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Studies on the Constituents of Pollen. X. On the Constituents of Pollen Grains of *Ambrosia elatior* Linné (2)

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The new sterols 4α,14-dimethyl-9,19-cyclocholestan-3β,24ξ-diol (IIIa) and 4α,14-dimethyl-9,19-cyclocholestan-3β,24ξ,25-triol (IVa) were isolated, together with lophenone, lophenol and cholest-7-en-3β-ol, from the pollen grains of *Ambrosia elatior* Linné. The structures were elucidated on the basis of spectroscopic studies and chemical evidence.

Keywords——*Ambrosia elatior* Linné; Compositae; pollen grains; lophenone; lophenol; cholest-7-en-3β-ol; 4α,14-dimethyl-9,19-cyclocholestan-3β,24ξ-diol; 4α,14-dimethyl-9,19-cyclocholestan-3β,24ξ,25-triol

Ambrosia elatior Linné, commonly known as short ragweed, is an allergenic plant, and many studies have been reported on allergy due to *A. elatior*.[1,2]

Heyl[3] and Inayama,[1] with their colleagues, reported the presence of flavonoids including quercetin glucoside and sesquiterpenes such as ambrosic acid in the pollen grains of *A. elatior*. In part II of this series,[4] we reported the isolation of triterpenoids such as α- and β-amyrin, and sterols such as campesterol and β-sitosterol, and others.

The prestent paper deals with the isolation of five sterols from pollen grains of *A. elatior*.

The constituents of the pollen grains were extracted with ether, and the extract was fractionated into acidic, phenolic and neutral fractions. Column chromatography of the neutral fraction gave five compounds (Ia, Ic, IIa, IIIa and IVa), whose structures were elucidated on the basis of spectroscopic data and chemical evidence.

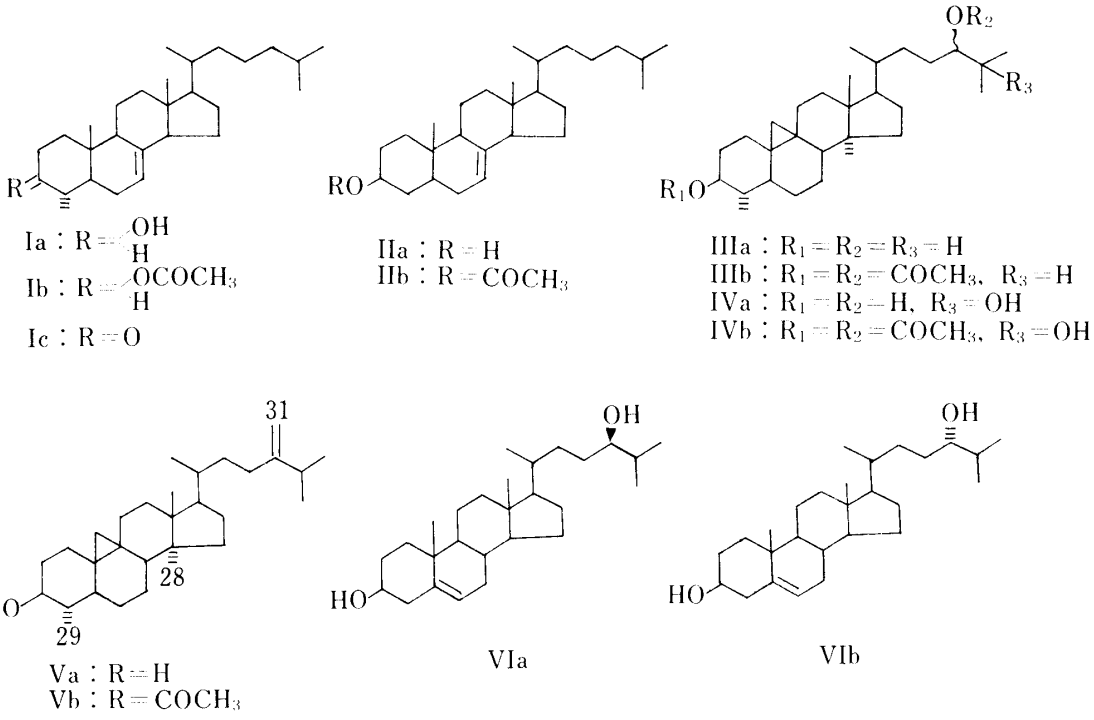

Chart 1

Compound Ia was obtained as colorless needles of mp 148—149 °C with a positive Liebermann–Burchard reaction. The molecular formula $C_{28}H_{48}O$ was established by high resolution mass spectrometry. The infrared (IR) spectrum showed absorptions due to a hydroxyl group and a double bond, while the proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR) and mass (MS) spectra were consistent with those of lophenol. Therefore, compound Ia was acetylated by the conventional method to give an acetate Ib as a white powder, mp 112—113 °C. The acetate Ib was identified as lophenyl acetate by mixed melting point determination and comparisons of TLC behavior and IR spectra with those of an authentic sample. Compound Ia was thus proved to be lophenol.[5]

Compound Ic was obtained as colorless plates of mp 119—120 °C with a positive Liebermann–Burchard reaction. Elemental analysis and MS of Ic showed its composition to be $C_{28}H_{46}O$. The IR spectrum showed absorptions due to a six-membered ring ketone at 1720 cm^{-1} and a double bond at 1630 cm^{-1}, while exhibiting no absorption due to a hydroxyl group. The ^{1}H-NMR spectrum of Ic exhibited signals due to six methyl protons at δ 0.56—0.98, and a 1H multiplet at δ 5.19 due to a trisubstituted double bond. In addition, comparison of ^{1}H- and ^{13}C-NMR spectra and MS revealed a close resemblance between Ic and Ia.

From these spectral correlations, Ic was presumed to be lophenone.[6] The identity of Ic as lophenone was proved by mixed melting point determination with an oxidized product of Ia, obtained by the conventional method with chromium trioxide as the oxidizing agent, and by comparison of IR spectra and TLC behavior.

Compound IIa was obtained as colorless needles of mp 121 °C with a positive Liebermann–Burchard reaction, and its formula, $C_{27}H_{47}O$, was confirmed by high resolution MS. Both the IR and ^{1}H-NMR[7] spectra revealed that IIa contained a secondary hydroxyl group (3400 cm^{-1}; δ 3.59), a trisubstituted double bond (1620 cm^{-1}; δ 5.15), and five methyl groups (δ 0.54—0.92). The MS and ^{13}C-NMR[8] spectra showed the formation of fragment ions m/z 161, 147 and 93 by retro-Diels–Alder cleavage in ring B, and signals at δ 117.5 (d) and 139.7 (s) indicated that IIa had a trisubstituted double bond at C-7.

Acetylation of IIa by the conventional method gave an acetate (IIb) as colorless needles, mp 94 °C. The ^{1}H-NMR spectrum of IIb exhibited an acetylmethyl signal at δ 2.00, indicating the formation of a monoacetate. The 1H multiplet at δ 3.59 and 4.70 for the methine proton of free hydroxyl and acetyl derivatives, respectively, is characteristic of the C-3 position when a 3β-hydroxyl group is present.[9]

From comparison of the IR, ^{1}H- and ^{13}C-NMR spectra of IIa and IIb with known data, compound IIa was identified as cholest-7-en-3β-ol.

Compound IIIa was obtained as colorless plates of mp 146—148 °C with a positive Liebermann–Burchard reaction, and high resolution MS gave the molecular formula $C_{29}H_{50}O_2$. Its IR spectrum showed absorption due to a hydroxyl group at 3260 cm^{-1}. The ^{1}H-NMR spectrum of IIIa exhibited signals due to six methyl protons at δ 0.87—1.25, an AB system centered at δ 0.13 and 0.40 due to cyclopropyl methylene group, and a methine signal at δ 3.26 due to two secondary alcohols.

MS showed the molecular ion peak (M^+) at m/z 430, with fragment ions of M^+-H_2O at m/z 412, and M^+-2H_2O–CH_3 at m/z 379. Further fragment peaks of ion **a** at m/z 304 and ion **b** at m/z 175 in IIIa were characteristic peaks, from which the steroidal skeleton was considered to have the same structure as cycloartenol[10] (Chart 2). Furthermore, the ^{13}C-NMR spectrum of IIIa coincided with that of Va except for the side chain.[11]

Acetylation of IIIa by the conventional method gave an acetate IIIb as colorless needles, mp 146—148 °C. The ^{1}H-NMR spectrum of IIIb exhibited a 6H singlet at δ 2.06 due to two acetylmethyl groups, indicating the formation of a diacetate. One of the hydroxyl groups was considered to be at C-3 on the basis of biogenetic considerations. Its configuration was inferred to be β from the width of the coupling constant of the methine proton, $W_{1/2}=14$ Hz, and by comparison of its ^{13}C-NMR spectrum with that of cycloeucalenyl acetate (Vb). The

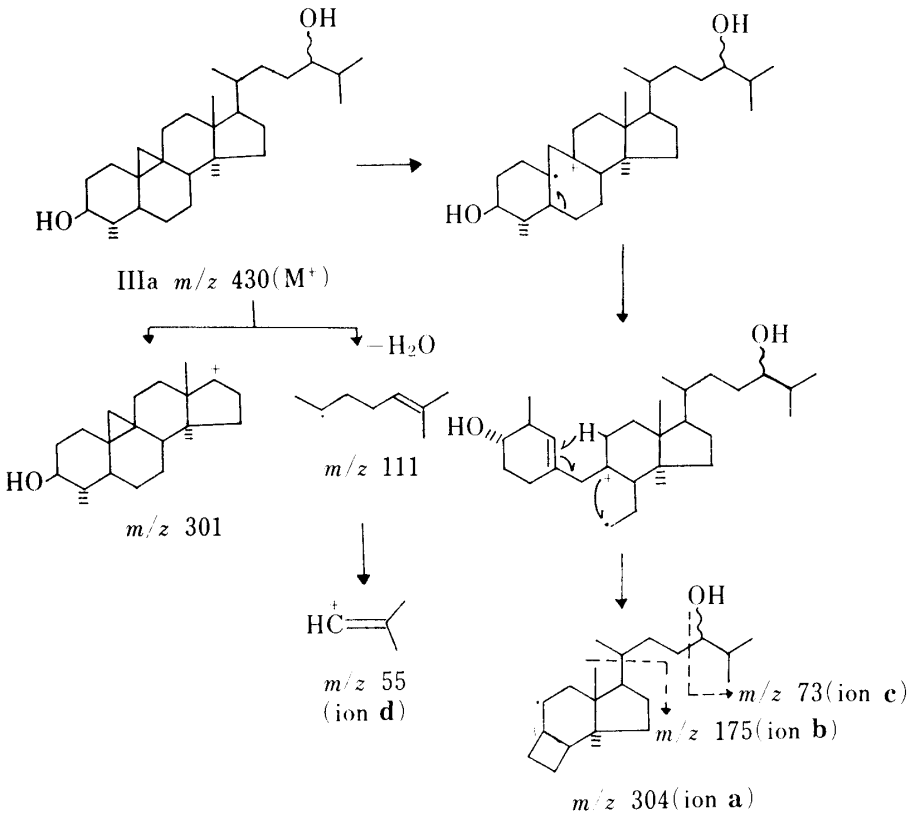

Chart 2

other hydroxyl group was inferred to be located at C-24 from the presence of fragment ions at *m/z* 73 (ion **c**) and *m/z* 55 (ion **d**) (Chart 2).

^{13}C-NMR chemical shift values for the side chain of IIIa were compared with those for the side chain of 24-hydroxy-cholesterol (VIa, VIb)[12] (Table II). Koizumi *et al.*[12] reported that characteristic differences between 24-*R* and 24-*S* isomers were apparent in both the C-20 and C-24 resonances, and those of 24-*R* compounds were always observed at higher field than those of 24-*S* isomers. In the spectra (Table II), the signals of C-24 in IIIa were observed at lower field than those of Va. Thus, the configuration at C-24 seemed to be *S*, but this could not be confirmed because of the absence of the C-24 epimeric compound. Consequently, the structure of compound IIIa was identified as 4α,14-dimethyl-9,19-cyclocholestan-3β,24ξ-diol.

Compound IVa was obtained as a white powder of mp 140 °C with a positive Liebermann–Burchard reaction. High resolution MS gave the molecular formula $C_{29}H_{50}O_3$. Its IR spectrum showed absorption due to a hydroxyl group at 3400 cm^{-1}. The ^{1}H-NMR spectrum of IVa exhibited signals due to six methyl protons at δ 0.86—1.21, an AB system centered at δ 0.13 and 0.40 due to a cyclopropyl methylene group, and a methine signal at δ 3.22 due to two secondary alcohols. The ^{13}C-NMR spectrum of IVa is in accordance with that of IIIa except that it showed a tertiary alcohol carbon (Table I). Similar fragmentation patterns were observed in the MS of IVa, which was compared with that of IIIa (Chart 3). High resolution MS suggests that IVa has one more hydroxyl group than IIIa.

Acetylation of IVa by the conventional method gave the acetate (IVb) as a white powder, mp 145—146 °C. The ^{1}H-NMR spectrum of IVb exhibited two 3H singlet signals, one at δ 2.04 and the other at β 2.09, indicating the formation of a diacetate. Its IR spectrum showed absorption due to a hydroxyl group at 3500 cm^{-1}. Furthermore, the ^{13}C-NMR spectrum showed a singlet signal at δ 72.5. From these findings, it is clear that one of the three hydroxyl groups is a tertiary hydroxyl group. A secondary hydroxyl group was located at C-3, and its configuration was estimated to be β by comparison of its ^{13}C-NMR spectrum with that of IIIb. The presence

Table I. ^{13}C-NMR Spectral Data for Sterols

Carbon	Va[a]	Vb[a]	IIIa	IIIb	IVa	IVb
C-1	30.7	30.4	30.9	30.5	30.8	30.5
C-2	34.8	30.9	34.9	31.0	34.9	30.9
C-3	76.3	78.5	76.6	78.8	76.5	78.7
C-4	44.5	41.4	44.6	41.6	41.6	41.5
C-5	43.2	43.3	43.4	43.5	43.4	43.4
C-6	24.6	24.6	24.7	24.7	24.7	24.7
C-7	28.0	28.0	28.1	28.0	28.1	28.0
C-8	46.7	46.7	46.9	47.0	46.9	46.9
C-9	23.5	23.6	23.5	23.9	23.5	23.6
C-10	29.5	29.3	29.6	29.4	29.4	29.4
C-11	25.1	24.9	25.2	25.1	25.1	25.0
C-12	35.2	35.3	35.4	35.4	35.4	35.3
C-13	45.2	45.2	45.4	45.4	45.4	45.3
C-14	48.7	48.7	49.0	49.0	48.9	48.9
C-15	32.8	32.8	32.5	32.9	32.9	32.8
C-16	26.9	26.9	27.0	27.0	27.0	26.7
C-17	52.0	52.0	52.2	52.1	52.3	52.1
C-18	17.7	17.7	17.8	17.8	17.9	17.8
C-19	26.9	26.9	27.3	27.0	27.2	26.9
C-20	36.0	36.0	36.4	36.2	36.4	36.3
C-21	18.3	18.3	18.5	18.4	18.5	18.5
C-22	35.0	35.0	32.9	31.9	33.6	32.8
C-23	31.3	31.3	31.1	28.0	28.8	26.4
C-24	156.2	156.1	77.5	79.2	79.6	80.9
C-25	33.7	33.7	32.9	29.7	73.1	72.5
C-26	21.8	21.9	16.7	17.4	23.3	25.0
C-27	21.8	21.9	19.1	18.8	26.5	25.0
C-28	19.1	19.1	19.1	19.2	19.1	19.1
C-29	14.4	14.4	14.4	14.4	14.4	14.4
C-30	—	—	—	—	—	—
C-31	105.6	105.6	—	—	—	—
3-$\underline{C}OCH_3$	—	170.1	—	170.0	—	170.9
3-CO$\underline{C}H_3$	—	21.2	—	21.3	—	21.3
24-$\underline{C}OCH_3$	—	—	—	170.0	—	171.3
24-CO$\underline{C}H_3$	—	—	—	21.2	—	21.1

a) Data taken from ref. 11).

Table II. ^{13}C-NMR Spectral Dara for Side Chains of Sterols

Carbon	C-20	21	22	23	24	25	26	27
24-*R* (VIa)[a]	35.6	18.8	31.9	30.4	76.6	33.4	17.2	18.6
24-*S* (VIb)[a]	35.8	19.0	32.1	30.6	77.0	33.0	16.7	18.8
IIIa	36.4	18.5	32.9	31.1	77.5	32.9	16.7	19.1

a) Data taken from ref. 12).

of another hydroxyl group was indicated as follows: MS showed fragment ions at *m/z* 301 ($C_{21}H_{33}O$) due to loss of the side chain, and at *m/z* 127 ($C_8H_{15}O$) due to loss of H_2O in the side chain, from which the side chain was inferred to possess secondary and tertiary hydroxyl groups. The tertiary hydroxyl group in the side chain was presumed to be located at C-20 or C-25. It was determined to be at C-25 because of the existence of the fragment ion at *m/z* 59 (ion **g**). Fragment ions at *m/z* 99 and *m/z* 85 corresponded to the ion **e** and ion **f** in the MS (Chart 3), respectively. The secondary hydroxyl group was determined to be at C-24.

In addition, comparison of ^{13}C-NMR data (Table I), disclosed a fair agreement of the signals

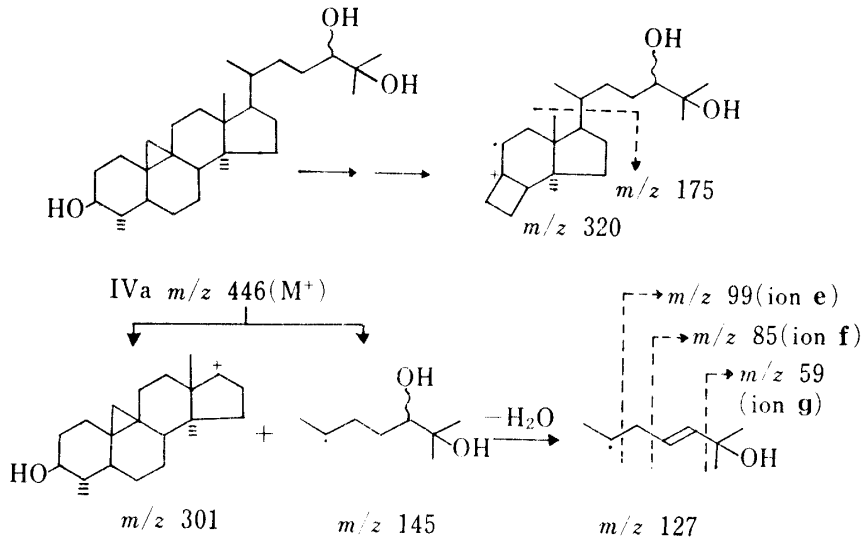

Chart 3

of IIIa and IVa except for those of C-24, C-26 and C-27, which were influenced by the hydroxyl group at C-25. The ^{13}C-NMR chemical shift of C-24 was displaced downfield, and those of C-23 and C-25 were displaced upfield when IVa was converted to IVb by acetylation.

From the above findings, IVa, except for the configuration at C-24, was identified as 4α,14-dimethyl-9,19-cyclocholestan-3β,24ξ,25-triol.

This is the first time that Ia, Ic, IIa, IIIa and IVa have been isolated from the pollen grains of *A. elatior*; IIIa and IVa are new compounds.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra were taken on Hitachi 295, JEOL JNM-4H-100, Hitachi R-900 and JEOL JMS-01-SG-2 spectrometers, respectively. Optical rotations were measured on a JEOL DIP-4 digital polarimeter. Column chromatography was performed on alumina (Wako, 300 mesh) and silica gel (Wako, C-200). TLC was carried out on precoated silica gel plates (Merck, silica gel 60), and the developing solvents were S-1 [benzene–$CHCl_3$ (1: 1)]; (Ic), S-2 [$CHCl_3$–AcOEt (20: 1)]; (Ia), S-3 [$CHCl_3$–AcOEt (4: 1)]; (IIa, IIIa) and S-4 [$CHCl_3$–AcOEt (1: 1)]; (IVa). The spots were detected with 10% H_2SO_4.

Extraction and Fractionation——Pollen grains (1362 g) collected in August, 1975, at Funabashi City, Chiba Prefecture, were extracted with ether in a Soxhlet apparatus for 24 h. The extract (225 g) was dissolved in ether and the ether solution was shaken with 5% $NaHCO_3$, 5% Na_2CO_3 and 5% NaOH. The ether layer was washed with water, dried over Na_2SO_4, and concentrated to give a neutral fraction (52 g) which was passed through a column packed with silica gel (60 g, upper layer) and alumina (600 g, bottom layer). The column was eluted successively with hexane, benzene, $CHCl_3$ and MeOH.

Isolation of Lophenol (Ia)——The fraction (2.0 g) eluted with benzene–$CHCl_3$ (7: 3) was repeatedly chromatographed on silica gel with benzene–$CHCl_3$ (7: 3). The eluted substance was recrystallized from acetone to give compound Ia (32 mg) as colorless needles, mp 148—149°C. $[\alpha]_D^{15.5}$ +1.44° (c=0.30, $CHCl_3$). MS m/z: 400 (M^+, 100%), 385 (20), 269 (28), 245 (11), 227 (6), 161 (12), 121 (17), 107 (15), 71 (9), 69 (18), 57 (17), 43 (33). High resolution MS m/z: Calcd for $C_{28}H_{48}O$: 400.3705. Found: 400.3676. IR ν_{max}^{KBr} cm^{-1}: 3350, 2940, 2860, 1630, 1463, 1448, 1378, 1100, 1045, 1015, 970, 830. ^{1}H-NMR ($CDCl_3$) δ: 0.53 (3H, s), 0.82 (3H, s), 0.86 (3H×2, d, J=6.6 Hz), 0.92 (3H, d, J=5 Hz), 0.98 (3H, d, J=6 Hz), 3.15 (1H, m, $W_{1/2}$=14 Hz), 5.18 (1H, m, $W_{1/2}$=9 Hz).

Acetylation of Lophenol (Ia)——Compound Ia (28 mg) was acetylated with Ac_2O (0.8 ml) in pyridine (2 ml). The product (23 mg) was recrystallized from acetone to give the monoacetate Ib as a white powder, mp 112—113°C. $[\alpha]_D^{15.5}$ +23.9° (c=0.20, $CHCl_3$). MS m/z: 442 (M^+, 100%), 427 (18), 382 (12), 269 (37), 161 (13), 107 (18), 71 (77), 57 (14), 43 (20). High resolution MS m/z: Calcd for $C_{30}H_{50}O_2$: 442.3811. Found: 442.3711. IR ν_{max}^{KBr} cm^{-1}: 2940, 1740, 1650, 1460, 1370, 1240, 1035, 970. ^{1}H-NMR ($CDCl_3$) δ: 0.56 (3H, s), 0.88 (3H, s), 0.90 (3H×3, d, J=5 Hz), 0.95 (3H, d, J=5 Hz), 2.05 (3H, s), 4.40 (1H, m, $W_{1/2}$=25 Hz), 5.17 (1H, m, $W_{1/2}$=11 Hz). The melting point showed no depression on admixture of Ib with an authentic

sample of lophenyl acetate, and the IR spectra and TLC behavior of the two samples were identical.

Isolation of Lophenone (1c)——The fraction (2.7 g) eluted with hexane–benzene (4: 1) was repeatedly chromatographed on silica gel and the product was recrystallized from acetone to give compound Ic (80 mg)as colorless plates, mp 119—120°C. $[\alpha]_D^{17}$ +6.8° (c=0.80, $CHCl_3$). *Anal.* Calcd for $C_{28}H_{46}O$: C, 84.35; H, 11.63. Found: C, 84.51; H, 11.83. MS m/z: 398 (M^+, 100%), 383 (25), 313 (2), 285 (48), 243 (25), 201 (6), 189 (4), 161 (15), 124 (4), 123 (9), 71 (10), 57 (21), 43 (33). IR ν_{max}^{KBr} cm^{-1}: 2950, 2860, 1720, 1630, 1468, 1445, 1380, 1235, 1170, 980. ^{1}H-NMR ($CDCl_3$) δ: 0.56 (3H, s), 0.82 (3H, s), 0.85 (3H, d, J=6 Hz), 0.92 (3H×2, d, J=6 Hz), 0.98 (3H, d, J=7 Hz), 5.19 (1H, m, $W_{1/2}$=10 Hz). The melting point showed no depression on admixture of Ic with an authentic sample of lophenone, and the IR spectra and TLC behavior of the two samples were identical.

Oxidation of Lophenol (Ia)——Compound Ia (38 mg) was dissolved in pyridine (1 ml) and allowed to stand with CrO_3–pyridine (100 mg in 3 ml) overnight at room temperature, then the mixture was poured into 50% MeOH. A white powder (35 mg) that appeared was collected and chromatographed on silica gel with hexane–benzene (4: 1). The product obtained was recrystallized from acetone to give lophenone (Ic) as colorless plates, mp 119—120°C. IR ν_{max}^{KBr} cm^{-1}: 2950, 2860, 1720, 1630, 1468, 1445, 1380, 1235, 1170, 980.

Isolation of Cholest-7en-3β-ol (IIa)——The fraction (4.4 g) eluted with benzene–$CHCH_3$ (1:1) was chromatographed on silica gel (100 g) and the product was recrystallized from acetone to give compound IIa (40 mg) as colorless needles, mp 121°C. $[\alpha]_D^{17}$ +0.20° (c=2.00, $CHCl_3$). MS m/z: 386 (M^+, 100%), 371 (30), 273 (22), 255 (59), 231 (22), 161 (22), 147 (24), 133 (26), 119 (27), 111 (4), 105 (28), 93 (30), 81 (32), 55 (36), 43 (40). High resolution MS m/z: Calcd for $C_{27}H_{46}O$: 386.3549. Found: 386.5439. IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 2880, 1620, 1465, 1440, 1370, 1095, 1050, 1040, 940, 845. ^{1}H-NMR ($CDCl_3$) δ: 0.53 (3H, s), 0.78 (3H, s), 0.85 (3H×2, d, J=6 Hz), 0.92 (3H, d, J=6 Hz), 3.59 (1H, m, $W_{1/2}$=15 Hz), 5.15 (1H, br s, $W_{1/2}$=10 Hz). ^{13}C-NMR ($CDCl_3$) δ: 139.7 (C-8), 117.5 (C-7), 71.1 (C-3), 56.3 (C-17), 55.1 (C-14), 49.5 (C-9), 43.4 (C-13), 40.3 (C-5), 39.6 (C-12, 24), 38.0 (C-4), 37.3 (C-1), 36.2 (C-20, 22), 34.2 (C-10), 31.5 (C-2), 29.7 (C-6), 28.0 (C-16, 25), 24.0 (C-23), 22.9 (C-15, 27), 22.6 (C-26), 216 (C-11), 18.9 (C-21), 13.1 (C-19), 11.9 (C-18).

Acetylation of Cholest-7-en-3β-ol (IIa)——Compound IIa (32 mg) was acetylated with Ac_2O (0.7 ml) in pyridine (0.5 ml). The product (24 mg) was recrystallized from acetone to give the monoacetate IIb as colorless needles, mp 94°C. $[\alpha]_D^{17}$ +1.6° (c=1.60, $CHCl_3$). MS m/z: 428 (M^+, 100%), 413 (17), 368 (9), 255 (51), 213 (29), 161 (13), 147 (21), 119 (22), 93 (21), 81 (38), 57 (22), 55 (29), 43 (26). High resolution MS m/z: Calcd for $C_{29}H_{48}O_2$: 428.3654. Found: 428.3718. IR ν_{max}^{KBr} cm^{-1}: 2950, 2860, 2840, 1740, 1465, 1445, 1365, 1250, 1030, 895. ^{1}H-NMR ($CDCl_3$) δ: 0.54 (3H, s), 0.83 (3H, s), 0.85 (3H×2, d, J=6 Hz), 0.92 (3H, d, J=6 Hz), 2.00 (3H, s), 4.70 (1H, m, $W_{1/2}$=17 Hz), 5.17 (1H, br s, $W_{1/2}$=10 Hz). ^{13}C-NMR ($CDCl_3$) δ: 170.7 ($CH_3\underline{C}O$–), 139.6 (C-8), 117.3 (C-7), 73.5 (C-3), 56.2 (C-17), 55.1 (C-14), 49.4 (C-9), 43.4 (C-13), 40.1 (C-5), 39.6 (C-12, 24), 36.9 (C-1), 36.2 (C-20, 22), 34.2 (C-10), 33.9 (C-4), 29.6 (C-6), 28.0 (C-16, 25), 27.6 (C-2), 24.0 (C-23), 23.0 (C-15), 22.8 (C-27), 22.6 (C-26), 21.5 (C-11, $\underline{C}H_3CO$–), 18.9 (C-21), 13.0 (C-19), 11.9 (C-19).

Isolation of 4α,14-Dimethyl-9,19-cyclocholestan-3β,24ξ-diol (IIIa)——The fraction (2.02 g) eluted with benzene–$CHCl_3$ (1: 3) was rechromatographed on silica gel (35 g) with benzene, and the product was recrystallized from EtOH to give compound IIIa (27 mg) as colorless plates, mp 146—148°C. $[\alpha]_D^{20}$ +11.7° (c=0.70, $CHCl_3$). MS m/z: 430 (M^+, 100%), 415 (17), 412 (82), 403 (7), 357 (5), 304 (38), 301 (26), 283 (24), 175 (16), 171 (4), 134 (16), 69 (37), 55 (36), 43 (59). High resolution MS m/z: Calcd for $C_{29}H_{50}O_2$: 430.3811. Found: 430.3785. IR ν_{max}^{KBr} cm^{-1}: 3260, 3040, 2960, 2860, 1456, 1373, 1110, 1095, 1048, 1018, 996. ^{1}H-NMR ($CDCl_3$) δ: 0.13 (1H, d, J=4 Hz), 0.40 (1H, d, J=4 Hz), 0.87 (3H, d, J=6 Hz), 0.91 (3H, d, J=6 Hz), 0.94 (3H, s), 0.98 (3H, s), 1.25 (3H×2, J=6 Hz), 3.26 (2H, m, $W_{1/2}$=23 Hz). ^{13}C-NMR: The results are shown in Table I.

Acetylation of 4α,14-Dimethyl-9,19-cyclocholestan-3β,24ξ-diol (IIIa)——Compound IIIa (20 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (1 ml). The product (20 mg) was recrystallized from acetone to give the diacetate IIIb as colorless needles, mp 152—154°C. $[\alpha]_D^{20}$ +49.0° (c=1.00, $CHCl_3$). MS m/z: 514 (M^+, 10%), 455 (36), 454 (100), 439 (27), 394 (12), 379 (15), 346 (12), 343 (7), 283 (28), 175 (32), 171 (4), 134 (16), 73 (28), 69 (37), 55 (36), 43 (59). IR ν_{max}^{KBr} cm^{-1}: 3025, 2860, 1730, 1460, 1370, 1240, 1115, 1095, 1032, 1015, 980, 960, 867. ^{1}H-NMR ($CDCl_3$) δ: 0.13 (1H, d, J=4 Hz), 0.40 (1H, d, J=4 Hz), 0.88 (3H, d, J=6 Hz), 0.90 (3H, d, J=6 Hz), 0.93 (3H, s), 0.98 (3H, s), 1.28 (3H×2, d, J=6 Hz), 2.06 (3H×2, s), 4.42 (1H, m, $W_{1/2}$=14 Hz), 4.58 (1H, m, $W_{1/2}$=14 Hz). ^{13}C-NMR: The results are shown in Table I.

Isolation of 4α,14-Dimethyl-9,19-cyclocholestan-3β,24ξ,25-triol (IVa)——The fraction (2.5 g) eluted with $CHCl_3$ was rechromatographed on silica gel with benzene, and the product was recrystallized from acetone to give compound IVa (48.5 mg) as a white powder, mp 104°C. $[\alpha]_D^{20}$ +23.4° (c=0.20, $CHCl_3$). MS m/z: 446 (M^+, 20%), 431 (7), 429 (22), 428 (65), 413 (31), 410 (5), 395 (19), 373 (3), 302 (29), 301 (24), 283 (20), 175 (56), 172 (30), 117 (11), 109 (67), 99 (7), 85 (12), 81 (69), 67 (36), 59 (100), 41 (39). High resolution MS m/z: Calcd for $C_{29}H_{50}O_3$: 446.3760. Found: 446.3747. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 2860, 1460, 1370, 1150, 1100, 1040, 1000. ^{1}H-NMR ($CDCl_3$) δ: 0.13 (1H, d, J=4 Hz), 0.39 (1H, d, J=4 Hz), 0.86 (3H, d, J=6 Hz), 0.88 (3H, s), 0.94 (3H, d, J=6 Hz), 0.96 (3H, s), 1.16 (3H, s), 1.21 (3H, s), 3.22 (2H, m, $W_{1/2}$=17.5 Hz). ^{13}C-NMR: The results are shown in Table I.

Acetylation of 4α,14-Dimethyl-9,19-cyclocholestestan-3β,24ξ,25-triol (IVa)——Compound IVa (40 mg) was acetylated with Ac_2O (1 ml) in pyridine (1 ml). The product (37 mg) was recrystallized from acetone

to give the diacetate IVb as a white powder, mp 145—146°C. $[\alpha]_D^{20}$ +45.9° (c=0.40, $CHCl_3$). MS m/z: 530 (M^+, 10%), 515 (2), 471 (24), 470 (60), 455 (22), 412 (7), 410 (14), 395 (12), 283 (28), 175 (33), 127 (13), 109 (34), 81 (49), 67 (20), 59 (66), 43 (100), 41 (21). High resolution MS m/z: Calcd for $C_{33}H_{54}O_5$: 530.3971. Found: 530.4013. IR ν_{max}^{KBr} cm^{-1}: 3500, 2970, 2930, 2860, 1740, 1720, 1460, 1370, 1260, 1235, 1020. ^{1}H-NMR ($CDCl_3$) δ: 0.14 (1H, d, J=4 Hz), 0.40 (1H, d, J=4 Hz), 0.83 (3H, d, J=6 Hz), 0.87 (3H, s), 0.88 (3H, d, J=6 Hz), 0.94 (3H, s), 1.19 (3H×2, s), 2.04 (3H, s), 2.09 (3H, s), 4.53 (1H, m, $W_{1/2}$=16 Hz), 4.75 (1H, d, J=10 Hz). ^{13}C-NMR: The results are shown in Table I.

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