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Biological-Active Triterpenes of Alismatis Rhizoma. IV.[1] The Structures of Alisol B, Alisol B Monoacetate and Alisol C Monoacetate — Some Reactions of the α-Hydroxy Epoxide of the Alisol B Derivatives[2]

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Studies on the structures of alisol B, alisol B monoacetate and alisol C monoacetate, the new biological-active triterpenes of Alismatis Rhizoma, are reported. The former two compounds have been correlated to alisol A (**1**) establishing their structures as shown **2** and **4**, respectively. The structure of alisol C monoacetate has been clarified to be 16-oxoalisol B 23-monoacetate (**21**) on the basis of correlation of the compound with **4**.

Stereochemical courses and mechanisms of some epoxide cleavage reactions of **2**, **4** and alisol B diacetate (**8**) are also described.

As reported in the earlier papers, alisol A and its monoacetate, the hypocholesterolemic triterpenes of Alismatis Rhizoma, were shown to have structure **1** and **3**, respectively.[1] In a continuation of the study on the new triterpenes of the drug, the present paper deals with the structures of alisol B, alisol B monoacetate and alisol C monoacetate. Stereochemical courses and mechanisms of some epoxide cleavage reactions of alisol B derivatives are also described.

Alisol B and Alisol B Monoacetate

Alisol B, $C_{30}H_{48}O_4$, showed a carbonyl band at 1705 cm^{-1} in the infrared (IR) spectrum. Acetylation of the compound gave the diacetate **8** showing the presence of two hydroxyl groups. The nuclear magnetic reasonance (NMR) spectrum of the parent substance displayed the signals assignable to two C<u>H</u>–OH protons at 3.14 and 3.77 ppm (δ). In the spectrum of the diacetate **8**, these protons showed downfield shift to 4.54 and 4.89 ppm, respectively. The diacetate **8** showed no hydroxyl band in the IR spectrum. Two secondary hydroxyl groups are there for present in alisol B (**2**).

Alisol B exhibited intense mass peaks at *m/e* 122, 150 (base peak), 311 and 329; these peaks were also noticed in the spectrum of alisol A (**1**), suggesting a close relationship between **1** and **2**. This was evident from oxidation of alisol B (**2**) with periodic acid yielding acetone and the tetranoraldehyde **9**, which were also obtained from alisol A (**1**) by the same oxidation. The resultse stablished that alisol B (**2**) has the tetracyclic nucleus identical with that of alisol A (**1**). Thus the side chain of **2** must bear one secondary hydroxyl group and the fourth oxygen of unknown function. Reduction of alisol B (**2**) with sodium borohydride afforded dihydroalisol B (**10**) which lacked the carbonyl band in the IR spectrum. Compound **10** gave the triacetate **12** upon acetylation, which showed no hydroxyl absorption in the IR

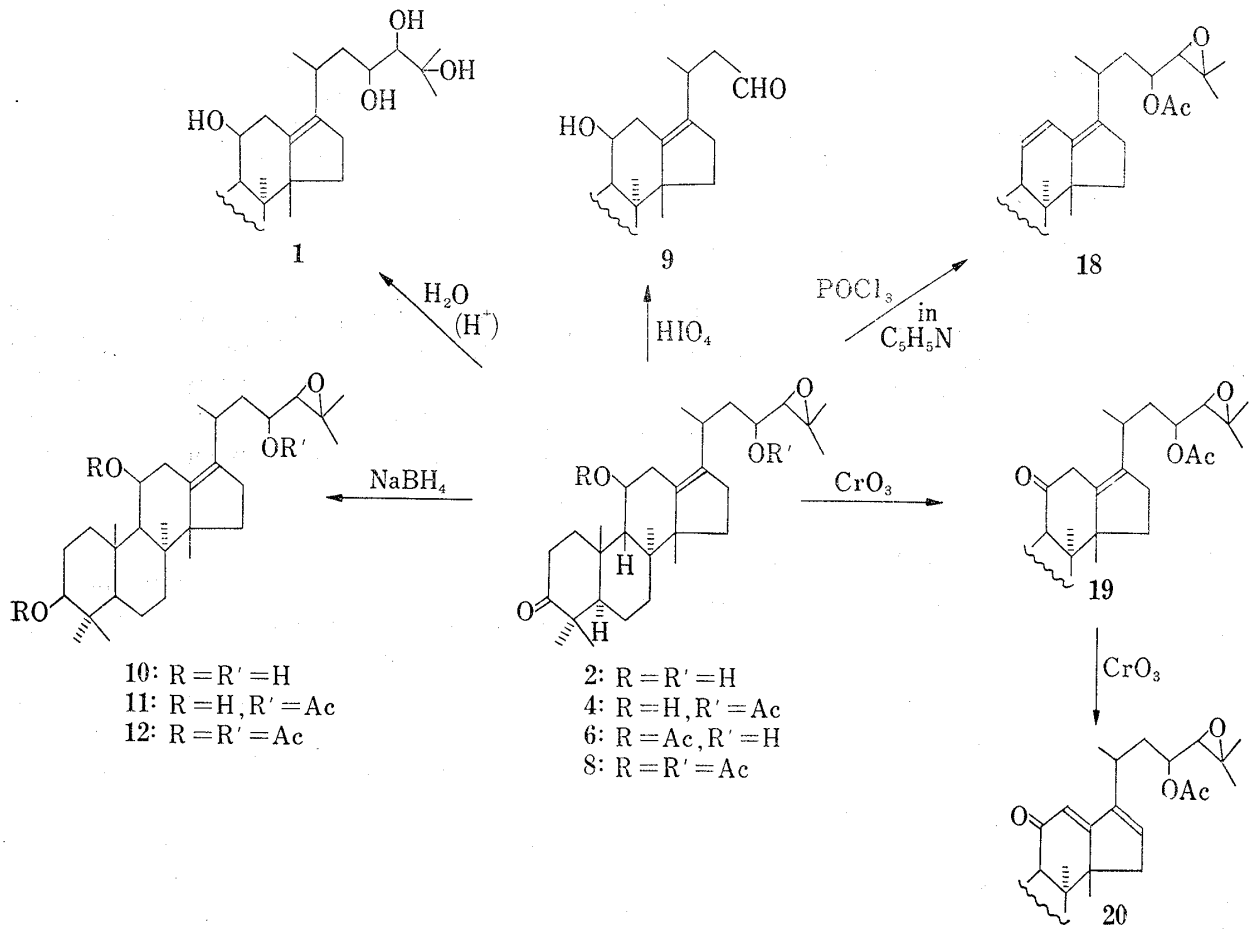
1) *a*) Part I: T. Murata, Y. Imai, T. Hirata and M. Miyamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1347 (1970); *b*) Part II: T. Murata and M. Miyamoto, *ibid.*, **18**, 1354 (1970); *c*) Part III: K. Kamiya, T. Murata and M. Nishikawa, *ibid.*, **18**, 1362 (1970).

2) A part of this work was preliminarily reported in *a*) T. Murata, M. Shinhara, T, Hirata, K. Kamiya, M. Nishikawa and M. Miyamoto, *Tetrahedron Letters*, **1968**, 103; *b*) *Idem.* T. Murata, M. Shinohara, T. Hirata and M. Miyamoto, **1968**, 849; *c*) Represented at the 11th Symposium on the Chemistry of Natural Products, Kyoto, Oct. 1967.

3) Location: *Juso-Nishino-cho, Higashiyodogawa-ku, Osaka.*

spectrum. These results indicated that no additional carbonyl group existed in the molecule of alisol B (**2**) other than the C_3-carbonyl.

The NMR spectrum of alisol B diacetate (**8**) showed two low field methyl singlets at 1.29 and 1.32 ppm besides the methyl signals (total 18H, six methyls) in the region of 1.00 to 1.12 ppm. The spectrum also revealed a one-proton doublet at 2.66 ppm (J=8.5 cps) together with the two CH–OAc protons at 4.54 (sextet, J=8.5 cps and 3.5 cps) and at 4.89 ppm (sextet, J=12.0 and 6.0 cps), respectively. The spin-decoupling experiment clarified that there existed a spin-spin coupling of J=8.5 cps between the signal at 2.66 ppm and the one at 4.45 ppm. The signal at 4.89 ppm can reasonably be accounted for by the C_{11}-proton because of its characteristic coupling constant values.[1b]



10: R=R'=H
11: R=H, R'=Ac
12: R=R'=Ac

2: R=R'=H
4: R=H, R'=Ac
6: R=Ac, R'=H
8: R=R'=Ac

Chart 1

Consideration of these NMR data and the formation of acetone upon periodate oxidation of alisol B (**2**) led us to the assumption that a 23-hydroxy-24,25-oxide moiety should be present in the molecule of **2**.[2b] Treatment of alisol B (**2**) with sulfuric acid or *p*-toluenesulfonic acid in aqueous dioxane afforded alisol A (**1**). This conversion almost certainly established the structure of the side chain of **2** except the stereochemistry at C_{24}.

Lithium aluminum hydride reduction of **2**, followed by acetylation yielded two products. The first of these ,the major one, was the tetrol tetraacetate (**13**) which was shown to be identical with the compound obtained from alisol A (**1**) by the following reactions: Alisol A triacetate (**7**) was dehydrated with thionyl chloride in pydirine to give the 25-anhydro compound **16**. The structure assigned to the latter was supported by its NMR spectrum which exhibited the signals due to the isopropenyl group at 1.69 (3H, singlet) and at 4.92 ppm (2H, singlet). In addition to this, compound **16** showned the IR bands at 3085, 1655 and 900 cm^{-1}, the charac-

teristic bands of an exomethylene double bond.[4] Compoumd **16** was catalytically reduced with palladium–charcol to afford the 25-deoxyalisol A derivative **17** which lacked the above signals and absorption bands. Reduction of compound **17** with sodium borohydride followed by acetylation gave the terol tetraacetate **13** which was indistinguishable from the one obtained from alisol B (**2**) in all respects. The correlation has now established the presence of the 23-hydroxy-24,25-oxide moiety in alisol B (**2**) as well as the stereochemistry at C_{23} and C_{24} which are shown to be identical with that of alisol A (*i.e.* 23*S*, 24*R*).

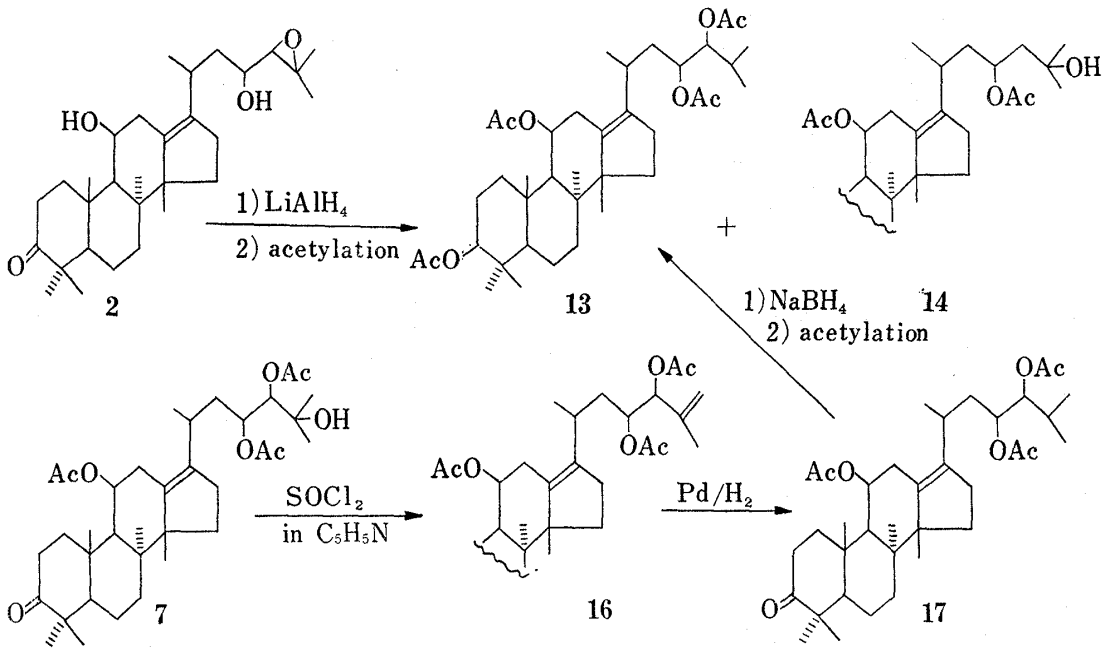


Chart 2

The second product in the lithium aluminum hydride reduction of alison B (**2**) was the triacetate of another tetrol. Structure **14** was allotted to it on the ground of its spectroscopic properties: Compound **14** showed a hydroxyl absorption at 3500 cm^{-1} in the IR spectrum, while **14** was unaffected by further acetylation at room temperature. No signal attributable to C<u>H</u>–OH protons was noticed in the NMR spectrum of **14**, thus indicating the presence of one tertiary hydroxyl group in **14**.

That the natural alisol B monoacetate is alisol B 23-monoacetate (**4**) was established by the fact that the NMR spactrum of the compound showed a sextet (J=8.5 and 3.5 cps) at 4.60 ppm and another sextet (J=12 and 6 cps) at 3.77 ppm; the former was reasonabley assigned to the CH–OAc proton at C_{23} and the latter sextet to the CH–OAc proton at C_{11}. In addition to this, partial deacetylation of alisol B diacetate (**8**) with sodium acetate in methanol yielded another monoacetate, alisol B 11-monoacetate (**6**) whose structure was proved by its NMR spectrum. Further reactions of alisol B 23-monoacetate (**4**) provided evidence not only for the presence of an acetyl group at C_{23} but also for the presence of double bond at $C_{13,17}$ in the molecule of **4**. Dehydration of **4** with phosphor oxychloride in pyridine afforded the 11,13 (17)-diene derivative **18** whose structure was supported by its NMR spectrum which showed signals due to the olefinic protons at 5.77 (1H, doublet, J=10.0 cps) and at 6.10 ppm (1H, double doublet, J=10.0 and 2.5 cps), respectively.[5] The ultraviolet (UV) spectrum, with λ_{max} 253 mμ, ε 1.89×10^4, was also consistent with the presence of a heteroannular diene.[6a,b] On the other hand, oxidation of compound **4** with a large excess of chromium trioxide–pyridine

4) L.J. Bellamy, "Infra-red Spectra of Complex Molecules," Second Edition, Methuen & Co., London, 1958, p. 34.

complex gave a conjugated dienone derivative **20**. The structure of compound **20** was supported by the spectral evidence: Its IR spectrum showed the bands at 1640, 1625 and 1600 cm^{-1} indicating the presence of a grouping CO–C=C–C=C in the molecule and the UV maximum at 292 mμ (ε 1.60×10^4) also confirmed the presence of a conjugated dienone group.[6b,c] Furthermore, the NMR signals (one–proton each) at 5.62 (singlet) and 6.12 ppm (triplet, J=2.5 cps) were attributed to the olefinic protons at C_{12} and C_{16}, respectively. The dienone **20** must be derived from the intermediate 11-oxo compound **19** as shown in Chart 3. Actually the compound **19** was obtained by oxidation of **4** with a nearly equimolar amount of chromium trioxide, and **19** eventually yielded the dienone **20** upon further oxidation.

High resolution mass spectrometry revealed that the mass peaks of alisol B (**2**) at *m/e* 150 (base peak) and at 122 corresponded to $C_{10}H_{14}O$ and C_9H_{14}, respectively. Since the latter is presumably due to the loss of carbon monooxide from the former, these peaks can well be assigned to the fragments which are consisted of the A ring of the molecule.[7]

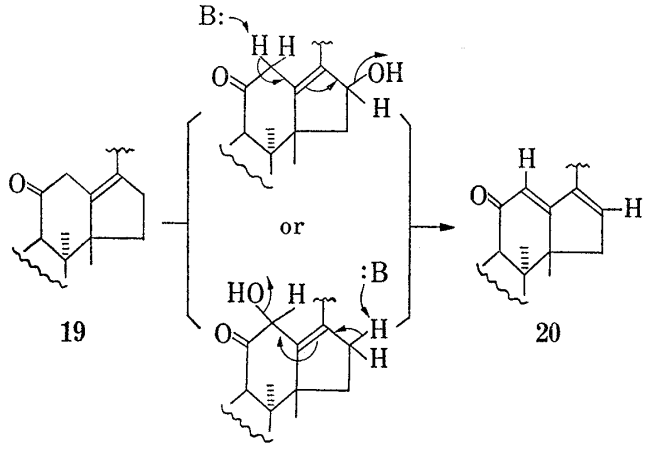
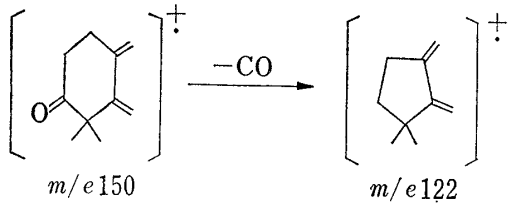


Chart 3



m/e 150 *m/e* 122

Alisol C Monoacetate

The structure of alisol C monoacetate has been established as **21** on the basis of correlation of the compound with alisol B 23-monoacetate (**4**): In comparison with alisol B 23-monoacetate (**4**), alisol C monoacetate (**21**) added intence IR bands at 1690 and 1642 cm^{-1} besides the bands at 1705 (ring ketone), 1745 (ester) and 3470 cm^{-1} (hydroxyl); the latter three being also noticed in the spectrum of **4**. The presence of one secondary hydroxyl bearing acetyl group in **21** was suggested by the three–proton singlet at 2.05 (CH_3COO) and a one–proton sextet–like signal at 4.49 ppm (CH–OAc) in the NMR spectrum of the compound. The spectrum also displayed a one–proton sextet at 3.96 ppm which was well attributable to the carbinyl proton at C_{11} as in the case of alisol B 23-monoacetate (**4**). Alisol C monoacetate (**21**) gave the diacetate **22** upon acetylation which now lacked the hydroxyl band in the IR spectrum.

A close similarity between the patterns of the CH–OAc proton signal in alisol C monoacetate (**21**) and of the C_{23}-proton signal in alisol B 23-monoacetate (**4**) suggested the existence of a 23-acetoxyl-24,25-oxide moiety in the molecule of **21**: The one–proton signal attributable to the C_{24}-epoxide proton appeared at 2.70 ppm as a doublet (J=9.0 cps) which changed into a singlet upon irradiation at 4.49 ppm (CH–OAc proton at C_{23}). The UV spectrum of **21** had the maximum at 246 mμ (ε 1.40×10^4), indicating the presence of an α,β-unsaturated ketone,[6b,c]

5) N.S. Bhacca and D.H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden–Day Inc., San Francisco, 1964, p. 85.
6) *a*) D. Arigoni, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **38**, 222 (1955); *b*) L. Fieser and M. Fieser, "Steroids," Reinhold Publishing Cooperation, New York, 1959, p. 15; *c*) H.H. Jaffe and M. Orehin, "Theory and Applications of Ultraviolet Spectroscopy," John Willey & Sons, Inc., New York, 1962, p. 196.
7) K. Biemann, "Mass Spectrometry Organic Chemical Applications," McGraw–Hill Co., New York, 1962, p. 334.

whereas its NMR spectrum revealed no signals assignable to olefinic protons. The UV maximum shifted to 286.5 mμ in the anhydro compound **23** which was obtained by treatment of **21** with thionyl chloride in pyridine. Treatment of **21** with potassium carbonate in hot methanol, followed by acetylation also yielded compound **23**. The NMR spectrum of the 11-anhydro compound **23** exhibited the two newly formed olefinic protons as two double doublets with a larger splitting of 10.0 cps at 6.11 and 6.47 ppm, respectively, characteristic of *cis*-olefinic protons signals.[5] The results can best be rationalized by assuming the presence of a 13 (17)-en-16-oxo moiety in alisol C monoacetate (**21**) and of an 11,13(17)-dien-16-oxo group in the molecule of the anhydro compound **23**. The bands at 1690 and 1642 cm^{-1} (CO–C=C) in the IR spectrum of **21** could reasonably be assigned to the α,β-unsaturated, five-membered ring ketone at this stage. Since alisol C monoacetate (**21**) had no signals ascribable to methyl group attached to a double bond, possibility of a 17(20)-en-16-oxo structure for the compound was excluded. Further, alisol C monoacetate (**21**) showed no aldehyde proton in its NMR spectrum. On the ground of above observations it was concluded that alisol C monoacetate had the structure **21**. Evidence supporting this conclusion was obtained by chromic acid oxidation of alisol B 11,23-diacetate (**8**) which yielded alisol C 11,23-diacetate (**22**).

Treatment of alisol C monoacetate (**21**) with *p*-toluenesulfonic acid in aqueous dioxane, followed by acetylation gave compound **24** which was shown to be identical with the one obtained from alisol A triacetate (**7**) by chromic acid oxidation thus demonstrating that the epoxide hydration of alisol C monoacetate (**21**) also took place with retention of the configuration at C_{24} as is seen later in the case of alisol B monoacetate (**4**).

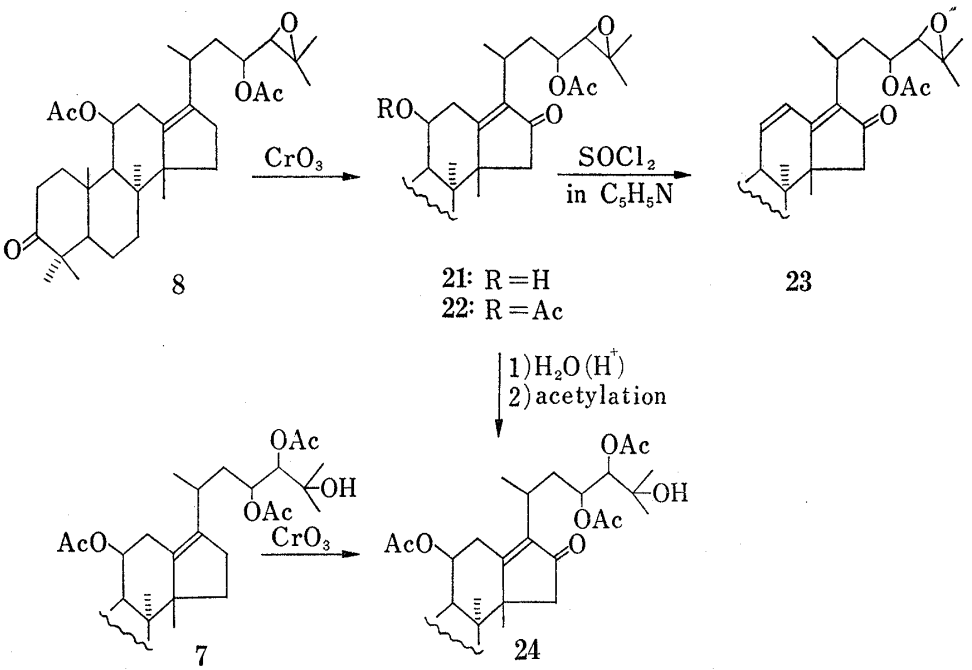
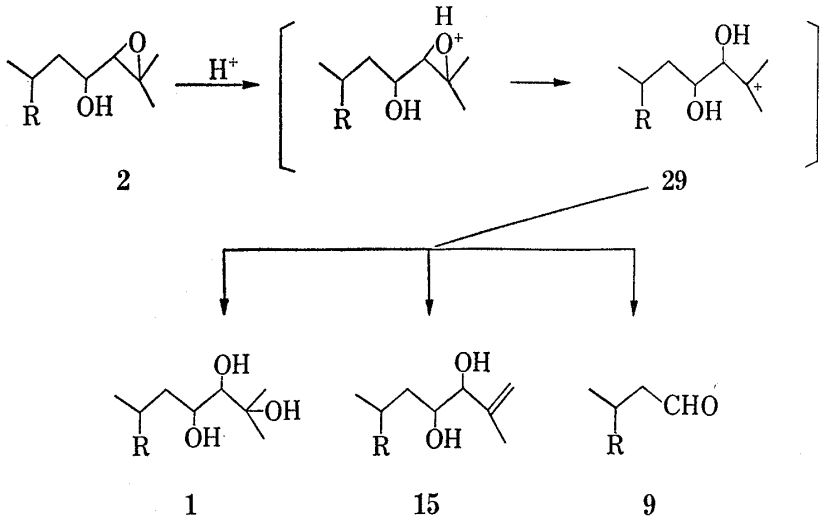


Chart 4

Stereochemical Courses in Some Epoxide Cleavage Reactions of the Alisol B Derivatives

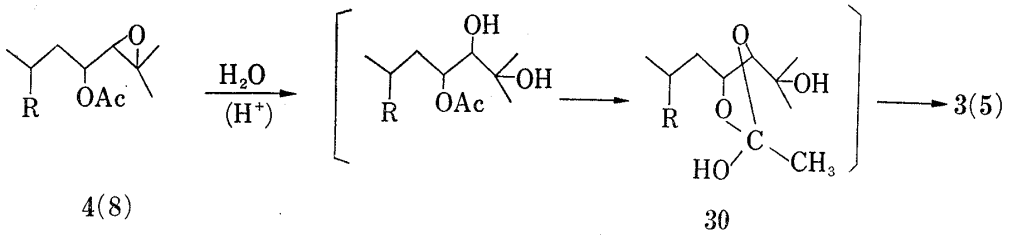
At this stage attention was turned to the stereochemical courses in some epoxide cleavage reactions of alisol B and related compounds. First, hydration of the epoxide in alisol B (**2**) with sulfuric, hydrochloric, oxalic or *p*-toluenesulfonic acid was shown to proceed with the configuration retention at C_{24} to give alisol A (**1**). The yield of this conversion was about 60%. Epi-alisol A (**25**), a corresponding epimer at C_{24} which was obtained by hydration of alisol B (**2**) in alkaline solution, could not be found in the above reaction mixture. In the light of knowledge on epoxide reaction mechanism, S_N2 nucleophilic attack in acid appears

to take place at C_{25} of **2**, because acid-catalysed cleavage should involve Walden inversion at the optical active center.[8] Treatment of alisol B (**2**) with acid alsoy ielded 25-anhydroalisol A (**15**) and the tetranoraldehyde (**9**) as the minor products, which are apparently derived from the carbonium ion **29**.[8]



R=the tetracyclic nucleus of the molecule

Chart 5



R=the tetracyclic nucleus of the molecule

Chart 6

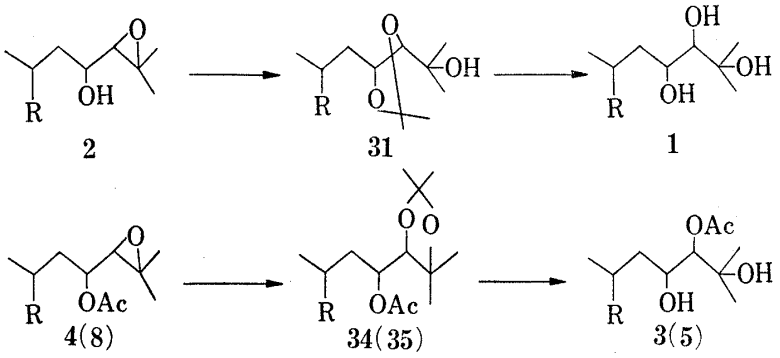
Epoxide hydration of alisol B 23-monoacetate (**4**) and alisol B 11,23-diacetate (**8**) in acid were demonstrated to undergo with acetyl migration from C_{23} to C_{24} hydroxyl group; thus the products were alisol A 24-monoacetate (**3**) and alisol A 11,24-diacetate (**5**), respectively. The reaction was also found to be almost stereospecific; only a trace of epi-alisol A triacetate (**28**) was obtained by acetylation of the mother liquor of **3** or **5**, followed by silica gel chromatography. The results are explicable by assuming that hydration of the epoxide must be followed by acetyl migration from C_{23} to C_{24} *via* the possible orthoester intermediate **30**,[9] to retain the configuration at C_{24}.

Acetonization of alisol B (**2**) with boron trifluoride–ether complex in acetone yielded alisol A (23, 24)-acetonide (**31**), while treatment of alisol B 23-monoacetate (**4**) and alisol B 11,23-diacetate (**8**) under similar condition afforded alisol A (24, 25)-acetonide-23-monoacetate

8) *a*) R.E. Parker and N.S. Isaacs, *Chem. Rev.*, **59**, 737 (1959); *b*) Rosowsky, "Heterocyclic Compounds with Three and Four Membered Rings," Part 1, Chapter 1 (Ethylene Oxides), ed. by A. Weissberger, Interscience Publishers, New York, 1964, p. 270.

9) R.U. Lemieux, "Molecular Rearrangements," ed. by P. de Mayo, Interscience Publishers, New York, 1964, p. 709.

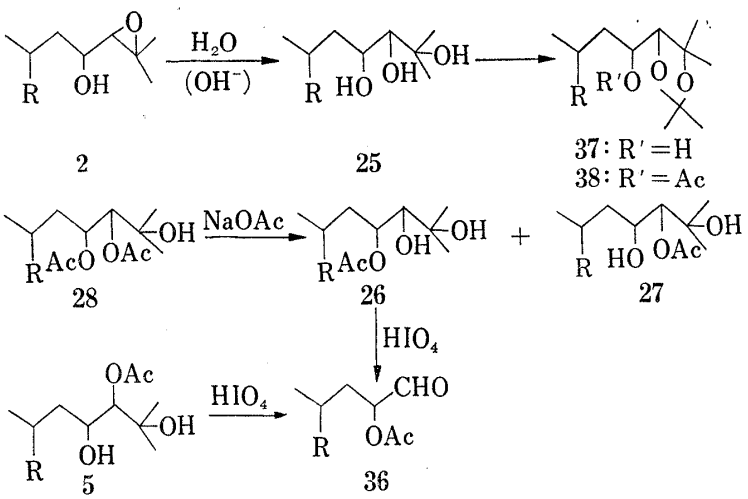
(**34**) and alisol A (24, 25)-acetonide-11,23-diacetate (**35**). Compounds **34** and **35** were identical with the ones obtained by acetonization of alisol A 24-monoacetate (**3**) and alisol A 11,24-diacetate (**5**), respectively.



R=the tetracyclic nucleus of the molecule

Chart 7

Prolonged treatment of alisol B or its acetate **4** or **8** with potassium carbonate in aqueous methanol gave a compound named epi-alisol A in a rather poor yield. Small amount of alisol A (**1**) also formed in this reaction. Treatment of **1** under this condition did not give epi-alisol A. Acetylation of epi-alisol A gave the triacetate **28**; the mass spectrum of the latter were superimposable with that of alisol A triacetate (**7**), whereas the physical constants of compound **28** are quite different from that of **7**. Since periodate oxidation of epi-alisol A (**25**) yielded acetone and the tetranoraldehyde **9**, it was suggested that compound **25** is an epimer of alisol A at C_{23} or/and C_{24}. Partial hydrolysis of epi-alisol A triacetate (**28**) with sodium acetate afforded two diacetates namely epi-alisol A 11,23-diacetate (**26**) and epi-alisol A 11,24-diacetate (**27**); the latter being a minor product. Oxidation of **26** with periodic acid gave the trisnoraldehyde diacetate (**36**) which was indistinguishable from the compound obtained from alisol A 11,24-diacetate (**5**) by the same oxidation. The results so far established the structure of epi-alisol A (**25**) as a C_{24}-epimer of alisol A, provided that the C_{23} configuration was unaltered during the periodate oxidation of alisol A 11,24-diacetate (**5**), which must undergo with acetyl migration from C_{24} to C_{23} hydroxyl group, as was seen in the acetonization of compound **5** yielding the 24,25-acetonide-11,23-diacetate **35**.[1b,9]



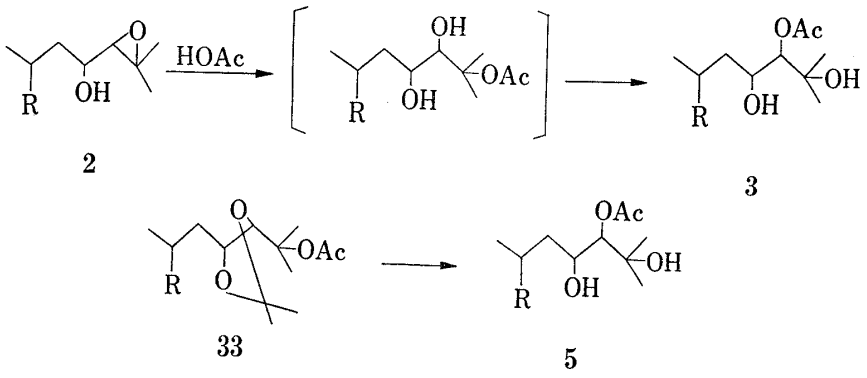
R=the tetracyclic nucleus of the molecule

Chart 8

On the basis of the fact that in hydration of some unsymmetrical epoxides, nucleophilic attacks in acid and base take place at the different carbons,[8] the structure assigned to epi-alisol (**25**) was supported by the difference in the hydration products of alisol B (**2**) in acid and alkaline solution. Acid-catalyzed ring opening of the epoxide yielded alisol A (**1**) to retain the configuration at C_{24} as stated above, whereas base-initiated cleavage led predominantly

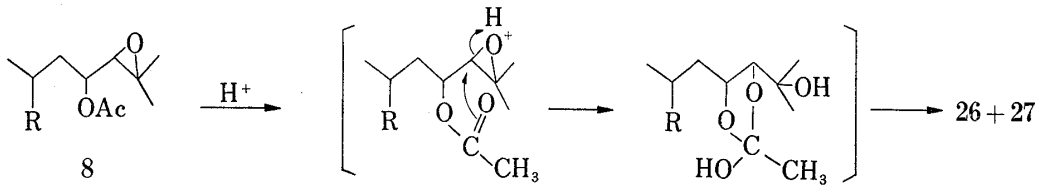
to an epimer of alisol A. Thus it is reasonable to conclude that nucleophilic attack occurred, contrary to the case of acid hydrolysis, mainly at C_{24} in alkaline solution to afford epi-alisol A, the C_{24}-epimer (23*S*, 24*S*; *erythro* configuration) of alisol A (**1**).[10] In contrast with alisol A, epi-alisol A (**25**) gave the (24,25)-acetonide **37** which without isolation was characterized as its diacetate **38**. The difficulty of formation of alternative (23,24)-acetonide of epi-alisol A would possibly be due to the *erythro* relationship at C_{23} and C_{24} of epi-alisol A, because the configuration would not facilitate the formation of a *cis*-acetonide at the positions.[11]

Stereochemical specificity is also manifest in acetolysis of alisol B (**2**) with hot glacial acetic acid to give rise to alisol A 24-monoacetate (**3**) in 60% yield, and alisol A (**1**), 25-anhydro-alisol A (**15**) and the tetranoraldehyde **9** as minor products. No derivative of epi-alisol A (**25**) was obtained from the reaction mixture. If nucleophilic attack occurred at C_{24}, epi-alisol A 24-monoacetate should have been obtained.[8b] The discrepancy may be solved by assuming that C_{25} was attacked initially in the acetolysis of **2** and that the resulting alisol A 25-monoacetate was converted into alisol A 24-monoacetate (**3**) through acetyl migration. Support for above assumption comes from deacetonization of alisol A (23,24)-acetonide 11,25-diacetate (**33**) with hot aqueous acetic acid which yielded alisol A 11,24-diacetate (**5**) as a sole product. The result shows that C_{25} acetyl group migrates spontaneously to C_{24} hydroxyl under acidic condition.



R=the tetracyclic nucleus of the molecule

Chart 9



R=the tetracyclic nucleus of the molecule

Chart 10

Treatment of alisol B 11,23-diacetate (**8**) with hot glacial acetic acid gave alisol A 11,24-diacetate (**5**), alisol A triacetate (**7**), epi-alisol A 11,23-diacetate (**26**) and epi-alisol A 11,24-

10) Epi-alisol A (**25**) was also obtained together with alisol A (**1**) from the unsaponifiable lipid fraction of Alismatis Rhizoma.[2a] Since **25** was not obtained from the natural lipid fraction, it was concluded that previously reported epi-alisol A (**25**) was an artefact during saponification. The compound showed a positive hypocholesterolemic action in the biological test; the efficacy being 20% at the dose level of 0.1%.[1a]

11) G.J. Breen, E. Ritchie, W.T.L. Sidwell and W.C. Taylor, *Australian J. Chem.*, **19**, 455 (1965).

diacetate (**27**) in decreasing yields. Above results show that hydration of the epoxide took place preferentially to acetolysis of the epoxide and that there formed a significant amount of two epi-alisol A diacetates (**26** and **27**); the latter fact would possibly be ascribed to the presence of acetyl group at C_{23} hydroxyl in **8**, which attacks the backside of C_{24} as a nucleophile,[12] as depicted in Chart 10.

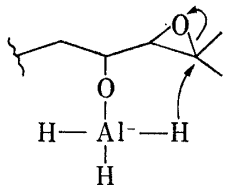


Chart 1

It is necessary at this point to return to the lithium aluminum hydride reduction of alisol B (**2**). Lithium aluminum hydride reduction is known generally to occur in a manner to give a more highly substituted alcohol, whereas some unsymmetrically substituted epoxides are shown to open in both directions.[8] The result obtained by lithium aluminum hydride reduction of alisol B (**2**) shows that nucleophilic attack had taken place at C_{25} preferentially to C_{24}. This is presumably due to the neighbouring group effect of the C_{23} hydroxyl as written in Chart 1.[2b]

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 noted. The NMR spectra were recorded 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; o, octet; m, multiplet. For column chromatography silica gel (Merck, ϕ 0.05—0.2 mm) was used; for thin-layer chromatography Silica Gel G (Merck) was used.

Alisol B (**2**)——Colorless prisms from AcOEt, mp 166—168°. Mass (*m/e*): 454 ($M^+ - H_2O$), 329, 311, 150 (base peak), 122. High resolution mass spectrometry (*m/e*): Calcd. for $C_{10}H_{14}O$, 150.1045. Found, 150.1014; Calcd. for C_9H_{14}, 122.1095. Found, 122.1140. IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 1705 (ring ketone). NMR (δ): 2.61 (1H, d, $J=8.0$ cps, C_{24}–H), 3.14 and 3.77 (1H, each broad signals, C<u>H</u>–OH×2).

Alisol B 23-Monoacetate (**4**)——Colorless prisms from AcOEt–*n*-hexane, mp 162—164°. IR ν_{max}^{Nujol} cm^{-1}: 3470 (OH), 1745 (acetyl), 1705 (ring ketone). NMR (δ): 2.06(3H, s, $OCOCH_3$), 2.72 (1H, d, $J=8.5$ cps, C_{24}–H), 3.77 (1H, broad sext, $J=12$ and 6 cps, C_{11}–H), 4.60 (1H, sext, $J=8.5$ and 3.5 cps, C_{23}–H).

A solution of **4** (50 mg) in 2% methanolic K_2CO_3 (5 ml) was refluxed for 1 hr. After evaporation of the solvent water was added to the residue, and the mixture was extracted with AcOEt. The AcOEt extract was washed with water, dried and evaporated to dryness. The residue (40 mg) was crystallized from AcOEt to give colorless prisms of **2** (25 mg), mp 166—168°, alone or in admixture with an authentic specimen of **2**. The IR spectra were also identical.

Alisol B Diacetate (**8**)——A solution of **2** or **4** (400 mg) in a mixture of Ac_2O (2 ml) and C_5H_5N (2 ml) was allowed to stand at room temperature for 17 hr. Work up in the usual manner gave the acetate **8** (200 mg). Recrystallization from MeOH afforded colorless plates, mp 143—145°, $[\alpha]_D^{23}$ +117.5°. No OH absorption was observed in its IR spectrum ($CHCl_3$). NMR (δ): 1.00—1.12 (total 18H, overlapped signals, $CH_3\times6$), 1.29 (3H, s, CH_3), 1.32 (3H, s, CH_3), 2.00 (6H, s, $OCOCH_3\times2$), 2.66 (1H, d, $J=8.5$ cps, C_{24}–H), 4.54 (1H, sext, $J=8.5$ and 3.5 cps, $C_{23}\langle^{H}_{OAC}$), 4.89 (1H, sext, $J=12.0$ and 6.0 cps, $C_{11}\langle^{H}_{OAC}$).

Compound **8** (2 g) in a mixture of MeOH (25 ml) and K_2CO_3 (2 g) was refluxed for 2 hr. After dilution with water, the mixture was extracted with AcOEt (200 ml). The AcOEt layer was washed with water, dried and evaporated to afford a colorless oil (2 g). Crystallization of the product from AcOEt gave colorless prisms of **2** (1.2 g).

Alisol B 11-Monoacetate (**6**)——To a solution of **8** (1 g) in MeOH (20 ml), anhydrous NaOAc (2 g) was added and the mixture was refluxed for 20 hr. After evaporation of the solvent the residue was extracted with AcOEt (80 ml). The AcOEt solution was washed with water (40 ml), dried and evaporated to afford a colorless oil (950 mg). The product was chromatographed on silica gel (50 g) and eluted with benzene–acetone (20:1) to recover 20 mg of the starting material **8**. Subsequent elution with benzene–acetone (10:1) gave 0.6 g of **6**, which was recrystallized from EtOH to yield colorless plates, mp 88—90°, $[\alpha]_D^{23}$ +113° ($c=0.2$, chloroform). IR ν_{max}^{KBr} cm^{-1}: 1745 (acetyl), 1710 (ring ketone). NMR (δ): 1.98 (3H, s, $OCOCH_3$), 3.14 (1H, sext, $J=8.0$ and 3.0 cps, $C_{23}\langle^{H}_{OH}$), 4.90 (1H, sext, $J=11.0$ and 6.0 cps, $C_{11}\langle^{H}_{OAC}$). *Anal.* Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.52; H, 9.75.

12) S. Julia and B. Fürer, *Bull. Soc. Chim. France*, **1966**, 1106.

Partial Acetylation of 2——To a solution of **2** (50 g) in C_5H_5N (150 ml), Ac_2O (150 ml) was added and the mixture was allowed to stand at room temperature for 30 min. The mixture was poured into ice–water and the resulting oil was dissolved in AcOEt. The AcOEt solution was washed with water, dried and evaporated to dryness. The residue (52 g) thus obtained was chromatographed on silica gel (750 g), and eluted with benzene–acetone (20:1) to give compound **8** (12 g). The column was then eluted with benzene–acetone (10:1) to afford a mixture (34 g) of **4** and **6**. Further elution with AcOEt recovered the starting material **2** (5 g). The monoacetate mixture above obtained was once recrystallized from MeOH to give the 23-monoacetate **4** (20 g), which was identified with the compound of natural origin through admixture and comparison of the IR spectra. The NMR spectrum of the mother liquor of **4** showed two sextets near 4.5 and 4.9 ppm, which were attributable to the CH–OAc protons at C_{23} of **4** and C_{11} of **6**, respectively. The intensity ratio C_{23}–H/C_{11}–H was approximately 4:1. No diacetate **8** was contained in the mother liquor portion according to TLC; acetylation of the mixture gave the diacetate **8**.

HIO_4 Oxidation of 2——To a solution of **2** (1 g) in dioxane (30 ml), $HIO_4 \cdot 2H_2O$ (1 g) and water (10 ml) were added and the mixture was warmed at 50° for 30 min. The volatile product was introduced into 2,4-dinitrophenylhydrazine–H_3PO_4 solution as in the case of the oxidation of alisol A.[1b] The yellow precipitates were collected, washed with water and dried to obtain 180 mg of acetone 2,4-dinitrophenylhydrazone. Recrystallization from MeOH furnished a pure hydrazone, mp 122—124° (decomp.), alone or in admixture with an authentic specimen.

Above reaction mixture was diluted with water and the resulting precipitates were collected, washed with water and dried. The product (700 mg) was recrystallized from CH_2Cl_2–AcOEt to give a pure sample of the tetranoraldehyde **9** (500 mg). The identity was confirmed by the direct comparison with the compound obtained from alisol A (**1**).

Dihydroalisol B (**10**)——To a stirred solution of **2** (3 g) in MeOH (30 ml), $NaBH_4$ (0.6 g) was added in small portion during 30 min at room temperature, and stirring was continued for further 30 min. After evaporation of the solvent the residue was treated with AcOEt (300 ml). The AcOEt solution was washed with water (3×50 ml), dried and evaporated. The residue was then crystallized from *n*-hexane–AcOEt to give 2.2 g of **10**. Recrystallization from AcOEt yielded colorless needles, mp 137—138°, $[\alpha]_D^{22}$ +46.5°. No carbonyl band was noticed in its IR spectrum. *Anal.* Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.81; H, 10.71.

Acetylation of compound **10** with Ac_2O–C_5H_5N and crystallization of the product from MeOH gave dihydroalisol B triacetate (**12**) as colorless needles, mp 195—197°. $[\alpha]_D^{23}$ +66.5°. Mass (*m/e*): 540 (M^+ –AcOH). *Anal.* Calcd. for $C_{36}H_{56}O_7$: C, 71.96; H, 9.40. Found: C, 72.09; H, 9.38.

Dihydroalisol B 23-Monoacetate (**11**)——Compound **4** (1.5 g), dissolved in a mixture of MeOH (6 ml) and AcOH (0.8 ml), was reduced with $NaBH_4$ (0.4 g) at room temperature for 2 hr. The reaction mixture was processed as above and the product was chromatographed on silica gel (50 g). Elution with benzene–acetone (6:1) gave the starting material **4** (480 mg) and dihydroalisol B 23-monoacetate (**11**) (550 mg), successively. Upon crystallization of the latter from MeOH, colorless needles of **11** were obtained. mp 108—110°, $[\alpha]_D^{22}$ +46.8°. Mass (*m/e*): 516 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1745 (acetyl). NMR (δ): 2.02 (3H, s, $OCOCH_3$), 2.64 (1H, d, J=8.5 cps, C_{24}–H), 3.19 (1H, q, J=9 and 6 cps, $C_3\langle^{H}_{OH}$), 3.70 (1H, sext, J=13.5 and 5.5 cps, $C_{11}\langle^{H}_{OH}$), 4.52 (1H, sext, J=8.5 and 3.5 cps, $C_{23}\langle^{H}_{OAc}$). *Anal.* Calcd. for $C_{32}H_{52}O_5 \cdot H_2O$: C, 71.76; H, 10.18. Found: C, 71.87; H, 10.18.

Acetylation of compound **11** with Ac_2O–C_5H_5N at room temperature gave the triacetate **12**, and treatment of **11** with K_2CO_3 in hot MeOH for 1 hr afforded **10**.

$LiAlH_4$ Reduction of 2 (Formation of 13 and 14)——To a stirred solution of $LiAlH_4$ (6 g) in tetrahydrofuran (200 ml), compound **2** (3 g) dissolved in tetrahydrofuran (30 ml) was added dropwise at room temperature and the mixture was then gently refluxed for 1 hr. After decomposition of the reagent with AcOEt, saturated aq. Na_2SO_4 was added and the mixture was extracted with ether. The ethereal extract was washed with water, dried and evaporated to dryness to give colorless powder (3.1 g). 1.3 g of the product was acetylated with Ac_2O (4 ml) and C_5H_5N (4 ml) at room temperature for 24 hr. The acetate mixture (**13** and **14**) thus obtained was chromatographed on silica gel (75 g). Elution with benzene–acetone (10:1) afforded colorless crystals (**13**) (1 g) and colorless oil (**14**) (0.5 g), successively. Recrystallization of **13** from MeOH gave colorless pillars, mp 177—178°, $[\alpha]_D^{22}$ +14.0°. Mass Spectro (*m/e*): 584 (M^+ –AcOH). IR ν_{max}^{Nujol} cm^{-1}: 1745 (shoulder) and 1730 (acetyl), no OH absorption. NMR (δ): 2.00, 2.05 and 2.07 (total 12H, $OCOCH_3$×4), *ca.* 4.6 (total 4H, overlapped signals, CH–OAc×4). *Anal.* Calcd. for $C_{38}H_{60}O_8$ (tetrol tetraacetate **13**): C, 70.77; H, 9.38. Found: C, 70.68; H, 9.59.

Compound **14** was unaffected by further acetylation at room temperature. After further chromatography under the same condition as described above, the compound was crystallized from MeOH to afford colorless plates, mp 87—88°. $[\alpha]_D^{24}$ +47.5°. Mass Spectro (*m/e*): 542 (M^+ –AcOH), 524 (542 $-H_2O$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (OH), 1740 (acetyl). NMR (δ): 1.99 (3H, s, $OCOCH_3$), 2.06 (6H, s, $OCOCH_3$×2), *ca.* 4.6 (3H, overlapped signals, CH–OAc×3). *Anal.* Calcd. for $C_{36}H_{58}O_7$ (tetrol triacetate **14**): C, 71.72; H, 9.70. Found: C, 71.23; H, 9.49.

Dehydration of Alisol A Triacetate (7) to 16——A solution of **7** (100 mg) in C_5H_5N (3 ml) was treated with $SOCl_2$ (0.2 ml) at −5° and the mixture was allowed to stand at 0° for 40 min. After dilution with ice-water, the product was extracted with AcOEt. The AcOEt solution was washed successively with water, aq. 2N HCl and water, and dried. After removal of the solvent the residue was chromatographed on silica gel (20 g) and eluted with benzene–acetone (12:1) to give 60 mg of **16**, which was recrystallized from MeOH to afford colorless needles, mp 165°. $[\alpha]_D^{23}$ +64°. Mass (*m/e*): 538 (M^+−AcOH). IR ν_{max}^{Nujol} cm^{-1}: 3085, 1655 and 900 (exomethylene), 1710 (ring ketone), 1745 and 1730 (acetyl). NMR (δ): 1.69 (3H, s, CH_3–C=C), 4.92 (2H, s, >C=CH_2). *Anal.* Calcd. for $C_{36}H_{54}O_7$: C, 72.21; H, 9.09. Found: C, 72.06; H, 8.87.

Catalytic Hydrogenation of 16 to 17——A solution of **16** (290 mg) in MeOH (20 ml) was shaken with 5% Pd–C (200 mg) at room temperature under one atomospheric H_2. In 30 min, 0.86 mole H_2 were absorbed and the consumption ceased. The catalyst was removed and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (20 g) and eluted with benzene–acetone (12:1). The product was crystallized from MeOH to give colorless plates of **17**, mp 196—197°. $[\alpha]_D^{24}$ +61.7°. *Anal.* Calcd. for $C_{36}H_{56}O_7$: C, 71.96; H, 9.40. Found: C, 71.80; H, 9.54.

$NaBH_4$ Reduction of 17 (Formation of 13)——To a stirred solution of **17** (70 mg) in EtOH (20 ml), $NaBH_4$ (50 mg) was added over 20 min at room temperature. After stirring for further 40 min the reaction mixture was processed as in the reduction of **2** or **4**, and the product was acetylated with Ac_2O–C_5H_5N at room temperature for 16 hr. The acetylation product (**13**) (80 mg) was recrystallized from MeOH to afford 40 mg of colorless pillars, mp 177—178°, $[\alpha]_D^{23}$ +13.5°, alone or in admixture with the compound obtained from alisol B (**2**). The IR spectra were superimposable.

CrO_3 Oxidation of Alisol B 23-Monoacetate (4)——a) Compound **4** (1 g), dissolved in C_5H_5N (10 ml) was oxidized with CrO_3 (1 g) in C_5H_5N (10 ml) at room temperature for 16 hr. After dilution with water the mixture was extracted with ether. The ethereal extract was filtered with the aid of Celite to remove a gel, and the filtrate was washed with water, dried and evaporated. The residue was then chromatographed on silica gel (50 g) and eluted with benzene–acetone (10:1) to give 700 mg of faint-yellow plates of **20**. Recrystallization from AcOEt, after further chromatography of the product, afforded faint-yellow plates, mp 203—205°, $[\alpha]_D^{23}$ −13.4°. Yield was 400 mg. Mass Spectrum (*m/e*): 510 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1750 (acetyl), 1710 (C_3-ketone), 1640, 1625 and 1600 (CO–C=C–C=C). UV λ_{max}^{EtOH} mμ (ε): 292 (1.6×10^4). NMR (δ): 2.01 (3H, s, $OCOCH_3$), 2.71 (1H, d, J=8.0 cps, C_{24}–H), 4.69 (1H, q, J=14.0 and 8.0 cps, C_{23}–H), 5 62 (1H, s, C_{12}–H), 6.12 (1H, t, J=2.5 cps, C_{16}–H). *Anal.* Calcd. for $C_{32}H_{46}O_5$: C, 75.26; H, 9.08. Found: C, 75.29; H, 9.10.

b) Compound **4** (1 g), dissolved in C_5H_5N (8 ml), was oxidized with CrO_3 (300 mg) in C_5H_5N (3 ml) at room temperature for 16 hr The reaction mixture was processed as a) above, and the product was chromatographed on silica gel (50 g) Elution with benzene–acetone (12:1) gave colorless crystals of **19** (450 mg) which was further purified by silica gel chromatography. Recrystallization from MeOH afforded colorless plates, mp 177—178°, $[\alpha]_D^{23}$ +130°. IR ν_{max}^{KBr} cm^{-1}: 1745 (acetyl), 1710 (C_3– and C_{11}–ketones). NMR (δ): 2.05 (3H, s, $OCOCH_3$), 2.69 (1H, d, J=8.5 cps, C_{24}–H), 2.87 (2H, s, $C_{12}<^{H}_{H}$), 4.60 (1H, sext, J=8.5 and 3.0 cps, C_{23}–H). *Anal.* Calcd. for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.58; H, 9.35.

CrO_3 Oxidation of 19 (Formation of 20)——To a solution of **19** (80 mg) in C_5H_5N (1 ml), CrO_3 (50 mg) in C_5H_5N (1 ml) was added and the mixture was allowed to stand at room temperature for 16 hr. The product was chromatographed on 10 g of silica gel which was eluted with benzene–acetone (10:1) to give 40 mg of **20**. Recrystallization from AcOEt afforded faint-yellow plates, mp 200—202°, identified with the compound **20** prepared as described under a) above by admixture and comparison of the IR spectra.

Dehydration of 4 to 18——To a solution of **4** (500 mg) in C_5H_5N (15 ml), $POCl_3$ (0.6 ml) was added dropwise with ice-cooling, and the mixture was then allowed to stand at room temperature for 1 hr. After dilution with water the mixture was extracted with AcOEt and the AcOEt extract was washed with water and dried. After removal of the solvent the residue was chromatographed on silica gel (30 g) and eluted with benzene–acetone (20:1) to give an oil of **18** (200 mg), which was crystallized from MeOH giving colorless plates, mp 160—163° (sintered at 155°). $[\alpha]_D^{24}$ +53°. UV λ_{max}^{EtOH} mμ (ε): 245 (1.67×10^4), 253 (1.89×10^4), 262 (1.24×10^4). NMR (δ): 2.04 (3H, s, $OCOCH_3$), 2.71 (1H, d, J=8.5 cps, C_{24}–H), 4.60 (1H, sext, J=8.5 and 4.5 cps, C_{23}–H), 5.77 (1H, d, J=10.0 cps) and 6.10 (1H, double d, J=10.0 and 2.5 cps) (C_{11}– and C_{12}–H). *Anal.* Calcd. for $C_{32}H_{48}O_4$: C, 77.37; H, 9.74. Found: C, 77.18; H, 9.52.

Alisol C Monoacetate (21)——Colorless pillars from MeOH, mp 232—233°. $[\alpha]_D^{24}$ +102.9°. Mass (*m/e*): 528 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 1745 (acetyl), 1705 (ring ketone at C_3), 1690 and 1642 (CO–C=C). UV λ_{max}^{EtOH} mμ (ε): 246 (1.40×10^4). NMR (δ): 2.05 (3H, s, $OCOCH_3$), 2.70 (1H, d, J=9.0 cps, C_{24}–H), 3.96 (1H, sext, J=11.0 and 6.0 cps, C_{11}–H), 4.49 (1H, broad sext-like signal, C_{23}–H).

Alisol C Diacetate (22)——Compound **21** (200 mg) was acetylated with Ac_2O (0.8 ml) and C_5H_5N (1 ml) at room temperature for 18 hr. Work up in the usual manner yielded 200 mg of the diacetate **22**. Recrystallization from MeOH gave colorless plates, mp 126°/142—144°. $[\alpha]_D^{25}$ +98.2°. IR ν_{max}^{KBr} cm^{-1}: 1745 (acetyl), 1700 (C_3– and C_{16}-ketones), 1650 (C=C). *Anal.* Calcd. for $C_{34}H_{50}O_7$: C, 71.55; H, 8.83. Found: C, 71.27; H, 8.57.

Dehydration of 21 to 23——To a solution of **21** (500 mg) in C_5H_5N (6 ml), $SOCl_2$ (0.25 ml) was added dropwise at −30°. After 30 min at 0°, the mixture was poured into ice–water and the resulting aqueous mixture was extracted with AcOEt. The organic layer was washed with water, dried and evaporated. The residue was chromatographed on silica gel (30 g) and eluted with benzene–acetone (12:1) to afford an oil, which was crystallized from MeOH to form colorless plates of **23**, mp 128—130° (sintered at 125°). $[\alpha]_D^{25}$ +47.8°. IR ν_{max}^{KBr} cm^{-1}: 1745 (acetyl), 1698 (C_3-ketone), 1688 and 1625 (C=C–C=C–CO). UV λ_{max}^{EtOH} $m\mu$ (ε): 286.5 (1.77×10^4). NMR (δ): 1.98 (3H, s, $OCOCH_3$), 2.68 (1H, d, J=8.5 cps, C_{24}–H), 4.58 (1H, o, J=10.0, 8.5 and 2.5 cps, C_{23}–H), 6.11 (1H, q, J=10.0 and 2.0 cps) and 6.47 (1H, q, J=10.0 and 3.0 cps) (C_{11}– and C_{12}–H). *Anal.* Calcd. for $C_{32}H_{46}O_5$: C, 75.26; H, 9.08. Found: C, 74.96; H, 8.88.

Oxidation of 8 to 22——To a stirred solution of **8** (500 mg) in AcOH (5 ml), CrO_3 (200 mg) dissolved in 95% AcOH (2.5 ml) was added with ice-cooling over 3 min. After 7 min at room temperature, the reaction mixture was diluted with water, and precipitates, which formed, were dissolved in ether. The ethereal solution was washed with water, dried and evaporated, and the residue was chromatographed on silica gel (30 g). Elution with benzene–acetone (20:1) recovered the starting material **8** (120 mg). The column was then eluted with benzene–acetone (15:1) to afford **22** (60 mg). Recrystallization from MeOH gave colorless plates, mp 126°/142—144°, undepressed in admixture with the compound obtained by acetylation of **21**. $[\alpha]_D^{24}$ +101.0°. The IR spectra of the two products were also identical.

Hydration of 21 with Acid (Formation of 24)——To a solution of **21** (600 mg) in a mixture of dioxane (10 ml) and H_2O (2 ml), *p*-toluenesulfonic acid (100 mg) was added, and the mixture was heated at 60° for 20 min. The product was submitted to column chromatography on silica gel (50 g). After elution with 200 ml of benzene–acetone (10:1), the column was eluted with benzene–acetone (5:1) to afford colorless crystals (350 mg). 160 mg of the crystals were acetylated with Ac_2O and C_5H_5N at room temperature for 17 hr, and the acetylation product was chromatographed on silica gel (15 g) using benzene–acetone (7:1) as an eluent to yield an oil of **24** (110 mg). Crystallization from aq. MeOH gave colorless thin plates, mp 175—176°, undepressed in admixture with the compound prepared by chromic acid oxidation of alisol A triacetate (**7**).[1b] IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490 (OH), 1735 (acetyl), 1690 (C_3– and C_{16}-ketones), 1642 (C=C). UV λ_{max}^{EtOH} $m\mu$ (ε): 242 (1.56×10^4). 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}– 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, 67.35; H, 8.34.

Hydration of 2 with Acids——a) To a solution of **2** (500 mg) in a mixture of dioxane (2 ml) and water (1 ml), *p*-toluenesulfonic acid (100 mg) was added and the mixture was allowed to stand at room temperature for 1 hr. After dilution with water (30 ml) the reaction mixture was extracted with AcOEt (2×20 ml) and the combined AcOEt extracts were washed with water, dried and evaporated to dryness. The residue (480 mg) was then acetylated with Ac_2O (3 ml) and C_5H_5N (3 ml) at room temperature for 18 hr. The acetate (630 mg) thus obtained was chromatographed on silica gel (20 g) and eluted with benzene–acetone (7:1) to afford 390 mg of alisol A triacetate (**7**). Recrystallization from CH_2Cl_2–MeOH gave colorless needles, mp 231—233°, $[\alpha]_D^{22}$ +55°. The melting point was undepressed in admixture with an authentic sample of **7**. The IR spectra were also identical.

b) To a solution of **2** (1.1 g) in a mixture of dioxane (4 ml) and water (2 ml), *p*-toluenesulfonic acid (100 mg) was added and the mixture was left to stand at room temperature for 1 hr. The reaction mixture was processed as above a), and the hydration product (1.2 g) was chromatographed on silica gel (25 g). Elution with benzene–acetone (4:1) afforded the tetranoraldehyde **9** (110 mg), the starting material **2** (205 mg) and 25-anhydroalisol A (**15**) (55 mg), successively. The column was then eluted with benzene–acetone (1:1) to give alisol A (**1**) (600 mg). Compounds **9** and **2**, obtained above, were identified with an authentic specimen, respectively through admixture and comparison of the IR spectra.

Recrystallization of **15** from AcOEt yielded colorless plates, mp 155—157°, $[\alpha]_D^{22}$ +104.3°. IR ν_{max}^{KBr} cm^{-1}: 3080, 1655 and 900 (exomethylene), 1695 (ring ketone). NMR (δ): 1.60 (3H, s, CH_3–C=C), 3.24 (1H, sext, J=8.0 and 3.0 cps, C_{23}–H), 3.66 (1H, d, J=8.0 cps, C_{24}–H), 3.80 (1H, m, C_{11}–H), 4.83 (2H, s, >C=CH_2). Acetylation of **15** with Ac_2O and C_5H_5N and work up in the usual way gave the triacetate **16** which was recrystallized from MeOH affording colorless needles, mp 165°. The acetate was identified with the compound prepared by dehydration of alisol A triacetate (**7**).

The IR spectrum of alisol A (**1**), obtained above, was identical with that of an authentic sample.

c) Compound **2** (500 mg), dissolved in a mixture of dioxane (2 ml) and water (1 ml), was hydrated with 10% aq. H_2SO_4 (0.05 ml) at room temperature for 1.5 hr. The product was acetylated and silica gel chromatography of the acetate (630 mg) gave 310 mg of pure compound **7**. No *epi*-alisol A triacetate (**28**) was noticed in TLC ($CHCl_3$–AcOEt (3:1) as a solvent).

Hydration of 4 with Acid——A mixture of **4** (1 g), dioxane (2 ml), water (1 ml) and oxalic acid (0.3 g) was heated at 70° for 2 hr. After dilution with water (20 ml), the resulting precipitates were collected, washed with water and dried. The product (1 g) was then washed on a filter with AcOEt leaving 700 mg of alisol A 24-monoacetate (**3**). Recrystallization from acetone gave colorless prisms, mp 194—196°, alone or in admixture with an authentic specimen of **3**. $[\alpha]_D^{22}$ +78.5°. The IR spectra were also superimposable.

Hydration of 8 with Acid——A mixture of **8** (1 g), dioxane (4 ml) and aq. 2N H_2SO_4 (0.5 ml) was heated at 60° for 15 min. After dilution with AcOEt (30 ml), the solution was washed with water (2×20 ml),

dried and evaporated. Upon scratching the product crystallized. Filtration and washing of the crystals with a small amount of AcOEt gave pure **5** (445 mg). The combined filtrate and washing were evaporated to dryness and the residue (520 mg) was chromatographed on silica gel (20 g). Elution with benzene–acetone (7:1) afforded a second crop of **5** (370 mg), which was recrystallized from AcOEt to yield 173 mg of pure **5**. Compound **5** thus obtained was identified with an authentic specimen by admixture and comparison of the IR spectra.

The mother liquor of the second crop of **5** was evaporated and the residue (190 mg) was acetylated with Ac_2O and C_5H_5N at room temperature for 6 hr. The acetylation product was chromatographed on silica gel (20 g) and eluted with benzene–acetone (8:1) to give alisol A triacetate (**7**) (150 mg) and epi-alisol A triacetate (**28**) (12 mg); the products were identified by IR spectral comparison with an authentic specimen, respectively.

Acetonization of 2 to 31——To a solution of **2** (1 g) in acetone (10 ml), BF_3 etherate (0.2 ml) was added and the mixture was allowed to stand at room temperature for 40 min. After neutralization with aq. $NaHCO_3$, the mixture was diluted with water (30 ml) and extracted with AcOEt (30 ml). The AcOEt layer was washed with water, dried and evaporated. The residue (1.18 g) was chromatographed on silica gel (30 g) and eluted with benzene–acetone (6:1) to give 690 mg of **31** as colorless powder. $[\alpha]_D^{22}$ +69.0°. The compound was identified by IR spectral comparison with the product prepared by acetonization of alisol A (**1**).[1b]

Acetonization of 4 to 34——To a solution of **4** (120 mg) in acetone (4 ml), BF_3 etherate (0.05 ml) was added at room temperature. After standing for 15 min, the mixture was diluted with AcOEt (30 ml) and the solution was washed successively with water, 5%. aq. $NaHCO_3$, and water. The organic layer was dried and evaporated to dryness and the residue (130 mg) was chromatographed on silica gel (20 g) using benzene–acetone (10:1) as an eluent to yield 100 mg of an oil. The oil was crystallized from aq. MeOH to afford colorless needles of **34**, mp 192—193°, alone or in admixture with the compound obtained through acetonization of alisol A 24-monoacetate (**3**).[1b] The IR spectra were also identical.

Acetonization of 8 to 35——To a solution of **8** (400 mg) in acetone (10 ml), BF_3 etherate (0.1 ml) was added and the mixture was allowed to stand at room temperature for 20 min. The reaction mixture was processed as above and the product (400 mg) was chromatographed on silica gel (25 g). Elution with benzene–acetone (13:1) gave a colorless oil of **35** (280 mg), which was crystallized from aq. MeOH to afford colorless needles, mp 154—155°. The melting point was undepressed in admixture with the compound prepared by acetonization of alisol A 11,24-diacetate (**5**).[1b] The IR spectra were also identical.

Hydration of 2 with Alkali (Formation of Epi-alisol A (25))——a) A mixture of **2** (1 g), MeOH (20 ml), water (16 ml) and K_2CO_3 (1 g) was refluxed for 30 hr. After dilution with water, the mixture was extracted with AcOEt and the extract was washed with water, dried and evaporated. The residue was chromatographed on silica gel (30 g) and eluted with benzene–acetone (5:1) to recover the starting material (700 mg). The column was then eluted with bezene–MeOH (2:1) to afford 100 mg of **25**, which was purified by further chromatography on silica gel (25 g) using benzene–acetone (2:3) as an eluant to give 70 mg of colorless powder of **25**. The compound did not crystallize. $[\alpha]_D^{22}$ +81.4°. *Anal.* Calcd. for $C_{30}H_{50}O_5$: C, 73.43; H, 10.27. Found: C, 73.70; H, 10.23. Treatment of alisol B 11,23-diacetate (**8**) under same condition yielded **25** in a similar yield.

Compound **25** was acetylated with Ac_2O–C_5H_5N at room temperature for 10 hr, and the product was recrystallized from MeOH to give colorless prisms of **28**, mp 192—194°. $[\alpha]_D^{22}$ +67.5°. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1740 (acetyl), 1690 (ring ketone). *Anal.* Calcd. for $C_{36}H_{56}O_8$: C, 70.10; H, 9.15. Found: C, 70.13; H, 9.04.

b) A mixture of **2** (500 mg), MeOH (20 ml), water (16 ml) and K_2CO_3 (250 mg) was heated at 150° for 16 hr in a sealed tube. The reaction mixture was processed as above a), and the product (440 mg) was chromatographed on silica gel (25 g), using benzene–acetone (1:2) as an eluent. Repeated chromatography under similar condition gave rise to epi-alisol A (**25**) (300 mg) and alisol A (50 mg). These products were indistinguishable from an authentic specimen in the IR spectra, respectively. The identity was also confirmed as their acetates **28** and **7**, respectively.

Isolation of 25 after Saponification of the Lipid Fraction of Alismatis Rhizoma——The neutral lipid fraction A-1 (3.3 kg),[1a] obtained from the crude drug (100 kg) of Korean origin, was dissolved in MeOH (40 liters). Anhydrous K_2CO_3 (2.4 kg) was added to the solution and the mixture was refluxed for 4 hr with stirring. After concentration water (40 liters) was added to the residue and the mixture was extracted with AcOEt (2×40 liters). The AcOEt layers were combined, washed with water, dried and evaporated to afford an unsaponifiable lipid fraction (2.15 kg). One-half of this fraction was chromatographed on charcoal (1.74 kg); the results are shown in Table I (Chromatography-1).

The fraction 4 in Table I was combined with the corresponding fraction obtained by the chromatography of another one-half of the unsaponifiable fraction (total 157 g), and then rechromatographed on 1.08 kg of silica gel; the results are given in Table II (chromatography-2).

The fractions 6 (20 g) and 7 (6 g) in Table II were combined and acetylated with Ac_2O (150 ml) and C_5H_5N (100 ml) at room temperature for 18 hr. Work up in the usual way afforded 25.4 g mixture of **7** and **28**, which was submitted to column-chromatography using 1.2 kg of silica gel. Elution with benzene–acetone (8:1) gave 5.7 g of **28**, 6.2 g of **7** and 8.9 g mixture of the two compounds. The mixture part was

Table I. Chromatography-1

Fraction	Eluent	Volume (liter)	Yield (g)	Alisol
1	benzene	40	813	**2**
2	benzene	40	40	**2**
3	benzene	40	19	**2**
4	AcOEt	150	78	**1, 25**
5	AcOEt–MeOH (1:1)	50	15	**1**

Table II. Chromatography-2

Fraction	Eluent	Volume (liter)	Yield (g)
1	benzene–acetone (5:1)	4.0	41.4
2	benzene–acetone (5:1)	3.5	26.9
3	benzene–acetone (5:1)	3.5	13.9
4	benzene–acetone (5:1)	3.5	10.0
5	benzene–acetone (3:1)	3.0	16.0
6	benzene–acetone (3:1)	6.0	22.1
7	benzene–acetone (3:1)	6.0	6.0
8	benzene–acetone (1:1)	6.0	8.8
9	acetone	6.0	3.4

rechromatographed under similar condition to afford additional **28** (2.0 g) and **7** (6.1 g). Total yields were 7.7 g of **28** and 13.3 g of **7**, respectively. Compounds **28** and **7**, thus obtained, were identified with an authentic sample, respectively, by admixture and comparison of the IR spectra.

HIO_4 Oxidation of 25——Compound **25** (350 mg), dissolved in a mixture of dioxane (8 ml) and water (4 ml) was oxidized with $HIO_4 \cdot 2H_2O$ (350 mg). Work up in the same way as in the case of alisol A (**1**) afforded 230 mg of **9**, mp 190°, and 85 mg of acetone 2,4-dinitrophenylhydrazone, mp 123—124° (decomp.). The products were identified with an authentic specimen, respectively by admixture and comparison of the IR spectra.

Partial Deacetylation of 28 (Formation of 26 and 27)——A mixture of **28** (3.5 g), MeOH (100 ml) and anhydrous NaOAc (1.8 g) was refluxed for 3 hr. After concentration of the mixture to about one-fourth volume, water was added to the residue and the resulting oil was extracted with AcOEt. The product (3.3 g), thus obtained, was chromatographed on silica gel (100 g). Elution with $CHCl_3$–AcOEt (10:1) recovered the starting material **28** (0.7 g) and subsequent elution with $CHCl_3$–AcOEt (3:1) gave a mixture (1.2 g) of **26** and **27** as an oil. Upon scratching the oil with a few drops of EtOH, crude crystals of **26** separated, which were collected and washed with EtOH. Yield was 600 mg. The product was purified through silica gel chromatography using $CHCl_3$-AcOEt (3:2) as an eluent to afford 480 mg of **26**, mp 106—108°. Recrystallization from MeOH raised the melting point to 108—110°. $[\alpha]_D^{23}$ +74°. NMR (δ): 1.92 (3H, s, $OCOCH_3$), 1.94 (3H, s, $OCOCH_3$), 3.41 (1H, d, J=2.0 cps, C_{24}–H), 4.58 (1H, broad sext, J=6 and 2 cps, C_{23}–H), 4.81 (1H, sext, J=12.0 and 6.0 cps, C_{11}–H). *Anal.* Calcd. for $C_{34}H_{54}O_7 \cdot \frac{1}{2}H_2O$: C, 69.95; H, 9.50. Found: C, 69.86; H, 9.13. Acetylation of **26** gave the triacetate **28**.

The mother liquor of the crude crystals of **26**, above mentioned, and the washing were combined and evaporated. Repeated silica gel chromatography of the residue using $CHCl_3$–AcOEt (2:1) yielded 150 mg of **27** as colorless powder which according to TLC ($CHCl_3$–AcOEt (1:1) as a solvent) was pure. The product did not crystallize. $[\alpha]_D^{22}$ +102°. NMR (δ): 1.93 (3H, s, $OCOCH_3$), 1.98 (3H, s, $OCOCH_3$), 3.43 (1H, sext, J=8.0 and 3.0 cps, C_{23}–H), 4.52 (1H, d, J=8.0 cps, C_{24}–H), 4.88 (1H, sext, J=11.0 and 6.0 cps, C_{11}–H). *Anal.* Calcd. for $C_{34}H_{54}O_7$: C, 71.04; H, 9.47. Found: C, 71.03; H, 9.36. Acetylation of **27** also afforded **28**. Compound **27** had an *Rf* value slightly larger than that of **26** in TLC. Treatment of **27** with glacial AcOH at 80° for 1 hr gave a mixture, which according to TLC was an approximately 3:1 mixture of **26** and **27**.

HIO_4 Oxidation of Epi-alisol A 11,23-Diacetate (26)——To a stirred solution of **26** (250 mg) in dioxane (1 ml), $HIO_4 \cdot 2H_2O$ (150 mg) in water (0.5 ml) was added at room temperature during 10 min. The product was chromatographed on silica gel (15 g) and eluted with benzene–acetone (10:1) to afford 170 mg of an oil. $[\alpha]_D^{23}$ +108.2° (c=0.17). The IR and NMR spectra of the oil were superimposable with that of compound **36** obtained by the same oxidation of alisol A 11,24-diacetate (**5**).[1b]

Acetonization of Epi-alisol A (25) to 37——To a solution of **25** (550 mg) in acetone (10 ml), BF_3 etherate (0.1 ml) was added at room temperature and the mixture was allowed to stand for 5 min. After neutralization with aq. $NaHCO_3$, the mixture was diluted with AcOEt. The AcOEt solution was then washed with water, dried and evaporated to leave an oil which was acetylated with C_5H_5N (5 ml) and Ac_2O (2 ml) at room temperature for 18 hr. The acetate thus obtained was chromatographed on silica gel (50 g) and eluted with benzene–acetone (12:1) to yield 400 mg of an oil, which upon scratching crystallized. Recrystallization from MeOH gave colorless prisms of **37**, mp 167—168°, $[\alpha]_D^{24}$ +92.7°. IR ν_{max}^{Nujol} cm^{-1}: 1750 and 1735 (acetyl), 1700 (ring ketone). No OH band was observed in the IR spectrum. NMR (δ): 1.98 (3H, s, $OCOCH_3$), 2.00 (3H, s, $OCOCH_3$), 3.64 (1H, d, J=7 cps, C_{24}–H), 4.71 (1H, broad sext, J=7 and 3 cps, C_{23}–H), 4.94 (1H, sext, J=11.0 and 6.0 cps, C_{11}–H). *Anal.* Calcd. for $C_{37}H_{58}O_7$: C, 72.27; H, 9.51. Found: C, 72.37; H, 9.48.

Acetolysis of Alisol B (2)——A mixture of **2** (2.0 g) and glacial AcOH (20 ml) was heated at 90° for 50 min. After evaporation of the solvent, AcOEt (6 ml) was added to the residue and crystals, which separated, were collected and washed with AcOEt to yield 840 mg of **3**. The filtrate and the washing were combined and after evaporation of the solvent, the residue was chromatographed on silica gel (20 g). The results of the chromatography are shown in Table III.

Table III. Chromatographic Separation of the Mother Liquor of 3

Fraction	Eluent	Volume (ml)	Yield (mg)	Constituent
1	benzene–acetone (7:1)	250	460	9
2	benzene–acetone (5:1)	200	170	
3	benzene–acetone (4:1)	350	750	3, 15
4	benzene–acetone (3:1)	200	55	3, 15
5	benzene–acetone (3:1)	200	65	1
6	benzene–acetone (1:1)	250	40	1

The fractions 3 and 4 of Table III were combined and treated with AcOEt. The resulting crystals were collected and washed with AcOEt to give a second crop (430 mg) of **3**. Total yield was 1.27 g. Recrystallization from CH_2Cl_2–acetone gave pure **3**, mp 194—196°, alone or in admixture with an authentic specimen of natural origin. The IR spectra of the two products were also identical.

The mother liquor of the second crop of **3** and the washing, described above, were combined and evaporated. The residue was then chromatographed on silica gel (20 g) and eluted with benzene–acetone (5:1) to afford 120 mg of **3** and 120 mg of **15**. The latter was again chromatographed to give pure **15** (70 mg), which was crystallized from AcOEt to yield colorless plates, mp 155—157°, alone or in admixture with the compound prepared by hydration of **2** with acid. The IR spectra of the two products were superimposable.

The fraction 1 of Table III was treated with a few drops of AcOEt to give 90 mg of crude **9**, which was recrystallized from CH_2Cl_2–AcOEt to afford colorless prisms, mp 190°, undepressed in admixture with the product obtained by HIO_4 oxidation of **2**.

The fractions 5 and 6 were combined and evaporated to dryness. The residue according to TLC was pure alisol A (**1**). The IR spectrum was identical with that of an authentic sample of **1**.

Acetolysis of Alisol B 11,23-Diacetate (8)——A mixture of **8** (35 g) and glacial AcOH (350 ml) was heated at 90° for 3 hr. After evaporation of the solvent the residue was treated with 20 ml of AcOEt to crystallize. The crystals were collected and washed with AcOEt, being shown to be mainly composed of the starting material **8**, alisol A 11,24-diacetate (**5**) and alisol A triacetate (**7**) together with epi-alisol A 11,23-diacetate (**26**) according to TLC, while the mother liquor and the washing contained epi-alisol A 11,24-diacetate (**27**) besides the compounds described above. The crystalline and filtrate parts were separately chromatographed on silica gel using benzene–acetone (20:1—5:1) as an eluent. After repeated chromatography the starting mateial **8** (2.18 g), **5** (12.14 g), **7** (8.52 g), **26** (3.01 g) and **27** (975 mg) were obtained. These compounds were identified with an authentic sample, respectively by IR spectral comparison.

Alisol A (23, 24)-Acetonide 11,25-Diacetate (33)——Alisol A (23, 24)-acetonide 11-monoacetate (**32**) (750 mg), dissolved in a mixture of Ac_2O (4 ml) and C_5H_5N (4 ml), was heated at 170° for 12 hr. After decomposition of the reaction mixture with ice–water, the resulting precipitates were collected and washed with water. Chromatography of the product on silica gel (30 g) using benzene–acetone (15:1) as an eluent yielded an oil of **33** (600 mg) which gave a single spot on TLC plate (For TLC benzene–acetone (5:1) was used). The compound did not crystallize. Its IR spectrum showed no OH band (CCl_4). NMR (δ): 1.92 (3H, s, $OCOCH_3$), 1.98 (3H, s, $OCOCH_3$), 3.59 (2H, broad s, C_{23}– and C_{24}–H), 4.92 (1H, sext, C_{11}–H). *Anal.* Calcd. for $C_{37}H_{58}O_7$: C, 72.27; H, 9.51. Found: C, 72.26; H, 9.36.

A solution of **33** (50 mg), prepared above, in 2% methanolic KOH (4 ml) was refluxed for 1 hr. After dilution with water, the mixture was extracted with AcOEt, and the AcOEt extract was washed with water and dried. After removal of the solvent the residue (45 mg) was identified with an authentic sample of alisol A (23, 24)-acetonide (**31**) by IR spectral comparison as well as by TLC using benzene–acetone (3:1) as a solvent. The product was then acetylated with Ac_2O–C_5H_5N at room temperature to afford the 11-monoacetate (**32**); the latter was identified with an authentic sample through admixture and IR spectral comparison.

Deacetonization of 33——A solution of **33** (400 mg) in aq. 80% AcOH (10 ml) was refluxed for 2.5 hr. After evaporation of the solvent the residue was dissolved in $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried and evaporated. The residue (370 mg) was chromatographed on silica gel (20 g) and eluted wth benzene–acetone (10:1) to recover the starting material **33** (90 mg). The column was then eluted with 300 ml of benzene–acetone (5:1) to afford crystals (250 mg) of alisol A 11,24-diacetate (**5**). Recrystallization of the crystals from MeOH gave pure **5**, mp 204—206°, alone or in admixture with an authentic sample of **5**. The mother liquor was evaporated and the residue was acetylated with Ac_2O–C_5H_5N at room temperature to yield alisol A triacetate (**7**).

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