (4H, s), 5.50 (1H, m). Further elution with the same solvent afforded 32 mg of 24,24-ethylenedioxycholest-5-ene-2β,3β-diol-24-one, mp 147—150°.

Cholest-5-ene-1α,3β-diol-24-one (14)——To the solution of **13** (60 mg) in MeOH (6 ml), 5% HCl (0.6 ml) was added and the mixture was stirred for 1 hr at 50°. The reaction mixture was extracted with ether. The extract was washed with aq. $NaHCO_3$ and then H_2O, dried, and evaporated to give a colorless solid. Recrystallization from acetone afforded 50 mg of **14**, mp 148—149°. IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1698 (C=O). NMR ($CDCl_3$) δ: 0.69, 1.05 (3H each, s), 1.02 (6H, s), 3.83 (2H, m), 5.58 (1H, m).

Cholesta-1,5-dien-24(*R*)-ol-3-one (4a) and Cholesta-1,5-dien-24(*S*)-ol-3-one (4b)——The same treatment of **3a** (113.6 mg) with *t*-BuOK and the usual work-up gave 86 mg of the corresponding **4a**, mp 113—116° (from ether–hexane). Similarly, **4b** (mp 96—97°) was also obtained from **3b**. The spectra (NMR and IR) of the both isomers (**4a**, **4b**) are practically indistinguishable. IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1687 (C=O). NMR ($CDCl_3$) δ: 0.73, 0.87, 0.97, 1.21 (3H each, s), 3.27 (1H, m), 5.45 (1H, m), 5.88 (1H, d, $J=10$), 7.10 (1H, d, $J=10$).

Cholesta-1,5-diene-3β,24(*R*)-diol (5a) and Cholesta-1,5-diene-3β,24(*S*)-diol (5b)——Reduction of **4a** (0.6 g) with $NaBH_4$ as in **12** afforded 447.3 mg of **5a**, mp 179—181° (from $CHCl_3$–MeOH). Similarly **5b** (mp 197—200°) was obtained also from **4b**. The spectra (NMR and IR) of the both isomers (**5a**, **5b**) were practically indistinguishable. NMR (50% $CDCl_3$–CD_3OD) δ: 0.72, 0.85, 0.95, 1.10 (3H each, s), 5.40 (1H, m), 5.51 (1H, d, $J=10$), 5.80 (1H, d, $J=10$).

Preparation of Cholest-5-ene-1α,3β,24(*R*)-triol (6a) and Cholest-5-ene-1α,3β,24(*S*)-triol (6b)——a) To the solution of **14** (162 mg) in MeOH (10 ml), $NaBH_4$ (81 mg) was added portionwise and the mixture was stirred for 1 hr at 0°. After decomposition of the excess reagent with aq. acetic acid, the reaction mixture was evaporated and the residue was extracted with ether. The extract was washed with H_2O, dried, and evaporated to give a colorless solid. Recrystallization from acetone–petr. ether afforded 160 mg of the triol (1 : 1 mixtures of **6a** and **6b**), mp 97—104°. NMR ($CDCl_3$) δ: 0.69, 0.85, 0.96, 1.01 (3H each, s), 3.30 (1H, m), 3.82 (2H, m), 5.55 (1H, m). MS *m/e*: 418 (M^+), 400, 385, 382.

b) Oxymercuration-demercuration of **5a** and **5b** as in the case of **13** afforded **6a** (mp 93—94.5°) and **6b** (mp 89—91°) as colorless crystals (from acetone petr. ether) respectively. The spectra (IR and NMR) of the both isomers (**6a**, **6b**) were identical with the foregoing sample.