[Chem. Pharm. Bull. 18(7)1354—1361(1970)]

UDC 547.597.02 : 582.536.04 : 615.322.011.05

Biological-Active Triterpenes of Alismatis Rhizoma. II.[1] The Structures of Alisol A and Alisol A Monoacetate[2]

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(Received January 23, 1970)

Chemical studies on the structures of alisol A (1) and its monoacetate (2), the new hypocholesterolemic triterpenes of Alismatis Rhizoma, are described.

In connection with the isolation of the new triterpenes alisols from the rhizome of *Alisma Plantago-aquatica* L. var. *orientale* SAMUELS., as described in the preceding paper,[1] the authors wish to report in the present paper the chemical studies on the structures of alisol A and its monoacetate which have been established as **1** and **2**, respectively finally by the use of X-ray crystallographic technique.

Alisol A (**1**), $C_{30}H_{50}O_5$, upon acetylation with acetic anhydride and pyridine at room temperature gave a triacetate **3**, which showed a hydroxyl band at 3500 cm^{-1} in the infrared (IR) spectrum ($CHCl_3$). The triacetate **3** regenerated **1** upon treatment with potassium carbonate in aqueous methanol. The secondary nature of the three acetylated hydroxyls was suggested by the nuclear magnetic resonance (NMR) spectrum of the triacetate **3** which revealed signals assignable to three CH–OAc protons at 4.60 (1H, doublet) and near 4.8 ppm (δ) (2H, overlapped signals). Prolonged acetylation of the triacetate **3** with hot acetic anhydride and pyridine afforded alisol A tetraacetate (**4**), which lacked the hydroxyl band in the IR spectrum but showed no additional CH–OAc proton signal in the NMR spectrum, thus suggesting the presence of one tertiary hydroxyl group in alisol A (**1**). In addition to this, no signal attributable to a carbinyl proton could be noticed in the NMR spectrum of alisol A triacetate (**3**). The fifth oxygen in alisol A (**1**) was assigned to a six-membered ring ketone by the following evidence: **1** exhibited a carbonyl band at 1705 cm^{-1} in the IR spectrum. The band disappeared in dihydroalisol A (**5**) which was obtained by sodium borohydride reduction of **1**. Dihydroalisol A (**5**) gave the tetraacetate (**7**) upon acetylation with acetic anhydride and pyridine at room temperature. Similarly, sodium borohydride reduction of alisol A triacetate (**3**) yielded dihydroalisol A triacetate (**6**), which afforded **3** upon oxidation with chromium trioxide–pyridine complex. The NMR signal due to the newly formed carbinyl C<u>H</u>–OH proton in **6** appeared at 3.17 ppm as a one-proton triplet-like signal. Alisol A and the triacetate **3** showed the positive Zimmermann test for 3-keto triterpene,[4] and both optical rotatory dispersion (ORD)and circular dichroism (CD) curves of **3** displayed strongly positive Cotton effect.[5] These data strongly suggested the presence of a 5α-3-ketone moiety like some steroids or triterpenes in the molecule of alisol A (**1**).

The NMR spectrum of **1** showed eight methyls in the region of 0.95 to 1.22 ppm. Oxidation of **1** with periodic acid in dioxane gave acetone and the tetranoraldehyde (**8**); the mole-

1) Part I: T. Murata, Y. Imai, T. Hirata and M. Miyamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1347 (1970).
2) Preliminary communications have been published. T. Murata, M. Shinohara, T. Hirata, K. Kamiya, M. Nishikawa and M. Miyamoto, *Tetrahedron Letters*, **1968**, 103; T. Murata, M. Shinohara, T. Hirata and M. Miyamoto, *ibid.*, **1968**, 849.
3) Location: *Juso-Nishino-cho, Higashiyodogawa-ku, Osaka.*
4) D.H.R. Barton and P. de Mayo, *J. Chem. Soc.*, **1954**, 887.
5) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Co., New York, 1960, p. 41, 89; L. Velluz, M. Legrand and M. Grosjean, "Optical Circular Dichroism," Verlag Chemie, GMBH, Weinheim, 1965, p. 79.

cular formula of the latter was confirmed by mass peak at *m/e* 400 (M+). The NMR spectrum of **8** showed the aldehyde proton at 9.58 (1H, triplet, J=2.4 cps, $-CH_2-C\underline{H}O$) and one-proton carbinyl C<u>H</u>–OH proton at 3.80 ppm as a sextet, together with six methyls in the region of 0.93 to 1.06 ppm.

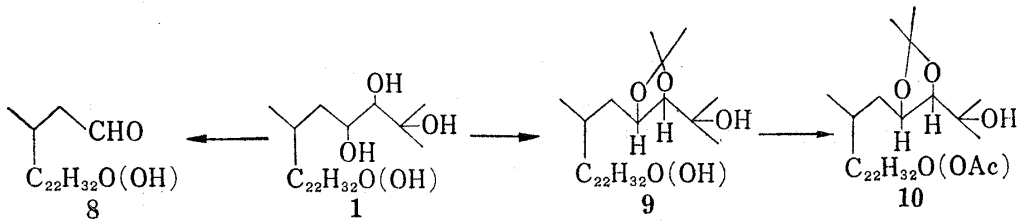


Chart 1

Treatment of alisol A with boron trifluoride–ether complex in acetone yielded the acetonide (**9**) which gave a monoacetate (**10**) upon acetylation with acetic anhydride and pyridine at room temperature. The acetonide monoacetate **10** exhibited the two methine protons involved in the acetonide moiety at 3.34 (doublet, J=9.0 cps) and 3.59 ppm (sextet), respectively. These results led us to the assumption that alisol A (**1**) would be a tetracyclic triterpene in which the isooctyl side chain bears the glycerol moiety, and that the tetracyclic nucleus has one secondary hydroxyl group and one six-membered ring ketone. That the secondary hydroxyl group in the ring part of the molecule of alisol A is involved in the part of structure >CH–CH(OH)–CH_2– and that this hydroxyl has an equatorial orientation can be deduced by the fact that the carbinyl proton appeared in the NMR spectrum of the tetranoraldehyde **8** as a sextet with the coupling constant values of 13.5 and 5.5 cps, demonstrating the presence of two large, equal spin–spin interactions between *trans* diaxial protons in above part structure. Support for this conclusion was derived from the NMR spectrum of Δ^4-androsten-11α-ol-3,20-dione which displayed the 11-axial proton signal at 3.80 ppm as a sextet (J=13.5 and 5.6 cps),[6] showing a close similarity to the sextet in the tetranoraldehyde **8**.

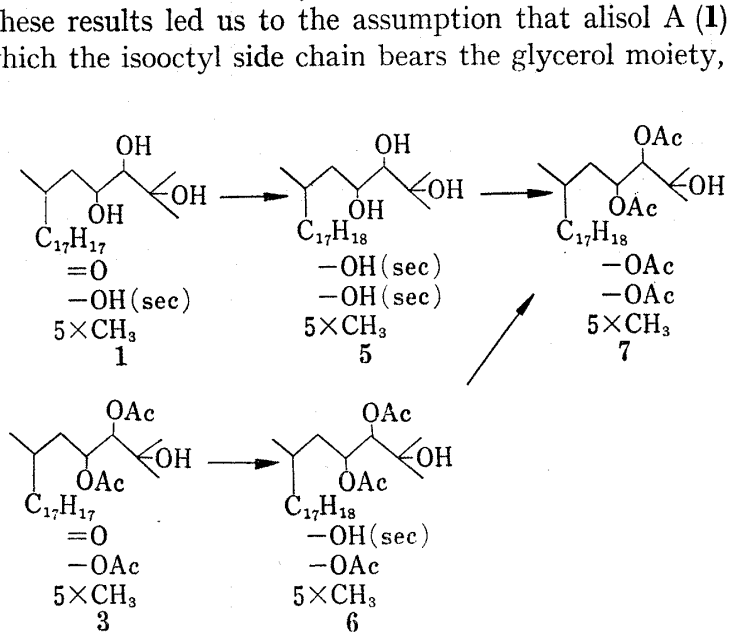


Chart 2

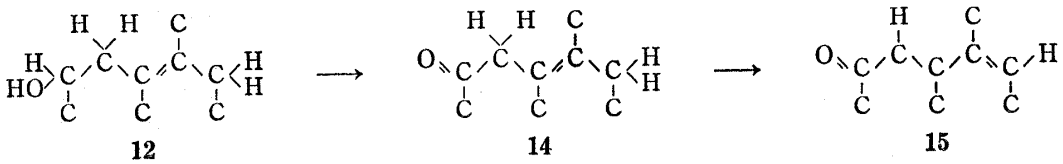
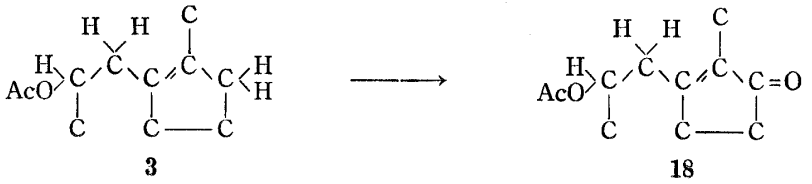


Chart 3

6) N.S. Bhacca and D.H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day Inc., San Francisco, 1964, p. 82.

The IR spectrum of alisol A (**1**) showed a weak absorption at 1655 cm^{-1}; however, no olefinic protons could be observed in the NMR spectrum. This suggested that one tetra-substituted double bond must be present in the tetracyclic nucleus of **1**. The position 8 (9) can almost certainly be excluded because CD curve of alisol A triacetate (**3**) is quite different from that of Δ^8-lanosten-3-one.[7] Chemical evidence for the presence of a double bond at 13 (17) position was provided by the following reactions: Alisol A (24, 25)-acetonide 23-monoacetate (**12**) which was obtained through acetonization of natural alisol A monoacetate (**2**), gave the dienone **15** upon oxidation with a large excess of chromium trioxide–pyridine complex. The partial structure assigned to the dienone **15** was supported by its spectral properties. The IR spectrum had strong bands at 1645, 1623 and 1604 cm^{-1}, and the ultraviolet (UV) spectrum with λ_{max} 292 mμ (ε 1.54×10^4) was also consistent with the presence of an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl group. The NMR spectrum exhibited two olefinic protons (one–proton each) at 5.72 (singlet) and 6.18 ppm (triplet, J=2.5 cps) which were reasonably attributable to the protons at C_{12} and C_{16}, respectively, of the molecule. The formation of the dienone **15** can best be interpreted by assuming that the oxidation proceeded through the 13(17)-en-11-oxo compound (**14**), a normal oxidation product.[8]

The result almost certainly established the existence of not only a double bond at 13 (17)[9] but also of a secondary hydroxyl group at 11 in the molecule of alisol A (**1**); the location of the hydroxyl is well agreeable with the NMR data which was assigned to the partial structure above described. Further evidence for the presence of a double bond at 13 (17) position of **1** was obtained by chromic acid oxidation of alisol A triacetate (**3**), yielding the product **18**, $C_{36}H_{54}O_9$ which had an UV maximum at 242 mμ, ε 1.63×10^4. Compound **18** added a new, relatively strong band at 1645 cm^{-1} in the IR spectrum. The spectrum also showed that intensity of the 1705 cm^{-1} band of **3** increased in **18**. In addition to this, no olefinic protons was noticed in the NMR spectrum of the oxidation product **18** as in the case of **3**. The results suggested that a methylene group α to a double bond was oxidized to afford an α,β-unsaturated



3 **18**

Chart 4

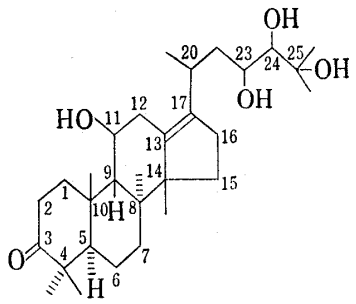
ketone. Since the CH–OAc proton signal due to the C_{11}-proton was found to remain unaffected in the NMR spectrum of **18**, though the signal overlapped with the C_{23}-proton signal to some extent, it can be concluded that the oxidation had not occurred at C_{12} but at C_{16}. Thus a 13(17)-en-16-oxo structure was assigned to the oxidation product **18**. The band at 1645 cm^{-1} (C=C) and the increasing of intensity of the 1705 cm^{-1} band (C=O) in the IR spectrum of **18** can now reasonably be attributable to the α,β-unsaturated, five-membered ring ketone. A 17(20)-en-16-oxo structure can be excluded because both compounds **3** and **18** revealed no signals assignable to a methyl group attached to a double bond.

7) Δ^8-Lanosten-3-one showed the CD (c=0.105, dioxane) $[\theta]^{26}$ (mμ): −40 (316) (negative maximum), 0 (302), +54 (288) (positive maximum). *Cf.* P. Witz, J. M. Lehn and G. Ourisson, *Bull. Soc. Chim. France*, **1963**, 1101.

8) The mechanism of the reaction **12**→**15** will be discussed in Part IV of the series.

9) For tetracyclic triterpenes which have a double bond at the position, see K. Christin, M. Dünnenberger, C.B. Roth, H. Heusser and O. Jeger, *Helv. Chim. Acta*, **35**, 1756 (1952); M.C. Dawson, T.G. Halsall and R.E.H. Swayne, *J. Chem. Soc.*, **1953**, 590; J.B. Barbour, W.A. Lourens, F.L. Warren and K.H. Watling, *ibid.*, **1955**, 2194; D. Arigoni, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **38**, 222 (1955).

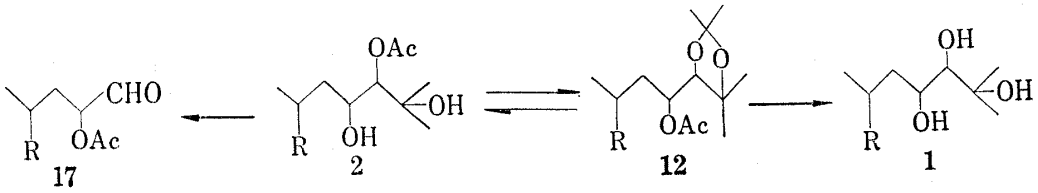
As for the configuration at C_{23} and C_{24} of the side chain,[10] the ease of formation of the (23,24)-acetonide (**9**) and the observed coupling constant value between the methine protons at C_{23} and C_{24} in the acetonide monoacetate (**10**) are indicative of the *threo* configuration at the positions: In acetonide of a 1,2-diol, *erythro* configuration leads to a *cis*-acetonide and *threo* configuration to a *trans* derivative. The formation of *cis*-acetonide would be more difficult than *trans* one. As noted above, alisol A (**1**) smoothly gave the acetonide (**9**) upon treatment with boron trifluoride–ether complex in acetone. This suggests that a *trans*-acetonide formed and that alisol A has the *threo* configuration. The recorded J values for the protons of the *trans* and *cis* acetonides of butane 2,3-diol are 8.5 and 5.35 cps, respectively; the former is the one estimated on the *quasi*-diaxial protons. The observed $J_{23,24}$ of 9.0 cps for the acetonide monoacetate (**10**) is more consistent with the *trans* derivative. This coincidence also supports the *threo* configuration at C_{23} and C_{24} of alisol A (**1**).



1

However, since above data led to no crucial evidence for the tetracyclic nucleus of alisol A, X-ray crystallography on the 11-bromoacetate of the acetonide **9** was carried out establishing the whole structure of alisol A as shown in **1**; the configuration of the side chain being 20*R*, 23*S* and 24*R*. It has now been shown that alisol A is the first triterpene of protostane series,[11] which has been accepted as an intermediate in the biosynthesis of lanosterol.[12] Further prominent structural feature lies in the fact that **1** has a double bond at 13 (17) position. So far as the authors know, alisol A is also the first naturally occurring triterpene which possesses a double bond at the position.[9,11]

Acetonization of natural alisol A monoacetate (**2**) with boron trifluoride–ether complex in acetone afforded the (24, 25)-acetonide 23-monoacetate (**12**); the structure of the latter was confirmed by its NMR spectrum which showed only one signal attributable to a methine proton involved in the acetonide moiety at 3.60 ppm as a doublet (J=7.0 cps). The spectrum



R=the tetracyclic nucleus of alisol A

Chart 5

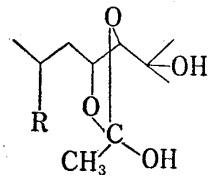
also revealed the C_{23}-proton at 4.85 ppm as a quartet (J=7.0 and 13.0 cps). The acetonide monoacetate (**12**) gave alisol A monoacetate (**2**) upon treatment with aqueous acetic acid, a fact which suggested that alisol A monoacetate (**2**) is a C_{23}-monoacetate. Further, oxidation

10) G.J. Breen, E. Ritchie, W.T.L. Sidwell and W.C. Taylor, *Australian J. Chem.*, **19**, 455 (1965) and the literatures cited therein.

11) T. Hattori, H. Igarashi, S. Iwasaki and S. Okuda, *Tetrahedron Letters*, **1969**, 1023.

12) A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955); W.O. Godtfredsen, W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni and A. Merela, *Tetrahedron*, **21**, 3505 (1965); E.J. Corey, W.E. Russey and P.R. Ortiz de Montellano, *J. Am. Chem. Soc.*, **88**, 4750 (1966); E.E. van Tamelen, J.D. Willet, R.B. Clayton and K.E. Lord, *ibid.*, **88**, 4752 (1966); J.D. Willet, K.B. Sharpless, K.E. Lord, E.E. van Tamelen and R.B. Clayton, *J. Biol. Chem.*, **242**, 4182 (1967); W.O. Godtfredsen, H. Lorck, E.E. van Tamelen, J.D. Willet and R.B. Clayton, *J. Am. Chem. Soc.*, **90**, 208 (1968); E.J. Corey, K. Lin and H. Yamamoto, *ibid.*, **91**, 2132 (1969).

of **2** with periodic acid afforded acetone and the trisnoraldehyde monoacetate (**17**). However, scrutiny of the NMR spectra of **2** and related compounds showed that **2** is not C_{23}-monoacetate but C_{24}-monoacetate; thus the NMR spectrum of the monoacetate **2** showed the CH–OAc proton at 4.61 ppm as a singlet. Alisol A 11,24-diacetate (**16**) which was obtained by partial deacetylation of alisol A triacetate (**3**), displayed the two CH–OAc protons at 4.88 ppm as a sextet (J=11.5 and 6.0 cps) and at 4.44 ppm as a doublet (J=1.0 cps), respectively; the former was apparently ascribable to the C_{11}-proton. The signal assignable to the carbinyl proton at C_{23} appeared at 3.72 ppm as a double doublet (J=8.0 and 4.0 cps). This shows that the spin–spin coupling between the C_{23}- and C_{24}-protons is very small. The formation of the (24, 25)-acetonide 23-monoacetate **12** and the trisnoraldehyde monoacetate **17**, above stated can reasonably be interpreted by assuming migration of the acetyl group from C_{24} to C_{23} hydroxyl groups during acetonization and periodate oxidation of alisol A monoacetate (**2**). Since alisol A (24, 25)-acetonide 23-monoacetate (**12**) gave alisol A (**1**) upon deacetylation followed by deacetonization, and the acetonide monoacetate **12** regenerated **2** upon deacetonization as mentioned above, it can be concluded that the configuration at C_{23} and C_{24} have been unaltered during acetyl migration between the C_{23} and C_{24} hydroxyls, which apparently proceeds through an orthoester intermediate: **20**[13]



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Experimental

All melting points were measured on Kofler block and uncorrected. The specific rotations were taken on $CHCl_3$ solutions, c=1.0% unless otherwise stated. The NMR spectra were reocrded with a Varian HA-100 NMR spectrometer on $CDCl_3$ solutions and calibrated against internal tetramethylsilane. Chemical shifts are expressed in ppm: s, singlet; d, doublet; t, triplet; q, quartet; sext, sextet; m, multiplet. For column chromatography silica gel (Merck, ϕ 0.05—0.2 mm) was used.

Alisol A (**1**)——Cololress powder, $[\alpha]_D^{23}$ +99.6°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705 (ring ketone); 1655 (weak band, C=C). NMR (δ): 0.95—1.22 (total 24H, overlapped signals, $8CH_3$).

Alisol A Monoacetate (**2**)——Colorless prismatic needles from acetone, mp 194—196°, $[\alpha]_D^{23}$ +86°. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH); 1745 (acetyl); 1705 (ring ketone). NMR (δ): 2.10 (3H, s, $OCOCH_3$); 4.61 (1H, s, C_{24}–H); *ca.* 3.8 (2H, overlapped signals, C_{11}–H and C_{23}–H).

Alisol A Triacetate (**3**)——Colorless needles from MeOH or CH_2Cl_2–MeOH, mp 231—233°, $[\alpha]_D^{21}$ +54.4°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH); 1745 (acetyl); 1705 (ring ketone). NMR (δ): 0.95—1.15 (total 24H, overlapped signals, $8CH_3$); 1.96 (6H, s, $2OCOCH_3$); 2.07 (3H, s, $OCOCH_3$); 4.60 (1H, d, J=3.0 cps, C_{24}–H); *ca.* 4.8 (2H, overlapped signals, C_{11}–H and C_{23}–H). ORD (c=0.217, dioxane) $[\alpha]^{26}$ (mμ): +55° (589), +805° (316) (peak), +712° (308) (shoulder), 0° (293), −643° (272) (peak), −505° (255). CD (c=0.217, dioxane) $[\theta]^{26}$ (mμ): +8500 (291) (positive maximum).

Alisol A Tetraacetate (**4**)——A solution of **3** (2.8 g) in a mixture of Ac_2O (2 ml) and C_5H_5N (3 ml) was heated at 95° for 2 hr. After cooling, the mixture was poured into ice–water, and the resulting precipitates were collected (2.9 g). The product was chromatographed on silica gel (60 g) and eluted with benzene–acetone (15:1) to give an oil (1.9 g), which was crystallized from MeOH to yield 1.2 g of **4**, colorless needles, mp 148—150°. $[\alpha]_D^{23}$ +56.7°. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (acetyl); 1710 (ring ketone). No OH band was noticed in the IR spectrum. NMR (δ): 1.89 (3H, s, $OCOCH_3$); 1.97 (6H, s, $2OCOCH_3$); 2.07 (3H, s, $OCOCH_3$); *ca.* 4.9 (2H, overlapped signals, 2CH–OAc); 5.06 (1H, d, J=2.5 cps, CH–OAc). *Anal.* Calcd. for $C_{38}H_{58}O_9$: C, 69.27; H, 8.87. Found: C, 69.16; H, 8.85.

Dihydroalisol A (**5**)——a) To a solution of **1** (50 mg) in EtOH (3.5 ml), $NaBH_4$ (40 mg) was added at room temperature and the mixture was stirred for 30 min. After neutralization with AcOH, water was added and the mixture was extracted with AcOEt. The AcOEt layer was washed with water, dried and evaporated. The residue (45 mg) was chromatographed on silica gel (15 g) and eluted with benzene–AcOEt (2:1) to afford 30 mg of colorless needles, which were recrystallized from aq. MeOH to give pure **5**, mp 134—137°. $[\alpha]_D^{22}$ +30.0° (c=1.0, dioxane). *Anal.* Calcd. for $C_{30}H_{52}O_5$: C, 73.12; H, 10.64. Found: C, 73.12; H, 10.74.

13) R represents the tetracyclic nucleus of alisol A; J.M. Sugihara, *Advances in Carbohydrate Chem.*, **8**, 1 (1953); R.U. Lemieux, "Molecular Rearrangements," ed. by P. de Mayo, Interscience Publishers, New York, 1964, p. 709.

b) A solution of **7** (120 mg) in 2.5% methanolic KOH (6 ml) was refluxed for 3 hr. After concentration, water was added to the residue and the mixture was extracted with AcOEt. The AcOEt extract was washed with water, dried and evaporated. The residue was then chromatographed on silica gel (15 g) and eluted with benzene–acetone (2:1) to yield 80 mg of **5**. Crystallization from aq. MeOH gave colorless needles, mp 134—137°, alone or in admixture with the compound prepared as (a) above. The IR spectra of the two products were also identical.

Sodium Borohydride Reduction of Alisol A Triacetate (3) **(Formation of Dihydroalisol A Triacetate** (6))——To a stirred solution of **3** (400 mg) in EtOH (70 ml), $NaBH_4$ (350 mg) was added in small portions during 45 min. After standing at room temperature for 1 hr, the mixture was neutralized with AcOH, diluted with water and extracted with AcOEt. The AcOEt extract was washed with water, dried and evaporated to dryness to leave 350 mg of an oil. Crystallization from MeOH gave colorless plates of **6**, mp 177—178°, $[\alpha]_D^{22}$ +3.5°. NMR (δ): 0.77 (3H, s, CH_3); 0.94, 0.96 and 0.99 (total 9H, $3CH_3$); 1.02 (9H, s, $3CH_3$); 1.16 (3H, s, CH_3); 1.97 (3H, s, $OCOCH_3$); 2.00 (3H, s, $OCOCH_3$); 2.10 (3H, s, $OCOCH_3$); 3.17 (1H, t-like signal, J=7 cps, C_3–H); 4.62 (1H, d, J=3.0 cps, C_{24}–H), 4.75 (2H, overlapped signals, C_{11}–H and C_{23}–H). *Anal.* Calcd. for $C_{36}H_{58}O_8$: C, 69.87; H, 9.45. Found: C, 69.88; H, 9.16.

CrO_3 Oxidation of 6 to 3——Compound **6** (70 mg), dissolved in C_5H_5N (1 ml) was oxidized with CrO_3–C_5H_5N complex prepared from 70 mg of CrO_3 and 1 ml of C_5H_5N for 1 hr. After dilution with water, the mixture was extracted with ether and the ethereal extract was washed with water, dried and evaporated. Treatment of the residue with MeOH afforded crude crystals of **3** (50 mg), which were recrystallized from MeOH to give a pure sample of **3**, mp 231—233°, alone or in admixture with an authentic sample. The IR spectra were also identical.

Dihydroalisol A Tetraacetate (7)——A solution of **6** (50 mg) in a mixture of Ac_2O (2 ml) and C_5H_5N (2 ml) was left to stand at room temperature for 6 hr. Work up in the usual way and recrystallization of the product from aq. MeOH gave 40 mg of **7**, mp 195—196°, $[\alpha]_D^{22}$ +10.3°. Mass (*m/e*): 600 (M^+—AcOH). IR ν_{max}^{KBr} cm^{-1}: 3600 (OH); 1745 (acetyl). NMR (δ): 1.98 (9H, s, $3OCOCH_3$); 2.07 (3H, s, $OCOCH_3$); 4.42 (1H, t-like signal, J=7 cps, C_3–H); 4.60 (1H, d, J=3.0 cps, C_{24}–H); *ca.* 4.8 (2H, overlapped signals, C_{11}–H and C_{23}–H). *Anal.* Calcd. for $C_{38}H_{60}O_9$: C, 69.06; H, 9.15. Found: C, 69.22; H, 9.02.

HIO_4 Oxidation of 1 (Formation of Acetone and the Tetranoraldehyde 8)——To a solution of **1** (120 mg) in dioxane (5 ml), $HIO_4 \cdot 2H_2O$ (500 mg) and water (2 ml) were added and the mixture was warmed at 45° for 30 min. During the reaction the mixture was bubbled with N_2-gas which was introduced into 2,4-dinitrophenylhydrazine·HCl solution. The resulting precipitates were collected, washed with water, dried and chromatographed on silica gel (12 g) using benzene as an eluent to afford 40 mg of acetone 2,4-dinitrophenylhydrazone. Recrystallization from MeOH gave orange needles, mp 123—124° (decomp.), undepressed in admixture with an authentic sample. *Anal.* Calcd. for $C_9H_{10}O_4N_4$: C, 45.38; H, 4.23; N, 23.52. Found: C, 45.07; H, 4.15; N, 23.53.

Above reaction mixture was diluted with water (30 ml) and extracted with AcOEt (2×50 ml). The combined AcOEt extracts were washed with water, dried and evaporated to dryness, and the residue was chromatographed on silica gel (20 g). Elution with $CHCl_3$–AcOEt (5:1) followed by $CHCl_3$–AcOEt (3:1) yielded colorless crystals of **8** (70 mg), which were recrystallized from AcOEt to give colorless prisms, mp 190°. $[\alpha]_D^{23}$ +129.4°. Mass (*m/e*): 400 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH); 1725 and 2770 (aldehyde); 1705 (ring ketone). NMR (δ): 0.93—1.06 (total 18H, $6CH_3$); 3.80 (1H, sext, J=13.5 and 5.6 cps, C_{11}–H); 9.58 (1H, t, J=2.5 cps, $-CH_2-\underline{C}HO$). *Anal.* Calcd. for $C_{26}H_{40}O_3$: C, 77.95; H, 10.07. Found: C, 77.70; H, 9.93.

Acetonization of 1 (Formation of the Acetonide 9)——To a solution of **1** (1.05 g) in acetone (20 ml), BF_3 etherate (0.2 ml) was added and the mixture was allowed to stand for 2 hr with ice-cooling. After dilution with water (50 ml), the mixture was extracted with AcOEt (70, 50 ml). The combined AcOEt extracts were washed with aq. $NaHCO_3$ followed by water, dried and evaporated. The residue was dissolved in benzene–AcOEt (5:1) and the solution was passed through a column of silica gel (130 g). Elution with the same solvent mixture afforded colorless powder of the (23, 24)-acetonide **9** (790 mg), which did not crystallize. $[\alpha]_D^{23}$ +69°. *Anal.* Calcd. for $C_{33}H_{54}O_5$: C, 74.67; H, 10.26. Found: C, 74.22; H, 10.10.

A solution of **9** (110 mg) in aq. 66% AcOH (7 ml) was heated at 95° for 2 hr. After evaporation of the solvent, the residue was acetylated with Ac_2O (1 ml) and C_5H_5N (0.5 ml) for 18 hr at room temperature. After working up in the usual manner, crude acetate was chromatographed on silica gel (15 g) and eluted with benzene–acetone (8:1) to yield 65 mg of alisol A triacetate (3). Recrystallization from MeOH gave a pure sample, mp 231—233°, undepressed in admixture with an authentic specimen of **3**.

Alisol A (23, 24)-Acetonide 11-Monoacetate (10)——Compound **9** was acetylated with Ac_2O–C_5H_5N at room temperature for 6 hr. Work up in the usual way and recrystallization of the acetate from MeOH afforded colorless pillars of **10**, mp 213—215°, $[\alpha]_D^{28}$ +75.5°. Mass (*m/e*): 572 (M^+); 557 ($M^+ - CH_3$). IR ν_{max}^{Nujol} cm^{-1}: 3480 (OH); 1735 (acetyl); 1705 (ring ketone). NMR (δ): 3.34 (1H, d, J=9.0 cps, C_{24}–H); 3.59 (1H, broad sext, J=9 and 5 cps, C_{23}–H); 4.94 (1H, sext, J=12.0 and 6.0 cps, C_{11}–H). *Anal.* Calcd. for $C_{35}H_{56}O_6$: C, 73.39; H, 9.85. Found: C, 73.19; H, 10.00. Deacetylation of **10** with hot methanolic K_2CO_3 gave compound **9**, which was reacetylated to yield compound **10**.

CrO_3 Oxidation of Alisol A Triacetate (3) (Formation of 18)——To a stirred solution of **3** (300 mg) in AcOH (7 ml), CrO_3 (80 mg), dissolved in 95% AcOH (1 ml), was added with ice-cooling over 5 min. After 40 min at room temperature, the reaction mixture was diluted with water, and the resulting aqueous suspension was extracted with AcOEt (40 ml). The AcOEt layer was washed with water (3×30 ml), dried and evaporated to dryness. The residue was submitted to column chromatography on silica gel (30 g), and elution with benzene–acetone (7:1) gave 220 mg of **18** as a colorless oil. Crystallization from aq. MeOH yielded colorless plates, mp 175—176° (turned to needles at 160°). $[\alpha]_D^{25}$ +47.8°. UV λ_{max}^{EtOH} mμ (ε): 242 (1.63×10^4). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH); 1745 (acetyl); 1705 (ring ketones at C_3 and C_{16}); 1645 (C=C). NMR (δ): 2.00 (3H, s, $OCOCH_3$); 2.05 (3H, s, $OCOCH_3$); 2.09 (3H, s, $OCOCH_3$); 4.69 (1H, d, J=4.0 cps, C_{24}–H); *ca.* 5.0 (2H, overlapped signals, C_{11}–H and C_{23}–H). *Anal.* Calcd. for $C_{36}H_{54}O_9\cdot\frac{1}{2}H_2O$: C, 67.59; H, 8.67. Found: C, 67.59; H, 8.88.

Alisol A (24, 25)-Acetonide 23-Monoacetate (12)——To a stirred suspension of **2** (500 mg) in acetone (12 ml), BF_3 etherate (0.2 ml) was added at room temperature. After stirring for 15 min, the mixture was diluted with AcOEt and the AcOEt solution was washed successively with water, aq. 5% $NaHCO_3$ and water, and dried. After removal of the solvent, the residue was chromatographed on silica gel (30 g). Elution with benzene–acetone (10:1) gave a colorless oil of **12** (430 mg) which was crystallized from aq. MeOH to yield colorless needles, mp 192—193°. $[\alpha]_D^{22}$ +61.8°. NMR (δ): 2.02 (3H, s, $OCOCH_3$); 3.60 (1H, d, J=7.0 cps, C_{24}–H); 3.77 (1H, sext, C_{11}–H); 4.85 (1H, q, J=13.0 and 7.0 cps, C_{23}–H). *Anal.* Calcd. for $C_{35}H_{56}O_6$: C, 73.39; H, 9.85. Found: C, 73.37; H, 10.01.

Acetylation of compound **12** with Ac_2O–C_5H_5N at room temperature for 16 hr afforded the diacetate **13**, which was recrystallized from aq. MeOH to give colorless needles, mp 154—155°, $[\alpha]_D^{22}$ +72.5°. *Anal.* Calcd. for $C_{37}H_{58}O_7$: C, 72.27; H, 9.51. Found: C, 72.26; H, 9.39. Compound **13** was also obtained by acetonization of alisol A 11,24-diacetate (**16**) by the procedure above described.

Alisol A (24, 25)-Acetonide (11)——To a solution of **12** (120 mg) in MeOH (7 ml), anhydrous K_2CO_3 (70 mg) was added and the mixture was refluxed for 80 min. After dilution with water, the mixture was extracted with AcOEt, and the AcOEt extract was washed with water, dried and evaporated to drynesss. Crystallization of the residue from aq. MeOH gave colorless needles of **11** (80 mg), mp 92—93°, $[\alpha]_D^{22}$ +75.0°. *Anal.* Calcd. for $C_{33}H_{54}O_5\cdot\frac{1}{2}H_2O$: C, 73.43; H, 10.27. Found: C, 73.27; H, 10.00.

A solution of **11** (100 mg) in aq. 80% AcOH (4 ml) was heated at 90° for 2 hr. After evaporation of the solvent, the residue was dissolved in AcOEt (20 ml). The solution was washed with water, dried and evaporated to give an oil of alisol A (**1**) (85 mg), whose IR spectrum was superimposable with that of an authentic sample of **1**. The product was acetylated to afford alisol A triacetate (**3**) and the identity was confirmed by admixture and IR spectral comparison.

Deacetonization of 12 and 13——A solution of **12** (100 mg) in aq. 80% AcOH (4 ml) was heated at 90° for 1.5 hr. After dilution with water, the resulting precipitates were collected, washed with AcOEt to give 60 mg of crude **2**. Recrystallization from CH_2Cl_2–AcOEt gave a pure sample, mp 194—196°, alone or in admixture with an authentic sample of **2**. The IR spectra of the two products were also identical.

Treatment of compound **13** under a similar condition afforded alisol A 11,24-diacetate (**16**), and the identity was confirmed by admixture as well as by IR spectral comparison.

HIO_4 Oxidation of Alisol A Monoacetate (2)——A solution of **2** (200 mg) in dioxane (10 ml) was oxidized with $HIO_4\cdot2H_2O$ (500 mg), and the reaction mixture was processed as in the case of alisol A (**1**). 2,4-Dinitrophenylhydrazone (60 mg) of the volatile product was identified with authentic acetone 2,4-dinitrophenylhydrazone by admixture and IR spectral comparison.

The corresponding nor-compound was chromatographed on silica gel (18 g) and eluted with $CHCl_3$–AcOEt (3:1) to afford a colorless oil of the trisnoraldehyde monoacetate **17** (100 mg) which did not crystallize. $[\alpha]_D^{23}$ +185°. Mass (*m/e*): 454 ($M^+ - H_2O$). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH); 1740 (acetyl and aldehyde); 1705 (ring ketone). NMR (δ): 2.10 (3H, s, $OCOCH_3$); 3.76 (1H, sext, J=12.0 and 6.0 cps, C_{11}–H); 4.62 (1H, double d, J=8.0 and 4.0 cps, C_{23}–H); 9.40 (1H, s, CHO). *Anal.* Calcd. for $C_{29}H_{44}O_5\cdot H_2O$: C, 70.98; H, 9.45. Found: C, 71.09; H, 9.02.

Partial Deacetylation of Alisol A Triacetate (3) (Formation of the Diacetate 16)——Compound **3** (5 g), dissolved in MeOH (200 ml), was refluxed with anhydrous NaOAc (2.5 g) for 2.5 hr. After evaporation of the solvent, the residue was extracted with AcOEt (300 ml), and the AcOEt solution was washed with water, dried and evaporated to leave colorless crystals (4.8 g). The product was then chromatographed on silica gel (200 g) and eluted first with benzene–acetone (10:1) to recover 2 g of the starting material. The column was then eluted with benzene–acetone (5:1) to yield alisol A 11,24-diacetate (**16**) (1.7 g), which was recrystallized from MeOH to give colorless needles, mp 204—206°. $[\alpha]_D^{23}$ +86.7°. NMR (δ): 1.98 (3H, s, $OCOCH_3$); 2.14 (3H, s, $OCOCH_3$); 3.72 (1H, double d, J=8.0 and 4.0 cps, C_{23}–H); 4.44 (1H, d, J=1.0 cps, C_{24}–H); 4.88 (1H, sext, J=11.5 and 6.0 cps, C_{11}–H). *Anal.* Calcd. for $C_{34}H_{51}O_7$: C, 71.04; H, 9.47. Found: C, 70.91; H, 9.42.

Reacetylation of compound **16** with Ac_2O–C_5H_5N at room temperature gave the triacetate **3**.

HIO_4 Oxidation of Alisol A 11,24-Diacetate (16)——A solution of **16** (300 mg) in a mixture of dioxane (4 ml) and water (1 ml) was oxidized with $HIO_4\cdot2H_2O$ (180 mg) at room temperature for 1 hr. After dilution

with water, the reaction mixture was extracted with AcOEt and the organic layer was washed with water, dried and evaporated to dryness. The residue (270 mg) was chromatographed on silica gel (15 g) and eluted with benzene–acetone (10:1) to afford a colorless oil of the trisnoraldehyde diacetate **19** (250 mg), which did not crystallize. $[\alpha]_D^{23}$ +107.2° (c=0.2). IR ν_{max}^{KBr} cm^{-1}: 1740 (acetyl and aldehyde); 2750 (aldehyde); 1710 (ring ketone). NMR (δ): 1.98 (3H, s, $OCOCH_3$); 2.06 (3H, s, $OCOCH_3$); 4.59 (1H, double d, J=8.0 and 4.0 cps, C_{23}–H); 4.86 (1H, sext, J=12.0 and 6.0 cps, C_{11}–H); 9.41 (1H, s, CHO). *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.03; H, 8.53.

CrO_3 Oxidation of Alisol A (24, 25)-Acetonide 23-Monoacetate (12)——Compound **12** (500 mg), dissolved in C_5H_5N (6 ml), was oxidized with CrO_3 (500 mg) in C_5H_5N (6 ml) at room temperature for 16 hr. After dilution with water, the mixture was extracted with ether and the ethereal extract was washed with water, dried and evaporated. The residue was chromatographed on silica gel (30 g) and eluted with benzene–acetone (40:3) to give a faint yellow oil of **15** (400 mg). Crystallization of the oil from aq. MeOH afforded pale-yellow needles, mp 165—166°. $[\alpha]_D^{24}$ −27.6°. IR ν_{max}^{KBr} cm^{-1}: 1745 (acetyl); 1703 (C_3-ketone); 1645, 1623 and 1604 (–CO–C=C–C=C–). UV λ_{max}^{EtOH} mμ (ε): 292 (1.54×10^4). NMR (δ): 2.03 (3H, s, $OCOCH_3$); 3.67 (1H, d, J=8.0 cps, C_{24}–H); 5.04 (1H, m, C_{23}–H); 5.72 (1H, s, C_{12}–H); 6.18 (1H, t, J=2.5 cps, C_{16}–H). *Anal.* Calcd. for $C_{35}H_{52}O_6$: C, 73.91; H, 9.22. Found: C, 73.80; H, 9.20.

Acknowledgement The authors wish to thank the members of analysis section for elementary analyses and physico-chemical measurements. Thanks are also due to Mr. M. Shinohara for his technical assistance.